

# DEFINITION OF THE UPPER REFERENCE LIMIT FOR THYROGLOBULIN ANTIBODIES ACCORDING TO NACB GUIDELINES. COMPARISON OF SIX AUTOMATED METHODS

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## Aim of the study

Serum thyroglobulin antibodies (TgAb) can interfere with thyroglobulin (Tg) immunometric assays, causing unpredictable effects on serum Tg results in patients with differentiated thyroid cancer (DTC). Moreover, in some limited clinical conditions, TgAb measurement is used for the diagnosis of autoimmune thyroid disease.

In view of this background, the definition of reference limits for TgAb is of considerable importance.

The aim of the study was to define the upper reference limit (URL) of TgAb, according to NACB guidelines (1) by using six automated immunometric methods.

## Methods

120 subjects were selected from a population survey in the province of Verona according to NACB criteria: males, younger than 30 years, biochemically euthyroid, with no goiter, no personal/family history of thyroid disease and without non-thyroid autoimmune diseases.

TgAb were determined with five chemiluminescent automated methods and one fluorimetric method: Advia Centaur XP (CEN, Siemens Healthineers), Cobas 6000 (COB, Roche Diagnostics), Liaison XL (LIA, Diasorin), Lumipulse G 1200 (LUM, Fujirebio), Maglumi 2000 Plus (MAG, Snibe) and Phadia 250 (PHA, Phadia AB), respectively (Table 1). All methods were traceable to the WHO 1<sup>st</sup> International Reference Preparation 65/93 and used International Units (IU), except for CEN, whose results were initially expressed in Arbitrary Units and subsequently corrected in IU (conversion factor = 2.8), as indicated in the package insert.

The normality of the distribution was assessed using the Shapiro-Wilk test. Since TgAb values were not normally distributed, data were reported as median and the non-parametric Kruskal-Wallis test was used for comparing groups. A two-sided value of  $p < 0.05$  was considered statistically significant.

The experimental URL (eURL) was established at 97.5<sup>th</sup> percentile. The difference between manufacturer's URL (mURL) and eURL was expressed as the ratio between them in percentage ( $\Delta\% = |mURL - eURL| / mURL * 100$ ).

Statistical analyses were performed by GraphPad Prism Software (San Diego, CA, USA).

| COMPANY              | INSTRUMENT        | METHOD/TRACER  | ABBR |
|----------------------|-------------------|--|------|
| Siemens Healthineers | Advia Centaur XP  | CLIA/Acridium esters   | CEN  |
| Roche Diagnostics    | Cobas 6000        | ECLIA/Ruthenium-triethylamine  | COB  |
| Diasorin             | Liaison XL        | CLIA/Isoluminol derivative   | LIA  |
| Fujirebio            | Lumipulse G1200   | CLEIA/Alkaline phosphatase-spiroadamantyl-methoxy-phosphoryloxy-phenyl-dioxetane | LUM  |
| Snibe                | Maglumi 2000 Plus | CLIA/N-(aminobutyl)-N-(ethyl)-isoluminol   | MAG  |
| Phadia               | Phadia Elia 250   | FIA/ $\beta$ -D-galactosidase; 4-methyl-umbelliferyl- $\beta$ -D-galactoside     | PHA  |

Table 1. Instruments and methods used to measure TgAb.

Abbreviations. ABBR: abbreviation; CLEIA: chemiluminescence enzyme immunoassay; CLIA: chemiluminescence immunoassay; ECLIA: electrochemiluminescence immunoassay; FIA: fluorimetric immunoassay.

## Results

Value distributions were not Gaussian with a positive skew.

Medians were statistically different from one method to the other ( $p < 0.05$ ), except for COB versus MAG, COB versus PHA, LIA versus PHA and MAG versus PHA (Table 2, Fig. 1).

URL ranged widely, depending on the method, with a 4-fold difference between minimum and maximum (Table 2, Fig. 2): 8.95 IU/mL (CEN), 18.65 IU/mL (LIA), 20.03 IU/mL (LUM), 23.38 IU/mL (PHA), 34.23 IU/mL (MAG), and 38.6 IU/mL (COB). Such cutoffs were far lower than those stated by the manufacturers, except for MAG (Fig. 2).

| Instrument/Assay       | No. | Median (IU/mL) | CV (%) | Min-Max (IU/mL) | eURL (IU/mL) | mURL (IU/mL) | Delta (%) |
|------------------------|-----|----------------|--------|-----------------|--------------|--------------|-----------|
| Advia Centaur XP/TgAb  | 120 | 3.43           | 60.44  | 0.25-18.57      | 8.95         | 21.40        | 58.22     |
| Cobas 6000/TgAb        | 120 | 12.56          | 52.83  | 9.68-70.88      | 38.60        | 115.00       | 66.43     |
| Liaison XL/TgAb        | 120 | 9.40           | 43.25  | 6.76-43.91      | 18.65        | 100.00       | 81.35     |
| Lumipulse G1200/TgAb   | 120 | 6.87           | 53.36  | 3.51-32.8       | 20.03        | 55.40        | 63.84     |
| Maglumi 2000 Plus/TgAb | 120 | 13.35          | 58.77  | 2.06-69.36      | 34.23        | 30.00        | 14.10     |
| Phadia Elia 250/TgAb   | 116 | 11.00          | 32.35  | 6.00-26.00      | 23.38        | 60.00        | 61.03     |

Table 2. Main statistical parameters of TgAb, measured by six different methods in the reference population.

The experimental URL (eURL) was established at 97.5<sup>th</sup> percentile. The difference between manufacturer's URL (mURL) and eURL was expressed as the ratio between them in percentage ( $\Delta\% = |mURL - eURL| / mURL * 100$ ).

Abbreviations. CV: coefficient of variation; eURL: experimental upper reference limit; Max: maximum; Min: minimum; mURL: manufacturer's upper reference limit; TgAb: thyroglobulin antibodies.

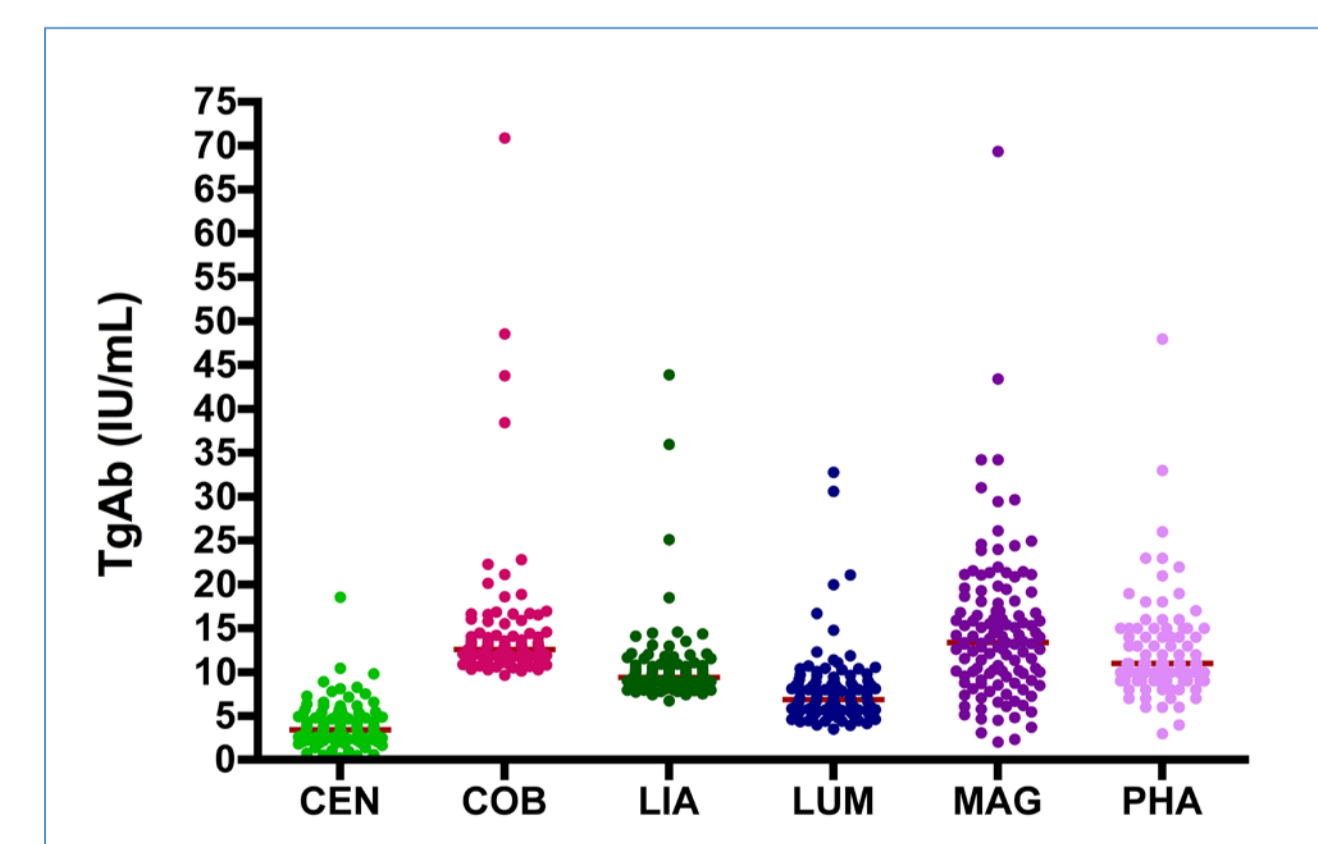


Figure 1. Distribution of TgAb values determined by six different methods in the reference population.

Medians were statistically different from one method to the other ( $p < 0.05$ ), except for COB vs MAG, COB vs PHA, LIA vs PHA and MAG vs PHA.

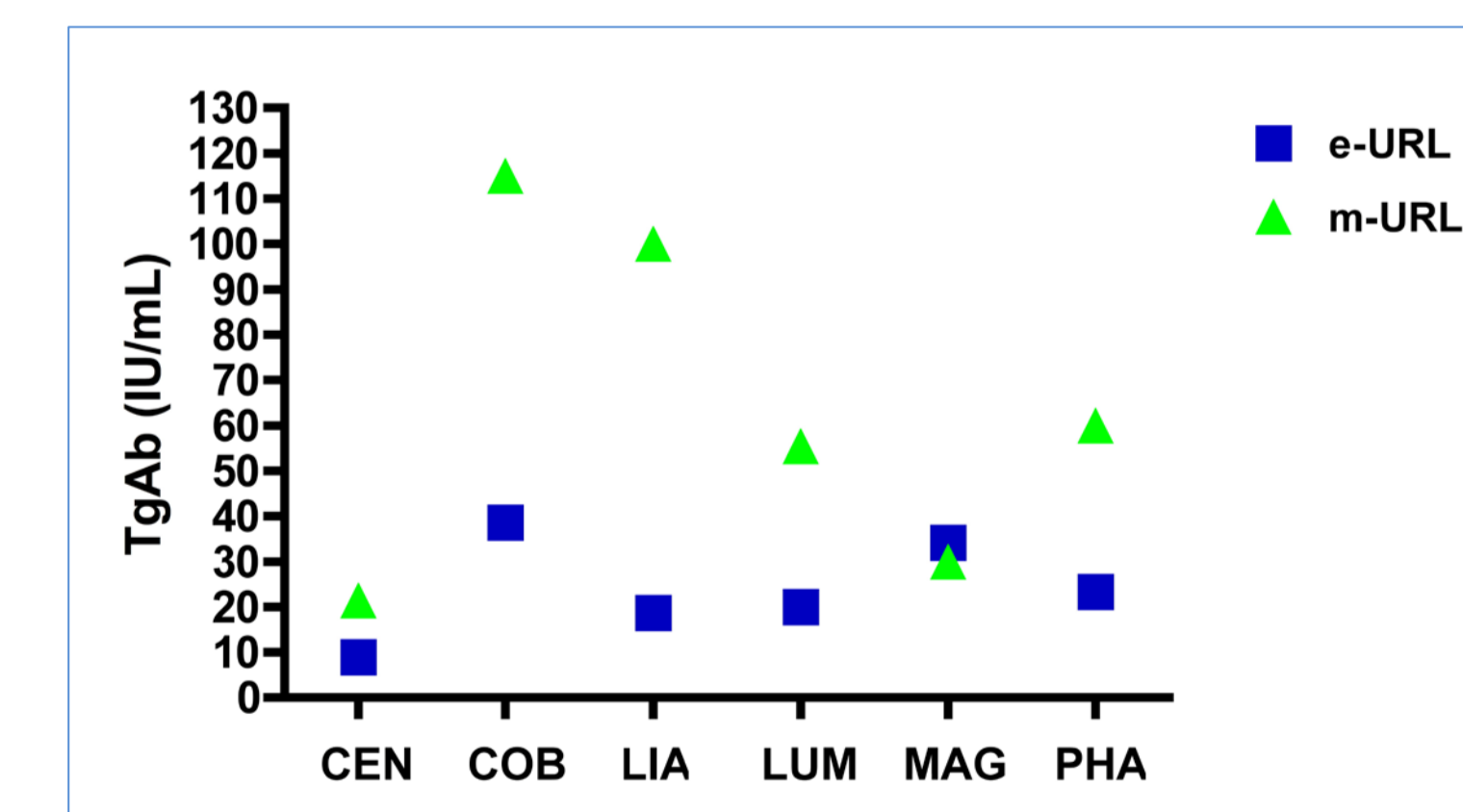


Figure 2. Difference between e-URL and m-URL.

Abbreviations. e-URL: experimental upper reference limit; m-URL: manufacturer's upper reference limit.

## Conclusions

NACB recommendations allowed a more precise definition of TgAb URL. Experimental TgAb URLs were method-dependent and lower than those reported in package inserts. The use of a lower cutoff than that proposed by manufacturers could lead to a better assessment of TgAb potential interference on the measurement of Tg, thus ensuring a greater clinical utility of Tg monitoring in DTC patients.

## References

1. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, LiVosli VA, Niccoli-Sire P, John R, Ruf J, Smyth PP, Spencer CA, Stockigt JR; Guidelines Committee, National Academy of Clinical Biochemistry. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. *Thyroid* 2003;13:3-126.