Original Article

Journal of Inborn Errors of Metabolism & Screening
2016, Volume 4: 1–5

DOI: 10.1177/2326409816669370
iem.sagepub.com
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Evaluation of the Genetic Screening Processor for the Performance of Newborn Screening Tests

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Abstract

The collection of dried blood spots (DBSs) on filter paper has been a powerful tool in newborn screening (NBS) programs and in other fields. However, filter paper has been associated with some level of imprecision due to the filter paper matrix effect. In order to minimize measurement variations, these interferences should be evaluated by NBS assays. The aim of this study is to evaluate the performance of genetic screening processor (GSP) equipment in comparison with a widely used AutoDELFIA and to discuss the limitations and advantages of this new technology in NBS. We evaluated the performance of 3 NBS assays in DBS using GSP in comparison with AutoDELFIA. To determine the inaccuracy and the intra-assay precision, a comparative study and a replication experiment were performed. In the comparative study, human thyroid-stimulating hormone (hTSH) assay showed the highest correlation coefficient, followed by 17α-OH-progesterone and immunoreactive trypsinogen (IRT) assays. The results of the present study suggest that the GSP equipment and kits are suitable for implementation and have acceptable performance for NBS routine. Genetic screening processor assay tends to underestimate hTSH and IRT concentrations in the clinically relevant range when compared to AutoDELFIA assays. More studies are necessary to reevaluate cutoff values. Furthermore, the equipment has advantages when compared with AutoDELFIA, such as methodology with more specificity, reduction in the processing time, and randomized routine. This helps promoting faster dynamic technical processes and faster report generation.

Keywords

newborn screening, GSP, validation, IRT, NTSH, 17OHP

Introduction

Dried blood spot (DBS) specimens have been used in newborn screening (NBS) and in many other clinical diagnosis due to their strong advantages compared to the conventional collection. These advantages include the minimal volume requirements, sample stability, ease of collection, transport and storage, and cost-effectiveness. Also, DBS has achieved the same level of precision and reproducibility of the standard methods of collecting blood such as vacuum tubes and capillary pipettes.[1] However, DBS used in NBS have some qualitative restrictions, which can lead to analytical error (AR) and erroneous results. Also, filter paper has been associated with some level of imprecision due to the filter paper matrix effect.

In order to minimize measurement variations, these interferences should be evaluated by NBS assays. There are several DBS standardized methods available for NBS. Of the immunoassays, fluorescence immunoassays (FIAs) are the most sensitive apart from radioimmunoassays (RIAs) due to the kind of label used.[2] They are still the most used method worldwide for the detection of some diseases in NBS, such as congenital hypothyroidism, congenital adrenal hyperplasia, and cystic fibrosis because of their ease of execution, availability, and cost-effectiveness. However, compared with immunoassays, tandem mass spectrometry (MS/MS technology) is more specific and accurate and consolidated the metabolic screening of

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Received March 23, 2016, and in revised form August 9, 2016. Accepted for publication August 11, 2016.

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many diseases, such as amino acid and acylcarnitine disorders. The limitations of this technology include issues with sample preparation and instrument standardization, besides the high machine cost associated with the laborious technique, requiring extensively trained laboratory personnel, making the MS/MS technology not affordable for many services. Liquid chromatography–tandem mass spectrometry has become the standard assay for the measurement of many steroid hormones during the serum confirmatory and follow-up phases.

In FIAs, some lanthanides, especially europium (III) and terbium (III), form highly fluorescent chelates with many different organic ligands. The sensitized fluorescence results from the ligand absorbing light, the energy of which is then transferred to the chelated metal ion. The metal ion emits the energy as narrowband, line-type fluorescence with a long Stokes shift (over 250 nm) and an exceptionally long fluorescence decay time (0.1-1 milliseconds). The use of europium as a label in time-resolved fluoroimmunoassays makes it possible to achieve highly sensitive measurements that incorporate the positive features of FIA but avoid the drawbacks of RIA.[2] AutoDELFIA is an example of this technology. Despite the total automation of pipetting, incubation, and measurement, it also has some drawbacks already described.[3]

The new integrated screening plate processor GSP from PerkinElmer for in vitro diagnostic in NBS is designed to incorporate all screening tests, except MS/MS. It is supposed to overcome most of the drawbacks due to changes in chemistry and handling process.[3,4]

So, the aim of this study is to evaluate the performance of the GSP equipment in comparison with a widely used AutoDELFIA and to discuss the limitations and advantages of this new technology in NBS.

Materials and Methods

The neonatal immunoreactive trypsinogen (IRT), human thyroid-stimulating hormone (hTSH), and 17α-OH-progesterone (17-OHP) were measured in DBS by a time-resolved fluoroimmunoassay GSP kits (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) on a GSP model 2021 (PerkinElmer) instrument and compared with results from AutoDELFIA Neonatal IRT, hTSH, and 17-OHP kits (PerkinElmer Life and Analytical Sciences) performed on an AutoDELFIA Model 1235 (PerkinElmer) instrument. This study was conducted in the Reference Newborn Screening Laboratory of Rio Grande do Sul State, Southern Brazil.

All concentrations are related to whole blood. Units are as follows: IRT ng/mL, hTSH μU/mL Sg, and 17α-OHP ng/mL.

For validation, we evaluated samples in routine collected in the standardized filter paper (Schleicher and Schuell 903) by heel prick from the newborn babies (age: 3-30 days) of the Rio Grande do Sul State, Southern Brazil, and forwarded to the reference laboratory for assays.

To determine the inaccuracy, a comparative study was performed. We evaluated 115, 105, and 109 DSB samples for IRT, hTSH, and 17-OHP, respectively. In AutoDELFIA, the cutoff values were 70 ng/mL for IRT, 9.0 μU/mL Sg for thyroid-stimulating hormone, and 15 ng/mL for 17-OHP.

To determine the intra-assay precision, a replication experiment was performed to IRT, hTSH, and 17OHP assays by obtaining test results on 20 samples of the same material, in a run. In the interassay, this sample was assayed in quadruplicate once per day for 5 days.

We used EP-Evaluator software (version 11) for statistical evaluation of the results. The random error, systematic error, and AR were calculated.

Table 1. Performance of GSP Kits for Newborn Screening.

Parameters	IRT	hTSH	17-OHP
Accuracy			
Slope	0.896	0.832	0.942
Intercept	−2.655	1.062	2.115
r^2	0.894	0.995	0.989
Precision			
Intra-assay			
Mean[a]	5.0	2.8	1.1
CV	6.0	9.4	14.2
Inter-assay			
Mean[a]	5.0	2.8	1.7
CV	4.4	8.0	13.9
Performance			
RE	7.3	13.2	22.9
SE	6.9	10.5	6.7
AR[b]	14.2	23.7	29.6

Abbreviations: AR, analytical error; CV, variability coefficient; hTSH, human thyroid-stimulating hormone; IRT, immunoreactive trypsinogen; r^2, correlation coefficient; RE, random error; SE, systematic error; 17-OHP, 17α-OH-progesterone.
[a]IRT, ng/mL; TSH, μU/mL Sg; 17α-OHP, ng/mL.
[b]Calculated considering $\alpha = 0.05$.

Results

The performance of IRT, hTSH, and 17-OHP in GSP assays are described in Table 1. Correlation plots and Bland-Altman plots are shown in Figures 1, 2 and 3 for IRT, hTSH, and 17-OHP in GSP assays, respectively.

Considering an allowable total error of 30%, the largest error index occurred at the concentration of 71.5 ng/mL, 13.60 μU/mL Sg, and 14.30 ng/mL for IRT, hTSH, and 17-OHP, respectively.

In order to evaluate cutoff values for GSP assays, κ coefficient was calculated. For IRT, the best agreement between the 2 techniques were observed in 60 ng/mL ($\kappa = 0.79$). Similarly, for hTSH and 17-OHP assays, the best concordance were observed in 8.0 μU/mL Sg ($\kappa = 0.86$) and in 13 ng/mL ($\kappa = 0.77$), respectively.

Discussion

In this study, we evaluated the performance of 3 NBS assays in DBS using GSP processor in comparison with AutoDELFIA. In the comparative study, hTSH assay showed the highest

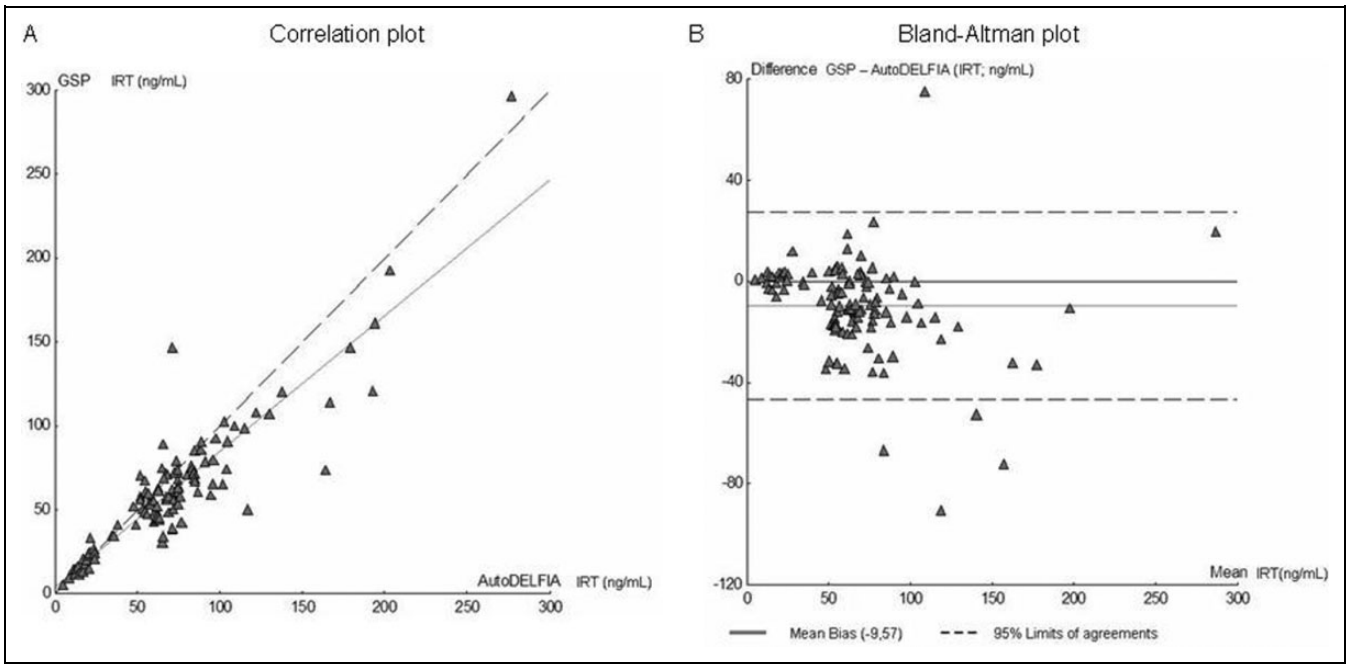

Figure 1. Comparison study between GSP and AutoDELFIA IRT assays: (A) correlation plot and (B) Bland-Altman analysis plot. GSP indicates genetic screening processor; IRT, immunoreactive trypsinogen.

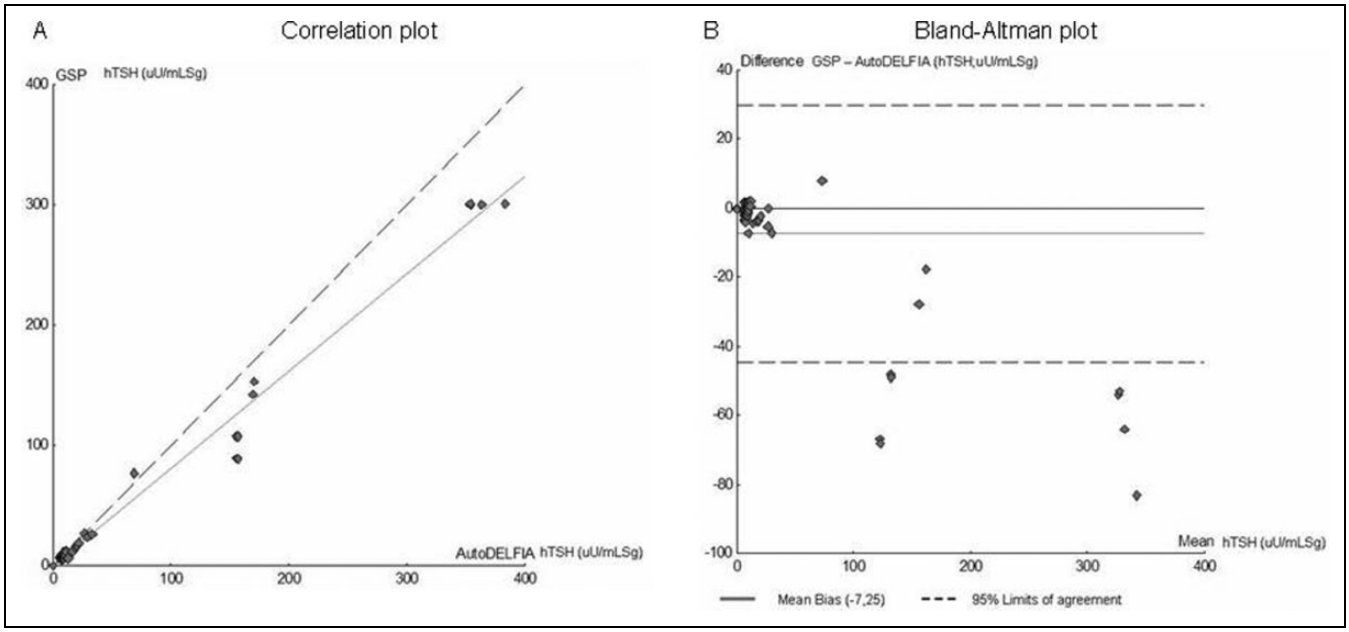

Figure 2. Comparison study between GSP and AutoDELFIA hTSH assays: (A) correlation plot and (B) Bland-Altman analysis plot. GSP indicates genetic screening processor; hTSH, human thyroid-stimulating hormone.

correlation coefficient, followed by 17-OHP and IRT assays. Previous study had demonstrated similar results.[3] Moreover, hTSH and IRT results in GSP had exhibited negative bias when compared to AutoDELFIA results. These differences may partly be explained by differences in calibration and chemistry improvements in GSP kits.[3] The main GSP chemistry changes are the new tracer antibody, shorter incubation time due to different antibody kinetics, and a new tracer chelate (N3 instead of N1 in AutoDELFIA). In the 17-OHP kit, we did not find these differences probably because both technologies have the same protocol.

In our study, for all evaluated kits, the highest result variation was found in concentration nearby cutoff values. These data could explain moderated agreement founded in concordance analysis. The choice of the cutoff values by this approach has several drawbacks such as (1) the limited

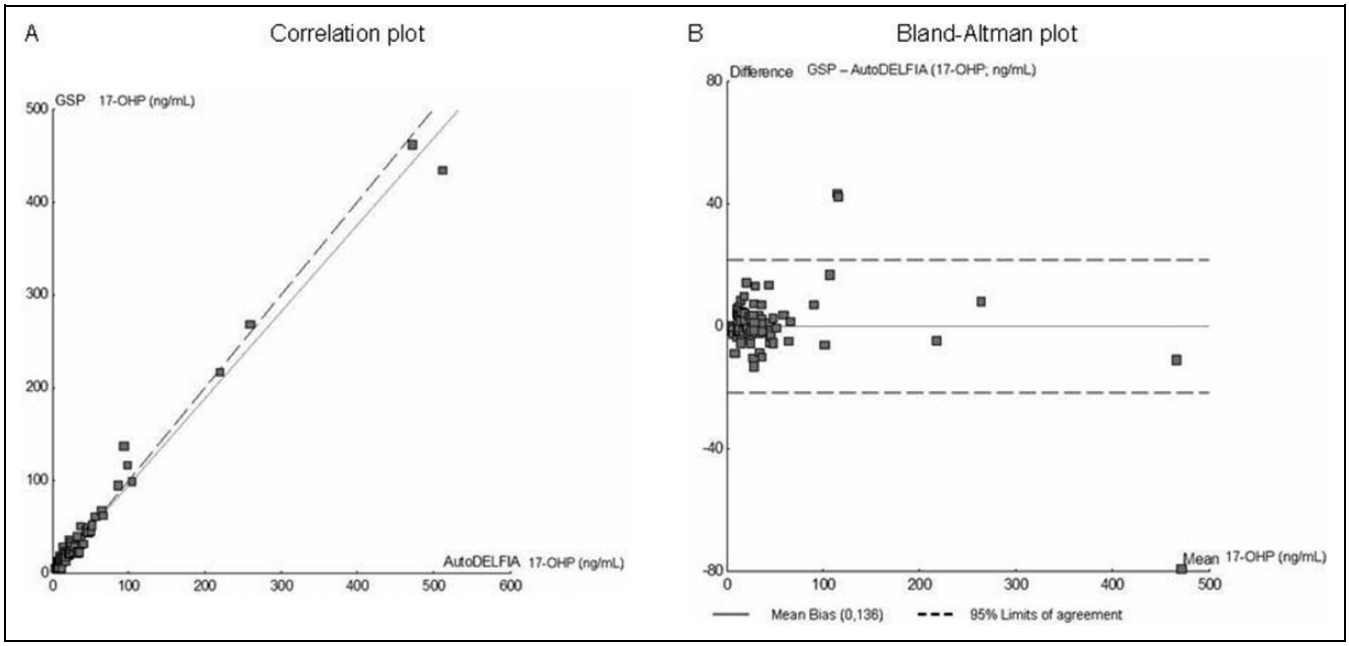

Figure 3. Comparison study between GSP and AutoDELFIA 17-OHP assays: (A) correlation plot and (B) Bland-Altman analysis plot. GSP indicates genetic screening processor; 17-OHP, 17α-OH-progesterone.

number of patients included in the correlation study and (2) the lack of knowledge of final diagnosis leading us to the assumption of no misclassification by AutoDELFIA kits. Also, the proposed threshold values must be validated by further clinical studies to avoid the increasing of false-negative results.[5-7]

Analyzing the data obtained in the precision studies, our results differ somewhat from those of Fingerhut and Torresani[3] who found the lowest CV for all kits evaluated. These differences may partly be explained by sample selection. Ideally, the sample concentration should be in a clinically relevant range. We utilized routine samples, which is a limitation of the present study.

Several factors can also contribute to analytic variation in DBS. Hematocrit and blood spot volume effects are some examples. The uniform absorbing properties of the filter paper can be defeated if blood is blotted or smeared onto the paper or if a drop of blood is placed on top of a previously collected drop. In addition, the volume of whole blood applied to filter paper as a blood spot can influence the volume of serum contained within a single disc punched out of that spot. Chromatographic effects are an additional potential source of error. It has been shown for specific analytes that concentration can vary across a single spot. Those effects should also be considered in the determination of the more adequate cutoff value, to avoid an increase in false-negative or false-positive results.[8]

Besides the specific differences in each assay, some advantages of GSP are developing a quicker and less labor-intensive assay, possibility to add plates while the instrument is running, high capacity with up to 26 plates and up to 13 techniques, traceability of consumables, operators and operations, easy-to-use software, and automatic elimination of the waste. Disadvantages are high need of deionized water (12.5 mL/s); quite heavy instrument (610 kg), may need special reinforcement of the floor; refrigeration noisy; and some mechanical and software problems during the evaluation: need for a trained technical assistance service.

Conclusion

In conclusion, the results of the present study suggest that the GSP equipment and kits could be implemented in NBS routine. However, new cutoff values need to be validated. Genetic screening processor methodology tends to underestimate hTSH and IRT concentrations in the clinically relevant range when compared to AutoDELFIA assays. Clinical studies are necessary to reevaluate cutoff values. The 17-OHP GSP kit showed the best performance for NBS.

Furthermore, the equipment has advantages when compared with AutoDELFIA, such as methodology with more specificity, reduction in the processing time, and randomized routine. This helps promoting faster dynamic technical processes and faster report generation.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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