

Tg-AB (ATAB-CT)
BL-06-CT-100

Bio-Line s.a.

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IMMUNORADIOMETRIC ASSAY FOR THE QUANTITATIVE DETERMINATION OF THE ANTI-THYROGLOBULIN AUTOANTIBODIES IN HUMAN SERUM

FOR IN VITRO DIAGNOSTIC USE ONLY

1. CLINICAL APPLICATIONS

Thyroid autoimmunity is more frequently registered in women. Antibody prevalence in women increases with age, rising from approximately 10% at the age of 18-24 up to 30% at the age of 55-65 for TgAb and from 15% at the age of 18-24 up to 24% at the age of 55-65 for TMAb.

2. PRINCIPLE OF THE ASSAY

The present method is based on a competitive radioimmunoassay (RIA). During the incubation, the monoclonal anti-Tg antibody on solid phase competes with the sample/calibrator anti-Tg autoantibodies for the specific sites of the Iodine¹²⁵ labelled Tg (tracer). After aspiration and washing, the radioactivity in the tubes is measured in a gamma counter. The degree of binding will be inversely proportional to the sample anti-Tg autoantibody concentration.

THE CALIBRATORS OF RIAZENCO TG AB ARE CALIBRATED AGAINST THE TgAb INTERNATIONAL REFERENCE PREPARATION MRC65/93.

3. REAGENTS PROVIDED WITH THE KIT

- The reagents are sufficient for 50 (100) determinations.
- Store the kit and reagents at 2-8°C.
- The expiration date of each reagent is shown on the label.

- 1 - Radioactive Tracer:** 1(2) vial(s) containing 11 ml of iodinated thyroglobulin, red color coded. Radioactivity content : ± 68.5kBq per vial. Preservative: NaN₃ (<0.1%).
- 2 - Calibrators: Zero Calibrator:** 1 vial containing 5 ml. Preservative: NaN₃ (<0.1%). Ready for use.
Calibrators 1 to 5: 5 vials containing 0.7 ml of anti-Tg (human serum), at the following concentrations: 20, 60, 200, 1000, 2000 IU/ml. Preservative: NaN₃ (<0.1%).
- 3 - Controls:** 2 vials containing 0.7 ml of anti-Tg (human serum). **For the exact value, refer to the value written on the Quality Control data sheet.** Preservative: NaN₃ (<0.1%).
- 4 - Coated Tubes:** 50 (100) tubes coated with mouse monoclonal anti-Tg antibodies. Unused tubes must be stored at 2-8°C, protected from moisture.
- 5 - Washing Solution (50 x concentrated):** 1 vial of 20 ml Tris-HCl buffer with detergent and preservative NaN₃ (<0.1%). Bring to 1000 ml with distilled water. The diluted washing solution is stable for 2 months at 2-8°C.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Plastic test tubes
- Test tube racks.
- Adjustable, automatic micropipettes with disposable tips.
- Vortex mixer.
- Graduated cylinder.
- Aspiration pump or automated washing device.
- Scintillation gamma counter.
- Distilled water.
- Orbital shaker adjustable at 150 rpm.

5. WARNINGS AND PRECAUTIONS

In order to obtain reproducible results, the following rules must be observed :

- Do not mix reagents of different lots.
- Do not use reagents beyond their expiry date.

- Use thoroughly clean glassware.
- Use distilled water, stored in clean containers.
- Avoid any contamination among samples; to this purpose disposable tips should be used for each sample and reagent.

In order to avoid personal and environmental contamination, the following precautions must be observed :

- Use disposable gloves while handling potentially infectious material and performing the assay.
- Do not pipette reagents by mouth.
- Do not smoke, eat, drink or apply cosmetics during the assay.
- All material of human origin used for the preparation of this kit has been tested and found negative for HBsAg, anti-HIV and anti-HCV. Since no test at present can guarantee complete absence of these viruses, all samples and reagents used for the assay must be considered potentially infectious; therefore, the assay waste must be decontaminated and disposed of, in accordance with established safety procedures. Disposable ignitable material must be incinerated; disposable non-ignitable material must be sterilized in autoclave for at least 1 hour at 121°C. Liquid wastes must be added with sodium hypochlorite at a final concentration of 3%. Let the hypochlorite act for at least 30 minutes. Liquid wastes containing acid must be neutralized with appropriate amounts of base, before treating with sodium hypochlorite.
- Avoid splashing and aerosol formation; in case of spilling, wash carefully with a 3% sodium hypochlorite solution and dispose of this cleaning liquid as potentially infectious waste.
- Some reagents contain sodium azide as preservative; to prevent build-up of explosive metal azides in lead and copper plumbing, reagents should be discarded by flushing the drain with large amounts of water.
- Acquisition, storage, use and disposal of radioactive material (liquid and solid) are subject to regulation and ordination of local authorities.

6. SPECIMEN COLLECTION

It is recommended to use serum. Highly lipemic or hemolyzed samples must be discarded. Keep samples at 2-8°C for 1 day; for longer periods it is advisable to freeze samples in aliquots at -20°C. Repeated freezing and thawing of samples should be avoided.

7. ASSAY PROCEDURE

- Bring all reagents and samples to warm up at room temperature.
 - Before use, mix samples by gentle agitation before use.
 - For all calibrators, a duplicate measure is recommended
- 1- Prepare plain tubes for Total Counts, and coated tubes for Calibrators, Samples and Controls.
 - 2- Pipette **20 µl** of each Calibrator, Control Serums and Samples into the corresponding tubes.
 - 3- Add **200 µl** of Radioactive Tracer into all tubes.
 - 4- Mix the tubes on vortex and incubate for **90 minutes** at room temperature on an orbital shaker set at 150 rpm.
 - 5- Aspirate the contents of each tube, except the tubes for Total Counts.
 - 6- Add **2 ml** of diluted washing solution to every tube, except tubes for Total Counts and aspirate thoroughly or decant the contents of all tubes on absorbent paper.
 - 7- Count the radioactivity bound to the tubes for 1 minute in a gamma counter. We suggest to control the background of the instrument before counting the assay. In order to avoid variations in the sensitivity of the system, the background must be reduced to a minimum or adjusted properly.

NOTE :

If high anti-Tg values (> 2000UI/ml) are expected for any patient sample, the original sample should be diluted. The dilution should be done using zero calibrator. **Do not use any buffers for this purpose.**

ASSAY SCHEME

| Tubes | Total Activity | Calibrators | Controls | Samples |
|----------------|----------------|-------------|----------|---------|
| Reagent | | | | |
| Calibrators | ---- | 20 µl | ---- | ---- |
| Control Serums | ---- | ---- | 20 µl | ---- |
| Samples | ---- | ---- | ---- | 20 µl |
| Tracer | 200 µl | 200 µl | 200 µl | 200 µl |

- Mix and incubate: 90 min R.T. shaking (150 rpm)
 - Aspirate and wash: 1 x 2 ml
 - Count

8. CALCULATION OF RESULTS

$$\text{Binding capacity} = \frac{B}{T} \% = \frac{\text{Bo Cpm}}{T \text{ Cpm}} \times 100$$

$$\text{Percent binding for Calibrators, Controls and Samples} = \frac{B}{\text{Bo}} \% = \frac{\text{Calibrator or Sample Cpm}}{\text{Bo Cpm}} \times 100$$

Draw the calibration curve on log/lin graph by plotting the B/Bo% obtained for each calibrator (y-axis) against the relative concentration (x-axis). Calculate the B/Bo% of each sample and read the anti-Tg concentration in IU/ml, by interpolating on the calibration curve.

EXAMPLE OF CALCULATION

The values reported below must be considered as an example and may not be used in place of experimental data.

| Description | Average cpm | B/Bo (%) | TG Ab conc. (IU/ml) |
|--------------------|-------------|----------|---------------------|
| Total Activity (T) | 57953 | - | - |
| Cal 0 | 28090 | 100 | 0 |
| Cal 1 | 24340 | 86.6 | 20 |
| Cal 2 | 16927 | 60.3 | 60 |
| Cal 3 | 9706 | 34.5 | 200 |
| Cal 4 | 4100 | 14.6 | 1000 |
| Cal 5 | 2048 | 7.3 | 2000 |
| CONTROL 1 | 10843 | 38.6 | 158.2 |
| CONTROL 2 | 5757 | 20.5 | 604.7 |
| P1 | 13998 | 49.8 | 91.5 |
| P2 | 6634 | 23.6 | 464.8 |
| P3 | 4270 | 15.2 | 942.9 |

9. REFERENCE VALUES

It is recommended that each laboratory determines its own reference interval. Values reported below are only indicative.

| | |
|-----------------------|--------------------------------------------------|
| Lower than 30 IU/ml | Negative for anti-Thyroglobulin auto-antibodies. |
| Between 30 - 70 IU/ml | Borderline |
| Higher than 70 IU/ml | Positive for anti-Thyroglobulin auto-antibodies. |

10. PERFORMANCES OF THE ASSAY

SPECIFICITY

No cross-reactions have been observed with anti-TPO autoantibodies present in human serum.

SENSITIVITY

ANALYTICAL SENSITIVITY

THE SENSITIVITY WAS CALCULATED BASED UPON THE CALIBRATION CURVE AND EXPRESSED AS THE MINIMAL DOSE SHOWING A SIGNIFICANT DIFFERENCE FROM THE ZERO CALIBRATION (MEAN VALUE - 2 S.D.). THIS DOSE IS 6 IU/ML.

FUNCTIONAL SENSITIVITY

The functional assay sensitivity is the lowest value which is measured with a precision of maximum 20% inter-assay variance. For the anti-thyroglobulin autoantibodies, this value is lower than 15 IU/ml

PRECISION

PRECISION WAS EVALUATED UPON INTRA- AND INTER-ASSAY VARIABILITY, AT DIFFERENT ANALYTE CONCENTRATIONS.

Intra-assay

| Serum | Mean (IU/ml) | ± | S.D. | C.V. % | N |
|-------|--------------|---|------|--------|----|
| A | 72.6 | ± | 6.0 | 8.3 | 20 |
| B | 345.7 | ± | 24.7 | 7.1 | 20 |
| C | 889.9 | ± | 51.0 | 5.7 | 20 |

Inter-assay

| Serum | Mean (IU/ml) | ± | S.D. | C.V. % | N |
|-------|--------------|---|-------|--------|---|
| A | 90.3 | ± | 8.4 | 9.3 | 9 |
| B | 390.0 | ± | 49.8 | 12.8 | 9 |
| C | 985.4 | ± | 104.9 | 10.6 | 9 |

ACCURACY

ACCURACY OF THE METHOD HAS BEEN CHECKED BY THE RECOVERY AND PARALLELISM TESTS:

RECOVERY TEST

Samples, mixed with equal volumes of each calibrator, were tested.

| | Expected (IU/ml) | Measured (IU/ml) | Recovery (%) |
|------------|------------------|------------------|--------------|
| S1 | - | 50.4 | - |
| S1 + CAL 0 | 25.2 | 32.0 | 127.0 |
| S1 + CAL 1 | 35.2 | 39.1 | 111.1 |
| S1 + CAL 2 | 55.2 | 59.6 | 108.0 |
| S1 + CAL 3 | 125.2 | 111.8 | 89.3 |
| S1 + CAL 4 | 525.2 | 392.4 | 74.7 |
| S1 + CAL 5 | 1025.2 | 1251.1 | 122.0 |
| S2 | - | 102.9 | - |
| S2 + CAL 0 | 51.5 | 63.9 | 124.1 |
| S2 + CAL 1 | 61.5 | 64.2 | 104.4 |
| S2 + CAL 2 | 81.5 | 84.7 | 103.9 |
| S2 + CAL 3 | 151.5 | 133.0 | 87.8 |
| S2 + CAL 4 | 551.5 | 367.2 | 66.6 |
| S2 + CAL 5 | 1051.5 | 1261.3 | 120.0 |

Parallelism Test

A serum with high analyte concentration was tested at different dilutions with the Zero Calibrator.

| Dilution | Expected (IU/ml) | Measured (IU/ml) | Recovery (%) |
|--------------|------------------|------------------|--------------|
| S1 undiluted | - | 1109 | - |
| 1/2 | 554.5 | 586.7 | 105.8 |
| 1/4 | 277.3 | 275.0 | 99.2 |
| 1/8 | 138.6 | 143.0 | 103.2 |
| 1/16 | 69.3 | 82.0 | 118.3 |

NB. Due to the heterogeneity of autoantibodies, for some patient samples a nonlinear dilution is possible

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