

PHILIPPINE JOURNAL OF PATHOLOGY

The Official Journal of the Philippine Society
of Pathologists, Inc.

IN THIS ISSUE:

FEATURE ARTICLE

National Reference Laboratory Surge Capacity Response to a Massive Nationwide Measles Outbreak in 2013-2014

Amado Tandoc III and Rex Centeno

ORIGINAL ARTICLES

Philippines Diagnostic Pathology Laboratory Benchmarking

Tony Badrick, Jozica Habijanac, Sam Yew Mah, Elizabeth Arcellana-Nuqui

Correlation of Tumor-associated Leukocytes with Prognosis of Colorectal Carcinoma based on Pathologic Stage

John Anthony Tindoc, Emilio Villanueva III, Nelson Geraldino

A Pilot Study on the Evaluation of Clinical Chemistry Laboratory Test Performance using Six Sigma Metrics

Pier Angeli Medina, Jenny Matibag, Sarah Jane Datay-Lim, Elizabeth Arcellana-Nuqui

CASE REPORTS

Gastric Pyloric Gland Adenoma: A Case Report, Review of Literature, and Diagnostic Challenges in the Philippine Setting

Kevin Elomina and Ignacio de Guzman Jr.

Histologic Transformation in an EGFR-Mutant Lung Cancer in a Filipino Patient treated with Afatinib: A Case Report and Review of Literature

Steffanie Charlyne Tamayo, Joanmarie Balolong-Garcia, Michelle Joanne Alcantara, Rubi Li, Daphne Ang, Jose Jasper Andal, Rex Michael Santiago

Targeted Sequencing of Mixed Neuroendocrine- Non-neuroendocrine Neoplasm of the Gallbladder Suggests a Monoclonal Origin: A Case Report

Flora Mae Sta. Ines, Anna Louise Anceno, Rachele Arah Salamat, Narciso Navarro Jr., Glenda Lyn Pua, Jose Jasper Andal, Daphne Ang

BRIEF COMMUNICATIONS

External Quality Assessment Scheme for Transfusion Transmissible Infections Among Blood Service Facilities in the Philippines, 2018

Kenneth Aristotle Punzalan, Rhoda Yu, Iza Mae Chamen



CONRAD
MANILA

CONRAD HOTEL
22nd-24th of April 2020



Warm greetings to all!

I would like to invite you all to our 69th PSP Annual Convention this coming April 22-24, 2020. This will be held at The Conrad Hotel, Manila.

I am sure that everyone will have fun while learning new things and updates in pathology and laboratory medicine with all our renowned speakers coming from different parts of the globe. This will also be a time to meet and greet our friends, and experience a fun-filled fellowship night.

Our supplies will be showcasing new machines and other equipment that will surely fill our desires to improve our laboratories.

I hope to see you all on our 69th Annual Convention.

Sincerely yours,

Robert D. Padua, MD, FPSP
President, Philippine Society of Pathologists, Inc.





Philippine Journal of Pathology
Vol. 4 No. 2 December 2019 | ISSN 2507-8364 (Online)
<http://philippinejournalofpathology.org>



This journal is **OPEN ACCESS**, providing immediate access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. The Philippine Journal of Pathology is licensed under a **Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License**, which allows sharing, copying and redistributing the material in any medium or format, and adaptation, in which the material can be built upon, under strict terms of giving appropriate credit to the authors and this journal, and use for non-commercial purposes.



PRESIDENT'S MESSAGE	3
EDITORIAL	4
FEATURE ARTICLE	
National Reference Laboratory Surge Capacity Response to a Massive Nationwide Measles Outbreak in 2013-2014	6
<i>Amado Tandoc III and Rex Centeno</i>	
ORIGINAL ARTICLES	
Philippines Diagnostic Pathology Laboratory Benchmarking	15
<i>Tony Badrick, Jozica Habijanac, Sam Yew Mah, Elizabeth Arcellana-Nuqui</i>	
Correlation of Tumor-associated Leukocytes with Prognosis of Colorectal Carcinoma based on Pathologic Stage	24
<i>John Anthony Tindoc, Emilio Villanueva III, Nelson Geraldino</i>	
A Pilot Study on the Evaluation of Clinical Chemistry Laboratory Test Performance using Six Sigma Metrics	31
<i>Pier Angeli Medina, Jenny Matibag, Sarah Jane Datay-Lim, Elizabeth Arcellana-Nuqui</i>	
CASE REPORTS	
Gastric Pyloric Gland Adenoma: A Case Report, Review of Literature, and Diagnostic Challenges in the Philippine Setting	38
<i>Kevin Elomina and Ignacio de Guzman Jr.</i>	
Histologic Transformation in an EGFR-Mutant Lung Cancer in a Filipino Patient treated with Afatinib: A Case Report and Review of Literature	43
<i>Steffanie Charlyne Tamayo, Joanmarie Balolong-Garcia, Michelle Joanne Alcantara, Rubi Li, Daphne Ang, Jose Jasper Andal, Rex Michael Santiago</i>	
Targeted Sequencing of Mixed Neuroendocrine- Non-neuroendocrine Neoplasm of the Gallbladder Suggests a Monoclonal Origin: A Case Report	48
<i>Flora Mae Sta. Ines, Anna Louise Anceno, Rachele Arah Salamat, Narciso Navarro Jr., Glenda Lyn Pua, Jose Jasper Andal, Daphne Ang</i>	
BRIEF COMMUNICATIONS	
External Quality Assessment Scheme for Transfusion Transmissible Infections Among Blood Service Facilities in the Philippines, 2018	54
<i>Kenneth Aristotle Punzalan, Rhoda Yu, Iza Mae Chamen</i>	
Instructions to Authors	60
Authorship Form	64
ICMJE Form for Disclosure of Potential Conflicts of Interest	65
Patient Consent Form	68
Equator Network Reporting Guidelines	69
OJS Guide to Authors	80
Peer Reviewers	90

AMADO O. TANDOC III
Editor-in-Chief

FRANCIS G. MORIA
Vice Editor-in-Chief

IVY A. ROSALES
MARICEL REGINO-RIBO
FARRAH KRISTINE FONTILA-SANTIAGO
ANN MARGARET V. CHANG
FRANCES SAURA-SANCHEZ
MARIE CHRISTINE F. BERNARDO
DAPHNE CHUA ANG
MA. LOURDES L. GOCO
MARY JANE CARIAS-MARINES
Associate Editors

Editorial Board

AGUSTINA D. ABELARDO
Cytopathology

JOSE M. CARNATE JR.
Head & Neck Pathology

MA. RIZALINA F. CHUA
Blood Banking

NELSON T. GERALDINO
Biochemistry

EVELINA N. LAGAMAYO
Medical Microbiology

RAYMUNDO W. LO
Immunology & Molecular Pathology

MIGUEL MARTIN N. MORENO II
Biosafety/Biosecurity

JANUARIO D. VELOSO
Hematopathology

SOCORRO C. YAÑEZ
Laboratory Quality Assurance

ROWEN T. YOLO
Surgical Pathology

Editorial Advisers

JOSE MA. C. AVILA
MARISSA A. ORILLAZA
MARITA V.T. REYES

Editorial Staff

MELISSA O. TANDOC
Editorial Coordinator

The Philippine Journal of Pathology (PJP) is an open-access, peer-reviewed, English language, medical science journal published by the Philippine Society of Pathologists, Inc. Committee on Publications. It shall serve as the official platform for publication of high quality original articles, case reports or series, feature articles, and editorials covering topics on clinical and anatomic pathology, laboratory medicine and medical technology, diagnostics, laboratory biosafety and biosecurity, as well as laboratory quality assurance. The journal's primary target audience are laboratorians, diagnosticians, laboratory managers, pathologists, medical technologists, and all other medical and scientific disciplines interfacing with the laboratory. The PJP follows the **ICMJE Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals**, **EQUATOR Network Guidelines**, and **COPE Guidelines**. The PJP does not charge any article processing or submission fees from authors. It does not charge any subscription fees or download fees to access content.





Greetings!

Welcome to the December 2019 issue of the Philippine Journal of Pathology. My congratulations to the very hardworking editorial team of PJP who remained steadfast in their determination to come up with this issue and to the unwavering support of the Board of Governors of the Philippine Society of Pathologists, Inc.

As the Philippine Journal of Pathology continues to publish the contributions of our members of their scholarly work, they help us improve our diagnostic ingenuity. Researches and case reports help us in our shrewdness in handling our daily work issues in the Pathology Laboratory.

Let us look forward to the many more issues of the PJP and hope that you can join us as one of its contributors. God speed to the Philippine Society of Pathologists, Inc. and the Philippine Journal of Pathology.

Wishing you all a Merry Christmas and a Prosperous 2020.

A handwritten signature in black ink, appearing to read 'R. Padua Jr.'.

Roberto D. Padua Jr., MD, FPSP, MHA
President, Philippine Society of Pathologists, Inc.

Tiny Sprouts, Heaps of Earth: PJP's Inclusion in the ASEAN Citation Index

“千里之行，始於足下”*

– Lao Tzu, Tao Te Ching, Chapter 64^{1,2}



In what is dubbed as a “21st Century Science Overload,” an average of a quarter of a billion new scientific papers are published each year.^{3,4} Traditional print journals are either shifting to a mixed publication model of print-online or a fully online platform as the world becomes more and more connected by the internet.⁵

Our indexing in ACI is a testament to the quality of our journal and a direct effect of continued support by the Philippine Society of Pathologists. We have met all the selection criteria: 3 years minimum age or at least 6 issues published regularly, citations in national and/or international databases, good diversity in authorship, good diversity in editorial board members, clear journal concepts and policy, uniform journal formats, comprehensive journal website with online submission, and abstract quality; all of these means that our journal is up to standards.

Consequently, the survival of medical journals lies in visibility and accessibility in a virtual sea of digital content. Despite the intention to publish, an invisible manuscript is as worse as an unpublished one. In an increasingly online world, this “visibility” is facilitated through indexing in databases that make it easy for other researchers to find one’s scientific outputs through the scientific “paper deluge.”⁴

The ACI database system was developed through initial funding support from the Thai Office of the Higher Education Commission (OHEC) in 2011, with the objective of increasing the visibility and discoverability of local scientific outputs by ASEAN member states. It is envisioned as a regional platform that shall eventually house all quality publications in ASEAN to stimulate and encourage knowledge sharing, improve journal quality in the ASEAN region, and facilitate indexing in Scopus, Web of Science, and other international indexes. Like the PJP, it is on its own journey of a thousand miles.

I am truly glad to share that our journey to indexing began, not just with a single step, but a leap: our inclusion in the ASEAN Citation Index (Figure 1).

The Tao Te Ching mentions that huge trees grow from tiny sprouts, and terraces nine-storys high are built from heaps of earth,² reminding us that even the longest and most difficult ventures have a starting point. For us, here in our own little corner in the world, hopefully it is one that would lead to a better publishing platform for Filipino pathologists and one that would contribute to a better scientific understanding of disease.

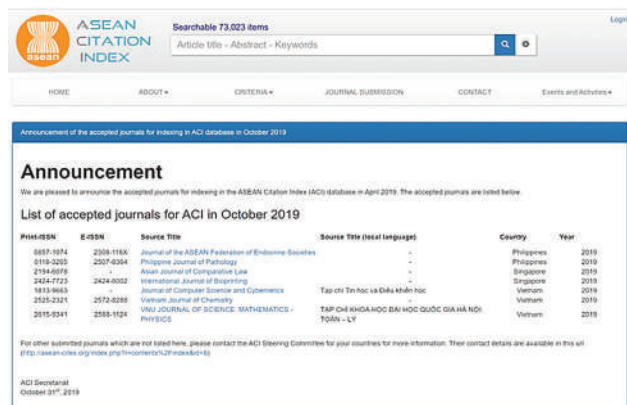


Figure 1. Screenshot of announcement of PJP’s acceptance to the ASEAN Citation Index website.

The ACI is a central regional database established to index bibliographic records of quality research outputs of scholarly journals published by ASEAN countries. The Philippines is a member of the ACI along with Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Singapore, Thailand, and Vietnam.

Amado O. Tandoc III, MD, FPSP
Editor-in-Chief

REFERENCES

1. A journey of a thousand miles begins with a single step. Wikipedia. Retrieved 3 December 2019. Available from https://en.wikipedia.org/wiki/A_journey_of_a_thousand_miles_begins_with_a_single_step.
2. Tao Te Ching. Retrieved 3 December 2019. Available from http://with.org/tao_te_ching_en.pdf.
3. Jinha AE. Article 50 million: an estimate of the number of scholarly articles in existence. Learn. Publishing. 2010;23(3): 258–63. <https://doi.org/10.1087/20100308>.
4. Boon S. 21st Century Science Overload. Canadian Science Publishing, 2017. Retrieved 3 December 2019. Available from <http://blog.cdnsiencepub.com/21st-century-science-overload/>.
5. Ware M, Mabe M. The STM report: an overview of scientific and scholarly journal publishing 4th ed. London: STM Association, 2015. Retrieved 3 December 2019. Available from: http://www.stm-assoc.org/2015_02_20_STM_Report_2015.pdf.

<https://doi.org/10.21141/PJP.2019.09>

* Chinese proverb literally translating to: “A journey of a thousand miles starts beneath one’s feet.”



ASEAN
CITATION
INDEX

ACI Secretariat: c/o 5th Floor, School of Energy Environment and Materials, King
Mongkut's University of Technology Thonburi
126 Prachautid Road, Bangmod, Thung Khru, Bangkok 10140, Thailand
Tel. and Fax +66 2470 8647 Email: aci.cites@gmail.com

31st October 2019

RE: Notification of the journal evaluation result for ACI

Title: Philippine Journal of Pathology
ISSN / E-ISSN : 0118-3265 / 2507-8364
Owner : the Philippine Society of Pathologists, Inc., Philippines

Dear Editor,

The title mentioned above has been evaluated for inclusion in ASEAN Citation Index (ACI) database by the ACI Secretariat. The review of this title is now completed and the ACI Steering Committee has advised **to accept** the title for ACI inclusion in April 2019. Congratulations! The reviewers also suggested that the journal should include more editorial board members from abroad to increase visibility.

To record all the bibliographic data and references of all the journal articles into the System, the following information is required :-

1. General information of the journal (the form is attached) ;
2. Journal Subject Categories for ACI (the list is attached) ;
3. PDF files of all issues of this journal published in 2019.

Please kindly send us the needed information to aci.submitting@gmail.com as soon as possible so that we can proceed with the indexing.

Thank you for your kind co-operation. We look forward to receiving all the requested information.

Your sincerely,

A handwritten signature in black ink, appearing to be 'Narongrit Sombatsompop', written over a horizontal line.

(Prof. Dr. Narongrit Sombatsompop)
Chairman of the ACI Steering Committee

National Reference Laboratory Surge Capacity Response to a Massive Nationwide Measles Outbreak in 2013-2014*

Amado Tandoc III and Rex Centeno

Research Institute for Tropical Medicine-Department of Health, Philippines

ABSTRACT

This management case documents the experience of the Research Institute for Tropical Medicine (RITM) National Reference Laboratory, when a massive nationwide outbreak of Measles occurred during the last quarter of 2013 to the whole of 2014. This was the largest infectious disease outbreak referred thus far to the Institute, with an unprecedented 40,000 blood specimens from all over the country received by the laboratory, overwhelming its testing capacity, and causing large backlogs. The incident revealed significant gaps in the laboratory's preparedness to respond to a sudden large surge of specimens.

The activation of a department-level Incident Command System was the most appropriate management approach to implement due to the urgency and scale of the surge of specimens. The response to the specimen surge was prioritized leading to temporary rearrangements in the organizational structure of the department in order to effectively and rapidly coordinate the staff and allocate resources.

Key words: measles, outbreak, surge capacity, outbreak response, incident command system, laboratory management

ISSN 2507-8364 (Online)
Printed in the Philippines.
Copyright© 2019 by the PJP.
Received: 6 September 2019.
Accepted: 29 October 2019.
Published online first: 29 November 2019.
<https://doi.org/10.21141/PJP.2019.10>

Corresponding author: Amado O. Tandoc III, MD, FPSP
E-mail: amado.tandocmd@gmail.com
ORCID: <https://orcid.org/0000-0002-2186-4989>

**This article is based on a Capstone Paper presented to the Development Academy of the Philippines in partial fulfillment of the author's academic requirements for the Diploma in Public Development Management.*

INTRODUCTION

Background

The Research Institute for Tropical Medicine (RITM) is one of the National Reference Laboratories (NRL) designated by the DOH, following the dissolution of the Bureau of Research and Laboratories in 2000. RITM was particularly assigned as the NRL for most infectious diseases, such as dengue, influenza, polio, measles, tuberculosis, bacterial enteric diseases, mycology, malaria, emerging bacterial diseases, and transfusion-transmissible infections, to include antimicrobial resistance surveillance. As NRL, RITM is tasked to provide laboratory support services to the DOH for patient management as well as public health, i.e., confirmatory testing for disease-specific surveillance, and outbreak investigations.

The National Measles Laboratory (NML), under the Department of Virology, is the specific NRL responsible for testing of serum specimens referred by the DOH measles surveillance (i.e., collected from suspect measles cases identified by the disease reporting units and referred by the local surveillance units to RITM). Accredited by the WHO and a member of the Global Measles-Rubella Laboratory Network, it is the only laboratory in the country performing measles confirmatory serologic testing for the whole Philippines using Enzyme-Linked Immunosorbent Assay (ELISA) platform.

Management Problem

The 2013-2014 Outbreak

From the 1st to 3rd quarter of 2013, measles outbreaks have already been noted in the National Capital Region, followed by Regions 3 and 4A, which further spread to Regions 5 and 6. By the 4th quarter, measles rapidly spread to almost all regions of the country, causing a



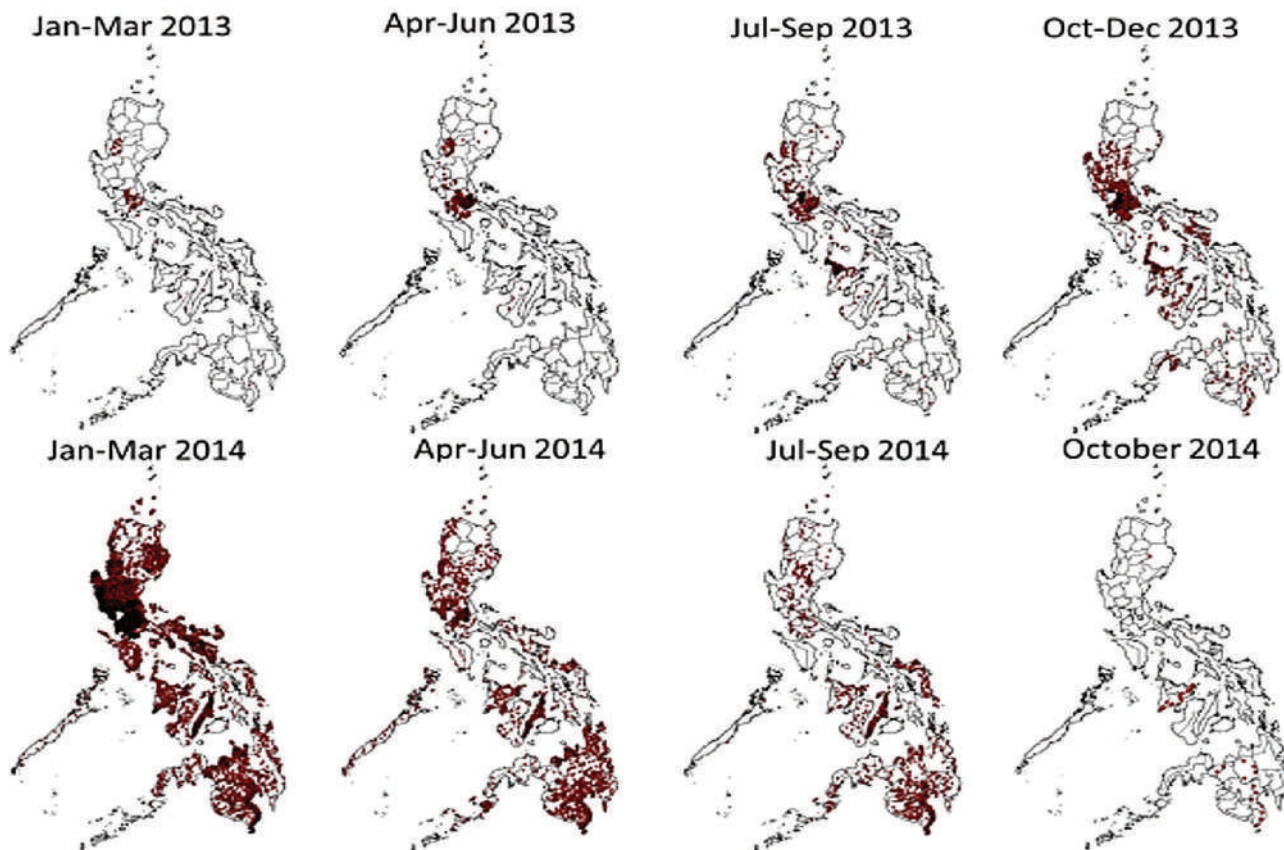


Figure 1. Distribution of measles cases by quarter 2013-2014 (Adapted from Silva, 2016).

sharp increase in measles reporting and investigation (Figure 1).¹ By December, specifically during the last 2 weeks of 2013, blood specimen referrals to the NML rose sharply and remained untested until the 1st two weeks of January 2014 due to the holiday season. This served as the “incident” which prompted laboratory management response.

Management Response

Rapid Baseline Capacity Review

A rapid baseline capacity assessment was conducted (Table 1). Based on the review, it was determined that the NML was not prepared to cope with 100% testing within the acceptable turnaround time of seven (7) days.

Activation of Incident Command System (ICS) at department-level and engagement of other support offices within the agency for improving internal surge capacity

Several bottlenecks were recognized in the set-up of the NML which contributed to the delays in testing. This included receipt and encoding of specimens, specimen processing, and data management/results reporting.

A whole-of-department approach was needed to augment the NML in addressing the incident (i.e., the surge of specimens). To do this, laboratory management decided to activate a department-level Incident Command System. Originally developed to address challenges in inter-agency responses to forest fires/wild fires in the

United States of America, the ICS is a standardized operational management approach to emergency response by providing command (i.e., leadership), control, and coordination mechanisms so that responders from multiple agencies can be effective.^{2,3} It is designed to be used from the time an incident occurs until the requirement for operational management has been completed and/or no longer needed. Its application has evolved to include all hazards situations, and this includes hospital emergencies, public health emergencies, and even outbreaks.^{4,5}

The ICS strategy called for rearrangements within the structure of the department’s organization to provide additional technical support in terms of logistics and procurement of supplies, capacity for testing and data management, in order to allow NML to scale up its operations.

The ICS activation was undertaken with the following objectives:

1. Provide additional manpower to NML to manage all of its operations/processes.
2. Maintain adequate supplies/reagents for continued testing.
3. Ensure generation of timely and accurate laboratory results.
4. Ensure accountability of all specimens and records (prevent loss of specimens/records)
5. Perform further testing and analysis of data as expected

Table 1. Baseline capacity review of National Measles Laboratory (2014)

Operational Aspect	Baseline Status (Pre-Outbreak)
Man/Human Resources	<ul style="list-style-type: none"> Two (2) technical staff and one (1) data encoder. Only the senior technical staff holds a <i>plantilla</i> position Technical staff can run up to a maximum of 320 tests a day (specimens, excluding controls) One (1) administrative staff doing the encoding of all specimens in the database
Material/Laboratory Reagents and Supplies	<ul style="list-style-type: none"> Limited number of reagents and supplies on hand and depended on WHO donations of Measles IgM testing kits¹ Testing kits were limited due to concurrent measles outbreaks experienced by other countries in the Western Pacific Region at the time
Machine/Laboratory Equipment/Facilities	<ul style="list-style-type: none"> ELISA equipment (washer, reader) and testing facilities shared with other NRLs in the Department
Method/Laboratory Processes	<ul style="list-style-type: none"> In the measles elimination phase, surveillance required 100% of specimens tested using ELISA. The Measles IgM ELISA test is semi-automated (i.e., several steps of the testing process are manually done). The laboratory was expected to provide testing results to DOH and WHO within seven (7) calendar days from receipt of specimens.[†] After testing, the technical staff also had to encode the results and release them to the disease reporting units, the DOH surveillance bureau, and the WHO Testing scheduled 3 times a week (Monday-Wednesday-Friday) Testing algorithm for each sample was sequential, consisting of Measles IgM, with repeat for Measles equivocals (i.e., below cutoff for Measles IgM positive result), followed by Rubella IgM for Measles IgM negatives and repeat for Rubella equivocals (i.e., below cutoff for Rubella IgM positive results)[‡] Technical staff were the ones sorting and processing the specimens prior to testing. Specimens collected using alternative methods (i.e., dried blood spots and oral fluid) necessitated extra steps before they could be tested Additional respiratory specimens (oral and nasopharyngeal swabs) were also submitted for viral culture and molecular tests which were also important in identifying the circulating measles genotype causing the outbreak No documented outbreak preparedness plan in place Technical staff attended meetings called by the DOH and had to be the ones to coordinate directly with the WHO to request for additional testing kits The NML was able to meet the laboratory testing standards set by the WHO for turnaround time and timeliness of reporting^{§,1-6} Reporting requirements to the DOH Epidemiology Bureau (DOH-EB), which runs the Measles surveillance were met Provision of laboratory results to the disease reporting units (DRUs) which reported the cases, as well as the surveillance officers in the DOH regional offices and local government unit health offices in the cities and provinces, were achieved within the 7-day turnaround time
Money/Funding	<ul style="list-style-type: none"> The NML derived its funding for operational expenses and personnel augmentation from the General Appropriations Act through the Department of Virology budget The laboratory, at the time, also received suballotments from the DOH surveillance bureau as well as immunization program for its work on vaccine-preventable disease surveillance Often, the suballotments were not received at the start of the year NML received some financial assistance from the WHO annually through a technical services agreement, as a WHO-accredited laboratory and member of the Measles Laboratory Network

¹ Measles IgM Enzygnost™ (Siemens, Marburg, Germany), as pre-evaluated and recommended by the WHO. The WHO regularly provided Measles testing kits to the Philippines and countries in the Western Pacific Region as part of its technical assistance.

[†] In 2012, to accelerate reporting in support of measles elimination, the Global Measles Laboratory network adopted four (4) calendar days among its member laboratories, as the prescribed turn-around time from the previous seven (7) calendar days. Between 2013-2014, the NML was transitioning to the four (4) calendar days turn-around time. For DOH measles surveillance purposes, the NML reported its results weekly.

[‡] Depending on the serologic status, a single serum specimen may undergo testing to as much as four times.

[§] Measles IgM test results are reported by the laboratory within 7 days for ≥80% of specimens.

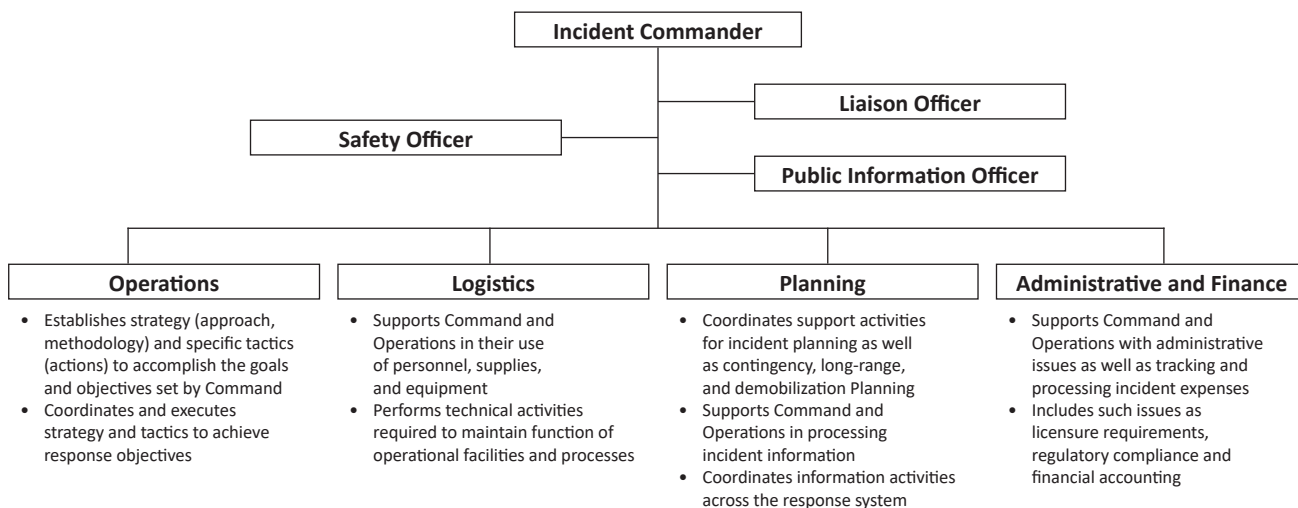


Figure 2. Incident Command System structure.

The general ICS layout was followed (Figure 2) with team leaders identified for the areas of Operations (Dengue NRL technical supervisor), Logistics (Polio NRL technical supervisor), and Finance/Administrative support (Influenza technical supervisor), and the department head as the Incident Commander and lead of Planning.

Non-NML members of each team were selected based on technical competencies vis-à-vis current load of duties and responsibilities (i.e., technical staff from the other NRLs

doing molecular work were assigned to do the PCR tests and genotyping, those doing viral culture were assigned to do culture work, et cetera). Laboratory aides and technicians were assigned to specimen reception, sorting, and processing. Administrative staff were assigned to support encoding.

At the outset, the ICS functioned through 24-hour operational periods and daily meetings during which team leaders reviewed and reported on the status of their

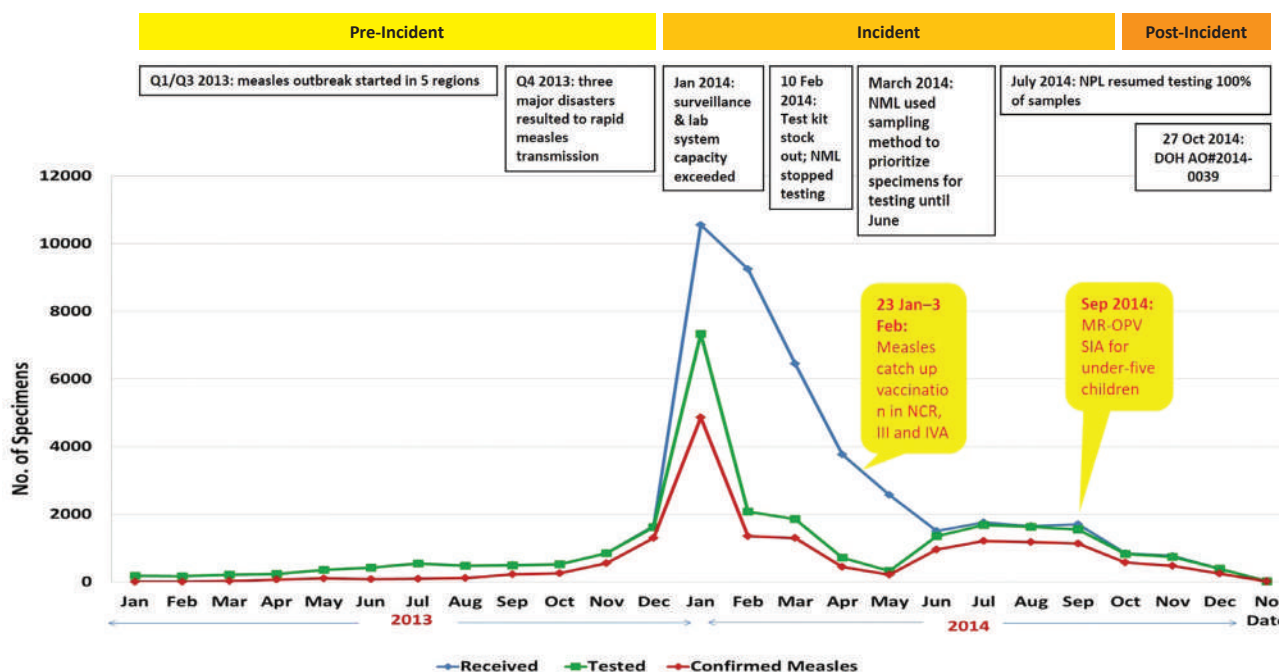


Figure 3. Measles Outbreak 2013-2014 and timeline of National Measles Laboratory Incident Command System.

respective areas, status is reviewed, objectives for the period are set, and action plans per area are discussed. Despite some adjustment challenges among the senior and junior staff who had regular daily activities and responsibilities, the measles outbreak laboratory response was tagged as the highest priority in the department. Other activities were put on hold, except for routine diagnostics (for RITM inpatients and outpatients) and testing of the other NRLs which were of a programmatic nature and cannot be deferred (e.g., viral culture and intratypic differentiation for poliovirus, realtime PCR for dengue serotype surveillance, viral culture, immunofluorescence assay and realtime PCR for influenza surveillance). The set-up increased oversight and facilitated measles laboratory operations.

Chronology of Management Response (Figure 3)

January 2014

1st round of ICS operational adjustments to increase testing capacity

Measles ELISA IgM testing shifted from the previous 3-day testing schedule of Monday-Wednesday and Friday to daily testing. Additional testing, such as virus isolation, conventional and realtime PCR, followed by genotyping were covered by medical technologists from the other NRLs of the department. This allowed the 2 technical staff of NML to focus exclusively on ELISA testing. Laboratory aides of other sections were assigned to specimen sorting and processing. Administrative staff were pulled out and re-assigned to assist in the encoding of case investigation forms which accompanied the specimens.

Release of specimen collection guidelines and laboratory testing updates

The ICS prepared and disseminated updated guidelines on laboratory testing of suspected measles cases to

standardize the collection of specimens from the field. The guidelines were released along with laboratory updates as of the 1st week of January 2014 and an advisory to the DOH and regional surveillance units regarding the status of the pending specimens for testing and the expected lag in provision of results from the turn-around time of seven days to two weeks.

Provision of specimen collection supplies

Red top blood tubes, dried blood spot filter papers, viral transport media, and respiratory swabs, were provided to the regional surveillance units, health offices, and other requesting agencies to improve specimen collection. Regions/areas with high rates of measles suspected cases were prioritized. Supply provision was distributed, tracked and monitored closely by the logistics team.

Internal agency support

The ICS sought the assistance of the Institutional Surveillance and Response Unit for centralization of results release, coordination of specimen referrals, and handling of inquiries from the surveillance units, health offices, and referring hospitals. Other departments were tapped for additional volunteers to assist in specimen sorting, processing, and testing. Moreover, the ICS requested the Administrative Division through General Services Department to provide accommodations for those who shall go on overtime duty, and the Finance Division to provide overtime compensation.

Strengthening data management

Recognizing the department's limitations in terms of big data management, the ICS sought and obtained the support of the WHO. The WHO extended technical assistance to RITM by deploying a data manager to the Institute to work with the Measles laboratory in analyzing laboratory testing results. The assigned staff from WHO facilitated the database cleaning and encoding in the

Philippine integrated disease surveillance and response software. The staff also generated the graphs and the GIS maps weekly and forwarded it to the immunization program manager for analysis. At RITM, the WHO staff assigned at the Measles Laboratory, was tasked to generate graphs, maps and identification of laboratory prioritization to be tested weekly to be analyzed by the measles technical officer. The WHO staff also facilitated the cleaning of the laboratory database used by the department. Data from the DOH surveillance bureau and NML were collected and analyzed by frequency, distribution, and location, incidence rate and Case Fatality Ratio. The process of unifying the databases of the surveillance system with the laboratory was fraught with difficulties as there was no common identification number for linking cases to specimens. The DOH conducted catch-up immunization in the National Capital Region, Region III and Region IV, the top 3 regions with the highest measles transmission based on the data generated.

Request for Additional Testing Kits

With supplies running out, the ICS requested WHO for additional ELISA testing kits. However, only a few kits were provided as even WHO was allotting kits to other countries in the Western Pacific Region which were also experiencing outbreaks concurrently.

February 2014

By the end of January and with more areas reporting measles cases throughout the country, the NML received a total of 10,616 samples, which was already 640% more than those received in December. Significant backlogs in testing piled up as the number of testing staff doing ELISA (i.e., the 2 NML staff) and equipment was no longer sufficient. The Incident Commander realized that the operational adjustments were not enough to address the laboratory testing.

By mid-February, the laboratory stopped testing due to stock out of the kits. WHO informed the Institute that it will provide 100 kits (enough for conducting 8,800 tests only) but that these will be delivered by first week of March. The ICS recognized the need to source testing kits aside from the donations of WHO, but had to identify funds and local suppliers if available.

By the end of February, another 10,016 samples were received. As the DOH, in collaboration with the local government units, were conducting outbreak response immunization activities, the need for laboratory confirmation further increased. Discussions with DOH and WHO began on strategies for maximizing use of the already depleted resources.

Request for Funding Support from DOH

The ICS facilitated the NML's request for additional funding to the DOH. The DOH approved the laboratory's request for additional funds in the amount of PhP 10M, however, this was transferred only in the 2nd quarter of 2014. The funds were utilized for hiring of contractual laboratory testing personnel (4 technical staff and 2 data encoders) as well as purchasing ELISA kits from the identified exclusive local distributor.

Request for Additional Equipment

The ICS also submitted a request for additional equipment to the WHO to address the limited ELISA equipment being shared by the NRLs of the department. The funds provided by the DOH were classified as Maintenance and Other Operating Expenses (MOOE) and not Capital Outlay (CO), and therefore could not be utilized to procure equipment, under government accounting laws.

March 2014

2nd Round of ICS Operational Adjustments

To maximize the limited number of WHO-donated ELISA kits and other resources available, and upon consultations with DOH and WHO, the ICS reviewed its operational adjustments and decided on the following:

- Changes in the original testing algorithm: samples for re-testing (such as equivocal) were no longer retested.
- Samples from epidemiologically-linked cases were no longer retested. If at least one case in the chain of transmission is laboratory confirmed, the other cases may already be considered as confirmed.
- Shift to priority testing of samples from the previous action plan of 100% testing.

To assist in the prioritization of samples for testing, WHO deployed additional technical staff to the NML for data management and analysis. Data of incoming referrals were analyzed, the index case was determined, and a few samples from the area of the confirmed index case were selected for testing. The premise of the priority testing was to ensure representative sampling to the barangay level if with few cases or at municipality level if cases are already confirmed in many barangays. The strategy also considered calamity areas (i.e., those provinces affected by Typhoon Haiyan/Yolanda) and the areas that were deemed as "urgent" or "priority" by the regional surveillance units as they are the ones who best know their respective areas. It was agreed with the RITM hospital management that all specimens taken from patients seen at the Institute shall also be included in the priority testing. All untested samples (i.e., those that were not selected in the priority testing strategy) were stored at RITM.

Coordination with DOH bureaus and stakeholders

It was challenging to communicate the continued backlogs in testing to the stakeholders, but even more so the shift in testing strategy, which meant that not all of the specimens they have been collecting were going to have laboratory results. Aside from the advisories it released, the ICS requested the support of the DOH central surveillance bureau to provide parallel information dissemination to the regions for cascading to their respective disease reporting units. Meetings with the regional surveillance units were taken as opportunities to provide updates on the laboratory testing.

Discussions with DOH and WHO began on coming up with guidelines for the local government units for measles outbreak and planning for appropriate response. This included clearer guidelines on specimen collection and sampling strategies in disease clusters identified at the barangay level, and highlighted the importance of

epidemiologic linkage to guide their action plans. This way, local surveillance units need not wait for 100% of specimens to be tested before making decisions on whether or not to conduct outbreak response immunization.

ICS also attended meetings with the immunization program to provide updates. Planning efforts were underway at the DOH at the time for mounting a country wide measles supplemental immunization activity (along with Rubella) by the 3rd quarter of the year. March ended with an additional 6,512 samples.

April-June 2014

From April to June, specimen referrals were still in the thousands but decreasing by month. The NML, using the revised algorithm and priority testing, was able to provide results—albeit still delayed by 2 weeks up to as much as 4 months—to the stakeholders. The revised operations proved useful in economizing the limited resources and increased the use of epidemiologic linkage as a public health tool. The ICS maintained the operational set-up and monitored the progress of testing.

July to December 2014

By July, ELISA kits purchased locally using the fund suballotment from DOH were delivered, and the ELISA/serology equipment requested to WHO for doubling testing capacity arrived. These included a microplate reader, washer, and pipettors.

Post-Outbreak

Final operational adjustment and ICS de-activation

The ICS, upon consultation with DOH and WHO, resumed 100% testing of all specimens received from July onwards while working on backlogs from earlier months. After reviewing the NML operations, the trends of specimen referrals, and the stock inventories, the ICS was finally deactivated in August 2014. There was no longer any need for additional shifts and the re-assigned staff returned to their normal operations. After the 2014 Measles-Rubella-Oral Polio Vaccine Supplemental Immunization Activity (MR-OPV SIA) in September, the number of cases further decreased until the end of the year.

By October, DOH issued Administrative Order 2014-0039 strengthening local government unit capacity for identifying measles outbreaks and planning for appropriate response, and declared the resumption to normal surveillance operations for measles elimination (i.e., 100% of suspected measles cases reported, investigated, and tested) by January 2015. The NML, with its additional contractual staff, was able to manage the specimens that were received until the end of the year.

Final Counts

At the end of the 2014, the NML received over 40,000 samples and tested 51% of them using its revised strategies (Table 2). Based on the data, 84% of the samples were received between January to June 2014 with the highest peak in January (Table 3). The final count is 3 times higher than the total specimen referrals the Institute tested for Influenza during the Influenza AH1N1 pandemic in 2009 in which 12,000 samples were tested, and is, by far, the largest number of specimens received by the Institute for a single outbreak to date.

DISCUSSION

Laboratories play a key role in generating information on health, whether for individual patient management, as in the case of routine diagnostic clinical laboratories, or for public health, in which case, laboratory services are utilized to support disease prevention and control programs.

In the context of public health systems, the laboratory is an integral component of disease surveillance, particularly in case investigation and confirmation.⁶ Prior to outbreaks, laboratory testing of specimens derived from routine surveillance systems allows confirmation of suspected cases and analysis of disease trends. During outbreaks, on the other hand, specimens are also confirmed to determine the cause of the epidemic, which, in turn, is utilized to implement control measures to stop transmission, determine other appropriate management measures, and guide allocation of resources. Laboratory testing is also used for programmatic monitoring of diseases targeted for control, elimination, or eradication, and contributes to evidence-based public health action towards healthier communities.⁷ It is very clear that much depends on the efficiency of the public health laboratory services and any delay in generating the information will also cause a delay in the public health action.

The NML, as the recognized public health laboratory for measles, despite being WHO-accredited for its consistent excellent performance in terms of quality assurance

Table 2. Summary of Referrals for Measles Testing and Results, 1 January – 31 December 2014

Category	Total (%)
Serum Referrals	40,861 (100%)
Tested	20,657 (50.6%)
Measles IgM Positive	13,932
Measles IgM Equivocal	1,675
Measles IgM Negative	5,000
Not Tested	20,144 (49.3%)
Rejected	50 (0.1%)

Table 3. Turn-around time (TAT) of Measles IgM Testing, January-December 2014

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Received	10565	9272	6450	3759	2569	1503	1761	1653	1698	848	776	395
Tested	7331	2065	1856	713	321	1351	1679	1621	1553	825	741	384
Unacceptable/ rejected samples	47	20	57	34	8	151	79	32	145	23	35	11
Not selected	3179	7128	4507	3004	2225	1	2	0	0	0	0	0
	10557	9213	6420	3751	2554	1503	1760	1653	1698	848	776	395
TAT (Average Calendar days)	34.8	68.9	99.0	98.9	82.0	220.0	82.0	95.3	57.3	22.1	15.1	20.4
Variance from Ideal TAT (7 days)	27.8	61.9	92.0	91.9	75.0	213.0	75.0	88.3	50.3	15.1	8.1	13.4

Note: Shaded portion reflects the period the ICS was active.

Table 4. Accomplishment of ICS Objectives

ICS Objectives	Operational Adjustments	Accomplishment
1. Provide additional manpower to NML to manage its operations.	<ul style="list-style-type: none"> • Re-assignment of staff • NML focused on outbreak testing 	Yes Yes
2. Maintain adequate supplies/reagents for continued testing	<ul style="list-style-type: none"> • Stock inventory monitoring • Allocative/priority distribution • Local sourcing • Acquisition of supplies/equipment 	Yes Yes Yes Yes
3. Ensure timely and accurate results	<ul style="list-style-type: none"> • 3-day testing to daily testing • Change in algorithm • TAT within 7 days 	Yes Yes No
4. Ensure accountability of all specimens and records (prevent loss of specimens/records)	<ul style="list-style-type: none"> • Re-assignment of staff • Centralization of encoding, reports and results release 	Yes Yes
5. Perform further testing and analysis of data as expected	<ul style="list-style-type: none"> • Other tests done by other NRLs 	Yes

and performance as part of measles surveillance, was ill-prepared in adjusting its operations to the surge of specimens caused by the nationwide outbreak. As the measles elimination target is looming in the horizon and the incidence has gone down,⁸ the operational level of the laboratory remained the same. The absence of an outbreak response plan, surge capacity, and contingencies clearly manifested itself in the resulting backlogs and inability to provide results. Ultimately, the onus of ensuring operational efficiency rested in the laboratory management.

Evaluation of Incident Response

The ICS served as the department's operational management strategy to mitigate the surge of specimens in the background of limited resources. From this perspective, *was the ICS strategy effective and efficient?* Effectivity ("doing the right things"), is about providing accurate laboratory results (quality), whereas efficiency, ("doing things right") is about providing the laboratory results within the expected turn-around time (timeliness). An indirect means of ascertaining the quality of laboratory testing is through the NML's performance in the WHO quality assurance schemes in which the technical staff have consistently performed excellently until the present. For the turnaround time, however, analysis showed significant variance from the standard WHO turn-around time for measles surveillance (Table 3).

Much emphasis is placed on the TAT as efficiency indicator as the earlier the information is provided to the surveillance officers and program officers, the earlier their actions and response are executed. It is evident that from January to August (shaded in Table 3)—the period during which the ICS was active—the TAT from specimen receipt to results ranged from 28 days to as much as 214 days beyond the 7 days ideal. It must be noted, however, that the suballotment support was only released in the 2nd quarter of the year, and consequently, the 4 additional medical technologists were only hired by July. There is also the stock out of kits in February which temporarily stopped the operations until March as the kits from WHO was likewise delayed, but on the other hand, no contingency plan was in place to address this risk.

In consultation with WHO, the regular TAT of 4 days applies only to regular surveillance and does not apply to periods of high transmission. Despite this, while it is understandable that during periods of high transmission the TAT may be adjusted beyond 4 days, very prolonged TATs such as in this outbreak, also exerted a negative impact to program implementation.

In retrospect, the backlogs, coupled with the challenges in the field (i.e., new surveillance officers were not yet trained on measles outbreak field investigation and epidemiologic linking, thus they were dependent on laboratory confirmation for initiating response activities), may have contributed, along with other factors to the spread and continued transmission of measles. Thus, despite the ICS strategy, in terms of TAT, operational efficiency is not achieved.

Have the objectives for the ICS activation been achieved, in the first place? Table 4 summarizes the ICS objectives, the specific activities per objective, and the assessment on the objectives' accomplishment. Due to the problems in TAT, objective 3 was not fully achieved; thus, only 4 out of the 5 ICS objectives at the outset, have been achieved. But this must be interpreted in the context of the kit stock out and the time the augmentation from DOH arrived.

The main objective of the ICS is the timely and accurate release of results. This is the core responsibility of the laboratory in the context of public health surveillance and action. The other ICS objectives are, in actuality, strategies to contribute to the main objective.

Key Management Lessons Learned

Looking at the Bigger Picture

In the course of daily operations, there is a tendency to miss the "bigger picture" or the context in which an organization operates and for what reason it is operating. In this case, the DOH central office and regional staff were all prioritizing the response efforts to Typhoon Haiyan/Yolanda, such that the extent of the silent spread of measles across provinces and regions was perceived only when it was just about to surge and the number of cases has exceeded thresholds. Even the laboratory was focused in testing specimens and releasing results, such that the increasing trend of laboratory confirmed cases was also missed. Operating units should make sense of the information that are being made available to them.

Operating in Silos vs Whole-of-Government Approach

Agencies should not *and cannot* operate in silos.¹ Open communication and coordination, sharing of information and transparency, integrative and collaborative problem-solving, must be the norm of public service agencies. The

1 To "operate in silos" is to work as isolated, independent units without sharing information and/or resources.

“us vs them” must be replaced with a “we” and “our” mentality. A vaccine-preventable disease outbreak, for example, is not *just* a DOH program concern, but also the concern of the surveillance bureau, the national laboratory, the surveillance officers and disease reporting units. This was our mindset when the ICS worked on standardized guidelines and provided standard specimen collection materials for the use of those in the field, collaborated with DOH and WHO in analyzing the data for priority testing, and worked with offices outside the Department during the surge response.

Adopting a VUCA Perspective as a Tool for Strategic Leadership

VUCA—volatility, uncertainty, complexity, ambiguity—are said to characterize the so-called “new normal,” or the context in which organizations should perceive their situation and future.⁹ VUCA has been used to highlight the importance of foresight and insight to strategic leadership.

Public health, with its unpredictability, scale, complexity, must be viewed through the VUCA lens. Thus, public health managers and practitioners, including those working in the public health laboratories, must never be complacent. There should be a proactive approach and attempt to forecast public health incidents by monitoring trends, scanning the environment, and analyzing data. In the context of RITM, this applies to emerging and re-emerging infectious threats and outbreak-prone diseases, as disease incidence, morbidity and mortality, may escalate rapidly.

Proactive Approach to Preparedness Planning

In addition, RITM recently achieved ISO 9001:2015 certification. The key difference between this standard and its 2008 predecessor is the establishment of an institutional systematic approach to risk identification and management which is applied throughout the agency’s business process from inputs to outputs. The standard is designed to shift the organization’s approach to management of the quality of its services, from being “reactive” to problems (correction) to being “pro-active” in addressing potential problems (prevention, mitigation, or elimination of risk, promotion of continual improvement).

This is very applicable to the public health laboratory function of the Institute’s NRLs. The laboratory management must place a premium on regularly assessing risk (analyzing data, monitoring global, regional disease trends), taking action on those risks (maintaining staff proficiency, ensuring good equipment condition and calibration, monitoring inventories and initiating procurement of buffer stocks), and contingency planning, at the department-level, division-level, and institution-level, to address risks.

CONCLUSION

The National Reference Laboratory’s response to the Measles outbreak of 2013 to 2014 was far from optimal as there were many operational challenges and limitations faced, factoring into the delay in laboratory testing.

Despite this, the response was appropriate by assuming command and control of the situation. Necessary decisions were made and objective interventions were introduced.

The Incident Command System is an appropriate operational management strategy during acute incidents that place a high demand on the organization’s limited resources. The experience with the ICS also led the management to important lessons, invest in preparedness for an even bigger outbreak, and challenged the leadership to think about ways of improving institutional resiliency.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

Laboratory operations were funded through the General Appropriations Act, suballotments from the Department of Health, and donations from the World Health Organization Western Pacific Regional Office.

ACKNOWLEDGMENTS

The authors thank Dr. Maria Lourdes Rebullida and Dr. Themistocles D. Padla, faculty of the Development Academy of the Philippines Public Management Development Program, who contributed to the discussion framework and analysis of the management case, as well as Dr. Celia Carlos of the Research Institute for Tropical Medicine (RITM) and Dr. Januario Veloso of National Kidney and Transplant Institute (NKTi) for reviewing, appraising, and providing inputs to the analysis and discussion when it was presented. The authors also thank the technical supervisors and staff of the Department of Virology of RITM, DOH Epidemiology Bureau, DOH Expanded Programme on Immunization, WHO country office and the Western Pacific Regional Office.

REFERENCES

1. Silva MWT. How the Philippines overcame the surveillance challenges elicited by the 2014 measles outbreak. Proceedings of the 25th Meeting of the Technical Advisory Group on Immunization and Vaccine-Preventable Diseases in the Western Pacific Region. Manila, Philippines: World Health Organization. 2016. Retrieved from <http://iris.wpro.who.int/bitstream/handle/10665.1/13512/RS-2016-GE-50-PHL-eng.pdf>.
2. Emergency Management and the Incident Command System – PHE (n.d.). Retrieved December 5, 2018, from <https://www.phe.gov/Preparedness/planning/mscc/handbook/chapter1/Pages/emergencymanagement.aspx>.

3. Incident Command System Resources | FEMA.gov. (n.d.). Retrieved December 3, 2018, from <https://www.fema.gov/incident-command-system-resources>.
4. Bochenek R, Kristjanson E, Schwartz B. (n.d.). Incident Management System (IMS) in the public health response: from SARS to pandemic H1N1, 41
5. Bruno T (n.d.). Using Incident Command System in Foodborne Outbreak Response, 42.
6. APHL. What is a public health laboratory? 2012. Retrieved from <https://vimeo.com/52548635>.
7. The role of the public health laboratory and the definition of public health laboratory services (n.d.). Retrieved October 27, 2018, from <https://www.apha.org/policies-and-advocacy/public-health-policy-statements/policy-database/2014/07/08/14/15/the-role-of-the-public-health-laboratory-and-the-definition-of-public-health-laboratory-services>.
8. Ylade MC. Epidemiology of measles in the Philippines. 2018;52(4):380-9.
9. Volatility, uncertainty, complexity and ambiguity. Wikipedia. 2018. Retrieved from https://en.wikipedia.org/w/index.php?title=Volatility,_uncertainty,_complexity_and_ambiguity&oldid=868117588.

Disclaimer: This journal is **OPEN ACCESS**, providing immediate access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. As a requirement for submission to the PJP, all authors have accomplished an **AUTHOR FORM**, which declares that the ICMJE criteria for authorship have been met by each author listed, that the article represents original material, has not been published, accepted for publication in other journals, or concurrently submitted to other journals, and that all funding and conflicts of interest have been declared. Consent forms have been secured for the publication of information about patients or cases; otherwise, authors have declared that all means have been exhausted for securing consent.

*Publish in the new PJP.
Visit our website:
<http://philippinejournalofpathology.org>*

Philippines Diagnostic Pathology Laboratory Benchmarking

Tony Badrick,¹ Jozica Habijanac,² Sam Yew Mah,³ Elizabeth Arcellana-Nuqui⁴

¹Royal College of Pathologists of Australasia Quality Assurance Programs, St Leonards, Sydney Australia

²Roche (Philippines) Inc., Manila, Philippines

³Roche Diagnostics Asia Pacific Ltd., Singapore

⁴Department of Laboratory Medicine and Pathology, The Medical City, Pasig City, Philippines

ABSTRACT

Introduction. To ensure continuous quality improvement, laboratories need to obtain data about best practice from peers. Data about analytical EQA is available but far less is available about other important aspects of laboratory performance. There is a Roche Diagnostics Survey of laboratories which provides benchmarking in key areas of laboratory performance.

Methodology. The Roche Diagnostics Survey included 1058 laboratories from 14 countries in the Asia Pacific Region with both developing and developed nations. The data were collected in 2017 but the survey has been collecting data each second year since 2011. Data was collected in the areas of quality, speed and cost.

Results. The results for the Philippines was compared with other countries in the Asia Pacific Region. Broadly it was found that 42% of all laboratories in the Region were accredited to ISO 15189 or ISO 9001 and that 50% of laboratories were in an External Quality Assurance (EQA) program. Compared to other countries in the survey, the Philippines laboratories had fewer sites with ISO 15189 and with Lean Six Sigma improvement deployment. There are six laboratories in the Philippines that are accredited to ISO 15189. There was a greater emphasis on customer satisfaction related Key Performance Indicators (KPIs) such as turnaround time monitoring, cost reduction and employee productivity.

Conclusions. Benchmarking can highlight the differences in the apparent quality of laboratory services compared to their peers and may lead to improvement. The benchmarking comparison has identified opportunities for Philippine laboratories to improve including obtaining ISO 15189 accreditation, implementing laboratory information systems and concentrating on Lean practices to improve productivity. The Roche scheme provides an ongoing (growing) large sample of benchmarks that can be used by participants to improve their performance and the performance of individual countries.

Key words: benchmarking, quality, cost of service, customer satisfaction, turnaround time

ISSN 2507-8364 (Online)

Printed in the Philippines.

Copyright© 2019 by the PJP.

Received: 6 September 2019.

Accepted: 13 October 2019.

Published online first: 19 October 2019.

<https://doi.org/10.21141/PJP.2019.11>

Corresponding author: Tony Badrick, PhD

E-mail: tony.badrick@rcpaqap.com.au

INTRODUCTION

Benchmarking is the process of measuring products, services, and practices against leaders in a field, allowing the identification of best practices that will lead to sustained and improved performance. Performance may be compared either in a generic way, in which there is a comparison of a process regardless of the industry, or in a functional way, in which there are comparisons within the same industry. The aim of benchmarking is to identify variation in performance of key indicators so that improvement can be undertaken. In pathology practice we are more used to quality assurance activities where results from samples are sent from an EQA organisation and the performance of laboratories are compared. Omdahl¹ defines benchmarking as a continuous improvement process in which a company:

- Measures the most relevant specific attributes of its own products, services, and practices, often including operations, performance, procedures, project, processes, strategies



- Compares its own performance against:
 - Best-in-class company performance
 - Companies recognized as industry leaders
 - The company's toughest competitors
 - Any known process that is significantly superior to that of the company's processes
- Determines how those companies achieved their significantly superior performance level
- Uses that information to improve its own performance
- Ultimately reaches the level of performance achieved by the benchmarked process (or a level above that process)
- Continually repeats the process in an iterative fashion

An example of benchmarking system is Q-Probes, which are part of the College of American Pathologists' (CAP) programme of studies in quality assurance.² Q-Probes aims to provide short-term, external peer-comparison studies that provide a one-time comprehensive assessment of key processes including pre- and post- analytical activities such as turnaround time (TAT) and customer satisfaction.

Benchmarking can lead to improvement in the quality of patient care, support for administrative accountability, assistance in making judgements about testing quality, facilitation of inter-provider comparisons over time and assessment of improvement effectiveness.³ Comparing broad organisational activities against peer laboratories, can be used to set priorities for quality improvement interventions. For example, when other similar laboratories have lower frequencies of process defects, e.g., shorter TAT, then the comparison suggests a focus for process improvement for laboratories with longer TATs.

Indicators of the extra-analytical phases of the Total Testing Process (TTP) have been developed in several countries, such as Australia and New Zealand,⁴ the United States,^{5,6} Brazil,⁷ and Spain/Catalonia,⁸ and other surveys and programs have been promoted in the UK,^{9,10} and Croatia.¹¹ In 2008 the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) launched a Working Group named "Laboratory Errors and Patient Safety" (WG: LEPS) to identify QIs and related quality specifications which (i) produce Benchmarks from comparing laboratories, (ii) promote error reduction, and (iii) increase patient safety. The IFCC has developed Model Quality Indicators (MQIs) which laboratories in several countries have evaluated and the WG: LEPS has reported preliminary results.^{12,13}

A Benchmarking program has been undertaken by Roche Diagnostics (Roche Diagnostics Asia Pacific) in the Asia Pacific Region with purpose to identify trends in laboratory management, to help laboratories identify areas for improvement and provide access to new ideas and procedures that drive further efficiency gains.

It was designed to collect information on three key areas of laboratory practice (quality, speed and cost) with a focus on, but not limited to, Clinical Chemistry and Immunoassay testing.¹⁴

The data collected is quite granular and provides information in each of the key areas (Table 1).

METHODOLOGY

The questionnaires were formulated based on the common performance indicators that are used in laboratories.

The survey is delivered online with the survey questionnaires usually completed by laboratory manager or laboratory director.

The survey is carried every alternate year or so and when the country specific report is ready, it is provided to the countries and they will share with the participating laboratories. In this country specific report, the performance of the individual laboratory (myLab) will be compared against the APAC peer group data:

- by APAC (based on all survey submission)
- by country
- by country group (developed/developing, based on IMF advanced economies grouping)
- by lab type (government hospital/private hospital/commercial laboratory/others)
- by lab size (small <250 / medium 251-1000 / large >1000 samples per day)

The surveys are sent to a wide range of laboratories and is not restricted to Roche customers, who represent 70-80% of respondents.

RESULTS

The survey started in 2011 with 181 laboratories in twelve countries and now includes 1058 participant laboratories in 14 countries (Figure 1). The laboratories are categorised by the following groups:

- Developed (18%) and developing (82%) countries based on International Monetary Fund advanced economies
- Government hospital laboratories (60%), private hospital laboratories (28%), private commercial laboratories (11%) and clinical research organisations laboratories (1%)

In general, it appeared that there were more (45%) medium laboratories (251-1000 samples per day) in the survey than large (29%) (>1000 samples per day) or small (<250 samples/day).

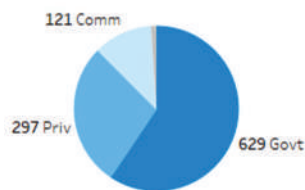
Table 1. Structure of the questionnaire

Quality	Cost	Speed
<ul style="list-style-type: none"> • External Quality Assurance (EQA) Program • International accreditation • Continuous improvement • IT infrastructure • KPIs used • Point-of-care testing 	<ul style="list-style-type: none"> • Instrument efficiency • Staff efficiency • Workspace efficiency 	<ul style="list-style-type: none"> • Turnaround Time (TAT) Monitoring • TAT Target • Urgent specimen handling

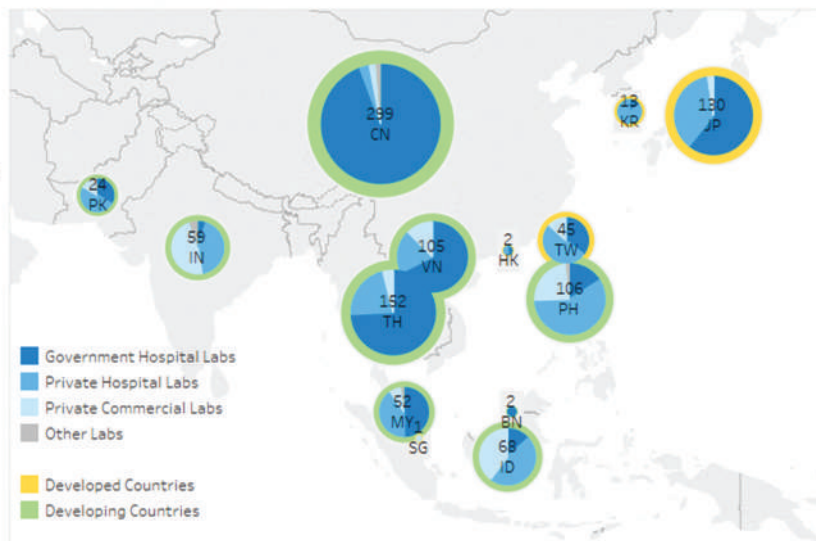
The Survey had a total of **1,058** participating labs from **14** countries

Survey data collected from 2015 to 2017

Labs per by Lab Type:



Labs per Country and by Lab Type:



Note: grouping of "developed" and "developing" countries based on IMF advanced economies

Figure 1. Participating laboratories by country, 2015-2017.

Overall in the 2017 APAC survey:

- 42% of laboratories were accredited to ISO 15189 or ISO 9001
- 50% of laboratories were in an EQA program
- 76% of laboratories had a TAT less than or equal to 60 minutes for stat chemistry
- 62% of laboratories had a TAT less than or equal to 60 minutes for stat immunoassay tests
- 33% of laboratories consolidate chemistry and immunoassay analysers
- 33% of laboratories utilise automation for pre-/post-analytic processes

There were 106 laboratories from the Philippines comprising the following types: private hospital 62 (58%); private commercial 25 (24%); government hospital 17 (16%); other 2 (2%). We will present the results under the broad headings of Quality, PoCT and TAT.

Quality

External Accreditation

The Philippines had fewer laboratories accredited to ISO 15189 than developed (41%) or developing (27%) countries. There is an intention for more laboratories to pursue this accreditation. Generally, across the APAC countries there were similar numbers of government and commercial laboratories accredited to this standard. Comparing with the number of laboratories with ISO 15189 accreditation in the developing countries of the APAC (27% have accreditation, 35% intend to achieve accreditation) the Philippines (3% and 25% respectively). The private hospital laboratories have the highest awareness of the need to implement ISO15189.

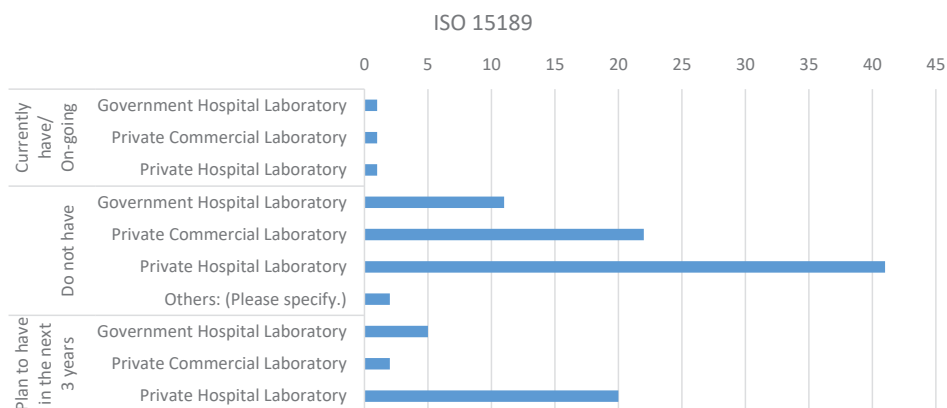


Figure 2. Philippine laboratories with ISO 15189 accreditation.

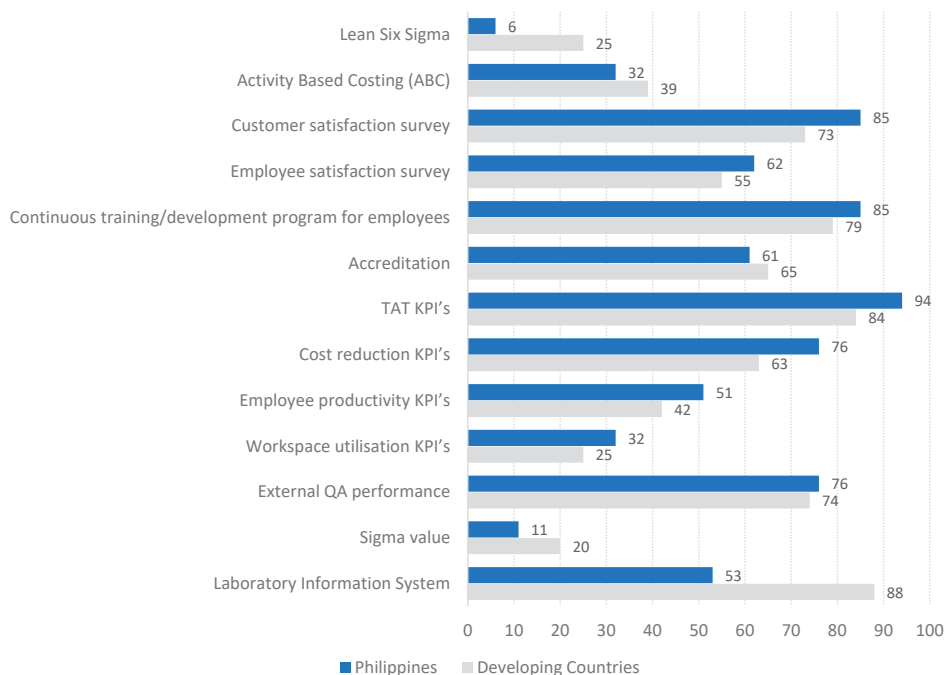


Figure 3. Laboratory KPIs comparing the Philippine laboratories to all developing countries in the APAC (Asia Pacific).

Key Performance Indicators

In Figure 3 we see that compared to other developing countries in APAC there are few differences between the Quality measures being used in the Philippines except for lean six sigma tools. In Figure 4 we see the deployment of lean six sigma by Philippine laboratory type. It can be seen that Laboratory Information Systems were more common in other developing countries. It also appears, that more Philippine laboratories implement customer satisfaction, TAT, employee satisfaction and training, and cost reduction as key measures compared to laboratories in developing countries.

The data in Figures 4 and 5 show the quality KPIs being used per Philippine laboratory type. It shows that with lean six sigma, the early adopters are the government hospitals with all sites planning to introduce this tool within three years. This is also the case with activity-based accounting. With the other quality KPIs perhaps the only apparent trend is that private commercial laboratories appear to be lagging compared to the other types of laboratory.

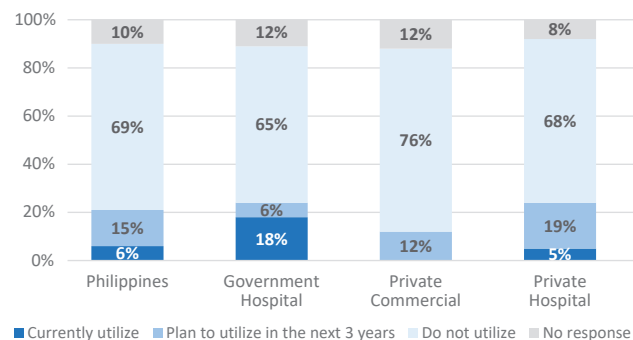


Figure 4. Deployment of lean six sigma in Philippine laboratories.

Government laboratories have the greatest lean six sigma utilization with private commercial the least, in fact nil at present. Private commercial laboratories have minimal implementation but there is an intention to utilize in the future.

In Figure 5 the deployment of ABC is shown indicating that this is greater in government hospitals.

Point of Care Testing (PoCT)

Laboratories were surveyed to determine where PoCT devices were deployed and what the role of the PoCT co-ordinator was. The results are given in Figures 6 and 7.

PoCT usage was high in the Philippine laboratories, higher than in other developing countries of the APAC at 55%. These devices are found throughout hospitals with the greatest numbers in the laboratories themselves. The role of the POCT co-ordinator is broad in the Philippines. In fact, it is broader than in other developing countries of the APAC countries where there is less emphasis on logistic management of these devices.

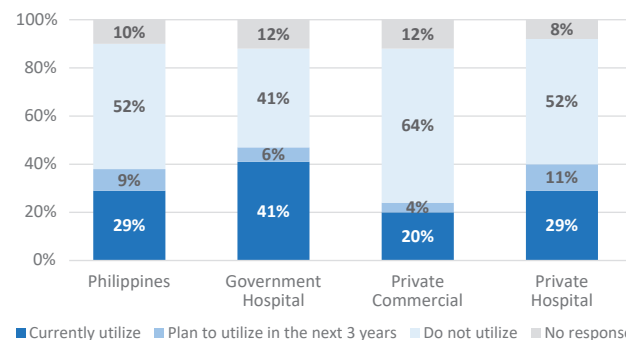


Figure 5. Deployment of activity based costing (ABC) in Philippine laboratories.

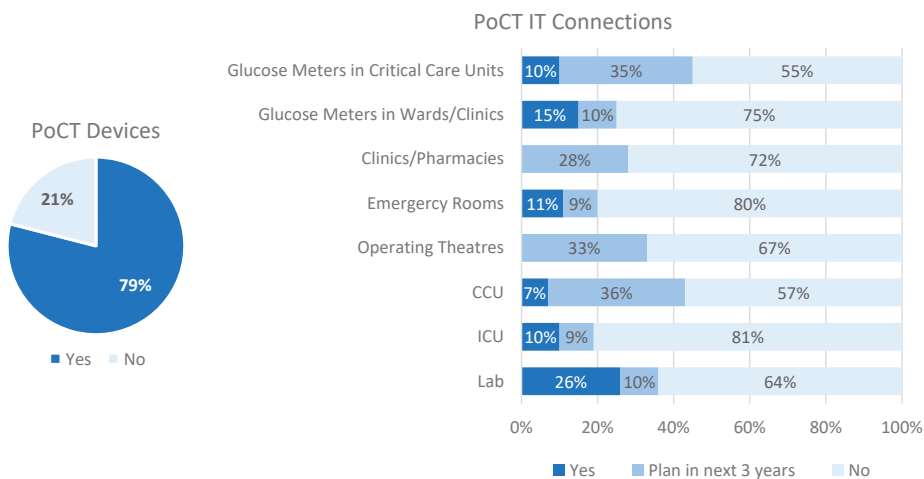


Figure 6. Deployment of point of care testing devices.

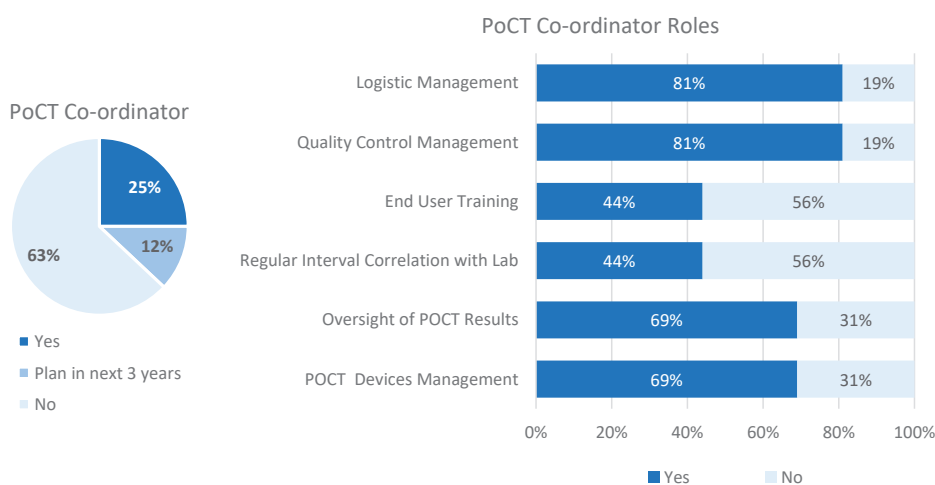


Figure 7. Role of the PoCT co-ordinator.

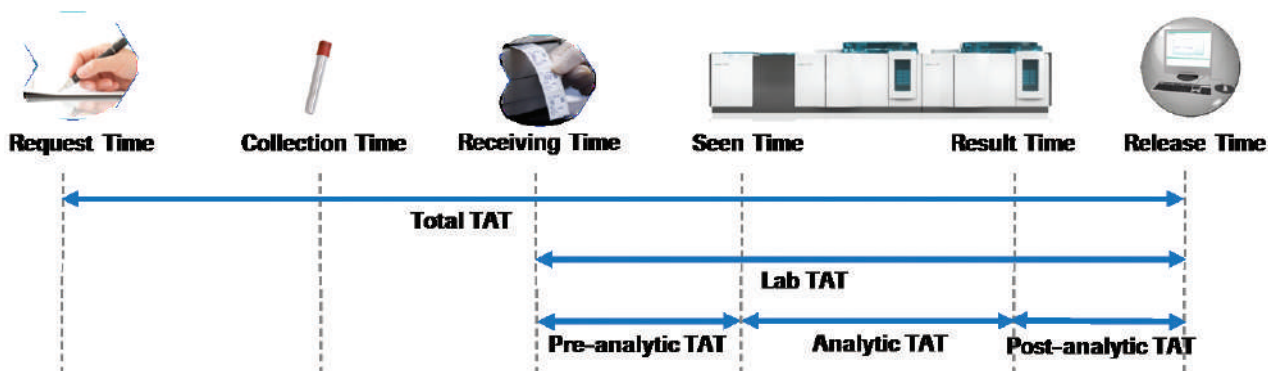


Figure 8. The definition of the various turnaround times.

Turnaround Time

The definition of TAT is varied so we have defined in Figure 8 the different TATs collected in the Survey.

In Figures 9 and 10, we present the Lab TAT for the stat and routine clinical chemistry and immunoassay samples.

The majority of laboratories have a TAT of 30-60 minutes for Stat specimens and 60-120 for routine specimens. There is a broad range of performance.

In Figure 11 is the total TAT for different categories of laboratories.

There are different ways a laboratory can deal with stat samples. There can be a dedicated stat laboratory, dedicated staff to deal with these samples and/or have instruments dedicated to these samples.

Figure 12 reveals that having dedicated stat laboratories is relatively common in private hospitals.

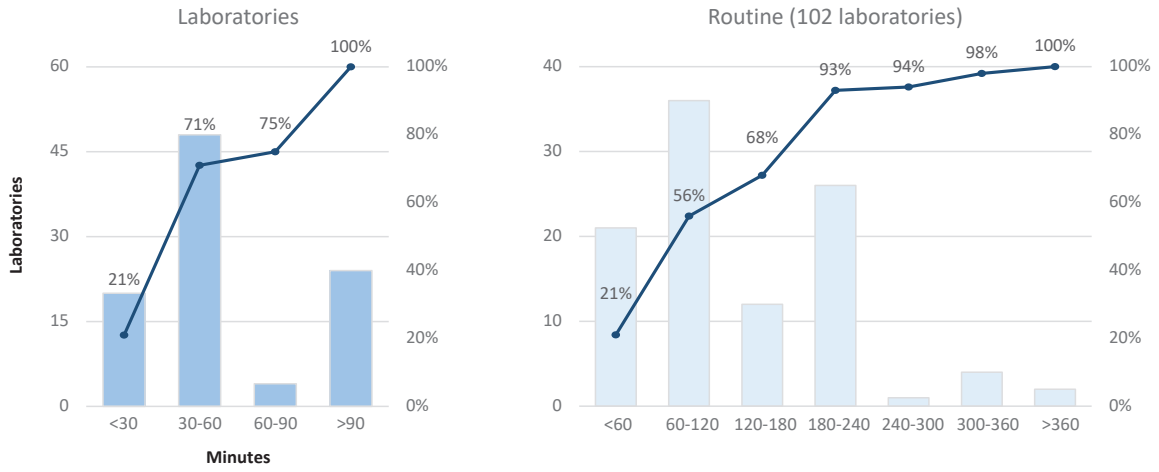


Figure 9. Laboratory TAT for Stat Samples for Clinical Chemistry samples.

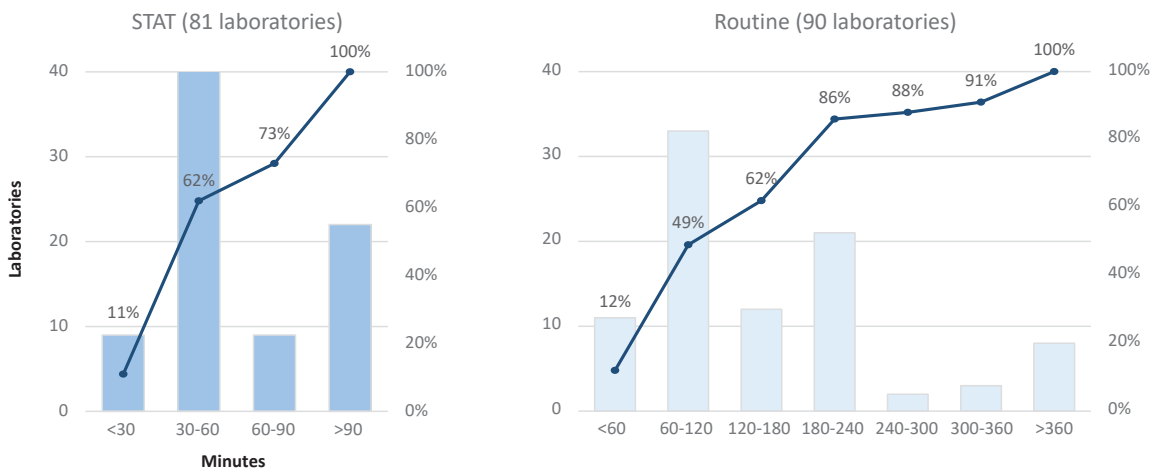


Figure 10. Laboratory TAT for stat samples for immunoassay samples.

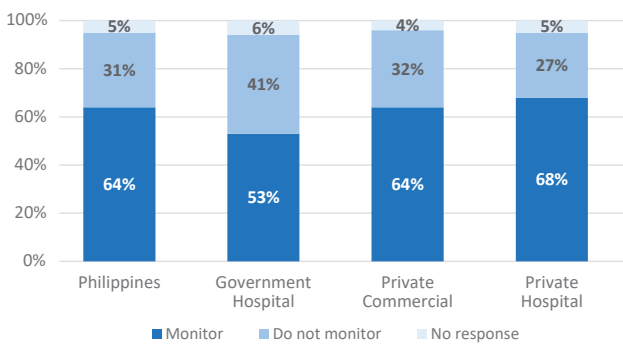


Figure 11. Laboratory total TAT for stat samples.

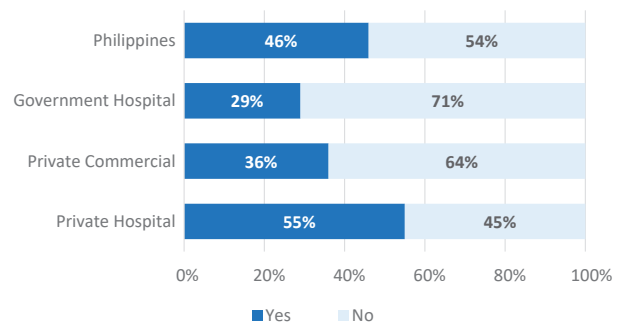


Figure 13. Laboratories with dedicated stat staff.

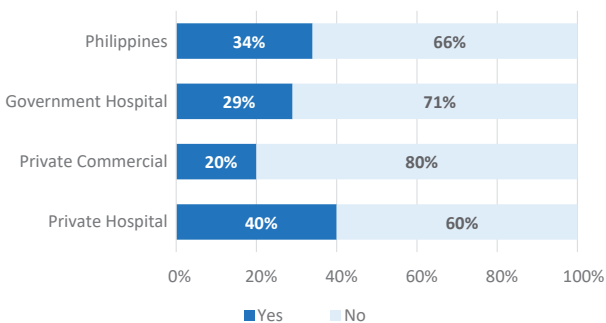


Figure 12. Laboratories with dedicated stat laboratories.

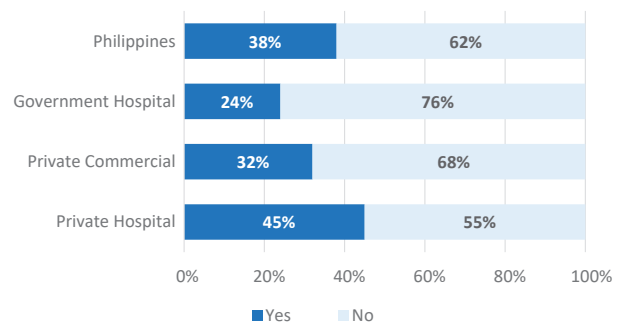


Figure 14. Laboratories with dedicated stat equipment.

DISCUSSION

These data represent key benchmarks for laboratories to enable improvement. As expected the Survey has revealed varying degrees of compliance with the implementation of best practice, however there are common themes.

It is important to benchmark against the correct peer group to get the best possible comparison and insights for improvement. When comparing within survey laboratories in APAC versus the Philippines, there is a very different population by type and size of laboratory. In Philippine survey majority of laboratories are private hospital laboratories (58%), while in APAC survey, it is government laboratories. In addition, most laboratories in Philippines are small, while medium-sized laboratories predominate in the APAC. Productivity in the larger laboratories will be much higher than in the small laboratories for example. Also, when comparing private and government laboratories it is important to take note, that private laboratories will be measuring customer satisfaction as a priority. That could explain the difference for some data, for example, ISO 15189, which is less prevalent in Philippines due to budget constraints of small laboratories, and these small laboratories are not audited by government. There is a common focus on meeting customer demands, apparent through the monitoring of TAT and customer satisfaction on the one hand, and performance of the laboratory in EQA on the other.

On continuous improvement program, it seems that the Philippines is ahead of Asia. However, we need to keep in mind that this is happening mainly in private hospital laboratories and their driver is to improve efficiency, speed and hence customer satisfaction. One interesting finding is that few laboratories in the Philippines are accredited to ISO 15189, despite the evidence that accreditation leads to improvement. The benefits of adopting ISO 15189 accreditation for laboratories are the reduction in patient and business risk, the encouragement of sharing of best practices and the stimulation of innovation. For payers and healthcare providers, accreditation is a tool that provides assurance that clinical lab services are safe, reliable and good value for patients. It also provides a mechanism for measuring quality improvements and supporting consistency.^{15,16}

Pursuant to a 2007 Executive Order¹⁷ mandating the institutionalization of Total Quality Management programs in all government agencies, there was an initiative from the Department of Health to implement ISO 15189 in government laboratories.¹⁸ Under Executive Order No. 605 the National Unit of Health Laboratories of the Department of Health - Health Facilities Development Bureau (DOH HFDB) targeted 50% of tertiary laboratories be accredited for ISO 15189 in five years. The Department of Trade and Industry (DTI) is mandated to conduct assessments for ISO 15189 accreditation however they have been constrained due to a lack of resources. These efforts are difficult to sustain due to a dearth of leadership in government to regulate the laboratory industry and a lack of resources and funds to implement Accreditation.

We note that CAP accreditation is in its initial phases in the country and that laboratories that participate in selective CAP proficiency such as Q-probes and performance improvement for pathologists are the larger commercial laboratories or private hospitals which can afford CAP fees. The perceived purpose of this is to distinguish themselves in the market and set themselves above the rest in terms of quality and standardized service to patients.

Government laboratories seem to be leading the sector with the use of improvement tools including activity based accounting, though the application of lean six sigma is low. Lean is not yet widespread, most likely due to space limitations and the fact that this technique has not yet been widely adopted in the market. It is likely that an increased awareness of Lean and attention to this area will lead to more efficient utilization of space. This is an opportunity for improvement for all laboratories.^{19,20}

It seems that Philippine laboratories measure employee satisfaction more, and the reason might be the scarcity of medical technologists in the Philippines. Employee satisfaction and the design of new career tracks in molecular pathology, mass spectrometry and genomics, could be some of the retention strategies for private laboratories as there is huge competition for health manpower resources in the Philippines. Due to that challenge, there is also greater need for training and re-training for the employees due to rapid turnover, which is also seen in data.

There is a low income subsidy implementation in the Philippines than in other developing countries in spite of the benefits in accuracy, efficiency and cost. This is probably due to problems with Internet connectivity, IT personnel in hospitals and a lack of funds. It is worth noting that the same Administrative Order¹⁷ that sought implementation of total quality management (TQM) and advocated ISO 15189 accreditation also promoted Laboratory Information Systems (LIS) to strengthen information management.

There is wide variation in the TAT of laboratories with both stat and routine samples. However, in general laboratories in the Philippines have similar TATs to their peers in the APAC Region. Perhaps Philippine laboratories seem to have a higher focus on TAT measurement which may reflect the business reality that customer satisfaction is key to their survival. TAT is a differentiating factor among the private laboratories and can lead to improved profitability.

This also could explain the STAT numbers observed among private Hospitals more focused on STATs. Analysis of the frequency and types of STAT requests may lead to development of guidelines for more rational utilization of laboratory services, influence ordering practices of physicians, and ultimately, reduce the costs of health care. There will be variation due to different capabilities of equipment and less optimized internal processes. However this is an area where laboratories impact directly on patient outcomes and hospital efficiency. This is one of the KPIs to deliver best service to Doctors and patients. Laboratories everywhere need to concentrate on this performance indicator.

Point of care testing in the Philippines is in varying stages of development and implementation. Although some forms of near-patient testing exist (e.g., glucose testing, blood gas, etc.), most hospitals that have this facility do not have a formal structure in place. Since a Department of Health directive^{18,19} tasking the laboratory director/pathologist oversight and supervision over PoCT, regardless of its location in the hospital, the organizational chart of the clinical laboratory has included PoCT and a designated POCT coordinator. Still, there are not a lot of Point of Care Coordinators (PoCC) who are supposed to be overseeing and managing PoCT program in their respective institutions and in general, they are limited only to private and internationally-accredited hospitals in Metro Manila. Since the number of PoCC in the country is very limited, one of the biggest challenges facing them is not having a support group or a network of like-minded individuals with shared interests with whom they could exchange ideas and best practices. This is despite the many responsibilities expected from a PoCC that include instrument selection and validation, device and operator management, logistics management, quality control management, etc. Often, PoCC would rely on web-based resources (i.e., online forums that are based in the US) to keep abreast on the latest developments in the PoCT space. Unlike other allied health professionals such as nurses and medical technologists, among others, that have local organizations that foster continuous professional development and provide a sense of community to its members, PoCC are left to rely on themselves. This may well be the reason why the tasks a PoCC perform is unpopular among laboratory staff and as a result interest level in the role remains low.

Connectivity of PoCT devices in hospitals that use them is another consideration. Often these instruments still operate as standalone units and rarely as integrated solutions that are able to interface with LIS/HIS, mostly due to cost implications. Hence, the value of having a connected hospital PoCT system is not fully utilized and this is certainly true in the case of glucose meters wherein manual operation continues to be the norm. In terms of device operations, lab technicians are by far the typical users of PoCT devices in the Philippines. This practice is really the opposite because in most countries the nurses are the end-users whilst the lab technicians are only tasked to do device quality control management.

There are data in the survey which show that the productivity of laboratories in the Philippines is much lower in all aspects, consolidated, non-consolidated, automated and non-automated, compared to APAC laboratories (Supplementary S6). This additional data also demonstrates that on average, there are only 5 parameters measured by sample, versus Asia laboratories average of 6-7. This is difficult to comment upon. In the Philippines where ordering physicians are keenly aware of budgetary constraints on patients, it is not unusual for chemistry requests to have fewer than six parameters, rather than the full chemistry panel of 20 or more analytes. The more common practice is to order symptom-directed and diagnosis-related or focused tests.

Limitations

Benchmarking processes suffer from the problem of ensuring participants measuring the same thing. Different units of measure or, if manual processes are used, the accuracy of any measure can impact on the value of the outcome. However, if a benchmarking scheme is used repeatedly then, over time, there seems to be agreement on the measures and the results do become useful. This survey has been in existence for nearly a decade and the results over that time have to be consistent, indicating some reproducibility and hence internal validity of the results. External validation i.e., extrapolation to other groups is another issue.

CONCLUSION

Benchmarking can highlight the differences in the apparent quality of laboratory services compared to their peers.

Furthermore, Q-Probe studies have demonstrated that Benchmarking does indeed lead to improvement in laboratory performance over time.⁶ When laboratories in the Philippines are compared against their APAC peers one of the major differences is the lack of ISO 15189 accreditation. ISO 15189 has been shown to lead to improvements in laboratory quality and this finding is an opportunity to improve patient outcomes in the Philippines. Other key differences between Philippine laboratories and their peers were the lack of LIS and lean six sigma implementations. Both of these will lead to fewer errors, better patient and business outcomes and better value for the health system.

In summary, as the value of benchmarking becomes better understood by laboratory professionals, its impact will grow. There are also local Benchmarking schemes^{20,21,22} but few global schemes. The Roche scheme provides an ongoing (growing) large sample of benchmarks that can be used by participants to improve their performance and the performance of individual countries.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

Jozica Habijanac is the Country Manager of Roche Philippines. Sam Yew Mah is the Consulting Team Manager of Lab Workflow Solutions, Roche Asia Pacific. The authors did not receive any honoraria for this work. Roche Diagnostics provided the Survey data. The interpretation of the data and the decision to publish were made by Prof. Tony Badrick and Dr. Elizabeth Arcellana-Nuqui.

FUNDING SOURCE

None.

REFERENCES

- Omdahl T. Quality dictionary. Terre Haute, IN: Quality Council of Indiana, 1997.
- Q-Probes College of American Pathologists. Quality management programs. <https://www.cap.org/laboratory-improvement/quality-management-programs>. Accessed July 5, 2019.
- Mainz J. Defining and classifying clinical indicators for quality improvement. *Int J Qual Health Care*. 2003;15(6):523-30. PMID: 14660535. <https://doi.org/10.1093/intqhc/mzg08>.
- Badrick T, Gay S, Mackay M, Sikaris K. The key incident monitoring and management system – history and role in quality improvement. *Clin Chem Lab Med*. 2018;56(2):264-72. PMID: 28771429. <https://doi.org/10.1515/cclm-2017-0219>.
- Nakhleh RE, Souers RJ, Bashleben CP, et al. Fifteen years' experience of a College of American Pathologists program for continuous quality improvement. *Arch Pathol Lab Med*. 2014;138(9):1150-5. PMID: 25171697. <https://doi.org/10.5858/arpa.2014-0148-OA>.
- Meier FA, Souers RJ, Howanitz PJ et al. Seven Q-Tracks monitors of laboratory quality drive general performance improvement: experience from the College of American Pathologists Q-Tracks program 1999-2011. *Arch Pathol Lab Med*. 2015;139(6):762-75. PMID: 26030245. <https://doi.org/10.5858/arpa.2014-0090-CP>.
- Shcolnik W, de Oliveira CA, de São José AS, de Oliveira Galoro CA, Plebani M, Burnett D. Brazilian laboratory indicators program. *Clin Chem Lab Med*. 2012;50(11):1923–34. PMID: 23096110. <https://doi.org/10.1515/cclm-2012-0357>.
- Kirchner MJ, Funes VA, Adzet CB, et al. Quality indicators and specifications for key processes in clinical laboratories: a preliminary experience. *Clin Chem Lab Med*. 2007; 45(5):672–7. PMID: 17484633. <https://doi.org/10.1515/CCLM.2007.122>.
- Barth JH. Clinical quality indicators in laboratory medicine. *Ann Clin Biochem* 2012; 49(Pt 1):9–16. PMID: 22042979. <https://doi.org/10.1258/acb.2011.011126>.
- Barth JH. Selecting clinical quality indicators for laboratory medicine. *Ann Clin Biochem* 2012;49(Pt 3):257–61. PMID: 22422153. <https://doi.org/10.1258/acb.2011.011159>.
- Simundic AM, Topić E. Quality indicators. *Biochem Med* 2008;18(3): 311–19. <https://doi.org/10.11613/BM.2008.027>.
- Plebani M, Sciacovelli L, Lippi G. Quality indicators for laboratory diagnostics: consensus is needed. *Ann Clin Biochem* 2011;48(Pt 5):479. PMID: 21733928. <https://doi.org/10.1258/acb.2011.011088>.
- International Federation of Clinical Chemistry and Laboratory Medicine. Laboratory errors and patient safety (WG-LEPS). <http://www.ifcc.org/ifcc-education-division/working-groups-special-projects/laboratory-errors-and-patient-safety-wg-leps/>. Accessed July 5, 2019.
- Badrick TC, Gutscher A, Sakamoto N, Loong DCM. Diagnostic laboratories in Asia Pacific region: investigation on quality characteristics and time of reporting. *Clin Biochem*. 2017;50(10-11):625-31. PMID: 28336392. <https://doi.org/10.1016/j.clinbiochem.2017.03.017>.
- Zima T. Accreditation of medical laboratories - system, process, benefits for labs. *J Med Biochem*. 2017;36(3):231–7. PMID: 30568539. PMID: PMC6287213. <https://doi.org/10.1515/jomb-2017-0025>.
- Tzankov A, Tornillo L. Hands-on experience: accreditation of pathology Laboratories according to ISO 15189. *Pathobiology*. 2017;84(3):121-9. PMID: 27923229. <https://doi.org/10.1159/000449254>.
- Republic of the Philippines, Executive Order No. 605 [Gloria M. Arroyo]: Institutionalizing the Structure, Mechanisms, and Standards to Implement the Government Quality Management Program. March 2007
- Republic of the Philippines, Department of Health Administrative Order 2012-0021 [Enrique T. Ona]: National Framework of the National Laboratory Health Network. October 2012.
- Republic of the Philippines, Department of Health Administrative Order 2007- 0027 [Francisco T. Duque III]: Revised Rules and Regulations Governing the Licensure and Regulation of Clinical Laboratories in the Philippines. February 2007.
- Hamilton L. Lean, lean six sigma and the clinical Lab. MLO 25 January 2018. <https://www.mlo-online.com/home/article/13009430/lean-lean-six-sigma-and-the-clinical-laboratory>. Accessed 13 July 2019.
- Inal TC, Goruroglu Ozturk O, Kibar, F, et al. Lean six sigma methodologies improve clinical laboratory efficiency and reduce turnaround times. *J Clin Lab Anal*. 2018;32(1). PMID: 28205271. <https://doi.org/10.1002/jcla.22180>.
- Galloway M, Nadin L. Benchmarking and the laboratory. *J Clin Pathol*. 2001;54(8):590-7. PMID: 11477112. PMID: PMC1731489. <https://doi.org/10.1136/jcp.54.8.590>.
- Neil A, Pfeffer S, Burnett L; BiPAC. Benchmarking in pathology: development of a benchmarking complexity unit and associated key performance indicators. *Pathology*. 2013;45(1):66-70. PMID: 23222245. <https://doi.org/10.1097/PAT.0b013e32835b77c4>.

Disclaimer: This journal is **OPEN ACCESS**, providing immediate access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. As a requirement for submission to the PJP, all authors have accomplished an **AUTHOR FORM**, which declares that the ICMJE criteria for authorship have been met by each author listed, that the article represents original material, has not been published, accepted for publication in other journals, or concurrently submitted to other journals, and that all funding and conflicts of interest have been declared. Consent forms have been secured for the publication of information about patients or cases; otherwise, authors have declared that all means have been exhausted for securing consent.

Correlation of Tumor-Associated Leukocytes with Prognosis of Colorectal Carcinoma based on Pathologic Stage*

John Anthony Tindoc,^{1,2} Emilio Villanueva III,^{1,3} Nelson Geraldino¹

¹Department of Laboratories, University of the Philippines – Philippine General Hospital

²Department of Laboratories, Silliman University Medical Center, Philippines

³Department of Pathology, University of the Philippines – College of Medicine

ABSTRACT

Objectives. To perform a pilot study investigating the presence of correlation between the different mean tumor-associated leukocyte counts and the prognosis of colorectal cancer based on pathologic stage.

Methodology. A cross-sectional study, involving colorectal carcinoma cases in the Philippine General Hospital from 2015-2016. Proportional allocation stratified random sampling was done, with pathologic stage (AJCC 7th Edition) as the stratifying variable, collecting a total of 59 samples. Tissue sections from the samples were evaluated for the different tumor-associated lymphocyte counts. Correlation coefficients were computed to determine their correlation with pathologic stage as surrogate marker for prognosis.

Results. Of the myriad populations counted within and around the tumor mass, total lymphocyte, cytotoxic T-cell (CD8+ T-cell), neutrophil, macrophage, and plasma cell populations have significant correlation with pathologic stage as surrogate marker for prognosis of colorectal carcinoma.

Conclusion. The immune system appears to have a significant role in the natural history of colorectal carcinoma. The tumor-infiltrating lymphocytic population and especially the CD8+ T-cell subset, neutrophils, and macrophages are correlated with better prognosis. The same observation can be seen with the peritumoral CD8+ T-cells, neutrophils, macrophages, and plasma cells.

Key words: colorectal adenocarcinoma, tumor-infiltrating lymphocytes, peritumoral leukocytes, prognosis

ISSN 2507-8364 (Online)

Printed in the Philippines.

Copyright© 2019 by the PJP.

Received: 2 September 2019.

Accepted: 11 November 2019.

Published online first: 29 November 2019.

<https://doi.org/10.21141/PJP.2019.12>

*Corresponding author: Emilio Q. Villanueva III, MD, MSPH, DPSP
Email: drevilthird@gmail.com*

**Presented at the Philippine Society of Pathologists Annual Convention
Research Presentation, EDSA Shangri-La, April 20, 2018*

INTRODUCTION

Recently interest on the role of the immune system in influencing prognosis of cancers is on the rise. It has been noted several decades ago that the immune system did have a role with cancer biology but interest receded. Today with the advent of more cancer drugs and the possibility of manipulating the immune system against the tumor cells, interest on the interaction between the immune system and cancer is on the rise. Local data regarding these interactions can provide useful information on the treatment and prognosis of Filipino cancer patients.

Over the course of the medical investigation of cancers, the emphasis has been on the nature of the tumor cells. Rightly so, a veritable caché of inherent characteristics of the rouge cells determines the course of the disease. A lot of therapeutic forays have been based on the results of the studies of the tumor characteristics. The initial chemotherapeutic agents were chosen on their ability to destroy tumor cells with characteristic rapid mitotic capability but with poor genetic proofreading and repair. However, normal cells, likewise, can approach the same velocity of cell replication in times of tissue repair or normal turn-over. This rendered the first chemotherapeutic agents very nonspecific and fraught with a lot of side-effects bringing into limelight the discussion of the dichotomy of quality versus length of life.



Eventually, research opened up the possibility of having therapeutic agents with lesser collateral tissue damage. This was made possible with the discovery of protein markers which are increased or mutated in tumor cells. A degree of greater specificity was achieved by engineering drugs which target the cells that express these markers. Hence, the advent of monoclonal antibodies. As promising as this technology may seem, the cancer cells possess an impeccable resilience by adapting to the treatment modality through myriad means – increase extracellular drug transport, gain of new mutations, and circumvention of apoptosis, among other things.

At the very beginning of the study on cancer biology, an early postulate on the nature of cancer was summarized with the “seed and soil hypothesis.” Today, an emerging interest is seen for the study of, not the seed, but of the soil. Cancer, like any disease, is an interplay of the pathogen and the host. It is in this context that we would begin to investigate the host response to cancer.

The tumor milieu is a complex environment. It rivals the complexity of the offending tumor cells. In the background environment, we see the body’s response to and how it interprets the rogue cells. The immune system does not appear to be ignorant of the presence of these cancer cells since histologic assessment has seen immune cells infiltrating the tumor environment. Several studies have begun to investigate the significance of these cells in the tumor environment. At the beginning of tumor formation is an accompanying inflammatory response that contributes to a pro-tumorigenic niche.^{1,2} This protumorigenic niche is developed over time through repetitive inflammation leading to accumulation of immune cells. Eventually, their tissue repair functions become maladaptive and the excessive response provides a focus of tumor development or metastasis.^{1,2} On each of the step of tumor growth and metastatic cascade, bone marrow derived cells have been observed to influence the tumor microenvironment as either susceptible or resistant to tumorigenic growth.¹ Although data on leukocyte infiltration, especially lymphocytic cell line, has initially shown mixed results; it would seem that further studies have revealed that these monotonous mononuclear lymphocytes are composed of distinct populations of cytotoxic and suppressor lymphocytes, among others.³⁻⁵ It is beginning to show that the populations of these cells rather than the general leukocyte population per se influence the prognosis of the patient.^{6,7}

As far as colorectal cancers are concerned, there are several studies pointing out the significance of the lymphocytic infiltration with regards to underlying mechanism of tumorigenesis and the prognosis.⁸ In fact, tumors arising from microsatellite instabilities tend to create a colorectal carcinoma with a distinctly heavy tumor lymphocyte infiltration and Crohn-like reaction with lymphoid nodules.⁹ Several studies have also shown the lymphocytic infiltration on colorectal carcinoma as an independent prognostic factor. Some even find it a stronger prognostic factor than TNM staging.¹⁰ More recent studies have investigated into the subpopulations of these lymphocytes to determine a sharper correlation

with prognosis, especially on the T-cell population which is inherently associated with cytotoxic immune response.⁶

A unique subpopulation of cytotoxic lymphocytes are the natural killer cells (NK cells). They differ from the T-cells in that they can attack cells with or without aid of antibodies. They do this by targeting cells with depressed expression of MHC class I molecules. NK cells have been an established arm in immunologic tumor surveillance. In recent studies, NK cell activity was shown to be depressed during post-tumor resection presumably due to “tissue stress.”¹¹

Macrophages play an active role in tumors. In fact their presence in the stroma of the tumor is dense to the point that they may compose up to 30% of the total tumor mass. These tumor-associated macrophages (TAMs) represent the source of most proteases and cytokines involved in tumor growth. A study on intimate macrophage-tumor cell interaction has been done where it is shown that the TAMs aid during tumor intravasation in the process of metastasis.¹¹

The myeloid lineage contributes several granulocytes in the circulating immune system. Of these, neutrophils and eosinophils have been objects of interest as far as their contribution to tumor growth is concerned. Early studies on neutrophils appeared to show a protumorigenic profile wherein they enhance angiogenesis and support metastatic seeding. Recent studies however begin to show contrasting results wherein tumor infiltrating neutrophils appeared to blunt metastatic colonization of the lung by breast carcinoma. In some other studies, the lymphocyte:neutrophil ratio in the tumor has also been suggested to be of prognostic import.¹¹

There is no local data investigating the interplay between tumor cells and immune response. All patient care decisions have been based on studies of foreign populations with the assumption that the results will hold true for Filipinos. Observing the interplay of local cancers and their hosts at the immunologic level can both influence future patient care decision making and lay the ground for future local studies into this subject. This study wants to determine the presence of correlation between the different tumor-associated leukocyte counts and the pathologic stage as surrogate marker for the prognosis of colorectal carcinoma.

METHODOLOGY

This is a pilot study investigating the correlation of the different tumor-associated leukocyte counts with pathologic stage as surrogate marker for prognosis of colorectal carcinoma. The study was carried out as a cross-sectional design, involving colorectal carcinoma cases from the Philippine General Hospital during 2015-2016. There is a total of 230 colorectal carcinoma cases with surgical resection specimens in the records filed by organ-system for the years 2015-2016. The different cases were grouped according to pathologic stage (AJCC 7th edition) with the following count and proportion of the cases in each stratum (Table 1).

Table 1. The count and proportion of the different colorectal carcinoma cases with surgical resection specimens for the years 2015-2016, and the number of samples for each stratum taken for the study

Stage	Sampled Population		Sample	
	Count	Proportion	Count	Proportion
I	29	12.61%	8	13.56%
IIA	63	27.39%	16	27.12%
IIB	0	0.00%	0	0.00%
IIC	5	2.17%	2	3.39%
IIIA	10	4.35%	3	5.08%
IIIB	75	32.61%	18	30.51%
IIIC	29	12.61%	7	11.86%
IVA	13	5.65%	3	5.08%
IVB	6	2.61%	2	3.9%

The minimum sample was computed using G*Power 3.1 and at least 27 samples is needed for the study to achieve a power of 0.80 and level of significance of 0.10 in detecting presence of correlation with an effect size of ±0.40, i.e., coefficient of $\rho > 0$ (one-tailed). Stratified random sampling was done, with pathologic stage as the stratifying variable. For each stratum, all cases were numbered from 1 to N, and simple random sampling by random number method using the random number generator function of Microsoft Excel was employed. The number of samples taken per stratum was proportional to that of the sampled population of the study (Table 1).

The paraffin blocks and the tissue slides of the samples were retrieved and reassessed. Hematoxylin and eosin staining for the tumor sections were used to assess by light microscopy, the intensity of the following tumor-associated leukocytes (TALs): total lymphocytes, neutrophils, eosinophils, and plasma cells. Additional tissue sections were obtained from the paraffin blocks and were subjected to immunohistochemical staining with CD4, CD8, CD56, and CD68 to assess the population of CD8+ T-cells, helper T-cells (CD4+ T-cells), NK cells, and macrophages, respectively. Tumor-infiltrating leukocyte count were recorded for each TALs by obtaining the average cell count over 10 high power fields on the intratumoral areas more than 1 low power field from the tumor border and with at least 80% tumor within the field. Likewise, peritumoral leukocyte count were recorded for each TALs by obtaining the average cell count over 10 high power fields on the peritumoral border within 1 low power field at the edge of the deepest site of tumor invasion.

Data analysis was done using Stata 15.1. The difference between median tumor-infiltrating and peritumoral counts of the different tumor-associated leukocytes was evaluated using Wilcoxon sign-rank test. Spearman's rho correlation coefficients of the different TAL counts and pathologic stage were computed and presence of correlation is identified when the 90% confidence interval did not intersect with the null value $\rho = 0$. It is noted that the 5-year survival rate of stage IIIA is higher than stage IIA, IIB, and IIC, as well as the 5-year survival rate of stage IIC than IIB. Therefore, for the variable of pathologic stage to be used as a surrogate marker for prognosis, the ordered ranks of the different stages were coded following an increasing 5-year survival rate (Table 2).

Table 2. The ranks used in the Spearman rank correlation analysis of TAL counts with 5-yr observed survival rates of the different colorectal cancer stages as surrogate marker for prognosis

Rank	Stage	5-yr survival rate
1	IVB	9.7%
2	IVA	15.2%
3	IIIC	46.9%
4	IIIB	70.3%
5	IIB	71.3%
6	IIC	73.8%
7	IIA	79.7%
8	IIIA	85.4%
9	I	86.3%

This study is limited to samples of patients with colorectal adenocarcinoma in our institution. Histologic variants were not specified. Immunohistochemical stains were done to assess the CD8+ and CD4+ T-cell subpopulations, but without segregation of CD4+ T-cells into Th1 and Th2 helper T-cells. No immunohistochemical staining for B-cells were done. Macrophages were not segregated as well into their M1 and M2 cytokine profiles. Neutrophils were likewise not segregated into their N1 and N2 cytokine phenotypes.

RESULTS

A total of 59 samples were included in the study. The number of samples included per stratum is summarized in Table 1.

There were significant differences between the median TAL counts present infiltrating the tumor from those at the periphery of the tumor. This difference exists across all populations of the enumerated TALs in the study. More TALs are found at the periphery of the tumor than within the tumor parenchyma (Table 3).

Table 3. The difference in counts (per hpf) of tumor-infiltrating leukocytes and peritumoral leukocytes

Leukocyte	Tumor-infiltrating		Peritumoral		p-value*
	Median	(IQR)	Median	(IQR)	
Total Lymphocyte	16.4	(16.1)	31.1	(16.5)	<0.0001
CD4+ T-cell	5.5	(6.5)	15.7	(14.3)	<0.0001
CD8+ T-cell	8.5	(8.8)	25.0	(15.1)	<0.0001
NK Cell	0.3	(0.4)	0.5	(1.1)	<0.0001
Neutrophil	6.2	(8.0)	12.4	(16.6)	<0.0001
Eosinophil	0.2	(0.8)	2.2	(5.1)	<0.0001
Macrophage	7.0	(5.3)	15.2	(18.7)	<0.0001
Plasma Cell	1.7	(3.0)	9.5	(14.1)	<0.0001

*Wilcoxon Sign-Rank Test

Tumor-infiltrating total lymphocyte count has a positive correlation with survival based on pathologic stage. The more lymphocytes seen infiltrating the intratumoral milieu, the better the prognosis the patient has (Table 4 and Figure 1A). Similar observations were made with tumor-infiltrating CD8+ T-cells, neutrophils, and macrophages (Table 4, and Figures 1B to 1D).

Table 4. Correlation coefficients of tumor associated leukocyte counts (per hpf) with good prognosis (increasing 5-year survival rates)

Leukocyte	Tumor-infiltrating		Peritumoral	
	<i>p</i>	[90% CI]	<i>p</i>	[90% CI]
Total Lymphocyte	*0.405	[0.207, 0.571]	*0.368	[0.165, 0.541]
CD4+ T-cell	0.127	[-0.092, 0.334]	-0.092	[-0.302, 0.127]
CD8+ T-cell	*0.419	[0.223, 0.582]	*0.324	[0.116, 0.505]
NK Cell	0.134	[-0.084, 0.341]	0.177	[-0.040, 0.379]
Neutrophil	*0.398	[0.199, 0.566]	*0.344	[0.138, 0.522]
Eosinophil	0.015	[-0.202, 0.231]	0.192	[-0.026, 0.392]
Macrophage	*0.372	[0.169, 0.545]	*0.481	[0.295, 0.631]
Plasma Cell	0.157	[-0.062, 0.361]	*0.586	[0.423, 0.712]

*Presence of correlation, $\rho > 0$

At the peritumoral border of the deepest site of invasion of the tumor, presence of positive correlation with prognosis can be seen with total lymphocyte count, CD8+ T-cells, neutrophils, macrophages, and plasma cells (Table 4 and Figures 2 to 3A). The more total lymphocytes, CD8+ T-cells, neutrophils, macrophages and plasma cells seen bordering the deepest site of tumor invasion, the better the prognosis of the patient. It is noted that it is the abundance of plasma cells at the peritumoral border that is correlated with prognosis rather than plasma cells infiltrating the tumor. Further investigation

on the peritumoral plasma cells showed that there is presence of correlation between peritumoral plasma cells and the depth of invasion (T criterion of TNM), $\rho = -0.432$, 90% CI [-0.593, -0.238]. There is a decrease in the peritumoral plasma cell count as the depth of invasion increases (Figure 3B).

The rest of the cellular population within and around the tumor infiltrate show a trend of decreasing counts as the prognosis and stage worsens; however, there is not enough evidence to show the presence of correlation.

DISCUSSION

It is interesting to note that the inflammatory response is starkly more prominent at the periphery of the tumor than within the tumor parenchyma. Inflammation is a response to proteins or markers that lead to the activation of the inflammatory cascade. As to why the response is much more prominent at the periphery is not fully understood. This study only corroborates that observation.

It is understood by previous studies that the degree of lymphocytic infiltration can have positive or negative correlation with the prognosis of certain tumors. The direction of the correlation however has been uncertain.

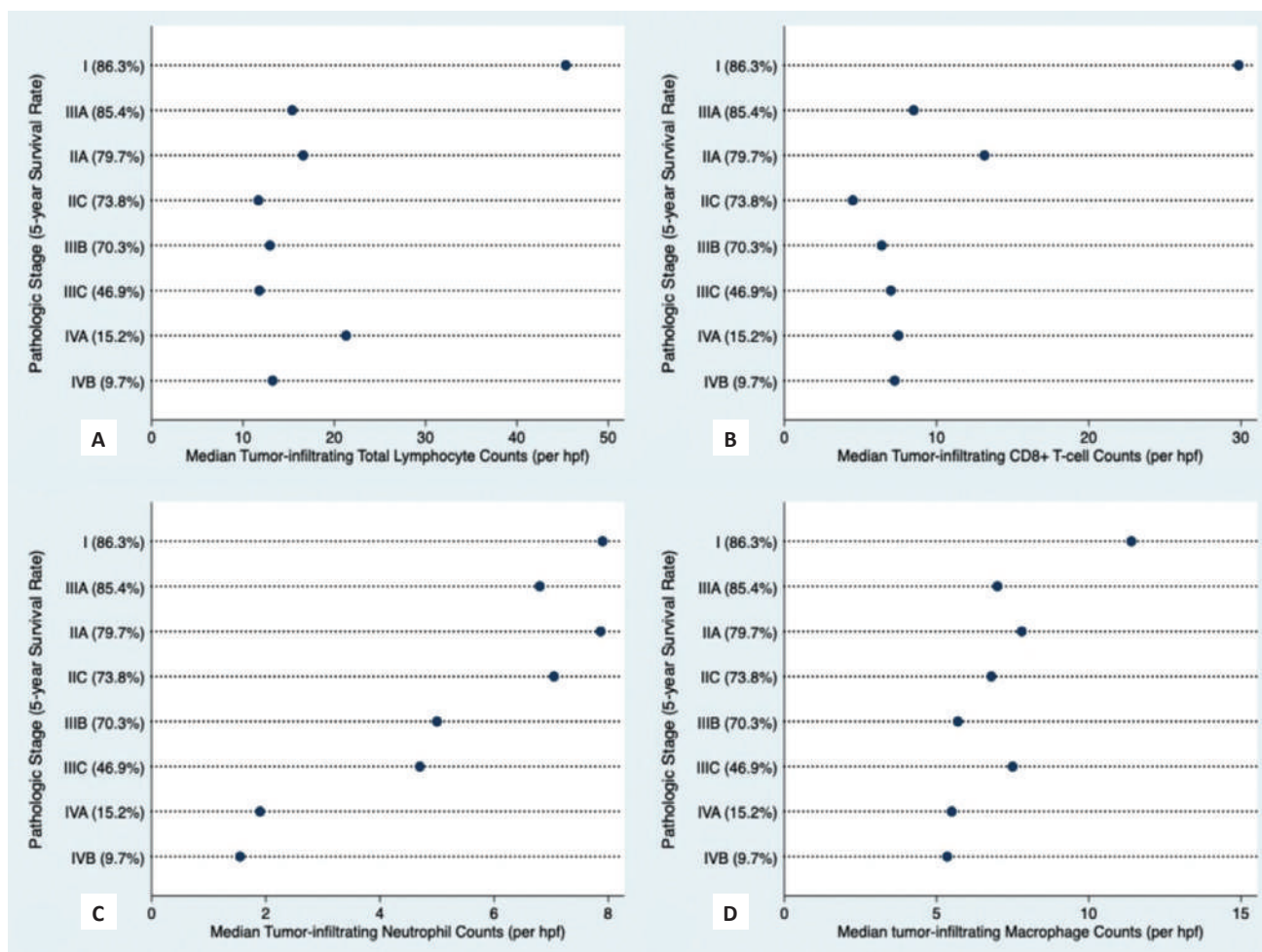


Figure 1. Median tumor-infiltrating leukocyte counts (per hpf) of different 5-year survival rates of the different pathologic stages: (A) total lymphocyte, (B) CD8+ T-cell, (C) neutrophil, and (D) macrophage.

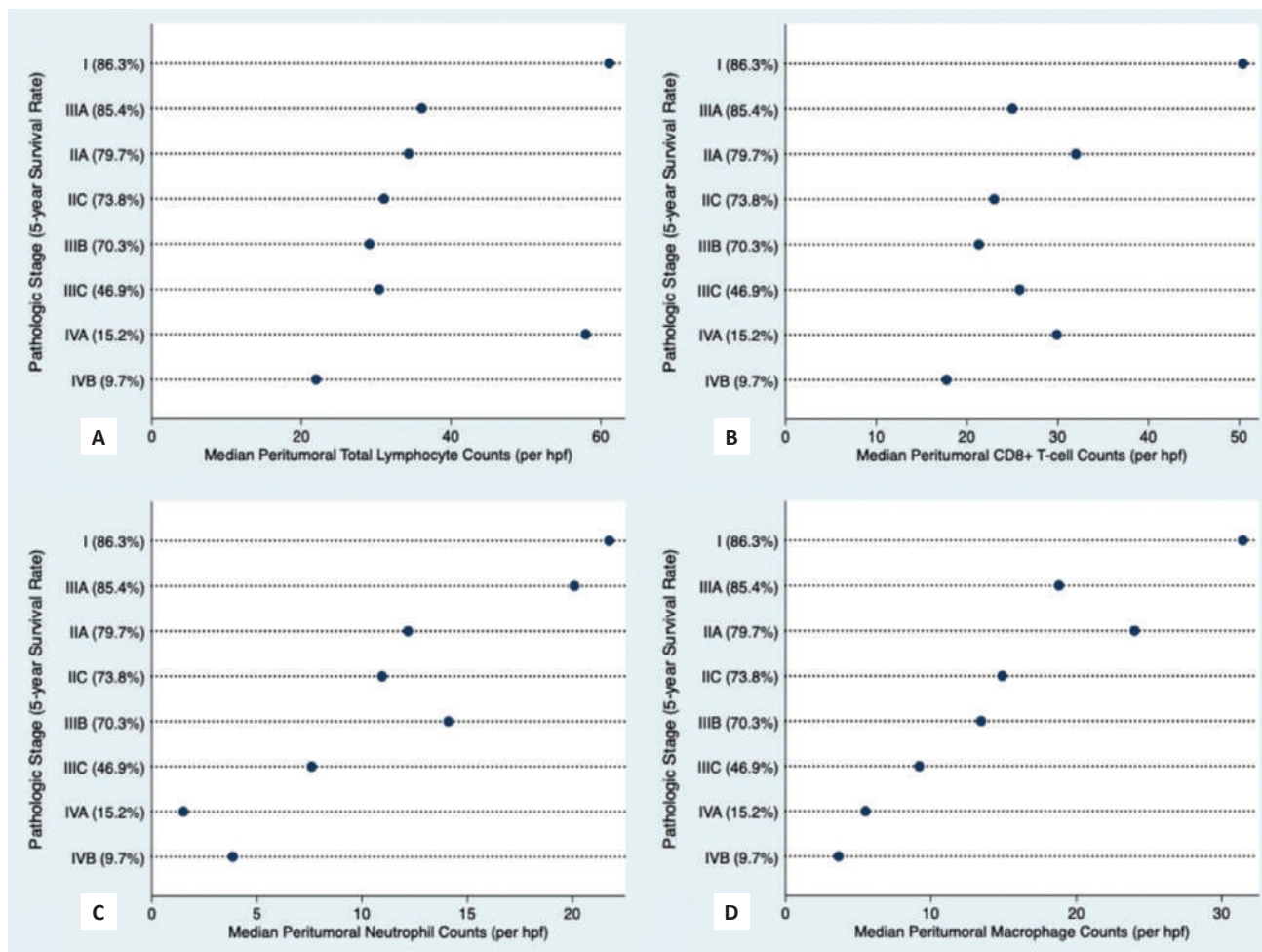


Figure 2. Median peritumoral leukocyte counts (per hpf) of different 5-year survival rates of the different pathologic stages: (A) total lymphocyte, (B) CD8+ T-cell, (C) neutrophil, and (D) macrophage.

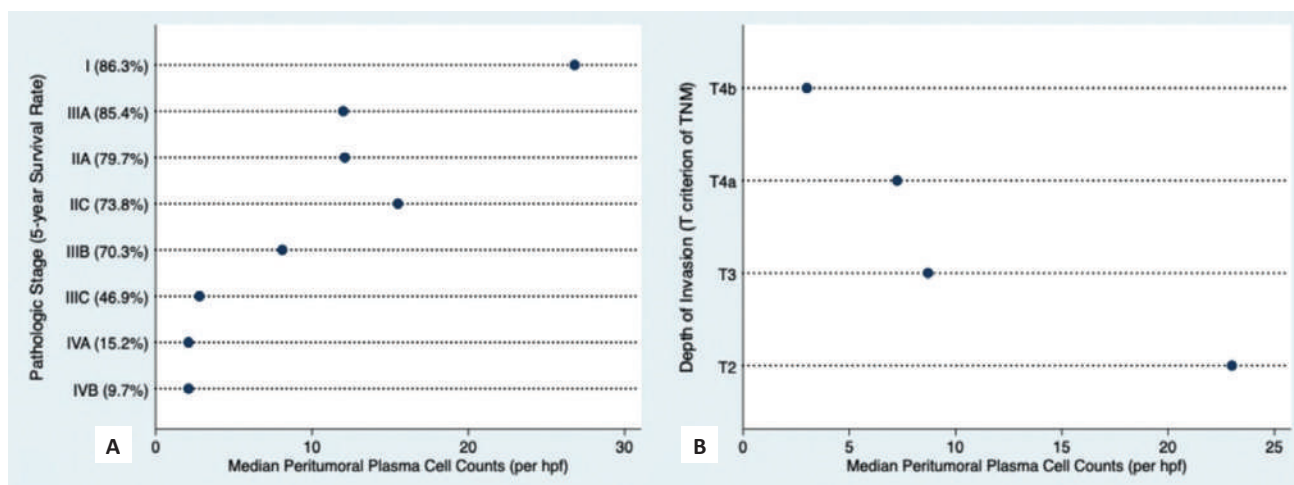


Figure 3. Median peritumoral plasma cell counts (per hpf) of different: (A) 5-year survival rates of the different pathologic stages, (B) depth of invasion (T criterion of TNM).

It is noted that cancer cells can recruit lymphocytes from the peripheral circulation into the cancer stroma to help promote its growth. It is in this context that increased densities of tumor infiltrating lymphocytes can prove disadvantageous to the host.¹¹ However there is an alternative view proposed and well-supported that

teaches that the lymphocytes are actually sent by the immune system to target and dispatch of the aberrant tumor cells.¹¹ This study supports the latter in that the correlative evidence points towards the view that cancers with lower stages and better prognoses have higher amounts of tumor infiltrating lymphocytes.

Lymphocytic populations despite their monotonous histologies are actually diverse. Subpopulations of the lymphocytes are categorized based on some antigens they display. In this study interest is focused on two subpopulations of T lymphocytes: CD4⁺ and the CD8⁺ T-cells. CD8⁺ T-cells are known for their function to destroy cells harboring pathogenic particles within the cytosol. It is because of this function that they are called cytotoxic T-cells. The evidence in this study points to a positive correlation of the infiltrating CD8⁺ T-cells with the increasing survival rates or good prognosis of colorectal cancer patients. CD4⁺ T-cell populations, on the other hand, have proven to be inconclusive and their association with the tumor is nebulous. This may be attributed to the fact that even this subpopulation can still be categorized into regulatory T-cells (Treg cells) and helper T-cells (Th cells). These two appear to have opposite effects on tumor progression. Treg cells are involved in blunting the activities of the CD8⁺ T-cells and are primarily involved in promoting peripheral immunotolerance – a mechanism which may be hijacked by the tumor. Th cells on the other hand secrete cytokines that promote the activation of CD8⁺ T-cells and would most likely help in limiting tumor growth. Differentiating the two populations would require more than labeling of the CD4 antigen.

TAMs likewise have been associated with both tumor growth and anti-tumor activities. This study shows however that TAMs around the tumor border and within the tumor are positively correlated with better prognosis. The disparity seen together with other studies may be attributable to macrophages having at least 2 subpopulations depending on the cytokine profile. M1 macrophages are known for their antitumor properties and with their capacity to help present tumor proteins to Th cells and also help in killing tumors by antibody-dependent cellular cytotoxicity. M2 macrophages on the other hand may be pro-angiogenic and promote tumor progression.

Formation of extrafollicular lymphoid structures can occur in areas surrounding tumor. These often produce a type of plasma cells that are short-lived and that do not acquire the ability to migrate to distant sites. These short-lived plasma cells can produce antibodies of presumably lower affinity and thus still help in antibody-dependent cell mediated cytotoxicity. This study supports the view that plasma cells provide antitumor effects as postulated. The evidence shows a positive correlation between peritumoral plasma cells and increasing 5-year survival rates. Also, it is noted that there is a negative correlation between peritumoral plasma cell counts and the depth of tumor invasion – as peritumoral plasma cell counts decreases, the depth of tumor invasion increases.

Neutrophils are the predominant granulocytic population in the body. They play a vital role in the early response and defense against invading microorganisms. Like the macrophages, the neutrophils can have different phenotypes that polarize their response to tumors.¹² This is the likely reason why there are divergent findings on the tumorigenic versus antitumor effects of neutrophils. Suffice it to say that at least two publications support

the antitumorigenic effects of neutrophils by blunting the metastatic colonization of renal cell carcinoma on the lung¹³ and by promoting cell death of disseminated breast cancer cells in the premetastatic lung.¹⁴

For other cellular constituents of the inflammatory infiltrate, the trends hinted of a decreasing numerical trend as prognosis worsens but do not have statistical significance.

CONCLUSION

The study demonstrates what has long been held in suspicion to be true: that there is a significant role played by the immune system in tumor progression. The intent of the study is to see if there is a correlation between the quantity and density of inflammatory cells with the colorectal cancer prognosis. True enough, some degree of understanding is elucidated in this study. There are mostly inconclusive trends, showing decreasing TAL cell counts as the prognosis worsens, seen with most inflammatory cells. However, four particular cell populations show a presence of positive correlation with prognosis: tumor-infiltrating and peritumoral CD8⁺ T-cells, neutrophils, and macrophages; and, peritumoral plasma cells. The tumor-infiltrating and peritumoral CD8⁺ T-cells are correlated with better prognosis. Similarly, the tumor-infiltrating and peritumoral neutrophils, and macrophages; and, peritumoral plasma cells are correlated with a better prognosis. The greater the quantity of these cells within the tumor, and at the peritumoral border of the deepest point of invasion, the better the prognosis of the colorectal carcinoma patient.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria. All authors have equally contributed to this work, proofread and approved the manuscript for publication.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

1. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. *Nat Rev Cancer*. 2009;9(4):239-52. PMID: 19279573. PMCID: PMC3251309. <https://doi.org/10.1038/nrc2618>.
2. Medzhitov R. Origin and physiological roles of inflammation. *Nature*. 2008;454(7203):428-35. PMID: 18650913. <https://doi.org/10.1038/nature07201>.
3. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer*. 2012;12(4):298-306. PMID: 22419253. <https://doi.org/10.1038/nrc3245>.
4. DeNardo DG, Andreu P, Coussens LM. Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. *Cancer Metastasis*

- Rev. 2010;29(2):309-16. PMID: 20405169. PMCID: PMC2865635. <https://doi.org/10.1007/s10555-010-9223-6>.
5. Kovacsovics-Bankowski M, Chisholm L, Vercellini J, et al. Detailed characterization of tumor infiltrating lymphocytes in two distinct human solid malignancies show phenotypic similarities. *J Immunother Cancer*. 2014;2(1):38. PMID: 25436113. PMCID: PMC4247679. <https://doi.org/10.1186/s40425-014-0038-9>.
 6. Reissfelder C, Stamova S, Gossmann C, et al. Tumor-specific cytotoxic T lymphocyte activity determines colorectal cancer patient prognosis. *J Clin Invest*. 2015;125(2):739-51. PMID: 25562322. PMCID: PMC4319435. <https://doi.org/10.1172/JCI74894>.
 7. Huh JW, Lee JH, Kim HR. Prognostic significance of tumor-infiltrating lymphocytes for patients with colorectal cancer. *Arch Surg*. 2012;147(4):366-72. PMID: 22508783. <https://doi.org/10.1001/archsurg.2012.35>.
 8. Tougeron D, Fauquembergue E, Rouquette A, et al. Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. *Mod Pathol*. 2009;22(9):1186-95. PMID: 19503063. <https://doi.org/10.1038/modpathol.2009.80>.
 9. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. *Cancer*. 2001;91(12):2417-22. PMID: 11413533.
 10. Ropponen KM, Eskelinen MJ, Lipponen PK, Alhava E, Kosma V-M. Prognostic value of tumour-infiltrating lymphocytes (TILs) in colorectal cancer. *J Pathol* 1997;182(3):318-24. PMID: 9349235. [https://doi.org/10.1002/\(SICI\)1096-9896\(199707\)182:3<318::AID-PATH862>3.0.CO;2-6](https://doi.org/10.1002/(SICI)1096-9896(199707)182:3<318::AID-PATH862>3.0.CO;2-6).
 11. Mendelsohn J, Howley P, Israel M, Gray J, Thompson C. *The Molecular Basis of Cancer*, 4th ed. Elsevier; 2014.
 12. Fridlender ZG, Sun J, Kim S, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell*. 2009;16(3):183-94. PMID: 19732719. PMCID: PMC2754404. <https://doi.org/10.1016/j.ccr.2009.06.017>.
 13. López-Lago MA, Posner S, Thodima VJ, Molina AM, Motzer RJ, Chaganti RS. Neutrophil chemokines secreted by tumor cells mount a lung antimetastatic response during renal cell carcinoma progression. *Oncogene*. 2013;32(14):1752-60. PMID: 22665059. PMCID: PMC3435490. <https://doi.org/10.1038/onc.2012.201>.
 14. Granot Z, Henke E, Comen EA, King TA, Norton L, Benezra R. Tumor entrained neutrophils inhibit seeding in the premetastatic lung. *Cancer Cell*. 2011;20(3):300-14. PMID: 21907922. PMCID: PMC3172582. <https://doi.org/10.1016/j.ccr.2011.08.012>.

Disclaimer: This journal is **OPEN ACCESS**, providing immediate access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. As a requirement for submission to the PJP, all authors have accomplished an **AUTHOR FORM**, which declares that the ICMJE criteria for authorship have been met by each author listed, that the article represents original material, has not been published, accepted for publication in other journals, or concurrently submitted to other journals, and that all funding and conflicts of interest have been declared. Consent forms have been secured for the publication of information about patients or cases; otherwise, authors have declared that all means have been exhausted for securing consent.

A Pilot Study on the Evaluation of Clinical Chemistry Laboratory Test Performance using Six Sigma Metrics

Pier Angeli Medina, Jenny Matibag, Sarah Jane Datay-Lim, Elizabeth Arcellana-Nuqui

The Medical City, Pasig City, Philippines

ABSTRACT

Introduction. Six sigma has been used over the years, initially in manufacturing industries to improve quality by reducing the number of wastes and defects. In the laboratory, it can also provide measurement of quality using the sigma scale.

Objective. The main objective of the study is to evaluate the performance of tests in two chemistry analyzers using the six sigma scale.

Methodology. A total of twenty (28) tests were evaluated on two Abbott Architect c8000 chemistry analyzers from September 2014 to July 2019 using results of quality control mean, coefficient of variation, bias and total allowable error to compute for the six sigma value. Both level one and level two third party quality controls were included in the evaluation.

Results. Results of the study showed the tests that were >6 sigma for both levels 1 and 2 throughout the 5 years. Di-Bil, CK, HLD, TG and UA were consistently >6 sigma for one machine while CK, Di-Bil, HDL, Mg, TG and UA were consistently >6 sigma for the other. Level 1 and Level 2 sigma scores were noted to be incongruent in some analytes as follows: ALB, ALT, K, TP for one instrument and ALB, ALP and AST for the other instrument. Electrolytes Ca, Cl, and Na were generally low (<3.0) for both machines with the exception of K which showed better sigma scores.

Conclusion. Using six sigma metrics allowed the laboratory to evaluate the performance of the chemistry tests objectively. Tests that are >6.0 sigma signifies world class performance and entail application of fewer Westgard rules with fewer number of runs while those that are <3.0 need method improvement or more stringent quality control measures. The findings show that we can use this for monitoring and performance evaluation for quality improvement.

Key words: bias, laboratory, quality control, quality improvement, six sigma, Westgard rules

ISSN 2507-8364 (Online)

Printed in the Philippines.

Copyright© 2019 by the PJP.

Received: 5 October 2019.

Accepted: 4 December 2019.

Published online first: 5 December 2019.

<https://doi.org/10.21141/PJP.2019.13>

Corresponding author: Sarah Jane L. Datay-Lim, MD

E-mail: sldataylim@themedicalcity.com

INTRODUCTION

Laboratory results are a keystone in the diagnostics and therapeutics of medicine. It is therefore important that measures are taken to assure the quality of processes that generate these results. Running control materials is a vital element to ensuring that all the machines are working at optimal levels before any of the patient results are released. Control results are normally plotted on a Levy-Jennings chart in order to easily visualize if they are within acceptable range. Mr. James O. Westgard established the “Westgard rules”, which are generally accepted guidelines applied to the Levy-Jennings charts to make decisions on the reliability of results.¹ However, laboratories are still faced with challenges of false rejection and inappropriate use of QC rules

Six sigma was first developed at Motorola in the 1980's to improve quality and reduce cost by eliminating defects. It was developed through statistical measurements and benchmarking using the DMAIC (Define, Measure, Analyze, Improve and Control) principle.² Since then, it has been applied not only in the manufacturing industries, but also in the medical field. It is particularly suitable in the laboratory where variation can be measured to



predict performance instead of counting the defects.³ Most studies involving the use of six sigma in the laboratory have shown benefit of using this method as part of the approach to quality management.^{4,5,6,7} Six sigma is a powerful tool for assessment of test performance in order to apply appropriate Quality Control (QC) rules and other recommendations such as number of runs and levels.

Hence, we analyzed internal quality control data of two (2) Abbott Architect c8000 series in the chemistry section of our laboratory from August 2014 to June 2019 to evaluate the performance of clinical chemistry analytes on the six sigma scale.

METHODOLOGY

Methods and sample

This is a descriptive study of all internal quality control samples of clinical chemistry tests done at The Medical City Department of Laboratory Medicine and Pathology, Ortigas Pasig City, Philippines, from August 2014 to June 2019.

All the Quality control data were extracted from two (2) Abbott Architect c8000 series clinical chemistry analyzers (Abbott Diagnostics, Chicago, IL, USA) per year. The machines are labeled “Instrument A” (c803024) and “Instrument B” (c803029).

Both Level 1 and Level 2 control data of the following analytes were included: Albumin (ALB), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Amylase, Aspartate Aminotransferase (AST), Total Bilirubin (Bil-T), Direct Bilirubin (Bil-D), Calcium (Ca), Chloride (Cl), Total Cholesterol (Chole), Creatine Kinase Total (CK), Complement 3 (C3), Carbon Dioxide(CO2) Glucose, Gamma- glutamyl Transpeptidase (GGT), High Density Lipoprotein (HDL), Iron, Lactate (Lac), Lactate Dehydrogenase (LDH), Low Density Lipoprotein (LDL), Lipase (Lip), Magnesium (Mg), Phosphatase (Phos), Potassium (K), Sodium (Na), Total protein (TP), Triglyceride (TG), Uric acid (UA), Blood Urea Nitrogen (BUN), Creatinine (Crea) and Unsaturated Iron Binding Capacity (UIBC). Quality control materials used were Level 1 and 2 Lymphocheck Biorad Assayed Chemistry Control (Bio-Rad, Marnes-la-Coquette, France) of the same lot number for a defined period of time (lyophilized).

Analysis

The sigma values were then determined for each test using the formula:

Sigma metrics (σ) = Total allowable error (TEa%) – Bias %/
Coefficient of variation (CV%)

Precision and Bias

The degree of precision can be determined through the computation of the coefficient of variation (CV%). It can be computed from our internal quality control (IQC) using the formula:

CV % = Standard of Deviation (SD)/ Mean * 100

On the other hand, Bias was computed using our data from External Quality Assurance Scheme (EQAS) using the formula:

Bias % = [(Laboratory mean – Peer group mean)/ Peer group mean] * 100

Total allowable error (TEa)

TEa combines both imprecision and bias of a method to calculate the impact on a test result and gives the tolerance limits of each analyte in the laboratory. There are different available TEa goals such as CLIA (Clinical Laboratory Improvements Amendments)⁸ from the US, Rili BAK (German Medical Council for the Quality Assessment of quantitative Analyses in Medical Laboratories, 2008 version; the inter-lab or “Ring Trials” values, in contrast to the intra-lab values) and the Ricos biological variability database (desirable target values, in contrast to the minimal or optimal target values).⁹ For this study, we used TEa from different sources (Table 1).

Table 1. Total allowable error (TEa) used to compute for six sigma derived from CLIA, Ricos BV and CAP

Test	TEa Source	TEa
Albumin	CLIA	10%
Alkaline phosphatase	CLIA	30%
Alanine aminotransferase	CLIA	20%
Amylase	CLIA	30%
Aspartate transaminase	CLIA	20%
Bilirubin, Direct	RICOS	44.50%
Bilirubin, Total	CLIA	20% or 6.84 umol/L
C3	RICOS	8.40%
Calcium	CLIA	0.2495 umol/L
Chloride	CLIA	5%
Cholesterol, Total	CLIA	10%
CO ₂	CAP	25%
Creatine Kinase, Total	CLIA	30%
Creatinine	CLIA	15% or 26.52 umol/L
Glucose	CLIA	10% or 0.333 mmol/L
Gamma-glutamyl transferase	RICOS	22%
High Density Lipoprotein (HDL)	CLIA	30%
Iron	CLIA	20%
Lactate	RICOS	30%
Low Density Lipoprotein (LDL)	CLIA	20%
Lactate Dehydrogenase	CAP	20%
Lipase	RICOS	29%
Magnesium	CAP	25%
Phosphorus	CAP	10.70% or 0.0969 mmol/L
Potassium	CLIA	0.5 mmol/L
Protein, Total	CAP	10%
Sodium	CLIA	4 mmol/L
Triglyceride	CLIA	25%
UIBC	Westgard	25%
Urea Nitrogen	CLIA	9% or 0.7142 mmol/L
Uric Acid	CLIA	17%

Legend: TEa – allowable total error; CLIA – Clinical Laboratory Improvement Amendments 1988; Ricos BV – Ricos biological variability database; CAP – College of American Pathologists

RESULTS

Monthly sigma was monitored since the start of the study and the cumulative yearly sigma was also calculated and summarized for the chemistry analytes for each of the chemistry instruments (Tables 2 and 3).

Table 2. Sigma metrics for Instrument A (c8000) from 2015-2019

Test	Level	2015	2016	2017	2018	2019
Albumin	1	4.3	3.3	7	6.9	3.2
	2	3.5	1.7	8.7	2.8	2.8
Alkaline phosphatase	1	4.4	*	5	19.6	4.1
	2	7.0	*	7.5	7.5	7.0
Alanine aminotransferase	1	3.1	-4.4	3.5	6.4	2.6
	2	7.1	9.4	9.2	6.6	4.7
Amylase	1	16.0	*	*	11.7	6.5
	2	17.9	*	*	15.1	7.2
Aspartate transaminase	1	6.1	7.6	6.7	0.4	4.0
	2	13.3	12.1	14.4	3	5.4
Bilirubin, Direct	1	8.4	14.3	8.8	14.2	9.4
	2	11.9	0.1	17.2	7.5	12.2
Bilirubin, Total	1	3.5	16.3	4	6.5	3.9
	2	6.1	8.4	4.1	2.6	3.0
C3	1	*	2.6	*	1.2	2.2
	2	*	0.7	*	1.5	2.0
Calcium	1	1.9	*	2.7	27.5	2.1
	2	1.8	*	2.9	2.9	1.6
Chloride	1	4.1	1.3	3.9	1.17	3.8
	2	4.3	1.3	3.8	2.5	3.0
Cholesterol, Total	1	2.0	2.6	8.1	9.4	3.7
	2	2.6	1.4	4.8	4.7	2.9
CO ₂	1	*	*	3.3	19.2	2.5
	2	*	*	1.7	2	1.9
Creatine Kinase, Total	1	9.0	8	8.8	6.1	5.5
	2	17.9	17.4	13.3	8.2	8.2
Creatinine	1	5.4	10.4	9.5	10.8	4.5
	2	5.9	6.8	6.3	6.2	5.3
Glucose	1	5.2	3.4	3.4	98.6	1.9
	2	6.8	2.5	4	4.2	2.1
Gamma-glutamyl transferase	1	8.8	*	4.8	12.4	3.2
	2	10.0	*	6.6	4.9	3.7
High Density Lipoprotein (HDL)	1	8.4	9.6	9.6	8.8	5.6
	2	8.0	9.3	8.3	7.4	4.4
Iron	1	7.5	*	6.9	6.7	8.0
	2	3.3	*	3.8	4.5	5.2
Lactate	1	*	*	6.1	11.4	5.7
	2	*	*	6	10.1	4.0
Low Density Lipoprotein (LDL)	1	5.9	6.1	10.3	6.4	2.0
	2	6.6	9.1	10	6.5	2.4
Lactate Dehydrogenase	1	3.5	*	4.2	3.2	1.9
	2	5.3	*	8	5.8	2.9
Lipase	1	6.1	*	3.8	4.9	3.0
	2	5.6	*	3.5	5.2	3.4
Magnesium	1	8.6	8.7	12.3	8.6	8.7
	2	9.6	6.3	11.6	11.3	10.5
Phosphorus	1	7.9	*	*	3.3	2.1
	2	8.5	*	*	3.1	2.9
Potassium	1	3.9	6.8	9.5	23.9	6.6
	2	4.4	4.7	5.6	4.4	4.3
Protein, Total	1	4.5	2.7	4.1	11.3	4.4
	2	5.8	3	3.2	4.1	3.8
Sodium	1	1.6	1.6	1.5	6.3	1.6
	2	2.1	2	2.6	1.6	1.7
Triglyceride	1	4.9	11	9	13.5	6.7
	2	3.5	10	6.5	13.1	5.3
UIBC	1	2.7	*	1.1	1.1	*
	2	4.2	*	2.3	2.6	*
Urea Nitrogen	1	2.6	3.8	3	5.4	2.7
	2	3.4	3.1	3	2.6	2.2
Uric Acid	1	16.8	8.3	8.4	12.3	4.1
	2	13.9	11.2	9.5	9	4.5

* Test unavailable on the instrument

Table 3. Sigma metrics for Instrument B (c8000) from 2015-2019

Test	Level	2015	2016	2017	2018	2019
Albumin	1	5.4	3.4	5.7	9.2	*
	2	4.2	0.2	3.8	2.4	*
Alkaline phosphatase	1	*	2.8	5.2	17.2	*
	2	*	5.7	8.9	8	*
Alanine aminotransferase	1	3.1	-6.5	3.6	6.3	*
	2	8.5	7.3	7.9	6.5	*
Amylase	1	*	9.8	15.6	10.2	*
	2	*	10	19.7	13.6	*
Aspartate transaminase	1	8.1	3.1	6.5	0.2	*
	2	16.7	10.5	12.4	3.3	*
Bilirubin, Direct	1	4.0	7.1	7.9	11.4	7.0
	2	5.8	-0.2	17.6	6.5	9.3
Bilirubin, Total	1	4.3	6.4	4.2	6.5	3.0
	2	*	3.3	6.3	2.7	*
C3	1	*	2.9	*	*	*
	2	*	1.5	*	*	*
Calcium	1	*	4.3	4.9	*	1.1
	2	*	2.8	6.3	*	1.2
Chloride	1	3.53	1.6	3.8	7.7	2.7
	2	4.41	1.1	4.2	3.3	2.7
Cholesterol, Total	1	8.2	6	7.2	9.2	3.1
	2	7.34	2.4	8.3	4.8	2.4
CO ₂	1	*	2.7	*	3.7	2.1
	2	*	1.3	*	2.3	1.5
Creatine Kinase, Total	1	*	*	9	5.5	*
	2	*	*	13.9	8	*
Creatinine	1	13.5	4.1	10.3	7.7	4.2
	2	5.8	3.4	8.3	7.8	5.1
Glucose	1	4.1	3.7	4.1	114.4	2.3
	2	5.1	3.6	6.4	4	3.2
Gamma-glutamyl transferase	1	*	3.9	*	14.3	*
	2	*	7.8	*	7.1	*
High Density Lipoprotein (HDL)	1	6.4	5.7	13.1	6.9	5.8
	2	7.1	5.6	10.6	7.8	6.3
Iron	1	*	5.1	5.2	4.1	6.6
	2	*	2.6	3.1	3.2	4
Lactate	1	*	3.5	*	10.6	*
	2	*	-5.4	*	10.5	*
Low Density Lipoprotein (LDL)	1	5.5	4.9	11.8	7.2	3.8
	2	5.4	7.3	10	8.7	3.8
Lactate Dehydrogenase	1	*	3.4	*	2.5	*
	2	*	5.1	*	4.6	*
Lipase	1	*	5.1	*	4.8	3.6
	2	*	5.6	*	5.4	4.3
Magnesium	1	13.8	6.9	12	5.4	7.8
	2	14.5	9.6	13.6	11.1	10.6
Phosphorus	1	*	1.9	5	*	0.9
	2	*	6.2	5.3	*	2.0
Potassium	1	3.7	4.1	8.2	14.7	5.4
	2	3.6	4.3	6.9	5.6	3.7
Protein, Total	1	6.1	4.1	6.8	10.8	3.6
	2	6.0	3.1	6	5.2	3.0
Sodium	1	1.9	1.8	1.2	4.2	1.4
	2	2.1	1.3	2.3	2.3	1.7
Triglyceride	1	18.9	10.2	14	12.4	6.1
	2	18.3	8.8	10.8	8.8	4.9
UIBC	1	*	1.5	*	1.2	1.6
	2	*	2	*	2.6	2.9
Urea Nitrogen	1	4.6	3.1	3.1	5.3	*
	2	5.7	2.8	3	3.1	*
Uric Acid	1	12.7	5.3	12.8	20.2	*
	2	10.4	11.3	13.2	8.2	*

* Test unavailable on the instrument

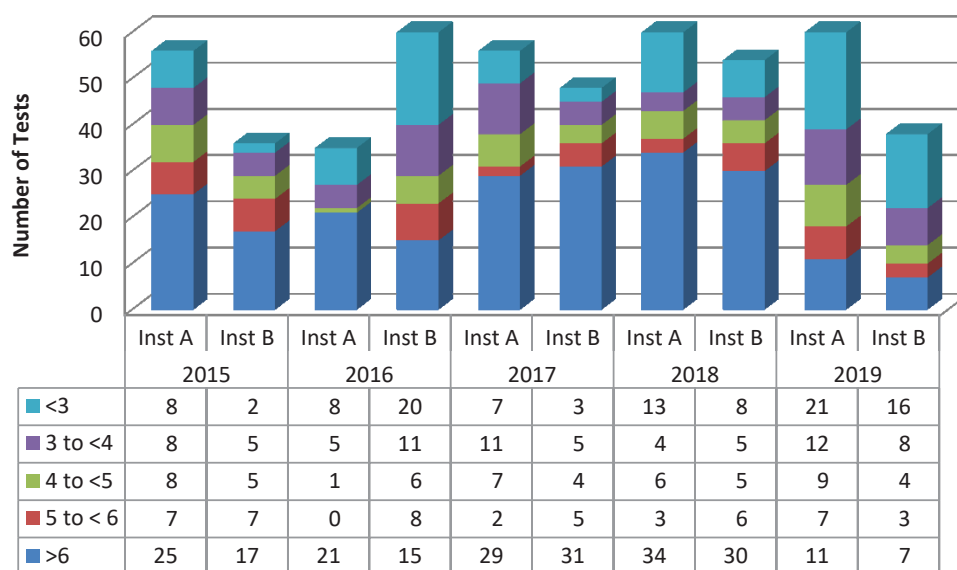


Figure 1. Sigma Performance of Chemistry tests in both machines showing the number of tests under the different sigma categories from 2015 to 2019.

the following percentage of tests that are more than six sigma as follows: 47.2% (2015), 25% (2016), 64.6 % (2017), 55.6 % (2018) and 18.42 % (2019). There are also noted differences between the two machines in terms of sigma performance percentage (Figure 1).

From 2015 to 2019, the percentage of tests that were >6 sigma, <3 sigma and those that fall in between show variations in number with 2016 and 2019 having the highest number of tests <3 sigma at 32.5 % (2016) and 35% (2019) for instrument A and 33.3 % (2016) and 42% (2019) for instrument B. All other years showed predominance of tests that were >6 sigma. There were also years with predominance of tests computed between >3 to <6 at 55.4% (2015) and 46.7% (2019) for instrument A and 47.2% (2015) and 41.7% (2016).

Tests that were >6 sigma for both levels 1 and 2 throughout the 5 years were noted. Di-Bil, CK, HLD, TG and UA were consistently >6 sigma for Instrument A. CK, Di-Bil, HDL, Mg, TG and UA were consistently >6 sigma for instrument B. Level 1 and Level 2 sigma scores were noted to be incongruent in some analytes as follows: ALB, ALT, K, TP for Instrument A and ALB, ALP and AST for Instrument B. Electrolytes Ca, Cl, and Na were generally low (<3.0) for both machines, with the exception of K which showed better sigma scores.

DISCUSSION

Six sigma means that six sigmas or standard deviations of process variation should fit within the tolerance limits. The measure of process performance is the number of defects per million (DPM) products or defects per million opportunities (DPMO).² Hence, an analyte that is computed to be six sigma is “world class” with a 3.4 DPM only, reflecting very few defects or errors. As sigma increases, consistency and steadiness of a test improves, which can reduce operating costs and wastes, and at the same time increase levels of customer satisfaction.⁴

The tests that were computed to be ≥ 6 sigma were identified (Tables 2 and 3). They require less stringent quality control monitoring using fewer QC runs with lower false rejection rates through application of selected Westgard rules. Highest percentages of tests >6.0 sigma were noted at 56.7 % (2018) for instrument A (Figure 2) and 64.6% (2017) for instrument B.

Three (3) sigma on the other hand, is the minimum acceptable quality at 66,807 DPM. Anything that is 3 sigma or below requires maximum QC or method improvement. More stringent quality control should be undertaken for these processes, such as application of more Westgard rules, more frequent monitoring, and additional QC runs for the day.

The observed difference in the sigma score percentages reflect the inherent nature of laboratory testing in a chemistry laboratory. Tests are affected by numerous factors such as the materials used, preventive maintenance schedules, equipment, and staff competency. Because quality is a continuous process, the sigma metrics represent only the performance at a given period of time. Sigma may change depending on the quality improvement strategies employed and the current conditions in the laboratory or equipment, among other factors. The observed improvements in the number of tests >6 sigma from 2015 to 2017 and 2018 can be attributed to the increased frequency of water filter changes, more intensive staff training. The fluctuations in the number of tests <3 sigma on the other hand, reflect the recorded periods of poor water supply, problems with room air conditioning, and instrument maintenance issues. The sigma of analytes in 2019 which showed significantly lower number of tests >6 sigma reflected the water crises which happened in the area together with issues in room temperature (air conditioning).

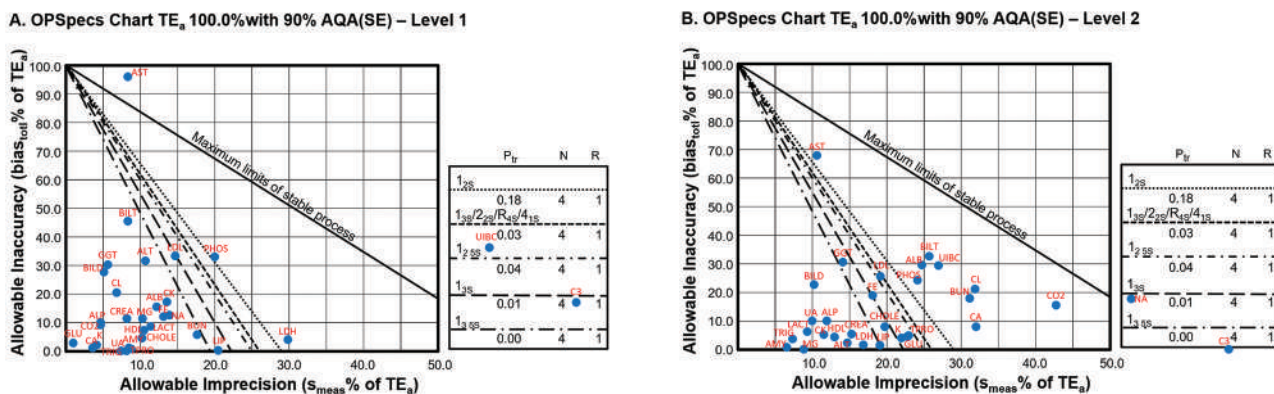


Figure 2. OPSpecs chart of Chemistry tests in 2018 for Instrument A showing the analytes under the different sigma categories: (A) Level 1 controls. (B) Level 2 controls. Routine operating specifications are presented in the form of an "OPSpecs chart," which describes the operational limits for imprecision and inaccuracy when a desired level of quality assurance is provided by a specific QC procedure. The allowable inaccuracy based on computed bias on the Y axis are plotted against the allowable imprecision on the X axis for each analyte. Note that those points on the left-most corner are those with the six sigma or "world class performance" while those that fall on the far right and even outside the chart are those with poor performance, with sigma less than 3.0. This helps visualize the performance of tests and make decisions regarding the QC plan.

Quality control policy of decreasing QC run for the tests that are >6 sigma as recommended by Westgard² was started May 2017. This policy of decreasing the number of runs to once a day for tests >6 sigma enabled our staff to focus on the problematic ones and improve efficiency in the laboratory.

Certain tests had constantly good performance (>6 sigma both levels), such as Di-Bil, CK, HLD, TG and UA for instrument A and CK, Di-Bil, HDL, Mg, TG and UA for instrument B. This consistency reflects the stability of the methods and its robustness despite other factors that more easily affected the other tests.

Electrolytes such as Ca, Cl, and Na were noted to have a sigma of <3.0 throughout the years. This is most likely due to the fact that the biological variation and total allowable error are very narrow for these tests. The coefficient of variation and bias for these tests were consistently very low, but overall sigma is <3.0 due to narrow TEa. Since sigma is computed with an equation, the variables play a role in its computed value. This brings to light the need to also look into computational variables when investigating poor sigma performance, as we do not want to cause unnecessary wastage of time resources, or manpower due to cause false rejection.⁷

Some tests reported significantly different sigma scores of its Level 1 and Level 2, one below or near 3.0 and the other >6.0 on certain years. Notable examples of such are Albumin and Bilirubin total for Instrument B (Table 3 and Figure 3). Albumin Level 1 sigma was 9.12 and Level 2 was 2.4 in 2018 at instrument B. This occurred in different tests throughout the years. It may be attributed to the methodologies having different detection performance at high and low levels. According to some studies, wide variations in sigma values for both the QC levels must be evaluated further, especially the method, and more strategies must be implemented to decrease or remove the discrepancy.⁴ The performance of the different levels cannot be averaged and must be

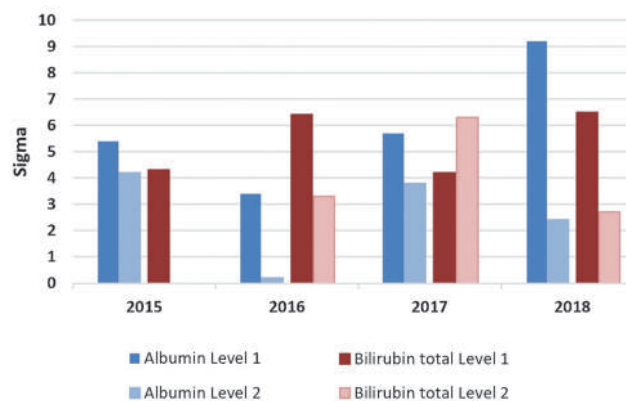


Figure 3. Discordant Sigma for levels 1 and 2 of select analytes (Instrument B). (Bilirubin total level 2 not available on instrument B in 2015).

addressed individually. This may also be the explanation for the difference in the performance of the two machines even if it is of the same brand and model.

Six sigma metrics provides a standard framework for measuring analytical quality but there are also issues with regards to the computation. It is said that one of its weakness is the bias which is usually based on the inter-laboratory peer group comparison using either third party controls or manufacturer controls. The controls may not be commutable and so the bias may only be relative. When we participate in EQA, we are compared with our peers and there are some who argue that peer group may not be sufficient to determine analytical quality.¹⁰ According to studies,^{11,12} realistic estimates of assay bias/ trueness require metrological standardization of all field assays and analysis of trueness controls. However, this may be difficult to apply because the gold standard reference materials are not always readily available for the clinical laboratories and likely too costly for routine use.

Source of TEa to compute sigma is a major factor to consider. One study demonstrated the impact of this by comparing the different common sources of TEa: biological variability, CLIA and RiliBAK.¹¹ They concluded that the most stringent was the biological variability but may not be appropriate for all tests. They recommended that laboratories choose TEa values from different sources which maybe the most appropriate for individual assays, as what was performed in this study.

Despite of the limitations, six sigma metrics may give laboratories a better understanding of the performance of their tests. This tool, in combination with a rational QC design for each analyte, can improve quality and reduce waste.²

CONCLUSION

In conclusion, computation of six sigma metrics allowed us to evaluate the performance of our chemistry tests on the six sigma scale. We were able to identify which are good performers and those that need monitoring and improvement. Tests that are >6.0 sigma require fewer Westgard rules and QC runs while those that are <3.0 sigma require more stringent quality control measures such as more Westgard rules application and QC runs. We recommend that six sigma metrics may be added to current quality improvement programs of the laboratory.

ACKNOWLEDGMENT

The authors thank the following: Mr. Sten Westgard, Ms. Ma. Lourdes Gatbonton, RMT, Ms. Leilani Cureg Soriano, RMT, Ms. Aileen Damasing, RMT and Abbott Diagnostics.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

1. Westgard JO. Basic QC practices, 3rd ed. Madison, WI. Westgard QC, Inc., 2010.
2. Westgard JO. Six sigma quality design and control. Madison, WI. Westgard QC, Inc., 2006.
3. Lippi G, Plebani M. A six-sigma approach for comparing diagnostic errors in healthcare- where does laboratory medicine stand? *Ann Transl Med.* 2018; 6(10):180. PMID: 29951502. PMCID: PMC5994534. <https://doi.org/10.21037/atm.2018.04.02>.
4. Singh B, Goswami B, Gupta VK, Chawla R, Mallika V. Application of sigma metrics for the assessment of quality assurance in clinical biochemistry laboratory in India: a pilot study. *Ind J Clin Biochem.* 2011;26 (2):131-5. PMID: 22468038. PMCID: PMC3107403. <https://doi.org/10.1007/s12291-010-0083-1>.
5. Chaudhary NG, Patani SS, Sharma H, Maheshwari A, Jadhav PM, Maniar MA. Application of six sigma for the quality assurance in clinical biochemistry laboratory – a retrospective study. *Int J Res Med.* 2013;2(3):17-20.
6. Lakshman M, Reddy BR, Bhulaxmi P, et al. Evaluation of sigma metrics in a Medical Biochemistry lab. *Int J Biomed Res.* 2015;6(3):164-71. <https://doi.org/10.7439/ijbr>.
7. Modi N, Shah T. Application of six sigma test in clinical biochemistry laboratory. *Int J Res Med.* 2017;6(2):75-8.
8. CLIA Requirements for Analytical Quality. Available at: <https://www.westgard.com/cli.htm>.
9. Ricós C, Alvarez V, Cava R et.al. Current databases on biological variation: pros, cons and progress. *Scand J Clin Lab Invest.* 1999;59(7):491-500. PMID: 10667686. <https://doi.org/10.1080/00365519950185229>.
10. Friedecky B, Kratochvila J, Budina M. Why do different EQA schemes have apparently different limits of acceptability? *Clin Chem Lab Med.* 2011;49:743-5. PMID: 21235390. <https://doi.org/10.1515/CCLM.2011.105>.
11. Hens, K, Berth M, Armbruster D, Westgard S. Sigma metrics used to assess analytical quality of clinical chemistry assays: importance of the allowable total error (TEa) target. *Clin Chem Lab Med* 2014;52(7):973-80. PMID: 24615486. <https://doi.org/10.1515/cclm-2013-1090>.
12. Guo X, Zhang T, Gao X, et al. Sigma metrics for assessing the analytical quality of clinical chemistry assays: a comparison of two approaches: electronic supplementary material available online for this article. *Biochem Med (Zagreb).* 2018;28(2):020708. PMID: 30022883. PMCID: PMC6039159. <https://doi.org/10.11613/BM.2018.020708>.






Disclaimer: This journal is **OPEN ACCESS**, providing immediate access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. As a requirement for submission to the PJP, all authors have accomplished an **AUTHOR FORM**, which declares that the ICMJE criteria for authorship have been met by each author listed, that the article represents original material, has not been published, accepted for publication in other journals, or concurrently submitted to other journals, and that all funding and conflicts of interest have been declared. Consent forms have been secured for the publication of information about patients or cases; otherwise, authors have declared that all means have been exhausted for securing consent.



BioRisk Association of the Philippines 2015, Inc.

SEC Reg. No.: CN201715954 / TIN: 009-668-420

2020 Calendar of Events and Representations

<p>January 20 to 24, 2020 8:00-5:00 PM</p>	<p>Tech Training for BRM and Strengthening Best Practices for Specimen Handling, Packaging and Transport for the Animal Sector Legaspi City, Albay</p>	<p>FULLY SPONSORED</p> 
<p>April 17, 2020 8:00-5:00 PM</p>	<p>Joint CPD Seminar Quezon City, Metro Manila</p>	
<p>April 29 to 30, 2020 8:00-5:00 PM</p>	<p>BEP and CRDF Global's 1st PhABOT Alumni Conference and Awards Night Clark, Pampanga <i>Organized by BRAP2015</i></p>	<p>FULLY SPONSORED</p> 
<p>May 20 to 22, 2020 8:00-5:00 PM</p>	<p>PAMET 25th Mid-Year Convention Baguio City, Benguet Province</p>	
<p>July 1 to 3, 2020 8:00-5:00 PM</p>	<p>BRAPs 4th National Convention and 2nd International Forum & 1st BRAP Recognition Night Awards Manila</p>	

Other events will be plotted after its final confirmation.

Please visit the BRAP website at <http://bioriskassociationphilippines.org> for details of the upcoming events. You may also email us at bioriskassociationphilippines@gmail.com or call during office hours +63.918.9292.660.

Gastric Pyloric Gland Adenoma: A Case Report, Review of Literature, and Diagnostic Challenges in the Philippine Setting

Kevin Elomina and Ignacio de Guzman Jr.

Department of Laboratory Medicine, De La Salle University Medical Center, Dasmariñas City, Philippines

ABSTRACT

Pyloric gland adenoma (PGA) is a rare neoplasm with definite malignant potential that is difficult to recognize because of its characteristically bland histology. We present a case of a 74-year-old female with chronic, intermittent symptoms referable to gastroesophageal reflux, bloatedness, and frequent flatus, with family history of gastric cancer. Initial endoscopy was done and biopsy revealed an inflammatory pseudopolyp. After six months, repeat endoscopy showed multiple polyps at the cardia, and biopsy of one of the visualized polyps was done. Microscopic sections of the polyp showed a neoplasm composed of discrete glands lined by simple cuboidal to columnar epithelial cells with amphophilic to eosinophilic cytoplasm without apical mucin caps, and mild nuclear atypia. Mild epithelial stratification was noted in some of the glands. PAS staining showed granular, cytoplasmic staining in tumor cells. Immunohistochemical staining with P53 showed focal, weak, nuclear staining in tumor cells. Staining with Ki67, MUC2, MUC5AC, and MUC6 was not done because the tissue had already been exhausted. The diagnosis of PGA with low-grade dysplasia has been made. The patient is apparently well, and is advised surveillance endoscopy at six-month intervals. PGA may be diagnosed in a limited resource setting, through thorough histologic examination, and use of special histochemical stains.

Key words: Pyloric gland adenoma, P53, Ki-67, GNAS, KRAS

ISSN 2507-8364 (Online)

Printed in the Philippines.

Copyright© 2019 by the PJP.

Received: 19 June 2019.

Accepted: 1 August 2019.

Published online first: 15 September 2019.

<https://doi.org/10.21141/PJP.2019.14>

Corresponding author: Kevin A. Elomina, MD

E-mail: kevin.elomina@gmail.com

INTRODUCTION

Adenomatous polyps of the stomach are established precursor lesions of gastric carcinoma. The WHO classification divides such lesions as to having an intestinal or a gastric phenotype. In practice, intestinal-type adenomas are more frequently encountered; on the other hand, gastric-type adenomas are rare, and are further subdivided into foveolar or pyloric gland adenomas (PGAs).¹

PGAs are rare, accounting for less than three percent of gastric polyps.²⁻⁴ The remarkably low incidence of this lesion may not necessarily be because of its rare occurrence, but may be attributed to difficulty in recognition because of the low degree of architectural disarray and cytologic atypia it usually demonstrates. In spite of its deceptively benign appearance, molecular analysis reveals that PGAs harbor several chromosomal abnormalities, as well as mutations in several oncogenes and tumor suppressor genes, which indicate that PGAs have an inherent malignant potential.^{5,6} To further this point, a good 30% of PGAs was found to be associated with malignant transformation;⁴⁻⁷ also, a few cases of PGAs are found in patients with familial adenomatous polyposis (FAP)^{2,6} and Lynch syndrome.^{2,3,6}

The rarity of PGAs, and the difficulty and clinical implications of its diagnosis make this case worth reporting. In addition, to our knowledge, there has not been a formally reported case of gastric PGA in the Philippines, to date; this may be secondary to its characteristically bland histology that complicates its



recognition. Nevertheless, we attempt to document a case of gastric PGA in an elderly female with family history of gastric cancer, and to provide valuable diagnostic insights that may help practicing gastroenterologists and pathologists in a limited resource setting.

CASE

A 74-year-old female presented with chronic, intermittent, epigastric discomfort especially when lying supine, with associated frequent belching relieved with short course of proton pump inhibitors (PPIs), and non-specific gastrointestinal complaints of bloatedness and frequent flatus. Past medical history was non-contributory. Family history revealed history of gastric cancer in her father.

She sought consult with a gastroenterologist and underwent esophagogastroduodenoscopy (EGD). Multiple, pale, flat polyps were noted at the cardia; and a slightly raised polyp measuring 0.5 cm in widest dimension, was noted at the proximal body. Biopsy of the raised polyp was performed,

which revealed an inflammatory pseudopolyp. She underwent repeat EGD after six months for surveillance, which revealed multiple, pale, flat polyps at the cardia and fundus, and an erythematous, slightly raised polyp with reticular gastric pits, measuring 0.5 cm in widest dimension, at the cardia. The said polyp was removed and was sent to histopathology.

Microscopic examination of the polyp showed a neoplasm composed of closely packed glands lined by simple cuboidal to columnar epithelium with some glands showing mild epithelial stratification. The cells do not form apical mucin caps and exhibit mild nuclear atypia. Mitotic figures are not seen (Figure 1). Staining with Periodic Acid Schiff (PAS) showed granular, cytoplasmic staining in tumor cells (Figure 2). Immunohistochemical staining with p53 showed focal, weak, nuclear staining in tumor cells (Figure 3). Unfortunately, the tissue had been exhausted due to its diminutive size, precluding further immunohistochemical staining with Ki67, MUC2, MUC5AC, and MUC6.

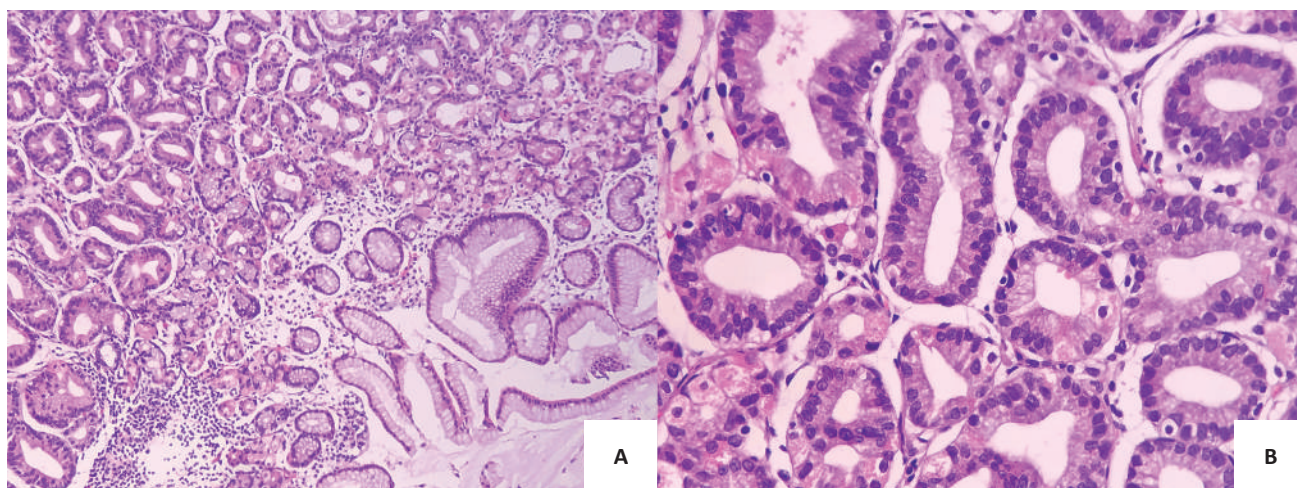


Figure 1. (A) Microscopic appearance of PGA showing discrete glandular structures under the non-neoplastic foveolar epithelium (H&E, 400X); (B) Some of the glands show low-grade dysplasia with mild epithelial stratification and nuclear atypia (H&E, 400X).

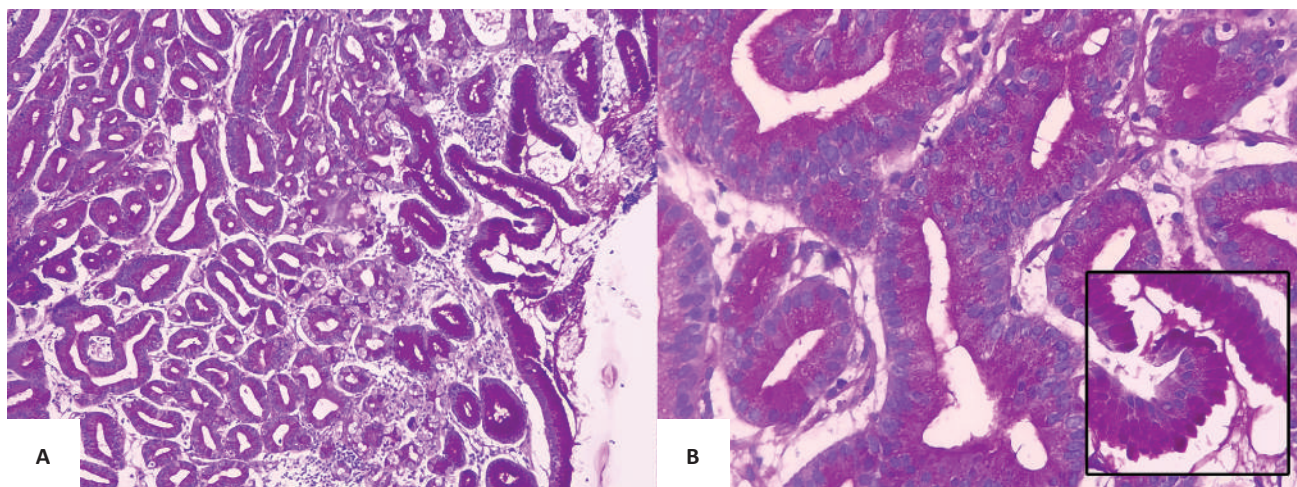


Figure 2. (A) Staining with PAS showing the difference in staining pattern of the neoplastic glands and foveolar epithelium (PAS, 100X); (B) The neoplastic glands show granular, cytoplasmic staining, in contrast with that of the foveolar epithelium, which shows diffuse staining of the well-formed apical mucin caps (inset) (PAS, 400X).

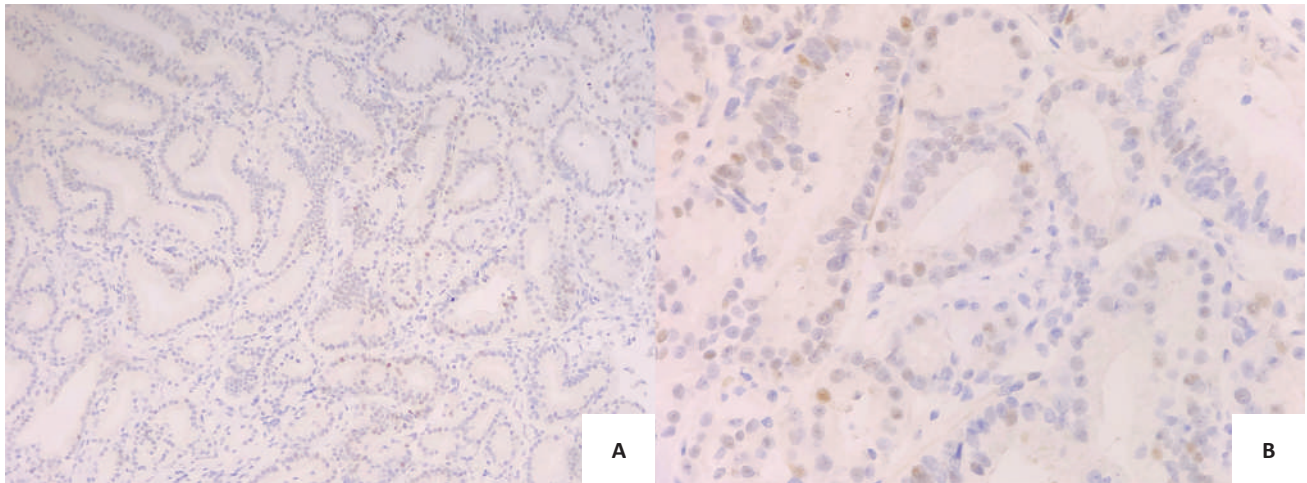


Figure 3. (A) Immunolabeling with P53 showing focal staining in tumor cells (P53, 100X); (B) Higher magnification shows weak, nuclear staining in tumor cells (P53, 400X).

Currently, the patient is apparently well, is not on any maintenance medications for her gastrointestinal complaints, and is advised close follow-up and surveillance of her gastric lesions, through EGD at six-month intervals.

DISCUSSION

Epidemiologically, PGAs are common in the elderly, usually in the seventh decade of life, with a slight female preponderance,^{2,4-8} which makes PGA an important differential diagnosis in elderly female patients with gastric polyps, especially when there is family history of gastric cancer, such as in our patient.

Sporadic PGAs are thought to arise in the setting of chronic mucosal injury, usually caused by *Helicobacter pylori* or autoimmune gastritis (AIG); the association between PGA and AIG partly explains the observed age and sex predilection.^{6,7} Between the two mentioned causes of chronic gastric injury, *H. pylori* infection is more common in our setting. In this case, *H. pylori* testing was not done, and only the lesion of interest was removed. Because of the association of sporadic PGAs with chronic mucosal injury, we therefore recommend that in cases where sporadic PGA is highly considered, biopsy of the background mucosa should also be performed aside from polypectomy, to document the presence of changes referable to chronic gastritis. One study showed that AIG is known to be associated with higher risk of high-grade dysplasia (HGD) and carcinoma.² The non-association of *H. pylori* with such risk may be explained by the high prevalence of AIG in their study population. In the local setting, where *H. pylori* is more prevalent than AIG, when applicable or indicated, *H. pylori* testing should also be performed. Syndromic PGAs, on the other hand, generally arise from normal mucosa.⁶ In cases where the patient is young and syndromic PGA is highly considered, biopsy of the background mucosa may also be performed, but is expected to have unremarkable findings.

The clinical significance of PGA lies in its malignant potential, which it owes to certain genetic alterations. Chromosomal aberrations such as gains in 17pq and

20q, and losses in 5q and 6q, have been documented in gastric PGAs; interestingly, these mutations are common in gastric adenocarcinomas. Activating *GNAS* mutations in amino acid residues 201 (R201C and R201H) and *KRAS* mutations in amino acid residues 14 (V14I) and 61 (Q61H) are considered characteristic of gastric PGAs; both mutations may be found in almost 40% of cases.⁵⁻⁷ *CTNNB1* mutation (S37F) has been identified in one case of gastric PGA and one esophageal PGA in one study.⁵ Recently, mutations in *SMAD4*, a tumor suppressor gene, have been initially identified in gastric PGAs; such mutations are also found in colorectal, pancreatic, and gastric carcinomas.⁵ Loss of mismatch repair (MMR) proteins has been reported in PGAs, but studies are conflicting.⁷

PGAs pose a diagnostic challenge to pathologists mainly because of its deceptively benign histomorphology. PGAs are classically characterized by discrete, tubular structures lined by a single layer of cells with abundant amount of eosinophilic cytoplasm with ground-glass appearance, without a well-formed apical mucin cap, and basally located, round nuclei, with or without visible nucleoli.¹⁻⁸ In our case, while most of the glands comprising the lesion conform to the said findings, we noted occasional foci of mild epithelial stratification and nuclear atypia; such findings point to low-grade dysplasia. The finding of dysplasia in this case makes the diagnosis of a neoplastic process more likely. Interpretation of dysplasia in PGAs is difficult because of the lack of a standardized grading scheme.⁶ Usually, authors provide operational definitions of grades of dysplasia confined within the purposes of their study. One study showed that PGAs commonly harbor dysplasia, usually of the high grade; and it assessed the degree of dysplasia based on the following classification: no dysplasia, low-grade (LGD), and HGD. Lesions with no dysplasia are composed of well-formed glands lined by a single layer of cells with basally located, round, non-atypical nuclei. Nuclear elongation and mild cytologic atypia typify LGD. Back-to-back glands with cribriforming, marked epithelial stratification, nuclear crowding, and cytologic atypia are characteristic of HGD.^{2,3} The said scheme is partly in congruence with that presented in the WHO classification; however, in the latter, the term 'negative

for dysplasia' is reserved for non-neoplastic lesions; and PGA is definitely not one of those, because of its known malignant potential. WHO provides the entity, 'indefinite for dysplasia', to which PGAs without histologic evidence of dysplasia, may be more appropriately classified.¹ Because of the frequency of dysplasia encountered in PGAs,^{2,5,6,8} as well as the clinical implications of this finding, we argue that in diagnosing PGAs, the degree of dysplasia should be reported following the WHO classification; and in cases of PGAs without histologic evidence of dysplasia, the more appropriate term would be 'indefinite for dysplasia.'

PGAs, particularly those that do not exhibit histologic evidence of dysplasia, may be difficult to differentiate from pyloric gland hyperplasia, but it is important to do so, because the former are premalignant lesions, while the latter is a benign process. PAS stain, which highlights gastric mucin may aid in differentiating the two entities. PGAs do not form apical mucin caps, and show granular cytoplasmic staining with PAS, while non-neoplastic pyloric glands have well-formed mucin caps, and show diffuse staining of the mucin cap with PAS.⁷ Differentiating PGAs from foveolar-type adenomas (FTAs) poses another diagnostic difficulty, and may be of importance, because of their distinct genetic alterations that may have an impact on their biologic behavior. Compared to PGAs, FTAs are characterized by glands lined by pseudostratified tall columnar epithelium composed of cells with well-formed apical mucin caps and elongated nuclei.⁶⁻⁸ While the two have distinct histomorphologic features, the possibility of hybrid differentiation and inconsistency of FTAs in forming apical mucin caps, may complicate diagnosis.^{1,8} Special histochemical stains, particularly PAS/Alcian Blue stain may be of help. FTAs show strong PAS staining highlighting their mucin caps, while PGAs only show granular cytoplasmic staining.⁶⁻⁸ Alcian blue stains acid mucins that are typically found in the intestine, and may help identify foci of intestinal differentiation in PGAs showing mixed phenotype. Our case showed the classic PAS staining pattern of PGAs, which reinforced our diagnosis, even in the absence of the recommended immunohistochemical stains. Our findings demonstrate the use of special histochemical stains, together with meticulous histologic examination, as a viable alternative in the pathologic examination of PGAs.

Immunohistochemistry (IHC) has two main uses in the pathologic workup of PGAs: to strengthen presumptive diagnosis; and to reinforce that dysplasia is present. In terms of IHC, generally, intestinal-type adenomas express MUC2, CDX2, and CD10, and are negative for gastric mucins MUC5AC and MUC6. FTAs express MUC5AC, and are negative for MUC6 and CD10, with low CDX2 expression; while, PGAs characteristically express MUC6.^{1,3-8} MUC5AC expression in PGAs is variable, but in its pure pyloric gland phenotype, is limited to the foveolar surface epithelium.^{5,6,8} Foci of intestinal differentiation may also be encountered in PGAs, and these are positive for stains for intestinal mucins.^{6,7} Mixed foveolar and pyloric gland adenoma (MFPGA) may be diagnosed only with IHC using the following criteria: MUC5AC and MUC6 expression in the neoplastic glands, with 20% to more than 90% of cells being positive for MUC6. Diagnosis of MFPGA may be important, as it is found to

be associated with higher risk of HGD and carcinoma in PGAs.² These findings underscore the value of IHC, not only in differentiating PGAs from FTAs and intestinal-type adenomas, but also in diagnosing MFPGA, which has a high risk for malignant transformation.

IHC stains for P53 and Ki-67 may be used to reinforce that dysplasia is present in PGAs; in which case, patient surveillance is necessary. One study showed that degree of dysplasia in PGAs positively correlates with the magnitude of P53 and Ki-67 expression; such that lesions without histologic evidence of dysplasia show scattered, weak, nuclear P53 staining, with 5-10% cells positive for Ki-67, while lesions with LGD show more intense staining than the former, with 20-35% of cells positive for Ki-67, and areas with HGD and carcinoma show more intense staining than those with LGD, with about 80% of cells positive for Ki-67.⁵ P53 expression in our case with LGD, matched that of lesions without histologic evidence of dysplasia; which suggests that P53 may not consistently correlate with the degree of dysplasia in PGAs. In such a case, the proliferative index may identify areas at risk for malignant transformation through increased Ki-67 expression.⁶ Meticulous assessment of H&E sections is central in the recognition of PGAs, and histochemical stains and IHC are necessary to support the diagnosis. While genetic testing is starting to be available in some centers in the Philippines, the cost of the test precludes its routine use in our setting.

The applicability of the recommendations presented with regard to the pathologic approach to PGAs may vary across institutions depending on the availability of the appropriate technology; the lack of additional IHC was the main weakness in the approach to this case. Nevertheless, the recognition of this neoplasm with a definite malignant potential, hiding within a deceptively benign histologic appearance, is still possible, through careful histologic examination with use of special histochemical stains, such as PAS.

CONCLUSION

PGAs should be an important differential diagnosis in elderly patients presenting with gastric polyps; particularly those with family history of gastric cancer. Management of PGA should include polypectomy with biopsy of the background gastric mucosa and *H. pylori* testing, especially in areas with high endemicity. Pathologic examination of PGAs should include routine histologic examination with close attention to the degree of dysplasia they harbor, and special histochemical stains such as PAS and Alcian blue stain if indicated, IHC stains for MUC5AC and MUC6 to establish diagnosis, and P53 and Ki-67 to reinforce that dysplasia is present. Pathologists should be aware that PGAs are neoplasms with definite malignant potential that intelligently hides in a deceptively innocuous histology.

ACKNOWLEDGMENT

The authors thank Dr. Paulo Giovanni Mendoza for sharing his expertise on this case, and Dr. Ma. Carmen Cagampan and Dr. David Saguil for bringing the case to Dr. Mendoza's attention.

ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

1. Lauwers GY, Carneiro F, Graham DY, Curado M-P, Franceschi S, Montgomery E, et al. Gastric carcinoma. In: Bosman FT, Jaffe ES, Lakhani SR, Ohgaki H, editors. WHO Classification of Tumors of the Digestive System. 4th ed. Lyon, France: International Agency for Research on Cancer; 2010.
2. Choi WT, Brown I, Ushiku T, et al. Gastric pyloric gland adenoma: a multicenter clinicopathologic study of 67 cases. *Histopathology*. 2018;72(6):1007–14. PMID: 29278427. <https://doi.org/10.1111/his.13460>.
3. Lee SE, Kang SY, Cho J, et al. Pyloric gland adenoma in Lynch syndrome. *Am J Surg Pathol*. 2014;38(6):784–92. PMID: 24518125 PMCID: PMC4014525. <https://doi.org/10.1097/PAS.0000000000000185>.
4. Cakar E, Bayrak S, Paşaoğlu E, Colak S, Bektaş H, Güneyi A. Pyloric gland adenoma: a case report. *Case Rep Gastroenterol*. 2013;7(3):392–5. PMID: 24163651. PMCID: PMC3806671. <https://doi.org/10.1159/000355342>.
5. Chlumská A, Waloschek T, Mukenšnabl P, Martínek P, Kašpírková J, Zámečník M. Pyloric gland adenoma: a histologic, immunohistochemical and molecular genetic study of 23 cases. *Cesk Patol*. 2015;(3):137–43. PMID: 26421956.
6. Vieth M, Montgomery EA. Some observations on pyloric gland adenoma: an uncommon and long ignored entity! *J Clin Pathol*. 2014;67(10):883–90. PMID: 25092673. <https://doi.org/10.1136/jclinpath-2014-202553>.
7. Pezhouh MK, Park JY. Gastric pyloric gland adenoma. *Arch Pathol Lab Med*. 2015;139(6):823–6. PMID: 26030253. <https://doi.org/10.5858/arpa.2013-0613-RS>.
8. Chen ZM, Scudiere JR, Abraham SC, Montgomery E. Pyloric gland adenoma: an entity distinct from gastric foveolar type adenoma. *Am J Surg Pathol*. 2009;33(2):186–93. PMID: 18830123. <https://doi.org/10.1097/PAS.0b013e31817d7ff4>.

Disclaimer: This journal is **OPEN ACCESS**, providing immediate access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. As a requirement for submission to the PJP, all authors have accomplished an **AUTHOR FORM**, which declares that the ICMJE criteria for authorship have been met by each author listed, that the article represents original material, has not been published, accepted for publication in other journals, or concurrently submitted to other journals, and that all funding and conflicts of interest have been declared. Consent forms have been secured for the publication of information about patients or cases; otherwise, authors have declared that all means have been exhausted for securing consent.

Histologic Transformation in an EGFR-Mutant Lung Cancer in a Filipino Patient Treated with Afatinib: Case Report and Review of Literature

Steffanie Charlyne Tamayo,¹ Joanmarie Balolong-Garcia,² Michelle Joane Alcantara,² Rubi Li,² Daphne Ang,¹ Jose Jasper Andal,¹ Rex Michael Santiago¹

¹Institute of Pathology, St. Luke's Medical Center Quezon City, Philippines

²Department of Medical Oncology, St. Luke's Medical Center Quezon City, Philippines

ABSTRACT

We report a case of a 64-year-old Filipino male who initially presented with chronic cough, easy fatigability, and weight loss. Work-ups lead to a diagnosis of lung adenocarcinoma with epidermal growth factor receptor (EGFR) exon 19 deletion. Patient was placed on targeted therapy with Afatinib. He was able to complete 17 months of targeted therapy with relatively stable disease before experiencing recurrence of easy fatigability. Work-ups then lead to a diagnosis of a high-grade neuroendocrine tumor consistent with small cell lung carcinoma (SCLC). Afatinib was then discontinued and the patient was started on Carboplatin and Etoposide. However, after only one cycle, the patient's symptoms progressed and the patient eventually expired. Histological transformation of EGFR-mutant adenocarcinoma to SCLC as a mechanism of resistance to targeted therapy has been documented in literature since 2006. However, to our knowledge, this is the first fully-documented case of histologic transformation occurring in a Filipino patient. As molecular targeted therapy and immunotherapy become standard-of-care in our country, it is of paramount importance that clinicians and pathologists are aware of the various mechanisms of resistance that can occur as a result of these treatments.

Key words: Lung cancer; adenocarcinoma; small cell carcinoma; receptor; epidermal growth factor; cell transformation, neoplastic

ISSN 2507-8364 (Online)

Printed in the Philippines.

Copyright© 2019 by the PJP.

Received: 21 June 2019.

Accepted: 24 July 2019.

Published online first: 15 September 2019.

<https://doi.org/10.21141/PJP.2019.15>

Corresponding author: Steffanie Charlyne A. Tamayo, MD

E-mail: scatamayo1031@gmail.com

INTRODUCTION

Lung cancer is still one of the major causes of cancer-related deaths worldwide. In recent years, the advent of molecular targeted therapy has drastically changed the treatment and prognosis of these patients. Herein we present a case of a 64-year old Filipino male with an Epidermal Growth Factor Receptor (EGFR)-mutant lung adenocarcinoma, which was treated with a tyrosine kinase inhibitor (TKI; Afatinib) and subsequently developed small cell carcinoma on progression.

CASE

We report a case of a 64-year-old Filipino male who initially presented with complaints of chronic cough associated with easy fatigability and weight loss. He had a 30-pack-year smoking history along with controlled hypertension and diabetes mellitus type 2. Family history was significant only for breast cancer. Physical examination showed decreased breath sounds at the right lung base. Chest x-ray revealed a hazy density at the right infraclavicular region and in the right lung base, which prompted further evaluation. Chest CT scan showed a right upper lobe nodule measuring 2 cm in widest diameter, innumerable bilateral pulmonary parenchymal and fissural nodules, an enlarged precarinal lymph node, and right-sided pleural effusion. There were no enlarged mediastinal or hilar lymphadenopathies. Upper abdomen CT scan, total body bone scan, and brain MRI were all negative for metastasis. CT-guided biopsy of the right lung nodule and thoracentesis of the right pleural effusion were done in another institution. Histopathology



of the right lung nodule showed lung adenocarcinoma while that of the pleural fluid showed adenocarcinoma. Immunohistochemical (IHC) stains were done on both specimens, showing positive staining for CK7 and TTF-1 and negative staining for CK20, CK5/6 and calretinin. Epidermal growth factor receptor (EGFR) mutation analysis using real-time polymerase chain reaction (real-time PCR) showed exon 19 deletion.

While awaiting results of EGFR, the patient was given first line metastatic treatment with chemotherapy using Pemetrexed and Carboplatin, which he completed for 6 cycles. Re-evaluation CT scans of the chest showed stable disease on the lung nodules. The patient was subsequently started on Afatinib. He was able to complete 17 months of targeted therapy with relatively stable disease, before again experiencing easy fatigability.

On work-up, re-evaluation CT scan of the chest showed an interval progression in the size and number of the multiple, confluent, non-calcified, pleural-based and parenchymal pulmonary nodules and masses in the right lung. The largest mass had a diameter of 12.5 cm. These findings prompted CT-guided fine needle aspiration biopsy of the right lung mass at our institution.

The moderately cellular smears and cell block showed atypical cells with large, round, hyperchromatic nuclei, inconspicuous nucleoli and scant cytoplasm. These were seen scattered singly and arranged in tight clusters and

in monolayered sheets (Figure 1). Nuclear molding was also appreciated on the cell block. Immunohistochemical studies revealed that these atypical cells were positive for TTF-1, synaptophysin, and CD56, and focally positive for chromogranin A (Table 1; Supplemental Figure 1). Ki-67 was high at more than 90%. The case was then signed out as a high grade neuroendocrine tumor, consistent with small cell carcinoma.

Table 1. Immunohistochemical stain results of the patient's right lower lobe pulmonary mass

Immunohistochemical Stain	Result
TTF-1	Positive
Synaptophysin	Positive
Chromogranin A	Positive, focal
CD56	Positive
Ki-67	> 90%

The specimen was also sent for EGFR mutation analysis and results showed the patient's original exon 19 deletion. PD-L1 was performed and showed a tumor proportion score of less than 1%. Afatinib was then discontinued and chemotherapy with Carboplatin and Etoposide was planned in October 2018. However, the patient continued to be symptomatic at this time with shortness of breath and episodes of desaturation. There was also concomitant pneumonia. He was given only one cycle of Carboplatin and Etoposide before his symptoms progressed. The patient eventually expired.

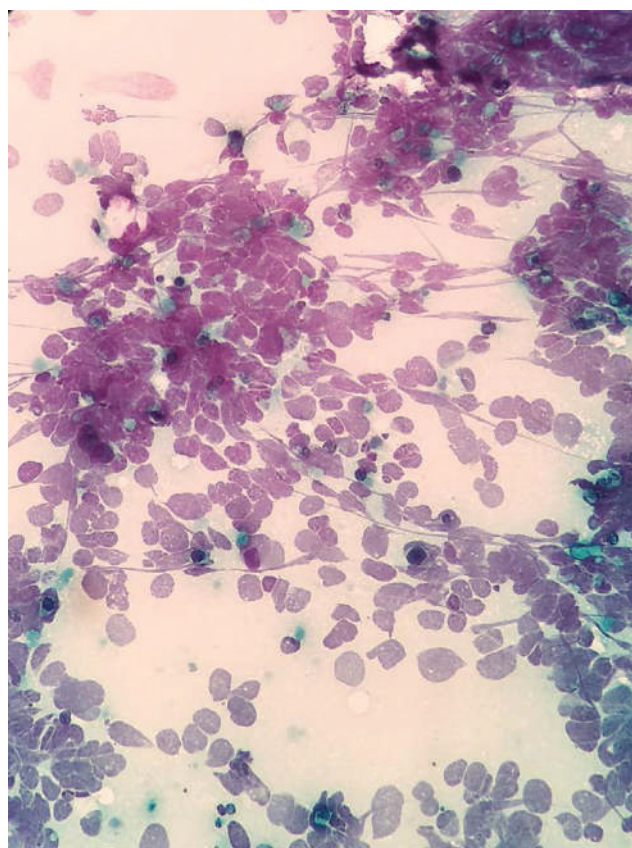
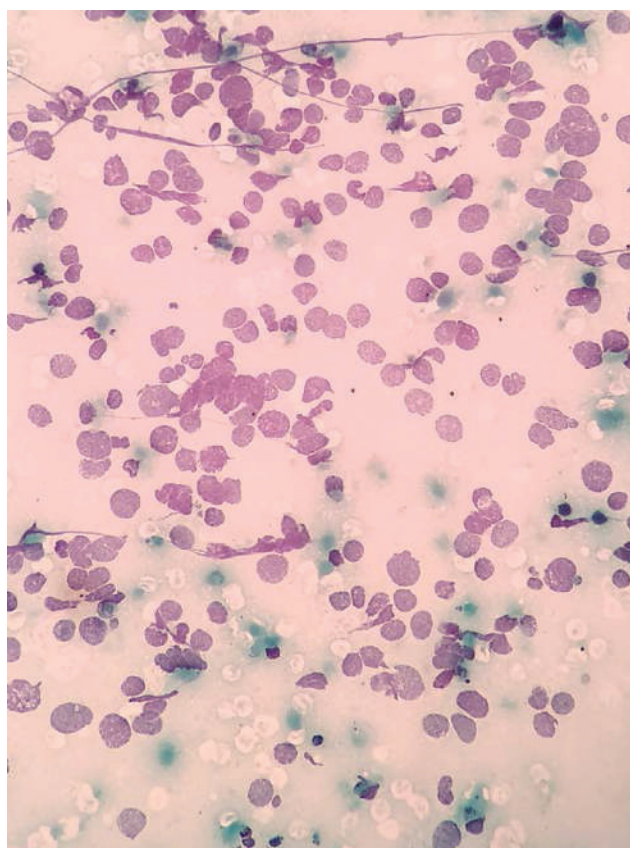


Figure 1. Fine needle aspiration biopsy of the patient's right lower lobe pulmonary mass. The smears showed atypical cells with large, round, hyperchromatic nuclei and scant cytoplasm. These were seen scattered singly and arranged in tight clusters. (Papanicolaou stain, 400X).

DISCUSSION

The biopsy that demonstrated small cell carcinoma may represent any of three possibilities: (1) a new primary; (2) combined small cell carcinoma with adenocarcinoma; and (3) histologic transformation of adenocarcinoma to small cell carcinoma as a mechanism of resistance to targeted therapy with tyrosine kinase inhibitors.

The existence of combined-histology lung cancers has been recognized and documented in large case series.^{1,2,3} However, such cases usually constitute only a small proportion of small cell lung carcinomas (SCLC). The initial diagnosis of pure non-small cell lung carcinoma (NSCLC) on biopsies in these cases may be due to the limited material submitted at initial diagnosis, which may not be representative of the entire tumor.⁴ Another possibility is that the SCLC component of these mixed tumors become more prominent after regression of the adenocarcinoma component in response to EGFR inhibitors.⁵

Erlotinib, gefitinib, and afatinib are three EGFR inhibitors that are widely used for the first-line treatment of lung cancers with EGFR-activating mutations. However, resistance to these inhibitors develops after an average time of 12 months.⁴ This is concordant with data from a 2017 case series done in Malaysia.⁶ In this case, resistance developed after 17 months of afatinib therapy, as evidenced by the increase in the number and sizes of the pulmonary and pleural-based nodules on imaging studies.

There are several mechanisms that may account for the development of resistance in tumors that have been treated with tyrosine-kinase inhibitors. These can be generally divided into two main categories: (1) primary or intrinsic resistance and (2) secondary or acquired resistance.

In primary resistance, there is an immediate inefficacy to EGFR-TKI. This is often attributed to a non-sensitive EGFR mutation, such as an exon 20 insertion that adds residues at the N-lobe of EGFR (M766 to C775).

In contrast, secondary or acquired resistance is defined by an initial response to EGFR-TKI with stable disease and the subsequent development of progression. The mechanisms of acquired resistance can be divided into three: (1) insurgence of secondary mutations in the EGFR gene, such as exon 20 T790M; (2) activation of alternative pathways that bypass the need for EGFR signalling; and (3) phenotypic or histologic transformation.⁷ Of these, the T790M mutation is the most commonly documented mechanism, accounting for 50-60% of cases.⁴ Histological transformation to SCLC is the least common mechanism, occurring in 3-10% of EGFR-mutant NSCLCs.⁸ It should be noted however, that the aforementioned mechanisms are not mutually exclusive; thus, a combination of the mechanisms may occur in the same patient.⁵

In the case presented in this report, the original EGFR exon 19 deletion was identified in the biopsy that showed small cell carcinoma. This finding effectively rules out the possibility of a new primary and favors that the prior adenocarcinoma on initial diagnosis is related to the small cell carcinoma. While there is the possibility that

the patient's tumor is of mixed histology right at the outset, given that the diagnosis of adenocarcinoma was based merely on biopsy material and not on a resection, it is believed that combined small cell carcinoma and adenocarcinoma would have a less dramatic response to EGFR inhibitors and would develop resistance much earlier during the course of treatment.⁴ In our case, the patient had stable disease for 17 months while he was on Afatinib therapy. Thus, given the clinical course of this patient, histologic transformation of adenocarcinoma to small cell carcinoma is the favored mechanism of resistance that developed in this tumor.

Histological transformation of EGFR-mutant adenocarcinoma to SCLC was first documented in 2006 in a 45 year old woman who had EGFR-mutant adenocarcinoma and who was subsequently treated with erlotinib for 18 months.⁹ Other case series have since demonstrated this occurrence, with the transformation to SCLC supported by histomorphology, positive immunohistochemical staining for synaptophysin, chromogranin, or NCAM, and/or retention of the tumor's original EGFR-activating mutation.^{3,4,6,8,10,11,12,13,14} Current data suggest that histological transformation to SCLC can occur in up to 14% of EGFR-mutant NSCLC as a mechanism of tyrosine kinase inhibitor resistance.¹² In Asia, a case series done in Shanghai enrolled 87 patients whose lung adenocarcinomas transformed to SCLC after TKI treatment.¹⁵ Among these patients, female gender and EGFR exon 19 deletion were found to be independent positive predictors for SCLC transformation.¹⁵ In contrast, the patient in this case report is male with an EGFR exon 19 deletion.

Genomic analyses have shown that *RBI* inactivation is a necessary step in SCLC tumorigenesis. Among patients with EGFR-mutant adenocarcinoma that transformed to SCLC, 100% had loss of *RBI*, suggesting that this inactivation is a vital step in transformation from adenocarcinoma to SCLC.⁴ In this case, however, testing for *RBI* inactivation was not performed. Other steps in this transformation pathway remain to be elucidated, but studies have suggested that the PI3K-AKT pathway also plays an important role in SCLC transformation.⁵

The cells of origin of SCLC and adenocarcinoma have traditionally been thought to be neuroendocrine cells and alveolar type II cells, respectively. However, studies done on murine models of lung cancer suggest that alveolar type II cells also have the potential to give rise not only to SCLC, but to EGFR-mutant adenocarcinoma as well.⁴ It has since been postulated that the presence of EGFR mutation and constitutively active EGFR signalling drives the proliferation and differentiation of alveolar type II cells. The use of EGFR tyrosine kinase inhibitors blocks this effect, and when additional genetic events such as *RBI* inactivation occur, these same alveolar type II cells might subsequently transform to SCLC.⁴

The clinical course of patients with EGFR-mutant adenocarcinomas that underwent histologic transformation to SCLC is poorly characterized. In the 2019 study by Marcoux *et al.*, the median time to transformation was 17.8 months.⁸ Treatment after transformation with platinum-

etoposide and taxanes yielded high response rates. The tumors were unresponsive to checkpoint inhibitors. Median overall survival since the time of SCLC transformation was 10.9 months.⁸ Another study showed a median progression-free survival after SCLC transformation of only two months when treated with tyrosine kinase inhibitor monotherapy and six months when treated with etoposide combined with cisplatin or carboplatin.¹⁵ In our case, the patient was only given one cycle of carboplatin and etoposide and expired soon after the diagnosis of SCLC was made.

In summary, we have presented a case of a 64-year old Filipino male with a known EGFR-mutant adenocarcinoma that was treated with Afatinib and subsequently developed resistance through phenotypic transformation to small cell carcinoma after 17 months of therapy. Targeted therapy has only recently become widely available in the Philippines and, to our knowledge, this is the first fully-documented case of histologic transformation occurring in a Filipino patient. As molecular targeted therapy and immunotherapy become standard-of-care in our country, it is of paramount importance that clinicians and pathologists are aware of the various mechanisms of resistance that can occur as a result of these treatments.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

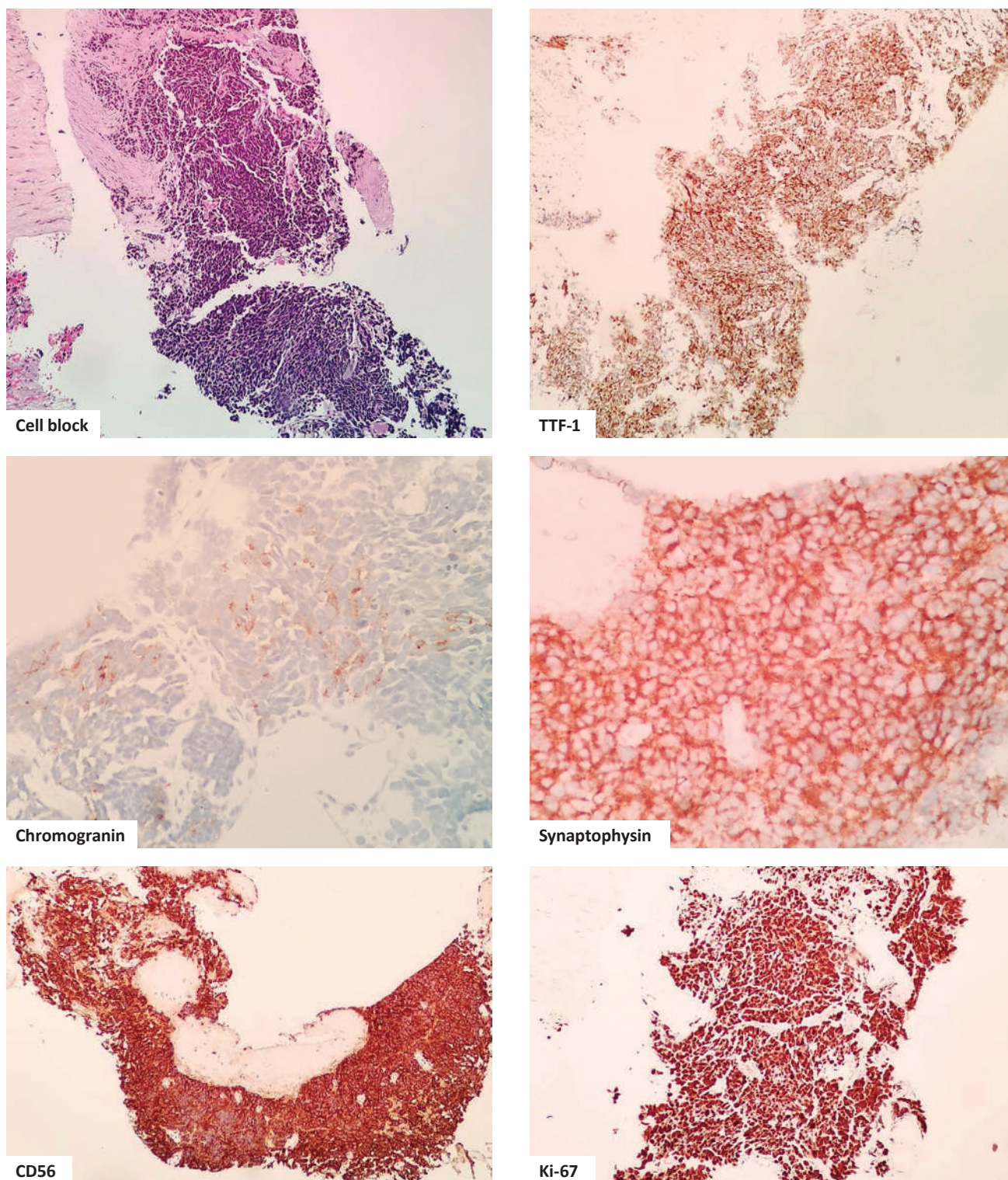
The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

- Adelstein DJ, Tomaszefski JF Jr, Snow NJ, Horrigan TP, Hines JD. Mixed small cell and non-small cell lung cancer. *Chest*. 1986;89(5):699-704. PMID: 3009096. <https://doi.org/10.1378/chest.89.5.699>.
- Mangum MD, Greco FA, Hainsworth JD, Hande KR, Johnson, DH. Combined small-cell and non-small-cell lung cancer. *J Clin Oncol*. 1989;7(5):607-12. PMID: 2540288. <https://doi.org/10.1200/JCO.1989.7.5.607>.
- Wallace AS, Arya M, Frazier SR, Westgate S, Wang Z, Doll D. Combined small-cell lung carcinoma: an institutional experience. *Thorac Cancer*. 2014;5(1): 57-62. PMID: 26766973. PMID: PMC4704284. <https://doi.org/10.1111/1759-7714.12059>.
- Oser MG, Niederst MJ, Sequist LV, Engelman JA. Transformation from non-small cell lung cancer to small cell lung cancer: molecular drivers and cells of origin. *Lancet Oncol*. 2015;16(4): e165-72. PMID: 25846096. PMID: PMC4470698. [https://doi.org/10.1016/S1470-2045\(14\)71180-5](https://doi.org/10.1016/S1470-2045(14)71180-5).
- Suda K, Murakami I, Sakai K, et al. Small cell lung cancer transformation and T790M mutation: complimentary roles in acquired resistance to kinase inhibitors in lung cancer. *Scientific Reports* 5. 2015; Article number 14447. <https://www.nature.com/articles/srep14447>. Accessed 24 April 2019.
- Chai CS, Liam CK, Pang YK. Case series of small cell lung cancer transformation post tyrosine kinase inhibitor failure. *Ann Oncol*. 2017;28(suppl 10):x153-4. <https://oncologypro.esmo.org/Meeting-Resources/ESMO-Asia-2017-Congress/Case-Series-of-Small-Cell-Lung-Cancer-Transformation-Post-Tyrosine-Kinase-Inhibitor-Failure-498P>. Accessed 30 April 2019.
- Morgillo F, Della Corte CM, Fasano M, Ciardiello F. Mechanisms of resistance to EGFR-targeted drugs: lung cancer. *ESMO Open*. 2016; 1(3): e000060. PMID: 27843613 PMID: PMC5070275. <https://doi.org/10.1136/esmoopen-2016-000060>.
- Marcoux N, Gettinger SN, O'Kane G, et al. EGFR-mutant adenocarcinomas that transform to small-cell lung cancer and other neuroendocrine carcinomas: clinical outcomes. *Journal of Clinical Oncology*. 2019;37(4):278-85. PMID: 30550363. <https://doi.org/10.1200/JCO.18.01585>.
- Zakowski MF, Ladanyi M, Kris, MG. EGFR mutations in small-cell lung cancers in patients who have never smoked. *N Engl J Med*. 2006;355(2):213-5. PMID: 16837691. <https://doi.org/10.1056/NEJMc053610>.
- Fallet, V, Ruppert, A, Poulot, V, et al. Secondary resistance to erlotinib: acquired T790M mutation and small-cell lung cancer transformation in the same patient. *J Thoracic Oncol*. 2012;7(6): 1061-3. PMID: 22588159. <https://doi.org/10.1097/JTO.0b013e31824fea45>.
- Kuiper JL, Heideman DA, Thunnissen E, et al. Incidence of T790M mutation in (sequential) rebiopsies in EGFR-mutated NSCLC-patients. *Lung Cancer*. 2014;85(1): 19-24. PMID: 24768581. <https://doi.org/10.1016/j.lungcan.2014.03.016>.
- Lin Q, Cai G, Yang K, Yang L, Chen C, Li, Y. Case report: small cell transformation and metastasis to the breast in a patient with lung adenocarcinoma following maintenance treatment with epidermal growth factor receptor tyrosine kinase inhibitors. *BMC Cancer*. 2016;16:593. PMID: 27488410. PMID: PMC4972970. <https://doi.org/10.1186/s12885-016-2623-4>.
- Varghese, AM, Zakowski, MF, Yu, HA, et al. Small-cell lung cancers in patients who never smoked cigarettes. *J Thorac Oncology*. 2014; 9(6):892-6. PMID: 24828667. PMID: PMC4199745. <https://doi.org/10.1097/JTO.0000000000000142>
- Watanabe S, Sone T, Matsui T, et al. Transformation to small-cell lung cancer following treatment with EGFR tyrosine kinase inhibitors in a patient with lung adenocarcinoma. *Lung Cancer*. 2013; 82(2): 370-2. <https://doi.org/10.1016/j.lungcan.2013.06.003>.
- Zhang, B, Xu, J, Wang, S, et al. Characteristics and outcome of small cell lung cancer patients (SCLC) transformed from adenocarcinoma after tyrosine kinase inhibitors treatment. *Annals of Oncology*. 2018; 29 (Suppl 9): ix150-60. <https://oncologypro.esmo.org/Meeting-Resources/ESMO-Asia-2018-Congress/Characteristics-and-outcome-of-small-cell-lung-cancer-patients-SCLC-transformed-from-adenocarcinoma-after-tyrosine-kinase-inhibitors-treatment>. Accessed 30 April 2019.



Supplemental Figure 1. Immunohistochemical studies done on the cell block. The tumor was positive for TTF-1, chromogranin A, synaptophysin, and CD56. Ki-67 was high (more than 90%). All controls showed appropriate immunoreactivity. (H&E, TTF-1, CD56, and Ki-67 at 100X magnification; chromogranin and synaptophysin at 400X magnification).

Disclaimer: This journal is **OPEN ACCESS**, providing immediate access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. As a requirement for submission to the PJP, all authors have accomplished an **AUTHOR FORM**, which declares that the ICMJE criteria for authorship have been met by each author listed, that the article represents original material, has not been published, accepted for publication in other journals, or concurrently submitted to other journals, and that all funding and conflicts of interest have been declared. Consent forms have been secured for the publication of information about patients or cases; otherwise, authors have declared that all means have been exhausted for securing consent.

Targeted Sequencing of Mixed Neuroendocrine–Non-neuroendocrine Neoplasm of the Gallbladder Suggests a Monoclonal Origin: A Case Report

Flora Mae Sta. Ines,¹ Anna Louise Anceno,² Rachele Arah Salamat,¹ Narciso Navarro Jr.,³ Glenda Lyn Pua,¹ Jose Jasper Andal,¹ Daphne Ang¹

¹Institute of Pathology, St. Luke's Medical Center, Global City, Philippines

²Institute of Radiology, St. Luke's Medical Center, Global City, Philippines

³Institute of Surgery, St. Luke's Medical Center, Global City, Philippines

ABSTRACT

Mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN) of the gallbladder is a rare tumor that is defined in the World Health Organization (WHO) 2019 digestive system tumor classification as the presence of a neuroendocrine neoplasm admixed with a non-neuroendocrine carcinoma, each component constituting at least 30% of the neoplasm. The exact pathogenesis of MiNENs remains unclear. We present a case of a 74-year-old Filipino woman who presented with nonspecific clinical and radiologic findings and subsequently underwent cholecystectomy. Histopathologic and immunohistochemical evaluation of the gallbladder confirmed the diagnosis of a mixed well-differentiated adenocarcinoma (30%) and large cell neuroendocrine carcinoma (70%). The adenocarcinoma and neuroendocrine carcinoma components were separately microdissected and submitted for targeted 15-gene sequencing using the Illumina Trusight Tumor 15 (TST15) panel. NGS identified a TP53 missense mutation leading to a stop codon in both components. The finding of similar molecular signatures in the two morphologically distinct components supports the hypothesis that MiNEN arises from a common precursor stem cell capable of divergent phenotypic differentiation.

Key words: gallbladder, MiNEN, molecular analysis

ISSN 2507-8364 (Online)

Printed in the Philippines.

Copyright© 2019 by the PJP.

Received: 28 August 2019.

Accepted: 25 September 2019.

Published online first: 29 September 2019.

<https://doi.org/10.21141/PJP.2019.16>

Corresponding author: Daphne C. Ang, MD

E-mail: daphnechuaang@gmail.com

INTRODUCTION

Gallbladder neuroendocrine neoplasms (NENs) account for 0.5% of all NENs and 2.1% of gallbladder cancers.¹⁻³ Of the different types of gallbladder NENs, large cell neuroendocrine carcinoma (LCNEC) is an aggressive and exceptionally rare tumor, with about 20 cases of both pure and mixed types reported since 2000.^{1,4-7} Primary mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN) of the gallbladder is even more rare. MiNENs are characterized by the neuroendocrine tumors or carcinomas with a non-neuroendocrine carcinoma component, each carcinoma type constituting at least 30% of the tumor. MiNENs were traditionally classified by Lewin into collision, composite and amphicrine types, on the basis of the association between, and the immunophenotype of the two components.^{4,7,8} We herein report a case of mixed adenocarcinoma and LCNEC of the gallbladder with immunohistochemical evaluation and molecular testing.

CASE

A 74-year-old Filipino woman with no known comorbidities experienced right upper abdominal pain and fever of three-day duration. Persistence of the abdominal pain prompted her to seek consult in the emergency department. Physical examination revealed direct tenderness in the right upper quadrant, a positive Murphy's sign and mild icterus. Blood tests were unremarkable and non-contributory. Initial impression was acute calculous cholecystitis. Endoscopic retrograde cholangiopancreatography (ERCP) revealed stenotic ampulla of Vater and an obstructed cystic duct.



Whole abdominal CT scan (Figures 1A and B) demonstrated a distended multiseptated gallbladder (maximum width of 4.3 cm) with a slightly hyperdense lumen (suggestive of bile sludge) and a 1.1 cm non-enhancing faintly hyperdense ovoid focus (suggest a low-density cholelithiasis versus sludge ball) within the body. Mild thickening of the gallbladder wall with extensive pericholecystic fat stranding (reflective of cholecystitis), and a focal defect along the left anteromedial portion of the gallbladder were also seen. There was moderate dilatation of the cystic duct, intra- and extrahepatic ducts and common bile duct, without discrete signs of calcified choledocholithiasis. The gastric pylorus, first and second portion of the duodenum, hepatic flexure/ascending colon all showed wall thickening/edema, likely reactive, and the pancreas is atrophic. There were no other significant pathologic lesions in the imaging studies.

Open cholecystectomy was then performed and the gallbladder was sent for routine histopathologic examination. Gross examination revealed the gallbladder (Figure 1C) to have a tan brown, dull and rough external surfaces, with multiple fine adhesions and thickened wall (1 cm.). The gallbladder lumen is completely filled with a cream tan, variegated, polypoid, soft to friable mass (6.1 cm in greatest dimension) that is loosely attached to the luminal surface, which had brown tan and flattened mucosa. No yellow flecks nor stones appreciated. No lymph nodes submitted for gross examination. Microsections disclosed a neoplasm with two distinct morphologies (Figures 2A-2C; Figure 3): a poorly differentiated carcinoma and a well differentiated adenocarcinoma, that are closely juxtaposed but with apparent transition. The poorly differentiated carcinoma (Figure 4A), which comprised the majority of the tumor, is composed of large, round to pleomorphic cells with vesicular nuclei, prominent nucleoli and scant to moderate amount of cytoplasm. These cells, arranged in solid sheets, palisades and rosette-like and pseudoglandular patterns, invaded up to the perimuscular connective tissue of the organ without serosal involvement. This component is associated with abundant mitotic figures (83/50 high power fields) with rare atypical mitoses, extensive geographic necrosis, and demonstrated a high (95%) Ki67 proliferation index (Figure 4F). The adenocarcinoma component, which constitute 30% of the tumor, is composed of atypical columnar epithelial cells with enlarged, hyperchromatic nuclei. The cells exhibit stratification and crowding and display glandular, cribriform and papillary formations. Lymphovascular and perineural invasion were seen, and the cystic duct margin was uninvolved by the tumor. The non-neoplastic mucosa showed intestinal metaplasia and chronic inflammatory infiltrates (Figure 2D).

Immunohistochemical studies

Immunohistochemical staining was performed as previously described (Roche BenchMark ULTRA and Leica BOND-MAX IHC/ISH systems) on a representative section to better characterize the poorly differentiated carcinoma component of the tumor. Immunoreactivity (Figures 4B-4E) of the poorly differentiated component to neuroendocrine markers [chromogranin A (LK2H10) and synaptophysin (SP11)], cytokeratin (AE1/AE3/PCK26) and CK19 (b170) confirmed the diagnosis of a large cell neuroendocrine carcinoma. Hence, a final diagnosis of

mixed well-differentiated adenocarcinoma and poorly differentiated (large cell) neuroendocrine carcinoma was reported. As per the staging of gallbladder carcinoma, the patient was stage IIA.

Molecular studies

The non-neuroendocrine (adenocarcinoma) and neuroendocrine carcinoma components were carefully microdissected from 5 μ m thick paraffin-embedded tissue slices. DNA from each component was extracted using the QIAamp DNA FFPE tissue kit (Qiagen), according to the manufacturer's instructions. Both components were individually submitted for next-generation sequencing (NGS) using the Illumina (San Diego, CA) Trusight Tumor 15 (TST15) kit and subsequently sequenced using Illumina MiSeq® system. Along with all the exons of TP53, selected regions of the following genes were sequenced: AKT1, BRAF, EGFR, ERBB2, FOXL2, GNA11, GNAQ, KIT, KRAS, MET, NRAS, PDGFRA, PIK3CA and RET. Mutational analysis identified a TP53 missense mutation (Figure 3) that leads to a stop codon (c.273G>A, p.Trp91Ter) in both components.

Follow-up

Patient was lost to follow up and was asymptomatic until seven months later she decided to consult her surgeon due to a palpable abdominal mass. She is scheduled to undergo another whole abdominal CT scan.

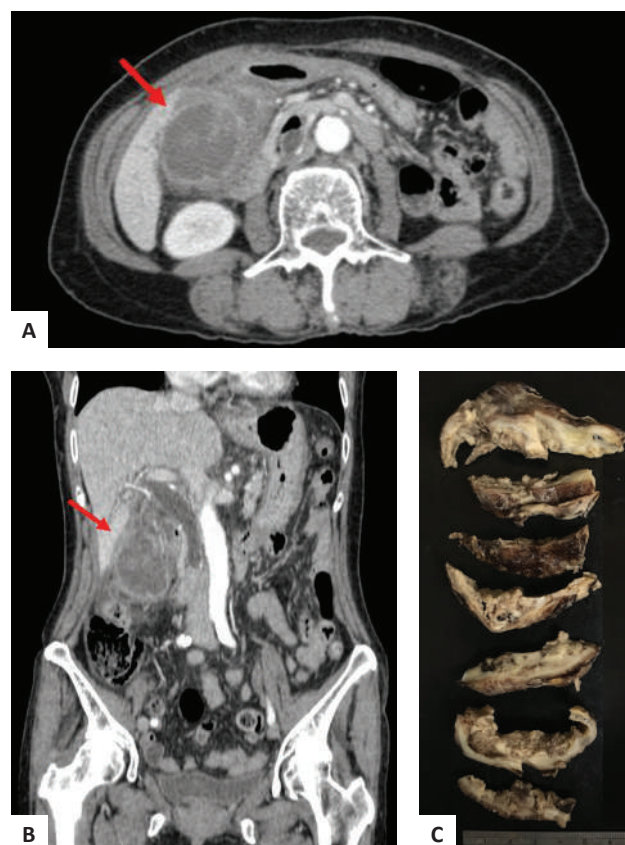


Figure 1. Whole abdominal CT scan, (A) axial and (B) coronal slices. The gallbladder (red arrow) is distended with hyperdense lumen suggestive of bile sludge. (C) The gallbladder wall is thickened with extensive pericholecystic fat stranding reflective of cholecystitis. The gallbladder has tan brown external surfaces with fine adhesions and thickened wall.

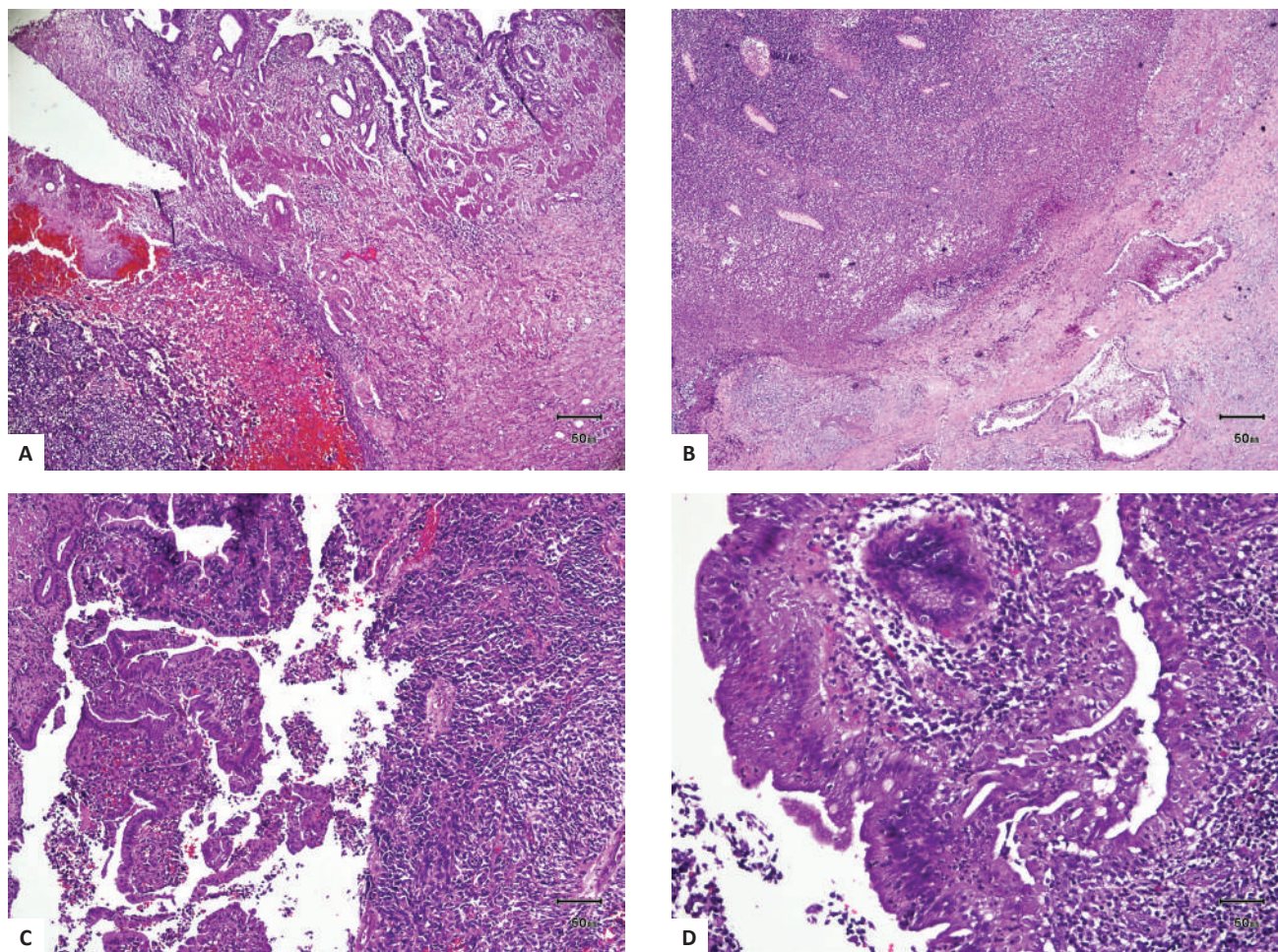


Figure 2. Hematoxylin & eosin stain [H&E]. (A and B) [H&E, 4X] and (C) [H&E, 10X] MiNEN showing juxtaposition of the well-differentiated adenocarcinoma and the poorly differentiated carcinoma components. (D) Intestinal metaplasia, as evidenced by the presence of scattered goblet cells in the mucosa, and chronic inflammatory infiltrates were seen in the non-neoplastic portion of the gallbladder [H&E, 20X].

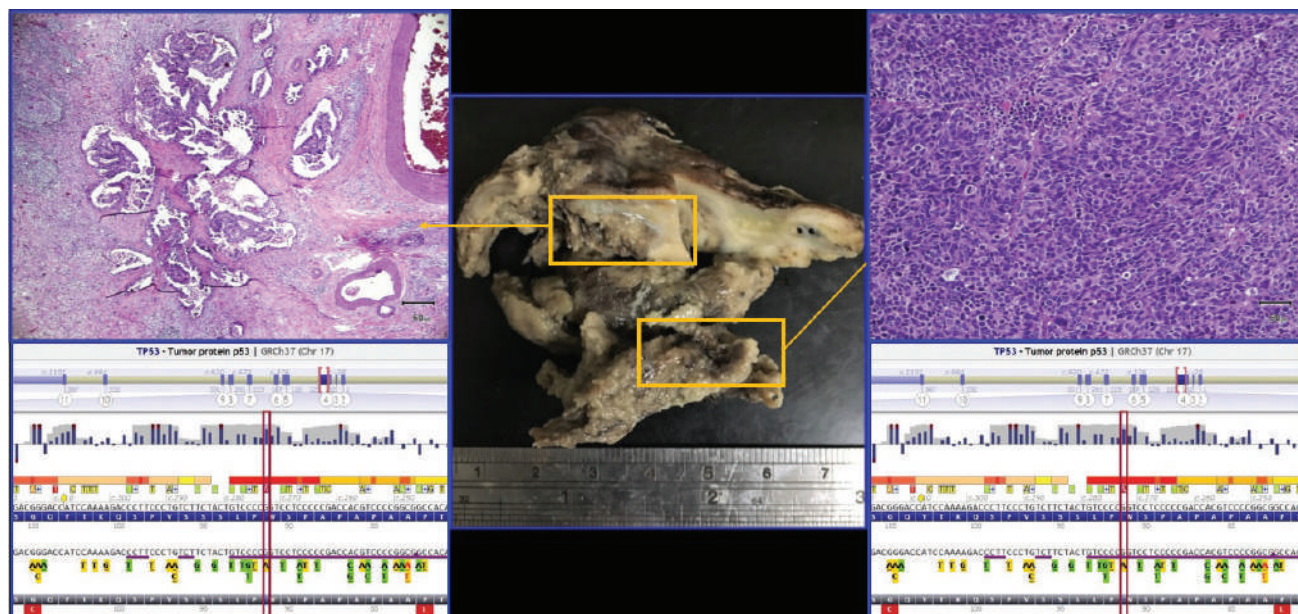


Figure 3. The adenocarcinoma component on the left exhibits cribriform, papillary and glandular patterns. The neuroendocrine component on the right is composed of solid sheets of large cells with hyperchromatic nuclei. Both components are intimately admixed with chronic inflammatory cells. Next generation sequencing revealed an identical TP53 missense mutation that leads to a stop codon (c.273G>A, p.Trp91Ter) in the two components [H&E, 4X].

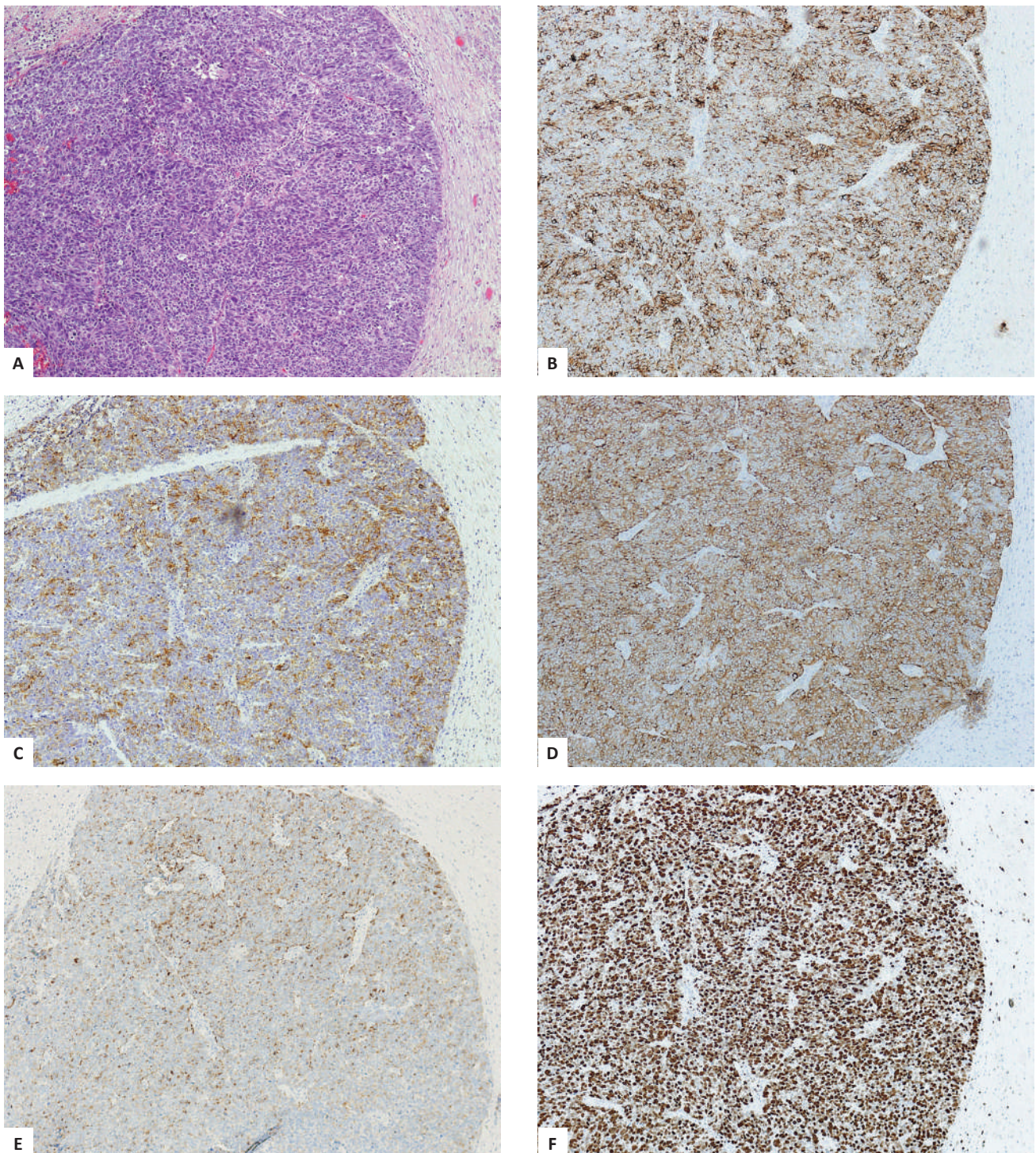


Figure 4. Hematoxylin & eosin stain [H&E]. **(A)** The poorly differentiated neuroendocrine component shows large, round to pleomorphic cells with vesicular nuclei, prominent nucleoli and scant to moderate amount of cytoplasm [H&E, 10X]. These cells are arranged in trabeculae and pseudoglandular formations. This carcinoma component displays immunoreactivity to CK [AE1/AE3, 10X] **(B)**, CK19 [10X] **(C)**, Synaptophysin [10X] **(D)**, and Chromogranin [10X] **(E)** A high (95%) Ki67 [10X] **(F)** Proliferation index is appreciated.

DISCUSSION

Neuroendocrine neoplasms of the gallbladder and bile ducts were subtyped in the WHO 2019 tumor classification based on the mitotic activity and Ki67 proliferation index. The categories include neuroendocrine tumor (NET) grade 1, NET grade 2, NET grade 3, large cell neuroendocrine carcinoma, small cell neuroendocrine carcinoma. Poorly differentiated neuroendocrine carcinoma (PDNEC) which include small cell and large cell neuroendocrine carcinoma (LCNEC), are characterized by brisk mitotic activity (>20 mitoses/ 10 HPFs) and Ki67 proliferation index of more than 20%, with or without necrosis. These neoplasms can occur in the pure form or may be admixed with other histologic components as in cases of mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN). Grossly, LCNEC of the gallbladder NENs appear as infiltrative polypoid, nodular or cauliflower-shaped masses with homogeneous cut surfaces, that invade the muscular wall, with or without extension to the serosa. On microscopic examination, LCNEC is composed of large polygonal cells about three times the lymphocyte diameter. These pleomorphic cells have low nuclear to cytoplasmic ratio, vesicular nuclei, conspicuous nucleoli and abundant cytoplasm. They exhibit peripheral palisading and grow in trabeculae, cords, sheets, pseudoglandular or rosette-like patterns.^{1,2,4,9,10} Although at a reduced extent and intensity as compared with well-differentiated tumors, PDNECs are generally immunoreactive to neuroendocrine markers (Synaptophysin, Chromogranin and CD56), a criterion required for the diagnosis of LCNEC. At the molecular level, PDNECs of the gastrointestinal and pancreaticobiliary tracts show TP53 and retinoblastoma gene (RB1) mutations. LCNEC are aggressive tumors that metastasize early, and are associated with a poor prognosis.^{2,9,4}

Mixed neuroendocrine-non-neuroendocrine neoplasm, previously referred to as mixed adenocarcinoma and neuroendocrine carcinoma (MANEC) in the 2010 WHO blue book, is defined as a tumor histologically composed of at least 30% of both glandular and neuroendocrine carcinoma components.^{1,4,5} The histologic components of MiNEN should be individually graded.^{4,7} The present case was composed predominantly of large cell carcinoma (70%), as confirmed by immunohistochemical evaluation, admixed with well-differentiated adenocarcinoma (30%). Chronic inflammation and intestinal metaplasia were noted in the background.

Endocrine cells are ubiquitously seen throughout the gastrointestinal tract but are absent in the normal gallbladder except for a few cells in the neck region.¹¹ This explains the low prevalence of NENs in the gallbladder (2%). Although the origin of MiNENs remain unclear, it has been hypothesized that these tumors may have been derived from a single pluripotent stem cell precursor that is capable of divergent phenotypic differentiation.⁶ Another proposed mechanism is through metaplastic change. Gallbladder mucosa that underwent gastric and intestinal metaplasia, which are commonly associated with chronic cholecystitis and cholelithiasis, express different types of neuroendocrine cells.¹⁰ These cells are postulated to follow the metaplasia-dysplasia-carcinoma sequence. An alternative view is that MiNENs may have

arisen from the transdifferentiation of adenocarcinoma cells. Evidence also support the association of chronic inflammation to gallbladder cancer.^{4,5,11,12} The hypothesis that MiNEN arises from a common precursor stem cell that undergoes differentiation into several distinct phenotypes is supported by the finding of similar immunohistochemical and ultrastructural profiles in both carcinoma components.¹³ The detection of an identical molecular genetic alteration (TP53 missense mutation leading to a stop codon) in the individual components of the above case further supports the hypothesis that the tumor arises from a common progenitor cell.

p53 is a tumor suppressor gene located on chromosome 17p, the phosphoprotein product of which is involved in the regulation of cell division, by acting as a transcription factor that modulates cyclin-dependent kinase activity. p53 gene mutations are the most frequent genetic abnormality in human cancers. Mutations can be detected using immunohistochemical staining of the p53 protein product or molecular studies such as somatic mutation profiling. In gallbladder cancers, high grade neoplasms exhibit a greater p53 positivity as compared with low grade tumors, and immunoreactivity to p53 might be associated with a shorter patient survival.¹⁴ A case of combined large cell neuroendocrine carcinoma and adenocarcinoma of the gallbladder displaying p53 overexpression and high Ki67 proliferative index was previously reported.¹⁵ Next generation sequencing of 15 gallbladder cancer cases, including adenocarcinoma, adenosquamous carcinoma and carcinosarcoma, also revealed that P53 mutations are the most common of the 26 mutations identified. Other mutations involved the following genes: TP53, STK11, CCNE1, MDM2, MYC, RICTOR, APC, ARID1A, AURKA, CDKN2A, CDKN2A/B, CRKL, FGF10, FGFR3-TACC, KRAS, MCL1, PPKARIA, SMAD4, SMARCA4, TSC2, BAP1, ERBB2, PIK3CA, and ZNF703.¹⁶

Acosta et al.,⁴ reviewed and summarized biliary MiNEN cases reported in the literature. Patients are diagnosed at a mean age of 64 years. The tumor is more commonly seen in women (female to male ratio of 2:1) and in Asian patients, with nonspecific epigastric or right upper quadrant abdominal pain as the most common presenting symptom. Two-thirds of biliary MANEC cases primarily arise from the gallbladder. Patients usually have locally advanced disease (T3 in more than 60%) and lymph node metastasis (half of the cases) at initial diagnosis. Majority of the cases reported were treated with surgery alone, others were treated with chemotherapy and/or radiotherapy.

Most gallbladder carcinomas (GBCs) are both clinically and radiologically unapparent as they mimic presentations of benign diseases such as cholecystitis.⁵ The initial symptoms of primary GBCs are nonspecific and as previously mentioned, patients most commonly present with right upper quadrant or epigastric pain. Histologic typing of GBCs and MiNENs is of utmost important since the treatment is tailored to the most aggressive component present in the tumor.^{4,8} Complete en-bloc surgical resection is the only curative treatment modality in GBCs. Patients with MiNEN generally fare better than those diagnosed with a pure biliary PDNEC.⁸ However, despite adequate surgical management, the

prognosis of biliary MiNENs remain generally poor and this is partly attributed to the delay in their diagnosis and treatment.^{1,4,8,10,12} Tumor recurrence is highly considered for this patient, hence, close clinical follow-up and monitoring is vital for prompt management.

CONCLUSION

We report a case of a 74-year-old Filipino woman who was diagnosed with MiNEN composed of a well-differentiated adenocarcinoma and a large cell neuroendocrine carcinoma component. Molecular analysis of the respective components revealed a similar molecular signature, confirming the common/monoclonal origin hypothesis and indicating that this entity is most likely derived from a pluripotent stem cell capable of divergent differentiation.

ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

- Liu W, Wang L, He XD, Feng C, Chang XY, Lu ZH. Mixed large cell neuroendocrine carcinoma and adenocarcinoma of the gallbladder: a case report and brief review of the literature. *World J Surg Oncol*. 2015;13:114. PMID: 25890047. PMCID: PMC4365805. <https://doi.org/10.1186/s12957-015-0533-6>.
- Bosman FT, Carneiro F, Hruban RH, Theise ND, eds. WHO classification of tumour of the digestive system, 5th edition. Lyon: IARC Press, 2019.
- Miyamoto R, Kikuchi K, Uchida A, et al. Large cell neuroendocrine carcinoma of the gallbladder: a case report and literature review. *Int Surg*. 2016;101:458-64. <https://doi.org/10.9738/10.9738/INTSURG-D-16-00122.1>.
- Acosta AM, Wiley EL. Primary biliary mixed adenoneuroendocrine carcinoma (MANEC): a short review. *Arch Pathol Lab Med*. 2016;140(10):1157-62. PMID: 27684986. <https://doi.org/10.5858/arpa.2015-0102-RS>.
- Jung J, Chae YS, Kim CH, et al. Combined adenosquamous and large cell neuroendocrine carcinoma of the gallbladder. *J Pathol Transl Med*. 2018;(52):121-5. PMID: 28994275. PMCID: PMC5859238. <https://doi.org/10.4132/jptm.2017.08.20>.
- Chen XS, Xie XF, Zhou JJ. Mixed large cell neuroendocrine carcinoma and adenosquamous carcinoma of gallbladder: a case report. *Int J Clin Exp Pathol* 2017;10(2):1882-5. <http://www.ijcep.com/files/ijcep0042195.pdf>.
- Skalický A, Vištejnová L, Dubová M, Malkus T, Skalický T, Troup O. Mixed neuroendocrine-non-neuroendocrine carcinoma of gallbladder: case report. *World J Surg Oncol*. 2019;17. <https://doi.org/10.1186/s12957-019-1598-4>.
- De Mestier L, Cros J. Digestive system mixed neuroendocrine-non-neuroendocrine neoplasms (MiNEN). *Ann Endocrinol (Paris)*. 2019;80(3):172–3. PMID: 31064662. <https://doi.org/10.1016/j.ando.2019.04.006>.
- Goldblum JR, Odze RD. *Odze and Goldblum Surgical Pathology of the GI Tract, Liver, Biliary Tract, and Pancreas*. Philadelphia, PA: Elsevier Saunders, 2015.
- Eltawil KM, Gustafsson BI, Kidd M, Modlin IM. Neuroendocrine tumors of the gallbladder: an evaluation and reassessment of management strategy. *J Clin Gastroenterol*. 2010;44(10):687-95. PMID: 20375728. <https://doi.org/10.1097/MCG.0b013e3181d7a6d4>.
- Mills SE. *Histology for Pathologists*. Fourth ed. Philadelphia, PA: Wolters Kluwer, 2012.
- Buscemi S, Orlando E, Damiano G, et al. "Pure" large cell neuroendocrine carcinoma of the gallbladder: report of a case and review of the literature. *Int J Surg*. 2016; 28(Suppl 1): S128-32. PMID: 26708705. <https://doi.org/10.1016/j.ijssu.2015.12.045>.
- Paniz Mondolfi AE, Slova D, Fan W, et al. Mixed adenoneuroendocrine carcinoma (MANEC) of the gallbladder: a possible stem cell tumor? *Pathol Int*. 2011;61(10):608-14. PMID: 21951672. <https://doi.org/10.1111/j.1440-1827.2011.02709.x>.
- Diamantis I, Karamitopoulou E, Perentes E, Zimmermann A. p53 protein immunoreactivity in extrahepatic bile duct and gallbladder cancer: correlation with tumor grade and survival. *Hepatology*. 1995;22(3):774-9. PMID: 7657282.
- Sato K, Imai T, Shiota Y, Ueda Y, Katsuda S. Combined large cell neuroendocrine carcinoma and adenocarcinoma of the gallbladder. *Pathology Res Pract*. 2010;206(6):397-400. PMID: 19945229. <https://doi.org/10.1016/j.prp.2009.10.008>.
- Javle M, Rashid A, Churi C, et al. Molecular characterization of gallbladder cancer using somatic mutation profiling. *Hum Pathol*. 2014;45(4):701-8. PMID: 24508317. PMCID: PMC4428571. <https://doi.org/10.1016/j.humpath.2013.11.001>.

Disclaimer: This journal is **OPEN ACCESS**, providing immediate access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. As a requirement for submission to the PJP, all authors have accomplished an **AUTHOR FORM**, which declares that the ICMJE criteria for authorship have been met by each author listed, that the article represents original material, has not been published, accepted for publication in other journals, or concurrently submitted to other journals, and that all funding and conflicts of interest have been declared. Consent forms have been secured for the publication of information about patients or cases; otherwise, authors have declared that all means have been exhausted for securing consent.

External Quality Assessment Scheme for Transfusion Transmissible Infections among Blood Service Facilities in the Philippines, 2018

Kenneth Aristotle Punzalan, Rhoda Yu, Iza Mae Chamen

Research Institute for Tropical Medicine-Department of Health, Philippines

ABSTRACT

External Quality Assessment Scheme (EQAS) is an important and vital component of a quality system to which a retrospective and periodic assessment of quality can be undertaken by an independent external agency.

The Transfusion Transmissible Infections–National Reference Laboratory (TTI-NRL) annually provides an EQAS program for transfusion transmissible infections to all blood service facilities in the Philippines as a requirement for the renewal of their license to operate and raise the quality standards of testing for infectious diseases.

A total of 188 participants registered in the 2018 test event and were given an EQAS panel comprised of a serology program (HVHT4120) and malaria program (MLRA415). Results from the participants were submitted through an online informatics system managed by OneWorld Accuracy Canada using the ISO 13528:2008 Robust Statistics method (Huber's Method). Results were analyzed and evaluated with the reference result from the TTI-NRL.

The HVHT4120 program generated 15,330 results and the MLRA415 generated 940 results. 97 results (0.63%) and 80 results (8.51%) were reported as aberrant from each program respectively and were either due to random or systematic errors.

The data generated from this test event are used for the improvement of the quality processes of each participant and the subsequent renewal of their license to operate as required by local health regulations.

Key words: EQAS, transfusion transmissible infections, blood safety, quality improvement

ISSN 2507-8364 (Online)
Printed in the Philippines.
Copyright© 2019 by the PJP.
Received: 30 June 2019.
Accepted: 13 August 2019.
Published online first: 12 September 2019.
<https://doi.org/10.21141/PJP.2019.17>

Corresponding author: Kenneth Aristotle P. Punzalan, RMT
E-mail: kenn.punzalan@gmail.com; tti.nrl@gmail.com
ORCID: <https://orcid.org/0000-0002-0564-2331>

INTRODUCTION

Quality assurance comprises all activities and programs that are planned, developed and practiced to establish the confidence that products or services meet customer expectations. An important and vital component of a quality system is assessment to which a retrospective and periodic assessment of quality can be undertaken by an independent external agency.¹

Participation in an external quality assessment scheme is an annual requirement for the licensure of blood service facilities in the Philippines as regulation is one of the objectives of the Department of Health to ensure access to quality services.² This also aims to stimulate performance improvements and raise the standards of testing.

Transfusion of safe blood involves a number of processes from donor selection until the administration to the recipient. The blood service facilities play a major role in the provision in the transfusion process and errors in screening donated blood can have serious implications for the recipients of these blood products.



This report evaluates the results of the participants of the external quality assessment scheme provided by the Transfusion Transmissible Infections – National Reference Laboratory in 2018.

METHODOLOGY

Panel Composition

The 2018 transfusion transmissible infections test event consists of two programs, (a) HVHT4120 for blood donor serology and (b) MLRA415 for malaria microscopy.

The HVHT4120 program consists of twenty (20) pooled plasma samples obtained from blood donors from different regions in the country. Each pooled sample was prepared by mixing similar volumes of at least two samples that had similar antibody and antigen profiles. All samples were subjected to filtration prior to aliquoting. The samples were aliquoted, and their homogeneity confirmed. The serology profile for HIV, Hepatitis B and C, Syphilis of each sample were identified using serological assays: chemiluminescence assay (ChLIA), enzyme immunoassay (EIA), Rapid Plasma Reagin (RPR), Particle Agglutination (PA) and a Differentiation/Supplemental Assay (SA).

The MLRA415 program consists of five (5) blood smears and the samples were obtained from malaria patients in Palawan. These were prepared by the National Reference Laboratory for Malaria and other Parasites of the Research Institute for Tropical Medicine.

Participants

The 2018 transfusion transmissible infections panel were distributed to 188 participants nationwide (Figure 1) and were charged a registration fee to cover expenses for the test event. 46% (n=87) of the participants are from private institutions, 40% (n=75) from government institutions and 14% (n=26) from the Philippine Red Cross (Figure 2).

Data Analysis

Participants were asked to enter assay results through the online informatics system developed and operated by Oneworld Accuracy Systems (OASYS™). Results reported by the participants for assay interpretations and final status were compared with the relevant reference results for qualitative evaluation. An assay interpretation that is different from the reference result is marked as aberrant.

ISO 13528:2005 Robust Statistics method (Huber's Method) was used to identify outlying results (numerical test results found to be statistically different from other test results reported by participants that tested the same sample in the same assay) for the created peer groups. A peer group is defined as a set of laboratories that utilize the same test format and assay test kit for screening TTI. The said method uses the mean as an estimator and outlying test results were removed from statistical calculation.

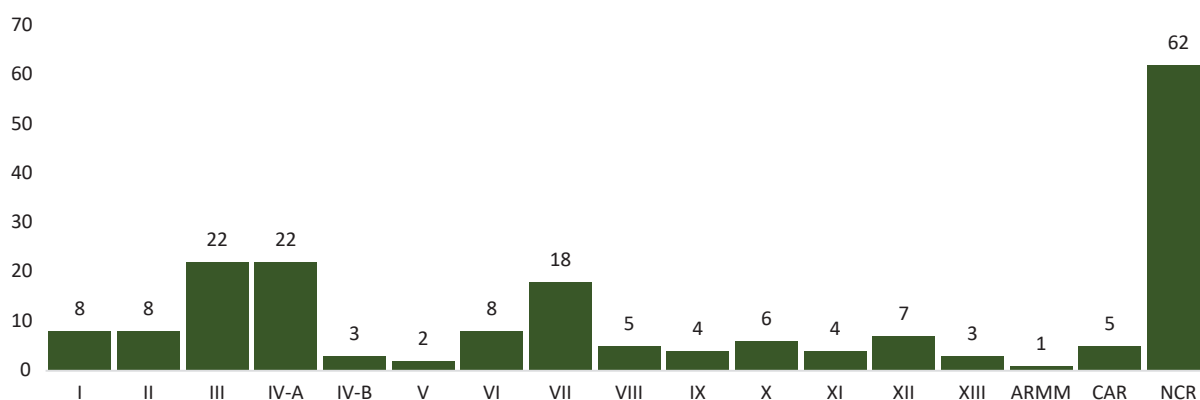


Figure 1. Regional distribution of participants, 2018 TTI-NRL EQAS.

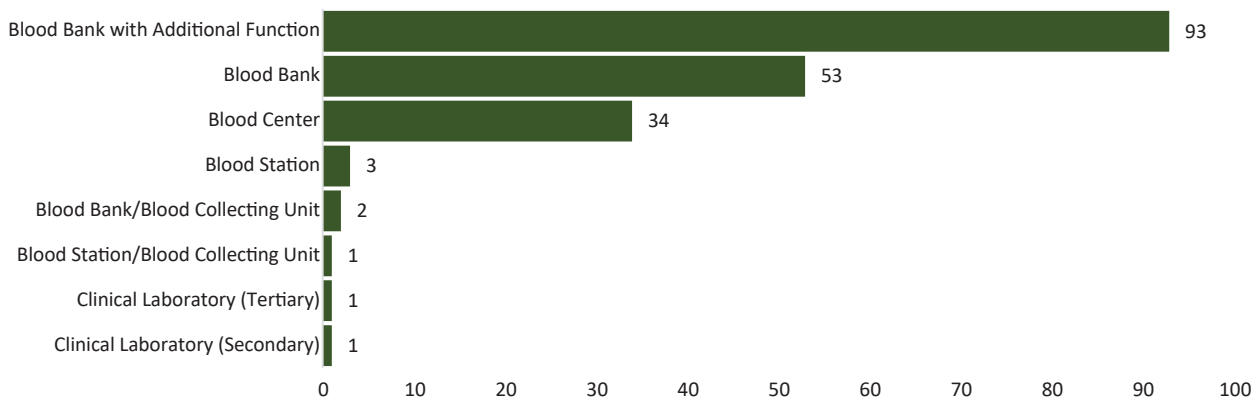


Figure 2. Distribution of participants according to License to Operate issued by the DOH-Health Facilities Services and Regulatory Bureau.

RESULTS AND DISCUSSIONS

A total of 15,330 results were generated from 75 assays for the HVHT4120 program and 940 results were generated through malaria microscopy for the MLRA415 program.

Data entry errors

One (1) participant reported a “reactive” test results but submitted a “negative” assay interpretation. Two participants reported a “negative” test result but submitted a “reactive” assay interpretation.

False positive results

Seven (7) participants reported false reactive results on known negative samples. Twenty-five participants reported false reactive results on samples with a different analyte present.

False negative results

Nine (9) participants reported false negative results on initial testing.

Educational sample (HBsAg and Anti-TP reactivity)

One (1) participant reported a “negative” result for Anti-TP on the HBsAg and Anti-TP reactive sample. One (1) participant reported a “reactive” test result for HIV Ag/Ab.

Educational sample (HIV p24 Antigen)

Four (4) participants reported a “reactive” result using a 3rd generation HIV assay. Seven (7) participants reported a “negative” result using a 4th generation HIV. Two (2) participants reported a different analyte present on the sample.

From the total number of results generated in the HVHT4120 program, 97 results (0.63%) were reported as aberrant.

Scoring Criteria

A participant shall be rated as an unsatisfactory performer in the HVHT4120 program if one of the following criteria are met:

- at least one false negative result
- at least twenty percent (20%) false positive results

Participants with aberrant results are given an investigation checklist to aid them in identifying errors and perform the corrective action needed. A 2nd set of the HVHT4120 program are given to participants if the unsatisfactory performance was due to a testing error. Eleven (11)

participants were given a second set of samples wherein three (3) participants reported aberrant results (2 false reactive results and 1 inconclusive result).

From the total number of results generated in the MLRA415 program, 80 results (8.51%) were reported as aberrant.

Figure 3 shows the participants’ rating according to the following grading scheme:

- EXCELLENT – 100% acceptable results on the initial panel (all final results were correctly identified in comparison with the reference results);
- VERY SATISFACTORY – Less than 100% acceptable results on the initial panel without being given a second panel for retesting;
- SATISFACTORY – 100% acceptable results on retesting of the second panel; or had an aberrant result in the initial panel due to a clerical error, given that the participant was able to identify this error through the EQAS investigation checklist;
- POOR – Participant did not follow minimum requirements as per Department of Health issuance (Department Circular No. 2013-0132) or less than 100% acceptable results on retesting of the second panel; or had an aberrant result in the initial panel due to a clerical error which the participant had failed to identify in the EQAS investigation checklist.

CONCLUSION

Majority of blood service facilities use serological assays to screen for malaria, the malaria EQAS program is limited to blood smears as majority of these assays require freshly collected samples. Participants are recommended to be equipped with the gold standard of malaria diagnosis which is microscopy.

Participation in the external quality assessment scheme for transfusion transmissible infections by all screening blood service facilities in the Philippines is critical and necessary to ensure the accuracy of results generated from serological tests.³ This shall enable the EQAS provider to assess and monitor the quality of laboratory results generated by the participants. The performance report given at the end of the cycle to the participants shall aid them in analyzing the essential corrective and preventive action for outliers and/or aberrant results and shall also compare their performance with other laboratories which shall improve their quality processes.

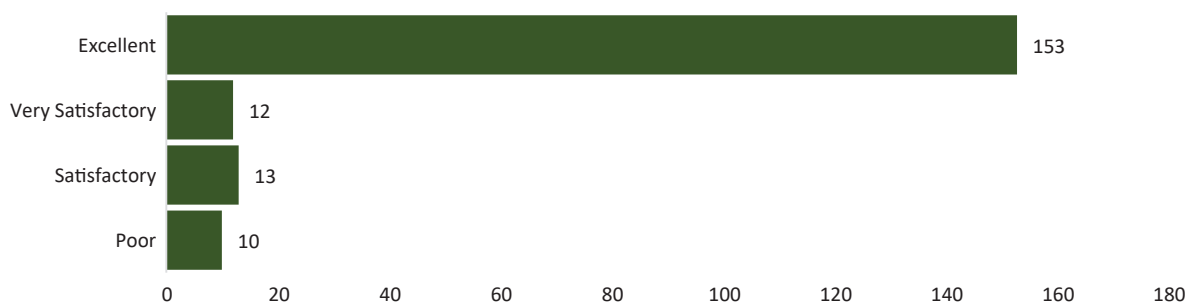


Figure 3. Participant rating distribution, 2018 TTI-NRL EQAS.

RECOMMENDATION

Participation in the external quality assessment scheme in the Philippines is a mandatory requirement for the renewal of the license to operate of all laboratories. The participants should take this as an opportunity to challenge their current quality management system as they should be adhering to the standards set by the Department of Health.

ACKNOWLEDGMENTS

The authors thank the TTI-NRL staff, Dr. Catherine Masangkay, Dr. Socorro Lupisan and Dr. Celia Carlos of the Research Institute for Tropical Medicine (RITM), the DOH Health Facility Development Bureau (HFDB), the DOH Health Facility Services and Regulatory Bureau (HFSRB), the DOH National Voluntary Blood Services Program (NVBSP), the National Council for Blood Services–Technical Committee, the RITM Department of Parasitology, Philippine Red Cross–National Blood Center (Port Area), Asian Hospital and Medical Center, OneWorld Accuracy–Canada and Joe Vincini from NRL Australia. The authors also thank all participating Blood Service Facilities for their support.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

1. World Health Organization, Regional Office for South-East Asia. Quality assurance in bacteriology and immunology, 3rd ed. (SEARO Regional Publication No. 47); 2012. http://apps.searo.who.int/PDS_DOCS/B4871.pdf?ua=1.
2. Philippine Department of Health. Revised rules and regulations governing the licensure and regulation of clinical laboratories in the Philippines. Administrative Order, 2007-0027; 2007. http://lcp.gov.ph/images/Admin_Order_2007_0027.pdf.
3. Bello-López JM, Castañeda-García C, Muñoz-Estrada C, Machorro-Peréz AJ. External quality control program in screening for infectious diseases blood banks in Mexico. *Transfus Apher Sci.* 2018; 57(1):97-101. PMID: 29452838. <https://doi.org/10.1016/j.transci.2018.01.004>.

Disclaimer: This journal is **OPEN ACCESS**, providing immediate access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. As a requirement for submission to the PJP, all authors have accomplished an **AUTHOR FORM**, which declares that the ICMJE criteria for authorship have been met by each author listed, that the article represents original material, has not been published, accepted for publication in other journals, or concurrently submitted to other journals, and that all funding and conflicts of interest have been declared. Consent forms have been secured for the publication of information about patients or cases; otherwise, authors have declared that all means have been exhausted for securing consent.

Workshop

shop

NRL
Asian
Workshop
on Quality
2020

First Announcement!

3-4 March 2020
Sheraton Manila Bay Hotel, Philippines

NRL is pleased to announce the NRL Asian Workshop on Quality 2020 will be held in Manila, Philippines from 3-4 March 2020. Built upon the previous success of NRL Quality Workshops in Indonesia in 2017, Malaysia in 2018 and Vietnam in 2019, the aim of this Workshop is to promote the importance and development of high quality infectious diseases testing in the Asian region. The two day programme will provide opportunity to gain valuable insight for participants and allow for discussions among peers on this very important and essential subject matter.

Specifically, the objectives of this Workshop include:

- Improving the quality of infectious diseases testing in Asia
- Education on the benefits of quality assurance programmes such as EQAS and QC
- Capacity building for laboratories
- Offering local staff the opportunity to present on their experiences around quality and testing
- Providing an interactive forum for delegates to liaise and connect with one another regarding common issues and challenges encountered



Sponsorship Open:	28 October 2019
Registrations Open:	20 November 2019
Cancellation Deadline:	17 February 2020
Online Registrations Close:	26 February 2020

REGISTER NOW

Workshop Details
E: events@nrlquality.org.au
www.nrlquality.org.au



Workshop

NRL
Asian
Workshop
on Quality
2020

3-4 March 2020
Sheraton Manila Bay
Manila, Philippines

Preliminary Programme

TUESDAY 3 MARCH

SESSION 1 BLOOD SAFETY
This session will focus on transfusion transmissible infections, blood donation safety and risk.

Morning Break

SESSION 2 REGISTRATION AND VALIDATION OF IVDs
This session will discuss the importance of IVD assessment for use in infectious diseases testing and best practice for the implementation of IVDs.

Lunch 

SESSION 3 TESTING ALGORITHMS
Themes in the session will include point-of-care/rapid testing algorithms as well as confirmatory/reference testing.

Afternoon Break

SESSION 4 QC
This session will focus on the important of QC, what QConnect limits are and feature QC case studies for delegates to troubleshoot and provide their assessment in an interactive forum.

WEDNESDAY 4 MARCH

SESSION 1 NEW TECHNOLOGIES
In this session, we will learn about novel technologies that are currently available to aid in testing for infectious diseases.

Morning Break

SESSION 2 EQAS
Presentations will focus on the importance of EQAS and the value of these programs.

Lunch 

SESSION 3 PANEL DISCUSSION
Our panel will be available to discuss and address your issues or queries regarding quality of infectious diseases testing.

Afternoon Break

SESSION 4 QMS
This session will include topics such as assessing competency, auditing and meeting regulatory requirements.

CLOSE 

*This is a proposed draft and therefore the order and session topics may change



Instructions to Authors

The **Philippine Journal of Pathology (PJP)** is an open-access, peer-reviewed, English language, medical and health science journal that is published continuously online and semi-annually in print by the Philippine Society of Pathologists, Inc. (PSP, Inc). All manuscripts must be submitted through the PJP Official Website (Open Journal Systems) (<http://philippinejournalofpathology.org>). All other correspondences and other editorial matters should be sent via electronic mail to philippinepathologyjournal@gmail.com.

Articles and any other material published in the PJP represent the work of the author(s) and do not reflect the opinions of the Editors or the Publisher. **Articles that do not subscribe to the Instructions to Authors shall be promptly returned.**

ARTICLE SECTIONS

The PJP welcomes manuscripts on all aspects of pathology and laboratory medicine, to include cytology, histopathology, autopsy, forensic pathology, clinical chemistry, clinical microscopy, medical microbiology, parasitology, immunology, hematology, blood banking, medical technology, laboratory diagnostics, laboratory biosafety and biosecurity, laboratory management, and quality assurance.

The PJP accepts original articles, review articles, case reports, feature articles, brief communications, autopsy cases, editorials, or letters to the Editor.

Original articles

The research must have received institutional review board approval that is explicitly stated in the methodology. The abstract should contain no more than 200 words with a structured format consisting of the objective/s, methodology, results and conclusion. A manuscript for original articles should not exceed 25 typewritten pages (including tables, figures, illustrations and maximum of 30 references) or 6000 words.

Reviews

Review articles, both solicited and unsolicited, provide information on the "state of the art." PJP reviews not only summarize current understanding of a particular topic but also critically appraise relevant literature and data sources, describe significant gaps in the research, and future directions. The abstract should be from 50 to 75 words and should not be structured. A manuscript for reviews should not exceed 15 typewritten pages (including tables, figures, illustrations and maximum of 50 references) or 4000 words.

Case Reports

This type of article pertains to single or multiple reports of well-characterized cases that are highly unusual, novel, or rare; or with a unique or variant presentation, evolution or course; or that represent an unexpected or uncommon association of two or more diseases or disorders that may represent a previously unsuspected causal relationship; or that are underreported in the literature. The abstract should be from 50 to 75 words and should not be structured. A manuscript for case reports should not exceed 10 typewritten pages (including tables, figures, illustrations and maximum of 15 references) or 3000 words.

Feature articles

The PJP may feature articles, either as part of an issue theme or a special topic on pathology by a local or international expert or authority. The abstract should be from 50 to 75 words and should not be structured. A manuscript for feature articles should not exceed 25 typewritten pages (including tables, figures, illustrations and maximum of 30 references) or 6000 words.

Autopsy Vault

The PJP highly welcomes articles on autopsy protocols of cases. The article must include a summary presentation of the history, evaluation and work-up, clinical course of a case, followed by the autopsy procedure performed, gross and

microscopic findings, discussion, learning points and conclusion. The PJP recognizes the instructional and educational value of articles under this section. The abstract should be from 50 to 75 words and should not be structured. A manuscript for the Autopsy Vault should not exceed 25 typewritten pages (including tables, figures, illustrations and maximum of 30 references) or 6000 words.

Images in Pathology

Images of unique, interesting, or highly educational cases encountered in hematology, cytology, histopathology, or medical microbiology, may be submitted under this section, and may include photomicrographs, gross pictures, machine read-outs, among others. A brief history, the photograph(s) and short discussion of the case. No abstract is required. A manuscript for Images in Pathology should not exceed 500 words, with maximum of 10 references. This is distinct from the Case Report which is a full write up.

Brief Communications

Brief Communications are short reports intended to either extend or expound on previously published research or present new and significant findings which may have a major impact in current practice. If the former, authors must acknowledge and cite the research which they are building upon. The abstract should be from 50 to 75 words and should not be structured. A manuscript for brief communications should not exceed 5 typewritten pages (including tables, figures, illustrations and maximum of 10 references) or 1500 words.

Editorials

Recognized leaders in the field of pathology and laboratory medicine may be invited by the Editor-in-Chief/Editorial Board to present their scientific opinion and views of a particular topic within the context of an issue theme or issues on scholarly publication. No abstract or keywords necessary.

Letters to the Editor

PJP welcomes feedback and comments on previously published articles in the form of Letters to the Editor. No abstract or keywords are necessary. A Letter to the Editor must not exceed 2 typewritten pages or 500 words.

Special Announcements

Special announcements may include upcoming conventions, seminars or conferences relevant to pathology. The Editors shall deliberate and decide on acceptance and publication of special announcements. Please coordinate with the Editorial Coordinator for any request for special announcements.

COVER LETTER

A cover letter must accompany each manuscript citing the complete title of the manuscript, the list of authors (complete names, position/designation and institutional affiliations), with one (1) author clearly designated as corresponding author, providing his/her complete institutional mailing address, institutional telephone/fax number, and work e-mail address. The **PJP Cover Letter Template** must be used.

PJP AUTHOR FORM

For submissions to the PJP to be accepted, all authors must read and sign the **PJP Author Form** consisting of: (1) the Authorship Certification, (2) the Author Declaration, (3) the Statement of Copyright Transfer, and (4) the Statement of Disclosure of Conflicts of Interest. The completely accomplished PJP Author Form shall be scanned and submitted along with the manuscript. No manuscript shall be received without the PJP Author Form.

GENERAL FORMATTING GUIDELINES

- Authors must use the standard PJP templates for each type of manuscript. These templates are aligned with the most current versions of the EQUATOR Network guidelines and checklists (<http://equatornetwork.org>).
- The manuscript should be encoded on the template using Microsoft Word (2007 version or later version), single-spaced, 2.54 cm margins throughout, on A4 size paper. Preferred fonts may include Century Gothic (template default), Times New Roman, or Arial.
- The manuscript should be arranged in sequence as follows: (1) Title Page, (2) Abstract, (3) Text, (4) References, (5) Tables, and (6) Figures & Illustrations.
- All the sheets of the manuscript should be labelled with the page number (in Hindu-Arabic Numerals) printed on the upper right corner.
- References should pertain directly to the work being reported. Within the text, references should be indicated using Hindu-Arabic numerals in superscripts.

SPECIFIC FORMATTING GUIDELINES

Title and Authors

- The title should be as concise as possible.
- A running title (less than 50 characters) shall also be required. The running title is the abbreviated version of the title that will be placed in the header. The running title should capture the essence of the manuscript title.
- The full name of the author(s) directly affiliated with the work should be included (First name, Middle initial and Last name). The order of authorship shall be the prerogative of the author(s).
- There are 4 criteria for authorship (ICMJE recommendations). These are captured in the **PJP Author Form**.
 - **Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND**
 - **Drafting the work or revising it critically for important intellectual content; AND**
 - **Final approval of the version to be published; AND**
 - **Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.**
- The highest educational attainment or title of the authors should be included as an attachment whenever appropriate (MD, PhD, et cetera).
- Name and location of no more than one (1) institutional affiliation per author may be included.
- If the paper has been presented in a scientific forum or convention, a note should be provided indicating the name of the forum or convention, location (country), and date of its presentation.

Abstract

- **For manuscripts under the “Original Article” section:** the abstract should contain no more than 300 words with a structured format consisting of the following standard headings: objective/s, methodology, results and conclusion.
- **For manuscripts under the “Feature Article,” “Review Article,” “Case Report,” “Brief Communications,” and “Autopsy Vault” sections:** the abstract should be no more than 200 words and need not be structured.
- Letters to the Editor and editorials do not require an abstract.

Keywords

At least three (3) keywords but no more than six (6), preferably using terms from the **Medical Subject Headings (MeSH) list of Index Medicus**, should be listed horizontally under the abstract for cross-indexing of the article.

Text

- The text should be organized consecutively as follows: **Introduction, Methodology, Results and Discussion, Conclusion** (IMRaD format), followed by **Disclosures, Acknowledgments** and **References**.
- All references, tables, figures and illustrations should be cited in the text, in numerical order.
- All abbreviations should be spelled out once (the first time they are mentioned in the text) followed by the abbreviation enclosed in parentheses. The same abbreviation may then be used subsequently instead of the full names.
- All measurements and weights should be in System International (SI) units.
- Under **Methodology**, information should be provided on institutional review board/ethics committee approval or informed consent taking (if appropriate).
- **Acknowledgements** to individuals/groups of persons, or institution/s who have contributed to the manuscript but *did not qualify as authors* based on the ICMJE criteria, should be included at the end of the text just before the references. Grants and subsidies from government or private institutions should also be acknowledged.

References

- References in the text should be identified by Hindu-Arabic Numerals in superscript on the same line as the preceding sentence.
- References should be numbered consecutively in the order by which they are mentioned in the text. They should not be alphabetized.
- All references should provide inclusive page numbers.
- Journal abbreviations should conform to those used in PubMed.
- A maximum of six authors per article can be cited; beyond that, name the first three and add “et al.”
- The style/punctuation approved by PJP conforms to that recommended by the International Committee of Medical Journal Editors (ICMJE) available at <http://www.icmje.org>. Examples are shown below:

One to Six Authors

Krause RM. The origin of plagues: old and new. *Science*. 1992;257:1073-1078.

Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the US. *JAMA*. 2001;286(10):1195-1200.

More than Six Authors

Rhynes VK, McDonald JC, Gelder FB, et al. Soluble HLA class I in the serum of transplant recipients. *Ann Surg*. 1993; 217 (5): 485-9.

Authors Representing a Group

Moher D, Schulz KF, Altman D; for the CONSORT Group. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. *JAMA*. 2001;285(15):1987-1991.

Book

Byrne, DW. Publishing your medical research paper: What they don't teach in medical school. Baltimore: Williams & Wilkins, 1998.

World Wide Web

Barry JM. The site of origin of the 1918 influenza pandemic and its public health implications. [Commentary]. *JTranslational Med*. January 20, 2004;2(3):1-4. <http://www.translational-medicine.com/content/2/1/3>. Accessed November 18, 2005.

Tables

- Cite all tables consecutively in the text and number them accordingly.
- Create tables preferably using Microsoft Excel with one table per worksheet.
- Tables should not be saved as image files.
- The content of tables should include a table number (Hindu-Arabic) and title in capital letters above the table.
- Place explanatory notes and legends, as well as definitions of abbreviations used below the table. For legends, use small letters (i.e., a, b, c, d).
- Each table must be self-explanatory, being a supplement rather than a duplicate of information in the text.
- Up to a maximum of five (5) tables are allowed.

Figures and Graphs

- Figures or graphs should be identified by Hindu-Arabic Numeral/s with titles and explanations underneath.
- The numbers should correspond to the order in which the figures/graphs occur in the text.
- Figures & graphs should not be saved as image files. For illustrations and photographs, see next section.
- Provide a title and brief caption for each figure or graph. Caption should not be longer than 15-20 words.
- All identifying data of the subject/s or patient/s under study such as name or case numbers, should be removed.
- Up to a maximum of five (5) figures and graphs are allowed.

Illustrations and Photographs

- Where appropriate, all illustrations/photographic images should be at least 800 x 600 dpi and submitted as image files (preferably as .png, .jpeg, .tif, .psd or .pdf files).
- For photomicrographs, the stain used (e.g. H & E) and magnification (e.g. 400X) should be included in the description.
- Computer-generated illustrations which are not suited for reproduction should be professionally redrawn or printed on good quality laser printers. Photocopies are not acceptable.
- All letterings for illustration should be of adequate size to be readable even after size reduction.
- Place explanatory notes and legends, as well as definitions of abbreviations used below the illustration/photograph.
- Up to a maximum of five (5) illustrations/ photographs are allowed.

N.B.: For tables, figures, graphs, illustrations and photographs that have been previously published in another journal or book, a note must be placed under the specific item stating that such has been adapted or lifted from the original publication. This should also be referenced in the **References** portion.

EDITORIAL PROCESS (Figure 1)

- The Editorial Coordinator shall review each submission to check if it has met aforementioned criteria and provide feedback to the author within 24 hours.
- Once complete submission is acknowledged, the manuscript undergoes Editorial Board Deliberation to decide whether it shall be considered or not for publication in the journal. Within five (5) working days, authors shall be notified through e-mail that their manuscript either (a) has been sent to referees for peer-review or (b) has been declined without review.
- The PJP implements a strict double blind peer review policy. For manuscripts that are reviewed, authors can expect a decision within ten (10) working days from editorial deliberation. There may be instances when decisions can take longer: in such cases, the Editorial Coordinator shall inform the authors.
- The editorial decision for manuscripts shall be one of the following: (a) acceptance without further revision, (b) acceptance with minor revisions, (c) major manuscript revision and resubmission, or (d) non-acceptance.
- Accepted manuscripts are subject to editorial modifications to bring them in conformity with the style of the journal. Copyediting and layout shall take five (5) working days, after which the manuscript is published online.
- All online articles from the last six (6) months shall be collated and published in print as a full issue.

EDITORIAL OFFICE CONTACT INFORMATION:

The Philippine Journal of Pathology

2nd Floor, Laboratory Research Division
Research Institute for Tropical Medicine
Filinvest Corporate City

Alabang, Muntinlupa City 1781

Editor-in-Chief: **Amado O. Tandoc III, MD, FPSP**

Telefax number: (+632) 88097120

E-mail: philippinepathologyjournal@gmail.com

Website: <http://philippinejournalofpathology.org>

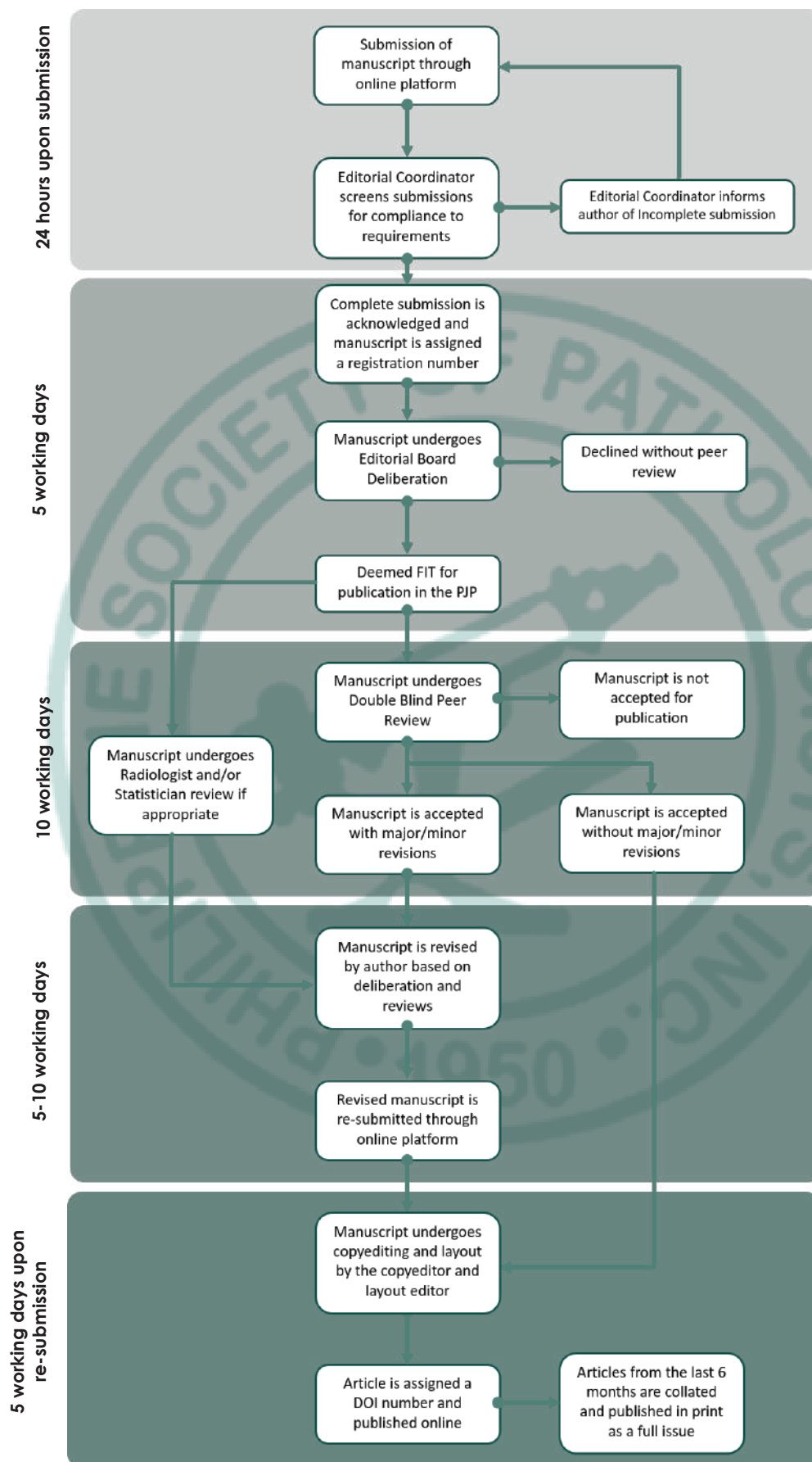


Figure 1. Editorial Process Flow.



PJP AUTHOR FORM

For submissions to the PJP to be accepted, all authors must read and sign this PJP Author Form consisting of: (1) the Authorship Certification, (2) the Author Declaration, (3) the Statement of Copyright Transfer, and (4) the Statement of Disclosure of Conflicts of Interest. The completely accomplished PJP Author Form shall be scanned and submitted along with the manuscript. No manuscript shall be received without the PJP Author Form.

COMPLETE TITLE OF MANUSCRIPT

AUTHORSHIP CERTIFICATION

- In consideration of our submission to the Philippine Journal of Pathology (PJP), the undersigned author(s) of the manuscript hereby certify, that all of us have actively and sufficiently participated in (1) the conception or design of the work, the acquisition, analysis and interpretation of data for the work; AND (2) drafting the work, revising it critically for important intellectual content; AND (3) that we are all responsible for the final approval of the version to be published; AND (4) we all agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

AUTHOR DECLARATIONS

- The undersigned author(s) of the manuscript hereby certify, that the submitted manuscript represents original, exclusive and unpublished material. It is not under simultaneous consideration for publication elsewhere. Furthermore, it will not be submitted for publication in another journal, until a decision is conveyed regarding its acceptability for publication in the PJP.
- The undersigned hereby certify, that the study on which the manuscript is based had conformed to ethical standards and/or had been reviewed by the appropriate ethics committee.
- The undersigned likewise hereby certify that the article had written/informed consent for publication from involved subjects (for case report/series only) and that in case the involved subject/s can no longer be contacted (i.e., retrospective studies, no contact information, et cetera), all means have been undertaken by the author(s) to obtain the consent.

AUTHOR STATEMENT OF COPYRIGHT TRANSFER

- Furthermore, the undersigned author(s) recognize that the PJP is an OPEN-ACCESS publication which licenses all published manuscripts to be used for building on and expanding knowledge, for non-commercial purposes, so long as the manuscripts are properly cited and recognized (Attribution-NonCommercial-ShareAlike 4.0 International Creative Commons License [CC BY-NC-SA 4.0]). The undersigned author(s) hereby, transfer/assign or otherwise convey all copyright ownership of the manuscript to the PJP.

AUTHOR DISCLOSURE OF CONFLICTS OF INTEREST

In order to ensure scientific objectivity and independence, the PJP requires all authors to make a full disclosure of areas of potential conflict of interest. Such disclosure will indicate whether the person and/or his/her immediate family has any financial relationship with pharmaceutical companies, medical equipment manufacturers, biomedical device manufacturers, or any companies with significant involvement in the field of health care. Place all disclosures in the table below. An extra form may be used if needed.

Examples of disclosures include but not limited to: ownership, employment, research support (including provision of equipment or materials), involvement as speaker, consultant, or any other financial relationship or arrangement with manufacturers, companies or suppliers. With respect to any relationships identified, author(s) must provide sufficiently detailed information to permit assessment of the significance of the potential conflict of interest (for example, the amount of money involved and/or the identification of any value of goods and services).

AUTHOR NAME	RELATIONSHIP	MANUFACTURER/ SUPPLIER/ COMPANY

All disclosures shall remain confidential during the review process and the nature of any final printed disclosure will be determined by the PJP. If there are no conflicts of interest to disclose, the author(s) should check the box below.

- I/We do not have any conflicts of interest to disclose.

Author Name	Signature	Date (MM/DD/YYYY)

ICMJE Form for Disclosure of Potential Conflicts of Interest

Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in six parts.

1. Identifying information.

2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Intellectual Property.

This section asks about patents and copyrights, whether pending, issued, licensed and/or receiving royalties.

5. Relationships not covered above.

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

Definitions.

Entity: government agency, foundation, commercial sponsor, academic institution, etc.

Grant: A grant from an entity, generally [but not always] paid to your organization

Personal Fees: Monies paid to you for services rendered, generally honoraria, royalties, or fees for consulting, lectures, speakers bureaus, expert testimony, employment, or other affiliations

Non-Financial Support: Examples include drugs/equipment supplied by the entity, travel paid by the entity, writing assistance, administrative support, etc.

Other: Anything not covered under the previous three boxes

Pending: The patent has been filed but not issued

Issued: The patent has been issued by the agency

Licensed: The patent has been licensed to an entity, whether earning royalties or not

Royalties: Funds are coming in to you or your institution due to your patent

ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name)

2. Surname (Last Name)

3. Date

4. Are you the corresponding author?

Yes No

5. Manuscript Title

6. Manuscript Identifying Number (if you know it)

Section 2. The Work Under Consideration for Publication

Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Are there any relevant conflicts of interest? Yes No

ADD

Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were **present during the 36 months prior to publication**.

Are there any relevant conflicts of interest? Yes No

ADD

Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? Yes No

ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 5. Relationships not covered above

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

- Yes, the following relationships/conditions/circumstances are present (explain below):
- No other relationships/conditions/circumstances that present a potential conflict of interest

At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

Section 6. Disclosure Statement

Based on the above disclosures, this form will automatically generate a disclosure statement, which will appear in the box below.

Generate Disclosure Statement

Evaluation and Feedback

Please visit <http://www.icmje.org/cgi-bin/feedback> to provide feedback on your experience with completing this form.



PATIENT CONSENT FORM

For case report and image submissions to the PJP to be accepted, the author/s must ensure that patients or patients' legal guardian/relative have provided informed consent to publish information about them in the journal. The completely accomplished PJP Patient Consent Form shall be scanned and submitted along with the manuscript. No case report and image shall be received without the PJP Consent Form.

Name of person described in article or shown in photograph: _____

Subject matter of photograph or article (brief description):

(The Subject matter of the photograph or article is hereafter termed as the "INFORMATION.")
Title of article:

I, _____, give my consent for this information
[please insert your full name]
about MYSELF/MY CHILD OR WARD/MY RELATIVE relating to the subject matter
[please underline correct description]
above to appear in the Philippine Journal of Pathology (PJP) subject to its
publication policies and ethical standards.

I have seen and read the material to be submitted to the PJP and thoroughly understand the following:

- The Information will be published in the PJP without my name. It is the obligation of the PJP to make all attempts, within its reasonable jurisdiction and authority, to ensure my anonymity.
- The Information may also be placed on the PJP website.
- The PJP shall not allow the Information to be used for advertising or packaging or to be used out of context (i.e., used to accompany an entirely different article or topic).
- I can withdraw my consent at any time before publication, but once the Information has already been sent to press, it is my understanding that it will not be possible to revoke the consent.

Signed: _____
[signature over complete name]

Date: _____

Witness:

Signed: _____
[signature over complete name]

Date: _____

Consolidated criteria for reporting qualitative research (COREQ): A 32-item checklist for interviews and focus groups

No	Item	Guide questions / description
DOMAIN 1: RESEARCH TEAM AND REFLEXIVITY		
Personal Characteristics		
1	Interviewer/facilitator	Which author/s conducted the interview or focus group?
2	Credentials	What were the researcher's credentials? E.g. PhD, MD
3	Occupation	What was their occupation at the time of the study?
4	Gender	Was the researcher male or female?
5	Experience and training	What experience or training did the researcher have?
Relationship with participants		
6	Relationship	Was a relationship established prior to study commencement?
7	Participant knowledge of the interviewer	What did the participants know about the researcher? e.g. personal goals, reasons for doing the research
8	Interviewer characteristics	What characteristics were reported about the interviewer/facilitator? e.g. Bias, assumptions, reasons and interests in the research topic
DOMAIN 2: STUDY DESIGN		
Theoretical framework		
9	Methodological orientation and Theory	What methodological orientation was stated to underpin the study? e.g. grounded theory, discourse analysis, ethnography, phenomenology, content analysis
Participant selection		
10	Sampling	How were participants selected? e.g. purposive, convenience, consecutive, snowball
11	Method of approach	How were participants approached? e.g. face-to-face, telephone, mail, email
12	Sample size	How many participants were in the study?
13	Non-participation	How many people refused to participate or dropped out? Reasons?
Setting		
14	Setting of data collection	Where was the data collected? e.g. home, clinic, workplace
15	Presence of non-participants	Was anyone else present besides the participants and researchers?
16	Description of sample	What are the important characteristics of the sample? e.g. demographic data, date
Data Collection		
17	Interview guide	Were questions, prompts, guides provided by the authors? Was it pilot tested?
18	Repeat interview	Were repeat interviews carried out? If yes, how many?
19	Audio/visual recording	Did the research use audio or visual recording to collect the data?
20	Field notes	Were field notes made during and/or after the interview or focus group?
21	Duration	What was the duration of the interviews or focus group?
22	Data saturation	Was data saturation discussed?
23	Transcripts returned	Were transcripts returned to participants for comment and/or correction?
DOMAIN 3: ANALYSIS AND FINDINGS		
Data analysis		
24	Number of data coders	How many data coders coded the data?
25	Description of the coding tree	Did authors provide a description of the coding tree?
26	Derivation of themes	Were themes identified in advance or derived from the data?
27	Software	What software, if applicable, was used to manage the data?
28	Participant checking	Did participants provide feedback on the findings?
Reporting		
29	Quotations presented	Were participant quotations presented to illustrate the themes / findings? Was each quotation identified? e.g. participant number
30	Data and findings consistent	Was there consistency between the data presented and the findings?
31	Clarity of major themes	Were major themes clearly presented in the findings?
32	Clarity of minor themes	Is there a description of diverse cases or discussion of minor themes?

EQUATOR stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.



CARE Checklist (2013) of Information to include when Writing a Case Report

Topic	Item no.	Checklist item description	Reported on page no.
Title	1	The words "case report" should be in the title along with the area of focus	_____
Key Words	2	2 to 5 key words that identify areas covered in this case report	_____
Abstract	3a	Introduction—What is unique about this case? What does it add to the medical literature?	_____
	3b	The main symptoms of the patient and the important clinical findings	_____
	3c	The main diagnoses, therapeutics interventions, and outcomes	_____
	3d	Conclusion—What are the main "take-away" lessons from this case?	_____
Introduction	4	One or two paragraphs summarizing why this case is unique with references	_____
Patient Information	5a	De-identified demographic information and other patient specific information	_____
	5b	Main concerns and symptoms of the patient	_____
	5c	Medical, family, and psychosocial history including relevant genetic information (also see timeline)	_____
	5d	Relevant past interventions and their outcomes	_____
Clinical Findings	6	Describe the relevant physical examination (PE) and other significant clinical findings	_____
Timeline	7	Important information from the patient's history organized as a timeline	_____
Diagnostic Assessment	8a	Diagnostic methods (such as PE, laboratory testing, imaging, surveys)	_____
	8b	Diagnostic challenges (such as access, financial, or cultural)	_____
	8c	Diagnostic reasoning including other diagnoses considered	_____
	8d	Prognostic characteristics (such as staging in oncology) where applicable	_____
Therapeutic Intervention	9a	Types of intervention (such as pharmacologic, surgical, preventive, self-care)	_____
	9b	Administration of intervention (such as dosage, strength, duration)	_____
	9c	Changes in intervention (with rationale)	_____
Follow-up and Outcomes	10a	Clinician and patient-assessed outcomes (when appropriate)	_____
	10b	Important follow-up diagnostic and other test results	_____
	10c	Intervention adherence and tolerability (How was this assessed?)	_____
	10d	Adverse and unanticipated events .	_____
Discussion	11a	Discussion of the strengths and limitations in your approach to this case	_____
	11b	Discussion of the relevant medical literature	_____
	11c	The rationale for conclusions (including assessment of possible causes)	_____
	11d	The primary "take-away" lessons of this case report	_____
Patient Perspective	12	When appropriate the patient should share their perspective on the treatments they received	_____
Informed Consent	13	Did the patient give informed consent? Please provide if requested	<input type="checkbox"/> Yes <input type="checkbox"/> No

EQUATOR stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.



PRISMA 2009 Checklist of Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Section / Topic	Item no.	Checklist item	Reported on page no.
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	_____
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	_____
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	_____
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	_____
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	_____
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	_____
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	_____
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	_____
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	_____
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	_____
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	_____
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	_____
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	_____
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	_____
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	_____
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	_____
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	_____
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	_____
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	_____
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	_____
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	_____
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	_____
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	_____
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	_____
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	_____
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	_____
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	_____

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med* 6(6): e1000097. doi:10.1371/journal.pmed1000097. For more information, visit: www.prisma-statement.org.

EQUATOR stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.



STROBE Statement - Checklist of Items that should be included in Reports of Observational Studies

Section / Topic	Item no.	Recommendation
TITLE		
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found
INTRODUCTION		
Background / rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
METHODS		
Study Design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data Sources / measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study Size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —Report numbers of outcome events or summary measures
Main Results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
DISCUSSION		
Key Results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
OTHER INFORMATION		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

* Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

EQUATOR stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.

Section and Topic	No.	Item
TITLE OR ABSTRACT		
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)
ABSTRACT		
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)
INTRODUCTION		
	3	Scientific and clinical background, including the intended use and clinical role of the index test
	4	Study objectives and hypotheses
METHODS		
Study design	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)
Participants	6	Eligibility criteria
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)
	8	Where and when potentially eligible participants were identified (setting, location and dates)
Test Methods	9	Whether participants formed a consecutive, random or convenience series
	10a	Index test, in sufficient detail to allow replication
	10b	Reference standard, in sufficient detail to allow replication
	11	Rationale for choosing the reference standard (if alternatives exist)
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory
Analysis	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test
	13b	Whether clinical information and index test results were available to the assessors of the reference standard
	14	Methods for estimating or comparing measures of diagnostic accuracy
	15	How indeterminate index test or reference standard results were handled
	16	How missing data on the index test and reference standard were handled
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory
	18	Intended sample size and how it was determined
RESULTS		
Participants	19	Flow of participants, using a diagram
	20	Baseline demographic and clinical characteristics of participants
	21a	Distribution of severity of disease in those with the target condition
	21b	Distribution of alternative diagnoses in those without the target condition
	22	Time interval and any clinical interventions between index test and reference standard
Test Results	23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)
	25	Any adverse events from performing the index test or the reference standard
DISCUSSION		
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability
	27	Implications for practice, including the intended use and clinical role of the index test
OTHER INFORMATION		
	28	Registration number and name of registry
	29	Where the full study protocol can be accessed
	30	Sources of funding and other support; role of funders

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on <http://www.equator-network.org/reporting-guidelines/stard>.

EQUATOR stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.



CHEERS Checklist - Items to include when Reporting Economic Evaluations of Health Interventions

Section / Item	Item no.	Recommendation	Reported on page no. / line no.
TITLE AND ABSTRACT			
Title	1	Identify the study as an economic evaluation or use more specific terms such as "cost-effectiveness analysis", and describe the interventions compared.	_____
Abstract	2	Provide a structured summary of objectives, perspective, setting, methods (including study design and inputs), results (including base case and uncertainty analyses), and conclusions.	_____
INTRODUCTION			
Background and objectives	3	Provide an explicit statement of the broader context for the study. Present the study question and its relevance for health policy or practice decisions.	_____
METHODS			
Target population and subgroups	4	Describe characteristics of the base case population and subgroups analysed, including why they were chosen.	_____
Setting and location	5	State relevant aspects of the system(s) in which the decision(s) need(s) to be made.	_____
Study Perspective	6	Describe the perspective of the study and relate this to the costs being evaluated.	_____
Comparators	7	Describe the interventions or strategies being compared and state why they were chosen.	_____
Time horizon	8	State the time horizon(s) over which costs and consequences are being evaluated and say why appropriate.	_____
Discount rate	9	Report the choice of discount rate(s) used for costs and outcomes and say why appropriate.	_____
Choice of health outcomes	10	Describe what outcomes were used as the measure(s) of benefit in the evaluation and their relevance for the type of analysis performed.	_____
Measurement of effectiveness	11a	<i>Single study-based estimates:</i> Describe fully the design features of the single effectiveness study and why the single study was a sufficient source of clinical effectiveness data.	_____
	11b	<i>Synthesis-based estimates:</i> Describe fully the methods used for identification of included studies and synthesis of clinical effectiveness data.	_____
Measurement and valuation of preference based outcomes	12	If applicable, describe the population and methods used to elicit preferences for outcomes.	_____
Estimating resources and costs	13a	<i>Single study-based economic evaluation:</i> Describe approaches used to estimate resource use associated with the alternative interventions. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any adjustments made to approximate to opportunity costs.	_____
	13b	<i>Model-based economic evaluation:</i> Describe approaches and data sources used to estimate resource use associated with model health states. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any adjustments made to approximate to opportunity costs.	_____
Currency, price date, and conversion	14	Report the dates of the estimated resource quantities and unit costs. Describe methods for adjusting estimated unit costs to the year of reported costs if necessary. Describe methods for converting costs into a common currency base and the exchange rate.	_____
Choice of model	15	Describe and give reasons for the specific type of decision analytical model used. Providing a figure to show model structure is strongly recommended.	_____
Assumptions	16	Describe all structural or other assumptions underpinning the decision-analytical model.	_____
Analytical methods	17	Describe all analytical methods supporting the evaluation. This could include methods for dealing with skewed, missing, or censored data; extrapolation methods; methods for pooling data; approaches to validate or make adjustments (such as half cycle corrections) to a model; and methods for handling population heterogeneity and uncertainty.	_____
RESULTS			
Study parameters	18	Report the values, ranges, references, and, if used, probability distributions for all parameters. Report reasons or sources for distributions used to represent uncertainty where appropriate. Providing a table to show the input values is strongly recommended.	_____
Incremental costs and outcomes	19	For each intervention, report mean values for the main categories of estimated costs and outcomes of interest, as well as mean differences between the comparator groups. If applicable, report incremental cost-effectiveness ratios.	_____
Characterising uncertainty	20a	<i>Single study-based economic evaluation:</i> Describe the effects of sampling uncertainty for the estimated incremental cost and incremental effectiveness parameters, together with the impact Consolidated Health Economic Evaluation Reporting Standards – CHEERS Checklist 3 of methodological assumptions (such as discount rate, study perspective).	_____
	20b	<i>Model-based economic evaluation:</i> Describe the effects on the results of uncertainty for all input parameters, and uncertainty related to the structure of the model and assumptions.	_____
Characterising heterogeneity	21	If applicable, report differences in costs, outcomes, or costeffectiveness that can be explained by variations between subgroups of patients with different baseline characteristics or other observed variability in effects that are not reducible by more information.	_____
DISCUSSION			
Study findings, limitations, generalisability, and current knowledge	22	Summarise key study findings and describe how they support the conclusions reached. Discuss limitations and the generalisability of the findings and how the findings fit with current knowledge.	_____
OTHER INFORMATION			
Source of funding	23	Describe how the study was funded and the role of the funder in the identification, design, conduct, and reporting of the analysis. Describe other non-monetary sources of support.	_____
Conflicts of interest	24	Describe any potential for conflict of interest of study contributors in accordance with journal policy. In the absence of a journal policy, we recommend authors comply with International Committee of Medical Journal Editors recommendations.	_____

EQUATOR stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.

Section / Topic	Item no.	Checklist item
TITLE AND ABSTRACT		
Title	1	Provide as accurate and concise a description of the content of the article as possible.
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.
INTRODUCTION		
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.
METHODS		
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals).
Experimental procedures	7	A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out. For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.
Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used.
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.
RESULTS		
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing (this information can often be tabulated).
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%). b. If any animals or data were not included in the analysis, explain why.
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.
DISCUSSION		
Interpretation/ scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.
Generalisability/translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.

The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines were developed as part of an NC3Rs initiative to improve the design, analysis and reporting of research using animals – maximising information published and minimising unnecessary studies. The guidelines were published in the online journal PLOS Biology in June 2010 and are currently endorsed by scientific journals, major funding bodies and learned societies. More information can be found on www.nc3rs.org.uk/ARRIVE

EQUATOR stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.

Revised Standards for Quality Improvement Reporting Excellence (SQUIRE 2.0)

No	Item	Guide questions / description
TITLE AND ABSTRACT		
1	Title	Indicate that the manuscript concerns an initiative to improve healthcare (broadly defined to include the quality, safety, effectiveness, patient-centeredness, timeliness, cost, efficiency, and equity of healthcare)
2	Abstract	a. Provide adequate information to aid in searching and indexing b. Summarize all key information from various sections of the text using the abstract format of the intended publication or a structured summary such as: background, local problem, methods, interventions, results, conclusions
INTRODUCTION		
WHY DID YOU START?		
3	Problem Description	Nature and significance of the local problem
4	Available knowledge	Summary of what is currently known about the problem, including relevant previous studies
5	Rationale	Informal or formal frameworks, models, concepts, and/or theories used to explain the problem, any reasons or assumptions that were used to develop the intervention(s), and reasons why the intervention(s) was expected to work
6	Specific aims	Purpose of the project and of this report
METHODS		
WHAT DID YOU DO?		
7	Context	Contextual elements considered important at the outset of introducing the intervention(s)
8	Intervention(s)	a. Description of the intervention(s) in sufficient detail that others could reproduce it b. Specifics of the team involved in the work
9	Study of the Intervention(s)	a. Approach chosen for assessing the impact of the intervention(s) b. Approach used to establish whether the observed outcomes were due to the intervention(s)
10	Measures	a. Measures chosen for studying processes and outcomes of the intervention(s), including rationale for choosing them, their operational definitions, and their validity and reliability b. Description of the approach to the ongoing assessment of contextual elements that contributed to the success, failure, efficiency, and cost c. Methods employed for assessing completeness and accuracy of data
11	Analysis	a. Qualitative and quantitative methods used to draw inferences from the data b. Methods for understanding variation within the data, including the effects of time as a variable
12	Ethical Considerations	Ethical aspects of implementing and studying the intervention(s) and how they were addressed, including, but not limited to, formal ethics review and potential conflict(s) of interest
RESULTS		
WHAT DID YOU FIND?		
13	Results	a. Initial steps of the intervention(s) and their evolution over time (e.g., time-line diagram, flow chart, or table), including modifications made to the intervention during the project b. Details of the process measures and outcome c. Contextual elements that interacted with the intervention(s) d. Observed associations between outcomes, interventions, and relevant contextual elements e. Unintended consequences such as unexpected benefits, problems, failures, or costs associated with the intervention(s). f. Details about missing data
DISCUSSION		
WHAT DOES IT MEAN?		
14	Summary	a. Key findings, including relevance to the rationale and specific aims b. Particular strengths of the project
15	Interpretation	a. Nature of the association between the intervention(s) and the outcomes b. Comparison of results with findings from other publications c. Impact of the project on people and systems d. Reasons for any differences between observed and anticipated outcomes, including the influence of context e. Costs and strategic trade-offs, including opportunity costs
16	Limitations	a. Limits to the generalizability of the work b. Factors that might have limited internal validity such as confounding, bias, or imprecision in the design, methods, measurement, or analysis c. Efforts made to minimize and adjust for limitations
17	Conclusions	a. Usefulness of the work b. Sustainability c. Potential for spread to other contexts d. Implications for practice and for further study in the field e. Suggested next steps
OTHER INFORMATION		
18	Funding	Sources of funding that supported this work. Role, if any, of the funding organization in the design, implementation, interpretation, and reporting

EQUATOR stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.

Section / Topic	Item no.	Description
ADMINISTRATIVE INFORMATION		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
INTRODUCTION		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
METHODS: PARTICIPANTS, INTERVENTIONS, AND OUTCOMES		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size
METHODS: ASSIGNMENT OF INTERVENTIONS (FOR CONTROLLED TRIALS)		
Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial

METHODS: DATA COLLECTION, MANAGEMENT, AND ANALYSIS

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)

METHODS: MONITORING

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

ETHICS AND DISSEMINATION

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

APPENDICES

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

**It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.*

EQUATOR stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.



CONSORT 2010 Checklist of Information to include when Reporting a Randomised Trial*

Section / Topic	Item no.	Checklist item	Reported on page no.
TITLE AND ABSTRACT			
	1a	Identification as a randomised trial in the title	_____
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	_____
INTRODUCTION			
Background and objectives	2a	Scientific background and explanation of rationale	_____
	2b	Specific objectives or hypotheses	_____
METHODS			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	_____
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	_____
Participants	4a	Eligibility criteria for participants	_____
	4b	Settings and locations where the data were collected	_____
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	_____
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	_____
	6b	Any changes to trial outcomes after the trial commenced, with reasons	_____
Sample size	7a	How sample size was determined	_____
	7b	When applicable, explanation of any interim analyses and stopping guidelines	_____
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	_____
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	_____
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	_____
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	_____
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	_____
	11b	If relevant, description of the similarity of interventions	_____
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	_____
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	_____
RESULTS			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	_____
	13b	For each group, losses and exclusions after randomisation, together with reasons	_____
Recruitment	14a	Dates defining the periods of recruitment and follow-up	_____
	14b	Why the trial ended or was stopped	_____
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	_____
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	_____
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	_____
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	_____
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	_____
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	_____
DISCUSSION			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	_____
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	_____
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	_____
OTHER INFORMATION			
Registration	23	Registration number and name of trial registry	_____
Protocol	24	Where the full trial protocol can be accessed, if available	_____
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	_____

* We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

EQUATOR stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.

PJP Online Journal System

User Guide for Authors

Getting Started

- From the PJP website (<http://www.philippinejournalofpathology.org>), navigate to 'For Authors'. (add screenshot of PJP landing page, circle 'for authors' on right column).



Select 'FOR AUTHORS'.

Information For Authors

Interested in submitting to this journal? We recommend that you review the [About the Journal](#) page for the journal's section policies, as well as the [Author Guidelines](#). Authors need to [register](#) with the journal prior to submitting or, if already registered, can simply [log in](#) and begin the five-step process.

- Log in
- New user:
 - If you are a new user of the PJP website, please register by clicking the link 'Not a user, Register with this site'.

Login

Username

Password

Remember my username and password

- [Not a user? Register with this site](#)
- [Forgot your password?](#)

- Complete the online form then select 'Register'. A confirmation email with your username and password will be sent to your email address.

Register

Fill in this form to register with this site.

[Click here](#) if you are already registered with this or another journal on this site.

PROFILE

Username *

The username must contain only lowercase letters, numbers, and hyphens/underscores.

Password *

The password must be at least 6 characters.

Repeat password *

Salutation

First Name *

Middle Name

Last Name *

Initials Joan Alice Smith = JAS

Gender

Affiliation

(Your institution, e.g. "Simon Fraser University")

Signature

Email * [PRIVACY STATEMENT](#)

Confirm Email *

Confirmation Send me a confirmation email including my username and password

Register as Reader: Notified by email on publication of an issue of the journal.

Author: Able to submit items to the journal.

Reviewer: Willing to conduct peer review of submissions to the site.

Identify reviewing interests (substantive areas and research methods):

* Denotes required field

- Existing user:
 - Log in to your OJS account using the username and password from original registration.
 - If you have forgotten your log in details, please click the 'Forgot the password?' and an email will be sent to your registered email address.

Login

Username

Password

Remember my username and password

- [Not a user? Register with this site](#)
- [Forgot your password?](#)

The Submission Process

- To start the submission process, click 'New Submission'

Active Submissions

ACTIVE ARCHIVE

ID	MM-DD SUBMIT	SEC	AUTHORS	TITLE	STATUS
No Submissions					

[Start a New Submission](#)
[CLICK HERE](#) to go to step one of the five-step submission process.

Step 1: Starting the submission

- From the drop-down menu, please select the most appropriate section to describe your submission article type. If you are not sure what section to select, click '[About](#)' to find out more information.

Step 1. Starting the Submission

1. START 2. UPLOAD SUBMISSION 3. ENTER METADATA 4. UPLOAD SUPPLEMENTARY FILES 5. CONFIRMATION

Encountering difficulties? Contact [Melissa O. Tandoc, RN](#) for assistance (+6326373162).

JOURNAL SECTION

Select the appropriate section for this submission (see Sections and Policies in [About](#) the Journal).

Section *

Please select a section...

- Original articles
- Reviews
- Case Reports
- Feature articles
- Autopsy Vault
- Images in Pathology
- Brief Communications
- Editorials
- Letters to the Editor
- Special Announcements

SUBMISSION CHECK

Indicate that this sub (comments to the ed

Instructions t

Cover Letter


- Include cov
- Indicate in
- Indicate all the authors (complete names and affiliations); and
- Indicate in the letter the corresponding author and provide complete contact information (institutional mailing address, work telephone, fax number, and work e-mail address).

- Please ensure the items listed in the checklist are ready then tick each box.

SUBMISSION CHECKLIST	
Indicate that this submission is ready to be considered by this journal by checking off the following (comments to the editor can be added below).	
<input checked="" type="checkbox"/>	Instructions to Authors Review the manuscript submission guidelines.
<input checked="" type="checkbox"/>	Cover Letter <ul style="list-style-type: none">• Include cover letter as an attachment;• Indicate in the letter the complete title of the work;• Indicate all the authors (complete names and affiliations); and• Indicate in the letter the corresponding author and provide complete contact information (institutional mailing address, work telephone, fax number, and work e-mail address).
<input checked="" type="checkbox"/>	Author Form <ul style="list-style-type: none">• Ensure all authors have qualified as authors based on ICMJE authorship criteria;• Ensure all authors have read and agreed to the Certification;• Ensure all authors have read and provided disclosure of conflicts of interest; and• Submit a scanned copy of the fully accomplished form.
<input checked="" type="checkbox"/>	Patient Consent Form <ul style="list-style-type: none">• Submit a scanned copy of the fully accomplished form (if indicated); and• If all attempts have been made and consent form is not signed, state so in the Cover Letter.
<input checked="" type="checkbox"/>	Title Page <ul style="list-style-type: none">• Full names of the authors directly affiliated with the work (First name and Last name), highest educational attainment;• Name and location of not more than 1 institutional affiliation per author; and• If presented in a scientific forum or conference, provide a footnote indicating the name, location and date of presentation.
<input checked="" type="checkbox"/>	Abstract <ul style="list-style-type: none">• Provide an abstract conforming with the format;• Structured for Original Articles, Review Articles: Objective/s, Methodology, Results, Conclusion;• Unstructured for Case Reports and Feature Articles; and• Do not place cross references within the abstract.
<input checked="" type="checkbox"/>	Keywords Provide 3-6 keywords (listed in MeSH)
<input checked="" type="checkbox"/>	Content <ul style="list-style-type: none">• Provide text/content in IMRAD format (Introduction, Methodology, Results and Discussion, Conclusion);• Make sure all abbreviations are spelled out once (the first time they are mentioned in the text) followed by the abbreviation enclosed in parentheses; the same abbreviation may then be used subsequently;

- Read the 'Copyright Notice' and add comments to the editor (optional). Select 'Save and continue'.

COPYRIGHT NOTICE



The Philippine Journal of Pathology is licensed under a [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License](http://creativecommons.org/licenses/by-nc-sa/4.0/). Based on works made open access at <http://philippinejournalofpathology.org>

The authors agree to the terms of this Copyright Notice, which will apply to this submission if and when it is published by this journal (comments to the editor can be added below).

JOURNAL'S PRIVACY STATEMENT

The names and email addresses entered in this journal site will be used exclusively for the stated purposes of this journal and will not be made available for any other purpose or to any other party.

COMMENTS FOR THE EDITOR

Enter text (optional)

✂ 📄 📁 | **B** *I* U | ☰ ☷ | 📧 🌐 🌐 HTML 🖨

Save and continue
Cancel

* Denotes required field

Step 2: Uploading the Submission

- Please follow the instructions on this page to upload your file, then select 'Save and continue'. This is where you upload the **manuscript only**. (You will be asked to upload other required documents at Step 4).

Step 2. Uploading the Submission

1. START **2. UPLOAD SUBMISSION** 3. ENTER METADATA 4. UPLOAD SUPPLEMENTARY FILES 5. CONFIRMATION

To upload a manuscript to this journal, complete the following steps.

1. On this page, click Browse (or Choose File) which opens a Choose File window for locating the file on the hard drive of your computer.
2. Locate the file you wish to submit and highlight it.
3. Click Open on the Choose File window, which places the name of the file on this page.
4. Click Upload on this page, which uploads the file from the computer to the journal's web site and renames it following the journal's conventions.
5. Once the submission is uploaded, click Save and Continue at the bottom of this page.

Encountering difficulties? Contact [Melissa O. Tandoc, RN](mailto:Melissa.O.Tandoc@pjpath.org) for assistance (+6326373162).

SUBMISSION FILE

No submission file uploaded.

Upload submission file

Choose File No file chosen

Upload
ENSURING A BLIND REVIEW

Save and continue
Cancel

Step 3: Entering the Submission's Metadata

- Complete author(s)'s information as much as you can. Fields marked with * are mandatory. If you have more than one author for your submission, click 'Add author' for each of these.

Step 3. Entering the Submission's Metadata

1. START 2. UPLOAD SUBMISSION 3. ENTER METADATA 4. UPLOAD SUPPLEMENTARY FILES 5. CONFIRMATION

AUTHORS

First Name *

Middle Name

Last Name *

Email *

ORCID iD

ORCID iDs can only be assigned by [the ORCID Registry](#). You must conform to their standards for expressing ORCID iDs, and include the full URI (eg. <http://orcid.org/0000-0002-1825-0097>).

URL

Affiliation

(Your institution, e.g. "Simon Fraser University")

Country

Competing interests [CI POLICY](#)

Bio Statement (E.g., department and rank)

- Please note the system will automatically select the first-recorded author as the principal contact for editorial correspondence. If you want to change this, choose the following option listed at the bottom of the author details for the author you want to be the principal contact.

Reorder authors to appear in the order they will be listed on publication.

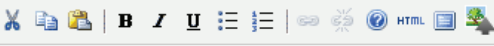
Principal contact for editorial correspondence.

- Complete 'Title', 'Abstract', 'Indexing' and 'Supporting Agencies' of your submission. Select 'Save and continue'. These can be pasted from a word document.

TITLE AND ABSTRACT

Title *

Abstract *



INDEXING

Provide terms for indexing the submission; separate terms with a semi-colon (term1; term2; term3).

Academic discipline and sub-disciplines

Subject classification

Keywords

Geo-spatial coverage

Chronological or historical coverage

Research sample characteristics

Type, method or approach

Language
English=en; French=fr; Spanish=es. [Additional codes.](#)

CONTRIBUTORS AND SUPPORTING AGENCIES

Identify agencies (a person, an organization, or a service) that made contributions to the content or provided funding or support for the work presented in this submission. Separate them with a semi-colon (e.g. John Doe, Metro University; Master University, Department of Computer Science).

Agencies

REFERENCES

Provide a formatted list of references for works cited in this submission. Please separate individual references with a blank line.

References

Step 4. Uploading Supplementary Files

1. START 2. UPLOAD SUBMISSION 3. ENTER METADATA 4. UPLOAD SUPPLEMENTARY FILES 5. CONFIRMATION

This optional step allows Supplementary Files to be added to a submission. The files, which can be in any format, might include (a) research instruments, (b) data sets, which comply with the terms of the study's research ethics review, (c) sources that otherwise would be unavailable to readers, (d) figures and tables that cannot be integrated into the text itself, or other materials that add to the contribution of the work.

ID	TITLE	ORIGINAL FILE NAME	DATE UPLOADED	ACTION
<i>No supplementary files have been added to this submission.</i>				

Upload supplementary file No file chosen [ENSURING A BLIND REVIEW](#)

SUPPLEMENTARY FILE

File Name [3-5-1-SP.docx](#)
 Original file name PJP Author Submission Checklist v.01.2015.docx
 File Size 175KB
 Date uploaded 2016-02-05 09:50 PM

Present file to reviewers (without metadata), as it will not compromise blind review.

Replace file No file chosen

* Denotes required field

- Once all files are uploaded, if you need to you can edit or delete them by clicking the links. To continue to next step, select 'Save and continue'.

Step 4. Uploading Supplementary Files

1. START 2. UPLOAD SUBMISSION 3. ENTER METADATA 4. UPLOAD SUPPLEMENTARY FILES 5. CONFIRMATION

This optional step allows Supplementary Files to be added to a submission. The files, which can be in any format, might include (a) research instruments, (b) data sets, which comply with the terms of the study's research ethics review, (c) sources that otherwise would be unavailable to readers, (d) figures and tables that cannot be integrated into the text itself, or other materials that add to the contribution of the work.

ID	TITLE	ORIGINAL FILE NAME	DATE UPLOADED	ACTION
1	submission checklist	PJP Author Submission Checklist v.01.2015.docx	02-05	EDIT DELETE

Upload supplementary file No file chosen [ENSURING A BLIND REVIEW](#)

Step 5: Confirming the Submission

- Please check that all required files have been uploaded and are listed on the 'File Summary'. Select 'Finish Submission' to submit your manuscript.

Step 5. Confirming the Submission

1. START 2. UPLOAD SUBMISSION 3. ENTER METADATA 4. UPLOAD SUPPLEMENTARY FILES 5. CONFIRMATION

To submit your manuscript to Philippine Journal of Pathology click Finish Submission. The submission's principal contact will receive an acknowledgement by email and will be able to view the submission's progress through the editorial process by logging in to the journal web site. Thank you for your interest in publishing with Philippine Journal of Pathology.

FILE SUMMARY

ID	ORIGINAL FILE NAME	TYPE	FILE SIZE	DATE UPLOADED
4	TEST SUBMISSION.DOCX	Submission File	329KB	02-05
5	PJP AUTHOR SUBMISSION CHECKLIST V.01.2015.DOCX	Supplementary File	175KB	02-05

- The principle contact of the submission will then receive an acknowledgement email.

Dear xxx:

Thank you for submitting the manuscript, "xxxxxx" to Philippine Journal of Pathology. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Manuscript URL:
<http://philippinejournalofpathology.org/index.php/PJP/.....>

Username: xxxxxx

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Amado O. Tandoc III, MD, DPSP
 Philippine Journal of Pathology

Philippine Journal of Pathology
<http://philippinejournalofpathology.org>

Status of Submission

- During the review and editing process, the principal contact can log in to the PJP website to check the status of the submission. Follow the log in instructions on Page (?) and then click the 'Active' tab.

Active Submissions

ACTIVE ARCHIVE

ID	MM-DD SUBMIT	SEC	AUTHORS	TITLE	STATUS
3	02-05	ORI	Smith	TEST SUBMISSION	IN EDITING

1 - 1 of 1 Items

[Start a New Submission](#)
[CLICK HERE](#) to go to step one of the five-step submission process.

- You will receive an email from the Editor-in-Chief after the peer review process which will indicate the outcome of the review and provide the reviewer's comments.

Dear xxx:

Your manuscript "xxxxxx" submitted to Philippine Journal of Pathology has undergone peer review. The manuscript has been accepted subject to major / minor revisions.

Please find attached the comments from the peer reviewers. Please take the following actions:

1. Review the manuscript according to the reviewers' comments using the track changes facility in Word.
2. Provide a response to each of the reviewers' comments in a separate Word document.
3. Upload both the revised manuscript and the response to the reviewers' comments.

The due date for these revisions is Friday, xx month. If you have any queries regarding this please contact me.

Thank you and kind regards,

Amado O. Tandoc III, MD, DPSP
Philippine Journal of Pathology

Philippine Journal of Pathology
<http://philippinejournalofpathology.org>

- Please make the required changes to manuscript and in a **separate** file provide responses to each of the reviewer's comments.
- These can then be uploaded onto the system.
 - Login (see instructions on Page (?))
 - Click 'Active' tab.
 - Click on your submission listed below 'TITLE'.
 - Select the 'Review' tab.
 - In the 'Editor Decision' section at the bottom of the page, you can upload your revised manuscript and responses to reviewer's comments.
 - Once you have uploaded your files, you can view them at the 'Author's Version' section.

PJP PEER REVIEWERS FOR 2019

Jose Jasper L. Andal, MD

St. Luke's Medical Center – Quezon City and Global City, Philippines

Mark Angelo C. Ang, MD, MoS

College of Medicine, University of the Philippines Manila

Ronald N. Araneta, MD

Hartford Hospital/Connecticut Children's Medical Center, USA

Elizabeth Y. Arcellana-Nuqui, MD

The Medical City, Pasig City, Philippines

Randell S. Arias, MD

Zamboanga City Medical Center, Philippines

Ruth Asirvatham, MD

University of Florida Health Pathology Laboratories, USA

Florido A. Atibagos Jr., MD

Philippine Heart Center

Jose Maria C. Avila, MD

University of the Philippines-Philippine General Hospital

Marife J. Bonifacio, MD

St. Luke's Medical Center – Quezon City and Global City, Philippines

Marie Christine F. Bernardo, MD

St. Luke's Medical Center – Quezon City and Global City, Philippines

Jose M. Carnate Jr., MD

College of Medicine, University of the Philippines Manila

Chrystalle Katte T. Carreon, MD

University of Pennsylvania/ The Children's Hospital of Philadelphia, USA

Ann Margaret V. Chang, MD

St. Luke's Medical Center – Quezon City and Global City, Philippines

Ma. Rizalina F. Chua, MT (ASCP)

Metropolitan Medical Center, Philippines

Leonides M. De Vera, MD

St. Louis University, Baguio City, Philippines

Arvin C. Faundo, MD

St. Luke's Medical Center – Global City, Philippines

Arnold Joseph M. Fernandez, MD

National Kidney and Transplant Institute, Quezon City, Philippines

Elizabeth M. Gillies, MD

University of Oklahoma Health Sciences Center, USA

Jan A. Graw, MD

Charite University, Berlin, Germany

Yael K. Heher, MD, MPH

Beth Israel Deaconess Medical Center, USA

Lisa Maria Hillen

Maastricht Universitair Medisch Centrum, Maastricht, The Netherlands

Marianette T. Inobaya, MSPH, PhD

Research Institute for Tropical Medicine, Alabang, Muntinlupa City, Philippines

Jundelle Romulo K. Jaliq, RN, MSPH, Biostatistics (c)

Veterans Memorial Medical Center, Philippines

Guia Elena Imelda R. Ladrera, MD

Department of Thoracic Oncology Lung Center of the Philippines

Evelina N. Lagamayo, MD

University of Santo Tomas, Manila, Philippines

Edna May Lasap-Go, MD

University of the Philippines-Philippine General Hospital

Catherine Jessica M. Lazaro, MD

The Medical City, Pasig City, Philippines

Frederick R. Llanera, MD

Philippine Heart Center

Raymundo W. Lo, MD

St. Luke's Medical Center – Quezon City,
Philippines

Manuelito A. Madrid, MD

Philippine Children's Medical Center,
Quezon City, Philippines

Herbert Manaois, MD

Delos Santos Medical Center, Quezon City,
Philippines

Paulo Giovanni L. Mendoza, MD

Makati Medical Center, Philippines

Prof. Emeritus Florinia E. Merca, PhD

University of the Philippines-Los Baños, Philippines

Edelwisa S. Mercado, PhD

Research Institute for Tropical Medicine,
Alabang, Muntinlupa City, Philippines

Miguel Martin N. Moreno II, MD, CBP, CIFBA

BioRisk Association of the Philippines 2015, Inc.

Francis G. Moria, MD

College of Medicine, St. Luke's Medical Center,
Philippines

Marissa A. Orillaza, MD

Institutional Ethics Review Board
Philippine Heart Center

Minnie Jane A. Pineda, MD

Philippine Heart Center

Glenda Lyn Y. Pua, MD

St. Luke's Medical Center – Quezon City and
Global City

Susan P. Quiaot, MD

Quezon City General Hospital, Philippines

Bernadette G. Reyna Asuncion, MD

National University of Singapore

Paula Andrea Rodriguez Urrego, MD

University Hospital Fundación Santa Fe de
Bogotá, Colombia

Ivy A. Rosales, MD

Massachusetts General Hospital, USA

Treah May Suacillo-Sayo, MD

Department of Pathology
Lung Center of the Philippines

Ava Kristy D. Sy, RMT, MSc

Research Institute for Tropical Medicine,
Alabang, Muntinlupa City, Philippines

Pedrito Y. Tagayuna, MD

The Medical City, Pasig City, Philippines

Linda D. Tamesis, MD

Far Eastern University-Nicanor Reyes Medical
Foundation Institute of Medicine

Carmela D. Tan, MD

Cleveland Clinic, USA

Amado O. Tandoc III, MD

Research Institute for Tropical Medicine,
Alabang, Muntinlupa City, Philippines

Enrico D. Tangco, MD

The Medical City, Pasig City, Philippines

Rogelio V. Tangco, MD

National Kidney and Transplant Institute,
Quezon City, Philippines

Merva Soluk Tekkeşin, DDS, PhD

Institute of Oncology, Istanbul University, Turkey

Felipe S. Templo Jr., MD

Philippine Heart Center

Edith S. Tria, MD

SLH Ministry of Health, Manila, Philippines

Anaclea P. Valdez, MD

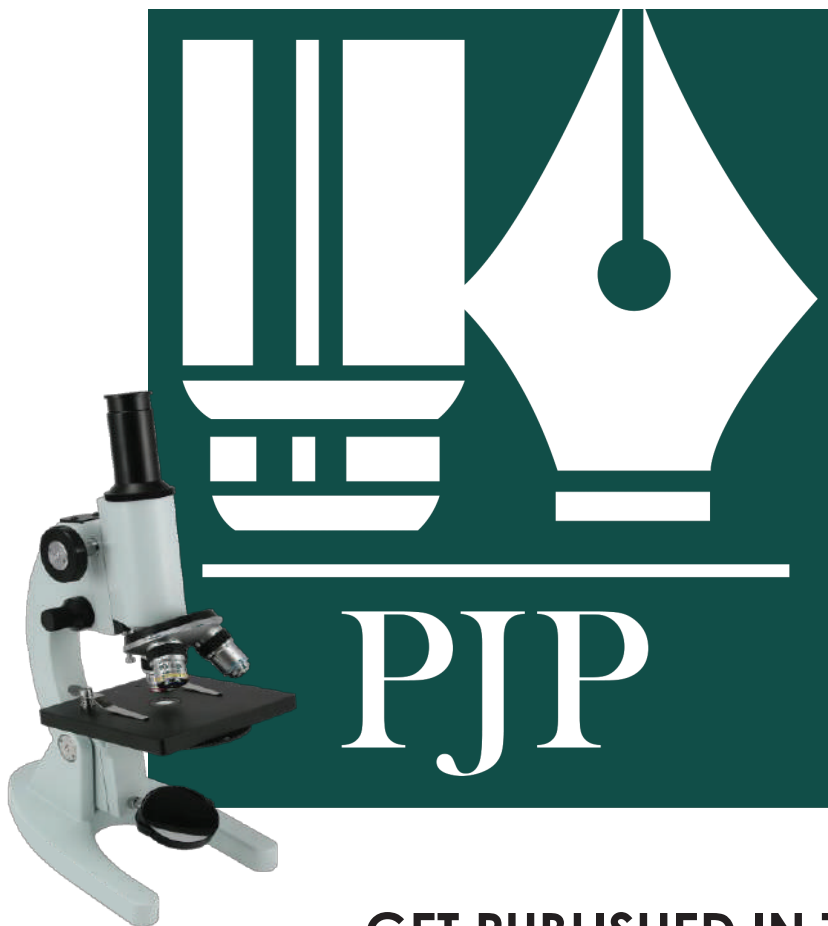
Daniel O. Mercado Medical Center, Tanauan,
Batangas, Philippines

+Demetrio L. Valle Jr., MD**Rowen T. Yolo, MD**

University of Santo Tomas, Manila, Philippines

Januario D. Veloso, MD

National Kidney and Transplant Institute,
Quezon City, Philippines



- *Online and Printed*
- *100% Open Access*
- *Peer Reviewed*
- *Continuous Publication model*
- *No Author Processing Fees*
- *Streamlined process from submission to publication*
- *24/7 web-based technical support*

GET PUBLISHED IN THE **NEW** PHILIPPINE JOURNAL OF PATHOLOGY!

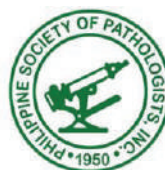
The Philippine Journal of Pathology (PJP) is an open-access, peer-reviewed, English language, medical science journal published by the Philippine Society of Pathologists, Inc. It shall serve as the official platform for publication of high quality original articles, case reports or series, feature articles, and editorials covering topics on clinical and anatomic pathology, laboratory medicine and medical technology, diagnostics, laboratory biosafety and biosecurity, as well as laboratory quality assurance.

The journal's primary target audience are laboratorians, diagnosticians, laboratory managers, pathologists, medical technologists, and all other medical and scientific disciplines interfacing with the laboratory. For instructions and more information, visit our Official Website at:

<http://philippinejournalofpathology.org>



PHILIPPINE JOURNAL OF
PATHOLOGY



Philippine Society
of Pathologists, Inc.



Philippine Journal of Pathology

Committee on Publications | Philippine Society of Pathologists, Inc.

E-mail: philippinepathologyjournal@gmail.com

Website: <http://philippinejournalofpathology.org>