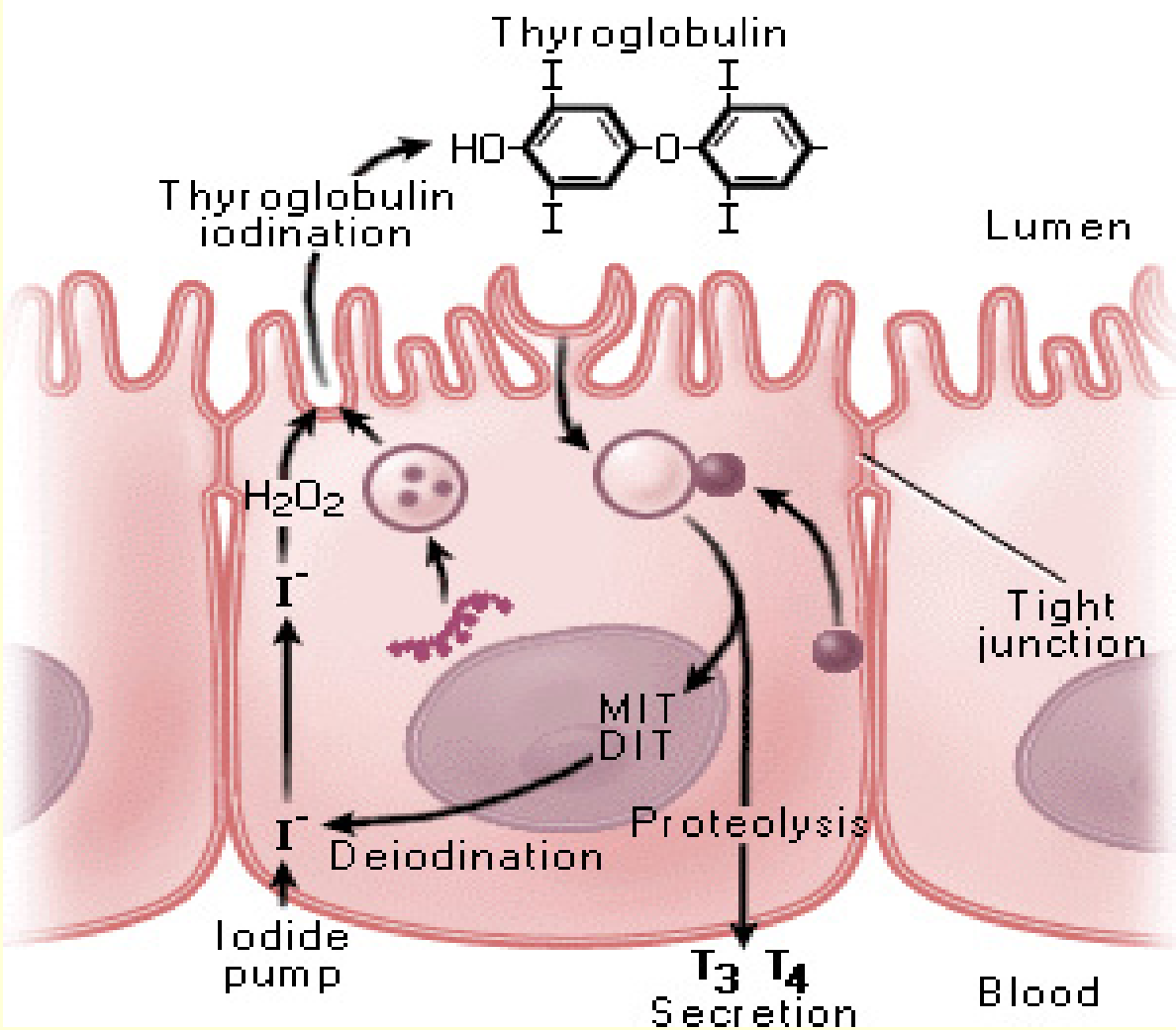
Thyroglobulin

- Large glycoprotein
- Two identical polypeptide chains
- 660 kDa
- Prohormone in the intra-thyroid T4 and T3 synthesis
- Produced only by normal thyrocytes or well-differentiated thyroid cancer (DTC) cells

Thyroglobulin



- Measurements can be made in:
  - Serum
  - Thyroid cyst fluids
  - Materials obtained by fine needle biopsy of thyroid nodules

- Thyroglobulin is elevated in:
  - Patients with goiter
  - Hyperthyroid conditions
- Thyroglubulin is low in:
  - Thyrotoxicosis factitia
  - Congenitale hypothyroidie
- The primary use of serum Tg measurement is, however, as tumor marker for patients affected by DTC

- Thyroid cancer is increasing:
  - Tg = very important biomarker
  - Tg = high reliability
- Aim of this presentation:
  - Focus on new insights of Tg measurements
  - Illustrate clinical impact of new Tg assays

Tg and DTC: commonly accepted key points

- Long term survival of DTC is > 90 %
  - But 33% develops relapse
    - 30 % of relapses can not be eradicated
    - 15% of the relapses die of the disease
  - Mortality is lower when recurrence is detected earlier
- Tg is a very specific tumor marker:
  - Tissue specific
  - Limited tissue distribution
  - Strong correlation between amount of DTC tissue and Tg levels

Tg and DTC: unsolved key points

There is no consensus on:

- The frequency of Tg determination
- The threshold values
- The necessity of Thyrogen stimulation or T4 withdrawal

Long-term DTC monitoring during L-T4 suppression

- Optimal management of DTC includes:
  - Appropriate surgery (total or near total thyroidectomy)
  - Radio-iodine ablation of residual tissue
  - L-T4 treatment to decrease TSH to a level that minimizes the risk of stimulating cancerous thyroid cells
- Long-term follow-up consists of:
  - Serum Tg measurements
  - Ultrasonography
  - Whole-body scanning

Serum Tg response to TSH stimulation

- Tg measurement after TSH stimulation is often considered the most sensitive index for residual tissue
- rh-TSH makes it possible to stimulate Tg without the need to induce hypothyroidism
- Well differentiated tumors typically display a **10-fold stimulation** of serum Tg

Serum Tg response to TSH stimulation

Conventional accepted cut-offs for disease-free patients:

- 5 µg/l during L-T4 withdrawal
- 2 µg/l after rh-TSH stimulation
- 1 µg/l during TSH suppressive therapy

Technical problems in Tg assays

- **Standardization** of the analytical procedure and assay comparability
- **Long-term stability** of assay to allow lifetime follow-up (high inter-assay precision over a period of 6–12 months)
- **Sensitivity** to allow Tg detection in the absence of normal thyroid tissue

Technical problems in Tg assays (2)

- **Working range** and high-dose hook effect
- Effect of **endogenous TgAb** (incidence in DTC is 15–30%)
- Different **Tg isoforms** released by thyroid tumors

Laboratory methods for Tg should not be changed unless a huge analytical advantage is obvious

Standardization problems

- The beween-lab variability is abnormally high:
  - 48 % CV for samples > 5 µg/l
  - 120 % CV for samples < 5µg/l
- If TgAb are present, the differences are even higher
- The widespread adoption of CRM-457 has only slightly reduced standardization problems

Standardization problems

- The difference can be explained by:
  - Different calibrators
  - Different specificity of the antibodies used
- According to the NACB guidelines, the use of different Tg methods to monitor DTC patients is precluded

EQC

- Modular/Elecsys Roche gives higher results as compared to other methods
- Immulite one/2000 DPC (IMM2) is more influenced by TgAb as compared to the other methods giving lower Tg values
- The clinical cut-off (1 µg/l during suppressive therapy) is not well measurable in terms of analytical performance with the conventionally used assays

Sensitivity and precision problems

- Sensitivity is critical:
  - To detect small amounts of Tg
  - To observe changes in follow-up patients
- Tg assays should have a functional sensitivity at least of 1 µg/l with a normal lower Tg limit of 3 µg/l

Tg sensitivity

Analytical sensitivity of Tg assays has been greatly improved, being between 5 and 10 µg/l with the first assays and **0.08 µg/l** (functional sensitivity of **0.2 µg/l**) recently obtained with a **commercial IRMA** (DYNOtest, Brhams Diagnostica GmbH, Berlin, Germany)

Type of assay[a]	Assay distributor	Analytical sensitivity (µg/l)	Functional sensitivity (µg/l)	TAT
ECLIA	Roche, Elecsys	0.10	1.0	18 min
ICMA	DiaSorin, Liaison	0.20	0.5	30 min
ICMA	Beckman, Access	0.01[b]	0.1[c]	40 min
ICMA	DPC, Immulite	0.50	0.9	90 min
ELISA	RSD, Ldt.	0.015	0.03	Overnight
IRMA	Brahams	0.08	0.2	Overnight
ILMA	Brahams	0.02	0.06	Overnight
IRMA	Nichols	0.07	0.5	Overnight

Tg sensitivity

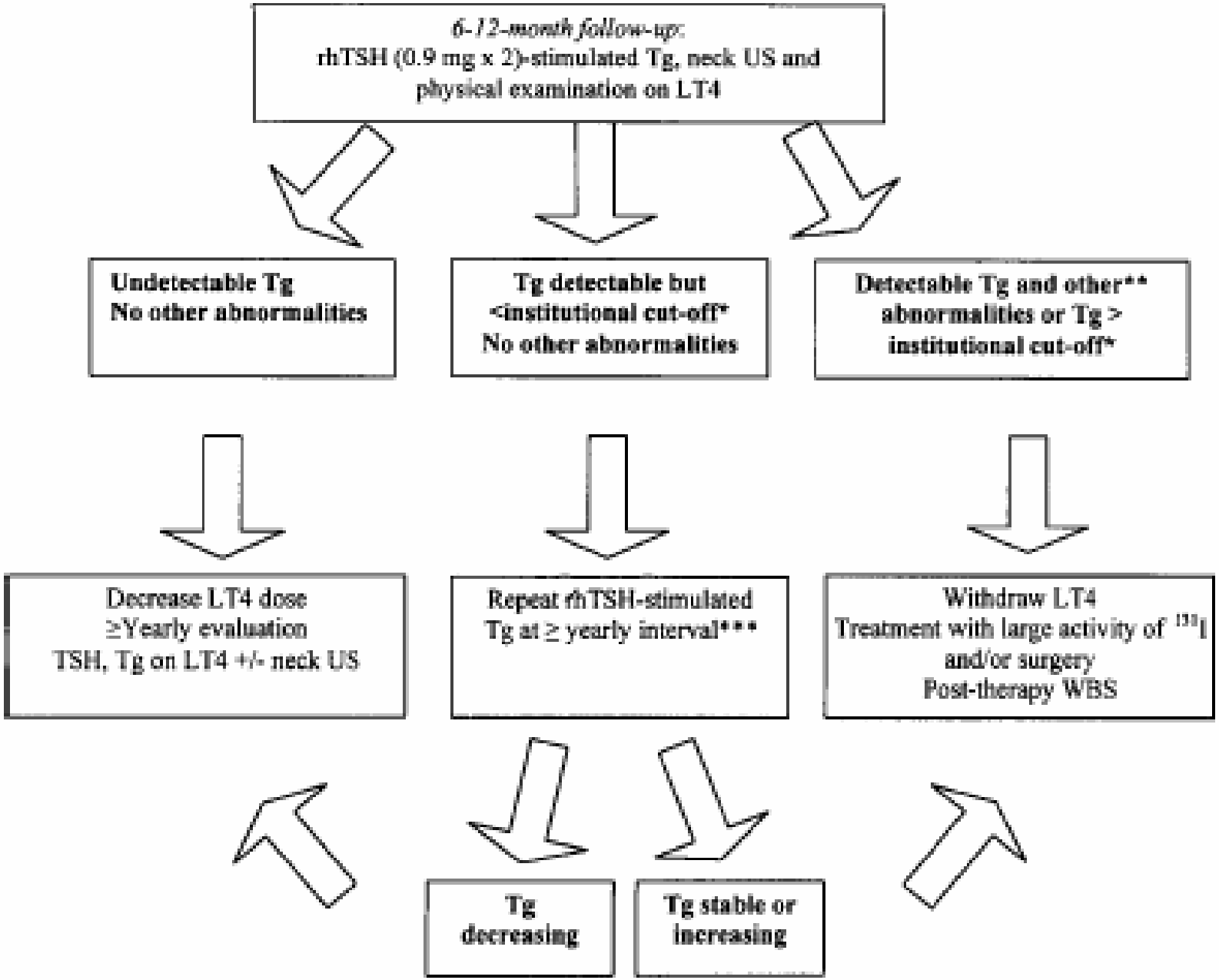
highly sensitive Tg assays will probably improve the value of Tg measurement in DTC patients

BUT:

how to deal with patients with low but measurable Tg levels without proven thyroid cancer?

European consensus paper (Schlumberger 2004)

- every center should define an institutional cut-off value for Tg levels
- the optimal strategy of the application of rh-TSH stimulation in the follow-up of DTC thyroid cancer has to be established
- comparison of long-term results in terms of recurrence rate and survival of different follow-up protocols is necessary to develop a well-founded protocol for treatment and follow-up on patients with DTC


6-12-month follow-up:
rhTSH (0.9 mg x 2)-stimulated Tg, neck US and physical examination on LT4
Undetectable Tg
No other abnormalities
Tg detectable but <institutional cut-off*
No other abnormalities
Detectable Tg and other** abnormalities or Tg > institutional cut-off*
Decrease LT4 dose
≥Yearly evaluation
TSH, Tg on LT4 +/- neck US
Repeat rhTSH-stimulated Tg at ≥ yearly interval***
Withdraw LT4
Treatment with large activity of ^{131}I and/or surgery
Post-therapy WBS
Tg decreasing
Tg stable or increasing

Will sensitive assays solve the problem?

- Some physicians would prefer “less sensitive” assays because it is sometimes difficult to verify the source of very low Tg concentration (< 0.5 µg/l) by additional imaging diagnostic, and “confusion” for the physician and the patients may occur
- Other people, however, argues in favour of a complete evaluation of patients with detectable serum Tg, irrespective of the concentration.

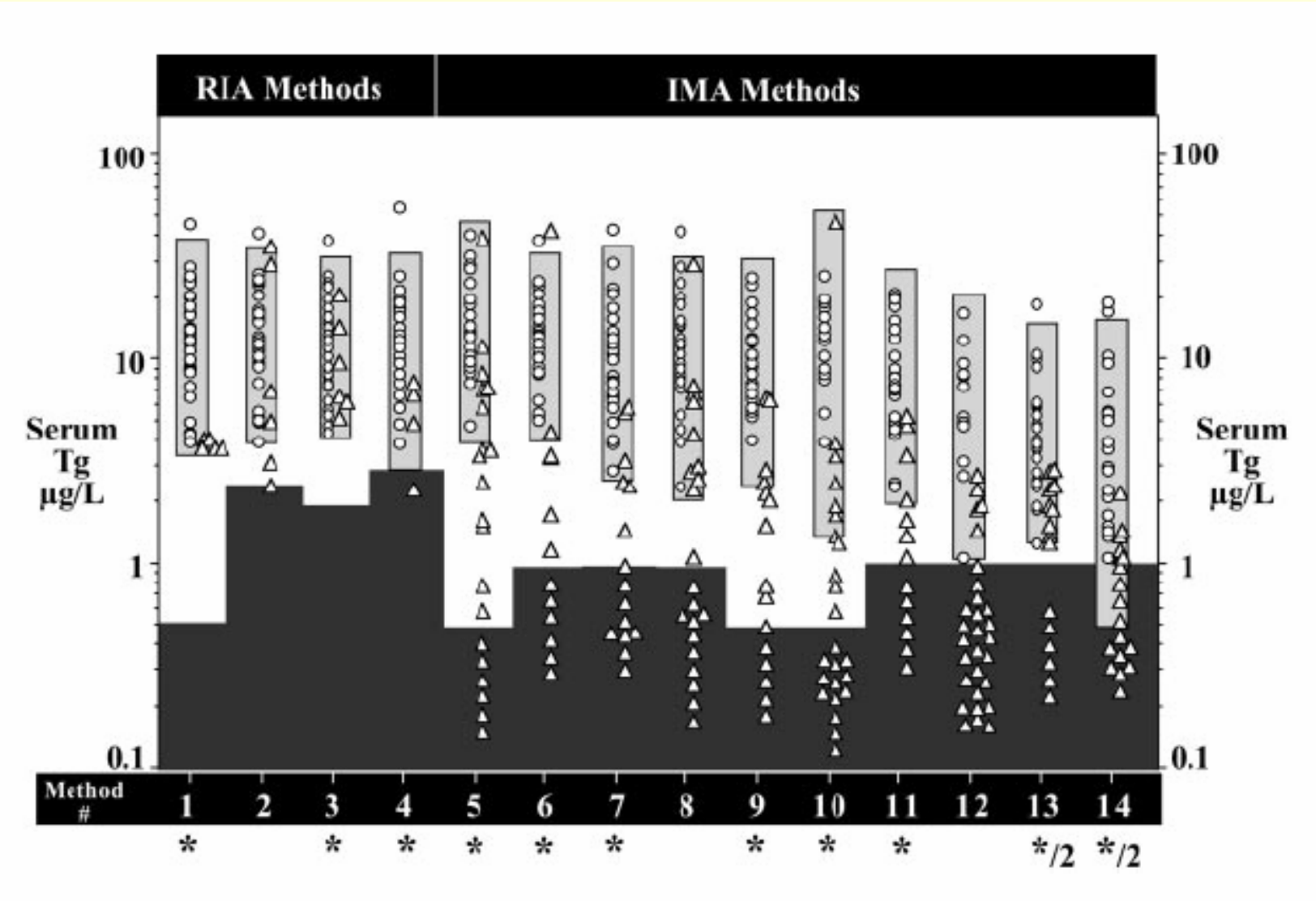
Antibody interference

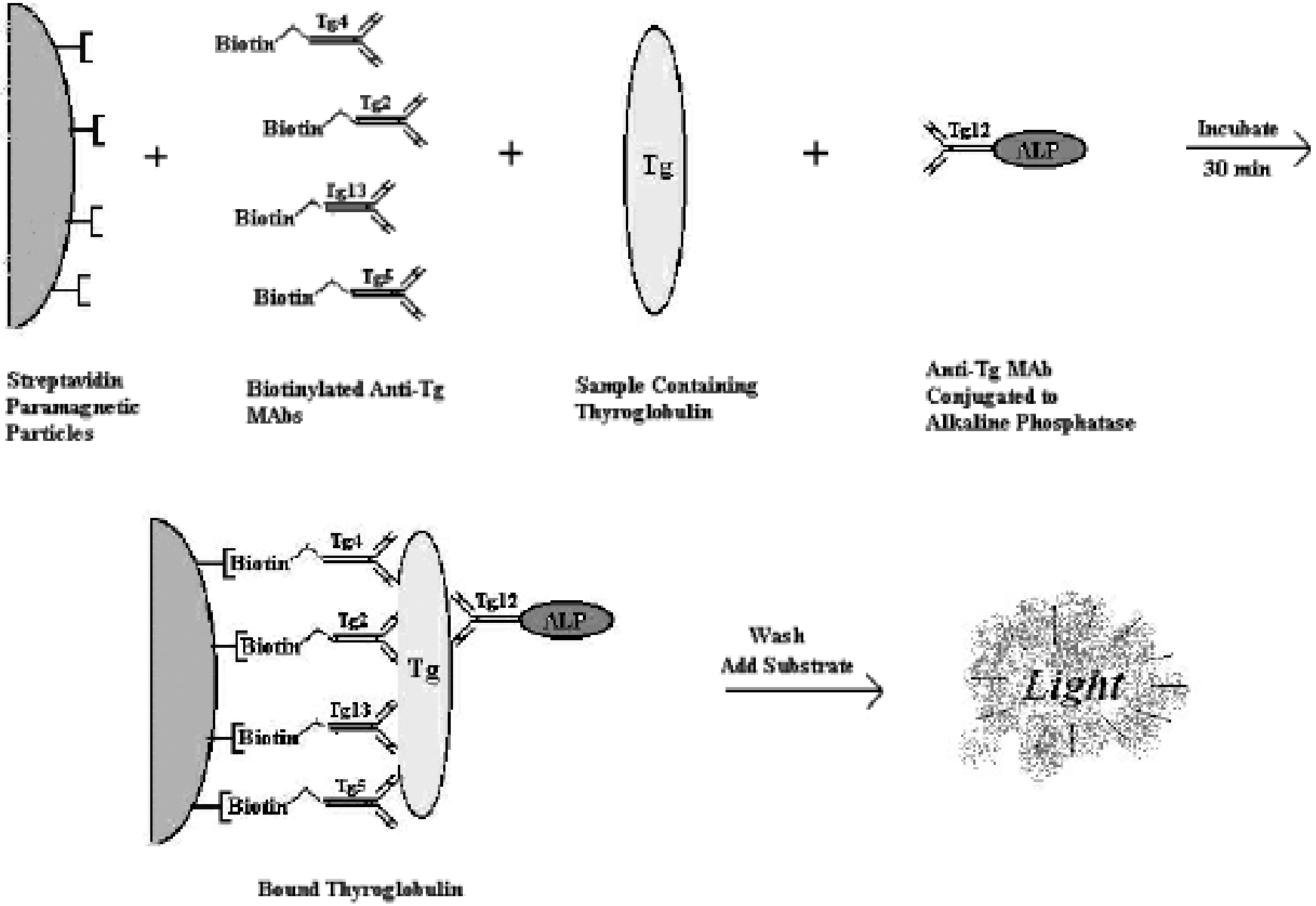
TgAb interference:

- no method is free from TgAb interference
- some methods appear more resistant than others
- RIA more robust than IMA (trend in US labs is to use RIA for TgAb positive patients)
- up to 30 % of the DTC population is positive
- TgAb should always be measured if Tg is measured (NACB guideline 2002)

Heterophile antibodies

heterophile blocking tubes (Scantibodies)


RIA Methods
IMA Methods
100
10
1
0.1
Serum Tg µg/L
Method #
1 2 3 4 5 6 7 8 9 10 11 12 13 14
* * * * * * * * * */2 */2


Biotin
Tg4
Biotin
Tg2
Biotin
Tg13
Biotin
Tg5
Tg
Tg12
ALP
Incubate
30 min
Streptavidin
Paramagnetic
Particles
Biotinylated Anti-Tg
MAbs
Sample Containing
Thyroglobulin
Anti-Tg MAb
Conjugated to
Alkaline Phosphatase
Biotin
Tg1
Biotin
Tg2
Biotin
Tg13
Biotin
Tg5
Tg
Tg12
ALP
Wash
Add Substrate
Light
Bound Thyroglobulin

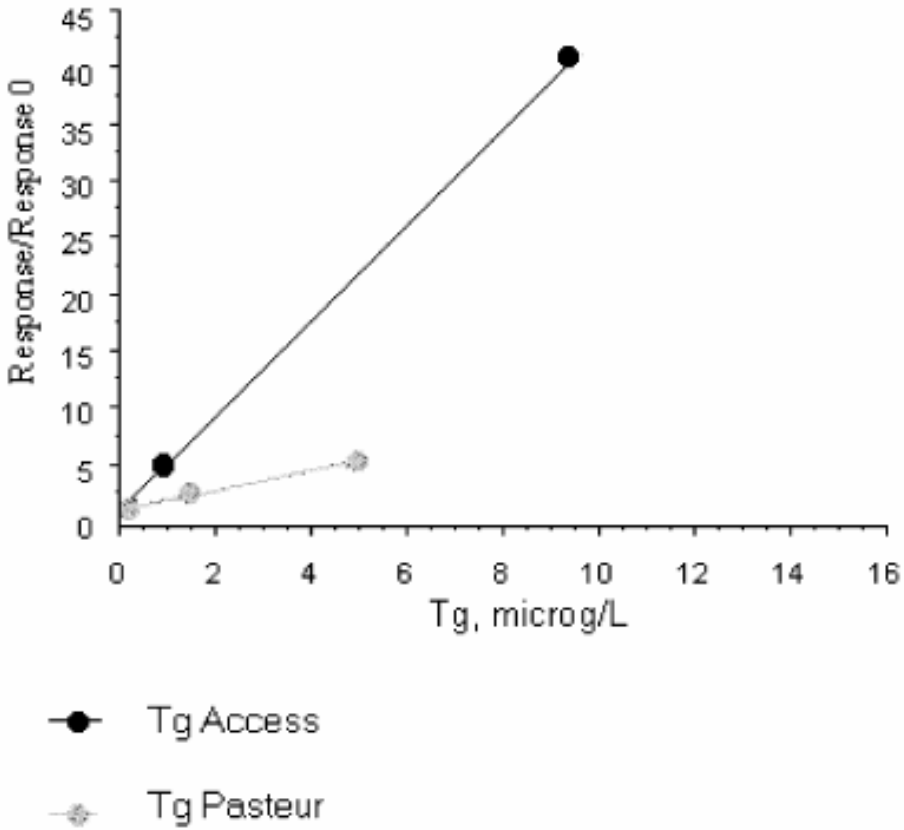


Figure 2 Representation of the first part of the calibration curves (first three points) of the Tg Access and Tg Pasteur assays. To compare the calibration curves of the two methods the ratio of the response/response at 0 µg/l is shown on the ordinate.

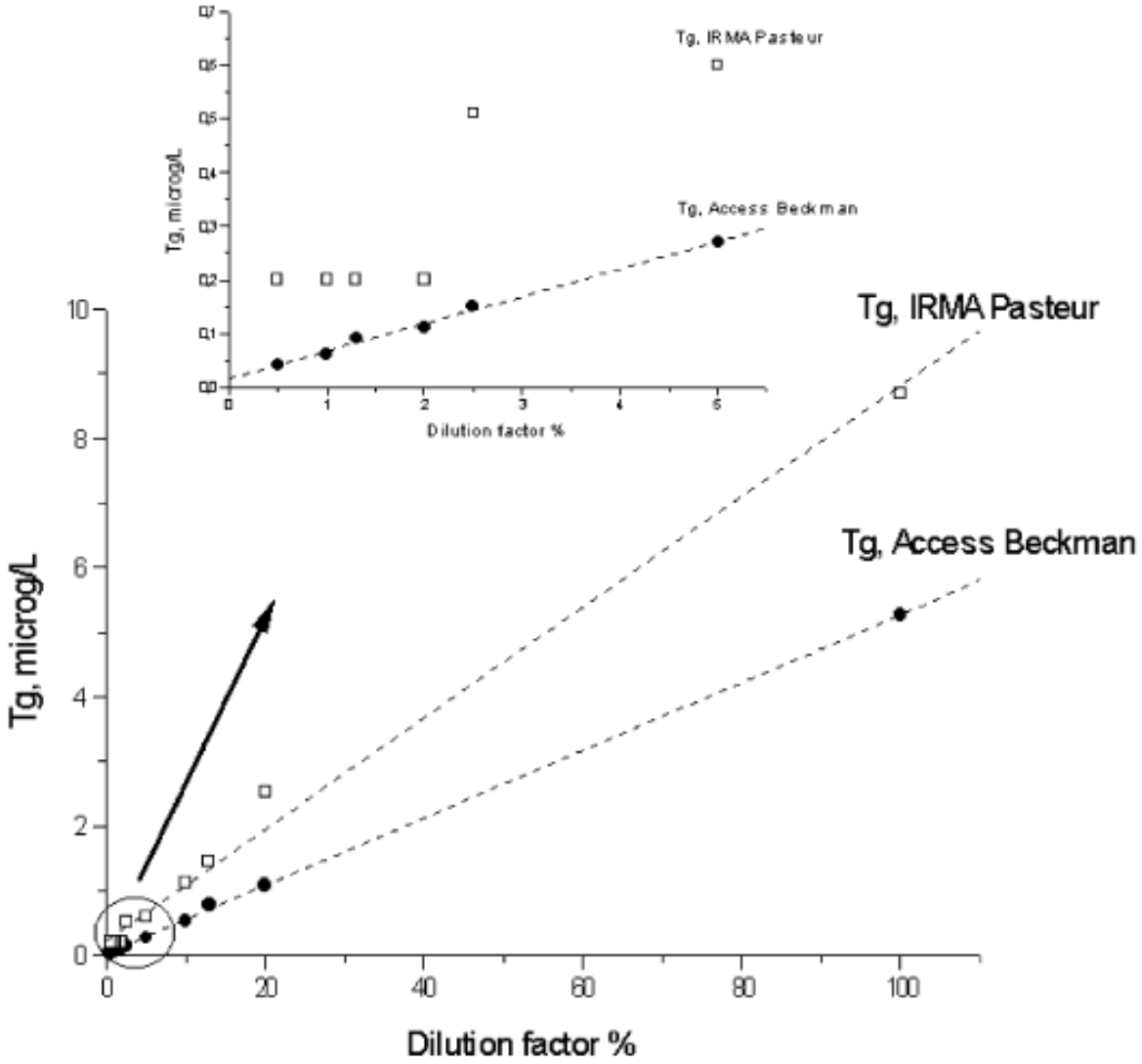
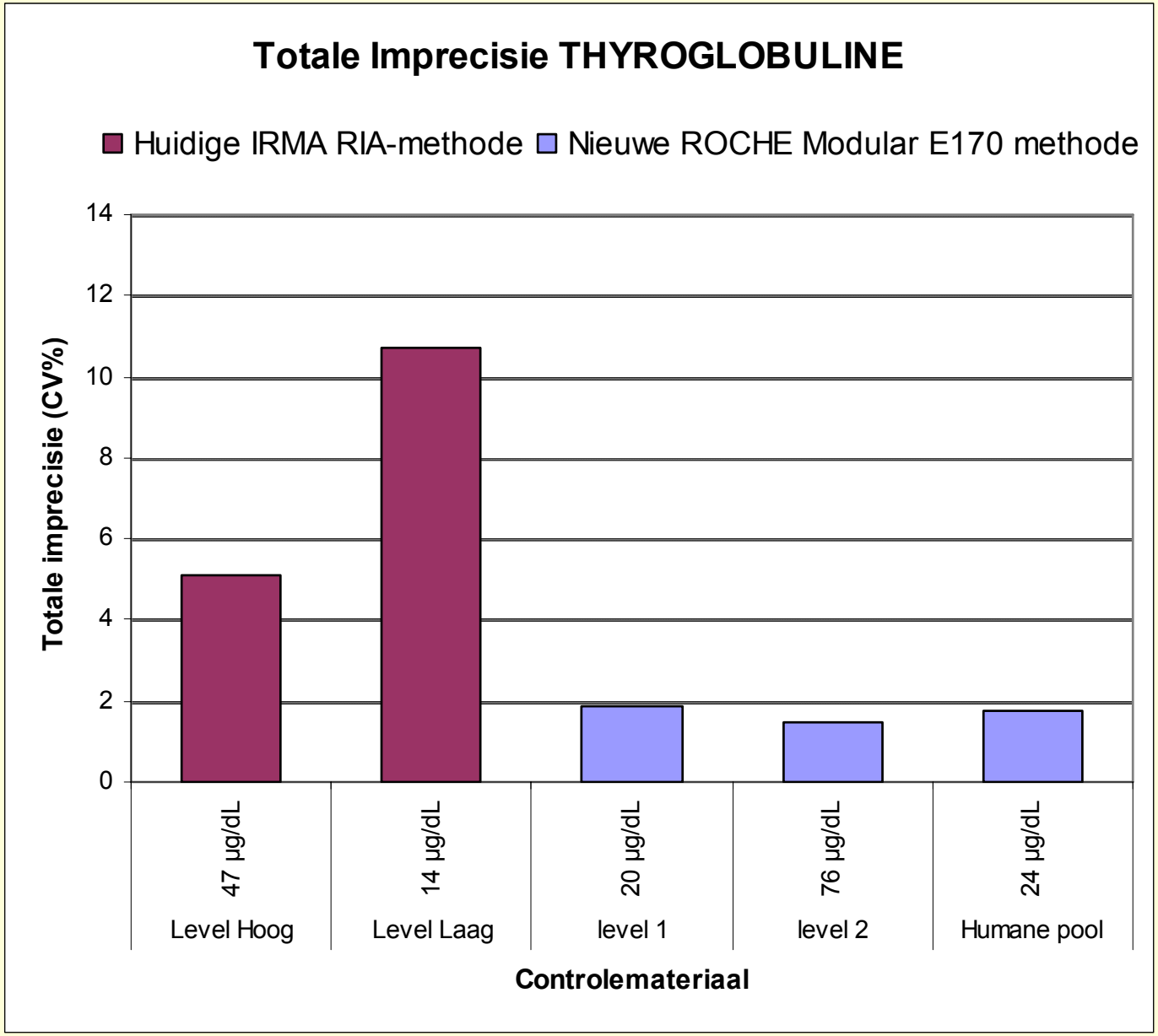
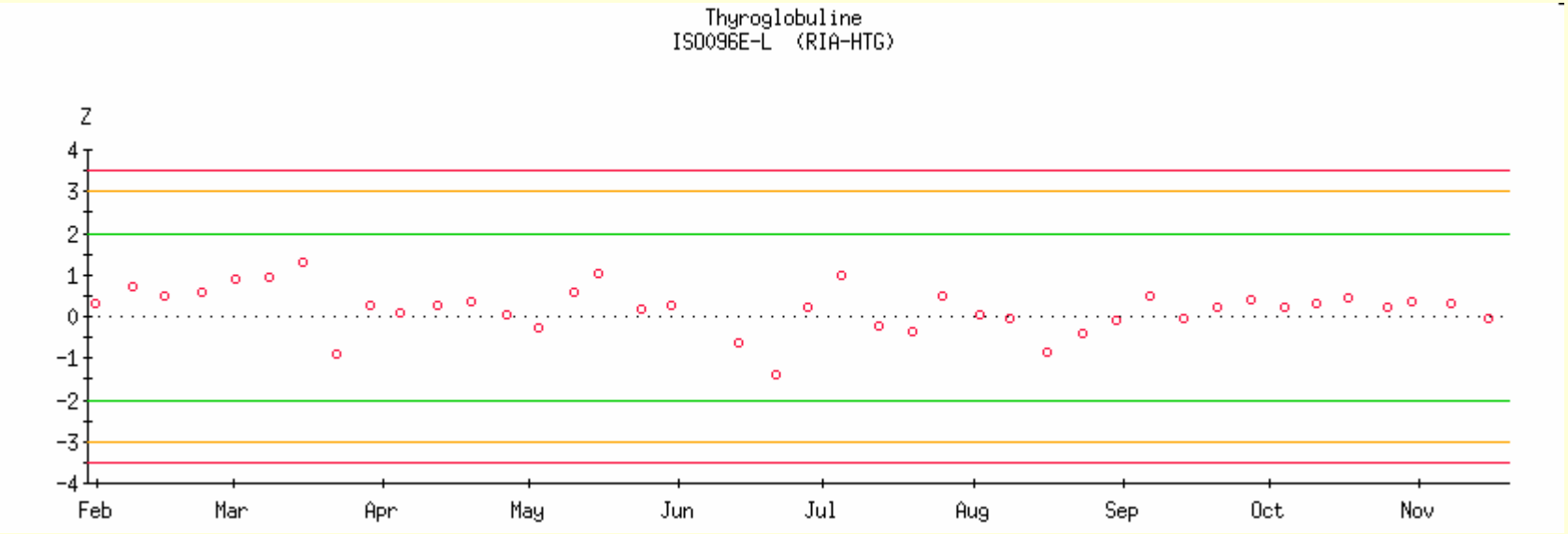


Figure 3 Dilution test for Tg Access (●) and Tg Pasteur (□) performed by progressively diluting the same sample pool at 5·27 µg/l (Access) and 8·69 µg/l (Pasteur) with Tg-free AbTg-free serum obtained from thyroidectomized and radio-ablated patients for DTC stage 1 treatment (see Materials and Methods). The dashed lines indicate linear regression.

Tg IRMA: imprecision

	REPRODUCTIBILITE INTRA-ESSAI n = 30			REPRODUCTIBILITE INTER-ESSAI n = 21	
SERUM	X (ng/ml)	CV (%)	SERUM	X (ng/ml)	CV (%)
A	1,22	7,7	F	0,8	16,7
B	8,2	3,1	G	7,9	7,0
C	43,8	2,6	H	43,3	3,1
D	116,0	1,4	I	111,0	2,0
E	373,0	1,8	J	299,0	3,0


Totale Imprecisie THYROGLOBULINE
Huidige IRMA RIA-methode
Nieuwe ROCHE Modular E170 methode
Totale imprecisie (CV%)
14
12
10
8
6
4
2
0
47 µg/dL
Level Hoog
14 µg/dL
Level Laag
20 µg/dL
level 1
76 µg/dL
level 2
24 µg/dL
Humane pool
Controlemateriaal


Thyroglobuline
ISO096E-L (RIA-HTG)
Z
4
3
2
1
0
-1
-2
-3
-4
Feb
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Apr
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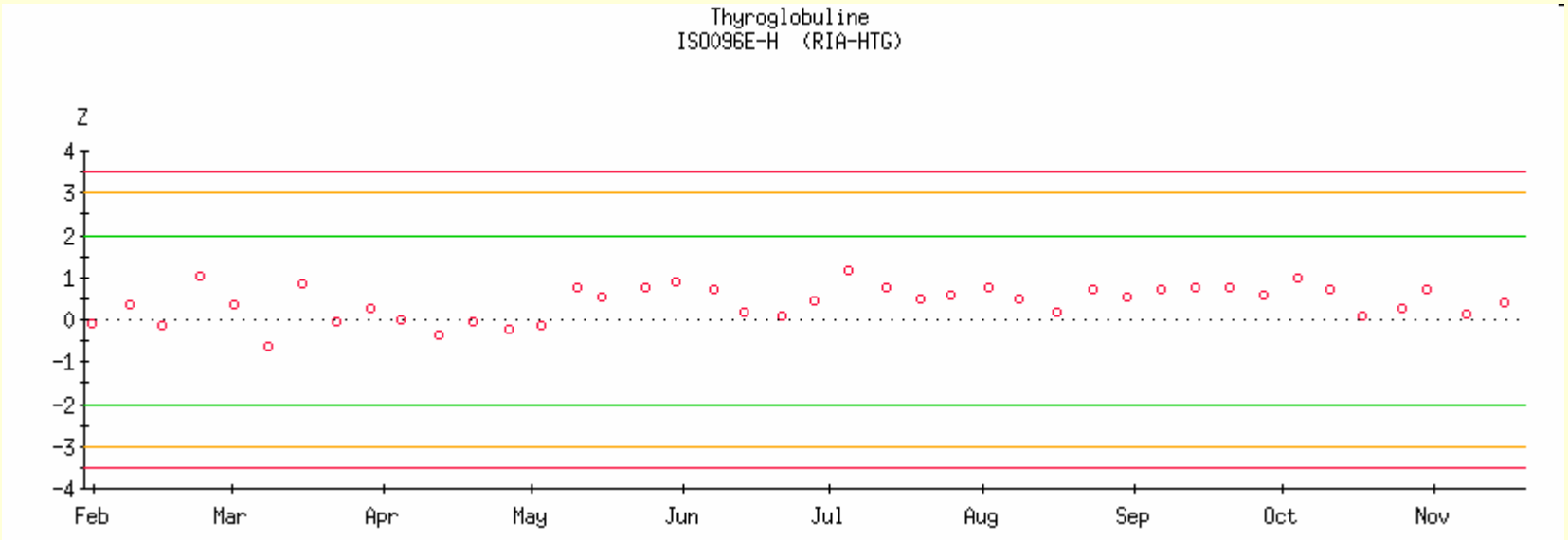
Nov


Thyroglobuline
ISO096E-H (RIA-HTG)
Z
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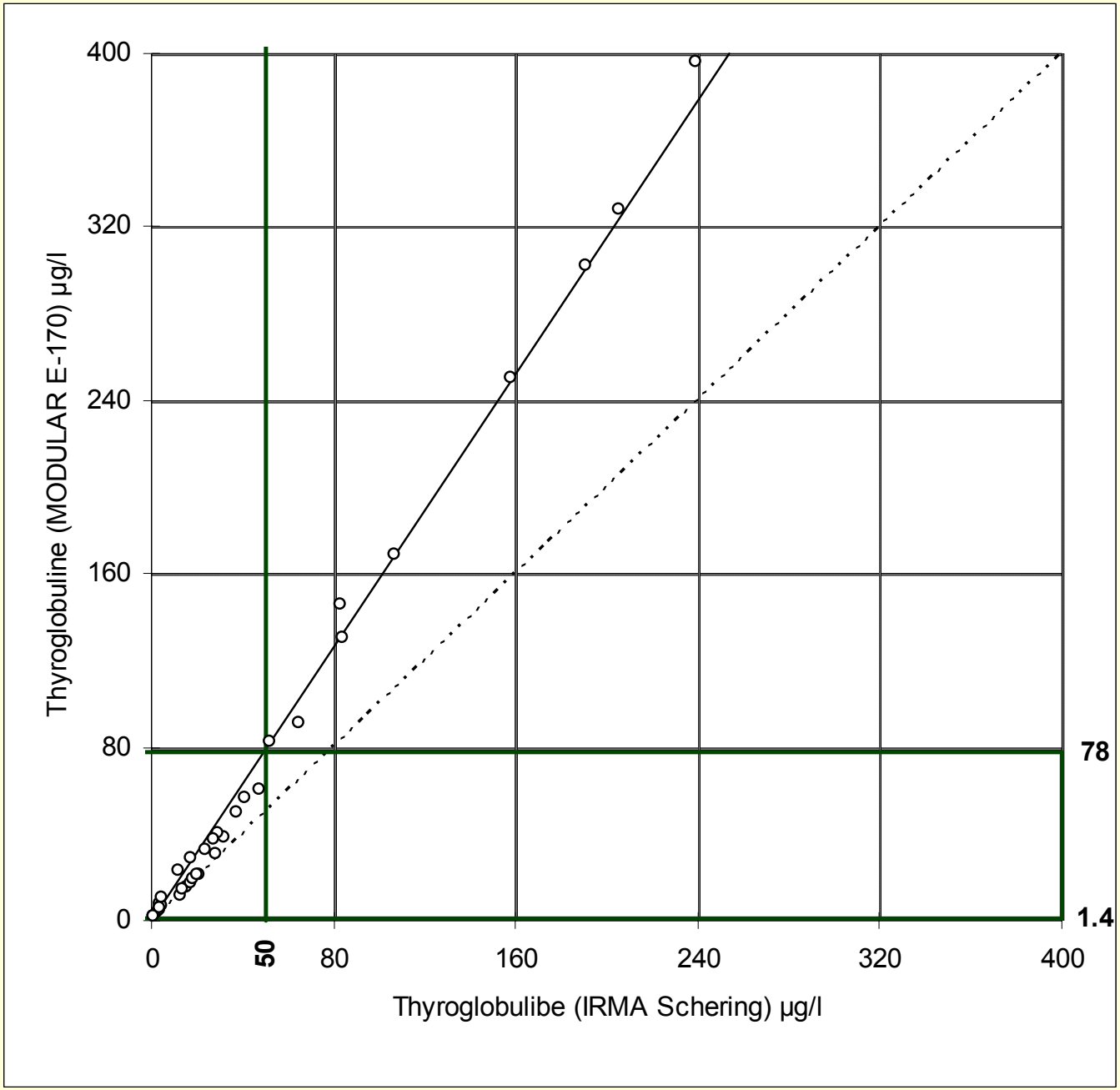

P/B Regression
Y = 1.581 * X - 0.663
md(95) = 7.401
N = 35, r = 0.998

TABLE 1. Tg standard CRM 457 was diluted in human Tg free serum and then measured with each method

Serial dilutions of CRM 457 (ng/ml)	Calculated values (ng/ml)					
	Tg-Kryptor	Immulite TG	Thyro	Tg Access	Tg Advantage	DYNOtest
200	95	188	245	185	112	100
100	52	81	125	95	55	50
20	9.6	14.6	24.1	17	13.2	10
5	2.9	3.8	7.6	4.4	3.7	2.8
1	0.8	0.8	1.8	1	1.2	0.7
0.5	0.2	<0.2	1.4	0.5	0.5	0.2
0.25	<0.17	<0.2	0.91	0.33	<0.3	<0.1
0.10	<0.17	<0.2	<0.2	0.20	<0.3	<0.1

J Clin Endocrinol Metab. 2007 Jul;92(7):2487-95. Schlumberger

Analytische gevoeligheid

Definitie: analytische gevoeligheid = detectielimiet = minimaal detecteerbare concentratie (concentratie overeenkomend met het signaal van de nulcalibrator + 2 SD)

Irma: 0,2 µg/l

E 170: 0,1 µg/l

Functionele gevoeligheid

Definitie: concentratie corresponderend met 20 % CV

Irma: 0,7 µg/l

E 170: 0.5 µg/l

Meetbereik

Irma:0,7- 500 µg/l
E170: 0,5 - 1000 µg/l

Hook effect

Irma: 28000 µg/l
Modular E 170: 120000 µg/l

IRMA	E170
0.500	1.110
< 0.2	0.216
< 0.2	0.110
0.400	0.172
0.400	0.405
0.500	0.107
0.600	0.173
0.500	0.143
< 0.2	0.196
0.600	0.286
0.400	0.451
0.700	0.146
0.800	0.283
0.600	< 0.1
0.700	< 0.1
0.600	< 0.1

IRMA	E170
0.200	0.128
0.200	< 0.1
< 0.2	< 0.1
< 0.2	< 0.1
0.200	0.308
< 0.2	< 0.1
< 0.2	< 0.1
< 0.2	< 0.1
< 0.2	< 0.1
0.200	< 0.1
20.2	< 0.1
0.3	0.443
0.3	0.662
0.3	0.883
1	3.300
0.4	ngs
0.5	0.933
0.3	0.505
0.4	1.160

Staal ID	TgAb	TG staal+S 1 (a)	TG staal+S 7 (b)	(b)-(a)	%recovery
st30	263	97.30	218.70	121.40	97%
		100.10	223.50	123.40	99%
st31	130	0.60	125.90	125.30	100%
		0.70	129.60	128.90	103%
st32	143	0.50	101.60	101.10	81%
		0.60	109.10	108.50	87%
st33	787	16.90	141.20	124.30	99%
		19.90	143.60	123.70	99%
st34	234	0.40	108.80	108.40	87%
		0.50	112.80	112.30	90%
st35	162	3.30	98.40	95.10	76%
		3.60	102.60	99.00	79%
st36	442	0.30	52.00	51.70	41%
		0.20	54.50	54.30	43%
st37	314	2.30	119.10	116.80	93%
		2.20	124.40	122.20	98%
st38	347	0.30	121.50	121.20	97%
		0.40	128.50	128.10	102%
st39	561	16.00	145.90	129.90	104%
		16.8	147.5	130.70	105%

Staal ID	TgAb	TG staal+S1 (a)	TG staal+S7 (b)	(b)-(a)	% recovery
st30	263	143.20	296.20	153.00	122%
		145.80	302.80	157.00	126%
st31	130	0.31	136.20	135.89	109%
		0.41	140.50	140.10	112%
st32	143	0.10	125.40	125.30	100%
		0.10	127.50	127.40	102%
st33	787	24.71	164.20	139.49	112%
		25.45	169.80	144.35	115%
st34	234	0.10	122.50	122.40	98%
		0.10	126.70	126.60	101%
st35	162	4.48	120.60	116.12	93%
		4.59	125.30	120.71	97%
st36	442	0.10	80.35	80.25	64%
		0.10	80.62	80.52	64%
st37	314	1.99	145.10	143.11	114%
		2.05	146.80	144.75	116%
st38	347	0.10	144.40	144.30	115%
		0.10	147.20	147.10	118%
st39	561	25.08	180.10	155.02	124%
		26.1	187.4	161.30	129%

Original article Schlumberger M *et al.* (2007) Comparison of seven serum thyroglobulin assays in the follow-up of papillary and follicular thyroid cancer patients. *J Clin Endocrinol Metab* **92:** 2487–2495

CONCLUSION

Use of an assay with improved functional sensitivity could allow early detection of changes in serum thyroglobulin without the need for TSH stimulation.

COMMENTARY

Penny M Clark

"Although clinical sensitivity and specificity were improved for LT4-suppressed thyroglobulin measurement, they still remained less favorable than for TSH-stimulated thyroglobulin measurement."

Clinical Endocrinology (2007) **67**, 321–323 doi: 10.1111/j.1365-2265.2007.02899.x

COMMENTARY

Will highly sensitive thyroglobulin assays change the management of thyroid cancer?

Ernest L. Mazzaferri

Department of Medicine, University of Florida, Gainesville, FL, USA and Department of Internal Medicine, Ohio State University, Columbus, OH, USA

Table 1. Basal, TSH stimulated Tg values and predictive values in 160 DTC patients

Tg Immulite							
Tg response after rh-TSH cut-off = 2 µg/l	Basal Tg < 0·9 µg/l n. pts	Basal Tg > 0·9 µg/l n. pts	Predictive value %	Tg response after rh-TSH cut-off = 1 µg/l	Basal Tg < 0·9 µg/l n. pts	Basal Tg > 0·9 µg/l n. pts	Predictive value %
True negative			NPV*	True negative			NPV*
148	148	0	100%	129	129	0	100%
True positive			PPV†	True positive			PPV†
12	10	2	16·6%	31	29	2	6·5%
Total 160	158	2		Total 160	158	2	
Tg Access							
Tg response after rh-TSH cut-off = 2 µg/l	Basal Tg < 0·1 µg/l n. pts	Basal Tg > 0·1 µg/l n. pts	Predictive value %	Tg response after rh-TSH cut-off = 1 µg/l	Basal Tg < 0·1 µg/l n. pts	Basal Tg > 0·1 µg/l n. pts	Predictive value %
True negative		NPV*	True negative				NPV*
152	137	15	90·1%	141	133	8	94·3%
True positive			PPV†	True positive			PPV†
8	0	8	100%	19	4	15	78·9%
Total 160	137	23	Total 160	137	23		

*NPV, negative predictive value; †PPV, positive predictive value.

Journal of Endocrinology (2004) **182,** 287–294
A. Iervasi

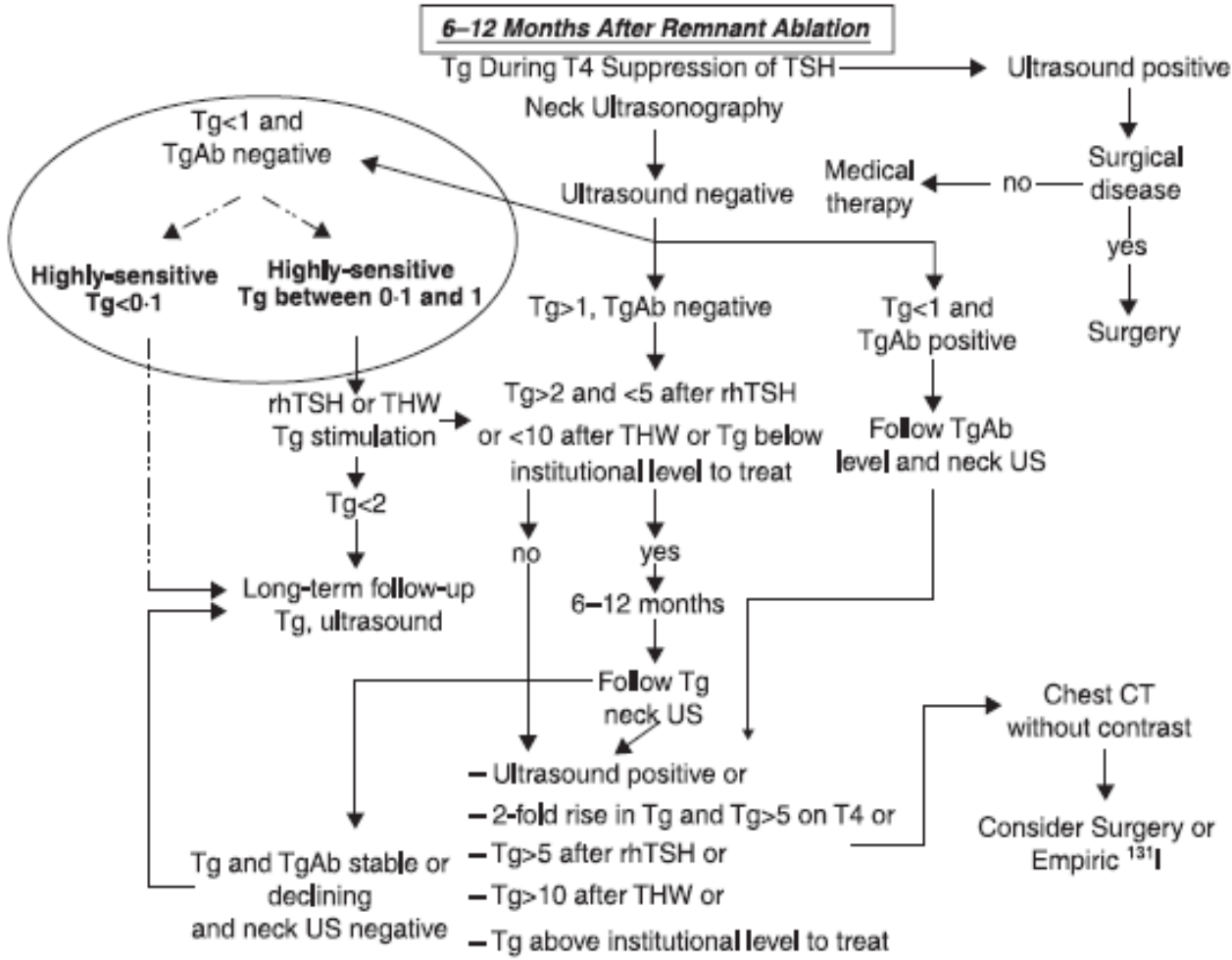

Fig. 3 Our modification (see circled area) of flow-chart for the follow-up of low-risk patients (from Cooper *et al.*[18]) proposed according to the results of the present study.

Suggestions for ROCHE R&D

- Improved standardisation
- Lower limit of detection
- More publications on/with this assay

Another dilemma…

"The measurement of esoteric hormones has moved increasingly from laboratories with meticulous quality control, clinical feedback, and long experience, to busy, shrinking-budget-driven general hospital laboratories or profit-driven private laboratories."