Factors that influence newborn CH screening

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The purpose of the national screening program for congenital hypothyroidism is to detect the disease at the earliest stage possible and to start treatment as soon as possible. In Romania, the incidence of CH is 1:3000-4000 new-borns. As a screening strategy, a heel prick blood sample is drawn at day 3-5 after birth. TSH is determined and if the initial value is above the cut-off value a duplicate test is performed. The cut-off value recommended by the Romanian Health Ministry is 20 mUI/L for TSH. If the result of the duplicate testing is above the cut-off value, the new-born is recalled and a second sample is tested using the same method. If the result from the second sample is above the cut-off value, the new-born is referred to a center for the diagnosis and treatment of the CH.

In order for the program to meet its purpose, all the phases of this process must function properly. A correct laboratory result depends on the sample being properly drawn and on the performance of the method. Also, the results reported as positive for the screening depends on the interpretation of the result by the laboratory personnel.

A sample that is correctly drawn is the first step for all types of matrices. Dry blood spot sampling has numerous advantages and the use of DBS is expanded in the last few years: endogenous substances, drug monitoring, molecular biology, genetic testing, epidemiological studies, and pharmacological studies are performed. In screening programs, the testing has expanded from 1-2 analytes to a complete panel of tests from one DBS through mass spectrometry.

A correct blood sampling on DBS is described, but is the responsibility of each laboratory to establish acceptance criteria. Those criteria are to be determined, since the influence of the sampling depends on the analyte. In pharmacology studies, the whole sample is used to eliminate the effects of sampling on the test result. In the UK national screening program samples that have a small quantity of blood (diameter smaller than 8 mm), compressed samples and multi spotted samples are rejected. The evaluation of the DBS was performed with a visual scale or by measuring the diameter of the DBS. A correct sampling depends on the drop of blood formed and on the angle of the drop with the filter paper and the patient's hematocrit. The evaluation of the samples and knowing the factors that influence the correct sampling may lead to measures that ensure a correct DBS. The hematocrit effect is the most cited effect on the results obtained from DBS. The hematocrit effect may be due to the dispersion of the blood on the filter paper, the quantity of serum in DBS and the recovery rate.

In our study, we evaluated the preanalytical, analytical and post-analytical phases of the total testing process of the TSH with an FEIA method. 11 studies were performed: studies 1-4 for the preanalytical phase (effect of blood volume, hematocrit effect, chromatographic effect and an assessment of the quality of the DBS with a device created for this purpose), studies 5-9 for the analytical phase (Total Error, Measurement Uncertainty, Six Sigma, effect of the calibration, effect of the temperature and biotin) and studies 10-11 for the postanalitycal phase (reference values and cut-off value and the use of average of normals).

The conclusions of each phase of the total testing process are listed:

1. Preanalytical phase

Performing TSH from DBS's that have a smaller quantity of blood may lead to lower TSH results. The difference from these DBS's is larger than the intraindividual biological variation and the accepted difference of 15%. In such cases, CH may remain undetected and false-negative cases may arise.

Most of the newborns have normal hematocrit values, but in newborns with lower than normal hematocrit values, TSH values may be higher than the real value of the analyte due to a higher quantity of serum in those DBS's. In patients with high hematocrit values, TSH may be lower than the actual value due to a smaller quantity of serum and to the heterogeneity of the DBS.

The chromatographic effect may be emphasized in samples that are obtained by heel prick than in samples that are obtained by volumetric devices.

After evaluation, the most frequent defects were: too small DBS and heterogeneity of the sample, the factors that have the most influence on the TSH value. The sampling procedure is not followed properly and the Sigma Score obtained (2.4) showed that minimum specifications for the process to be considered stable are not met. The evaluation with a device is preferable to the evaluation of the DBS's quality by laboratory personnel.

To the NSP for CH function properly the criteria for the DBS to be rejected must take into consideration the recall rate of the program. Considering the most frequent defects of the DBS's, it is recommended that newborns with results near the cut-off value +/- measurement uncertainty with a DBS that is too small must be recalled for a new blood sample. DBS that are heterogenous should be rejected without TSH being performed. In Maternity Wards that have a high defect rate training of the personnel should be scheduled more often.

2. Analytical Phase

Sigma score should be determined at each change in the procedure: new reagent lot, new calibration, new Bias results available after proficiency testing round, staff changes.

The rules applied for the SQC should be according to the Sigma Value. According to our results, internal QC samples should be performed in duplicate randomly distributed on the test plate and the results must be within 2.5 DS.

Measurement Uncertainty should be recalculated with each new calibrator lot available and the recall decision should be made considering MU. Although the recalibration effect is statistically significant it is included in the MU value.

To eliminate the effect of the incubation temperature all tests should be performed using the same protocol. Biotin values obtained in newborns samples are lower than the values stated by the CLIA manufacturer. Although FEIA results from whole blood for TSH are lower than the values obtained from serum with the CLIA method, biotin influence is unlikely. Biotin's influence may be suspected in newborns that are being treated for acidemia with high doses of biotin. In those cases, samples must be obtained before biotin is administered.

3. Postanaytical phase

Regardless of the method used for reference values, the upper limit of the reference interval for TSH from DBS was below 10 mUI/L. The recommended cut-off value for mature newborns in days 3-5 after birth is 10 mUI/L.

Using the average of normals may increase systematic error detection. These errors are not always detected by internal QC samples. An increase in the average of normals value may lead to a higher than recommended recall rate.