KALADRUG-R: Clinical SOP#C

Blood sample and filter paper for PCR and DNA extractions

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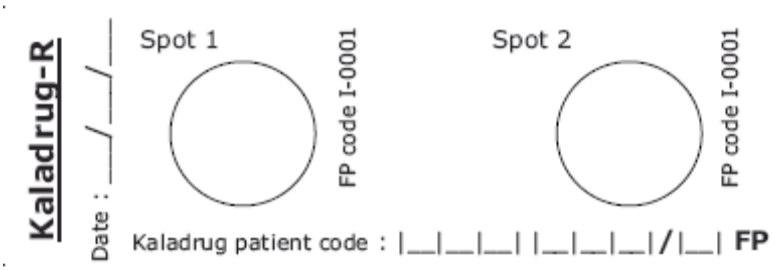
Note: TAPVAL tubes are no longer used in the Kaladrug-R protocols as per decision clinical meeting 15/02/2010. **IMPORTANT: also includes NEW DNA extraction protocols!**

A. Material required

1. Syringes 2ml + needle 21G, box for safe needle disposal + cool box
2. EDTA vacutainer for blood samples
3. Kaladrug-R Whatman filter paper #3 (example below) + silica-gel



4. Pipette + filter tip 0-200µl
5. Protective gloves
6. Equipment for DNA extraction

B. Collection of blood samples

1. Label the EDTA vacutainer tubes and the filterpaper according to cSOP with Kaladrug-R study code, and write the Kaladrug-R code in a clinical sample logbook (see labelling in cSOP#E for Nepal and cSOP#F for India).
2. Wear the protective gloves.
3. Draw min. 1 mL blood from patient.
4. Spot 2 separated blood drops on the filter paper, using the needle used to draw blood or using filter tips if the filter paper is spotted later. Let the blood spots dry but not in the sun.
5. Safely discard the needle.

6. Store the blood in the icebox and transport immediately to the laboratory.
7. In the laboratory, store the labelled EDTA vacutainer at -70^0C, this blood will be used for DNA extraction for PCR. (see point D. blood sample DNA extraction)
8. Store the blood spotted filter paper at -20°C in a plastic bag containing silica-gel. DNA can be extracted from the dried blood spots as described in point E. filter paper DNA extraction.

C. Remark

To avoid contamination it is necessary to use new material for each patient and each blood transfer

D. DNA extraction from blood samples using QIAamp protocol (QIAmp DNA Mini Kit, Qiagen; cat.no: 51304)

9. Equilibrate all samples to room temperature
10. Add 20 µ µl Qiagen Proteinase K (20mg/ml) into the bottom of a 1.5 mL microcentrifuge tube.
11. Add 200 µL blood to the microcentrifuge and add 200 µL Buffer AL to the sample
12. Mix thoroughly (vortex 15sec).
13. Incubate for 10 min. at 56°C.
14. Add 200 µl ethanol (100%) and mix thoroughly (vortex 15 sec).
15. Carefully apply the mixture from step 6 to the QIAamp Mini spin column, centrifuge at 6000g for 2 min. Place the QIAamp spin column in a clean 2 ml collection tube and discard the filtrate.
16. Add 500 µl AW1 buffer, centrifuge 2 min (6000g) and discard the filtrate.
17. Add 500 µl AW2 buffer, centrifuge 3 min at full speed and discard the filtrate.
18. Place the spin column in a clean 1.5 ml microcentrifuge tube, add 50 µl AE buffer, incubate for 5 min. at room temperature.
19. Centrifuge 1 min (6000g)
20. Store eluted DNA at –20°C, make sure the tube is labelled properly and register the extracted DNA sample (+ used controls) in the DNA extraction register of the lab.

E. DNA extraction from filter paper using QIAamp protocol (QIAamp DNA Mini Kit, Qiagen; cat.no:51304)

1. Place 3 punched-out circles from a dried blood spot into a 1.5 ml microcentrifuge tube and add 180 µl of Buffer ATL.
2. Incubate at 85°C for 10 min.
3. Add 20 µl proteinase K stock solution. Mix by vortexing, and incubate at 56°C for 1 h.
4. Add 200 µl Buffer AL to the sample. Mix thoroughly by vortexing, and incubate at 70°C for 10 min.
5. Add 200 µl ethanol (96–100%) to the sample, and mix thoroughly by vortexing.

6. Carefully apply the mixture from step 5 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
7. Add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.
8. Add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
9. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube, and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 150 µl Buffer AE or distilled water. Incubate at room temperature for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.