

## The Heartland Good Spot/Bad Spot Project



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## Newborn Screening Workshops

- Conducted in the spring for the last 3 years
- 2009 Meeting - Unsatisfactory specimens were a topic of interest
- Two states in the Heartland Region are not permitted to test UNSATs by their CLIA inspectors
- Unsatisfactory (UNSAT) due to poor specimen collection
  - Insufficient blood (incomplete saturation or spots too small)
  - Layered, oversaturated and/or clotted blood

## Goals of Project

- To evaluate if there is a definite value in analyzing UNSAT specimens
  - Most true disorders are still easily detectable
  - Allows for quick action of certain positive cases
- To determine the extent to which UNSAT specimens produce false positive or false negative results

## Project Methods

- Two studies were conducted for this project
- Study #1 focused on analysis of residual blood spots of true/confirmed positive cases
- Study #2 analyzed routine specimens which had both good and bad blood spots on the filter paper

## Project Methods Study #1

- Residual blood spots from 135 confirmed positive specimens were analyzed
- Data were collected for the key markers/analytes for the confirmed disorder
- Original values and study values were recorded, including data for simulated UNSAT blood spots

## Project Methods Study #1

- For both studies, all parallel testing was performed on the same day, using the same reagents, instruments and kits lot numbers
- Simulated extreme poor quality specimens were tested along with a good blood spot for each specimen

## Project Methods Study #1

- Simulated incomplete saturation
  - One spot was punched from the edge of the circle with approximately 50% white showing
- Simulated layered or oversaturation
  - Obtain two normal punches to be placed in the same well of the test plate
- Control Specimen
  - To test for storage induced changes, one regular punch was taken

## Project Methods Study #2

- Over several months
  - utilized routine specimens that had one or two poor quality circles
- After regular testing was completed for these specimens
  - one punch was taken from a good circle and one punch from a poor quality circle to run in parallel

## Project Methods Study #2

- Collected Data on key markers/analytes
  - TSH, GALT, Phe, C8, C3, 17-OHP, IRT, HB, BT
- Analyte values were then compared between the good spot and the bad spot for each specimen

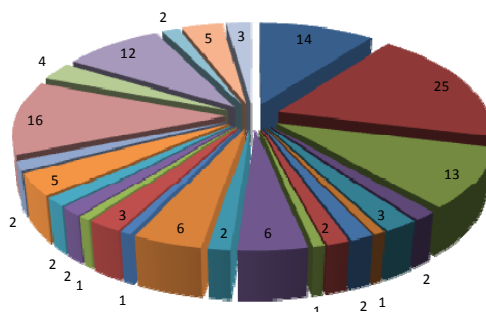
## Project Methods Study #2

- Incomplete saturation
  - Punch one spot from the poor quality area of the blood spot with the goal of punching about 50% white area
- Layered or oversaturation
  - Punch one spot from the poor quality area of the blood spot where it is visibly darker, crusted, or too concentrated with blood
- Control Specimen
  - Punch one spot from a good area of the blood spot

## Results of Study #1

### 135 Confirmed Disorders Used for Study # 1

■ CAH, 14	■ CF, 25	■ CH, 13	■ CIT, 2	■ CUD, 3
■ DG, 1	■ Bart's, 2	■ FE, 2	■ F-Only, 1	■ FS, 6
■ FSA, 2	■ FSC, 6	■ FSX, 1	■ GALT, 3	■ GA-1, 1
■ H-Phe, 2	■ HCY, 2	■ IVA, 5	■ LCHAD, 2	■ MCAD, 16
■ MMA, 4	■ PKU, 12	■ PROP, 2	■ SCAD, 5	■ VLCAD, 3



## Results of Study #1

### Incomplete Saturation Comparison

- 117 of 135 remained positive
- 9 cases converted to a borderline result
- Cases converted using the decision schemes of the state conducting the testing and may not have converted using other Heartland State's algorithms

## Results of Study #1

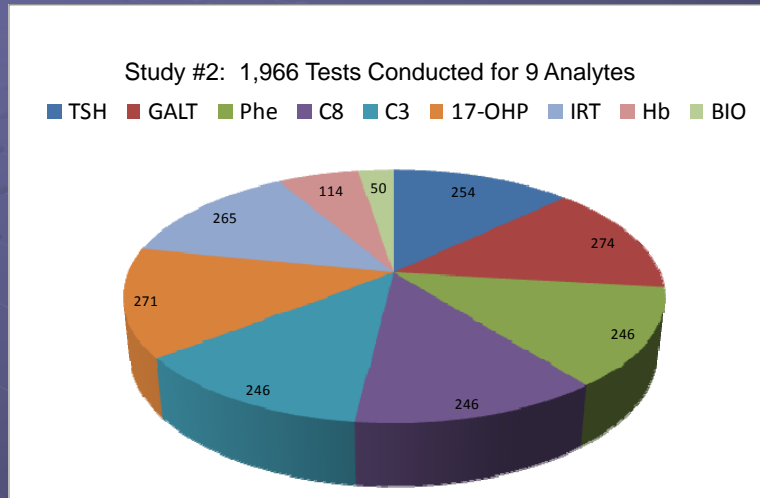
9 Confirmed Disorders Converted to Borderline with Undersaturation	
1 Citrullinemia (Mild)	Citrulline dropped from 102 to 71
2 Hypothyroidism	One TSH dropped from 50 to 35, and the other dropped from 65 to 45
1 CAH	17-OHP dropped from 75.5 to 66.7
1 MCAD (variant)	C8 dropped from 1.08 to 0.50; C8/C10 went from 1.87 to 1.4
1 VLCAD	C14:1 dropped from 1.09 to 0.71; C14:1/C16 went from 0.48 to 0.55
1 LCHAD	C16OH dropped from 0.30 to 0.15; C16OH/C16 went from 0.11 to 0.09
1 MMA (Cbl-C)	C3 dropped from 7.74 to 5.71; C3/C2 remained at 0.25
1 Propionic acidemia	C3 dropped from 7.32 to 5.64; C3/C2 went from 0.48 to 0.53

## Results of Study #1

### Oversaturation Comparison

- No specimens converted to borderline
- 4 specimens converted to normal
  - 3 cases of Carnitine Uptake Deficiency
  - 1 D/G Galactosemia
- 3 cases of Classic Galactosemia remained positive

## Results of Study #2



## Results of Study #2

- None of the incomplete saturation samples converted to abnormal
- 43 of the oversaturated samples converted to abnormal
  - 41 of those were analyzed by one state which was forced to punch two spots into well (similar to Study #1)



## Discussion

Working as a Region enabled the ability to

- Produce more confirmed positive cases for inclusion into the study
- Produce more routine poor quality cases for inclusion into the study
- Smooth out certain biases that may exist in how one state may determine UNSAT status
- Smooth out variables that may exist on how one state may set and use cutoffs or interpret result status

## Conclusion

- Hemoglobinopathy testing was invulnerable to the UNSAT scenarios that were utilized
- HB results remained consistent throughout both studies
- Hemoglobinopathies are one of the most common disorders screened for in newborn screening

## Conclusion

- Analytical ratios commonly used in the MS/MS profiles were also very resilient to changes in sample concentrations resulting from incomplete and oversaturated samples
- Very few routine specimens from Study #2 converted to borderline and positive, proving that analyzing UNSATs on a routine basis does not cause the laboratory to be inundated with false positive result predicaments

## Conclusion

- Very few true positives converted to borderline or normal status
- Those that did convert were milder forms of the disorder and would still have been abnormal in some states
- Ratios remained the same for primary MS/MS markers even though some of the primary markers converted to borderline in the Incomplete Saturation scenario
  - This could keep the sample in the higher risk range for many screening labs

## Conclusion

- Analyzing UNSAT specimens to look for obvious high risk NBS disorders can be accomplished without causing undo havoc in the NBS laboratory and provide what may be the only chance available to provide life saving intervention for a child with a true disorder

## Conclusion

- Our goal is not to report UNSAT specimens
- Our goal is to identify babies that are at risk for those disorders in which waiting several days for a second specimen may mean death or permanent disability

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