



Association des Assistants en
Biologie Clinique
A.S.B.L.

Interférences dans les dosages thyroïdiens : l'histoire se répète...

Dr Henry Paridaens

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4^{ème} Congrès

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Interference in thyroid-stimulating hormone determination

Mauro Imperiali^{*}, Paola Jelmini^{*}, Biagio Ferraro^{*}, Franco Keller^{*}, Roberto della Bruna^{*}, Marco Balerna^{*} and Luca Giovanella[†]

Phantoms in the Assay Tube: Heterophile Antibody Interferences in Serum Thyroglobulin Assays

CAROL M. PREISSNER, DENNIS J. O'KANE, RAVINDER J. SINGH, JOHN C. MORRIS, AND STEFAN K. G. GREBE

Clin Lab. 2012;58(11-12):1305-7.

TSH-assay interference: still with us.

Verdickt L¹, Maiter D, Depraetere L, Gruson D.

B. K. King, G. Klee, J. McDonald, N. Baumann. *Mayo Clinic, Rochester, MN,*

Background: Heterophile antibody, or human anti-animal antibody, interference is a known limitation of immunoassays. Prevalence of heterophile antibody interference has been reported to be between 1-2% in the general population, but most of these antibodies do not cause assay problems. It is difficult to detect heterophile interference

A196

2011 AACC Annual Meeting Abstracts

PLAN

- 1.Introduction – cas clinique
- 2.Comment rechercher une interférence ?
- 3.Conclusions



INTRODUCTION – CAS CLINIQUE

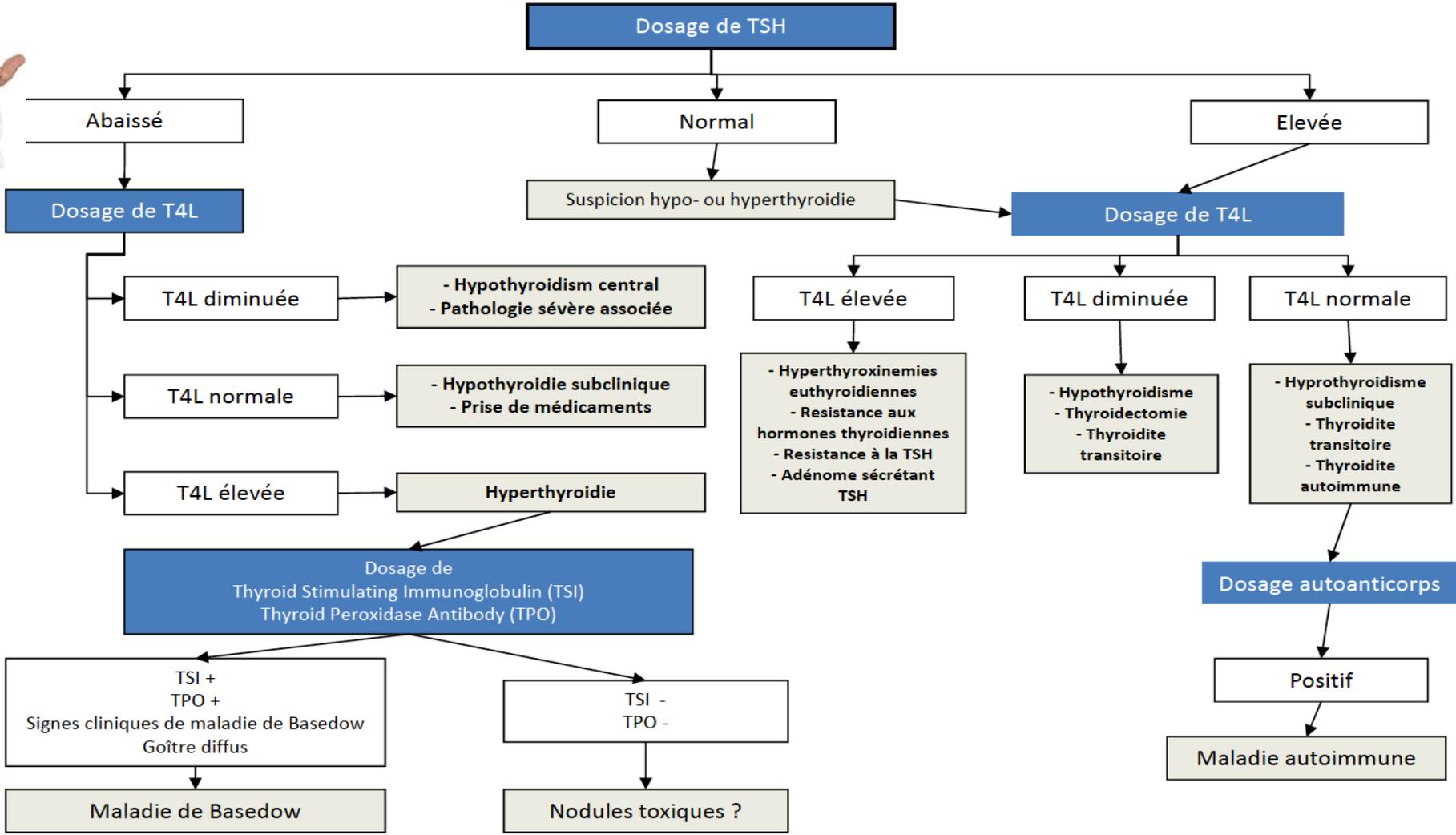
- ♀ de 35 ans
- Bilan biologique thyroïdien préalable à une seconde procédure de fécondation in vitro
 - **TSH : 1,08 mU/l (0,27-4,20 mU/l)**
 - **FT4 : < 0,5 mU/L (12,0-22,0 mU/l)**
 - **FT3 : < 0,6 pmol/L (3,1-6,8 mU/l)**
- Taux normaux des autres paramètres endocriniens (FSH, LH, prolactine, œstradiol, progestérone, IGF-1)
- Pas de symptômes d'hypothyroïdisme
- Pas d'antécédent médico-chirurgicaux hormis une infection à *Mycoplasma pneumoniae* et une chirurgie dans l'enfance pour sténose d'une valve pulmonaire
- Mère d'un enfant de 18 mois déjà né par IVF
- ATCDS familiaux : hyperparathyroïdisme et mère hypothyroïdisme
- Non fumeuse, alcool occasionnellement
- R/ : cocktails vitaminiques (vitamine C, guarana, tuarine), acide folique et supplément en fer.





Indications de dosage: Pathologie thyroïdienne

Perturbation métabolique
(intolérance à la chaleur ou au froid, perte ou gain de poids, dépression, anxiété, fatigue, etc...)



A LA RECHERCHE D'UNE EXPLICATION





VITROS[®] 5600
iSystem
Integrated



CHU
— UCL —
NAMUR

Paramètres	VITROS 5600	Roche 8000® e602
Principe de dosage	Immunodosage direct par compétition	ECLIA (compétition)
Réactif luminogène	Dérivé du luminol et un sel de peracide	Ruthénium
Interférences connues	Aspirine, bilirubine, phénytoïne, dipyrone, furosémide, acide méfénamique, phénylbutazone, PTU (Propyl-thiouracile), salicylate de sodium, D-Thyroxine L-thyroxine, TRIAC (acide L-3,3',5 triiodothyroacétique)	Tout médicaments pouvant affecter la capacité de liaison des protéines de transport (influence médicamenteuse, affections non thyroïdiennes ou hyperthyroxinémie/dysalbuminémie familiale), lévothyroxine, furosémide
Intervalle de référence	TSH : 0,27-4,20 mU/l FT4 : 12,0-22,0 pmol/l FT3 : 3,1-6,8 pmol/l)	TSH : 0,46-4,68 mU/l FT4 : 10,0 -28,2 pmol/l FT3 : 4,3 -8,1 pmol/L
Limites de détection (LOD)	TSH : 0,005 mU/L FT4 : 0,5 pmol/l FT3 : 0,6 pmol/l	TSH : 0,014 mU/l FT4 : 0,9 pmol/l FT3 : 0,8 pmol/l
Limites de blanc	0,18 pmol/l 0,12 pg/lml	0,4 pmol/L



Résultats comparaison de méthode

Table 1: Comparison of TSH, FT4 and FT3 values between the Roche Cobas e602[®] and Ortho Vitros 5600[®] immunoassays.

	Roche Cobas E602	Ortho Vitros 5600
TSH, mU/L	1.08 (0.27–4.20)	0.97 (0.46–4.68)
FT4, pmol/L	<0.5 (12.0–22.0)	16.8 (10.0–28.2)
FT3, pmol/L	<0.6 (3.1–6.8)	5.5 (4.3–8.1)



Epreuve de dilution

- Dilution du sérum de la patiente à des titres de 1/1, 1/2, 1/4, 1/8 avec le diluant universel : E170Cobas e601.
- **Recovery Reference Values (RRV)**

Table 2: Hormonal results obtained from the dilution tests (1/2, 1/4, and 1/8) and before and after treatment with heterophilic tubes (HBT).

	Total T3, nmol/L	Total T4, nmol/L	FT3, pmol/L	FT4, pmol/L
1	1.7 (1.3–3.1)	141.4 (66.0–181.0)	<0.6 (3.1–6.8)	<0.5 (12.0–22.0)
1/2	1.3	65.4	0.4	4.0
Recovery, %	76.47	46.23	66.67	800
RRV, %	48.39–76.92	50.28–76.52	63.24–125.80	69.09–100.83
1/4	1.2	35.4	1.6	8.1
Recovery, %	70.59	25.04	266.67	1620
RRV, %	38.71–69.23	27.24–44.39	54.41–103.23	56.82–85.95
1/8	1.0	20.6	2.3	8.8
Recovery, %	58.82	14.57	383.33	1760
RRV, %	32.26–69.23	16.74–28.48	45.59–90.32	50.91–75.00



PEG procedure

- Polyethylene glycol procedure (PEG6000 25% w/w)
- Recovery > 100% : indication de la possible présence d'un agent interférent.

PATIENT CONTRÔLE	TSH (mU/l)	FT4 (pmol/l)	FT3 (pmol/l)
Pre-PEG	0,98	17,8	4,2
Post-PEG	0,65	16,5	3,8
Recovery (%)	65,9	92,7	90,5
PATIENT	TSH (mU/l)	FT4 (pmol/l)	FT3 (pmol/l)
Pré-PEG	0,71	5,2	0,8
Post-PEG	0,46	15,8	4,4
Recovery (%)	64,8	303,8	550,0



Non-specific Activities against Ruthenium Crosslinker as a New Cause of Assay Interference in an Electrochemiluminescent Immunoassay

Takao Ando¹, Jun-ichi Yasui¹, Naoko Inokuchi², Toshiro Usa¹, Kiyohito Ashizawa¹, Shimeru Kamihara² and Katsumi Eguchi¹

"... concentrations normales ou élevés de TSH en présence de concentrations sériques en hormones thyroïdiennes (FT3 et FT4) élevées ..."

Martina Zaninotto*, Costanza Tognon, Roberta Venturini, Corrado Betterle and Mario Plebani

Interference in thyroid hormones with Roche immunoassays: an unfinished story

" ... valeurs augmentées de FT3 et FT4 alors que la TSH restait normale .."



HBT : Heterophilic Blocking Tubes

Table 2: Hormonal results obtained from the dilution tests ($1/2$, $1/4$, and $1/8$) and before and after treatment with heterophilic tubes (HBT).

	Total T3, nmol/L	Total T4, nmol/L	FT3, pmol/L	FT4, pmol/L
Before HBT	1.6	143.5	<0.6	<0.5
After HBT	2.2	143.8	1.8	7.0

- → FT3 et FT4 augmentent sans atteindre les intervalles de référence.
- ⇔ Totales T3 et T4 : peu/pas de changements.



Envoi d'un aliquot à



Diagnostics



Investigation Report

Affected Product	Assay FT4II, FT3III	Analyzer cobas e 602 module
Short Issue Description	Interference Analysis	

As a first step, the values of the FT4 and FT3 parameters - that were generated at customer site - were confirmed. Values below the Limit of Blank of both assays were measured.

Upon further analysis of the sample with a research toolbox, an interfering factor against the Ruthenium label of the FT4 and FT3 assays was detected.

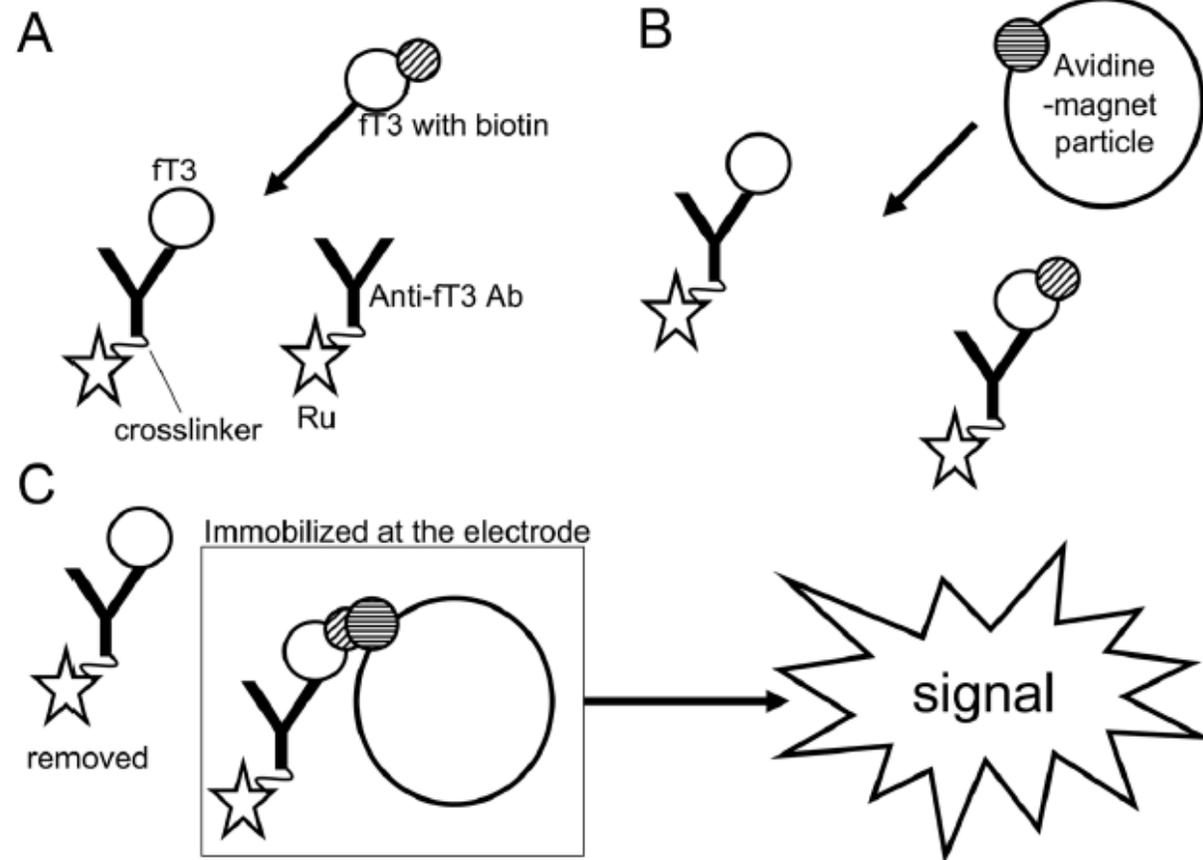
The objective was to check for the presence of a possible interfering factor.

As a first step, the values of the FT4 and FT3 parameters - that were generated at customer site - were confirmed. Values below the Limit of Blank of both assays were measured.

Upon further analysis of the sample with a research toolbox, an interfering factor against the Ruthenium label of the FT4 and FT3 assays was detected.



What Is Ruthenium?



CONCLUSIONS

- La nature exacte du facteur interférant avec le ruthénium n'a jamais pu être déterminée.
- → *vraisemblablement des anticorps impliquant FT3 et FT4 (cfr PEG procedure)*
- Comparaison de méthode : pas d'utilisation de streptavidin-biotin
- → *Excès de biotine → saturation des anticorps pour le FT4 et FT3 → interférence négative.*

- **Importance de garder en tête la possibilité de ces interférences**
- **Importance du dialogue entre cliniciens et le laboratoire**



a
falsely negative interference for FT3 and FT4 involving
ruthenium label was, to the best of our knowledge, never
reported.



Interference in thyroid-stimulating hormone determination

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DE GRUYTER

Clin Chem Lab Med 2016; x(x): xxx-xxx

Letter to the Editor

Julien Favresse^a, Henry Paridaens^a, Nathalie Pirson, Dominique Maiter and Damien Gruson*

Massive interference in free T4 and free T3 assays misleading clinical judgment

LE PROCESSUS DE PUBLICATION ...

**MANUSCRIPT
SUBMITTED**



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**MANUSCRIPT
ACCEPTED**

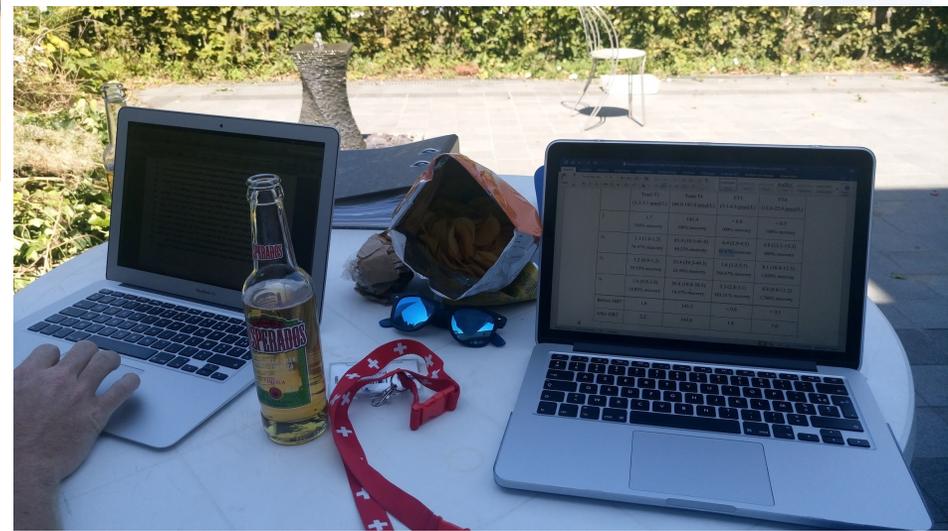


Dernière soumission : 22 août 2016



- Article M...ce 21.06
- Article M...21 06_DM
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- Author's...tion Henry
- Case Report .docx
- Case Rep...roid.docx
- Envoi Mont-Godinne
- Interferen...eport.doc
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- Interféren...t T4I .xlsx
- Massart ABC.pdf
- Massive i...R3 13.07
- Massive i...R4 17.08
- Montage vidéo
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- Massive i...s R4.docx
- Retour au...INAL.doc





Clinical Chemistry and Laboratory Medicine

À : FAVRESSE Julien, Jours fériés au Canada

Répondre à : heike.jahnke@degruyter.com

CCLM.2016.0255.R4 - Decision Accept

Clinical Chemistry and Laboratory Medicine

À : FAVRESSE Julien, Jours fériés au Canada

Répondre à : heike.jahnke@degruyter.com

CCLM.2016.0255.R2 - Decision Revise with Modifications

GRUSON Damien

À : Henry Paridaens

RE: CCLM.2016.0255.R1 - Decision Revise with Modifications

Clinical Chemistry and Laboratory Medicine

À : FAVRESSE Julien, Jours fériés au Canada

Répondre à : heike.jahnke@degruyter.com

CCLM.2016.0255.R3 - Decision Accept with Minor Revision



La R4 fut la bonne 😊



Letter to the Editor

Julien Favresse^a, Henry Paridaens^a, Nathalie Pirson, Dominique Maiter and Damien Gruson*

Massive interference in free T4 and free T3 assays misleading clinical judgment

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Keywords: free triiodothyronine (FT3); free thyroxine (FT4); interference; ruthenium; thyroid-stimulating hormone (TSH).

To the Editor,

A 35-year-old woman underwent a biological evaluation showing abnormalities in her thyroid hormone tests before a second in vitro fertilization (IVF) procedure. Her medical history only revealed a *Mycoplasma pneumoniae* infection as well as an early childhood surgery for a critical pulmonary valve stenosis. She was the mother of an 18-month-old child born from a first IVF-induced pregnancy. Family history showed hyperparathyroidism and hypothyroidism in her mother. The patient was asymptomatic, non-smoker, and drank alcohol only occasionally. Her treatment consisted of vitamins (a blend of vitamin C, guarana, and taurine), folic acid, and iron supplementation. On her thyroid work-up, thyroid-stimulating hormone (TSH), free thyroxine (FT4), and free triiodothyronine (FT3) were first measured with our routine assays, and a two-site electrochemiluminescent immunoassay (ECLIA) with ruthenium label for TSH and a competitive ECLIA also with ruthenium

label for FT3 and FT4, both ran on the Cobas 8000® e602 module (Roche Diagnostics®, Basel, Switzerland). For TSH, the limit of detection (LOD) was 0.005 mU/L and within-run precision was 8.6% and 4.0% at concentrations of 0.03 and 3.96 mU/L, respectively; for FT4, LOD was 0.5 pmol/L and within-run precision was 5.0% and 2.7% at concentrations of 1.6 and 87.8 pmol/L, respectively; for FT3, LOD was 0.6 pmol/L and within-run precision was 3.0% and 1.1% at concentrations of 1.5 and 46.8 pmol/L, respectively). The TSH level was normal at 1.08 mU/L [reference range (RR), 0.27–4.20 mU/L], whereas FT4 (RR, 12.0–22.0 mU/L) and FT3 (RR, 3.1–6.8 mU/L) concentrations were under the LOD of our method (<0.5 and <0.6 pmol/L, respectively). The results are shown in Table 1. The circulating levels of other hormones [follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, estradiol, progesterone, insulin growth factor 1 (IGF-1)] were in the normal ranges.

As the patient had no symptoms of hypothyroidism and no element was found to explain the extremely low levels of FT4 and FT3, an interference involving our assays was suspected. To confirm and characterize this interference, different procedures were undertaken. All those procedures were realized on the same patient's sample except for the PEG precipitation test.

We first challenged the patient's results against a different method available in another laboratory. This latter