Neonatal TSH Screening Assay (ELISA)

BornSafe™
TSH

Enzyme immunoassay for the quantitative determination of Thyroid Stimulating Hormone (TSH) in New born dried blood spots

(FOR IN VITRO DIAGNOSTIC USE ONLY)

1. INTENDED USE

Born Safe™ Neonatal TSH Screening ELISA is an enzyme immunoassay for the quantitative determination of human thyroid stimulating hormone in dried blood samples spotted on Whatman S&S 903 filter paper. This kit is particularly suitable for screening for primary hypothyroidism in newborns. Elevated results indicate the need for further study to assess congenital hypothyroidism.

2. SUMMARY AND EXPLANATION OF THE ASSAY

Human thyroid stimulating hormone (thyrotropin) is a glycoprotein hormone with a molecular weight of 28,000 Daltons, synthesized in and secreted from the anterior lobe of the pituitary gland. It stimulates the production of thyroid hormones by the thyroid gland. Thyroid Stimulating Hormone (TSH) is responsible for providing the primary stimulus for the synthesis and secretion of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). This glycoprotein hormone is secreted in the anterior pituitary gland, under the control of thyrotropin releasing factor (TRH), produced in the hypothalamus. The thyroid hormones produced under the direction of TSH exert a negative feedback on the pituitary gland, which regulates the secretion of TSH[1]. This negative feedback relationship between the thyroid and pituitary glands implies that TSH is always elevated in primary hypothyroidism, often to very high levels. It is, therefore, the most sensitive test of hypothyroidism, including patients whose T4 values are still within the normal range[2].

Early fetal development of the thyroid gland seems to be independent of the pituitary. TSH-secretion from the pituitary does not start until the end of the first trimester of pregnancy after which the secretion of TSH increases rapidly and reaches a level higher than the maternal level. TSH and thyroid hormones are not transported through the placenta. Consequently the pituitary-thyroid functions of the fetus are independent of those of the mother[3].

Immediately after birth a rapid increase in TSH concentration - known as the TSH surge - is seen in neonatal blood. The concentration stabilizes to a lower level a few days after birth, if the baby's thyroid is functioning normally[4].

An elevated TSH concentration in infant blood is the earliest available laboratory manifestation of primary hypothyroidism. Due to its high specificity and sensitivity, TSH testing is the screening method of choice for the detection of neonatal hypothyroidism. Since a clinical diagnosis is difficult to establish and the condition needs early medical attention, large scale laboratory screening programs have been implemented in many countries to detect neonatal hypothyroidism[5]. Congenital hypothyroidism is probably the single most common preventable cause of mental retardation. Transient and mild hypothyroidism can also occur, especially in very preterm or very low birth weight infants[6,7]. Studies have shown that the early clinical diagnosis and subsequent treatment of this disorder, usually within the first two weeks after birth, tends to prevent irreversible mental retardation, neurologic dysfunction or disabling[5,8,9,10].

It has been suggested that the most effective method of assessing the infant's thyroid function is a combination of a T4 and TSH screening program. This is due to the fact that TSH screening may miss hypothyroidism of the secondary type, while some T4 determinations may miss minimal hypothyroidism. Therefore, the combination of T4 and TSH affords the clinician with the best possible overview of the infant's thyroid state[5,9,11]. Infants suspected of marginal or borderline hypothyroidism by virtue of the blood spot screening procedures should have confirmation test, performed by using serum T3, T4, and TSH determinations as well as other thyroid tests prior to initiating therapy, such as thyroid controlled stimulation by TSH, scintigraphy or sonography.

Concentrations of TSH and T4 have been shown to vary with demographic variations, infant age, gender, weight, and prematurity. Therefore, it is important that each laboratory determines its own normal and cut-offs with infant age taken into account[6].

In this method, TSH dried whole blood calibrator, patient specimen or control is first added to a anti-TSH coated well. Elution reagent is added and the reactants mixed. Reaction between the anti-TSH and the TSH in the dried blood spot forms a complex that is immobilized to the well.

After the completion of the first elution/incubation period, the enzyme conjugate is added to the Ag-Ab complex immobilized on the plastic surface. The enzyme labeled anti-TSH antibody binds to the TSH making a sandwich complex with two antibodies bound to the antigen during a second incubation. The microplate is washed to remove unreacted enzyme. Finally, the activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

3. PRINCIPLE OF THE ASSAY

Born Safe™ Neonatal TSH Screening ELISA is an immunoenzymatic assay to quantitate human thyroid stimulating hormone (TSH) in newborn dried blood spots.

Born Safe ™ Neonatal TSH Screening ELISA is a sandwich ELISA.

The essential reagents required for a sequential immune-enzymatic assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess**, and native antigen.Upon mixing of dried blood spots with Elution Reagent, reaction results between the eluted antigen and immobilized anti-TSH antibody to form an antigen-antibody complex. The interaction is illustrated by the following equation:

$$Ag_{(TSH)} + {}^{Imm}Ab_{(m)} \underset{k\text{-}a}{\overset{ka}{\rightleftharpoons}} Ag_{(TSH)} \text{--} {}^{Imm}Ab$$

^{Imm}Ab = Immobilized Antibody (Excess Quantity),$Ag_{(TSH)}$ = TSH protein, Antigen (Variable Quantity)

$Ag_{(TSH)}$ --^{Imm}Ab =Antigen-Antibody complex (Variable Quantity)

Ka= Rate Constant of Association, K-a = Rate Constant of Dissociation

After a suitable incubation period, unreacted reactants and remnants are washed off via wash step. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen- antibody complex on the surface of the wells.

$$^{Enz}Ab + Ag_{(TSH)} \text{--} {}^{Imm}Ab \underset{k\text{-}b}{\overset{kb}{\rightleftharpoons}} {}^{Enz}Ab \text{--} Ag_{(TSH)} \text{--} {}^{Imm}Ab$$

^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)

$^{Enz}Ab – Ag_{(TSH)} – {}^{Imm}Ab$ = Antigen-Antibodies Complex

kb = Rate Constant of Association, k-b = Rate Constant of Dissociation

Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate ELISA reader.

The enzyme activity on the well is directly proportional to the TSH concentration in the dried blood spot. By utilizing several different dried spots of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4. PRESENTATION

REF	Pack Size
408010096	96 Assays

5. KIT COMPONENTS:

Reagents:.

1. **TSH Calibrator Level C0 to C5 –Dried Blood Spots in Aluminium pouch (One row of six spots levels - 1 x 6):** Six (6) levels of TSH Antigen in dried blood spots at approximate concentrations of 0(C0), 5(C1), 25(C2), 50(C3), 105(C4) and 150(C5) µIU/ml spotted on S&S Whatman 903 filter paper. Store at 2-8°C. Desiccant is included.
   Note 1: The Lot Specific calibrators are whole human blood based, were calibrated against the WHO (NIBSC) 3rd, International Standard (81/565) and 5th ISNS Reference Preparation for Neonatal Screening (5th ISNS-RPNS), RIVM, Bilthoven, Netherlands.
   Note 2: **The exact TSH values are printed on the lot specific quality control certificate included in the kit.(values mentioned here are for illustration purpose only)**
2. **TSH Control Level L1 and L2 –Dried Blood Spots in Aluminium pouch (One row of two spots – L1 x L2)**: Two levels of whole human blood controls with different concentrations of TSH antigen spotted on S&S type 903 filter paper. Store at 2- 8°C. Desiccant is included.
   Note 1: The controls are whole human blood based, were manufactured to fall within significant clinical ranges using WHO 3rd, international standard (81/565)12.
   Note 2: **The exact controls values are measured and printed on the lot specific quality control certificate included in the kit.**
3. **TSH Enzyme Reagent – 14.0 ml**
   One vial containing enzyme labelled monoclonal antibody to human TSH in buffer, and preservative. Store at 2-8°C.
4. **Extraction Reagent – 14.0 ml**
   One vial containing protein, stabilizers and surfactant in buffer, and preservative. Store at 2-8°C.
5. **Substrate Solution – 15.0 ml**
   One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.
6. **Stop Solution – 7.0 ml**
   One vial containing a mineral acid. Store at 2-8°C.
7. **Wash Solution – 22.0 ml**
   One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
8. **Coated Microwells**: One 96-well microplate coated with anti-TSH antibody and packaged in an aluminium bag with a drying agent. Store at 2-8°C.
9. **Sealing tapes– 3 nos**
10. **Product Instructions--** Details of the Kit and have testing methodology

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.

Note 3: Above reagents are for a single 96-well microplate.

Materials	Quantity (96T)	Physical State
TSH Calibrator Level C0 to C5	1 set (6 levels)	Ready to use
TSH Control Level L1 and L2	1 set (2 levels)	Ready to use
TSH Enzyme Reagent	1 vial (14.0 ml)	Ready to use
Extraction Reagent	1 vial (14.0 ml)	Ready to use
Substrate Solution	1 vial (15.0 ml)	Ready to Use
Stop Solution	1 vial (7.0 ml)	Ready to use
Wash Solution	1 vial (22.0 ml)	Concentrated X 40
Coated microwells	96 (12 x 8)	Ready to use
Sealing tapes	3 sheets	Ready to use
Pack Insert	1 No.	
Protocol Sheet	1 No.	
Microwell Holder	1 No.	

6. STORAGE AND STABILITY OF THE KIT

1. Store the kit and reagents at 2-8°C. Calibrators and Controls should be stored protected from moisture and light in the original bag with desiccant. Stable at 2-8°C until expiry date stated on the label. Make sure that the plastic bag remains sealed during storage.
2. **We recommend that the Blood Spots (Calibrators and Controls) should be preferably stored at -20°C with desiccants when not in use for prolonged periods.**
3. Unopened reagents will retain reactivity until expiration date shown on the label. Do not use reagents beyond this date.
4. Microtiter wells must be stored at 2- 8°C. Once the foil pouch has been opened care should be taken to seal it tightly again.
5. Equilibrate all kit components to ambient temperature (18-25°C) before use.
6. Avoid extended exposure to heat and light.

7. MATERIALS REQUIRED BUT NOT PROVIDED

1. Dispensers for repetitive deliveries of 0.050ml, 0.100ml, and 0.350ml volumes with a precision of better than 1.5% (optional)
2. Microplate washer or a squeeze bottle (optional)
3. Microplate reader with 450nm and 620nm wavelength absorbance capability. (The 620nm filter is optional)
4. Absorbent paper for blotting the microplate wells.
5. Aluminium foil wrap or microplate cover for incubation steps.
6. Timer
7. Storage container for storage of wash buffer
8. Distilled water or deionized water
9. 1/8"Blood spot puncher for dispensing 3.2 mm dried blood spots.
10. Orbital plate Shaker (100 to 1100 rpm) with orbital diameter 2mm.
11. Blood collection cards [Whatman S&S 903 recommended;CLSI NBS01-A6 compliant]

8. WARNINGS AND PRECAUTIONS

A thorough understanding of the pack insert is mandatory before performing the test for the first time. Adherence to the protocol specified herein is necessary to ensure optimal performance of the product. Any deviation from the assay procedure may affect the results.

Operating: **In order to obtain reproducible results, the following rules must be observed:**

1. Do not mix reagents of different lots.
2. Do not use reagents beyond their expiry date.
3. Use thoroughly clean glassware.
4. Use distilled water, stored in clean containers.
5. Avoid any contamination among samples; for this purpose, disposable tips should be used for each sample and reagent.
6. Keep all reagents at normal refrigerator temperature (2-8°C) in closed containers when not in use, but ensure that all reagents are equilibrated to 18-25°C before use. Keep Blood Spot Standards and Controls at normal refrigerator temperature (2-8°C) in the original foil pouch containing desiccant when not in use, but ensure that spots are equilibrated to 18-25°C before use.
7. The strips, calibrators and internal controls are packed in an outer aluminium pouch containing a desiccant. Immediately after removal of strips, the remaining strips should be resealed or closed with a Scotch tape in the outer bag along with the desiccant and stored at 2-8°C. It is important to ensure the desiccant remains in the bag. **If the desiccant has turned pink, ensure to replace the pink coloured desiccant (inactivated) with Blue coloured desiccant (activated)**
8. Do not use reagents from other manufacturers along with the kit reagents for a given test run.
9. Do not interchange reagent vials and their screw caps to avoid cross-contamination. Use a clean, fresh, disposable pipette tip for each reagent or specimen manipulation.
10. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.

Safety: **In order to avoid personal and environmental contamination, the following precautions must be observed:**

1. Use disposable gloves while handling potentially infectious material and performing the assay.
2. Do not pipette reagents by mouth.
3. Do not smoke, eat, drink or apply cosmetics during the assay.
4. All material of human origin used for the preparation of this kit tested negative for HBsAg, anti-HIV and anti-HCV. Since no test at present can guarantee complete absence of these viruses, all samples and reagents used for the assay must be considered potentially infectious. Therefore, the assay waste must be decontaminated and disposed off, in accordance with established safety procedures.
5. Disposable ignitable material must be incinerated; disposable non-ignitable material must be sterilized in autoclave for at least 1 hour at 121°C. Liquid wastes must be added with sodium hypochlorite at a final concentration of 3%. Let the hypochlorite act for at least 30 minutes. Liquid wastes containing acid must be neutralized with appropriate amounts of base, before treating with sodium hypochlorite.
6. Avoid splashing and aerosol formation; in case of spilling, wash carefully with a 3% sodium hypochlorite solution and dispose of this cleaning liquid as potentially infectious waste.

9. SPECIMEN COLLECTION AND HANDLING

Blood samples should ideally be collected between the third and the fifth day of life (48 to 120 hours after birth) and should be taken directly from a heel prick onto filter paper. Neonatal screening programs differ from one another in the type of specimen required, the recommendation is a blood spot, approximately 12.7 mm (½ inch) in diameter, collected by heel prick and spotted onto filter paper (Whatman Schleicher & Schuell 903). The specimen collection device must comply with national regulations. A method based on dried blood samples requires skillful collecting, handling and transport of samples.

The collection technique is described in detail in CLSI document LA4-A5, and the main points are listed below.

- Blood from the new-born's heel should be collected **ONLY** from the medial (closest to the body center-line) or lateral portion (furthest from the body center-line) of the planter surface (walking surface).
- Blood collection from other areas of the infant's foot, *e.g.* arch, may result in nerve, tendon or cartilage injury.
- Clean the skin with an alcohol swab and allow to air-dry.
- Puncture the infant's heel with a sterile lancet or with a heel incision device to the depth of approximately 2.0 mm. Puncturing deeper than 2.0 mm on small infants may cause bone damage.
- Wipe away the first drop of blood. Gently touch the filter paper against a large drop of blood and, in one step, allow a sufficient quantity of blood to soak through to completely fill a pre-printed circle on the filter paper. Examine both sides of the filter paper to make sure that the blood has penetrated and saturated the paper. Excessive milking or squeezing the puncture may cause haemolysis of the specimen or an admixture of tissue fluids with the specimen. Do not layer successive drops of blood in the collection circle (this causes caking).
- Allow the blood specimen to air-dry in a horizontal position for at least 4 hours at ambient temperature (18-25°C). Do not heat or stack the specimens during the drying process.
- Arrange transport of the collection card to the screening laboratory within 24 hours of collection.
- Store in sealed paper envelopes or containers that will provide protection from moisture, light, heat and contact with other materials
- The sample discs should be punched from similar areas on each individual blood spot. Do not punch sample discs from areas that include printed marks or that are near the edges of the blood spot.
- Be sure that the required information on the specimen collection card has been completed. The minimum pre-printed information required on the collection device includes:
  - last name (and first, if available), sex, birth date (optional: time of birth), birth weight and age of the infant; (indicate if < 24 h), and patient identification number
  - the first and last name of the mother
  - date of specimen collection (optional: time of collection)
  - the name and address of the submitter (optional: birth facility)
  - the name and phone number of the physician (health care provider)
  - the name of the new born screening program and address
  - each card should have a unique serial number and an expiration date.
- Specimens should not be placed in hermetically sealed containers (e.g. plastic or foil bags). If required, sufficient desiccant packages must be included. Humidity and moisture are detrimental to the dried blood spot specimen.
- Before placing the specimens in a container for transport, the dried blood spots on the collection cards should be separated by a physical barrier from the blood spots on the cards in the stack immediately above and below. The blood spots can also be protected by a fold-over cover attachment or by placing glassine paper between the specimens.

10. REAGENTS PREPARATION

Wash Buffer

Empty contents of wash solution concentrate in 858 ml of distilled or deionized water in a suitable storage container. Store at room temperature 2-30°C for up to 60 days.

Note1: Do not use reagents that are contaminated or have bacteria growth.

Note2: Do not use the substrate if it looks blue.

11. ASSAY PROCEDURE

A. Before proceeding with the assay, bring all reagents and patient samples to room temperature (20 - 27° C). Test Procedure should be performed by a skilled individual or trained professional.

1. Obtain the required number of microwells for each calibrator, control and patient sample to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C with activated desiccant.
2. Punch out a 3.2 mm blood dot out of each calibrator, control and specimens into the assigned wells. (**Note**: Do not punch blood dots from areas that are printed or that are near the edge of the blood spot).
3. Add 100µl of Extraction Reagent to all the wells.
4. Shake the microplate gently for 20-30 seconds to mix. (**Note**: Make sure that all blood dots are fully submerged in the liquid and not stuck to the walls of the microwells).
5. Cover with a sealing tape and rotate for 60 minutes at ambient temperature using a laboratory rotator set at 900rpm.
6. Discard the contents of the microplate by decantation, blot and tap the plate dry with absorbent paper. **Note**: Make sure all the blood dots are removed at this point. There should be no dots left in the microwells.
7. Add 350 µl of diluted wash buffer (see Reagent Preparation Section) to each well, decant (tap and blot) or aspirate. Repeat the same Two (2) additional times for a total of Three (3) washes. Follow the manufacturer's instruction while using automatic or manual plate washer for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash buffer. Decant the wash and repeat two (2) additional times.
8. Add 100 µl of TSH Enzyme Reagent to each well.
9. Cover the microplate with a sealing tape and rotate for 60 minutes at ambient temperature using a laboratory rotator set at 900rpm.
10. Repeat Step # 6 and then Step #7.
11. Add 100 µl of substrate solution to each well.
12. Cover the microplate with an aluminium foil and incubate for 15 minutes at ambient temperature (in dark without shaking).
13. Add 50 µl of stop solution to each well and gently mix for 15-20 seconds. **Note**: Always add reagents in the same order to minimize reaction time differences between wells.
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within fifteen (15) minutes of adding the stop solution.

B. CERTIFICATION/TRACEABILITY TO REFERENCE MATERIAL

The Lot Specific Blood Spot Standards and Controls provided with the Neonatal TSH Screening Assay kit is traceable to the WHO (NIBSC) 3rd, International Standard (81/565) and 5th ISNS Reference Preparation for Neonatal Screening (5th ISNS-RPNS), RIVM, Bilthoven, Netherlands.

12. CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of thyrotropin in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate blood reference versus the corresponding TSH concentration in µIU/ml on graph paper (average the duplicates of the blood references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of TSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µIU/ml) from the horizontal axis of the graph.

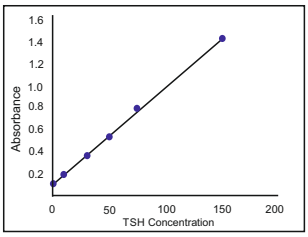
Note: Computer data reductions software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained. **A four parameter logistic curve is recommended to calculate the results (Do not use linear regression curve)**.

EXAMPLE 1

The data presented in Example 1 and Figure 1 are for illustration only and should not be used in lieu of a dose response curve prepared with each assay. A four parameter logistic curve is recommended to calculate the results.

Calibrator	Concentration(µIU/ml)	Absorbance
C0	0.5	0.058
C1	10.1	0.161
C2	30	0.32
C3	50.1	0.5
C4	75	0.762
C5	150	1.394

Fig.1



Note: **TSH values are expressed in whole blood units**. For conversion of units, use the following relationship: (µIU/ml blood = µIU/ml serum / 2.2)

13. QUALITY CONTROL

Internal controls (L1-L2) included in the kit should be routinely monitored to check that measured concentrations stay within the stated values. These controls provide valuable information regarding if the kit is working according to manufacturer specifications.

The assay run is acceptable if the mean concentration for each Control is within the range quoted by the manufacturer for each Control.

The assay is unacceptable if values for either of the Blood Spot Controls fall out with the specifications and patient sample results should not be reported. An investigation into the reasons for the assay failure must be undertaken immediately. Each laboratory should initiate and document appropriate quality assurance control systems for monitoring and sustaining the accuracy of test results. Users may wish to include further in-house controls and/or Reference materials if available, in addition to Controls L1 and L2. Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. The stability and storage conditions of these additional controls and the criteria for assay acceptance/rejection should be determined by each user laboratory. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

Participation in External Quality Control programs e.g. CDC Infant Screening Performance Surveillance Program is also recommended.

Note:In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator C5 should be >0.7
2. **Values in this kit are expressed in µIU/ml whole blood.**

14. EXPECTED VALUES AND INTERPRETATION CRITERIA

Please note that the values mentioned in this section should only be used as a guideline, and each laboratory should establish its own specific cut-off and reference range based on the specimens from the laboratory routine population and also a procedure for the follow up of newborns from which a 'presumptive positive' specimen was received The measurement of hTSH from dried blood spots as an aid in diagnosing congenital hypothyroidism is based on the use of a cut-off value, which distinguishes between euthyroid and hypothyroid neonates.

Test results may vary based on infant age at the time the blood is drawn as well as other conditions. Caution must be exercised in correlating the laboratory result to clinical status with specimens from new born less than 48 hours after birth, premature and low birth weight newborns and hospitalised sick newborns. A review of various published NBS programs study outcomes and referring recommended guidelines of national and international organizations such as ICMR, IAP, ISPAE, AAP & CDC, following guidelines are suggested; For term neonates of 2 to 6 days old, following cut-off values are suggested.

Suggested Cut off Values:

Interpretation	Concentration (µIU/ml)
Presumptive Negative	<10 µIU/ml
Borderline	(Repunch)10 – 20 µIU/ml
Presumptive Positive	>20 µIU/ml

Elevated TSH values without proper congenital hypothyroidism have been reported in certain conditions:

- transient neonatal hypothyroidism seen in newborn infants in areas of iodine deficiency. This condition is usually more severe in premature infants.
- transient benign hyperthyrotropinemia in newborn infants may occur in response to intrauterine drug exposure, intrauterine iodine exposure or intrauterine iodine deficiency in endemic goiter areas.

15. LIMITATIONS OF THE PROCEDURE

As with any other in vitro screening test, the test results obtained using Neonatal TSH Screening Assay, should be used as an aid to other medically established procedures and results interpreted in conjunction with other clinical data available to the clinician.

Born Safe™ Neonatal TSH Screening ELISA is a screening method for measuring the TSH concentration in newborn dried blood spot specimens.

Elevated results are not diagnostic per se of primary congenital hypothyroidism, but indicate the need for further study of the newborn from which a presumptive positive specimen was received.

- To ensure accurate and reliable results, make sure that all blood spot disks are within the extraction solution during the incubation period.
- Strict adherence to the protocol is advised to obtain reliable results. Any modification or change made to the kit or the assay procedure are under the responsibility of the user.
- This assay is designed to be used with dried blood specimens that are exclusively collected on Whatman's Filter Paper 903.
- Do not use cord blood.
- Demographic variations, infant weight, age, prematurity and twinning can affect the TSH concentrations. Laboratories should be aware of all these factors.

Conditions which are known to cause anomalous analytical assay results are:

- sample spot not uniformly saturated with blood
- sample spots punched too close to the edge of the blood spot
- poorly collected and improperly dried specimens
- non-eluting blood spot due to deterioration of sample caused by exposure to heat and humidity contamination of blood spot filter paper with fecal material.

16. COMPLAINTS

Complaints can be accepted in written format (preferably on the manufacturer's complaint form). All details of the test kit, as well as the test results, can be included. A copy of the complaint form is available from Tulip Diagnostics Pvt Ltd. upon request.

17. REFERENCES

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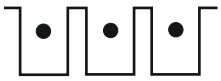
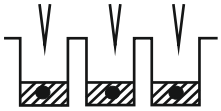
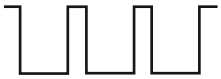
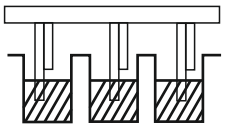
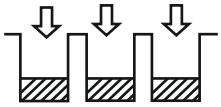
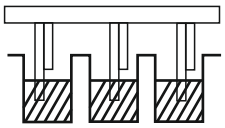
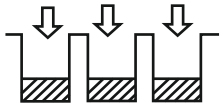
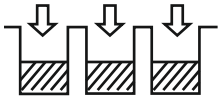
Summary Protocol

BornSafe™
TSH

REAGENT PREPARATION

Reconstitution of Wash Solution	 858 ml D/W + 22 ml (Wash Solution)	Dilute 22 ml of Wash Solution in 858 ml Deionized/Distilled water

ASSAY PROCEDURE

1. Punch out Calibrators, Controls and patient's sample in coated microwells		One blood spot into each well of anti-TSH coated microwell strips
2. Add 100 µl Extraction Reagent and Shake gently for 20-30 secs		Ensure that each disc is fully immersed in Extraction Reagent and not stuck to the microwell walls
3. Incubate		Cover with sealing tape and incubate for 60 min at RT (20°-26°C) on an orbital plate shaker (900 rpm)
4. Discard the contents and remove the discs		Decant the well content to remove blood dots completely, blot and tap the microwell dry with absorbent paper
5. Wash		Add 350µl wash buffer. Decant or aspirate. Repeat **2 more** times
6. Add 100µl TSH Enzyme Reagent		Cover with sealing tape and incubate for 60 min at RT (20°-26°C) on an orbital plate shaker (900 rpm)
7. Discard the contents		Decant the well content completely, blot and tap the microwell dry with absorbent paper
8. Wash		Add 350µl wash buffer. Decant or aspirate. Repeat **2 more** times
9.Add 100µl Substrate solution		Cover the microwells with aluminium foil or store in dark to incubate for 15 min at RT (20°-26°C)
10. Add 50µl Stop Solution NOTE: Add reagents in the same order to minimize reaction time differences between wells		Place the plate in a microplate reader and read at 450 nm and using reference wavelength of 620nm-630nm
11. Read/ Measure. Results should be read within 15 min of adding the stop solution.		Gently mix for 15-20 secs for uniform colouration.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	LOT Batch Number / Lot Number
Manufacturer	IVD *In vitro* Diagnostic Medical Device	This side up	Caution
Use by	REF Catalogue Number	Σ Contains sufficient for <n> tests	

Manufactured by:
Zephyr Biomedicals
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