

Automation of the *in situ* Dried Blood Spot Screening Assay for Severe Combined Immunodeficiency

2016 NBS and Genetic Testing Symposium

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Molecular Quality Improvement Program

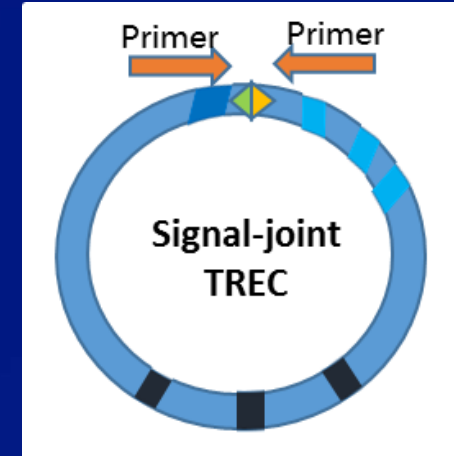
Newborn Screening and Molecular Biology Branch,

Division of Laboratory Sciences

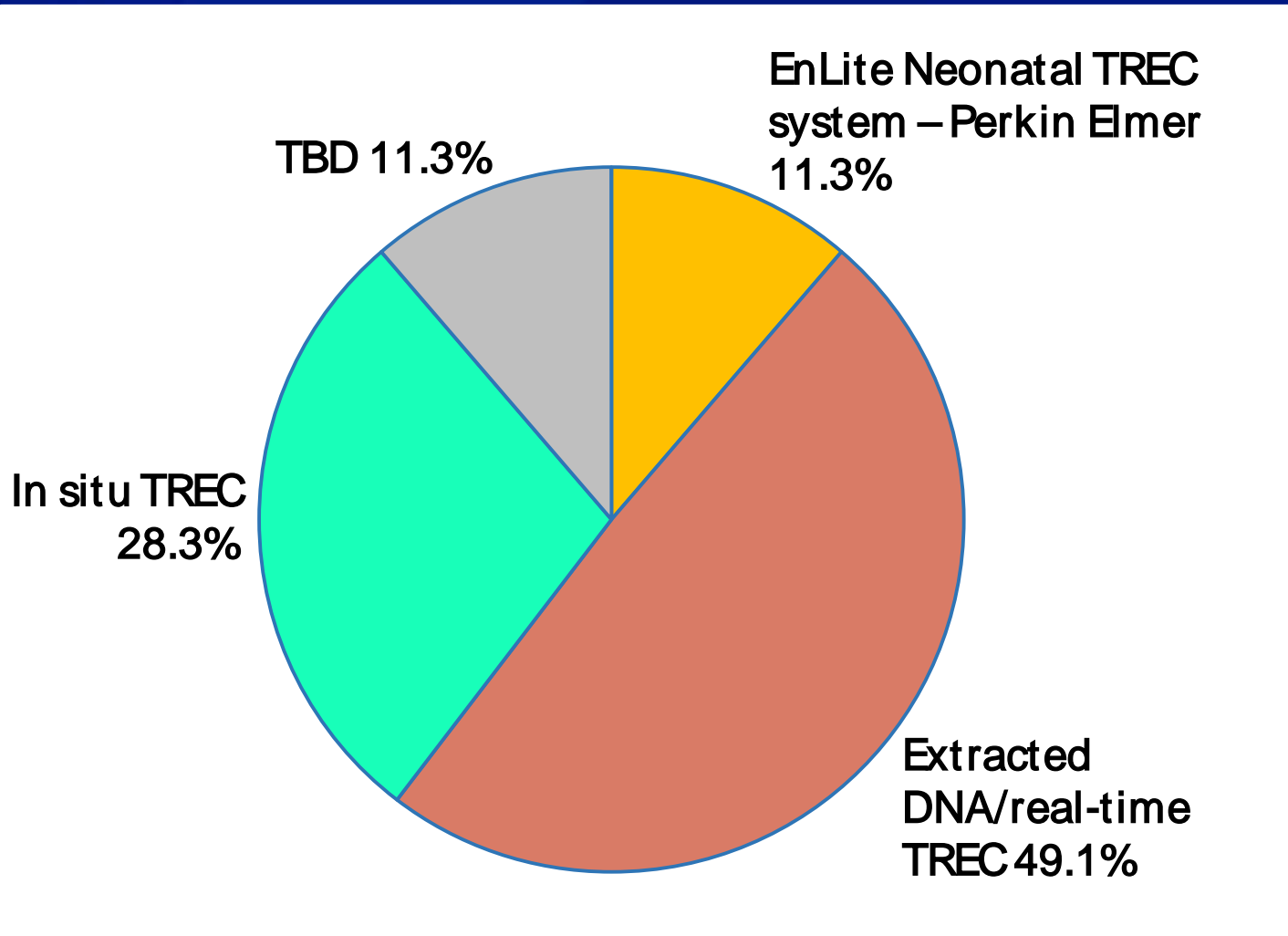
NCEH, CDC

Molecular Test Identifies Babies with Severe Combined Immunodeficiency (SCID)

- ❑ Mutation in one of 25+ genes
 - Non-functional immune system
 - Curable by a bone marrow transplant in the first few months of life
 - Early treatment prevents premature death and reduces medical costs associated with severe infections
- ❑ SCID detected by screening for T cell receptor excision circle (TREC)
 - Extrachromosomal DNA excised during T cell maturation
 - Excised DNA circularizes and the signal joint is unique in the human genome providing a target for detection
 - Immune defect that affects T cell production in newborns will cause a decrease or absence of TRECs



Methods Chosen by U.S. State Programs to Detect SCID



NBS Molecular Resources Website to Find Methods and Automation Currently Used in NBS

The screenshot shows the APHL website interface. At the top, there is a navigation bar with links for 'ABOUT APHL', 'CONFERENCES', 'EDUCATION', 'MEMBERSHIP', 'MY CAREER', 'POLICY', 'PROGRAMS', 'RESOURCE CENTERS', and 'GLOBAL HEALTH'. A search bar is located in the top right corner. The main content area is titled 'Newborn Screening and Genetics' and features a sub-section for 'Molecular Resources' with an image of a newborn baby. Below this, there is a section for 'NBS Automation Methods' which is highlighted with a red box. This section contains a list of links: 'Find the Liquid Handling Instrument that Fits Your Needs', 'Liquid Handling Options', 'Automation Methods', and 'Questions to Ask When Purchasing a Liquid Handler'. The text explains that this section is used to search for automation instruments and provides access to restricted information for NBS lab directors and their delegates.

www.aphl.org

NBS AUTOMATION METHODS

This section can be used to search the different types of automation instruments that are available, learn about how automation is currently being used in NBS programs and find questions to ask when purchasing a liquid handler. Access to the Automation Methods are restricted to NBS lab directors and their delegates.

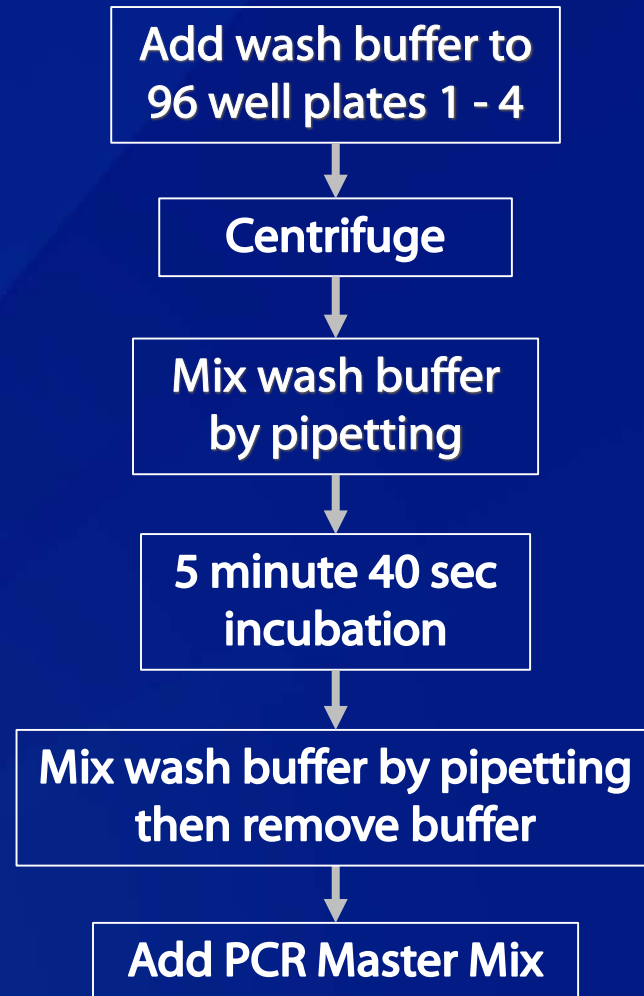
Find the Liquid Handling Instrument that Fits Your Needs:

[Liquid Handling Options](#)

[Automation Methods](#)

[Questions to Ask When Purchasing a Liquid Handler](#) (Click headers to expand)

In situ Assay for Severe Combined Immunodeficiency Automated Method Flow Chart



Deck layout for automated in situ TREC assay



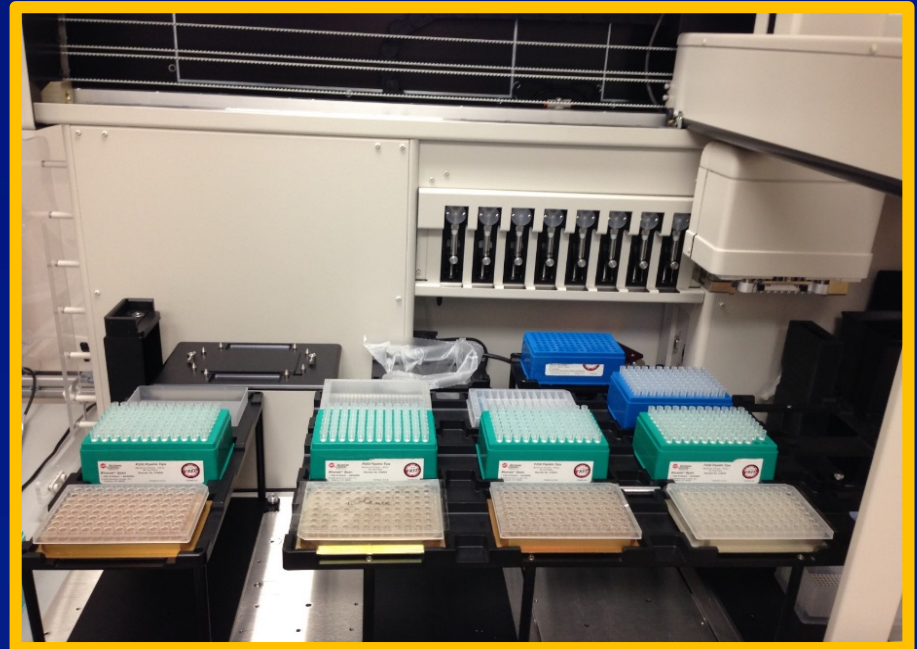
Automated In situ TREC assay Timeline: Addition of Wash Buffer

0:00 ~~2:42~~ 2:12

30:00 min



- 125µl Generation Solution 2 added to all 96 well plates (1 to 4)
- Instrument pauses for Centrifugation
 - Centrifuge at 2250g for 5 seconds



Automated *in situ* TREC assay Timeline: Mixing with 96 Well Head

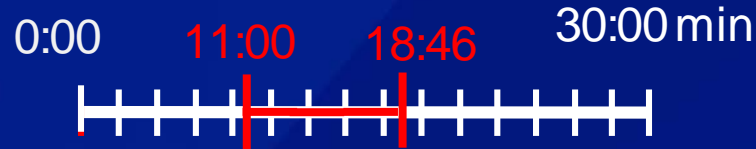
0:00 4:42 11:00 30:00 min



- Wash using 96 well head using a “tip mix”
 - Each plate has a designated tip box
 - Punches are rarely lost due to intricate tip movement



Automated In situ TREC assay Timeline: Removal of Second Wash



- ❑ All plates undergo a second “tip mix”
- ❑ Solution 2 is removed – punches are ready for PCR master mix



Automated *in situ* TREC assay Timeline: Addition of PCR MasterMix

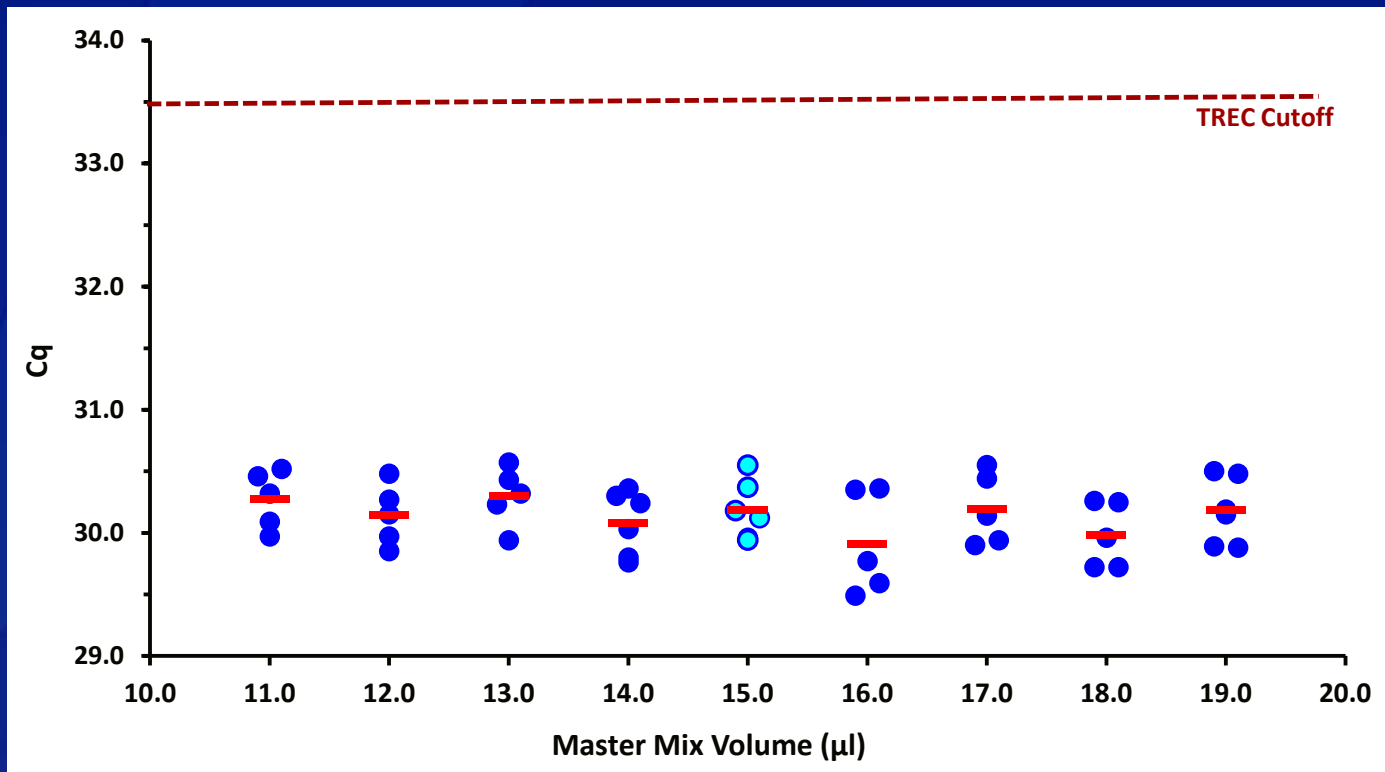


- ❑ Master Mix added in a multi-dispense steps
 - 15µl dispensed per well by column
- ❑ Selective tip pipetting head allows master mix dispense into partial plates
 - Eliminates unnecessary waste of master mix
- ❑ Seal plates for run



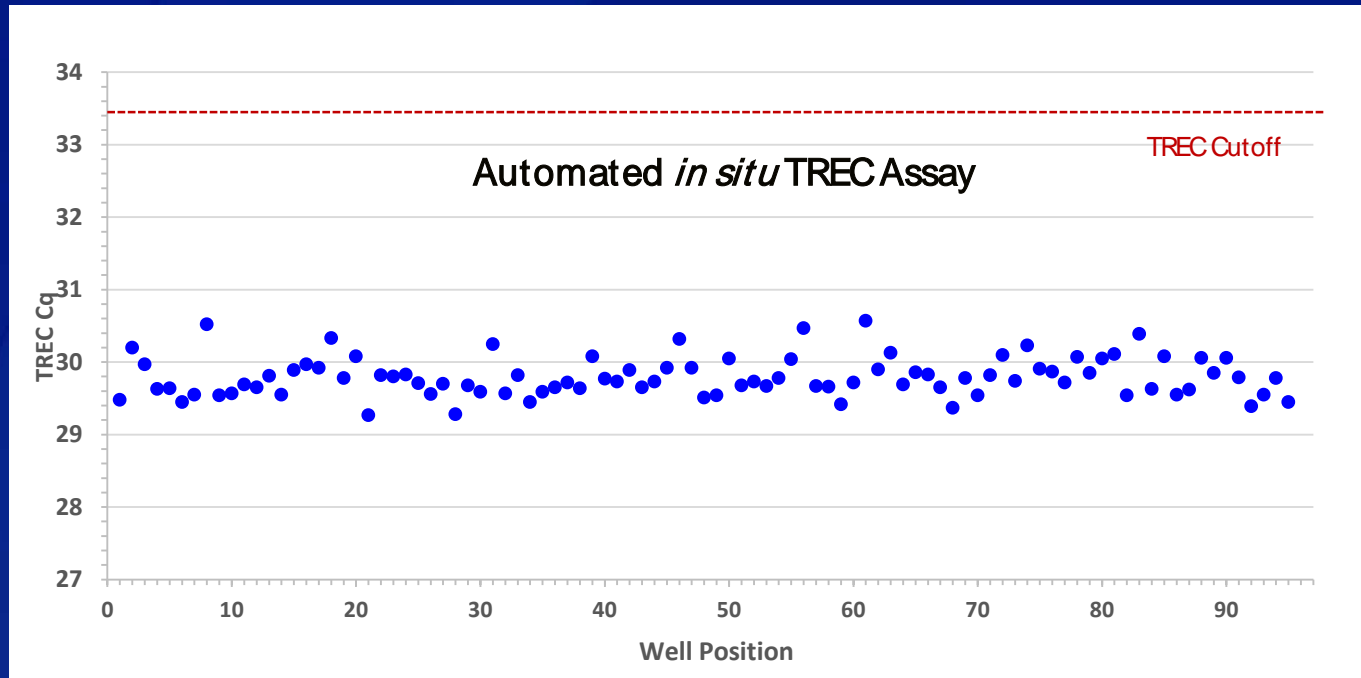
PCR Master Mix Volume Variation ($\pm 4 \mu\text{l}$) Does Not Affect *in situ* TREC Assay Results

- Replicate 2.0 mm punches of same sample prepared
- Different volumes of PCR Master Mix manually added



- No significant difference in Cq values between 11 – 19 μl ($p > 0.22$)

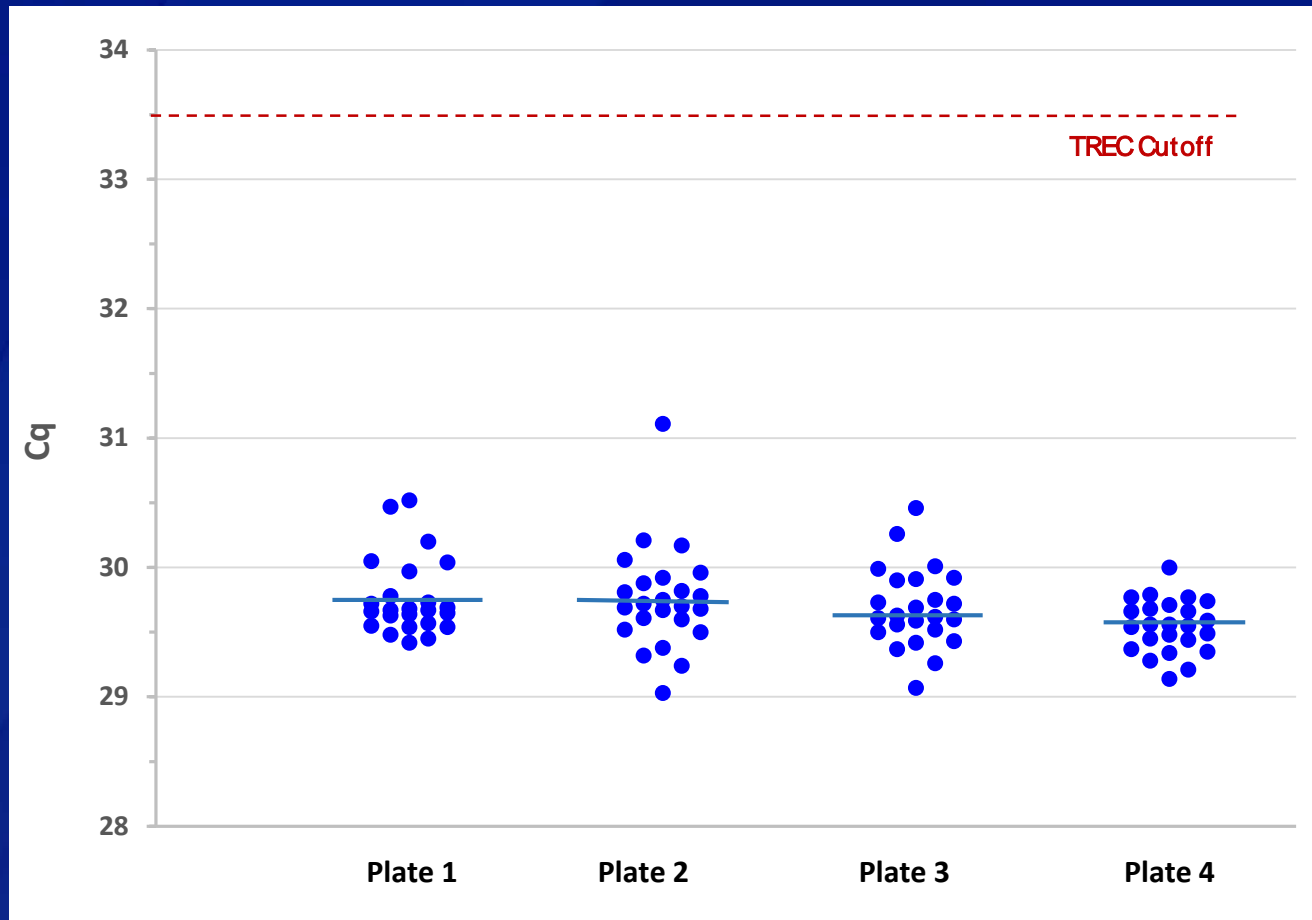
Intra-Plate Uniformity Comparable to Manual Results



95 punches of a sample were processed on one plate

	Automated	Manual
Mean Cq	29.80	29.79
CV%	0.91	1.18

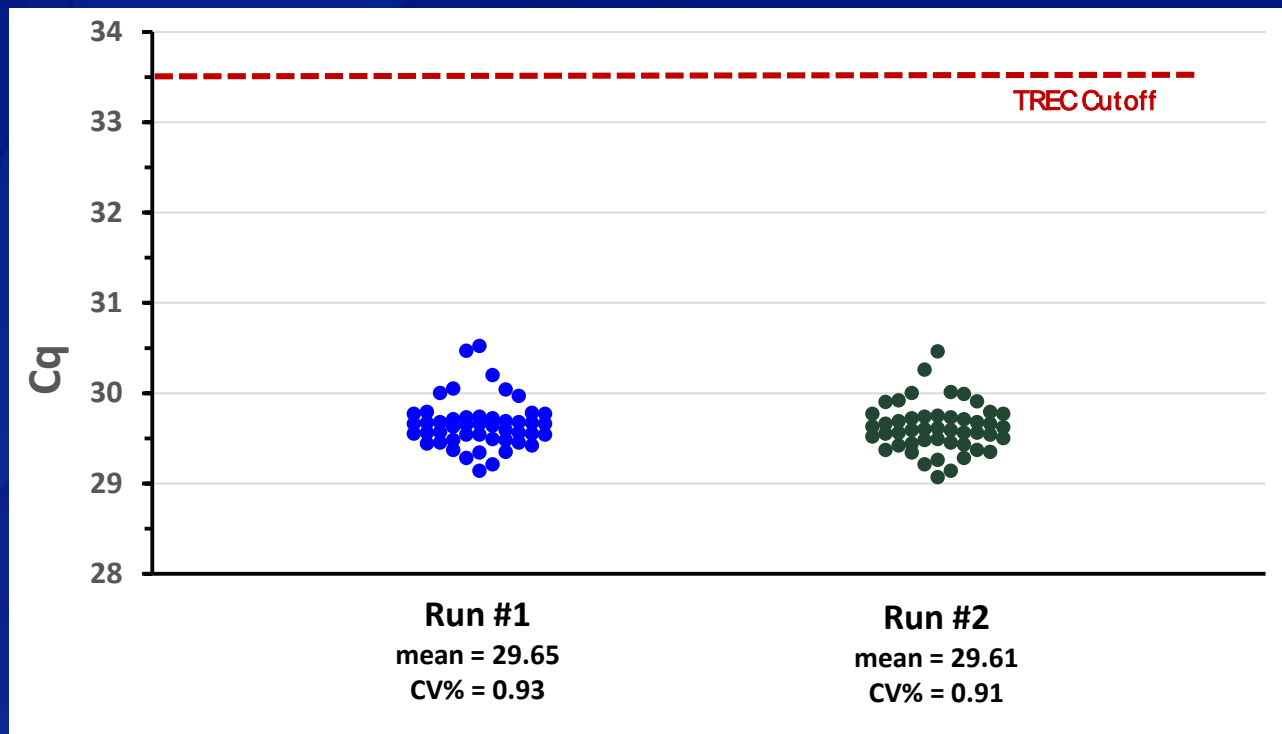
Consistent Results Across Plates Processed Simultaneously



24 punches of a sample were processed on each of 4 plates within the same robotic run

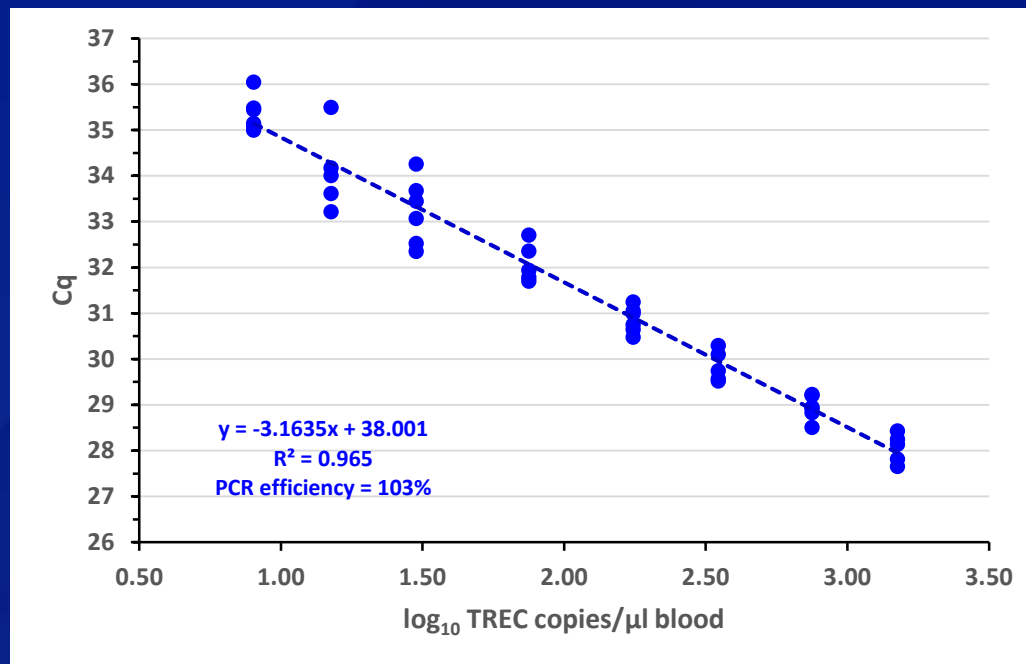
Reproducible Results Between Runs

- 96 replicate punches from a DBS sample distributed among 4 plates
- Same assay was repeated after 2 months



Linearity of Results Over an Extensive Range of TREC Levels

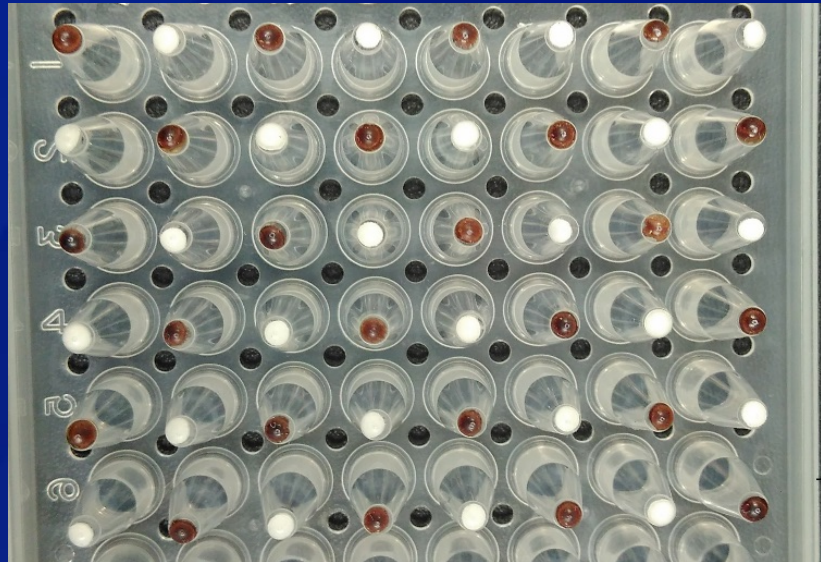
- Replicate punches of 8 DBS samples with 2-fold decreasing levels of TREC (1500 - 8 copies/ μ l blood)



- Results demonstrated linear regression correlation, with PCR efficiency close to 100%

No Cross-Contamination Between Samples

- ❑ DBS punches arrayed with blank punches in a checkerboard pattern in each of three PCR plates
- ❑ Processed with robotic system and tested for TREC and RNase P



- ❑ No false positive results in any of the wells containing blank punches

Summary of Automated *in situ* TREC Method

- Beckman NX can process up to 4-96 well plates in 30 min
 - Method validated on 1.5mm punch in Virginia (see poster #1)
 - Method has also been transferred to the PE Janus in New Jersey
- Automated In situ TREC method is robust and accurate
 - Variable PCR Master Mix volume ($\pm 4 \mu\text{l}$) does not affect results
 - Intra-plate uniformity is comparable to manual method
 - Results are consistent across plates processed simultaneously and between runs
 - Assay gives linear results over an extensive range of TREC levels
- No Cross-Contamination Between Samples

Acknowledgements

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For more information please contact Centers for Disease Control and Prevention

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E-mail: cdcinfo@cdc.gov Web: www.cdc.gov

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