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Thyretain® TurboTSI

Stimulating Reporter BioAssay

For the detection and quantification in serum of thyroid stimulating autoantibodies to the thyroid stimulating hormone (TSH) receptors (TSHRS) on the thyroid.

For *in vitro* diagnostic use.

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

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INTENDED USE

Thyretain Turbo TSI Stimulating Reporter BioAssay is intended to detect and quantify in serum thyroid stimulating autoantibodies to the thyroid stimulating hormone (TSH) receptors (TSHRs) on the thyroid. The detection of these stimulating autoantibodies, in conjunction with other clinical and laboratory findings, may be useful as an aid in the differential diagnosis of patients with Graves' disease (GD).

SUMMARY AND EXPLANATION OF THE TEST

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.

Hyperthyroidism is characterized by an excessive synthesis and secretion of thyroid hormones. In the majority of cases this overproduction of hormones is caused by a class of autoantibodies to TSHR which mimic the action of TSH. These stimulating autoantibodies are referred to as thyroid stimulating immunoglobulins (TSI) and, when present, are indicative of GD.

In a normal functioning system, homeostasis is maintained 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 TSH. The TSH, in turn, stimulates the thyroid to produce and release thyroid hormone until levels in the blood return to normal. Thyroid hormone exerts negative feed-back 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 (T_4) and triiodothyronine (T_3). T_3 is formed through deiodination of T_4 and is the most active of the thyroid hormones, regulating most bodily processes

The TSI present in patients with GD mimic TSH causing an over-production of both hormones, leading to hyperthyroidism.
GD, one of the most common forms of hyperthyroidism, has an incidence of approximately 5 in 10,000 people per year, affecting 13 million, and targets women seven times as often as men.[1] Although there is currently no cure for GD, it is treatable by anti-thyroid drug therapies, radioactive iodine ablation or surgical removal of the thyroid gland, as cited by American Association of Clinical Endocrinologist guidelines.[2] Though the presence of TSI in serum of patients known to have GD is significant to the disease, the direct screening for this autoantibody has not been used as a primary tool in its diagnosis. The diagnosis of GD is typically derived from a panel of diagnostic tests, which includes the measurement of serum levels of TSH, T_3, T_4, and thyroid receptor antibodies (TRAb). There are two types of TRAb, however, TSI and Thyroid Blocking Immunoglobulins (TBI). The TBI binds to the TSHR and prevents or inhibits the stimulation and secretion of thyroid hormones by TSH, leading to hypofunctioning of the thyroid or hypothyroidism. The measurement of serum TRAb is flawed by its inability to distinguish TSI from TBI.

Thyretain Turbo TSI Stimulating Reporter BioAssay (Turbo TSI) is a cell-based assay (or "bioassay") which utilizes a genetically engineered cell line capable of specifically detecting serum TSI.

PRINCIPLE OF THE PROCEDURE

The Thyretain Turbo TSI Stimulating Reporter BioAssay utilizes a patented bioassay technology to detect and quantitate TSI in human serum. Genetically engineered Chinese hamster ovary (CHO) cells, both chimeric form of TSH receptor (TSHR Mc4) and GloSensor (GS) reporter are cryogenically preserved and provided in measured aliquots.

Serum samples are mixed with freshly thawed GS-TSHR Mc4 cells in a white, opaque bottom, multiple well plate. The TSAb present in the samples will increase the intracellular cAMP level through the antibody and TSHR interaction. Upon binding of cAMP the GS reporter, which is a mutant form of firefly luciferase fused with cAMP binding domains, produces increased visible light output that can be measured as relative light units (RLU) by a luminometer.

A quantitative standard curve, using standards traceable to WHO international standards (IS), is generated with each test run. The average relative light units (RLU) from each standard (y-axis) and the concentration in IU/L (x-axis) are used to plot a standard curve. The RLU value for the specimens is then used to determine the quantity of thyroid stimulating autoantibody present.

REAGENTS AND MATERIALS PROVIDED

Thyretain Turbo TSI Stimulating Reporter BioAssay kit contains the following:

- Turbo TSI Kit panel 45-1005
- Turbo TSI Cells 45-9648.TSI
- Turbo TSI Negative Control (Neutral Cap) 45-102001
- Turbo TSI Low Positive Control (White Cap) 45-101001L
- Turbo TSI Mid Positive Control (Brown Cap) 45-101001M
- Turbo TSI High Positive Control (Black Cap) 45-101001H
- Turbo TSI cAMP Reagent 45-105005
- Turbo TSI Standard A (Red Cap) 45-206001A
- Turbo TSI Standard B (Orange Cap) 45-206001B
- Turbo TSI Standard C (Yellow Cap) 45-206001C
- Turbo TSI Standard D (Blue Cap) 45-206001D
- Turbo TSI Standard E (Purple Cap) 45-206001E

MATERIALS REQUIRED BUT NOT PROVIDED

- –70°C or lower freezer or liquid nitrogen Dewar
- Luminometer capable of reading a 96 multi-well plate
- Luminometer Calibrator Plate
- Calibrated Pipettes
  - Multi-Channel 20 µL to 200 µL
  - Single 5 µL to 20 µL
  - Single 100 µL to 1000 µL
  - Sterile Pipette Tips
- Sterile Transfer Pipette
- 96-Well White, Flat Bottom Assay Plate (Costar, 3912)
- Microplate Adhesive Film (USA Scientific, 2920-0000)
- Sterile Reagent Reservoirs (VWR, 41428-954)
- Water Bath, 35°C to 37°C
- Vortex Mixer
- Timer
- Household Bleach

REAGENT STORAGE INSTRUCTIONS

Turbo TSI Cells (FreshFrozenCells®)	Store at –70°C or lower
Turbo TSI Negative Control (CTRL)	
Turbo TSI Low Positive Control	
Turbo TSI Mid Positive Control	
Turbo TSI High Positive Control	
Turbo TSI cAMP Reagent	
Turbo TSI Standard A	
Turbo TSI Standard B	
Turbo TSI Standard C	
Turbo TSI Standard D	
Turbo TSI Standard E	

STABILITY

Reagents and components will retain their full potency through the expiration date shown on the kit box label when stored at recommended temperatures.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use
- This Kit contains materials of human (e.g., human serum) 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.[7]
- All specimens and materials used to process them should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel.

- 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.
- The Turbo TSI Cells 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 Turbo TSI Cells, Controls, Standards, and cAMP Reagent are for one-day use following thawing and cannot be re-frozen once thawed.
- The Controls are supplied at working strength. Any dilution of these reagents will decrease sensitivity.
- The Standards are supplied at working strength. Any dilution of these reagents will affect assay results.
- Each multi-well plate should be used only once. Do not re-use previously assayed plate.

SPECIMEN COLLECTION AND PREPARATION

Proper collection and handling of the patient specimen are the most important factors in successful TSI 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 Thyretain Turbo TSI Stimulating Reporter BioAssay.

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 7 days at 2°C to 8°C before testing. If testing does not occur before 7 days the specimen may be aliquoted and frozen at –20°C 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.

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.

PROCEDURE

Preliminary Comments and Precautions

- Adhere to the recommended volumes and times in the following procedure to ensure that accurate results are obtained.
- 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.

Thyretain Turbo TSI Procedure

- Thaw one vial of each cAMP Reagent, Standard Panel and Controls for 7 to 10 minutes in a 35°C to 37°C water bath. Ensure all reagents are equilibrated to room temperature (20°C to 25°C).
- Vortex samples, standards and controls for 5-10 sec. Add 5 µL Standards, Controls and samples to the bottom corner of each well in a white 96-well plate in singlet.
- Thaw one vial of Turbo TSI Cells for 3 to 5 minutes in a 35°C to 37°C water bath until just thawed.
- *Note: The cell suspension should be added to the cAMP within 30 minutes of thawing
- Transfer the entire volume of the cells with a transfer pipette to the bottle containing 5 mL of cAMP Reagent.
- Mix by inverting the bottle several times.

- Dispense 50 µL per well of the Turbo TSI cell suspension (step 3) per well using a 20 µL to 200 µL (or 50 µL to 300 µL) multi-channel pipette.
- ***Note:** The cell suspension should be added to the plate within 30 min after combining with the cAMP Reagent
- Seal the plate with a microplate adhesive film. Gently shake the plate laterally right to left on the benchtop 5 to 6 times.
- Incubate the plate at room temperature) for 60 minutes.
- Read the plate in a GloMax or equivalent luminometer. Transfer the Patient and Raw data to the Turbo TSI Analysis Tool. Results are reported as IU/L calculated based the proprietary algorithm.
- The following Plate Layout is required (Singlet Testing):

Plate Map												
	1	2	3	4	5	6	7	8	9	10	11	12
A	STD A	Neg Ctrl	Pat 8	Pat 16	Pat 24	Pat 32	Pat 40	Pat 48	Pat 56	Pat 64	Pat 72	Pat 80
B	STD B	Pat 1	Pat 9	Pat 17	Pat 25	Pat 33	Pat 41	Pat 49	Pat 57	Pat 65	Pat 73	Pat 81
C	STD C	Pat 2	Pat 10	Pat 18	Pat 26	Pat 34	Pat 42	Pat 50	Pat 58	Pat 66	Pat 74	Pat 82
D	STD D	Pat 3	Pat 11	Pat 19	Pat 27	Pat 35	Pat 43	Pat 51	Pat 59	Pat 67	Pat 75	Pat 83
E	STD E	Pat 4	Pat 12	Pat 20	Pat 28	Pat 36	Pat 44	Pat 52	Pat 60	Pat 68	Pat 76	Pat 84
F	H POS Ctrl	Pat 5	Pat 13	Pat 21	Pat 29	Pat 37	Pat 45	Pat 53	Pat 61	Pat 69	Pat 77	Pat 85
G	M POS Ctrl	Pat 6	Pat 14	Pat 22	Pat 30	Pat 38	Pat 46	Pat 54	Pat 62	Pat 70	Pat 78	Pat 86
H	L POS Ctrl	Pat 7	Pat 15	Pat 23	Pat 31	Pat 39	Pat 47	Pat 55	Pat 63	Pat 71	Pat 79	Pat 87

QUALITY CONTROL

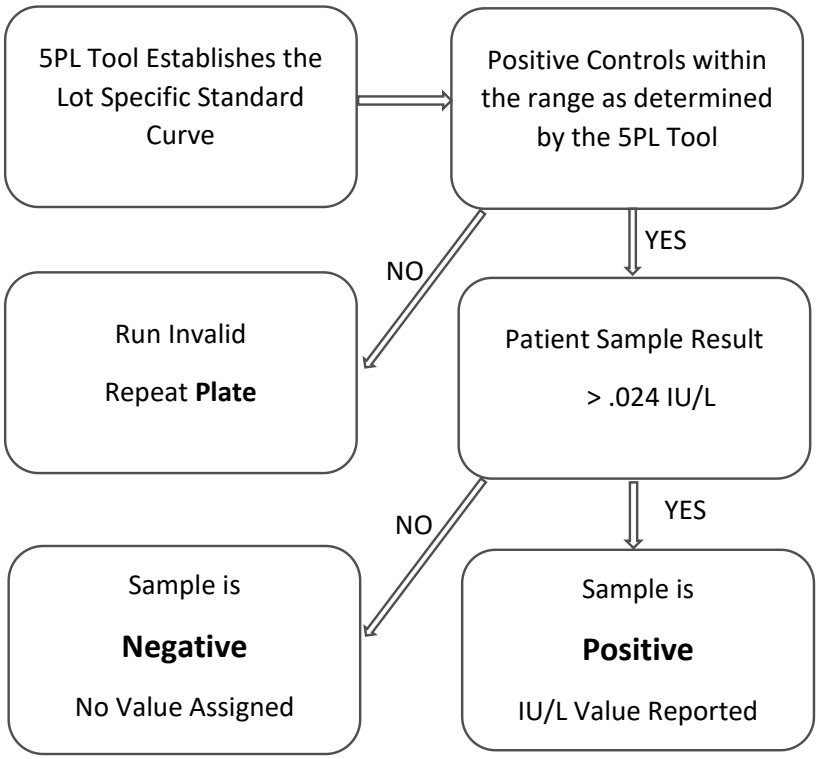
- The Negative and three Positive Controls must be included and results calculated with each plate of specimens to confirm the assay performance.
- Positive Control values must be within ranges specified on the Certificate of Analysis (COA) provided in the kit. The Positive Control range may change with each lot. Please check the value for the Positive Control on the Positive Control reference range label prior to evaluating control validity.
- If assay Controls fail to perform correctly (i.e., above or below established range), results for that plate are considered invalid. Contact Quidel Technical Support if an assay run is invalid.

RESULTS

Interpretation of Results (See Decision Matrix below)

- Results are reported as IU/L calculated using the Quidel Excel-5PL analysis tool.
- The Quidel Excel-5PL analysis tool will return a IU/L value for positive samples. A positive result is one in which the Specimen is > 0.0241 IU/L.
- The Quidel Excel-5PL analysis tool will return a result of "Negative" if the calculated IU/L values is at or below the assay cutoff of 0.0241 IU/L.

Decision Matrix



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 TSI.
- Incubation times or temperatures other than those cited in the test instructions may give erroneous results.
- Detection of TSI can vary depending upon the specimen quality and subsequent handling. A negative result does not exclude the possibility of the presence of TSI. 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 Turbo TSI Stimulating Reporter BioAssay is intended for the detection and quantitation of TSI. 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 TSI. 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.
- False-positive results may occur when serum TSH levels are > 350 mU/L. Specimens with TSH levels above this range should be disqualified for use.

EXPECTED VALUES

In a study of two hundred and seventeen (217) healthy blood donors, all yielded results below 0.0241 IU/L or considered negative by this assay. However, each laboratory should establish its own reference ranges.

SPECIFIC PERFORMANCE CHARACTERISTICS

The LOD for the Thyretain Turbo TSI Stimulating Reporter BioAssay is calculated to be 0.018 IU/L based on the following calculations:

Limit of Blank (LOB = Result at position [$N_B(p/100) + 0.5$])
A total of one (1)-hundred and thirty-six (136) blank measurements were carried out and ordered according to the concentration value of IU/L obtained from low to high. The Turbo TSI LoB was determined by calculating the 95th percent of the blank distribution according to the CLSI guideline (EP-17-1). The calculated TSI concentrations for all the samples were ranked from "lowest" to "highest" and the Turbo TSI LoB was determined using the formula below:

$$LoB = [NB\ (p/100) + 0.5] = [136*0.95 + 0.5] = 0.009\ IU/L.$$

Limit of Detection (LOD = LOB + $c_\beta\, SD_S$)
Thirty-six (36) measurements of a low positive were carried out and the standard deviation calculated. Based on the formula above the LOD has been calculated to be 0.018 IU/L [0.009 +(1.649 x 0.006)].

Cross-reactivity by Endogenous Substances

Cross-reactivity was determined for each of the 21 substances in Table 1 by spiking and testing normal human serum with different concentrations of each substance. The passing concentration for each substance is reported. No cross-reactivity was observed with any of the 21 substances when present at normal physiological concentration ranges for healthy adults.

Table 1. Summary of Cross-reactivity Studies on Thyretain Turbo TSI

Cross Reactant	Normal Physiological Range in Healthy Adults Concentration	Passing Concentration
Luteinizing Hormone	1-20 IU/L	60 IU/L
Thyroid Stimulating Hormone	0.5 - 4.5 mIU/L	5 mIU/l
Follicle Stimulating Hormone	1.4 - 116.3 IU/L	300 IU/L
Human Chorionic Gonadatropin (hCG)	0.1 - 8000 IU/L	50,000 IU/L
Hemoglobin	200 mg/dL	5 mg/dL
Bilirubin	0.2 - 1.3 mg/dL	40 mg/dL
Estrogen (Estradiol)	Up to 438 pg/mL at ovulation	4.5 ng/mL
Progesterone	Up to 214 ng/mL in late pregnancy	2.2 µug/mL
Prolactin	Up to 386 ng/mL in pregnancy	3.9 ug/mL
Levothyroxine (artificial T4)	Normal: 40-120 ng/mL Up to 160 ng/mL in Pregnancy	1.6 ug/mL
Liothyronine (artificial T3)	0.8-2.0 µg/mL	20 ug/mL
Selenium Supplement	10-15 ug/mL	2 ug/mL

Table 1. Summary of Cross-reactivity Studies on Thyretain Turbo TSI

Cross Reactant	Normal Physiological Range in Healthy Adults Concentration	Passing Concentration
Antithyroid Drugs (6-propyl-2-thiouracil)	9.1 µg/mL 1h after oral administration	91 ug/mL
Lithium	Normal dose serum levels: 22.5-45 µg/mL	75 ug/mL
Interferon α 2B	0.07-0.44 ng/mL	4.4 ng/mL
Interleukin-2 human	1.4 µg/mL after infusion	14 ug/mL
Iodine	0.125 µg/mL	1 ug/mL
Tyrosine Kinase Inhibitors (Sunitinib malate)	62.9-101 ng/mL	200 ug/mL
Tyrosine Kinase Inhibitors (Imatinib mesylate)	2.59 µg/mL	26 ug/mL
Ipilimumab	N/A	580 µg/mL
Alemtuzumab	N/A	264 µg/mL

Cross-Reactivity with other Disease States

Samples from patients with autoimmune diseases other than GD and other unrelated disease states were tested for cross-reactivity with Turbo TSI (Table 2): Of the 182 samples tested, 176 samples tested negative (< 0.0241 IU/L). Only six (6) samples (1/22 Diabetes, 1/50 Celiac Disease, and 4/21Thyroid Cancer samples) resulted in Turbo TSI positive results (> 0.0241 IU/L). No further information is available for these six (6) samples.

Table 2. Cross-Reactivity with Other Disease States

Disease State Serum	N	Percent Samples < Cutoff (Negative)
HAMA (human anti-mouse antibody)	1	100%
RF (rheumatoid factor)	1	100%
aTPO (anti-thyroid peroxidase0	1	100%
aTG (anti-thyroglobulin)	1	100%
Rheumatoid Arthritis	20	100%
Lupus	20	100%
Type 1 Diabetes Mellitus	22	95%
Celiac Disease	50	98%
Pregnancy: Chronic Hypertension	20	100%
Pregnancy: Preeclampsia	21	100%
Thyroid Cancer	21	81%
Hashimoto's Disease	4	100%

Interference by Endogenous Substances

Interference was determined for each of the exogenous 21 substances in Table 3 by spiking and testing Low TSI positive human serum (< 0.15IU/L) with different concentrations of each substance. The passing concentration (high concentration of substance at which the sample remained positive) for each substance is reported in Table 3.

Table 3. Interference by Endogenous Substances

Interfering substance	Normal Physiological Ranges in Healthy Adults or Normal Serum Concentration in Adults Prescribed Substance	Passing Concentration
Hemoglobin	5 mg/dL	200 mg/dL
Bilirubin	0.2 - 1.3 mg/dL	40 mg/dL
LH	1-20 IU/L (Immulite 0.5 IU/L)	60 IU/L
TSH	0.5 - 4.5 mIU/L (Immulite 0.14 mIU/L)	5 mIU/L
FSH	1.4 – 116.3 IU/L (Immulite 0.75 IU/L)	150 IU/L
hCG	0.1 - 8000 IU/L (Immulite 100 IU/L)	50,000 IU/L
Estrogen (Estradiol)	Up to 438 pg/mL at ovulation	4.5 ng/mL
Progesterone	Up to 214 ng/mL in late pregnancy	2.2 µg/mL
Prolactin	Up to 386 ng/mL in pregnancy	3.9 µg/mL
Levothyroxine (artificial T4) (Levothyroxine sodium)	Normal: 40-120 ng/mL Up to 160 ng/mL in Pregnancy	0.2 µg/mL
Liothyronine (artificial T3)	0.8-2.0 µg/mL	20 µg/mL
Selenium Supplement (seleno-L-methionine)	Normal: 0.10-0.15 µg/mL Toxic: 2 µg/mL	2 µg/mL
Antithyroid Drugs (thionamides) (6-propyl-2-thiouracil)	Average Serum Level: 9.1 µg/mL 1h after oral administration	91 µg/mL
Lithium (lithium carbonate)	Normal dose serum levels: 22.5-45 µg/mL Not recommended to exceed 75 µg/mL	75 µg/mL
Interferon-alpha (Interferon α 2B human)	0.07-0.44 ng/mL	4.4 ng/mL
Interleukin-2 (Interleukin-2 human)	Approximately 1.4 µg/mL after infusion	14 µg/mL
Ipilimumab	58.1 µg/mL at steady state (repeated administration)	290 µg/mL
Alemtuzumab	No steady state found, highest level recorded 26.4 µg/mL	264 µg/mL
Iodine	Mean healthy level 0.125 µg/mL	1 µg/mL
Tyrosine Kinase Inhibitors (Sunitinib malate)	62.9-101 ng/mL at steady state (repeated administration)	200 mg/mL
Tyrosine Kinase Inhibitors (Imatinib mesylate)	2.59 µg/mL at steady state (repeated administration)	26 µg/mL

Interference by other Disease States

Samples from patients with autoimmune diseases other than GD and other unrelated disease states were tested for interference with Turbo TSI. Interference was determined for each mixing each disease state sera with a Low TSI positive human serum (< 0.15IU/L) in a 1:1 ration and testing the combined sample with Turbo TSI. (See Table 4): Of the 58 samples tested, the TSI positive sample remained positive in the presence of the disease state sera.

Table 4. Passing Rates for Various Disease State Sera

Disease State Serum	N	Percent Pass (>Cutoff=0.0241 IU/L)
HAMA (human anti-mouse antibody)	1	100%
RF (rheumatoid factor)	1	100%
aTPO (anti-thyroid peroxidase)	1	100%
aTG (anti-thyroglobulin)	1	100%
Rheumatoid Arthritis	5	100%
Lupus	5	100%
Type 1 Diabetes Mellitus	5	100%
Gliadin Ab IgA	10	100%
Tissue Transglutaminase IgA	10	100%
Celiac Disease	5	100%
Pregnancy: Preeclampsia	5	100%
Thyroid Cancer	5	100%

Assay Cutoff

The Turbo TSI cutoff was determined using one-hundred and ninety-eight (198) serum samples that were classified as true positive or true negative for TSI by the FDA-cleared Thyretain TSI Reporter BioAssay. Data from the study was analyzed by generating a ROC curve and a ROC table via the JMP software. The Turbo TSI cutoff was determined to be 0.0241 IU/L which yielded the highest true positive rate (98.7% sensitivity) together with the lowest false positive rate (93.5% specificity) against the Thyretain TSI assay results.

Precision and Repeatability

A Precision Panel was prepared by diluting two (2) high TSI positive serum samples to yield five (5) TSI concentrations spanning the dynamic range of Turbo TSI. Three replicates of each were tested on one plate. The assay was repeated in this manner by two operators over 12 days, resulting in 72 measurements for each Precision Panel member. The Thyretain Turbo TSI assay was assessed for precision and repeatability at the end of 12 days by combining quantitative results (IU/L) of 72 measurements for each panel member and control.

Repeatability

For each test concentration, the Repeatability Standard Deviations (Sr) were calculated according to the formula below for one operator. The %CV was then calculated by dividing the standard deviations by the mean calculated concentrations. Results are summarized in Table 5 below.

$$s_r = \sqrt{\frac{\sum_{d=1}^{D}\sum_{r=1}^{n}(x_{dr} - \bar{x}_d)^2}{D(n-1)}}$$

D = total number of days (12)

n = total number of replicates per day (3)

x_{dr} = result for replicate r on day d

$\bar{x}_d$ = average of all replicates on day d

Table 5. Repeatability Precision Summary

Repeatability Summary					
Sample ID	Day	Replicate	Average IU/L	Within-Run SD (S_r)	Within-Run %CV
S1	12	3 per day	0.059	0.007	11.7%
S2	12	3 per day	0.146	0.017	12.0%
S3	12	3 per day	1.074	0.057	5.3%
S4	12	3 per day	3.734	0.204	5.5%
S5	12	3 per day	6.177	0.397	6.4%

Between-Day Precision

For each test concentration, the between-day Standard Deviations (Sd) were calculated according to the formula below. The %CVs were also calculated by dividing the standard deviations b the mean calculated concentrations. Results are summarized in Table 6 below.

$$s_b = \sqrt{\frac{\sum_{d=1}^{D}(\bar{x}_d - \bar{\bar{x}}^2)}{D-1}}$$

D = total number of days (12)

$\bar{x}_d$ = average of all replicates on day d

$\bar{\bar{x}}_d$ = average of all results

Table 6. Between-Day Precision Summary

Between-Day Precision					
Sample ID	Days	Runs	Average (IU/L)	Between-Day SD (s_b)	Between-Day %CV
S1	12	2 per day	0.061	0.007	11.5%
S2	12	2 per day	0.151	0.016	10.6%
S3	12	2 per day	1.079	0.059	5.5%
S4	12	2 per day	3.722	0.351	9.4%
S5	12	2 per day	6.315	0.798	12.6%

Within-Laboratory Precision

Within-Laboratory precision was calculated for each test concentration by calculating the within-laboratory Standard Deviations (SL) from the previously calculated repeatability and between-day standard deviations. The %CVs were also calculated by dividing the standard deviations by the calculated means of each concentration. Results are summarized in Table 7 below.

$$s_L = \sqrt{\frac{n-1}{n} \times s_r^2 + s_b^2}$$

n = total number of replicates per day

s_r = Repeatability Standard Deviation

s_b = Between Day Standard Deviation

Table 7. Within-Laboratory Precision Summary

Within Laboratory Precision							
Sample ID	Days	Runs	Average (IU/L)	Repeatability (s_r)	Between Day (s_d)	Within Lab (s_L)	Within Lab %CV
S1	12	2 per day	0.061	0.007	0.007	0.009	15.0%
S2	12	2 per day	0.151	0.017	0.016	0.022	14.3%
S3	12	2 per day	1.079	0.057	0.059	0.075	6.9%
S4	12	2 per day	3.722	0.204	0.351	0.388	10.4%
S5	12	2 per day	6.315	0.397	0.798	0.862	13.6%

The overall within-laboratory precision was ≤ 15%CV for all samples

Assay Reproducibility

A Reproducibility Panel was prepared by diluting two high TSI positive serum samples to yield five TSI concentrations spanning the dynamic range of the Turbo TSI standard curve. To demonstrate reproducibility of the assay, each of three trained sites performed testing on the panel[1] described in Table 8 below twice a day (by different operators) over a 5-day span.

Table 8. Reproducibility Panel Variation and Accuracy

Reagent	Target Concentrations (IU/L)	Expected Overall Variation	Expected Accuracy
S1	0.057	<20%	100%
S2	0.224	<15%	100%
S3	1.588	<15%	100%
S4	3.271	<15%	100%
S5	5.204	<15%	100%

Table 9 reports the results for each of the sites over their proficiency training period. Study Site 3 requested two technicians be trained.

Table 9. Performance by site and overall variability (reported as Average IU/L and Coefficient of Variation)

Site 1		Site 2		Site 3		OVERALL	
SAMPLE S1		SAMPLE S1		SAMPLE S1		SAMPLE S1	
0.06	25.1%	0.06	19.8%	0.05	13.5%	0.06	19.8%
SAMPLE S2		SAMPLE S2		SAMPLE S2		SAMPLE S2	
0.22	12.3%	0.21	11.25%	0.20	11.0%	0.21	10.5%
SAMPLE S3		SAMPLE S3		SAMPLE S3		SAMPLE S3	
1.50	6.6%	1.68	11.1%	1.57	9.1%	1.58	9.6%
SAMPLE S4		SAMPLE S4		SAMPLE S4		SAMPLE S4	
3.18	8.3%	3.25	11.0%	3.18	8.0%	3.21	8.3%
SAMPLE S5		SAMPLE S5		SAMPLE S5		SAMPLE S5	
5.26	7.9%	5.03	21.4%	5.18	8.1%	5.16	13.1%

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

All Study Sites performed the study using manufactured panel samples. Each site's data was analyzed cumulatively to determine the Reproducibility of the panel samples. The overall coefficient of variation (CV%) for Samples 1, 2, 3, 4, and 5 were 19.8%, 10.5%, 9.6%, 8.3%, and 13.1%, respectively.

Clinical Sensitivity and Specificity

The clinical sensitivity and specificity for the device was determined in a study performed in 2019 with a total of 295 specimens evaluated by both the subject (Thyretain Turbo TSI Stimulating Reporter BioAssay) and comparator (Thyretain TSI Reporter BioAssay) devices. All specimens were handled in accordance with the procedure in the instructions for use for Turbo TSI and the comparator device Package Insert.

Tables 10 shows the age and gender distribution for individuals studied.

Table 10. Age and Gender Distribution

Age Range	Number Positive/Total Specimens		
	Male	**Female**	**Total**
< 18 yrs.	0/2	11/18	11/20
19 to 40 yrs.	4/13	21/79	25/92
41 to 65 yrs.	11/33	29/94	40/127
66 > yrs.	1/18	13/38	14/56
Grand Total	16/66	74/229	90/295

All specimens were analyzed for sensitivity and specificity and the results are summarized in Table 11.

Table 11. Summary Results

295 specimen results		Thyretain TSI Reporter BioAssay		
		+	–	Indeterminate
Turbo TSI	+	**79**	**11**	**0**
Turbo TSI	–	**4**	**201**	**0**
	Invalid	**0**	**0**	
		95% Confidence Interval		
Sensitivity	95.2% (79/83)	88.3% - 98.1%		
Specificity	94.8% (201/212)	90.9% - 97.1%		

*All fifteen (15) discrepant samples were tested with the TRAb assay, and the results are summarized in Table 11. Of the four (4) Thyretain TSI positive/TSI Turbo negative samples, three (3) were TRAb positive and one (1) was TRAb Negative. Of the eleven (11) Thyretain TSI Negative/TSI Turbo positive five (5) were TRAb positive and six (6) were TRAb Negative.

The ability of the subject device to detect TSI using a cell-based system was compared to the comparator device's bioassay. The Thyretain Turbo TSI Stimulating Reporter BioAssay demonstrated clinical Sensitivity of 95.2% (95%CI: 88.3% to 98.1%) when compared the Thyretain TSI Reporter BioAssay. The Thyretain Turbo TSI assay demonstrated clinical Specificity of 94.8% (95%CI: 90.9% to 97.1%) when compared to Thyretain TSI Reporter BioAssay.

ASSISTANCE

If you have any questions regarding the use of this product or to report a product problem, please contact Quidel Technical Support at 1.800.874.1517 (in the U.S.) or technicalsupport@quidel.com. If outside the U.S., further information can be obtained from your distributor, or directly from Quidel at one of the numbers listed below. Reference quidel.com to see more options for Support.

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GLOSSARY

REF Catalog number	CE CE mark of conformity
EC REP Authorized representative in the European Community	LOT Batch code
 Use-by date	 Manufacturer
 Temperature limit	 Do not reuse
 Consult e-labeling instructions for use	IVD *In vitro* diagnostic medical device
 Keep away from sunlight	 Contains sufficient for <n> tests
CONTROL + Positive control	CONTROL − Negative control