

# **CERTAIN ASPECTS OF F-POINT FIBRINOGEN IVD ASSAY ANALYTICAL PERFORMANCE**

**ACCURACY, PRECISION & LIMITS OF DETECTION**



# OBJECTIVES

## TASKS

- I. Within-run Accuracy and Precision
- II. Between-run Accuracy and Precision
- III. Limit of Detection (LoD) and Lower Limit of Quantification (LLOQ)

## GUIDANCE

*"Guideline on bioanalytical method validation". EMEA/CHMP/EWP/192217/2009 Rev. 1  
Corr. 2. – European Medicines Agency, 2011.*

## QC SAMPLES:

Analytical standards are made from the set of four artificial fluids using the following mixing formula:

$$(M:B:P)+S$$

where M, B, P are the volumes of corresponding fluids and S is amount of final fibrinogen concentration spike (g/L)

<b>M</b>	• Matrix: The Model 046, Blood Mimicking Fluid (CIRS Inc.);
<b>B</b>	• Buffer: Imidazole (HYPHEN Biomed)
<b>P</b>	• 222101 Plasma Calibrator (HYPHEN Biomed)
<b>S</b>	• Spike: Fibrinogen, Plasminogen-Depleted, Human Plasma (95% clottable, MERCK Inc.)



QC levels		Prep Protocol {M:B} (v/v) + S (g/L)
QC **	0.81	{0.2:0.8} + 1.0
QC Low	1.59	{0.2:0.8} + 2.0
QC Med	3.17	{0.2:0.8} + 4.0
QC High	4.72	{0.2:0.8} + 6.0



## QUALITY CONTROL PROCEDURE

4 Quality Control (QC) Samples -> Number of runs -> F-Point Response -> Back-calc. concentrations -> Accuracy as mean difference with nominal values and Precision as CV

### BETWEEN-RUN

Quality Control	conc X		back-calc conc X	Accuracy, %	mean % diff	CV%	acceptance criteria, +/- %	
QC **	0.81	Day1	0.9	119.3	19.3	9.8	20.0	✓
		Day2	0.9					
		Day3	1.1					
QC Low	1.59	Day1	2.1	113.2	13.2	12.0	15.0	✓
		Day2	1.7					
		Day3	1.6					
QC Med	3.17	Day1	2.9	95.7	-4.3	3.1	15.0	✓
		Day2	3.1					
		Day3	3.1					
QC High	4.72	Day1	4.6	98.9	-1.1	2.0	15.0	✓
		Day2	4.8					
		Day3	4.6					

### WITHIN-RUN

Quality Control	conc X		back-calc conc X	Accuracy, %	mean % diff	CV%	acceptance criteria, +/- %	
QC **	0.81	Replicate1	0.9	116.0	16.0	15.9	20.0	✓
		Replicate2	0.8					
		Replicate3	0.8					
		Replicate4	1					
		Replicate5	1.2					
QC Low	1.59	Replicate1	2.1	110.7	10.7	10.5	15.0	✓
		Replicate2	1.6					
		Replicate3	1.6					
		Replicate4	1.7					
		Replicate5	1.8					
QC Med	3.17	Replicate1	2.9	90.2	-9.8	4.7	15.0	✓
		Replicate2	3					
		Replicate3	2.9					
		Replicate4	2.6					
		Replicate5	2.9					
QC High	4.72	Replicate1	4.6	106.4	6.4	4.8	15.0	✓
		Replicate2	5					
		Replicate3	5.2					
		Replicate4	5.3					
		Replicate5	5					



# LIMIT OF DETECTION & LOWER LIMIT OF QUANTIFICATION

## LOD

6 blank Samples -> F-Point -> Mean and noise evaluation

->  $LoD = 3.3 * St.Dev + Mean$

	CS sample	fibrinogen, g/L	back-calc. value, g/L
1	Blank	0	0.00
2	Blank	0	0.15
3	Blank	0	0.10
4	Blank	0	0.00
5	Blank	0	0.15
6	Blank	0	0.00
mean :			0.066
stdev :			0.069
LoD :			0.293

LOD:  
0.3 g/L

## LLOQ

Increment candidates:  $LoD$ ,  $1.5 * LoD$ ,  $2.0 * LoD$ , etc ... -> F-Point ->

Assessment of Accuracy & Precision ->  $LLOQ = \min \text{ accepted conc}$

Candidate Sample	conc x	back-calc. value, g/L		mean% diff	CV%	Acceptance criteria%, +/-
LoD	0.3	within-run	5 runs	31.0	29.4	20
		between-run	3 days	26.1	11.7	20
1.5*LoD	0.45	within-run	5 runs	24.4	22.7	20
		between-run	3 days	23.3	11.0	20
2*LoD	0.6	within-run	5 runs	15.3	21.1	20
		between-run	3 days	19.1	10.5	20
2.5*LoD	0.75	within-run	5 runs	16.0	15.9	20
		between-run	3 days	19.3	9.80	20

LLOQ:  
0.75 g/L