Thyroid Function Tests: From Clinical Utility To Laboratory Stewardship

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2025 ASCLS-ND Spring Symposium

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Disclosure

I have NO financial disclosures or conflicts of interest with the presented content

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Learning objectives

- Review the clinical utility and analytical methodology for thyroid function tests
- Identify different types of immunoassay interferences affecting thyroid function test results
- Discuss the appropriate test utilization and laboratory stewardship opportunities

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Outline

- Clinical utility and analytical methods
- Immunoassay interferences
  - ✓ Case 1: Spurious elevation of TSH in a patient with Hashimoto's Thyroiditis
  - ✓ Case 2: False indication of thyrotoxicosis in a patient with inherited syndrome
- Troubleshooting tips and best practices

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Clinical Utility & Analytical Methods

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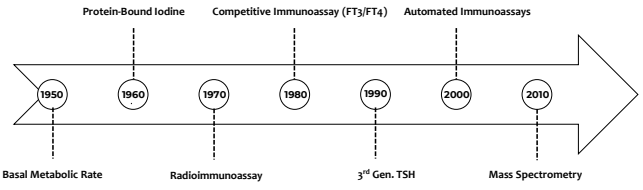
Historical Perspective



Spencer CA *et al. Thyroid.* 2023

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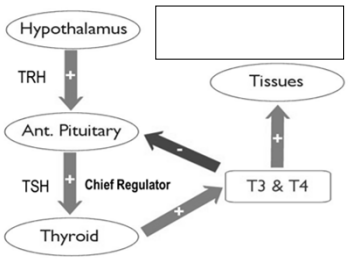
Hypothalamic-Pituitary-Thyroid axis regulates metabolism



Contemporary Practice in Clinical Chemistry, AACC Press

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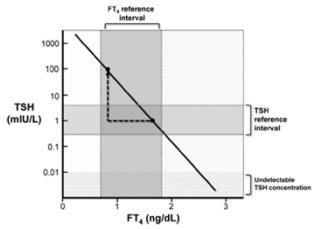
Inverse log-linear relationship between TSH and FT4



Contemporary Practice in Clinical Chemistry, AACC Press

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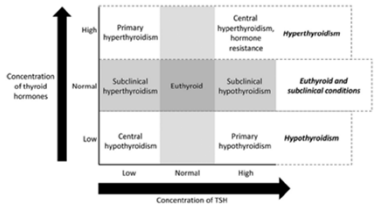
Interpretation of thyroid function tests



Contemporary Practice in Clinical Chemistry, AACC Press

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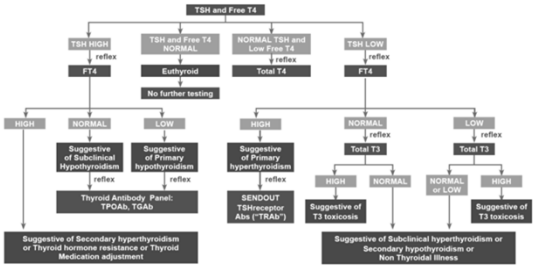
TSH is central to the diagnostic work-up of thyroid diseases



TCH Algorithm

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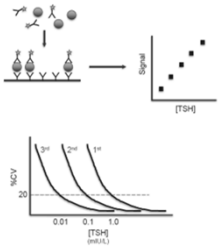
TSH is measured by sandwich immunoassay

- Two-site sandwich immunoassay: signal is directly proportional to concentration
- Analytical Sensitivity: Lowest concentration at which 20% CV can be achieved
  - ✓ 1st generation: 1 mIU/L
  - ✓ 2nd generation: 0.1 mIU/L
  - ✓ 3rd generation: 0.01 mIU/L



Clinical Chemistry Trainee Council

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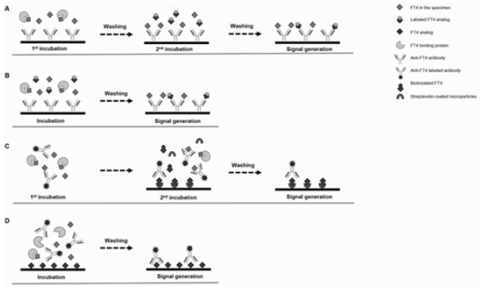
Free T4 is measured by competitive immunoassay



D'Aurizio F. et al., *Crit Rev Clin Lab Sci.* 2022

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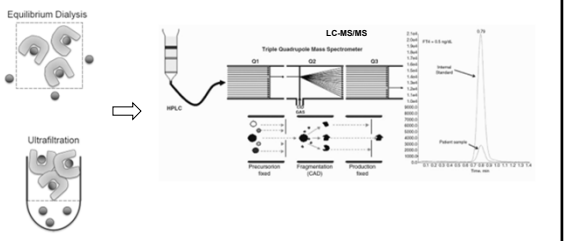
Reference laboratory method for FT4 measurement



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Immunoassay Interferences

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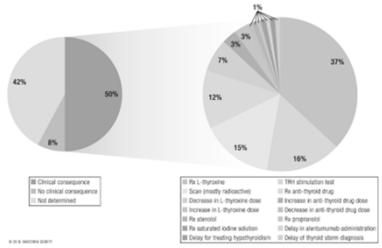
Clinical impact of immunoassay interferences in TFTs



Negative consequences in **50% cases**

Favresse J *et al. Endocr. Rev.* 2018

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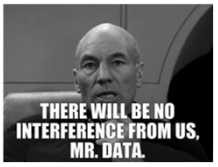
CLSI Definition

- **Interference:** Cause of medically significant difference in the measurand test result due to the effect of another component or property of sample
- **Mechanisms:**
  - Chemical artifacts
  - Detection artifacts
  - Physical artifacts
  - Enzyme inhibition
  - Non-selectivity
  - Cross-reactivity
  - Water displacement
  - Additive artifact



CLSI EP07-ED3. 2018

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Major interferences affecting thyroid function tests

Interference	TSH	fT4	fT3	other	Prevalence
Heterophile antibodies	↑ or ↓	↑ or ↓	↑ or ↓	Most assays may be affected	0.05–6%
Macro TSH	↑	–	–	–	0.6–1.6% in patients with subclinical hypothyroidism
Biotin	N or ↓	↑	↑	Anti-TSHR ↑, anti-TPO ↑, anti-TG ↑ and other hormonal assays	Not estimated in general population, related to the dosage of biotin intake
Anti-streptavidin antibodies	N or ↓	↑	↑	Anti-TSHR ↑, anti-TPO ↑, anti-TG ↑	Few cases described
Anti-ruthenium antibodies	↑ or ↓	↑ or ↓	↑ or ↓		Few cases described
Thyroid hormones autoantibodies	–	↑	↑	–	1.8%

Other interferants: Thyroid transport protein variants, TSH variants and drugs

Packzckowska K *et al. Endokrynol Pol.* 2020

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Case 1: Spurious elevation of TSH in a patient with Hashimoto's thyroiditis

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Clinical presentation

- A 16-year-old female with a past medical history of Down syndrome, congenital heart disease and **Hashimoto's thyroiditis** presented to the ED with acute respiratory failure and septic shock secondary to *E.coli* bacteremia
- Progressively ill in ICU, requiring ECMO and CRRT
- Received multiple pRBC transfusions, antibiotics and electrolyte replacement
- **Clinically euthyroid** with good compliance for **levothyroxine (150 µg/day)**
- No signs of goiter, thyromegaly or thyroid tenderness
- Thyroid function was closely monitored

Chokkalla AK *et al. Arch Clin Med Case Rep.* 2023

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Clinical presentation cnt'd

Analyte	At 6-years of age	Before Hospitalization	ICU Admission	Reference Range
TSH (µIU/mL)	559	0.86	**175.8**	0.5-3.4
Free T4 (ng/dL)	-	1.2	1.9	0.8-2.0
Total T4 (µg/dL)	2.3	-	11	6.4-13.3
T3 (ng/dL)	-	-	65	115-195
TPO antibody (IU/mL)	655.4	-	-	<20
Tg antibody (IU/mL)	<20	-	-	<20

- **Abnormally high TSH** and a normal free T4 on day 12 post-ICU admission
- Endocrine team consulted the laboratory for potential interferences

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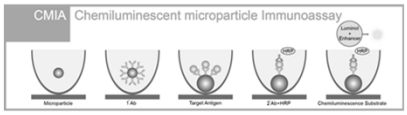
TSH immunoassay methodology

- TSH was measured on Abbott Architect i2000 SR analyzer
- 3rd generation chemiluminescent microparticle immunoassay
- Two-step sandwich format
- **Capture**: anti-β TSH antibody, **Detection**: anti-α TSH acridinium conjugate
- Analytical measurement range: 0.01 – 100 µIU/mL



Abbott Architect Package Insert

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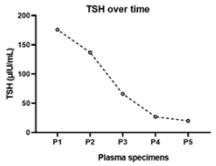
Serial TSH measurements showed a downtrend in <24 hours

Specimen	TSH (µIU/mL)
P1 (10:28 AM)	176
P2 (12:32 PM)	137
P3 (09:26 PM)	66
P4 (1 day later)	27
P5 (2 days later)	20



Potential interferants: heterophile antibodies or macro-TSH?

Chokkalla AK *et al. Arch Clin Med Case Rep.* 2023

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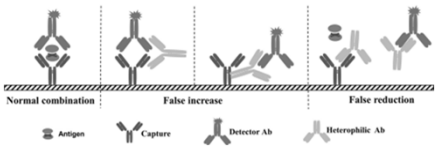
Interference due to heterophile antibodies

- Non-specific antibodies that interact weakly with immunoassay antibodies
- Prevalent in individuals with autoimmune diseases, undergoing monoclonal therapy or pet exposure
- Dilution test is the gold standard for investigating heterophile antibody interference



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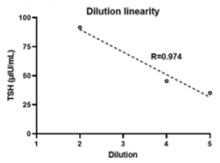
Dilution test ruled out heterophile antibody interference

Dilution	TSH (µIU/mL)
Neat	>100
1/2	183.3
1/4	181.6
1/5	175.8



Dilution test showed a linear decrease in TSH concentration

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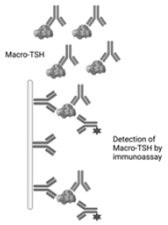
Interference due to macro-TSH

- Large (>150 kDa) inactive form of TSH complexed to immunoglobulins, mainly IgG and IgA
- Estimated prevalence of 0.6% to 1.6%
- Poor renal clearance
- Abbott Architect immunoassay is comparatively less reactive to macro-TSH than Roche Elecsys or Siemens Centaur platforms
- Macro-TSH is transferred transplacentally to neonates.



Favresse J *et al. Endocr. Rev.* 2018

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PEG precipitation and gel filtration chromatography are gold-standard for investigating macro-TSH interference

Author (Ref.)	Analyte Affected (mIU/L)	No.	Clinical Consequence	Methodology Used To Detect Interference[a]
Spitz *et al.*, 1981 (23)	TSH ↑ (40–115)	1	TRH, levodopa, dexamethasone	**AC**, dilution test, **GFC**
Tamaki *et al.*, 1995 (24)	TSH ↑ (16.4–53.9)	3	ND	**Method comparison, binding of ^{125}I-labeled TSH, incubation with high TSH sample, GFC[b], AC**
Halsall *et al.*, 2006 (18)	TSH ↑ (213–308)	2	ND	**Method comparison, dilution test, adsorption of serum IgG, GFC, incubation with high TSH sample**
Mendoza *et al.*, 2009 (17)	TSH ↑ (38.1)	1	No	Method comparison, **dilution test**, blocking antibodies, **SEC**
Verhoye *et al.*, 2009 (25)	TSH ↑ (5.1–22)	3	Rx L-thyroxine (2 patients/3)	**PEG**, HBT, RF, **method comparison** (1 patient/3), **dilution test** (2 patients/3), **protein A absorption**, **GFC**
Sakai *et al.*, 2009 (26)	TSH ↑ (96–274)	1	ND	**PEG, method comparison, GFC, adsorption of serum IgG**
Rix *et al.*, 2011 (20)	TSH ↑ (~50–103)	2	No	**PEG, method comparison, GFC**
Loh *et al.*, 2012 (16)	TSH ↑ (232)	1	No	**Method comparison, dilution test**, RF, **PEG**, **HBT, incubation with high TSH sample, GFC**
Mills *et al.*, 2013 (15)	TSH ↑ (10.2–33.6)	3	ND	**PEG, GFC, method comparison**
Hattori *et al.*, 2015 (14)	TSH ↑ (9.0–716)	11	Rx L-thyroxine (6 patients/11)	**PEG, GFC, adsorption of serum IgG**, dilution test

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Macro-TSH is the most likely source of interference

Precipitation with PEG recovered **~40% TSH**

Sample	TSH (µIU/mL)
Pre-PEG	47
Post-PEG	19

Serum IgA is markedly elevated

Antibody	Specimen#1	Specimen#2	Reference Range
IgA (mg/dL)	**327**	**339**	66-295
IgG (mg/dL)	1140	1150	641-1353
IgM (mg/dL)	97	96	40-180
IgE (mg/dL)	15	22	<100

Chokkalla AK *et al. Arch Clin Med Case Rep.* 2023

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Case 2: False indication of thyrotoxicosis in a patient with inherited syndrome

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Clinical presentation

- A 60-year-old male with no significant past medical history and a 10 kg weight gain over the past few months was referred to the endocrinology department due to elevated free T4 in the context of normal TSH
- No signs of thyrotoxicosis, goiter or orbitopathy and no structural alterations in pituitary gland by sella MRI

Analyte	Prior to referral	At the time of referral	Reference Range
TSH (µIU/mL)	1.44	0.75	0.3-4.2
Free T4 (pmol/L)	**29.57**	**32.3**	12-22
Total T4 (nmol/L)	-	148	66-181
Free T3 (pmol/L)	4.6	-	3.1-6.8
Total T3 (nmol/L)		1.55	1.3-3.1

Alegre E *et al. Clin. Chem.* 2022

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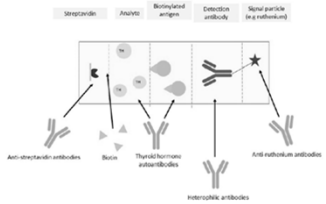
Free T4 immunoassay methodology

- Free T4 is quantified with Elecsys module (Roche Cobas 8000)
- One-step competitive immunoassay with electrochemiluminescence detection

Potential interferants



Alegre E *et al. Clin. Chem.* 2022

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Interference due to biotin

- Water-soluble vitamin, acts as a co-factor for carboxylase enzymes
- High-dose supplementation (>1 mg/day) in patients with multiple sclerosis, peripheral neuropathy, lipid disorders, carboxylase and biotinidase deficiencies
- High circulating biotin (10 ng/mL) seen in ~7% of emergency room patients interferes with biotinylated immunoassays
- Positive interference in competitive assays and negative interference in sandwich assays
- Current guidelines recommend collecting specimens at least 8 hours after abstaining from supplements
- Patient did not take biotin supplements

Dasgupta A. *Adv. Clin. Chem.* 2022

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Heterophile antibodies and anti-ruthenium antibodies are less likely source of interference

Analyte	After HBT treatment	By Centaur platform	Reference Range
TSH (µIU/mL)	0.77	0.647	0.55-4.78
Free T4 (pmol/L)	**35.08**	**36.7**	11.4-22.6
Total T4 (nmol/L)	**182**	**171**	58-140
Free T3 (pmol/L)	5.18	6.5	3.5-6.5
Total T3 (nmol/L)	1.54	1.7	0.9-2.7

Treatment with heterophile blocking tubes (HBT) and an alternate assay platform devoid of ruthenium yielded similar results

Alegre E *et al. Clin. Chem.* 2022

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Interferences involving thyroid hormone binding proteins

- >99% of circulatory T4 and T3 are bound to albumin, thyroxine-binding globulin and transthyretin
- Drugs such as aspirin, furosemide, carbamazepine, phenobarbital, phenytoin and heparin displaces T4 and T3 from binding proteins
- Genetic variants of binding proteins alters the affinity
- Gene testing for albumin in our patient showed R218H variant

Analyte	At the time of referral	Reference Range
Albumin (g/dL)	4.95	3.2-5.2
SHBG (nmol/L)	30.3	20.6-76.7
ACE (IU/L)	5	13.3-63.9

Alegre E *et al. Clin. Chem.* 2022

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Familial dysalbuminemic hyperthyroxinemia

- Inherited syndromes characterized by high serum concentrations of total T4
- Prevalence: 1 in 10,000 individuals
- Common genetic variants: R218H, R218P, R218S and R222I
- Increased binding affinity of albumin for T4
- Individuals with FDH are euthyroid due to normal free T4
- Most immunoassay platforms provide erroneously high free T4 due to increased sequestration of labelled T4 analog
- Buffer chloride content contributes to falsely elevated free T4 in FDH

Kragh-Hansen U *et al. Front. Endocrinol.* 2017

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Interference due to thyroid hormone autoantibodies

- Immunoglobulins (mostly IgG) directed against T3 and T4
- Highly prevalent in patients with autoimmune diseases (~40%)
- Deplete the labelled analog causing the false elevation of thyroid hormones
- Affects one-step but not two-step immunoassays
- Troubleshooting involves radioimmunoprecipitation with PEG

Favresse J *et al. Endocr. Rev.* 2018

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Summary and Best Practices

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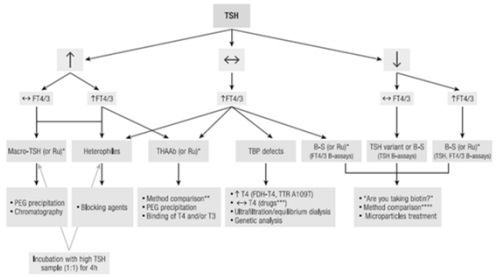
Troubleshooting algorithm for thyroid immunoassay interferences



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What is "appropriate" test utilization?

Using the **right test**…

…use **TSH** as the initial test for evaluating thyroid dysfunction

…for the **right patient**…

…avoid routine thyroid testing in **asymptomatic or hospitalized patients** without clinical signs

…at the **right time**

…monitor TSH in thyroid disease patients after at least **6 weeks** of initiating treatment

Choosing Wisely Campaign

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Lab stewardship opportunities

Methodology

- Define assay limitations in policy documents
- Case-based exercises for laboratory staff

Troubleshooting Aids

- Written procedures
- Reagents: PEG and HBT
- Reference lab tests

Clinician Collaboration

- Endocrinology rounds
- Trainee involvement
- Clinical Decision Support

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Summary

1. **TSH and FT4 are front-line screening tests for thyroid dysfunction**
2. **Troubleshooting of immunoassay interferences involve dilution test, PEG precipitation, HBT and correlation with reference methods**
3. **Laboratorians possess vast opportunities to actively engage in diagnostic stewardship**

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Thank You ☺

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