QUALITY CONTROL OF MALARIA MICROSCOPY SURVEILLANCE, MPUMALANGA PROVINCE MALARIA CONTROL PROGRAMME, SOUTH AFRICA, JANUARY – DECEMBER 2019

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Summary

The prompt diagnosis and treatment of malaria is the most effective way to prevent a mild case from developing into severe disease and potentially, death. All malaria patients must be treated as soon as possible following microscopy and/ or rapid diagnostic test confirmation of malaria. Light microscopy of Giemsa-stained blood smears remains the gold standard for diagnosing clinical malaria.

The South African National Malaria Quality Assurance Guidelines stipulate that microscopy results should be cross-checked for quality assurance purposes. The South African malaria control programme operates primarily in the three malaria-endemic provinces of Mpumalanga, KwaZulu-Natal and Limpopo. Malaria microscopy quality control (QC) was initiated in 2018 for the Mpumalanga provincial malaria control programme (MCP). On at least a monthly basis, smears were sent to the Parasitology Reference Laboratory (PRL) at the National Institute for Communicable Diseases (NICD) for QC. Twenty percent of the received smears and any additional positive smears underwent microscopy quality control; this included microscopy and PCR. The PRL received 4 738 smears in 2019 and microscopy quality control was done on 969 of these. The majority (939) were reported as negative by the MCP. The PRL found two false positive results and three false negative results. Mpumalanga Province MCP microscopy had a sensitivity of 88.9% and a specificity of 99.8% as compared against PRL microscopy. When microscopy results were compared to PCR results, the majority of smears (94%, 781/834) were concordant between the Mpumalanga Province MCP microscopy, PRL microscopy and PRL PCR. This quality control exercise shows that malaria parasite microscopy in the Mpumalanga Province MCP performs well. There is however room for microscopy skills improvement within the Mpumalanga province MCP. The PRL/NICD has recommended that all provincial MCP malaria microscopists attend the Malaria Microscopy Refresher Course offered by the PRL. They will also benefit greatly from the implementation of a proper quality management system.

Background

Malaria is a parasitic disease caused by *Plasmodium* species, and is usually transmitted by an infected female *Anopheles* mosquito.[1] In South Africa, human malaria is caused by four species of the *Plasmodium* parasite*: Plasmodium falciparum*; *P. malariae*; *P. ovale* and *P. vivax*.[2] *Plasmodium knowlesi* is recognised as the fifth human malaria parasite[2] but has not yet been reported in South Africa.

Malaria is preventable and curable but if not diagnosed and treated early, can be fatal. In 2018, an estimated 228 million cases of malaria occurred worldwide with 405 000 reported deaths.[1] The majority of malaria cases (213 million or 93%) and deaths (94%) were in the World Health Organization (WHO) African Region.[1] Children aged under 5 years are the most vulnerable group affected by malaria, accounting for 67% (272 000) of all malaria deaths worldwide.[1]

Ten percent of the population in South Africa is estimated to be at risk of contracting malaria.[3] In South Africa, malaria is seasonal, occurring mainly between September and May, with cases usually peaking after the Christmas and Easter holidays.[4] Over 90% of the reported malaria cases are caused by *P. falciparum*.[4] Malaria has been a notifiable medical condition (NMC) in South Africa since 1956.[5] According to South Africa's National Health Act 61 of 2003, all malaria cases should be reported within 24 hours of diagnosis.[6] Light microscopy of Giemsa-stained blood smears remains the gold standard for diagnosing clinical malaria.[7] Prompt diagnosis and treatment is the most effective way to prevent a mild case of malaria from developing into severe disease and potentially, death.[1]

Between 2000 and 2012, South Africa reduced the burden of malaria by ~90% (64 622 vs. 6 846 cases, respectively) and mortality by ~80% (459 vs. 91 deaths, respectively).[7] In 2012, South Africa adopted a malaria elimination strategy.[4] The broad objectives of the national malaria elimination strategy include strengthening case surveillance, preventing infections and eliminating the parasite reservoir.[5]

An essential component of a malaria surveillance system is the accurate parasitological diagnosis of malaria cases. The WHO Malaria Surveillance, Monitoring and Evaluation Reference Manual recommends that diagnosis should be made with either quality-assured malaria microscopy or WHO-recommended rapid diagnostic tests (RDTs).[8] The WHO Malaria Microscopy Quality Assurance Manual states that: "Blood film microscopy remains the only inexpensive, easily used test for direct measurement of the presence of parasites, distinguishing the infecting parasite species and providing a means of quantifying parasite load."[9] These attributes of malaria microscopy make it an extremely useful tool in any malaria control programme.

South Africa has a decentralised malaria control programme (MCP) with activities at the provincial level.[5] The national malaria programme at the National Department of Health (NDOH) defines policies and guidelines and provides technical support to provinces.[5] The provincial MCPs operate in the three malaria-endemic provinces of KwaZulu-Natal, Mpumalanga and Limpopo. All malaria-positive cases are provided with treatment within 24 hours and treatment is only prescribed when cases are confirmed.[5] Since 2000, all suspected malaria cases in South Africa have been confirmed using microscopy and/or RDTs.[5]

There are two broad types of malaria case surveillance activities conducted by the provincial MCPs in South Africa: passive case detection (PCD) and active case detection (ACD). During the PCD process, patients visit health facilities and, once they are confirmed as having malaria, the health worker notifies the case as per the NMC requirements.[5] Therefore PCD is detection of malaria cases among people who approach a health facility or a community health worker on their own initiative to seek treatment, usually related to fever.[8] On the other hand, ACD locates both symptomatic and asymptomatic malaria cases, who will also be treated according to national malaria treatment guidelines.[5]

The aim of ACD surveillance is to prevent onward malaria transmission by identifying new infections and potential sources of infections.[5] ACD surveillance is further classified into reactive case detection (RACD) and proactive case detection (PACD).[8] RACD is usually a response to an index case that may have been notified through the NMC process. RACD will screen for symptoms and test for malaria in the household of the index case and/or people in the community potentially linked to the index case[8] (Figure 1).

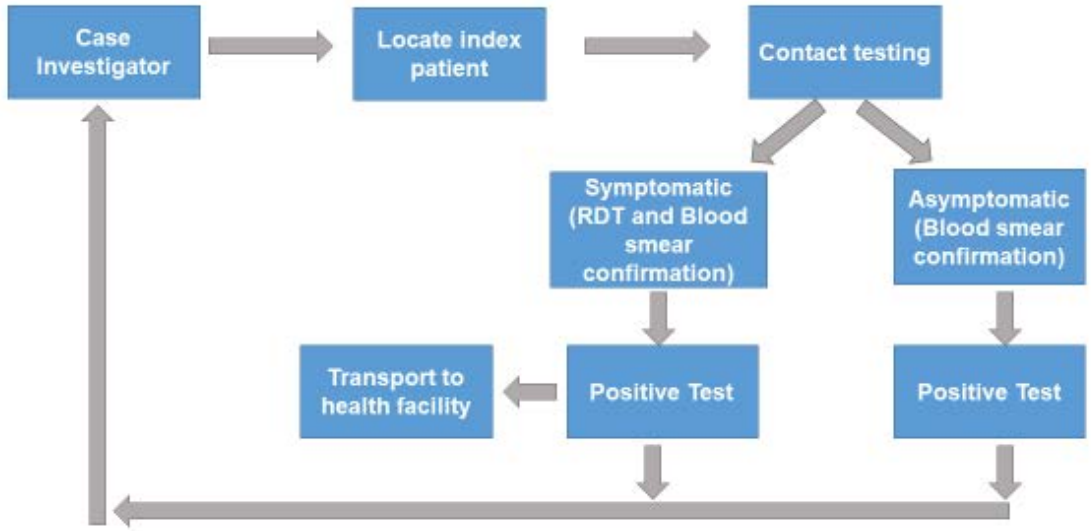

Figure 1. Adapted flow diagram demonstrating the general reactive case investigation and detection plan.[5]

PACD is triggered by the strong likelihood of malaria transmission in a defined area or among high-risk groups of people.[5,8] There is usually limited access to healthcare facilities. PACD is performed regularly at specific times (mainly during the malaria transmission season) to confirm active local transmission in target populations and to detect malaria cases early.[8]

There are dedicated teams of malaria surveillance officers and case investigators working between the health facilities and the communities on a daily basis.[5] The duties of the malaria surveillance teams include conducting malaria-related health education, taking blood smears, collecting malaria-related data in the field and assisting

the team leaders with conducting case investigations.[5] The MCPs have malaria microscopists who work independently of the National Health Laboratory Service (NHLS).[10]

The South African National Malaria Quality Assurance Guidelines stipulate that microscopy results should be cross-checked as quality control (QC) for quality assurance purposes.[11] This is required as South Africa is moving towards malaria elimination, with the target of zero locally-transmitted malaria cases.[12] To assist with malaria microscopy surveillance in South Africa, the Parasitology Reference Laboratory (PRL) at the National Institute for Communicable Diseases (NICD), a specialised institution of the NHLS, has assisted with the required QC for the provincial malaria control programmes. QC was initiated for Mpumalanga Province in 2018, as this MCP's slide procedures were mostly aligned to the processing QC requirements.

Methods

As per the routine malaria testing procedures described above for active surveillance, case investigator teams from the Mpumalanga Province MCP actively screened people who lived with or near to a person who had tested positive for malaria. Once these people were located, the case investigators and their team members collected basic demographic information using a registry form. Blood was collected using the finger prick method with a disposable lancet, an RDT was performed (when indicated), and thick and thin blood smears were prepared on site.

The forms and smears were sent to one of four testing stations in Mpumalanga Province for staining and/microscopy. The testing stations were located in Thulamashe and Cunningmoore in Bushbuckridge District, Masoyi in Mbombela districts and Tonga in Nkomazi District. At the testing stations, the dried blood smears were stained with Giemsa. Thin blood films were first fixed with 100% methanol. MCP microscopists then examined the blood films for *Plasmodium* spp. using light microscopy. Microscopy results were recorded on the forms and any positive results were communicated to the case investigators for further action. On at least a monthly basis, smears were sent to PRL/NICD with copies of the corresponding Mpumalanga Province MCP forms for microscopy QC.

At NICD, smears were checked against the accompanying forms. Any missing smears or forms were noted. Twenty percent (every 5th slide) of the received smears were then selected for QC. Any additional site positives that were not part of the 20% blinded reads were also selected for QC. Each of the selected smears was read by two microscopists independently. The thick blood smears were scanned under 100x magnification first, then under 1000x magnification using immersion oil. At least 100 to 150 high power fields (1000x magnification) were looked at before declaring a smear negative for malaria, where possible. A third microscopy read was done if there were discrepant results between the first two microscopy reads or when compared to the microscopy results from the Mpumalanga Province MCP microscopists. All smears received were also assessed for quality macroscopically and microscopically. Quality areas looked at were thick smear fixing by methanol or heat, presence of a feathered edge on thin smears, presence of stain precipitate, or washing-off of the blood smears. Following microscopy and checking of any discrepant microscopy results, DNA was extracted from the blood smears and a conventional malaria multiplex PCR, which detects the four common human

malaria species in South Africa, was performed.[13] Results were collated, analysed and reports sent to the Mpumalanga Province MCP.

Results

In 2019, 4 738 smears were received. Figure 2 shows the summary of the number of smears that were received by month. Nineteen batches were received (there were a few months where multiple batches were received). Due to occasional delays, smears were not always received in the month after they were completed by the province. Therefore, the number of smears received each month at NICD does not accurately reflect the number of smears done in the previous month from the Mpumalanga Province MCP.

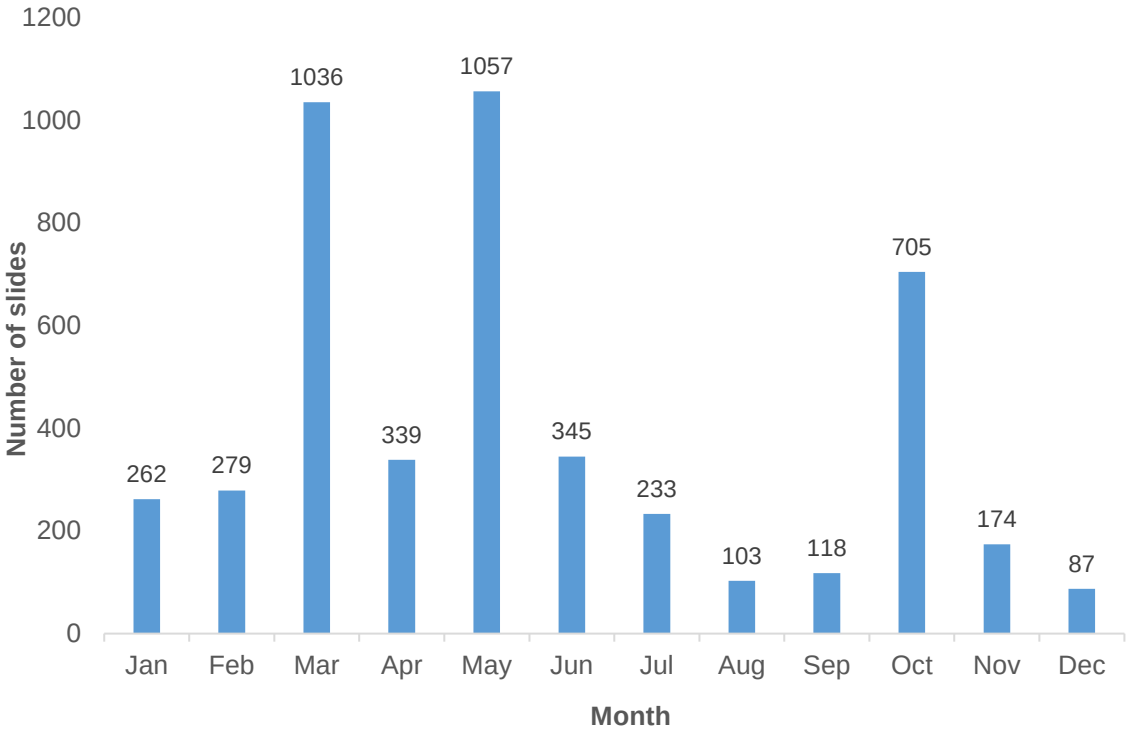

Figure 2. Numbers of malaria smears received by the National Institute for Communicable Diseases for quality control from the Mpumalanga Province malaria control programme, 2019.

Of all the smears received, the Mpumalanga province MCP reported 32 (0.7%) as positive and 4 665 as negative; there were 41 smears that had missing microscopy results or missing forms. All smears received without paperwork (n=37) were assumed to be negative for the purpose of this comparison and were included in the analysis. Table 1 shows a summary of the results, comparing microscopy between the Mpumalanga Province MCP and NICD for the smears that were quality controlled. The Mpumalanga Province MCP microscopy had a sensitivity of 88.9% and a specificity of 99.8%.

Table 1. Summary of the comparison of malaria parasite microscopy between Mpumalanga Province malaria control programme (MCP) and the National Institute for Communicable Diseases (NICD).

		NICD microscopy result	
		Positive	**Negative**
Mpumalanga Province MCP microscopy result	**Positive**	24	2
	Negative	3	936

Of the 969 slides that were microscopically quality controlled by NICD, Mpumalanga Provincial MCP reported 26 smears as positive and 939 as negative. Unfortunately, due to difficulty in interpreting the MCP forms, six smears reported by the Mpumalanga Provincial MCP as positive were accidentally discarded before microscopy QC was done. The results are explained further in Table 2. There were four cases that did have paperwork but had 'RDT positive' recorded as a microscopy result. For these four cases, one was confirmed as *P. falciparum* (1 032 p/µl) and the other three were found to be negative by microscopy QC. These results were excluded from the analysis.

Table 2. Comparison of malaria parasite microscopy results between the Mpumalanga Province malaria control programme (MCP) and the National Institute for Communicable Diseases (NICD).

Mpumalanga Province MCP microscopy result	NICD microscopy result	Number	Comment on the Mpumalanga MCP microscopy result
Positive	Positive	21	100% agreement
Negative	Negative	936	100% agreement
P. falciparum	*P. malariae*	1	Incorrect species identification
Relapsing malaria species	*P. ovale*	1	Acceptable response
Relapsing malaria species	*P. falciparum*	1	Incorrect species identification
Relapsing malaria species	Negative	1	False positive
"Positive"	Negative	1	False positive
Negative	*P. falciparum*	3	False negative

The three false negative slides had low parasitaemias: 16 parasites/microliter (p/µl), 546 p/µl and 417p/µl. To put this in context of percentage parasitaemia, an average of 50 000 p/µl is considered 1% parasitaemia.[14] Therefore, these three false negatives all had percentage parasitaemias of 0.01% or less.

All slides received were also assessed for smear quality (Figure 3). The major problems noted were the lack of a feathered edge on the thin blood films, partial washing off of the blood films and partial autofixing of the thick blood films.

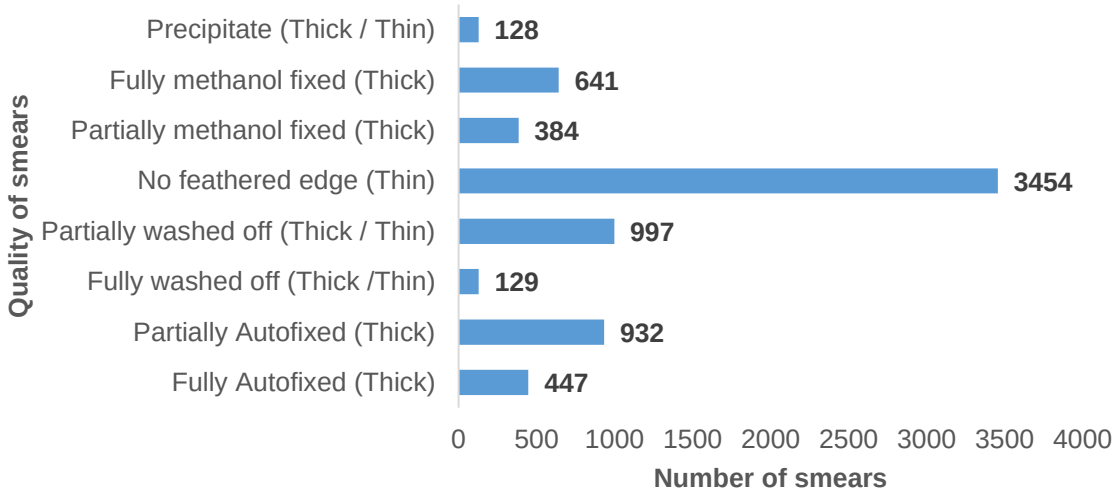

Figure 3. Results of the assessment of the Mpumalanga Province malaria control programme blood smear quality, 2019.

Molecular testing was initiated from slides received at NICD in March 2019. Seventeen of the 19 batches (851 slides) had PCR results available to be compared to the microscopy results and 118 slides were excluded. The majority of slides (94%, 781/834) had concordant results between the Mpumalanga Province MCP microscopy, NICD microscopy and NICD PCR (Table 3). Thirteen slides were quality-controlled microscopically, but accidently discarded before molecular testing could be performed. The four cases whose microscopy results were recorded as 'RDT Pos', were negative on PCR.

Table 3. Comparison between the National Institute for Communicable Diseases (NICD) PCR results and the microscopy results from the Mpumalanga Province malaria control programme (MCP) and the NICD.

Mpumalanga province MCP microscopy result	NICD microscopy result	NICD PCR result	Number
Negative	Negative	Negative	775
Positive	Positive	Positive	6
Positive	Positive	Negative	9
Positive	Negative	Negative	2
Negative	Positive	Negative	1
Negative	Positive	Positive	2
Negative	Negative	Positive	39

Discussion, conclusions and recommendations

The microscopy quality control results for 2019 show good sensitivity and excellent specificity by the Mpumalanga Province MCP microscopists. Very few false positives and false negatives were detected; nevertheless, there is room for improvement of the microscopy skills at the testing stations. Only six of the Mpumalanga province MCP positive smears had quantitation recorded. Quantitation should be determined for all *P. falciparum*-positive samples. Only asexual stages (trophozoites and schizonts) should be counted, as the asexual stages of the parasite contribute to the clinical symptoms of the disease. If gametocytes of *P. falciparum*

are observed, it is important to record this observation, as gametocytes (sexual stages) contribute to the ongoing transmission of malaria.

Concerning the quality issues, we note that it is difficult to prepare perfect smears in the field, i.e. from a fingerprick and often without a table. In April 2018, case investigators were provided with slide boards to assist them in the field. To prevent autofixing of the thick smears, the field teams were requested to deliver the smears to the testing stations for staining in the quickest time possible. The staff at the testing stations were also trained to take care while rinsing the stain off the slides to prevent the smears from washing off. There were major issues with the completion of forms: some data were illegible, missing or filled in incorrectly. These concerns were reported to the province. They will also benefit greatly from implementing a proper quality management system.

The NICD has requested that the batches be sent as soon as possible. Ideally, they should be sent in the month after the testing station has processed the smears. This will allow for a quicker feedback response to the province. As a preventive measure, NICD will store smears that have not undergone microscopy QC for at least six months after receipt.

It is expected that more samples would be identified as positive by PCR as compared to microscopy, as PCR is generally more sensitive than microscopy at very low parasitaemias.[7] However, there were 10 NICD-confirmed microscopy-positive results that were PCR negative. Low concentration of the *Plasmodium* spp. DNA, the sample type that the DNA was extracted from (blood smears) or the presence of inhibitors in the sample, are possible reasons for this.

It is recommended that all the MCP microscopists be given an opportunity to attend the Malaria Microscopy Refresher Training (MMRT) course offered by PRL, NICD. At the end of the course an evaluation will be conducted to determine if the malaria microscopists qualify to attend the National Competence Assessment in Malaria Microscopy (NCAMM). The NCAMM will be a formal certification of their level of skill in malaria microscopy (based on parasite detection, identification and quantification). A certificate of competence reflecting their grading (highest grade A, lowest grade D) will be awarded at the end of the NCAMM and will be valid for two years. This objective assessment of competence will be done according to the Malaria Microscopy Quality Assurance Manual (version 2) published by the World Health Organization.[9]

Acknowledgements

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