

Installation of AutoDELFI A: A new addition in Bangladesh for the Screening of Congenital Hypothyroidism in Newborn Babies

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ABSTRACT

Newborn Screening for the diagnosis of Congenital Hypothyroidism (CH) was first started in Bangladesh in 1999 by the Immunoradiometric assay (IRMA). Dissociation enhanced lanthanide fluorescent immunoassay (DELFI A) was started in 1986 due to its superior sensitivity, speed, and non-radioactive methodology. Auto DELFI A 1235 automatic immunoassay system was introduced in the In vitro Division of National Institute of Nuclear Medicine & Allied Sciences (NINMAS) under the ADP Project “Screening of Congenital Hypothyroidism in Newborn Babies (Phase 2)”. It is an automatic system that can minimize the pipetting error. In this experiment, dried blood spots (DBS) were punched into an antibody-coated microtitration well with an automated Panthera Puncher and analyzed by AutoDELFI A. Six different standards of 0.8, 10.3, 24.5, 49.3, 93.2, 239 μ U/mL were used and the calibration curve was drawn by using these standards. Standard error and Standard deviation were found 12.4 and 30.5. Limit of detection (LOD) and Limit of Quantification (LOQ) of X variables were 0.16 and 0.47. The average of the percent recovery was 96.7. The R² value of the calibration curve was 0.9985. The accuracy of the standard samples was 96.7 \pm 10.6. For this assay two Quality Control (QC) of 16.39 μ U/mL and 63.09, μ U/mL were used. All of the measured values from the calibration curve were close to \pm 1 standard deviation. All these findings indicate appropriateness of Auto DELFI A System for the CH screening from dried blood spots in the newborn screening laboratory of the In vitro division of NINMAS.

Key words: Newborn Screening, Congenital Hypothyroidism, Auto DELFI A, Limit of detection, Limit of Quantification

Bangladesh J. Nucl. Med. Vol. 23 No. 1 & 2 2020

Doi: <https://doi.org/10.3329/bjnm.v23i1-2.57703>

INTRODUCTION

Newborn children with Congenital Hypothyroidism (CH) show no or some signs that is unrecognized or mild. But, if it is not treated earlier it may cause mental impairment and growth retardation in newborns. In many countries, neonatal thyroid screening programs are done for early diagnosis and treatment of CH. The incidence of CH is around 1:3000 to 1:4000 infants (1).

The first CH screening was done by Dussault, in Quebec-Canada in 1972. They found 7 hypothyroid babies among 47000 babies for 3 years (2). In the meantime, radioisotope tagged antibodies for determining TSH in DBS was started in the USA and Europe. In the initial stage, the method introduced by Dussault was just a confirmatory test (3). Newborn screening for the diagnosis of CH first started in Bangladesh in 1999, using Immunoradiometric assay (IRMA) (4).

A time-resolved fluoroimmunoassay named Dissociation Enhanced Lanthanide Fluorescent Immuno Assay (DELFI A) was started in 1986 for its sensitivity, speed, and non-radioactive methodology (5,6). DELFI A is a time-resolved fluorescence (TRF) intensity technology that can detect the presence of a hormone using lanthanide chelate labeled reagents like Europium, Samarium, and Terbium instead of any radioisotope. Sensitivity is

increased because of the dissociation-enhancement principle. There is a big difference between excitation and emission peaks that can decrease the signal to noise ratio. The Europium chelate is excited at 320 or 340 nm and Fluorescence is measured at 615 nm (7,8).

Despite the accuracy of the test programs, nearly 5% of CH cases may remain undetected in any screening program. The cause of failure may be sampling or unsatisfactory analysis (9). For solving these problems, a software based automated analyzer is required which can analyze the samples without any man-made error of analysis. Auto DELFIA 1235 automatic immunoassay system can analyze the samples only by using software without any manual punching or pipetting error (10).

MATERIALS AND METHODS

(a) Reagents

Reagents were bought from PerkinElmer Life and Analytical Sciences, Wallac Oy, Mustionkatu 6, Turku, Finland. Six different standards of 0.8, 10.3, 24.5, 49.3, 93.2, 239 μ U/mL were used. All of them were prepared by using Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, and < 0.1% sodium azide as a preservative. Standards have been calibrated against WHO international standards. Anti-human alpha-fetoprotein (hAFP)-Eu tracer was mixed with Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, and <0.1% sodium azide as a preservative. Wash concentrate of Tween 20 was added with Tris-HCl buffered (pH 7.8) salt solution and Germall II as a preservative. The enhancement solution was the mixture of Triton X-1002, acetic acid, and chelators.

(b) Instruments

Panthera Puncher, Plate processor 1235 automatic immunoassay system, and specimen gate software were paralleled for analysis. Panthera Puncher is a next-generation automatic punching machine that can punch 9 plates at a time and punched information can be exported to plate processor AutoDELFI A manager 3.0. After analyzing by AutoDELFI A workstation 3.0, all of the results go to the specimen gate.

DBS were punched into an antibody-coated microtitration well with an automated Panthera Puncher™. After completing punching, the plate was ejected from the Panthera puncher. All of the sample information on the plate were exported to AutoDELFI A software through the LIS system. If any sample was not exported to Auto DELFI A worksheet, information can manually be given through the plate generator. When plates were inputted to the AutoDELFI A workstation, AutoDELFI A manager 3.0 demand sufficient reagents and liquids. After the addition of all of the required reagents amount, command is given to Auto DELFI A manager 3.0, for analyzing the samples, and the report comes to the result viewer and finally to the specimen gate for generating the final report.

DATA ANALYSIS

Obtained data were analyzed by using Microsoft excel. Regression, standard error and percent recovery was calculated from excel equations.

RESULTS AND DISCUSSION

(a) Calibration curve: A standard calibration curve (Figure 1) was drawn by using six different standards of 0.8, 10.3, 24.5, 49.3, 93.2, 239 μ U/mL (Table 1). Table 2 shows the regression analysis of calibration curve.

Table 1: NTSH counts of standard samples

Serial No.	Blood Concentration	NTSH Counts
1	0.8	1253
2	10.3	7550
3	24.5	17379
4	49.3	36550
5	93.2	66008.5
6	239	155325.5

Figure 1: Calibration curve of the standard known samples

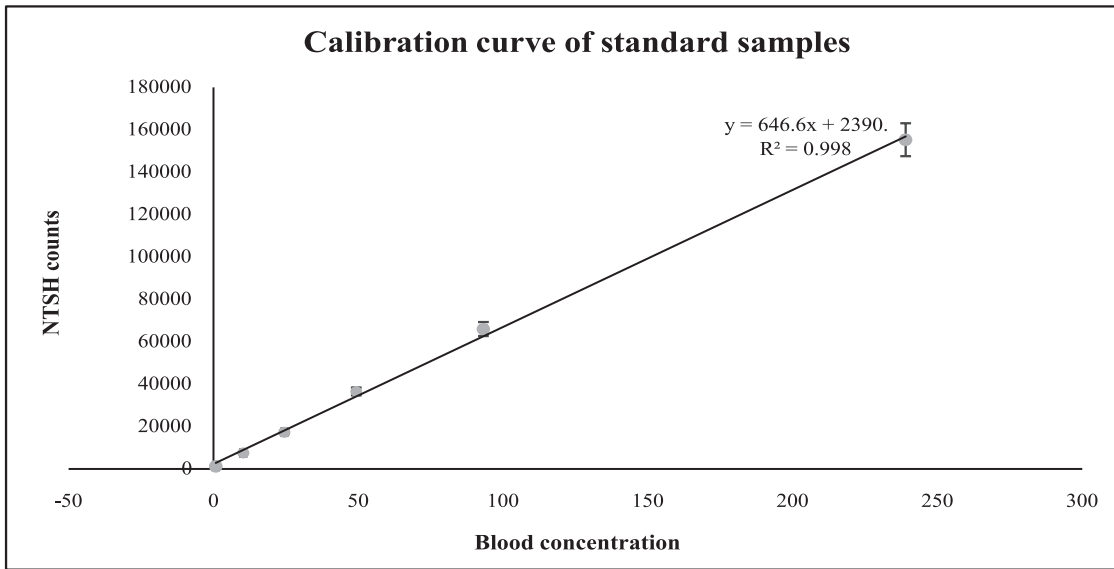


Table 2: Regression analysis of calibration curve

Serial no.	Topic	Value	
		Intercept	X variable
1	Standard error	1334.02	12.4
2	Standard deviation	3267.70	30.5
3	Limit of Detection (LOD)	16.7	0.16
4	Limit of Quantification (LOQ)	50.53	0.47

The limit of detection is the lowest analyte concentration that can be analyzed from samples. In this method, LOD is quite lower which indicates higher sensitivity of the analytical method. An analytical method is simply not capable of measuring the analytical concentration to zero. There is some limit of Blank. In this assay, the LOD of X variable means TSH concentration in the blood and is 0.16 and for intercept means NTSH count is 16.7.

LOQ is another important analytical term in the assay of biological samples for the determination of TSH. The LOQ of this assay for the X variable is 0.47. It indicates that biological samples with lower amounts of TSH can be analyzed with good accuracy and precision. For the DBS collected from the umbilical cord blood, the cut off value was set to 20 μ U/mL.

Table 3: Percent of recovery of known standard samples in the calibration curve

Serial no.	Standard Concentration	NTSH counts	Found Concentration	% Recovery
1	0.8	1253	-1.76	-219.8
2	10.3	7550	7.98	77.47
3	24.5	17379	23.18	94.61
4	49.3	36550	52.82	107.15
5	93.2	66008.5	98.38	105.56
6	239	155325.5	236.5	98.95

In the calibration curve, the percent recovery of all X coordinates was approximately 100 percent except for one value, which was quite close to the LOQ value. Except for this value, the average of the percent recovery was 96.7 (Table 3). R^2 value of the calibration curve was 0.9985. The value of R^2 lies between -1 to +1. For this calibration curve, the value of R^2 was quite closer to 1 which indicates a good calibration curve for unknown sample analysis. The accuracy of the standard samples was 96.7 ± 10.6 .

(b) Quality control:

Quality control (QC) is very important for every assay because it represents whether the assay is working well or not. For this assay, two QC of 16.39 $\mu\text{U/mL}$ and 63.09 $\mu\text{U/mL}$ was used. These two QC values (Figure 2 and Figure 3) were measured by using standard calibration curves and were repeated the same sample to 144 times. All of the values were close to ± 1 standard deviation.

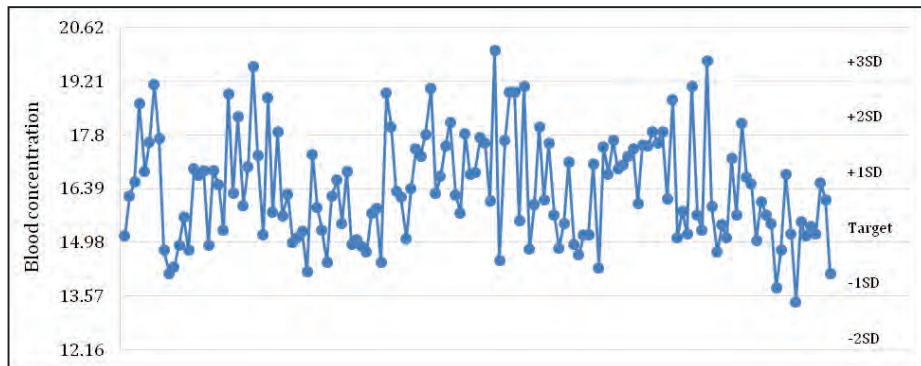


Figure 2: Variation of quality control 1

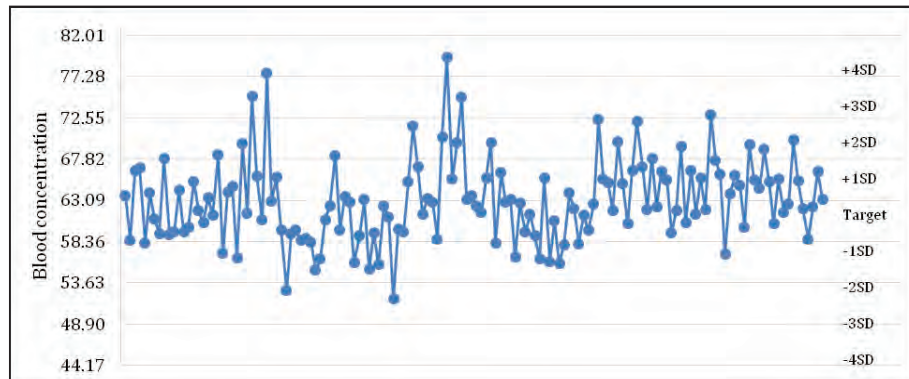


Figure 3: Variation of quality control 2

The standard calibration curve was drawn repeatedly twelve times (Figure 4). The target value of the intercept was 652.86. Every time this NTSH intercept value was near to the target value in the range of ± 2 standard deviations.

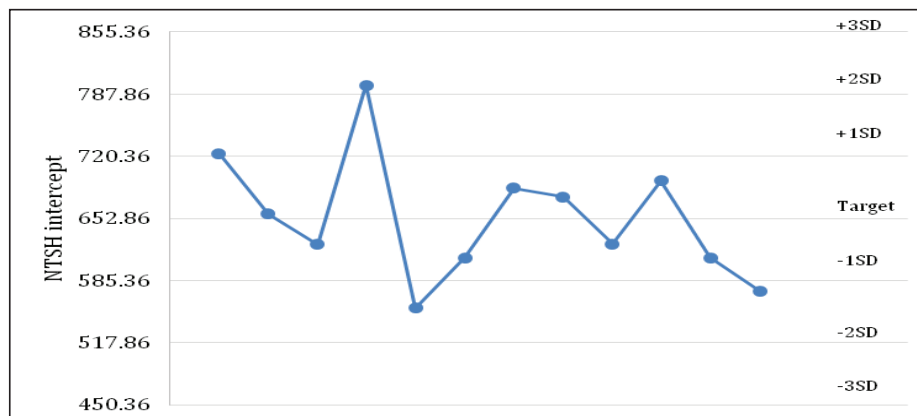


Figure 4: Graphical representation of the intercept variation of twelve celebration curves.

The target value of the NTSH slope was 704.50 (Figure 5). All of the values of slope in the different calibration curve were near to the target value in the range of ± 2 standard deviations.

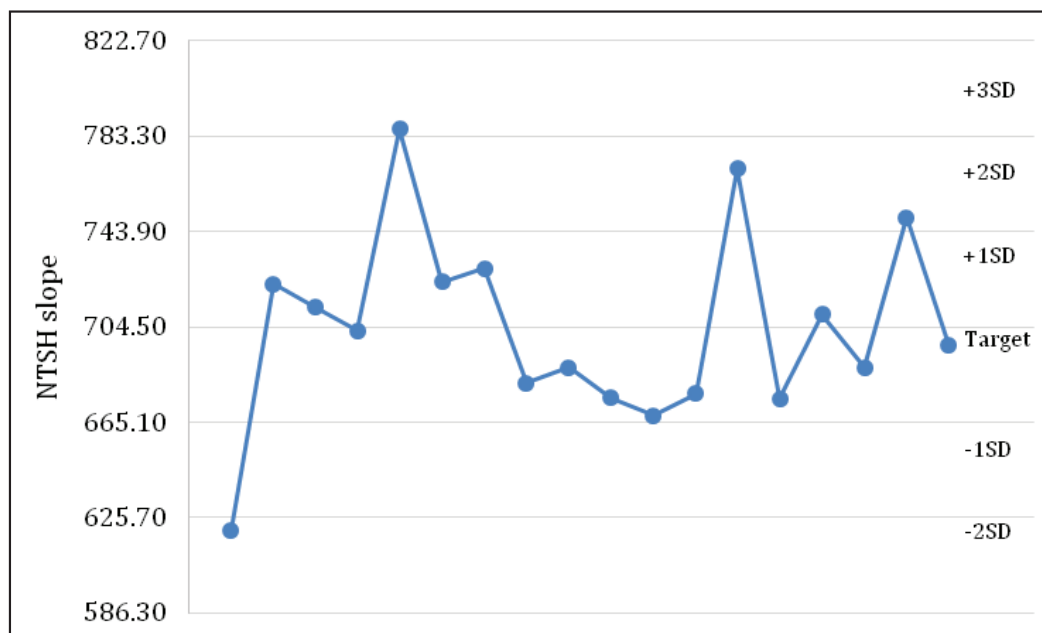


Figure 5: Variation of NTSH slope in 18 calibration curve.

CONCLUSION

The above finding stated the suitability of AutoDELFLIA System for the CH screening from dried blood spots (DBS) in the newborn screening laboratory of the In vitro division of NINMAS and it can detect a very low range of thyroid-stimulating hormone concentrations up to 0.16 $\mu\text{U/mL}$. Auto DELFLIA 1235 automatic immunoassay system was introduced with the funding from the ADP Project “Screening of Congenital Hypothyroidism in Newborn Babies (Phase 2)”, jointly implemented by Ministry of Science & Technology (MOST) and Bangladesh Atomic Energy Commission (BAEC). This system is also the latest and first-time addition in the field of newborn screening research and service in Bangladesh.

Acknowledgment

We are thankful to all laboratory staff of the In vitro Division of NINMAS for their immense participation in the ongoing congenital hypothyroidism screening program, despite of the heavy regular workload. We are also thankful to the respected directors Institute of Nuclear Medicine & Allied Sciences (INMAS) for their continuous support for the collection of DBS samples from all over the country. We also want to pay our special gratitude to the Ministry of Science and

Technology (MOST) of the Government of the People’s Republic of Bangladesh for funding the Project.

REFERENCES

1. Büyükgebiz A. Congenital hypothyroidism clinical aspects and late consequences. *Pediatr Endocrinol Rev.* 2003;1 Suppl 2: 185–90; discussion 190.
2. Deladoey J, Ruel J, Giguere Y, Van Vliet G. Is the incidence of congenital hypothyroidism really increasing? A 20-year retrospective population-based study in Quebec. *The Journal of Clinical Endocrinology & Metabolism.* 2011 Aug 1;96(8):2422-9.
3. Dilli D, Özbaş S, Acican D, Yamak N, Ertek M, Dilmen U. Establishment and development of a national newborn screening programme for congenital hypothyroidism in Turkey. *Journal of clinical research in pediatric endocrinology.* 2013 Jun;5(2):73.
4. Website. [Cited 7 Sep 2020]. Available: https://www.researchgate.net/publication/51439695_Newborn_screening_in_Bangladesh
5. Rose SR, Brown RS, American Academy of Pediatrics, American Thyroid Association, Public Health Committee. Update of newborn screening and therapy for congenital hypothyroidism. *Pediatrics.* 2006 Jun 1;117(6):2290-303.
6. Büyükgebiz A. Newborn screening for congenital hypothyroidism. *Journal of clinical research in pediatric endocrinology.* 2013 Mar;5(Suppl 1):8.
7. McDade TW, Williams S, Snodgrass JJ. What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers

- into population-based research. *Demography*. 2007 Nov 1;44(4):899-925.
8. National Library of Medicine (US). Index Medicus. US Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Library of Medicine; 2004.
 9. Marr A, Yokubynas N, Tang K, Saleh D, Wherrett DK, Stein R, Bassilious E, Chakraborty P, Lawrence SE. Transient vs Permanent Congenital Hypothyroidism in Ontario, Canada: Predictive Factors and Scoring System. *The Journal of Clinical Endocrinology & Metabolism*. 2022 Mar;107 (3):638-48.
 10. Rose SR, Brown RS, American Academy of Pediatrics, American Thyroid Association, Public Health Committee. Update of newborn screening and therapy for congenital hypothyroidism. *Pediatrics*. 2006 Jun 1;117(6):2290-303.