BIOMARKERS ASSAY DEVELOPMENT: DO YOU KNOW YOUR CONTEXT OF USE?

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EVERY STEP OF THE WAY

WHAT'S GOING TO BE PRESENTED?

1. Introduction
2. Define the Context of Use (COU)
3. Case studies
   - COU defined, unsuccessful project
   - COU defined, successful project
   - COU unknown

INTRODUCTION

Team of scientists

- Develop/validate ligand-binding assays (LBA) to quantify biomarkers
- Oversee biomarker sample analysis study phase
- Collaborate with clinical pathologists and clients to provide input on study design and assay development

CONTEXT OF USE

In LBA development, the COU helps guide the design of the method in 2 ways:

What do we want to achieve (Objective)?

- Detect Increases/Decreases?
- Detect slight or substantial variations in analyte concentration?

Limitations

- Sample type
- Species
- Quantity of sample available
- Required sensitivity
- Timeline

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CASE STUDIES

CASE STUDY 1: D-DIMER IN RAT PLASMA

Objective: Develop method to quantify increases in D-Dimer concentration in rat as a safety biomarker

Communication:

- Timelines: 14 weeks before start of study
- Lack of communication during method development

Limitations:

- Volume: 100 µL
- No Rat specific kit from reputable vendor

CASE STUDY 1: D-DIMER IN RAT PLASMA

Kit 1: Human D-Dimer kit from Abcam (ELISA)

Limitation:

- No Rat specific kit from reputable vendor

➢ **Issue:**

- Antibodies did not cross-react. All results were <LLOQ

CASE STUDY 1: D-DIMER IN RAT PLASMA

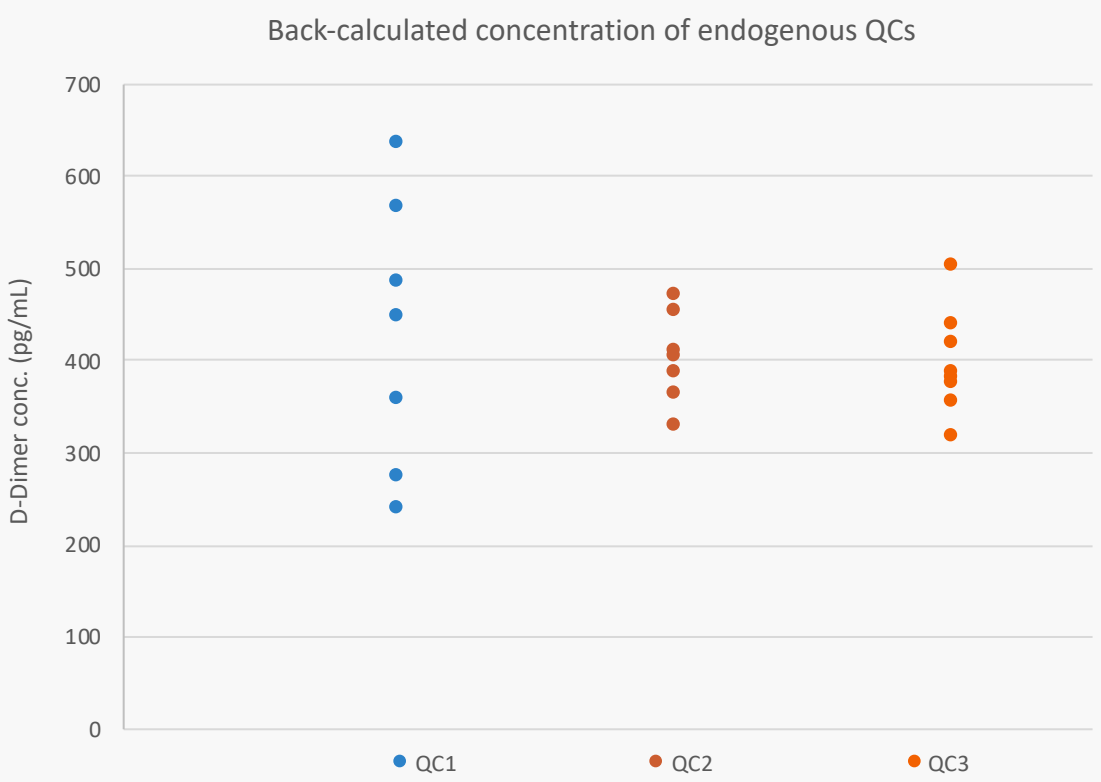
Kit 2: Rat specific D-Dimer kit from Kamiya Biomedical (ELISA)

Limitation:

- No Rat specific kit from reputable vendor. Quality of kit reagents?

➢ **Issue:**

- Inter-assay accuracy was terrible



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CASE STUDY 1: D-DIMER IN RAT PLASMA

Kit 3: Asserachrom® D-Dimer kit from Stago (ELISA)

Limitation:

- **No Rat specific kit from reputable vendor**

➢ **Human kit cross-reacted:**

- Range of endogenous values: 1.48 to 3.11 ng/mL

CASE STUDY 1: D-DIMER IN RAT PLASMA

Kit 3: Asserachrom® D-Dimer kit from Stago

Limitation:

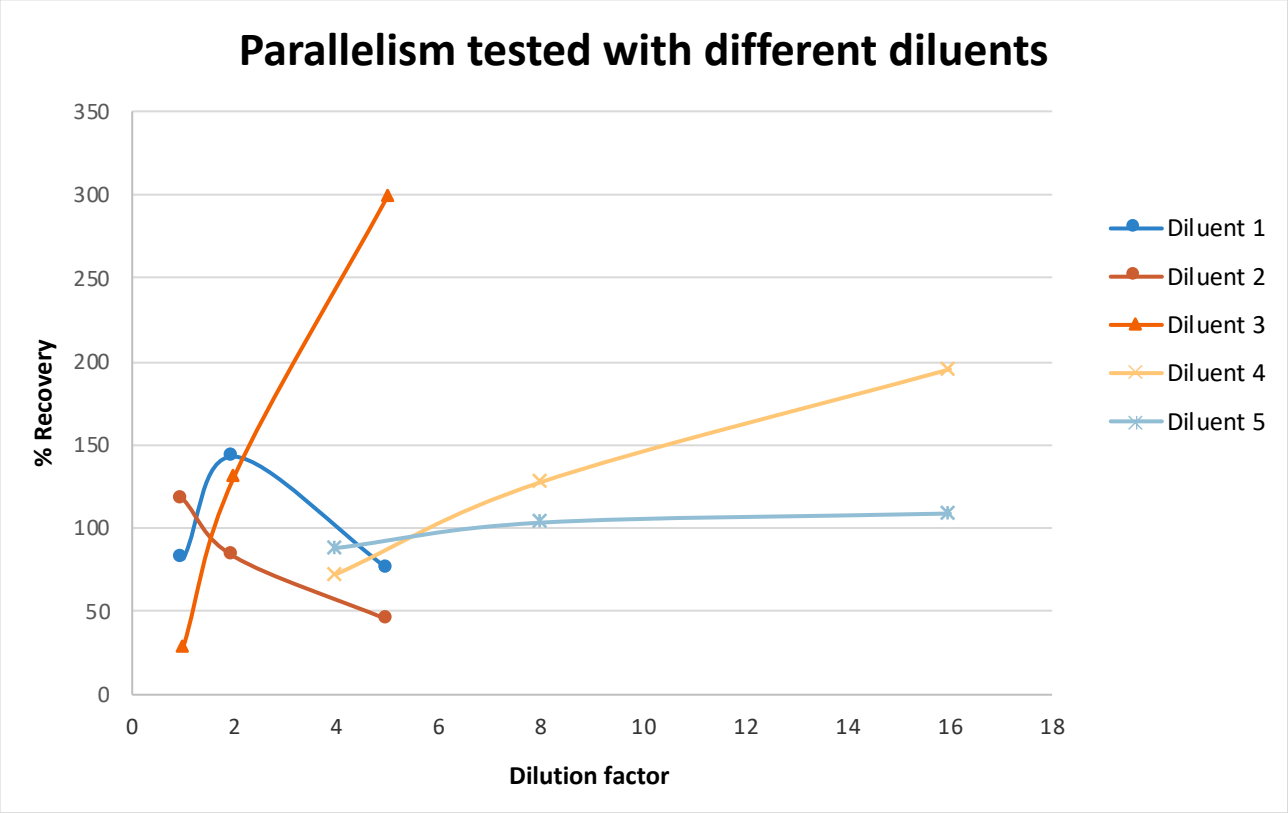
- **Volume: 100 µL**

➢ **Issues:**

- Volume too high: 200 µL/well - method modified to load 50 µL/well
- High %Difference between duplicate wells
- No parallelism proven with diluent provided with the kit
- After almost a year, no method was validated

CASE STUDY 1: D-DIMER IN RAT PLASMA

Kit 3: Asserachrom® D-Dimer kit from Stago



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CASE STUDY 1: D-DIMER IN RAT PLASMA

Additional development was done internally:

- 200 µL/well used- decrease in %Difference between duplicate wells
- Parallelism proven from 4 to 8-fold
- Volume required: 120 µL for duplicate analysis

CASE STUDY 2: COMPLEMENT FACTORS IN HUMAN AQUEOUS HUMOUR

Objectives: Develop the following methods for quantification of substantial increases in human aqueous humour

Ba fragment	Total C5	Factor B	Total C3
C3a fragment	C5a fragment	Factor H	Factor I

Communication:

Weekly with scientists

Limitations:

- **Sample type: Aqueous Humor from Live Patients**
- **Volume: 20-25 µL**
- Timelines: 10 weeks before sample analysis

CASE STUDY 2: COMPLEMENT FACTORS IN HUMAN AQUEOUS HUMOUR

Limitation:

- **Sample type: Live Patients Aqueous Humor (AH)**

➢ **Issues:**

- Matrix available for qualification: Cadaver AH vs Live Patients AH for sample analysis.
- Cadaver AH analyte concentration > Live Patients AH
- Parallelism range qualified not suitable to analyze Live Patients AH

CASE STUDY 2: COMPLEMENT FACTORS IN HUMAN AQUEOUS HUMOUR

Biomarker	Method	Cadaver AH (ng/mL)	Live Patients AH (ng/mL)
Ba fragment	ELISA	74.65	18.34
C3a fragment		308.51	6.62
C5	Luminex® (Panel 1)	505.31	41.83
C5a		0.20	0.02
Factor I		983.15	229.11
Factor B	Luminex® (Panel 2)	2995.23	530.22
Total C3		50176.33	1538.32
Factor H		1659.84	97.80

4 to 46-fold Decrease

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CASE STUDY 2: COMPLEMENT FACTORS IN HUMAN AQUEOUS HUMOUR

Limitation:

- **Sample type: Live Patients Aqueous Humor (AH)**

➢ **Solutions:**

- In-study parallelism assessment
- Better Minimal Required Dilution

Biomarker	Cadaver AH Parallelism (Fold)	Live Patients AH Parallelism (Fold)
Ba fragment	150 to 2000	40 to 2560
C3a fragment	50 to 1600	40 to 1600
C5	10 to 20	5 to 20
C5a	10 to 30	5 to 30
Factor I	10 to 30	5 to 640
Factor B	300 to 4800	100 to 4800
Total C3	2400 to 19200	100 to 19200
Factor H	150 to 4800	25 to 4800

CASE STUDY 2: COMPLEMENT FACTORS IN HUMAN AQUEOUS HUMOUR

Limitation:

- **Sample Volume: 20-25 µL**

➢ **Issue:**

- Limited volume to perform analysis on 4 different methods:
  - 2 Luminex® Methods
  - 2 ELISA Methods

CASE STUDY 2: COMPLEMENT FACTORS IN HUMAN AQUEOUS HUMOUR

Limitation:

- **Sample Volume: 20-25 µL**

➢ **Solution:**

- Simultaneous sample analysis using a common dilution buffer

Biomarker	Required Volume (µL)	MRD (Fold)	Required Sample Volume (µL) (Individual)	Required Sample Volume (µL) (Simultaneous)
Ba Fragment	70	5	14	19
C3a Fragment	70	100	2	
Panel 1	225	40	6	
Panel 2	225	40	6	
Total (µL)	N/A		**28**	**19**

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CASE STUDY 3: CTX IN RAT SERUM

Objective: Revalidate the RatLaps C-terminal telopeptide of type-1 collagen ELISA from Immuno Diagnostic Systems (IDS)

- IDS informed us : Capture antibody → Replaced

Communication:

Internal Study

Limitations:

- **Unknown COU**
- **Adequate sensitivity required**

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CASE STUDY 3: CTX IN RAT SERUM

Limitations:

- **Unknown COU**
- **Adequate sensitivity required**

➢ **Issues:**

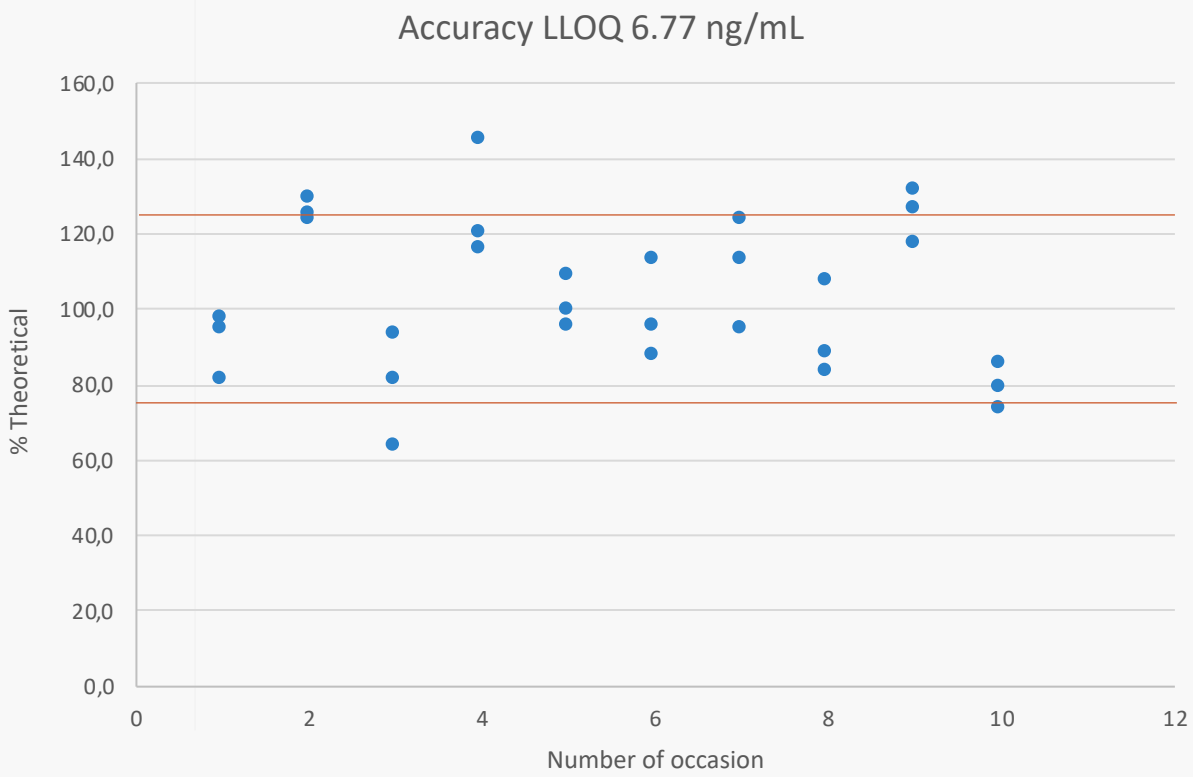
- Assay could be used for safety assessment or efficacy studies
- Increases / Decreases in concentration could be expected
- New capture antibody was less sensitive

CASE STUDY 3: CTX ACCURACY LLOQ

Limitation:

- **Adequate sensitivity required**

- LLOQ previously validated: 2.07 ng/mL
- LLOQ first attempted: 6.77 ng/mL
- AC %Theoretical: 75-125%
- Only 60% occasions met AC
- Next STD: 10.30 ng/mL



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CASE STUDY 3: CTX ENDOGENOUS VALUES IN RAT

Limitation:

- **Adequate sensitivity required**

Historical data range: all samples were quantifiable

Age	n	Average Males (ng/mL)	Average Females (ng/mL)
8 weeks	10	67.66	62.37
19 weeks	15	24.89	13.92
32 weeks	15	19.39	10.46

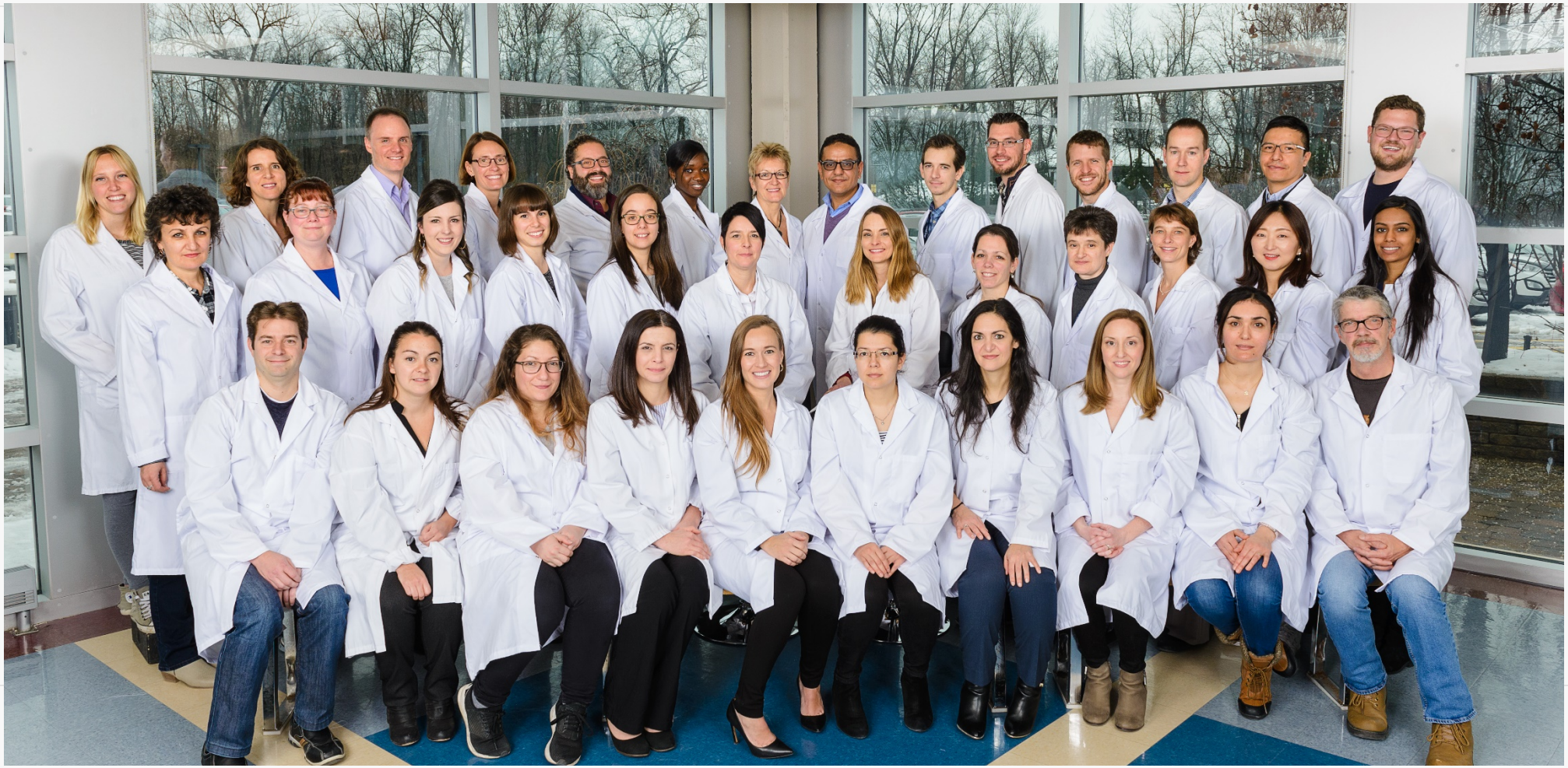
➢ **Solution:**

- AC %Theoretical increased from 75-125% to 70 to 130% at LLOQ level

CONCLUSION

- In LBA development, the COU plays a critical role in determining the design of an assay.
- The extent of the restrictions will greatly impact the ease with which a method can be developed.
- Good communication with the right people will improve chance of success.
- When the COU is unknown, it is important to consider the biological context under which a biomarker is typically investigated.

ACKNOWLEDGMENTS



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