

# Anti Thyroglobulin IgG

**Enzyme Immunoassay (ELISA) for the  
quantitative determination of IgG antibodies  
against TG  
in human serum and plasma**

- for "in vitro" diagnostic use only -



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REF.: TG.CE  
96 Tests

## Anti Thyroglobulin IgG

### A. INTENDED USE

The device TG.CE is an Enzyme ImmunoAssay (ELISA) intended to be used for the quantitative determination of IgG autoantibodies against Thyroglobulin in human plasma and sera.

For in vitro diagnostic use only.

### B. INTRODUCTION

Autoimmunity is the failure of an organism to recognize its own constituent parts as *self*, which allows an immune response against its own cells and tissues. Any disease that results from such an aberrant immune response is termed an *Autoimmune Disease*.

Thyroid autoantibodies are frequently found in patients with autoimmune thyroid disease. The two most common of these autoantibodies are antibodies to Thyroglobulin and Thyroid peroxidase.

Antibodies to Thyroglobulin and Thyroid Peroxidase will be found in cases of Hashimoto's disease, myxedema and Grave's Disease.

### C. PRINCIPLE OF THE TEST

Microplates are coated with a recombinant human thyroglobulin antigen. In the 1<sup>st</sup> incubation, the solid phase is treated with diluted samples and anti TG IgG are captured, if present, by the solid phase.

After washing out all the other components of the sample, in the 2<sup>nd</sup> incubation bound anti TG IgG are detected by the addition of anti hIgG antibody, labelled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti TG IgG antibodies present in the sample. IgG in the sample may be quantitated by means of a standard curve calibrated on WHO standard preparation for anti TG antibodies (code 65/093).

### D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

#### 1. Microplate: MICROPLATE

12 strips x 8 breakable wells coated with recombinant human thyroglobulin antigen. Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

#### 2. Calibration Curve: CAL N°...

6x2.0 ml/vial. Ready to use and blue colour coded.

The standard curve derive from human *TG IgG* positive plasma calibrated on WHO standard preparation for anti-TG antibodies (code 65/093) and ranging: CAL1 = 0 IU/ml // CAL2 = 50 IU/ml // CAL3 = 100 IU/ml // CAL4 = 300 IU/ml // CAL 5 = 500 IU/ml // CAL6 = 1000 IU/ml.

Contains human serum proteins, bovine proteins, Tris buffer pH 7.8 +/-0.1, , 0.09% Na-azide and 0.1% Kathon GC as preservatives.

#### 3. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

#### 4. Enzyme conjugate: CONJ

1x60ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated goat polyclonal

antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

#### 5. Chromogen/Substrate: SUBS TMB

1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H<sub>2</sub>O<sub>2</sub>).

*Note: To be stored protected from light as sensitive to strong illumination.*

#### 6. Sulphuric Acid: H2SO4 0.3 M

1x15ml/vialIt contains 0.3 M H<sub>2</sub>SO<sub>4</sub> solution.

Attention !: Irritant (Xi R36/38; S2/26/30)

#### 7. Specimen Diluent: DILSPE

2x60ml/vial. Ready to use and violet-blue color coded.

It contains bovine proteins, Tris buffer pH 7.8 +/-0.1, , 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the samples.

#### 8. Plate sealing foils n°2

#### 9. Package insert n°1

### E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000, 100 and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and possibly with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

### F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2.8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.

8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

#### G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°.8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8µ filters to clean up the sample for testing.

#### H. PREPARATION OF COMPONENTS AND WARNINGS

##### Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of manufacturing. In this case call Dia.Pro's customer service. Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°.8°C. When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

##### Calibration Curve:

Ready to use component. Mix carefully on vortex before use.

##### Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

*Note: Once diluted, the wash solution is stable for 1 week at +2°.8°C.*

##### Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

If this component has to be transferred use only plastic, possibly sterile disposable containers.

##### Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

Do not expose to strong illumination, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, possible sterile disposable container

##### Sample Diluent:

Ready to use component. Mix carefully on vortex before use.

##### Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Legend: R 36/38 = Irritating to eyes and skin.

S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

#### I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration +

dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

4. Incubation times have a tolerance of  $\pm 5\%$ .
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and ideally with a second filter (620-630nm) for blanking purposes. Its standard performances should be (a) bandwidth  $\leq 10$  nm; (b) absorbance range from 0 to  $\geq 2.0$ ; (c) linearity to  $\geq 2.0$ ; repeatability  $\geq 1\%$ . Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

#### L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dilute all the content of the 20x concentrated Wash Solution as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
7. Set the ELISA incubator at  $+37^{\circ}\text{C}$  and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
8. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.

9. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

#### M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000  $\mu\text{l}$  Sample Diluent + 10  $\mu\text{l}$  sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Then dispense 100  $\mu\text{l}$  of Calibrators in duplicate. Then dispense 100  $\mu\text{l}$  of diluted samples in each properly identified well.
4. Incubate the microplate for **45 min at  $+37^{\circ}\text{C}$** .

**Important note:** Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.

5. Wash the microplate with an automatic washer reported previously (section I.3).
6. Pipette 100  $\mu\text{l}$  Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

**Important note:** Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.

7. Incubate the microplate for **45 min at  $+37^{\circ}\text{C}$** .
8. Wash microwells as in step 5.
9. Pipette 100  $\mu\text{l}$  Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature ( $18-24^{\circ}\text{C}$ ) for 15 minutes**.

**Important note:** Do not expose to strong direct illumination. High background might be generated.

10. Pipette 100  $\mu\text{l}$  Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and possibly at 620-630nm (background subtraction), blanking the instrument on A1 or B1 or both.

#### General Important notes:

1. If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.
2. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background

## N. ASSAY SCHEME

Method	Operations
Calibrators	100 µl
Samples diluted 1:101	100 µl
<b>1<sup>st</sup> incubation</b>	<b>45 min</b>
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
<b>2<sup>nd</sup> incubation</b>	<b>45 min</b>
Temperature	+37°C
Wash step	4-5 cycles
TMB/H <sub>2</sub> O <sub>2</sub>	100 µl
<b>3<sup>rd</sup> incubation</b>	<b>15 min</b>
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme for Quantitative Analysis is reported below:

		Microplate											
		1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S3										
B	BLK	CAL4	S4										
C	CAL1	CAL5	S5										
D	CAL1	CAL5	S6										
E	CAL2	CAL6	S7										
F	CAL2	CAL6	S8										
G	CAL3	S1	S9										
H	CAL3	S2	S10										

Legend: BLK = Blank CAL = Calibrator S = Sample

## O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.050 OD450nm value
CAL 1 0 IU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 50 IU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 6 1000 IU/ml	OD450nm > 1.000

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
<b>Blank Well</b> > 0.050	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
<b>CAL 1</b> <b>0 IU/ml</b> > 0.150 OD450nm after blanking  coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one);

	4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
<b>CAL 2</b> <b>50 IU/ml</b>  OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
<b>CAL 6</b> <b>1000 IU/ml</b>  < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

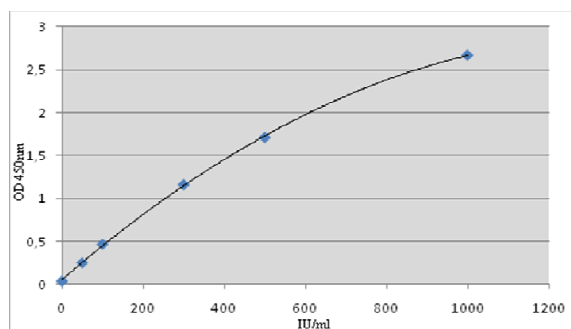
Should one of these problems have happened, after checking, report to the supervisor for further actions.

## P. RESULTS

If the test turns out to be valid, use an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).

Then on the calibration curve calculate the concentration of anti TG IgG antibody in samples.

An example of Calibration curve is reported below.



### Important Note:

Do not use the calibration curve above to make calculations.

## Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 50 IU/ml are considered Normal for anti TG IgG antibody. Samples with a concentration higher than 50 IU/ml are elevated for anti anti TG IgG antibody.

IU/ml	Interpretation
≤ 50	Normal
≥ 50	Elevated

It is recommended that each laboratory establishes its own normal and pathological ranges.

### Important notes:

- Results of this test alone are not enough to provide a clear diagnosis of a autoimmune disease. Other diagnostic tests should be carried out, especially in combination with others autoantibodies. The pattern of different antibody combinations and their concentration together with the whole clinical picture of the patient are helpful diagnostic tools in the assessment of rheumatoid autoimmune diseases.
- Positive results should be verified concerning the entire clinical status of the patient.
- Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.
- When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.

## R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted on panels of positive and negative samples with reference to a CE marked reference kit.

### 1. Limit of detection

The limit of detection of the assay has been calculated by means of the WHO standard preparation for anti-TG antibodies (code 65/093), in order to provide the device with a constant and excellent sensitivity.

The limit of detection has been calculated as mean OD450nm Calibrator 0 IU/ml + 5 SD.

The table below reports the mean OD450nm values of this standard when diluted and then examined in the assay.

WHO IU/ml	Lot P1 OD 450nm	Lot P2 OD 450nm	Lot P3 OD 450nm
1000	2.207	2.244	2.738
500	1.497	1.507	1.810
300	0.922	0.926	1.186
100	0.365	0.365	0.461
50	0.199	0.199	0.240
0	0.014	0.013	0.017

### 2. Diagnostic Sensitivity and Specificity:

The diagnostic sensitivity has been tested in a Performance Evaluation trial on panels of samples classified positive by a CE marked reference kit.

The diagnostic **sensitivity** was studied on at least 50 samples, positive with the reference kit. Positive samples were collected from patients with a clinical history of autoimmune disease.

The diagnostic **specificity** was determined on panels of more than 50 negative samples from normal individuals and blood

donors, classified negative with the reference kit, including potentially interfering specimens.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.

The anti TG IgG test kit is specific only for autoantibodies directed to the respective antigen. No cross-reactivity have been observed.

The Performance Evaluation provided the following values :

<b>Sensitivity</b>	≥ 98 %
<b>Specificity</b>	≥ 98 %

### 3. Precision:

A study conducted on three samples of different anti IgG TG reactivity, examined in 16 replicates in three separate runs has shown CV% values ranging 4-20% depending on the OD450nm readings.

### 4. Accuracy

The assay accuracy has been checked by the dilution. Any "hook effect", underestimation likely to happen at high doses of analyte, was ruled out.

## S. LIMITATIONS

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an autoimmune disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

False positivity has been assessed as less than 2% of the normal population.

## T. REFERENCES

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