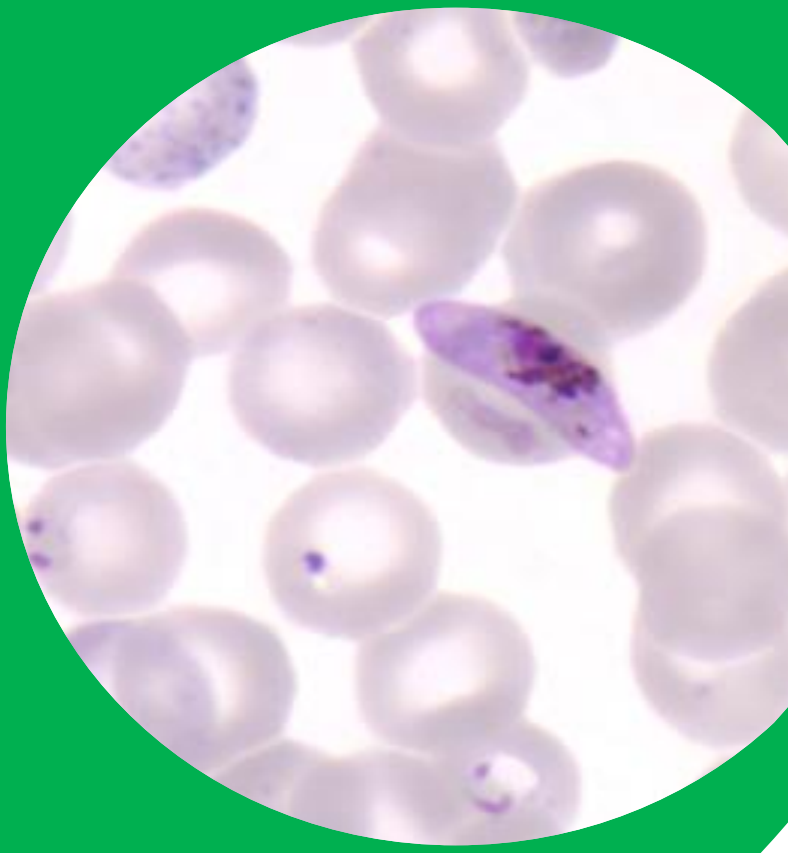
Guideline for National External Quality Assessment Scheme for Malaria Diagnosis



National Malaria Reference Laboratory

Royal Centre for Disease Control

Ministry of Health

3rd Edition

Contents

Preface

The 3rd edition of Guideline on National External Quality Assessment Scheme (NEQAS) for malaria diagnosis is prepared based on the World Health Organization (WHO) guidelines in Quality Assurance (QA) for malaria microscopist and align with the national malaria surveillance guideline.

This revised guideline primarily focuses and guide both reference laboratory and participating laboratories in conducting the NEQAS activities to improve quality performance in malaria diagnostic services. This guideline will help district laboratories staffs in improving and enhanced their competency skills on malaria microscopist and moreover, guide to maintain and improve their IQC which will provide as supporting document to help in malaria elimination and certification by the WHO.

WHO recommends that all the suspected malaria cases must be either diagnosed by quality assured Microscopy or Malaria Rapid Diagnostic Tests (RDTs) before administration of anti-malarial drugs. Since the case management of malaria is highly dependent on accurate and timely diagnosis, especially when country is in elimination phase, where the malaria incidence is comparatively low and positivity rate is below certain threshold, skills of malaria microscopists for the district laboratories on malaria microscopy will also gradually decrease and competency level might be compromised due to few positive blood slides or laboratory staff hardly encounters positive malaria slides

Therefore, guideline provides QA for malaria diagnosis is considered as core component in sustaining and maintaining high competency level in microscopy skills which is an important step towards achieving high quality of laboratory performance.

For the successful completion of this guideline, the National Malaria Reference Laboratory (NMRL) would like to sincerely acknowledge all the officials involved in the revision of this guideline.

Acknowledgement

National malarial Reference Laboratory (NMRL) under Royal Centre for Disease Control (RCDC) in collaboration with Vector Borne Disease Control programme (VDCP) would like to extend heartfelt gratitude to all the contributors of the first and second edition of National External Quality Assessment Scheme (NEQAS) Guideline of Malaria Diagnosis.

We are grateful to the Global Fund and World Health Organisation for their constant guidance and financial support. Their continuous support and cooperation have been invaluable in the development of this guidelines.

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Acronym

BF	Blood Film
CQI	Continuous Quality Improvement
DBS	Dried Blood Spot
EDTA	Ethylene Diamine Tetra Acetic Acid
EQAS	External Quality Assessment Scheme
IEQAS	International External Quality Assessment Scheme
IQC	Internal Quality Control
MP	Malaria Parasite
NEQAS	National External Quality Assessment Scheme
NMRL	National Malaria Reference Laboratory
PL	Participating Laboratory
PCR	Polymerase Chain Reaction
Pf	Plasmodium falciparum
Pv	Plasmodium vivax
QA	Quality Assurance
QC	Quality Control
RCDC	Royal Centre for Disease Control
RDT	Rapid Diagnostic Test
SOP	Standard Operating Procedure
WBC	White Blood Cell

Glossary

Agreement

It is a combination of sensitivity and specificity that describes the number of correct answers given or the amount of agreement between reference laboratory readers and the participant's answers, so both true negatives and true positives are counted toward this measurement

Competency

The skill of Microscopists for performing an accurate examination and reporting of a malaria blood film.

Controller

Term used to describe the supervisory laboratory or microscopists responsible for rechecking slides.

Corrective action

A suitable remedial action taken by the concerned laboratory after identifying the error to prevent future recurrence

Continuous Quality Improvement

The continuous, systematic, and sustainable process to enhance the quality of malaria diagnosis

External Quality Assessment Scheme

A system by which a laboratory's performance is checked objectively by an external agency or reference laboratory

False negative

A positive blood smear that is misread as negative

False positive

A negative blood smear that is misread as positive

Feedback

Communication of the results of an external quality assessment with identification of errors and recommendations for remedial action.

Major error

This type of error is considered the most critical since it has the highest potential impact on patient management and can result in an incorrect diagnosis or improper management of a patient. Major errors in context to malaria microscopy may include false Positive, false Negative and P. falciparum reported as P. vivax.

Minor error

This type of error is considered less serious but some impact on patient management. Minor errors in context to malaria microscopy may include P. vivax reported as P. falciparum.

Microscopist

A person who uses a microscope to read blood films to aid or confirm the diagnosis of malaria and reports on their findings.

National Malaria Reference Laboratory

National reference laboratory is the apex malaria laboratory in country which organizes and maintains network of malaria laboratories, develops guidelines for standardizing malaria diagnosis, conducts quality assurance program and oversees training.

Performance standard

A level of performance that is considered acceptable and that all laboratories and test should meet or exceed. Performance standards make it possible to identify laboratories that are not performing satisfactorily

Panel testing

A system in which a reference laboratory sends blood films to participating laboratories for examination, and the laboratory receiving the slides is not informed of the correct results unit it has reported its findings back to the reference laboratory

Quality assurance

The maintenance and monitoring of the accuracy, reliability and efficiency of laboratory services. QA address all the factors that affect laboratory performance, including test performance (internal and external QC), the quality of equipment and reagents, workload, workplace conditions, training and supervision of laboratory staff and continuous quality improvement. It includes procedures put in place to ensure accurate testing and reporting of results

Quality control

Assessment of the quality of a test or a reagent. QC also encompasses external QC and reagent QC. External QC is a system in which routine blood slides are cross checked for accuracy by supervisor or reference laboratory. Reagent QC is a system for formal monitoring of the quality of the reagents used in a laboratory

Quality improvement

A process in which the components of microscopy and RDT diagnostic services are analyzed to identify and permanently correct and deficiencies. Data collection, data analysis and problem solving are used.

Rapid diagnostic test

Rapid diagnostic tests (RDTs) are immuno-chromatographic tests for detecting parasite- specific antigens in blood sample.

Rechecking

Sending MP slides from the participating laboratory to a reference laboratory for rereading. These guidelines recommend that rechecking is always blinded, ensuring that the controller does not know the results from the participating laboratory.

Slide positivity rate

The proportion of positive results, detected by microscopists, among all slides examined over a defined period

Slide negativity rate

The proportion of negative results, detected by microscopists, among all the slides examined over a defined period

Standard Operating Procedure

Written description of all standards including the control tests to be carried out for the laboratory procedure.

Validation

Action of proving that a procedure, process, system, equipment or method used in manufacturing or controlling a product works as expected and achieves the intended result.

1. Background:

This National External Quality Assessment Scheme (NEQAS) guideline is developed to support laboratories personnel, both laboratory technicians and malaria technicians in the health centers. Its purpose is to provide a better understanding of the technical requirements regarding on operation of NEQAS activity both to organizing laboratory and the participating laboratories.

World Health Organization (WHO) recommends that all the suspected malaria cases must be either diagnosed by Microscopy or quality assured Rapid Diagnostic Tests (RDTs) before administration of anti-malarial drugs. Since the case management of malaria is highly dependent on accurate and timely diagnosis, especially when country is in elimination phase, where the malaria incidence is comparatively low and positivity rate is below certain threshold, skills of malaria microscopists for the district laboratories on malaria microscopy will also gradually decrease and competency level might be compromised due to few positive blood slides or laboratory staff hardly encounters positive malaria slides. The evaluation of laboratory performance is achieved using an appropriate QC material to the objectives of the scheme. Moreover, through NEQAS system, not only help in accessing the laboratories performance but also the performance of laboratory equipment, quality on laboratories reagent and consumables were evaluated. It is considered as core component in sustaining and maintaining high competency level in microscopy skills which is an important step towards achieving high quality of laboratory performance.

NEQAS for malaria diagnosis is to provide standard procedure on quality assurance (QA) to maintain high competency level of the microscopists in malaria microscopy, is an important tool for assessing performance of clinical laboratories providing malaria diagnosis. The malaria diagnosis by laboratories must be accurate, reliable and timely.

Beside NEQAS, this guidelines help the reference laboratory on how to perform lot testing of malaria RDTs to check and improve the quality of RDT use in district health centers.

National Malaria Reference Laboratory (NMRL) under the Royal Centre for Disease Control (RCDC) is mandated to assess and oversee to monitor the performance of malaria diagnosis by the laboratories across the country.

2. Objectives

The objectives of NEQAS is to;

2.1. Assess the performance of participating laboratories (PL) on malaria diagnosis and implementation of QA system

2.2. Determine competency among the microscopists within the PL by comparing the results of the panel slide

2.3. Detect errors and provide feedback for corrective action on panel testing and blinded rechecking.

2.4. Provide training and CME to maintained high level of staff competency and laboratory performance in malaria diagnosis.

3. NEQAS structure

3.1. Organizing laboratory

NMRL will be the organizing laboratory for conduction of NEQAS activities.

3.2. Roles and responsible for organizing laboratory

3.2.1. Conduct malaria blinded rechecking of slides received from participating laboratories

3.2.2. Provide NEQAS panel testing (5 Slides per round)

3.2.3. Conduct on-site visit(s) to identify gaps or deficiencies and implement corrective actions(s) where appropriate.

3.2.4. Prepare technical report, provide feedback and recommendations to PL for corrective action

3.2.5. Follow-up on implementation of corrective actions.

3.2.6. Compile and provide summary report to the relevant stake holders

3.2.7. Review and revise NEQAS guideline at periodically as and when required.

3.3. Participating laboratory

The participating laboratories are National, regional referral hospital, district hospitals, primary healthcare centers (PHC) and private diagnostic centers.

3.4. Roles and responsibilities of participating laboratory

3.4.1. All the laboratories performing malaria diagnosis are mandated to participate in the NEQAS program.

3.4.2. All the staff participating in NEQAS program should be well-versed with the guidelines and the associated relevant documents.

3.4.3. Perform NEQAS as per the guidelines and associated SOP or instruction.

3.4.4. Submit report to NMRL within the deadline

3.4.5. Take corrective actions as indicated by the NMRL in the feedback report

4. NEQAS Design

NEQAS for malaria diagnosis comprises of 3 main components

4.1. Blinded rechecking

4.2. Panel testing

4.3. On-site monitoring & supervision

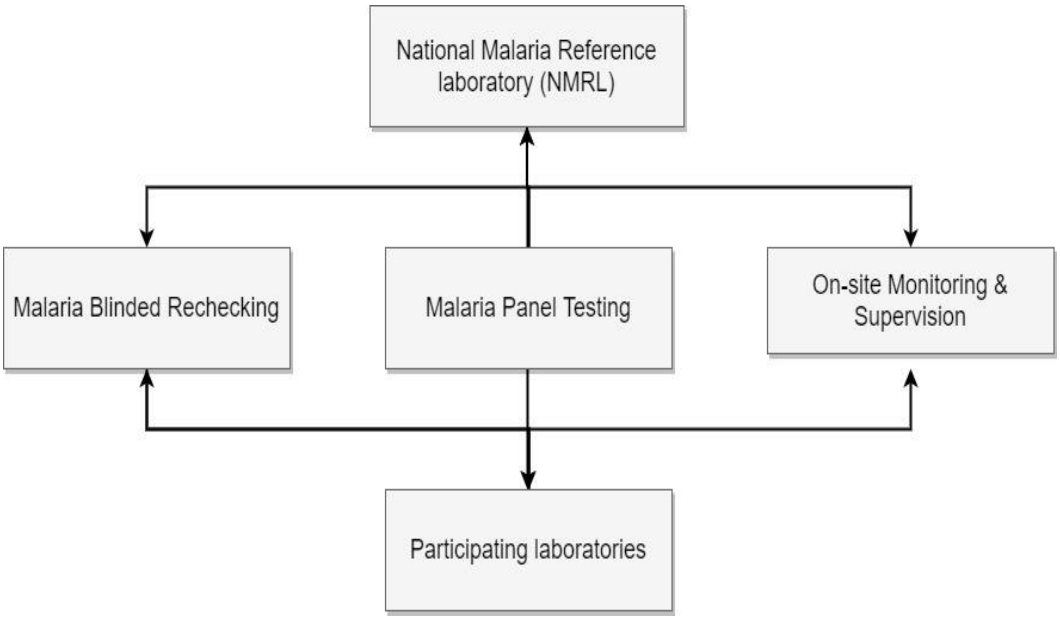


Fig: 1 NEQAS components for Malaria Diagnosis

5. Panel Testing

Panel Testing (PT) refers to the process by which participating laboratory carry out malaria microscopy on a set of prepared slides received from the NMRL. The staff must demonstrate their competency on malaria parasite detection, species identification, stages identification and parasite density determination in concordance to pre-determine results of NMRL.

Panel slide sets are prepared, characterized and validated by minimum of 2 microscopist (WHO certified level 1 or level 2) and confirm with molecular techniques if available.

Every PL Staff must examine the panel slides and submit their results to the NMRL within a month from the date of PT sample receipt in their respective laboratory. The results reported by the PL staff on panel slides must be compared with the pre-determined results of the NMRL and feedback on the result is provided within the stipulated time frame.

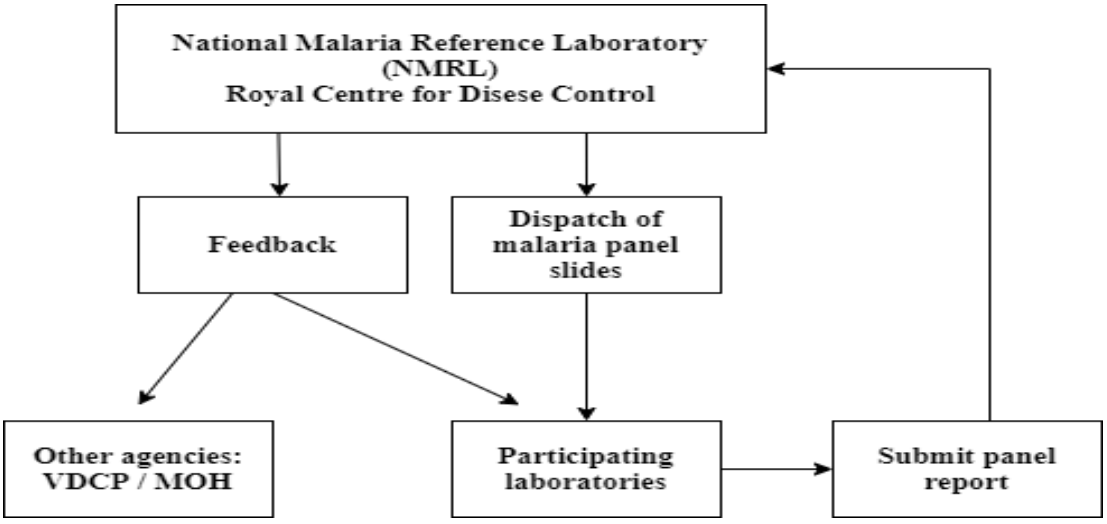


Fig: 2 Flow chart on malaria panel testing

5.1. Frequency of PT slide

NEQAS for PT will be conducted annually

5.2. Design of Panel Testing

The slides for PT must be prepared with standard method where all slides are characterized and validated by WHO certified level 1 or 2.

The PT slides set comprises of:

5.2.1. Positive and negative stained slides

5.2.2. Slide prepared with species present in the region with differential diagnosis including different stages and parasite densities

5.2.3. Same characterized blood sample (for species, stages and parasitaemia) to ensure that the evaluation is comparable when sets of the same type are used to evaluate different laboratories microscopist.

5.3. Packaging and shipment

Slides are packed in slide mailer and shipped through a courier service. The reporting formats, instruction letters and other additional information are packed separately within the shipment box / envelope. NMRL shall notify PL on shipment of PT slides.

5.4. Role of Reference laboratory (Organizer)

5.4.1. Provide NEQAS panel (5 slides per round)

5.4.2. Coordinate testing in participating laboratories

5.4.3. Collate and analyze results

5.4.4. Communicate results to the laboratories and their respective health authorities with beneficial commentaries

5.4.5. Coordinate the schedule of the return of the selected slides.

5.5. Role of PL

5.5.1. Receive and examine the testing panels (and notify the provider /organizer if none has been received).

5.5.2. PL should acknowledge the receipt of PT slides and examine as per instruction provided.

5.5.3. These panel samples are attempted to simulate clinical patient samples and should be treated in the same manner as the latter when handling them.

5.5.4. The panel slides should be examined blinded by individual microscopist and record their finding independently in Individual Results Sheet (RS1). If there are more than one microscopist / technician in the participating laboratory, each of them should read and analyze the test smear independently (in Blinded manner) and record his/her results in a separate individual results sheet 1 (RS1).

5.5.5. Each reader should NOT compare or share their results among themselves before they submit their answer sheets to their incharge / enter in data base.

5.5.6. After all the microscopists have submitted their individual results., the laboratory has to establish a single or unified lab result using the results sheet 2 (RS2). The unified lab results may be established by any of the following:

- Consensus or agreement reading of the microscopists after consultation among themselves.
- Reading of the most experienced microscopist in the lab

5.5.7. Submit the results either thorough email or hard copy.

5.5.8. Strictly comply with the deadline set by reference lab in the reporting back of results to prevent any delay in the analysis and release of reports.

5.5.9. The PT slides shipped from the NMRL must be returned after the examination.

5.6. Analysis and feedback

Data entry and analysis will be conducted at NMRL, and feedback will be provided within the stipulated time frame.

5.7. Results analysis:

Results of the panel testing are analyzed using 2 x 2 table and results are recorded as positive or negative as given below (Table 1).

		Reference lab results		
		Positive	Negative	Total
Participating lab's result	Positive	A	B	A+B
	Negative	C	D	C+D
	Total	A+C	B+D	A+B+C+D

Table 1: Result Recording as Positive or Negative on 2 x 2 table format for analyzing panel testing

- A = number of panel slides reported as *positive* by reference laboratory and participating laboratory *(True positive)*
- B = number of panel slides reported as *positive* by the participating laboratory but found to be *negative* by the reference laboratory (false *positives*)
- C = number of panel slides reported as *negative* by the participating laboratory but found to be *positive* by the Reference laboratory (false *negatives*)
- D = number of panel slides reported as *negative* by both the participating laboratory and reference laboratory *(True Negative)*

- **Sensitivity:**

Sensitivity of the panel testing is proportion of true positive among the panel slides

$$\text{Sensitivity} = \frac{TP}{(TP + FN)} \times 100$$

- **Specificity:**

Proportion of the panel slides which are true negative among the panel slides

$$\text{Specificity} = \frac{TN}{(TN + FP)} \times 100$$

- **Percentage slide agreement**:

This refers to assessment of the slides with positive or negative findings for the presence of malaria parasite.

$$\%\ \text{Slide agreement} = \frac{TP + TN}{(TP+FP+FN+TN)} \times 100$$

- **% False Positive:**

Percentage of negative slide that is misread as positive

$$\%\ \text{False positive} = \frac{FP}{(FP + TN)} \times 100$$

- **% False Negative:**

  Percentage of positive slide that is misread as negative

$$\% \text{ False negative} = \frac{FN}{(FN + TP)} \times 100$$

		Reference laboratory		
		Pf seen	***Pf not seen***	**Total**
Participating laboratory	***Pf seen***	A	B	A+B
	Pf not seen	C	D	C+D
	Total	A+C	B+D	A+B+C+D

Table 2: Result recording for monitoring the accuracy of the differentiation of Pf and non-Pf

- A = number of panel slides reported as containing *P. falciparum* (either as a single or mixed infection) by both participating laboratory and reference laboratory
- B = number of panel slides reported as *P. falciparum* seen by the PL but *P. falciparum* was not seen by the reference laboratory (incorrect species identification)
- C = number of slides reported as *P. falciparum* not seen by the participating laboratory but seen by the cross checker – reference laboratory (incorrect species identification)
- D = number of slides reported as *P. falciparum* not seen by both readers

		Reference laboratory		
		Pv seen	***Pv not seen***	**Total**
Participating laboratory	***Pv seen***	A	B	A+B
	Pv not seen	C	D	C+D
	Total	A+C	B+D	A+B+C+D

Table 3: Result recording for monitoring the accuracy of the differentiation of Pv and non-Pv

- A = number of panel slides reported as containing *P. vivax* (either as a single or mixed infection) by both PL and reference laboratory
- B = number of panel slides reported as *P. vivax* seen by the PL but *P. vivax* was not seen by the reference laboratory (incorrect species identification)
- C = number of panel slides reported as *P. vivax* not seen by the PL but seen by the reference laboratory (incorrect species identification)
- D = number of slides reported as *P. vivax* not seen by both readers

- **Concordance of species:**

  This refers to assessment of the results for identification of each species present in the positive slides in the set. In the case of slides with mixed parasites, all species should be identified; if the laboratory being evaluated identifies only one of the species, only half of the value of the correctly evaluated slide will be counted.

$$\text{Species concordance} = \frac{\text{Total no of species correctly identified by participating laboratory}}{\text{Total no species identified by reference laboratory}} \times 100$$

- **Concordance of stages:**

  This refers to assessment of the results on identification of the sexual and asexual stages of *Plasmodium* present in the positive slides.

$$\text{Stages concordance} = \frac{\text{Total no of stages correctly identified by participating laboratory}}{\text{Total no stages identified by reference laboratory}} \times 100$$

- **Parasite density:**

  This refers to assessment of the results on recognition of the exact quantity of parasites on the positive slide, expressed in parasites per microliter

$$\text{Parasite Density} = \frac{\text{No of parasites}}{\text{No of WBCs}} \times 100$$

In the analysis of Parasite Density concordance between the participating laboratory and reference laboratory, a slide shall be considered concordant if the number of parasites reported by the participating laboratory is ± 50% of the value reported by the reference laboratory.

$$\text{PD concordance} = \frac{\text{Total no of parasite count correctly determined by participating laboratory}}{\text{Total no parasite count determined by reference laboratory}} \times 100$$

5.8. Scoring system

The scores for all examined slides were score as follow:

Reference Laboratory	Participating laboratories	Score
NMPS slides	Reported as NMPS	3 points
PF slide	Reported as PF	3 points
Pf slide	Reported as PV	1 point
PV slide	Reported as PV	3 points
PV slide	Reported as PF	1 point
All malaria stages	Identifies all malaria stage	3 points

All malaria stages	Missed in identify malaria stage	1 point
Parasite density determination	+-25% from the true value of reference lab	3 points
Parasite density determination	+-50% from the true value of reference lab	1 point

5.9. Scoring

Performance	Score	Action
Excellent	≥ 90 %	• Congratulate staff for exemplary performance
Very good	80 – 90 %	• Staff should be congratulate for performance and told to maintain it
Good	70 – 80%	• Staff should be congratulate for good performance and the need for further improvement. • Check staff competency • Consider on the job training based in staff's weakness • Check the quality of microscope
Poor	≤ 70 %	• Staff should be informed of poor and the need for immediate action for improvement • Check staff competency • Consider on the training based on staff's weakness • Check the quality of microscope • Regular follow up for corrective action

Table 4: Grading of Laboratory Performance Based on Result of Panel testing

Level	Parasite Detection	Species Identification	Parasite Quantification
Level A	≥ 90%	≥ 90%	≥ 50%
Level B	80 – < 90%	80 – < 90%	40 – < 50%
Level C	70 – < 80%	70 – < 80%	30 – < 40%
Level D	< 70%	< 70%	< 30%

Table 5: Criteria for certifying the Microscopy level

6. Blinded rechecking

Blinded rechecking refers to the process by which a certain percent of the slides collected from the PL is re-examined by the NMRL. It determines whether a laboratory is providing accurate results and detects errors in laboratory performance due to competency of microscopist, faulty equipment, poor quality reagent and consumables. In this process, slides are cross checked for

- Quality of blood film prepared
- Quality of staining
- Concordance of the result

At the end of each round of rechecking process, feedback, including parameters score and recommendation on quality improvement is provided.

6.2. Design of blinded rechecking

Blinded rechecking slides received from PL are re- examined monthly by NMRL. All slides received must be treated as blinded and is re-checked by first controller. If any discrepancy is observed, the slide is submitted for further recheck by a second controller.

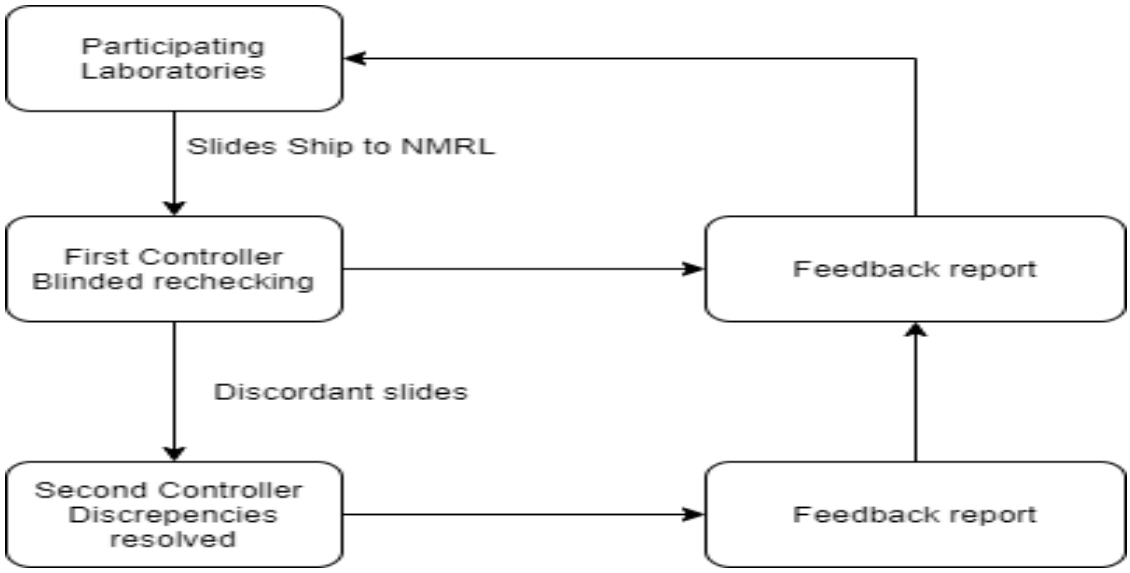


Fig: 3 Blinded re-checking flowcharts

6.3. Storage of slides at PL

The routine slides examined by PL should be stored systematically in a slide box as per the Blood examination serial (BES) number. Before storing, immersion oil must be completely removed from the slides by placing the smeared side on a piece of tissue paper overnight. All the stored slide box must be placed away from direct sunlight to protect it from excessive heat. To protect slides from humidity, place desiccant or silica gel inside the slide box.

6.4. Systemic slide selection techniques

To avoid selection bias, slides for rechecking must be selected from the laboratory register and not directly from the slide storage boxes. Minimum of 10% negative slides must be selected by PL as per the SOP and sent to NMRL for re-checking on monthly basis. This is done for the evaluation performance on quality of blood films prepared and quality of stain. On the other hand, all the positive slides should be sent to NMRL for evaluation of discordance agreement on parasite detection, species identification; stages and parasite density.

6.5. Slide shipment

All the slides selected for blinded rechecking must be packed in a slide box or a slide mailer depending on number of slides with adequate adsorbent tissue. Ship the slides to the NMRL on monthly basis within the first week of the next month. For example, the cross-checking slide for the month of January should be sent within the first week of February. PL must submit cross checking form, both form A and B separately along with the cross-checking slides.

6.6. Slide rechecking

The first controller at NMRL must re-examine (blinded) the slide as per the SOP for blinded rechecking. The slides must be cross-checked for the presence or absence of the parasites. In case of a positive finding, the parasite species and stages must be identified and determine the parasite density. Additionally, the MP slides must be checked for the quality of the film and stain. The controller should note all the errors identified in the worksheet and use this information to provide feedback to PL.

6.7. Analysis of results and feedback:

Once the slides have been re-checked and information recorded, the results are compared to the initial PL results. If any discrepancy is observed, the slide should be further rechecked by a second controller. The false positive, false negative, percentage agreement on species identification, stages identification, parasite density, quality of blood film and quality of staining will be recorded to calculate the overall performance.

The NMRL issues a performance report, as part of feedback, to all the PL through email. For any discrepancy result observed, NMRL should provide additional feedback, including likely explanations for the discrepancy and suggestive corrective actions. Any discrepant results and corrective action undertaken should be documented by PL and made available for review during the supervisory visit.

6.8. Types of Error:

Rechecking is not a method for validating individual patient diagnosis, but rather of assessing overall laboratory performance, detecting unacceptable levels of errors so that corrective action can be taken and providing continuous motivation for good performance.

Results of controller	Results being reported by PL	Error type
Negative	Positive	Major error (False positive)
Positive	Negative	Major error (False negative)
Pv	Pf	Minor error (Identification error)
Pf	Pv	Major error (Identification error)
PC	PC count not within +- 50%	Wrong PC determination

Table 6: Types of error identified while cross checking

6.9. Discrepant results:

Discrepancies between the result of PL and the results of the first controller should be resolved by a second controller. The result of the second controller is considered "final" and establishes whether the error was made by the PL or first controller. If in case the PL disagree on both controller's findings, opportunity should be provided to the PL for molecular analysis. Once the molecular analysis is performed, its findings must be considered as the final result.

6.10. Evaluation and interpretation of blinded rechecking:

		Reference laboratory		
		Positive	**NMPS**	**Total**
Participating laboratory	**Positive**	A	B	A+B
	NMPS	C	D	C+D
	Total	A+C	B+D	A+B+C+D

Table 7: Result Recording as Positive or Negative on 2 x 2 table format

- A= number of slides reported as positive by both laboratories (True positive)
- B= number of slides reported as positive in routine testing by the laboratory but found to be negative by the cross checker (False positive)
- C= number of slides reported as negative in routine testing by the laboratory but found to be positive by the cross checker (False negative)
- D= number of slides reported as negative by both readers (True negative)

6.11. Results are analyzed as

- **Sensitivity:**

  Sensitivity of the panel testing is proportion of true positive among the panel slides

$$\text{Sensitivity} = \frac{TP}{(TP + FN)} \times 100$$

- **Specificity:**

  Proportion of the panel slides which are true negative among the panel slides

$$\text{Specificity} = \frac{TN}{(TN + FP)} \times 100$$

- **Percentage slide agreement**:

  This refers to assessment of the results on recognition of the slides with positive or negative findings for the presence of malaria parasite.

$$\text{Percentage slide agreement} = \frac{TP + TN}{(TP+ FP+ FN+TN)} \times 100$$

- **False positive rate (% False positive):**

  Percentage of negative slides that are misread as positive

$$\text{False positive rate (\% false positive)} = \frac{FP}{(TP+ FP)} \times 100 = \frac{B}{(A +B)} \times 100$$

- **False negative rate (% False negative):**

  Percentage of positive slides that are misread as negative

$$\text{False positive rate (\% false positive)} = \frac{FN}{(TN+ FN)} \times 100 = \frac{B}{(C +D)} \times 100$$

		Reference laboratory		
		P. falciparum seen	***P. falciparum not seen***	**Total**
Participating laboratory	***P. falciparum seen***	A	B	A+B
	P. falciparum not seen	C	D	C+D
	Total	A+C	B+D	A+B+C+D

Table 8: Result recording for the accuracy of the differentiation of Pf and non- Pf

- A= number of slides reported as containing *P. falciparum* (either as a single or mixed infection) by both readers

- B= number of slides reported as *P. falciparum* seen by the participating laboratories but *P. falciparum* was not seen by the reference laboratory (incorrect species identification)
- C= number of slides reported as *P. falciparum* not seen by the participating laboratory but seen by the cross checker – reference laboratory (incorrect species identification)
- D= number of slides reported as *P. falciparum* not seen by both readers

Notes:

1. *For specific species, % agreement is calculated from only positive slides reported by the facility*
2. *Species identification % agreement can be calculated for all malaria parasite species including mixed infections*

$$\% \text{ Species identification agreement} = \frac{A + D}{(A+B+C+D)} \times 100$$

6.12. Grading of performance on blinded rechecking

Grade	% Slide agreement	Action
Excellent	≥ 90 %	• Congratulate staff for exemplary performance
Very Good	80<90 %	• Staff should be congratulated for very good performance and told to maintain their performance • Identify any breach for improvement
Good	70<80 %	• Staff should be congratulated for good performance and the need for 'further improvement' • Conduct on site supervision • Check staff competency • Check reagent quality and the microscope • Consider on the training based on staff weakness
Poor	≤ 70%	• Staff should be informed of poor performance and the need for 'immediate action for improvement' • Conduct on site supervision • Check staff competency • Check reagent quality and the microscope • Consider on the training based on staff weakness • Regular follow up for corrective action

Table 9: Grading performance of slide rechecking cycle

Notes:

1. *'Error' stand for any positive result reported as negative, or any negative result reported as positive.*
2. *Any EQA performance persistently static or a progressive decreasing pattern in the percentage agreement is an alarming sign that indicates the corrective action has not been effective and should be reviewed immediately.*
3. *Any EQA performance above the previous once is encouraging and still needs follow ups.*

7. On- site supervision

On-site supervision is the ideal way to obtain a realistic assessment of the skills practiced in the testing laboratory/facility, to provide problem solving strategies and corrective action, and assess the need for training. The supervision includes assessment of test performance, provision of on-site training and strengthening of services. To assess the performance of malaria microscopy and RDT by PL, periodic supervisory visits from NMRL is essential. This is done to obtain a realistic picture of laboratory conditions and practices for malaria microscopy and RDT use. On-site supervision for malaria microscopy and RDT should include a comprehensive assessment of laboratory organization, equipment, adequacy and storage of supplies, reagent quality, availability and usage of SOPs, reading and reporting of results and infection control measures using a standard supervisory checklist (Annexure).

The on-site supervision is conducted once a year by NMRL staff during which sufficient time is allotted for the visit to include observation of all the work associated with malaria microscopy, including preparing films, staining, reading of films by the laboratory personnel and examining a few stained *positive* and *negative* films by supervisors to observe the quality of film preparation and staining as well as condition of microscope.

On-site feedback must be provided for corrective action and additional resources required should be recommended to respective health facility. A consolidated summary report must be submitted to concerned health center for action.

7.2. General activities to be considered for on-site supervision (SOP)

- Make a schedule for the site visits
- Prepare necessary materials like the checklist and feedback report
- Conduct on site supervision
- Review the previous site supervision feedback (if available)
- Provide EQA feedback, investigate any poor performance, corrective action and follow up

7.3. Procedure for on-site supervision of laboratories for malaria microscopy

7.3.1. Task to be done by supervisory team:

Based on M & S checklist, laboratory performance is evaluated on the following parameters

- Laboratory management
- Infrastructural and facilities
- Supply of equipment and lab consumables

- Equipment maintenance
- Management of laboratory reagent and test kits
- Internal quality system
- External quality system
- Infection control and waste management
- Checklist for performance evaluation of laboratory for competency includes
- Competency for on-site examination of MP panel slide
- Observation of procedure on malaria diagnosis

Malaria RDTs Lot Testing Programme:

As a complement to malaria RDTs which are all WHO pre-qualified product, NMRL carried out assessment of the diagnostic performance of RDTs. It is designed to detect lots of RDTs that perform poorly before they are sent to and used in the field and sometimes to verify unexpected or unusual rates of negative test results reported from the field.

WHO recommends that all RDT should be checked either before or after shipment. Lot testing ensures that diagnostic products that supplied to health centers meet performance expectations and, if lot testing is conducted after shipment, that RDT performance has not been adversely affected during transport.

Malaria RDTs are affected by various conditions of manufacture, storage and use can impair their accuracy and reliability.

To provide guidelines for the testing of RDTs using Quality Control (QC) samples to assess whether the sensitivity and specificity of the RDT batch is acceptable for use in the field.

According to WHO- FIND protocol, eligible products should meet the minimum criteria recommended as :

- For the detection of P. falciparum in all transmission settings, the panel detection score against P. falciparum samples should be at least 75% at 200 parasites/µL.
- For the detection of P. vivax in all transmission settings, the panel detection score against P. vivax samples should be at least 75% at 200 parasites/µL.
- The false-positive rate should be < 10%.
- The invalid rate should be < 5%.

Flow diagram of immediate RDT QA procedure

1. Quality control selection

Malaria RDTs received for lot testing will be tested for quality checking using quality control panel prepared from wild type malaria positive control.

1.1. All the RDTs of same lot are tested with use of QC sample consists of Pf, Pv and Negative control.

1.2. QC sample for both Pf and Pv is diluted at 200 and 2000 p/ul.

2. Requirement of RDT for initial and long-term lot testing:

A total quantity of 69 RDTs (41 + spare 28) from each lot of supply is required for the complete QA testing including spare RDT-kits as shown below;

Immediate QA	13 RDTs
3 months	7 RDTs
6 months	7 RDTs
9 months	7 RDTs
12 months	7 RDTs
Total	41 RDTs plus spare kits (Minimum 28 RDTs)

3. Lot testing procedure:

3.1. Request form

To apply for lot testing, the requester (participating laboratories) completes a lot testing request form and sends it by e-mail / in hard copy to the lot-testing laboratory.

3.2. RDT sample

It is recommended that all purchased lots be tested to ensure that they perform well. The number of sample RDTs required for lot testing depends on the type of RDT and the expiry date of the product. Usually, a sample of 100 P. falciparum-only RDTs or 150 combination P. falciparum and pan-specific (or P. vivax-specific) RDTs is required from each lot. Random sampling of RDTs from different parts of the pallets is the recommended sampling technique.

3.3. Lot testing

Upon receipt of RDTs, a rapid initial assessment is made against panels of high- and low-density parasite-positive and parasite-negative blood. These reference panels are prepared according to the same standard operating procedures and have similar characteristics to the panels used in the product testing programme. The remaining RDTs are stored under controlled conditions at 37 °C and are retested every after 3 months.

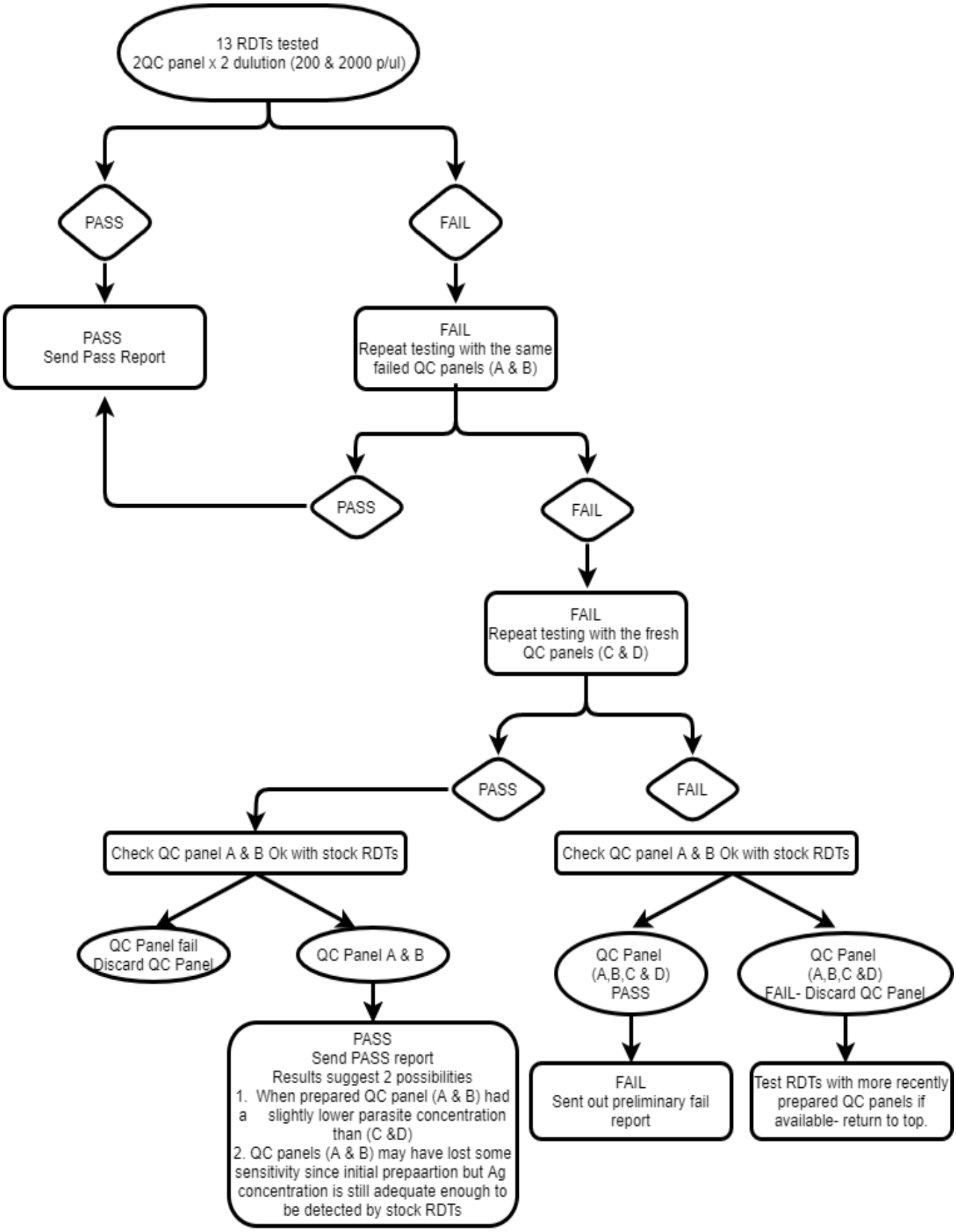
3.4. Reporting of lot testing results

A malaria RDT lot-testing quality control report is generated, with a guide for interpreting observations, and is sent by e-mail to the requester, usually within 7 working days of receipt of the RDTs at the lot-testing laboratory. If test anomalies are

detected, the photographs of the test results may be sent with the final report.
Lot testing is performed against a smaller panel of parasite-positive and parasite-negative samples than product testing. Therefore, lot testing is not designed to detect small differences in RDT performance but to detect major deficiencies in a production lot, including the device and/or buffer. Because of the small size and variable antigen concentration in samples of the same parasite density, RDTs that fail initial testing are assessed against reference stock RDTs (high quality) and against another sample.
If there are no failures, a "pass" report is issued. If the RDT fails confirmatory testing, a "fail" report is issued.

- **Pass.**
  The tested RDTs detected antigens at a threshold sufficient for clinical use in the field. The corresponding RDT lot is considered to have passed the quality control assessment.
- **Fail**
  The tested sample RDTs failed the initial quality control assessment and also failed confirmatory testing at the lot-testing centre. It is recommended that this particular RDT lot not be used in the field.

4. Algorithm for lot testing of malaria RDTs:



Annexure 1: MP Panel Testing Reporting form

National Malaria Reference Laboratory

Royal Centre for Disease Control

National External Quality Assessment Scheme in Malaria Diagnosis

MP Panel Slide Test Reporting Form

Panel Round: …… **Date of received (DD/MM/YY): ……. / ……. /.....**

Test parameters (Microscopy result)	Test Results				
	Slide No.				
Plasmodium species					
Plasmodium Stages					
N0. of Parasite count					
No. of WBCs counted					
Parasite Density					

Name of the Health Centre: …… **Date of examination (DD/MM/YY): …. / ….. / …..**

Examined by: …….... **Designation: …….**

Name and signature of the Head of Healthcare Centre: ……

(Note: To be used by the participating laboratory to report the findings of the MP panel testing)

Annexure 2: MP Panel Testing Feedback form

National Malaria Reference Laboratory

Royal Centre for Disease Control

National External Quality Assessment Scheme in Malaria Diagnosis

MP Panel Testing Feedback

Name of participant: …….. Designation: ……

Health Center: …… Panel round: ……

Parameters	Score
Sensitivity	
Specificity	
% of the agreement (malaria infection detection)	
% of the agreement (parasite species identification)	
% of the agreement (parasite stages identification)	
% of the agreement (parasite density calculation)	
Average score	

Recommendation:

Panel Rechecked by:

Controller 1

NMRL, RCDC

Report verified:

Controller 2

NMRL, RCDC

Annexure 3: MP Slide Cross checking form- A (Confidential)

National Malaria Reference Laboratory

Royal Centre for Disease Control

National External Quality Assessment Scheme in Malaria Diagnosis

MP slide cross-checking form

Name of the Health Centre: ..... **Dzongkhag: .....**

MP cross-check slides for the month of: .....… **Year: .....**

MP cross- check slides shipped by: ..... **Designation: .....**

A total number of MP slides screened: …… **Microscopy: ……. RDTs: ……**

- **Total Positive slides: ….....**
- **Total Negative slide: ….....……..**

Participating laboratories results

B/S No.	Date of collection	Result										
		Microscopy results				Parasite stages observed				No of parasite count	No of WBC count	Parasite density
		NMPS	Pf	Pv	Mix	Ring	Troph	Schizont	Gametocyte			

To be completed by NMRL, Royal Centre for Disease Control

Date of received (DD/MM/YY): ……..... / ……..... / ……...

A total number of slides received: ……...…….....

A total number of slides missing: ……...…….....

Received by: .....

(Name & signature)

Annexure 4: MP Slide Cross checking form B

National Malaria Reference Laboratory

Royal Centre for Disease Control

National External Quality Assessment Scheme in Malaria Diagnosis

MP slide cross-checking form

Name of the Health Centre: ..... **Dzongkhag: .....**

MP cross-check slides for the month of: ..... **Year: .....**

MP cross-check slides shipped by: ..... **Designation: .....**

A total number of MP slides screened for the month: …….

Participating laboratories results

BS no	Date of collection	Remarks

To be completed by National Malaria Reference Laboratory

Date of received (DD/MM/YY): …….. / ……. / ……..

A total number of slides received: ……...…….....

A total number of slides missing: ……...…….....

Received by: .....

(Name & signature)

Annexure 5: MP Slide Cross checking feedback form

National Malaria Reference Laboratory

Royal Centre for Disease Control

National External Quality Assessment Scheme in Malaria Diagnosis

Monthly MP slide cross-checking feedback

Name of health centers: ……. **Month: …….**

Parameters	Score
Sensitivity	
Specificity	
% of the agreement (malaria infection detection)	
% of the agreement (parasite species identification)	
% of the agreement (parasite stage identification)	
% of the agreement (parasite density calculation)	
% of the agreement (film quality)	
% of the agreement (stain quality)	
Average score	

Comment or recommendations:

Reported by:

National Malaria Reference Laboratory,

Royal Center for Disease Control.

Annexure 6: Checklist for monitoring and supervisory for laboratories

National Malaria Reference Laboratory

Royal Centre for Disease Control

National External Quality Assessment Scheme in Malaria Diagnosis

Checklist for monitoring and supervisory

Name of the Health Centre: …….

Name & Designation of Lab In charge: …… **Date of visit: …… / …… / …….**

Staff Details:

Name of staff	Designation	Training related to malaria			
		Frequency	Days	Last date	Topic

General condition of Laboratory:

YES: 1, NO: 0

S.no	Checklist Items with categories	Rating		
		Yes	No	Remarks
	1. Organization and Management			
1.1	**Organization:** Laboratory have organization and management structure			
1.2	Laboratory conducts staff coordination meeting			
1.3	Laboratory maintain minutes on every meeting and compile in designated file			
1.4	Is there appropriate platform for information sharing for rapid communication			
1.6	**Internal audit:** Laboratory conducts internal audit laboratory performance on malaria diagnosis.			
1.7	Laboratory maintains record of internal audit and file in designated file.			
1.9	Capacity Development Laboratory conducts and organizes CME on malaria diagnosis.			
1.10	Laboratory maintained record on CME conducted			
	2. Laboratory Design			
2.1	**Availability of infrastructure:** Is working area spacious with adequate Slabs / working bench to carry out lab work.			
2.2	Washbasin / sink			

2.3	Designated are for staining of malaria slides			
2.4	Storage space for supplies and materials			
2.5	**Availability of Facilities:** Adequate furniture's, A/C & fans, computers with internet connection			
3. Equipment, lab consumables & reagents				
3.1 Microscope:				
3.1.1	No of microscope available in laboratory			
3.1.2	No of functional microscope in laboratory			
3.1.3	Proper set up of the microscope (stable bench away from staining area and vibration producing equipment)			
3.1.2	The microscope lamp has sufficient power to provide good illumination when set at x100 objective			
3.1.6	Maintenance logbook for microscope available in the lab			
3.1.7	Are any maintenance and cleaning activities recorded in Microscope logbook			
3.2 Microscope Slides				
3.2.1	Microscope slides are of good quality, free from scratches or surface aberrations			
3.2.2	Transparent with adequate thickness			
3.2.3	Microscopes slides do not have fungal contamination			
3.2.4	Microscope slides are thoroughly cleaned before use.			
3.2.6	In areas with high humidity, microscope slides are protected against fungal contamination			
3.3 Staining reagents				
3.3.1	All required staining reagent are available Giemsa stain & Buffered water / Distilled water			
3.3.2	All staining reagents are within the recommended expiry date			
3.3.3	Staining solutions are stored as per the manufacturer's recommendation / SOP			
3.3.4	SOPs are available for preparation of working stain solution			
3.3.5	Laboratory performed quality control on stain			
3.3.6	Troubleshoot staining problem			
3.4 Lab consumables				
3.4.1	**Supply:** Adequate supply of laboratory logistics related to malaria microscopy			
3.4.2	**Laboratory inventory maintained** Goods received and use of lab consumables			
3.4.3	**Methanol:** Analytical grade with acetone free			
3.4.4	**Immersion oil:** Supplied of good quality recommended for microscopy			
3.4.5	**Microscope cleaning:**			
	• Gauze / tissues for cleaning slides			
	• Lens cleaning paper			
3.4.6	**Microscopy staining accessories:**			
	• Staining rack / Suitable staining jar			

	• Drying rack			
	• Timer			
	• Slides boxes for storage			
	• Reagent bottles			
	• pH meter or litmus paper			
	• Tally counter			
	• Filter paper			
3.5 Malaria RDTs				
3.5.1	RDT supplied is of good quality control and is of WHO pre-qualified product			
3.5.2	Internal QC is performed for each box is being removed for use			
3.5.3	Adequate supply of malaria RDT kits within the expiry dates- (Update on test kits)			
3.5.4	Arrange & usage of test kit in the order of expiry dates (FIFO)			
3.5.5	Does laboratory maintain the room temperature log			
3.5.6	All the temperature log is daily recorded			
3.6 Anti-malarial Drugs				
3.6.1	Adequate supply of antimalarial drugs available in store / pharmacy unit			
3.6.2	All the antimalarial drugs are within the expiry dates			
4. Equipment Maintenance				
4.1	**Temperature monitoring:** Documentation of daily temperature monitoring of refrigerators (if using) and testing area available			
4.2	All the temperature log is daily recorded			
5. Internal Quality Control system				
5.1 SOPs and Guidelines				
5.1.1	Written Sop for malaria diagnosis is available in lab			
5.1.2	SOPs is followed strictly and implemented			
5.2 QC for staining				
5.2.1	Is the giemsa working stain solution freshly prepared before each staining (within 4 h)			
5.2.2	Is freshly prepared giemsa working solution is filtered before each staining			
5.2.3	Are the solution and chemical bottles kept in cool, dry place and away from sunlight?			
5.2.4	Is the stock Giemsa stain bottle properly closed when it is not in use (Screw tight)?			
5.2.5	Is buffered water / Distilled water pH 7.2 (+- 0.2) used to dilute the giemsa stain			
5.2.6	Is giemsa working solution used is diluted in correct dilution.			
5.2.7	Is internal QC performed regularly with known positive and negative slides during staining.			
5.3 QC for Blood Film Preparation & slides reading				
	Quality of thick Smear:			

5.3.1	Adequate size (1 cm – diameter) with circular shape			
5.3.2	Is thickness of the smear (new paper readable) is correct.			
5.3.3	Visibility of labels			
5.3.4	Smear are not washed off			
5.3.5	**Quality of Thin smear:** Shape of thin smear			
5.3.6	Length of the thin smear			
5.3.7	Blood smear are of a correct thickness			
5.3.8	**Slide reading time:** Examine a minimum of 10 minutes per slide,			
5.3.9	Examines a minimum of 100 fields before reporting negative smears			
5.3.10	**Test result:** Do you report the MP result in species?			
	Do you report MP result in parasite's stage?			
	Do you report the parasites count in the result?			
5.3.11	Are examined slides stored and archived properly			
6. External Quality assessment Scheme				
6.1	Does the laboratory timely submit the slides for blinded cross checking			
6.2	Does laboratory participate in malaria proficiency testing scheme			
6.3	Does laboratory undertaken the corrective action based on the feedback report provided by reference laboratory • Blinded rechecking			
	• Panel testing			
	• On-site monitoring and supervision			
7. Documentation				
7.1	Are technical manuals and bench aids available in the laboratory			
7.2	Are equipment maintenance logbook available in the laboratory			
7.3	Are internal QC log sheet available in the laboratory • QC for stain			
	• QC for lab Consumable and test kits			
7.4	Are blinded rechecking feedback report is documented and file properly in record file			
7.5	Are panel testing feedback report is documented and file properly in record file			
7.6	Are onsite monitoring and supervision feedback report is documented and file properly in record file			
7.7	Record on corrective action on error is performed and recorded properly in the file			
7.8	System of filing the test kit /reagent leaflet in maintained record file			
8 Infection Control and Waste Management				
8.1	**Infection control information:** Is national guideline available for infection control?			
8.2	Posters or signs to discourage people entering the laboratory in place			
8.3	Is there a hand washing poster in front of the washbasin?			
8.4	infectious and non-infectious waste clearly labelled			

8.5	**Hand washing System:** Availability of hand rubs solution in all appropriate place			
8.6	**Decontamination of work area**: Decontamination log for each work area; right and appropriate concentration of decontaminating agent used			
8.7	**Personal Protection:** All relevant personal protective equipment available and used			
8.8	**Segregation of laboratory wastes:** Colour coded waste • bins for sharps, • non-infectious and • infectious wastes			

Name and signature of the evaluator:

**Date (DD/MM/YY): ……..... / ……..... / ……..... **

Annexure 7: Checklist for on-site evaluation for participants

National Malaria Reference Laboratory

Royal Centre for Disease Control

National External Quality Assessment Scheme in Malaria Diagnosis

Checklist for on-site evaluation and monitoring of participants

Name of the Health Centre: …….. Department/Unit: ……..……

Name & Designation of participant…….. Date of visit: …….

A number of years in service: …….. No of malaria training/workshop attended: ……..

Date of the last training/workshop attended: ……..

	B: Participants Technical Performance			
S.no.	**Checklist**	**Yes**	**No**	**Remarks**
	1. Competency in General Knowledge or Procedures			
1.2	**Blood collection procedures:** • **For venous blood** 1. Selection of the correct sites 2. proper disinfection of the venepuncture sites 3. correct use of tourniquets 4. correct venipuncture angle 5. correct blood volume 6. proper dispensing of the blood			
1.3	**Film preparation:** 1. Correct technique 2. adequate size 3. shape and thickness of the film 4. visibility of the labels			
1.4	**Staining procedures:** 1. Adequate and proper air drying 2. proper fixation 3. correct steps of staining 4. correct timing 5. free of precipitates and artefacts			
1.5	**Knowledge of QA/QC:** 1. The adequate concept of IQC and EQAS 2. Sensitivity 3. Specificity 4. false positive 5. false negative			
1.6	**Knowledge of RDT:** 1. RDT principle 2. test performance 3. test interpretation			
	Competency in on site malaria reference slide examination			
	Total no. of panel slides…… No. of positive panel slides…… No of negative panel slides……..			
S. no	**Checklist**	**Score**		Remarks
2.1	Sensitivity			

2.2	Specificity			
2.3	% of agreement- malaria detection			
2.4	% of agreement- parasite species identification			
2.5	% of agreement- parasite stage identification			
2.6	% of agreement- parasite density calculation			
Overall performance %				

Name & Signature of the Evaluator

Note: For final rating, the average performance percentage will be calculated