Poster P17.005.C

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Inspiring Genomic Excellence

GenQA Genomics Quality Assessment

Member of UK NEQAS consortium

Delivery of a multi-distribution external quality assessment for severe combined immunodeficiency newborn screening.

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Introduction

Newborn screening for severe combined immunodeficiency (SCID) by measurement of T-cell receptor excision circles (TRECs) continues to be evaluated and implemented globally. This requires concomitant implementation of external quality assessment (EQA) distributed regularly throughout the year. GenQA has successfully developed and delivered a two year pilot EQA consisting of multiple sample distributions.

Methods

Newborn blood spot cards were prepared using blood samples with either normal or absent TRECs and validated to confirm the levels of TRECs using commercially available TREC assays prior to the global distributions. Participants were requested to use their normal testing methodology and results were submitted online using a *proforma*. Assessment was performed by independent expert advisors against peer ratified criteria.

Pilot year 1 (2020) 8 samples across 2 distributions

Pilot year 2 (2021) 12 samples across 6 distributions

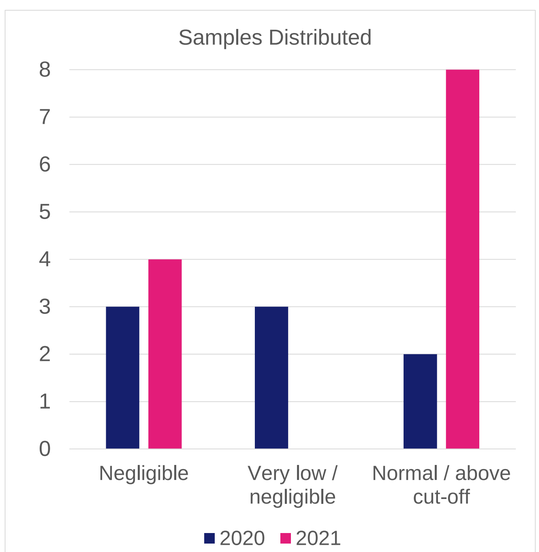


Figure 1: TREC level for samples distributed in 2020 & 2021

Figure 2: **EQA blood spots prepared using standard UK newborn screening cards**

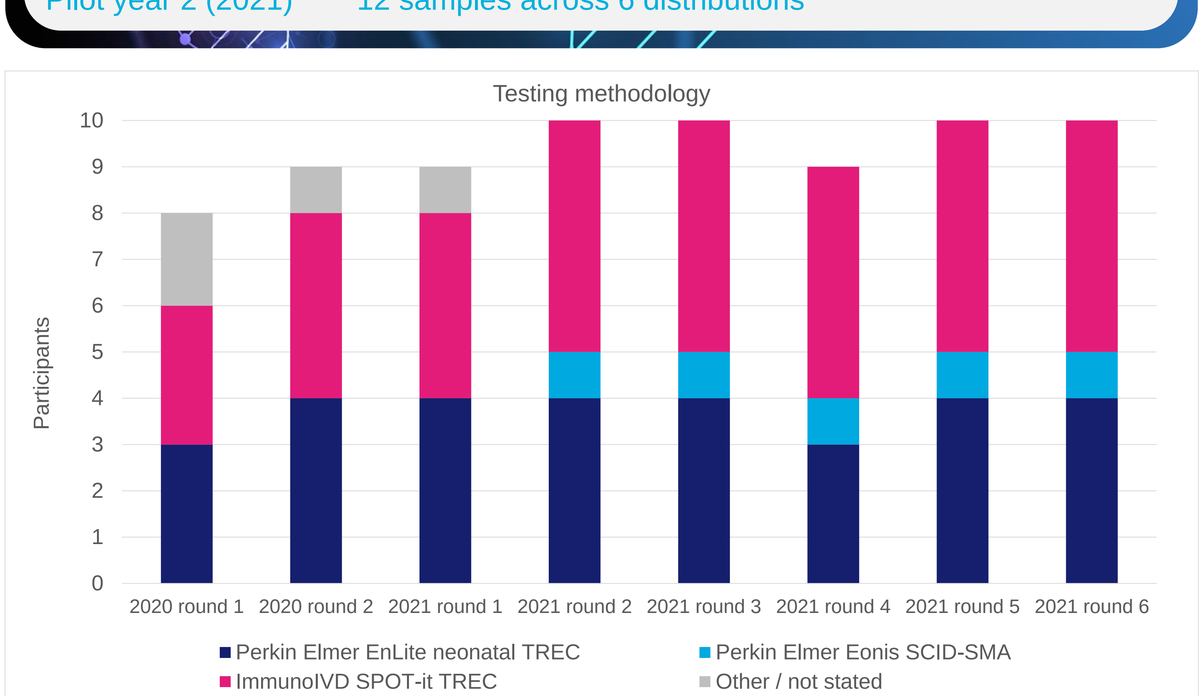


Figure 4: testing methodology utilised by participants in 2020 & 2021

Sample stability

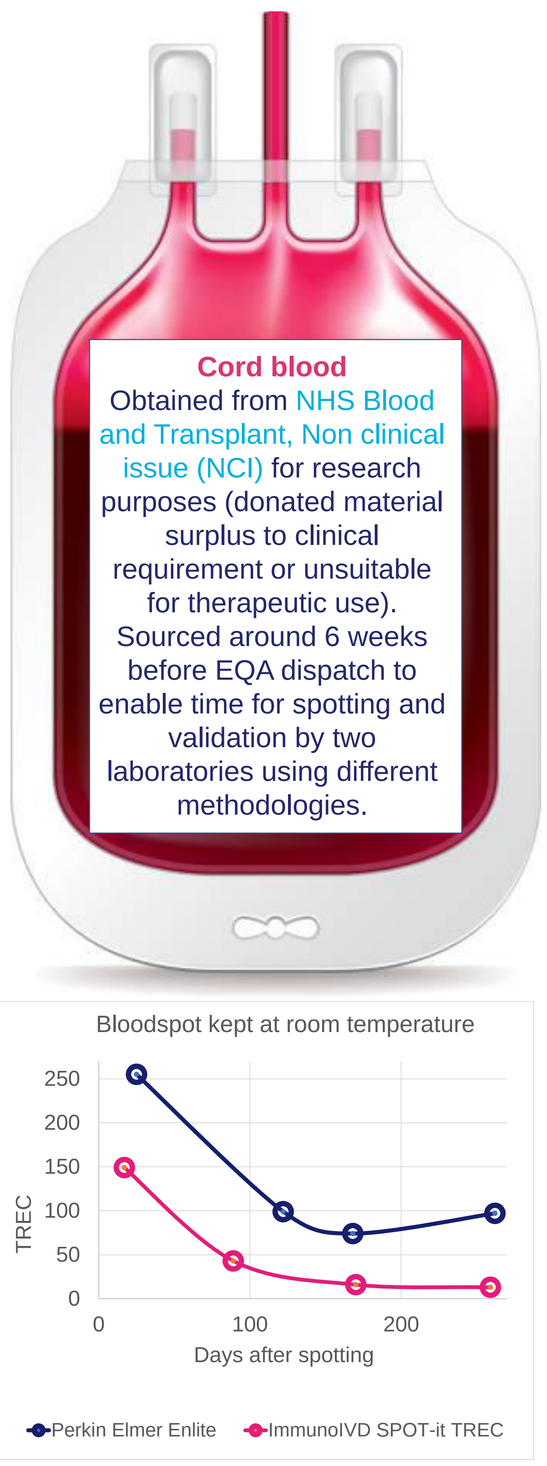
Previous EQA experience

GenQA has extensive experience of providing DNA-based EQA for cystic fibrosis and medium-chain acyl-CoA dehydrogenase deficiency for molecular newborn screening purposes. Blood samples are frozen at -80^{o}C until required, then spotted and validated for EQA distribution. Samples are relatively stable when stored at room temperature for extended periods with samples being re-validated successfully after several years of storage.

TREC stability

This strategy was unsuccessful for SCID with significant decline in TREC values observed over a period of several months whereby samples with normal TREC levels had dropped below laboratory cut-off levels. This decline in TREC levels was not consistently duplicated in control gene (generally *ACTB*) levels. The decline in TREC values was more apparent with the ImmunoIVD SPOT-it TREC kit however this may merely be due to the lower numerical values reported using this kit.

To solve the issue, cord-blood was sourced and spotted without freezing (see figure for further information). Blood with deficient TREC levels was sourced by the Haematology Unit at Great Ormond Street Hospital and spotted on site.



Cord blood
Obtained from NHS Blood and Transplant, Non clinical issue (NCI) for research purposes (donated material surplus to clinical requirement or unsuitable for therapeutic use). Sourced around 6 weeks before EQA dispatch to enable time for spotting and validation by two laboratories using different methodologies.

Figure 3: **Initial stability studies**
(more comprehensive work currently ongoing)
TREC levels appear to drop over time in bloodspot samples stored at room temperature.
Ongoing studies include samples stored at -20^{o}C with desiccant.

Results

Participants were split evenly between using the two commercially available TREC assays (as shown above) with some variation in cut-off levels and the units used to describe results as would be expected for locally validated methods.

Marking deductions are shown below; the marking criteria were refined over the pilot period. By the end of 2021 round 6, most participants were including essential information such as assay units, cut-off levels and reference gene results.

Figure 5: Marking deductions applied in 202 & 2021

Deductions for absence of measurement units or reference gene results were introduced from 2021.

There were 10 participants for the EQA in both 2020 and 2021.

Conclusion

The development of a comprehensive newborn screening EQA is challenging when samples need to be sourced from patients. An EQA format consisting of regular distributions throughout the year of samples suitable for SCID newborn screening analysis has been successfully implemented. GenQA are planning to offer this as an accredited EQA with performance criteria in 2023.

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Conflicts of interest: The authors have no conflicts of interest to declare.