

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k063437

B. Purpose for Submission:

New device

C. Measurand:

Thyroid Stimulating Hormone (TSH)

D. Type of Test:

Quantitative enzyme immunoassay

E. Applicant:

Monobind, Inc.

F. Proprietary and Established Names:

AccuBind Neo-TSH Microwell Elisa Assay

G. Regulatory Information:

1. Regulation section:

21 CFR §862.1690, Thyroid stimulating hormone test system

2. Classification:

Class II

3. Product code:

JLW, Radioimmunoassay, thyroid-stimulating hormone

4. Panel:

Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

See Indication for use below.

2. Indication(s) for use:

The Monobind AccuBind Neo-TSH Microwell Elisa Assay is an *in vitro* diagnostic test system for the quantitative determination of thyroid stimulating hormone (TSH) in human whole blood dried on Whatman 903 filter paper. It is intended to be used to screen newborns for congenital hypothyroidism.

3. Special conditions for use statement(s):

For Prescription Use Only.

This kit is not to be used for confirmatory testing, prenatal testing or to monitor therapy. This kit will not detect secondary hypothyroidism due to failure of the hypothalamus nor will it detect tertiary hypothyroidism due to pituitary failure.

A definitive clinical diagnosis should not be based on the results of a single test but should be made by the physician after all of the clinical and laboratory findings have been evaluated. An additional diagnostic procedure, using serum as the sample, should be performed to confirm a diagnosis of congenital hypothyroidism.

4. Special instrument requirements:
A microplate spectrophotometer.

I. Device Description:

For the assay: NTSH Enzyme Reagent (enzyme-labeled affinity-purified polyclonal goat anti-TSH IgG in Tris buffer with red dye and stabilizers), NTSH Biotin Reagent (anti-TSH monoclonal IgG labeled with biotin in Tris buffer with green dye and stabilizers), one streptavidin-coated 96-well microplate, Wash Solution (surfactant in phosphate buffered saline with preservative), Substrate Solution (tetramethylbenzidine and hydrogen peroxide in acetate buffer) and Stop Solution (0.4N HCl).

Neo-TSH Calibrators: Six levels of TSH antigen in dried blood spots (plus preservative) at approximate levels of 0 (A), 4 (B), 18 (C), 40 (D), 100 (E), and 250 (F) μ IU/ml dried on Whatman 903 filter paper.

Neo-TSH Controls: Three levels of TSH antigen in dried blood spots (plus preservative) on Whatman 903 filter paper.

All products that contain human components have been found to be non-reactive for Hepatitis B surface antigen, HIV 1 & 2 and HCV antibodies by FDA licensed reagents.

J. Substantial Equivalence Information:

1. Predicate device name(s): DPC Neonatal TSH RIA Kit (125 I)
2. Predicate 510(k) number(s): k772192
3. Comparison with predicate:

Similarities		
	Predicate device (k772192)	Proposed device
Intended Use	It is intended for <i>in vitro</i> diagnostic use as an aid in screening for thyroid disorders in neonates.	Same
Test principle	Quantitative immunoassay	same
Number of calibrator levels	Six	same
Sample type	Blood spot on Whatman 903 filter paper	same

Differences		
	Predicate device (k772192)	Proposed device
Reaction tube/location	Polystyrene tube	Streptavidin coated 96-well microplate
Instrument for quantitating labeled TSH	Gamma counter	Spectrophotometer
Antibody label	125 I	biotin

K. Standard/Guidance Documents Referenced (if applicable):

None referenced.

L. Test Principle:

The Monobind AccuBind Neo-TSH Microwell Elisa Assay is a solid phase two-site immunoenzymometric assay based on the direct sandwich technique in which two specific antibodies are directed against two separate antigenic determinants on the hTSH molecule. 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 anti-TSH and the TSH in the dried blood spot forms a complex that binds to the streptavidin coated to the well due to the inherent affinity of streptavidin and biotin. After the completion of the first incubation period, excess reactants are washed off via a wash step and the enzyme conjugate (another specific anti-TSH antibody linked to an enzyme) is added to the Ag-Ab complex deposited 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 substrate to produce color. The color is measured with a microplate spectrophotometer. 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The inter- and intra-assay precision of the AccuBind Neo-TSH Microwell Elisa Assay was determined by analyses of three different levels of three pooled human whole blood spotted controls. The number mean values, standard deviations (SD) and coefficient of variation for each of these control sera are presented below:

Intra-assay: twenty replicates of each of three pooled human blood spotted controls (low, medium, and high levels of TSH) were assayed in the same assay. An intra-assay precision of 5.1 – 8.6% was obtained:

	Low pool	Medium pool	High pool
Number (n)	20	20	20
Mean	10.6	43.3	87.1
1 SD	0.91	3.61	4.42
% CV	8.6	8.3	5.1

Inter-assay: one duplicate of each of three pooled human blood spotted controls (low, medium and high levels of TSH) was assayed in 10 assays done

over a period of six months that involved five different sets of reagents and three different technicians. An inter-assay precision of 6.0-10.8% was obtained.

	Low pool	Medium pool	High pool
Number (n)	10	10	10
Mean	11.05	42.22	85.10
1 SD	1.20	3.76	5.11
% CV	10.80	8.90	6.00

b. *Linearity/assay reportable range:*

The claimed range for this assay is 2 - 250 uIU/ml.

A pool of human blood was purchased and hematocrit was adjusted to 55%. An aliquot of this sample was assayed using Monobind's liquid phase TSH Elisa test (k971980) to give the baseline of TSH present in the sample. This original sample was spiked with TSH (standardized against WHO 2nd IRP 80/558) to 300 uIU/mL and then used to dilute the spiked sample to various concentrations across the measuring range before spotting on filter paper. Four replicates of each concentration were measured.

Linear regression statistics:

$$y = 1.0139x - 0.4243$$

$$r^2 = 0.996$$

Target value (µIU/ml)	Observed value (µIU/ml)	% Recovery
300	289.36	96.4
250	255.03	102.0
200	194.84	97.42
150	143.21	95.47
100	92.72	92.72
50	50.75	101.5
25	25.72	102.9
12.5	12.15	97.2
6.25	6.42	102.7
2.0	1.91	95.5

The recovery at each dilution was within 10% of the expected value.

Hook effect:

Two different sera were taken and spiked to different levels with TSH stock solution made from the WHO 2nd IRP 89/558. Results showed no hook effect for the Monobind AccuBind Neo-TSH Assay when concentrations up to 10,000 uIU/ml TSH were tested.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Controls and calibrators: For the calibrators and controls, a master dose response curve is generated using master calibrators referenced to the WHO 2nd IRP 80/558 standard for TSH.

Controls and calibrators are created by adding purified, lyophilized TSH to a pooled whole blood sample with low TSH value (and preservatives) to the various desired TSH levels. These samples are spotted on Whatman 903 filter paper. Values for each control and calibrator are assigned by using the master curve described above.

Stability: Study protocols, preliminary data and acceptance criteria for stability testing were provided for the Monobind AccuBind Neo-TSH Elisa Assay at room (20-25°C), refrigerated (2-8°C) and elevated (37°C) temperatures and found to be acceptable. Based on the accelerated stability data, twelve month stability at 2-8°C is claimed for the Assay kit. Real-time studies will continue to confirm and extend the dating.

d. Detection limit:

The Limit of the Blank (LoB) was determined after 16 tests of the '0' level NeoTSH Calibrator. CLSI guideline EP17 was not followed to evaluate the LoB. The LoB (the mean + 2 standard deviations) was calculated to be 0.5 μ IU/ml.

The low limit of the claimed assay measuring range (2 uIU/mL) was determined based on the linearity study described above.

e. Analytical specificity:

Cross reactivity between potential interfering substances and this assay was tested by spiking high levels of these substances into a pooled blood sample and calculating changes in assay results with and without the substance added. No cross reactivity was observed with hLH (1,000 mIU/ml), hFSH (1,000 mIU/ml), hCG (10,000 mIU/ml) or hPRL (1,000 ng/ml). No effect on the performance of the assay was observed with up to 200 μ g/ml of bilirubin, 36 – 55% hematocrit, or in samples enhanced with 4% additional packed red blood cells.

Testing to evaluate whether there is end-of-run effect (due to time delay in reagent dispensing) with the AccuBind Neo-TSH Microwell Elisa Assay showed that there was no drift in the measurement of the control material whether these samples were placed in the beginning, middle, or end of the microwell plate.

Additional potential effects on TSH are noted in the package insert and literature is provided as references to support some of this information:

1. TSH concentration, in the circulation, is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. Thus, thyrotropin concentration alone

is not sufficient to assess clinical status.

2. TSH values may be elevated by pharmacological intervention. Domperidone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels¹.
3. A decrease in thyrotropin values has been reported with the administration of propranolol, methimazol, dopamine and d-thyroxine¹.

¹Young, D.S., Pestaner, L.C., and Gilberman, U., "Effects of Drugs on Clinical Laboratory Tests", *ClinicalChemistry*, 21, 3660 (1975).

f. *Assay cut-off:*
Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

264 patient samples from 3-7 day old infants were used tested on both the predicate device, DPC RIA kit, and the AccuBind Neo-TSH Microwell Elisa Assay. Samples were obtained from the NJ Department of Health laboratory; patients were referred to this laboratory for routine thyroid testing mandated by universal screening for congenital hypothyroidism. 103 samples from 3-7 day old infants considered hypothyroid from testing on the predicate device were also tested on the proposed device. These patient samples were referred to the laboratory for thyroid status check.

Regression statistics:

$$y = 1.0182x + 0.3306$$

$$r^2 = 0.9783$$

range = 0.64- 26.6 (normal samples); 29.0 – 264.7 (hypothyroid samples)

		Predicate device		
		0 – 20 μIU/ml	20 – 40 μIU/ml	> 40 μIU/ml
Proposed device	0 – 20 μIU/ml	161	2	-
	20 – 40 μIU/ml	2	17	2
	> 40 μIU/ml	-	1	79

b. *Matrix comparison:*
Not applicable.

3. Clinical studies:

a. *Clinical Sensitivity:*
Not applicable.

b. *Clinical specificity:*
Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable.

4. Clinical cut-off:

The sponsor references the guideline for newborn screening for congenital hypothyroidism from the American Academy of Pediatrics for interpretation of screening results as follows:

Category	TSH μ IU/ml Serum
Slightly Elevated	20 – 40
Positive (Hypothyroid)	> 40

5. Expected values/Reference range:

Recommended guidelines for newborn screening for congenital hypothyroidism have been published by the American Academy of Pediatrics. For infants 2-6 days old, these recommendations categorize TSH concentrations as “normal”, “elevated” or “only slightly elevated” relative to values of 20 and 40 uIU/mL. According to the AAP guidelines, “any infant with a low T4 and TSH concentration greater than 40 mU/L is considered to 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.”

A study of 158 newborn normal specimens (0-7 days old) was completed and the following range was observed: 0.7-25.52 μ IU/mL.

Age (Days)	Days (0 – 3)	Days (0 – 5)	Days (0 – 7)	Number & % < 20 μ IU/ml)
Total (n)	50	86	158	153 (97.4%)
Range(μ IU/ml)	(0.55 – 17.20)	(0.55 – 21.40)	(0.55 – 25.52)	
Mean (μ IU/ml)	7.54	8.66	8.75	
At 95% (n)	0.57-15.11 (48)	0.57-18.7 (81)	0.57-25.52 (156)	
At 90% (n)	0.52-13.98 (46)	0.52 – 14.65 (81)	0.52-18.7 (157)	

The manufacturer recommends that each laboratory determine their own in-house range using the method with a population indigenous to the area in which the laboratory is located.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.