Standard Operating Procedure

Title	DNA extraction from rapid diagnostic tests (RDT) by Chelex Method
SOP code	SOP_LAB_Pf_62_v01
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1 Objectives

Describe the procedures for the extraction of DNA from blood onto nitrocellulose strips in rapid diagnostic tests (RDT) using the Chelex-based method.

2 Definitions

RDT: Rapid Diagnostic Test
PBS: Phosphate-buffered saline

3 Responsibilities

This procedure is designed for use by appropriately-equipped laboratories. Training is required to perform the procedure successfully. Trained researchers/laboratory technician are responsible of the correct execution of the SOP. The principal investigator and co-principal investigator are responsible for approval and evaluation of the procedures

4 Related SOPs

- SOP_LAB_BCN_59_v01_EN (2017): DNA extraction from dried blood spots by Chelex method
- SOP_LAB_BCN_64_v01_EN (2023) Extraction of DNA from dried blood spots (DBS) and rapid diagnostic tests (RDT) by Chelex method in 96 well plates
- SOP_LAB_BCN_19_v02_EN (2018): Detection and quantification of *Plasmodium falciparum* by Real Time PCR

5 Procedures

5.1 Materials required

a. Reagents

- PBS**.**
- Chelex® 100 sodium form (C7901, Merck).
- Nuclease free water
- Bleach
- Ethanol

b. Consumables

- Filter tips (10 µL, 200 µL and 1000 µL)
- Wide opening pipette tips: 200µl (Ref: 541152-965-008-09, Cultek).
- 1.5 mL centrifuge tubes
- Disposable gloves
- Fine tip marker pens

- 50ml tubes
- Plastic sterile Pasteur pipettes

c. Reference Samples

- Plasmoidum infected whole blood in RDT nitrocellulose strips (RDT).
- Uninfected blood on RDT as negative controls

d. Equipment

- Vortex, mini-centrifuge
- P1000, P200, P20, P10 pipettman
- Dry bath
- Scissors

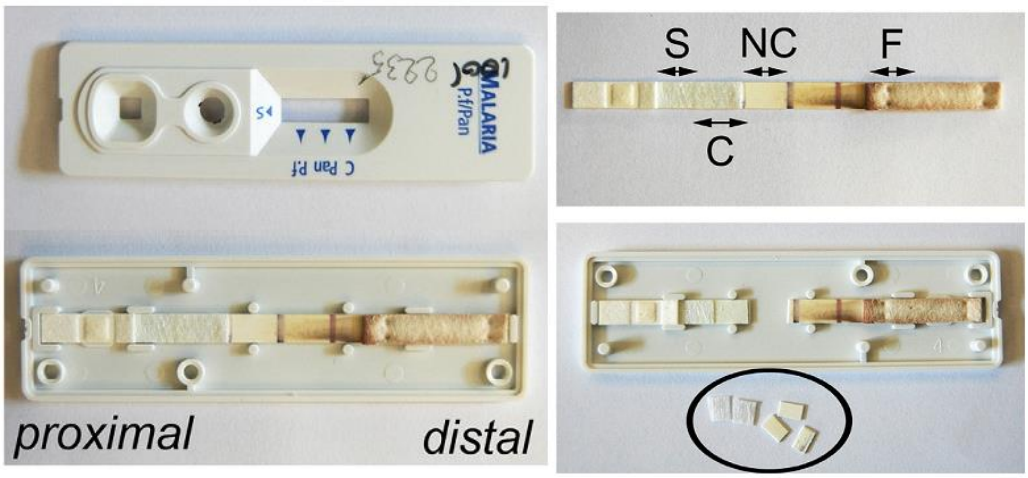
5.2 Preparation of solutions and buffers (before starting the procedure)

- 20% Chelex: weigh 2g of Chelex 100 sodium form and mix into 10ml of H_20_{dd}.Store at 4ºC.
- 5% bleach in distilled water
- 70% ethanol in distilled water
- Filtered PBS**.**

5.3 Working procedure

a. Clean the scissors and forceps after processing each RDT by dipping 1min in 5% bleach and 1min in 70% etanol using 50ml falcon tubes or similar containers, and let them dry.
b. Open the RDT cassette using sterile scissors and forceps, and remove the nitrocellulose strip.
c. Cut the conjugate pad (C) together with the proximal part of the nitrocellulose strip (NC) (see Figure 1)

Figure 1:



d. Put the pieces of the nitrocellulose strip in the bottom of a 1,5ml eppendorf.
e. If extracting DNA from multiple RDT cassettes, clean the scissors and forceps after processing each RDT by dipping 1min to 5% bleach and 1min to 70% ethanol.
f. Add 1 ml PBS to the 1,5ml eppendorf with the pieces of nitrocellulose. Ensure the sample are soaked in buffer. Vortex 5s.
g. Incubate at room temperature for 10 min.
h. Centrifuge at 14,000 rpm for 2 min and discard supernatant using a clean pipette tip for each sample.

i. Add 1 ml PBS. *Vortex 5s.*
j. Centrifuge at 14,000 rpm for 2 min and discard supernatant using a clean pipette tip for each sample.
k. Create a 1:2 solution of Chelex in nuclease-free water (for example, take 1mL 20% Chelex + 2mL H2O)
l. Add 100µl of 1:2 Chelex solution to the eppendorf using a wide opening pipette tip (200µl)
Note: Mix very well the Chelex solutions before use it and do it quickly- Chelex solution is very heterogeneous and Chelex beads tend to settle on the bottom very quickly.
m. Incubate at 95 ºC for 10 min.
n. Centrifuge at 14,000 rpm for 1 min.
o. Transfer supernatant into a clean microcentrifuge tube.
Note: Try not to transfer Chelex beads as it may inhibit the qPCR.
p. Store supernatants at +4ºC and do the qPCR. If storing samples for longer than a day, store at -20 ºC.

6 References

- Cnops, L., Boderie, M., Gillet, P., Van Esbroeck, M., and Jacobs, J. (2011). Rapid diagnostic tests as a source of DNA for Plasmodium species-specific real-time PCR. Malaria Journal *10*.
- WWARN (2014). Preparation of Rapid Diagnostic Tests (RDTs) for DNA extraction. 1.1 1–7.