Lesson learned: Development of National Archive of Malaria Slides (NAMS) in the DR Congo

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ABSTRACT

The accuracy of malaria diagnosis by microscopy has been a challenge in health facilities in the DRC due to inadequate training, quality assurance, and maintenance of microscopy skills. In order to improve quality assured malaria microscopy in the DRC, MalariaCare supported the Institut National de Recherche Biomédicale (INRB) and National Malaria Control Program to develop the National Archive of Malaria Slides (NAMS). This slide bank will be used for microscopy training, supervision and proficiency testing.

51 blood samples of *P. falciparum*, non-falciparum, mixed infections and negatives were collected in the DRC from May 2016 through June 2017. The NAMS protocol was developed by the INRB in collaboration with MalariaCare following the WHO protocol and approval by an ethics board. As there was no *P. vivax* present during blood collection, 200 validated *P. vivax* slides were purchased externally. All slides were developed at the INRB and barcode labels were applied to each slide. Two slides per donor were selected for slide validation by six WHO-accredited level 1 microscopists in the DRC and Mali. Each blood donor was validated by PCR to confirm malaria infection and species. The quality of each slide was validated.

Microscopy detection of infection showed high sensitivity (98%) and specificity (100%) when compared to PCR, whereas microscopy species detection was 84% (range: 74% - 90%) concurrent with PCR. Among 51 blood donors, there were 3 discrepancies between the PCR and microscopy results and these were excluded from the slide bank. The remaining 48 donor samples were accepted and used to produce 18,369 fully validated malaria slides.

The DRC NAMS will be used for national and regional malaria microscopy training and can be used throughout the country to perform proficiency testing and on-site supervision. The NAMS will be an important tool in the DRC's efforts to improve the quality of clinical care and also will assure the ability to develop new generations of laboratory specialists as the country moves towards improved case management and control of malaria.

METHODS

- The NAMS protocol was developed by the INRB from 2014-2015 in collaboration with MalariaCare following the WHO protocol and approval by the ethics board in 2016.
- The laboratory technicians were trained by MalariaCare experts for the development of the NAMS, including in blood collection, slide preparation, slide staining, slide validation by microscopy and PCR, and on the NAMS Access database for slide management.
- 51 blood samples, including negative, *P. falciparum, P. ovale, P. malariae*, mix *P. falciparum/P. ovale* and mix *P. falciparum/malariae*, were collected from May 2016 through June 2017.
- 4 ml blood samples were collected in EDTA containers from each volunteer patient (aged 7–50 years) from 2 sites in DRC (Kinshasa and Nkamba, both in the Ngombe Matadi health zone in Kongo Central province). Each volunteer donor gave consent before having blood drawn. Blood spots were prepared from the same blood sample for PCR validation.
- 100–300 blood smears were produced per sample. The slides were prepared and stained at the INRB for the blood samples collected in Kinshasa, and at Nkamba health zone for blood samples collected in Nkamba for rare species. Barcode labels were applied to each slide at the INRB.
- As there was no *P. vivax* present during blood collection, 200 validated *P. vivax* slides were purchased externally.
- The quality of each slide was validated by microscopy and PCR:
  - Two slides per donor were selected for slide reading validation by six WHO-accredited level 1 microscopists in the DRC and Mali, except two donors who had only 1 slide for validation.
  - Each blood donor was validated by PCR to confirm malaria infection and species.
- The NAMS Access database system for slide management has been provided to the INRB and the NAMS was completed by September 2017.

RESULTS

- Of the 51 blood donors, there were 3 slides with discrepancies between the PCR and microscopy results. These slides were excluded from the slide bank.
- Slides with parasites that could not be detected by microscopy are likely due to low density infection, as microscopy is limited to detection at ten parasites per µl, while PCR is sensitive down to one parasite per µl.
- The remaining 48 donor samples, were negative or contained single species *P. falciparum, P. ovale, P. malariae*, or mixed *P. falciparum/P. ovale or P. falciparum/malariae* and were used to produce 18,369 fully validated malaria slides.
- Overall, microscopist competency was good, showing high sensitivity (98%) and specificity (100%) when compared to PCR, and microscopy species detection at 84% (range: 74% - 90%) concurrent with PCR.

Table 1: Summary of the microscopy findings for malaria parasite detection compared with PCR result (using PCR as gold standard)

Microscopy		PCR			
Microscopist[a]	N of slides	Positivity rate[b]	Agreement[b]	Sensitivity[b]	Specificity[b]
A	100	82%	98%	98%	100%
B	100	82%	98%	98%	100%
C	100	82%	98%	98%	100%
D	100	82%	98%	98%	100%
E	100	82%	98%	98%	100%
F	100	82%	98%	98%	100%

Table 2: Summary of the microscopy findings for malaria species identification compared with PCR result (using PCR as gold standard)

Microscopy		PCR	
Microscopist[a]	N of slides	Match[b]	Errors[c]
A	100	90%	10%
B	100	88%	12%
C	100	85%	15%
D	100	86%	14%
E	100	74%	26%
F	100	78%	22%

[a]Microscopist certified WHO Level 1

[b]Comparison between PCR and microscopy, using PCR as a gold standard

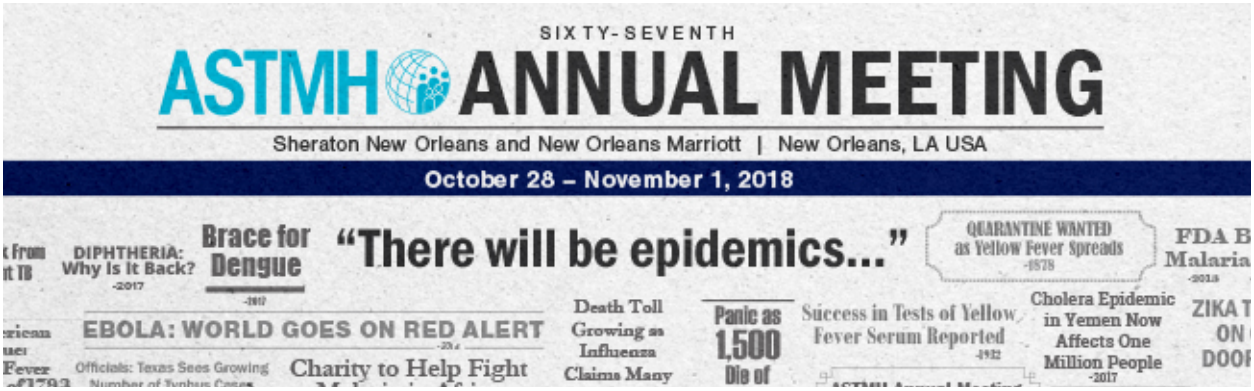
[c]Error rate refers to the number that does not match for malaria species between microscopy and PCR result

CONCLUSIONS

- The process of NAMS development in the DRC took 3 years to complete, with delays due to difficulty in finding non-*falciparum* species. *P. malaria* and *P. ovale* samples were ultimately collected in the DRC; however, it was necessary to procure *P. vivax* slides outside the country.
- Countries that develop a NAMS must establish and adhere to realistic timelines to complete all aspects of the process: protocol development, ethics approval, blood collection, slide preparation, PCR validation, slide reading validation, storage and labeling. Our experience shows this process takes 2 to 3 years.
- Countries that have a paucity of non-*falciparum* species present should procure these slides outside the country from an established research institution.
- In countries with internal capacity, the time and effort costs must be weighed with the benefits from learning the methodology before deciding whether a NAMS should be developed internally or procured from an external institution.
- The NAMS will be an important and essential tool in malaria control programs' efforts to improve the quality assurance of malaria diagnostics and of clinical care. It will also ensure the ability to develop new generations of laboratory specialists as the country moves towards improved case management and control of malaria.

ACKNOWLEDGEMENTS

- US President's Malaria Initiative
- United States Agency for International Development
- Ministry of Health/NMCP/INRB in DR Congo
- World Health Organization



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Abstract:

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