Newborn Screening for Galactosemia: A Review of 5 Years of Data and Audit of a Revised Reporting Approach

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BACKGROUND: Availability of the galactose-1-phosphate uridyltransferase (GALT) assay for newborn (NB) screening has improved identification of classic galactosemia. Previously defined critical cutoffs for total galactose (Gal), typically 1.110 mmol/L (20 mg/dL), are still in use in laboratories measuring total Gal for the diagnosis of nonclassic galactosemias. Urgent notification/referral to a treatment center follows, although few of the NBs will need treatment.

METHODS: We reviewed all NB galactosemia-screening results and their corresponding clinical outcomes over a 5-year period (first phase, 1.32 × 10^6 NBs) and then over a 2-year period (second phase, 274,960 NBs). Each NB was screened for Gal and GALT. When Gal was increased and/or GALT was deficient, testing for percentage galactose-1-phosphate and/or DNA testing for common GALT mutations were performed.

RESULTS: Of 209 reported positive results, 89% did not indicate GALT deficiency. These non–GALT-deficient results represented mostly clinically benign cases with a Gal threshold of ≥1.110 mmol/L (≥20 mg/dL). The positive predictive value of a GALT cutoff of ≤40 μmol/L was 83%. After a protocol change that redefined a critical result as a GALT value ≤40 μmol/L and/or a Gal value ≥1.665 mmol/L (≥30 mg/dL), results were monitored for an additional 2 years. The new protocol dramatically reduced the number of urgent calls/referrals and reduced the total number of referrals by nearly half.

CONCLUSIONS: Use of a GALT cutoff of ≤40 μmol/L/L and a Gal cutoff of ≥1.665 mmol/L (≥30 mg/dL) for urgent notification/referral dramatically reduces false positives and unnecessary follow-up, thereby reducing the stress on healthcare resources.

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Newborn (NB) screening for galactosemia is done primarily to detect clinically devastating galactosemia due to defective function of galactose-1-phosphate uridyltransferase (GALT) (1). Increases in blood galactose (Gal) are also observed in other conditions, however: in the relatively rare galactokinase (GALK) deficiency, which can have serious sequelae; in partial GALT deficiency, which has no clinical consequences; and in UDP-galactose-4-epimerase (GALE) deficiency, which has 1 common benign form and 1 extremely rare untreatable form with severe clinical outcomes (2, 3). Additionally, there are other transient galactosemias of unknown cause and other known benign variants that are routinely flagged in NB screening. Recently, Sakurai et al. reported that congenital portal-systemic shunt, rather than hereditary galactosemias, is the most common cause of persistent hypergalactosemia in NB screening in Hiroshima, Japan (4). The Duarte/classic compound heterozygote variant (D/G galactosemia) is often the cause of hypergalactosemia and is frequently flagged as positive in NB-screening programs. The urgent reporting of an increased Gal concentration, with or without GALT deficiency, sets off a chain of events: immediate notification of the Department of Health, primary care physicians, and/or metabolic specialists; bringing the NB in for observation; confirmatory testing; and possible diet change. These events are often nonproductive, wasteful of medical resources, and traumatic for families.

To assess the specificity of galactosemia screening, we compiled NB-screening data in our laboratory from January 1, 2001, to March 1, 2006, for ≥1.3 × 10^6 NBs.
These data included Gal and GALT measurements for all screened NBs. Samples with an increased Gal concentration and/or low GALT activity were also assayed for galactose 1-phosphate (Gal-1-P) and subjected to qualitative DNA testing for 4 common mutations. Whenever possible, clinical outcomes were obtained. The database summarized here for the initial phase of the study was provided as a starting point for a series of discussions in 2006 that were headed by the Pennsylvania Department of Health (PA DOH) and carried out in conjunction with the laboratory and directors of the 2 Pennsylvania galactosemia treatment centers. The goal of the discussions was to analyze these data within the clinical contexts in order to match the reporting of galactosemia with the clinical phenotype. Combining the patterns provided by mining the large amount of data with the clinical experience of the treatment centers suggested changes in the reporting and subsequent revision of some follow-up protocols. For nonclassic galactosemias, our data suggested that the new protocol would produce fewer urgent referrals, conserve time and effort, reduce financial costs, and reduce emotional stress for a substantial number of families. We implemented the recommended reporting and protocol changes in 2006 and have tracked outcomes for 2 years (2007 and 2008). We report our combined results for both phases of the study.

**Materials and Methods**

Whatman 903-type filter papers (previously Schleicher & Schuell 903) were used for all collections and all assays.

**TOTAL Gal**

Total Gal (free Gal plus Gal-1-P) was measured by first extracting a filter paper punch (3/16th in) of a blood spot from each NB. The Gal enzymatic assay uses the Astoria-Pacific automated SPOTCHECK continuous-flow assay system as modified by Hoffman et al. (5). Alkaline phosphatase converted the Gal-1-P in the sample to Gal in the first incubation step. The Gal conversion to galactonolactone by the NADH–coupled Gal dehydrogenase reaction was then monitored by the change in NADH fluorescence. The mean total Gal concentration for a representative Pennsylvania NB population is 0.200 mmol/L (3.6 mg/dL) [nonparametric range of 2 SDs, 0.128–0.483 mmol/L (2.3–8.7 mg/dL)].

**MODIFIED Gal**

Modified Gal was measured in samples with an increased total Gal concentration by repunching the blood spot and reanalysis with the Astoria-Pacific SPOTCHECK system, but without the alkaline phosphatase incubation of the first sample. Subsequent incubation with Gal dehydrogenase yielded NADH fluorescence due to the free Gal in the sample. The Gal-1-P concentration was measured by subtracting the total Gal concentration from the modified Gal concentration. Percentage Gal-1-P was calculated as the Gal-1-P concentration divided by total Gal concentration. The expected Gal-1-P percentage is >25% of the total Gal concentration. If the total Gal concentration is increased, a Gal-1-P value >25% suggests possible GALE deficiency, whereas a Gal-1-P value <25% suggests possible GALK deficiency.

**GALT Analysis**

GALT activity was measured by first extracting a filter paper punch (3/16th in) of a blood spot from each NB. The assay used was the Astoria-Pacific SPOTCHECK continuous-flow assay system as modified by Sturgeon et al. (6). The conversion of UDP-glucose in a series of enzymatic steps to 6-phosphogluconate was coupled to NADP+-NADPH reduction with fluorescence detection. A linear calibration curve was constructed from known NADPH concentrations (in micromoles per liter). Results were expressed in micromoles per liter of fluorescence measured as a direct indication of enzymatic activity. For in-house QC samples, we obtained CVs of 17% for the low-concentration pool (mean, 40 μmol/L) and 12% for the typical-concentration pool (mean, 277 μmol/L). The reference interval for GALT in the NB population is approximately 150–500 μmol/L. A GALT value ≤32 μmol/L is typical in GALT-deficient galactosemia, whereas GALT activity values of 41–120 μmol/L are common with clinically benign mutations. We use “GALT-deficient” throughout this report to denote the laboratory observation of a low enzymatic activity, irrespective of the mutations detected.

**DNA Analysis**

Three classic mutations in the GALT (galactose-1-phosphate uridylyltransferase) gene (Q188R, K285N, and L195P), the S135L mutation, and the Duarte variant (N314D) were detected as previously described (7). DNA analysis was performed for all GALT-deficient samples (i.e., GALT ≤40 μmol/L).

**Criteria for Reporting Positives**

Before September 2006, the PA DOH defined a positive result for non–GALT-deficient galactosemia as a Gal concentration ≥1.110 mmol/L (≥20 mg/dL). The protocol for reporting positive results included immediate notification of the Newborn Screening Program and notification of the primary care physician with a recommendation for immediate referral of the NB to a galactosemia treatment center. Samples with a Gal concentration <1.110 mmol/L (<20 mg/dL) but ≥0.8325 mmol/L (≥15 mg/dL) were reported as “inconclusive” on the next working day, along with a request for a
Reduced Urgent Reporting in Noncritical Galactosemia Cases

repeat collection. The revised reporting protocol was implemented on September 1, 2006. In the revised protocol, only cases with a GALT value ≤ 40 μmol/L or with a Gal value ≥ 1.665 mmol/L (≥ 30 mg/dL) were reported as positive. Cases with a GALT value > 40 μmol/L and a Gal value ≥ 0.8325 mmol/L (≥ 15 mg/dL) but < 1.665 mmol/L (< 30 mg/dL) were reported as inconclusive, along with a request for repeat collection within 72 h. If the NB was on a Gal-free diet before sample collection, he/she was automatically referred to a treatment center. Otherwise, a repeat collection would be made and analyzed. If the repeat Gal value was ≥ 1.110 mmol/L (≥ 20 mg/dL) (or GALT ≤ 40 μmol/L), the second report stated the results as inconclusive and recommended referral of the NB to the treatment center.

**COMPARATIVE AUDIT OF THE NEW REPORTING PROTOCOL**

In phase 2 of this study, we reviewed galactosemia-reporting results for all NBs in Pennsylvania during the years 2005, 2007, and 2008. All cases reported as positive or inconclusive were investigated. Occurrences of false positives/negatives were documented, and the impact of the changed protocol was assessed by comparing the 2007 and 2008 results with those for 2005 (before the changes in reporting and protocol). This study was performed with the approval of the PA DOH Institutional Review Board.

**Results**

In the first phase of this study, we carried out an audit of all positive reports of galactosemias to categorize the laboratory results and then compare them with clinical outcomes. Results were presented to the Pennsylvania Newborn Screening Advisory Committee, and recommendations for changes in reporting were determined. The second phase was a 2-year study (2007-2008) of outcomes that used the revised reporting and follow-up protocol.

Fig. 1 illustrates the distribution of all of our screen-positive samples for the period January 1, 2001, to March 1, 2006, with respect to test results. For the period surveyed, 209 samples had a Gal value ≥ 1.110 mmol/L (≥ 20 mg/dL) and/or a GALT value ≤ 40 μmol/L; 23 of these samples had a GALT activity ≤ 40 μmol/L. Of the 186 screened samples with a GALT value > 40 μmol/L and a Gal concentration ≥ 1.110 mmol/L (≥ 20 mg/dL), 176 had a typical Gal-1-P percentage (> 25%). Twenty of these 176 cases had a total Gal concentration ≥ 1.665 mmol/L (≥ 30 mg/dL), values consistent with possible GALE deficiency, and 10 cases had an abnormally low Gal-1-P percentage (≥ 25%). Only 1 of these cases had a Gal value > 1.665 mmol/L (> 30 mg/dL), which is consistent with possible GALK deficiency.

Table 1 summarizes the screening and DNA results for the 23 cases screened between January 1, 2001, and March 1, 2006, that had GALT values ≤ 40 μmol/L. In 14 cases, 2 copies of DNA mutations responsible for GALT deficiency were identified (11 homozygotes, 3 compound heterozygotes). In 4 cases, only 1 DNA mutation was identified (heterozygous G; all individuals with clinical diagnoses of GALT-deficient galactosemia). Case no. 4 was a compound heterozygote with an unidentified exon 10 variant. Case no. 10 was a confirmed case of classic disease that featured no detected mutational markers but did have an apparent partial gene deletion at a location remote from the mutation-probe sites tested. Three cases were D/G heterozygotes, and 1 sample had no detectable mutations (confirmed as classic disease by the symptoms).

Table 2 summarizes the 20 cases in our initial 5-year study that had Gal concentrations ≥ 1.665 mmol/L (≥ 30 mg/dL), GALT values > 40 μmol/L, and Gal-1-P values > 25% (possible GALE deficiency). The cases are grouped by the race of the NB to highlight the observation that the presence of a Duarte mutation in 6 cases was exclusively associated with Caucasian NBs, all with GALT values below the reference interval. For the African American NBs, GALE deficiency was diagnosed in 5 cases. In 2 cases, no remarkable abnormality was noted in the NB period, and in 7 cases (all non-
Caucasian), no further follow-up was recorded. Except for the 6 cases with the Duarte mutation, all NBs with Gal concentrations >1.665 mmol/L (30 mg/dL) and GALT values >40 μmol/L were non-Caucasian.

The analysis of the data in Tables 1 and 2 led to recommendations (see Discussion) for a revised Gal cutoff of 1.665 mmol/L (30 mg/dL) for reporting non–GALT-deficient positives and for revised follow-up protocols for positive and inconclusive screening results (Fig. 2). In the revised protocol, positive screens would require urgent notification and referral to the treatment center, with full dietary restrictions and evaluation/confirmatory testing. Inconclusives (next-day calls) would not require urgent notification or dietary restrictions until after rescreening, and referral would occur only if the rescreening results were abnormal. The new cutoff and revised protocols were then implemented.

Table 3 compares the galactosemia-screening outcomes obtained in this study’s second phase for all NB screens in Pennsylvania for the revised protocol (2007 and 2008) with the screening outcomes obtained with the previous protocol (2005). The total number of NBs increased only slightly, whereas the number of positives requiring urgent calls and referral decreased from 30 cases in 2005 to 5 cases in 2007 and 2 cases in 2008. Inconclusives (next-day calls) remained relatively constant, whereas the number of reported second samples with increased values rose from 10 in 2005 to 18 in 2007 and 20 in 2008, a result that reflects the higher number of cases initially called as inconclusive that continued to have increased Gal values.

**Discussion**

The screening data for galactosemia presented in Fig. 1 reveal that only 13% of all positive cases reported during the period 2001–2006 were associated with a GALT value ≤40 μmol/L. A review of all cases with GALT values ≤40 μmol/L (Table 1) provides the following

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gal, mmol/L</th>
<th>GALT, μmol/L</th>
<th>DNA</th>
<th>Notes</th>
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<td>1</td>
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<td>16</td>
<td>Cpd het</td>
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<td></td>
</tr>
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<td>1.30</td>
<td>38.3</td>
<td>Het D/G</td>
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* To convert Gal concentrations to milligrams per deciliter, divide by 0.0555.

b Cpd het, 2 different GALT mutations (compound heterozygote); Homo G, 2 GALT mutations (homozygous); Het G, 1 copy of GALT mutation, other allele unidentified; Het D/G, Duarte/GALT mutation heterozygotes; WT, wild type (no mutations detected).
observations: We found no GALT-deficient galactosemia when the GALT value was \( \geq 40 \) \( \text{mol/L} \). On the basis of either DNA or clinical confirmation, we found 19 cases of GALT-deficient galactosemia, a frequency of approximately 1 in 68,400. The sensitivity for detecting GALT deficiency was 100% with the GALT cutoff of \( \geq 40 \) \( \text{mol/L} \); the positive predictive value with this cutoff was 83% (19 of 23 cases). No GALT-deficient cases were missed.

Three cases reported as positive with GALT values \( \geq 40 \) \( \text{mol/L} \) were heterozygous D/G. The vast majority of NBs who are D/G heterozygotes have GALT values in the interval of 41–150 \( \text{mol/L} \) (data not shown) and typically show improvement in GALT activity in the weeks after initial screening, as with the NB in case no. 9 in Table 1. GALT DNA analysis is ancillary to screening and potentially a diagnostic aid. When mutations are found, such analysis is most useful for differentiating clinically true GALT deficiency from D/G galactosemia for the purpose of treatment. Treatment of D/G galactosemia is controversial; however, Ficicioglu et al. recently reported that long-term clinical and

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**Table 2.** NBs with Gal values \( \geq 1.665 \) mmol/L (\( \geq 30.0 \) mg/dL) and GALT values \( \geq 40 \) mmol/L.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gal, mmol/L*</th>
<th>GALT, ( \mu \text{mol/L} )</th>
<th>Gal-1-P</th>
<th>Race</th>
<th>Clinical</th>
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<td>A</td>
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</tr>
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<td>A</td>
<td>FNA</td>
</tr>
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<td>B</td>
<td>FNA</td>
</tr>
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<td>FNA</td>
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<td>11</td>
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<td>289</td>
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<td>B</td>
<td>Epimerase deficiency (profound)</td>
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<td>1.80</td>
<td>118</td>
<td>N</td>
<td>O</td>
<td>FNA</td>
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</tbody>
</table>

* To convert Gal concentrations to milligrams per deciliter, divide by 0.0555.

* N, within reference interval; A, Asian; FNA, further follow-up data not available; B, African American; C, Caucasian; Het D/G, Duarte/GALT mutation heterozygote; Het D, Duarte mutation only detected; O, other.

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**Fig. 2.** Flow diagram for evaluating NBs for galactosemia with a GALT cutoff value \( \leq 40 \) \( \mu \text{mol/L} \) for reporting GALT-deficient galactosemia and a Gal cutoff \( \geq 1.665 \) mmol/L (\( \geq 30 \) mg/dL) for urgent reporting of other types of galactosemia.

WRI, within reference interval.

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developmental outcomes in children with D/G galactosemia are good, regardless of any diet changes in the first year of life, and that there was no relationship between clinical/developmental outcomes and the concentrations of erythrocyte Gal-1-P and urine galactitol (8). The GALT cutoff of 40 μmol/L is sufficiently low to exclude most compound heterozygote D/G cases without missing true GALT-deficient galactosemias.

Of the 19 confirmed GALT-deficient galactosemias, we identified 2 GALT mutations in 14 cases (11 homozygotes, 3 compound heterozygotes), and 1 mutation in 4 cases. In 1 case, none of the mutations we tested for were present. With the 4 mutations tested, we expect to confirm 70%–80% of the mutations responsible for GALT deficiency with DNA analysis.

The vast majority (87%) of screen positives for galactosemia in the 5-year study were not GALT deficient. GALT-deficient galactosemias are expected to represent 95% of all true clinically important cases; that is, the frequencies of GALK deficiencies and clinically important GALE deficiencies are small by comparison. Therefore, few of these 186 cases are likely to represent clinically important cases of either of these deficiencies. By determining the Gal-1-P percentage in samples with Gal concentrations ≥1.100 mmol/L (≥20 mg/dL), we were able to segregate these 186 cases into those with possible GALK deficiency (Gal-1-P <25%) and those with a possible GALE deficiency (Gal-1-P ≥25%). We identified 10 cases of possible GALK deficiencies, of which only 1 case (GALT, 299 μmol/L; total Gal, 3,996 mmol/L (72 mg/dL]) was a confirmed positive. Early differentiation of possible GALK-deficient NBs from possible GALE-deficient NBs with the Gal-1-P percentage is an important additional step in guiding clinical decision-making in these cases.

The remaining 176 cases had results consistent with GALE deficiency. To investigate the possible GALE deficiencies, we carried out a follow-up of the subset of the 176 cases that had total Gal values ≥1665 mmol/L (≥30 mg/dL) (n = 20; see Table 2). The cases are grouped by race to highlight that all of the cases with a Duarte mutation were Caucasian NBs and that all of the remaining cases were non-Caucasian NBs. As expected, NBs who are Duarte heterozygotes or compound heterozygotes often have GALT activities less than the lower limit of 2 SDs (150 μmol/L). All of the confirmed GALE deficiencies (including 1 profound deficiency) were African American NBs. Benign GALE deficiency has been reported to occur with a frequency of approximately 1 in 6200 African Americans and 1 in 64,000 Caucasians (9). On the basis of these numbers, benign GALE deficiency should occur with a frequency comparable to that of classic galactosemia in our population mix. Our identification of only 6 cases may be partially attributed to an inability to obtain final diagnoses for some of the NBs listed in Table 2. This result also suggests that some NBs with benign GALE deficiencies have Gal values <1.665 mmol/L (<30 mg/dL).

In March 2006, the second phase of the study was formulated. We presented our data in a conference call with representatives from the laboratory, the PA DOH Newborn Screening Program, and galactosemia treatment centers. We reviewed the numbers of cases and their apparent clinical classifications and requested input from the treatment center directors in assessing the appropriateness of the classifications, in defining the degree of urgency of notification and treatment for each category from their perspective, and in providing us with input on the treatment protocols.

The clinical input regarding urgency of reporting and treatment was central to our final recommendations. The focus of the discussion was primarily on the 156 cases among the group with possible GALE deficiency that had been called as positive and referred to galactosemia treatment centers. The treatment center directors commented that in their experience true GALK and GALE deficiencies are associated with very high Gal values, usually >3.330 mmol/L (>60 mg/dL). They proposed that a screening cutoff of 1.665 mmol/L (30 mg/dL) was a reasonably conservative value for detecting clinically important GALE or GALK deficiency. They further proposed that all NBs with Gal values <1.665 mmol/L (<30 mg/dL) and GALT values >40 μmol/L be considered as nonurgent cases from a notification/treatment perspective. A repeat collection would be sufficient follow-up in these cases, with a persistent abnormal Gal result in the second sample being the signal for referral to a treatment center. One caveat was that any NB with a Gal value ≥0.8325 mmol/L (≥15 mg/dL) who had been on Gal-free formula before sample collection would be automatically referred to a treatment center.

### Table 3. Outcomes comparison: new protocol vs previous protocol.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total NBs, n</th>
<th>Positives (urgent calls), n</th>
<th>Inconclusive (next-day call), n*</th>
<th>Increased Gal, second samples (delayed referral), n</th>
<th>Total referrals, n</th>
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<td>2008</td>
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* GALT > 40 μmol/L; 0.8325 mmol/L (15 mg/dL) ≤ Gal < 1.665 mmol/L (30 mg/dL).
With 1.665 mmol/L (30 mg/dL) as a Gal cutoff, 165 NBs in our first-phase study (156 possible GALE cases plus 9 possible GALK-deficiency cases) with Gal values <1.665 mmol/L (<30 mg/dL) could have been reported as inconclusive (unless the NB was on a Gal-free diet before collection). Urgent calls with urgent referral to a galactosemia treatment center could have been avoided in these cases.

We developed a flow diagram that reflects the suggested changes (Fig. 2). This flow diagram consolidates all samples with GALT values ≤40 μmol/L and Gal values ≥1.665 mmol/L (≥30 mg/dL) under positive reporting and consolidates all samples with GALT values >40 μmol/L and Gal values ≥0.8325 mmol/L (≥15 mg/dL) but <1.665 mmol/L (<30 mg/dL) as inconclusive. These suggested changes in definitions for positive and inconclusive classification were matched with the proposed revisions in clinical protocols for each classification: A positive screening result would be defined as a GALT-deficient galactosemia or as a Gal value ≥1.665 mmol/L (≥30 mg/dL) and would require urgent notification, referral to a treatment center, full dietary restriction, and appropriate confirmatory testing. An inconclusive screening result would be defined as an initial GALT value >40 μmol/L and a Gal concentration ≥83.25 mmol/L (15 mg/dL) but <1.665 mmol/L (30 mg/dL) and would not require urgent notification or dietary restriction, but it would require a repeat collection. Referral would then occur only if the NB was on a Gal-free diet before the first collection or if the result of the repeat screen was abnormal. Also included in Fig. 2 is the decision point for the NB-screening counselors to contact the primary care provider in the inconclusive arm to determine if the NB had been on a Gal-free diet before collection of the blood spot. If so, the NB would then be referred immediately to a treatment center.

Table 3 demonstrates that the number of positives (urgent calls) in Pennsylvania decreased substantially from 2005 to 2007 and 2008. Of the 7 reported positives in 2007 and 2008, 3 were GALT-deficient galactosemias, 2 were GALE deficiencies, 1 was a D/G compound heterozygote variant, and 1 was lost to follow-up. For 2008, one of the 2 reported positives was GALT deficient. The other was identified as having 1 copy of a GALT mutation, but this NB was lost to follow-up. An audit of positive urgent calls in 2005 showed that of the 30 reported positives, 5 were true positives (2 GALT cases, 2 GALE cases, and 1 GALK-deficiency case), 7 cases were D/G galactosemia, 12 were ultimately diagnosed as galactosemia negative, 4 were carriers, and 2 were lost to follow-up. Clearly, unnecessary urgent calls on NBs with increased Gal values were eliminated by the protocol change.

The number of NBs with increased Gal values in the second sample (delayed referrals) nearly doubled from 2005 to 2007 and 2008 because of the classification of NBs as inconclusive who would have previously been reported as positive [i.e., Gal values ≥1.110 mmol/L (≥20 mg/dL) and GALT values >40 μmol/L]. Nevertheless, the total number of referrals has been substantially reduced because of the normalization of Gal results with repeat evaluation in many cases.

Eighteen cases in 2007 and 22 cases in 2008 were initially called as inconclusive but had abnormal results in repeat screens. Such cases are delayed referrals under the revised protocol. A review of the final diagnoses in the 2007 cases revealed 8 classic variants (3 D/G heterozygotes, 3 single-copy heterozygotes, 2 Duarte carriers), 1 GALE deficiency, 1 GALE carrier, and 8 cases negative for galactosemia. In 2008, there were 10 variants (5 D/G compound heterozygotes, 3 single-copy heterozygotes, 1 Duarte homozygote, 1 unidentified), 9 cases negative for galactosemia, and 2 cases with a final diagnosis pending. Thus, the repeat screening allowed appropriate identification and referral of cases with persistent Gal increases.

In summary, urgent referrals with the revised protocol during the years 2007 and 2008 were reduced to 5 and 2 cases, respectively, compared with 30 cases in 2005. This dramatic reduction was entirely due to reclassification of urgent reporting for nonclassic galactosemia cases with GALT values >40 μmol/L and Gal values ≥1.110 mmol/L (≥20 mg/dL) but <1.665 mmol/L (≤30 mg/dL) from positive to inconclusive. Total referrals were reduced from 41 in 2005 to 23 in 2007 and 24 in 2008. This reduction was due to normalization of Gal results in repeat testing of the reclassified cases. The reduction in referrals represents a substantial savings in terms of the emotional stress on parents, the NB being spared from unnecessary referral, and the costs to the healthcare system.

**Conclusion**

The primary goal of NB screening for galactosemia is to identify GALT-deficient galactosemia. Semiquantitative GALT analysis with an appropriate cutoff identifies these cases with few false positives. When NB screening includes Gal quantification, the vast majority of reported galactosemias in NBs are nonclassic, however, and these NBs rarely require urgent treatment. The traditional Gal cutoff for galactosemia [1.110 mmol/L (20 mg/dL)] is not optimal for urgent follow-up if the GALT value is >40 μmol/L and leads to unnecessary urgent notification and initiation of treatment protocols, which can be avoided with selective monitoring of second samples. By combining the accumulated data for comprehensive galactosemia testing with the clini-
cal expertise of metabolic specialists in the treatment
centers, the PA DOH Newborn Screening Program was
able to review and improve follow-up protocols for
nonclassic galactosemia, thereby saving money, time,
and stress on an already overburdened system.

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acquisition of data, or analysis and interpretation of data; (b) drafting
or revising the article for intellectual content; and (c) final approval of
the published article.

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References

1. Cuthbert C, Klapper H, Elias L. Diagnosis of inher-
ited disorders of galactose metabolism. Curr Protoc
Hum Genet 2008;56:17.5.1–29.
2. Bosch AM, Bakker HD, van Gennip AH, van Kem-
pen JV, Wanders RJ, Wijburg FA. Clinical features
of galactokinase deficiency: a review of the liter-
3. Openo KK, Schulz JM, Vargas CA, Orton CS, Ep-
stein MP, Schnur RE, et al. Epimerase-deficiency
galactosemia is not a binary condition. Am J Hum
Genet 2006;78:89–102.
4. Sakura N, Mizoguchi N, Ono H, Nishimura Y, Naito
K. Congenital porto-system shunt as the major
5. Hoffman GL, Laessig RH, Hassemer DJ, Makowski
ER. Dual-channel continuous-flow system for de-
termination of phenylalanine and galactose; appli-
cation to newborn screening. Clin Chem 1984;30:
287–90.
method for screening galactosemia. In: Technicon
7. Dobrowolski SF, Banas RA, Suzow JS, Berkley M,
Naylor EW. Analysis of common mutations in the
galactose-1-phosphate uridyl transferase gene:
new assays to increase the sensitivity and speci-
ficity of newborn screening for galactosemia. J Mol
8. Ficicioglu C, Thomas N, Yager C, Gallagher PR,
pilot study of biochemical and neurodevelopmen-
tal assessment in children detected by newborn
9. Alano A, Almashanu S, Chinsky JM, Costeas P,
Blitzer MG, Wulfberg EA, Cowan TM. Molecular
characterization of a unique patient with
epimerase-deficiency galactosaemia, J Inherit