Laboratory Conundrums (Conundra?)

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Thyroglobulin

Case Scenarios

- A 17 year old woman with MEN 1 has had total thyroidectomy and remnant ablation and has an ongoing Tg of <1 ng/mL and TgAb<20 kIU/L with no uptake seen on ^{123}I scintigraphy.
- Lab changes Tg methods from Siemens Immulite to Roche Cobas and the Tg becomes detectable at 0.1 ng/mL while the TgAb 340 kIU/L and remains stable over 6 months.

Case Scenarios

- Imaging is normal.
- Do you:
  - Wait until Tg goes above 0.2 or 1 ng/mL?
  - Monitor the TgAb as a surrogate?
  - Send for multi-epitope RIA at USC?
  - Send for tandem mass spectrometry?

Case Scenarios

- What do you think might going on here?
  - If the same patient had TgAb = 450 kIU/L and Tg < 0.1 ng/mL?
  - Enlarged lymph nodes are seen on U/S and are seen to have uptake on ^{123}I scintigraphy.
  - After Thyrogen stimulation: Tg <0.1 ng/mL by both immunoassay and mass spectrometry.

Case Scenarios

- What do you think might going on here?
  - If the same patient had TgAb >4000 kIU/L and Tg = 0.8 ng/mL.
  - U/S and scintigraphy are negative
  - However, Tg by mass spectrometry is <0.1 ng/mL and there is absolutely no evidence of signal on chromatograms.

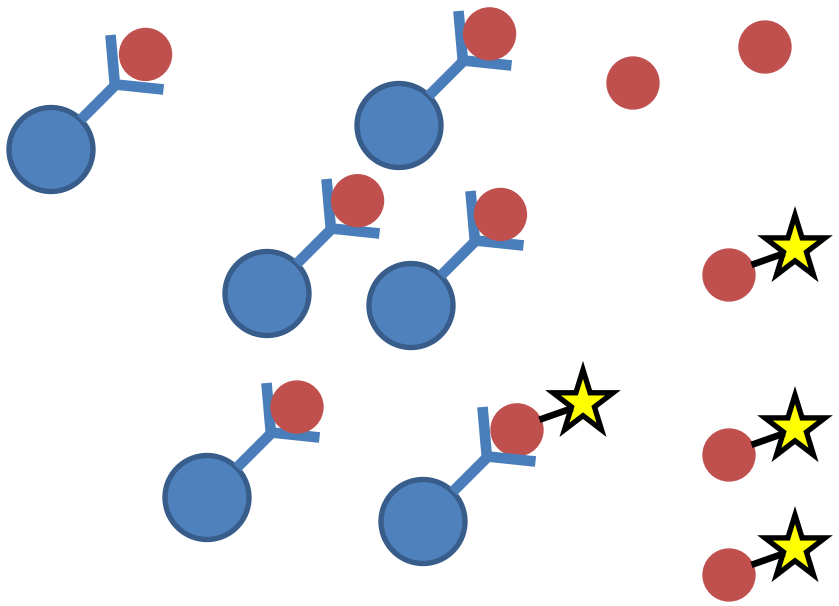
Background

- Thyroglobulin (Tg) is used as a tumor marker for papillary/follicular thyroid Ca – particularly after thyroidectomy and radioactive iodine ablation.
- The therapeutic target for Tg is <0.2 ug/L.

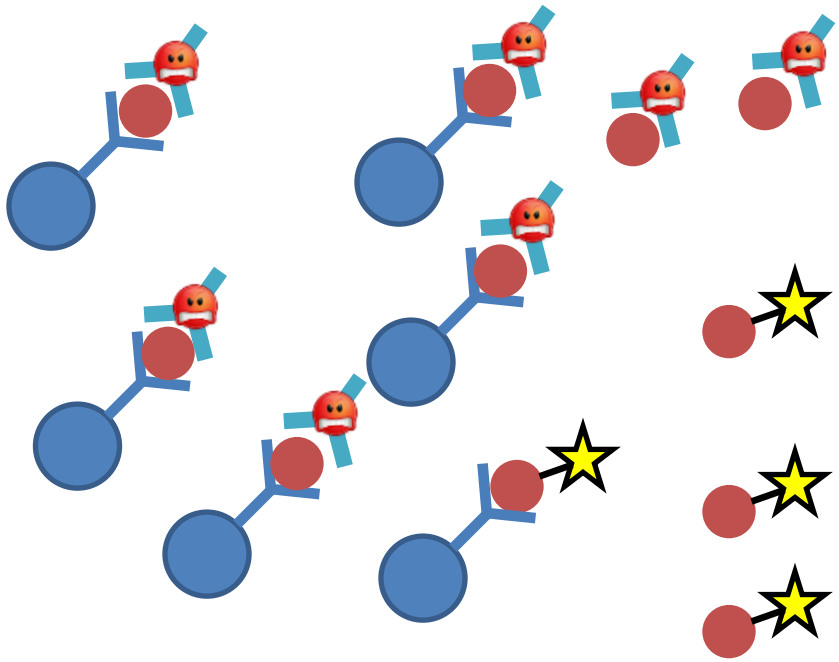
Background

- It is known that antithyroglobilin Abs (ATg) interfere (negatively) with the detection of thyroglobulin.
- The extent of the effect is method-specific with Immulite being the most affected.
- When a patient has positive ATg but has Tg that is below the limit of detection, the question is always raised about factitiously low results.

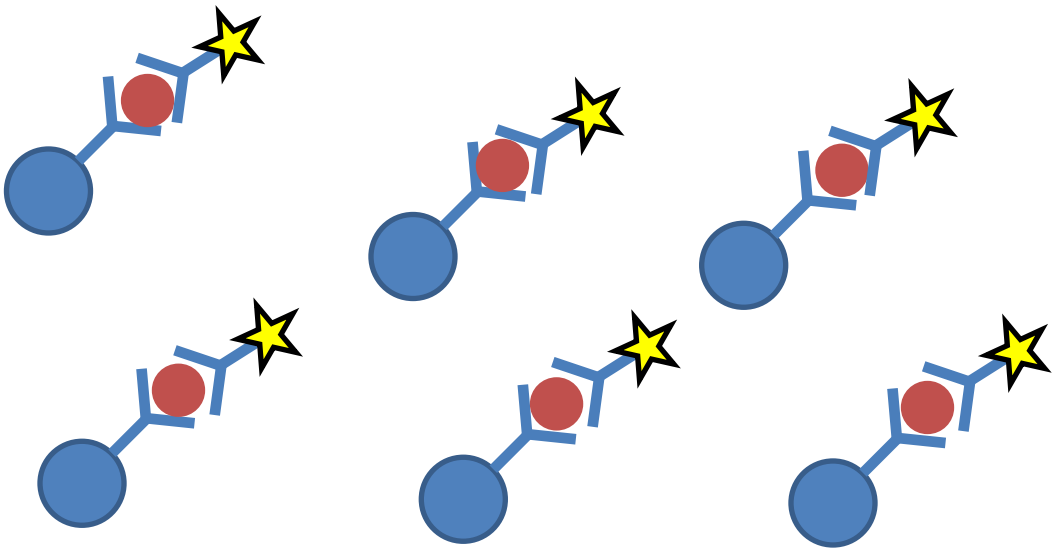
Competitive Immunoassay



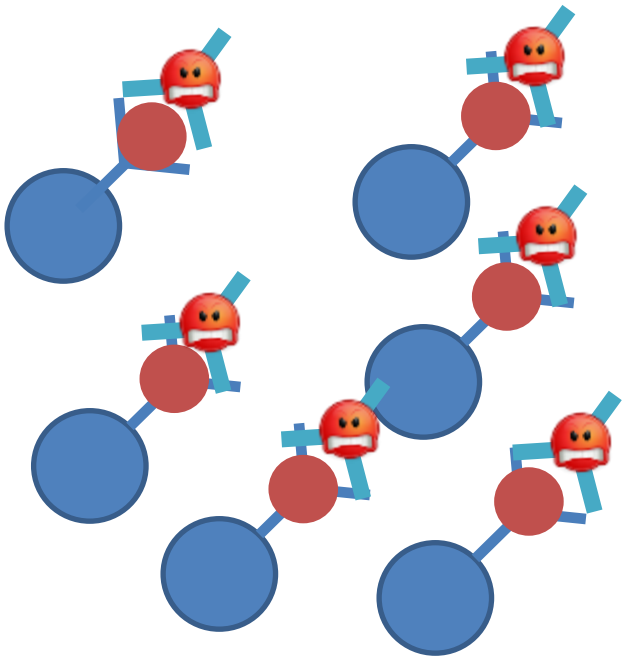
Antibodies? Possibly unproblematic.

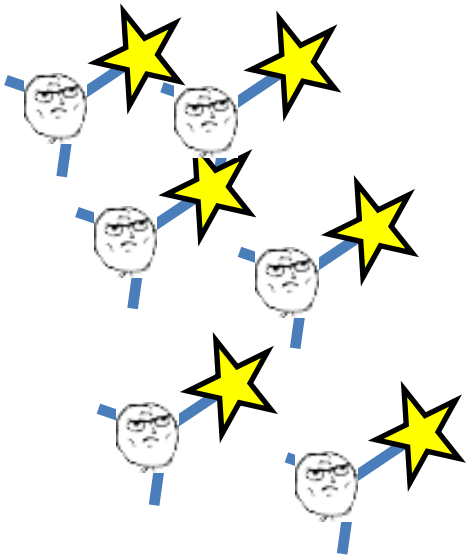


Sandwich Immunoassay

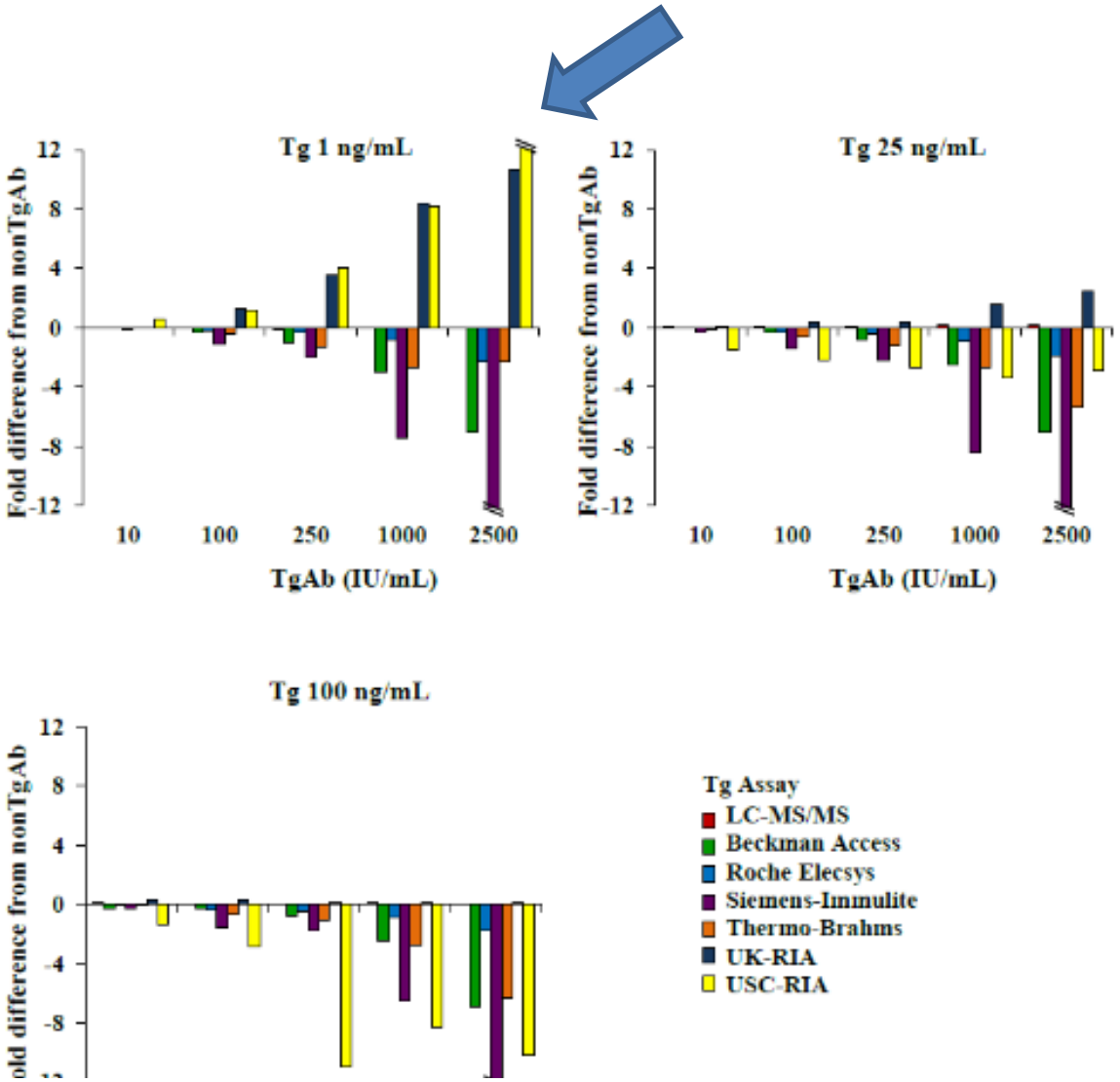


Antibodies – more likely problematic





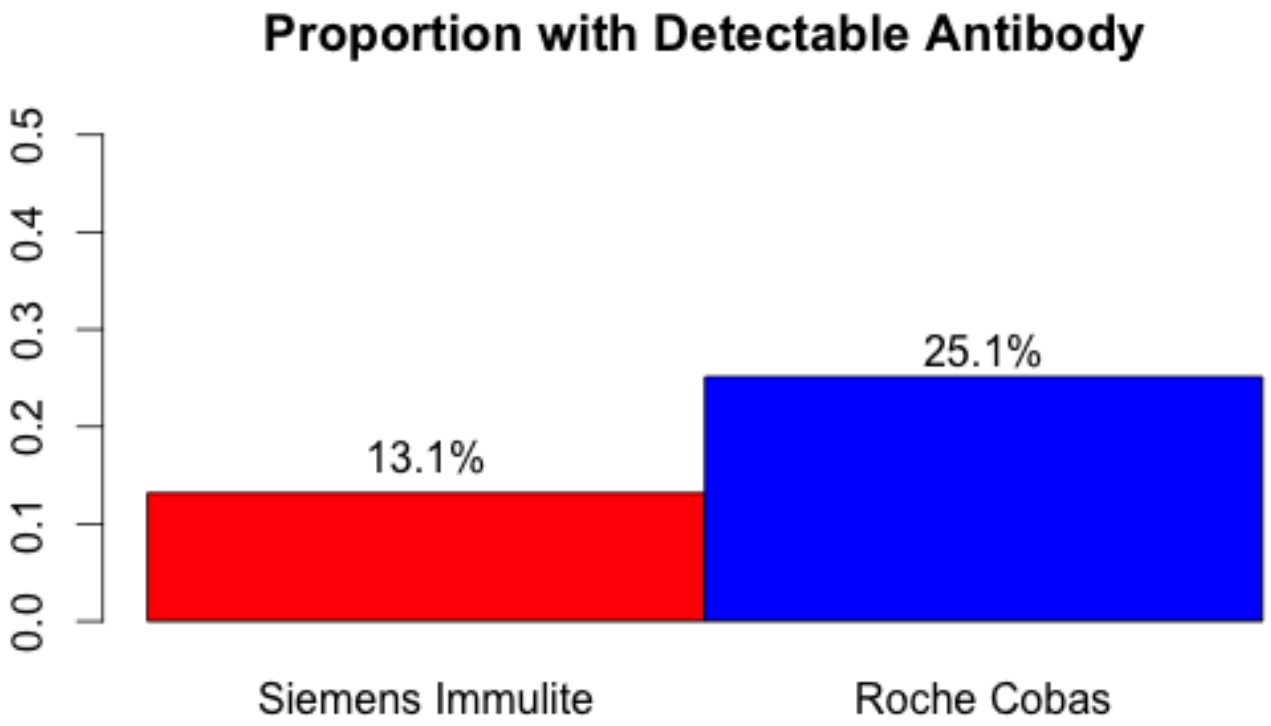
But Problems Persist



J Clin Endocrinol Metab, August 2015, 100(8):E1074–E1083

Antibodies Induce ***and*** Reduce Tg signal

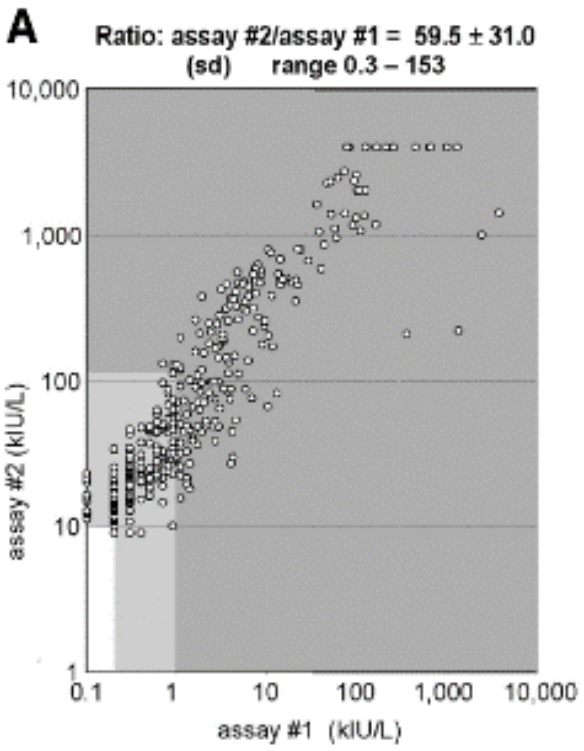
ATg Detection Rate



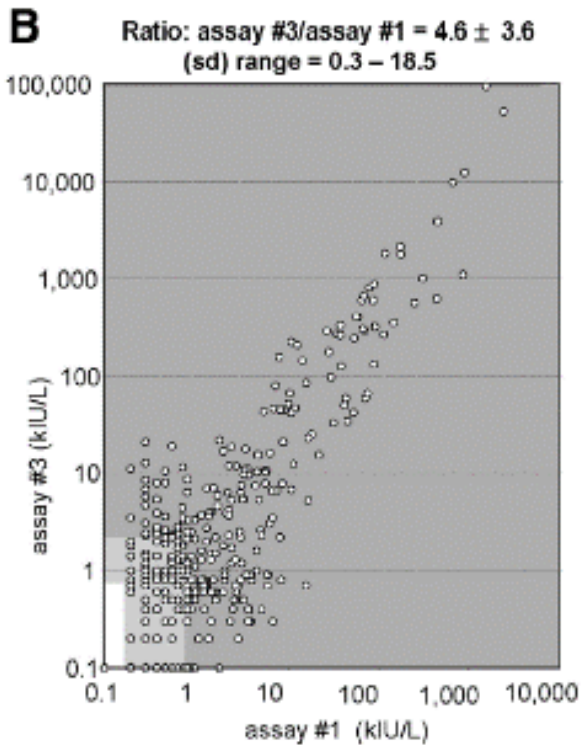
Data from our routine clinical Laboratory 2016 calendar year

ATg assay dependent paranoia

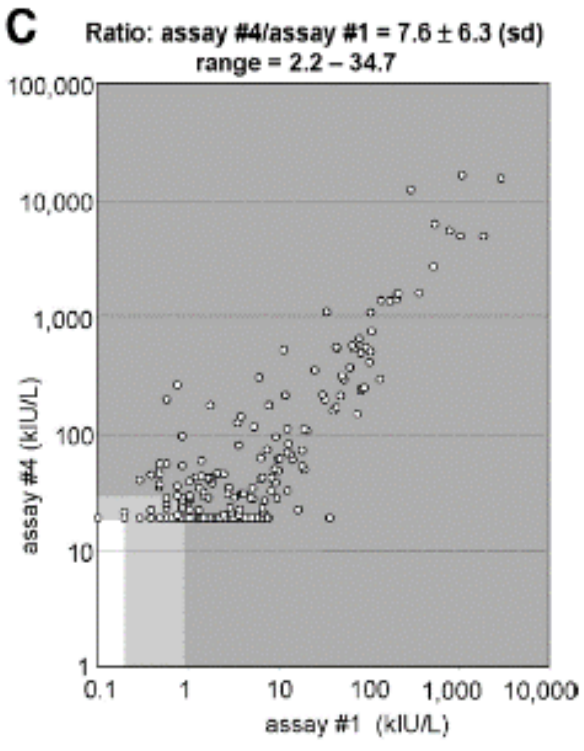
Roche



Beckman



Immulite



J Clin Endocrinol Metab, May 2011, 96(5):1283–1291


ANTITHYROGLOBULIN COMPARISON
HORRIBLE
imgflip.com

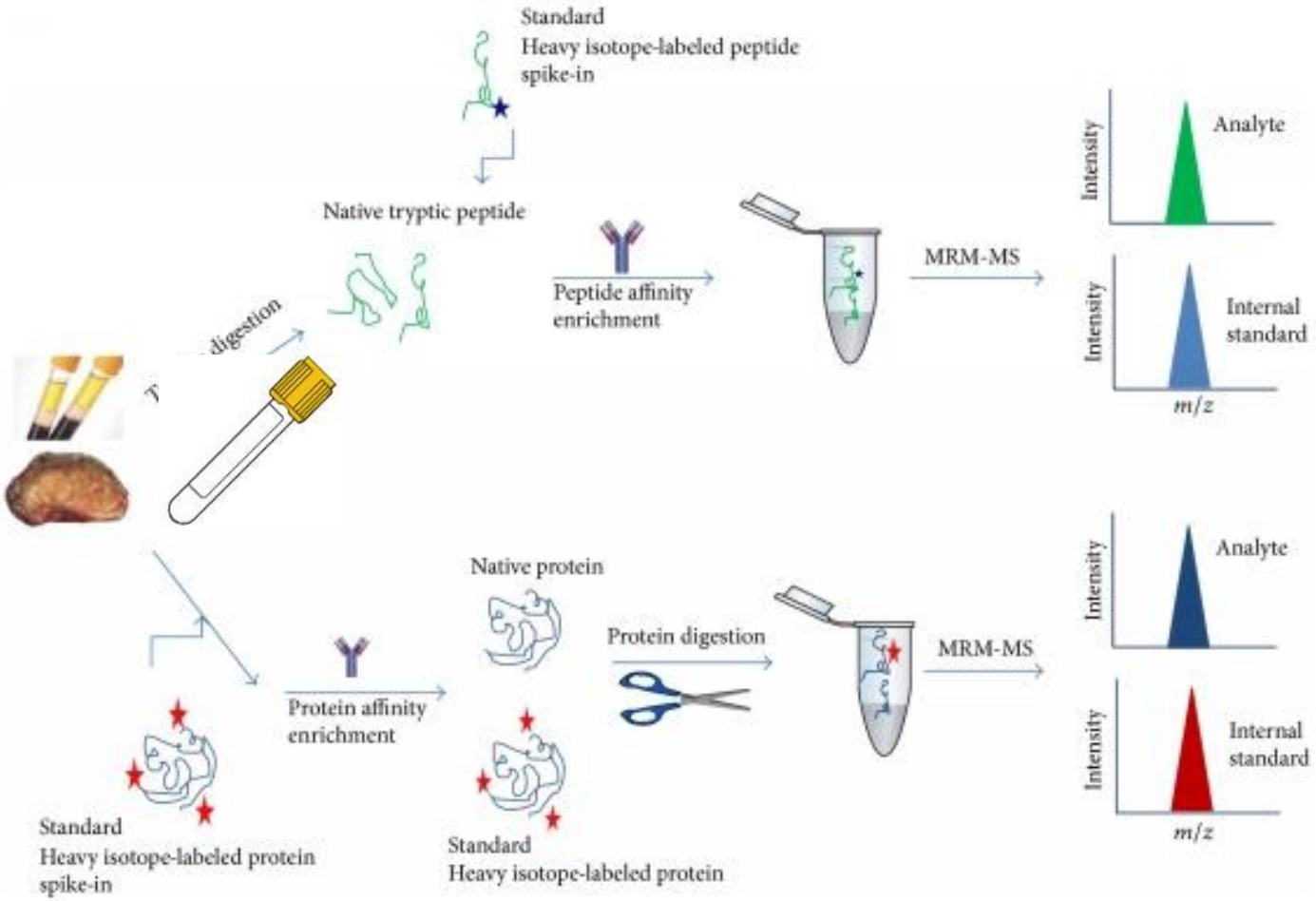
Strategies

- If ATgs are positive and Tg < 0.1 ug/mL (or thereabouts) there are three strategies:
  1. Use ATg as a surrogate
  2. Use a multi-epitope competitive RIA (Spencer)
  3. Use trypsin digest and SISCAPA LC-MS/MS

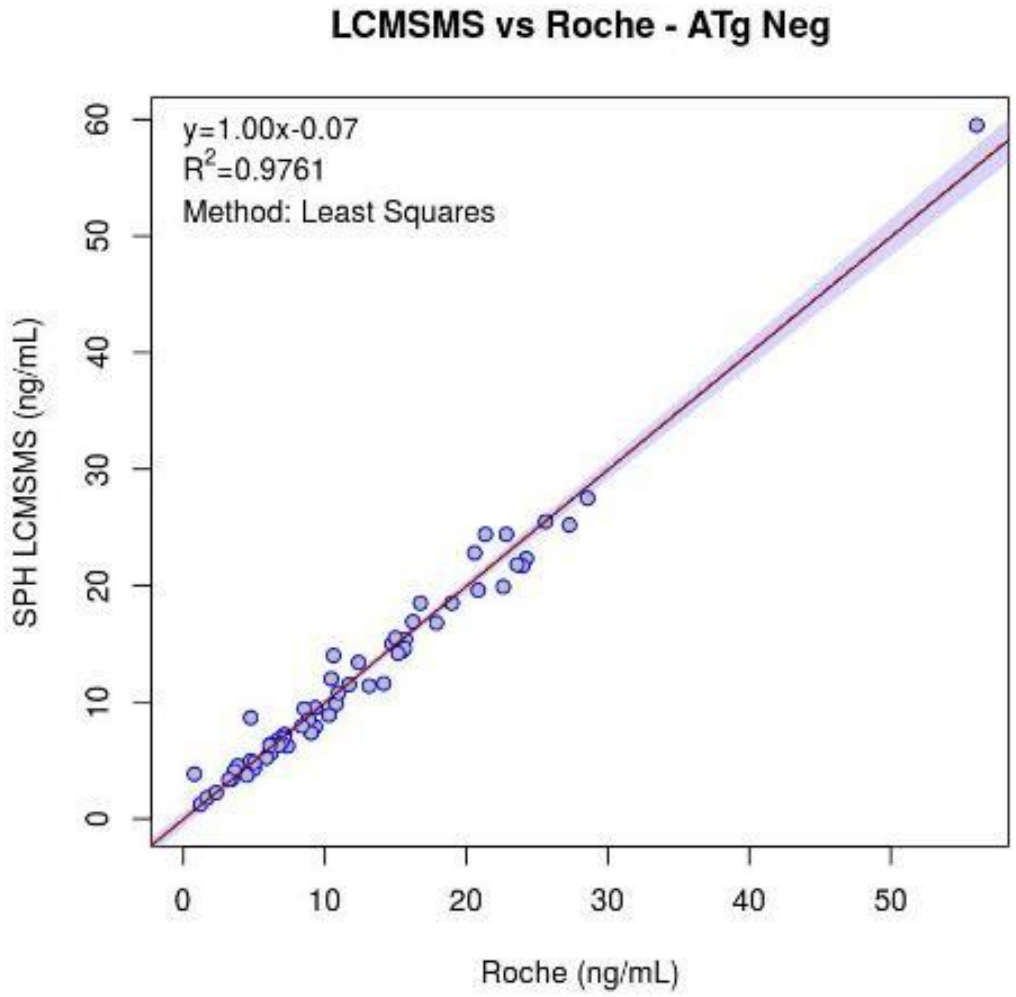
Mass Spectrometry

- Mass spectrometry has offered a means of measuring Tg accurate in the presence of TgAb.
- Works by tryptically digesting the antibody away and digesting the thyroglobulin also.
- Then we measure a peptide from Tg as a surrogate for Tg.

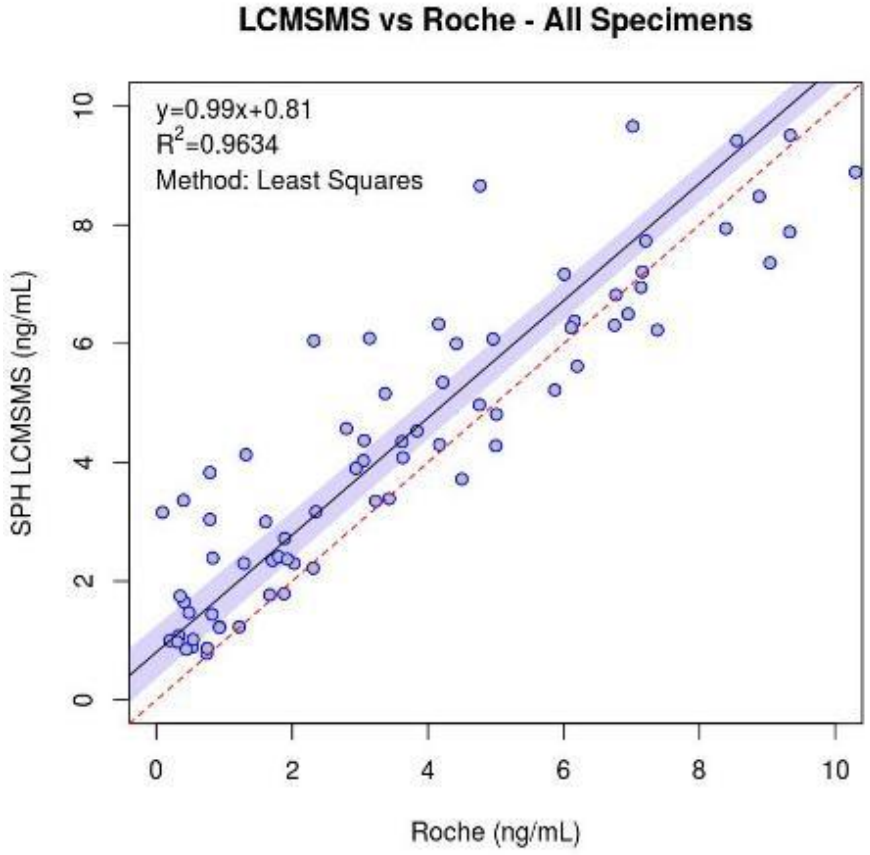
How Does it Work?



How does MS perform against IA?
ATg *Negative* Patients

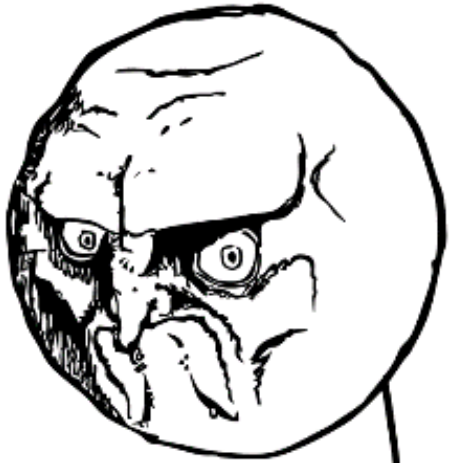


How does it perform against IA?
ATg *Positive* Patients



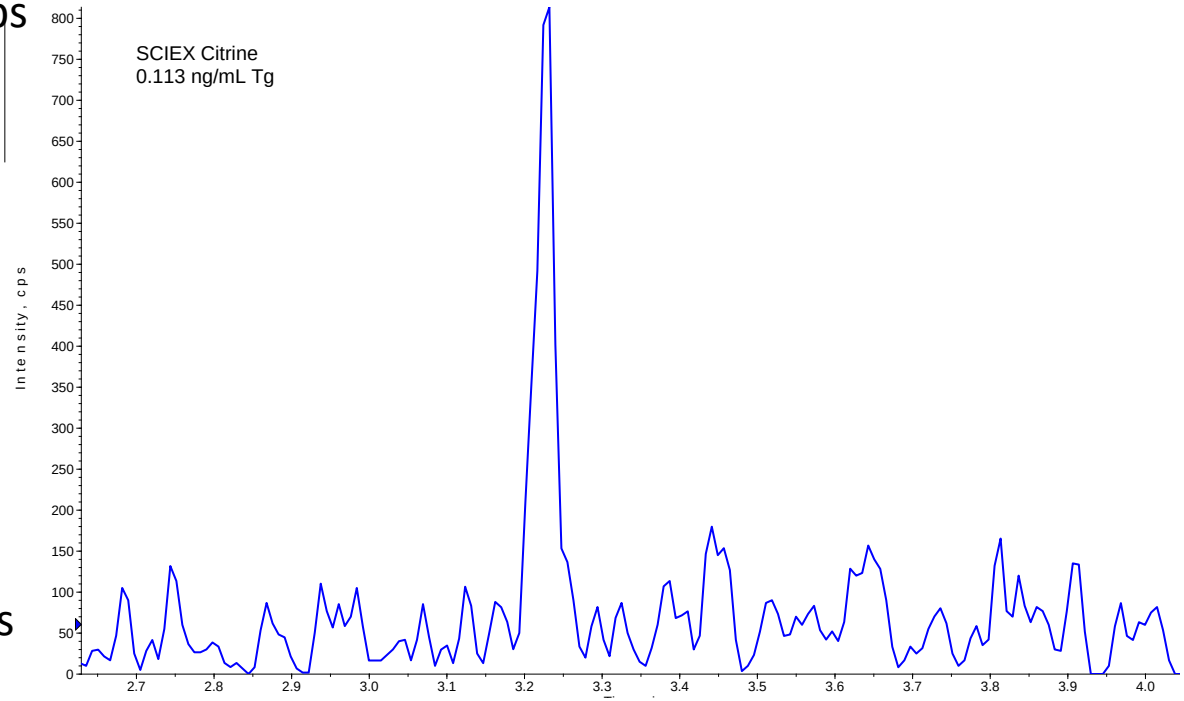
Rad Oncs
Endos
H&N Surgeons

Immunoassay
Manufacturer



Tg = 0.11 ng/mL, ATg = <20 kIU/L

Signal: 800 cps

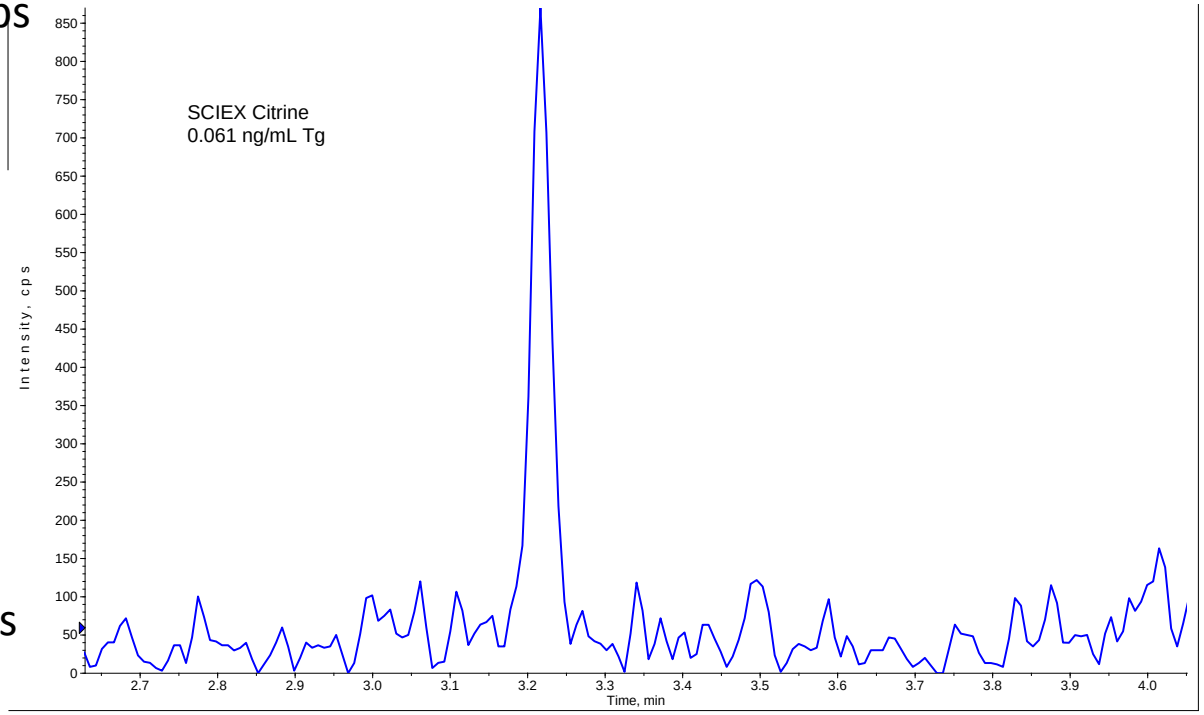


Noise: 50 cps

SCIEX Citrine Mass Spectrometry System
SISCAPA Tg Workflow

Tg = 0.06 ng/mL, ATg = 178 kIU/L

Signal: 850 cps

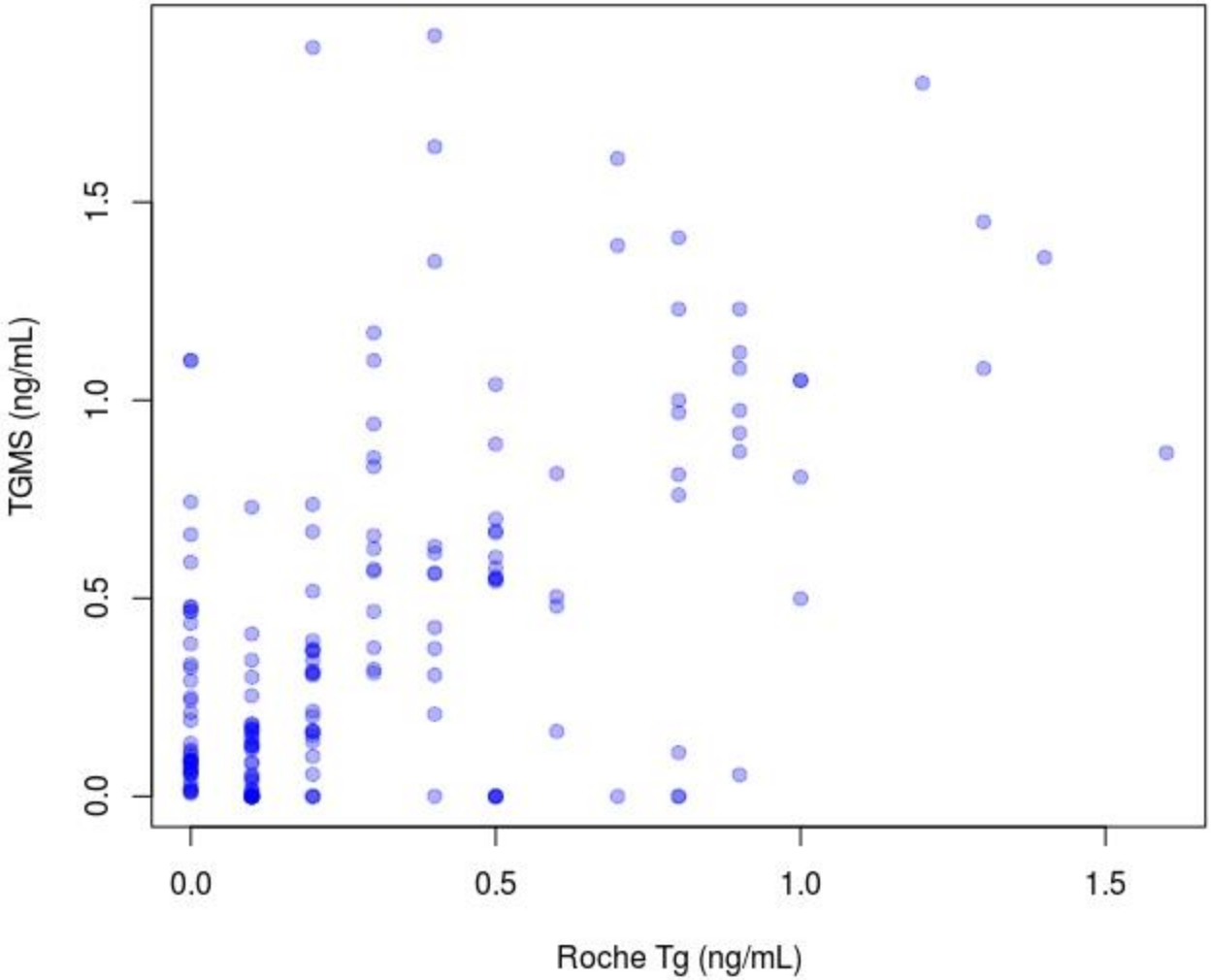


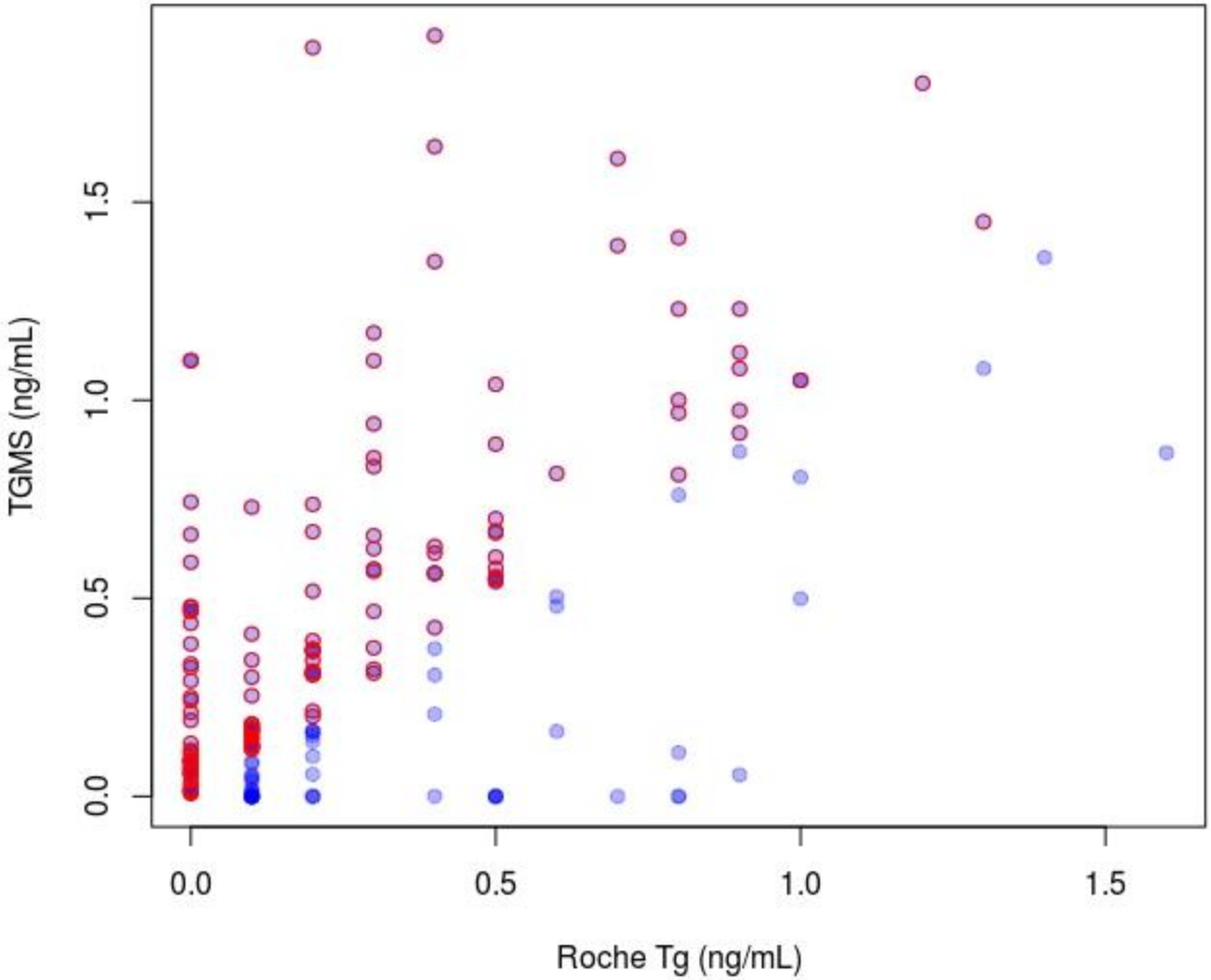
Noise: 50 cps

SCIEX Citrine Mass Spectrometry System
SISCAPA Tg Workflow

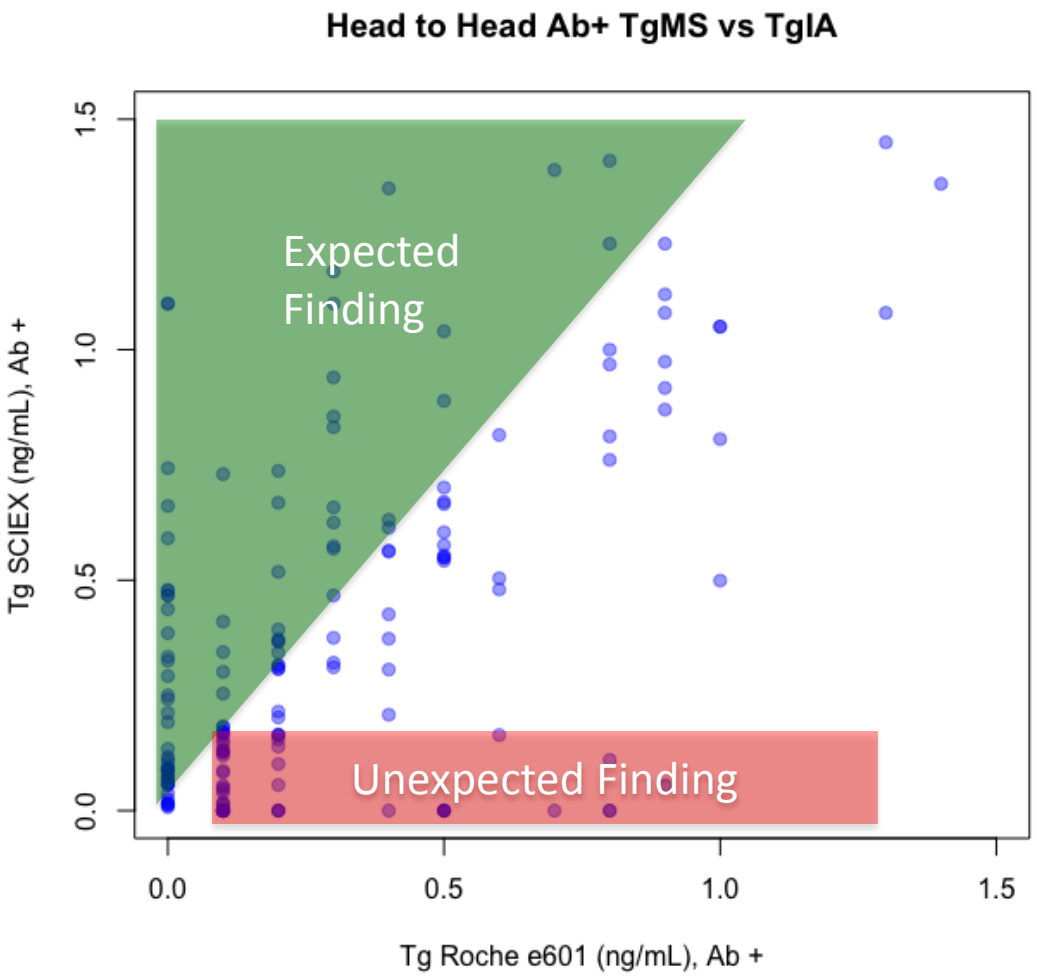
1 year head to head

- 534 analyses
- All ATg > 20 kIU/L
- Almost all Tg < or = 1 ug/L


TGMS (ng/mL)
0.0
0.5
1.0
1.5
0.0
0.5
1.0
1.5
Roche Tg (ng/mL)


TGMS (ng/mL)
0.0
0.5
1.0
1.5
0.0
0.5
1.0
1.5
Roche Tg (ng/mL)

Very Interesting Phenomenon



Descriptive Statistics

- 135 of the 534 (25%) showed TGMS > TGIA
- 63 of the 534 (11.8%) showed a discrepancy between TGMS and TGIA > 0.3 ug/L
- 23 (4.3%) specimens were undetectable by TGIA and detectable by TGMS.
  - Median 0.39 ug/L, range 0.1 – 2.52 ug/L
- 23 (4.3%) were undetectable by TGMS and detectable by TGIA

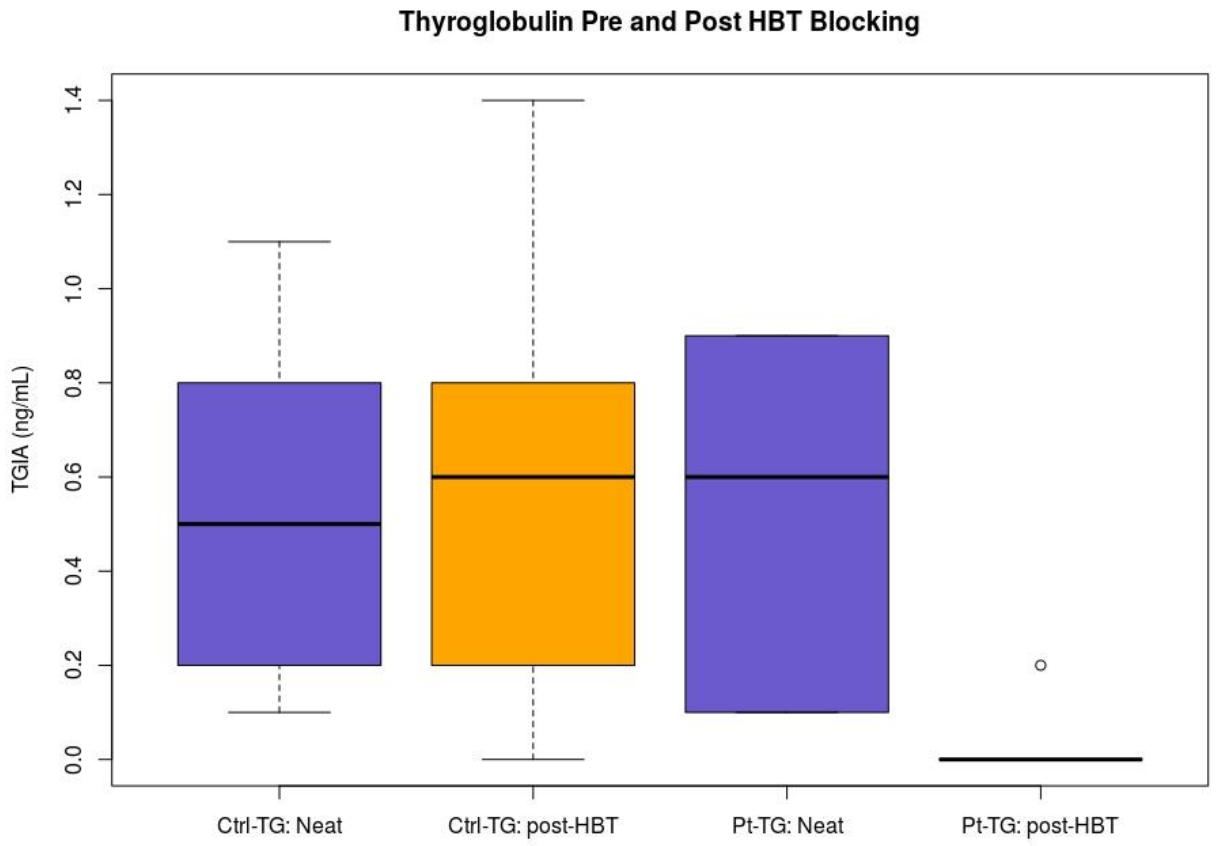
So what about those confusing specimens?

Sample	TGIA	ATG	TGMS	TGIA post HBT
1	0.1	25	<0.1	<0.1
2	0.8	27	<0.1	0.2
3	0.4	23	<0.1	<0.1
4	0.1	23	<0.1	<0.1
5	0.9	>4000	<0.1	<0.1
6	0.1	76	<0.1	<0.1
7	0.2	22	<0.1	<0.1
8	0.9	736	<0.1	<0.1
9	0.9	>4000	<0.1	<0.1
10	0.9	493	<0.1	<0.1

Tiebreaking

- 10 of these 23 specimens showing TGIA +ve and TGMS –ve were available for reanalysis.
- These were heterophile blocked and 9 of 10 became undetectable.
- The 10th went from 0.8 to 0.2 ug/L to better match the mass spec result.
- 31 control specimens with Tg < 1 were analyzed
  - 9 with Ab+, 21 with Ab-
- Control specimens were not affected by HBT blocking.
  - If anything the Tg goes up.

Controls Show Modest Increase postHBT

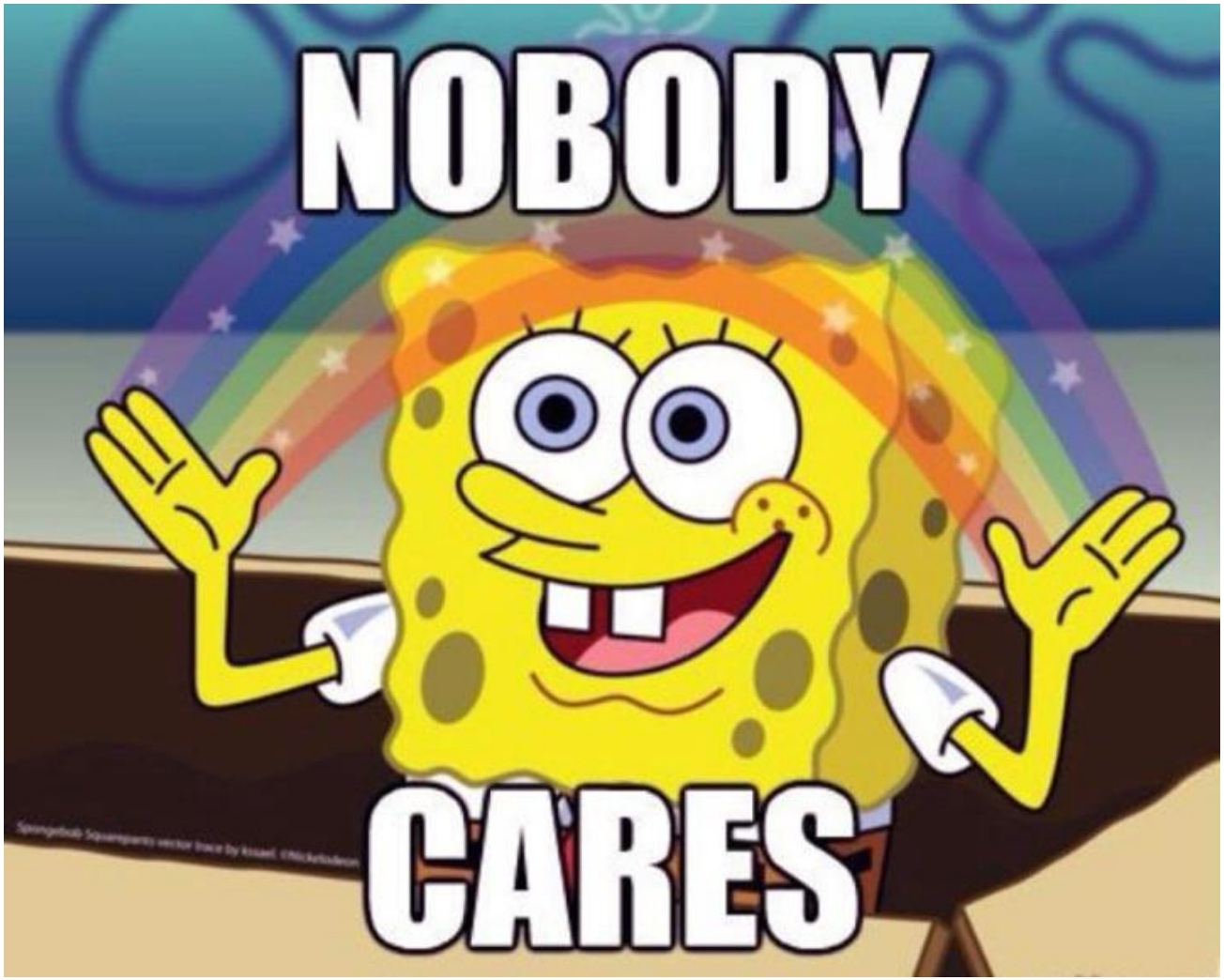


Conclusions

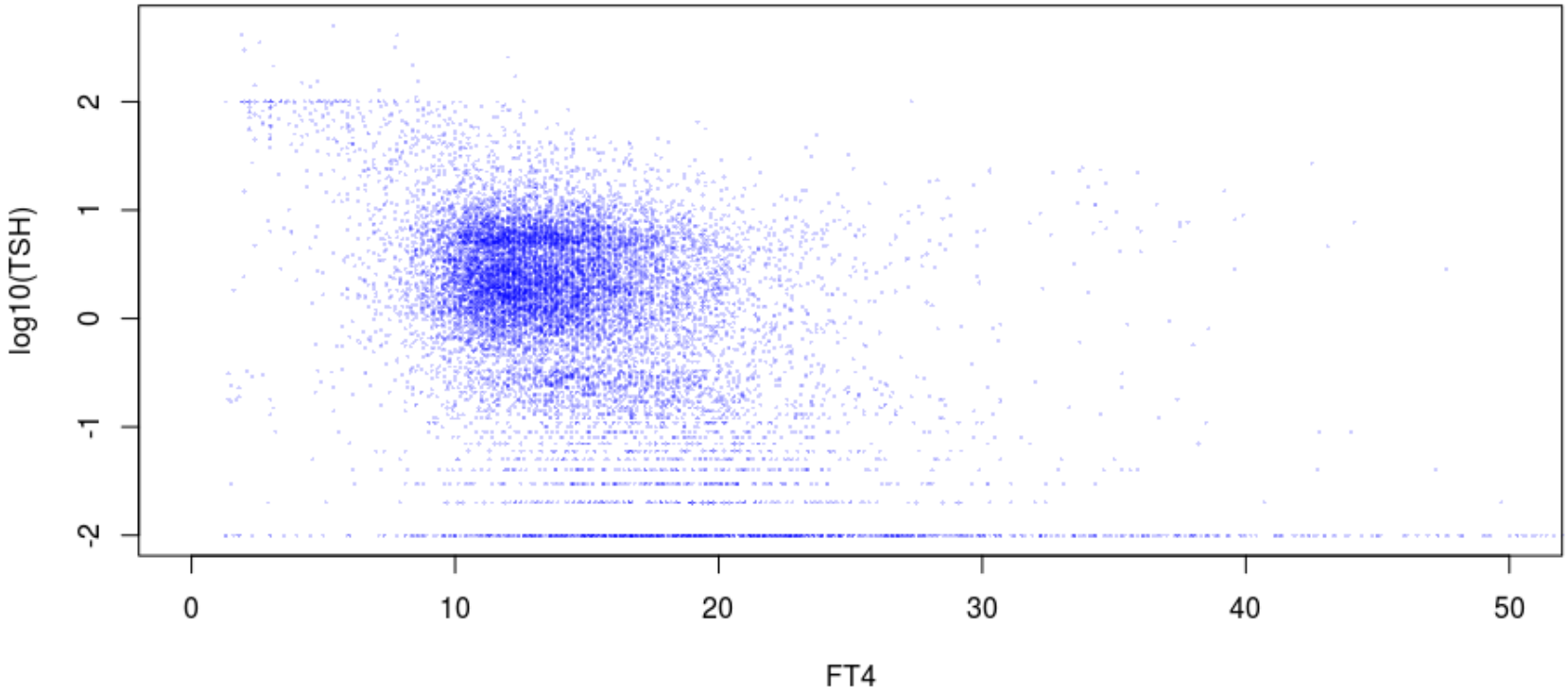
- 25% of all specimens are TgAb+
- We are reflexing those with Tg < 1 and TgAb > 20 to LC-MS/MS
  - About 25% of these will show TGMS > TGIA
  - About 5% of these have falsely undetectable results by IA.
  - About 5% show falsely detectable results by IA.

Thyroid Function Reference Intervals

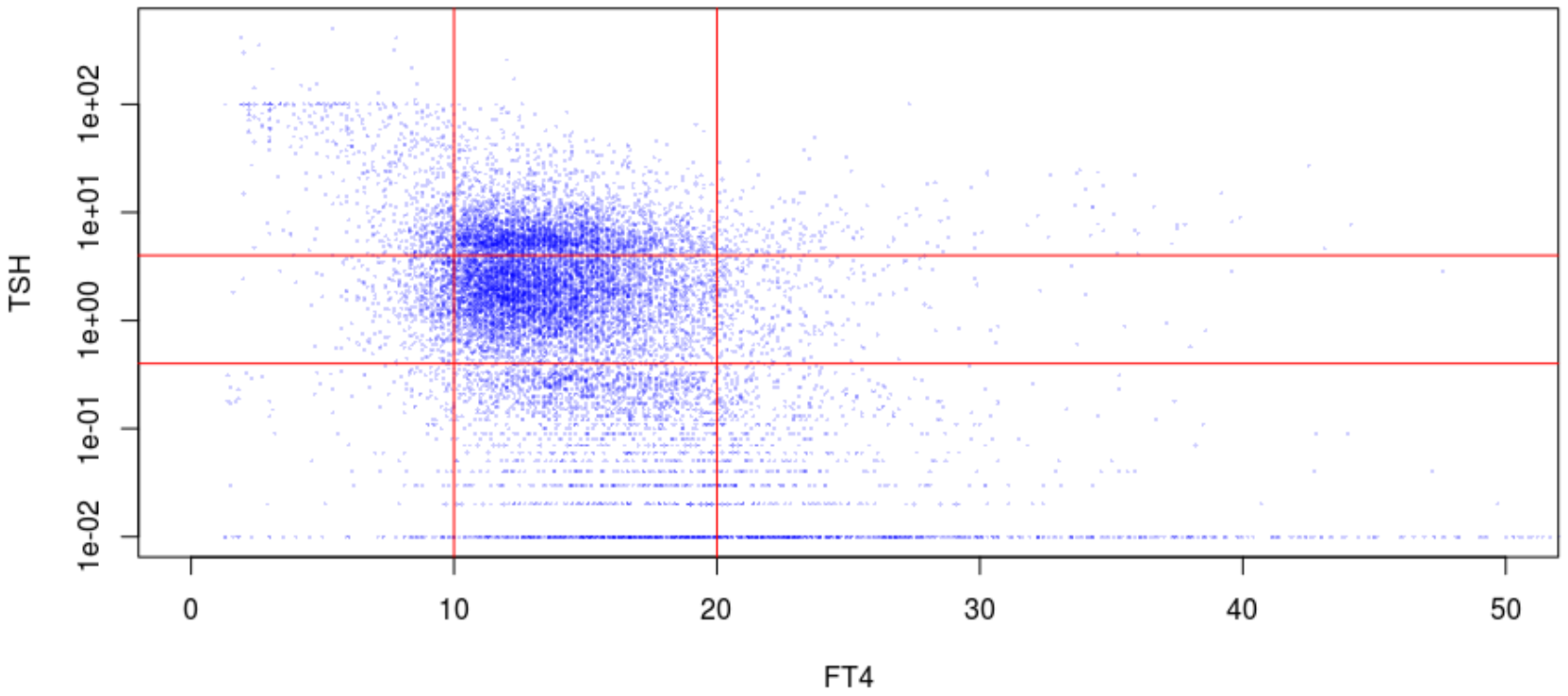
It’s a dry topic



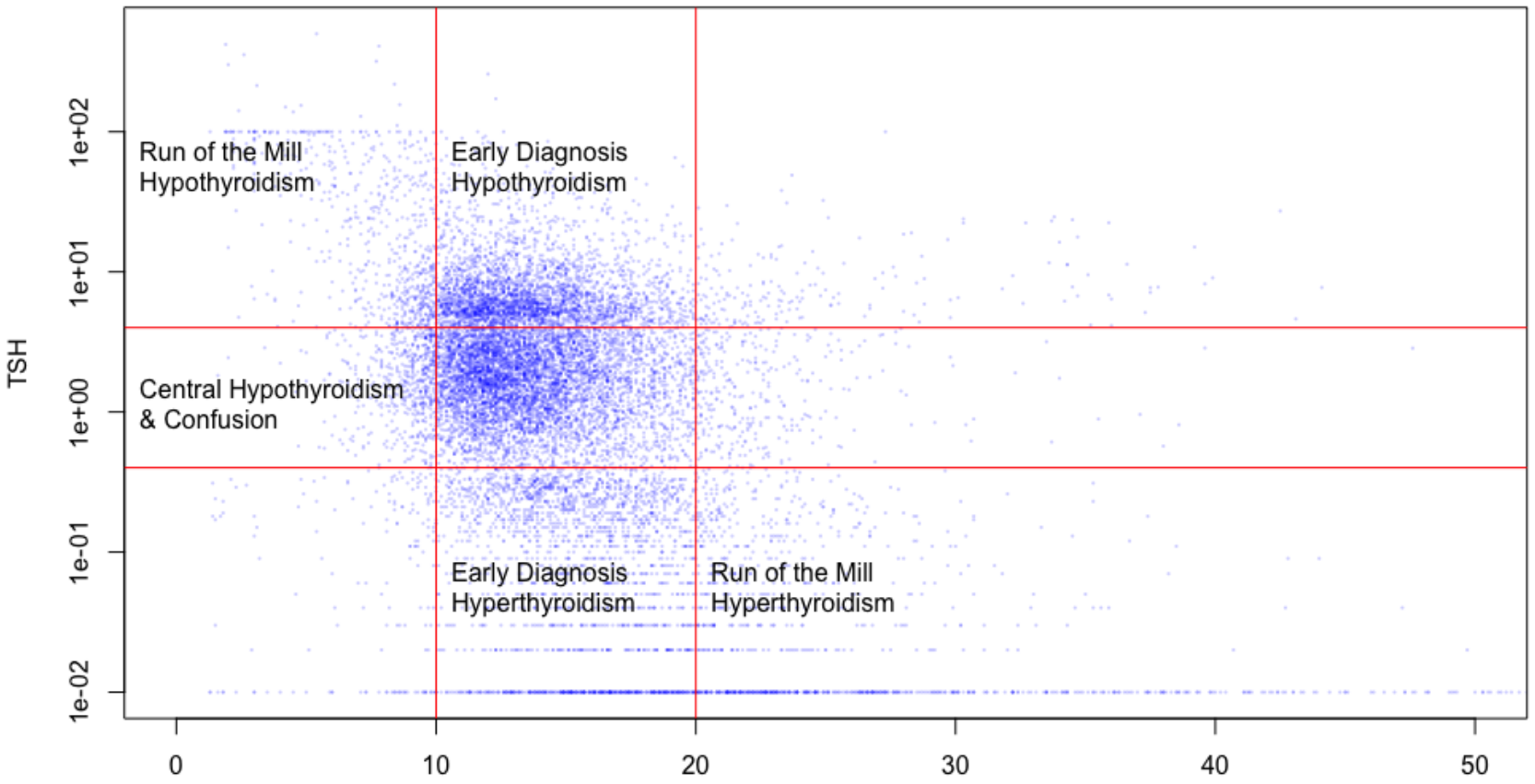
TSH Log-Linear



TSH Log-Linear



TSH Log-Linear



Common Adult Referral

- TSH and fT4 ordered simultaneously as a screening test .
  - Usually Abbott instrumentation but not exclusively.
  - TSH comes back fully normal.
  - fT4 comes back borderline low.
- Referral follows.
- Investigations on other platforms may show same or normal.
- Patient is found clinically euthryoid with intact pituitary function.
- Final conclusion is that this is an artifactual of the reference interval.

Common Pediatric Referral

- TSH is marginally low or marginally high.
- fT4 Probably normal.
- Patient is clinically euthyroid.
- Final conclusion is that this is an artifactual of the reference interval.

Reference Intervals: No Worky?

Female Reference Intervals

Age	Lower Limit	Upper Limit	Samples	Lower CI	Higher CI	95th Percentile
4 days - < 6 months	0.73	4.77	139	(0.367 - 0.98)	(4.27 - 5.54)	
6 months - <14 years	0.7	4.17	640	(0.61 - 0.82)	(4.04 - 4.43)	
14 - < 19 years	0.47	3.41	259	(0.25 - 0.57)	(3.15 - 3.45)	

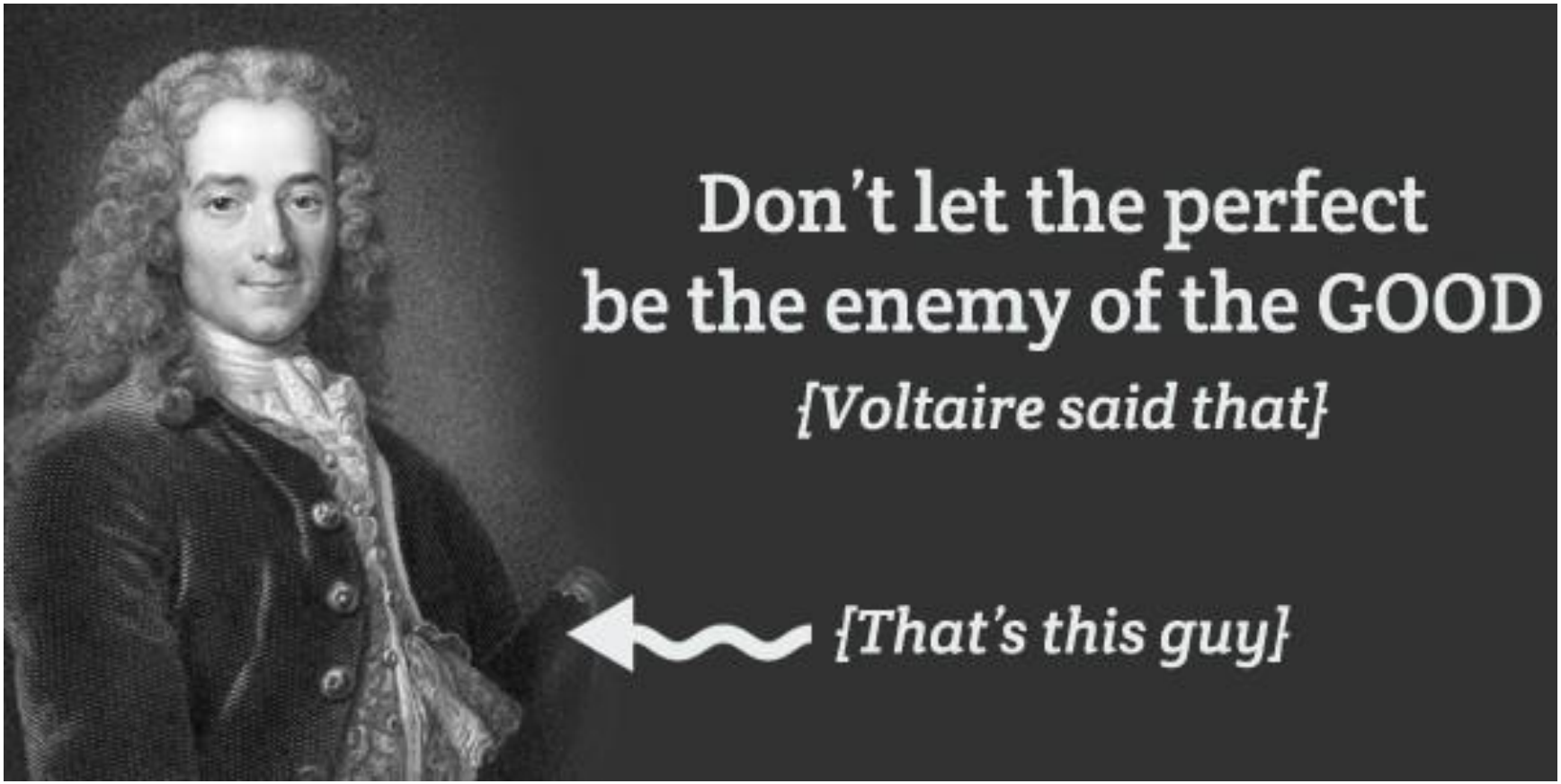
Male Reference Intervals

Age	Lower Limit	Upper Limit	Samples	Lower CI	Higher CI	95th Percentile
4 days - < 6 months	0.73	4.77	139	(0.367 - 0.98)	(4.27 - 5.54)	
6 months - <14 years	0.7	4.17	640	(0.61 - 0.82)	(4.04 - 4.43)	
14 - < 19 years	0.47	3.41	259	(0.25 - 0.57)	(3.15 - 3.45)	

Toronto Sick Kids

2016/01/22 to 2016/03/21	2016/03/22 to present
Abbott Architect	
	3d-<15d: 13.0-52.2
	15d-<30d: 10.5-30.0
	30d-<4m:10.5-22.7
	4m-<1y:10.0-22
	1y-<19y:10.0-17.6
4d-<6m: 0.73-4.77	0 -<6 d: 3.2-19
6m-<14y: 0.70-4.17	6 d-<1 m: 1.70-9.10
14y-<19y: 0.47-3.41	1m -<3 m: 0.50-6.30
	3m-<6 m: 0.50-4.77
	6 m-<1 y: 0.61-4.58
	1y- <14 y: 0.73-4.09
	14y-<19y: 0.47-4.00

Perfection: No Worky



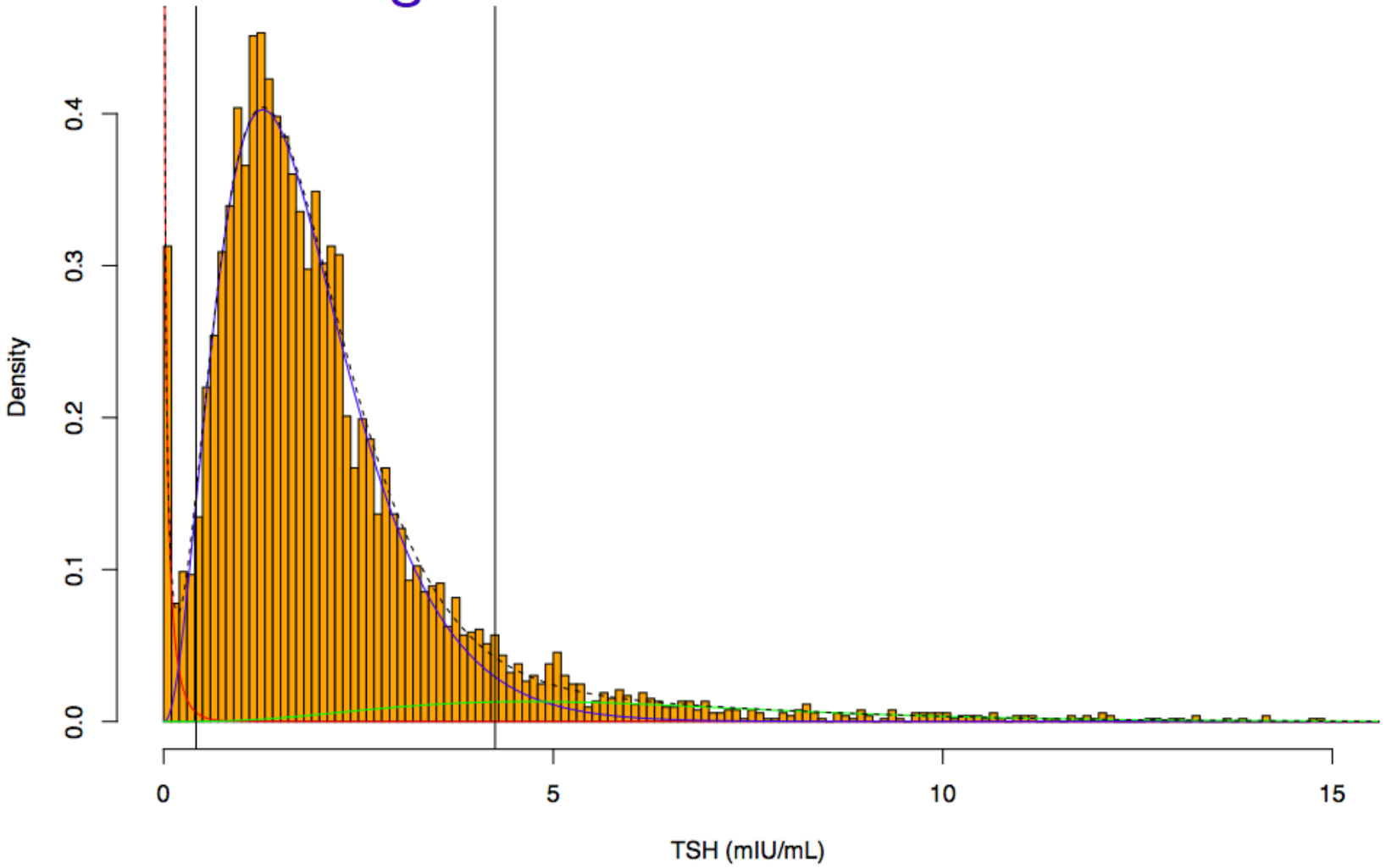
Other Strategies

- Practical and arbitrary
  - Decide on a clinical decision limit and report in such a way as to not produce over-referral.
    - Happens to be the FIT testing strategy
  - Pick the distribution centile corresponding to 99% instead of 97.5%.
  - Report from the lower 90% CI of the lower limit of normal to the upper 90% CI of upper limit of normal.

Data Mining Strategies

- These represent a simple sanity check of reporting limits.
- Generally considered dodgy.
- Extract routine results from the laboratory information system: 19500 results from the last 3 years.
  - Analyses on children aged 12–19 were initially considered on whom TSH < 15 mIU/L.
  - Gender breakdown: F 3028 M 2246

Distribution Fitting

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## [1] 0.42
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```
## [1] 4.25
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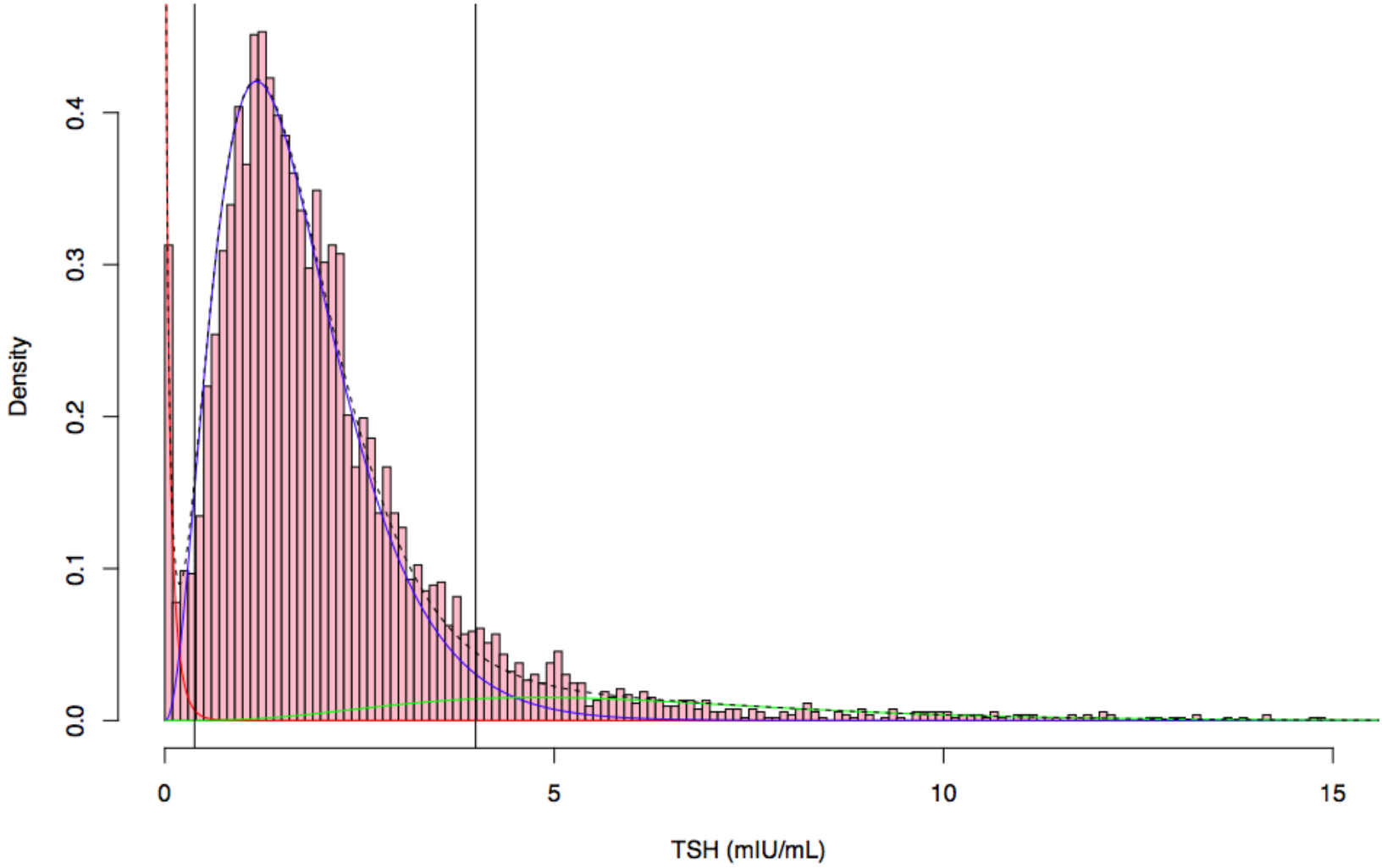
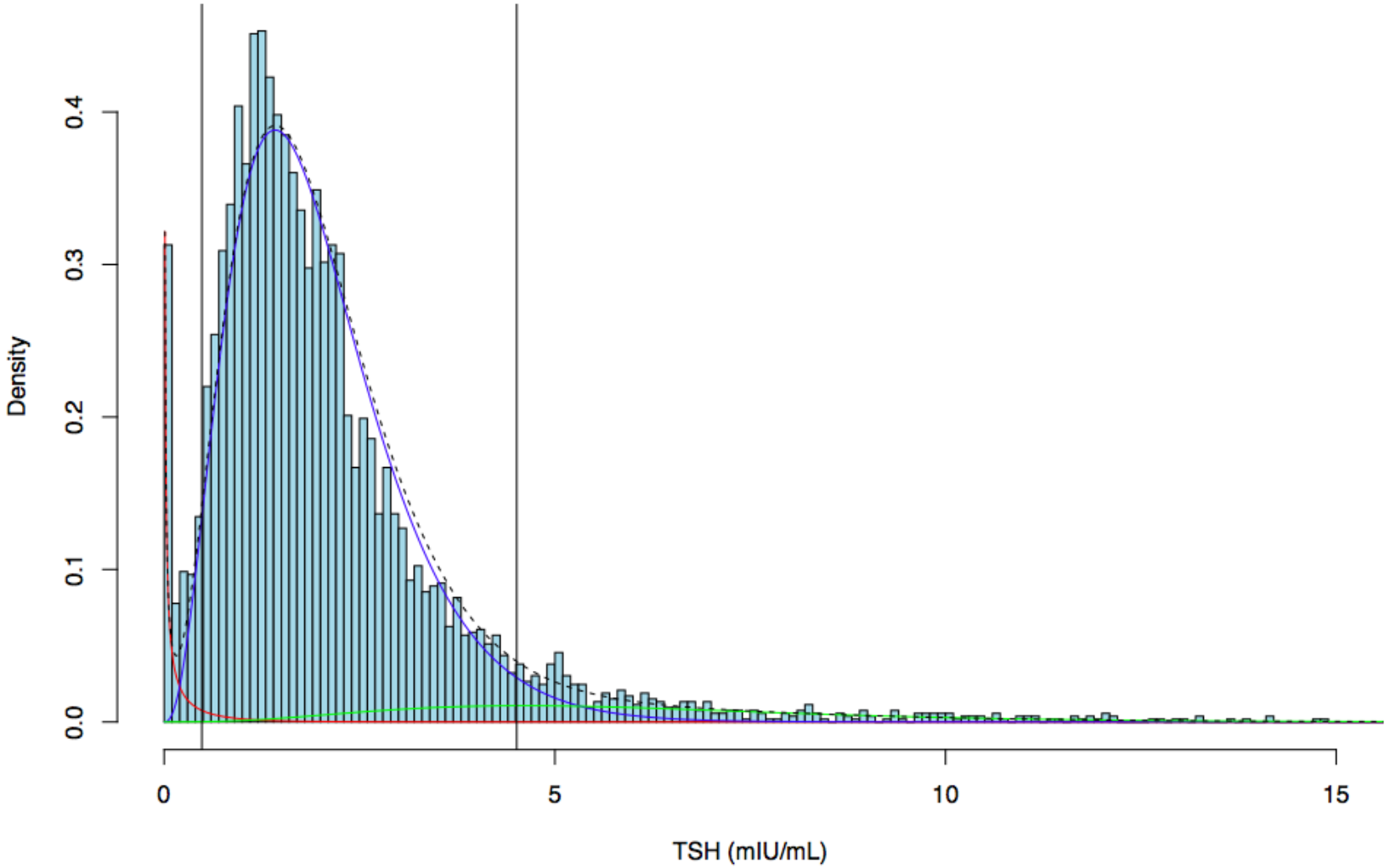
Females




```
## [1] 0.38
```

```
## [1] 3.99
```

Males




```
## [1] 0.48
```

```
## [1] 4.51
```

Conclusions

- TSH reference intervals appear to be too narrow based on the resulting referral rates.
- Strategies to mitigate the problem are to widen the reference intervals based on a specific statistical strategy or based on an agreed-upon clinical decision limit.

Spontaneous Hypoglycemia

Case

- 28 yo first nations male
- Lives at home w family in difficult social situation
- Type I DM
  - Rx Aspart (Novorapid), Glargine (Lantus)
- Admitted for spontaneous hypoglycemia and stabilization of insulin management.
- Moved from Aspart to Lispro (Humalog) in hospital.

Case

- On the ward, the patient was found to have ongoing episodes of hypoglycemia despite sequential decreases in insulin dosing.
- Had witnessed hypoglycemic seizure on the ward. Plasma glucose ~ 1.0 mmol/L.
- Is there another cause of hypoglycemia in this patient?

Case 4

- Differential Diagnosis Spontaneous Hypoglycemia (Adult)
  - Insulin administration
  - Insulin secretagogue administration – glyburide, gliclazide (Diamicron). nateglinide (Starlix), repaglinide (Gluconorm)
  - Drugs of abuse/medications: alcohol, cocaine, beta blocker, quinine
  - Insulinoma
  - Insulin Autoantibody Syndrome (Hirata Disease)
  - Non-Islet cell tumor hypoglycemia (IGF II paraneoplastic)
  - Post gastric bypass
  - Activating Abs to Insulin receptor (analogue of Grave's Disease)
  - Failed production: liver failure, unmasked inborn error of metabolism.
  - Consumptive: sepsis, shock, starvation
  - Failed counter-regulation: primary/secondary adrenal failure, panhypopituitarism

Case

- Urinary LC-MS/MS screen did not reveal the presence of any oral hypoglycemic agents.
- A serum and EDTA plasma specimen was collected during a hypoglycemic event to measure cortisol and ACTH respectively.
  - Cortisol > 500 nmol/L
  - ACTH ~ 15 pmol/L N<12 pmol/L
- Endocrinologist began to be suspicious of the extent and frequency of hypoglycemic events.

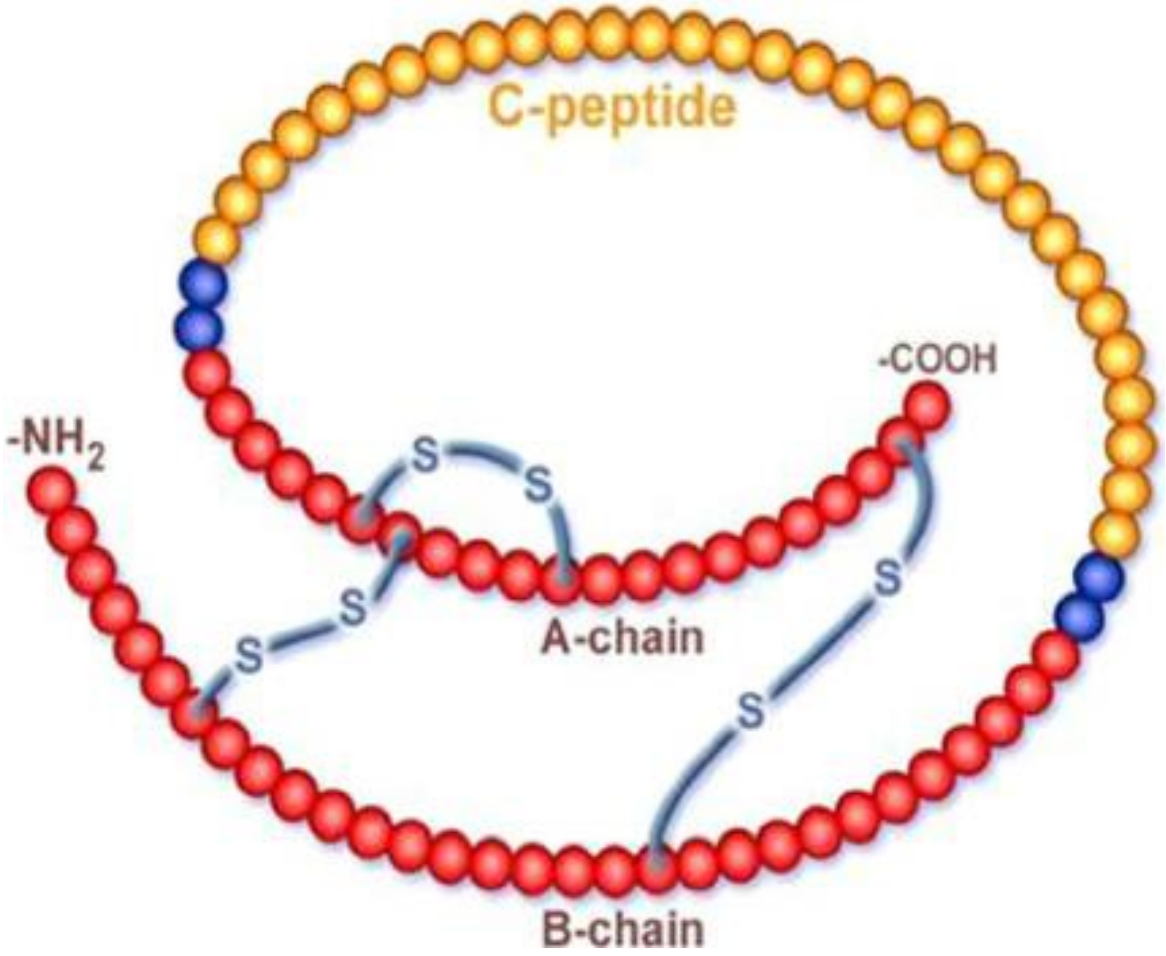
Case

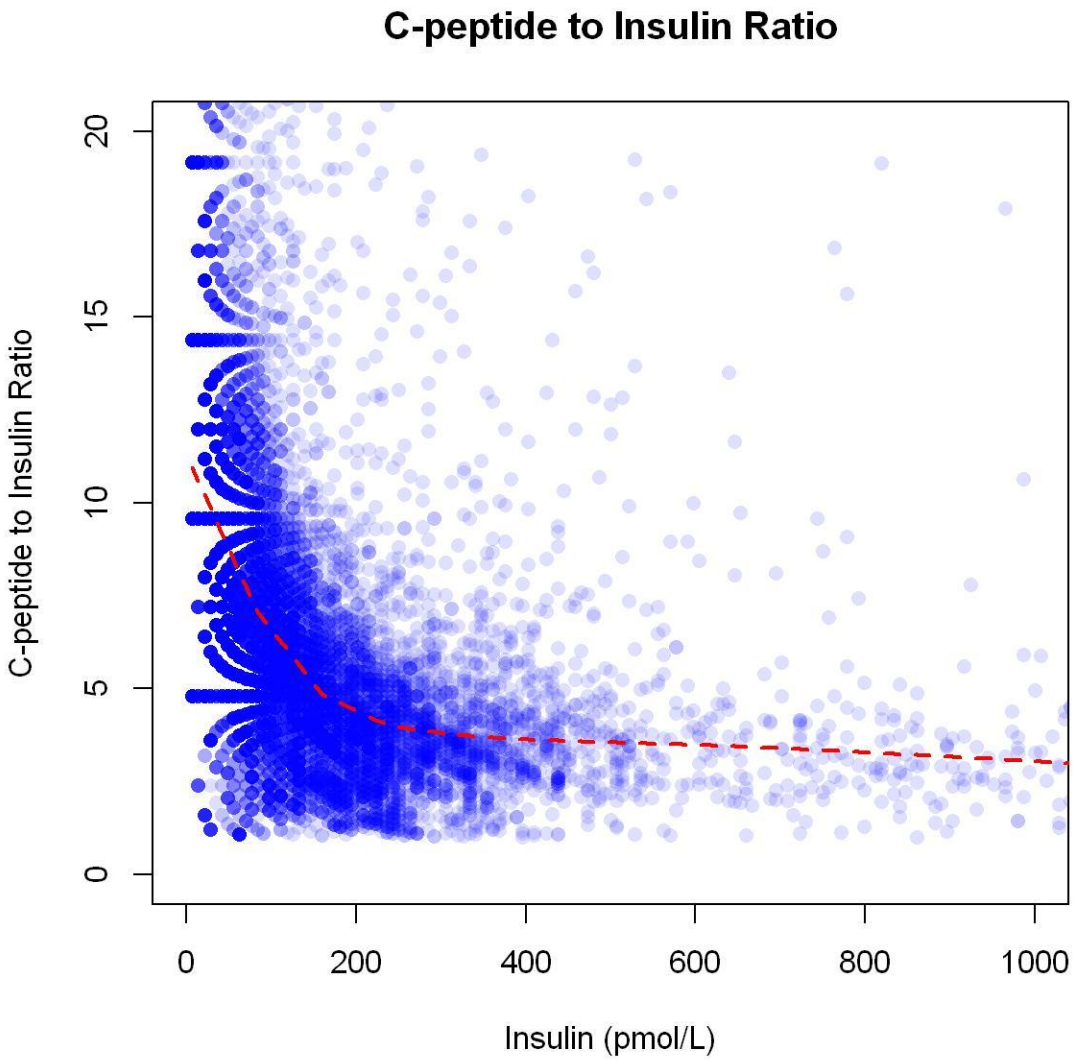
- Sidebar:
  - Why obtaining the hypoglycemic specimen is so important.
  - Not appreciated by many clinicians.
- Fortunately, the ACTH specimen was run at our hospital and we had this specimen. Glucose was measured and confirmed to be 2.0 mmol/L
- Insulin analogue analysis.

Case

- Recall:
- Rx in community: glargine and aspart
- Rx in hospital: glargine and lispro

Proinsulin




C-peptide to Insulin Ratio
C-peptide to Insulin Ratio
0
5
10
15
20
0
200
400
600
800
1000
Insulin (pmol/L)

Problem Is Bigger Than this

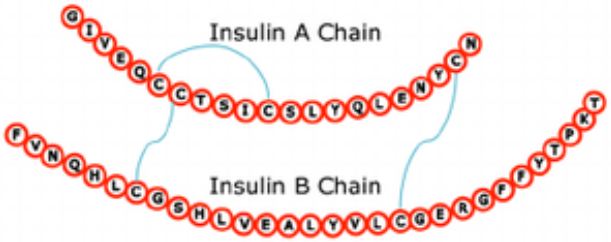
Table 1. Cross-reactivities of insulin analogs.

	Cross-reactivity, %					
					IMMULITE 2000	
Analog and concentration	**Access**	**Advia Centaur**	**Coat-A-Count**	**E170**	**Lot 122**	**Lot 151**
Insulin aspart						
30 mIU/L	85.3	120	36.7	<0.7	14.7	9.3
100 mIU/L	80.0	124	57.0	<0.2	17.4	5.4
300 mIU/L	84.3	135	54.7	<0.07	40.7	8.0
1000 mIU/L	77.1	125	34.4	<0.02	40.7	13.0
Mean	81.7	126	45.7		28.4	8.9
Insulin glargine						
30 mIU/L	91.7	129	32.0	<0.7	<6.7	8.3
100 mIU/L	85.0	140	46.0	<0.2	3.4	2.8
300 mIU/L	78.7	152	35.7	<0.07	11.1	1.6
1000 mIU/L	79.7	150	27.1	<0.02	13.2	1.8
Mean	83.8	143	35.2		9.2	3.6
Insulin lispro						
30 mIU/L	78.7	86.7	37.0	<0.7	14.7	10.3
100 mIU/L	77.0	89.0	52.0	<0.2	18.0	6.3
300 mIU/L	79.3	92.3	49.7	<0.07	42.3	8.4
1000 mIU/L	80.2	89.2	33.4	<0.02	39.3	12.3
Mean	78.8	89.3	43.0		28.6	9.3

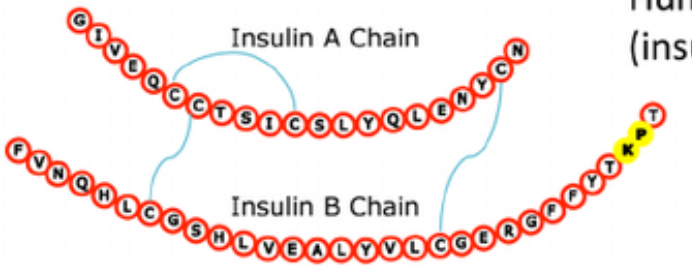
How Can Insulins Be Distinguished?

- We want to be able to quantify the synthetic insulin analogues.
- We want to be able to unequivocally identify the analogue.
  - We don't want confusion related to low cross reactivity.
- Realizing that the results may have significant legal implications, we want to eliminate the possibility of heterophile interference etc.

Human Insulin
MW 5808



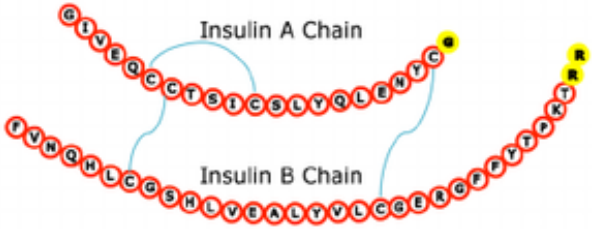
Humalog
(insulin lispro)



lysine proline switch

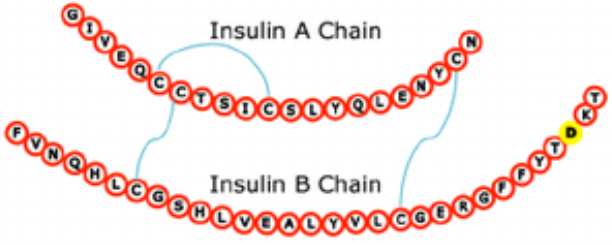
Insulin glargine
(Lantus®)
Avg MW 6063

asparagine replaced
with glycine

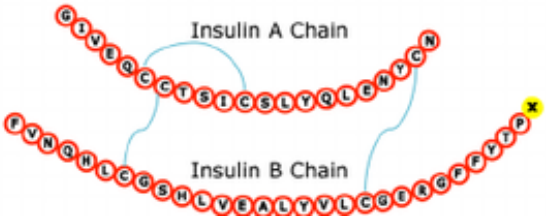


chain extension with
arginine

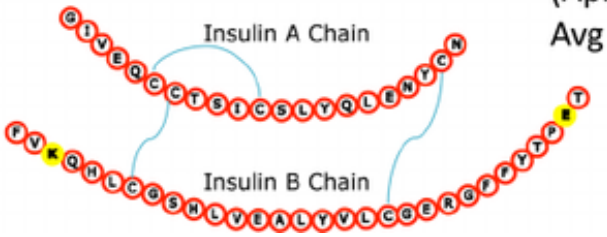
Insulin aspart
(Novalog®)
Avg MW 5826



Insulin detemir
(Levemir®)
Avg MW 5917



Insulin glulisine
(Apidra®)
Avg MW 5823



Sample Prep

Optimized Sample Preparation Procedure

- 1 mL human serum

- 25 uL of 500 uU/mL Bovine insulin (internal standard)

- 5 uL of 5 g/L dextran sulfate + 0.5M $MgCl_2$

-500 uL of Monoclonal anti-insulin mouse Ab-coated magnetic beads --> incubate at RT for 1 hour, on rocker

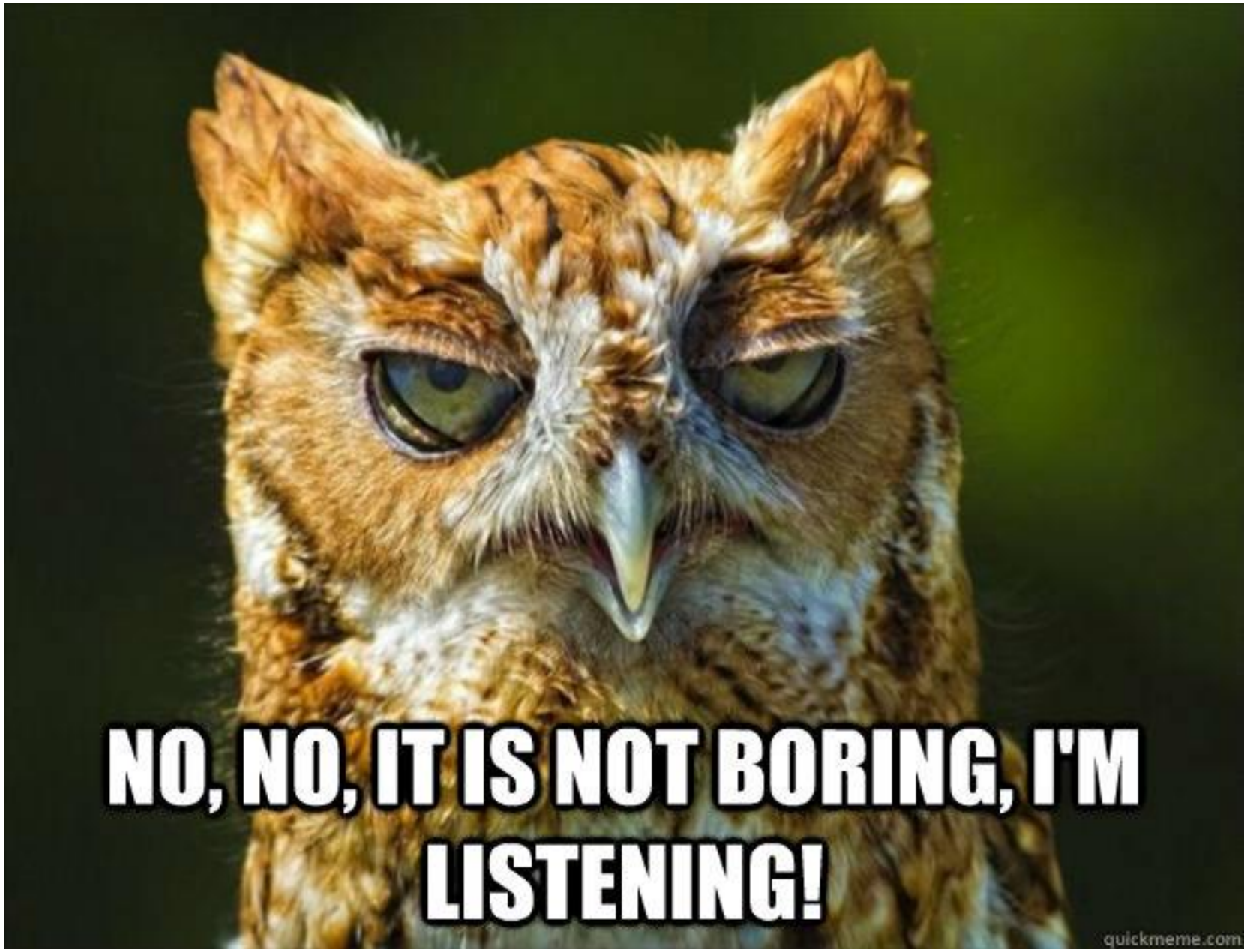
-transfer entire contents of tube to 96-well filter plate

- wash 3x 1 mL PBS – eluant goes to waste

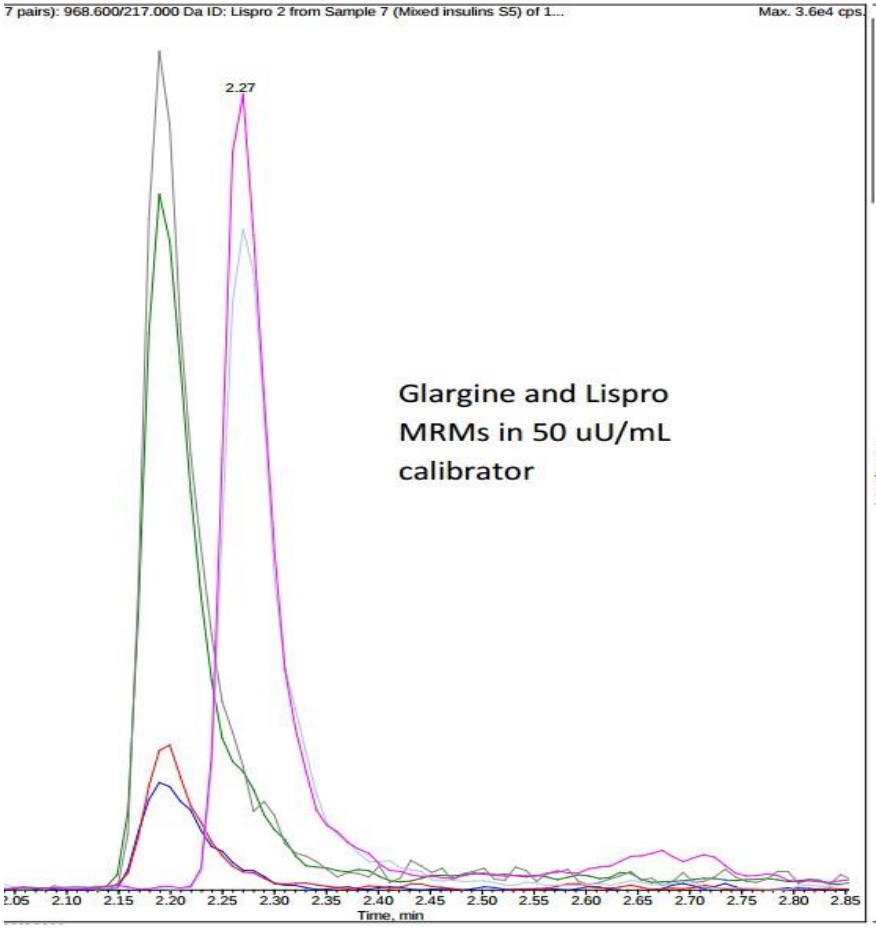
- elute 2 x 100uL of 1% acetic acid to a BSA-treated 96 well plate

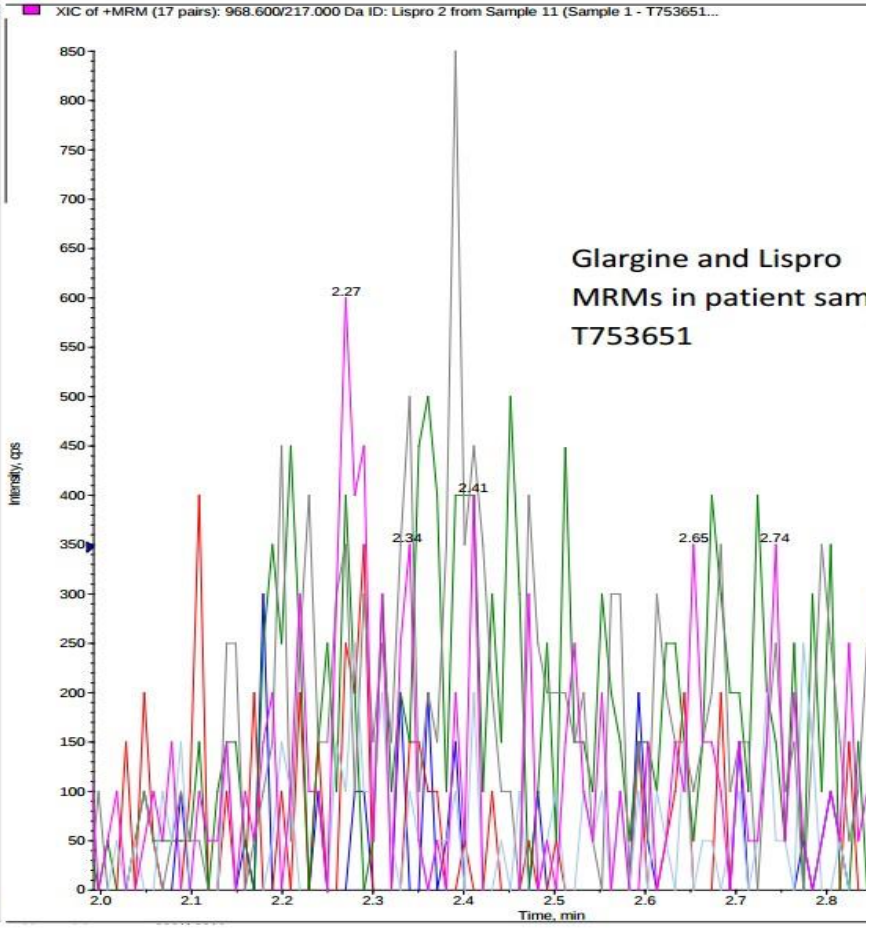
Chromatography

Extracted Ion Chromatogram for all insulins

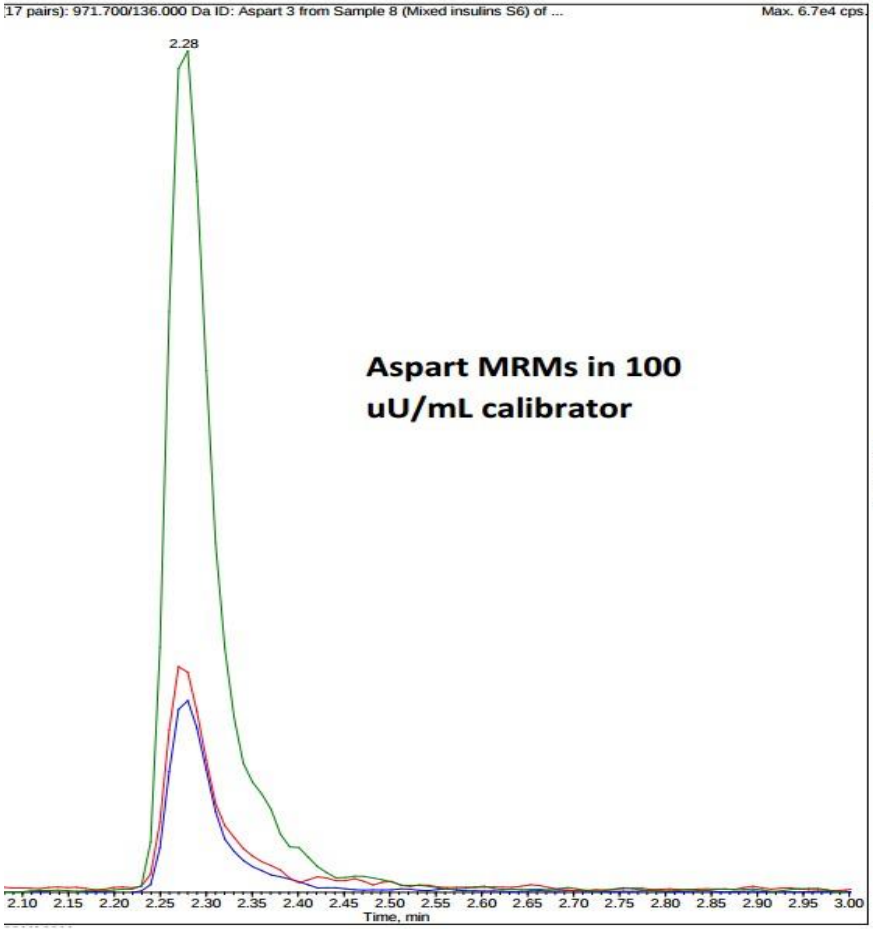

NO, NO, IT IS NOT BORING, I'M LISTENING!
quickmeme.com

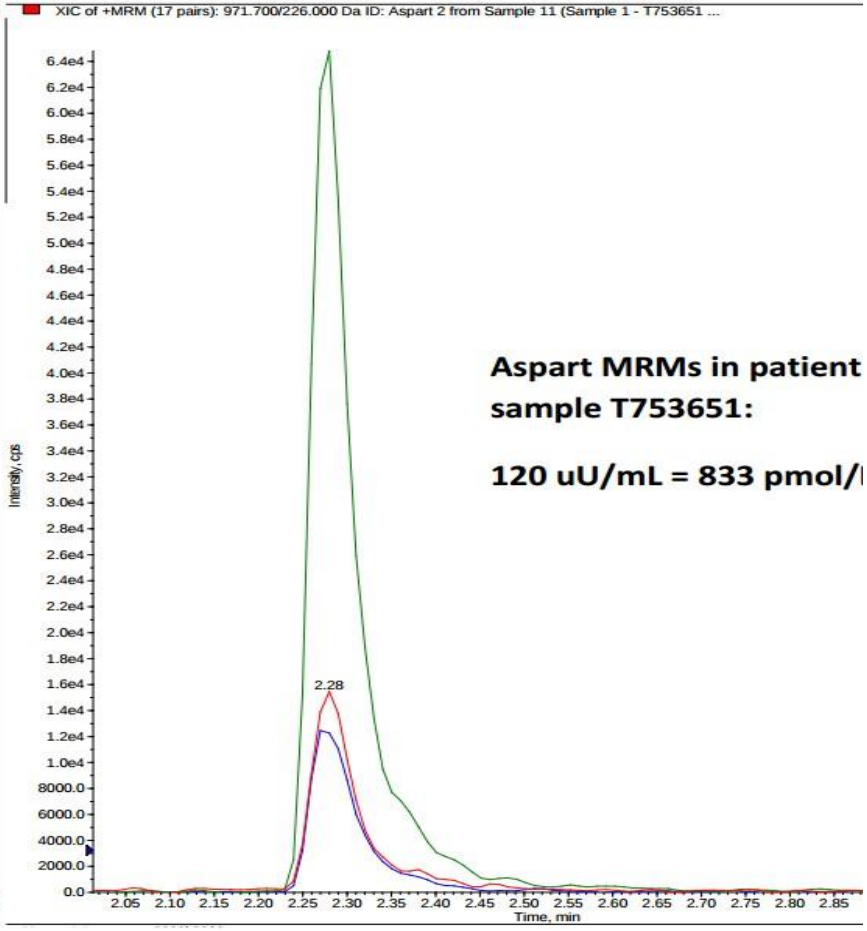
Case


7 pairs): 968.600/217.000 Da ID: Lispro 2 from Sample 7 (Mixed insulins S5) of 1...
Max. 3.6e4 cps.
2.27
Glargine and Lispro
MRMs in 50 uU/mL
calibrator
Time, min


XIC of +MRM (17 pairs): 968.600/217.000 Da ID: Lispro 2 from Sample 11 (Sample 1 - T753651...
Intensity, cps
2.27
2.34
2.41
2.65
2.74
Glargine and Lispro
MRMs in patient sam
T753651
Time, min

Case


17 pairs): 971.700/136.000 Da ID: Aspart 3 from Sample 8 (Mixed insulins S6) of ...
Max. 6.7e4 cps.
2.28
Aspart MRMs in 100 uU/mL calibrator
Time, min


XIC of +MRM (17 pairs): 971.700/226.000 Da ID: Aspart 2 from Sample 11 (Sample 1 - T753651 ...
Intensity, cps
2.28
Aspart MRMs in patient sample T753651:
120 uU/mL = 833 pmol/
Time, min

Case

- Patient was administering the insulin he was Rx'd at home after each of his on ward administrations.
- He was gently confronted and psychiatry was consulted.

Cortisol: How High?

(How wide, How deep)

Case: Non-classical CAH

- Previously well 15 y F
- 2 year history of oligomenorrhea, dysmenorrhea, mild hirsutism, acne
  - Puberty: Axillary and pubic hair at 11 y
  - Menarche 12 y
  - Review of systems otherwise unremarkable
- Family history:
  - Non-consanguineous parents (French Canadian/German)
  - Mother: menarche at 13 y, irregular menses, infertility (IVF)

Case: Non-classical CAH

- PCOS screening done by Pediatrician
  - Normal estradiol, LH, FSH, lipids, A1C
  - Elevated androgens
  - Elevated morning 17-OHP: 201.8 nmol/L (reference interval < 8.6, LC/MS)
- **Referred to Pediatric Endocrinology**

Physical examination

- Vitals: normal for age
- Height: 45.5%-ile, −0.11 SD (Parental heights: 20–90th %-ile)
- BMI: 88.8%-ile, 1.22 SD
- Acne: Face, back and shoulders
- Muscular build
- GU exam: Tanner stage 5, no clitoromegaly

Baseline laboratory investigations

Test	Result	Reference Interval
DHEAS (µmol/L)	11.0	< 10.8
Androstenedione (nmol/)	7.2	0.1–6.7
Total testosterone (nmol/L)	2.8	< 1.8
Free testosterone (pmol/L)	76	< 30
Bioavailable testosterone (nmol/L)	1.8	1.8
SHBG (nmol/L)	14.4	20.0–180.0
Plasma renin activity (ng/L/s)*	0.41	< 1.50
Aldosterone (pmol/L)*	197	70-660
Sodium (mmol/L)	144	135–145
Potassium (mmol/L)	4.2	3.5–5.0

* Tandem Mass Spectrometry method

250 microgram Cosyntropin stimulation test

	Cortisol (nmol/L)		17-hydroxyprogesterone (nmol/L)	
Time (min)	Immunoassay	Tandem Mass Spectrometry	Immunoassay	Tandem Mass Spectrometry
0	322	296	62.6	53.7
60	410	361 (ion 1) 353 (ion 2)	Insufficient sample	Insufficient sample

Reference intervals:

Expected stimulated cortisol > 500 nmol/L

17-hydroxyprogesterone > 50 nmol/L is indicative of CAH

History of Adrenal Insufficiency Testing

- 1925: investigators described the infusion of Ringer's solution as a means to prolong the life of adrenalectomized dogs.[1]
- 1933: oral sodium chloride treatment brought about marked clinical improvement in classical Addisonian patients.[2]
- 1933: Classic electrolyte disturbances are seen in a dog model.[3]

1. Stewart GN, Rogoff JM. Studies on adrenal insufficiency. Proc Soc Exper Biol Med 1924-25;22:394.
2. Loeb RF. Effect of sodium chloride in treatment of a patient with Addison's disease. Proc Soc Exper Biol Med. 1933;30:808.
3. Harrop GA, Soffer LJ, Ellsworth R, Trescher JH. Plasma electrolytes and electrolyte excretion during suprarenal insufficiency in the dog. J Exp Med 1933;58:17-38

Clinical Definition

- 1933 Harrop formed a Clinical Definition
- Weakness and fatigability, nausea and weight loss
- Pigmentation (followed by serial paintings in the absence of photography!)
- Orthostasis common but not mandatory

Harrop GA, Weinstein A, Soffer LJ, Trescher JH. The diagnosis and treatment of Addison's disease. JAMA 1933;100:1850-1855

Wilder's Test

- In 1936 Mayo Clinic described "Wilder's Test" .
- Hospitalization of the patient for 6 to 9 days.
- Low salt diet and K loading (20 mg/lb/day).
- It was observed that this procedure "reliably provoke[d] adrenal crisis" and some later reports even described fatalities
- Urine Cl is monitored -> first lab test

Cutler HH, Power MH, Wilder RM. Concentrations of chloride, sodium and potassium in urine and blood: their diagnostic significance in the diagnosis of Addison's disease. JAMA 1938;111:117-22.

RPK Test

- Observation during Wilder test – water loading (given to enhance urine output for analysis) – urine output was less than input in positive tests
- Theory that adrenal products must be needed for hepatic degradation of the ADH
- Urine collection 2230 – 0730h after being NPO at 1800h
- 20 cc / kg H2O at 0830 with hourly urine volumes x 4 hours after
- “negative” if any single hour’s output exceeds the overnight volume
- “positive” if any single hour’s output is less than the overnight volume

ACTH Stim

A TEST FOR ADRENAL CORTICAL INSUFFICIENCY

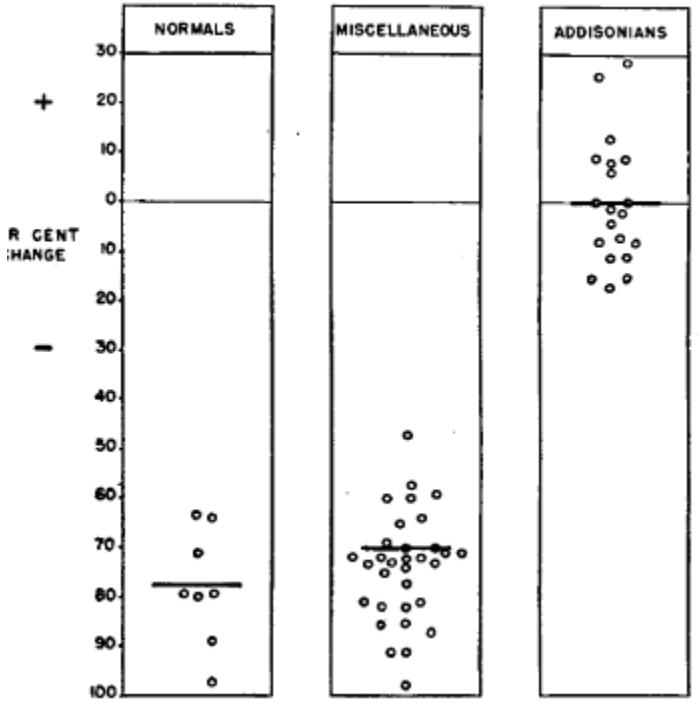
The Response to Pituitary Andrenocorticotropic Hormone

George W. Thorn, M. D.
Peter H. Forsham, M. D.
F. T. Garnet Prunty, M. D.
and
A. Gorman Hills, M. D.
Boston

J. A. M. A.
July 17, 1948

The beginning of ACTH stim

- Purified bovine ACTH (Armour Labs)
- Compounds E&F (hydrocortisone, hydrocortisol) identified and synthesized but no assay yet
- Measured the decrease in eosinophil count at 4 hours post ACTH (IM)
- > 50% decrease defined as normal
- “one seldom encounters an equivocal effect of ACTH on eosinophils”
- “Does not depend on electrolyte regulating hormones”



1965 – fluorometric serum cortisol assay

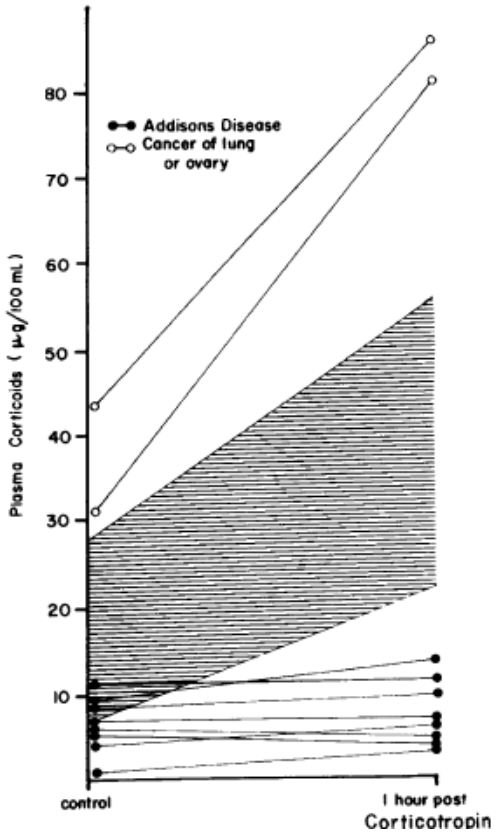
- Plasma cortisol now becomes the test of choice

Rapid Intravenous Administration of Corticotropin as a Test of Adrenocortical Insufficiency

Byron U. Musa, MD, and J. Thomas Dowling, MD

The one-hour response of plasma-fluorescent corticoid concentrations to 25 units of corticotropin has allowed complete separation of a group of seven patients with adrenal insufficiency from another group of 31 patients with findings suggestive of adrenal insufficiency but having, in fact, normal adrenal function. From the standpoint of time required for the analytical procedure, availability of reagents, and apparatus requiring no modification, the laboratory techniques described are suitable for a small clinical laboratory.

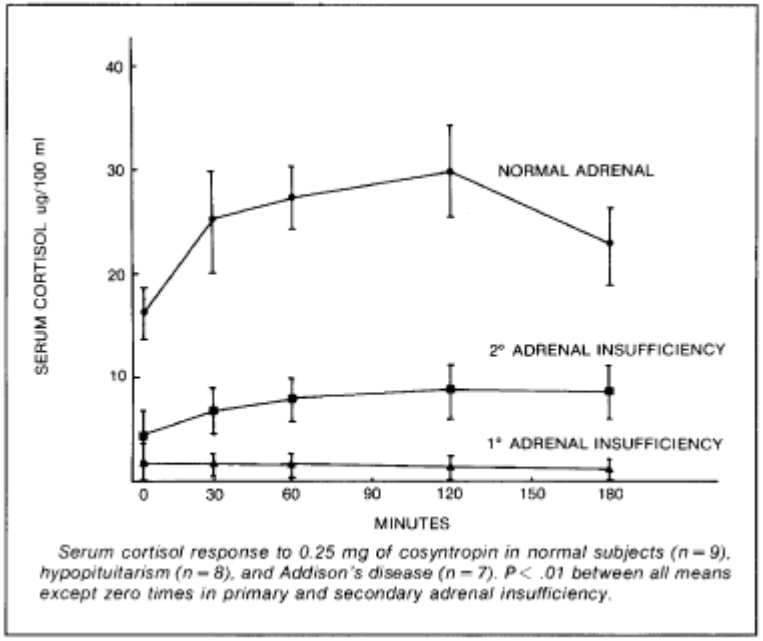
7 AD
29 controls,
Reference diagnosis not stated.



JAMA, Aug 21, 1967 • Vol 201, No 8

1966 – cosyntropin now a standardized source (UCLA)

Screening for Adrenocortical Insufficiency With Cosyntropin (Synthetic ACTH)



Serum cortisol response to 0.25 mg of cosyntropin in normal subjects (n = 9), hypopituitarism (n = 8), and Addison's disease (n = 7). P < .01 between all means except zero times in primary and secondary adrenal insufficiency.

Competitive protein binding assay
Normal mean: 834 nmol/l
Normal – 2SD: 420 nmol/l

Normals = 9 patients

Arch Intern Med/Vol 128, Nov 1971

Fast Forward

Rapid Adrenocorticotropic Hormone Test in Practice

Retrospective Review

MICHAEL E. MAY, Ph.D., M.D.
ROBERT M. CAREY, M.D., F.A.C.P.
Charlottesville, Virginia

Retrospective analysis of the rapid adrenocorticotropic hormone (ACTH) test in a large adult population shows a marked interdependence of the basal cortisol concentration, peak cortisol concentration, and increase in cortisol concentration. Repetition of the rapid ACTH test in the same patient does not improve diagnostic accuracy. A significant number of falsely abnormal rapid ACTH test results were observed (in comparison to continuous ACTH infusion as a reference test). This supports the use of the rapid ACTH test as a screening test, but not as a diagnostic test for adrenocortical failure. It is proposed that a peak cortisol level greater than or equal to 20 μg/dl (550 nmol/liter) is a sufficient single criterion for normal adrenal function as assessed

COMMON DIAGNOSTIC TESTS
Series Editors: Alan Garber, MD, PhD, and Harold Sox, MD

ACADEMIA AND CLINIC

Diagnosis of Adrenal Insufficiency

Richard I. Dorin, MD; Clifford R. Qualls, PhD; and Lawrence M. Crapo, MD, PhD

Background: The cosyntropin stimulation test is the initial endocrine evaluation of suspected primary or secondary adrenal insufficiency.

Purpose: To critically review the utility of the cosyntropin stimulation test for evaluating adrenal insufficiency.

Data Sources: The MEDLINE database was searched from 1966 to 2002 for all English-language papers related to the diagnosis of adrenal insufficiency.

Study Selection: Studies with fewer than 5 persons with primary or secondary adrenal insufficiency or with fewer than 10 persons as normal controls were excluded. For secondary adrenal insufficiency, only studies that stratified participants by integrated tests of adrenal function were included.

Data Extraction: Summary receiver-operating characteristic (ROC) curves were generated from all studies that provided sensitivity and specificity data for 250-μg and 1-μg cosyntropin tests; these curves were then compared by using area under the curve (AUC) methods. All estimated values are given with 95% CIs.

Data Synthesis: At a specificity of 95%, sensitivities were 97%, 57%, and 61% for summary ROC curves in tests for primary adrenal insufficiency (250-μg cosyntropin test), secondary adrenal insufficiency (250-μg cosyntropin test), and secondary adrenal insufficiency (1-μg cosyntropin test), respectively. The area under the curve for primary adrenal insufficiency was significantly greater than the AUC for secondary adrenal insufficiency for the high-dose cosyntropin test ($P < 0.001$), but AUCs for the 250-μg and 1-μg cosyntropin tests did not differ significantly ($P > 0.5$) for secondary adrenal insufficiency. At a specificity of 95%, summary ROC analysis for the 250-μg cosyntropin test yielded a positive likelihood ratio of 11.5 (95% CI, 8.7 to 14.2) and a negative likelihood ratio of 0.45 (CI, 0.30 to 0.60) for the diagnosis of secondary adrenal insufficiency.

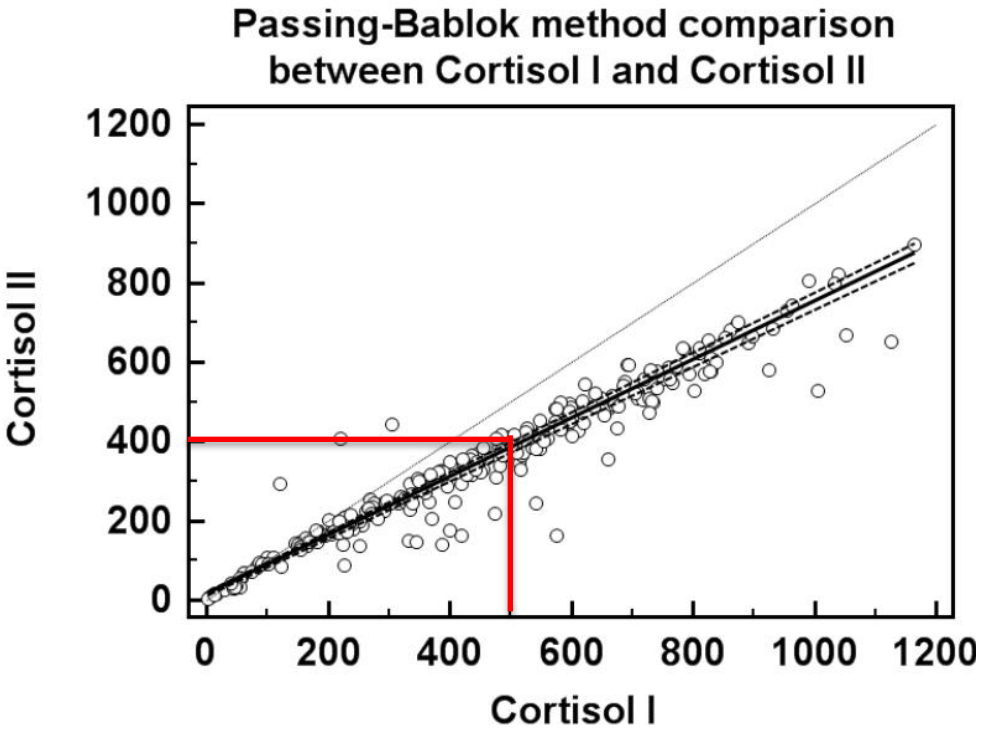
Conclusions: Cortisol response to cosyntropin varies considerably among healthy persons. The cosyntropin test performs well in patients with primary adrenal insufficiency, but the lower sensitivity in patients with secondary adrenal insufficiency necessitates use of tests involving stimulation of the hypothalamus if the pretest probability is sufficiently high. The operating characteristics of the 250-μg and 1-μg cosyntropin tests are similar.

Ann Intern Med. 2003;139:194-204. www.annals.org
For author affiliations, see end of text.

500-550 nmol/L

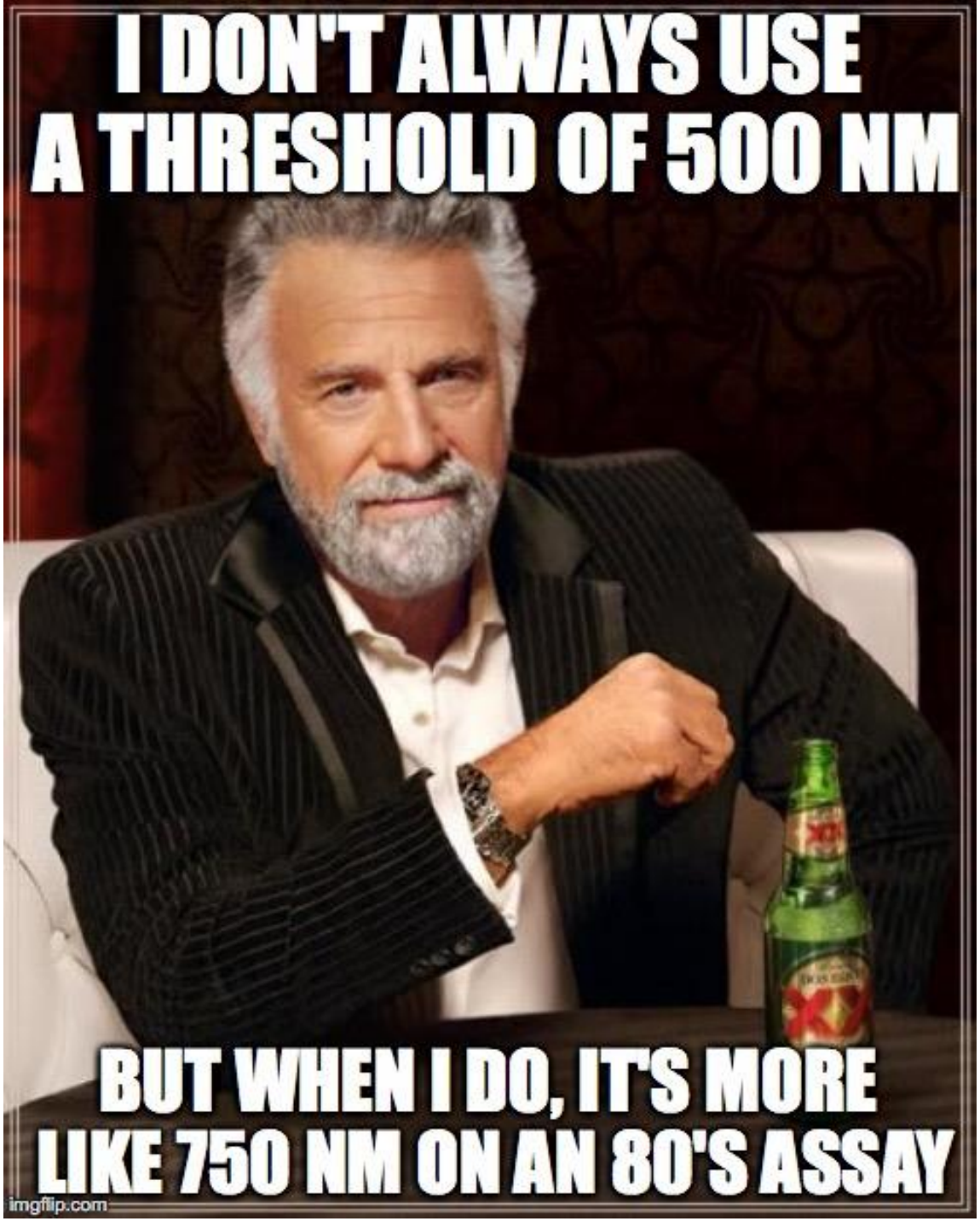
- Is established on the basis mixtures of fluorometric assays, radioimmunoassays and homogeneous assays.
- Suppose we grant that 500 nmol/L is correct.
  - What does 500 nmol/L mean on a modern assay?
  - How do modern assays compare between one another?

Roche Cortisol I and II



- Two modern format assays made by the same company differ by 30%.
- That is, 500 is now 370…
- Let that sink in…

Kline GA, Buse J, Krause RD. Clinical implications for biochemical diagnostic thresholds of adrenal sufficiency using a highly specific cortisol immunoassay. Clinical biochemistry. 2017 Jun 1;50(9):475-80.


I DON'T ALWAYS USE A THRESHOLD OF 500 NM
BUT WHEN I DO, IT'S MORE LIKE 750 NM ON AN 80'S ASSAY
imgflip.com

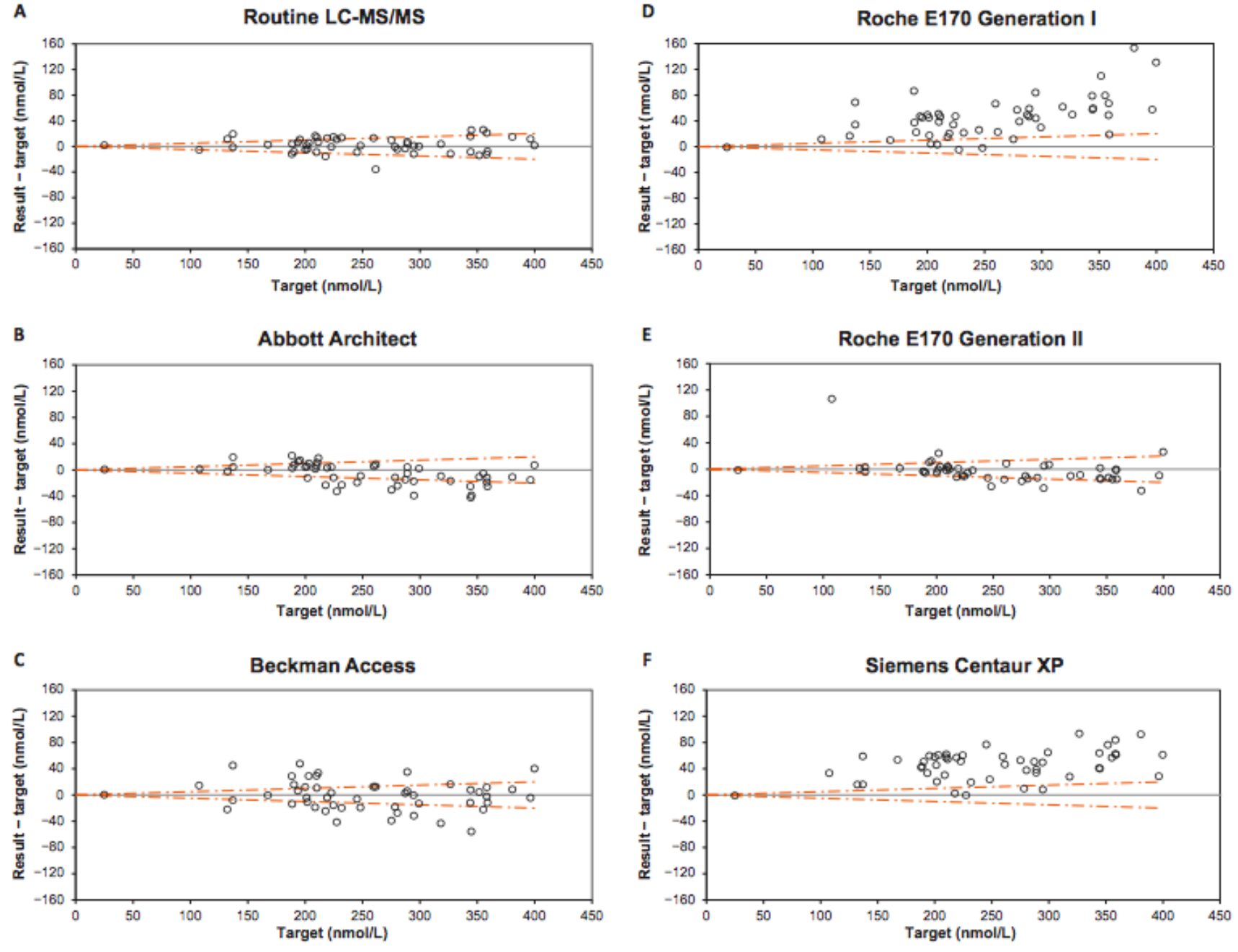


Fig. 1. Bland-Altman bias plots depicting the performance of the assays investigated relative to the cRMP in the male cohort (n = 51).

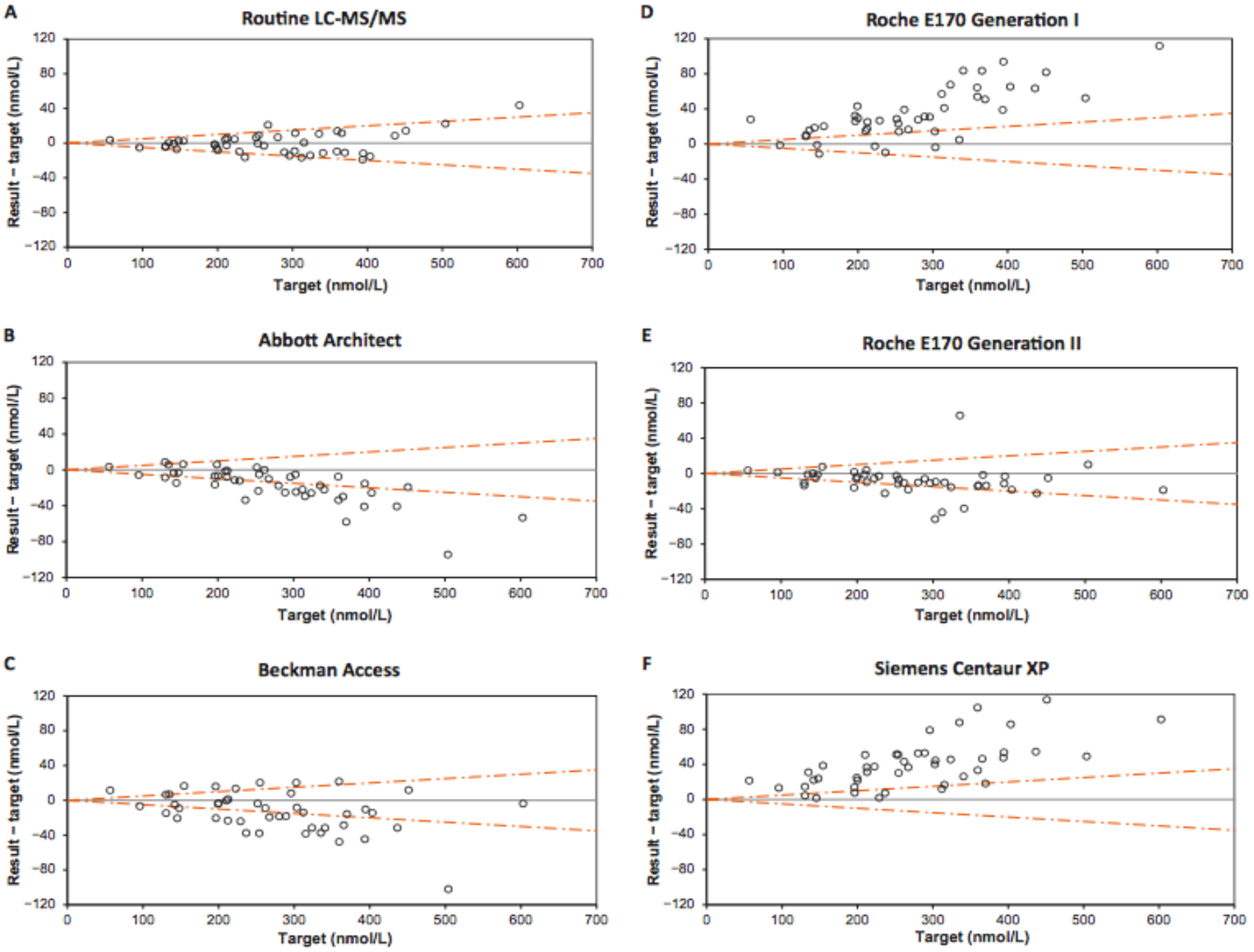


Fig. 2. Bland-Altman bias plots showing the performance of the assays investigated relative to the cRMP in the nonpregnant female cohort (n = 45).

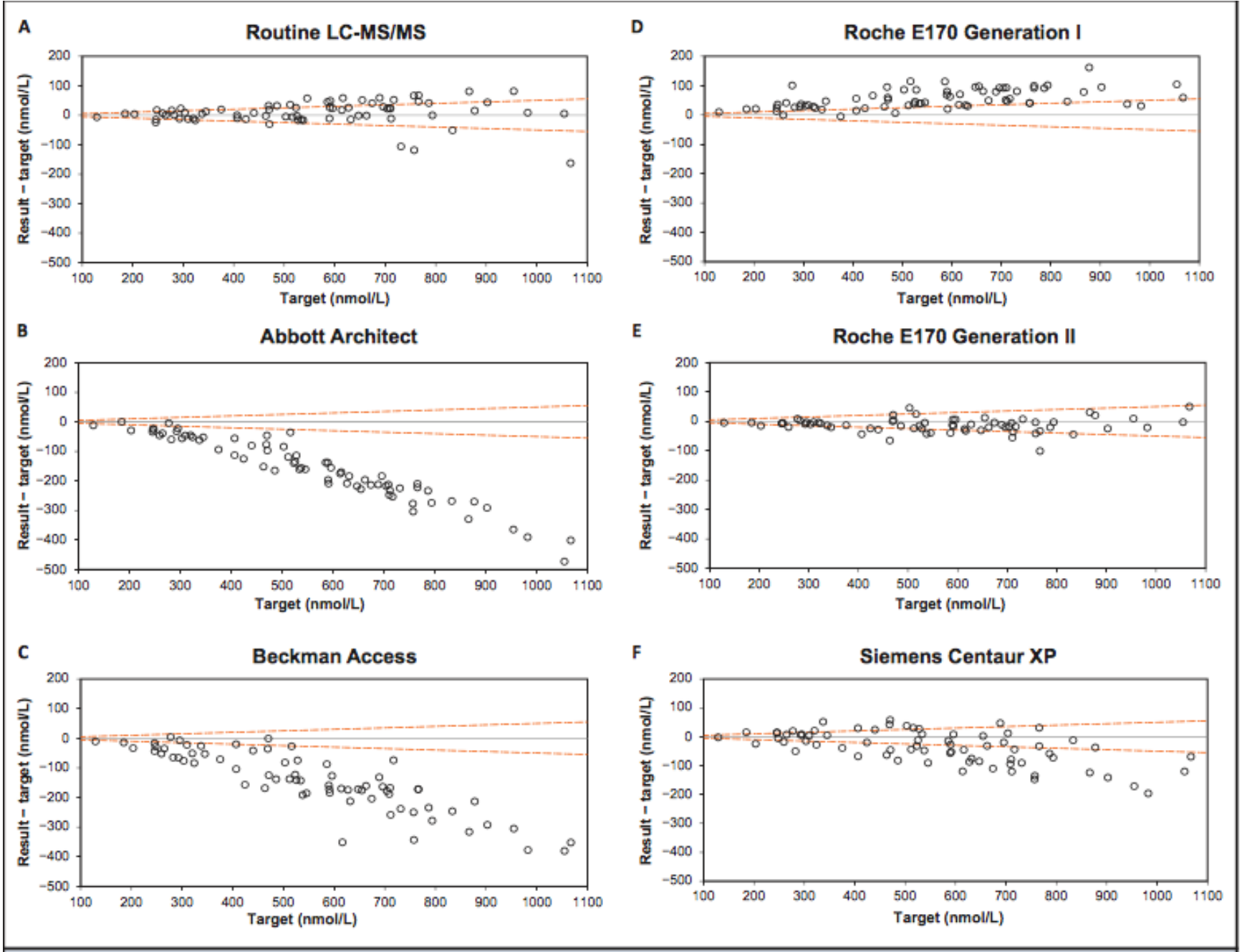


Fig. 3. Bland–Altman bias plots displaying the performance of the assays investigated relative to the cRMP in the pregnant cohort (n = 72).

Conclusions

- ~350 is the new 500 nmol/L from a purely analytical standpoint.
- Cortisol immunoassays have much improved performance over yesteryear but still demonstrate large differences between methods which has not been accounted for in guidelines.
- The risk is overtreatment and over commitment of patients not-at-risk for Addisonian crisis to lifelong steroid replacement.

Acknowledgments

- Dr. Gregory Kline for many helpful discussions on what constitutes sufficient cortisol response in ACTH stimulation testing.
- Grace Van Der Gugten for her many efforts to develop difficult LC-MS/MS assays and keep them running.
- The technologists of St. Paul's Hospital for their enthusiasm to try new things – even very difficult workflows such as Tg by LC-MS/MS.