Unsatisfactory Newborn Screening Specimens: Interpretations, Studies and Current Trends

- Presenters
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- Dr. Lisa Kalman-Center for Disease Control

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The web conference is co-sponsored by

- The Newborn Screening Quality Assurance Program (CDC)
- APHL's Quality Assurance/ Quality Control/ Proficiency Testing Subcommittee of the Newborn Screening and Genetics in Public Health Committee
- The National Laboratory Training Network

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Objectives

- Pretest-assess the current status of evaluating dried blood spot specimens
- Review of testing of inadequate specimens and equivalent adequate specimens
- Summary of inadequate specimen handling

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We are going to show you a series of dried blood spot specimens.

Through live online audience participation, please determine the suitability for testing of each specimen by clicking on the single best assessment in each of the polling windows.

Results will follow each polling.

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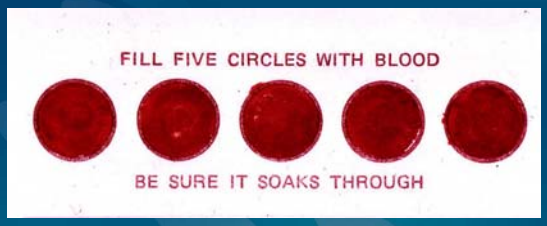
Specimen # 1



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Poll: Specimen #1

Is this specimen satisfactory for testing?

- Yes
- No, blood is clotted
- No, blood is layered
- No, blood is contaminated
- No, capillary tube was used to spot the blood
- No, incomplete saturation
- No, other

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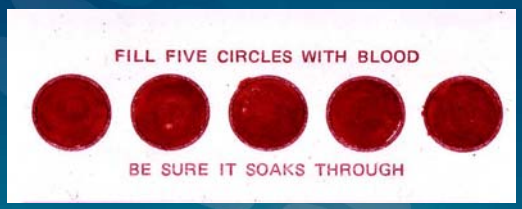
Specimen #1 Assessment

Uneven blood saturation- colored in circles with capillary



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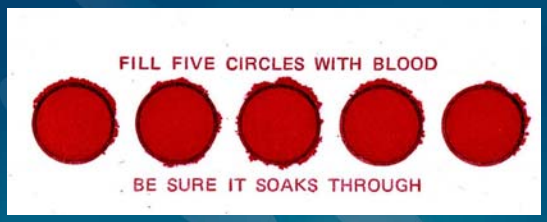
Specimen #2



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Poll: Specimen #2

Is this specimen satisfactory for testing?

- Yes
- No, blood is clotted
- No, blood is layered
- No, blood is contaminated
- No, capillary tube was used to spot the blood
- No, incomplete saturation
- No, other

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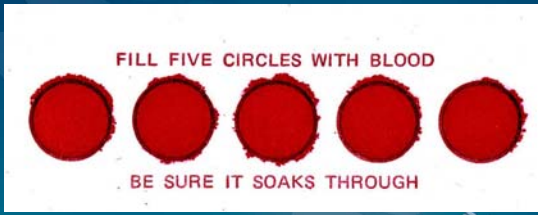
Specimen #2 Assessment

Properly collected specimen



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Specimen #3

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Poll: Specimen #3

Is this specimen satisfactory for testing?

- Yes
- No, blood is clotted
- No, blood is layered
- No, blood is contaminated
- No, capillary tube was used to spot the blood
- No, incomplete saturation
- No, other

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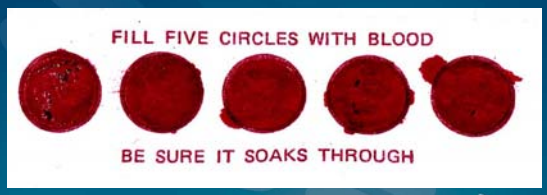
Specimen #3 Assessment

Blood Clot on Specimen



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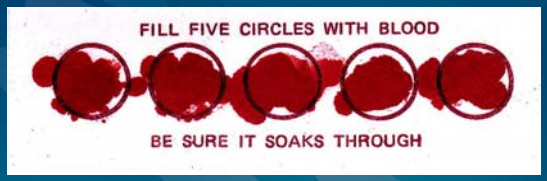
Specimen #4



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Poll: Specimen #4

Is this specimen satisfactory for testing?

- Yes
- No, blood is clotted
- No, blood is layered
- No, blood is contaminated
- No, capillary tube was used to spot the blood
- No, incomplete saturation
- No, other

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Specimen #4 Assessment

Circles not filled

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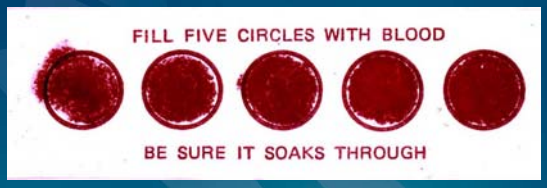
Specimen #5



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Poll: Specimen #5

Is this specimen satisfactory for testing?

- Yes
- No, blood is clotted
- No, blood is layered
- No, blood is contaminated
- No, capillary tube was used to spot the blood
- No, incomplete saturation
- No, other

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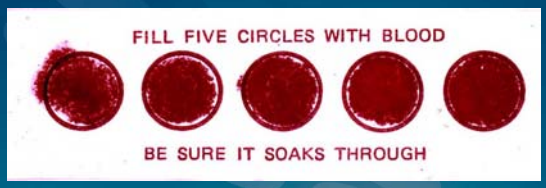
Specimen #5 Assessment

Poor saturation-possibly due to high hematocrit



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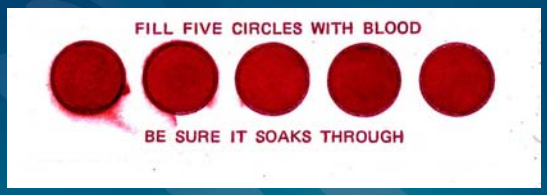
Specimen #6



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Poll: Specimen #6

Is this specimen satisfactory for testing?

- Yes
- No, blood is clotted
- No, blood is layered
- No, blood is contaminated
- No, capillary tube was used to spot the blood
- No, incomplete saturation
- No, other

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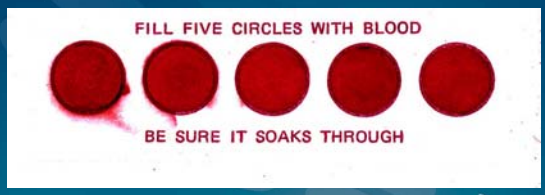
Specimen #6 Assessment

Contamination



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Specimen #7

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Poll: Specimen #7

Is this specimen satisfactory for testing?

- Yes
- No, blood is clotted
- No, blood is layered
- No, blood is contaminated
- No, capillary tube was used to spot the blood
- No, incomplete saturation
- No, other

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Specimen #7 Assessment

**Incomplete saturation-
too little blood applied**

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Specimen #8

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Poll: Specimen #8

Is this specimen satisfactory for testing?

- Yes
- No, blood is clotted
- No, blood is layered
- No, blood is contaminated
- No, capillary tube was used to spot the blood
- No, incomplete saturation
- No, other

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Specimen #8 Assessment

Smeared blood- Pressed too hard against the heel

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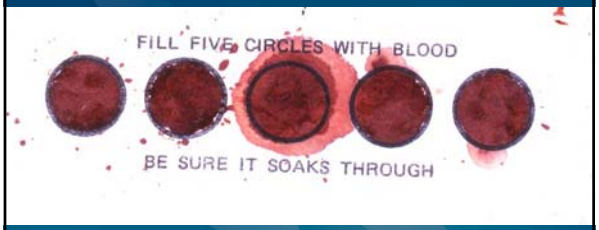
Specimen #9



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Poll: Specimen #9

Is this specimen satisfactory for testing?

- Yes
- No, blood is clotted
- No, blood is layered
- No, blood is contaminated
- No, capillary tube was used to spot the blood
- No, incomplete saturation
- No, other

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Specimen #9 Assessment

Improper use of capillary tube - blood clotted before application causing serum rings

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Specimen #10

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Poll: Specimen #10

Is this specimen satisfactory for testing?

- Yes
- No, blood is clotted
- No, blood is layered
- No, blood is contaminated
- No, capillary tube was used to spot the blood
- No, incomplete saturation
- No, other

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Specimen #10 Assessment

Folds and creases through the blood circles

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The dried blood spot specimens for the polling slides were generously provided by
Gary Hoffman
Wisconsin State Laboratory of Hygiene

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Invalid Specimen Study

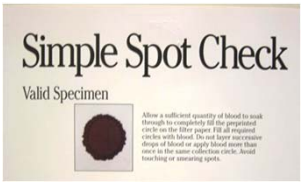
Newborn Screening Program
NYSDOH

Joe Orsini, PhD
Ken Pass, PhD

Full credit to the guy with the idea, and the talent and energy to make it happen:

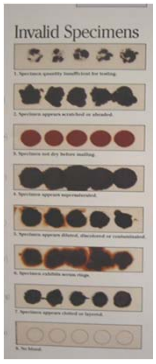
Joe Orsini, PhD

Valid Sample



- Completely fills circle
- Un-layered
- Un-smeared
- Homogeneous

Invalid Samples



1. Insufficient Quantity ✓
2. Appears Scratched or abraded
3. Not dried prior to mailing
4. Supersaturated
5. Diluted, discolored, or contaminated ✓
6. Exhibits serum rings ✓
7. Clotted or layered ✓
8. No blood

✓ addressed in this study

Invalid Samples: NYS 2004*

Invalid Type	Samples	Percentage
Quantity insufficient	492	15.4 %
Not dry before mailing	15	0.47 %
Diluted, discolored, or contaminated	215	6.7 %
Exhibits serum rings	1015	31.8 %
Appears clotted or layered	543	17.0 %

*Note, all specimens on card were identified as invalid, list does not include all invalid types counted.

Methodology

Example for invalid/valid pair: diluted, discolored or contaminated

- Evaluate a single patient sample with valid/invalid DBS Pairs
- Punch sample pairs from invalid and valid sample
- Performed CAH, TSH, T4, Hgb, Biotinidase, Galactosemia, and MS/MS (AA/AC) tests
- Analyze data with Excel (Mean, Std. Dev., t-test, etc.)
- Compare data to valid sample pairs

CAH, TSH, T4 – immunoassay
PKU, MCADD – msms
Hgb – electrophoresis
Galactosemia – Beutler
Biot. Def – Wolff test

Study Method: Pros and Cons

Pros

- Use real world specimens
- Invalid and valid samples collected at same time from same specimen
- Random sampling of DBS
- Invalid/valid sample pairs analyzed at same time

Cons

- Dependent on available samples
- Not all invalid types were available for this study
- Sometimes difficult to assign invalid sample type
- Unlikely for there to be both an abnormal baby and invalid sample type on same specimen

Testing Summary

Qualitative tests	Quantitative tests
Heminoglobinopathies	CAH (17-OHP)
Biotinidase	CH (T4* and TSH)
Galactosemia	MCADD (C8*)
	PKU (phenylalanine*)

* Example data shown, other tests showed similar data and are not presented on slides.

Invalid Samples - Study

Invalid Type	Samples
Insufficient quantity	24
Diluted, discolored, contaminated	69
Exhibits serum rings	61
Specimen clotted or layered	180

Insufficient quantity

Note: on front side of card, all samples look acceptable

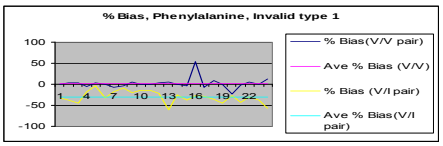
Insufficient quantity
Phenylalanine

Phenylalanine (MS/MS)

Valid/Valid Sample Pair	Valid	Valid	% Bias(V/V pair)
N	24	24	
Ave	1.25	1.26	2.11
Std Dev	0.51	0.55	13.04
min	0.68	0.67	-23.81
max	3.17	3.55	54.4
T-Bias (Bias(V/Bias)) =	2.76E-10		
T-test paired (S1V/S2V) =	0.663		
T-test paired (V/I)	5.45E-10		

Valid/Invalid Sample Pair	Valid	Invalid	% Bias (V/I pair)
N	24	24	
Ave	1.1	0.8	-29.8
Std Dev	0.24	0.25	14.40
min	0.79	0.33	-60.2
max	1.51	1.32	-3.2

High negative bias



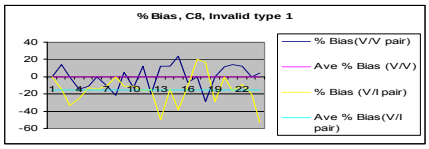
Insufficient quantity
MCADD/C8

MCADD/C8 (MS/MS)

Valid/Valid Sample Pair	Valid	Valid	% Bias(V/V pair)
N	24	24	
Ave	0.11	0.11	-0.09
Std Dev	0.049	0.053	13.3
min	0.060	0.050	-28.57
max	0.25	0.26	23.529
T-Bias (Bias(V/Bias)) =	0.00134		
T-test paired (S1V/S2V) =	1.00		
T-test paired (V/I)	0.00184		

Valid/Invalid Sample Pair	Valid	Invalid	% Bias (V/I pair)
N	24	24	
Ave	0.067	0.070	-15.3
Std Dev	0.031	0.017	17.3
min	0.050	0.030	-53
max	0.19	0.10	20

High negative bias



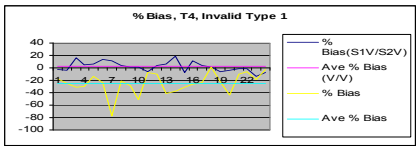
Insufficient quantity
Thyroxine

Thyroxine

Valid/Valid Sample Pair	Valid	Valid	% Bias(V/V pair)
N	24	24	
Ave	10.2	10.3	2.04
Std Dev	4.01	3.71	8.08
min	5.2	5.4	-14.1
max	24.9	23.1	18.4
T-Bias (Bias(V/Bias)) =	7.72E-08		
T-test paired (S1V/S2V) =	0.473		
T-test paired (V/I)	8.26E-08		

Valid/Invalid Sample Pair	Valid	Invalid	% Bias (V/I pair)
N	24	24	
Ave	14.5	10.9	-25.0
Std Dev	1.96	3.08	17.4
min	11.01	2.60	-77.6
max	17.663	15.619	1.7

High negative bias



Insufficient quantity summary

Marker	% Bias
Phenylalanine	-29.8
MCADD/C8	-15.3
CAH	-25.8
TSH	-19.5
Thyroxine	-25

- Overall negative bias
- Expected result with less blood
- T-test indicates high probability that invalid data sets are different from valid data sets ($P<0.05$)
- Potential for qualifying abnormal specimen as normal (opposite for T4)

Insufficient quantity Decision making

Marker	Valid	Invalid	Cutoff	Report Valid	Report Invalid
CAH	10.6	7.5	≥ 50	Normal	Normal
PKU	1.49	1.05	≥ 4	Normal	Normal
MCADD	0.13	0.08	≥ 0.8	Normal	Normal
T4 (CH)	12.5	2.8	≤ 5	Normal	**Abnormal**
TSH (CH)	19.7	14.9	≥ 18	Retest	**Normal**

Diluted, Discolored, Contaminated Sample

Diluted, discolored, contaminated
Phenylalanine

Phenylalanine (MS/MS)

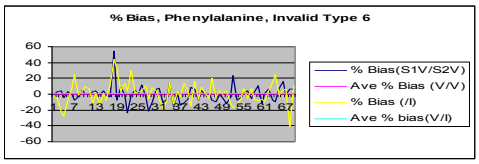
Valid/Valid Sample Pair

	Valid	Valid	% Bias(V/V pair)
N	69	69	
Ave	1.32	1.31	0.02
Std Dev	0.540	0.561	10.65
min	0.68	0.67	-23.81
max	3.51	3.75	54.41

Valid/Invalid Sample Pair

	Valid	Invalid	% Bias (V/I pair)
N	69	69	
Ave	0.99	1.00	1.4
Std Dev	0.205	0.235	13.80
min	0.53	1	-41.07
max	1.89	1.91	44.23

T-Bias (Bias(V/Bias)) =	0.526
T-test dependent (S1V/S2V) =	0.724
T-test dependent (V/I)	0.543



Diluted, discolored, contaminated:
MCADD/C8

MCADD C8(MS/MS)

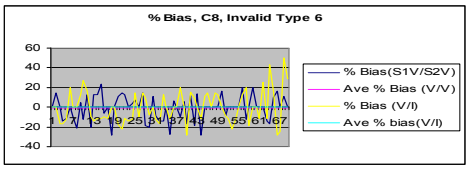
Valid/Valid Sample Pair

	Valid	Valid	% Bias(V/V pair)
N	69	69	
Ave	0.123	0.123	-0.96
Std Dev	0.0896	0.0951	12.45
min	0.05	0.05	-28.57
max	0.52	0.59	23.529

Valid/Invalid Sample Pair

	Valid	Invalid	% Bias (V/I pair)
N	69	69	
Ave	0.085	0.084	-0.26
Std Dev	0.0234	0.0229	16.07
min	0.05	0.05	-28.57
max	0.17	0.15	50

T-Bias (Bias(V/Bias)) =	0.777
T-test dependent (S1V/S2V) =	0.883
T-test dependent (V/I)	0.487



Diluted, discolored, contaminated
Thyroxine

Thyroxine

Valid/Valid Sample Pair

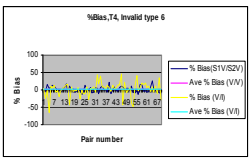
	Valid	Valid	% Bias(V/V pair)
N	69	69	
Ave	9.05	9.18	2.02
Std Dev	3.50	3.29	8.61
min	1.20	3.20	-14.1
max	24.90	23.1	26.7

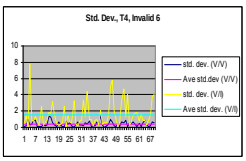
Valid/Invalid Sample Pair

	Valid	Invalid	% Bias (V/I pair)
N	69	69	
Ave	15.1	15.3	1.56
Std Dev	4.005	4.876	20.41
min	5.90	5.47	-66.3
max	23.90	26.13	45.5

Ave Std Dev large for V/I pair

T-Bias (Bias(V/Bias)) =	0.863
T-test paired (S1V/S2V) =	0.142
T-test paired (V/I)	0.620





Diluted, discolored, contaminated Summary

- No difference in average bias for any marker
- MS/MS analytes – Valid/Invalid pairs indistinguishable
- Thyroxine: large variation in bias (-66 to 46), potential for false positives and negatives

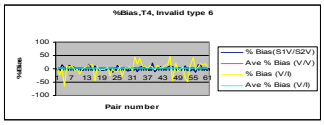
Thyroxine

Valid/Valid Sample Pair

	Valid	Valid	% Bias(V/V pair)
N	61	61	
Ave	9.11	9.26	2.06
Std Dev	3.61	3.41	8.25
min	1.2	3.2	-14.1
max	24.90	23.10	22.5

Valid/Invalid Sample Pair

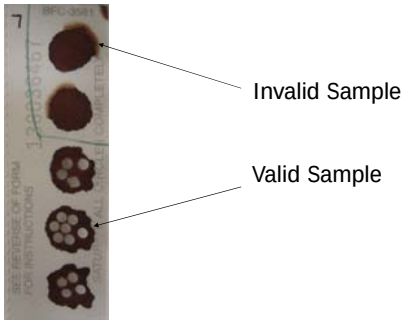
	Valid	Invalid	% Bias (V/I pair)
N	61	61	
Ave	14.999	15.169	1.51
Std Dev	3.71	4.77	20.8
min	6.99	5.62	-66.3
max	23.90	26.13	45.5



Diluted, discolored, contaminated Decision making

Marker	Valid	Invalid	Cutoff	Result Valid	Result Invalid
CAH	24.9	20.3	≥ 50	Normal	Normal
PKU	0.97	1.21	≥ 4	Normal	Normal
MCADD	0.17	0.14	≥ 0.8	Normal	Normal
TSH (CH)	10.3	5.8	≤ 5	Normal	Normal
TSH (CH)	17.4	20.6	≥ 18	Normal	**Abnormal**

Serum Rings



Serum rings
Phenylalanine

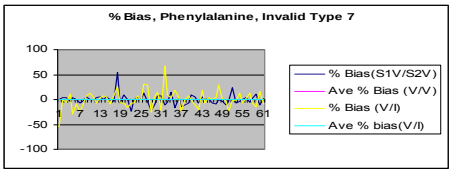
Phenylalanine (MS/MS)

Valid/Valid Sample Pair

	Valid	Valid	% Bias(V/V pair)
N	61	61	
Ave	1.29	1.27	-0.371
Std Dev	0.46	0.46	10.9
min	0.66	0.67	-23.8
max	3.17	3.55	54.4
T-Bias (Bias(V/Bias) =	0.849		
T-test dependent (S1V/S2V) =	0.430		
T-test dependent (V/I)	0.924		

Valid/Invalid Sample Pair

	Valid	Invalid	% Bias (V/I pair)
N	61	61	
Ave	1.05	1.05	0.21
Std Dev	0.19	0.24	17.6
min	0.64	0.37	-54.3
max	1.470	1.73	68.0



Serum rings
MCADD/C8

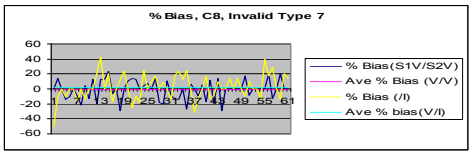
MCADD/C8(MS/MS)

Valid/Valid Sample Pair

	Valid	Valid	% Bias(V/V pair)
N	61	61	
Ave	0.13	0.13	-1.19
Std Dev	0.093	0.099	12.61
min	0.05	0.05	-28.6
max	0.52	0.59	23.5
T-Bias (Bias(V/Bias) =	0.163		
T-test dependent (S1V/S2V) =	0.824		
T-test dependent (V/I)	0.318		

Valid/Invalid Sample Pair

	Valid	Invalid	% Bias (V/I pair)
N	61	61	
Ave	0.081	0.083	2.55
Std Dev	0.0258	0.03098	16.56
min	0.050	0.04	-50.0
max	0.17	0.21	42.9



Serum rings
Thyroxine

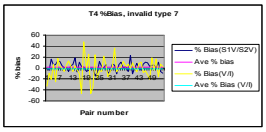
Thyroxine

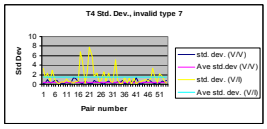
Valid/Valid Sample Pair

	Valid	Valid	% Bias(V/V pair)
N	61	61	
Ave	9.11	9.26	2.1
Std Dev	3.61	3.41	8.25
min	1.20	3.20	-14.1
max	24.9	23.1	22.5
T-Bias (Bias(V/Bias) =	0.129		
T-Values Paired(S1V/S2V) =	0.117		
T-Values Paired (V/I) =	0.509		

Valid/Invalid Sample Pair

	Valid	Invalid	% Bias (V/I pair)
N	61	61	
Ave	16.0	15.5	-1.7
Std Dev	3.94	4.11	17.1
min	7.80	8.08	-46.8
max	26.0	27.2	47.5





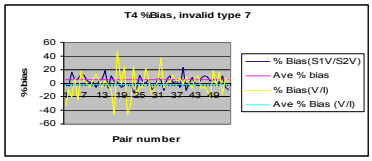
Serum Rings Summary

- MS/MS analytes – Valid/Invalid pairs indistinguishable
- Thyroxine: large variation in bias for V/I pair compared to V/V pair (see min/max)
- No major difference in average bias for any marker

Thyroxine

Valid/Valid Sample Pair	Valid	Valid	% Bias(VV pair)
N	54	54	
Ave	9.27	9	2.25
Std Dev	3.65	3.45	7.97
min	1.20	3.20	-14.1
max	24.90	23.10	22.59

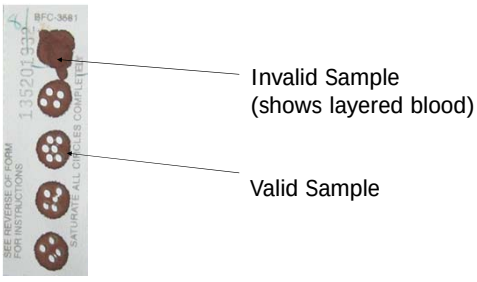
Valid/Invalid Sample Pair	Valid	Invalid	% Bias (VI pair)
N	54	54.00	
Ave	15.99	16	-1.20
Std Dev	4.16	4.305	17.75
min	7.6	8.00	-46.66
max	28.04	27.24	47.52



Serum Rings Decision making

Marker	Valid	Invalid	Cutoff	Result Valid	Result Invalid
CAH	49.5	15.4	≥ 50	Normal	Normal
PKU	1.03	1.73	≥ 4	Normal	Normal
MCADD	0.16	0.11	≥ 0.8	Normal	Normal
T4	10.3	16.9	≤ 5	Normal	Normal
TSH (CH)	19.1	11.9	≥ 18	Retest	**Normal**

Clotted or Layered



Clotted or layered
Phenylalanine

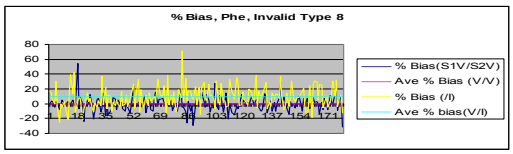
Phenylalanine (MS/MS)

Valid/Valid Sample Pair	Valid	Valid	% Bias(V/V pair)
N	180	180	
Ave	1.29	1.30	-0.14
Std Dev	0.95	1.02	10.4
min	0.62	0.61	-31.27
max	11.1	11.9	54.4

Valid/Invalid Sample Pair	Valid	Invalid	% Bias (V/I pair)
N	180	180	
Ave	1.08	1.17	8.00
Std Dev	0.22	0.22	14.5
min	0.52	0.60	-25.2
max	1.69	1.79	71.8

T-Bias (BiasV/Bias) =	7.77E-11
T-test dependent (S1V/S2V) =	0.926
T-test dependent (V/I)	4.69E-13

Note T-test show V/I data sets high probability of data sets being different



Clotted or layered
MCADD/C8

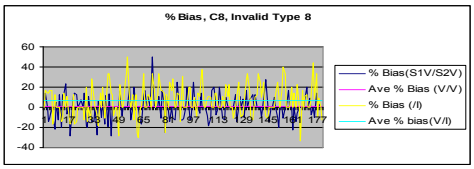
MCADD/C8 (MS/MS)

Valid/Valid Sample Pair	Valid	Valid	% Bias(V/V pair)
N	180	180	
Ave	0.11	0.11	0.48
Std Dev	0.071	0.073	12.0
min	0.04	0.04	-26.6
max	0.52	0.59	50.0

Valid/Invalid Sample Pair	Valid	Invalid	% Bias (V/I pair)
N	180	180	
Ave	0.086	0.090	6.66
Std Dev	0.024	0.026	14.9
min	0.05	0.05	-33.3
max	0.21	0.22	50.0

T-Bias (BiasV/Bias) =	1.88E-05
T-test dependent (S1V/S2V) =	0.954
T-test dependent (V/I)	3.51E-07

Note T-test show V/I data sets high probability of data sets being different



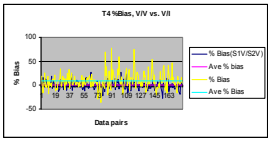
Clotted or layered
Thyroxine

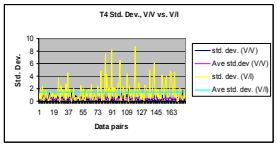
Thyroxine

Valid/Valid Sample Pair	Valid	Valid	% Bias(V/V pair)
N	180	180	
Ave	9.09	9.12	0.64
Std Dev	3.07	2.95	8.92
min	1.2	2.9	-27.9
max	24.9	23.1	26.9

Valid/Invalid Sample Pair	Valid	Invalid	% Bias (V/I pair)
N	180	180	
Ave	15.4	16.8	9.28
Std Dev	3.3	4.4	17.2
min	7.24	7.32	-35.7
max	27.325	32.9	77.3

T-Bias (BiasV/Bias) =	6.81E-09
T-test dependent (S1V/S2V) =	0.683
T-test dependent (V/I)	5.55E-11





Clotted or layered Summary

Marker	Average % Bias
17-OHP	9.8
Phenylalanine	8.8
MCADD/C8	6.7
TSH	14.2
Thyroxine	9.3

- Overall Positive bias
- Some specimens w/ large neg. bias results
- Potential to have false positives and false negatives
- Expected due to layering of blood

Clotted or layered: Decision making

Marker	Valid	Invalid	Cutoff	Result Valid	Result Invalid
CAH	50.2	**39.3***	≥ 50	Retest	**Normal**
PKU	0.76	0.96	≥ 4	Normal	Normal
MCADD	0.09	0.11	≥ 0.8	Normal	Normal
T4	18.5	20.1	≤ 5	Normal	Normal
TSH	7.7	19	≥ 18	Normal	**Retest**
TSH	19.1	11.9	≥ 18	Retest	**Normal**

*Invalid result lower than valid specimen result

Summary

Qualitative tests: valid/invalid samples indistinguishable

Disorder(s)	Invalid Type	Valid Sample	Invalid Sample
Biotinidase	Insufficient Quantity	24N	24N
	Diluted, discolored, or contaminated	69N	69N
	Serum Rings	61N	61N
	Clotted or layered	180N	180N
Galactosemia	Insufficient Quantity	24N	24N
	Diluted, discolored, or contaminated	69N	69N
	Serum Rings	61N	61N
	Clotted or layered	180N	180N
Hemoglobin	Insufficient Quantity	24N	24N
	Diluted, discolored, or contaminated	67N, 1AS, 1AF	67N, 1AS, 1AF
	Serum Rings	57N, 2AC, 1AS, 1AF	57N, 2AC, 1AS, 1AF
	Clotted or layered	173N, 2AFE, 2FST, 2AS, 1AF	173N, 2AFE, 2FST, 2AS, 1AF

N - Normal, AC - C-trait , AF - A>F , AFE - fetal variant ,
AS - Sickle trait , FST - J, N, or Bart's variant

Quantitative Data: Effect on Decision Making

Test	17-OHP	Phe	C8	T4	TSH	Galac	Biotin	Hgb
signal	↑	↑	↑	↓	↑	↑	↑	P/A
QNS	FN	FN	FN	FP	FN	N/A	N/A	N/A
Diluted	FN/FP	FN/FP	FN/FP	FN/FP	FN/FP	N/A	N/A	N/A
Rings	FN/FP	FN/FP	FN/FP	FN/FP	FN/FP	N/A	N/A	N/A
Clotted/ layered	FN/FP	FN/FP	FN/FP	FN/FP	FN/FP	N/A	N/A	N/A

P/A = present/absent, FN = False Neg., FP = False Positive

Conclusions

Quantity Insufficient

- Bias low results, may be false negative or false Positive (T4)
- Qualitative results were unaffected (however, only 24 points and no positive specimens were tested)

Decision making data summary:

Marker	Valid Result	Invalid Result	"Cutoff(s)"	Descrepancy
T4	12.5	2.8	5	N vs. Abn.
TSH	19.7	14.9	18	Retest vs. N

Conclusions

Diluted, discolored, or contaminated

- MS analytes largely unaffected
- Qualitative results unaffected (however, no positive specimens were tested)
- T4 and TSH results show large variation in measured bias for V/I pair

Decision making data summary:

Marker	Valid Result	Invalid Result	"Cutoff(s)"	Descrepancy
TSH	16.0	18.1	18	N vs. Retest
TSH	19.8	16.3	18	Retest vs. N
TSH	27.9	14.6	18/20	Abnormal vs. normal
TSH	18.5	25.8	18/20	Retest vs. Abnormal

Conclusions

Serum Rings

- MS analytes largely unaffected, however there are affected samples (see below)
- Qualitative results unaffected (however, no positive specimens were tested)
- T4 and TSH results show large variation in measured bias for V/I sample pair

Decision making data summary:

Marker	Valid Result	Invalid Result	"Cutoff(s)"	Descrepancy
Leu/Iso, Leu/Phe	3.14, 3.06	3.43, 3.5	3, 3.5	N vs. Retest
C5DC	0.06	0.2	0.2	N vs. abnormal
CAH	49.5	15.4	50	N vs. N near limit
TSH	19.1	11.9	18	Retest vs. N
TSH	18.1	14.9	18	Retest vs. N

Conclusions

Clotted or layered

- Bias high results, may be false negative (T4) or false Positive
- Qualitative results were unaffected (however, no positive specimens were tested)

Decision making data summary:

Marker	Valid Result	Invalid Result	"Cutoff(s)"	Descrepancy
CAH	50.2	39.3	50	Retest vs. N
TSH	18.1	16.2	18	Retest vs. N
TSH	19.1	16.7	18	Retest vs. N
TSH	7.7	19	18	N vs. retest
TSH	16.8	19.7	18	N vs. Retest
TSH	15.5	22.9	18/20	N vs. abnormal
TSH	17.4	20.6	18/20	N vs. abnormal

Conclusions

- Quantity Insufficient
  - Bias Low results for quantitative tests (false neg. - except T4)
  - Qualitative tests unaffected
- Diluted, discolored, or contaminated
  - MS analytes largely unaffected
  - T4 and TSH results show large variation in bias for V/I pair (+/-)
  - Qualitative results unaffected
- Serum Rings
  - MS analytes largely unaffected
  - T4 and TSH results show large variation in bias for V/I pair (+/-)
  - Qualitative results unaffected
- Clotted or layered
  - Generally bias high results, may be false negative (T4) or false positive
  - Some bias low results (where blood has not been layered)
  - Qualitative results unaffected

Urban Legend vs. Reality

Invalid ok	Invalid **NOT** ok
Galactosemia	CH
Hgb	CAH
Biotinidase	MCAD
	PKU

Thank you.

The Effect of Unsatisfactory Specimens on Newborn Screening

Lisa Kalman, PhD
Newborn Screening Quality Assurance Program, CDC

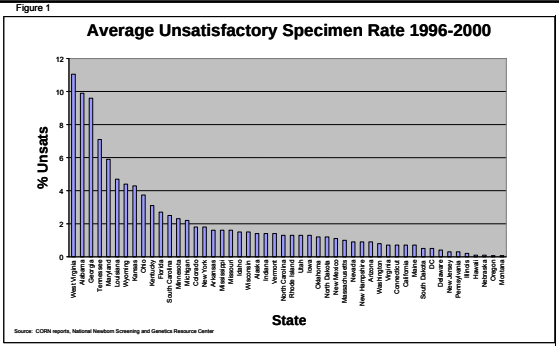
How do unsat rates, criteria and practices differ between states?

?

National Unsat Survey

- Data on state unsat rates was collected from the 1996-2000 National Newborn Screening Reports. The average unsat rate for each state was calculated from the available data.
- Data on state unsat practices (2001) was collected by email and phone survey.

Figure 1



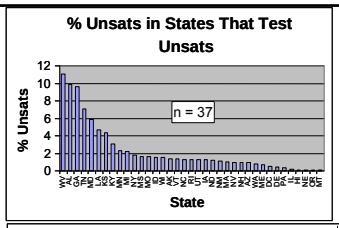
Unsatisfactory Specimen Rates 1996-2000
Mean = 2.2%
Median = 1.3%

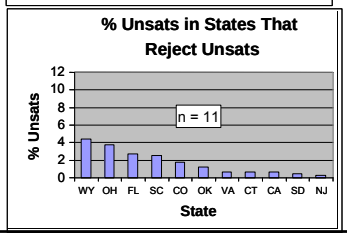
Why Do States Have Different Unsat Rates?

?

Do states that do not test unsats have a lower unsat rate?

Idea – Perhaps hospitals will collect better specimens if they know that unsats will not be tested…





Data from 49 of 51 programs

TX tests one class of unsats

There is not a significant difference in unsat rates between states that test or do not test (reject) unsat specimens.

States That….

	Reject Unsats	Test Unsats
Mean	1.7% Unsats (S.D=1.3)	2.3% Unsats (S.D. = 2.8)
Median	1.2% Unsats	1.3% Unsats

Does the number of unsat critera affect the state unsat rate?

Perhaps states with more unsat criteria have higher unsat rates?

Unsat Criteria

- 48 of 51 programs reported their unsat criteria
- The number of unsat criteria per state varied from 6 to 20. The median was 9.
- Most states adopt some or all of the 8 Schleicher and Schuell criteria.
- States also list additional criteria
- We found that the number of unsat criteria is apparently not related to the state unsat rate.

Summary of Unsat Rate Variation Study

We were unable to identify reasons for the variation in state unsat rates.

Do unsats delay newborn screening result reporting ?

- Examine effect of unsats in two states with different…
  - % Unsats
  - # of Births/yr
  - Screening schemes (1 vs 2 required specimens)
  - Follow-up procedures

The effect of unsatisfactory specimens on newborn screening in two states

- Data from unsat specimens and satisfactory specimens of matched controls were collected from 2001 newborn screening records in two states
- State 1 requires 1 specimen/baby, high # births/yr, high % unsats
- State 2 requires a second screen after day 7, low # births/yr, low % unsats

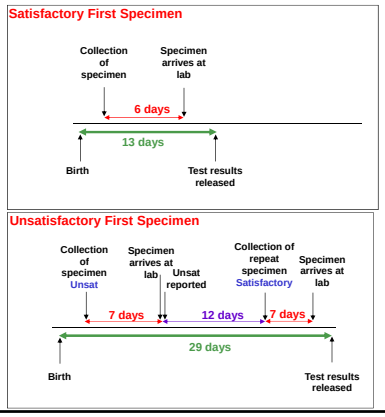
Data collected or calculated from unsats and age/weight/date matched controls included...

- Unsat type (for unsat specimens)
- Age at collection
- Birth weight
- Mailing times
- Date of receipt at lab
- Time between specimens
- Age when result from first satisfactory specimen is available

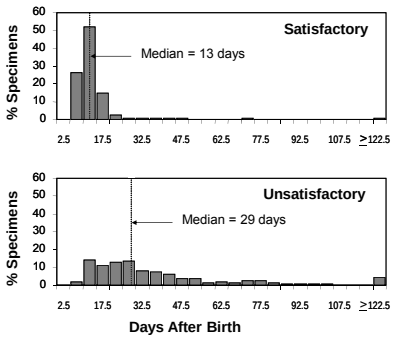
Number of specimens analyzed.....

- State 1: n = 1505 unsats/matched controls (representing 5 weeks of 2001)
- State 2: n = 359 unsats/matched controls (representing most of 2001)
- Some unsats were excluded from analysis due to difficulties with control matching

State 1 - One required specimen - Median number of days



State 1 – Ages when results from a satisfactory specimen are available



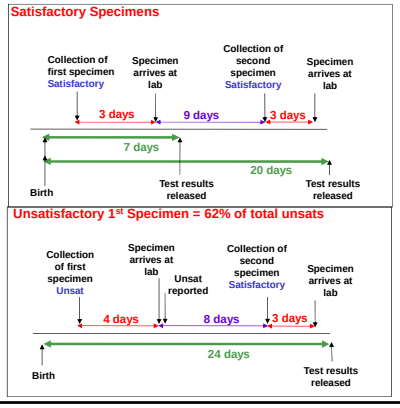
State 1 -

- 66% of patients with unsats eventually got a satisfactory repeat specimen
- 34% of patients with unsats were not documented to have a satisfactory repeat specimen

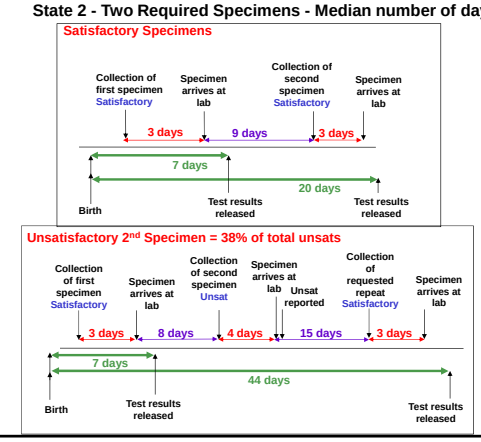
Of the 34% with no satisfactory repeat:

- 59% had no repeat specimen
- 41% had only unsat repeats

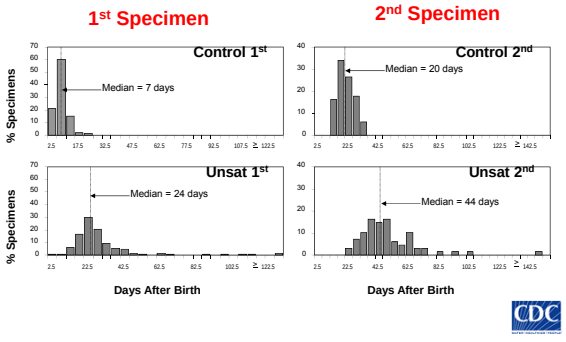
State 2 - Two Required Specimens - Median number of days



State 2 - Two Required Specimens - Median number of days



State 2 – Ages when results from a satisfactory specimen are available



State 2 -

- 91% of babies with and unsat 1st specimen (62% of all unsats) had a satisfactory repeat specimen
- 9% of patients with unsat 1st specimens were not documented to have a satisfactory repeat specimen

Of these 9% without satisfactory repeat:

- 67% had no repeat specimen
- 33% had unsat repeats

State 2 (cont).....

- 5.6% of all patients with unsats (1st or 2nd) were not documented to have any satisfactory specimen

(9% of 62% = 5.6%)

Conclusions – State 1

- Babies with unsat 1st specimens got valid results 16 days later (median difference) than children with a satisfactory 1st specimen (29 vs 13 days)
- 34% of patients with unsats were never documented to have a satisfactory repeat specimen – this state does not follow up unsat specimens

Conclusions – State 2

- Babies with unsat 1st specimens got valid results 18 days later (median difference) than children with a satisfactory 1st specimen (24 vs 7 days)
- The impact of an unsat 2nd specimen is lessened since these patients had results from a satisfactory 1st specimen
- 5.6% of all patients with unsat specimens were never documented to have a satisfactory specimen - this state has aggressive follow up procedures and a mandatory second specimen

Bottom Line

- The delay and possible false negative results caused by unsats could potentially cause harm to affected children
- Need to scientifically analyze unsat specimens to determine which categories are really unsuitable for testing
- Need to improve specimen collection (education)
- Need electronic specimen linking and tracking
- Need aggressive follow up of unsats

Others involved in these studies....

- Scott Grosse, CDC
- Owen Devine, CDC
- Harry Hannon, CDC
- Brad Therrell, NNSGRC

Special Thanks to:
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For more information on the "Unsatisfactory Specimens" Web Conference

- Nancy Meredith – nkm1@cdc.gov
- Jelili Ojodu - jojodu@aphl.org

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APHL

2005 Newborn Screening and Genetic Testing Symposium

- October 24 – 27, 2005
- Hilton Portland & Executive Towers Hotel
- Emphasis on state issues, education, follow up, new disorders, hot topics, Cystic fibrosis, data, and MS/MS
- Pre conference workshops on QA/QC and Follow up
- For more information, visit http://www.aphl.org
- Contact: Terry Reamer, treamer@aphl.org

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