

Monoclonal Antibodies Cross-Reactive with Thyroglobulin(TG)and Thyroid Peroxidase (TPO). Possibility to Apply Them for Detection of Antigens in Human Samples.

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Background

- **Cross-reactive anti-TG and anti-TPO antibodies** were described in the sera of patients with thyroid disorders, in hyperimmune animal sera and among mouse monoclonal antibodies (1, 2, 3).
- Nature and usefulness of such antibodies is not clear in spite of the wide clinical application of separate anti-TG and anti-TPO kits.

Materials and Methods

Mouse monoclonal antibodies were produced against human TG – purified by Sepharose-4B gel-filtration eluate of thyroid gland and against membranes of thyrocytes – washed with PBS cells solubilised by Tween-20/ether treatment – Membrane Antigen (MA).

Both of the antigens were not 100% purity but consisted of mainly native antigens. So the antigens were enough immunogenic and active in ELISA for the production of 200-300 Mab positive hybridoma clones per a fusion of mouse spleenocytes with Sp-2/0 myeloma cells.

Characterization of thyroid Membrane Antigen (MA) as a substance for detection of mouse monoclonal antibodies – determination of working dilution of antigen in indirect ELISA with goat anti-mouse IgG-HRP conjugate.



- - control

Anti-TPO Mab 6h7

Anti-T4 Mab 1h1

Anti-T3 Mab 3a6

Anti-transferin receptor Mab 7-1

Anti-AFP receptor Mab 2B8

Anti-CA 125 Mab 4a8

- Control of anti-mouse IgG-HRP conjugate.



Dilutions of antigen

1:20 :40 :80 :160 :320 :640 :1024 : 2,048 :4,096 :8,192:16,384:32,768

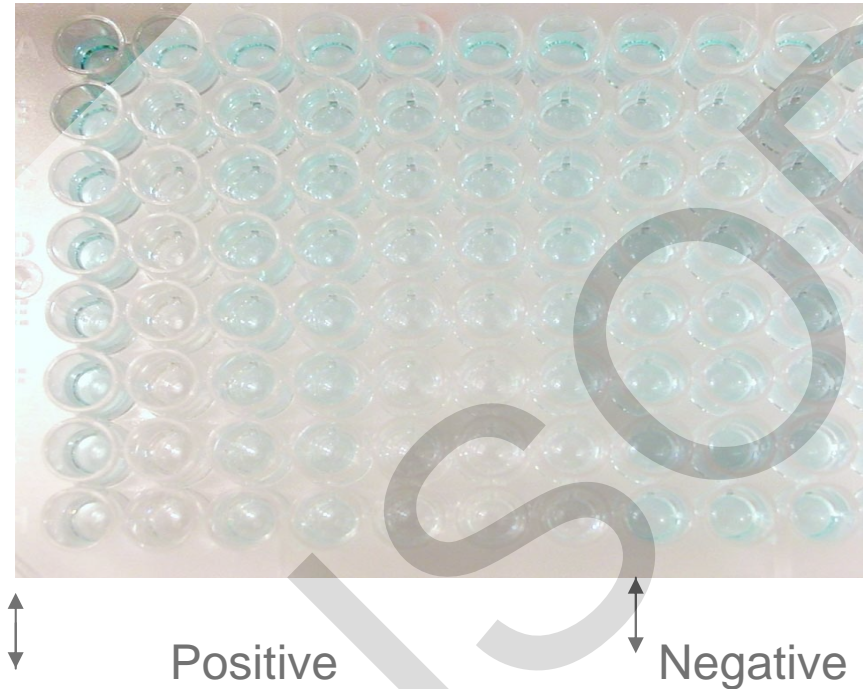
Fig.1

Immunologic procedures for the detection and characterization of monoclonal antibodies were modifications of ELISA:

- 1/ Indirect ELISA with antigens adsorbed in Maxisorb (Nunk) EIA plates and goat anti-mouse IgG –Horse Radish Peroxidase (HRP) conjugates as detector, Fig.1.
 - 2/ Competition ELISA with blocking of antigen – Mab's – HRP conjugates by polyclonal sera or Monoclonal antibodies (in the courses of specificity approval and/or Mab's epitop mapping) Fig.2.
 - 3/ Double Mab's sandwich ELISA with purified Mab's as capture and Mab's – HRP conjugates as detector Fig.3.
- As Reference Materials were used TG-Ab, TPO-Ab and TG kits from ORGENTEC Diagnostika GmbH (Germany) and Commercial TG and TPO antigens from HyTest Co.

(Finland).

Competition of human antibodies with HRP-conjugate of anti-TG/TPO Mab 3F2 in direct ELISA.



:2187 Positive human sera have 100 –
:729 3000 IU of anti -TPO and anti-TG
:243 antibodies according to ELISA
determination by ORGENTEC
GmbH kits.

:81 Dilutions of the sera were
:27 incubated in the plate with
:9 membrane thyroid antigen and
:3 after washing the plate was
incubated with HRP-Mab
conjugate in working dilution –
A405 = 0,5 – 0,7.

Fig.2

HRP-conjugates: 14B6,16A1,2A8, 11-16,12g10,1c10 3A6 1E2 5C5 6A9 7E7 1A1

Ag's 1 2 1 2 1 2 1 2 1 2 1 2

1 2 1 2 1 2 1 2 1 2 1 2

Capture Mab's



2E11
5C5
3F2
1B10
3F1
3E4
15G4
16A1

2A6
2A8
14B6
14F6
6E6
7F7
6B2
6A8

4H5
3H2
11-14
12G10
1C10
18E1
11-21
11-16

12H4
2D2
1E2
1D2
4D9
4E1
1A1
1F1

Pairs analysis in double Mab's Sandwich ELISA.

Antigen N1 – whole human serum – pooled sample of anti-TG negative sera.

Antigen N2 – lizate of thyroid tissue.

Fig.3

Results

- After primary screening all positive clones were tested in competition ELISA with TG/TPO positive human serum and the most interesting clones were recloned and produced as ascetic fluids in Balb/c mice. Mab's of 28 hybridomas were purified by Na_2SO_4 precipitation or prG –chromatography. HRP conjugates were produced by Na-perjodate method and Mab's were characterized by different ELISA's. Results are shown in table.1. 14 different epitops were determinate by competition ELISA and specificity of the Mab were studied with reference materials. According to HyTest Co. reference material we have 7 TPO specific clones(1,2 and 3 epitops), 7 TG specific clones (epitops 4 and 5) and 14 cross-reactive. According to Orgentec kits we have 25 cross-reactive clones and 3 non specific.

Analysis of Mab's pairs with two antigens – whole human serum (1) and thyroid tissue lysate (2) have shown that many combinations of Mab's have the ability to detect some substance in the both antigens with $A_{405} > 1.0$. 33 combinations were Ag1+2 positive and 30 were Ag 2 positive. All Mab's : TPO specific, TG specific and cross-reactive Mab's can be used in pairs for double Mab's systems. It means that thyroid antigens in serum or gland are more complicated than two separate molecules – TG and TPO. More detailed analysis of every combination is needed to proof Mab's specificity and applicability for diagnostics.

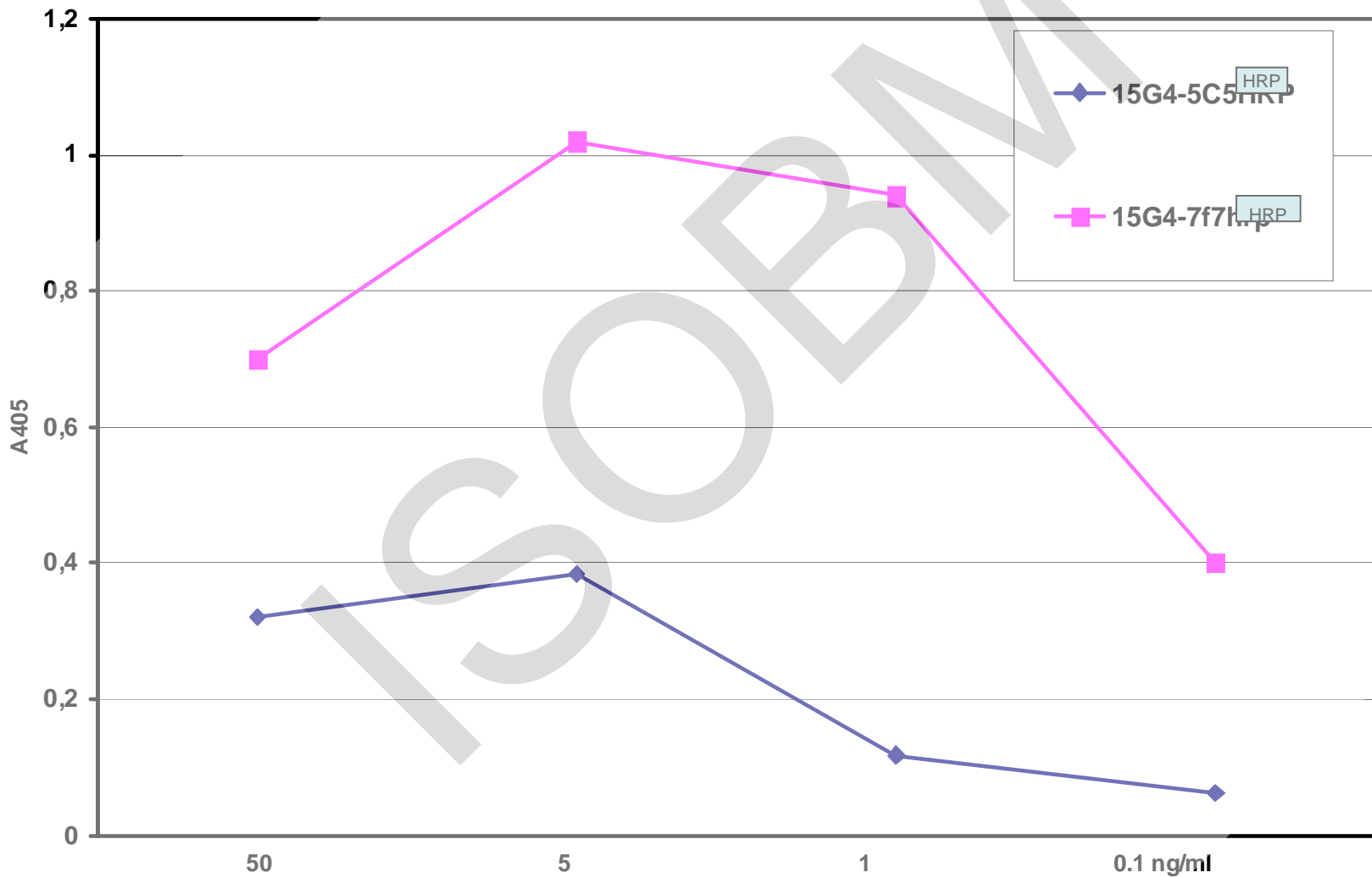
Epitop N	1	1	2	3	3	3	3	3	4
Clone	1A1	1B10	1E2	2E1	2E11	5C5	3F2	3H2	
Subclass Ig	1	2a	2a	2a	1	2a	1	1	
Activity with antigens:									
TPO HyTest Co/	+	+	+	+	+	+	+	+	-
TPO Orgentec GmbH, Ab kit	-	-	-	+	+	+	+	+	+
TG Hytest Co	-	-	-	-	-	-	-	-	+
TG Orgentec GmbH, Ab kit	-	-	-	--+	--+	--+	--+	--+	+
Competition with human serum – +TPO/TG Ab's - positive titer	:640	:20	:20	:40	:640	:640	:40	:1280	
---- polyclonal anti-TG conjugate – recovery test TG kit (Orgentec)-% \pm 5%	18	19	19	4	14	23	18	7	
---- anti-T ₄ Mab – HRP conjugate, >10%		+					+		
Capture ability with 50 ng of TG and polyclonal conjugate (Orgentec) - A ₄₀₅ (0.300 for TG kit)	0.15	0.0	0.1	0.26	0.1	0.15	0.17	0.32	
NN	1	2	3	4	5	6	7	8	

4	4	4	4	5	5	6	6	7	8	9	10
4H5 2a	4D9 2a	7F7 2a	6E6 1	2A8 1	2A6 2a	6B2 1	6A9 2b	11-16 2a	11-21 1	12H4 1	14F6 1
-	-	-	-	-	-	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
:640	:20	:320	:40	:20	:40	<:20	<:20	:20	:40	:80	:320
20	18	27	14	20	20	0	10	7	0	4	40
+			+			+	+				+
0.15	0.12	0.26	0.02	0.01	0.02	0.12	0.06	0.23	0.04	0.27	0.30
9	10	11	12	13	14	15	16	17	18	19	20

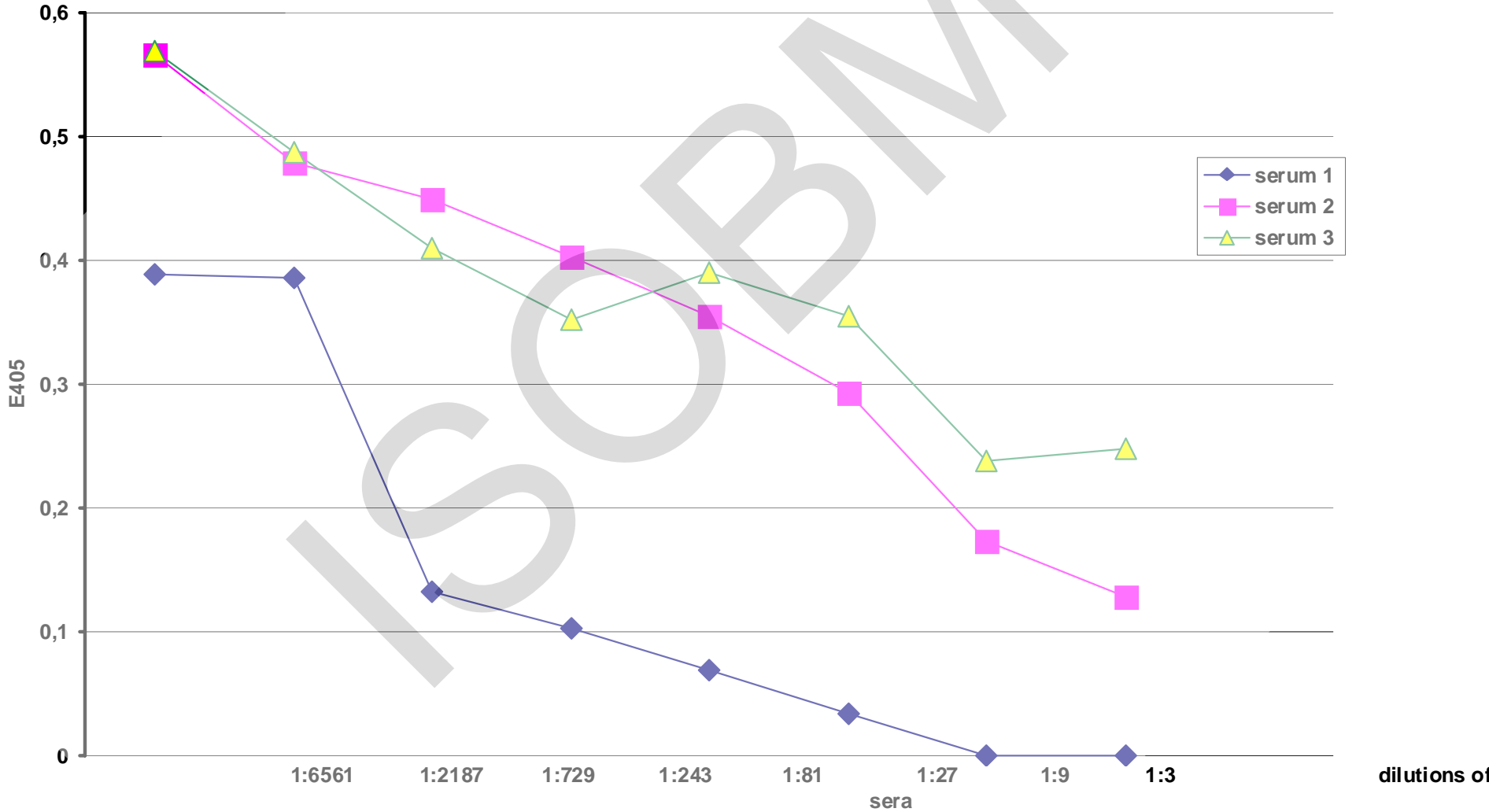
10	11	11	12	13	13	14	14
14B6 1	11-4 1	12G10 1	18E1 1	15G4 1	16A1 2a	1C10 M	1D2 M
+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+
:640	<:20	:160	:40	:20	<:20	:20	<:20
21	16	34	0	35	7	12	10
+	+	+	+	+			
0,08	0,06	0,08	0,30	0,26	0,20	0,04	0,02
21	22	23	24	25	26	27	28

Table 1.

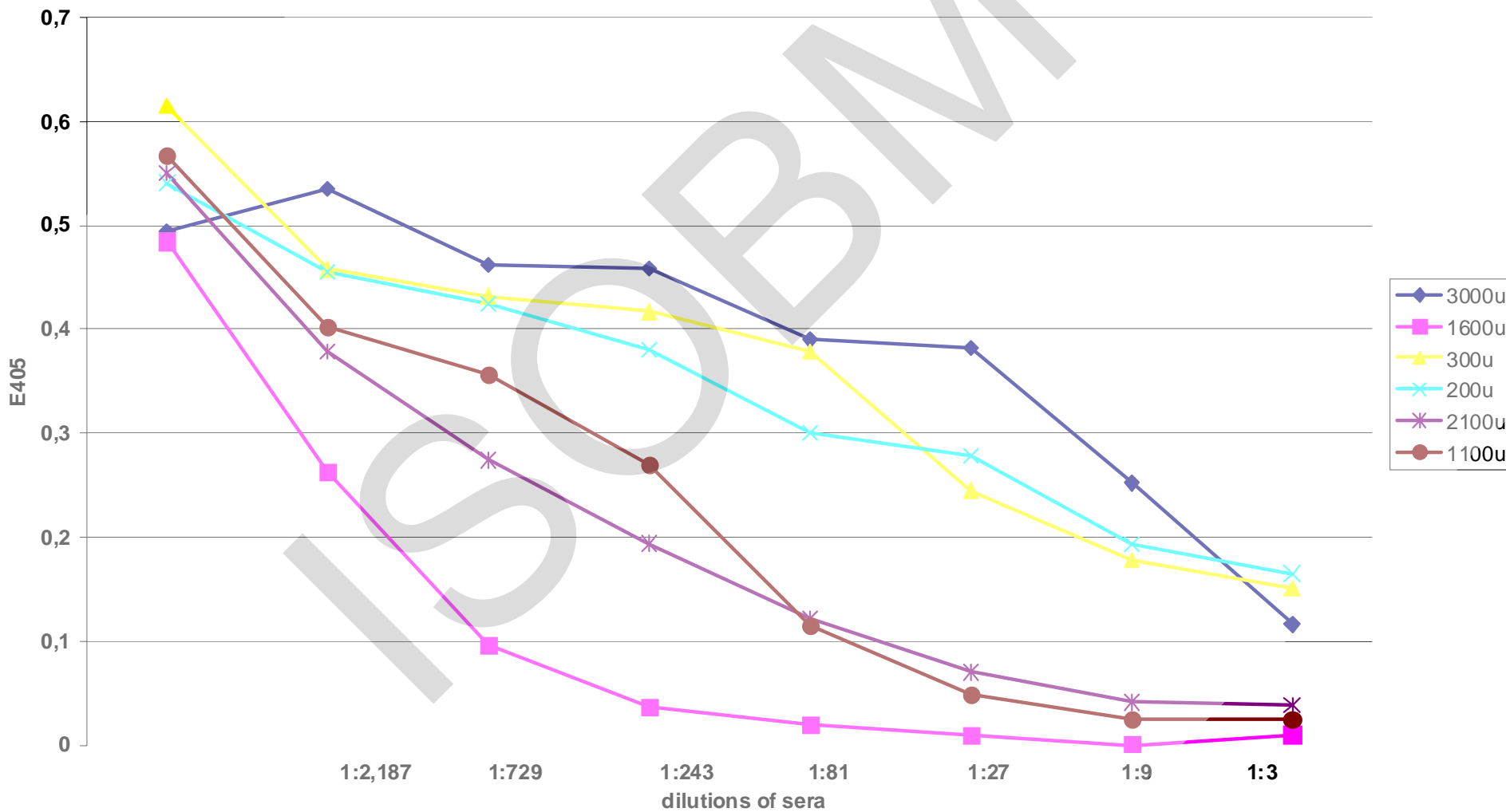
Detection of reference TG (50 ng,Orgentec) by Mab's pairs



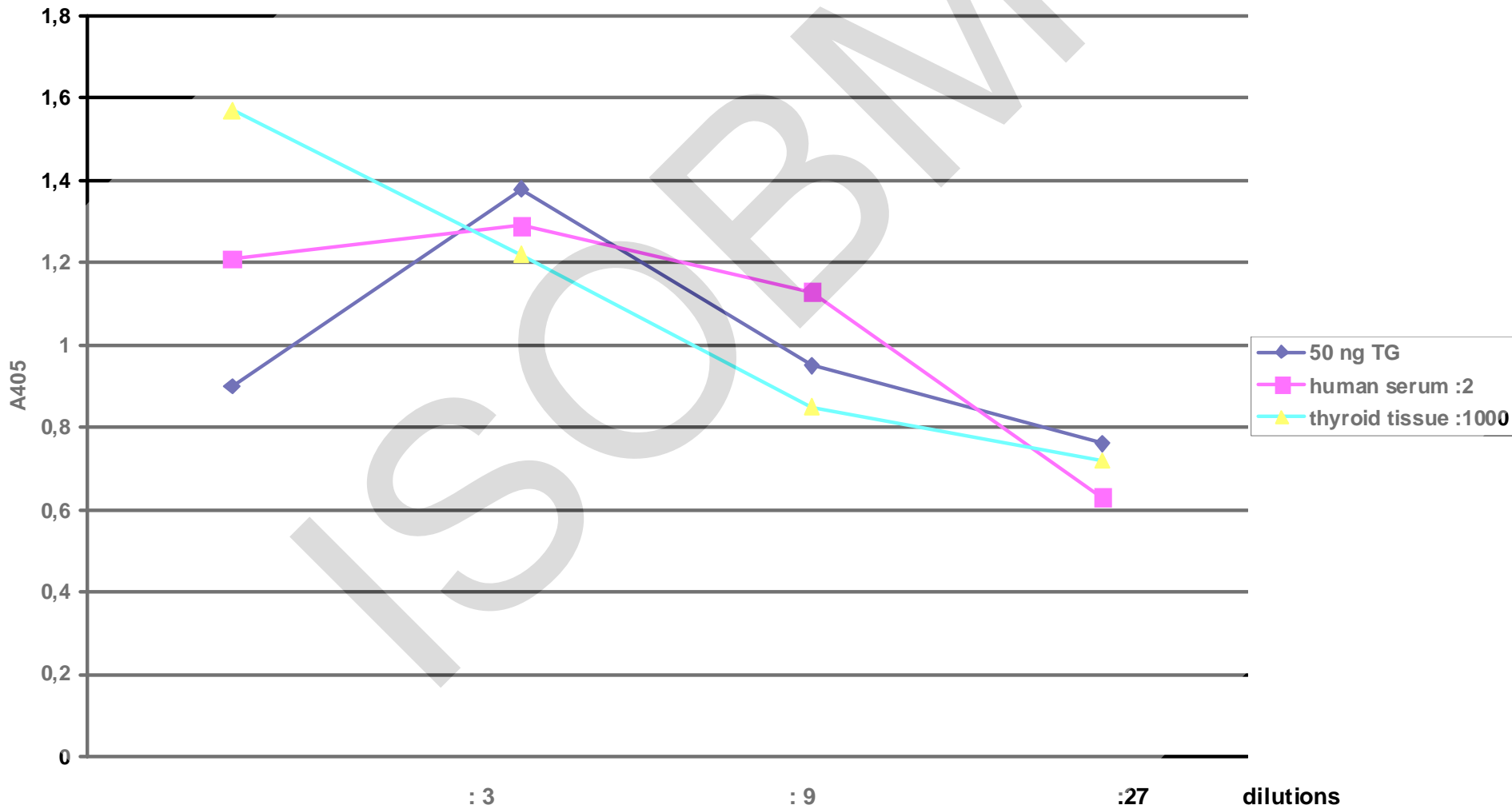
Competition of Mab 5C5-HRP with human anti-TPO antibodies in positive sera



Competition ELISA of human anti-TPO/TG sera with 3F2-Mab HRP conjugate



5C5 -7F7 HRP Double Mab's activity with human samples



Conclusions:

TG and TPO probably exist as one complex of several subunits.

Serum TG secreted from the thyroid gland is a mature form of this complex and intracellular form (microsomal antigen, TPO) is an immature precursor.

Human and animal immune response against thyroid cells don't discriminate these two targets but pathology caused by auto-antibodies could depend upon epitopes of the TG-TPO complex.

Development of Mab's based, epitope-specific test-systems for the determination of human anti-thyroid antibodies would be useful tools for the understanding of mechanisms of variable thyroid pathology.

References

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3. **Duthoit C, Estienne V, Durand-Gorde JM , Carayon P, Ruf J.**
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