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Determination of Total Galactose from Dried Blood Spots—Extensive Assay Evaluation of a CE-Marked Test-Kit

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ABSTRACT

Most newborn screening laboratories use CE-marked or FDA-approved test-kits, like in routine clinical chemistry. National regulations require only minimal evaluation from the customer, if the test-kits are used as specified by the manufacturer. The microtiter-based kit-concept is often based on the perception, that the laboratory always processes whole microtiter plates. However, in the daily routine, this is rather a rare exception, which leads to much higher costs per newborn, compared to the costs per assay in the test-kits. In addition, the amount of wasted resources is quite high. Performance of the Neonatal Total Galactose kit from Perkin Elmer was tested. We have determined specificity, limit of detection (LOD), limit of quantitation (LOQ), intra and inter assay variation, recovery, stability of measuring signal and reagents. Results were also compared with the Astoria Pacific Spot Check System. In addition, we had (by chance) the opportunity to test 2 kits, which were already expired for more than 3 years. LOD was 165 - 306 µmol/L and LOQ 475 - 703 µmol/L, depending on the definition of LOD/LOQ. Mean recovery was 112.8%, intra assay CVs were 11.3, 7.3, 4.0, and 3.0, and inter assay CVs 28.7, 15.9, 7.8, and 9.3, at 220, 590, 1200, and 2060 µmol/L respectively. Reconstituted and mixed reagents must be used within some hours, and were unstable even if stored at −20˚C. However, if the reconstituted galactose substrate reagent and galactose oxidase reagent were only mixed according to the daily requirements, and the rest stored separately at −20˚C, they were stable for at least 12 days. The performance of the expired test-kits did not differ from the others. The performance of the Total Galactose kit is comparable to other tests used for newborn screening. However, we could significantly reduce the costs per newborn and reduce unnecessary production of waste, by thorough validation and modification of the assay procedures.

Keywords: Newborn Screening; Galactosemia; Total Galactose

1. Introduction

Newborn Screening for Galactosemia was introduced in the 1970s [1]. While the classical "Beutler-Test" can only detect newborns with classical galactosaemie [2], the determination of total galactose [3,4] will also detect newborns with galactokinase deficiency and uridine diphosphate galactose-4-epimerase deficiency.

Most newborn screening laboratories use CE-marked or FDA-approved test-kits, like in routine clinical chemistry. National regulations require only minimal evaluation from the customer if the test-kits are used as specified by the manufacturer. The microtiter-based kit-concept is often based on the perception, that the laboratory always processes whole microtiter plates. However, in the daily routine of newborn screening laboratories, this is rather a rare exception, which leads to much higher costs per newborn, compared to the costs per assay in the test-kits. In addition, the amount of wasted resources is quite high. We have thoroughly tested the performance of the Neonatal Total Galactose kit from Perkin Elmer. We have determined specificity, LOD, LOQ, intra- and inter-assay variation, recovery, signal stability of the fluorescent reaction product, and stability of reagents. Results were also compared with the Astoria Pacific Spot Check System. In addition, we had (by chance) the opportunity to test 2 kits, which were already expired for more than 3 years.

2. Materials and Methods

Total Galactose was determined from whole blood, dried on filterpaper, with the Neonatal Total Galactose test-kit from Perkin Elmer. For the determination of intra and inter assay variation various kit controls were used (concentrations up to 726 µmol/L). In addition, dried blood samples with galactose concentrations above 1000 µmol/L were prepared by adding galactose to whole blood of a healthy adult volunteer. After thorough mixing, the blood was spotted onto filterpaper and let dry for several hours. Control samples were stored at −18˚C. The *Galactose-Oxidase/Substrate-Reagent* has to be mixed from reconstituted *Galactose-Oxidase-Reagent* and *Galactose-Substrate-Reagent*. One vial of lyophilized *Galactose-Oxidase-Reagent* has to be dissolved with 6 mL of reconstitution buffer and one vial of *Galactose-Substrate-Reagent* has to be dissolved with 2 mL of reconstitution buffer. This results in 8 mL of *Galactose-Oxidase/Substrate-Reagent* which is sufficient for one microtiterplate. Reconstituted and mixed reagents must be used within one hour, and were unstable even if stored at −20˚C. Due to the instability of reconstituted *Galactose-Oxidase/Substrate-Reagent*, we used a modified assay procedure. After reconstitution of *Galactose-Oxidase-Reagent* and *Galactose-Substrate-Reagent* only the necessary amount of *Galactose-Oxidase/Substrate-Reagent* is mixed. A whole vial of *Galactose-Oxidase-Reagent* and *Galactose-Substrate-Reagent* for a whole microtiterplate, and *x* * 750 µL of reconstituted *Galactose-Oxidase-Reagent* and *x* * 375 µL of reconstituted *Galactose-Substrate-Reagent*. With *x* being the number of rows on the microtiterplate that contain samples. Then all mixed *Galactose-Oxidase/Substrate-Reagent* is pooled for the whole daily batch. With this procedure one calibration curve on the first plate is sufficient for the daily batch, in contrast to the kit instructions, which call for a calibration curve on each plate. Residual reagents can be stored at −20˚C, and will be used the next working day.

The performance of the PE-kit for routine newborn screening was compared with the results of the Astoria Pacific Spot Check Kit.

3. Results

When the Total Galactose Kit was used according to our modified assay procedure, reagents are stable for at least 12 days (**Figure 1**). The fluorescent signal of the reaction product, after stopping the reaction, was stable for more than 3 hours (**Figure 2**).

Values for LOD, LOQ are shown in **Table 1**, and values for intra- and inter-assay-variation are shown in **Table 2**, at various concentration levels. Mean recovery was 112.8%. The correct determination of LOD and LOQ is difficult. Whole blood samples without endogenous galactose are hard to obtain and the measurement of plain white filterpaper gives significantly higher values than kit-calibrator A ($n = 30$, $p < 0.001$). This is also reflected by the very high values of the statistically achieved LOD[4], LOQ_1, and LOQ_2.

Our data show, that the use of the unweighted linear regression algorithm, as proposed by the manufacturer, results in an underestimation of the galactose concentrations above 1000 µmol/L (**Figure 3**). The use of a spline function for calibration curves gives much more reliable results.

Comparison of the Total Galactose kit from PE with the Astoria Pacific Spot Check Kit under routine conditions showed no perfect correlation. The best curve fit was achieved with a quadric equation (**Figure 4**).

The performance of the test-kits, that were expired for more than 3 years, showed no difference to still valid test

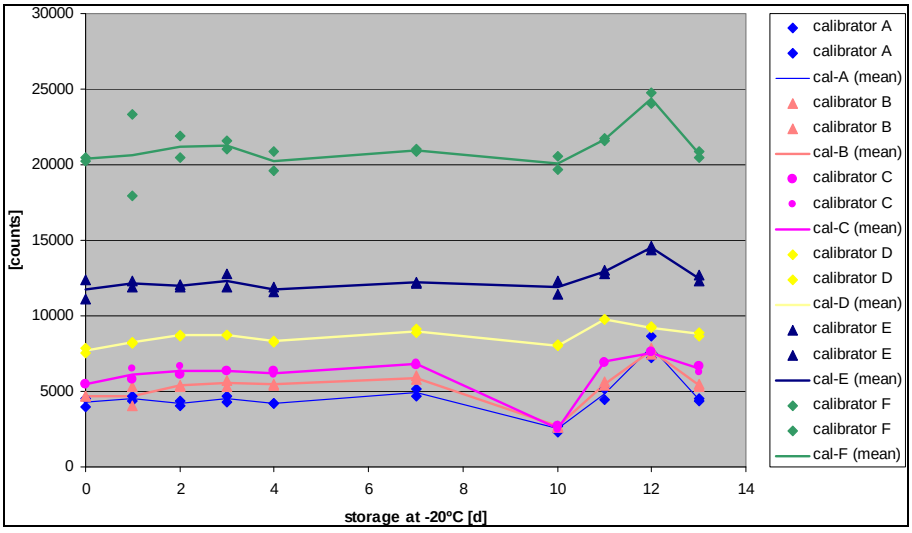

Figure 1. Stability of reagents stored at −20˚C according to our modified assay procedure.

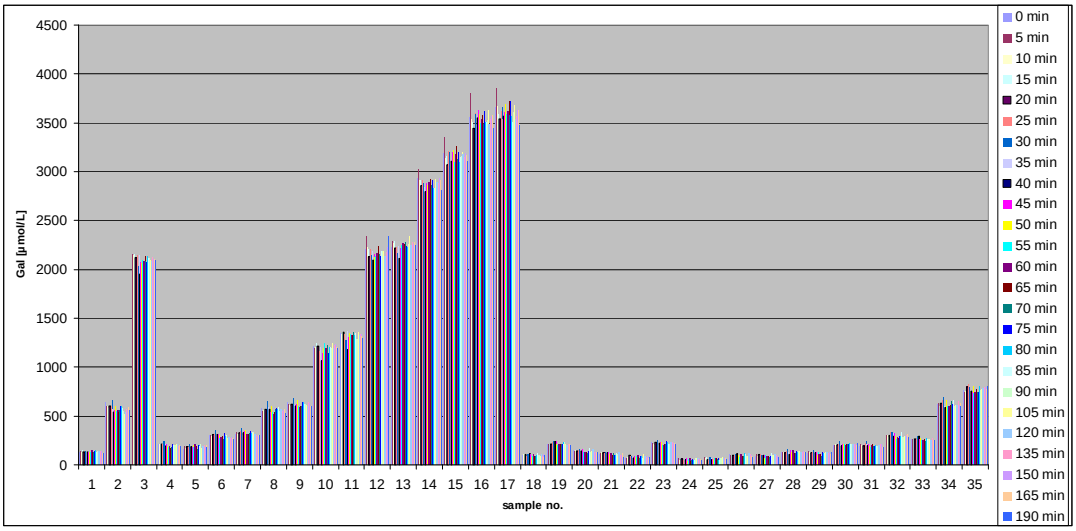

Figure 2. Signal stability of the fluorescent reaction product. After adding stop solution and shaking, 35 samples with galactose concentrations between 50 - 3600 µmol/L were repeatedly measured up to 190 min.

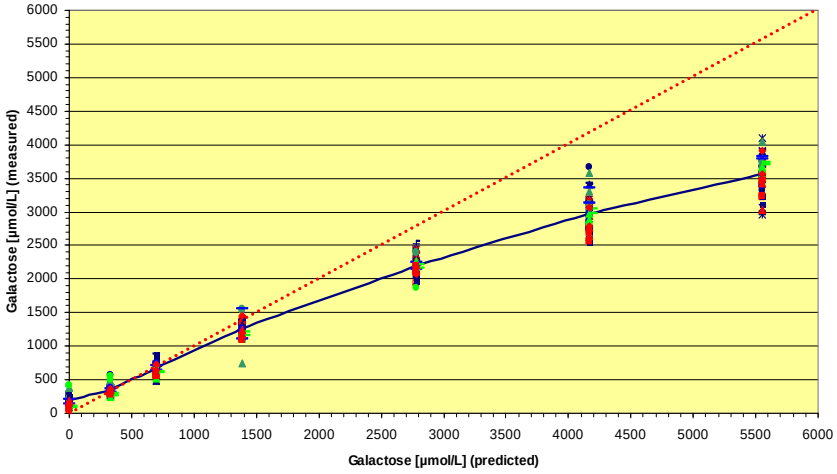

Figure 3. Comparison of measured and predicted galactose concentrations, up 5556 µmol/L. Galactose concentrations were calculated with the proposed linear regression algorithm.

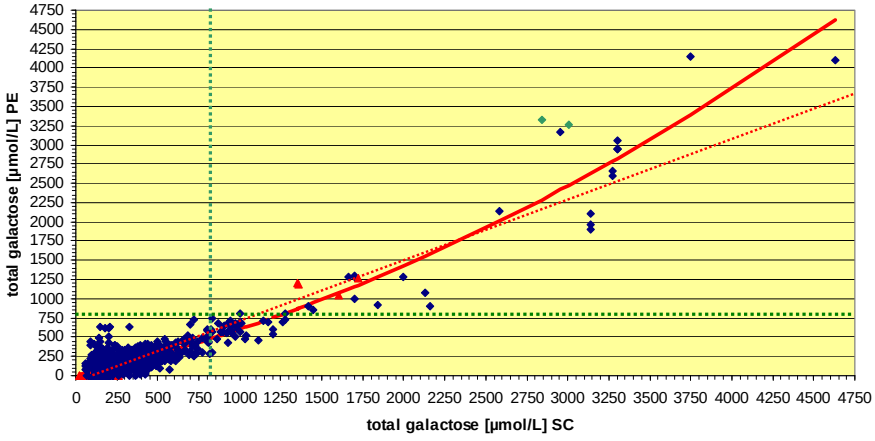

Figure 4. Comparison of the Total Galactose test-kit from PE with the Astoria Spot Check kit under routine conditions. 3650 newborn samples (◆), 20 CDC quality control samples (▲), and 2 samples from a newborn with classical galacto-saemia (◆). Linear regression (••••), and calculated quadric correlation (—).

Table 1. Limit of detection and limit of quantitation of the Total Galactose assay.

	Values from kit-inserts				Values determined			
Analyte	LOB[1]	LOD[2]	LOQ[3]	Linearity	LOD[4]	LOQ_1	LOQ_2	LOQ_3
Total Galactose [µmol/L]	39	72	*No information*	72 - 3108	306	476	703	<83

LOB[1] = limit of blanc; defined as the 95th centile of a distribution of blank samples. LOD[2] = limit of detection; according to NCCLS document EP17-A. LOQ[3] = limit of quantitation; defined as the lowest concentration with a total CV equal to or less than 20%. LOD[4] = limit of detection; defined as the mean of a sample without analyte + 3 SD (blanc filterpaper). LOQ_1 = limit of quantitation; defined as the mean of a sample without analyte + 6 SD (blanc filterpaper). LOQ_2 = limit of quantitation; defined as the mean of a sample without analyte + 10 SD (blanc filterpaper). LOQ_3 = limit of quantitation; defined as the lowest concentration with a total CV equal to or less than 20%.

Table 2. Intra- and inter-assay variation at various galactose concentrations.

	Intraassay variation (n = 10)					Interassay variation (n = 30)								
Galactose [µmol/L]	226	357	457	592	726*	1215	1509	2059	177	283	479	604	1397	2262
c.v. [%]	11.3	7.9	7.4	7.1	11.6	4.0	4.8	3.0	25.7	10.3	15.9	7.3	8.3	9.3

*Determination of total galactose with the PE-kit is not influenced by EDTA up to 100 mM. Mean value of 18 control samples with EDTA concentrations from 0 - 100 mM: 719 µmol/L, c.v. 9.8%.

kits (data not shown).

4. Discussion

Newborn screening laboratories have to measure all screening samples on the day of arrival in the laboratory. Therefore it is extremely rare that the total sample number of a daily routine, including calibrators and controls, will exactly (or nearly) fill a whole microtiterplate, or two, three, etc. But since the reagents in the Total Galactose kit from Perkin Elmer are packed for one plate, respectively, sticking to the procedures outlined in the kit-insert will result in a significant waste of reagents, and hence increase of costs per newborn. This can be up to 50% subject to the number of samples per day. In addition, the stated expiry date seems to be unnecessarily short, which could also lead to unnecessary waste of reagents in small screening laboratories.

Our data show that a thorough in house validation even of CE-marked test-kits can significantly improve the overall performance of a test, including economical aspects.

5. Acknowledgements

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