BIOASSAYS FOR TSH RECEPTOR ANTIBODIES ARE MORE SENSITIVE THAN AUTOMATED BINDING ASSAYS

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CONTEXT AND OBJECTIVE:

TSH receptor (TSHR) antibodies (Ab) can be measured with binding or bio-assays. Sensitivity and specificity of five binding and two bio-assays were compared.

METHODS:

TSHR blocking (TBAb) -and stimulating (TSAb) Ab were measured with reporter bioassays. Blocking activity was defined as percent inhibition of luciferase expression relative to induction with bovine TSH alone (cut-off >40% inhibition). TSAb was reported as percentage of specimen-to-reference ratio (SRR% >140%). TSHR-binding inhibitory immunoglobulins (TBII) were measured with two ELISA (Kronus and Dynex), as well as with three automated assays (Kryptor, Cobas, and Immulite).

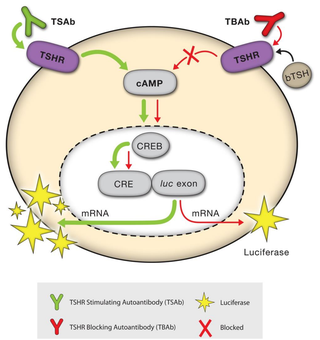


Figure 1: Principle of the TSAb and TBAb bioassays: TSAb bind to the TSHR which are expressed in genetically engineered cell lines, resulting in an increase of intracellular cAMP production. Subsequently, the transcription factor CREB (cAMP response element-binding) binds to CREs (cAMP response elements) and stimulates the transcription of the reporter luciferase gene (*luc*). The activation of the cAMP signaling pathway results in the production of chemiluminescent signals which can be quantified in a luminometer. In contrast, TBAb inhibit bTSH-induced stimulation of luciferase.

RESULTS:

A total of 80 patients (median age 42 years, range 23-73 years, 64 (80%) female) with autoimmune thyroid diseases, involving 60 patients with Graves' disease (GD), 20 patients with Hashimoto's thyroiditis (HT) and 20 euthyroid healthy controls (C) were included. C tested negative in all assays (specificity 100%) while all 60 hyperthyroid patients with GD were positive in the TSAb bioassay (sensitivity 100%). Among the GD patients, 20 showed low TSAb positivity (SRR% 140-279), but were TBII-positive in only 20 (100%), 7 (35%), 9 (45%), 11 (55%), and 18 (90%) using the Kronus, Dynex, Kryptor, Cobas and Immulite, respectively. In 20 moderate TSAb-positive (SRR% 280-420) patients, TBII tested positive in 20 (100%), 14 (70%), 13 (65%), 16 (80%), and 19 (95%), respectively. The high (SRR% >420) TSAb positive patients were TBII positive in all testings. All 20 hypothyroid HT patients tested TBAb positive (sensitivity 100%) in the bioassay while they tested TBII-positive in 20 (100%), 18 (90%), 20, 20, and 18, respectively. Results obtained with two luminometers correlated for TSAb-positive (r=0.99, p<0.001), TBAb-positive (r=0.88, p<0.001), and C (r=0.86, p<0.001). None of the binding assays differentiated between TSAb and TBAb.

Table 1: Demographic and serological data of all investigated study groups

	Healthy controls	Patients with Hashimoto's thyroiditis (HT)	Patients with Graves' disease (GD)		
		TBAb+	Low TSAb+	Moderate TSAb+	High TSAb+
Gender (F / M)	10 / 10	20 / 0	15 / 5	12 / 8	17 / 3
Age, y					
Mean ± SD	30 ± 11	37 ± 7	41 ± 9	45 ± 10	54 ± 11
Median (min - max)	24 (22 - 57)	35 (31 - 59)	42 (23 - 57)	40 (33 - 68)	52 (31 - 73)
TSH (0.4 - 4.9 mU/L)					
Mean ± SD	1.9 ± 0.7	10 ± 0.9	0.1 ± 0.2	0.01 ± 0.1	0.01 ± 0.01
Median (min - max)	1.8 (0.8 - 3.5)	8.5 (5 - 21)	0.1 (0.01 - 0.2)	0.01 (0.01 - 0.1)	0.01 (0.01 - 0.01)
fT3 (2 - 4.4 pmol/L)					
Mean ± SD	3.5 ± 0.5	1 ± 0.4	4.9 ± 0.4	5.8 ± 0.8	6.8 ± 2.1
Median (min - max)	3.45 (2.6 - 3.4)	0.9 (0.5 - 2)	5 (4.5 - 9.1)	5.7 (4.8 - 12.2)	6.2 (5.4 - 17.2)
fT4 (0.9 - 1.7 ng/dl)					
Mean ± SD	1.4 ± 0.1	0.6 ± 0.2	2.4 ± 0.5	4.5 ± 0.3	6 ± 1.3
Median (min - max)	1.3 (1.2 - 1.5)	0.5 (0.1 - 0.7)	2.1 (1.7 - 3)	3 (1.9 - 13.5)	4.8 (2.4 - 13.6)
Tg-Ab (< 4.1 IU/ml)					
Mean ± SD	1 ± 0.7	38.4 ± 20.2	251.7 ± 357.7	65.7 ± 145.6	69.6 ± 160.3
Median (min - max)	1.2 (0 - 3.5)	33.3 (20 - 67.1)	95 (1.3 - 1000)	13.2 (1 - 417.6)	8.7 (1.5 - 432.8)
TPO-Ab (< 6 IU/ml)					
Mean ± SD	2 ± 0.9	395.5 ± 404.3	371.2 ± 416.3	404.1 ± 362.1	321.4 ± 358.7
Median (min - max)	1.8 (0 - 4.6)	215 (152 - 1000)	223 (3 - 1000)	292 (8 - 1000)	140 (28 - 1000)

The patients with GD were divided in low (SRR% 140 – 279), moderate (SRR% 280 – 420, two cut-offs) and high (SRR% > 420, three cut-offs) positive TSAb levels based on the measurements in the TSAb bioassay. Thyroid-associated orbitopathy (TAO) was present in 1/20 (5%), 9/20 (45%), and 20/20 (100%) low, moderate and high TSAb positive patients with GD, respectively. TAO was absent in all patients with HT and in controls.

Table 2: Prevalence of TSHR-Ab in healthy controls

	TSAb bioassay		TBAb bioassay		Binding Assays				
	TSAb positive	TSAb negative	TBAb positive	TBAb negative	Elisa (Kronus)	Elisa DS2 Dynex (TecoMedical)	Kryptor (ThermoFisher)	Cobas e411 (Roche)	Immulite 2000 Xpi (Siemens)
Healthy controls (n=20)	0	20	0	20	0	0	0	0	0

Figure 2: Prevalence of TSHR-Ab positivity in **low** TSAb positive GD patients (SRR 140-280%) measured with two different luminometers (Glomax, Tecan) in the TSAb bioassay

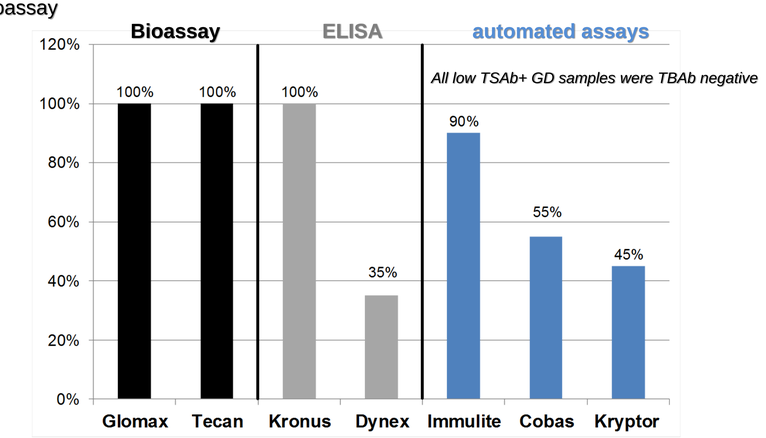
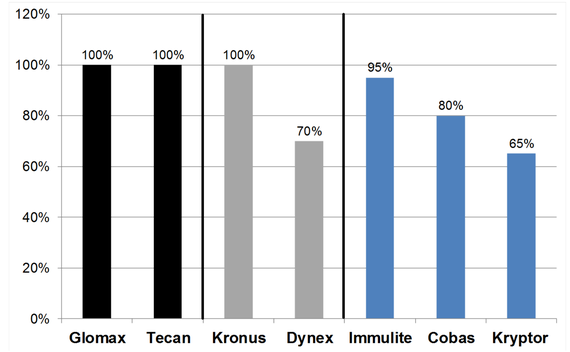
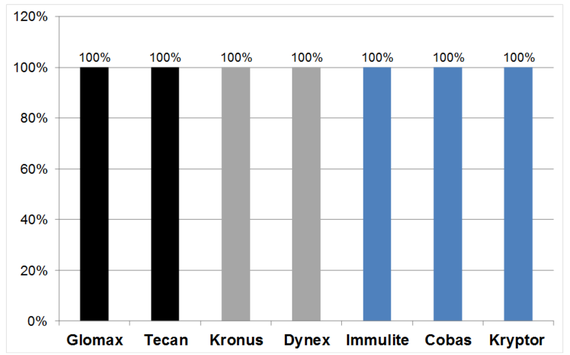


Figure 3: Prevalence of TSHR-Ab positivity in **moderate** TSAb positive GD patients (SRR 280-420%) measured with two different luminometers (Glomax, Tecan) in the TSAb bioassay



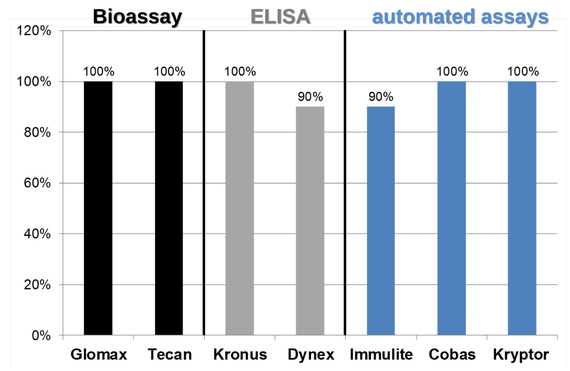
All moderate TSAb+ GD samples were TBAb negative

Figure 4: Prevalence of TSHR-Ab positivity in **high** TSAb positive GD patients (SRR > 420%) measured with two different luminometers (Glomax, Tecan) in the TSAb bioassay



All high TSAb+ GD samples were TBAb negative

Figure 5: Prevalence of TSHR-Ab positivity in TBAb+ HT samples measured with two different luminometers (Glomax, Tecan) in the TBAb bioassay



All TBAb+ HT samples were TSAb negative

CONCLUSIONS:

Bioassays for TSHR Ab are more sensitive than the automated binding assays and exclusively differentiate between stimulatory and blocking antibody activity.