12th European Bioanalysis Forum Open Symposium

20-22 November 2019

Launchpad

Inspire: Biomarker Assay Validation – are we ready for launch?

Biomarker Assay Validation?
BRINGING CONTEXT OF USE INTO PRACTICE

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IMMUNOLOGIX LABORATORIES
Designed by Scientists, Run by Scientists

Scope

- Biomarkers in Perspective
- Assay performance and validation vs Same COU
  (for different biomarkers)
- Assay performance & validation vs Changing COU
  (for same biomarker)
- Conclusions

Setting the Scene: Biomarker History in Perspective!

- Its not a new Science! - Earliest recording – **4000BC**
- Written ID of “Biomarker”, “COU” and “genetic” link – **2nd Century AD**
- “Validation” of several quantitative methods dates back to **1904**
- International Validation recommendations in biomarker assays – **1960’s**
- Guidance in Bioanalysis - **2001**

Biomarker Assay Validation? Bringing Context of Use into practice

Important Messages…….

1. Don’t try to reinvent the wheel…

…or suffer the consequences..

2. If it ain’t broke…..don’t fix it

Biomarker Assay Validation? Bringing Context of Use into practice

CRITICAL PATH INSTITUTE

Points to Consider Document:
Scientific and Regulatory Considerations for the Analytical Validation of Assays Used in the Qualification of Biomarkers in Biological Matrices

June 11, 2019

Biomarker Assay Collaborative Evidentiary Considerations
Writing Group, Critical Path Institute (C-Path)

https://c-path.org/wp-content/uploads/2019/06/evidconsid-whitepaper-analyticalsectionv2019.pdf

What exactly, is Context of Use?

- BEST resource 2016:
  - The Context of Use (COU) is “A statement that fully and clearly describes the way the medical product development tool is to be used and the medical product development related purpose of the use”

- Or, more succinctly…
  - Context of Use = The ‘Purpose’ in Fit-For-Purpose

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Context of Use

- PK assays, COU is always the same : fixed validation and acceptance criteria makes sense
- What about when the COU is the same for different Biomarker assays

Assay performance and validation vs Same COU *(for different biomarkers)*

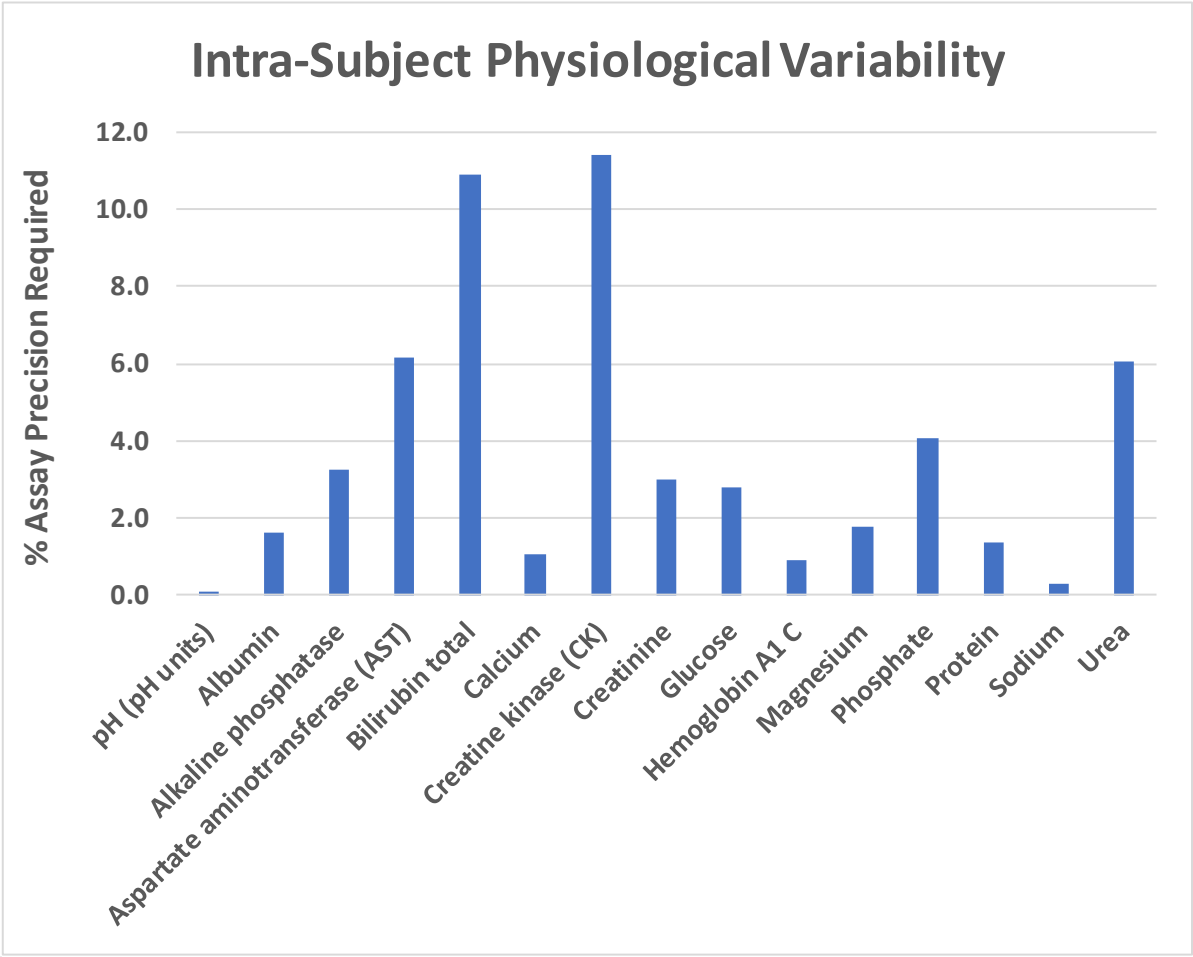
COU = CAN WE DETECT CLINICALLY SIGNIFICANT CHANGE

FACTORS IMPACTING UPON ASSAY PERFORMANCE REQUIREMENTS & ACCEPTANCE:

- **PHYSIOLOGICAL VARIABILITY**

Biomarker Assay Validation? Bringing Context of Use into practice

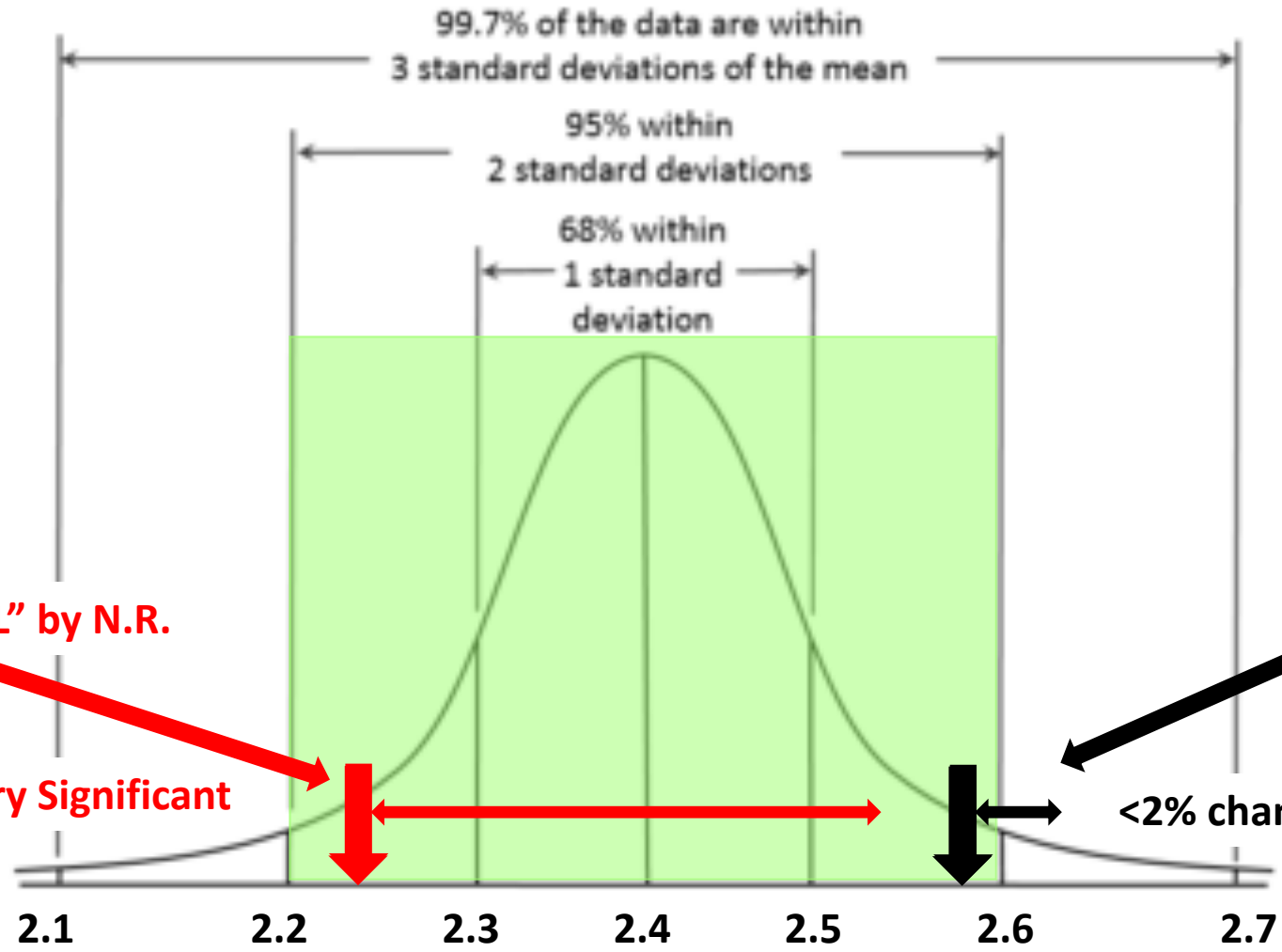
Analyte	Physiological Variability		Assay Performance Requirements	
	Intra-Subject	Inter-Subject	Precision	Total Error
	CVI	CVg	I(%)	TE(%)
pH (pH units)	0.2	---	0.1	---
Sodium	0.6	0.7	0.3	0.7
Calcium	2.1	2.5	1.1	2.6
Protein	2.8	4.7	1.4	3.6
Albumin	3.2	4.8	1.6	4.1
Hemoglobin A1 C	1.9	5.7	0.9	3.0
Magnesium	3.6	6.4	1.8	4.8
Glucose	5.6	7.5	2.8	7.0
Creatinine	6.0	14.7	3.0	8.9
Alkaline phosphatase	6.5	26.1	3.2	12.0
Phosphate	8.2	10.8	4.1	10.1
Urea	12.1	18.7	6.1	15.6
Aspartate aminotransferase (AST)	12.3	23.1	6.2	16.7
Bilirubin total	21.8	28.4	10.9	26.9
Creatine kinase (CK)	22.8	40.0	11.4	30.3



http://www.westgard.com/guest17.htm

Biomarker Assay Validation? Bringing Context of Use into practice

Serum Calcium example



Smallest Clinically Significant Change is a better way of evaluating on a longitudinal basis during a clinical trial than just using NORMAL RANGE.

Flagged "NORMAL" by N.R.

13.3% change – Very Significant

Flagged "HIGH" by N.R.

<2% change = not significant

Biomarker Assay Validation? Bringing Context of Use into practice

Serum IP-10	Serum IL-8	Serum TNFa

Serum IP-10	Serum IL-8	Serum TNFa

Research Biomarkers

Same COU (% reduction)

but vastly different responses to drug effect

= Different assay performance characteristics & acceptance requirements

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Context of Use

- PK assays, COU is always the same : fixed validation and acceptance criteria makes sense
- Biomarker assays

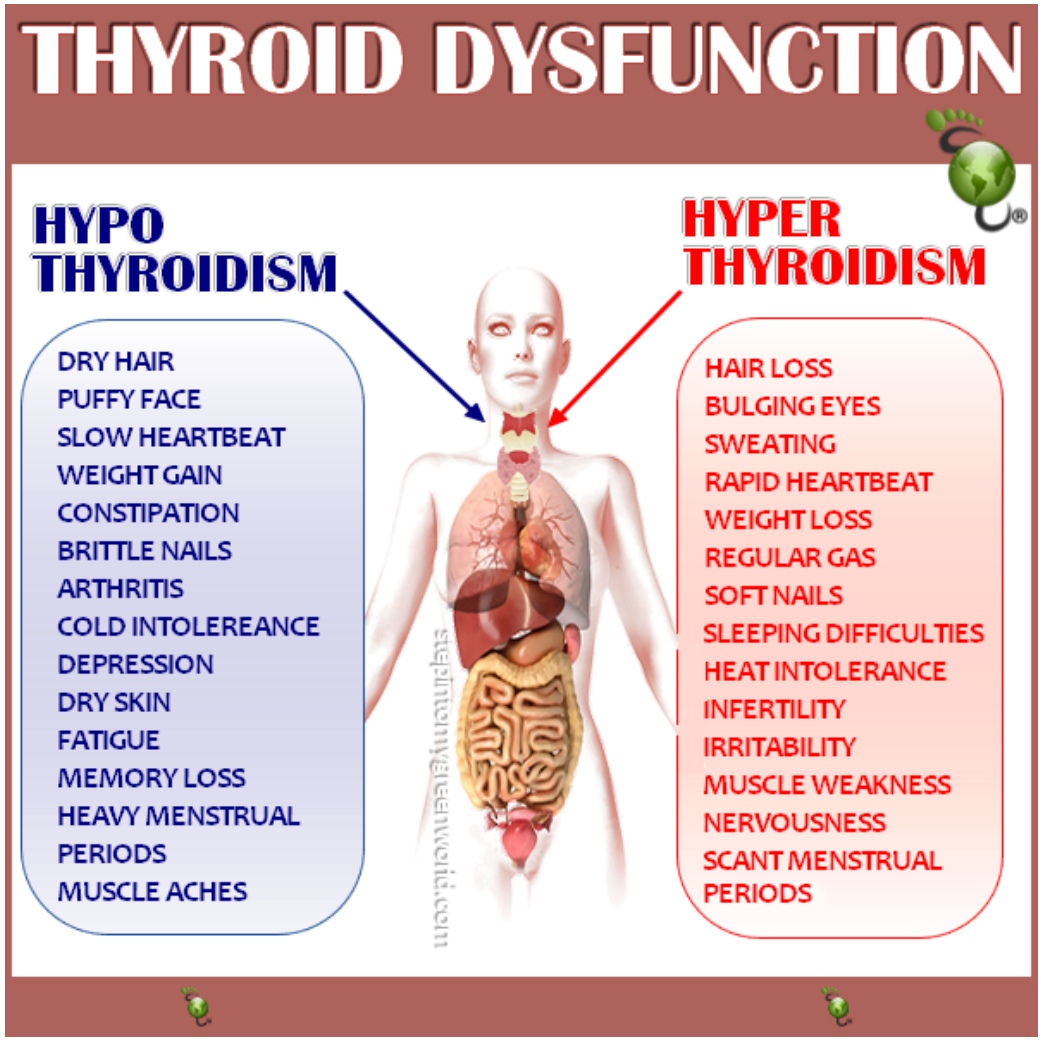
the SAME biomarker being measured…..different COU

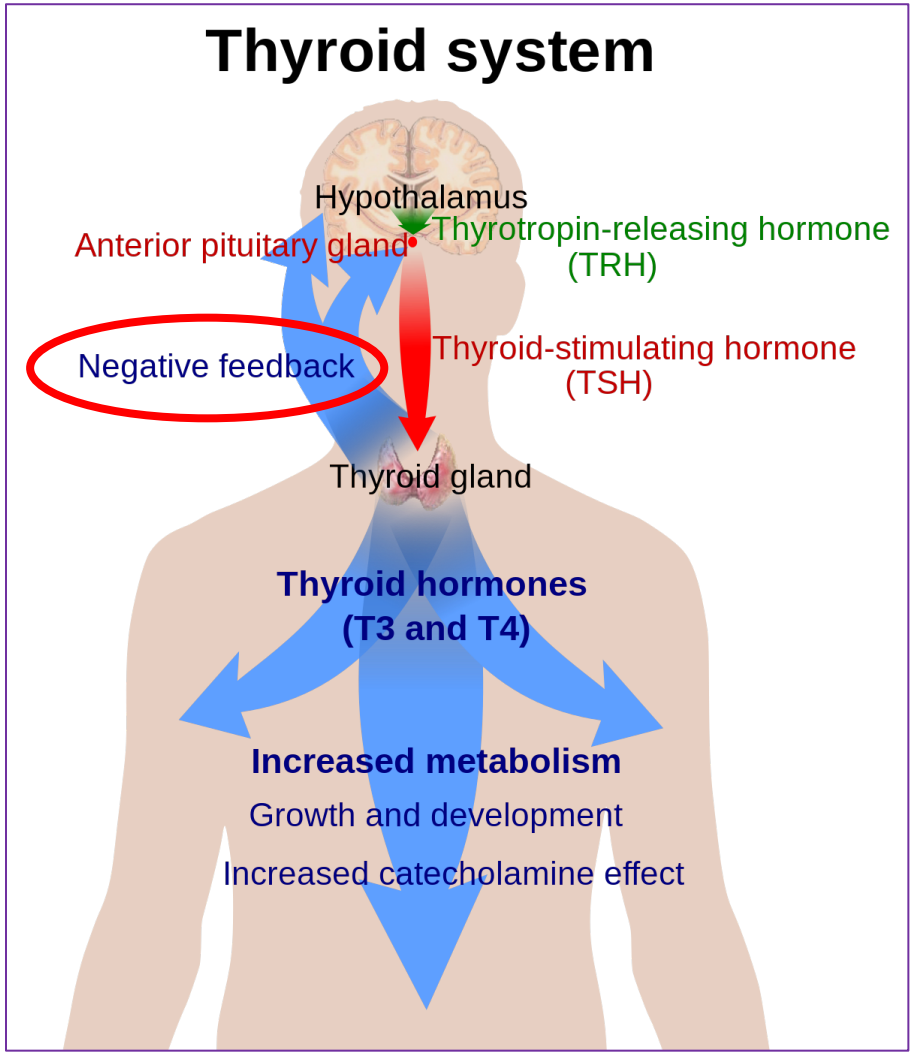
The Evolution of Thyroid Stimulating Hormone Assays:

…..from a diagnostic used to identify hypothyroidism to

delivering Truly Personalised Healthcare/Therapy (achieved late 1980's)

Biomarker Assay Validation? Bringing Context of Use into practice

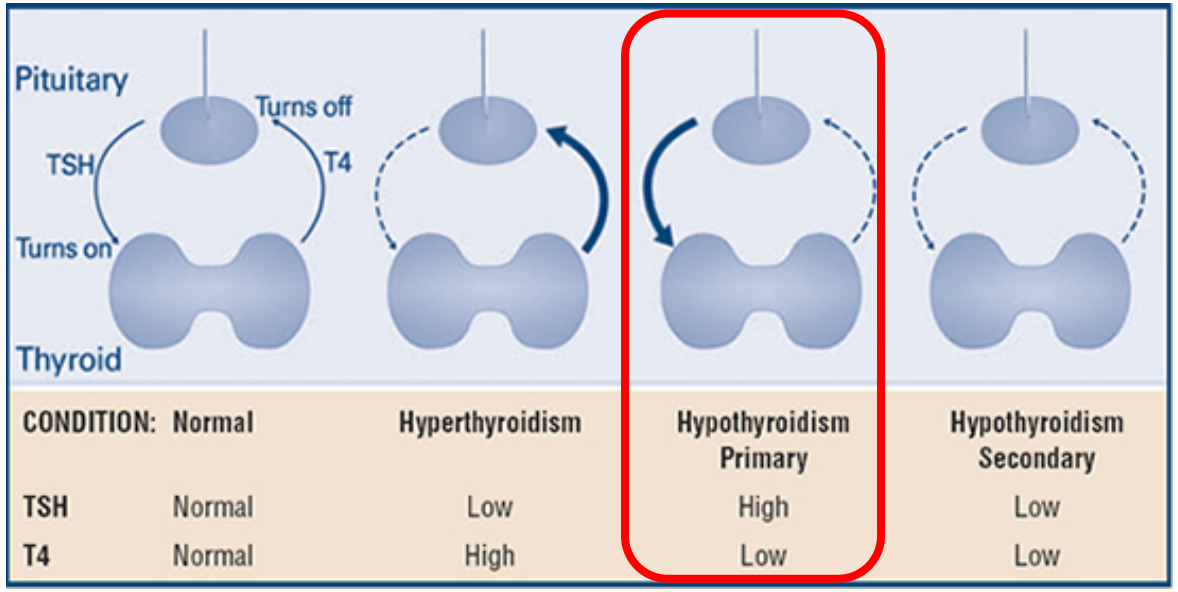




Biomarker Assay Validation? Bringing Context of Use into practice

DATE	DISEASE	TECHNIQUE / BM
1950's	Thyroid Disease	Radio-isotopic Assays (not RIA) – "PBI"

PBI (Protein-Bound Iodine) was really a "proxy" for Total Thyroid Hormones – Thyroxine (Tetra-iodothyronine (T4)) + Tri-iodothyronine (T3)



High T4 = Hyperthyroid
….but could not differentiate sufficiently between NORMALS and **HYPOthyroidism**

Due to NEG feedback on pituitary thought investigating TSH may be more sensitive due to physiological response (↑TSH)

Biomarker Assay Validation? Bringing Context of Use into practice

1960 – 1990 = SAME BIOMARKER (TSH), EVOLVING COU'S

1950's	1960's	1970's	1980-90's
Analytical Methods			
PBI (T4+T3)	TSH – 1st Gen. (Sens = 1 – 2 mIU/L)	TSH – 2nd Gen (Sens = 0.1 – 0.2 mIU/L)	TSH – 3rd Gen (Sens = 0.01 – 0.02 mIU/L)
Radio-isotopic (not "RIA")	CPB (pAb & radiolabel)	Sandwich RIA & EIA 2 x pAb's → pAb + mAb	FIA, new EIA, CIA mAbs

Changing Critical Reagents = ALL new versions re-validated for COU's already in use and new specific COU's

Biomarker Assay Validation? Bringing Context of Use into practice

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1950's	1960's	1970's	1980-90's
Analytical Methods			
PBI (T4+T3) Radio-isotopic (not "RIA")	TSH – 1st Gen. (Sens = 1 – 2 mIU/L) CPB (pAb & radiolabel)	TSH – 2nd Gen (Sens = 0.1 – 0.2 mIU/L) Sandwich RIA & EIA 2 x pAb's → pAb + mAb	TSH – 3rd Gen (Sens = 0.01 – 0.02 mIU/L) FIA, new EIA, CIA mAbs
Context of Use (COU)			
?Hyper / Hypothyroidism	1) Replace T4/T3 as first-line test for Hypo (>5) *(develop data for hyper by studying low concs.)*	2) Differentiate normal & 1˚ hyperthyroid (<0.1) *(continue to develop data at low concs for other possible COU's)*	3) Monitor T4 repl. Rx Guide ↑↓ dose or ↓↓ 4) Monitor suppressive Rx in thyroid cancer 5) Monitor for **2˚ Hypopituitarism**
Method Validation			
	QC @ 5 & "HIGH" PARA, LOD, Stability LQC- IACV @ 1 & 2 ≤20%	QC @ 4 & 5 & "HIGH" PARA, LOD, Stability IACV @ 0.1 & 0.2 ≤20% Repl. Rx interference	QC @ 4 & 5 & "HIGH" PARA, LOD, Stability Add QC @ 0.1 IACV @ 0.01 & 0.02 ≤20% Repl. Rx interference Suppressive Rx interference

Biomarker Assay Validation? Bringing Context of Use into practice

Context of Use (COU)

?Hyper / Hypothyroidism	1) Replace T4/T3 as first-line test for Hypo *(develop data for hyper)*	2) Differentiate normal & 1˚ hyperthyroid (continue to dev. data)	3) Monitor T4 repl. Rx Guide ↑↓ dose 4) Monitor suppressive Rx in thyroid cancer 5) Monitor for **2˚ Hypopituitarism**

COU - ?Drug Development

detect presence of Biomarker X (yes/no)	analytically detect qualitative changes in level of Biomarker X (identify trends)	analytically detect ≥2-fold changes in levels of Biomarker X (relative quantification)	detect clinically meaningful changes in levels of Biomarker X (clinically qualified)

Validation - ?Drug Development

Fully validated to each specific C.O.U.

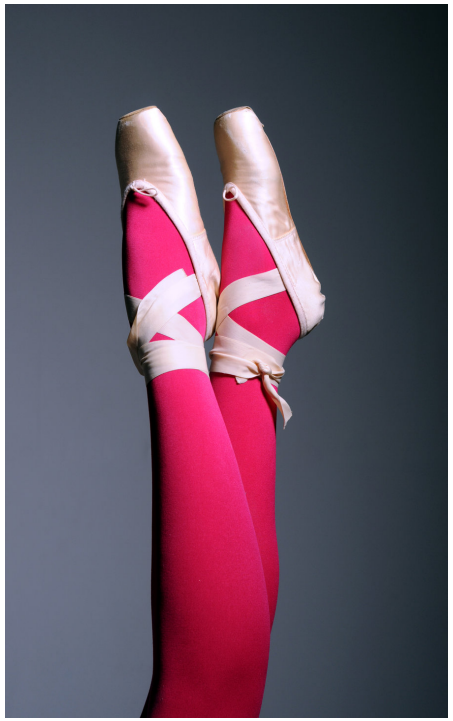
So : FFP and COU….

A picture is worth a thousand words…….

….Courtesy of Lauren Stevenson

Biomarker Assay Validation? Bringing Context of Use into practice

Fit-for-Purpose…



COU is specific…

…COU is the “P” in FFP

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Fit-for-Purpose…








"Fully Validated Assay"

Without Context of Use

- Bring the right ball to the game
- Biomarkers require sound science

- Not divination

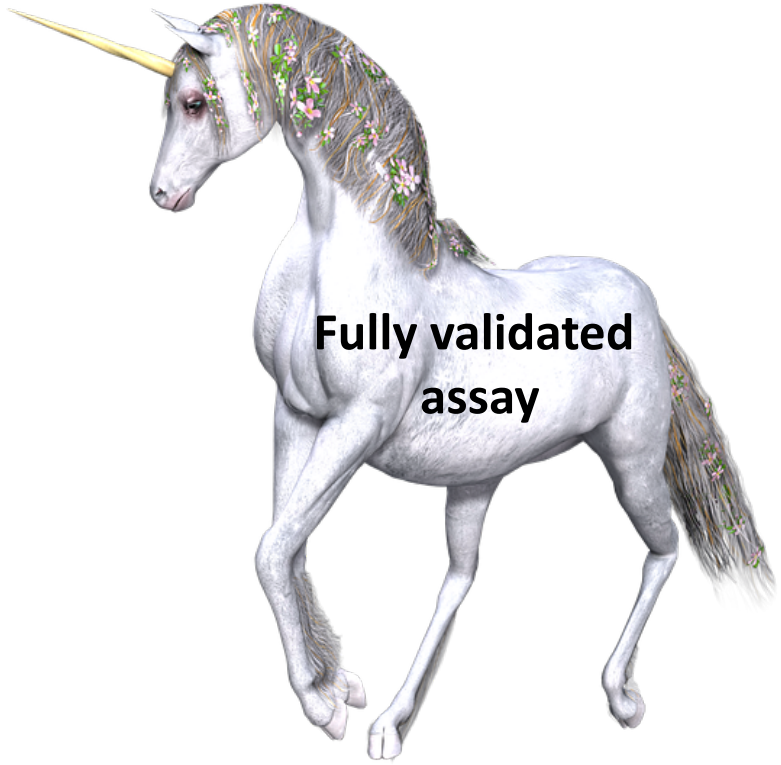
"Fully Validated Assay"

Questions

- Is a fully validated assay not fit-for-purpose?
- Is there an assumption that a 'fully validated assay' fits all purposes?

Without context of use…There is no such thing as a fully validated assay

Without context of use….a standalone 'full validation' is merely an analytical characterization of the assay, against which proposed contexts would later need to be applied



Mythical Creature

Scope

- Biomarkers in Perspective
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- Assay performance & validation vs Changing COU *(for same biomarker)*
- Conclusions

Context of Use is REQUIRED to "Fully" Validate your assay

- Context of Use = The 'Purpose' in Fit-For-Purpose
- Fit-for-Purpose ≠ lesser quality
- Fit-for-Purpose = exquisitely suited for the Purpose (COU)

Biomarker Assay Validation? Bringing Context of Use into practice

- All biomarker assays should be (fully) validated for their COU, regardless of their application
  - 'Exploratory' is not a context of use
  - COU: Specifically, how will the data be used? Starts with a question…

Question ⇨ COU ⇨ Assay development & validation

With Context of Use: Fully validated to….

COU 1: Fully validated to detect presence of Biomarker X (yes/no)

COU 2: Fully validated to analytically detect qualitative changes in level of Biomarker X (identify trends)

COU 3: Fully validated to analytically detect ≥2-fold changes in levels of Biomarker X (relative quantification)

COU 4: Fully validated to detect clinically meaningful changes in levels of Biomarker X (clinically qualified)

The Challenge of Biomarker Assay Guidance

- Consider:
  - Why is there separate guidance for BMV (PK) and Immunogenicity?
  - Their contexts of use are different and specific
  - The assay validations are customized for their COUs
- So….do we need guidance for each biomarker assay COU?

Avoiding Misguided Guidance

- We need to think about this differently

- Prescriptive approaches with a priori acceptance criteria cannot address the needs of numerous and varied COUs

- Let science be our guidance
  - Start with questions
  - Demand and define COU
  - Build and properly characterize the assay
  - Justify its suitability

Fully validated for COU

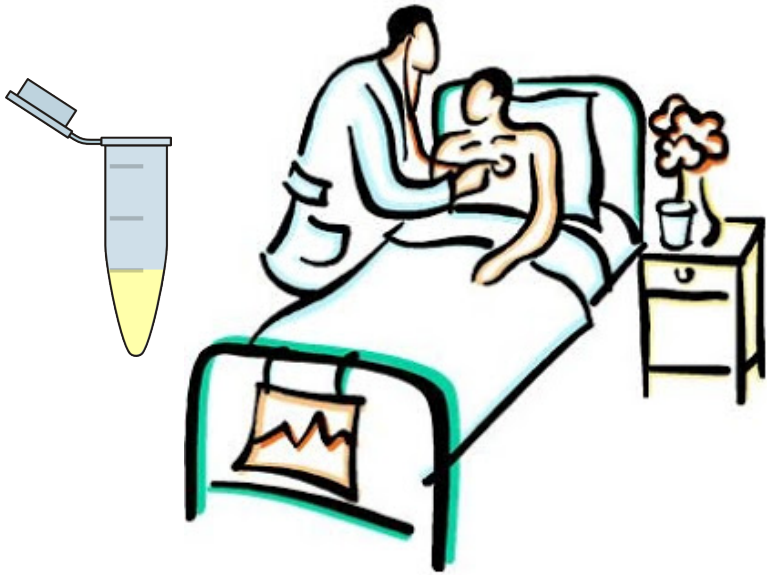
Conclusions…

- Demand COU: No context, no assay
- Without COU there is no such thing as a fully validated biomarker assay
  - Analytical characterization only
- Let science be our guidance – considerations not criteria

Stop looking for this
It's also mythical

Important messages….

1) Biomarker Science - Its not new
2) Ensure good science – be a Biomarker Scientist
3) Learn about true analytical QC
4) NEVER forget about the patients



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- Westgard Multirules QC - https://www.westgard.com/mltirule.htm

Biomarker Assay Validation? Bringing Context of Use into practice

Critical thought partners: Lauren Stevenson (ILX) & Devangi Mehta (ILX)

If there's no scientific rationale, it's not science

Demand Context of Use