8.0 REAGENT PREPARATION:

1. Wash Buffer

Dilute 10x wash solution to 1000x with distilled or deionized water. Store at 2-8°C for up to 60 days.

Note 1: Do not use the substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacterial growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents and patient samples to room temperature (20 - 27°C).

**This test can only be performed by a skilled individual or trained professional**

1. Assemble the required number of microplates 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 1/8” blood dot out of each calibrator, control, and patient sample (do not punch blood dots from areas that are printed or that are near the edge of the blood spot).

3. Combine either 0.050 ml (50µl) of N-TBG Elution Buffer or 0.050 ml of N-TBG Biotin Reagent to each well.

4. Place the dried blood samples at 2-8°C for 2-3 weeks if stored in zip-lock, moisture resistant bags with desiccants.

5. Place the dried blood samples at 2-8°C for 2-3 weeks if stored in a dry blood dot carrier (Cat# 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 spot (DBS) in a moisture resistant 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 thyroid hormone screening 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.

6. SPECIMEN COLLECTION AND PREPARATION

The sampling from neonates is performed by lancelet of the infants and then spotting enough whole blood on S&S filter paper card (Cat# 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 spot (DBS) in a moisture resistant barrier plastic bag with desiccant and send to the laboratory.

Note 2: Do not use reagents that are contaminated or have bacterial growth.
11. Add 350µl 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.

12. Add 0.100 ml (100µl) of substrate solution to each well.

13. Draw the best-fit curve through the plotted points. 

*Note: Always add reagents in the same order to minimize reaction time differences between wells.*

15. 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.

9.1 ALTERNATIVE TEST PROCEDURE – OVERNIGHT

1. Assemble the required number of microwells for each specimen. (Note: Do not punch blood dots from areas that are printed on or that are near the edge of the blood spot).

2. Add 0.050 ml (50µl) of N-TBG Elution Buffer to all the wells.

3. Add 350µl of wash buffer (see Reagent Preparation Section), mix for 15-20 seconds.

4. Add 0.100 ml (100µl) of substrate solution to each well.

5. Read within fifteen (15) minutes of adding the stop solution.

11. Add 350µl 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.

12. Add 0.100 ml (100µl) of substrate solution to each well.

13. Draw the best-fit curve through the plotted points. 

*Note: Always add reagents in the same order to minimize reaction time differences between wells.*

15. 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.

11. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

14.2 Sensitivity

The N-TBG AccuBind® ELISA test system has a sensitivity of 29.6. A total of 50µl to 150µl was evaluated by adding measured concentrations of the interfering substance to a serum matrix. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of thyroxine needed to displace the same amount of conjugate.

14.4 Specificity

The cross-reactivity of the antibody used for N-TBG AccuBind® ELISA was determined by analyses on human sera, and the cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of thyroxine needed to displace the same amount of conjugate.

15.0 REFERENCES


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