Thyretain® TBI

Blocking Reporter BioAssay

For the rapid qualitative detection and differentiation of blocking autoantibodies to the thyroid stimulating hormone (TSH) receptors (TSHRs) in patients suspected of having autoimmune thyroid disease (AITD).

For *in vitro* diagnostic use.

A symbols glossary can be found at quidel.com/glossary.

CONTENTS

INTENDED USE 2

SUMMARY AND EXPLANATION OF THE TEST 2

PRINCIPLE OF THE PROCEDURE 3

MATERIALS PROVIDED 3

MATERIALS REQUIRED BUT NOT PROVIDED 3

REAGENT STORAGE INSTRUCTIONS 4

WARNINGS AND PRECAUTIONS 4

SPECIMEN COLLECTION AND PREPARATION 5

SPECIMEN TRANSPORT AND STORAGE 6

ASSAY PROCEDURE 6

Preliminary Comments and Precautions 6

Thyretain TBI Procedure 6

QUALITY CONTROL 9

CALCULATION OF RESULTS 10

EXAMPLE RUN: 10

Calculation of Results 10

INTERPRETATION OF RESULTS 11

LIMITATIONS OF PROCEDURE 11

EXPECTED VALUES 11

SPECIFIC PERFORMANCE CHARACTERISTICS 11

Eighty eight (88) blank measurements were carried out to determine LOB for the TBI assay. Because the data was non-Gaussian, the LOB was determined using the method as recommended in the CLSI guideline (EP17-A). 12

Cross-reactivity and Interference by Endogenous Substances: 12
Cross-Reactivity with Other Autoantibodies: 13
Assay Cutoff 13
Intra-Assay Precision 13
Inter-Assay Precision (Intra-Day) 14
Inter-Assay Precision (Inter-Day) 14
Assay Reproducibility 14
CUSTOMER AND TECHNICAL ASSISTANCE 15
REFERENCES 15
4. CLSI. PROCEDURES FOR THE COLLECTION OF DIAGNOSTIC BLOOD SPECIMENS BY VENIPUNCTURE; APPROVED STANDARD, 5TH EDITION. CLSI DOCUMENT H3-A6 (ISBN 1-56238-650-6). CLSI, 940 WEST VALLEY ROAD, SUITE 1400, WAYNE, PENNSYLVANIA 19087-1898 USA, 2003. 15
GLOSSARY 16

INTENDED USE

Thyretain TBI Blocking Reporter BioAssay, is intended for the qualitative detection of blocking autoantibodies to the thyroid stimulating hormone (TSH) receptors (TSHRs) in patients suspected of having autoimmune thyroid disease (AITD).

SUMMARY AND EXPLANATION OF THE TEST

In a normal functioning system, metabolic homeostasis is mainly controlled by the hypothalamic-pituitary-thyroid axis. The hypothalamus senses low circulating levels of thyroid hormone and responds by releasing thyrotropin-releasing hormone (TRH). The TRH stimulates the pituitary to produce and secrete thyroid-stimulating hormone (TSH). TSH, in turn, stimulates the thyroid to produce and release thyroid hormone until levels in the blood return to normal. Thyroid hormone exerts negative feedback control over the hypothalamus as well as the anterior pituitary, thus controlling the release of both TRH from hypothalamus and TSH from anterior pituitary gland.

The two major hormones produced by the thyroid are thyroxine (T4) and triiodothyronine (T3). T3 is formed through the deiodination of T4 and is the most active of the thyroid hormones, regulating most bodily processes. The synthesis and secretion of thyroid hormone by the thyroid gland is controlled by thyroid stimulating hormone (TSH), also called thyrotropin. TSH, secreted by the anterior pituitary, binds to the thyroid stimulating hormone receptors (TSHR), or thyrotropin receptors (TR), on the cells of the thyroid gland stimulating the synthesis and secretion of thyroid hormones. Therefore, the TSHR plays a key role in controlling thyroid function. It is also a major auto-antigen in thyroid autoimmune diseases. Those autoantibodies include thyroid-stimulating immunoglobulin (TSI), TSHR agonist, and thyroid-blocking immunoglobulin (TBI), TSHR antagonist.

Autoimmune thyroiditis (AT) is a form of hypothyroidism caused by the presence of autoantibodies against the TSHR, and is characterized by a deficiency of thyroid hormone. In autoimmune thyroiditis, TBI binds to the TSHR and prevents or blocks the TSH from binding to its own receptor. This event stops the intracellular signaling cascade required for the production and secretion of thyroid hormones.

Autoimmune diseases involving the presence of TBI occur much less frequently than those involving TSI. However, it has been reported that during treatment, the circulating autoantibody population can change from TSI to TBI.[1] In addition, the presence of TBI during pregnancy can lead to severe consequences as the autoantibodies can cross the placental barrier. Therefore, it is important to provide a diagnostic method to detect the thyroid blocking antibodies (TBAb) that can aid physicians in patient care. Currently, no specific TBI detecting method is available. The typical panel of AT diagnostic methods includes the measurement of serum levels of Thyroid stimulating hormone (TSH), Free T3, Free T4, and anti-thyroid antibodies (Tg) and thyroid peroxidase antibody (TPO) tests.

Thyretain TBI Blocking Reporter BioAssay is a competitive cell-based assay (or "bioassay") which utilizes a genetically engineered cell line, expressing a chimeric TSHR and firefly luciferase reporter gene, capable of detecting serum TBI.

PRINCIPLE OF THE PROCEDURE

The Thyretain TBI Blocking Reporter BioAssay utilizes a patented bioassay technology to detect TBI in human serum. Genetically engineered Chinese hamster ovary (CHO) cells, expressing a chimeric form of the human TSHR and a cyclic adenosine monophosphate (cAMP) induced luciferase reporter gene, are cryogenically preserved and provided in measured aliquots. The cells are seeded and grown for 15 to 18 hours to a confluent monolayer in a 96-well plate.

Patient sera, Reference Control, Negative Control and Positive Control are mixed with Working Solution containing bovine thyroid stimulating hormone (bTSH) at 1:11, added to the cell monolayers and allowed to react with the cells for 3 hours. During this induction period, TBI, if present in the patient serum, binds to the chimeric human TSHR on the cell surface and blocks the binding of the bTSH. This blocking event inhibits the signaling cascade resulting in the decreased production of intra-cellular cAMP. The suppressed production of cAMP is evidenced by reduced production of luciferase. At the conclusion of the 3-hour induction period the cells are lysed. Luciferase levels are then measured using a luminometer. A significant decrease in luminescence as compared to the assay Reference Control indicates the presence of TBI in the sample.

MATERIALS PROVIDED

- Ten CHO Mc4 FreshFrozenCells®, 1.0 mL each.
- 100 mL Cell Attachment Solution (CAS)
- 100 mL Growth Medium (GM)
- 200 mL Reaction Buffer (RB)
- Control Set (10):
  - TBI Reference Control, 0.1 mL each.
  - Positive Control, 0.1 mL each.
  - Negative Control, 0.1 mL each.
  - Working solution, 9 mL each.
- Luciferase Assay Reagent Set (5 each)
  - Luciferase Substrate, 1 vial, lyophilized.
  - Luciferase Assay Buffer Solution, 1 vial, 10 mL.

MATERIALS REQUIRED BUT NOT PROVIDED

- –70°C or lower freezer or liquid nitrogen Dewar
  Note: A chest freezer is preferred over an upright freezer. Assay failure is a potential issue due to repeated exposure of the cells and panel to temperature changes over time when an upright freezer is used.
- Humidified, 5% CO_2, 35°C to 37°C Incubator
- Bio-safety Cabinet Class II
- Luminometer capable of reading a 96 multi-well plate (MWP)

- Luminometer calibrator plate
- Microscope, inverted
- Calibrated Pipettes
  - Multi-Channel 20 to 200 µL
  - Single 20 to 200 µL
  - Single 100 to 1000 µL
  - Sterile Pipette Tips
  - 5, 10, 25-mL Sterile Serological Pipettes
- Sterile Transfer Pipette
- Pipetman® variable volume pipetters
- 96 MWP, Black, Clear Bottom (Costar #3603)
- Sterile Reagent Reservoirs
- Sterile Absorbent Pad
- Water bath, 35°C to 37°C
- Sterile screw-cap tubes (15 or 50 mL)
- 13-mm Test Tubes
- Vortex Mixer
- Timer
- Household Bleach

Pipetman is a registered trademark of Gilson S.A.S.

REAGENT STORAGE INSTRUCTIONS

<table>
<tr><th colspan="2">Reagent Storage Conditions</th></tr>
<tr><td>1. CHO Mc4 FreshFrozenCells</td><td rowspan="2">Store at –70°C or lower</td></tr>
<tr><td>2. Control Set (Positive, Negative, Reference, and Working Solution)</td></tr>
<tr><td>3. Cell Attachment Solution
Note: The solution may show precipitation if stored at refrigerated temperatures. The material may be dissolved when the solution is warmed in a 35°C to 37°C water bath. It is recommended that this solution be stored at room temperature to avoid precipitation.</td><td>Store at 2°C to 30°C.</td></tr>
<tr><td>4. Growth Medium</td><td rowspan="2">Store at 2°C to 8°C.</td></tr>
<tr><td>5. Reaction Buffer</td></tr>
<tr><td>6. Luciferase Assay Reagent Set</td><td>Store at –20°C or lower.</td></tr>
</table>

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- This Kit contains materials of human (e.g., human serum) and bovine (e.g., bTSH) origin. All bovine materials have been certified to be of United States origin. All human serum controls have been tested for HBsAg, HIV-1, -2 and HCV antibodies and found to be negative. Despite this screening, all human serum controls and patient samples should be considered potentially hazardous and handled with extreme care.
- No known test method can offer complete assurance that infectious agents are absent; therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease. It is recommended that reagents and human specimens should be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[2]
  - Cell cultures may have some potential to be hazardous. Personnel working with these cultures must be properly trained in safe handling techniques[3] and have experience with tissue culture before attempting this procedure.
  - All procedures must be conducted in accordance with the CDC 5th edition Biosafety in Microbiological and Biomedical Laboratories, 2007, and CLSI Approved Guideline M29-A, Protection of Laboratory

Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.

- All specimens and materials used to process them should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel.
  - Biosafety Level 2 or other appropriate biosafety practices should be used when handling these materials.
  - Decontamination of specimens and cultures is most effectively accomplished using a 1:10 dilution of household bleach.
  - Although Control reagents have been shown to be non-infectious (Positive and Negative Controls and Working Solution) and of United States origin (Reference Control), the same precautions taken in handling and disposing of other infectious materials should be employed in their use.
- Never pipette reagents or clinical samples by mouth; avoid all contact of clinical samples with broken skin.
- Avoid splashing and the generation of aerosols with clinical samples.
- Use aseptic technique and sterile equipment and materials for all cell culture procedures.
- The CHO Mc4 FreshFrozenCells must be properly stored (–70° C or below) at all times to maintain optimum performance. The swift transfer of freezer vials to and from the freezer or liquid nitrogen storage is mandatory. Repeated exposure to temperature fluctuations may affect cell viability and/or assay performance.
- The CHO Mc4 FreshFrozenCells are single-use only and cannot be re-frozen once thawed.
- Extreme care should be taken to ensure that the level of CO_2 in the incubator is accurately calibrated to 5%. Prolonged exposure to excessively high (>5.5%) or low (<4.5%) CO_2 conditions could affect assay performance.
- The handling and preparation of the CHO Mc4 FreshFrozenCells and cell culture reagents must be performed using aseptic technique, unless otherwise noted.
- All reagents should be pre-warmed to ambient temperature before use. This includes Growth Medium, Reaction Buffer, and Luciferase Assay Reagent Set.
- The TBI Reporter Controls are supplied at working strength. Any dilution of these reagents will decrease sensitivity.
- Reagents should be used prior to their expiration date.
- Each multi-well plate should be used only once. Do not re-use previously assayed plate.
- Microbial contamination of the CHO Mc4 FreshFrozenCells and cell culture reagents may cause a decrease in sensitivity.
- Use of other reagents than those specified with the components of this Kit, especially those that contain sodium azide, may lead to erroneous results.
- The Growth Medium contains the pH indicator phenol red. Repeated exposure of the medium to the air may cause an increase in pH, evidenced by an increasingly deep red color. Limit the exposure of the medium to air as pH levels above 7.9 may affect the assay performance.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com

SPECIMEN COLLECTION AND PREPARATION

Proper collection and handling of the patient specimen are the most important factors in successful TBI detection. Specimen collection and processing should be attempted only by personnel trained in such procedures. Care should be taken during all specimen collection and handling to avoid generation of aerosols.

Serum is required for the TBI Reporter. For additional specimen collection and processing recommendations please refer to CLSI Document H3-A6.[4]

SPECIMEN TRANSPORT AND STORAGE

Serum specimens should be transported to the laboratory at 2°C to 8°C using cold packs, wet ice, foam refrigerant, or other coolants. The specimen should be processed and tested as soon as possible or stored for up to 72 hours at 2°C to 8°C before testing. If testing does not occur before 72 hours the specimen may be aliquoted and frozen at –20ºC for up to 2 months. Extended storage, beyond 2 months, should occur at temperatures that are –70°C or lower or in a liquid nitrogen Dewar.

- Repeated freezing and thawing of serum samples should be avoided since this may affect specimen biological activity, leading to erroneous results.
- Samples may go through a maximum of three (3) freeze/thaw cycles.
- Samples collected for retrospective analysis should be aliquoted upon receipt and immediately frozen.

All potentially infectious agents should be transported according to International Air Transport Association (IATA), International Civil Aviation Organization, (ICAO), Titles 42 and 49 of the US Code of Federal Regulations, or other regulatory requirements, as may be applicable.

ASSAY PROCEDURE

Preliminary Comments and Precautions

1. Adhere to the recommended volumes and times in the following procedure to ensure that accurate results are obtained.
2. When medium is decanted from the cells, it is important that all medium is removed. Decant medium with enough force to completely remove it from each well. Visually examine the wells for medium removal before proceeding.
   Note: It is imperative that all medium be removed from the wells before moving to the next step in the procedure.
3. To prevent aspiration of water from the water bath into the vial, do not allow the water bath level to reach the junction of the vial/cap.
4. Aseptic technique must be used throughout the first 15 to 18 hours of the procedure. Microbial contamination increases the risk of assay failure.
5. Do not allow the monolayers to dry between steps; this can be avoided by handling one plate at a time.
6. Uniform heating of the cells is a requirement. The plates are to be placed side-by-side in the incubator rather than stacked. Stacking the plates will cause poor assay performance and greatly increase the risk for both inter- and intra-plate variation as well as assay failure.
7. The plates must be carefully handled in order to avoid uneven distribution of the cells. Use of non-vibrating surfaces is a necessity to ensure uniform distribution of the cells in the wells.
8. A confluent monolayer is one where cells are in contact with each other forming a continuous sheet of adherent cells on the bottom of the plate well. The confluency of the monolayer is assessed prior to use with a microscope at 100X magnification.

Thyretain TBI Procedure

Day 1: Carry out aseptically

1. Calculate the number of plates needed to perform the assay – each plate can support 21 specimens tested along with the Positive, Negative, and Reference controls. All testing is performed in duplicate.
2. Aliquot 5 mL per plate of Growth Medium (GM) into a suitable, sterile container (i.e., 50-mL centrifuge tube).
3. Place aliquoted GM into a 35°C to 37°C water bath 5 minutes prior to use.
4. Add 100 μL of Cell Attachment Solution (CAS) using a multi-channel pipette to each of the 48 inner wells of each plate, and treat for 1 to 10 minutes at 20°C to 25°C.

5. Decant the CAS from the wells onto a sterile absorbent pad inside the biological hood.
6. Thaw one vial of CHO Mc4 FreshFrozenCells per plate in a 35°C to 37°C water bath for 2 to 4 minutes.
7. Transfer the entire volume of thawed cells to the pre-warmed GM using a sterile transfer pipette. Rinse transfer pipette into the GM by aspirating and expelling 3 times.
8. Close the tube and mix the cell suspension by inverting several times.
9. Pour the suspension to a sterile reagent reservoir.
10. Add 100 μL of the cell suspension to each of the inner 48 wells of each plate using a 100-μL multi-channel pipette. It is necessary to mix the suspension in the reagent reservoir by pipetting up and down frequently to ensure the cells stay in suspension and are uniformly distributed among the wells.
11. Incubate all seeded plates for 15 to 18 hours in a humidified, 5% CO_2, 35°C to 37°C incubator. In order to avoid microbial contamination, handle the plates so that the lid is not opened outside of the Bio-safety Cabinet.

Day 2: Steps 1-16 can be carried out on the benchtop

1. Place Reaction Buffer (RB), Working Solution (WS), Positive Control, Negative Control and Reference Control in a 35°C to 37°C water bath 5-10 minutes prior to use.
2. Remove the plate(s) from the incubator.
3. Examine the monolayers in each well microscopically using a magnification of 100X to 200X.
    a. Plates exhibiting signs of microbial contamination should be discarded.
    b. Monolayers must be confluent to be used in the assay. Mark any wells for disqualification based on sub-confluence.
    ***Note**: Plates can remain in the incubator for up to 18 hours or until confluency is reached (a minimum of 15 hours). Plates that fail to reach confluency should be disqualified from use.*
    c. Individual wells containing piled or layered cells should be disqualified from use based on over-confluence.
4. Return plate(s) to the incubator following examination.
5. Calculate the amounts of serum specimen, Reference control, Positive control, Negative control, Reaction Buffer (RB), and Working Solution (WS) needed to perform the assay as follows:
    a. Prepare a 1:11 dilution of each specimen as follows:
        i. Label a 13-mm test tube with patient identifier.
        ii. Add 300 μL of WS to each tube.
        iii. Vortex serum specimen vigorously for 15 seconds.
            a. If frozen, thaw specimens in a 35°C to 37°C water bath for 7 to 10 minutes.
        iv. Add 30 μL of each specimen to the WS in the appropriately labeled tube.
        v. Vortex vigorously for 15 seconds.
    b. Prepare a 1:11 dilution of Reference Control in the same manner as outlined in 5.a:
        i. Thaw the appropriate volume of Reference Control in a 35°C to 37°C water bath for 7 to 10 minutes.
        ii. Label a 13-mm test tube with control identifier.
        iii. Add 300 μL of WS to the tube.
            a. The Reference Control must be included per plate.
        iv. Vortex Reference Control vigorously for 15 seconds.
        v. Add 30 μL of Reference Control to the WS in the appropriately labeled tube.
        vi. Vortex vigorously for 15 seconds.
    c. Prepare a 1:11 dilution of Positive Control and Negative Control in the same manner as outlined in 5.a:
        i. Label two 13-mm test tubes with control identifier.
        ii. Add 300 μL of WS to each tube.
            a. The Positive Control and Negative Control must be included per plate.
        iii. Vortex Positive Control and Negative Control vigorously for 15 seconds.
        iv. Add 30 μL of Positive Control and Negative Control to the WS in the appropriately labeled tube.

v. Vortex vigorously for 15 seconds.

Steps 6 through 16 below are to be performed **one plate at a time**. They should also be performed sequentially in order to avoid drying of the cell monolayer.

6. Remove a plate from the incubator.
7. Decant the GM from the plate into an appropriate waste container.
8. Rinse the cells by adding 100 μL of pre-warmed RB to each well using a multi-channel pipette. The medium should be gently dispensed down the side of the well in order to avoid disruption of the monolayer.
9. Decant the RB into an appropriate waste container.
10. Add 100 μL of pre-warmed RB to each well using a multi-channel pipette. The medium should be gently dispensed down the side of the well in order to avoid disruption of the monolayer.
11. Add 100 μL of the diluted Positive, Negative and Reference controls, and specimens (prepared above) to the appropriate RB containing wells (in duplicate) using a 20 to 200-μL pipette. The plate layout indicated below is recommended.

	1	2	3	4	5	6	7	8	9	10	11	12
A	X	X	X	X	X	X	X	X	X	X	X	X
B	X	X	Ref	PC	NC	S1	S2	S3	S4	S5	X	X
C	X	X	Ref	PC	NC	S1	S2	S3	S4	S5	X	X
D	X	X	S6	S7	S8	S9	S10	S11	S12	S13	X	X
E	X	X	S6	S7	S8	S9	S10	S11	S12	S13	X	X
F	X	X	S14	S15	S16	S17	S18	S19	S20	S21	X	X
G	X	X	S14	S15	S16	S17	S18	S19	S20	S21	X	X
H	X	X	X	X	X	X	X	X	X	X	X	X

12. Incubate each plate for 3 hours in a humidified, 5% CO_2, 35° to 37°C incubator.
13. Remove an appropriate number of Luciferase Assay Reagent Set kits from the freezer approximately 30 minutes prior to use. Two sets provide enough substrate for 5 plates.
    a. Thaw the Luciferase Assay Buffer Solution (buffer) in a 35°C to 37°C water bath for 15 minutes.
    b. Store the Luciferase Substrate at 20°C to 25°C until used.
14. Pour the buffer (white bottle) into the lyophilized Luciferase Substrate (amber bottle). Replace the cap, and invert gently 6 times to mix.
15. Transfer the substrate solution to a reagent reservoir and cover to keep the substrate solution in the dark. Minimize exposure of the substrate solution to light as this may cause a decrease in activity.
16. Process each plate separately to completion as follows:
    Note: The lysis process for the next plate should not be carried out until the previous plate is completed and enough time has elapsed to allow for the previous plate to be analyzed in the luminometer.
    a. Decant the contents of the plate into an appropriate waste container.
    b. Remove the remaining volume of medium from the wells by blotting the plate upside down on an absorbent pad.
    c. Add 75 μL of Luciferase Substrate to each well using a multi-channel pipette.
    d. Cover the MWP and allow to stand at 20°C to 25°C for 10 minutes.
    Note: It is critical to maintain the cell lysis temperature above 20°C. The test result will be affected if the lysis temperature falls to 19°C or less.
    e. Read the plate in a luminometer that is programmed to read the inner 48 wells of the plate at an integration time of 1 second per well.

QUALITY CONTROL

1. Positive, Negative and Reference Controls should be run and calculated with each plate of specimens to confirm the assay performance.
2. A reference range is provided with each Positive Control and Negative Control Set which establishes the maximum and minimum acceptable values when Percent Inhibition (%I) is calculated. The Negative Control should yield a % I value less than 22%. The Positive Control range may change with each lot of the positive

control. Please check the value for the positive control on the reference range label prior to evaluating control validity.

3. If assay controls fail to perform correctly (i.e., above or below established range), results for that plate are considered invalid. Contact Quidel/Diagnostic Hybrids, Inc. Technical Support if an assay run is invalid.

CALCULATION OF RESULTS

Example Run:

X	X	X	X	X	X	X	X	X	X	X	X
X	X	13963	6506	14060	5608	6811	2610	6729	4626	X	X
X	X	13693	6294	13795	5361	6374	2324	5882	4717	X	X
X	X	2796	4393	6184	5363	4844	3435	2892	3075	X	X
X	X	2540	4622	6144	5609	4867	3501	2789	2850	X	X
X	X	7226	7510	10018	7226	7382	10006	9428	2111	X	X
X	X	7057	8361	10684	8035	8151	10570	9580	1996	X	X
X	X	X	X	X	X	X	X	X	X	X	X

Calculation of Results

1. The average Relative Light Unit (RLU) and coefficient of variation (CV) % of each control is calculated using the values from the duplicate wells.

Example Run:

Control Type	RLU Value 1	RLU Value 2	Average RLU	% CV	CV% Acceptable
Positive Control	6506	6294	6400	2.3%	Yes
Negative Control	14060	13795	13928	1.3%	Yes
Reference Control	13963	13696	13830	1.4%	Yes

The CV% of the positive, negative and reference controls should be ≤15% within each plate.

2. Calculate the average RLU and (CV) % for each specimen.

Example Run:

Sample ID	RLU Value 1	RLU Value 2	Average RLU Value	CV%	CV% Acceptable
Sample 1	4393	4622	4508	3.6%	Yes
Sample 2	6184	6144	6164	0.5%	Yes
Sample 3	7226	7057	7142	1.7%	Yes
Sample 4	10018	10684	10351	4.5%	Yes

The CV% for each specimen should be ≤15%.

3. Calculate the percent inhibition (%I) for each control and specimen using the following calculation:

% Inhibition = (Reference RLU - Sample RLU)/(Reference RLU) x 100

Example Run:

Sample ID	Average RLU Value	CV%	%I
Positive Control	6400	2.3%	54%
Negative Control	13928	1.3%	0.7%
Sample 1	4508	3.6%	67%
Sample 2	6164	0.5%	55%
Sample 3	7142	1.7%	48%
Sample 4	10351	4.5%	25%

INTERPRETATION OF RESULTS

- A specimen must have a CV of less than 15% between the duplicate RLU values to be considered valid. All specimens with an invalid (Null) result must be repeated.
- A patient inhibition result greater-than or equal to (≥) 34% I is reported as Positive for the presence of TBI.
- A patient result less than ≤ 34 % I is reported as Negative for the presence of TBI.

LIMITATIONS OF PROCEDURE

- This assay requires **serum samples only**. Use of plasma or whole blood may result in assay failure.
- Serum must be free of particulate matter before analysis can commence. The presence of particulate matter may affect the sensitivity of the assay.
- The testing of serum that is visibly icteric, hemolytic or lipemic may lead to decreased sensitivity in the detection of TBI.
- Incubation times or temperatures other than those cited in the test instructions may give erroneous results.
- Detection of TBI can vary depending upon the specimen quality and subsequent handling. A negative result does not exclude the possibility of the presence of TBI. Results of the test should be interpreted in conjunction with information available from other clinical information, such as physical symptoms and thyroid hormone testing, as recommended by the American Thyroid Association (ATA).
- The Thyretain TBI Blocking Reporter BioAssay is intended for the qualitative detection of TBI. It is not intended for use in monitoring a patient's treatment. The effects of various drug therapies on the performance of this Kit have not been established.
- This is a functional bioassay for the detection of serum TBI. Sample dilutions are not advisable as there is a non-linear relationship between antibody concentration and signal (Relative Light Unit, RLU).
- Performance of the Kit can only be assured when components used in the assay are those supplied by Quidel.

EXPECTED VALUES

In a study of 160 healthy blood donors, all yielded inhibition results ≤ 34 % and considered negative by this assay. However, each laboratory should establish its own reference ranges.

SPECIFIC PERFORMANCE CHARACTERISTICS

A multi-center study was performed to evaluate the Thyretain TBI Blocking Reporter BioAssay using two hundred six (206) frozen serum specimens from patients diagnosed with and without autoimmune thyroid disease (AITD). The AITD specimens included those specifically diagnosed with Graves' disease (eighty [80] specimens) and non-Graves' disease (eighty-eight [88] specimens). Sera from thirty-eight (38) normal patients were also included. The inclusion of Graves' disease patients was to demonstrate the Thyretain TBI Blocking Reporter BioAssay's utility in identifying patients potentially undergoing switching between TBAb (thyroid blocking antibody) and TSAb (thyroid stimulating antibody)

Three (3) panels were created from the randomized serum specimens and tested at three (3) sites: Site 1 tested seventy-one (71) specimens; Site 2 tested sixty-eight (68) specimens; Site 3 tested sixty-seven (67) specimens. The testing sites were blinded to the diagnosis associated with the serum.

Each specimen was tested at a central location for the presence of human antibodies to the TSH receptor . This testing was performed concurrently with each site's testing of the panel members. The central location was blinded to the diagnosis associated with the serum.

The gender and age demographics for the patients are listed in Table 1.

Table 1
Age and Gender Distribution

Gender	Female	Male
Total	169	37
Age		
≤ 18 yrs.	10	1
19 to 40 yrs.	80	19
41 to 65 yrs.	69	15
≥ 66 yrs.	10	2

Table 2 below summarizes the comparison of Thyretain TBI Blocking Reporter BioAssay and Kronus TSH Receptor Autoantibody (TRAb) ELISA kit for the specimens from diagnosed AITD (both Graves' and non-Graves') and normal patients. Thirty-nine (39) of the specimens were positive for TSI and have been removed from analysis. The data presented is for the remaining one hundred sixty-seven (167) specimens.

Table 2
Combined Sites Result Summary AITD Disease Patients

167 specimen results		**Comparator Device (KRONUS TRAb)**	
		+	**–**
Subject Device (TBI Blocking Reporter BioAssay)	**+**	63	7
	–	4	93
		95% Confidence Interval	
Positive Percent Agreement	94.0%	85.6 to 97.7%	
Negative Percent Agreement	93.0%	86.3 to 96.6%	

Limit of Blank
Eighty eight (88) blank measurements were carried out to determine LOB for the TBI assay. Because the data was non- Gaussian, the LOB was determined using the method as recommended in the CLSI guideline (EP17-A).

TBI LOB = Results at position $[N_B(p/100)+0.5)]=[88*0.95+0.5]=84.1$. According to the data, % I value is 13% for the samples at both 84th and 85th positions, therefore the LOB of the TBI assay is **13% I**

Limit of Detection
The calculated LOD for the TBI assay was determined to be 22% I according to the CLSI guideline (EP17-A).

TBI LOD = LOB + 1.645 Std Dev (low concentration samples) = 13% I +1.645 ×5.6% I = **22% I**

This LOD was verified by performing 20 measurements of a contrived positive specimen diluted to the claimed LOB concentration and the confirmed TBI LOD value is 22% I.

Cross-reactivity and Interference by Endogenous Substances:

Cross-reactivity was determined for the following substances by spiking and testing TBI-containing human serum with different concentrations of luteinizing hormone up to 625 mIU/mL, human chorionic gonadatrophin up to 40,625 mIU/mL, follicle stimulating hormone up to 2,000 mIU/mL and thyroid stimulating hormone up to 0.35 mIU/mL (Table 3).

Interference was not observed for the following substances by spiking and testing TSI-containing human serum with different concentrations of bilirubin up to 36.6 mg/dL, hemoglobin up to 250 mg/dL and lipids up to 1168 mg/dL.

Table 3
Summary of Cross-reactivity Studies on the TBI Reporter

Hormone	WHO_ NIBSC Code	Starting Concentration (mIU/mL)	Ending Concentration (mIU/mL)	Normal Physiological Range in Healthy Adults Concentration (mIU/mL)	Cross-reactivity	Interference
Luteinizing Hormone (LH)	80/552	10,000	2.4	5 to 20	No	No
Human Chorionic Gonadatropin (hCG)	75/589	650,000	158.7	0.1 to 8,000	No	No
Follicle Stimulating Hormone (FSH)	83/575	8,000	3.9	1.4 to 116.3	No	No
Thyroid Stimulating Hormone (TSH)	03/192	9,500	0.000039	0.0003 to 0.0030	No	No
Plasma or serum Hemoglobin	N/A	2000 mg/dL	250 mg/dL	5 mg/dL hemoglobin	No	No
Bilirubin	N/A	100 mg/dL	36.6 mg/dL	0.2 – 1.3 mg/dL	No	No
Cholesterol	N/A	10000 mg/dL	1168 mg/dL	< 0.2 g/dL	No	No

* Based on testing standards for various reference laboratories and institutions such as LabCorp of America, AACE and Mayo Foundation.

Cross-Reactivity with Other Autoantibodies:

The TBI Reporter was tested on 20 samples with autoimmune diseases other than autoimmune thyroid disease (AITD): 10 Rheumatoid Arthritis (RA) and 10 Systemic Lupus Erythematosus (SLE). All samples tested negative for cross-reactivity and interference.

Assay Cutoff

Patient serum, when tested using the TBI Reporter, demonstrating an inhibition result greater-than or equal to (≥) 34% I is reported as Positive for the presence of TBI. A total of eighty-two (82) sera were tested using the TBI assay by multiple operators. The percent inhibition was calculated for each sample. The average and standard deviation for each operator's results were calculated, and if the assay was repeated by two operators, the average between the two operators was determined. The percent inhibition that would result in negative qualitative results for 95%, 98% and 99% of the tested results (average plus 1.65, 2.05 and 2.33 standard deviations, respectively) was reported as possible values for the assay cutoff.

A distribution analysis was performed on the average calculated % Inhibition values for serum samples. A normal curve was fit to the data, and the Shapiro-Wilk test was used to determine that the data did not deviate significantly from a Normal distribution ($p=0.1804$). Based on these results, 98% will fall below 33.5% Inhibition. The cutoff chosen for the TBI Assay is 34% I.

Intra-Assay Precision

A Precision Panel was prepared by spiking normal serum with TSHR blocking antibody at two concentrations. The "medium" or "mid" concentration contained 80 ng/mL of TSHR blocking antibody, and the "low" concentration contained 40 ng/mL of TSHR blocking antibody. Six replicates of each Control and contrived specimen were tested by the TBI assay on one plate. The assay was repeated by one user over 25 days.

Inter-Assay Precision (Intra-Day)

For each test concentration, the within-run standard deviations (Sr) and %CVs were calculated (Table 4).

Table 4
Intra-Day Precision

Sample ID	Average % Inhibition	Within-Run Standard Deviation (S_r)	Within-Run %CV
Low conc. [40 ng/mL]	45%	2.6%	5.7%
Medium conc. [80 ng/mL]	71%	1.5%	2.1%

Inter-Assay Precision (Inter-Day)

For each test concentration, the between-day standard deviations (Sr) and %CVs were calculated (Table 5).

Table 5
Inter-Day Precision

Sample ID	Average % Inhibition	Between-Day Standard Deviation (S_r)	Between-Day %CV
Low conc. [40 ng/mL]	45%	7.4%	16.4%
Medium conc. [80 ng/mL]	71%	4.5%	6.3%

Assay Reproducibility

To demonstrate competency with and reproducibility of the assay, each trained site performed testing on the panel[a] described in Tables 6 and 7 below, twice a day over an 8-day span.

Table 6
Reproducibility Panel Variation and Accuracy

Reagent	Concentration	Expected Result	Expected Accuracy
Specimen A	25 ng/mL	Negative	100%
Specimen B	45 ng/mL	Positive	100%
Specimen C	65 ng/mL	Positive	100%

Table 7
Reproducibility Results

Panel Member ID	Site 1			Site 2			Site 3			Combined Site Data		
	Rate of Detection	AVG I%	% CV	Rate of Detection	AVG I%	% CV	Rate of Detection	AVG I%	% CV	Rate of Detection	AVG I%	% CV
TBI Negative (25 ng/mL)	0/30	22	34	0/30	24	26	0/30	27	18	0/90	24	27
TBI Low Positive (45 ng/mL)	30/30	42	11	30/30	45	19	30/30	46	12	90/90	44	15
TBI Moderate Positive (65 ng/mL)	30/30	54	11	30/30	45	19	30/30	58	9	90/90	56	12

---

[a] The panel consisted of four specimens created to meet the requirements for precision as set forth in both CLSI (EP12-A2) and FDA guidance documents (http://www.fda.gov/cdrh/ode/odecl051.html)

Panel Member ID	Site 1			Site 2			Site 3			Combined Site Data		
	Rate of Detection	AVG I%	% CV	Rate of Detection	AVG I%	% CV	Rate of Detection	AVG I%	% CV	Rate of Detection	AVG I%	% CV
TBI Positive Control	30/30	49	9	30/30	47	21	30/30	47	10	90/90	48	14

CUSTOMER AND TECHNICAL ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at (800) 874-1517 (toll-free in the U.S.) or (858) 552-1100 (outside the U.S.), Monday through Friday, between 8:00 a.m. and 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

REFERENCES

1. McLachlan S., Rapoport B, 2013, Thyrotropin-Blocking Autoantibodies and Thyroid-Stimulating Autoantibodies: Potential Mechanisms Involved in the Pendulum Swinging from Hypothyroidism to Hyperthyroidism or Vice Versa. THYROID, Vol 23, 14-24
2. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.
3. *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th edition, 2009, CDC-NIH manual. [http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm]
4. CLSI. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard, 5th Edition. CLSI document H3-A6 (ISBN 1-56238-650-6). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2003.

40-35010 – Thyretain TBI Blocking Reporter BioAssay

MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

Diagnostic Hybrids, Inc. – also trading as Quidel Corporation
2005 East State Street, Suite 100
Athens, OH 45701 USA
quidel.com

PI4035011EN00 (10/20)

GLOSSARY

REF

Catalogue number

CE mark of conformity

EC REP

Authorized Representative
in the European Community

LOT

Batch code

Use by

Manufacturer

Temperature limitation

Intended use

Rx ONLY

Prescription use only

Consult e-labeling
instructions for use

Biological risks

IVD

For *In Vitro* diagnostic use

Contains sufficient for 105 determinations

CONT

Contents/Contains