Malaria Diagnostics - Functions of the malaria reference laboratory in SADC Regions

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Welcome to the Functions of the malaria reference laboratory in SADC Regions Module
Module Objectives

At the end of this module, participants will be able to:

➢ List the activities necessary for a state of the art malaria diagnostic reference laboratory;
➢ Discuss the differences between routine diagnostic lab support vs. elimination support;
➢ Discuss the methods used for malaria diagnostics;
➢ Describe the advantages and disadvantages of routine laboratory methods for malaria diagnosis;
➢ Discuss specialized laboratory techniques related to elimination activities.
At the end of this module, participants will be able to:

- Discuss the importance of counting malaria parasites and the differences in parasite counting on thin blood films vs. on thick blood films;
- List the aims of EQA (external quality assessment)/PT (proficiency testing) programmes;
- Discuss the need for malaria slide banks;
- Discuss test validation and test evaluation;
- Discuss the importance of training and training requirements.
Malaria reference laboratory: diagnosis

Routine diagnostic lab support
- Clinical service
  - Training
  - EQA/PT schemes
  - Slide bank
  - Slide cross-checking programmes
  - Test validation

- Conventional PCR
  - Quality systems
  - Test evaluation
  - RDTs, Microscopy

Elimination support
- Surveillance
  - Field/near field diagnostics e.g. LAMP
  - Supersensitive molecular methods
  - Serology in low-transmission areas
  - Drug sensitivity testing

- National Health Laboratory Service
Purpose
- Detection, identification, enumeration, and sometimes, drug resistance testing of malaria parasites
- Other blood parasites, incidentally/simultaneously
- For clinical care, research, and surveillance for control programmes

Methods
- Microscopy
- Rapid antigen diagnostic tests (RDTs)
- Molecular methods
- Serology
Advantages and disadvantages of routine laboratory methods for malaria diagnosis

<table>
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<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>Microscopy of Giemsa-stained smears</td>
<td>- Cheap &lt;br&gt; - Accurate species identification &lt;br&gt; - Accurate parasite load determination &lt;br&gt; - Detects other blood parasites e.g. filariae, trypanosomes</td>
<td>- Requires intense training and skill &lt;br&gt; - Time-consuming &lt;br&gt; - Technically demanding &lt;br&gt; - Limited sensitivity</td>
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Advantages and disadvantages of routine laboratory methods for malaria diagnosis (continued…)

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<td>Rapid antigen tests</td>
<td>- Rapid&lt;br&gt;- Adequate sensitivity compared with average microscopy result&lt;br&gt;- Unskilled persons can be taught to use, with adequate training</td>
<td>- Expensive, relative to slides&lt;br&gt;- Some <em>P. falciparum</em> strains produce variant or no HRP-2, causing false-negatives&lt;br&gt;- False-positives may occur in autoimmune diseases&lt;br&gt;- Pan-specific versions’ sensitivity may be low&lt;br&gt;- Quality varies substantially between manufacturers&lt;br&gt;- Temperature stability is a frequent problem&lt;br&gt;- Only detects malaria&lt;br&gt;- HRP-2 persists after successful treatment – can’t monitor treatment outcome&lt;br&gt;- Prozone problems – rare but real&lt;br&gt;- Parasite load quantitation not possible</td>
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## Advantages and disadvantages of routine laboratory methods for malaria diagnosis (continued…)

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| PCR and other nucleic acid amplification (NAA) methods | - Highly specific and sensitive  
- Can cater for multiple species  
- Real time quantitation possible  
- Scalable and automatable  
- Ultra-high sensitivity in some versions (e.g. 50 x conv. PCR, 2500 x microscopy) | - High capital cost  
- Time-consuming unless automated  
- Not suitable for small labs or in the field  
- Contamination potential  
- Lack of standardisation  
- Performance may be constrained by type of specimen e.g. DBS  
- Fashionable, but not always feasible |
NICD Microscopy

NICD microscopy:
431980(1), 431980(2), 432692: *P. vivax* or *P. ovale*
432736: negative

Sender disputed results: claimed all *P. falciparum*
Traditional vs. molecular diagnosis: relative importance?

- WHO recommends:
  - Quality-assured RDTs and microscopy are primary diagnostic methods in all epidemiological settings
  - NAA methods only appropriate in low transmission settings** (<10% prevalence), and should not divert resources from routine diagnosis, control and surveillance
  - NAA for submicroscopic infections useful in epidemiological research and special interventions
  - Special NAA methods to detect gametocytes are not necessary for routine control or surveillance, but may be useful in special studies
  - Standardisation and EQA of NAA methods is required
  - Standardisation of serological methods is required

*Report available on the WHO-GMP website at the following URL: www.who.int/malaria/mpac/mpac_mar2014_diagnosis_low_transmission_settings_report.pdf

High and Low Transmission Setting

High transmission setting

Low transmission setting
LAMP Assay

- Loop-mediated isothermal amplification
- 4 primers (2 outer, 2 inner) therefore very specific (2 additional loop primers can speed up the reaction)
- Polymerase has strand-displacement activity
- Constant temperature means no thermal cycler needed, just a water bath/heating block
- Potential for field or near-field use
- Different detection methods (turbidity, fluorescence)
- Less prone to inhibitors than PCR
- DNA is amplified $10^9$-$10^{10}$ times in 15-60 minutes
LAMP Assay (continued…)
Standard Procedures using LAMP Method

1. Prepare master mix.
2. Add DNA/RNA samples
3. LAMP amplification
4. Detection
   - visual detection (by fluorescence)
   - real-time turbidity detection

DNA/RNA extraction from samples

Samples

DNA/RNA extraction

Amplification

Detection

(Reference time)

About 30 min

About 60 min
Where transmission intensity is low, usual lab methods are too insensitive to detect all infections

Low frequency of positives = logistical difficulties

Serology is more sensitive in highly seasonal transmission areas, as antibodies persist

Can assess transmission intensity and changes in transmission

Therefore, assess progress towards elimination
Drug Efficacy Monitoring

- WHO recommends regular drug efficacy monitoring
- The gold standard is a clinical trial – but trials are expensive and labour intensive
- A more cost-effective, less labour-intensive method is the routine surveillance for validated molecular markers of antimalarial drug resistance
- All that is required are either dried filter paper blood spots or used positive RDTs
- Parasite DNA is extracted and subjected to molecular analysis to detect markers associated with chloroquine, SP and lumefantrine resistance

Spread of Chloroquine resistance
Enumeration: Why count malaria parasites?

- Severity indicator
- Response to treatment
- High transmission areas: clinical assessment of fever
- Case definition for clinical drug or vaccine trials e.g. fever + 2500 parasites/μl
In falciparum malaria, parasitaemia is one indicator of likely severity of infection.

- Correlates roughly with clinical features and prognosis.
- >4 or 5% parasitaemia or > 250,000 parasites/µl: risk of severe malaria.
- Low parasitaemia does not exclude severe disease!
Parasite Counting

On thin blood films

- This method is based on the proportion of infected red blood cells on the thin blood film.
- Expressed as percent of red blood cells infected.
- 3 different methods: graticule, continuous RBC estimation, RBC multiplication factor (RMF)

On thick blood films

- This method is based on the number of asexual parasites on the thick blood film counted against the patient’s white cell count (WCC).
- When the patient’s WCC is not known an average of 8 000 may be used.
- Expressed as parasites / μl of blood.
- Parasite counts on thick blood films are not commonly done in SA.
Counting

Continuous estimation

Graticule: Miller squares

Thick film counts

Move on to the next acceptable field and repeat the steps above. For high parasitaemias (>2 parasites per field) – continue until a minimum of 2000 RBCs have been counted. For low parasitaemias (~1-2 parasites per field) – continue until a minimum of 4000 RBCs have been counted. Note: the more RBCs counted, the more accurate the count!

Example: HPF number | RBCs in HPF | Infected RBC
--- | --- | ---
1 | 308 | 15
2 | 321 | 18
3 | 306 | 16
4 | 297 | 15
5 | 301 | 11
6 | 323 | 13
7 | 306 | 10
8 | 2162 | 96

No of RBC in small square: 5X10 = 50 RBC in large square.
No of infected RBC in large square: 8
Don’t count this cell
Quality Assurance systems in the malaria laboratory

Definitions

- Quality: meeting specified standards
- Quality assurance: monitoring entire process to ensure that required standards are met
- Quality control: process of checking the performance of reagents, supplies and equipment to ensure required standards are met
- Quality assessment: evaluation of quality, objectively and ideally, independently
Components of Quality Assurance

- Staff training and competency
- SOP and document control
- Specimen logging, work flow, processing
- Reagent preparation and quality control
- Testing, reading, reporting results
- Equipment maintenance
- Test validation, audits, corrective actions
- EQA/PT scheme participation
- Slide cross-checking programmes
What is External Quality Assessment (EQA) or Proficiency Testing (PT)?

- An objective measure of the best quality performance of a laboratory
- Performed by an organisation outside of the laboratory being assessed i.e. an independent evaluation
- Usually involves panels of slides sent to blinded participants
- Can be applied to any diagnostic method
Aims of EQA/PT Programmes

- To build laboratory capacity, and thereby improve clinical care and public health
- To obtain an objective measure of the diagnostic ability of participants
- To improve scientific knowledge and give credibility to surveillance data, clinical trials and other research
- To assist laboratories to comply with accreditation requirements

Trend in performance in blood smear microscopy, NHLS proficiency testing programme, 2004-2010
\( n = 134 - 242 \)
Standard slide archive is needed for the continuous training and assessment of malaria microscopists and laboratories.

A national reference laboratory should set up a slide bank of well-characterised malaria blood films to support in-country training and assessment programmes.
Test Validation

- Validation (verification) of performance of diagnostic products is mandatory
- Necessary to confirm performance claims in a specific setting e.g. an individual laboratory
- Ascertain whether there are local factors that influence the quality of results
- Applies to all new tests; also when current test reagents or methods change
- Usually involves comparison using a sufficient number of reference samples
Comparison between samples identified using multiplex PCR and light microscopy

Figure 1: Comparison between samples identified using multiplex PCR and light microscopy. NPS = no parasites seen.

Discussion
The results show that the multiplex PCR is reproducible because three laboratories were able to run the same assay and get the same results. Dried blood spots and whole blood were validated as legitimate sample types for this assay. Two different DNA extraction methods, one manual (Qiagen DNA mini kit), the other automated (Roche MagNA Pure Compact), have both been validated for use on whole blood.

There was nine percent non-concordance between *Plasmodium* identification by microscopy (the gold standard) and the results from the multiplex PCR. Of these disparate results, two
General assessment of quality and suitability of a test or product for a certain purpose, for example:

- Batch QC testing of RDTs before release could be a reference lab function
- Evaluation of RDTs for national tender, to decide on a particular product
- Evaluation of new methods or equipment for national control programmes or lab services e.g. new tests, microscopes
Training

- Technical – technicians/technologists
  - Microscopist certification
  - Clinical, routine laboratory
  - Control programmes
  - Surveillance

- Research
  - Applied, practical
  - Postgraduate students
Training Requirements

- Finances
- Suitable trainers
- Venue
- Equipment
- Consumables
- Disposables
- Slide bank
Debate the statement below. You will be divided into 2 groups with opposing viewpoints. Each group will have 10 minutes to prepare and 5 minutes for its spokesperson to present the case, then there will be a brief general discussion.

‘Malaria microscopy is old-fashioned and outdated. It should be replaced by molecular methods as a matter of urgency.’
A national malaria reference laboratory supports functions in diagnostic and surveillance areas of a malaria control programme.

Some of these functions are common to both areas, i.e. providing lab tests (microscopy, RDTs, PCR), quality systems, test evaluation.

Other reference lab functions in the diagnostic area are:
- Administering proficiency testing and slide rechecking schemes
- Setting up and administering malaria slide banks
- Training
- Test validation at bench level

Other reference lab functions in the surveillance area are:
- Supporting diagnostics for epidemiological surveys
- Providing drug efficacy monitoring through sensitivity testing
- Providing supersensitive nucleic acid-based diagnostics when required e.g. in low transmission situations, elimination phase
- Likewise, serological testing in low transmission situations might be appropriate
References & Links

Links

- *Report available on the WHO-GMP website at the following URL: www.who.int/malaria/mpac/mpac_mar2014_diagnosis_low_transmission_settings_report.pdf*
Wrap Up: Outcomes

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Questions