A National Survey of Newborn Screening Policies Concerning Samples Submitted Post-Transfusion – The 50 States and The District of Columbia

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Reasons for Examining The Post-Transfused Sample Handling Policy of the Louisiana OPH Newborn Screening Program

Clinicians advised new practice approaches to Congenital Hypothyroidism requires reliable detection in the first days of life

Recognition that transfused blood may interfere with testing assays

Awareness of the large population of premature & low-birth weight infants receiving transfusion

Dissatisfaction with our established sample policy
Methods of Examining Post-Transfused Policy

- Research the Scientific Literature
- Evaluate the Magnitude of the Problem
- Survey other US Programs
Results of Evaluation

Scientific Literature Review:

Evidence Supports Early Treatment of CH

Very Few Studies Concerning Transfusion Effects on Newborn Screening

Some Reports of Missed Cases/Transfusion Interference with Newborn Screening
Recent Clinical Studies Have Stressed the Need for Early Treatment in Congenital Hypothyroidism

1. Children’s Hospital, Rotterdam, The Netherlands Study – 2000

Defined early treatment as before 13 days after birth

“optimal treatment includes achievement of euthyroidism before the third week of life by initiation of therapy before 13 days”

Recent Clinical Studies Have Stressed the Need for Early Treatment in Congenital Hypothyroidism

2. Department of Pediatrics – Portland, Oregon HSU – 1999

Emphasis on early recognition & treatment

“The goals of treatment are to raise the serum T4 as rapidly as possible into the normal range”

Recent Clinical Studies Have Stressed the Need for Early Treatment in Congenital Hypothyroidism


Normalization of TSH by 3 months of age

“Early TSH normalization is necessary to allow for normal development”

Reports of Missed Cases Due to Transfusion

Missed Galactosemia –


Reports of Missed Cases Due to Transfusion

Missed Hemoglobinopathy –


In this patient, the initial presence of a small amount of Hb S is followed by its subsequent disappearance with transfusion. After transfusion, the reappearance of a small percentage of Hb S is shown.

Fig. 1. Plasma $T_4$ during exchange transfusion in immature newborns with a birth weight between 1900–3500 g (group A, solid line) and in those with a birth weight below 1900 g (group B, dotted line). Group A included 24 infants sampled during the procedure, 18 infants were sampled also during the post-transfusion period. In group B there were 11 and 8 infants, respectively. The $T_4$ levels in plasma of donors are also shown. Data are presented as X ± S. E. I, II and III — sampling at the start, in the middle and at the end of the procedure, respectively. At the start, higher plasma $T_4$ levels were found in newborns in group A ($P < 0.001$) as well as in B ($P < 0.01$) than in donors. $T_4$ levels decreased profoundly during the procedure in group A and increased to an intermediate level 24 h later ($P < 0.001$ paired “t” tests). Plasma $T_4$ decreased also in group B during the procedure ($P < 0.02$ paired “t” test) and remained constant thereafter. The difference between groups A and B at the interval 24 h after the procedure was highly significant ($P < 0.01$).
Exchange Transfusion - TSH Response in normal & premature, low-birth weight infants


Fig. 2. Plasma TSH in the same groups of infants shown in Fig. 1. At the start of exchange transfusion, plasma TSH was significantly higher in group A (solid line) and B (dotted line) \((P < 0.01\) and \(0.05\), respectively, Mann-Whitney “U” test) than in donors, and decreased significantly during the procedure in both groups \((P < 0.05\), Wilcoxon matched-pairs signed-ranks test). Twenty four hours after the transfusion, plasma TSH was higher in group A than in B \((P < 0.05\), Mann-Whitney “U” test), although the Wilcoxon test did not confirm intra-group significance of post-transfusion changes.
Figure. The effect of ET on serum thyroid hormone concentrations: \( A \), T, (top panel), FT, (middle), and TSH (bottom), and \( B \), T, (top), rT, (middle), and TBG (bottom). Results are expressed as mean ± SEM (*\( P < 0.001 \) and **\( P < 0.025 \) compared to donor; †\( P < 0.001 \), ‡\( P < 0.05 \), and ‡‡ \( P < 0.005 \) increase or decrease). Solid lines represent results in six infants normal except for ABO or Rh incompatibility; dashed lines represent values in four sick preterm infants. Donor values for former group are depicted as open bars, those for latter group as shaded bars.
Neonatal Transfusions

“300,000 neonates undergo transfusions annually”

“Of the 38,000 premature, low-birth weight neonates … born annually 80% will require multiple red blood cell transfusions” (1980’s)

“The usual volume of red cells (RBCs) transfused is small (5 – 10 ml/Kg body weight)”

Categories of Louisiana OPH Newborn Screens by Blood Component

SERUM BASED TESTS

PKU - Phenylalanine (amino acid elevated from precursor accumulation)
- 3.0 mg/dL is a presumptive positive
- 2.5 to 2.9 mg/dL is an equivocal result (repeated)
- 2.0 mg/dL or higher is also repeated
- 1.9 mg/dL or lower is a presumptive negative

CH - T4 (Thyroxine) (decreased hormone levels)
- T4 “cutoff” values:
  - > 48 hrs. old – 4 micrograms/deciliter
  - < 48 hrs. old – 6 micrograms/deciliter

TSH (Thyroid Stimulating Hormone) (elevated)
- lower 12% of each day’s T4 analysis
- > 30 microunits/ml is a presumptive positive
- > 25 microunits/ml is repeated

Biotinidase Enzyme Activity
- inadequate enzyme activity by qualitative test (colorimetric)
Categories of Louisiana OPH Newborn Screens
by Blood Component

RED BLOOD CELL BASED TESTS

Hemoglobins (abnormal vs. normal)

Galactose–1-Uridyltransferase (GALT)
inadequate enzyme activity by quantitative test
< 2.4 U/gHgb is a presumptive positive (repeated)
2.5 U/gHgb to 3.5 U/gHgb is an equivocal result (repeated)
> 3.5 U/gHgb is a presumptive negative

Note: Loss of transfused red blood cells from senescence = 0.83% / day
All post-transfused samples were deemed unacceptable and submitter notified “unacceptable due to transfusion”

Required re-submit 7 days after last transfusion and 90 days after transfusion.

However – samples were tested and only abnormal results were notified by telephone
Magnitude of Transfusion Submit Impact
Louisiana OPH Newborn Screening Post-transfused Sample Submit

3 Year History Post-TX Submits

- Total IEF Samples
- Post-Transfused Submit
- Unsatisfactory Submits

Months

Samples

Values range from 0 to 700.
Louisiana OPH Newborn Screening Post-transfused Sample Submits

% of IEF Test Submits Post-TX

- X-axis: Months
- Y-axis: Percent IEF - Post-Tx

Graph showing the percentage of IEF test submits post-transfusion over a period of months, with fluctuations ranging from 0 to 25%.
Louisiana OPH Newborn Screening Post-transfused Sample Submits

Total Rejected vs. Post-Tx vs. Unsats

- Samples
- Months
- Unsats
- Post-Tx
- Tot. Rej.
Louisiana OPH Newborn Screening Post-transfused Sample Submits

Post-Tx as % of Total Rejected

% of Total Rejected

Months

0 10 20 30 40 50 60 70 80 90
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35

Years

21
Louisiana OPH Newborn Screening Post-transfused Sample Submits

3 Yr Hx - Post-Tx vs. Unsats

Post-Transfused Submits
Unsatisfactory Sampling
Louisiana OPH Newborn Screening Post-transfused Sample Submits

% of Total Samples Submitted Post-Transfused

- Months: 23, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35
- % of Total Mean Monthly samples
- mean # samples/month = 7263

- % of Total = Post-Transfused
Categories of Resubmit Policies from National Survey

Resubmission Policy Analysis

- Test All Samples
- No Resubmit (serum tests)
- 1 day
- 2 days
- 3 days
- 4 days
- 6 days
- 7 days
- 8 to 15 days
- 14 days
- 21 days
- 1 month
- 2 months
- 3 months
- 4 months

percent of all programs
## Survey Of NBS Program Post-transfusion Policies

<table>
<thead>
<tr>
<th>State</th>
<th>Post-transfusion Policy</th>
<th>3 months for Hgb &amp; GALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama (AL)</td>
<td>CH - 72 hours, also for PKU &amp; CAH</td>
<td>3 months after last transfusion for Hgb, Biotinidase, &amp; GALT</td>
</tr>
<tr>
<td>Alaska (AK)</td>
<td>Test all samples</td>
<td>3 months after last transfusion for Hgb, Biotinidase, &amp; GALT</td>
</tr>
<tr>
<td>Arizona (AZ)</td>
<td>Test all samples - suggest retest if transfused</td>
<td>3 months after last transfusion for Hgb, Biotinidase, &amp; GALT</td>
</tr>
<tr>
<td>Arkansas (AR)</td>
<td>Samples are screened as requested by physician -</td>
<td>3 months after last transfusion for Hgb, Biotinidase, &amp; GALT</td>
</tr>
<tr>
<td>California (CA)</td>
<td>Test all samples. Retest during a 6-day period AFTER the final transfusion.</td>
<td>Hgb at 90 days</td>
</tr>
<tr>
<td>Colorado (CO)</td>
<td>Test all samples - suggest retest in 72 hours if transfused</td>
<td>Suggest 90 days for Hgb &amp; GALT</td>
</tr>
<tr>
<td>Connecticut (CT)</td>
<td>Test all samples - recommend 2nd test all screens</td>
<td>3 months for Hgb's, total galactose, GALT, 1st screen notes UNSAT - TRANS. For these only</td>
</tr>
<tr>
<td>Delaware (DE)</td>
<td>Test for all analytes; T4 &amp; TSH are reported.</td>
<td>3 months for Hgb's, total galactose, GALT, 1st screen notes UNSAT - TRANS. For these only</td>
</tr>
<tr>
<td>District of Columbia (DC)</td>
<td>Neogen (Pediatric) contract - all tested and results reported total galactose &amp; GALT assayed request resubmit</td>
<td>Hgb are tested by IEF &amp; confirmed by DNA testing - genomic proof. Soon DNA will be only screen.</td>
</tr>
<tr>
<td>Florida (FL)</td>
<td>Test all samples</td>
<td>4 months after transfusion for GAL &amp; Hgb</td>
</tr>
<tr>
<td>Georgia (GA)</td>
<td>Test all samples but recommend resubmit -1 week, 2weeks</td>
<td>2 months for GAL &amp; Hgb</td>
</tr>
</tbody>
</table>
## Survey Of NBS Program Post-transfusion Policies

<table>
<thead>
<tr>
<th>State</th>
<th>Testing Protocols</th>
<th>Turnaround Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hawaii (HI)</td>
<td>No resubmit for CH, PKU, MSUD, GALT (contract with Oregon)</td>
<td>3 months for Hgb &amp; GALT by Beutler assay</td>
</tr>
<tr>
<td>Idaho (ID)</td>
<td>No resubmit for CH, PKU, MSUD, GALT (contract with Oregon)</td>
<td>3 months for Hgb &amp; GALT by Beutler assay</td>
</tr>
<tr>
<td>Illinois (IL)</td>
<td>request a repeat and usually get it by 2 weeks</td>
<td>90 days for Gal &amp; Hgb</td>
</tr>
<tr>
<td>Indiana (IN)</td>
<td>Test all samples - report values - T4 &amp; TSH on all specimens if low T4 &amp; high TSH - immediate retest, if low T4 &amp; NORMAL TSH - 40 days for retest - total galactose is done for Tx - Hgb reported as Tx - abn. PKU &amp; Biotinidase repeat 24hrs</td>
<td>2 to 4 months and GALT is tested this time</td>
</tr>
<tr>
<td>Iowa (IA)</td>
<td>test all screens and report all - except for GALT</td>
<td>8 weeks post-Tx for GALT &amp; Hgb</td>
</tr>
<tr>
<td>Kansas (KS)</td>
<td>test all samples - if transfused (A&gt;F) - repeat at 7 days (galt),14 days (PKU), 21 days (CH)</td>
<td>report Hgb A &gt; Hgb F - suggest retest</td>
</tr>
<tr>
<td>Kentucky (KY)</td>
<td>Test and report abnormals; otherwise report as inconclusive.</td>
<td>REPEAT AFTER TRANSFUSION: [T4, TSH, PKU: 72 HRS.] [GAL: 60 - 65 DAYS] [Hgb:120 DAYS]</td>
</tr>
</tbody>
</table>

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**T4, TSH, PKU: 72 HRS.**

- **GAL: 60 - 65 DAYS**
- **Hgb:120 DAYS**
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<tr>
<th>State</th>
<th>Policy Description</th>
<th>Timeframe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louisiana (LA)</td>
<td>Test &amp; report post-tx samples with a recommendation to repeat sample submission at 2 days post-transfusion, esp. useful for T4 &amp; TSH &amp; 7 days post-tx, esp. useful for PKU &amp; Biotinidase detection.</td>
<td>3 months for Hgb &amp; GALT</td>
</tr>
<tr>
<td>Maine (ME)</td>
<td>request a repeat for Tx&lt;48, Fact sheet given</td>
<td>repeats at 2-3 months after last transfusion</td>
</tr>
<tr>
<td>Maryland (MD)</td>
<td>test all and report results</td>
<td>4 months after transfusion for GAL &amp; Hgb &amp; Biotinidase</td>
</tr>
<tr>
<td>Massachusetts (MA)</td>
<td>request a repeat for Tx&lt;48, Fact sheet given</td>
<td>repeats at 2-3 months after last transfusion</td>
</tr>
<tr>
<td>Michigan (MI)</td>
<td>Test all - report all - state invalid due to Tx. Use A &gt; F to catch unmarked Tx samples</td>
<td>90 days for Hgb and &amp; Galt. Confirm GALT with total galactose.</td>
</tr>
<tr>
<td>Minnesota (MN)</td>
<td>test all samples</td>
<td>GALT &amp; Hgb at 90 days</td>
</tr>
<tr>
<td>Mississippi (MS)</td>
<td>repeat after 4 days for PKU, TSH, CAH,</td>
<td>10 &amp; 90 days for GALT 90 for Hgb</td>
</tr>
<tr>
<td>Missouri (MO)</td>
<td>test all samples - report results - Tx invalid GALT &amp; Hgb</td>
<td>GALT &amp; Hgb at 90 days</td>
</tr>
<tr>
<td>Montana (MT)</td>
<td>Test all samples</td>
<td>repeat 90 – 120 days</td>
</tr>
<tr>
<td>Nebraska (NE)</td>
<td>test all samples, reports results, for CH &amp; CAH no disqualifier statement is attached, If total galactose is tested - no disqualifier, if GALT - report invalid due to Tx.</td>
<td>Suggest repeat Hgb at 4 months</td>
</tr>
<tr>
<td>Nevada (NV)</td>
<td>Test all - report all results with disclaimer sheet and suggest 48 hrs. after Tx repeat CH/PKU,</td>
<td>60 to 90 days for biotinidase, GALT, Hgb</td>
</tr>
<tr>
<td>New Hampshire (NH)</td>
<td>No resubmit for CH, PKU, MSUD, GALT (contract with Oregon)</td>
<td>3 months for Hgb &amp; GALT by Beutler assay</td>
</tr>
</tbody>
</table>
## Survey Of NBS Program Post-transfusion Policies

<table>
<thead>
<tr>
<th>State</th>
<th>Action</th>
<th>Repeat Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Jersey (NJ)</td>
<td>request a repeat for Tx&lt;48 Fact sheet given</td>
<td>repeats at 2-3 months after last transfusion</td>
</tr>
<tr>
<td>New Mexico (NM)</td>
<td>test all samples report results for CH, PKU, CAH without comment, as same for total galactose, GALT &amp; Hgb indeterminate</td>
<td>GALT &amp; Hgb 120 days</td>
</tr>
<tr>
<td>New York (NY)</td>
<td>Mandatory second screen at 8 - 15 days, Reported as unsatisfactory with both tests - run all tests</td>
<td>90-120 post transfusion and all 6 screens are done</td>
</tr>
<tr>
<td>North Carolina (NC)</td>
<td>Request a repeat 24 hrs for serum based tests (PKU, CH, MSUD, HCY, CAH)</td>
<td>Repeat 30 days for HgB, GALT, BIO</td>
</tr>
<tr>
<td>North Dakota (ND)</td>
<td>contracted to Iowa test and report - except GALT</td>
<td>8 weeks post-Tx for GALT &amp; Hgb</td>
</tr>
<tr>
<td>Ohio (OH)</td>
<td>test is invalid, no report repeat 2 weeks</td>
<td>Hgb &amp; GALT at 90 days</td>
</tr>
<tr>
<td>Oklahoma (OK)</td>
<td>test all, all results reported, rescreened at 1 week, T4 in 7 to 14 days</td>
<td>90 to 120 days for Hgb</td>
</tr>
<tr>
<td>Oregon (OR)</td>
<td>No resubmit for CH, PKU, MSUD, GALT</td>
<td>3 months for Hgb &amp; GALT by Beutler assay</td>
</tr>
<tr>
<td>Pennsylvania (PA)</td>
<td>NEOGEN CONTRACT: all tested and results reported total galactose &amp; GALT assayed request resubmit</td>
<td>HgB are tested by IEF &amp; confirmed by DNA testing - genomic proof. Soon DNA will be only screen.</td>
</tr>
<tr>
<td>Rhode Island (RI)</td>
<td>request a repeat for Tx&lt;48 Fact sheet given</td>
<td>repeats at 2-3 months after last transfusion</td>
</tr>
</tbody>
</table>
## Survey Of NBS Program Post-transfusion Policies

<table>
<thead>
<tr>
<th>State</th>
<th>Testing and Reporting Requirements</th>
<th>Follow-up Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Carolina (SC)</td>
<td>tests all samples - if abnormal repeat ASAP</td>
<td>request a repeat at 2 months</td>
</tr>
<tr>
<td>South Dakota (SD)</td>
<td>test all samples and results reported, collected before discharge or before 7 days of age. If within 48 hrs. of transfusion, CH &amp; PKU must have repeat sample.</td>
<td>Galactosemia at 3 months after last transfusion</td>
</tr>
<tr>
<td>Tennessee (TN)</td>
<td>repeat after 4 days for PKU, TSH, CAH,</td>
<td>10 &amp; 90 days for GALT 90 for Hgb</td>
</tr>
<tr>
<td>Texas (TX)</td>
<td>Test all samples, &quot;Responsibility of the care provider properly collect sample&quot;</td>
<td></td>
</tr>
<tr>
<td>Utah (UT)</td>
<td>test all samples and report results, if first screen is post-Tx then require repeat 120 days after last transfusion</td>
<td>Mandatory 2nd screen at 7 - 28 days old.</td>
</tr>
<tr>
<td>Vermont (VT)</td>
<td>request a repeat for Tx&lt;48 Fact sheet given</td>
<td>repeats at 2-3 months after last transfusion</td>
</tr>
<tr>
<td>Virginia (VA)</td>
<td>test all samples - report results</td>
<td>60 days for Hgb &amp; GALT</td>
</tr>
<tr>
<td>Washington (WA)</td>
<td>test all samples &amp; report Tx - Hgb invalid</td>
<td>120 days for Hgb</td>
</tr>
<tr>
<td>West Virginia (WV)</td>
<td>test all samples &amp; report Tx - Hgb invalid</td>
<td>120 days for Hgb</td>
</tr>
<tr>
<td>Wisconsin (WI)</td>
<td>all testing is done on transfused specimens - UNSAT goes with the report</td>
<td>If we know that the transfusion was not a RBC containing type, the hemoglobin results are reported as acceptable.</td>
</tr>
<tr>
<td>Wyoming (WY)</td>
<td>recommends repeat PKU</td>
<td></td>
</tr>
</tbody>
</table>
Categories of Resubmit Policies

Resubmission Policy Analysis

percent of all programs

Test All Samples
No Resubmit (serum tests)
1 day
2 days
3 days
4 days
6 days
7 days
8 to 15 days
14 days
21 days
1 month
40 days
2 months
3 months
4 months
New Policy for Louisiana OPH Newborn Screening – July 2003

Test all samples and provide results (formal report)

Recommend repeat submits at 2 days post-transfusion, 7 days post-transfusion, and 90 days post-transfusion

“Important Notice – transfusion may alter ALL newborn screening results” on Report mailer
LA NBS Report With Transfusion Notice

LOUISIANA D. H. H.
Office of Public Health
DIVISION OF LABORATORY SERVICES
CENTRAL LABORATORY
325 Loyola Ave. Room 709
New Orleans, Louisiana 70112

Date: 01/05/2004

Lab Number: 0033584089
Form #: 03791277
Sex: F
Race: W

Dt. Coll: 12/19/2003
Dt. Recv.: 12/24/2003
Dt. Reported: 01/05/2004

Duplicate Mailer

Test          Result/Analysis
--------------
T4            T4 NORMAL
PKU           PKU NORMAL
Galactose     GALACTOSE NORMAL
BIO           BIOTINIDASE NORMAL
HB            HGB NORMAL

IMPORTANT NOTICE: Transfusion may alter ALL Newborn Screening results.
REPEAT TESTING RECOMMENDED:
2 days after last transfusion
AND 7 days after last transfusion
AND 90 days after last transfusion

IF SPECIMEN IS COLLECTED WITHIN 48 HOURS OF BIRTH,
PLEASE REPEAT TEST.
Continued growing population of premature, low-birth weight neonates requiring transfusion

Little success in non-invasive physiologic tests for neonates to date – still will need blood drawn

Little success with artificial blood substitutes or erythropoietin therapy – still will need blood transfusions

Metabolic Screening by MS/MS will markedly complicate this already difficult picture
Newborn Screening and Blood Transfusion

Need for Studies of the Effect of Transfusion on NBS

Need to Coordinate Laboratory Results with Clinical Picture

Most Decisions Will be Based on Clinical Judgment

Collaboration Between Clinicians & Laboratorians
Contact Me = pfhooper@dhh.la.gov
Acknowledgements:
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Ken Pass & The Wadsworth Center
Brad Therrell & The National Newborn Screening & Genetic Resources Center
David Jinks & Illinois Public Health
Alan Bergum – New Jersey Public Health & many other helpful scientists….
All the Members of the Newborn Screening Community Participating in the Survey