Datasheet for ABIN7232277

NN-TSH ELISA Kit

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Overview

Quantity:	192 tests
Target:	NN-TSH
Reactivity:	Chemical
Method Type:	Competition ELISA
Detection Range:	0-250 µIU/mL
Minimum Detection Limit:	0 µIU/mL
Application:	ELISA

Product Details

Purpose:	The Quantitative Determination of Thyrotropin Concentration in Human Whole Blood by a Microplate Immunoenzymometric assay.
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	97%
Sensitivity:	1.0 µµU/mL

Target Details

Target:	NN-TSH
Abstract:	NN-TSH Products
Target Type:	Chemical

Target Details

Background:	Determination of hypothyroidism within the first few days of birth has been recognized as the single most important diagnostic test in neonates by the American Thyroid Association. The need for its early detection and treatment has resulted in the establishment of screening centers by federal and state health departments. A program of early screening of neonates for congenital hypothyroidism was started in Quebec, Canada in the early seventies. They used dry blood spots on filter paper as the sampling device. Very soon the program was followed by other major public health institutions in Canada and the US. By 1978, almost one million infants had been screened and an incidence rate of congenital hypothyroidism was established to be approximately 1 in 7000 births. Congenital hypothyroidism is probably the single most common preventable cause of mental retardation. Diagnosis and treatment of congenital hypothyroidism within the first 1-2 months after birth appears to be necessary in order to prevent severe mental retardation. Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28,000 daltons and secreted from the anterior pituitary, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism. Increase in serum concentrations of TSH, which is primarily responsible for the synthesis and release of thyroid hormones, is an early and sensitive indicator of decrease thyroid reserve and in conjunction with decreased thyroxine (T4) concentrations is diagnostic of primary hypothyroidism. The expected increase in TSH concentrations demonstrates the classical negative feedback system between the pituitary and thyroid glands. That is, primary thyroid gland failure reduces secretion of the thyroid hormones, which in turn stimulates the release of TSH from the pituitary. In this method, TSH dried whole blood calibrator, patient specimen or control is first added to a streptavidin coated well. Elution buffer containing biotinylated monoclonal antibodies are added and the reactants mixed. Reaction between the biotinylated x-TSH and the TSH in the dried blood spot forms a complex that binds with the streptavidin coated to the well. After the completion of the first elution/incubation period, the enzyme conjugate is added to the Ag-Ab complex deposited on the plastic surface. The enzyme labeled x-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.

Application Details

Comment:	Quality Control:

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. Quality control charts should be maintained - follow the performance of the supplied reagents. Pertinent statistical methods should be employed - 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 - determine the reason for the variations.

Limitations of procedure: A. Assay Performance

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of reagents should not extend beyond ten (10) minutes - avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen (s) should not be used.
4. If more than one (1) plate is used, it is recommended - repeat the dose response curve.
5. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence - eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure - remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results. 8. Use components from the same lot. No intermixing of reagents from different batches. 9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Diagnostic Automation, Inc. may be yield inaccurate results. 10. All applicable national standards, regulations and laws including but not limited to, good laboratory procedures , must be strictly followed - ensure compliance and proper device usage. 11. It is important - calibrate al the equipment e.g. Pipettes, Readers, Washers and/or automated instruments used with this device, and - perform routine preventative maintenance.

Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient sera and should not be sole basis for therapy, particularly if the results, conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Diagnostic Automation, Inc.

	5. If computer controlled data reduction is used - interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10 % of the assigned concentrations. 6. TSH concentration, in the circulation, is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary - TRH. Thus, thyrotropin concentration alone is not sufficient - assess clinical status. 7. TSH values may be elevated by pharmacological intervention. Domperiodone, amiodazon, iodide, phenobarbital, and phenytoin have been reported - increase TSH levels. 8. A decrease in thyrotropin values has been reported with the administration of propranolol, methimazol, dopamine and d-thyroxine (4). 9. Genetic variations or degradation of intact TSH into subunits may affect the biding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results among various assay systems due - the reactivity of the antibodies involved. Expected ranges of values: Recommended guidelines for newborn screening for congenital hypothyroidism have been published by the American Academy of Pediatrics (AAP). For infants 2 - 6 days old, these recommendations categorize TSH concentrations as "normal," "elevated", or "only slightly elevated" relative - values of 20 and 40 ?g/mL (i.e. per milliliter of serum). According - the AAP guidelines, "any infant with a low T4 and TSH concentration greater than 40 mU/L is considered - have primary hypothyroidism until proved otherwise." Furthermore, "in cases in which the screening TSH concentration is only slightly elevated, above 20 mU/L but less than 40 mU/L, another filter paper specimen should be obtained for a subsequent test." In order - determine the applicability of these ranges - the Neonatal TSH Elisa, a limited study of 142 newborn normal specimens (3-7 d) was done and the following range was observed. Range 0.7 ?L/mL - 34 ?L/mL It is important - keep in mind that establishment of a range of values which can be expected - be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous - the area in which the laboratory is located.
Sample Volume:	50 µL
Assay Time:	3 h
Plate:	Pre-coated
Reagent Preparation:	1 .Wash Buffer Dilute contents of wash solution to 1000 mL with distilled or deionized water in

	a suitable storage container. Store at room temperature 2-30 °C for up to 60 days. Note 1: Do not use reagents that are contaminated or have bacteria growth. Note: 2 Do not use the substrate if it looks blue.
Sample Preparation:	The sampling from neonates is performed by lancing the heels of the infants and then spotting enough whole blood on S&S filter paper card (Type# 903) to fill the marked circle. Allow the filter paper to dry at room temperature overnight away from heat and moisture. Enclose the dry blood specimen (DBS) in a moisture barrier plastic bag with desiccant and send to the laboratory. The specimen should be collected 3-7 days post partum, Physical data including age and weight of the infant, whether a multiple birth, or a premature birth etc should accompany the sample. It is important for the clinician to know these facts in order to properly assess the thyroid status of the infant. The dried blood samples are stable at 2-8 °C for 2-3 weeks if stored in zip-lock, moisture resistant bags with desiccants.
Assay Procedure:	Before proceeding with the assay, bring all reagents and patient samples to room temperature (20 - 27° C). 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. 2. Punch out a 1/8" 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 0.100 mL (100µ of NTSH Biotin 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 microplate cover and rotate for 90 minutes at ambient temperature using a laboratory rotator set @ 150rpm. (Note: see alternative overnight incubation). 6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot 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 wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. An automatic or manual

	plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times. 8. Add 100 µL of NTSH Enzyme Reagent to each well. 9. Cover the microplate and rotate for 45 minutes at ambient temperature using a laboratory rotator set @ 150 rpm. (Note: see alternative overnight incubation). 10. Repeat wash step #7. 11. Add 0.100 mL (100µ of substrate solution to each well. 12. Cover the microplate and rotate for 45 minutes at ambient temperature using a laboratory rotator set @ 150 rpm. (Note: see alternative overnight incubation). 13. Add 0.050 mL (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 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a microplate reader. The results should be read within fifteen (15) minutes of adding the stop solution. Alternative overnight procedure: 1. Substitute overnight incubation (12-16 h) for the 90 minutes with rotation (Step 5). No rotator is required. Seal the plate(s) with plastic wrap. 2. All other steps remain the same.
Calculation of Results:	The within and between assay precisions of the Neo-Natal TSH test system were determined by analyses on three different levels of pooled whole blood samples. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Tables 2 and 3. Accuracy: This Neo-Natal TSH test system was compared with a reference immunochemiluminescence assay. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from I µLu/mL - 142 µLu/mL). The total number of such specimens was 156. The least square regression equation and the correlation coefficient were computed for the Neo-Natal TSH Elisa in comparison with the reference method. The data obtained is displayed in Table 4. Only slight amounts of bias between the Neo-Natal TSH test system and the reference method are indicated by the closeness of the mean values. The least square regression

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equation and correlation coefficient indicates excellent method agreement. C.

CALCULATION OF RESULT

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 serum reference versus the corresponding TSH concentration in µLü/mL on linear graph paper (do not average the duplicates of the serum 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 µLü/mL) from the horizontal axis of the graph. In the following example, the average absorbance (0.682) intersects the dose response curve at 51.1 µLü/mL TSH concentration (See Figure 1). EXAMPLE Sample I.D. Well Number Abs (A) Mean Abs (B) Value (mIU/mL) Cal A A1 0.024 0.027 0 B1 0.030 Cal B C1 0.069 0.068 7 D1 0.067 Cal C E1 0.156 0.153 18 F1 0.150 Cal D G1 0.369 0.361 45 H1 0.353 Cal E A2 0.937 0.947 110 B2 0.957 Cal F C2 2.056 2.027 250 D2 1.998 Control E2 0.220 0.218 26.2 F2 0.216 Control G2 0.776 0.811 95.3 H2 0.846 Patient A3 0.533 0.543 66.3 B3 0.533 Rgirel 1.000 c o <0 -Q < 0.900 0.000 0 50 100 150 200 SO 300 NT5H\lues in fJU mL *The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay. Q.C.

PARAMETERS

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

1. The absorbance (OD) of calibrator F should be > 1.2

2. Four out of six quality control pools should be within the established ranges.

Restrictions:	For Research Use only

Handling

Storage:	4 °C
Expiry Date:	12-14 months