

Establishing Quality Assurance Protocols for Low Frequency Events in a High Throughput SCID NBS Laboratory

**2011 Newborn Screening and
Genetics Testing Symposium
San Diego, CA**

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202,298 infants screened for SCID

4 SCID

~1 : 50,000

Surveillance: No known false negative

Through Guthrie date 10/25/2011

Assay and Laboratory Development



Implementing SCID NBS with Multiplexed Assays in an Integrated Program Approach

CDC National Center for Environmental Health

Grant # IV01-EH000362-03

New England Newborn Screening Program Staff

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GRANT COMPONENTS

- ASSAY DEVELOPMENT
- SCREENING IMPLEMENTATION
- **ALGORITHM REFINEMENT**
- TECHNOLOGY TRANSFER
- PUBLICATIONS

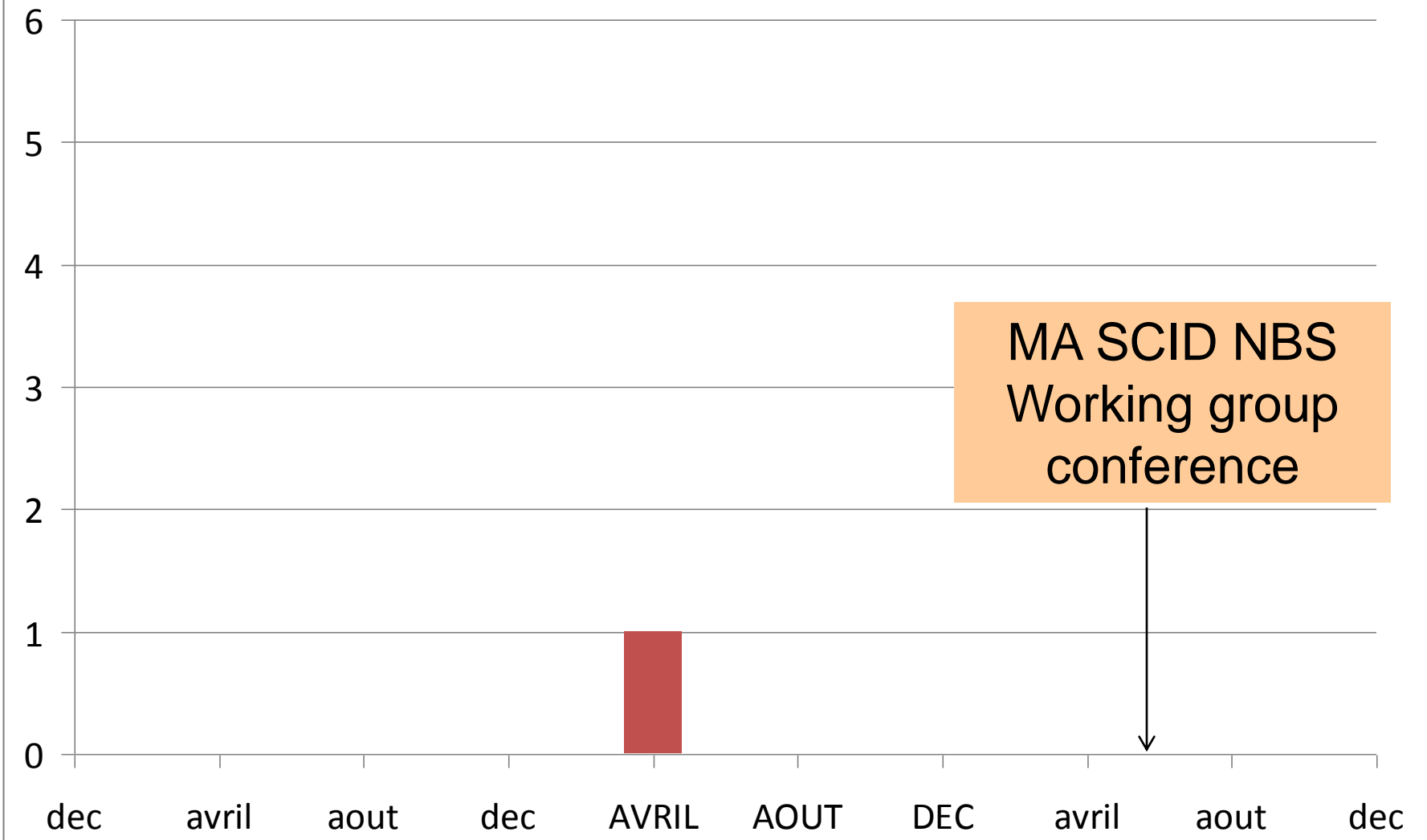
US PILOT STUDIES

INTERIM FINDINGS

Combined data from CDC and NICHD-funded pilots
(through April 2011)

| State | WI | MA | NY | CA | PR | LA | TOT |
|------------|---------|---------|---------|---------|--------|--------|---------|
| screened | 243,707 | 161,707 | 136,635 | 358,000 | 29,000 | 31,464 | 960,513 |
| SCID | 4 | 1 | 4 | 5 | 0 | 0 | 14 |
| rate (1/x) | 61,000 | 161,707 | 34,000 | 72,000 | | | 69,000 |

SCID NBS Diagnoses



Are we generating false negative results?

If so, what could be the cause?

Review data:

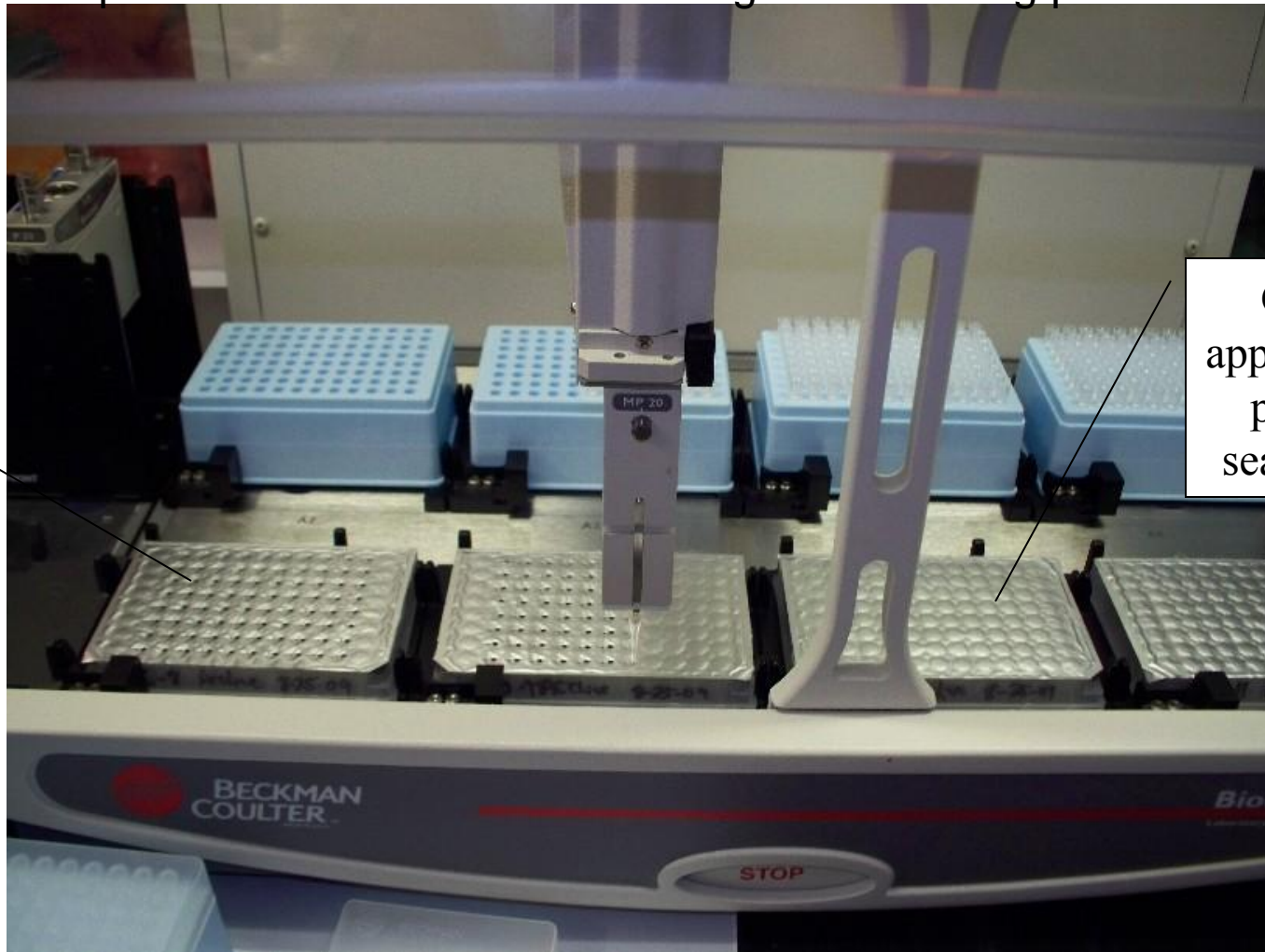
What is the potential for

Contamination?

Imprecision of the assay?

96-Well DNA Sample Plates on Biomek Deck

Individual wells remain hermetically sealed until punctured with the pipette tip for transfer to the 384-well reagent-containing plate.



Pierced wells

Convex appearance of perfectly sealed wells

Laboratory Algorithm

Components that might fail a run if copies detected

- No template controls, DNA from “no TREC” samples
- “blank spots” that are prepped with clinical specimens

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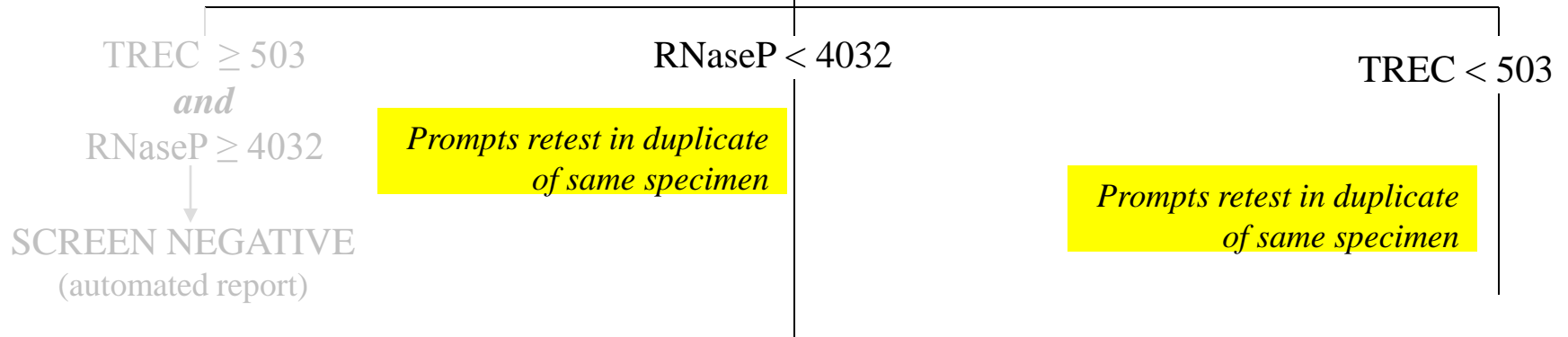
Other “blank spots”

SCID NBS Algorithm

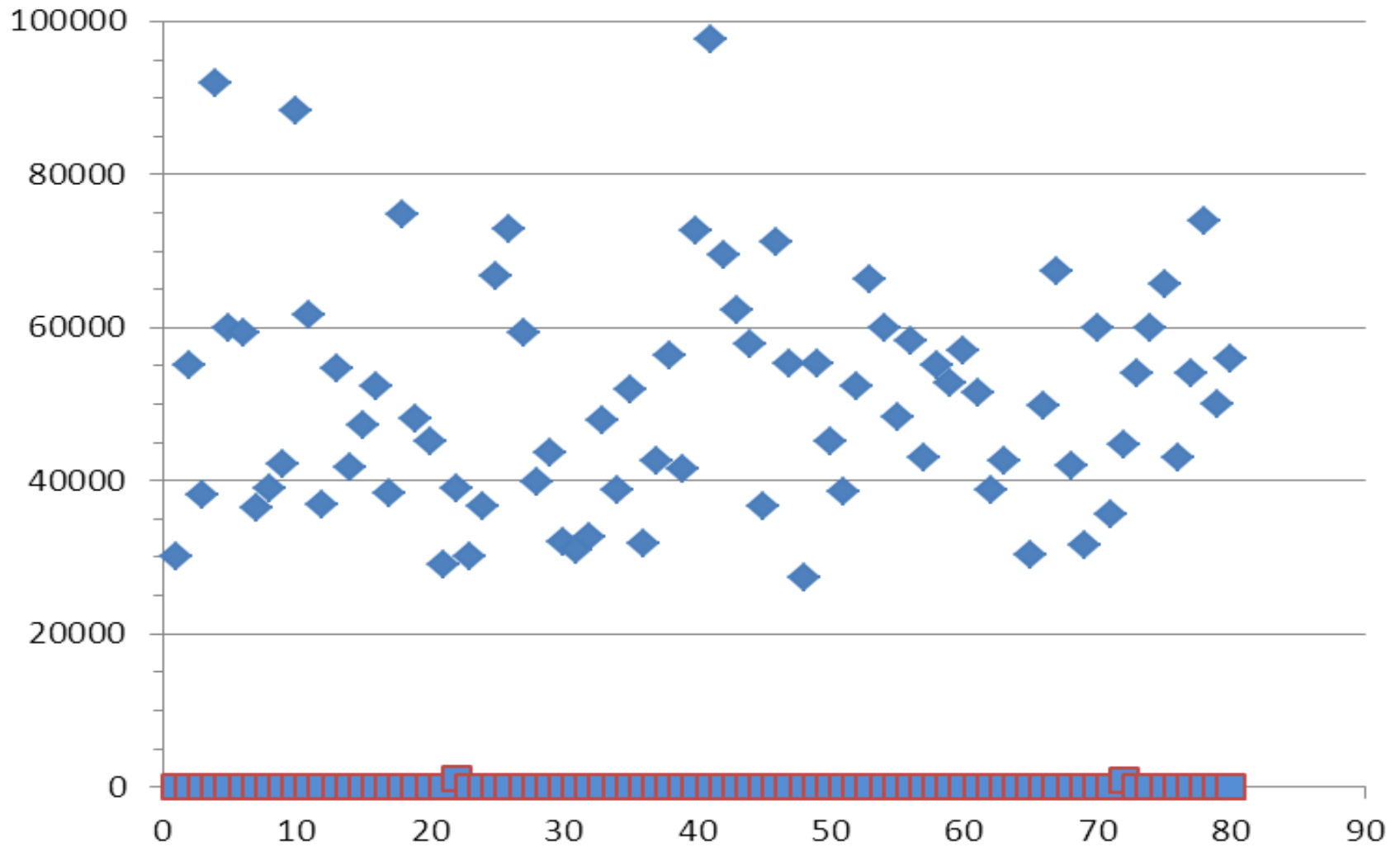
(All TREC & RNaseP Values are copies/ul)

Dried Blood Spot Specimen

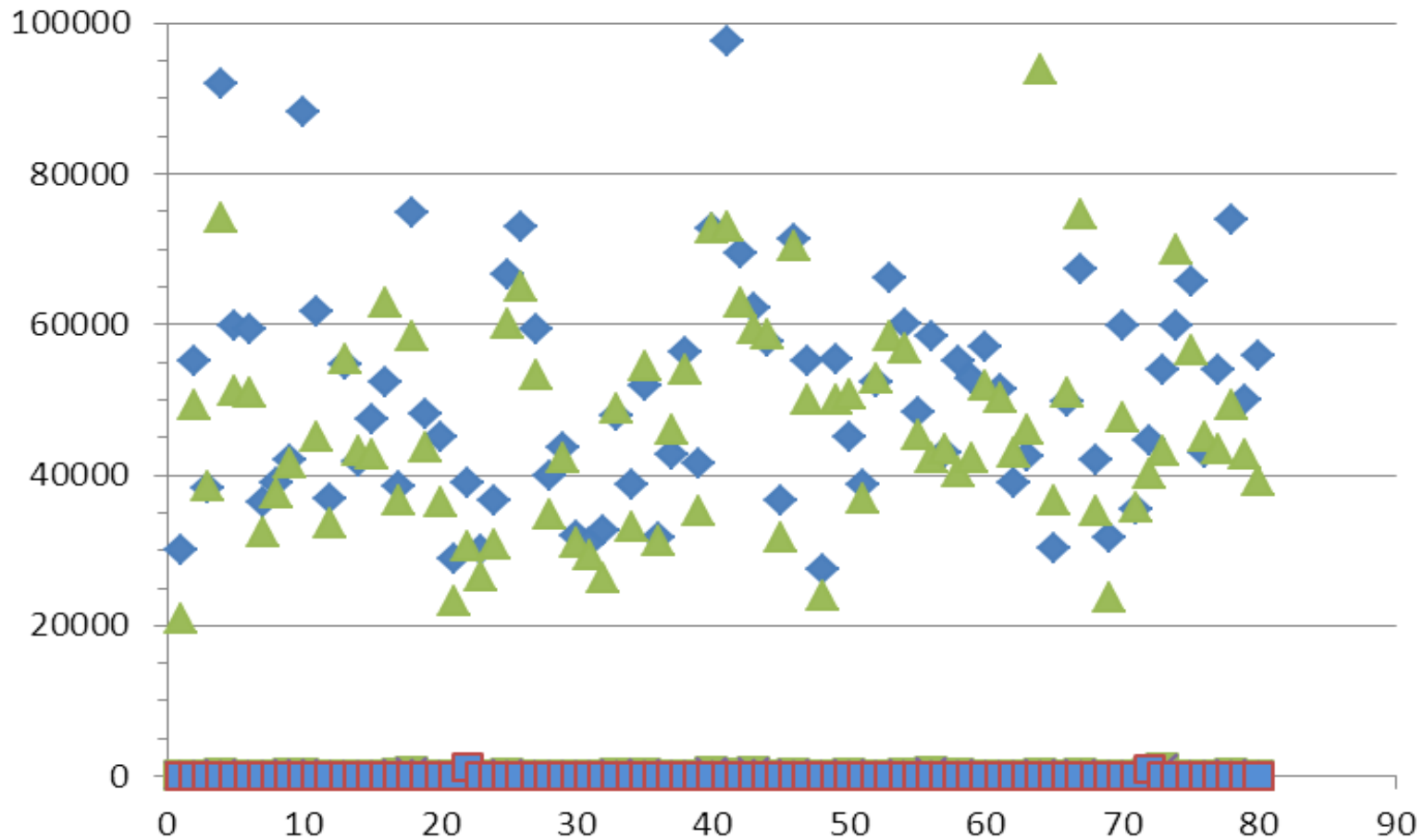
Multiplex assay for TREC and RNaseP



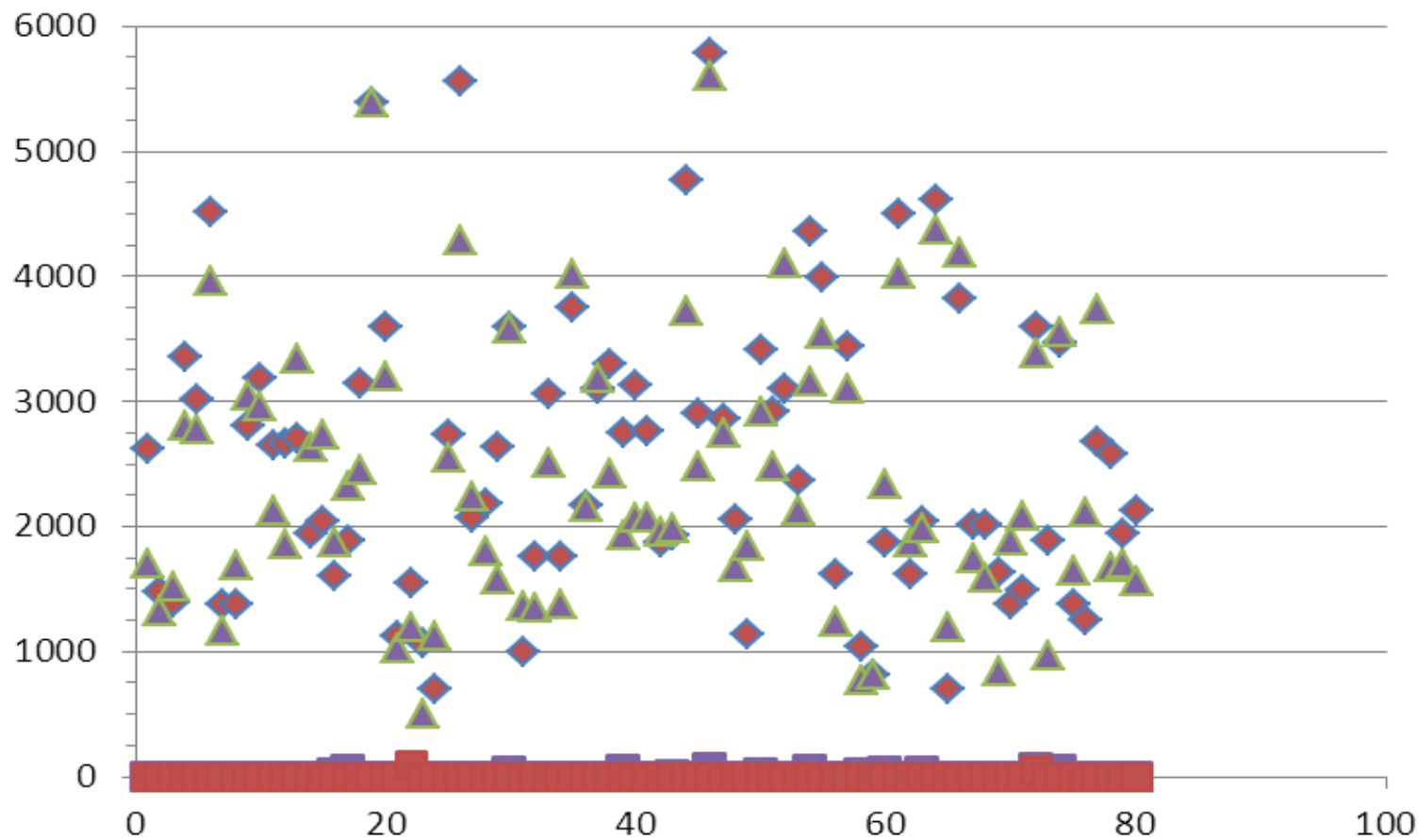
RNaseP Values for 80 BSD-punched Guthrie spots and Companion Blanks



RNaseP Values for 80 **BSD** or **Hand-** **punched Guthrie spots and** **Companion Blanks**

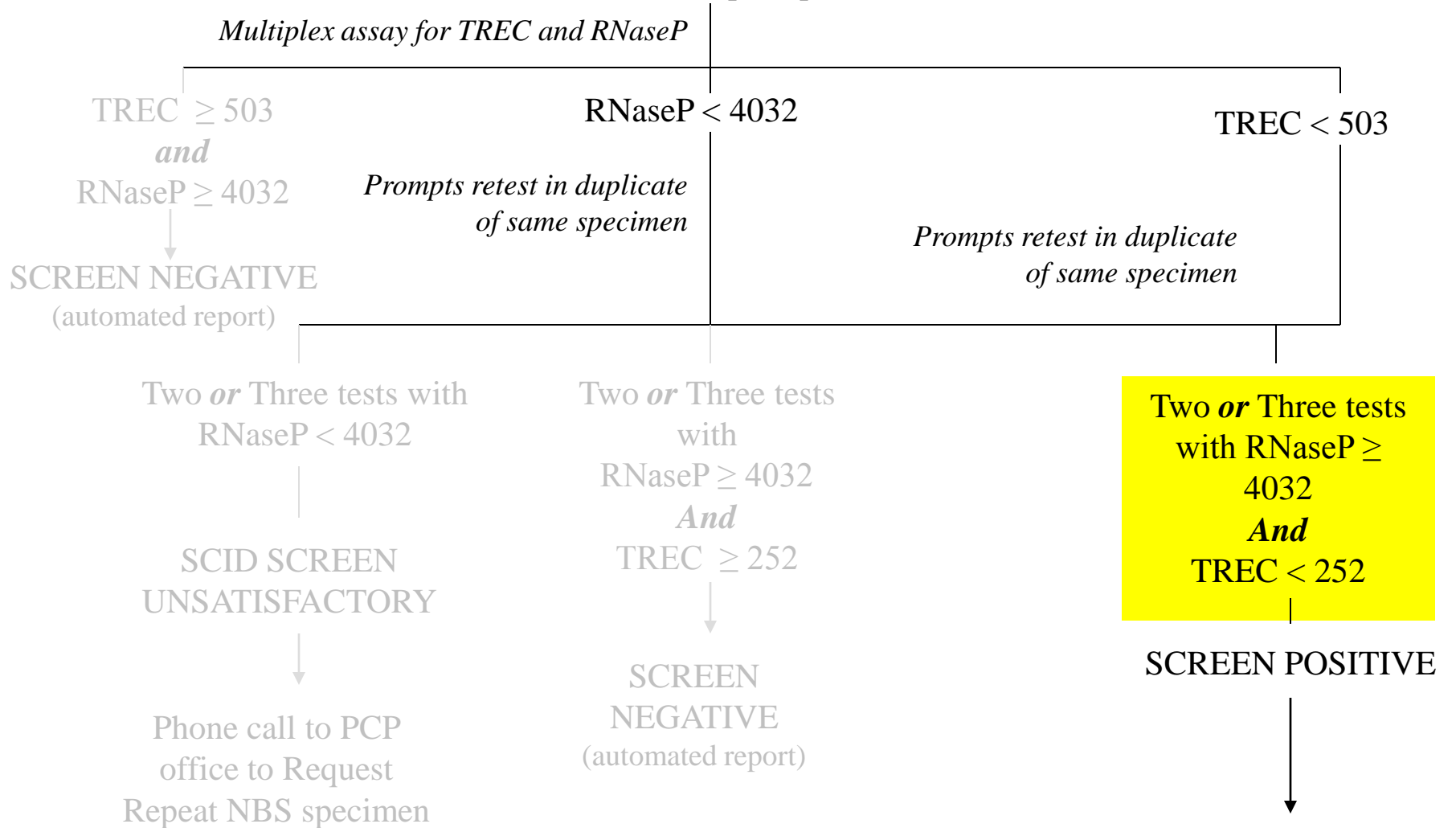


TREC Values for 80 **BSD**-or **Hand-** punched Guthrie Spots and Companion Blanks



SCID NBS Algorithm

(All TREC & RNaseP Values are copies/ul)
Dried Blood Spot Specimen



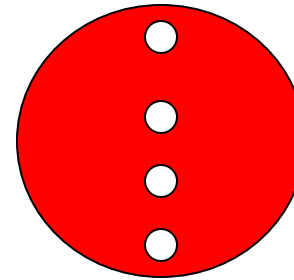
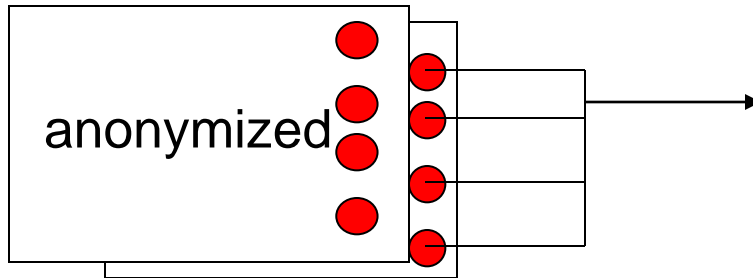
PHONE CONSULT with PCP and recommendation for repeat NBS and/or Flow Cytometry followed by fax of Screen Positive report packet

Precision of the Assay

Considerations

- intrinsic to the assay
 - within eluate
 - Between instruments, calibration curves
- intrinsic to the sample
 - Across a spot; DNA preparation

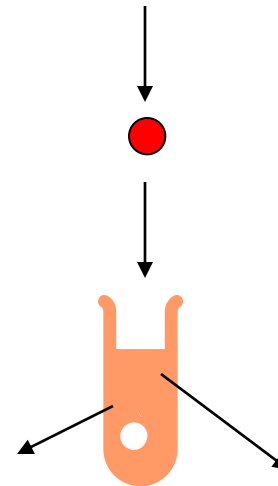
20
cards



Per
NBS card

16 punches

16 eluates



Per
eluate 10 reactions



Instruments 1 and 2

160 data points per NBS card

Precision of the Assay – eluates

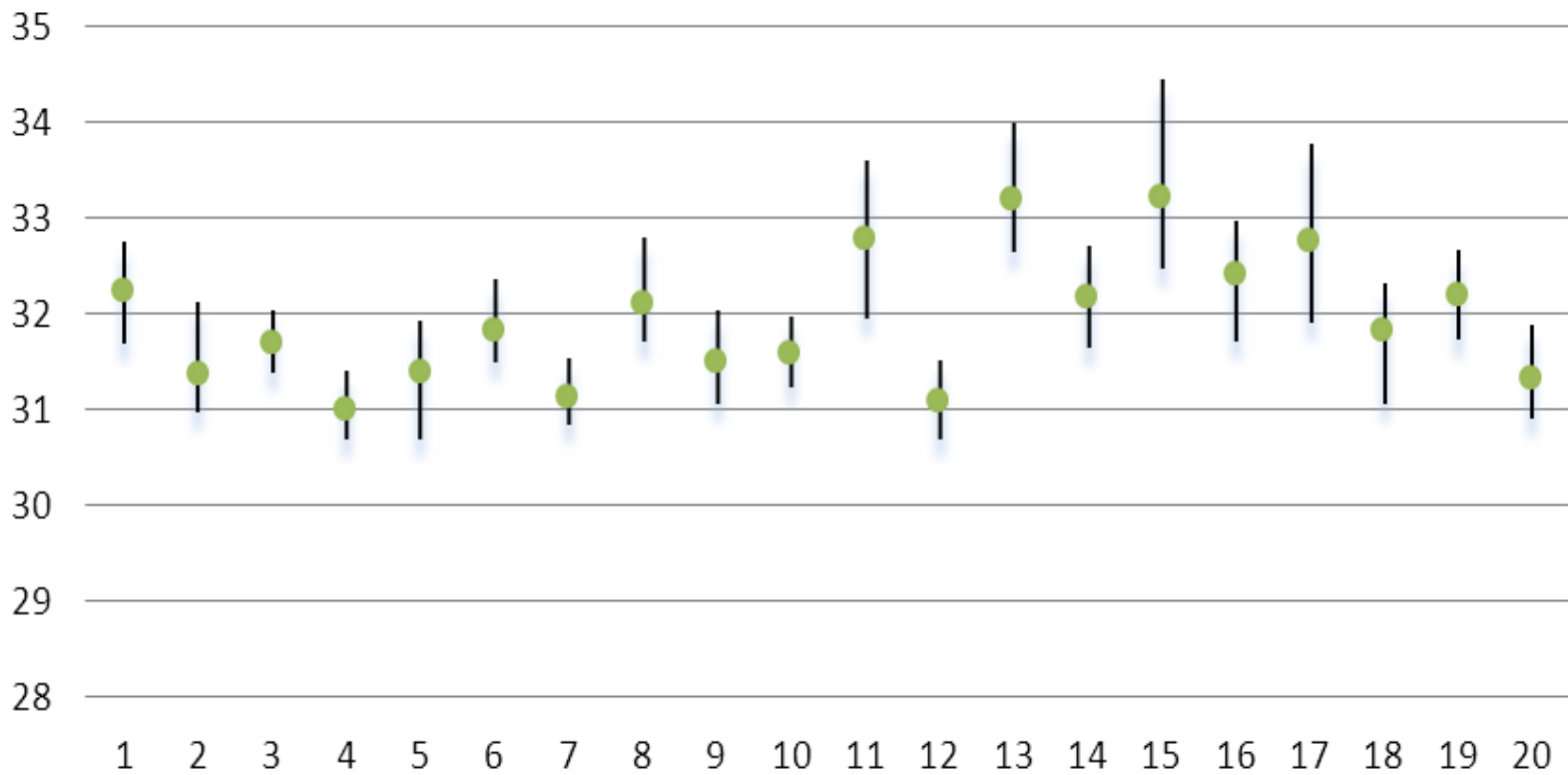
(sample from 320 eluates)

| | Cq values | Cq values | |
|----------------|-----------|-----------|---------------|
| | 31.51 | 31.27 | |
| | 31.14 | 31.11 | |
| 471 copies /ul | 31.13 | 31.24 | 463 copies/ul |
| | 30.88 | 30.96 | |
| | 31.07 | 30.82 | |

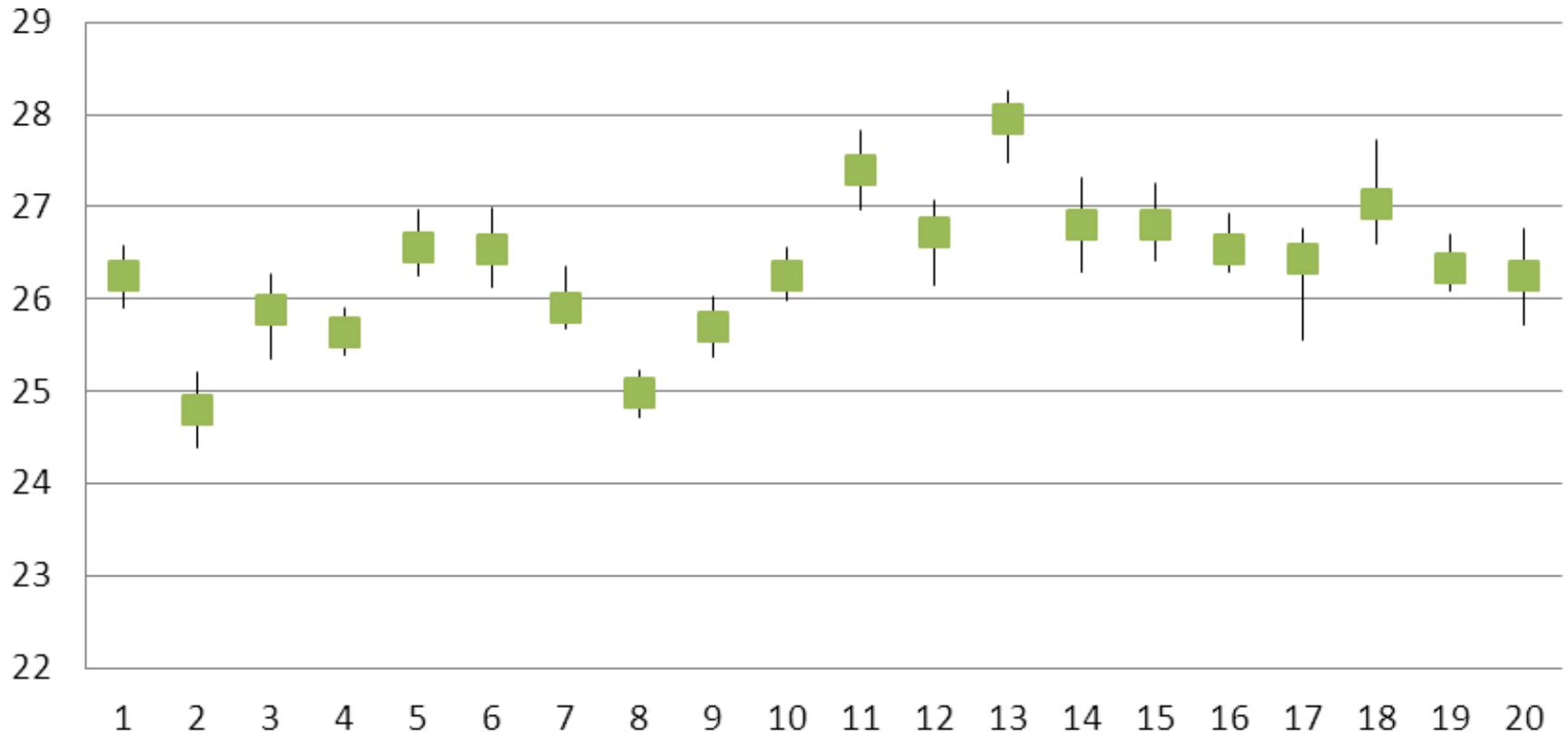
Mean SD all eluates 0.21

Precision of the Assay – across a spot

TREC Cq Values from Sampling across 20 Guthrie Spots Min, Max, Mean for 16 Punches



RNase P Cq Values from Sampling across 20 Guthrie Spots Min, Max, Mean for 16 Punches



Thoughts

Confirms High Quality Robust Assay

It's always a good thing to check...for things that might be reasonably preventable.

Punching considerations

Use of replicate data within algorithm

SCID NBS Diagnoses

