DRG

DRG® Galactose ELISA (EIA-1487)

CE

Revised 12 July 2006 (Vers. 1.1) **IVD**

INTENDED USE

The DRG Galactose Elisa is designed for the quantitative determination of galactose in neonatal blood spots. It is for in-vitro diagnostic use as an aid in screening newborns for elevated galactose levels as seen in Galactosemia.

CLINICAL PHYSIOLOGY

Galactose is a hexose sugar absorbed from the intestine. Under normal conditions, it is liberated by the digestion of lactose and is converted to glucose as well as fructose in the body (1). In a rare hereditary syndrome known as classical Galactosemia, there is a genetic defect in the conversion of galactose to glucose. This condition is due to an inherited deficiency of galactose-1-phosphate uridyl transferase (G-1-PUT) and affects approximately 1 in 40,000 newborns (2). G-1-PUT is responsible for the conversion of galactose-1-phosphate (G-1-P) to glucose-1-phosphate, and ultimately to glucose (3). Galactose and G-1-P accumulate in the blood and urine of affected individuals (4). Additionally, there are two separate disorders of galactose metabolism of clinical importance, galactokinase deficiency and uridine diphosphate galactose-4-epimerase (UDP) deficiency. Galactokinase deficiency mainly causes cataracts which regress without complications provided a galactose-free diet is started soon after birth, and UDP deficiency appears extremely rare. Both deficiencies result in elevated galactose and G-1-P in blood of affected individuals.

Classical Galactosemia, left untreated, is characterized by enlargement and subsequent damage to the liver and brain, cataract formation, and failure to thrive in infants (5,6). Affected infants may die in the neonatal period due to *Escherichia coli* sepsis, or later due to cirrhosis of the liver (7). This is a result of the toxicity of galactose and its metabolites, which accumulate in the body. Management of these patients is dependent upon the elimination of dietary galactose and lactose, which results in striking regression of all symptoms and signs (6,8). The value of early detection of Galactosemia has been clearly established by the fact that early therapy minimizes or obviates the deleterious effects of the disorder. Despite apparent improvement in galactose tolerance with age, strict adherence to galactose-free diet has been advocated throughout adulthood. Treatment for the clinically significant deficiencies of transferase and galactokinase consists of a diet as free of lactose as possible, obtained by the exclusion of milk and milk products (9). If begun within the first four or five days of life, clinical manifestations are almost always prevented (6).

Newborn screening for Galactosemia allows determination of galactose and G-1-P in plasma or whole blood during the first days after birth. Prompt recognition of this disorder and institution of treatment are essential in saving lives and in preventing the development of cataracts and mental retardation (10). Several methods have been instituted since the early 1960's to screen Galactosemia. These include a bacterial inhibition assay similar to that used for PKU screening (10, 11), as well as those based on metabolite accumulation (galactose/G-1-P) or measurement of transferase activity (11, 12-16). Additionally, galactose dehydrogenase and alkaline phosphatase have been used in measurements of galactose and G-1-P in neonatal blood spots for screening. The measurements of these metabolites (result read as total galactose) will detect all three galactose disorders. The diagnosis of a classic galactosemic patient is confirmed by direct assay of G-1-PUT activity, which is low or absent. The DRG® Galactose EIA is an enzyme assay, which follows an acid extraction of the galactose, contained in a 3/16" blood spot. A simple color reaction occurs demonstrating rapid and quantitative determination of galactose levels in neonates.

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CLINICAL APPLICATIONS

Newborn Screening: The DRG® Galactose EIA is designed as an aid in screening newborns for Galactosemia. Presumptive positive results should be confirmed by analysis with another method.

FACTORS AFFECTING NORMAL VALUES

Newborn Age: In patients affected with Galactosemia, galactose concentrations rise in the hours after birth. To clearly differentiate affected from unaffected individuals, it is recommended that sampling should occur 24-48 hours after birth.

Newborn Diet: A diet containing lactose, such as breast milk or formula, is required for galactose accumulation and facilitates identification of Galactosemia in newborns.

PRINCIPLE OF TEST

DRG International, Inc. has developed a rapid enzyme assay for the quantitation of total galactose in whole blood spotted on filter paper. In this assay, a blood spot is extracted with TCA Extraction Solution in a special semi-porous microwell plate. Following extraction, all 96 extracts are simultaneously transferred to a conventional microwell plate by means of a special vacuum manifold. A neutralizing buffer is added to each acid extract, followed by addition of a combination enzyme-substrate reagent. This rapidly oxidizes the galactose to galactonolactone, reducing NAD to NADH* in the process. The colorless substance is oxidized to a colored end-product with an absorbance maximum at 570 nm. The absorbance read is directly proportional to the amount of galactose in the sample in whole blood units. The DRG® Galactose EIA is simple to perform, rapid and easily automated. The basic assay is diagramed below:

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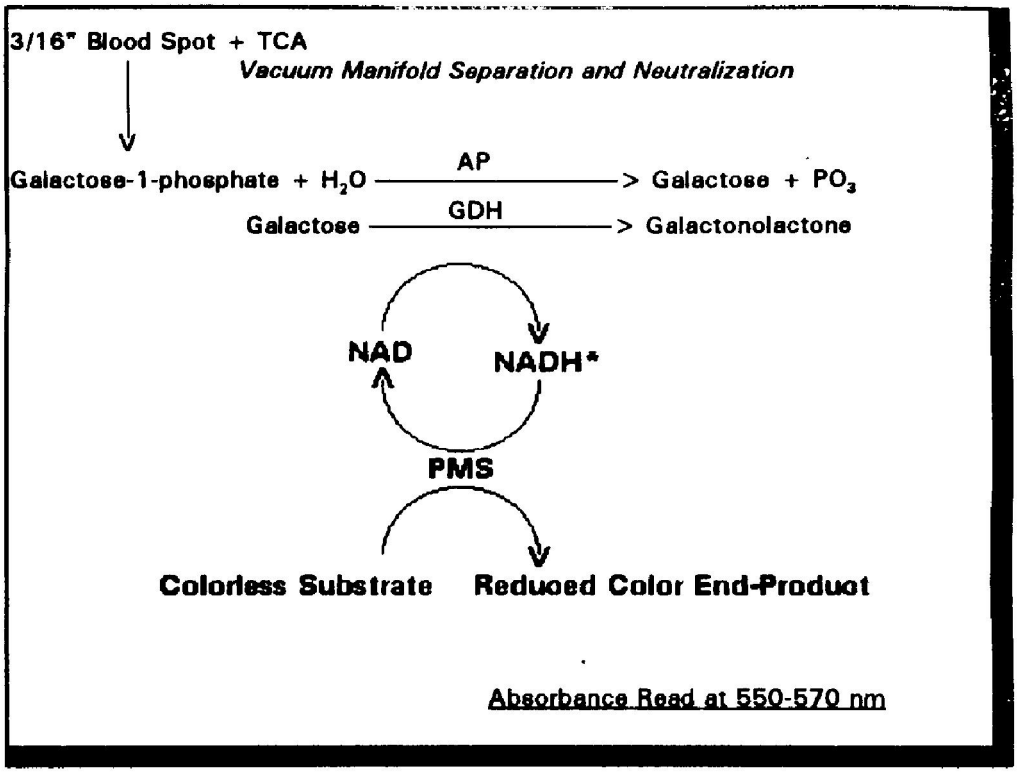


REAGENT AMOUNT AND LABEL COLOR CODE: (2 or 10 plate kit)

Component	Label Color	Volume or Quantity	
		Single Kit	Bulk Kit
Membrane-Transfer Plate	Tan	2 plate	10 plates
Collection-Reaction Plate	Tan	2 plate	10 plates
PHE/GAL Blood Spot Standards	Green	1 card	3 cards
PHE/GAL Tri-Level Blood Spot Controls	Green	1 card	3 cards
TCA Extraction Solution	Yellow	22 mL	110 mL
Galactose Neutralizing Solution	Yellow	11 mL	55 mL
Reagent A	Red	11 mL	55 mL
Galactose Reagent B	Green	11 mL	55 mL

REAGENTS DESCRIPTION AND PREPARATION

These reagents contain sodium azide, which has a tendency to build up in lead or copper plumbing forming potentially explosive metal azides. Always flush large quantities of water through the plumbing after the disposal of these reagents.

A. Membrane-Transfer Plate
96-well porous microwell plates.
Storage: Room temperature or at 2-8°C with kit
Stability: Refer to expiration date on plastic bag.

B. Collection-Reaction Plate
96-wall uncoated microwell plates.
Storage: Room temperature or at 2-8°C with kit
Stability: Refer to expiration date on plastic bag.

C. PHE/GAL Blood Spot Standards
Five standards are provided. These standards are prepared in a bovine whole blood matrix and are spotted on Schleicher and Schuell Filter Paper #903. Refer to foil package for concentration.
Storage: 2-8°C. For long-term storage greater than 7 days, store below –15°C. Reseal bag after use.
Stability: Refer to expiration date on foil bag.

D. PHE/GAL Blood Spot Controls
Three controls are provided. These controls are prepared in a bovine whole blood matrix and are spotted on Schleicher and Schuell Filter Paper #903. Refer to foil package for concentration.
Storage: 2-8°C. For long-term storage greater than 7 days, store below -15°C. Reseal bag after use.
Stability: Refer to expiration date on foil bag.

E. TCA Extraction Solution
Trichloroacetic acid (TCA) in deionized water is provided in ready-to-use form.
Storage: 2-8°C
Stability: Refer to expiration date on kit vial.

F. Galactose Neutralizing Solution
Alkaline phosphatase and bovine serum albumin in TRIS buffer is provided in ready-to-use form.
Storage: 2-8°C
Stability: Refer to expiration date on kit vial.

G. Reagent A
Substrate buffer with sodium azide as preservative is provided.
Storage: 2-8°C. For long-term storage greater than 7 days, store below -15°C.
Stability: Refer to expiration date on kit vial.

H. Galactose Reagent B
NAD and β-galactose dehydrogenase (GDH) in buffer with bovine calf serum and sodium azide as preservative is provided. GDH is a *Pseudomonas fluorescens* bacteria from *Esherichia coli.* The enzyme contains no material of human, animal or plant origin.
Storage: 2-8°C. For long-term storage greater than 7 days, store below 15°C.

Stability: Refer to expiration date on kit vial.

LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

A. The reagents supplied in this kit are for *In-Vitro Diagnostic Use Only.*
B. Strict adherence to the protocol is advised to obtain reliable results. Any modifications made to the reagents or assay procedures are the responsibility of the user.
C. A standard curve must be established for each run. A run should consist of a maximum of two consecutive plates. For larger assays, the timing of pipetting should be lagged to ensure uniform plate processing.
D. This assay is designed to be used with samples, which are exclusively collected on Schleicher and Schuell (S&S) Filter Paper #903. Changes in filter paper lots may affect patient results. All laboratories should record lot numbers of S&S #903 filter paper to monitor possible changes.
E. The DRG® Galactose EIA is to be used as an initial screening test for the quantitation of galactose levels in whole blood spots. Individual screening laboratories are responsible to further test those patients with increased galactose levels using documented tests for Galactosemia.
F. Several variables not directly related to serum levels of galactose may affect the determined value. These include hematocrit, moisture content of the paper, rate of blood deposition, and lot of filter paper utilized. For these reasons, uniform collection techniques coupled with careful examination of the blood spots before analysis is recommended.
G. Samples from neonates known to be exposed to tetracycline antibiotics may be slightly elevated and should be retested by another method (non-bacterial assay).
H. Extrapolated values outside the range of calibrators (see foil packet for concentration) are approximate and should be reported as a "value less than low standard" or a "value greater than high standard".
I. Blood spot cards must be checked for visual inconsistencies prior to performing analysis. The blood volume spotted on card must be enough to have completely saturated the circle printed on the filter paper and must be completely dried. Torn or otherwise disrupted filter paper is not acceptable, nor are caked clotted specimens.

SPECIMEN COLLECTION AND HANDLING

Infant screening programs may differ from one another in the amount of sample required. For neonatal screening, a sample collected from a 5/8" diameter blood spot preprinted on Schleicher and Schuell Filter Paper #903 obtained from a heel stick is suggested. The following summary is described in detail in NCCLS publication LA4-T.

A. Collect the blood from the heel of an infant usually 24-72 hours postpartum. Sampling times may vary from center to center.
B. Wash the heel with soap and water and wipe dry. Swab area with alcohol and allow to air dry.
C. Puncture infant's heel with a sterile lancet with a tip no longer than 2.5 mm and wipe away first drop of blood. Allow another drop of blood of adequate volume to form, and gently touch the specimen card to the droplet in the center of the Schleicher and Schuells Filter Paper #903 card. The blood volume must be enough to completely fill at least two circles on filter paper. View the card from the opposite side as the blood penetrates the filter paper. Avoid excessive squeezing of the heel as it may cause hemolysis and dilute the sample. Avoid tearing or disruption of the filter paper surface.

D. Place the filter paper card horizontally on a clean surface and allow to air dry for at least 6 hours at room temperature. Avoid direct sunlight.
E. Be sure the required information on the specimen card has been completed, including the infant's name, time and date of birth, and time and date of collection. Also indicate pre- or post-term, the infant's weight, and whether or not the infant was a twin, or other multiple birth.
F. Place each specimen in its own paper envelope and transport to the laboratory within 24 hours of drying.
G. The receiving laboratory should store the sample at 2-8°C in a moisture-proof environment shielded from direct light.
H. Galactose has been reported to be stable in blood spots stored for one year. However, not all components are as stable, and neonatal blood spot storage must be considered on an analyte-by-analyte basis.

EQUIPMENT AND REAGENTS REQUIRED

In addition to the materials provided with this kit, the following supplies are required:

A. A 3/16" or 1/8" diameter paper punch.
B. Plate reader able to read absorbency at 550-570 nm.
C. Multi- and single-channel micropipettes calibrated to 50-100 µL.
D. Rotary horizontal shaker.
E. Vacuum manifold. (Requires a standard hose bench top vacuum outlet or equivalent eternal vacuum source).

ASSAY PROCEDURE

A. Assay Preparation

1. Prior to beginning assay, label each collection-reaction plate to match its complementary membrane plate. Additionally, each laboratory should check the time for complete transfer of extract to the collection plate and use that time for extract transfer if greater than 30 seconds.
2. Following the neutralization step, quantitatively transfer the contents of Galactose Reagent B vial to Reagent A vial and mix well. Transfer mixture between vials A and B a few times to ensure adequate mixing. If less than 22.0 mL of Reagent A-B is needed per assay, mix equal volumes of Reagent A and Reagent B for desired volume. Reagent A-B is to be used immediately after preparation. Do not store or re-use.

B. Assay Steps

1. For the five (5) STANDARDS, three (3) CONTROLS, and variable unknowns: Punch one 3/16" (or two 1/8") blood spot, into the appropriate wells of the membrane transfer plate. Be sure to leave an empty well for the reagent blank.
2. Add 100 µL of TCA Extraction Solution to each well.
3. Rotate mix for ***60 minutes*** at room temperature.
4. Simultaneously transfer acid extracts to the empty collection-reaction plate using the vacuum manifold.
5. Add 50 µL of Neutralizing Solution to each well and shake gently by hand for 10 seconds.
6. Incubate for ***60 minutes*** at room temperature.
7. Add 100 µL of Reagent A-B to each well and shake plate gently by hand for 10 seconds.
8. Incubate ***30 minutes*** at room temperature.

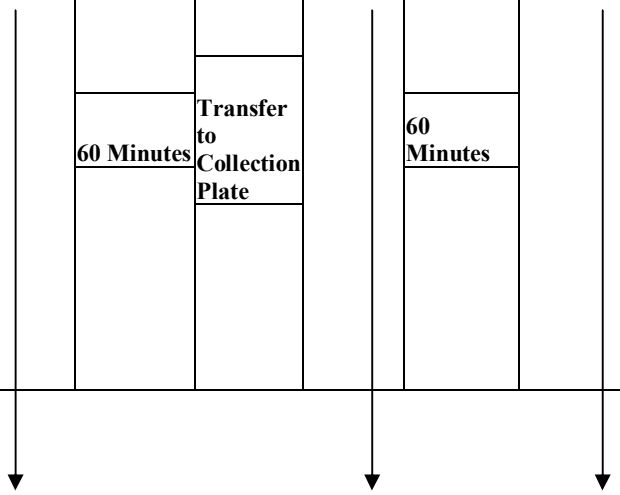
9. Read at 550-570 nm and plot on linear graph paper [(O.D. – Blank) vs. dose (mg/dL)].

QUALITY CONTROL

"Blood Spot Controls" containing a low, intermediate and high level of galactose are included in the kits. They should be included in each assay run as unknowns in order to monitor the performance and reliability of the assay. Likewise, external blood spot controls containing galactose at three different levels (low, intermediate, high) should be routinely included in each run. Analysis of the results obtained should be done according to acceptance criteria established by the individual laboratory.

PROTOCOLS

Wells	3/16" Blood Spot Sample	TCA	Shake	Vacuum	Neut. Sol.	Incubate	Reagent A-B	Incubate	Read
A_1 B_1	Reagent Blank	100 uL			50 uL		100 uL		
C_1 D_1	Standard 1								
E_1 F_1	Standard 2								
G_1 H_1	Standard 3			Transfer to Collection Plate					
A_2 B_2	Standard 4					60 Minutes		30 Minutes	550 - 570 nm
C_2 D_2	Standard 5		60 Minutes						
E_2 F_2	Control I								
G_2 H_2	Control II								
A_3 B_3	Control III								
C_3 D_3	Unknown 1								
E_3 F_3	Unknown 2								
Etc.	Etc.								



CALCULATIONS

Average the absorbency duplicates for all standards, controls and patients. Subtract the averaged reagent blank absorbency from each of the averages obtained above. This yields the *net absorbance* (Abs.). Construct the standard curve by plotting the net absorbance (y-axis) versus the concentration of the galactose standards (x-axis) using linear graph paper and a weighted linear cure fit. This yields the *standard curve*. Using the standard curve, determine the galactose concentration of each patient sample. Read patient samples (Abs – Blank) directly off cure as mg/dL galactose in whole blood. Computer-assisted data reduction may be used to calculate results. A weighted linear curve fit using a Blank subtracts is recommended. To program your automated data reduction system, please contact your software manufacturer. For additional information on the Iso-Data reduction systems used at DRG International, Inc., please contact the Technical Service Department at (908) 233-2079.

Unit conversion (mg/dL to mmol/L):
To convert mg/dL to mmol/L, multiply by 0.056.
To convert mmol/L to mg/dL, multiply by 18 or divide by 0.056.

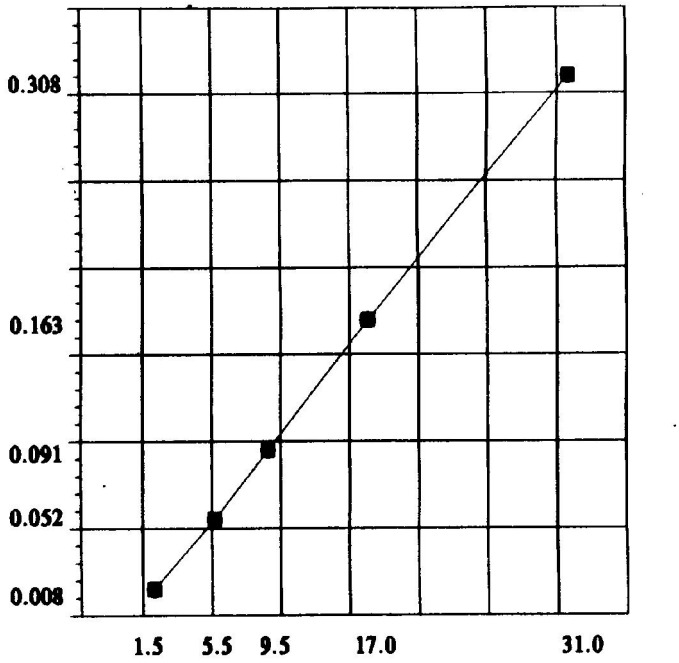
SAMPLE ASSAY AND STANDARD CURVE
These calculations are for example only. The user must construct a standard curve each time the assay is run.

Sample	ABS	ABS-BL	Galactose (mg/dL)
Blank	**0.061**		
Std 1	**0.069**	**0.008**	
Std 2	**0.113**	**0.052**	
Std 3	**0.152**	**0.091**	
Std 4	**0.224**	**0.163**	
Std 5	**0.369**	**0.308**	
Control I	**0.125**	**0.064**	**6.93**
Control II	**0.182**	**0.121**	**12.57**
Control III	**0.298**	**0.237**	**23.99**

SAMPLE STANDARD CURVE
The following is a sample standard curve and related information as illustrated by a computer assisted data reduction program.

Y Axis: ABS-NB
X Axis Conc. (Galactose)
Cure Fit: Weighted Linear
Graph: Linear vs. Linear
Slope: 0.01058
Y Int: -0.007218
R Factor 0.999840
%CV 2.14

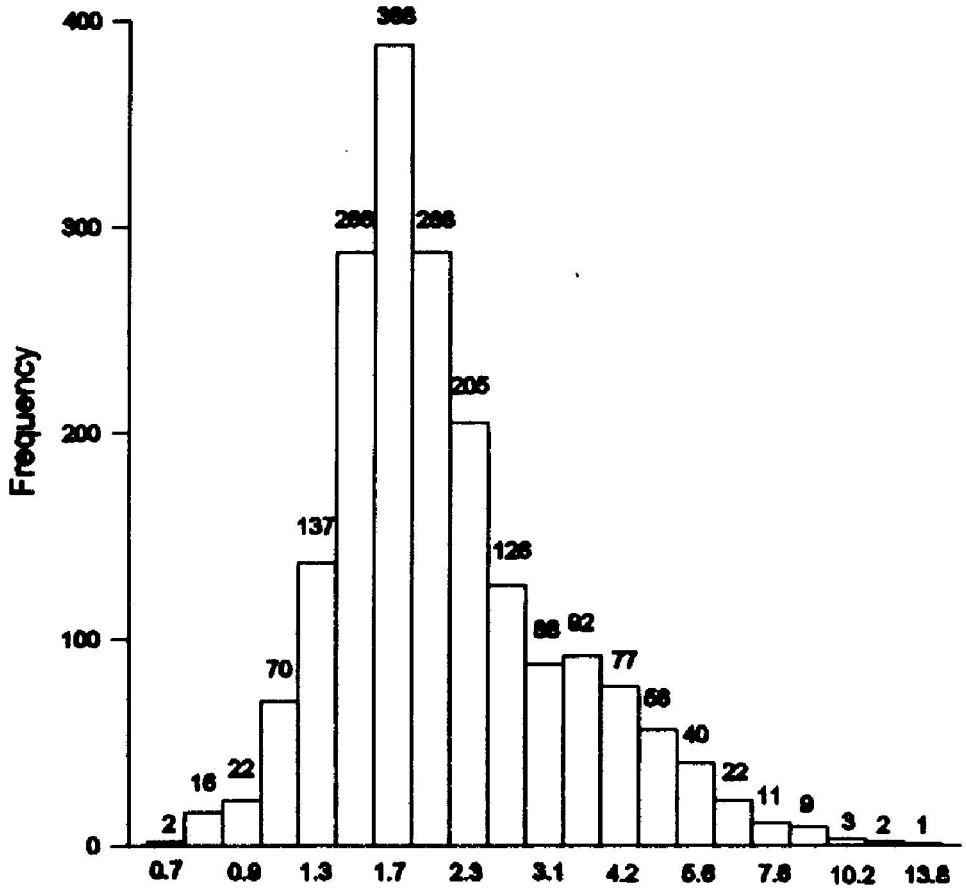
STD	Response	Conc.
1	0.0080	1.50
2	0.0515	5.50
3	0.0905	9.50
4	0.1625	17.00
5	0.3080	31.00



EXPECTED VALUES
Neonates (24-48 hours): Up to 5.0 ml/dL

Assay values of normal neonates would not be expected to exceed 5.0 mg/dL galactose based on the upper limit of the 95% confidence interval. This data was established at a State screening facility (n = 1947). As with any diagnostic test, differences in physiological ranges may be encountered from laboratory to laboratory due to patient demographics, laboratory techniques, and population sampling. This range should only be used as a guideline. We recommend each laboratory establish its own range using a sufficient number of characterized patient specimens to result in precise estimates of the normal range.

Normal Range Analysis
The DRG® Galactose EIA was compared with a commercially available quantitative method for galactose determination. After In-transformation, data from this study (n = 1947) approximate a Gaussian (normal) distribution. The calculated mean and standard deviation are 2.07 mg/dL, and 1.57 mg/dL, respectively. The actual observed range of the assay in the database is 0.69-to 14.19-mg/dL galactose. The In-transformation of the data is shown.



CUT-OFF DERIVATION

The table below illustrates the statistically derived cut-off for galactose levels in the DRG® Galactose EIA and a comparison assay at the 95th, 99th and the 99.9th percentile. These data are based on results generated at an established testing facility.

	DRG® Galactose EIA (Mg/dL)	COMPARISON ASSAY (mg/dL)
95th percentile	5.0	5.9
99th percentile	7.5	8.1
99.9th percentile	11.8	12.5

REFERRAL RATE ANALYSIS

The table below illustrates the variable presumptive positive rate observed in the DRG® Galactose EIA utilizing a broad range of cut-off values for galactose. The data below was generated at a State testing facility utilizing a population based upon random sampling over 7 days. Definition of a lower cut-off may enhance screening security through retesting of presumptive positive samples.

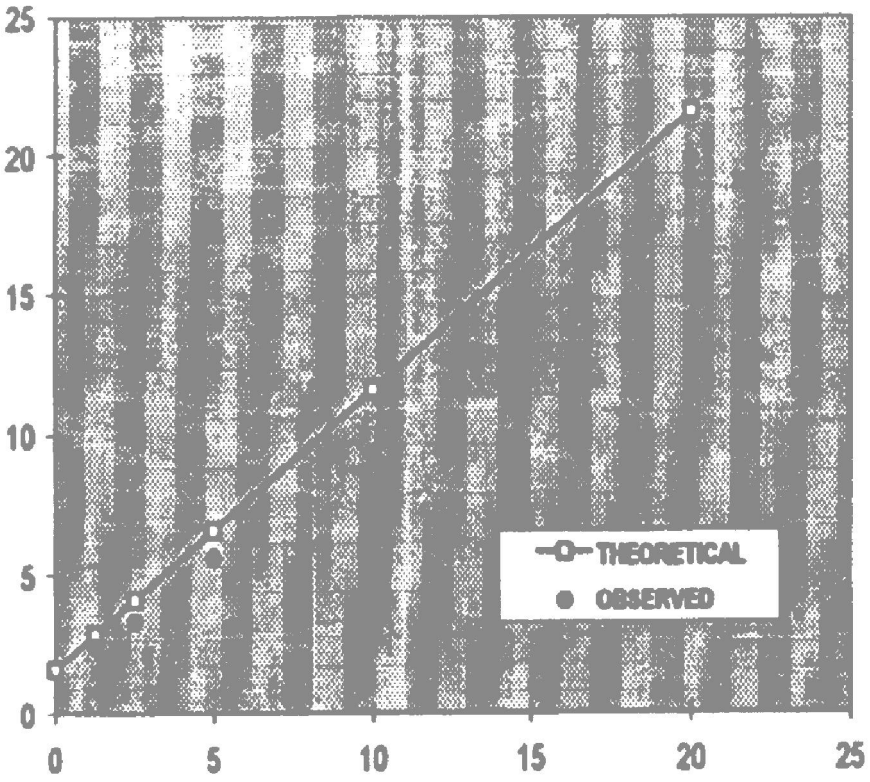
DRG® Galactose EIA Cut-off (mg/dL	Expected Presumptive Positive Rate (%)
3.5	12.18
4.0	7.21
4.5	4.27
5.0 (95% of Normal Range)	2.56
5.5	1.52
6.0	0.93
6.5	0.57
7.0	0.35
7.5 (99% of Normal Range)	0.22

PERFORMANCE CHARACTERISTICS

Parallelism (linearity of dilutions)

The parallelism study was conducted to evaluate the linearity of the DRG® Galactose EIA. Samples were prepared by adding a serially diluted galactose solution to human blood. Aliquots of each concentration were spotted into Schleicher & Schuell #903 filter paper and allowed to air-dry overnight. The samples were then assayed per protocol. The results shown illustrate the linearity of the DRG® Galactose EIA. Results shown represent actual values multiplied back by the indicated dilution factor and adjusted for endogenous galactose. Actual recovered values of galactose (mg/dL) were plotted against the expected results.

Sample	[GAL] (MG/dL)
1	**36.6**
1:2	**38.2**
1:4	**32.5**
1:8	**33.4**



RECOVERY

Recovery samples were prepared by spiking a galactose solution in human blood at various concentrations. Aliquots of each sample were spotted onto Schleicher & Schuell #903 filter paper and allowed to air-dry overnight. The samples were then assayed per protocol. The results shown illustrate the expected recoveries for the DRG® Galactose EIA in a selection of human blood samples.

Endogenuous GAL (mg/dL)	Spiked GAL (mg/dL)	Expected GAL (mg/dL)	Observed GAL (mg/dL)	n	Percent Expected
--	0	--	1.6	2	--
1.6	1.25	2.85	2.4	2	84.2%
1.6	2.5	4.1	3.2	16	78.0%
1.6	5.0	6.6	5.4	16	81.8%
1.6	7.5	9.1	7.7	14	84.6%
1.6	10.0	11.6	9.8	16	84.5%
1.6	20.0	21.6	19.9	2	92.1%
			Average Percent Expected		84.2%

FUNCTIONAL SENSITIVITY

Forty-six duplicates of the blank were assayed to determine the minimum quantity of galactose detectable by this assay. By adding the standard deviations to the mean of the blank, the minimum detectable dose of galactose was found to be below the lowest standard. Although lower doses are distinguishable from zero, it is recommended that, based on the functional sensitivity of the assay, all values which fall below the lowest standard (Standard 1), should be reported as a "value less than low standard".

PRECISION

Intra-Assay Variation

Intra-assay precision was calculated by assaying 10 replicates each of three-tri-level samples:

	Level I N = 10	Level II N = 10	Level III N = 10
Mean	6.79	11.57	22.08
S.D.	0.33	0.68	0.70
% C.V.	4.9	5.9	3.2

Inter-Assay Variation

Inter-assay precision was calculated by evaluating the same tri-level samples in multiple (n = 75) assay runs over the course of one week.

	Level I N = 76	Level II N = 76	Level III N = 76
Mean	6.42	11.56	22.16
S.D.	0.42	0.66	1.64
% C.V.	6.5	5.7	7.4

Variation at the Cut-off

The cut-off precision study was conducted to determine the intra-assay variation at two different suggested cut-off values, 5.0 and 7.5 mg/dL (95th and 99th percentile, respectively). Twelve replicates of each human blood sample were assayed. Dose C.V.'s were calculated.

	95th Percentile N = 12	99th Percentile N = 12
Mean	**5.43**	**7.73**
S.D.	**0.334**	**0.638**
% C.V.	**6.5**	**8.2**

Relative Presumptive Positive Analysis

The table classified 1943 of the 1947 cases of the Normal Range Study according to normal and presumptive positive results for the 95th percentile cut-off. Based on statistical analysis, the discordance observed between the two is due to random method variation,. No confirmed positives were encountered in this population.

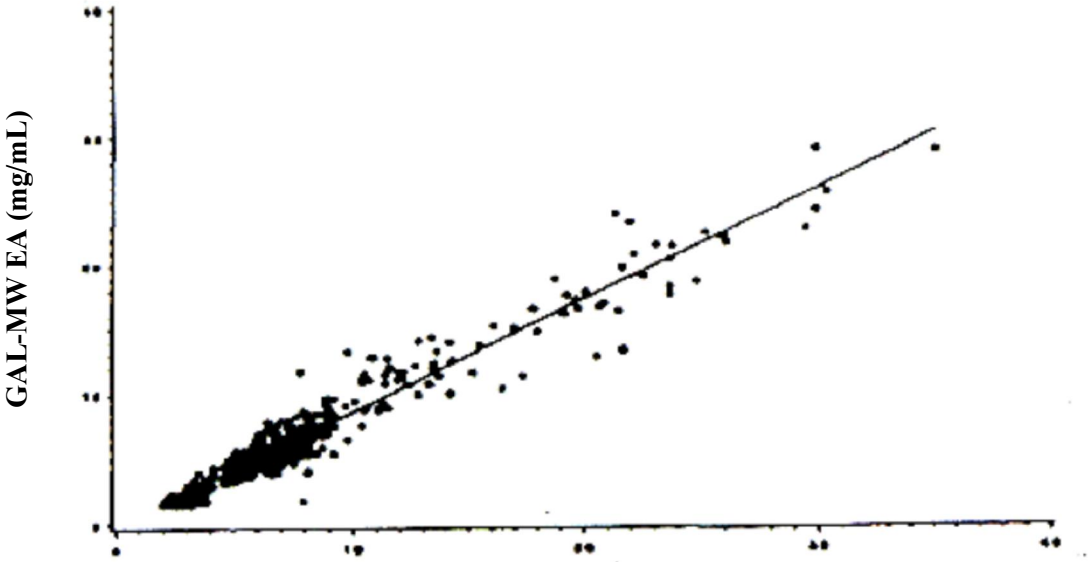
DRG® Galactose EIA	COMPARISION ASSAY		
≥ 5.0 Mg/dL	**≥5.9 mg/Dl n = 66**	**< 5.9 mg/dL n = 31**	**Total n = 97 4.99%**
< 5.0 mg/dL	**n = 29**	**n = 1817**	**n = 1946 95.01%**
TOTAL	**n = 95 4.89%**	**n = 1848 95.11%**	**n = 1943 100%**

PATIENT SAMPLE CORRELATION

Results from samples with values distributed throughout the quantitative range of this assay were compared with those obtained with a commercially available method. The observed range of the samples was 1.51 through 28.96 mg/dL galactose. The correlation coefficient was 0.970 (slope = 0.864, y-intercept = -0.0525 mg/dL).

Of the 447 patient samples selected from a Statewide screening program for galactosemia, 60 samples fell outside the range o the low and high standards. Thus, 387 samples were included in the clinical evaluation of the DRG® Galactose EIA. The patient samples were stratified over a broad range of measurements and were chosen from samples known to contain low, intermediate and elevated galactose levels. The 60 samples which fell outside the quantitative range of one or both of the assays compared, were not included in the regression analysis.

Data from the three strata were also evaluated separately by group. Statistical analysis indicates no significant difference in slopes (p≥ 0.40). The intercepts in this analysis vary from 0.299 to 4.214 mg/dL galactose. The individual data is presented below.



INTERFERING SUBSTANCES

The following substances were added to a blood spot standard in order to determine their effects on the result. No effect was noted except for D-Galactose and Galactose-1-phosphate, as expected. Except for a slight increase with the addition of tetracycline, no unexpected interferences were observed.

	Baseline GAL (mg/mL)	Amount Added	Observed GAL (mg/dL)
D-Galactose	8.47	10 mg/dL	21.98
L-Galactose	9.34	100 mg/dL	9.45
Galactose-1-phosphate	8.47	10 mg/dL	14.80
Fructose-6-phosphate	8.47	100 mg/dL	0.52
Glucose-1-phosphate	8.47	200 mg/dL	9.42
Glucose-6-phosphate	8.47	200 mg/dL	8.97
NAD	9.34	100 mg/dL	9.93
NADH	8.47	100 mg/dL	9.81
Glucose	8.47	200 mg/dL	9.01
Lactose	8.47	10 mg/dL	8.71
Fructose	8.47	100 mg/dL	9.18

Xylose	8.47	100 mg/dL	9.39
Maltose	8.47	100 mg/dL	9.23
Ribose	8.47	100 mg/dL	9.44
Arbinose	8.47	100 mg/dL	9.13
Galactitol	8.47	100 mg/dL	8.89
Galactose dehydrogenase	8.47	100 mg/dL	8.84
Amoxycillin	4.0	100 mg/dL	4.7
Ampicillin	4.0	100 mg/dL	4.0
Erythromycin	4.0	100 mg/dL	4.0
Gentamycin	4.0	100 mg/dL	4.2
Penicillin	4.0	100 mg/dL	4.5
Tetracycline	4.0	100 mg/dL	6.9
Bilirubin	4.1	20 mg/dL	4.4
Hemoglobin	4.0	100 mg/dL	4.9
Triglycerides	9.34	125 mg/dL	10.41
Cysteine	8.58	10 mg/dL	9.07
Creatinine	8.47	10 mg/dL	9.31
Uric Acid	8.47	10 mg/dL	9.50
Ascorbic Acid	8.47	2.5 mg/dL	8.57

ALTERNATE PROCEDURE
(For manual transfer of extracts)

For the five standards, three controls and variable unknowns:

1. Punch one 3/16" (or two 1/8") blood spots, in duplicate into appropriate well of plate. Be sure to leave empty wells for reagent blank.
2. Add 200 µl of TCA Extraction Solution to each well.
3. Rotate mix for 60 minutes at room temperature.
4. Using a single channel or multichannel pipet, transfer 100 µl of each extract to the corresponding well of a new plate.
5. Add Neutralizing Solution and Reagent A-B as indicated in package insert.

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