Plasma Renin Activity

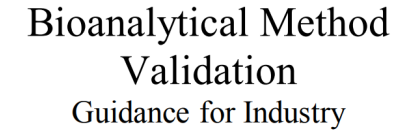
A Non-Standard Approach for a Non-Standard Biomarker Assay

Fit-For-Purpose Validations

EMA 2012

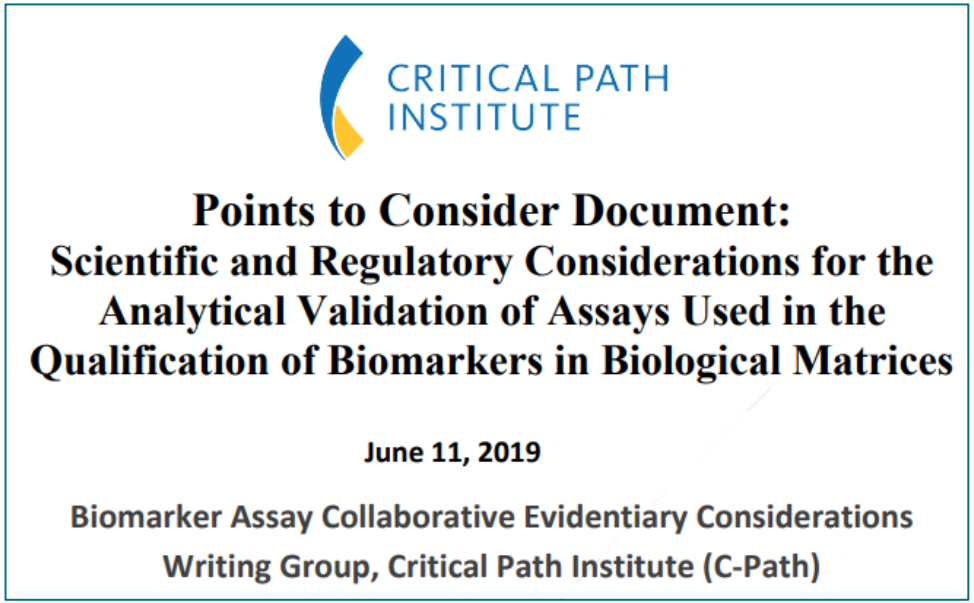
"Methods used for determining quantitative concentrations of biomarkers used in assessing pharmacodynamic endpoints are **out of the scope** of this guideline"

FDA 2018



"Biomarkers can be used for a wide variety of purposes during drug development; therefore, **a fit-for-purpose (FFP) approach** should be used when determining the appropriate extent of method validation"

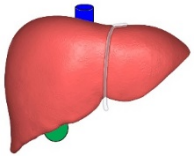
Fit-For-Purpose Validations



"…only the analytical elements directly relevant to the biomarker of interest and its Context of Use (COU) in drug development should be considered"

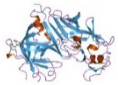
The Renin-Angiotensin-Aldosterone System

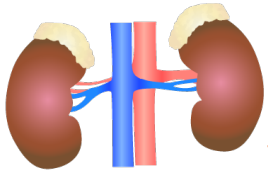
The RAA system is the hormonal system which regulates blood volume, blood pressure and osmoregulation



Angiotensinogen

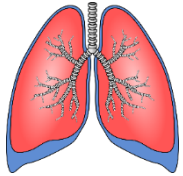
Renin





Angiotensin I

ACE



Angiotensin II

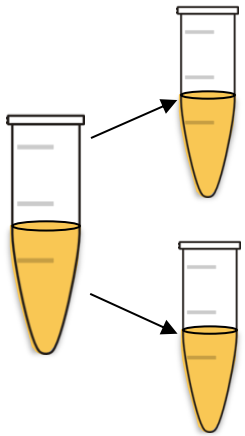
Aldosterone Secretion

Key regulators of:

- Vasoconstriction
- Sodium retention
- Inflammation

Different enzymatic reactions in this system may be the target of drug mediated inhibition for the treatment of cardiovascular and kidney diseases

How do you measure activity?



Incubate aliquot 1 with generation buffer at +4ºC

Incubate aliquot 2 with generation buffer at +37ºC

- Quench
- Sample clean-up
- Quantify Ang I

Activity Sample Incubated at +37ºC

Baseline Sample Incubated at +4ºC

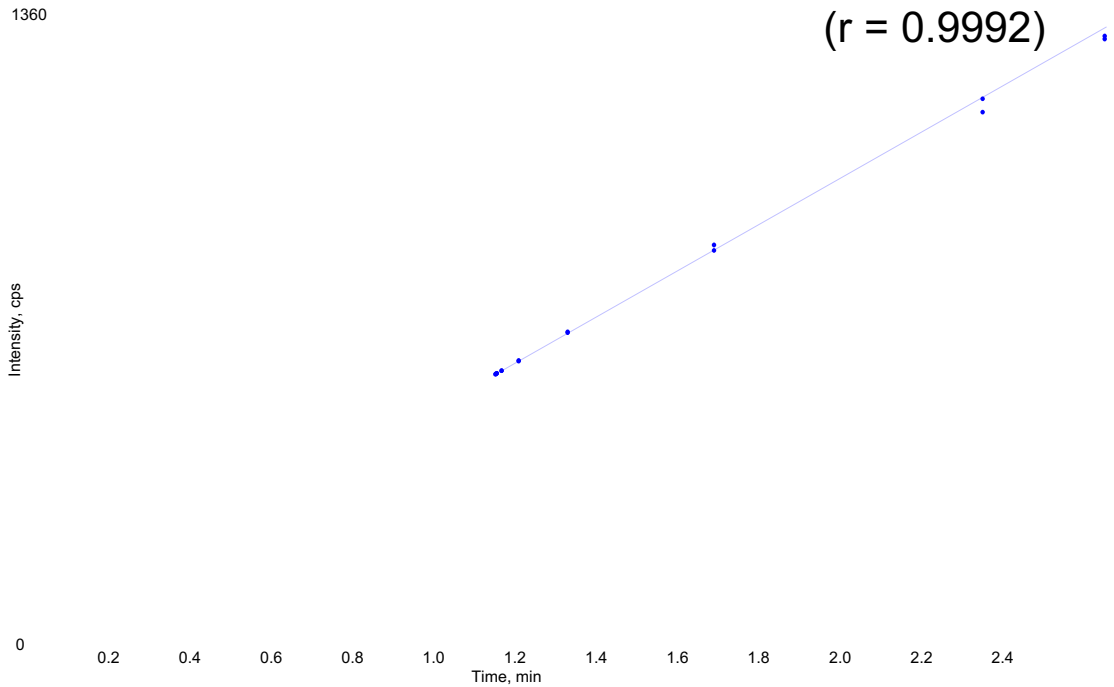
3.6e4

0.0 1.2 3.0

Time, min

$$\frac{AngI\ conc\ in\ +37ºC\ sample - AngI\ conc\ in\ +4ºC\ sample}{3\ (Incubation\ period)} = PRA\ ng/mL/hour$$

Surrogate Analyte[2] : Angiotensin I



Well characterised Ang I and Ang I SIL reference materials available

1% BSA in Tris Buffer used as surrogate matrix for calibrators

SPE sample clean-up

Chromatography developed using incubated QCs

ESI+ on Sciex API 5000

Calibration Range 0.2 – 100 ng/mL

Reference Range & Dilutions

Reference intervals vary between labs and with age, gender, race, diet, posture

- 0.167 – 40.0 ng/mL/hr

Dilution of PRA samples should be avoided

Undiluted Sample	Baseline Sample	Activity Sample
Ang I conc (ng/mL)	2.4	7.2
PRA (ng/mL/hr)	1.60	

Activity is disrupted

- This has an impact on activity QCs

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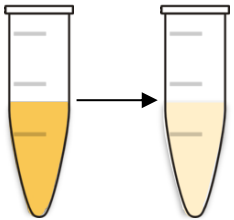

Not Corrected for Dilution Factor	Baseline Sample	Activity Sample
Ang I conc (ng/mL)	0.5	0.8
PRA (ng/mL/hr)	0.10	

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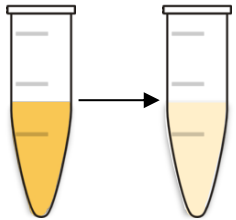

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PRA (ng/mL/hr)	0.10	

Corrected for Dilution Factor	Baseline Sample	Activity Sample
Ang I conc (ng/mL)	2.6	4.1
PRA (ng/mL/hr)	0.51	

Activity is disrupted

- This has an impact on activity QCs

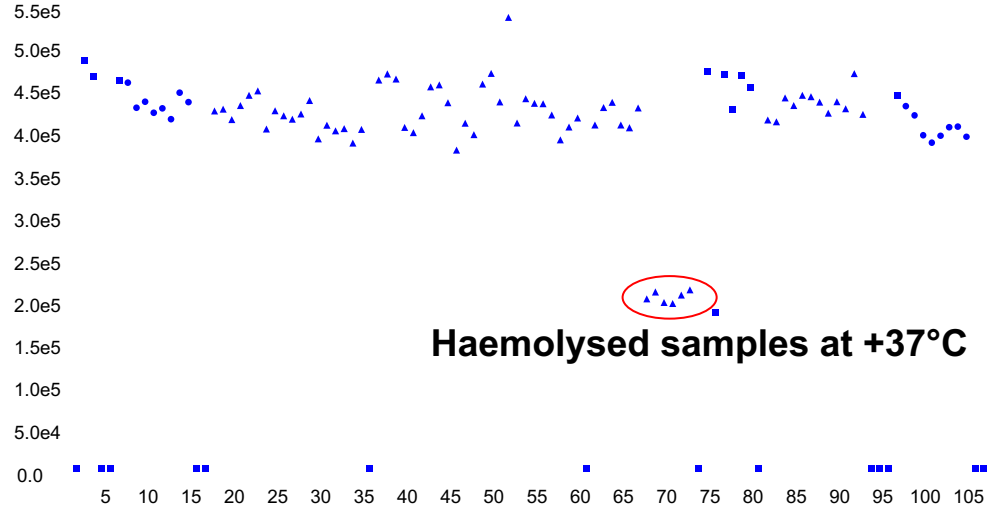
Matrix Effects

Parallelism – Angiotensin I Only

Use of 20% Intralipid for hyperlipidaemic plasma

- Cannot distinguish between abnormal PRA in an individual vs the potential impact of hyperlipidaemic matrix

Matrix effect in 3% haemolysed samples



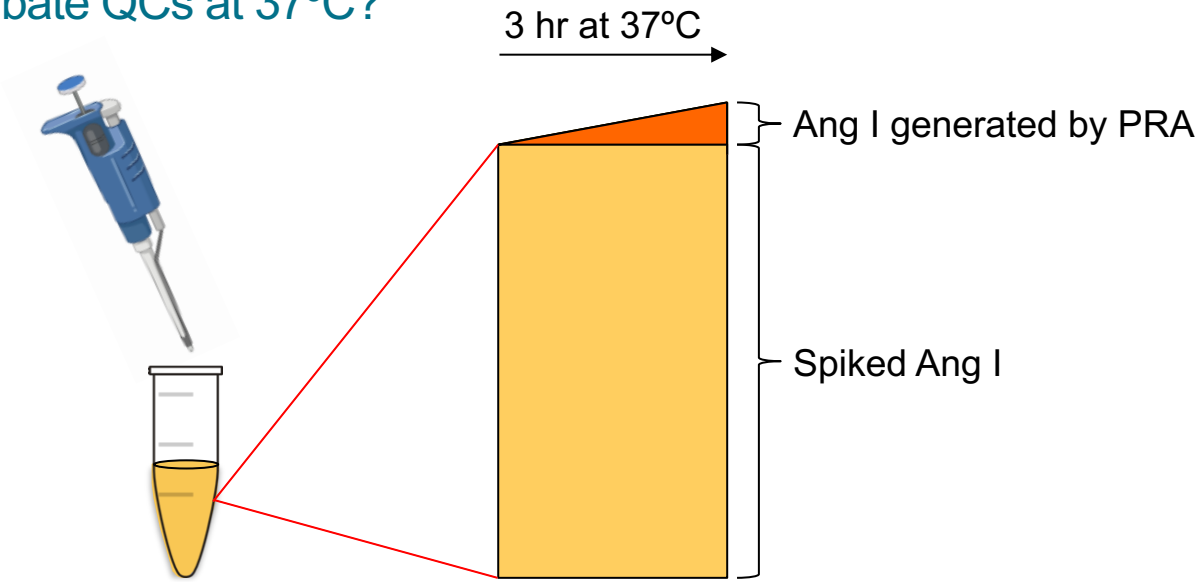
Haemolysed Plasma	
PRA (ng/mL/Hr)	1.86
% Difference vs Un-haemolysed Sample	39.6

Demonstrating Control: Angiotensin I

Surrogate LLOQ, spiked plasma QC Med and QC High

Incubated on ice for 3 hours

Why not incubate QCs at 37ºC?



- Intra- and Inter-batch accuracy was ≤ 7.1%, precision ≤ 4.8%

Is that a good control of renin activity?

The goal is reproducible PRA not Ang I…

Analytical Run	PRA ng/mL/hr
Run 1	1.27
Run 2	1.29
Run 3	1.21
Run 4	1.25
Run 5	1.33
Mean	1.27
SD	0.04
%CV	3.52

Assessed the precision of the incubation process in an endogenous pool across 5 analytical runs

- Intra-batch precision for **Ang I** in activity samples ≤6.5% CV
- Inter batch precision for PRA 3.5%

Accuracy?

Another Way to Demonstrate Control

Low, medium and high activity levels

BioRad QC Level 2	PRA ng/mL/hr
Run 1	2.87
Run 2	2.55
Run 3	2.59
Run 4	2.49
Run 5	2.80
Mean	2.63
SD	0.2
%CV	6.5

Acceptance criteria

- Clinical acceptance criteria?
- 4-6-X?

Context of Use!

“Stability can only be attained by inactive matter”

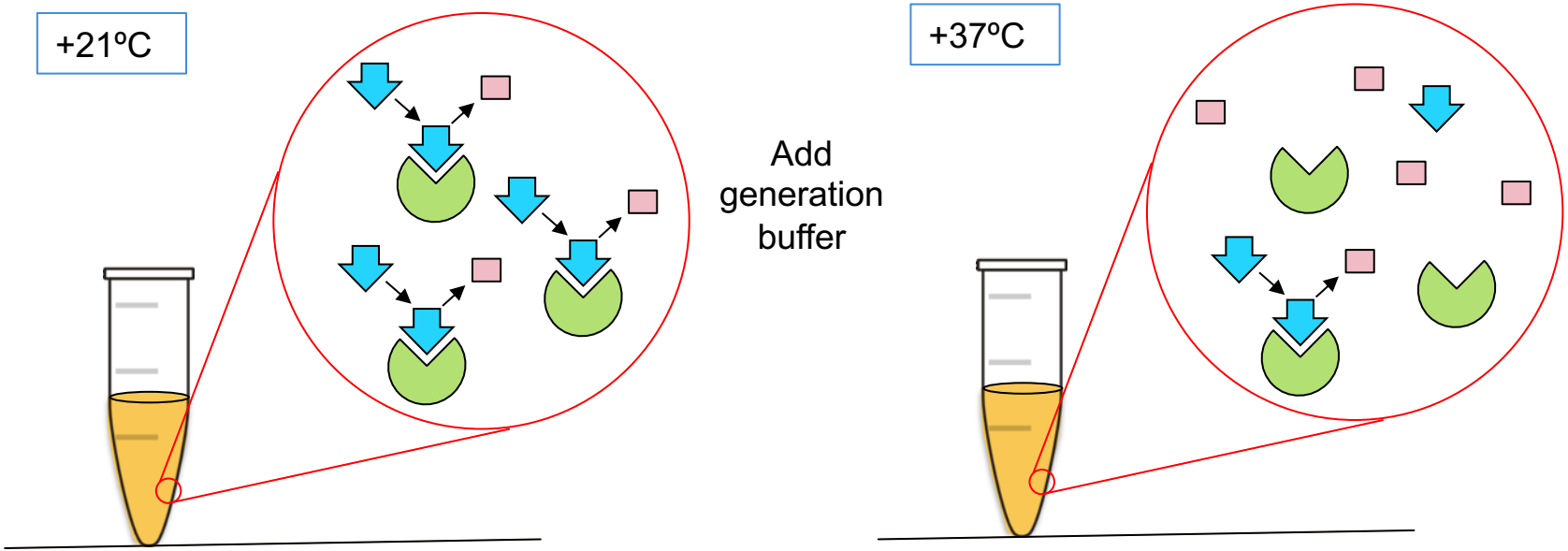
Marie Curie

“Currently, pre-analytical errors account for up to 70% of all mistakes made in laboratory diagnostics”

*“Quality Indicators to Detect Pre-Analytical Errors in Laboratory Testing” Mario Plebani

Stability and Pre-Analytical Factors

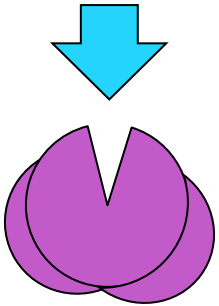
Enzyme activity at ambient temperature



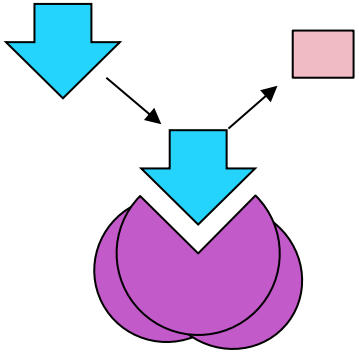
Substrate being used up before incubation

Stability and Pre-Analytical Factors

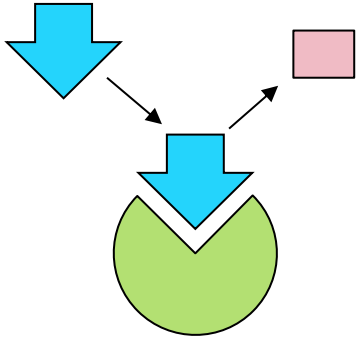
Cryo-activation of pro-renin



Pro-renin in frozen, ambient, and physiological temperatures



Pro-renin between -5°C and +4°C



Renin

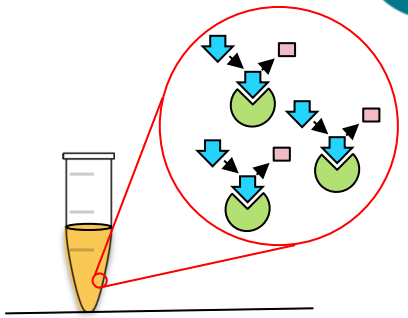
May result in apparent PRA in sample which is higher than in vivo

Stability and Pre-Analytical Factors

Did we see spontaneous generation of Ang I at RT?

+67% Ang I in baseline sample after 24 hours

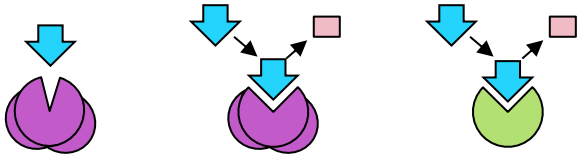
-16% decrease in renin activity



Did we see cryo-activation of pro-renin?

-0.8% change in renin activity after 24 hours at +4ºC

-3.4% change in renin activity after 4 freeze thaws



Pre-analytical factors addressed?

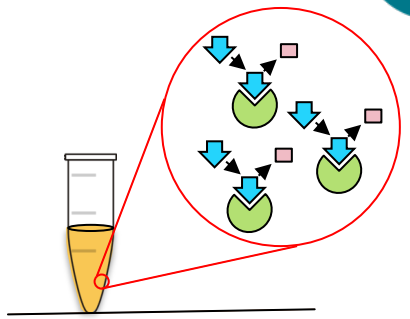
You Need to Factor in the Individual!

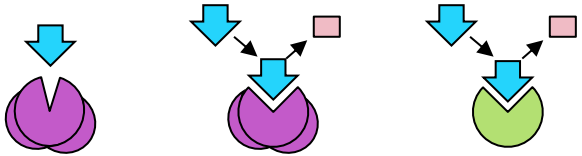
Did we see spontaneous generation of Ang I at RT?

- +67% Ang I in +4ºC sample after 24 hours
- -16% decrease in renin activity
- 0.7% change in PRA for Individual 1
- -29% change in PRA for Individual 2
- -14% change in PRA for Individual 3

Did we see cryo-activation of pro-renin?

- -0.8% change in renin activity after 24 hours at +4ºC
- -3.4% change in renin activity after 4 freeze thaws
- No change in renin activity after 24 hours at +4ºC
- 12% change in PRA for Individual 1 after 4 F/T
- 13% change in PRA for Individual 2 after 4 F/T
- 4.0% change in PRA for Individual 3 after 4 F/T



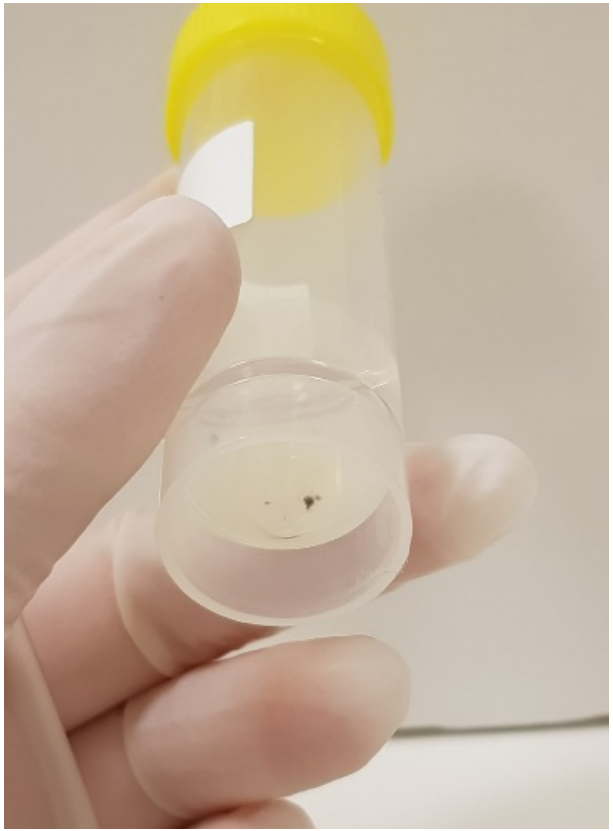


…Unwelcome Friends

Validated up to 1 month stability for Ang I in surrogate matrix however….

Created a growth medium for something else

- appeared at higher concentrations first!



Final Thoughts

Fit-for-purpose validation doesn't mean fewer assessments…

- 5x the stability work to ensure pre-analytical sample handling appropriate

Context of use

- What's the best way to demonstrate control?
- Is my acceptance criteria appropriate for the end use?

Do It Yourself

- The literature is a guide not a gospel

Acknowledgements

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