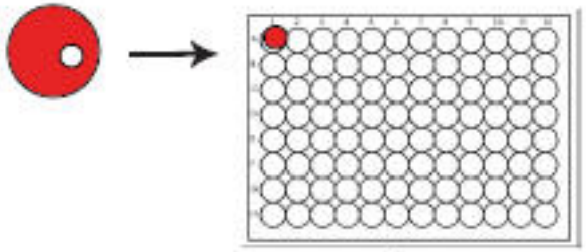
"An improved method for DNA extraction from Dried Blood Spots for T receptor excision (TREC) analysis and other Newborn Screening assays"

Heather Wood, M.S.
Michigan Department of Community Health
Bureau of Laboratories

Overall Analysis Scheme

Punch DBS into 96 well plate.



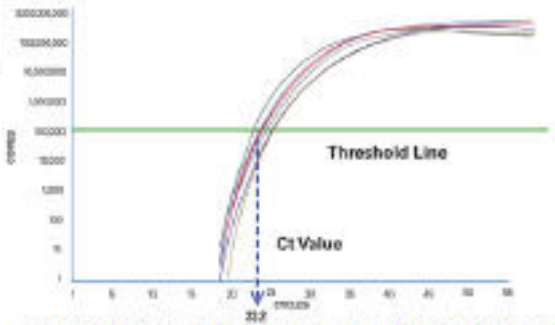
3 mm DBS is punched into a 96 well plate

Automated DNA Extraction

http://dna.uga.edu/image/sized/images/page-images/epMotion-5075

Partially automated DNA extraction using an epMotion 5075

Quantitative PCR

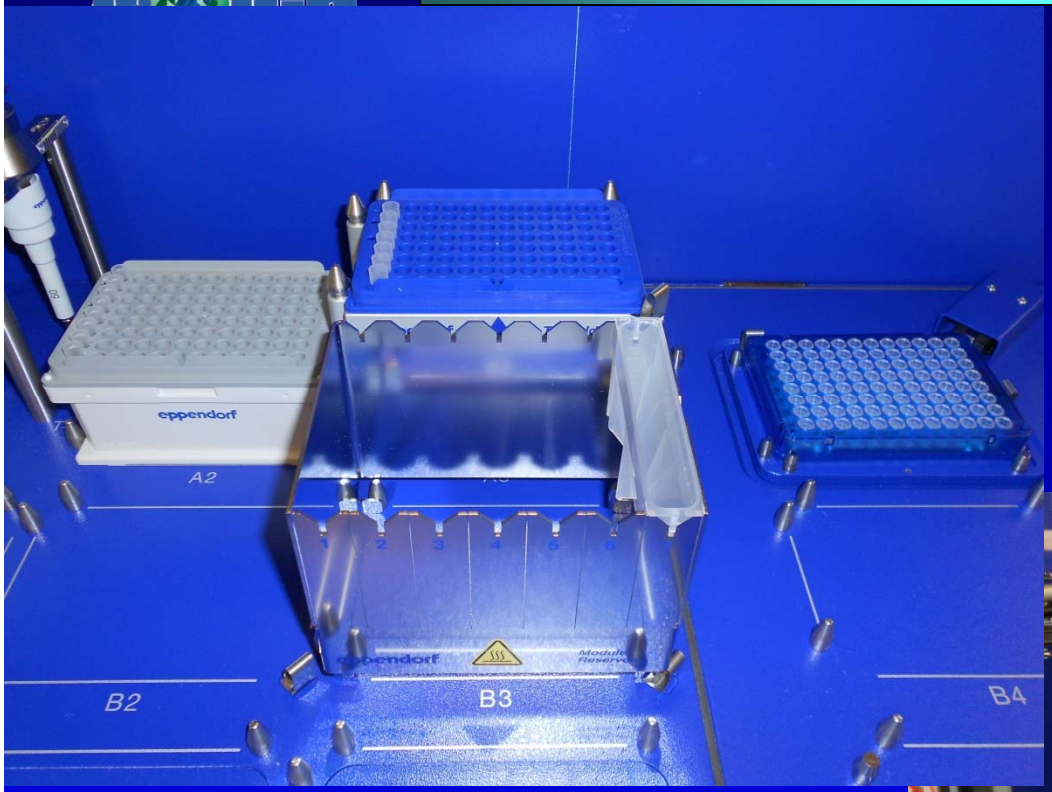


http://www.appliedbiosystems.com/absite/us/en/home/applications-technologies/real-time-pcr/real-time-pcr-vs-traditional-pcr.html

Automated set up of real time qPCR in a 384 well format using the epMotion 5075

Duplex qPCR amplification and analysis (TREC and beta actin) on a 7900HT.

epMotion 5075



Prevent Disease – Promote

Extraction method used from Oct. 1, 2011 to Sept 1, 2012

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Solution 1

WASH

Shake 10 min room temperature 500 rpm

WASH

Shake 10 min room temperature 500 rpm

Solution 2

WASH

Shake 10 min room temperature 500 rpm

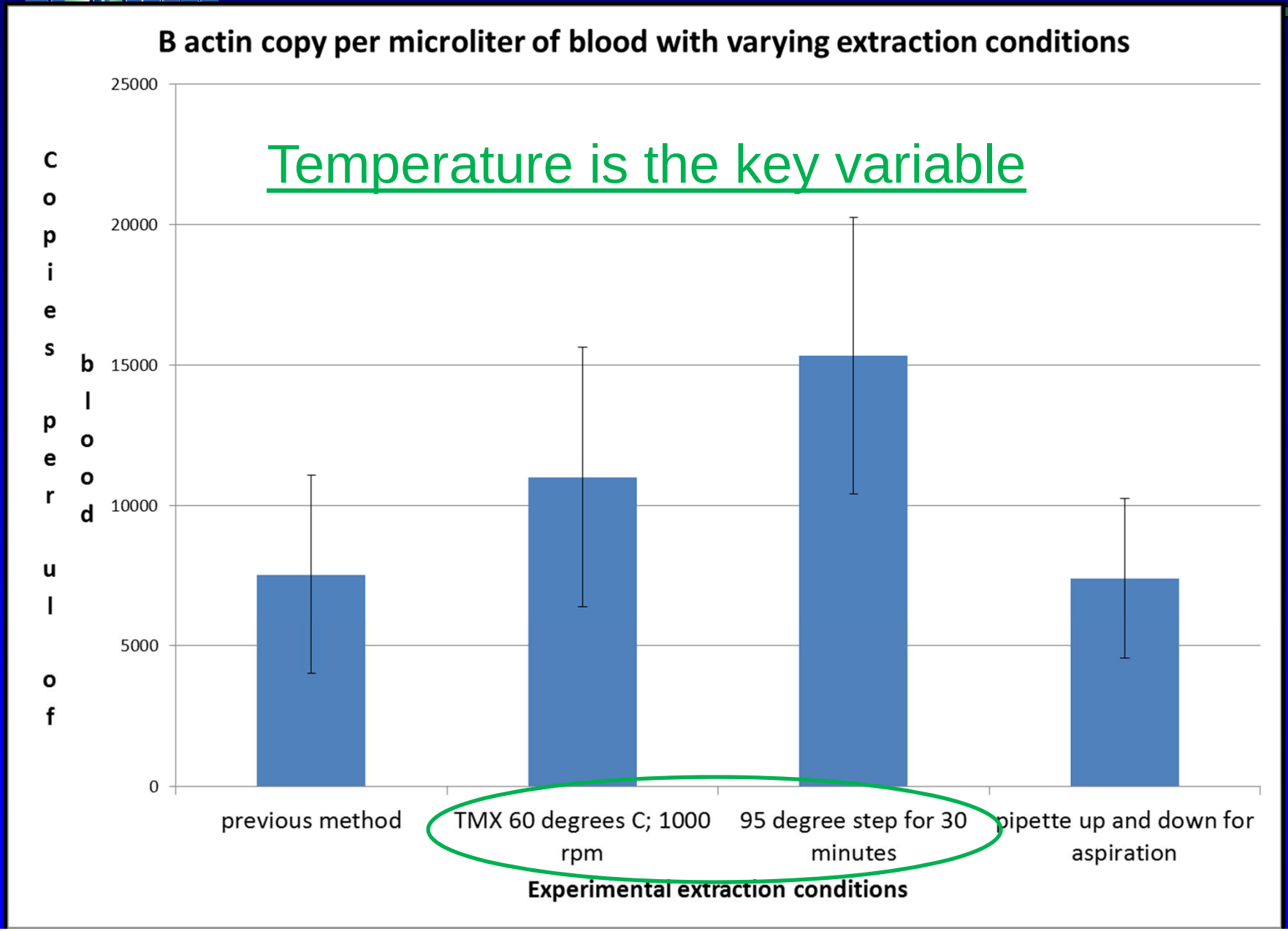
ELUTE into 50 µl

Incubate 95 degrees for 15 min

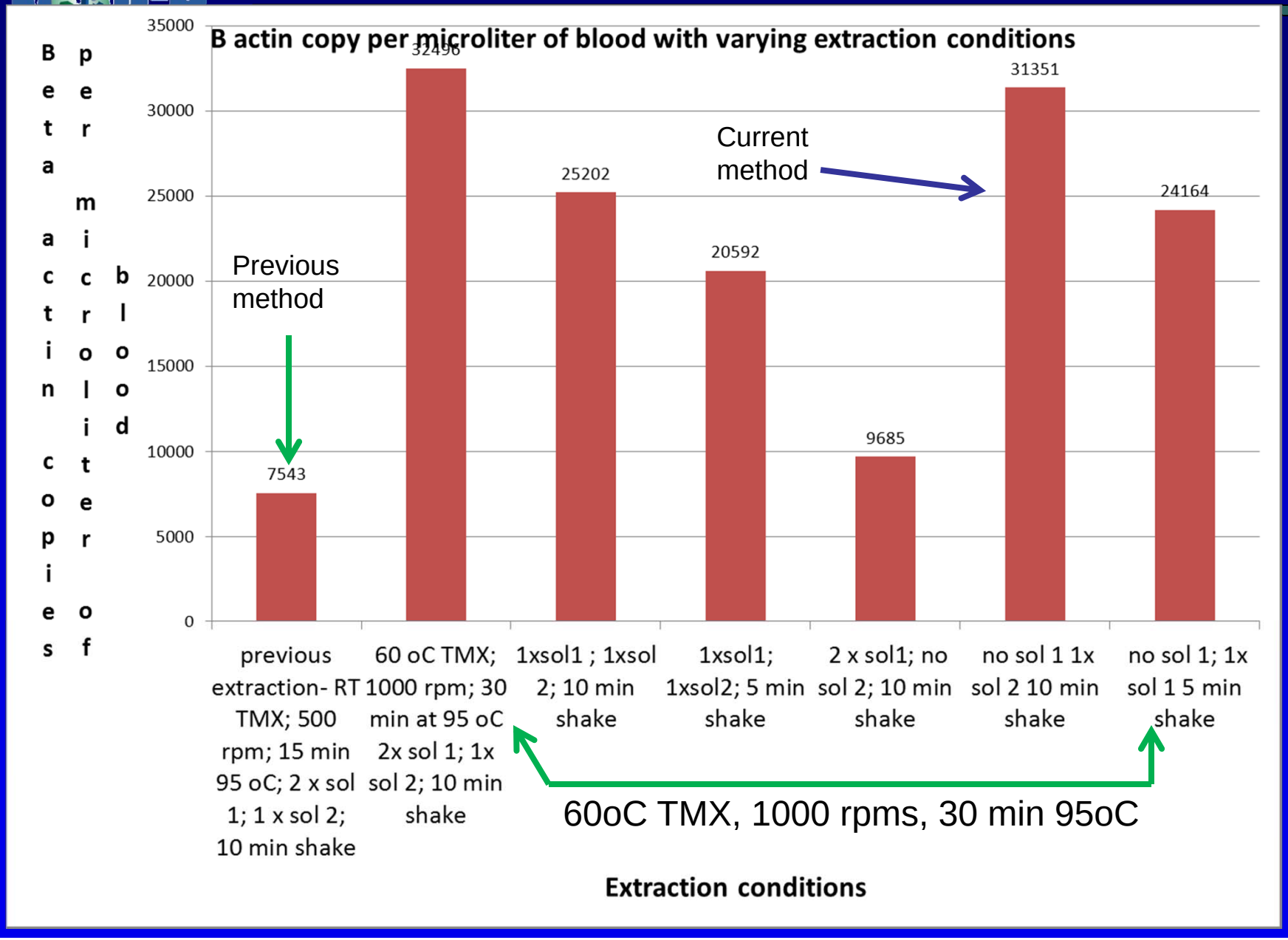
Reasons to change the TREC method in MI

- High repeat rate in the lab
- High “abnormal” rate reported (≈0.4%).
- Too many tips used
- Extraction method was time consuming.
- CDC SCID grant aims included:
  - » A new extraction method that is faster, less expensive, and more efficient.
  - » Utilizing the DNA extract for other molecular methods in the MI NBS lab (cystic fibrosis).

Summary for extraction conditions tested



Summary for extraction conditions tested



Comparison of the two DNA extraction methods

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Previous method

WASH

Shake 10 min room temperature 500 rpm

WASH

Shake 10 min room temperature 500 rpm

WASH

Shake 10 min room temperature 500 rpm

ELUTE into 50 µl

Incubate 95 degrees for 15 min

Solution 1

Solution 2

Current method

~~WASH~~

~~Shake 10 min~~ ~~500 rpm~~

~~WASH~~

~~500 rpm~~

WASH

Shake 10 min 60 degrees C 100 rpm

ELUTE into 50 µl

Incubate 95 degrees for 30 min

Comparison of the two DNA extraction methods

	Previous Method	Current Method
Time on epMotion	75 minutes	20 minutes
Tips used per 96 well plate	404	130
Average repeat rate in lab	10%	<3%

Summary of cost savings

- Reduction in consumables ≈ $0.24 per sample.
- Less storage space needed for tips, less autoclaving ≈ $5,000 a year.
- No more solution 1 used ≈ $23,000 a year.
- Less time spent doing the bench work. Able to reduce down to one FTE technician. ≈ $80,000 a year (benefits and salary).

Summary of cost savings

- Lower repeat rate in the lab from nearly 10% to down to less than 3%. Equates to over $15,000 dollars in savings (reagents and consumables).
- Estimated cost savings by utilizing DNA extract for Cystic Fibrosis testing ≈ $50,000 (reagents, consumables, and technician time).
- Additional measures saves nearly $30,000 a year in lab savings.

New Method Validation completed in 2012; go live date September 1, 2012

- Compared a new and improved DNA extraction method to the former DNA extraction method used for TREC analysis and for Cystic fibrosis.
- Compared Gene Expression master mix to Environmental master mix.

Comparison of master mixes from ABI

Heparin in Units/mL of blood	B actin quantity per µl of blood		Gene Expression Master Mix
			Environmental Master Mix
10	5655	24218	
20	3695	16878	
30	3837	21307	
40	4383	22231	
50	6275	22710	
60	5885	21982	
70	8225	21511	
80	8753	20668	
90	7936	24722	
100	9025	22756	
200	5516	22163	
300	5083	23771	
400	3024	23820	
500	1751	25621	
600	1060	21690	
700	829	18954	
800	379	19744	
900	554	18603	
1000	190	17244	

*Each data point is an average of 6 DBS replicates with various heparin levels with 2 master mixes.

Environmental master mix much better!

This DNA extraction works with the following molecular assays:

- **Methods run by MI**
  - qPCR (duplex of TREC and β-actin)
  - Rnase P
  - Cystic Fibrosis InPlex ®- (full validation)
  - Melting curve analysis of spinal muscular atrophy (SMA)- (several thousand samples)
  - Biotinidase sequencing (run by Henry Ford Hospital)

This DNA extraction works with the following molecular assays:

- **Methods run by CDC's NSMBB**
  - RNAse P qPCR
  - Microsatellite short tandem repeat (STR) analysis
  - Sequencing
    - CF exon 11 (501 bp) and 12 (477 bp)
    - HBA2 (1350 bp)
    - HBA1 (1622 bp)
    - HBB (1932 bp)
    - GALT (3974 bp)
    - CAH (5624 bp) *Note: A faint 5624 bp amplicon was present, however not enough to perform sequencing assay.

Where do we go from here?

- Implement “cherry” picking using epMotion 5075 from extracted DNA to use for CF mutation analysis.
- Host other NBS labs for training and assist with SCID startup
- Investigate other primary and secondary targets.

Acknowledgements

- MI NBS laboratory
- This work was partially funded by a research cooperative agreement from CDC (Grant # 01EH000936) and does not represent the official view of CDC.
- MAP team members
- NY & WI laboratories
- CDC/NCEH/NSMBB
- Other NBS labs