HHS Public Access

Author manuscript

*Clin Biochem*. Author manuscript; available in PMC 2016 April 01.

Published in final edited form as:

*Clin Biochem*. 2015 April ; 48(6): 437–442. doi:10.1016/j.clinbiochem.2014.12.009.

# Galactose-1-phosphate Uridyltransferase Dried Blood Spot Quality Control Materials for Newborn Screening Tests

**Barbara W. Adam**[a], **Sharon R. Flores**[a], **Yu Hou**[b], **Todd W. Allen**[c], and **Victor R. De Jesus**[a]

[a]Centers for Disease Control and Prevention (CDC), 4770 Buford Highway NE, Atlanta, GA 30341, USA

[b]Genetic Disease Laboratory Branch, California Department of Public Health, 850 Marina Bay Pkwy, Richmond, CA 94804, USA

[c]Astoria-Pacific, Inc., 15130 SE 82nd Drive, P.O. Box 830, Clackamas, OR 97015, USA

## Abstract

**Objectives—**We aimed to prepare dried-blood-spot (DBS) quality control (QC) materials for galactose-1-phosphate uridyltransferase (GALT), to evaluate their stability during storage and use, and to evaluate their performance in five DBS GALT test methods.

**Design and Methods—**We prepared and characterized GALT-normal and GALT-deficient DBS materials and compared GALT activities in DBSs after predetermined storage intervals at controlled temperatures and humidities. External evaluators documented the suitability of the DBS QC materials for use in five GALT test methods.

**Results—**GALT activity losses from DBSs stored in low (<30%) humidity for 14 days at 45°C, 35 days at 37°C, 91 days at room temperature, 182 days at 4°C, and 367 days at −20°C were 54%, 53%, 52% 23%, and 7% respectively. In paired DBSs stored in high humidity (>50%) for identical intervals, losses were: 45°C—68%; 37°C—79%; room temperature—72%, and 4°C—63%. GALT activities in DBSs stored at 4°C were stable throughout 19 excursions to room temperature. Twenty-five of 26 external evaluators, using five different GALT test methods, classified the GALT-deficient DBSs as “outside normal limits”. All evaluators classified the GALT-normal DBSs as “within normal limits”.

**Conclusions—**Most of the GALT activity loss from DBSs stored at elevated or room temperature was attributable to the effects of storage temperature. Most of the loss from DBSs stored at 4°C was attributable to the effects of elevated humidity. Loss from DBSs stored at −20°C was insignificant. The DBS materials were suitable for monitoring performance of all five GALT test methods.

## INTRODUCTION

Galactosemia is a rare inborn metabolic condition with a calculated incidence of 1:53,261 in the United States (US) [1]. Galactose is formed by enzymatic hydrolysis of lactose (milk sugar) and is converted to glucose by a series of enzymatic reactions. Although a deficiency in any one of three enzymes involved in the conversion of galactose to glucose—galactokinase, galactose-1- phosphate uridyltransferase (GALT), or galactose-4’-epimerase—can lead to galactosemia, GALT deficiency is the most common [2].

GALT deficiency patients usually show no signs of galactosemia at birth, but after ingestion of lactose, most present in the neonatal period with a life-threatening illness characterized by food intolerance, vomiting, diarrhea, jaundice, enlarged liver and spleen, lethargy, and muscle hypotonia. Early treatment by removal of all galactose from the diet is lifesaving [2, 3].

In the US, dried-blood-spot (DBS) samples obtained from heel pricks are collected from more than 98% of all newborns [4] and used in screening tests for treatable inborn disorders. GALT deficiency is one of the core disorders in the US recommended uniform screening panel [5, 6]. All US state newborn screening programs include GALT-deficiency tests in their newborn screening panels [7], and all US state, regional, and state-associated contract newborn screening laboratories participate voluntarily in the GALT component of the Newborn Screening Quality Assurance Program (NSQAP) of the Centers for Disease Control and Prevention (CDC). In addition to domestic laboratories, newborn screening laboratories in more than 70 foreign countries participate in NSQAP [8].

NSQAP routinely conducts research for development of unique DBS quality control (QC) materials that assist laboratories with monitoring performance of their newborn screening tests. NSQAP collaborated with the Genetic Disease Laboratory Branch of the California Department of Public Health to develop DBS QC materials for monitoring the performance of GALT screening tests and to classify these materials as GALT-normal or GALT-deficient.

NSQAP QC materials must be suitable for all screening tests that newborn screening laboratories use. All US screening laboratories use one of five GALT assays: in-house Beutler-Baluda based qualitative GALT tests [9]; one of two GALT kits from PerkinElmer Life and Analytical Sciences—the Neonatal GALT kit or the GSP Neonatal GALT kit—or one of two Astoria-Pacific International SPOTCHECK GALT kits—the Uridyltransferase 50 hour Reagent Kit or the Neonatal GALT Microplate Reagent Kit. NSQAP used PerkinElmer kits to evaluate the performance of NSQAP DBS materials by those methods and collaborated with Astoria-Pacific to evaluate the performance of NSQAP candidate GALT QC materials using the SPOTCHECK kits. The candidate QC materials were also evaluated by 26 newborn screening laboratories selected to include all five GALT test methods used in the US. The materials were not evaluated by test methods that are used by NSQAP participants in other countries but are not available in the US.

In this report, procedures for production of GALT-normal, GALT-intermediate, and GALT-deficient DBS QC materials are described. GALT activities in DBS materials stored at predetermined temperatures and humidities for preselected intervals are compared to illustrate the effects of exposure to heat, humidity, and temperature-fluctuations on the stability of GALT activity in DBSs. Analytic results are presented to show that the NSQAP's DBS candidate QC materials give comparable performance in all newborn screening GALT tests that US laboratories use. This information is intended to guide laboratories in the storage and use of DBS materials for routine QC of their GALT screening tests.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of paired GALT-normal and GALT-deficient whole blood portions

Freshly collected ABO-compatible units of packed red blood cells and serum were purchased from a blood bank (Tennessee Blood Services). The red cell units were combined and washed three times with 0.9% sterile saline solution (www.fishersci.com) before adjustment to a hematocrit of ≥95% by aspiration of the final saline wash. The serum units were combined and clarified by serial filtration through disposable sterilization units of pore sizes 0.45 and 0.22 μm (Nalgene), diluted 1:1 with 0.9% saline solution, and used to reconstitute the packed red cells to a hematocrit of 50±1%. After thorough mixing, half of the blood batch was transferred to overnight storage at 4°C.

The remaining half of the batch was transferred to a volumetric flask, submerged in a 53.5°C water bath, and continuously mixed with a mechanical stirring arm for two hours to inactivate its GALT content. The flask of heat-treated blood was placed in an ice-water bath and stirred to reduce the blood temperature to less than 20°C before transfer to overnight storage at 4°C.

This procedure was carried out twice to prepare two independent batches of paired GALT-normal and GALT-deficient blood portions. The first batch was used to create two sets of DBS dose-response materials for internal and external evaluations of candidate blood matrixes for DBS QC materials. The second batch was used to prepare a 3-member set of DBS GALT QC materials.

### 2.2. Preparation of DBS dose-response materials

Two sets of dose-response materials were made by combining pre-determined amounts of paired GALT-normal and GALT-deficient (heat-treated) blood portions and then dispensing these blood mixtures as 100 μL spots onto sheets of horizontally suspended Grade 903 (GE Healthcare Biosciences) filter paper. Before preparation of each set of dose-response materials, the GALT-normal and GALT-deficient blood portions were brought to room temperature (20°–25°C) and filtered through gauze to remove any clots that might have formed during storage. All blood portions were gently stirred before and during dispensing from handheld automated pipettes. The blood spots were dried overnight under ambient conditions before the sheets of DBSs were separated by sheets of weighing paper (www.fishersci.com) and placed in Bitran Series S liquid-tight zip-closure specimen bags (www.fishersci.com) with desiccant packets (polylam.com) and 30–50% humidity indicator cards (www.desiccare.com) to ensure that storage humidity was maintained below 30%. The sealed bags of DBS materials were stored at −20°C to await analysis and characterization.

The initial set of DBS dose-response materials (Set A) was made by combining aliquots of GALT-normal and GALT-deficient blood to produce intact-cell blood portions containing 100%, 50%, 40%, 30% and 0% GALT-normal blood and using these portions to produce the DBS materials. Immediately after production of Set A dose-response materials, the residual 100% GALT-normal and 100% GALT-deficient blood portions were stored for two weeks at −20°C to hemolyze the erythrocytes. After hemolysis, aliquots of the GALT-normal and GALT-deficient blood were combined to produce lysed-cell blood portions containing

100%, 50%, 40%, 30%, 20%, 10% and 0% GALT-normal blood. These portions were used to produce Set B dose-response materials.

### 2.3. Production of DBS GALT QC materials

The second batch of paired whole blood portions was brought to room temperature, filtered through gauze and used to create a 3-member set of blood spot QC materials consisting of a GALT-normal member, a GALT-deficient member, and an intermediate member made from a 30:70 combination of the GALT-normal and GALT-deficient blood. The blood portions were stirred gently before and while a multi-channel liquid handling system dispensed them in 100 μL aliquots onto horizontally suspended sheets of Grade 903 filter paper. The sheets of blood spots were dried overnight under ambient conditions before being separated with weighing paper, packaged in zip-closure bags with desiccant and humidity indicator cards, and stored at −20°C.

### 2.4. Measurements of GALT activity in DBS materials

NSQAP used Neonatal GALT Kit (PerkinElmer Life and Analytical Sciences) to measure GALT activity of all DBS materials. The Genetic Disease Laboratory Branch of the California Department of Public Health used Astoria-Pacific Uridyltransferase 50 hour Reagent Kits to validate GALT activities of the 3-member set of DBS QC materials. Astoria-Pacific International used both SPOTCHECK kit tests—the Uridyltransferase 50 hour Reagent Kit and the Neonatal GALT Microplate Reagent Kit—to evaluate the suitability of the DBS dose-response materials and the 3-member set of DBS QC materials for use in those tests. For simplicity, only NSQAP's analytic results are reported here.

### 2.5 Characterization of GALT levels and validation of homogeneity of the DBS materials

Duplicate analyses of the first, middle and last sheets of each level of the two sets of DBS dose-response materials (intact and lysed red cells) were performed on each of 5 days. The acquired data were used in previously described statistical procedures [10] for estimating initial marker level in small DBS production batches.

Characterization data from analyses of the 3-member set of DBS GALT QC lots were subjected to the statistical protocols NSQAP routinely uses for determining the homogeneity of marker distribution and initial marker level in large DBS production batches. To assess the homogeneity of every 621-sheet DBS QC production lot, 12 DBS punches were taken from each of six sheets (sheets 1, 10, 100, 300, 500 and 621) and analyzed in a single run. Evaluations of each homogeneity data set were based on the ratio of among- and within-sheet variability from a one-way random effects analysis of variance. Homogeneity was deemed sufficient as long as the upper limit of the confidence interval for the ratio was less than 3 [11]. To estimate the average initial GALT activities of the three lots of DBS QC materials, two DBS disks from each lot were analyzed in each of 20 runs. The cumulative results were subjected to multi-rule Shewhart control analysis according to NSQAP's institutional guidelines for duplicate analyses of three different QC lots per run [12].

### 2.6 Degradation studies design

Accelerated degradation studies of GALT activity were performed to measure separately the contributions of storage temperature and humidity to changes in levels of GALT activity in DBS samples. Members of each degradation study sample set were stored for pre-determined intervals under prescribed test conditions before removal to optimal storage (−70°C and <30% humidity) to await analysis. The Day 0 member of each DBS sample set, in a zip-closure bag with relative humidity maintained below 30%, remained at −70°C storage throughout the study to serve as a storage stability control for its sample set.

Paired sets of identical DBSs, taken from the 100% GALT-normal member of the intact-cell dose-response materials, were stored at each of four preselected temperatures—45°C, 37°C, room temperature, and 4°C—in Bitran Series S storage bags containing cards that indicated by color-change humidities below 30% (blue) or above 50% (pink). One DBS of each pair was stored with humidity controlled to below 30% by enclosing desiccant packets with the DBSs and zip-sealing the bags before storage. The other DBS of each pair was stored without desiccant in an open Bitran bag in a high-humidity chamber which was monitored by periodic hygrometer readings to maintain relative humidity above 90%. Additionally, a single sample set was stored at −20°C and low humidity. At predetermined intervals throughout the degradation studies, DBSs from all sample sets were transferred to optimal storage at −70°C. Immediately before this transfer, desiccant packets were added to the bags containing DBSs that had been stored at high humidity, and those bags were zip-sealed to ensure that the DBSs were stored at optimal humidity. The GALT activity levels of all samples in each complete sample set were measured in triplicate in a single analytical run.

Geometric means of triplicate measurements of initial (Day 0) GALT activity and GALT activity remaining on the last day of each degradation study were used to determine the percentage of the initial GALT activity level that was lost during the study. GALT losses from DBSs stored at low humidity were attributed to the effects of the elevated storage temperature. The percentage loss of activity from each sample set stored at low humidity was subtracted from the percentage loss of activity sustained by its paired sample set stored at high humidity, and the difference was attributed to the effects of elevated storage humidity.

### 2.7 Stability of GALT activity during the “active life” of DBS QC materials

Typically, supplies of NSQAP DBS QC materials for routinely monitoring the performance of laboratory tests are stored at 4°C and brought to room temperature each time DBS disks are punched for analysis. Reserve supplies of the DBS QC materials are stored at −20°C and brought to room temperature at infrequent intervals to replenish the supplies of QC materials for routine use. To estimate the effect of temperature fluctuations on GALT activity, DBS materials were stored at 4°C or −20°C and subjected to repeated brief excursions to room temperature. The sheets of DBS materials for each of these studies were from a single GALT-normal DBS production batch and were stored in zip-closure bags with humidity controlled to below 30%. At pre-determined intervals throughout month-long studies, the sets of stored DBS materials were transferred to room temperature for 30 to 60 minutes before the storage bags were opened and DBSs from each storage study sample set were

harvested. After each DBS harvest, the remaining portions of the DBS sheets were replaced in their zip-closure storage bags and returned immediately to their respective storage study temperatures. The harvested DBSs from each sample set were protected with weighing paper and packaged in zip-closure bags containing desiccant packets and a humidity indicator card. These bags were zip-sealed and immediately transferred to storage at −70°C to await analysis. The GALT activity levels of all samples in each complete sample set were measured in triplicate in a single analytical run. Geometric means of triplicate GALT activity measurements were used to assess the effects of temperature fluctuations on GALT activity in DBS QC materials.

### 2.8. External evaluation of NSQAP's DBS dose-response materials by all GALT methods that US newborn screening laboratories use

Identical 15-member panels of DBS materials were prepared from the two sets of DBS GALT dose-response materials and DBS samples from a GALT-normal blood donor, a GALT-deficient blood donor, and a previously distributed proficiency testing specimen from NSQAP's DBS library. These panels were analyzed by laboratories that used the Astoria-Pacific 50 Hour Reagent Kit (6 laboratories), Astoria-Pacific Neonatal Microplate Reagent Kit (4 laboratories), Beutler-type non-kit methods (5 laboratories), PerkinElmer Neonatal Kit (5 laboratories), and PerkinElmer GSP Neonatal Kit (6 laboratories). All laboratories reported clinical classifications of the samples.

## 3. RESULTS

### 3.1. Characterization of dose-response materials

GALT activities in both sets of DBS dose-response materials were proportional to the percentage of GALT-normal blood contained in the whole blood matrixes of the pools (Table 1). Average initial GALT activities of all dose-response pools that contained at least 30% GALT-normal blood were above the 3.0 enzyme activity units/gram hemoglobin (U/g Hb) cutoff value used by NSQAP to classify specimens as presumptive positive or negative for GALT deficiency. The average GALT activities of dose-response materials made from blood that had been frozen to hemolyze the red cells were about 10% lower than the average activities of the corresponding materials made from freshly prepared blood with intact red cells.

### 3.2 Characterization of initial GALT levels and validation of homogeneity of GALT distribution in the 3-member set of DBS QC materials

The distribution of GALT activity in each of the three lots that comprised the set of DBS QC materials was found to be homogenous. (Among-card variance of every lot was less than its within-card variance.) The average GALT activities of the three QC lots and the standard deviations and 95% confidence limits around those averages were: GALT-normal lot—8.2 ± 0.9 U/g Hb (6.4–10.0 U/g Hb); GALT-intermediate lot—3.5 ± 0.3 U/g Hb (2.9–4.1 U/g Hb); and GALT-deficient lot—1.5 ± 0.1 U/g Hb (1.3–1.7 U/g Hb).

### 3.3 Stabilities of GALT in blood spots stored at low and high humidities for predetermined intervals at preselected temperatures

The losses of GALT activity after each storage interval at five temperatures (45°C, 37°C, room temperature, 4°C, and −20°C) are shown in Figure 1 as the remaining percentages of their respective initial (Day 0) activities. All paired DBS samples stored at low relative humidity retained more of their initial GALT activity than did their paired samples stored at high humidity. Losses of GALT activity that occurred during low humidity storage (and were attributed to effects of the storage temperatures) were subtracted from the losses that occurred during high humidity storage to find the percentage of losses attributable to the effects of high storage humidity. Losses attributable to high relative humidity ranged from 14% loss from DBSs stored at 45°C to 40% loss from samples stored at 4°C (Table 2).

### 3.4 Stability of GALT activity during the “active life” of DBS QC materials

Each point in Figure 2 represents the geometric mean of triplicate measurements of GALT activity in DBS QC materials that were stored at 4°C and subjected to 0–23 excursions to room temperature (Figure 2A) or were stored at −20°C and subjected to 0–14 excursions to room temperature (Figure 2B). During a month-long study, DBSs stored at 4°C showed a decline in GALT activity after the 19$^{th}$ excursion to room temperature. DBSs stored at −20°C showed no decline in GALT activity after the 14 excursions to room temperature that comprised the freeze/thaw study.

### 3.5. External evaluation of candidate GALT QC materials

All 26 participants in the external evaluation reported qualitative (clinical) assessments of “within normal limits” or “outside normal limits” for each of the 15 samples in the evaluation test panel (Table 3). Twenty-five of 26 participants classified the presumptive GALT-deficient sample in the Set A dose-response materials (made from intact-cell blood) as “outside normal limits”; all participants classified the presumptive GALT-deficient sample in dose-response materials Set B (made from hemolyzed blood) as “outside normal limits”. All participants classified the presumptive GALT-normal samples (made from 100% GALT-normal blood) in both sets of dose-response materials as “within normal limits”.

## 4. DISCUSSION

NSQAP routinely conducts research for development of unique DBS QC materials that assist laboratories with monitoring the performance of their newborn screening tests. Development of QC materials for DBS GALT tests was challenging because: 1) GALT is associated with red blood cells and GALT-deficient red cells are not available commercially; 2) DBS samples must be stored under carefully controlled conditions to avoid loss of GALT activity [13]; 3) the DBS QC materials must be suitable for use in all GALT tests that newborn screening laboratories use; and 4) GALT reporting units for quantitative data are not standardized among all GALT test methods.

The Genetic Disease Laboratory Branch of the California Department of Public Health implemented the innovative use of a 1:1 serum:saline solution to reconstitute packed red cells to 50% hematocrit before heat-treatment to reduce GALT activity. This innovation

reduced non-specific background fluorescence of the blood matrix thus enabling production of DBS QC materials that were suitable for monitoring the performance of their GALT tests. NSQAP followed California's protocol for preparation of the blood matrix but used a more rigorous heat-treatment to produce GALT-deficient DBS QC materials with lower GALT activities than were desired by the California laboratory.

Historically, NSQAP QC materials for newborn screening tests have been made from blood with hemolyzed red cells. For historical reference, the lysed-cell blood matrix for GALT QC materials was evaluated. The lower GALT activities found in DBS materials made from the lysed-cell blood are consistent with the finding that the serum absorption volumes of disks punched from DBSs made from lysed cell blood are lower than those of identical punches taken from DBSs made from intact-cell blood [16]. Although both the intact-cell and the lysed-cell blood matrixes produced potentially acceptable GALT-normal and GALT-deficient candidate DBS QC materials, the higher GALT activity of the intact-cell blood matrix made it the more attractive choice for use in the DBS GALT QC materials.

In addition to GALT-normal and GALT-deficient QC materials, the NSQAP set of materials also contained an intermediate control for monitoring the performance of DBS tests near the GALT activity cutoff level used to classify test results as presumptive positive or negative for GALT deficiency. The mean GALT activities, standard deviations and confidence limits that were derived from the NSQAP characterizations of the three DBS GALT QC materials are for reference only. Each laboratory participating in the NSQAP must use its GALT test method to establish its own mean values and confidence limits for the QC materials [17].

Understanding the susceptibilities in DBS samples to temperature related and moisture related degradation is important for maintaining sample integrity for high quality measurements. Accelerated degradation studies were used to measure the contributions of temperature and humidity to the degradation of GALT activity in stored DBS materials. Declines in enzyme activity increased as storage temperature increased from 4°C to 45°C. Although exposure to elevated humidity accelerated losses of GALT activity during studies carried out at 45°C, 37°C and room temperature, most of these enzyme activity losses were attributable to the effects of exposure to the storage temperatures. In contrast, most of the loss of GALT activity in DBS stored at 4°C was attributable to the effects of exposure to elevated humidity. GALT stability in DBSs stored at −20°C was only studied at low humidity because of difficulty in controlling humidity in the frozen state. The findings that GALT lost substantial activity during storage at elevated temperatures and low or high humidity are consistent with a previously reported controlled study of GALT stability [13] and with the observation that population means of GALT activities in newborn screening samples were more than 20% lower in summer than in winter [18].

GALT activity in QC materials used to monitor assay performance must be stable. The GALT activity in NSQAP DBS QC materials stored at 4°C declined after 19 excursions from 4°C to room temperature. This finding suggests that NSQAP GALT QC materials stored at 4°C for routine use in laboratories with 5-day work-weeks should be replaced at 3-week intervals to maintain sample integrity.

Clinical classifications reported by participants in the external evaluations of dose-response materials made from 100% GALT-normal blood were concordant. One evaluator that used a qualitative method classified a 100% GALT-deficient intact-cell DBS material as “within normal limits”; all others classified it as “outside normal limits”. The members of the sets of dose-response materials for which clinical assessments were most evenly divided between positive and negative classifications were presumed to be the members with GALT activity closest to newborn screening programs’ cutoff values—i.e., they were presumed to be candidate intermediate controls. In dose-response materials made from blood with intact red cells, the presumptive-borderline intermediate control contained 30% GALT-normal blood. Because the GALT DBS QC materials were also made from blood with intact red cells, 30% GALT-normal blood was used to prepare an intermediate DBS QC material that was targeted to the cutoff value.

Building a reliable QC system for monitoring the performance of newborn screening tests is critical for understanding and controlling the variables that may influence the outcomes of these tests. Implementation of a robust analytic QC system contributes to confidence in the newborn screening test results. The special studies reported here provide essential QC production and storage guidelines to scientists contemplating production of in-house DBS QC materials for use with non-kit GALT tests or for adding intermediate controls to supplement GALT-normal and GALT-deficient DBS QC materials provided by GALT kit producers. The NSQAP QC materials for DBS GALT tests can strengthen existing QC systems by providing a secondary level of control to transcend production lots of primary QC materials and monitor long-term assay performance.

## ACKNOWLEDGEMENTS

The authors thank the state, regional, and state-associated contract newborn screening laboratories that participated in evaluations of NSQAP candidate DBS QC materials in 2012 and 2013.

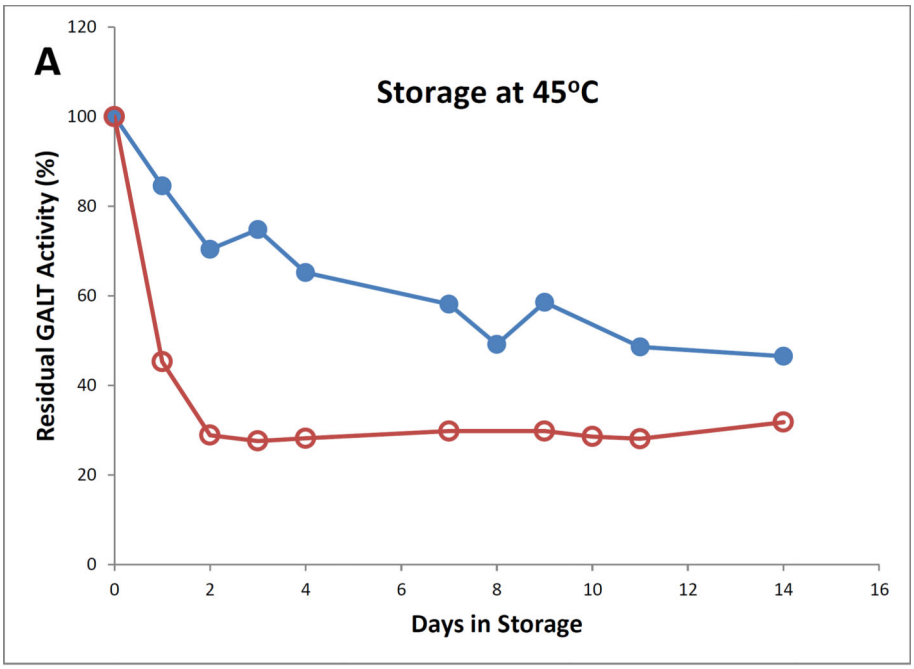
The use of trade names and commercial sources is for identification only and does not imply endorsement by the US Department of Health and Human Services.

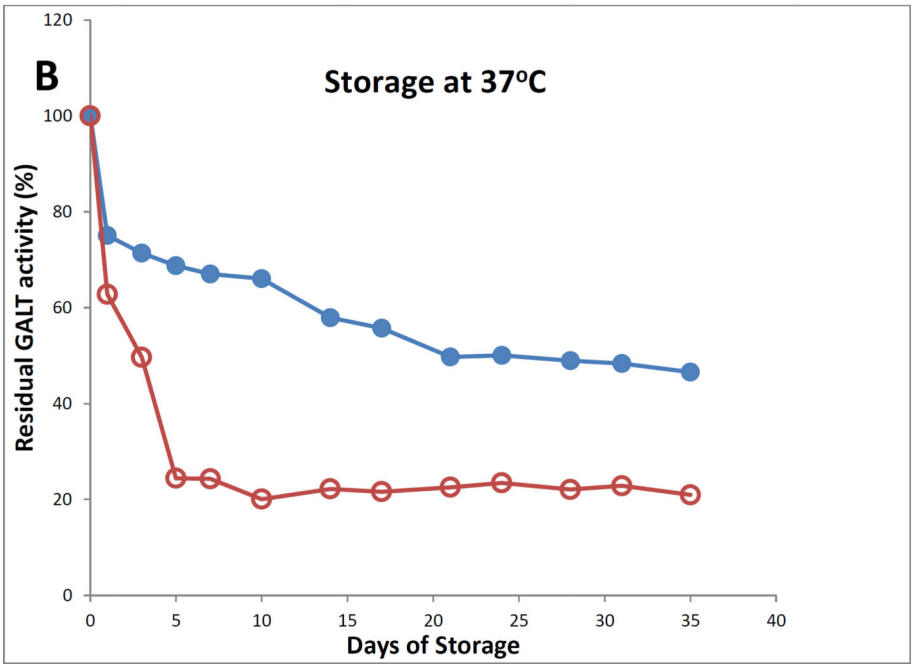
The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

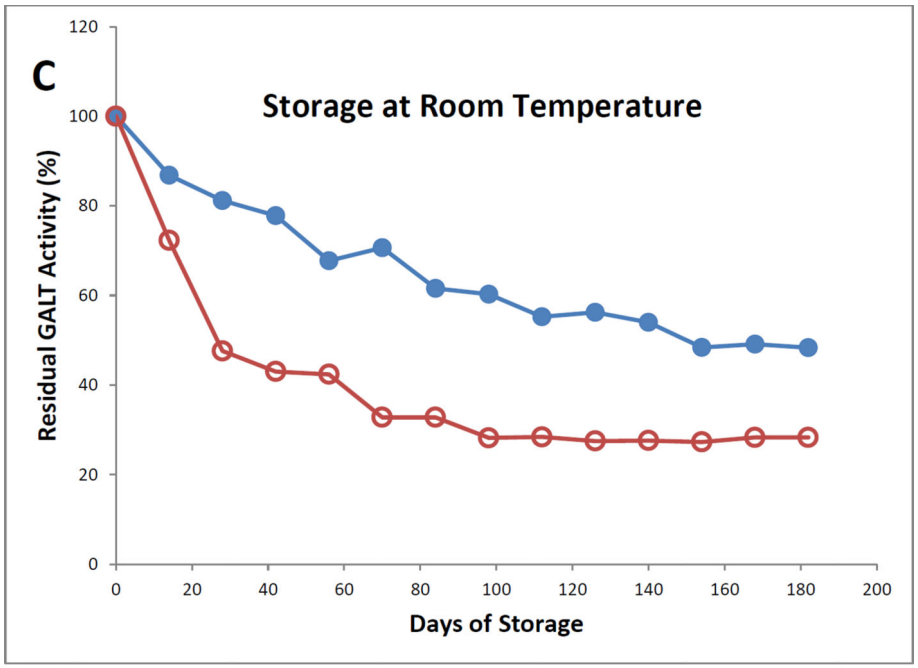
## REFERENCES

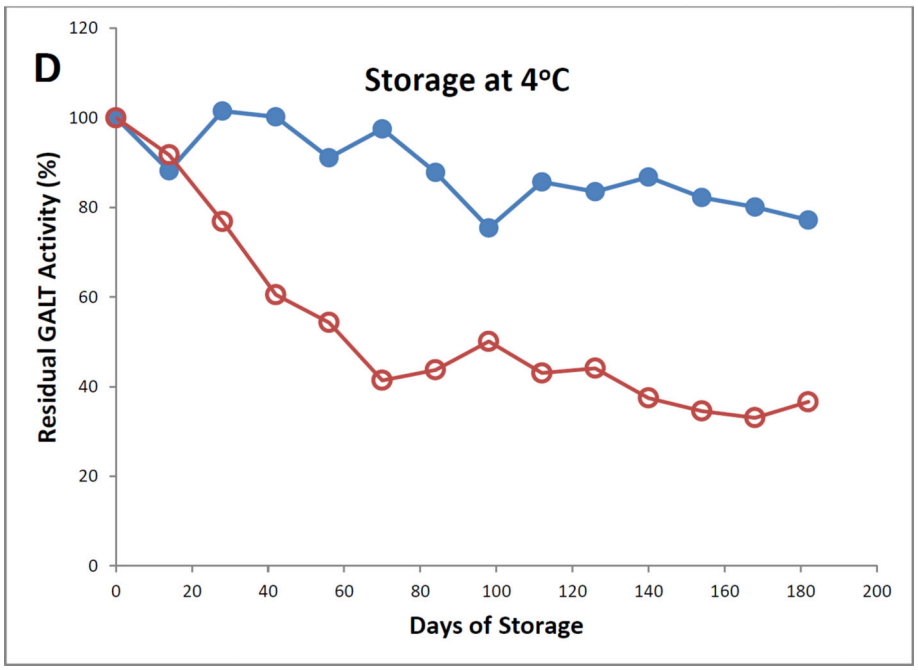
1. Therrell BL, Hannon WH. MRDD Research Reviews. 2006; 12:236–245.
2. Kaye CI. the Committee on Genetics. Newborn Screening Fact Sheet. Pediatrics. 2006; 118:e934–e963. [PubMed: 16950973]
3. Bosch AM. Classical galactosemia revisited. J Inherit Metab Dis. 2006; 29:516–525. [PubMed: 16838075]
4. Bell, CJ. Newborn Screening Quality Assurance Program 2010 annual summary report. Bell, CJ., editor. Atlanta GA: Centers for Disease Control and Prevention; 2011.
5. Watson MS, Mann MY, Lloyd-Puryear MA, Rinaldo P, Howell RR. Newborn screening: toward a uniform screening panel and system–executive summary. Pediatrics. 2006; 117S:S296–S307.
6. Newborn screening: toward a uniform screening panel and system. Genet Med. 2006; 8(5):S12–S252. as authored by the American College of Medical Genetics (ACMG) and commissioned by the Health Resources and Services Administration (HRSA).
7. [Accessed September 30, 2014] Conditions Screened by State. http://www.babysfirsttest.org/newborn-screening/states

8. Zobel, S. Newborn Screening Quality Assurance Program 2013 annual Summary Report. Zobel, S., editor. Atlanta, GA: Centers for Disease Control and Prevention; 2014.
9. Beutler E, Baluda MC. A simple spot screening test for galactosemia. J Lab Clin Med. 1965; 68:137–141. [PubMed: 4380286]
10. Adam BW, Chafin DL, De Jesus VR. Stabilities of hemoglobins A S in dried blood spots stored under controlled conditions. Clin Biochem. 2013; 46:1089–1092. [PubMed: 23701845]
11. Fearn T, Thompson M. A new test for 'sufficient homogeneity'. Analyst. 2001; 126:1414–1417. [PubMed: 11534616]
12. Caudill SP, Schleicher RL, Pirkle JL. Multi-rule quality control for the age-related eye disease study. Statist Med. 2008; 27:4094–4106.
13. Adam BW, Hall EM, Sternberg M, Lim TH, Flores SR, O'Brien S, Simms D, Li LX, De Jesus VR, Hannon WH. The stability of markers in dried-blood spots for recommended newborn screening disorders in the United States. Clin Biochem. 2011; 44:1445–1450. [PubMed: 21963384]
14. Flores, SK.; Adam, BW.; Cuthbert, CD.; De Jesus, VR. Laboratory update on the preparation of simulated galactose-1-phosphate uridyltransferase-deficient quality control dried blood spot material; Proceedings of the 2013 Newborn Screening and Genetic Testing Symposium; 2013 May 5–10; Atlanta (GA). Association of Public Health Laboratories; 2013.
15. Beutler E. Galactosemia Screening and Diagnosis. Clin Biochem. 1991; 24:293–300. [PubMed: 1959220]
16. CLSI. Blood Collection on Filter Paper for Newborn Screening Programs: Approved Standard. 6th ed. Wayne, PA: CLSI; 2013.
17. Slazyk, WE.; Hannon, WH. Quality Assurance in the newborn screening laboratory. In: Therrell, BL., Jr, editor. Laboratory Methods for Neonatal Screening. Washington (DC): American Public Health Association; 1993. p. 23-46.
18. Freer DE. Observations on heat/humidity denaturation of enzymes in filter paper blood spots from newborns. Clin Chem. 2005; 51:1060–1062. [PubMed: 15845800]





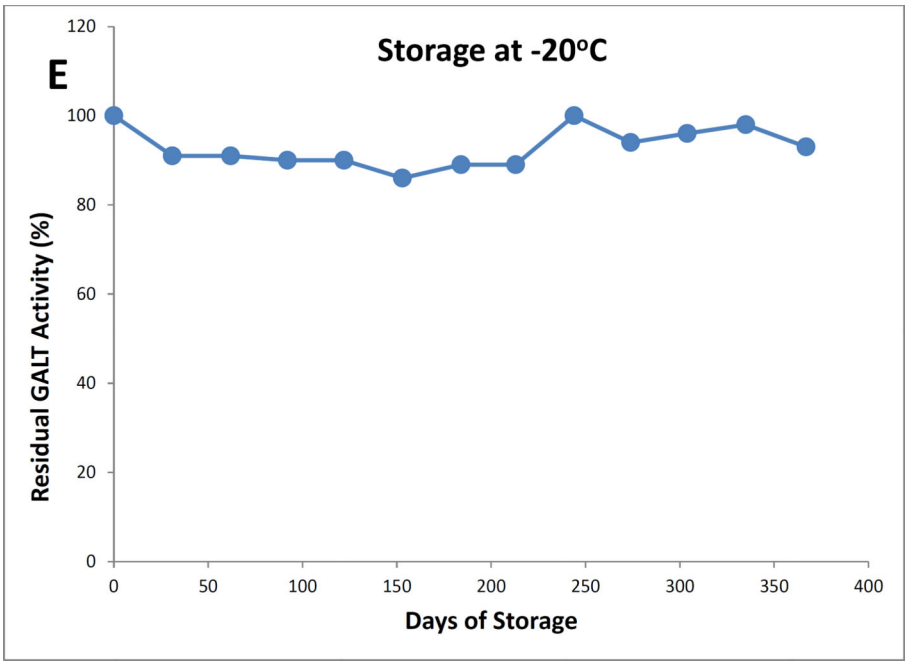



**Figure 1.**

Recoveries of galactose-1-phosphate uridyltransferase (GALT) from dried blood spots stored at controlled temperatures for predetermined intervals in low-humidity and high-humidity environments. Samples stored at −20°C were stored at low humidity only. ● = low humidity ○ = high humidity

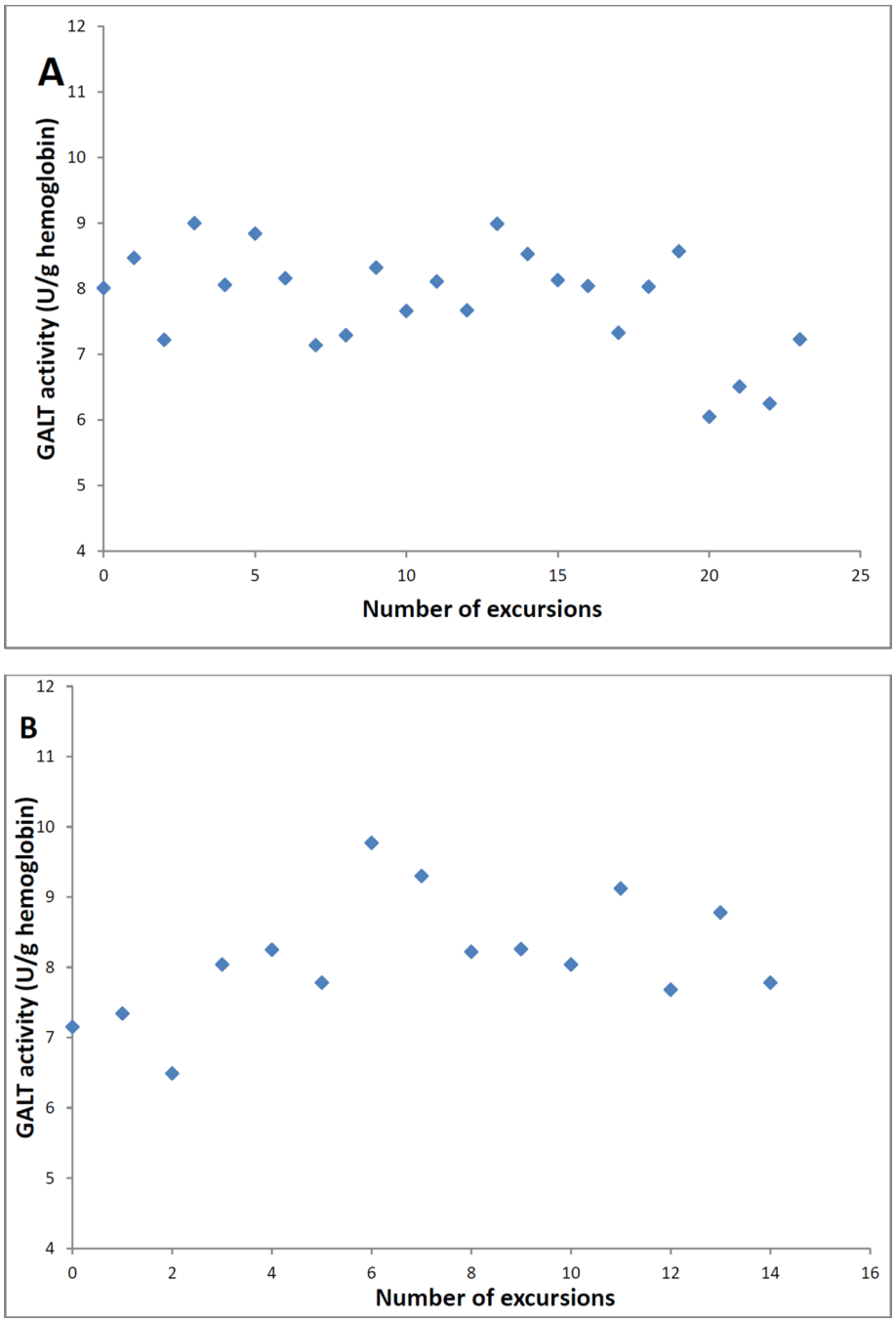



**Figure 2.**

Galactose-1-phosphate uridyltransferase (GALT) activities recovered from dried blood spots stored with relative humidity controlled to less than 30% and subjected to repeated excursions to room temperature from (A) 4°C or (B) −20°C

**TABLE 1**

Summary of Galt Dose-Response Curve Characterization Data

| GALT-normal blood (%) | MEAN[A] | | STANDARD DEVIATION[A] | | CV (%) | |
|---|---|---|---|---|---|---|
| | Intact[B] | Lysed[C] | Intact[B] | Lysed[C] | Intact[B] | Lysed[C] |
| 0 | 1.6 | 1.7 | 0.16 | 0.08 | 9.6 | 4.8 |
| 10 | ***[D] | 2.1 | *** | 0.15 | *** | 7.0 |
| 20 | *** | 2.6 | *** | 0.14 | *** | 5.1 |
| 30 | 3.8 | 3.4 | 0.26 | 0.28 | 7.0 | 8.1 |
| 40 | 4.6 | 4.2 | 0.35 | 0.22 | 7.7 | 5.3 |
| 50 | 5.1 | 4.7 | 0.40 | 0.38 | 7.7 | 8.1 |
| 100 | 8.1 | 7.6 | 0.77 | 0.47 | 9.6 | 6.1 |

[A] GALT activities are shown as U/g Hb.

[B] Dose-response materials made from blood with intact red cells.

[C] Dose-response materials made from blood with lysed red cells.

[D] Asterisks (***) indicate pools not included in the intact-cell set of dose-response materials.

## Table 2

Percentages[A] of galactose-1-phosphate uridyltransferase (GALT) activity lost from dried blood spots stored in low-humidity and high-humidity environments[B]

| Storage temperature | Study length | Loss during storage at high humidity | Loss during storage at low humidity | Loss attributable to to effects of high humidity |
|---|---|---|---|---|
| (°C) | (days) | (%) | (%) | (%)[C] |
| 45 | 14 | 68 | 54 | 14 |
| 37 | 35 | 79 | 53 | 26 |
| RT[D] | 91 | 72 | 52 | 20 |
| 4 | 182 | 63 | 23 | 40 |

[A] Percentages shown were derived from geometric means of triplicate determinations.

[B] Low relative humidity <30%; high relative humidity >50%.

[C] Derived by subtracting loss during storage at low humidity from loss during storage at high humidity

[D] RT=room temperature

## TABLE 3

Descriptions and Clinical Classifications of Candidate DBS Galt QC Materials

| Sample Number | Sample Description | GALT-normal | GALT-deficient | Clinical classifications | |
|---|---|---|---|---|---|
| | | | | WNL[F] | ONL[G] |
| 1 | Dose-response Set A[A] | 100% | 0% | 26 | 0 |
| 2 | Dose-response Set A | 50% | 50% | 22 | 4 |
| 3 | Dose-response Set A | 40% | 60% | 17 | 9 |
| 4 | Dose-response Set A | 30% | 70% | 14 | 12 |
| 5 | Dose-response Set A | 0% | 100% | 1 | 25 |
| 6 | Positive control [B] | NA | NA | 1 | 25 |
| 7 | Dose-response Set B[C] | 100% | 0% | 26 | 0 |
| 8 | Dose-response Set B | 50% | 50% | 17 | 9 |
| 9 | Dose-response Set B | 40% | 60% | 14 | 12 |
| 10 | Dose-response Set B | 30% | 70% | 8 | 18 |
| 11 | Dose-response Set B | 20% | 80% | 4 | 22 |
| 12 | Dose-response Set B | 10% | 90% | 1 | 25 |
| 13 | Dose-response Set B | 0% | 100% | 0 | 26 |
| 14 | Blind QC sample[D] | NA | NA | 26 | 0 |
| 15 | Negative control[E] | NA | NA | 26 | 0 |

[A] Made from blood with intact red cells

[B] GALT-deficient patient sample

[C] Made from blood with hemolyzed red cells

[D] An archived GALT-normal proficiency testing specimen

[E] A GALT-normal patient sample

[F] WNL = within normal limits

[G] ONL = outside normal limits