Newborn Screening by Tandem Mass Spectrometry in Texas

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Texas Newborn Screening

- Texas mandates 2 screens for each infant
- Collection Goals:
  - 1st screen at 24 – 48 hours of age &
  - 2nd screen at 1 – 2 weeks of age
- Receive ~700,000 specimens annually
  - Unsatisfactory specimen rate is ~ 0.5%

Texas Newborn MS/MS Screening

- Screen for Core RUSP Panel
- Use multiple reaction monitoring mode and only measure markers needed to detect core panel disorders
- Use different cutoffs for 1st and 2nd screen specimens
- Reagent rental contract includes technical support
- 10 Waters Quattro Micro Mass Instruments

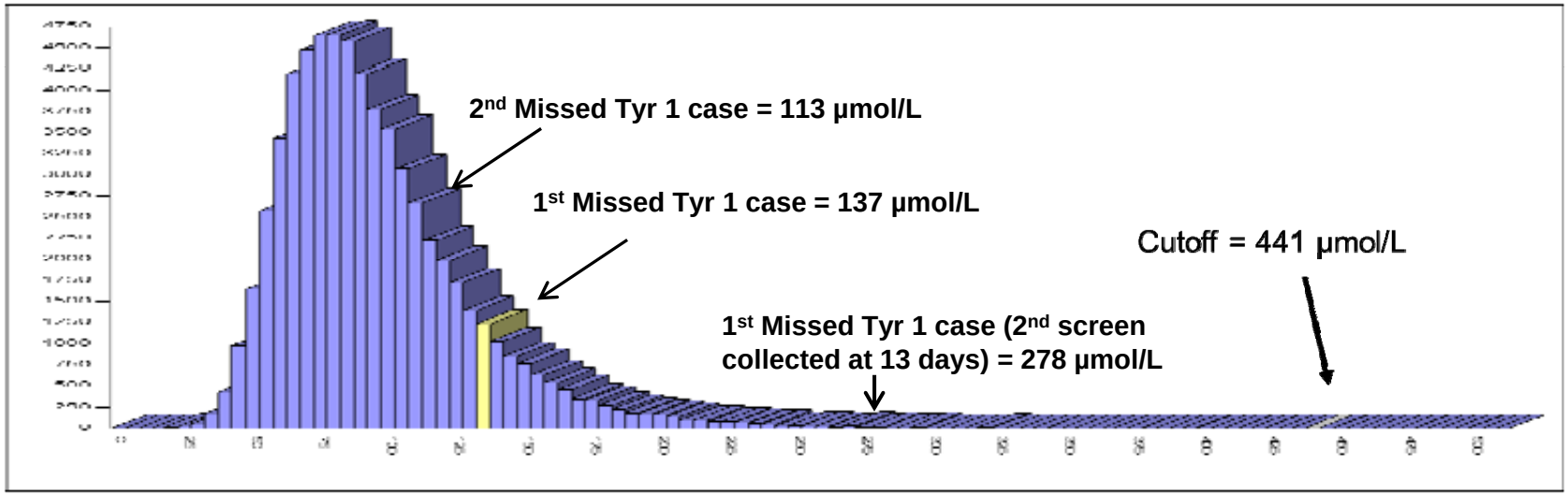
Texas MS/MS Methods

- December 2006 – March 2010 used PerkinElmer NeoGram Derivatized Kit
  - Initial cutoffs based on 20,000 1st screen specimens and 20,000 2nd screen specimens
- Decided to make the switch to non-derivatized method because:
  - 2 missed Tyrosinemia Type1 (TYR1) cases with very normal tyrosine values
  - Ability to measure succinylacetone, amino acids and acylcarnitines in one assay
  - Simplification of sample preparation steps

Tyrosinemia Type I

Limitations of screening for TYR 1 using tyrosine as a primary marker

- High false positive rate expected if cutoff is set low enough to detect affected infants



Texas MS/MS Methods

- Discussed the idea of changing with the Texas Metabolic Specialists in late 2009
  - Reviewed TYR 1 issue, isobar issue, lower recovery of some analytes observed during participation in PerkinElmer study
  - Approved by Metabolic Specialists & Medical Director
- April 12, 2010 implemented PerkinElmer NeoBase Non-Derivatized Kit
  - Follow kit insert – transfer off blood spots

NeoBase Non-derivatized MS/MS Kit Validation

- Validation Study – February - March 2010
- Initial cutoffs based on 10,000 1st screen specimens and 10,000 2nd screen specimens
- Recovery of some analytes was lower (Met, Phe, C0) and some analytes was higher (C5DC, C5OH and Leu)
- All cutoffs were recalculated – generally Texas cutoffs are based on percentile values

Workload – since non-derivatized assay implemented

	1st screen	2nd screen
2010 - Apr-Dec	296,974	277,601
2011	379,255	356,671
2012 - Jan-Apr	118,131	113,129
Total	794,360	747,101

Issues Encountered after Switch

- Issue 1 – Increase in PKU abnormals
  - To troubleshoot - tested abnormal samples with derivatized method, Phe normal – probable contamination issue
  - Most affected samples submitted by midwives
  - Contacted midwife, during discussion noted use of alcohol swabs with pain killer – benzocaine
  - Confirmed benzocaine has same mass as phenylalanine
  - Communicated with midwives/midwifery groups added a note to NBS collection kit to avoid use of alcohol swabs with benzocaine
- Issue 2 – Missed Succinylacetone Proficiency (PT) Samples
  - Initial Cutoff = 3.5 µmol/L
  - Due to poor recovery - samples spiked at 10 µmol/L ranged from normal to abnormal
  - Adjusted Cutoff = 3.0 µmol/L
  - Succinylacetone values of 3 TYRI confirmed positive specimens analyzed during verification study were 7.0, 4.5, and 8.3 µmol/L respectively

Issues Encountered after Switch

- Issue 3 - Free Carnitine Drift
  - Cutoff = 8.0 µmol/L
  - Missed one PT sample – value just above cutoff
  - A noticeable variation in patient mean and control values when instruments are recently cleaned vs when instruments are dirty
  - Still a challenge – but has led to more rigorous monitoring of patient mean and control values

- Issue 4 – Instruments became dirty sooner
  - More frequent troubleshooting due to bad total ion chromatograms, lowered analyte intensities and control flags out of range
  - Required changes to preventative maintenance schedule

Changes in Preventative Maintenance

- Daily cleaning of front end is more rigorous – cones are dirtier
- More frequent drops in analyte intensities due to blocked cones and stainless steel capillaries
- Tips of capillaries are damaged more often due to “burning/blackening” and have to be replaced more frequently
- More frequent increases in pump pressure due to blocked filter frits - frits replaced every 2-3 weeks versus monthly
- LC pump heads become “dirty” – debris builds up due to oxalic acid – wipe these down every few days to keep them clean.
- More frequent preventative maintenance and troubleshooting by service engineers.

Changes to Texas MS/MS Screening

- Addition of succinylacetone – TYR1 reporting algorithm adjusted
- Simpler sample preparation method – reduced plate handling – 1 less FTE needed
- Less peripheral equipment needed – 6 less specimen evaporators, 3 less plate sealers, and 3 less 9-plate incubators
- No corrosive chemicals
- Have to be more aware of timing of plate preparation due to 2 hour incubation so that plates can be added to the queue and analyzed continuously

Outcomes with Non-Derivatized Method

	Der	Der	Der	Der/NDer	NDer
Disorder	2007	2008	2009	2010	2011
Tandem Mass Spectrometry (21)	2,581	2,039	1,931	2,620	2,561
Amino Acid Disorders	767	625	748	667	563
Fatty Acid Oxidation Disorders	781	534	649	1057	853
Organic Acid Disorders	1033	880	537	896	1145
Total Specimens Tested	792,000	791,000	785,453	758,000	736,000

- Non-derivatized kit implemented April 10, 2010
  - Amino Acid presumptive positives dropped – TYR algorithm updated to incorporate succinylacetone result
  - Fatty Acid presumptive positives increased – more conservative cutoffs implemented initially – updated in October 2010
  - Organic Acid presumptive positives increased – beginning slow process of adjusting cutoffs as diagnosed cases are reported