Using the Clinical Laboratory: Practical Pearls for the Pediatric (Endocrine) Practitioner

Jon Nakamoto, M.D., Ph.D.
Regional Medical Director, Quest Diagnostics Nichols Institute
Assoc. Clin. Prof. (Voluntary) of Pediatrics & Endocrinology, UCSD

DISCLOSURE STATEMENT
Speaker: Jon Nakamoto, M.D., Ph.D.

Dr. Nakamoto has disclosed he following relevant financial relationships. Any real or apparent conflicts of interest related to the content of this presentation have been resolved. There will be no discussion of unapproved or off-label, experimental, or investigational use.

Affiliation/ Financial Interest	Organization
Employee & stockholder	Quest Diagnostics Nichols Institute

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Section 1: Choose the right test!

- Know which tests to be "fussy" about

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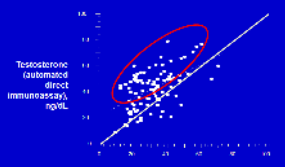
Be a discerning lab test orderer!

- Steroids are difficult to measure accurately at low levels
  - Seek out sensitive & specific assays when measuring: e.g.,
    - Testosterone in females or prepubertal males
    - Estradiol in males or prepubertal females



The results circled in red are falsely elevated when measured on a "direct" immunoassay

Or use tandem mass spectrometry (LC-MS/MS)

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Choose the right test for your patients

Immunoassays for Testosterone in Women: Better than a Guess?

(applies to prepubertal children as well)

Recent developments in the field of mass spectrometry have provided the accuracy and sensitivity to evaluate very-low-abundance steroids such as testosterone in female and pediatric patients. In this issue of *Clinical Chemistry*, Taieb et al. (*1*) present the most comprehensive evaluation of automated testosterone immunoassays to date. They compared 10 commercially available immunoassays with isotope-dilution gas chromatography–mass spectrometry (ID-GC/MS) and reached the inescapable conclusion that testosterone immunoassay results for specimens from females are inaccurate. Similar data have been reported for individual testosterone immunoassays previously (*2*), but Taieb et al. (*1*) are the first to show that for every commercially available testosterone assay studied, the values are in error—by a factor of 2 on average and in some cases by a factor of almost 5. Are assays that miss target values by 200–500% meaningful? Guessing would be more accurate and additionally could provide cheaper and faster testosterone results for females—without even having to draw the patient's blood.

monly measured in population studies" (*3*). The testosterone assay that Dorgan et al. were comparing with MS included an extraction and column purification. Many people believe that liquid–liquid extraction combined with column purification before RIA analysis provides accurate results for testosterone in specimens from females. However, we have previously demonstrated that RIAs that include extraction and column purification steps do not agree well with ID-GC/MS (*4*). An important limitation in the study by Dorgan et al. (*5*) is that for female specimens they tested only sample pools (low, mid, and high). Determining how the assay would work on individual patient samples is not possible when pooled samples are used. This is a critical flaw, because clinicians are concerned about the concentration of testosterone in an individual; in contrast, when pooled samples are analyzed, any cross-reacting substances in an individual sample are diluted in the rest of the pool. In Fig. 1 of their report, Taieb et al. (*1*) show that there is a wide degree of scatter when an extraction chromatography RIA is com-

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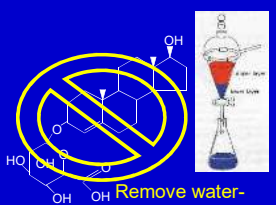
Samples need extra steps to avoid 'cross-reactivity'



Testosterone glucuronide

Remove water-soluble inactive conjugates via organic extraction

Remove similar steroids via chromatography

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Commonly-used "direct" immunoassays don't have these extra steps

	Extraction	Chromatography	Detection
Direct immuno-assay			Immunoassay
E/C Immuno-assay	Solvent	Column	Immunoassay
MS/MS assay	HTLC	HPLC	MS/MS

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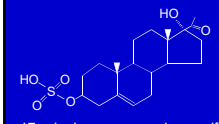
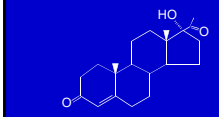
Cross-reactivity is a big problem for infants in particular (example: 17-hydroxyprogesterone)



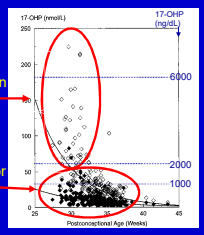
17-α-hydroxypregnenolone sulfate



17- α-hydroxyprogesterone

Non-extraction assay

Extraction RIA or LC-MS/MS assay



(Al Saedi *et al*., Pediatrics 1996; 97:100-2)

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Section 1: Choose the right test!

- Know which tests to be "fussy" about
- When do you really need a "gold standard" test?

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When should you order a more complicated "gold standard" assay?

- Most "free T4" assays are not true free T4 assays, but they're good enough in many cases
  - Most tend to have a slight low bias
  - If you know your patient has unusually high or low (or absent) TBG, you probably don't want to use these assays
- You can reserve the more complicated (and more expensive, with a longer turnaround time) "free T4 by dialysis" assay for the difficult cases

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Section 1: Choose the right test!

- Know which tests to be "fussy" about
- When do you really need a "gold standard" test?
- How do you approach insurance companies who will only cover the cheapest test (even if it's the wrong one!)

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Getting insurance companies to cover "the right tests"

- Insurers usually want to do the right thing, but need "proof"
- If you get more than one denial for a particular test, it's time to prepare a "justification packet" that can be used for all future appeals
- These packets can be prepared by a team of interested clinicians *and* your lab(s)
- Be sure to send this to the *medical director* of the insurance company/payor

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Section 2: Help your lab avoid “QNS”

** When you have a sample you suspect may not have enough volume, call the lab (don’t hesitate to ask for the lab director) to alert them of this issue. If they know ahead, they may be able to work wonders

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Section 2: Help your lab avoid “QNS”

- Ask your lab to consider “pediatric-friendly” methodologies

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- Ask your lab to consider “pediatric-friendly” methodologies
- Ask your lab to establish ‘absolute pediatric minimum volumes’

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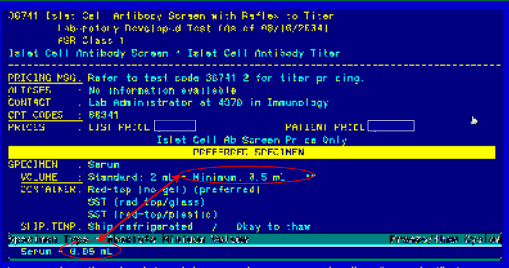
Labs should establish “absolute [pediatric] minimum volumes”



But remember: the absolute minimum volume may only allow “one shot” at a result – if, for example, the assay doesn’t meet quality control requirements, you may not get a result.

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Section 2: Help your lab avoid “QNS”

- Ask your lab to consider “pediatric-friendly” methodologies
- Ask your lab to establish ‘absolute pediatric minimum volumes’
- Ask your lab to validate special procedures to minimize the chances of a “QNS” outcome

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Special procedures to minimize “QNS” outcomes

- If the lab knows ahead that the specimen may be low volume, they can use special handling procedures
- Diluting a sample can help
  - You’ll lose some sensitivity at the lower end
  - The lab has to “validate” formally that a dilution is OK before they can do this and report a result
- The lab may know of alternate methods that don’t require as much sample volume
- The lab may need to consult with you to prioritize which tests/results are the most important to try for

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Section 3: Are my patient's results correct?

- Could there be antibody interference? What do I ask my lab to do to check for this?

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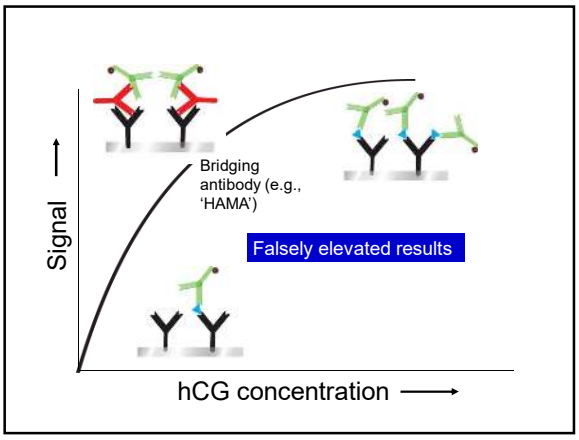

Antibody interference: practical aspects

When should one consider the possibility of interference?

- When the results aren't clinically concordant
- *When you're going to make a critical clinical decision* on the basis of a single immunoassay

What can one do?

- Fastest: ask your lab to have the sample re-tested using a different manufacturer's platform (e.g., Beckman vs Siemens)
- If available, have the lab re-test using a "HAMA" (heterophilic antibody) blocking agent prior to assay
- I do not see great value in assays that measure HAMA
- Some labs may be willing to assay serial dilutions (if validated)
- As mass spectrometry becomes more available for some proteins, consider comparison using this different method – less prone to interference from HAMA and autoantibody

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Section 3: Are my patient's results correct?

- Could there be antibody interference? What do I ask my lab to do to check for this?
- Why it's critical to contact your lab ASAP if you're not sure of a result

Because if the lab still has remaining sample, they can do all kinds of additional tests to corroborate the original result. Most of the specialized testing labs keep the sample for an addition 2-4 weeks, but there may be pressure in the future to reduce this retention period

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Section 3: Are my patient's results correct?

- Could there be antibody interference? What do I ask my lab to do to check for this?
- Why it's critical to contact your lab ASAP if you're not sure of a result
- What is the issue with biotin supplementation and lab results?

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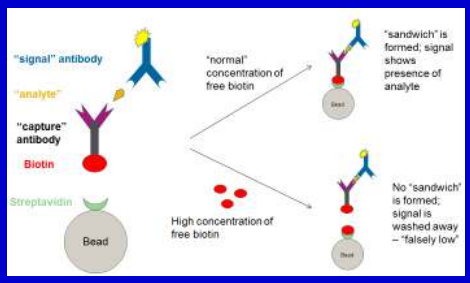
Why biotin interferes with (some) immunoassays



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Potential biotin interference: a summary

- High levels of biotin (associated with mega-dosing) *might* cause:
  - Falsely low results for some (not all) "sandwich" immunometric assays using biotin-streptavidin capture mechanisms
    - Typical: assays for larger molecules (PTH, TSH, LH, PRL, Tg, PSA)
  - Falsely high results for some (not all) "competitive" immunoassays
    - Typical assays for small molecules like thyroid hormones, steroids, or vitamin D metabolites
- Again, this applies only to immunoassays – and not all of them
  - E.g., Roche Elecsys platform assays are affected, but the Siemens Immulite platforms assays are not – check with the labs that you use.
- Half-life of biotin is only 2 h, but to be safe, it's recommended to be off biotin supplements for 72h before an affected immunoassay is done.

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Bonus: Research & Regulatory considerations

- Why it's so important for clinical research to plan properly about what assays you're going to use, and how you're going to collect samples…
- Regulations that limit what a lab can do for you

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Bone marker Blunders

- An investigator wants to measure bone formation as a therapeutic marker
- Osteocalcin is chosen because its assay is more available than BSALP
- Samples are collected from patients attending both AM and PM clinics
- The decision is made to measure full-length osteocalcin, not fragments

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Bone marker Blunders: epilogue

- The data show no consistent effect of the therapy on bone formation markers, and the clinical trial is scrapped
- The following year, another group demonstrates a clear positive effect of the same therapy on bone formation markers in a high impact journal
- What happened with the first study?
  - While both osteocalcin and BSALP show a diurnal rhythm, BSALP has a longer half life and less total variation in a day
  - Osteocalcin collection should therefore have been standardized to one time of day
  - The decision to use full-length osteocalcin, which only has a 5 minute serum half-life, meant that the speed of processing the samples affected the measured results!

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Moral of the story!

Find an assay expert and call her/him *before* you start your research, not after….

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Bonus: Research & Regulatory considerations

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Regulatory restrictions on what a lab can do for you…

The lab can't…

- …do anything that could be viewed as giving away free testing in exchange for business due to "anti-kickback" legislation
  - However, the lab is entitled to do corroborative testing (sending out a sample for additional testing), since this is part of ensuring an accurate result
- …do any maneuvers (like dilutions) unless they've been previously 'validated'
- …test samples that are out of official stability, unless the laboratory director has other evidence that the results will be accurate
- …test sample types that have not been formally validated (e.g., testing PTHrP in breast milk)

**Note: "Research" (as most of us define it) doesn't exempt you or the lab from these requirements

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Prelude: Ambitious Accuracy Assumptions

A fingerstick test for over 200 different substances → $9B valuation

What challenges would you expect for the following tests?

ACT1	ACTH (Corticotropin)
AND1	Androstenedione
EST2	Estradiol
IGF1	IGF-1 (Insulin-like Growth Factor 1)
TFS1	Testosterone, Free
TES2	Testosterone, Total
FT4	Thyroxine, Free (Free T4)
VTD1	Vitamin D 25-OH

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Most Important Take-home Messages

- Learn what you can about the tests you use most often, so you know how to get the right tests for your patients
- Always consider common interferences (antibody interference, substances like biotin) in your differential diagnosis
- Identify a "go-to" lab person (at your hospital and at the outside labs you use) to help you with test selection and with trouble-shooting/confirmation
- Contact the lab quickly when you need to confirm a result, or have doubts (maybe even before the sample arrives, if you're trying to avoid a QNS)

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Questions?

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