

## The National External Quality Assessment Scheme for Diagnostic Medical Parasitology in the Philippines, 2009–2015

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### ABSTRACT

**Background.** The Research Institute for Tropical Medicine (RITM)–National Reference Laboratory (NRL) for Malaria and Other Parasites, mandated by the Department of Health–Philippines (DOH), administers an annual Proficiency Test (PT) in diagnostic medical parasitology to clinical laboratories throughout the Philippines through the National External Quality Assessment Scheme (NEQAS). The PT in Parasitology aims to monitor and evaluate the capability of Philippine laboratories in the identification of blood and intestinal parasites, and the estimation of malaria parasite density in malaria-infected blood films. As of 2018, participation in the NEQAS is an annual requirement by the Department of Health–Health Facilities and Services Regulatory Bureau (DOH-HFSRB) for each clinical laboratory to obtain a license to operate.

**Objective.** This report aims to summarize the results of the PT for Parasitology and assess the performance of participating laboratories in malaria and fecal parasite microscopy from 2009 to 2015.

**Methodology.** RITM–NRL oriented clinical laboratories in the NEQAS in 2008. Laboratories submitted their accomplished enrolment forms to RITM–NRL and paid fees to enroll in the PT in 2009 to 2015. Participating laboratories identified the species of malaria in blood films and the parasite/s in formalin-preserved fecal specimens. Estimation of parasite density in malaria blood films was performed as well.

**Results.** One thousand five hundred forty (1,540) laboratories participated from 2009 to 2015. Mean and median scores in all seven years were below the cut-off score of 80. *Schistosoma japonicum* was the most difficult to identify with only 7.7% of laboratories having correct identification result. Majority of participants from 2010 to 2014 gave malaria parasite density estimates outside the acceptable range.

**Conclusion.** Most participating laboratories performed poorly in the proficiency tests over the last seven years. Training and refresher courses for laboratorians are recommended in order to address the poor performance in the laboratory diagnosis of parasitic infections, especially the endemic and uncommon ones, in the country

*Key words: laboratory proficiency testing, external quality assessment, medical parasitology, malaria, schistosomiasis, helminthiasis, protozoan infections*

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### INTRODUCTION

Parasitic infections caused by a diverse range of helminths and protozoans affect millions of people living in the Philippines. Around 25 million Filipinos are at risk of soil transmitted helminthiasis (STH), with a prevalence rate of six to 97 percent among Filipino children aged six to 12.<sup>1</sup> Also, 12 million are at risk of schistosomiasis, with 2.5 million Filipinos directly exposed to the infection.<sup>1</sup> In addition, around 33 million Filipinos are at risk of malaria.<sup>1,2</sup> Control and elimination of these diseases depend on accurate and reliable diagnosis, of which diagnostic medical parasitology laboratories are responsible. In the Philippines, medical parasitology laboratories typically employ microscopy to demonstrate parasites in stool, blood, or other specimens.<sup>3</sup> In order to ensure accurate and reliable diagnosis, laboratories must carry out quality assurance through a quality



management system, which encompasses documentation, implementation of standard operating procedures (SOPs), practice of quality control (QC), and participation in external quality assessment schemes (EQAS).<sup>4</sup>

The National External Quality Assessment Scheme (NEQAS) for Parasitology is one of the measures by the Department of Health (DOH) to assess the reliability of laboratory diagnosis and maintain quality assurance of licensed medical parasitology laboratories in the country. DOH, through the Department Order No. 393-E s. 2000, designated the Research Institute for Tropical Medicine (RITM) as the National Reference Laboratory (NRL) for Malaria and other Parasites,<sup>5</sup> which maintains DOH-approved external quality assessment program by administering annual proficiency testing (PT) to diagnostic medical laboratories through NEQAS. The DOH Administrative Order No. 2007-0027 and Memorandum No. 2009-0086, required every diagnostic medical laboratory throughout the country to participate in the NEQAS,<sup>6-8</sup> which allows each to obtain a license to operate (LTO) from the DOH Health Facilities and Services Regulatory Bureau (HFSRB, formerly Bureau of Health Facilities and Services).

This paper reports the results of the proficiency tests for diagnostic medical parasitology administered to participating laboratories in 2009–2015. The proficiency test was conducted to assess the capability of laboratorians to identify and quantify malaria parasite density in malaria blood films; and identify species of parasitic helminths and protozoans in formalin-preserved fecal suspensions.

## METHODOLOGY

### Laboratory participation

RITM–NRL conducted orientation seminars on NEQAS implementation to diagnostic medical parasitology laboratories in 2008 and 2009. Laboratories required by DOH–HFSRB to participate in the annual proficiency test submitted their enrolment forms and paid fees before the scheduled testing event.

### Preparation of blood and fecal specimens

The parasites used as analytes were obtained from blood or stool of infected patients with adequate number of parasites exhibiting characteristic morphological features. Malaria parasite-infected blood samples were collected from consenting individuals from malaria endemic areas in Palawan province. Thick and thin blood films were prepared for malaria microscopy.<sup>9</sup>

For the identification of intestinal helminths and protozoans, infected stools were collected and examined through Direct Fecal Smear (DFS), Kato-Katz technique<sup>10,11</sup> and Formalin-Ether Concentration Technique (FECT).<sup>10,12,13</sup> Samples were preserved in 10% formalin<sup>3</sup> for storage and validated with DFS and FECT prior to preparation for packaging. Validated samples were pooled and the resulting concoction was validated with DFS and FECT. Around 500  $\mu$ L of the concoction was transferred to each polypropylene vial.

### Quality control and validation of parasite species

Two (2) trained microscopists from RITM–NRL for Malaria and other Parasites performed quality control of blood films and formalin-preserved fecal samples in vials; validation of these analytes was done through blinded crosschecking. All blood films were ensured to be stained properly and to contain consistent malaria parasite species identity and parasite density. Likewise, fecal samples were ensured to contain parasites with consistent species identity and with intact and recognizable morphological features. In addition, the identities of *Plasmodium* species were confirmed through nested polymerase chain reaction and agarose gel electrophoresis.<sup>14</sup> In cases of discrepancies between the results of the two blinded examinations, a third microscopist who is a senior staff of the NRL would re-examine and validate the results.

### Packaging of analytes

Analytes sent to participating laboratories were packed based on the international standard of transporting biohazard materials (IATA).<sup>15</sup> Microscope slides were secured in plastic slide mailers. Each polypropylene vial was labeled and sealed with Parafilm M® (Bemis Co., Inc., Oshkosh, Wisconsin, USA), wrapped in a paper towel, and placed inside a 100 mm  $\times$  115 mm resealable propylene resin bag. The vials and slide mailers were encased in 600 mL polypropylene canister (Philtop Industries Inc., Valenzuela City, Metro Manila) and placed inside a 120 mm  $\times$  115 mm  $\times$  190 mm corrugated box (Thousand Oaks Packaging Corp., Parañaque City, Metro Manila) with necessary attachments and labels. The package included proficiency testing guidelines and answer sheets.

### Proficiency testing and scoring

During the testing event, participants received their package and were asked to identify the parasite or parasites in the fecal sample and in stained thick and thin blood films by microscopy. In 2010 to 2014, malaria parasite counting was included in the proficiency test. Each participant was asked to estimate the malaria parasite density by performing parasite counting on the thick and thin blood films. Results were submitted to RITM–NRL within 15-working days after the package had been received in the laboratory.

In 2009, the percentage score for parasite identification was calculated with a right-minus-wrong scheme which was modified to the percentage method in 2010. Parasite identification was calculated by determining the number of correctly identified organisms over the number of organisms in the actual analyte and additional organisms answered by the participant but excluded in the list of actual organisms in the analyte. For malaria parasite counting, the percentage score was calculated by determining the counts within  $\pm$  20% of the actual parasite count over the number of analytes given.

### Statistical analysis

Graphs were generated using Matplotlib version 2.0.0 pyplot module in Python<sup>16</sup> and statistical analyses were done using SciPy version 0.19.0 scipy.stats module.<sup>17</sup> Kruskal–Wallis one-way analysis of variance was performed to determine the differences between annual proficiency test scores.

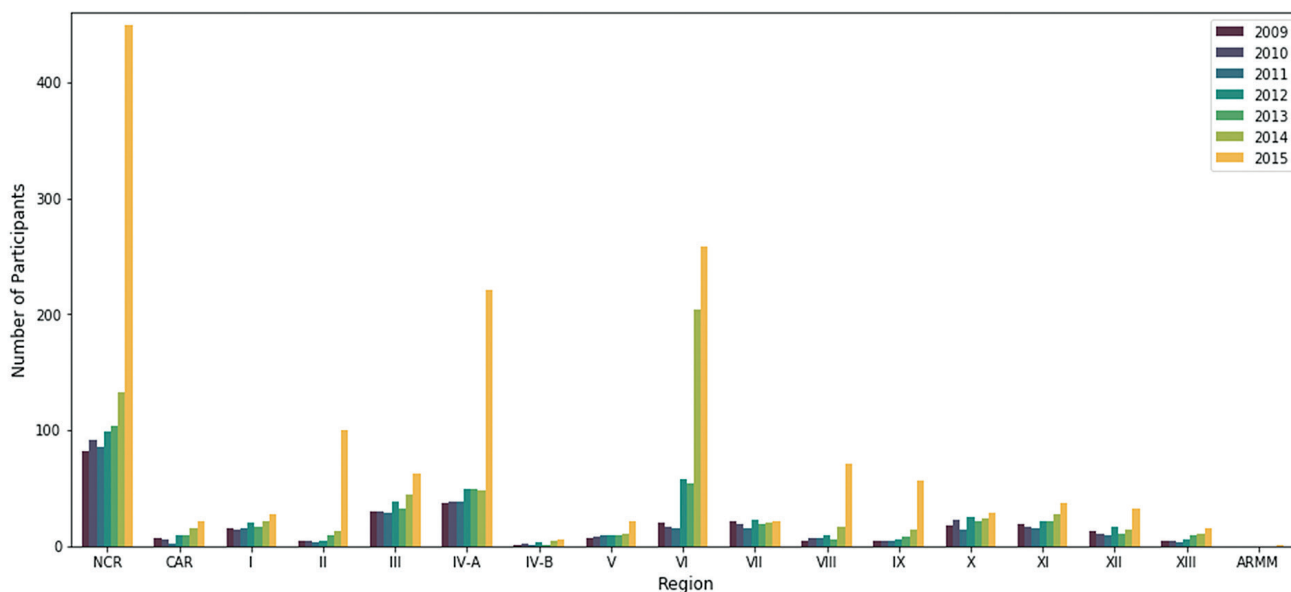


Figure 1. Number of participating laboratories from different regions in the Philippines in the PT for parasitology, 2009–2015.

RESULTS AND DISCUSSION

A total of 1,540 laboratories participated in the PT for parasitology in 2009-2015, of which 82% (1263/1540) were private and 18% (277/1540) were government facilities (Figure 1). In terms of laboratory type, the total number of participants is composed of 30.3% (467/1540) tertiary, 59.8% (921/1540) secondary, and 9.9% (152/1540) primary level clinical laboratories. National Capital Region holds the highest number of participating laboratories within the 7-year period (463/1540); followed by Western Visayas (Region VI; 284/1540); and CALABARZON (Region IV-A; 226/1540). Notably, one laboratory in the Autonomous Region in Muslim Mindanao or ARMM participated, for the first time, in the PT during 2015.

Scores ranged from zero to 100 in all years, except in 2009 where scores ranged from -125 to 100 because of the right-minus-wrong grading scheme (Figure 2). The mean scores and sample standard deviation per year were: 66.7 (41.0) in 2009, 70.3 (23.5) in 2010, 54.0 (24.1) in 2011, 52.7 (27.1) in 2012, 66.0 (23.6) in 2013, 60.3 (23.5) in 2014, and 61.9 (21.2) in 2015. Annual median scores were 75.0 in 2009 and 2010, 50.0 in 2011 and 2012, 66.7 in 2013, 62.5 in 2014, and 62.5 in 2015 (Figure 3). Mean and median scores in all years were below the cut-off score of 80.0. Annual PT scores were significantly different from each other ( $H = 192.14$ ;  $p$ -value =  $8.93 \times 10^{-39}$ ) based on the Kruskal–Wallis test.

Within the 7-year period, participants found the blood fluke, *Schistosoma japonicum*, to be the most difficult to identify—only 7.7% (15/196) of the laboratories that received the analyte identified it correctly. Following the schistosome was the intestinal protozoan, *Blastocystis hominis*, which 38.7% (592/1528); the pinworm, *Enterobius vermicularis* (42.3%; 202/478); the commensal and nonpathogenic amoebae, *Endolimax nana* (42.3%; 85/201) and *Entamoeba coli* (50.2%; 821/1635) (Table 1).

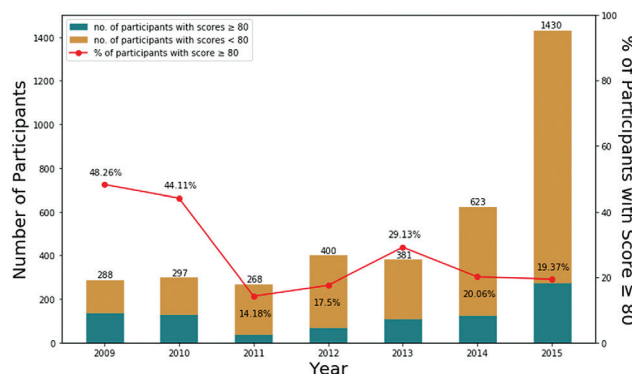


Figure 2. Annual number of participants and proportion of those who obtained scores of 80 and above in parasite identification, in the 2009–2015 PT for parasitology.

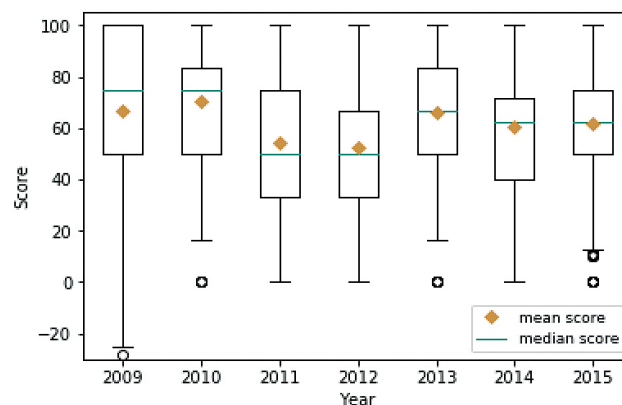


Figure 3. Annual distribution, mean, and median of scores in the 2009-2015 PT for parasitology.

**Table 1.** Parasite species used as analytes in the 2009–2015 PT for Parasitology and frequency of correct identification

Organism and Authority	Total Frequency	Frequency of Correct ID and Percentage
<i>Schistosoma japonicum</i> Katsurada, 1904	196	15 (7.7%)
<i>Blastocystis hominis</i> Brumpt, 1912	1528	592 (38.7%)
<i>Enterobius vermicularis</i> Linnaeus, 1758	478	202 (42.3%)
<i>Endolimax nana</i> Wenyon & O'Connor, 1917	201	85 (42.3%)
<i>Entamoeba coli</i> (Grassi, 1879), Casagrandi & Barbagallo, 1895	1635	821 (50.2%)
<i>Plasmodium vivax</i> Grassi & Feletti, 1890	1004	634 (63.2%)
<i>Plasmodium falciparum</i> Welch, 1897	5388	3598 (66.8%)
Hookworm		
<i>Ancylostoma duodenale</i> (Dubini, 1843)	1222	836 (68.4%)
<i>Necator americanus</i> (Stiles, 1902)		
<i>Taenia</i> spp. Linnaeus, 1758	1085	752 (69.3%)
<i>Giardia lamblia</i> (Lamb, 1859) Kofoid & Christiansen, 1915*	1621	1159 (71.5%)
<i>Entamoeba histolytica</i> Schaudinn, 1903	589	454 (77.1%)
<i>Trichuris trichiura</i> Linnaeus 1771	962	843 (87.6%)
<i>Ascaris lumbricoides</i> Linnaeus, 1758	1168	1033 (88.4%)
<i>Hymenolepis diminuta</i> Rudolphi, 1819	1	1 (100.0%)
<i>Plasmodium malariae</i> Feletti & Grassi, 1889	2	2 (100.0%)

\* also known as *Giardia duodenalis* Stiles, 1902 and more recently as *Giardia intestinalis* Kulda & Nohýnková, 1995

*S. japonicum* eggs are small with typically round to oval shape measuring 70–100  $\mu\text{m}$  by 55–65  $\mu\text{m}$ . Each egg contains a miracidium enclosed in a thin transparent shell with a small lateral spine, which usually is not clearly visible and often obscured by fecal debris adhering to the shell or by wrong orientation.<sup>10</sup> Moreover, detection of *S. japonicum* eggs is enhanced by concentration of formalin-preserved fecal sample by FECT.<sup>18</sup>

The cyst-like form of the stramenopile *B. hominis* is generally round and measures around 6–40  $\mu\text{m}$ . This form has a large central body that appears to be a large vacuole with a thin band, surrounded by multiple nuclei. To maximally recover the cyst-like forms, fecal samples must be concentrated through FECT before examining through a microscope.<sup>19</sup> Lysis of trophozoites and central body forms after exposure to water prior to fixation yield false-negative results<sup>20</sup>. In addition, concentrated wet mount preparations often fail to display the distinguishable features of the parasite so smears permanently stained with trichrome or iron hematoxylin are preferably prepared.<sup>10</sup>

*E. vermicularis* eggs are typically recovered from the perianal area using a swab or using the “sticky tape” method, where a clear adhesive tape is put on the perianal area in the morning before bathing or defecation. The eggs are elongated, measuring 50–60  $\mu\text{m}$  in length by 20–32  $\mu\text{m}$  in width; and are asymmetrical, with one side flattened and the other side convex. They are colorless and the shells are thin.<sup>19</sup>

*E. coli* and *E. nana* are nonpathogenic amoebae but they can colonize the intestine when a person ingests mature cysts in fecally contaminated food and water. The *E. coli* trophozoite, which measures around 15–50  $\mu\text{m}$  in diameter, contains a single nucleus with large karyosome, and coarse and irregular peripheral nuclear chromatin. Its cytoplasm appears to be coarsely granular and often vacuolated, and sometimes includes bacteria, yeasts, and other materials but not red blood cells. On the other hand, the *E. nana* trophozoite, which measures around 6–12  $\mu\text{m}$  in diameter, has a nucleus with large, irregular karyosome and does not have a peripheral nuclear chromatin. Its cytoplasm appears to be granular and

vacuolated and sometimes contains bacteria but no red blood cells. *E. coli* cysts are usually spherical with 10–35  $\mu\text{m}$  in diameter. Each mature cyst usually contains five or more nuclei while immature cysts have two to four nuclei. Each of the nuclei contains large, discrete, and usually eccentric karyosomal chromatin and coarsely granulated peripheral chromatin. Additionally, the cytoplasm of immature cysts usually appears to be diffuse and contains glycogen mass, which stains reddish brown with iodine, and chromatoid bodies with splintered ends. In contrast, *E. nana* cysts, which are also usually spherical but are smaller, measures around 5–10  $\mu\text{m}$  in diameter. Each cyst typically contains four nuclei with large, blot-like, and usually central karyosomal chromatin and no peripheral chromatin. Its cytoplasm usually contains diffuse glycogen and occasionally concentrated glycogen mass in young cysts.<sup>10,19</sup>

Artifacts in the stool such as fungal spore, algal spore, mite egg, plant cell, and pollen grain may be mistaken as helminth eggs. In addition, epithelial cells and white blood cells in stool may be mistaken as amoebae. Moreover, Howell–Jolly bodies and nucleated red blood cells in blood films may be mistaken as malaria parasites<sup>19</sup>. Laboratorians performing diagnostic parasitology should be able to recognize details that differentiate parasite components and non-parasite artifacts.

In 2009, participants were asked to perform malaria parasite count in malaria positive blood films merely as an initial survey to assess the capability of laboratories to determine the parasite density in blood but scoring was not done. Scores in malaria parasite count in 2010 to 2014 were below 50% owing to the majority of participating laboratories giving malaria parasite density estimates outside the acceptable range (Table 2). As a result, NEQAS removed malaria parasite counting in the proficiency test in 2015 since majority of laboratories were incapable of estimating malaria parasite density in blood films.

Participation in 2015 (1430 participants) rose to 230% from that in 2014 (623 participants). In addition, the number of participants in 2015 comprised around 93% (1430/1540) of all participating laboratories throughout seven years. Overall, only 19.4% (277/1430) obtained a

**Table 2.** Number of blood films with acceptable estimated malaria parasite density

Year	No. of Slides	No. and Percentage of Acceptable Results
2011	254	76 (28.5%)
2012	387	53 (13.3%)
2013	366	119 (31.2%)
2014	606	251 (40.3%)

**Table 3.** Passing rate of participating laboratories per region

Region	Percentage of Passers in 2015
Davao Region (XI)	45.9% (17/37)
Central Luzon (III)	42.9% (27/63)
Caraga (XIII)	40.0% (6/15)
CAR	38.1% (8/21)
Northern Mindanao (X)	37.9% (11/29)
Soccsksargen (XII)	31.3% (10/32)
Central Visayas (VII)	27.3% (6/22)
Western Visayas (VI)	23.3% (60/258)
Ilocos Region (I)	22.2% (6/27)
Zamboanga Peninsula (IX)	16.1% (9/56)
Eastern Visayas (VIII)	15.5% (11/71)
Cagayan Valley (II)	15.0% (15/100)
CALABARZON (IV-A)	14.0% (15/100)
NCR	13.1% (59/450)
Bicol Region (V)	4.8% (1/21)
MIMAROPA (IV-B)	0.0% (0/6)
ARMM	0.0% (0/1)
Overall	19.4% (277/1430)

passing score of 80 and above (Table 3) and all regions got below 50% passing rate. Four regions in Mindanao (Davao, Caraga, Northern Mindanao, and SOCCSKSARGEN) outperformed all regions in Visayas and most regions in Luzon in the 2015 PT based on the percentage of passers. Southern Luzon regions (CALABARZON, National Capital Region, Bicol, and MIMAROPA), plus ARMM had the least percentage of passers. It should be noted that MIMAROPA and ARMM, both of which had zero passing rates in 2015, consisted of malaria- and schistosomiasis-endemic provinces.<sup>21,22</sup>

Belizario et al. noted several reports describing low rates of parasite recognition by laboratorians in different areas in the Philippines.<sup>4</sup> Lack of resources and limited training opportunities in medical parasitology make for the poor performance of laboratorians in the diagnosis of parasitic diseases. Belizario et al. also proposed that emerging parasites should also be included in the proficiency testing.<sup>4</sup>

## CONCLUSION

Overall, majority of participating laboratories performed poorly in both identification of parasites in preserved fecal samples and in malaria blood films, including estimation of malaria parasite density. More training opportunities in medical parasitology, especially in malaria microscopy, must be prioritized by the government. Government regulatory agencies may also consider setting cut off scores for the licensing of diagnostic parasitology laboratories. Laboratorians should also be evaluated on their capability to identify uncommon and emerging parasites like schistosomes, filarial nematodes, pathogenic protozoans, etc. since many of these diseases are endemic in the country.

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## STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

## AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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