Preparation of Rapid Diagnostic Tests (RDTs) for DNA extraction v.1.1

Procedure

Molecular Module

WorldWide Antimalarial Resistance Network (WWARN)

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Version History

Version number	Revision(s) & reason for amendment	Release date
1.1	Addition of information from Cnops et al 2011	20/05/2011
1.0	Creation of procedure	01/03/2011

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Contents

1. Purpose

This procedure describes storage and processing of malaria Rapid Diagnostic Tests (RDTs) for DNA extraction.

2. Scope

Malaria-infected blood captured in the matrix of RDTs can be used as a source of *Plasmodium* DNA for PCR and other molecular genetic techniques. This procedure is limited to the RDTs identified in Section 5.3, and the noted extraction methods, validated by the co-authors. This procedure is intended for laboratories that routinely process malaria-infected blood samples and perform DNA extraction for molecular work.

3. Abbreviations

RDT	Rapid Diagnostic Test
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction

4. Duties and Responsibilities

N/A

5. Materials and Equipment

5.1 RDT Storage

- Zip-locking bags or equivalent
- Desiccant pouches
- Disposable latex or nitrile gloves
- Ink stamp, pencil or permanent ink pen

5.2 RDT Processing

- Scissors
- Forceps
- Ethanol (95%) or another sterilizing agent

- Deep 96-well plates or 1.5 mL microcentrifuge tubes for placement of RDT segments

5.3 RDT brands validated using this procedure

- CareStart™ Malaria *Pf/Pv* Combo (Access Bio, Inc., USA)
- CareStart™ Malaria HRP-2 (Access Bio, Inc., USA)
- ICT Malaria Pf/Pv (AMRAD ICT, Australia)
- Binax NOW ICT malaria P.f/P.v **(**Binax Inc. USA
- First Response® (Premier Medical Corporation Ltd., India)
- OptiMAL (Flow, Inc., USA)
- Paracheck Pf® (Orchid Biomedical Systems, India)
- ParaHIT® total (Span Diagnostics , India)
- OptiMAL-IT (DiaMed AG, Switzerland)
- SD Bioline Malaria Ag Pf FK50 (Standard diagnostics Inc., Korea)
- SD Bioline Malaria Ag Pf/Pan FK60 (Standard diagnostics Inc., Korea)
- Advantage Pan MAL card (J. Miltra & co. Pvt. Ltd., India)
- ICT Malaria Pf **(**ICT diagnostics, South Africa)
- Core™ Malaria PAN/Pf (Core Diagnostics, UK)
- Hexagon Malaria Combi (Human, Germany)

The co-authors have evaluated this RDT storage and processing procedure with only the RDTs named in Section 5.3. It should be noted that DNA extraction from RDTs with a plastic cover on top of the nitrocellulose membrane is more difficult than from RDTs without this plastic cover (Cnops et al 2011). It is likely that RDTs produced by other manufacturers, not named above, could be processed with equal success. Researchers are encouraged to contact molecular@wwarn.org with information on successful DNA extraction from RDTs not shown on the above list to allow WWARN to extend this procedure.

6. Procedure

DNA extraction using elution in water by heating at 95°C (Cnops et al 2011), chelex (Ishengoma et al 2011), or phenol/chloroform has successfully been performed from processed RDTs. Qiagen® Qiamp DNA Mini Kits were evaluated but did not yield optimal real-time PCR results when compared to DNA extracted by the elution method (Cnops et al 2011). Other extraction methods or commercial kits may be effective but have not been validated by the co-authors of this document at the time of writing. Please contact molecular@wwarn.org if you have specific questions regarding DNA extraction from RDTs or to share information that could be used to update this procedure.

6.1 RDT Storage

- RDTs and their matrices should always be handled with gloves. Matrices and/or storage covers should be labeled in permanent ink or pencil with patient and sample information before storage.

- To ensure optimal DNA integrity, the RDT matrix should be dried at room temperature and either stored with desiccant pouches in zip-locking bags.

- Dried RDTs can be stored at room temperature for 3 to 6 months (Ishengoma et al 2011, Veron and Carme 2006). Longer storage may also be possible (Cnops et al 2011, Veron and Carme 2006), although DNA quality may be reduced depending on the degradative effects of temperature and humidity.

- Full drying of RDT matrices with plastic covers may not be possible. Non-dried RDTs have been held at 4° C for up to one week prior to successful DNA extraction. For longer storage of non-dried RDTs, freezing at -20 °C or -80 °C is highly recommended; however, RDTs should be sealed in zip-locking bags with desiccant to prevent exposure to condensation.

6.2 RDT Processing

DNA migrates from the site of blood sample application on the RDT matrix, but not as far as the antibody lines used in detection. Experimental testing has shown that the DNA is concentrated in the region approximately half way between the blood application site and the result lines (See Figure 1). The half of the RDT where the blood sample was applied can be used in the extraction reaction.

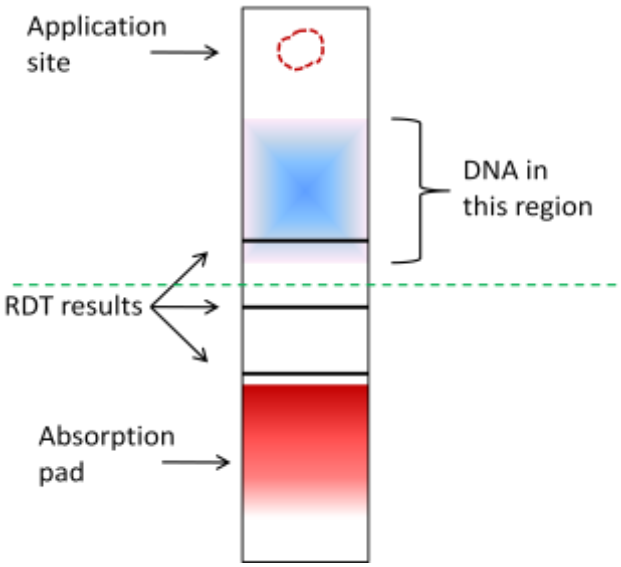

Figure 1. Migration of DNA and proteins in RDT. Blood sample migrates from site of application to absorption pad. The dotted line separates top half of RDT for use in DNA extraction from bottom half. Image source: Meera Venkatesan, WWARN, adapted from original image by Rinki Deb and Colin Sutherland, LSHTM, UK.

I. Open the RDT cassette using sterile scissors and forceps, and remove the nitrocellulose strip.

II. Cut the nitrocellulose strip in half, placing the half containing the blood sample application site (see Figure 1) into a tube for DNA extraction. Cutting the half nitrocellulose strip into smaller pieces can increase DNA recovery.

III. Clean the scissors and forceps after processing each RDT by dipping in 95% ethanol, thoroughly drying over a flame, and wiping with a clean tissue.

7. References

Cnops L, Boderie M, Gillet P, Van Esbroeck M, Jacobs J. Rapid diagnostic tests as a source of DNA for Plasmodium species-specific real-time PCR. *Malaria Journal* 2011; 10:67.

Ishengoma DS, Lwitiho S, Madebe RA, Nyadonde N, Persson O, Vestergaard LS, Bygbjerg IC, Lemnge MM, Alifrangis M. Using rapid diagnostic tests as source of malaria parasite DNA for molecular analyses in the era of declining malaria prevalence. *Malaria Journal* 2011; 10:6.

Veron V, Carme B. Recovery and use of *Plasmodium* DNA from malaria rapid diagnostic tests. *Am J Trop Med Hyg*. 2006; 74:941.