Standard Operating Procedure

Title	Extraction of DNA from dried blood spots (DBS) and rapid diagnostic tests (RDT) by Chelex method in 96 well plates
SOP code	SOP_LAB_BCN_64_v02_EN
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1 Objectives

Describe the procedures for the extraction of DNA from whole blood onto filter paper (dried blood spots, DBS) or onto nitrocellulose strips in rapid diagnostic tests (RDT) in 96 well plates using the Chelex-based method.
This protocol was adapted from "*96-Well DNA/Protein Extraction Protocol (Chelex+TWEEN20)"* , University of California San Francisco, EPPICenter, USA, 2021.

2 Definitions

DBS: dried blood spot.
PBS: phosphate-buffered saline.
RDT: rapid diagnostic test

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: DNA extraction from blood spots dried in filter paper by Chelex method.
- SOP_LAB_Pf_62_v01_EN: DNA extraction from rapid diagnostic tests by Chelex method.
- SOP_LAB_BCN_19_v02_EN: Detection and quantification of *Plasmodium falciparum* by Real Time PCR.

5 Procedures

5.1 Materials required

a. Reagents

- 0.5% Tween-20 (Ref: P1379, Merck) in 1X PBS.
- 1X filtered PBS**.**
- Chelex® 100 sodium form (C7901, Merck).
- Nuclease free water
- 5% bleach in distilled water
- 70% ethanol in distilled water

b. Consumables

- Pipette tips: 20µl and 200µl.
- 8x Wide opening pipette tips: 200µl (Ref: 541152-965-008-09, Cultek).

- 1x PCR plate 96 wells, non-skirted (Ref: AB0600L, Thermo Scientific).
- 1x LoBind PCR plate 96 wells (Ref: 0030129504, Eppendorf)
- 1x Wilmut rack and caps (W052960CL, W999601, Wilmut) [optional]
- 3x Adhesive plate foil, transparent (Ref: AB0558 (Fisher Scientific)
- 1x Deep well plate 2mL (Ref: 60180-P134, Thermo Scientific)
- 2x 50mL falcon tubes.
- 1x Plastic reservoir.
- 2x Clean filter papers

c. Reference Samples

- Whole blood drops onto Whatman filter paper (DBS)
- Whole blood in RDT nitrocellulose strips (RDT).
- Positive control: RBC pellet from lab strains stored as either DBS or RDT. Negative control: uninfected blood on DBS or RDT

d. Equipment

- Water bath.
- Thermomixer with plate adapter (Ref: 5382000015, 5363000039, Eppendorf).
- Dispenser.
- Vortex.
- Plate spinner (Ref: 521-1648, VWR)
- Multichannel pipettes: 200µl, 20µl.
- Monochannel pipettes: 1000µl, 200µl, 20µl.
- 3x Punchers of 5mm diameter (Hole punch plier 10/115, 5 mm. Ref: GE10/115 5MM - HANS HILSCHER GMBH)
- Water bath weight (Ref: 214-0007, VWR).

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

- 0.5% Tween-20 in 1X PBS preparation:
  - Preparation of a 10% stock: in a 50mL falcon tube add 2 mL of 100% Tween-20 and 18 mL of 1X PBS. 20ml are enough for 4 extraction plates. Store at RT
  - Preparation of 0.5% working solution: in a 50mL flacon tube add 2.5mL of 10% Tween-20 and 47.5mL of 1X PBS. 50ml are enough for 0.5 extraction plates (prepare 100ml for 1 extraction plate)
- 20% Chelex preparation:
  - Weight 10 g of Chelex resin and add it into a 50mL falcon tube, then add 50 mL of nuclease-free water and vortex vigorously.50ml are enough for 10 extraction plates. Store at 4ºC.

5.3 Working procedure

The procedure is described for one 96-well plates at a time. If working with two plates, allow double the amount of time for the punching phase on Day 1.

Day 1:

1. Select samples to be extracted and draft the design of a 96 well plate in an Excel template. Print the plate design and the list of samples
2. Punch 1x5mm of DBS or cut the nitrocellulose strip of the RDT (SOP_LAB_BCN_59_v01_EN) and add it into the corresponding well of a Deep Well plate.
3. Clean the puncher with working solutions in the following order: 1) bleach, 2) water and 3) EtOH between punches. Dry thoroughly the puncher with paper and make 2 punches in a clean filter paper.
4. Add 1x positive control and 1x negative control in each plate.
5. Prepare Tween-20 solution, if necessary.
6. Add 1 mL of 0.5% Tween-20 in PBS.
7. Make sure DBS are submerged before sealing the plate with a transparent foil seal.

8. Secure the plate to a Thermomixer using the plate adaper and shake overnight at 300rpm and 15ºC. Alternatively, incubate overnight in the fridge with frequent vortexing if possible (e.g. two times before leaving the lab and first-thing in the morning when arriving)

Day 2:

1. Prepare two additional plates for centrifuge balancing: one deep-well plate containing 1mL of water per well, and one plate containing 150µl of water per well (this step can be ignored if extracting two sample plates at the same time)
2. Prepare Chelex solution, if necessary, and store at 4ºC
3. Remove the plate from the thermomixer.
4. Centrifuge the plate at 300 rpm for 1min.
5. Using a multichannel p200 pipette, aspirate all the PBS/Tween from the wells and discard. When pipetting up, move the tips back and forth gently along the bottom of the wells to dislodge the DBS and aspirate all the liquid underneath.
6. Add 1 mL of PBS/well (no Tween-20 in this PBS), seal the plate with transparent foil seal, vortex briefly and incubate at 4ºC for 30 min.
7. Turn on the water bath at this time and set temperature to 95ºC**. The water level should reach half the height of the deep-well plate (add or remove water accordingly).**
8. Take Chelex out from the fridge.
9. Centrifuge the plate at 800 rpm for 1 min and aspirate as much fluid as possible. Check underneath the plate to visualize any remaining liquid. Afterwards, use the tip to press the filter paper down into the lower third of the well without packing it excessively.
10. Vortex the 20% Chelex solution.
11. Create a 1:2 solution of Chelex in nuclease-freee water (for one plate, take 5mL 20% Chelex + 10mL H2O) in a reservoir.
12. With a multichannel pipette take one column of wide opening pipette tips (200µl) and use it to transfer 150µl of 1:2 Chelex into the first column to the plate. Make sure to hover above the wells and drop the liquid without touching the rim. Repeat the procedure for the remaining columns without changing tips. Make sure Chelex is not settling at the bottom of the reservoir, **resuspend it frequently**.
13. Seal the plate with an aluminium seal foil. Centrifuge briefly at 300 rpm to collect all liquid at the bottom.
14. Extract the DNA by incubating the plate in the water bath at 95ºC for 15 minutes. Use a weight (e.g.: beaker with water) to make sure the plate stays at the bottom of the bath without moving. Vigorously vortex the plate every 5 minutes throughout the incubation process.
15. After incubation, centrifuge the plate at 1500 rpm for 5 minutes.
16. Using a multichannel pipette and 200µl barrier-tip, transfer as much solution as possible from the wells of the deep well plate plate into a new labelled PCR plate. Do not worry if Chelex is carried over. Gently move the tip along the bottom of the well to dislodge the DBS and ensure all liquid is aspirated. Visually inspect underneath the deep well plate to make sure there is no liquid left.
17. Seal the transfer plate.
18. Spin the transfer plate for 10 minutes at high speed, 1500 rpm.
19. Label the selected storage plate. For storage in PCR 96-well plates use a Low-bind plate. For biobanking in barcoded tube racks use a Wilmut plate or similar.
20. Transfer a minimum of 100 µl of supernatant per well into the labelled plate. Avoid disturbing the Chelex beads at the bottom by pipetting slowly and from the middle/top with a P20 multichannel repeatedly.
21. Seal the final plate. Use an aluminium seal for low-bind PCR plates, or Wilmut tapes for Wilmut barcoded racks.
22. In the case of Wilmut barcoded racks, proceed to scan and register barcodes in the corresponding database.
23. Proceed to parasite quantification by qPCR and/or store DNAs at -20ºC.

6 References

"*96-Well DNA/Protein Extraction Protocol (Chelex+TWEEN20)"* , University of California San Francisco, EPPICenter, USA, 2021