DRG® TSH (neonatal) ELISA (EIA-1483)

IVD

Revised 5 May 2011 rm (Vers. 3.1)

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

The DRG Neonatal hTSH ELISA kit is specifically designed to quantitate human thyroid stimulating hormone from neonatal blood spot samples collected on Schleicher and Schuell's filter paper #903.

1.1 INTRODUCTION AND BACKGROUND

Clinical Physiology

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 by the anterior pituitary, 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, which regulates secretion of TSH. (1)

Clinical Applications

As a result of the negative feedback relationship between the thyroid and pituitary glands, 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. (1,2)

Primary congenital hypothyroidism, caused by athyroidism and hypoplasia, occurs in 1 out of every 3,000 to 7,000 infants. (3) It is probably one of the most preventable causes of mental retardation. Studies have shown that the early clinical diagnosis and subsequent treatment of this disorder, usually within the first month after birth, tends to prevent irreversible mental retardation. (4,5)

Recent data suggests that the most effective method of assessing the infant's thyroid function is a combination of a T4 and TSH screening program. (4,5,6) This is due to the fact that some TSH screenings may miss hypothyroidism of the secondary type, while some T4 determinations may miss minimal hyperthyroidism. Therefore, the combination of T4 and TSH affords the clinician with the best possible overview of the infant's thyroid state. 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.

Concentrations of TSH and T4 have been shown to vary due to demographic variations, infant age, weight, and prematurity. Therefore, it is important that each laboratory determine its own normals and cutoffs with infant age taken into account.

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2 PRINCIPLE OF NEONATAL TSH-MW ELISA

The Neonatal TSH ELISA kit employs an enzyme-linked immunosorbent assay (ELISA) technique to quantitate Human Thyroid Stimulating Hormone in a blood spot sample. In ELISA assays, two complementary antibody configurations are generated against different portions of the same antigen. In the Neonatal TSH ELISA kit, one antibody is bound to the microwell and the other antibody is labeled with an enzyme. When an antigen is present, it simultaneously binds both antibodies in a „bridge“ or „sandwich“ fashion. This entire complex remains bound to the well. After washing out “unbound” enzyme, a specific substrate is added and converted to a colored and-product and the reaction is rapidly terminated with stopping solution. The absorbance is read for each well at 450 nm and the results plotted as concentration of TSH in µIU/ml vs Abs. on graph paper.

In our procedure, a disk is punched from a blood spot collected on Schleicher and Schuell’s filter paper #903. This disk is placed into the antibody well along with an eluting buffer. After overnight incubation, the eluting buffer and blood spot are aspirated out, the well washed and enzyme-labeled antibody added. After a second incubation, the well is washed and substrate is added. The enzyme reaction is rapidly terminated with stopping solution and the absorbance read. A standard curve is then constructed from which unknown concentrations of TSH can be calculated.

3 REAGENTS INCLUDED IN KIT

A. **TSH-ELISA Enzyme Conjugate Concentrate [CONJ ENZ]** 0.5 ml
Horseradish peroxidase labeled murine monoclonal anti-human TSH antibody.
For use, pour the entire contents of the Enzyme Diluent vial into the vial of Enzyme Conjugate Concentrate, cap vial, and invert gently several times to mix.
Stability of diluted enzyme reagent is 1 week (7 days) at 2-8°C.
Stability of sealed Enzyme Conjugate Concentrate is as indicated on the label.
NOTE: Care should be taken when removing the vial cap that no enzyme concentrate clinging to the cap is lost. If more than one vial of enzyme reagent is required, pool and mix all diluted enzyme prior to use.

B. **Enzyme Diluent [DILUENT ENZ]** 22.0 ml
Stability, when used with enzyme concentrate, is 1 week at 2-8°C.

C. **Eluting Buffer [BUFFER ELUTION]** 40.0 ml
Stability of Buffer is as per label at 2-8°C.

D. **Wash Buffer Concentrate [BUFFER WASH]** 50.0 ml
Dilute 10 times (to 500 ml) with distilled water prior to use.
Stability of diluted wash buffer is per kit shelf life at 2-8°C.

E. **Color Substrate (TMB) [SUB COLOR]** 22.0 ml
Stability after opening is 1 week at 2-8°C.

F. **Stopping Solution [SOLN STOP]** 22.0 ml
Stability is as per label at 2-8°C.

G. **Anti-Human TSH Coated Strip Plate [ANTI WELLS]** 2 each
Stability of unopened plate is as per label or 1 week at 2-8°C after opening sealed foil pouch.

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H. **Calibrators (8 levels) [STD 1-8], Controls (3 levels) [CONTROL 1-3]** 1 card each
Whole blood spiked with human TSH calibrated against the W.H.O. 80/558 second I.R.P. spotted on filter paper.
Concentrations are as indicated on the label.
Stability of unopened calibrator card is as indicated on label.
Stability of calibrator after opening pouch is 1 week at less than –15°C.

NOTE: Actual calibration levels may change between lots, the label on the current lot of calibrators should be consulted for calibration values to be used in calculations.

Warning: Human Based Material

All components of this kit should be handled as if capable of transmitting infectious diseases such as Hepatitis and HIV. Any components of human origin have been tested and found negative for the presence of Hepatitis B surface antigen and HIV antibody by FDA approved techniques. However, not test can guarantee the absence of agents capable of transmitting disease and all reagents in this kit should be handled with standard laboratory precautions. This kit is designed to be used by trained laboratory personnel only.

4 CALIBRATION AND STANDARDIZATION

The quantity of TSH in the patient's sample (blood spot) is calculated from a calibration curve prepared from a known amount of TSH calibrated against the WHO 2nd I.R.P. of hTSH 80/558.

5 SAFETY AND EFFICACY (SEE FIGURES 4 & 5)

The Neonatal TSH ELISA kit has been used on patient samples and has been validated and evaluated against other commercial kits. The ELISA correlation was found to be excellent when compared with TSH levels obtained from a state screening program (Figure 4) and when compared to our currently marketed Neonatal TSH IRMA (Figure 5).

6 ASSAY PROTOCOL

6.1 Reagent Preparation and Equipment

1. Plate Reader able to read absorbance at 450 nm.
2. Multi-channel and single micropipettes calibrated to 100, 200 and 300 µl.
3. Automated plate washer (optional).

6.2 Collection and Handling of Blood Specimen

Infant screening programs may differ from one another in the amount of sample required. A heel stick sample collected on Schleicher & Schuell filter paper #903 is suggested.

The following summary is described in detail in NCCLS publication LA4-A2.

1. Collect the blood from the heel of an infant usually 24 to 72 hours postpartum. Sampling times may vary from center to center.
2. Wash the heel with soap and water and wipe dry. Swab area with alcohol and allow to air dry.
3. Puncture infant's heel with sterile lancet and wipe away first drop of blood. Make sure the tip of lancet is not longer than 2.4 mm. Allow another drop of blood of adequate volume to form and gently touch the specimen card to the droplet in the center of the pre-printed circle on the filter paper card. The blood volume must be enough to completely fill at least two circles on the card. View the card from the opposite side as the blood penetrates the filter paper. Avoid excessive squeezing of the heel as it may cause hemolysis and also dilute the sample with tissue fluid. Avoid tearing or disrupting of the filter paper surface.
4. Place the filter paper card horizontally on a clean surface and allow to air dry for at least 3 hours at ambient temperature (15°C to 22°C). Avoid direct sunlight.
5. Place each specimen in its own paper envelope and transport to the laboratory within 24 hours of drying.
6. The receiving laboratory should store the sample at 2-8°C for short-term storage in a moisture-proof environment shielded from direct light. For long-term storage, samples should be stored at below –20°C in a moisture-proof environment.

6.3 Assay Protocol* (Duplicates Recommended)

* To be used for a maximum of two consecutive plates. For larger assays, the timing of pipetting should be lagged to ensure uniform plate processing.

1. For each calibrator, control and unknown, in duplicate: punch one 1/8" (3 mm) blood soaked filter paper spot into the appropriate wells of strip plate.
2. Add 200 µl of eluting buffer to each well.
3. Cover plate, mix gently by hand (30 seconds) and incubate overnight at room temperature.
4. Aspirate content of all wells (including blood spots).

5. Wash 3 times with 200 µl (or 300 µl) wash buffer and aspirate or "flick" plate to dryness.
6. Add 100 µl diluted enzyme conjugate, and incubate one hour at room temperature.
7. Aspirate (or flick) plate and wash 3 times with wash buffer as in (5) above.
8. Add 100 µl of color substrate (TMB) to each well.
9. Incubate 15 minutes at room temperature.
10. Add 100 µl of stopping solution to each well.
11. Read absorbance at 450 nm.

6.4 Calculations and Interpretations

1. Average the absorbance duplicates for all standards, controls, and patients.
   Subtract the averaged "0 µU/ml" absorbance from each of the averages obtained above. This yields the net absorbance.
2. Plot the net absorbance (y-axis) vs. the concentration of the TSH standards (x-axis) using log-log graph paper and a linear curve fit. This yields the standard curve.
3. Using the standard curve, determine the TSH concentrations of each patient sample. Read net sample absorbances directly off curve as µU/ml TSH (serum equivalent). A sample assay and calibration curve are provided in Figures 1a and 1b.
4. Samples with TSH levels beyond the limits of the standard curve should be reported as either "less than" or "greater than....".

6.5 Suggested Normal Range

Neonates 0-3d: less than 20 µU/ml.

The normal range for TSH in 0-3d term neonates has been established extensively by others and by us in our Neonatal TSH IRMA Assay. Agreement between the Neo-TSH ELISA and IRMA is as indicated in the product performance section (7).

In a recent evaluation of the Neo-TSH ELISA kit in which 50 "normal" neonatal blood spot specimens were submitted from a state screening lab for our evaluation, the observed normal range was 0-20 µU/ml.

The suggested normal range and cut-off is a guideline only. Each laboratory should establish specific cut-offs and ranges based on performance of the assay in their laboratory and with their constituent demographic population.

6.6 Quality Control

External Blood Spot Controls containing TSH at three different levels (low, intermediate, high), should be routinely included in each assay run. Control results should be recorded and evaluated by established protocols, e.g. Westgard, J.O. et al. Clinical Chemistry 27: 493-501, 1981.

Internal (Kit) Controls: Likewise tri-level controls included with the kit should be routinely monitored for adherence to stated values. Kit controls provide valuable information regarding how the kit is performing to manufacturer specifications.

7 ASSAY CHARACTERISTICS, ACCURACY AND RELIABILITY

7.1 Figures 1a and 1b

illustrate a typical Neonatal TSH ELISA assay.
Figures 1a and 1b are for illustration only and should not be used to calibrate actual patient results.

7.2 Figure 2 – Precision

Figure 2a illustrates the within-assay variability of different samples containing purified TSH.

Figure 2b illustrates the between-assay variability for different samples containing added or endogenous TSH.

7.3 Figure 3 – Recovery

Figure 3 illustrates the percent recoveries of samples to which known amounts of TSH were added.

7.4 Figure 4 – Clinical Correlation

Figure 4 displays the correlation between TSH results reported on neonates from a state screening program, using a commercial IRMA kit, with TSH levels determined on the same specimens with the new TSH ELISA. As indicated, the correlation ($r = 0.9762$, $P < 0.001$) is highly significant.

7.5 Figure 5 – Comparison of DRG Neonatal TSH ELISA with DRG Neonatal TSH IRMA Kit.

Figure 5 illustrates TSH values obtained from our current Neonatal TSH IRMA, compared to the new DRG Neonatal TSH ELISA. The correlation ($r=0.937$, $P<0.001$) is again, excellent.

7.6 Figure 6 – Specificity

Specificity of the DRG Neonatal TSH ELISA is as indicated in Figure 6, relative to our calibration with W.H.O. 80/558. No other interfering substances have been identified.

7.7 Sensitivity – Minimum Detectable Dose

The minimum detectable dose of the DRG Neonatal TSH ELISA is 5 µU/ml, as determined by repetitive analysis of the zero calibrator.

7.8 Limitations of the Procedure

1. To ensure accurate and reliable results, make sure all blood spot disks are within the reaction solution during the incubation period.
2. Strict adherence to the protocol is advised to obtain reliable results. Any modifications or changes made to the kit or the assay procedure are the responsibility of the user.
3. This assay is designed to be used with samples which are exclusively collected on Schleicher & Schuell's Filter Paper #903.
4. Demographical variations, infant weight, age, prematurity and twinning can affect the TSH concentrations. Laboratories should be aware of all these factors.

7.9 Antibody Constituents

The DRG Neonatal TSH ELISA kit uses a combination polyclonal-monoclonal "capture antibody configuration on the microwell plate and a complementary HRP-labeled monoclonal antibody as tracer. The polyclonal and monoclonal "capture antibodies are selected for their "beta" and "intact" specificity for human TSH. All monoclonals are produced from mice immunized with human TSH and/or its subunits. The polyclonal antibody was raised in TSH immunized rabbits.

Figure 1a

SAMPLE ASSAY – Neonatal TSH ELISA
Calibration Curve

Standard No.	Concentration (µlU/ml	Net Abs. (450 nm)	Assayed Range (µlU/ml)	TSH (µlU/ml)
BLANK	0.0	0.0950		
1	4.0	0.0340		
2	12.0	0.0990		
3	28.0	01603		
4	60.0	0.3453		
5	120.0	0.6553		
6	233.0	1.2430		
7	455.0	2.0233		
Level I		0.1025	4.00 - 16.0	12.96
Level II		0.1655	15.0 - 29.0	20.31
Level III		0.3235	35.0 - 56.0	47.17

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Figure 1b
Sample Standard Curve

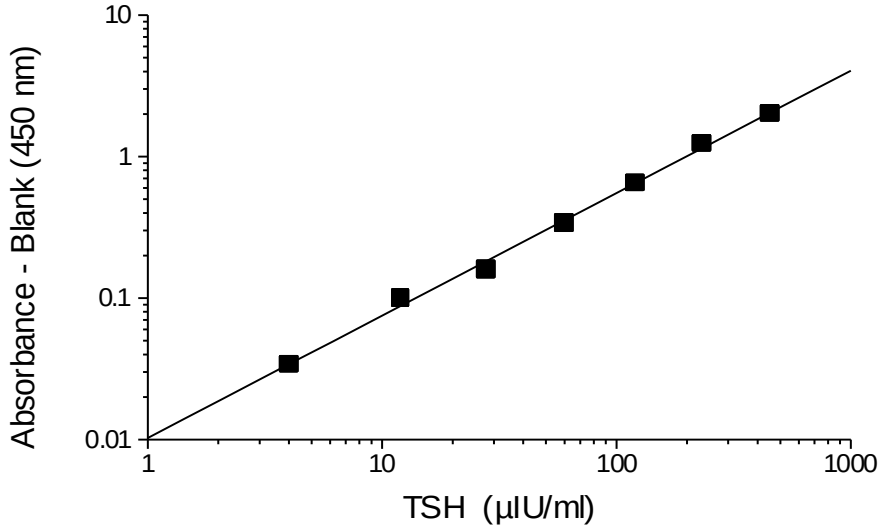

Figure 2

PRECISION
a: Intra-Assay

Sample	N	X µIU/ml	S.D.	C.V. %
A	11	13.4	2.1	15.7
B	11	23.0	2.0	8.7
C	11	45.0	6.3	14.0
D	11	82.0	7.9	9.6
E	11	149.0	8.7	5.8

b. Inter-Assay

Sample	N	X µIU/ml	S.D.	C.V. %
A	12	12.5	1.2	9.6
B	12	22.5	1.8	8.0
C	12	43.3	6.2	14.3
D	12	82.2	7.5	9.1
E	12	149.0	6.4	4.3
F	12	270.0	15.0	5.6

Figure 3.

RECOVERY

Sample	N	TSH Equivalent µIU/ml	TSH Recovered µIU/ml	% Recovery
A	12	24.0	23.0	95.8
B	12	45.0	45.0	100.0
C	12	88.0	82.0	93.2
D	12	156.0	149.0	95.5
E	12	274.0	266.0	97.1

Mean Recovery – All Samples X = 96.3%

Figure 4

Comparison of DRG Neonatal TSH ELISA with Results Reported by a State Screening Program* for Hypothyroidism

Sample	Reported (µIU/ml)	DRG ELISA (µIU/ml)
1	369	294
2	>439	390
3	22	18
4	69	74
5	47	40
6	50	50
7	76	95
8	61	74
9	42	55
10	47	35
11	371	234
12	50	35
13	235	293
14	39	24
15	60	53
16	>439	412
17	50	38
18	76	80
19	330	275
20	428	354
21	70	50
22	79	53
23	69	48
24	43	25
25	48	35
26	66	38
27	73	52
28	22	12
29	49	35
30	73	55

r = 0.9762

$p < 0.001$

*Method Used by State is a Commercial IRMA TSH

Figure 5

Comparison of DRG Neonatal TSH ELISA with DRG Neonatal TSH IRMA Kit (Catalog No. RIA-1452)

Sample	DRG IRMA	DRG ELISA
1	25.9	16.0
2	43.4	28.0
3	68.2	81.0
4	24.8	26.0
5	37.7	48.0
6	73.9	94.0
7	36.2	35.0
8	34.5	24.0
9	41.3	38.0
10	60.9	52.0
11	31.0	35.0
12	26.5	25.0
13	54.0	55.0
14	293.4	354.0
15	49.4	35.0
16	355.0	294.0
17	55.0	50.0
18	236.0	293.0
19	55.2	55.0
20	>439.0	275.0
21	43.1	40.0
22	59.5	74.0
23	60.8	48.0
24	97.2	95.0
25	46.3	35.0
26	<11	<11

$r = 0.9370$

$p < 0.001$

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Figure 6

SPECIFICITY

Peptide Hormones	% Cross Reactivity
TSH (80/558)	100
LH	<0.0001
HCG	<0.0001
Prolactin	<0.02
FSH	<0.003

8 REFERENCES / LITERATURE

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