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

A. 510(k) Number:
k072458

B. Purpose for Submission:
New device

C. Measurand:
Thyroid stimulating hormone (TSH)

D. Type of Test:
Quantitative immunoassay

E. Applicant:
Golden Bridge International, Inc.

F. Proprietary and Established Names:
GBI TSH Neonatal Screening Kit

G. Regulatory Information:

1. Regulation section:
   21 CFR 862. 1690

2. Classification:
   Class II

3. Product code:
   JLW

4. Panel:
   Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:
   The GBI TSH Neonatal Screening Kit is designed for the quantitative determination of Thyroid Stimulating Hormone (TSH) concentrations in neonatal

blood samples that have been collected onto Whatman 903 specimen collection paper. The results are used to screen newborns for congenital hypothyroidism.

3. Special conditions for use statement(s):
   For prescription use.

   The package insert contains information that the kit is not to be used for confirmatory testing, prenatal testing or to monitor therapy. Additional diagnostic procedures, using serum as the sample, should be performed to confirm a diagnosis of congenital hypothyroidism.

4. Special instrument requirements:
   None

I. Device Description:

The GBI TSH Neonatal Screening Kit consists of the following reagents:

TSH Capture Microplate-Goat polyclonal anti-alpha hTSH antibody coated onto 96-well microplate
TSH Elution Buffer-Borate buffer containing bovine albumin, surfactant, and preservatives
Anti-TSH PO Conjugate-Mouse monoclonal β-specific anti-TSH antibody conjugated to horseradish peroxidase in borate buffer containing bovine albumin, surfactant and preservatives
TSH Diluent Buffer- Borate buffer containing bovine albumin, surfactant, and preservatives
PO Wash Buffer (20X)-Concentrated solution of phosphate buffered saline containing a surfactant and preservatives.
PO Color Reagent- A colorless solution of 3,3′, 5,5′-Tetramethylbenzidine (TMB) and hydrogen peroxide
PO Color Stopper- A solution containing < 1% hydrochloric acid
TSH Dried Blood Standards and Controls-
Prepared from human whole blood, adjusted to a hematocrit of 55% and spotted onto Whatman 903 specimen collection paper. The six Standards contain concentrations of added TSH at approximately zero, 7.5, 15.0, 30.0, 60.0, and 120.0 µIU/ml serum equivalent. The three Controls contain approximate TSH concentrations of 15, 30 and 80 µIU/ml serum equivalent.
All human source materials used in the preparation of kit components was tested and found to be non-reactive for the presence of HBsAg, anti-HIV 1 and 2, and HCV by FDA approved methods.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Accuwell TSH ELISA

2. Predicate K number(s):
k001145

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	Quantitative determination of Thyroid Stimulating Hormone (TSH) concentrations in neonatal blood samples that have been collected onto Whatman 903 specimen collection paper. The results are used to screen newborns for congenital hypothyroidism.	Same
Recommended reference range	Normal < 20 μIU/ml Borderline 20 – 40 μIU/ml Positive (Hypothyroid) > 40 μIU/ml	Same

Differences		
Item	Device	Predicate
Reportable range	2.4-120 μIU/ml	2.9-160 μIU/ml
Analytical sensitivity (limit of blank)	2.4 μIU/ml	2.9 μIU/ml
Detection method	Light absorbance of final reaction read at 450 nm with spectrophotometer	Light absorbance of final reaction read at 450 nm with spectrophotometer

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

Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline- Second Edition (CLSI EP5-A2)

L. Test Principle:

A polyclonal goat anti-hTSH (human) antibody is immobilized onto each well of the 96-well microplates provided with the kit. Sample discs punched from dried whole blood spot standards, controls and neonate specimens are added to the coated wells. An elution buffer is also added. The plate is incubated to elute TSH from the sample

disc and to allow capture of the eluted TSH by the antibody immobilized onto the microplate wells. Following incubation the plates are washed to remove the sample discs as well as the eluate.

A second antibody, a ß-specific anti-hTSH monoclonal conjugated to the enzyme horseradish-peroxidase (HRP), is then added to the wells and incubated. The eluted TSH of the sample already captured by the microplate-bound antibody is bound by the enzyme-conjugated monoclonal antibody added. An antibody-TSH-antibody bridge, or "sandwich", forms that is bound to the surface of the microplate wells. Any unbound complexes are removed with subsequent plate washings.

The final stage of the assay is the detection of the microwell-bound complexes by the addition of a color developing reagent. The enzyme (HRP) portion of the bound "sandwich" reacts with the color developer, 3, 3', 5, 5'-Tetramethylbenzidine (TMB) in the presence of hydrogen peroxide (H_2O_2). The TMB/ H_2O_2 liquid is converted from colorless to blue. The degree of color change is directly proportional to the amount of TSH antigen that is bound in the well. The color development is terminated with the addition of a color stopper that converts the blue to yellow.

The results are measured with a microplate reader at a wavelength of 450 nm. The absorbance measured is directly proportional to the concentration of TSH in the sample. A standard curve is generated by plotting the light absorbance of each standard versus its known TSH concentration. The concentrations of TSH in the unknown samples are determined by interpolation from this standard curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The sponsor states that precision was evaluated following the Clinical and Laboratory Standards Institute (CLSI) Protocol EP5-A2.

Three Centers for Disease Control (CDC) Neonate Screening Quality Control Program samples (CDC 611, 612, and 613) and two other dried blood spot samples C1 and C2 with TSH values ranging from approximately 14 to 87 µIU/mL were tested in duplicate, one run per day, for 20 days, for a total of n=40. Since the testing was performed using dried blood spots, the extraction procedure was captured in the testing process. The imprecison for these samples ranged from 7.9-13.9 % coefficient of variation (% CV). The results are presented in the table below.

Sample ID:		**C1**	**C2**	**CDC611**	**CDC612**	**CDC613**
Precision Parameter	*Count*	*40*	*40*	*40*	*40*	*40*
	Mean	**14.6**	**28.3**	**30.6**	**46.3**	**86.8**
	SD	1.2	2.5	3.7	4.4	12.1

	%CV	7.9	8.7	11.9	9.4	13.9
	Min	12.5	23.5	23.3	37.5	71.4
	Max	18.0	34.0	40.4	59.1	122.5
Within-Run Standard Deviation:	**Sr =**	**0.9**	**1.3**	**2.6**	**4.2**	**6.5**
Between-Day Standard Deviation:	**Sdd =**	**1.0**	**2.3**	**2.6**	**1.2**	**10.3**
Within Device Standard Deviation:	**ST =**	**1.2**	**2.5**	**3.7**	**4.4**	**12.2**

b. *Linearity/assay reportable range:*

The reportable range is stated to be 2.4-120 µIU/mL. The claim is supported by data from the sponsor's linearity study and detection limit study. Samples were prepared from a commercially obtained, human, analyte-stripped serum-sample, supplemented with purified analyte weighed gravimetrically to produce a 1.5 µIU/mL, 25 µIU/mL, and 200 µIU/mL TSH concentrations. The range of low-level, mid-level and high-level samples were prepared by making multiple volume combinations of these. Then each prepared serum-sample was then combined with lysed, commercially obtained, human, red blood cells to achieve the equivalent of a 55% hematocrit. The resulting samples were then spotted onto blood collection filter paper and dried.

Observed values were compared to the expected values and the percent (%) recovery ranged from 88 to 114%, with a mean recovery of 95%.
Linear regression analysis resulted in the following equation:
$y= 0.8997x + 0.6036$, $r^2= 0.999$.

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

Calibrators and controls are prepared from commercially available TSH analyte that is traceable to WHO 3rd IRP of human TSH 81/565. The target TSH levels for the controls 15, 30, and 80 µIU/mL are suitable given the clinical decision decision points for Normal, Borderline, and Abnormal (Hypothyroid).

Targeted values are verified by testing in the TSH assay standardized with CDC-issued TSH calibrators and Reference Standards traceable to WHO 3rd IRP of human TSH 81/565.

Control values and expected ranges are assigned by repeat analysis on multiple TSH assays.

To support shelf-life claims, dried blood spots used to prepare calibrator and

control materials are stored at 2-8°C for 18, 19, and 20 months. The sponsor's stability testing protocol and acceptance criteria were reviewed and determined to be adequate.

d. Detection limit:

The TSH test kit zero standard was used to prepare dried blood spots (n=20) that were then punched and tested in a single assay run. The sponsor defines Analytical Limit at Low Levels as the calculated concentration in µIU/mL serum equivalents that corresponds to the mean of the absorbance value of the zero standard plus two times the standard deviation (+2SD). This value was calculated to be 2.4 µIU/mL.

e. Analytical specificity:

The cross-reactivity of three structurally related compounds, Follicle Stimulating Hormone (FSH-WHO 2nd IRP HMG), Luteinizing Hormone (LH-WHO 1st IRP 68/40)), and human Chorionic Gonadotrophic hormone (hCG-WHO 2nd IS 61/6)), were evaluated. Each compound was added to TSH-free whole blood which had been adjusted to 55% hematocrit and then spotted on filter paper to create dried blood spots. FSH concentrations up to 500 µIU/mL, LH up to µIU/mL, and hCG concentration up to 100,000 µIU/mL were undetectable (< 2.4 µIU/mL) in the assay.

Interference by elevated lipid, bilirubin (conjugated and unconjugated) and hemoglobin was evaluated. The levels of lipid and bilirubin tested were based on recommendations in CLSI EP7-A. The sponsor defined interference as an observed test value is greater than ± 1 standard deviation of the mean of the matching control value.

Lipid at a concentration of 1350 mg/dL was added to whole blood specimens containing approximately 16, 46, and 85 µIU/mL TSH and tested along with the same specimens without added interferant. Specimens containing 1350 mg/dL lipid had TSH values within ± 1 standard deviation of the mean of the matching control value and did not adversely affect the interpretation of "Normal" or "Hypothyroid" (Abnormal) results.

Conjugated and unconjugated bilirubin at a concentration of 20 mg/dL were added separately to whole blood specimens containing approximately 14, 27, and 68 µIU/mL TSH and tested along with the same specimens without added interferant. Specimens containing 20 mg/dL bilirubin had TSH values within ± 1 standard deviation of the mean of the matching control value and did not adversely affect the interpretation of "Normal" or "Hypothyroid" (Abnormal) results.

The effect of hemoglobin up to 18.7 g/dL was evaluated. This level is the highest expected hemoglobin level in neonates, according to the sponsor's search of the literature. Specimens with TSH concentration of approximately 13, 27 and 68 µIU/mL were tested with and without additional hemoglobin, 17 and 18.7 g/dL respectively. Specimens containing 18.7 g/dL had TSH values within ± 1 standard deviation of the mean of the matching control value and did not adversely affect the interpretation of "Normal" or "Hypothyroid" (Abnormal) results.

The labeling contains a limitation that any physiological state that will cause marked alterations in hematocrit and/or protein concentration may adversely affect the validity of the TSH concentration obtained.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A total of 995 neonatal dried blood spot specimens were obtained from a state public health laboratory's routine screening program bank of specimens. Nine-hundred eighty (980) were randomly selected from the (previously screened and presumed) normal population of banked specimens; and 15 were selected from patients confirmed as positive for hypothyroidism.
Testing of the samples was performed with the GBI TSH assay and the predicate method. The results are shown in the following table:

Comparison of Result Interpretations Obtained by the GBI TSH EIA versus a Predicate TSH EIA

Population N=995			**GBI Results**			
	Published Cut-off Ranges		Normal	Borderline	Positive	(Predicate) **Totals:**
			< 20	20 - 40	> 40	
Predicate Results	Normal	< 20	**967**	7	**0**	**974**
	Borderline	20 - 40	2	**4**	**0**	**6**
	Positive	> 40	0	0	**15**	15
	(GBI) **Totals**:		**969**	**11**	**15**	**995**

Linear regression analysis of samples with numeric values within the reportable range of the devices (n=832) yielded the following equation:

y (GBI TSH) = 1.07x (predicate) + 0.27, $r^2 = 0.92$

Of the fifteen (15) confirmed hypothyroid samples, the majority exceeded the reportable range of the device. To further demonstrate performance at the Positive cut-off of 40 µIU/ml additional testing of fifteen (n=15) spiked samples ranging in TSH value from 40-60 µIU/ml was performed on the GBI TSH test and the predicate device. The results are summarized in the table below:

Sample ID #:	**Predicate Result (µIU/mL)**	**GBI TSH Result (µIU/mL)**
1	53.3	44.7
2	45.4	42.1
3	48.7	46.3
4	52.6	50.7
5	53.1	50.5
6	50.9	46.1
7	44.8	40.1
8	43.4	43.2
9	60.2	47.0
10	56.2	53.7

Sample ID #:	Predicate Result (µIU/mL)	GBI TSH Result (µIU/mL)
11	49.2	49.3
12	46.8	45.2
13	54.2	53.6
14	51.1	59.3
15	50.3	57.1

b. Matrix comparison:

The device is to be used only with neonatal whole blood.

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):

Testing of archived Centers for Disease Control (CDC) proficiency program samples was performed with the GBI TSH test. Results with the GBI TSH test were similar to the predicate method and other commercially available methods as well as to CDC expected ranges.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The sponsor references the recommendations of the Academy of Pediatrics and the American Thyroid Association Committee on Public Health for interpretation of screening results as follows:

Category	TSH µIU/ml Serum
Normal	< 20
Borderline	20 – 40
Positive (Hypothyroid)	> 40

Their applicability to the GBI TSH assay was confirmed by comparison to the calculated rank and percentile values obtained from the normal neonate test population, n=980.

TSH Result (uIU/ml serum equivalent)			
GBI TSH EIA	95th Percentile	97.5th Percentile	99th Percentile
N = 980	15.2	17.3	20.4

The package insert states that a variety of factors will determine the normal range for neonatal TSH concentrations. Demographic variations, the age and weight of the infant at sample collection, multiple births and infants born prematurely are all factors which can affect the cut-off values for normal concentrations of TSH in a neonatal screening program. For these reasons each laboratory should establish normal ranges and cut-off values for their individual application.

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.