

# **Trials of RDTs**

## **Guidelines for the Field Evaluation of Rapid Diagnostic Tests for Malaria**

### **Introduction**

#### **Factors that influence RDT trial results**

##### *Transport /storage of RDTs*

Most RDT products state that storage should be at 4- 30°C. This is difficult in a field trial in the tropics and impossible for many potential end-user health workers. RDTs are sensitive to moisture (humidity) and high temperatures. Such exposure may reduce the intensities of the control and test lines and reduce sensitivity. If tests are read hours after preparation, negative test lines on some RDTs may appear positive due to accumulation of blood products or other factors. All products should be read within the time specified by the manufacturer.

##### *Local epidemiology*

Sensitivity and specificity is influenced by the parasite density of cases, and predictive values are influenced by the underlying parasite prevalence in the group recruited to the study (frequency of true negatives, as with any diagnostic study). The the sample population (the recruitment criteria) needs careful definition for the findings to be interpreted. This depends on the aims of the study, and could therefore range from "patients fitting routine criteria for treatment by health-workers" (with the criteria defined) to recruitment of known parasitaemic patients and non-parasitaemic controls. It is preferable for parasite density to be recorded, if this can be done accurately, and sensitivity and specificity expressed in terms of this.

##### *Test Preparation and Interpretation.*

Significant variation between technicians in both RDT preparation and interpretation may occur:

- RDT sensitivity varies with the blood volume used when parasite density is low (up to the manufacturers' specified volume);
- blood flow and antigen concentration may vary with the volume of reagent added to the test strip or well;

- product instructions may need translation to improve understanding of technicians;
- changes to the layout of product instructions may result in significant variations in test sensitivity.

Multiple blinded readings and rotation of technicians will reduce bias. Timing of readings needs to be documented (i.e. 'Strictly according to manufacturer recommendations' or otherwise stated). The method of obtaining blood and transferring it to the strip (i.e. direct contact or pipette provided) and volume used (if different from recommendations) needs documenting, together with any technical problems encountered in preparation.

The suitability of product instructions can be documented both quantitatively (sensitivity, specificity, proportion of observed mistakes) and qualitatively (preferences of end-users). This may be the most useful data in assessing the suitability of RDTs for use in a particular area or by certain end-users.

#### *Standards for comparison*

Most studies employ microscopy as a 'gold standard'. PCR may be more sensitive for detection and species identification, but high cost may limit use. It may be useful for assessing discordant results. Comparison with both can allow estimation of relative benefits of RDT compared to present microscopy-based diagnosis.

#### *Recent treatment*

Recent treatment may reduce true specificity (persistent antigen after parasite death), or apparent specificity with regard to standard microscopy (reduction of parasite density below microscopy threshold but antigen still detectable). Documentation of recent treatment, exclusion from study, or urine testing for drug residues can help control this. Comment on availability of treatment in the area of the study assists interpretation of results.

#### *Demography*

Immunity may increase with age and recent infection and affect sensitivity and specificity by reducing the parasite density. In pregnancy, sensitivity and specificity relative to microscopy can be affected by increased sequestration of the parasite.

## **Suggested minimum standards for efficacy trials of malaria rapid diagnostic tests**

The following should be identifiable to all individual cases:

1. Details of RDT kits used:
  - 1.1. manufacturer (company name, actual site of manufacture)
  - 1.2. batch number (includes strip, reagents, wells)
  - 1.3. date of manufacture
  - 1.4. date of expiry
  - 1.5. Whether product is under trial or commercially available
2. Record general description of test kits:
  - 2.1. packaging type (sealed individually, multiple strips in same canister etc.)
  - 2.2. state and type of packaging, and whether canisters of test strips have been opened previous to the first patient seen. (RDTs in damaged packaging should not be used)
  - 2.3. inclusion of desiccant with strips
  - 2.4. inclusion of lancets/ capillary tubes etc needed to perform the test (or otherwise note the items used).
3. Description of previous storage /transport conditions since manufacture:
  - 3.1. duration of storage
  - 3.2. general temperature and humidity at storage (monitoring of temperature and humidity if available). RDTs should be stored away from direct sunlight.
  - 3.3. time to complete use from opening of canister (when this packaging is used).
4. Description of trial site:
  - 4.1. climatic conditions (mean local temperature and humidity).
  - 4.2. workplace conditions (type of facility, lighting used for reading RDTs.)
  - 4.3. local malaria situation
5. Description of trial subjects:
  - 5.1. criteria for patient selection (symptoms and signs, relation to normal selection for treatment, exclusion criteria).
  - 5.2. demographics (age, sex)
  - 5.3. recent anti-malarial therapy
6. Description of technique used:
  - 6.1. time of strip package opening to time of use
  - 6.2. blood extraction (venous or capillary)
  - 6.3. blood transfer to strip (device provided by manufacturer or pipette etc)
  - 6.4. time taken to obtain reading (per manufacturer guidelines, or reason if longer).
7. Record each line on strip separately, including control. Record of intensity is not necessary.
8. Record organization of RDT readers /technicians
  - 8.1. one or multiple readers
  - 8.2. blinding to microscopy, other RDT readers, and preferably to clinical presentation (latter may not be possible in some circumstances).
  - 8.3. same technician/reader per RDT type, or alternating
  - 8.4. if possible, identify technicians/readers for later comparison.
  - 8.5. training/experience of technicians in this RDT use (including recency of training, validation of quality of training).
  - 8.6. any significant/recurrent problems encountered in kit preparation (including opening of packaging, obtaining blood etc.).
  - 8.7. record any variation from the exact RDT preparation technique detailed in the manufacturers insert.

9. Consider formal independent qualitative appraisal of 'ease of use' of product by each technician.
10. Microscopy:
  - 10.1. Reagents used.
  - 10.2. Time from preparation to staining.
  - 10.3. pre-qualification and training of microscopists
  - 10.4. blinding
  - 10.5. Criteria for counting parasites and assessment of slide negativity, parasite density.
11. Consider collecting dried blood on filter paper or EDTA samples etc. to allow for later clarification through microscopy /PCR. The criteria for settling discordant results (e.g. PCR, ELISA, independent microscopist) should be formulated beforehand and clearly stated.
12. Sampling size, data analysis. Results should include sensitivity, specificity, positive predictive value, negative predictive value. The kappa statistic can give a useful guide to agreement between readers.
13. Ethical considerations and approval, including treatment guidelines and informed consent.

Footnotes:

1. Note should be made in trial records of whether lot numbers, dates and product labels are on box, or individual test canisters, sachets, cards etc. It must be possible to trace aberrant results back to details of manufacture.

2-4, 6. The quality of the result may be affected by capillary tubes, pipettes etc. used to collect blood through variation in the droplet placed on the kit. Opening of sachets at a significant time prior to RDT preparation can reduce quality.

11. It can be useful to have samples for confirmation of discordant results at a later date. This applies particularly to samples negative by RDT, positive by microscopy. This may not always be possible, particularly if capillary blood (finger-prick) is used, but a drop of blood dried on filter paper and stored with desiccant (obtained from the RDT kits) in a sealed container may be sufficient.

Blinding: RDT strips need to be read and recorded independently of the microscopy result (individual results and trends) and vice versa. Any repeat microscopy must be done blinded to RDT results and first microscopy.

Re-reading of RDTs: If RDT results are checked later, the time delay between first and second readings should be noted. Reliance on readings beyond the period specified in the product instructions should be avoided.