

**Calibration and Specificity:
Fit-for-Purpose
Method Development
for Successful Biomarker
Measurement**

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Measurement of biomarker A in matrix B



Validated assay ↔ method set-up

Immunoassay kit ↔ individual reagents

Reference material is essential

Topics

1. Certified standard is not available
2. Certified standard is available
3. Matrix effect

Certified standard is not available

AMH = Anti-Müllerian Hormone

- Endogenous glycoprotein
- Levels drop following puberty
- Application: investigation ovarian reserve
- Biomarker

Analysis method: ELISA

Manufacturer kit: DSL

Example: AMH kit

ENGLISH

11016-B / 2008 APR 30

ACTIVE[®] MIS/AMH ELISA
DSL-10-14400

Revision date: May 1, 2006 p.

FOR RESEARCH USE ONLY--NOT FOR USE IN DIAGNOSTIC PROCEDURES

These instructions for use (IFU) are intended for Professional Use and must be read completely before product use.

I. INTENDED USE

The DSL-10-14400 ACTIVE[®] Mullerian Inhibiting Substance/Anti-Mullerian Hormone (MIS/AMH) Enzyme-

IV. REAGENTS SUPPLIED

A. Anti-MIS/AMH-Coated Microtitration Strips:

One strip holder, containing 96 microtitration wells with anti-MIS/AMH IgG immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

B. MIS/AMH Standard A/Sample Diluent:

One vial, 20 mL each, labeled A, containing concentrations of 0 ng/mL MIS/AMH in a protein-based buffer with a non-mercury preservative. Refer to vial labels for exact concentrations. Store unopened at 2-8°C until the expiration date.

C. MIS/AMH Standard B-G:

Six vials, 0.5 mL each, labeled B-G containing concentrations of approximately 0.05, 0.10, 0.25, 1.8, 7.5, and 15 ng/mL MIS/AMH in a protein-based buffer with a non-mercury preservative. Refer to vial labels for exact concentrations. Store unopened at 2-8°C until the expiration date.

D. MIS/AMH Controls:

Two vials, 0.5 mL each, labeled Levels I and II containing low and high concentrations of MIS/AMH in a protein-based buffer with a non-mercury preservative. Refer to vial labels for exact control ranges. Store unopened at 2-8°C until the expiration date.

Certified standard is not available

- One kit manufacturer
- Recombinant kit standard
- No kit independent control

Considerations

Is assay fit-for-purpose?



Intended use of the assay?



Measure biological variability of
study population

Immunoassay for biomarker measurement

- Precision important
- Within-run + Run-to-run variation
- Kit lot-to-lot variation
 - Choice of QC-samples
 - Pool for monitoring kit lot variation
 - Bridging

Certified standard is available

- Significant differences exist between calibration standards
- Reference ranges
- Impact?

Insulin: Measurement of control samples by kit standards and common standards ($\mu\text{IU/mL}$)

Kit	STD	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5
RIA 1	KIT	82.6	74.0	65.9	96.5	97.8
	WHO	46.3	40.5	36.2	48.2	42.8
RIA 2	KIT	39.2	45.1	55.7		71.4
	WHO	54.1	43.4	48.7		52.0
RIA 3	KIT	49.8	53.2	49.5		
	WHO	47.8	49.5	53.3		
RIA 4	KIT	53.9	53.5	61.4	54.2	
	WHO	48.2	45.4	47.6	50.2	
RIA 5	KIT	49.8	74.6	63.5		
	WHO	52.7	54.6	46.7		
RIA 6	KIT	42.8	52.6	41.1		
	WHO	48.2	49.9	48.8		

Range kit STD: 41.1 – 97.8

Range WHO STD: 36.2 – 54.6

Source: Clinical Chemistry, Vol. 28, No. 12

Precision

Insulin: Results of analysis of variance

Variation	KIT STD	WHO STD
Between kit	23.0%	6.2%
Within kit	18.2%	7.6%

Conclusion

- Between-kit variation reduced by using World Health Organisation standard
- Future: Standardisation of assay kits

Today: impact of different standards

- Central lab

- All samples of one study analysed in one lab

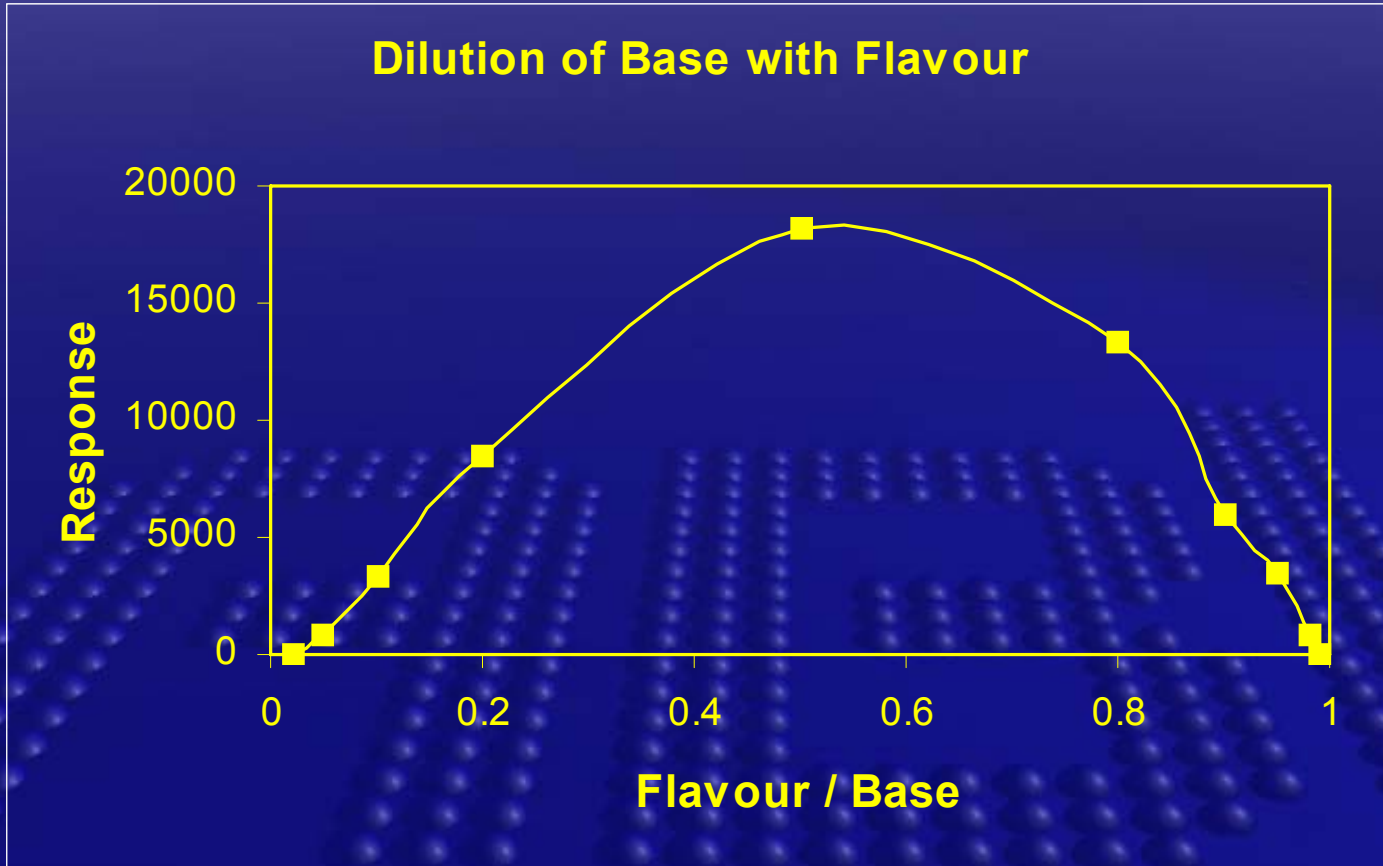
- Assay fit-for-purpose

- Measure intra- and inter-subject variation

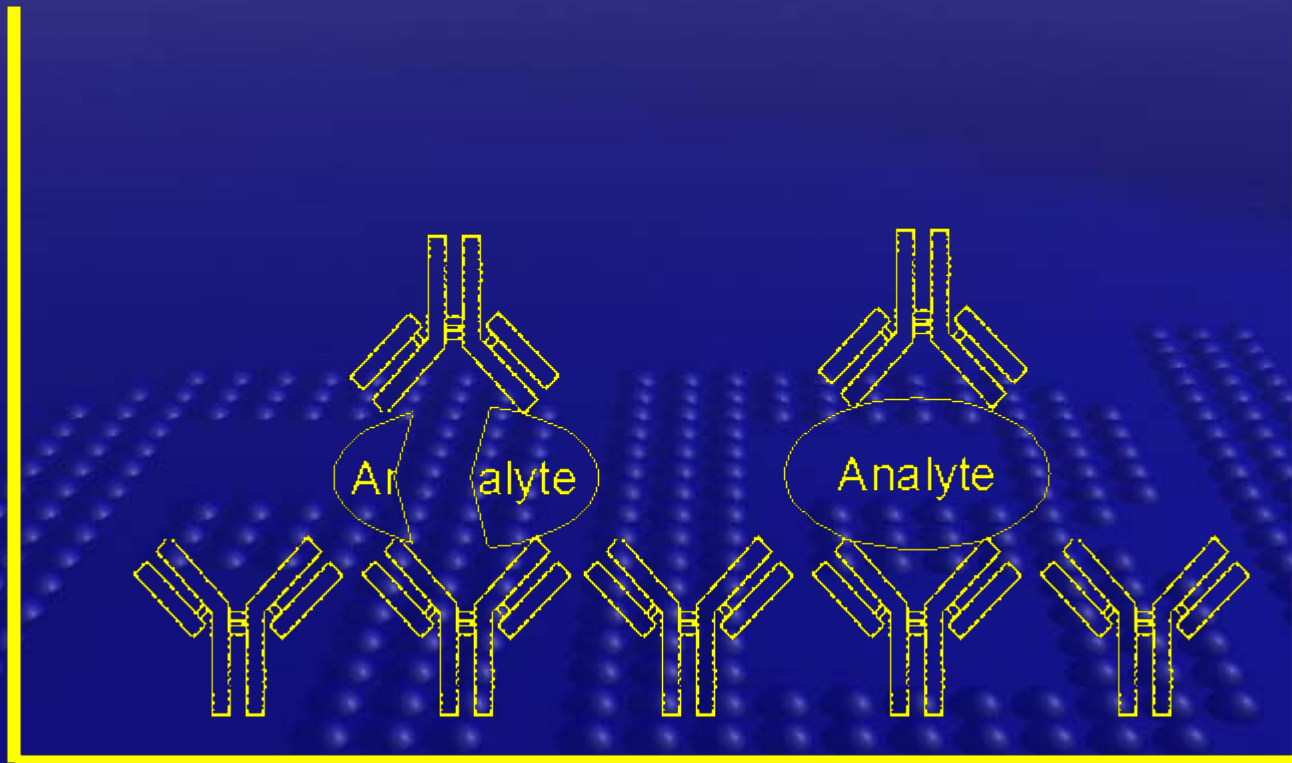
Matrix effect

- Determination of analyte (18kD) in product
- Preferred situation: analyte not present in matrix
 - 1) Product = base + flavour
 - 2) Base
 - 3) Flavour

Dilution



False positive response



False positive response

Action 1: Filtration (filter 10kD)

- Result: high response measured
- Conclusion: false positive response measured

Action 2: Dialysis (membrane 8kD)

- Result: after dialysis specific signal $<$ LLOQ (pH)
- Conclusion: after dialysis degradation of complex structurally similar to analyte

Immunoassay for biomarker measurement

- RIA / IRMA
- EIA / ELISA
- FIA / IFMA
- Time Resolved FIA
- CLEIA

Sensitive methods



Summary

Intended use of the assay:

- Pharmacokinetic / Pharmacodynamic

Biomarker:

- Measure intra- and inter-subject variation

Example AMH + Insulin:

- Standardisation calibration

Example matrix effect:

- What is the assay detecting
- Know your biological system

Conclusion

Immunoassay for biomarker
measurement

Measure intra- and inter-subject
variation



Assay is fit-for-purpose