Multistate Analysis of Single Tests or Routine Second Testing in Newborn Screening for Hypothyroidism and Congenital Adrenal Hyperplasia

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The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention or the Association of Public Health Laboratories and should not be construed to represent any agency determination or policy.

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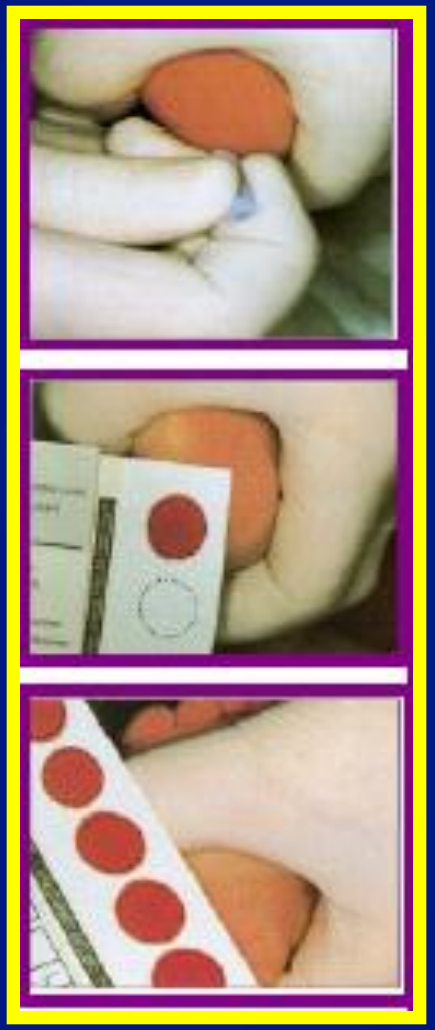
Routine Newborn Screening

- When newborn screening began (1960s)
- Specimens obtained at 48-96 hours after birth
- Decreased proportion of false negative results
  - Inadequate nutritional intake
  - Delay in elevation of TSH



Early Hospital Discharge

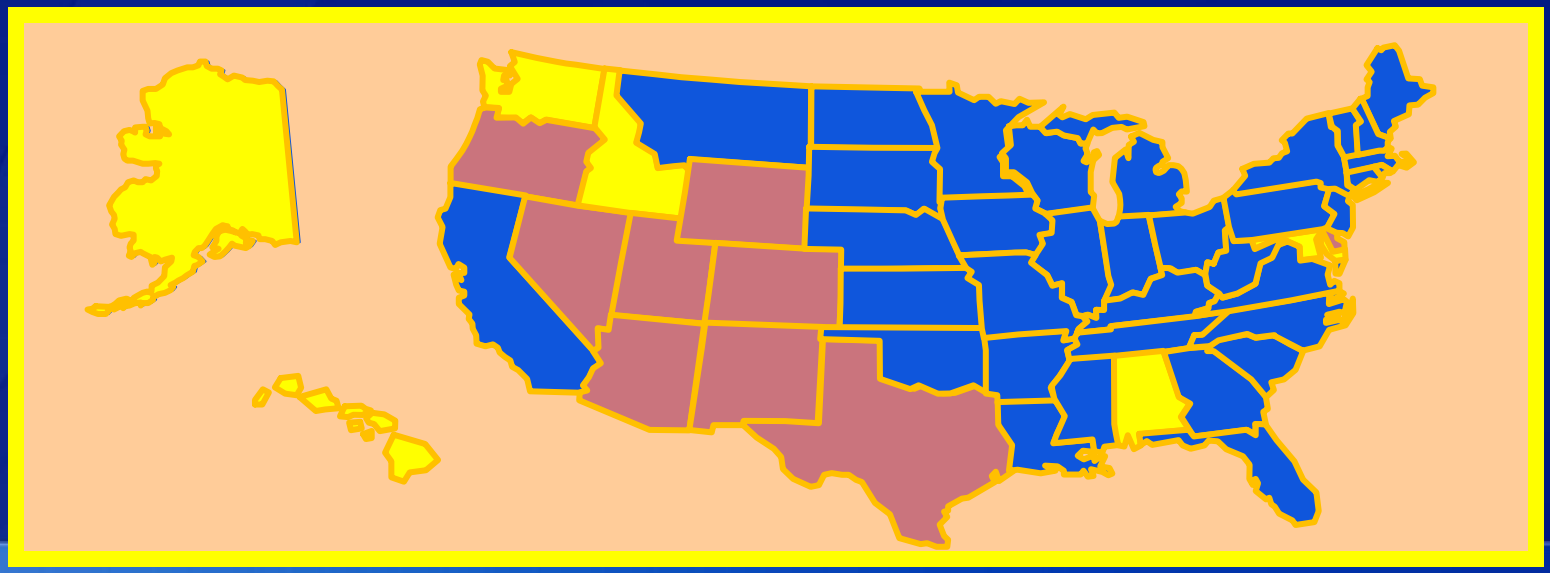
- Pressures to decrease health care costs
- Early discharge of mothers and newborns before 48 hours of life
- The AAP and others have addressed this issue
- Early hospital discharges still occur frequently, thus impacting newborn screen

Required Second Screens

- Nine states have a mandated second screen collected on all newborns at 8-14 days of age
  - Thought to reduce the chance of missing cases of clinically significant disorders
  - Collected on all infants, regardless of first NBS result
  - Arizona, Colorado, Delaware, Nevada, New Mexico, Oregon*, Texas, Utah, and Wyoming (*AK, HI, ID)
  - Account for approximately 17.3% of all U.S. births

Required Second Screens

- Three states have a recommended second screen collected on at least 85% of newborns at 8-14 days of age
  - Alabama, Maryland, Washington
  - Account for approximately 5.1% of all U.S. births
- The total percent of the U.S. population with a routine second screen is approximately 22.4%



Utility of Second Screens

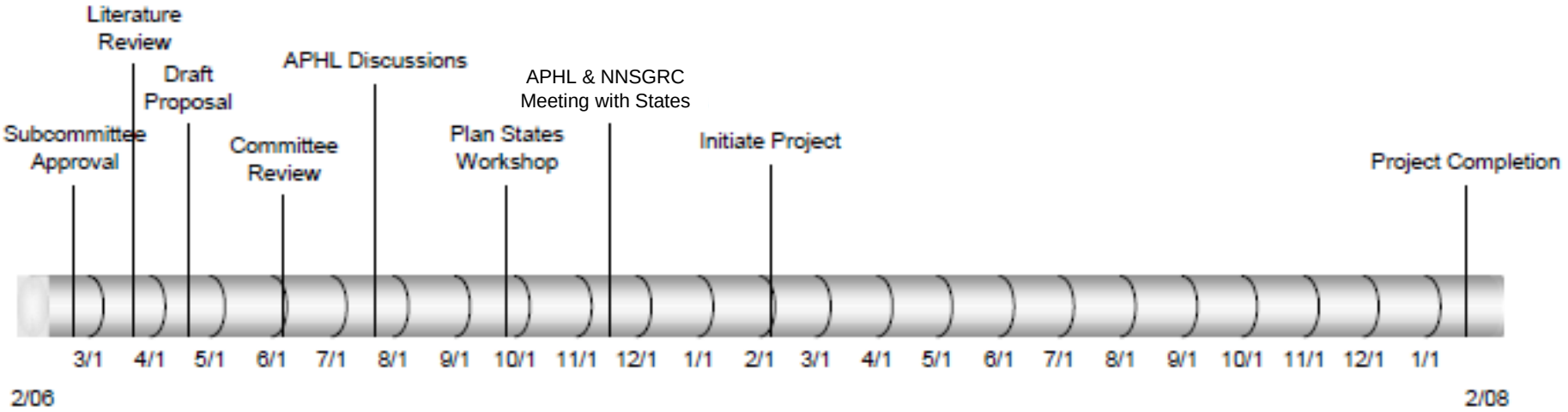
- Is a required second screen the appropriate means to detect cases that would otherwise be missed?
- Are there biochemical or laboratory-based practices that impact whether or not a case is detected on the first screen?
- Does the second screen detect treatable cases and prevent negative outcomes?
- Is the second screen a reasonable, cost-effective public policy?

Routine Second Testing Study

- In February, 2006, a project was proposed to the Laboratory Standards & Procedures Subcommittee of the SACHDNC
  - “Scientific literature indicates that cases of congenital hypothyroidism and CAH that are missed on the initial screen are detected on the routine second screen”
  - “Most newborn screening programs do not support the operation of a routine second screen”
  - “To better understand the justification for a routine second screen, we are proposing a study to investigate the effect of the routine second screen”

Routine Second Testing Study

- Timeline



Routine Second Testing Study

- Protocol development meeting
  - Meeting held in Silver Spring, MD on December 4-5, 2006: Issues in Requiring Routine Second Testing in Newborn Screening
  - Sponsored by NNSGRC and APHL
  - NBS laboratory and/or follow-up representation from
    - AZ, CO, DE, NV, NM, OR, TX, UT (not WY)
    - AL, MD, WA
    - CA, MA, WI

  - Endocrinologists: AZ, CO, DE, IA, MA, OR, PA, TX, WA
  - Representatives: HRSA, NNSGRC, CDC, FDA, APHL, SACHDNC, Pediatrix, CARES Foundation

Routine Second Testing Study

- During the meeting had presentations by panels of endocrinologists on experiences from NBS programs on second screens for
  - Congenital hypothyroidism (CH)
  - Congenital adrenal hyperplasia (CAH)
- Discussed participation by state NBS laboratories and follow-up programs in a
  - 1-year prospective study
  - 5-year retrospective study (2003-2008)

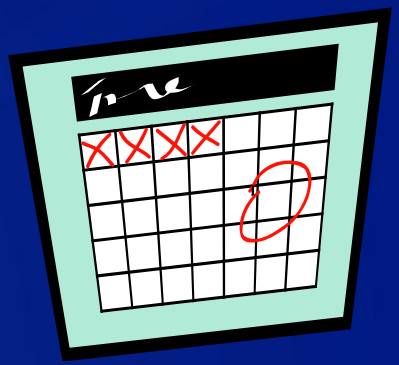
Routine Second Testing Study

- During the meeting and subsequently by e-mail and conference calls, decided upon the data elements to be reported
  - Demographics
  - Laboratory data
  - Clinical data

- All states present at the meeting verbally agreed to participate and to provide data elements on confirmed cases of CH and CAH for both studies, pending IRB approvals

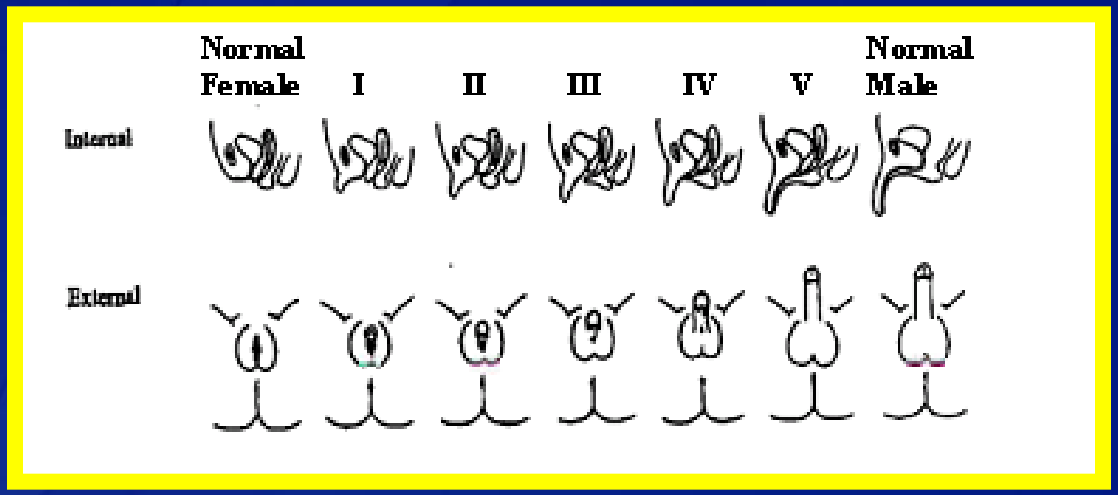
Routine Second Testing Study Data Elements

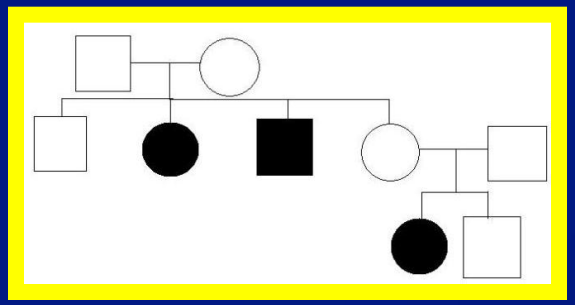
- Demographics
- Factors affecting screening test result
- Laboratory testing factors



Routine Second Testing Study Data Elements

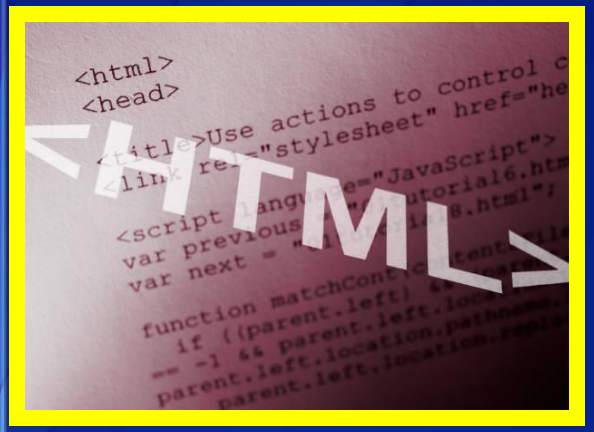
- Infant positive (first screen, second or subsequent screen, not detected by NBS)
- Clinical factors for Congenital Hypothyroidism
- Clinical factors for CAH





Routine Second Testing Study Data Repository

- Web-based data repository to be developed by APHL
- Individual-level anonymous data were to be submitted to APHL for analysis



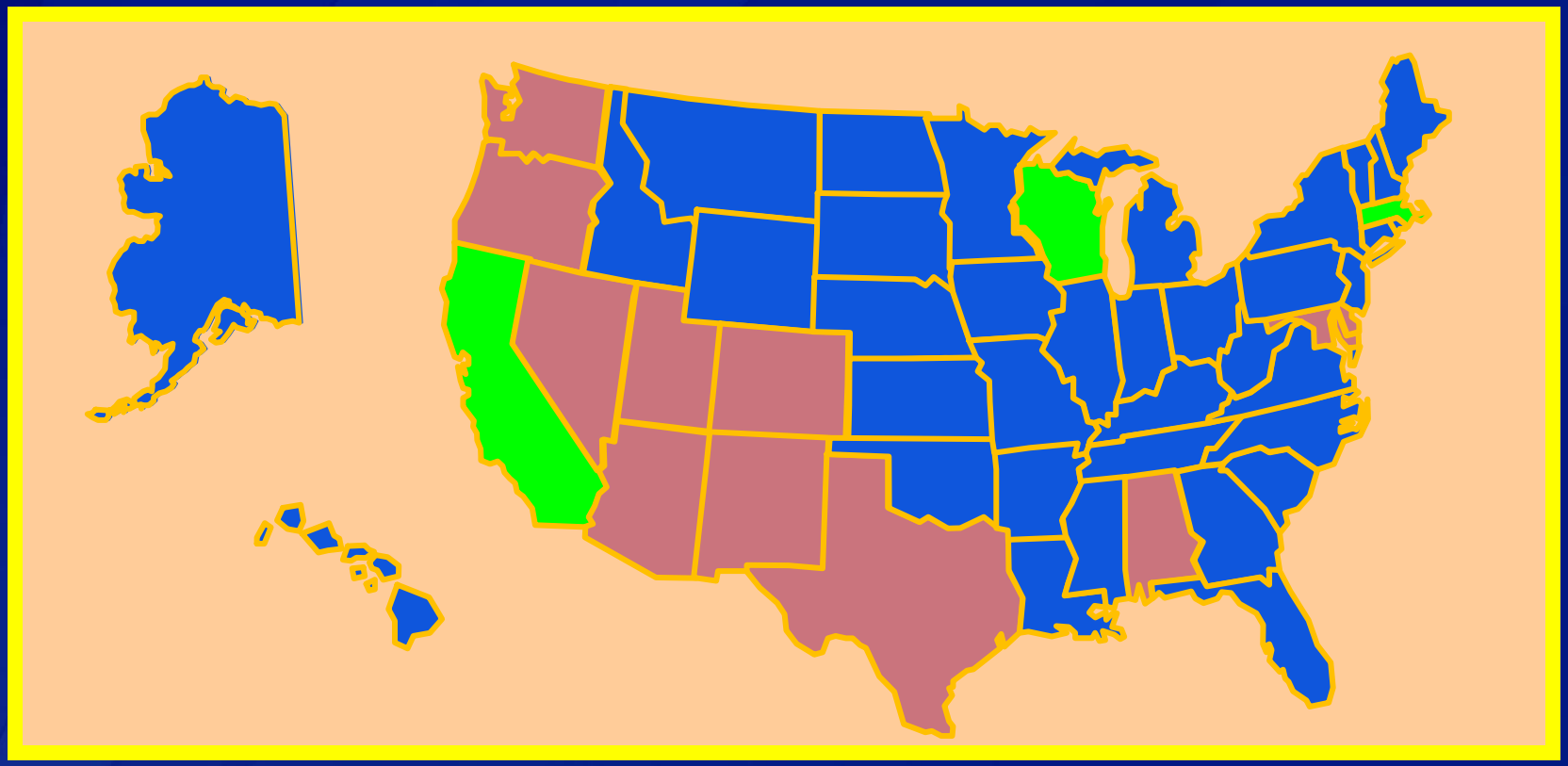
Routine Second Testing Study

- The Laboratory Standards & Procedures Subcommittee was updated on the project on December 18, 2006
  - Retrospective study (3-5 years of cases) expected to begin in February 2007 with data collection and submission over a 6 month period
  - Protocol for a prospective study (1 year of cases) to be refined, based on experience with the retrospective study

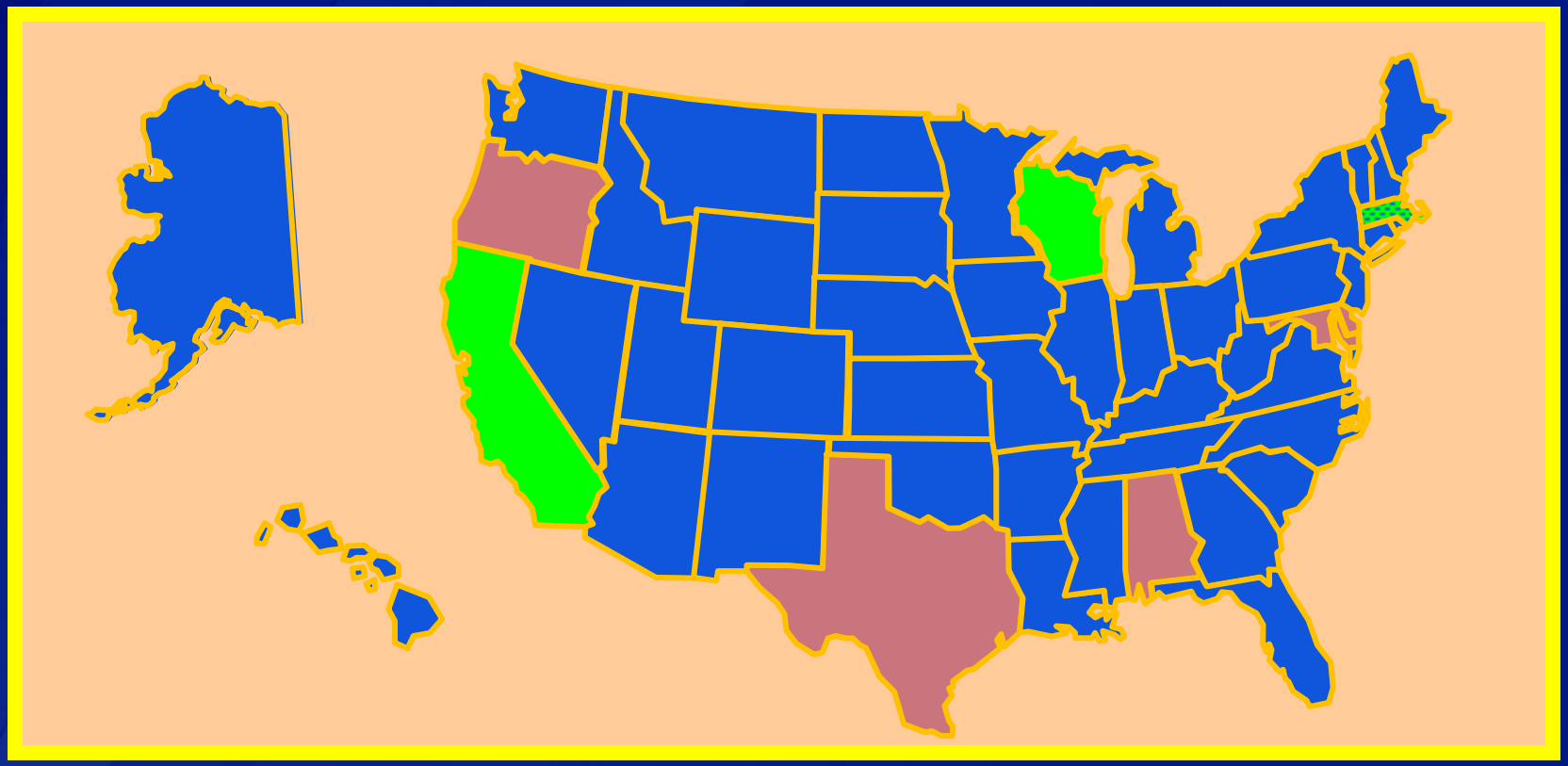
Routine Second Testing Study

- Although there was unanimity at the meeting in December, 2006 about proceeding with the study
  - Enthusiasm waned
  - People became busy with other tasks
  - Laboratory director/staff changes occurred
  - IRB approvals bogged down the process (could not obtain approval for enough states to participate in the 1-year prospective study)
  - Development of the data repository took more time and effort than expected
  - No dedicated resources for data collection (APHL did ultimately provide some funds to state NBS programs to support the activity)

States Eligible for Inclusion in the Routine Second Testing Study



States that Contributed Cases for the Routine Second Testing Study



Routine Second Testing Study

- A presentation of the initial data analysis results was made to the Laboratory Standards & Procedures Subcommittee in February, 2012
  - Since February
    - Analyses have been refined and additional variables evaluated
    - Multivariate analyses have been performed
    - Cases from Alabama were included in the Study in August, 2012
    - Working toward including cases from Massachusetts

Routine Second Testing Study
Source of Cases by Year

	2003	2004	2005	2006	2007	2008	2009	2010	2011
AL									
CA									
DE							Congenital Hypothyroidism		
MD									
OR									
TX									
WI									

	2003	2004	2005	2006	2007	2008	2009	2010	2011
AL									
CA									
DE							Congenital Adrenal Hyperplasia		
MD									
OR									
TX									
WI									

Routine Second Testing Study

- Screening Algorithms: Congenital Hypothyroidism

	T4 and TSH	T4 and if Abnormal, then TSH	TSH
One-Screen States			
California			
Wisconsin			
Two-Screen States			
Alabama			
Delaware			
Maryland			
Oregon			
Texas			

Routine Second Testing Study

- Summary data for Congenital Hypothyroidism

	One-Screen States (CA, WI)		Two-Screen States (AL, DE, MD, OR, TX)		Total	
Case Identified	**N**	**%**	**N**	**%**	**N**	**%**
First Screen	1091	96.80%	1307	80.23%	2398	87.01%
Second Screen	XXXX	XXXX	238	14.61%	238	8.63%
Targeted Second	25	2.22%	XXXX	XXXX	25	0.91%
Unknown	2	0.18%	69	4.24%	71	2.58%
Not Detected by NBS	9	0.80%	15	0.92%	24	0.87%
TOTAL	1127	100.00%	1629	100.00%	2756	100.00%

Routine Second Testing Study

- Summary data: Primary Congenital Hypothyroidism

	One-Screen States (CA, WI)		Two-Screen States (AL, DE, MD, OR, TX)		Total	
Case Identified	**N**	**%**	**N**	**%**	**N**	**%**
First Screen	1027	98.18%	1041	88.37%	2068	92.99%
Second Screen	XXXX	XXXX	137	11.63%	137	6.16%
Targeted Second	19	1.82%	XXXX	XXXX	19	0.85%
TOTAL	1046	100.00%	1178	100.00%	2224	100.00%

Routine Second Testing Study

- Summary data: Primary Congenital Hypothyroidism

	One-Screen States (CA, WI)		Two-Screen States (AL, DE, MD, OR, TX)		Total	
Case Identified	**N**	**%**	**N**	**%**	**N**	**%**
First Screen	1027	98.18%	1041	88.37%	2068	92.99%
Second Screen	XXXX	XXXX	137	11.63%	137	6.16%
Targeted Second	19	1.82%	XXXX	XXXX	19	0.85%
TOTAL	1046	100.00%	1178	100.00%	2224	100.00%

Routine Second Testing Study

- Summary data: Primary Congenital Hypothyroidism
- What characteristics are predictive of a case being identified on the first vs. the second screen in 2-screen states?

For Primary CH, characteristics predictive of being identified on the first vs. the second screen:

Characteristic	Odds Ratio	Lower CI	Upper CI
Texas	Reference		
Maryland	0.33	0.19	0.56
Oregon	0.44	0.26	0.75
White	Reference		
Black	0.40	0.22	0.73
Asian/Pacific Islander	0.21	0.11	0.37
Male	Reference		
Female	1.51	1.05	2.16

For Primary CH, characteristics predictive of being identified on the first vs. the second screen:

Characteristic	Odds Ratio	Lower CI	Upper CI
BW 2500-3999 gm	Reference		
BW <1000 gm	0.42	0.20	0.88
No Blood Transfusion	Reference		
Transfusion Prior to First Screen	0.43	0.19	0.97
Age at Collection <24 hr	Reference		
Age at Collection ≥24 and <48 hr	2.02	1.03	4.98
Time from Collection to Assay ≤3 days	Reference		
Time from Collection to Assay >3 days	2.06	1.26	3.35

For Primary CH, characteristics predictive of being identified on the first vs. the second screen:

Characteristic	Odds Ratio	Lower CI	Upper CI
White	Reference		
Black	0.40	0.22	0.73
Hispanic	1.33	0.84	2.13
Asian/Pacific Islander	0.21	0.11	0.37
Other	0.49	0.20	1.18

	First Screen		Second Screen	
Characteristic	N	%	N	%
White	365	46.97%	40	31.01%
Black	71	7.17%	19	14.73%
Hispanic	474	47.88%	38	29.46%
Asian/Pacific Islander	48	4.85%	25	19.38%
Other	32	3.23%	7	5.42%

Primary Hypothyroidism Cases in Relation to Screening Cutoffs

	First Screen Positive TSH % Above Cutoff (Arithmetic Mean)	Second Screen Positive TSH % Above Cutoff (Arithmetic Mean)
White	1345.4	536.4
ALL	1393.2	464.4

Primary Hypothyroidism Cases in Relation to Screening Cutoffs

	First Screen Positive TSH % Above Cutoff (Arithmetic Mean)	Second Screen Positive TSH % Above Cutoff (Arithmetic Mean)
White	1345.4	536.4
Hispanic	1553.3	504.2
ALL	1393.2	464.4

Primary Hypothyroidism Cases in Relation to Screening Cutoffs

	First Screen Positive TSH % Above Cutoff (Arithmetic Mean)	Second Screen Positive TSH % Above Cutoff (Arithmetic Mean)
White	1345.4	536.4
Hispanic	1553.3	504.2
Black	749.6	490.7
ALL	1393.2	464.4

Primary Hypothyroidism Cases in Relation to Screening Cutoffs

	First Screen Positive TSH % Above Cutoff (Arithmetic Mean)	Second Screen Positive TSH % Above Cutoff (Arithmetic Mean)
White	1345.4	536.4
Hispanic	1553.3	504.2
Black	749.6	490.7
Asian/Pacific Islander	1129.1	271.8
ALL	1393.2	464.4

Routine Second Testing Study

- Summary data: Primary Congenital Hypothyroidism

	One-Screen States (CA, WI)		Two-Screen States (AL, DE, MD, OR, TX)		Total	
Case Identified	**N**	**%**	**N**	**%**	**N**	**%**
First Screen	1027	97.25%	1041	87.11%	2068	91.87%
Second Screen	XXXX	XXXX	137	11.46%	137	6.09%
Targeted Second	19	1.80%	XXXX	XXXX	19	0.84%
Unknown	2	0.19%	12	1.01%	14	0.62%
Not Detected by NBS	8	0.76%	5	0.42%	13	0.58%
TOTAL	1056	100.00%	1195	100.00%	2251	100.00%

Routine Second Testing Study

- Summary data: Primary Congenital Hypothyroidism

Characteristic	One-Screen States (CA, WI) Incidence	Two-Screen States (AL, DE, MD, OR, TX) Incidence	Z-Test P-Value
ALL	1/1,926	1/2,278	<0.0001

Routine Second Testing Study

- Summary data: Primary Congenital Hypothyroidism

Characteristic	One-Screen States (CA, WI) Incidence	Two-Screen States (AL, DE, MD, OR, TX) Incidence	Z-Test P-Value
ALL	1/1,926	1/2,278	<0.0001
White	1/2464	1/2865	0.0477
Hispanic	1/3665	1/4429	0.0011
Black	1/1616	1/1971	0.3421
Asian/Pacific Islander	1/2112	1/1479	0.0198

Routine Second Testing Study

- Summary data: Primary Congenital Hypothyroidism
- Comparing cases identified on the single screen in 1-screen states vs. cases identified on the first screen in 2-screen states

Cases Identified in 1-Screen Compared to 2-Screen States*		
More Likely	**Less Likely**	**X^2 P-Value**
Female		0.0345
Breastfeeding and Formula	Formula Only	<0.0001
	Birth Weight <1000 gm	0.0027
	Transfused Prior to Screening	0.0007

Routine Second Testing Study

- Summary data: Primary Congenital Hypothyroidism
- Comparing cases identified on the single screen in 1-screen states vs. cases identified on the first screen in 2-screen states

Cases Identified in 1-Screen Compared to 2-Screen States*		
More Likely	**Less Likely**	**X^2 P-Value**
Age of Collection at <24 hrs		<0.0001
Time from Collection to Assay ≤ 3 days		<0.0001

*No difference in NICU Admission at First Screen

Routine Second Testing Study

- Summary data for Congenital Adrenal Hyperplasia

	One-Screen States (CA, WI)		Two-Screen States (AL, DE, MD, OR, TX)		Total	
Case Identified	N	%	N	%	N	%
First Screen	88	88.89%	165	60.00%	253	67.65%
Second Screen	XXXX	XXXX	99	36.00%	99	26.47%
Second Tier Test	5	5.05%	XXXX	XXXX	5	1.34%
Unknown	0	0.00%	7	2.55 %	7	1.87%
Not Detected by NBS	6	6.06%	4	1.45%	10	2.67%
TOTAL	99	100.00%	275	100.00%	374	100.00%

Routine Second Testing Study

- Summary data for Congenital Adrenal Hyperplasia

	One-Screen States (CA, WI)		Two-Screen States (AL, DE, MD, OR, TX)		Total	
Case Identified	N	%	N	%	N	%
First Screen	88	94.62%	165	62.50%	253	70.87%
Second Screen	XXXX	XXXX	99	37.50%	99	27.73%
Second Tier Test	5	5.38%	XXXX	XXXX	5	1.40%
TOTAL	93	100.00%	264	100.00%	357	100.00%

Routine Second Testing Study

- Summary data for Congenital Adrenal Hyperplasia
- What characteristics are predictive of a case being identified on the first vs. the second screen in 2-screen states?
- Only AL and TX identified cases on the second screen, so the analyses were limited to these two states

For CAH, characteristics predictive of being identified on the first vs. the second screen:

Characteristic	Odds Ratio	Lower CI	Upper CI
White	Reference		
Hispanic	0.44	0.24	0.80
Age at Collection <48 hr	Reference		
Age at Collection ≥48 hr	1.94	1.13	3.33
Classical Salt-Wasting	Reference		
Classical Simple Virilizing	0.05	0.02	0.14
Non-Classical	0.02	0.01	0.06

For CAH, characteristics predictive of being identified on the first vs. the second screen:

Characteristic	Odds Ratio	Lower CI	Upper CI
Classical Salt-Wasting	Reference		
Classical Simple Virilizing	0.05	0.02	0.14
Non-Classical	0.02	0.01	0.06

	First Screen		Second Screen	
Characteristic	N	%	N	%
Classical Salt-Wasting	92	77.31%	9	9.78%
Classical Simple Virilizing	12	10.08%	23	25.00%
Non-Classical	15	12.61%	60	65.22%

Second Screen Cases	Treated	Not Treated	Unknown	Total
Classical Salt-Wasting	9 (100%)	0	0	9
Classical Simple Virilizing	19 (83%)	3	1	23
Non-Classical	20 (33%)	38	2	60

Routine Second Testing Study

- Summary data for Congenital Adrenal Hyperplasia

CAH Type	One-Screen States (CA, WI) Incidence	Two-Screen States (AL, DE, MD, OR, TX) Incidence	Z-Test P-Value
Salt-Wasting	1/23,461	1/19,513	0.2005
Simple Virilizing	1/125,685	1/58,974	0.0114
Non-Classical	1/219,949	1/33,593	<0.0001

Routine Second Testing Study

- Summary data for Congenital Adrenal Hyperplasia
- Comparing cases identified on the single screen in 1-screen states vs. cases identified on the first screen in 2-screen states

Cases Identified in 1-Screen Compared to 2-Screen States*		
More Likely	**Less Likely**	**X^2 P-Value**
Age of Collection at <48 hrs		0.0002
Time from Collection to Assay ≤ 4 days		<0.0001

*No difference in Race/Ethnicity, Sex, Birth Weight, Feeding Status, NICU Admission at First Screen, or Type of CAH

Cases Not Detected by NBS

Hypothyroidism Type	1-Screen States	2-Screen States	Total
Primary	8	5	13
Secondary	1	4	5
TBG Deficiency	0	1	1
Uncertain	0	4	4
TOTAL	9	14	23

CAH Type	1-Screen States	2-Screen States	Total
Classical Salt Wasting	4	0	4
Classical Simple Virilizing	2	2	4
Non-Classical	0	2	2
TOTAL	6	4	10

Summary

- Among the states evaluated as part of this study
- In 2-screen states, ~12% of Primary Congenital Hypothyroidism and ~38% of Congenital Adrenal Hyperplasia cases (includes 9% of all Classical Salt-Wasting CAH cases) were detected on the 2nd screen
- If the 2-screen states performed only a single screen according to their current screening procedures, these cases would presumably not be detected by NBS
- All of the Primary Congenital Hypothyroidism and more than half (48/89) of the Congenital Adrenal Hyperplasia cases detected on the 2nd screen were treated, indicating that they were “clinically significant”

Summary

- In 2-screen states, the characteristics predictive of cases detected on the first vs. the second screen
- Primary Congenital Hypothyroidism
  - The only significant predictor was Race/Ethnicity (Black and Asian/Pacific Islander infants were more likely detected on the second screen)
  - Race/Ethnicity differences were perhaps attributable to physiologic differences in how Primary CH manifests; additional analyses will evaluate this
- Congenital Adrenal Hyperplasia (AL, TX)
  - The only significant predictor was Type of CAH (Classical Simple Virilizing and Non-Classical cases were more likely detected on the second screen)

Summary

- Incidence, comparing 1-screen and 2-screen states
  - Primary CH: Significantly higher in 1-screen states
    - Mostly attributable to higher incidence among Hispanics in 1-screen compared to 2-screen states
    - Incidence rate differences could be the effect of genetic or environmental differences in these populations in 1-screen and 2-screen states
    - Would require other types of studies (outside the scope of the Routine Second Screen Study)
  - Salt-Wasting CAH: Statistically equivalent in 1-screen and 2-screen states
  - Simple Virilizing and Non-Classical CAH: Significantly higher in 2-screen states

Summary

- Several characteristics, in addition to Race/Ethnicity among Primary CH cases, distinguish the cases detected on the first screen in 1-screen vs. 2-screen states
- Primary Congenital Hypothyroidism
  - Feeding Status, Birth Weight, Transfusion Status, Age at Collection, Time from Collection to Assay
- Congenital Adrenal Hyperplasia
  - Age at Collection, Time from Collection to Assay

Summary

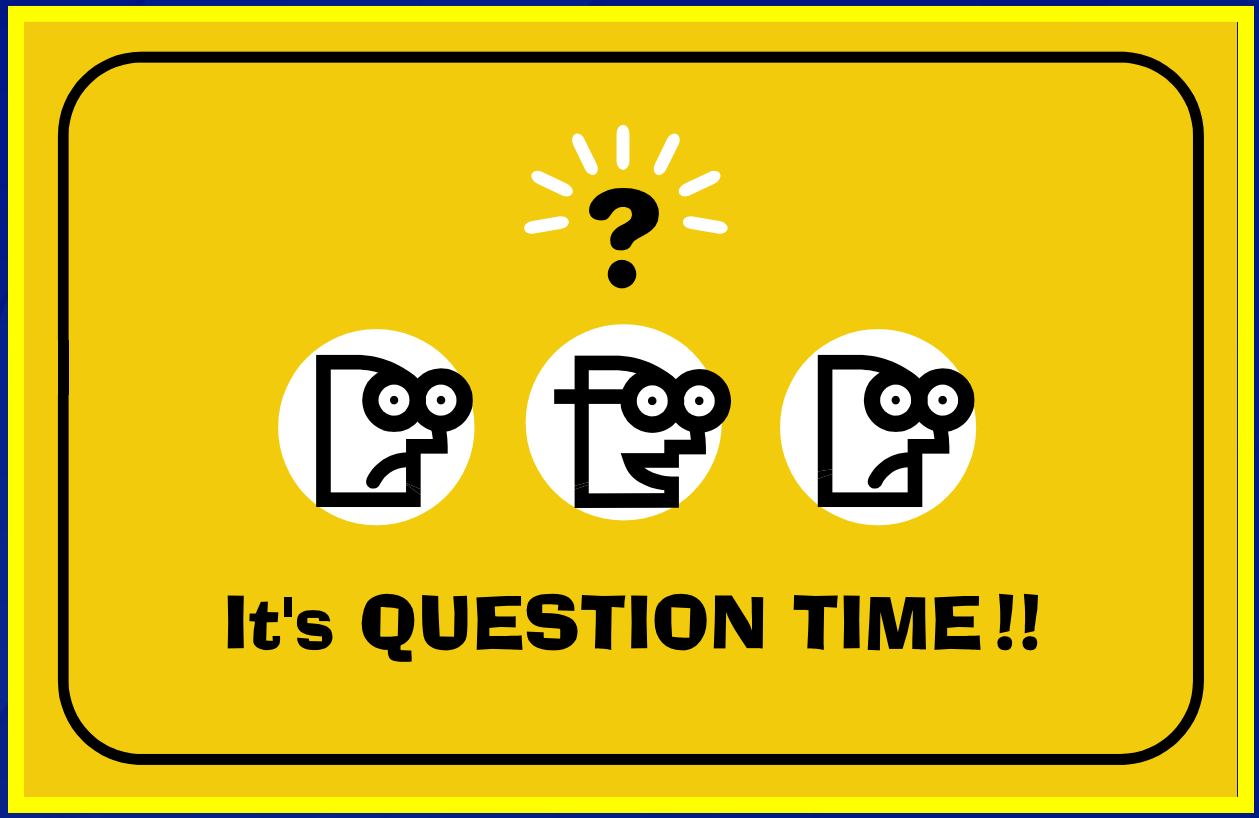
- Several characteristics, in addition to Race/Ethnicity among Primary CH cases, distinguish the cases detected on the first screen in 1-screen vs. 2-screen states
- Primary Congenital Hypothyroidism
  - Feeding Status, Birth Weight, Transfusion Status, Age at Collection, Time from Collection to Assay
- Congenital Adrenal Hyperplasia
  - Age at Collection, Time from Collection to Assay

Routine Second Testing Study

- Limitations of the Study
  - This was a retrospective study, so data were incomplete for certain variables; state labs reported only the data they had on hand
  - Final diagnosis, particularly for hypothyroidism, was not necessarily determined after adequate follow-up (differentiate between permanent and transient CH)
  - Different screening algorithms between 1-screen and 2-screen states limit the ability to make conclusions about detection of certain types of hypothyroidism
  - Results are biased by states that contributed largest number of cases

Routine Second Testing Study

- Strengths of the Study
  - Only comparative study between one-screen and two-screen states
  - Much larger sample size than any previous study
    - 2756 cases of Hypothyroidism
    - 374 cases of CAH
    - From among 4,687,800 births
  - Numerous laboratory and medical variables available for analysis
  - Analysis of individual-level data improves the ability to tease out specific associations


It's QUESTION TIME!!

SUPPLEMENTAL INFORMATION

Utility of Second Screens

LaFranchi, et al., 1985: Screening for congenital hypothyroidism with specimen collection at two time periods: results of the Northwest Regional Screening Program

- Infants undergoing screening, 1975-1984

	Primary CH 1st Screen	Primary CH 2nd Screen
# of Infants Detected	163	19
Population at Risk	811,917	484,604
Incidence	1:4,981	1:25,505

Utility of Second Screens

LaFranchi, et al., 1985: Screening for congenital hypothyroidism with specimen collection at two time periods: results of the Northwest Regional Screening Program

- Comparing infants detected on first vs. second screen

  Infants on first screen had:
  - Lower mean filter paper T4 level
  - Higher mean TSH concentration
  - Different sex ratio (2-to-1 female to male on first screen and slight male predominance on second screen)
  - Higher incidence of gestational age >42 weeks
  - Higher birth weight

Utility of Second Screens

LaFranchi, et al., 1985: Screening for congenital hypothyroidism with specimen collection at two time periods: results of the Northwest Regional Screening Program

- Cost to screen newborns for CH:
  - Approximately $1.25 per infant
  - Infants identified on first screen: $6,376 to detect each infant
  - Infants identified on second screen: $31,881 to detect each infant
- In 1977, the FGAO estimated the lifetime medical and institutional care costs for one infant with untreated hypothyroidism to be $330,000

Utility of Second Screens

Levine and Therrell, 1986: Second testing for hypothyroidism

- Letter to the editor regarding LaFranchi, et al. paper
- Evaluated NBS for hypothyroidism in Texas, 2/1/80-1/1/85

	Primary CH 1st Screen	Primary CH 2nd Screen
# of Infants Detected	393	21
Population at Risk	1,500,000	NS
Incidence	1:3,817	1:40,000

Utility of Second Screens

Levine and Therrell, 1986: Second testing for hypothyroidism

- Incidence of cases detected on second screen (1:40,000) was lower than reported by LaFranchi et al. (1:25,505)
  - Perhaps due to differences in laboratory protocol relative to
    - Cut-off values
    - Retest protocol
- Cost to detect a case was higher ($80,000 based on a cost per test of $2.00)
- Conclusion: Detection of cases on the second test is cost-effective, preventive medicine

Utility of Second Screens

Doyle, et al., 1995: Factors which influence the rate of receiving a routine second newborn screening test in Washington state

- During 1978-1992, 21 newborns were identified with a newborn screening condition only on a routine second NBS test
  - 3 cases of PKU (4% of 73 total)
  - 16 cases of CH (8% of 209 total)
  - 2 cases of CAH (5% of 38 total)

Utility of Second Screens

Maniatis, et al., 2006: Congenital hypothyroidism and the second newborn metabolic screening in Colorado, USA

	Primary CH 1st Screen	Primary CH 2nd Screen
# of Infants Detected	185	42
Population at Risk	494,324	471,877
Incidence	1:2,703	1:11,111

Utility of Second Screens

Maniatis, et al., 2006: Congenital hypothyroidism and the second newborn metabolic screening in Colorado, USA

- Comparing infants detected on first vs. second screen
  Infants on first screen had:
  - Different sex ratio (1.8:1 female predominance on first screen and 1.2:1 male predominance on second screen)
  - No significant difference for gestational age or birth weight

Utility of Second Screens

Maniatis, et al., 2006: Congenital hypothyroidism and the second newborn metabolic screening in Colorado, USA

- Cost to screen newborns for CH:
  - Infants identified on first screen: $6,108 to detect each infant
  - Infants identified on second screen: $25,684 to detect each infant
  - Overall cost per case (combining first and second screens): $9,730 to detect each infant

Utility of Second Screens

Brosnan, et al., 1998: A comparative cost analysis of newborn screening for classic congenital adrenal hyperplasia in Texas

- 15 infants with classical CAH* diagnosed in Texas in 1994 (incidence of 1:21,701)

	1st Screen	2nd Screen	Clinical Diagnosis	Total
Salt-wasting*	5	0	6	11
Simple virilizing*	1	2	1	4
Nonclassical	0	5	0	5

Utility of Second Screens

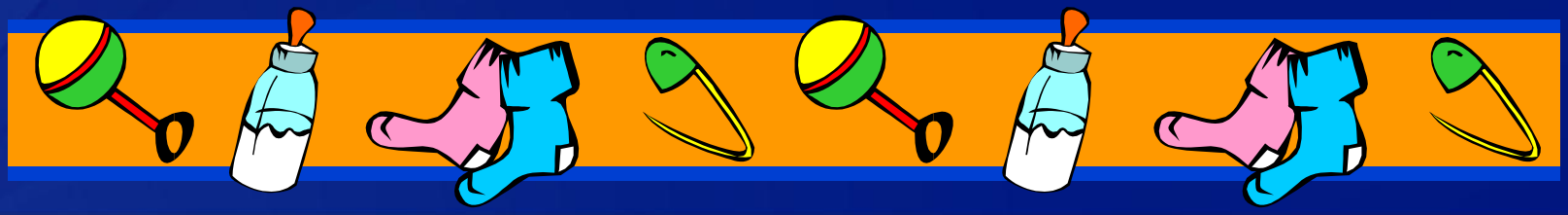
Brosnan, et al., 1998: A comparative cost analysis of newborn screening for classic congenital adrenal hyperplasia in Texas

- Cost to screen newborns for CAH (all costs, including screening test, follow-up testing, physician fees, and hospitalization):
  - Infants detected clinically: $11,312 per diagnosis
  - Infants identified on first screen: $115,169 per diagnosis
  - Infants identified on second screen: $242,865 per diagnosis
  - Overall cost per case: $147,093 for each diagnosed infant

Utility of Second Screens

Brosnan, et al., 1998: A comparative cost analysis of newborn screening for classic congenital adrenal hyperplasia in Texas

- Conclusions:
  - If the goal is early diagnosis of infants with the severe salt-wasting form of CAH, a single screen is effective
  - If the goal is to detect infants with the simple virilizing form of the disorder who may benefit from treatment, the second screen is necessary, but not as cost-effective as the first screen



Utility of Second Screens

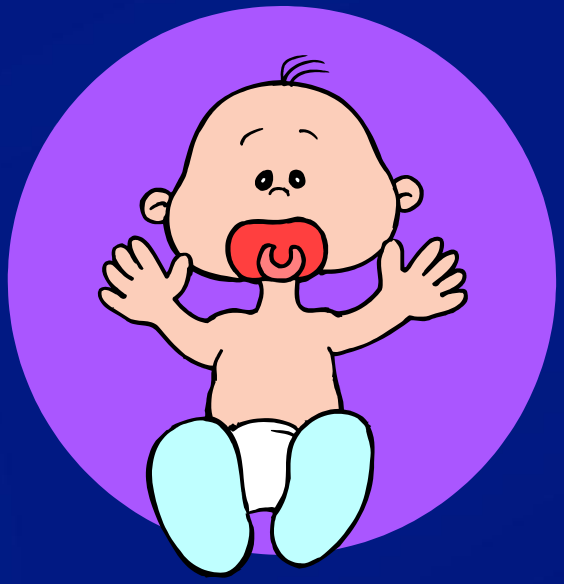
Varness, et al., 2005: Newborn screening for congenital adrenal hyperplasia has reduced sensitivity in girls

- In Wisconsin, 2000-2003, 8 infants not identified by NBS for CAH were subsequently diagnosed with 21-hydroxylase deficiency
  - 7 of the 8 infants were female
  - The male was identified at 54 months because of precocious adrenarche
  - 4 had laboratory evidence of salt-wasting, but none were treated for a salt-wasting crisis

Utility of Second Screens

Varness, et al., 2005: Newborn screening for congenital adrenal hyperplasia has reduced sensitivity in girls

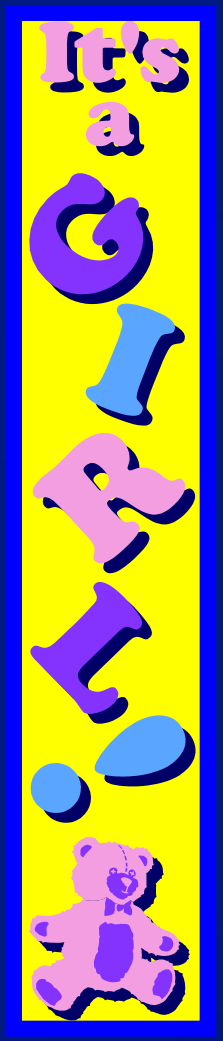
- Based on the false negative cases, the overall sensitivity of the screening test was 73%, but for females was only 60% while for male infants the sensitivity was 83%
- Since unrecognized false-negative cases could exist, the sensitivity could be even lower



Utility of Second Screens

Varness, et al., 2005: Newborn screening for congenital adrenal hyperplasia has reduced sensitivity in girls

- All false-negative infants identified had the simple virilizing form of CAH
- Conclusions: The goal of screening is to prevent life-threatening salt-wasting crisis and avoid male assignment to female infants
  - "In spite of occasional missed cases, these goals are being met with the use of a single screening test and current thresholds."



Routine Second Testing Study

- Summary data for Congenital Hypothyroidism
  - Primary: Low T4, Elevated TSH
  - Secondary (Central): Low T4, Normal to low TSH
  - CH of Prematurity: Low T4, Normal to low TSH in a preterm infant; often is treated
  - Transient: Initial confirmed case with subsequent normalization of analytes when off of treatment
  - Uncertain: Initial testing suggestive of hypothyroidism, but the specific type was either not communicated to the laboratory or could not be determined from the available data

Routine Second Testing Study

- Summary data for Congenital Hypothyroidism

  Cases detected by NBS

	One-Screen States		Two-Screen States		Total	
CH Type	N	%	N	%	N	%
Primary	1048	93.74%	1190	73.73%	2238	81.92%
Secondary	0	0.00%	79	4.90%	79	2.89%
TBG Deficiency	0	0.00%	42	2.60%	42	1.54%
T4 Resistance	0	0.00%	1	0.06%	1	0.03%
CH of Prematurity	1	0.09%	209	12.95%	210	6.69%
Transient	41	3.67%	4	0.25%	45	1.65%
Uncertain	28	2.50%	89	5.51%	117	4.28%
TOTAL	1118		1614		2732	

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