Standard Operating Procedures Clinical Protocol V: DNA Isolation

** NOTE: The following procedure is to be performed wearing laboratory coat, gloves, eye protection, and mask.

DNA isolation will be performed using the QIAamp DNA mini kit from QIAGEN (Cat no. 51304). This protocol is a summary of the one given with the kit. Please refer to vendor's protocol for additional information.

Important points before starting:

•All centrifugation steps are carried out at room temperature.

•Use carrier DNA if the sample contains less than 10 000 genome equivalents.

•200µL of whole blood yields 3 to 12µg of DNA. Preparation of buffy coat is recommended if a higher yield is required.

Things to do before starting:

•Equilibrate samples to room temperature.

•Heat a water bath or heating block to 56oC for use in step 4.

•Equilibrate Buffer AE or distilled water to room temperature for elution in step 11.

•Ensure that buffer AW1, buffer AW2 and QIAGEN Protease have been prepared according to the instructions on page 17.

•If a precipitate has formed in Buffer AL, dissolve by incubating at 56oC.

PROCEDURE:

1.Pipet 20µL QIAGEN Protease (or proteinase K) into the bottom of a 1.5mL microcentrifuge tube.

2.Add 200µL sample to the microcentrifuge tube. Use up to 200µL whole blood, plasma, serum, buffy coat or body fluids, or up to 5x106 lymphocytes in 200µL of PBS.

a.If RNA-free genomic DNA is required, 4µL of an RNase A stock solution (100mg/ml) should be added to the sample before addition of buffer AL.

3.Add 200µL of Buffer AL to the sample. Mix thoroughly by pulse-vortexing for 15 sec.

4.Incubate at 56oC for 10 minutes.

5.Briefly centrifuge the 1.5mL microcentrifuge tube to remove drops from the inside of the lid.

6.Add 200µL ethanol (96-100%) to the sample, and mix again by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the 1.5mL microcentrifuge tube to remove drops from the inside of the lid.

7.Carefully apply the mixture from step 6 to the QIAamp Mini spin column (in a 2mL collection tube) without wetting the rim.

8.Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 minute. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp Mini spin column is empty. (N.B. When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.)

9.Place the QIAamp Mini spin column in a clean 2ml collection tube and discard the tube containing the filtrate.

10.Carefully open the QIAamp Mini spin column and add 500µL Buffer AW1 without wetting the rim.

11.Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 minute.

12.Place the QIAamp Mini spin column in a clean 2 ml collection tube and discard the collection tube containing the filtrate.

13.Carefully open the QIAamp Mini spin column and add 500µL of Buffer AW2 without wetting the rim.

14.Close the cap and centrifuge at full speed (20 000 x g or 14 000 rpm) for 3 minutes.

15.Place the QIAamp Mini spin column in a new 2mL collection tube and discard the old collection tube with the filtrate.

16.Centrifuge at full speed for 1 minute.

17.Place the QIAamp mini spin column in a clean, properly identified 1.5mL microcentrifuge tube and discard the collection tube containing the filtrate.

18.Carefully open the QIAamp Mini spin column and add 200µL of Buffer AE or distilled water.

19.Incubate at room temperature for 5 minutes.

20.Centrifuge at 6000 x g for 1 minute.

21.Discard the column and close the cap of microcentrifuge tube.

22.Store the sample at -80oC until use.

For complete information, refer to the protocol “QIAamp DNA Mini and Blood Mini Handbook”, version of November 2007, p.27-29.
