

# DEFINITION OF THE UPPER REFERENCE LIMIT FOR THYROID PEROXIDASE AUTOANTIBODIES ACCORDING TO THE NACB GUIDELINES: COMPARISON OF FIVE DIFFERENT AUTOMATED METHODS. PART A

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## Background-Aim

Autoantibodies against thyroid peroxidase (TPOAb) are diagnostic hallmarks of autoimmune thyroid diseases. The estimation of TPOAb upper reference limit (URL) is a controversial issue because of method variability and different criteria to define the reference population. According to the NACB guidelines, TPOAb URL should be established from 120 subjects with the following features: male, younger than 30 years, biochemically euthyroid, without history of thyroid disease and non-thyroid autoimmune diseases (1).

The aim of the study was to investigate the validity of the NACB guidelines by comparing TPOAb URLs obtained from 120 healthy males (M) and 120 healthy females (F).

## Methods

In an Italian population survey, 7970 subjects were screened for thyroid disease (family/personal history, function tests and neck US). Among them, 120 M and 120 F were selected. Their sera were tested for TPOAb concentration by using 12 automated immunometric methods. In this communication, we reported the results of 3 chemiluminescent methods: Advia Centaur XP (CEN, Siemens HD), IMMULITE 2000 XPI (IMM, Siemens HD), Cobas e411 (COB, Roche Diagnostics) and 2 fluorimetric methods: Kryptor Compact Plus (KRY, Thermo Fisher BRAHMS) and Phadia 250 (PHA, Phadia AB) (Table 1).

URL was established at 99th percentile. The non-parametric Mann-Whitney U test was used to compare TPOAb levels in M and in F within the same method. A two-sided value of  $p < 0.05$  was considered statistically significant.

Company	Platform	Method/Tracer	Antigen	Abbreviation
Siemens HD	Advia Centaur XP	CLIA/Acrininium esters	natTPO	CEN
Siemens HD	Immolute 2000 XPI	CLIA/Adamantyl dioxetane phosphate	natTPO	IMM
Roche Diagnostics	Cobas e411	CLIA/Ruthenium-triitolamine	recTPO	COB
Thermo Fisher BRAHMS	Kryptor Compact Plus	FIA/Europium cryptate-XL 665	natTPO	KRY
Phadia AB	Phadia Elia 250	FIA/ $\beta$ -D-galactosidase; 4-methyl-umbelliferyl- $\beta$ -D-galactoside	recTPO	PHA

Table 1. Companies, platforms and methods involved in the study.

Abbreviations. CLIA: chemiluminescence immunoassay; FIA: fluorimetric immunoassay; TPO: thyroid peroxidase.

## Results

Value distributions were not Gaussian with a positive skew both in M and in F (Figure 1). A statistically significant difference between medians in M and in F was observed for PHA (medians: 2.6 IU/mL and 3.1 IU/mL, respectively) and COB (medians: 5.0 IU/mL and 6.2 IU/mL, respectively) but not for CEN, IMM and KRY (Table 2) (Figures 2-6). URLs were different according to the method and the gender. Such URLs are generally lower than those stated by the manufacturers (Table 2).

## Conclusions

TPOAb URLs were method- and gender-dependent and they were similar or lower than those proposed by manufacturers, which do not distinguish between sexes. Therefore, unlike what is indicated by the NACB guidelines, laboratories have the opportunity to use gender-specific reference intervals.

## Acknowledgments

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## Reference

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Platform	Sex	No.	Mean (C.I. 95%)	SD	Median	CV	p	URL	Package insert cut-off
CEN	M	120	9.5 (8.5-10.5)	5.4	9.3	57.0	0.3	20.5	20.0
	F	120	10.3 (9.2-11.3)	6.0	10.7	58.3		25.1	
IMM	M	120	8.2 (7.1-9.3)	6.0	6.4	73.6	0.8	28.7	35.0
	F	120	8.9 (7.6-10.1)	7.0	6.7	79.6		29.0	
COB	M	120	5.3 (4.6-6.0)	3.9	4.4	74.9	<0.05	18.2	34.0
	F	120	6.9 (6.0-7.8)	4.9	6.2	71.6		27.6	
KRY	M	120	2.7 (2.5-2.9)	1.4	2.6	50.6	0.6	6.4	10.5
	F	120	2.6 (2.3-2.8)	1.2	2.4	48.6		6.9	
PHA	M	120	2.8 (2.6-3.1)	1.4	2.6	50.9	<0.05	8.3	25.0
	F	120	3.7 (3.1-3.8)	1.8	3.1	51.8		10.0	

Table 2. Main statistical parameters of TPOAb, measured by the five different methods in males and females.

Median, URL and package insert cut-off are expressed in IU/mL; CV is expressed in %. Abbreviations. M: males; F: females; CV: coefficient of variation; URL: upper reference limit.

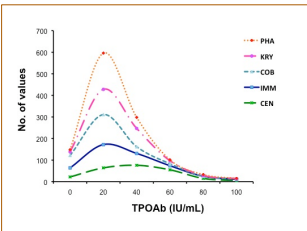


Figure 1. Frequency distribution of TPOAb values determined by the five methods in all the subjects (120 males and 120 females).

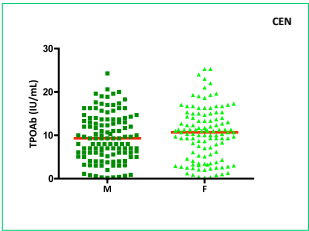


Figure 2. Comparison between values of TPOAb in males and in females (Advia Centaur XP). Red line: median.

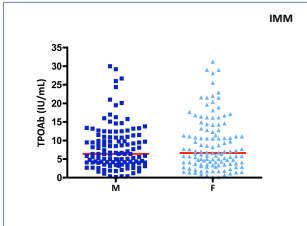


Figure 3. Comparison between values of TPOAb in males and in females (Immolute XPI). Red line: median.

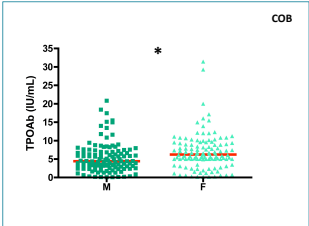


Figure 4. Comparison between values of TPOAb in males and in females (Cobas e411). \*  $p < 0.05$ , red line: median.

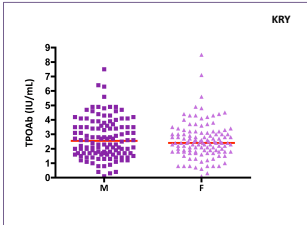


Figure 5. Comparison between values of TPOAb in males and in females (Kryptor Compact Plus). Red line: median.

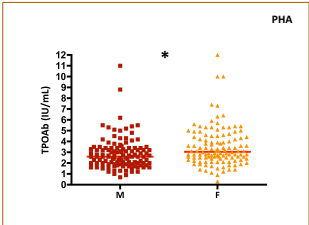


Figure 6. Comparison between values of TPOAb in males and in females (Phadia 250). \*  $p < 0.05$ , red line: median.