

# Second tier testing for disorders identified by tandem mass spectrometry in the Mountain States Region

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## Abstract

The objective of this study was to improve the specificity and sensitivity of newborn screening for metabolic disorders and for congenital adrenal hyperplasia (CAH). Second tier tests targeting analytes different from those of standard newborn screening were performed on the same sample used for the primary analysis. We evaluated at a regional level the effectiveness of second tier tests to decrease the false positive results and the cost of the screening. Five states from Region 6 (Mountain States Region) participated in this study (Utah, Colorado, Montana, Texas, Wyoming). Samples with abnormal results for CAH or elevated C3-carnitine, tyrosine, and methionine from each state were sent to ARUP laboratories in Utah for second tier tests. The tests performed were steroid profile by MS/MS, quantification of methylmalonic and methylcitric acids, succinylacetone, and total homocysteine. The results obtained were electronically transmitted to the submitting states. Each state, for the duration of the study, continued their follow-up activities according to their individual protocols. Results of the second tier tests were compared with the final outcome of the additional studies performed. To date we have tested 2,905 blood spots with one or more of the second tier tests. 2.7% of the samples tested showed an abnormality on the second tier result needing further action (repeat screen or confirmatory tests). The concordance between the results of the second tier tests and the confirmed positive cases was 100% for the samples analyzed. These results indicate that second-tier testing, when available, can significantly reduce the false-positive rate of newborn screening while maintaining high sensitivity. A cost effectiveness analysis is in progress.

## Introduction

Screening for metabolic disorders by MS/MS is a very powerful method that allows the simultaneous detection of multiple analytes. However, some of these analytes (markers) are elevated not only in metabolic disorders, but also with common iatrogenic factors and this can result in false positives. Secondary markers are helpful, but not always an option or sensitive/specific enough. The use of second tier tests, tests run on the same sample but targeting different markers and/or using a different methodology, can increase both sensitivity and specificity of screening. The implementation of these tests requires resources that are not easily available within a Public Health Laboratory. In addition, the efficiency is increased when a large number of samples are processed, reducing the costs of the testing. For these reasons, we have evaluated the impact of second tier testing on the sensitivity and specificity of newborn screening in our region and the feasibility of a regional center performing second tier tests.

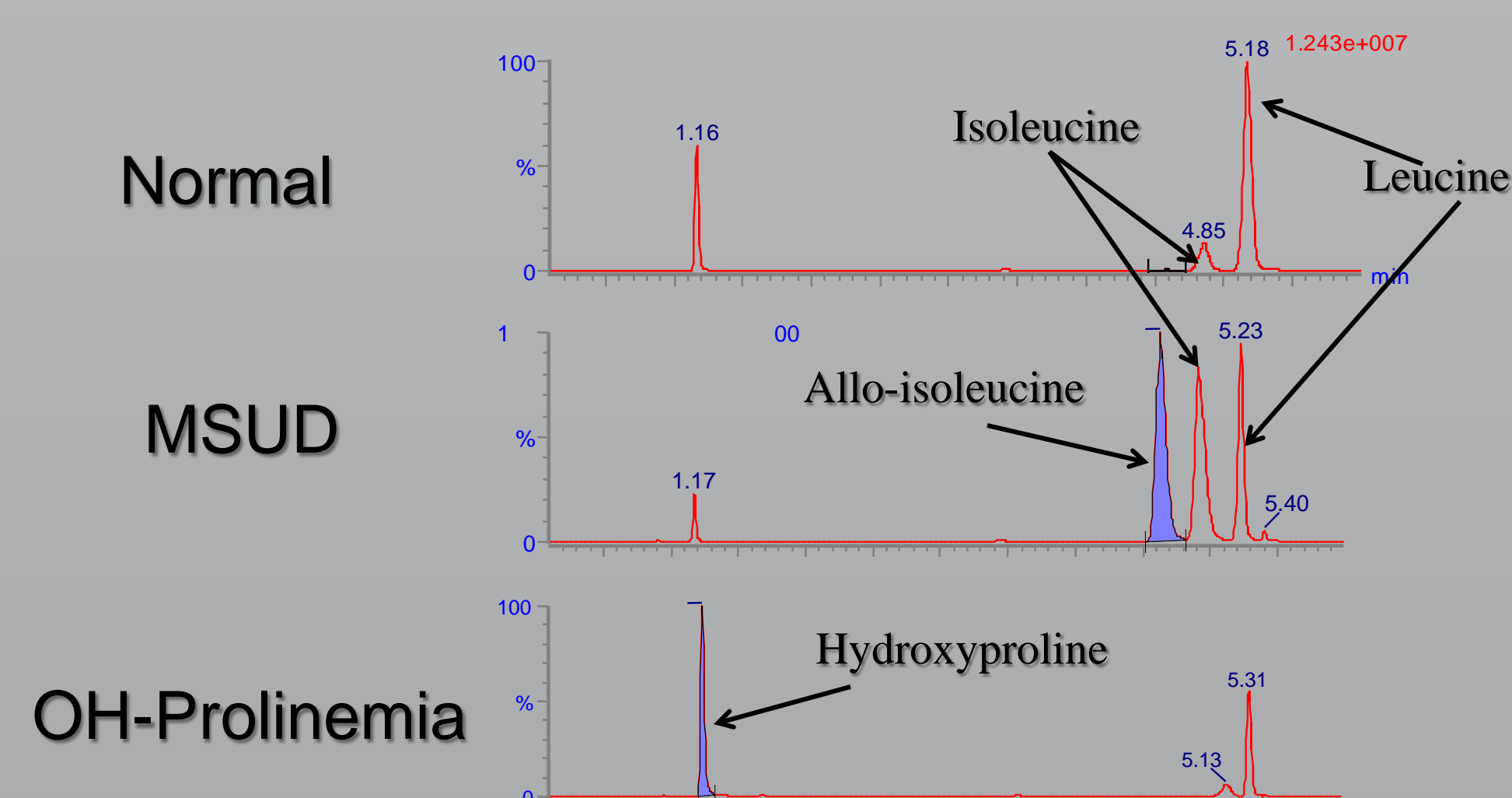


Figure 1. Separation of allo-isoleucine, leucine, isoleucine, and hydroxyproline by UPLC-MS/MS in dried blood spots.

## Methods

### Samples

This study was approved by the IRB of the University of Utah and of the Department of Health of each participating state. Deidentified dried blood spots (DBS) with abnormal screening results from each participating state were sent to ARUP Laboratories for second tier testing. The second tier tests performed were: 1) steroid profile for CAH (Congenital Adrenal Hyperplasia); 2) methylmalonic and methylcitric acids (elevated C3); 3) total homocysteine (elevated/low methionine); 4) allo-isoleucine and hydroxyproline (elevated leucine/isoleucine); 5) succinylacetone.

### Analytical methods

The extraction of the individual analytes was performed according to published procedures. The detection and quantitation of the analytes was performed using WATERS Quatro Premiere UPLC-MS/MS. Examples of chromatograms obtained are given in Figures 1 and 2. Outcome data were unknown to technologists during testing.

### Data analysis

Participating states did not modify their follow-up protocol. Outcome data from the follow-up of abnormal results are compared with the results obtained with the second tier tests. False positive and false negative rates are compared. We have also estimated the cost-effectiveness of adding second tier testing to newborn screening starting with the second tier test for CAH.

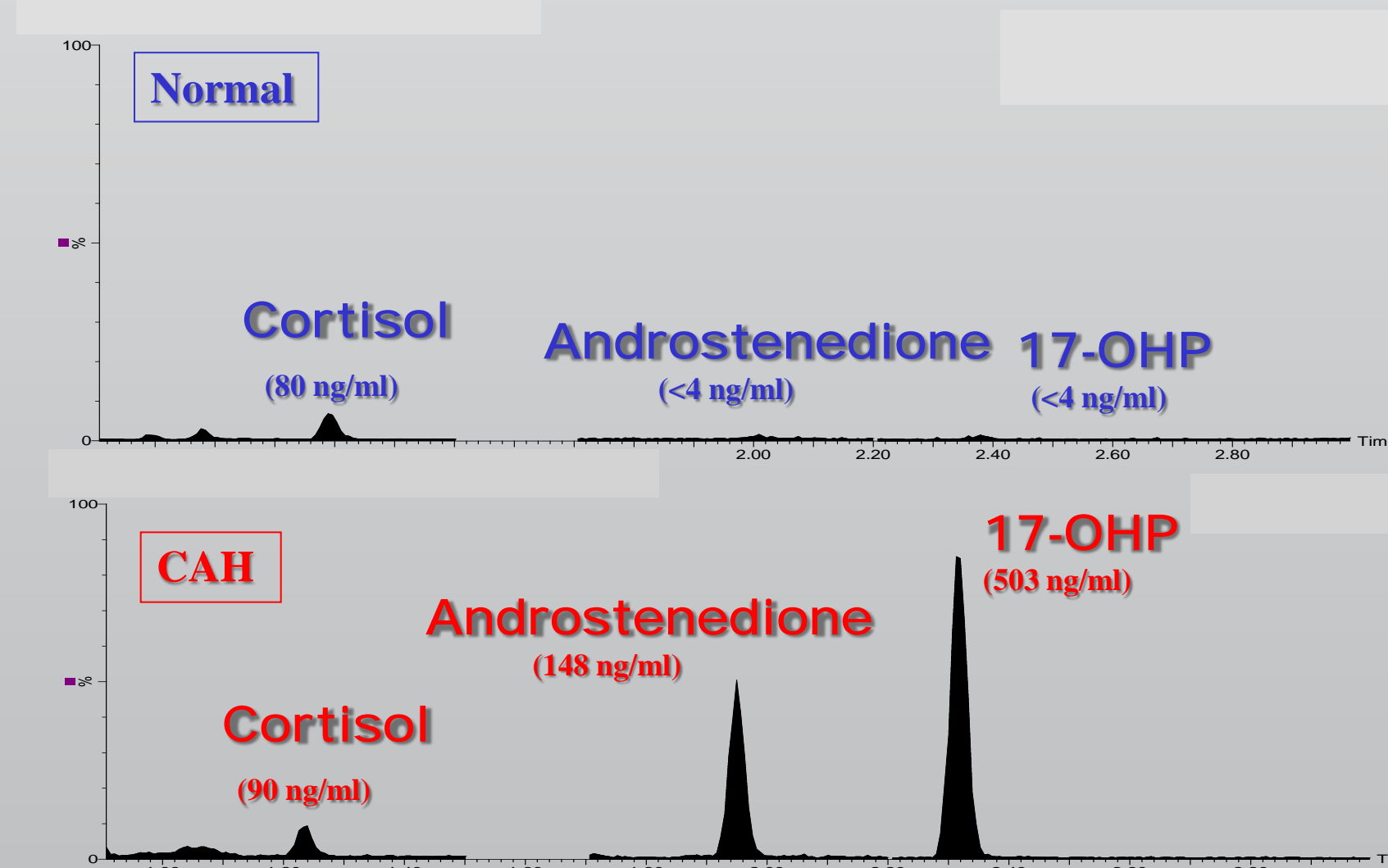


Figure 2. Steroid profiles by UPLC-MS/MS obtained from dried blood spots.

## Results

- A total of 3,693 second tier tests have been performed up to October 15, 2011; of these only 124 yielded abnormal results after second tier testing (3.4%) (Fig. 3).
- The majority of the second tier tests performed (55.2%) were for CAH, as expected, given the high positivity rate of the primary screen.
- Second tier tests for MMA/MCA and homocysteine represented 19.2% and 19.9% of the total tests respectively.
- Only 2% of the total tests were performed for allo-isoleucine, while 4% were performed for succinylacetone. The low percentage of tests for succinylacetone reflects the fact that most of the states participating in this project had already implemented or were implementing universal screening for succinylacetone.
- DBS from Low Birth Weight infants (LBW), defined as infants with a birth weight less than g 2500, were a significant percentage of the samples requiring second tier tests for CAH and for total homocysteine (due to elevated methionine) (Fig. 4).
- Among the samples analyzed so far, there was 100% concordance between the second tier test result and the results of the follow-up testing (Table 1).

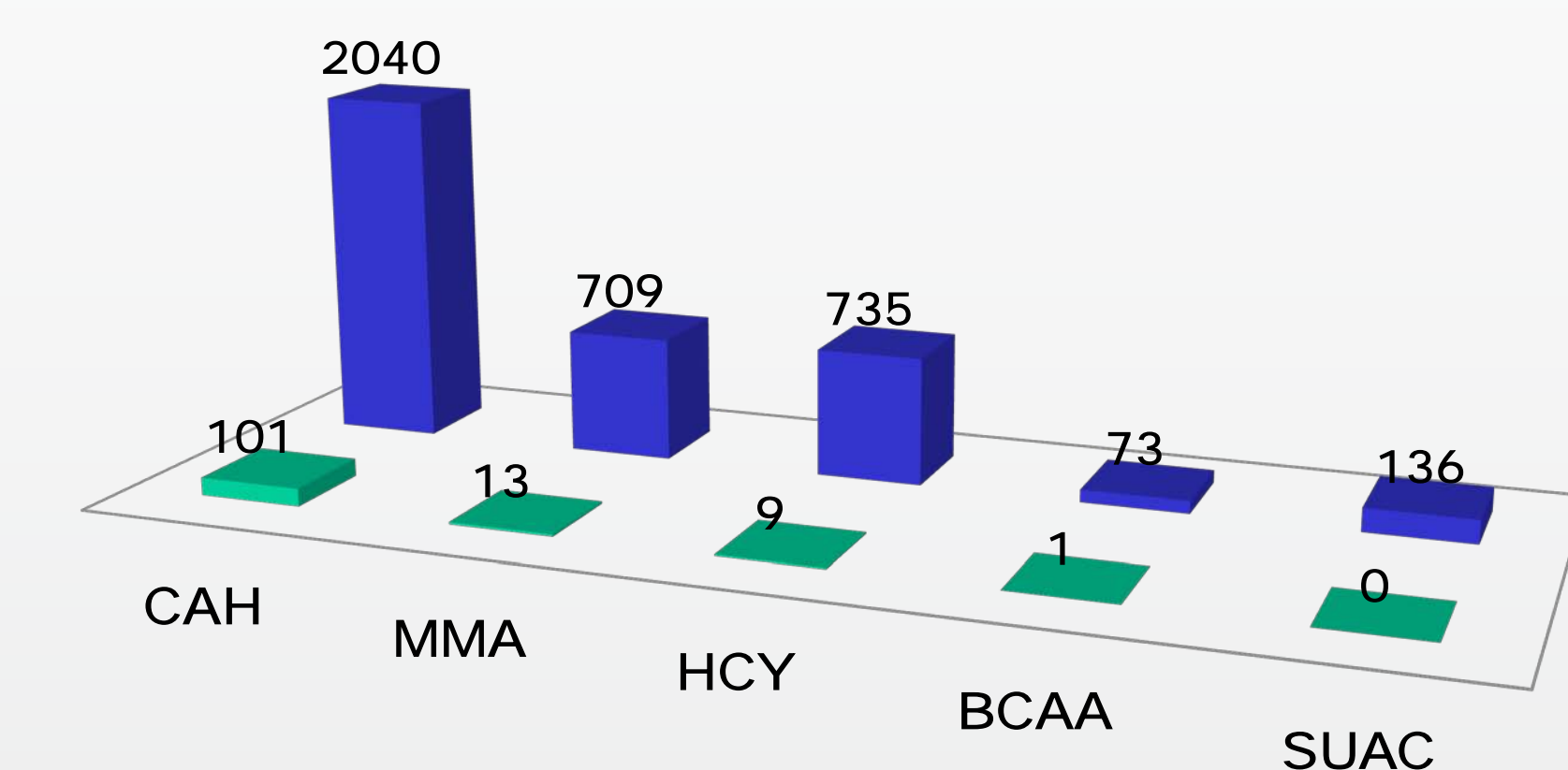


Figure 3. Second tier tests performed up-to-date. Blue columns represent the total number of tests, the green columns represent the number of abnormal second tier results.

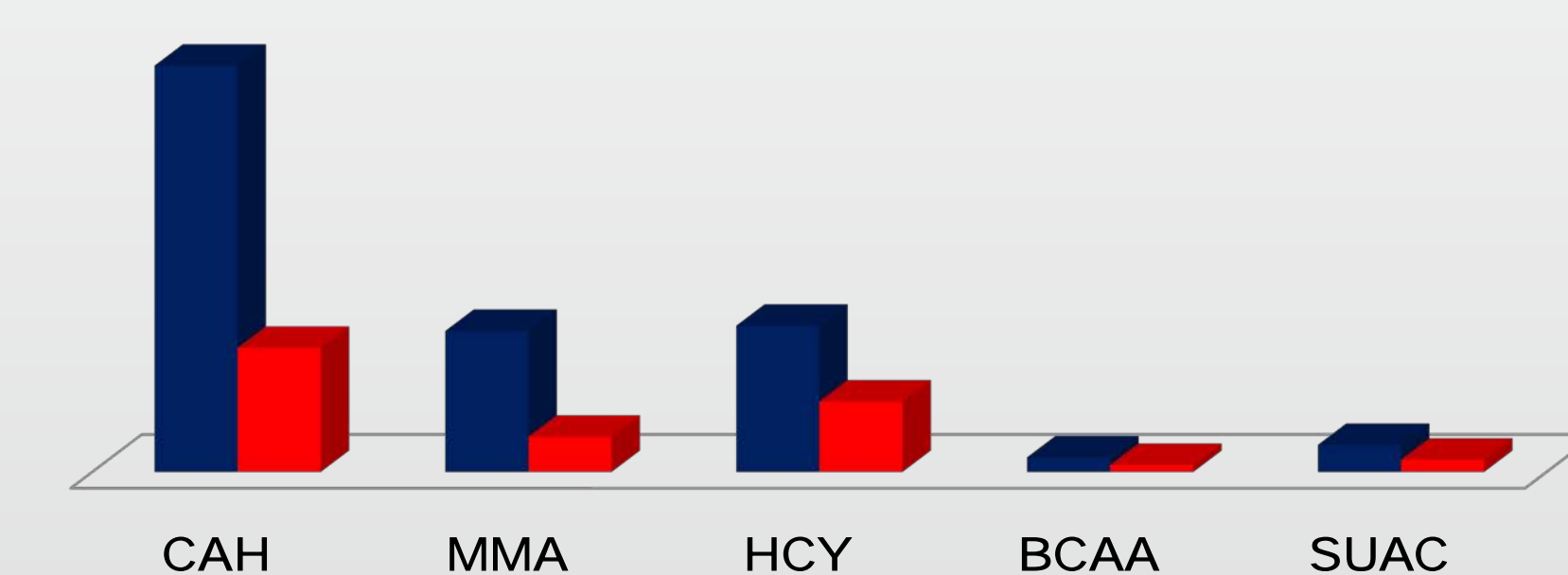


Figure 4. Total number of second tier tests performed (blue columns) compared to second tier tests performed in LBW infants (red columns).

	Abnormal Result		Normal Result		Total
	TP	FP	FN	TN	
CAH	3	44	0	1767	1815
MMA	3	4	0	601	608
HCY	0	2	0	486	488
BCAA	0	0	0	51	51
SUAC	0	0	0	66	66

Table 1. Comparison of results from second tier tests and routine follow-up test. TP = True positives; FP = False positives; FN = False negatives; TN = True negatives.

## Conclusions

- Second tier tests are effective in reducing false positives, especially in LBW infants.
- From the data analyzed so far, no false negatives resulted from the use of second tier tests.
- Using only one cohort of data, there was a net saving greater than \$2.5/baby screened when second tier test for CAH was implemented. For this study we used a "cost-effectiveness tool" developed by Dr. S. Guh and Dr. S. Grosse (CDC).
- Analysis of the data is still on-going to determine the false negative/positive rate and the cost-effectiveness for each second tier test.

## Acknowledgements

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