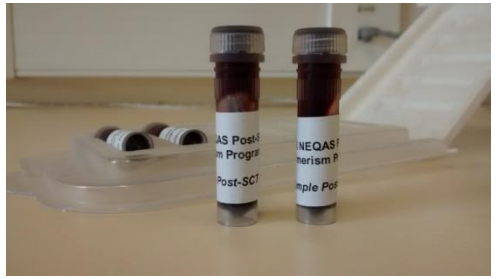
UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals
NHS Foundation Trust

Extracting Nucleic Acids from UK NEQAS LI Samples



Annie Tapley

What are UK NEQAS LI samples?

- Cultured cell lines (commercial or research)
- Blood donated from consenting patients/diagnostic waste
- Blood products (whole blood/buffy coat) NHSBT

- Bulking agents

- Samples are shipped as lyophilised material or fresh blood (usually the Chimerism programme only)

Lyophilisation

- We freeze dry our samples a bit like ‘space food’ (please don’t eat our samples!)

- This process stabilises our samples enough to be posted worldwide to our participants - Saldanha *et al* (2007)

Sample Stability

- If samples cannot be processed promptly upon receipt, they should be stored at 2-8 ^{o}C and remain lyophilised.
- This will ensure their stability until rehydration which revives the cells ready for laboratory processing but also revives RNases
- This is why we ask for sample processing to be performed immediately upon rehydration

Sampling Handling

There are minimal changes required when handling UK NEQAS LI lyophilised samples:

1. They will need rehydrating in water (injection grade or better) - swirl and leave for a minute

2. There is no need for the red cell lysis step present in a lot of extraction methods (even if our samples are red in colour, lyophilisation will have lysed any red cells present)

Sample Handling

3. Spin column style methods of DNA/RNA extraction often have a cell number limit

4. Sample cell numbers are provided in the accompanying paperwork - only approximate due to cell loss during lyophilisation/polyploidy/highly expressing

5. Dilute appropriately with PBS

RNA extraction

- Acid guanidinium thiocyanate-phenol-chloroform extraction is the method used in-house by UK NEQAS LI to extract RNA
- It can be added directly to the lyophilised cells but will remain too viscous to do anything with unless they are left alone for at least five minutes
- Gentle but persistent pipetting will produce a homogenous solution ready for processing

RNA extraction

- In-house extractions usually yield 40µl of RNA at concentrations of 600-1000ng/µl. More than enough template to synthesise cDNA from

- We have purchased a silica spin column based kit to get more hands on experience to aid in troubleshooting with participants

- UK NEQAS LI post cells instead of DNA/cDNA to assess **proficiency in extraction** as well as assay methodologies as this can have a fairly major impact on results

- We don't want this to prevent any participant from performing as well as you could so please don't hesitate to get in contact with any questions

Don’t Panic!

Everyone has ‘off days’. No one is immune to human error/accidents

- Ask for repeat samples
- Ask for advice

We would be very grateful for your troubleshooting to be fed back to us so we can in turn help others

Questions?

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