

CERTAIN ASPECTS OF F-POINT FIBRINOGEN IVD ASSAY ANALYTICAL PERFORMANCE

ACCURACY, PRECISION & LIMITS OF DETECTION

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

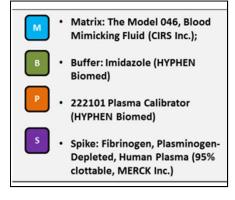
"Guideline on bioanalytical method validation". EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2. – European Medicines Agency, 2011.

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)





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

ACCURACY AND PRECISION

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					✓
QC Low	1.59	Day3 Day1	2.1	113.2	13.2	12.0	15.0	
		Day2 Day3	1.7 1.6					V
QC Med	3.17	Day1	2.9	95.7	-4.3	3.1	15.0	
		Day2 Day3	3.1					V
QC High	4.72	Day1	4.6	98.9	-1.1	2.0	15.0	
		Day2 Day3	4.8 4.6					V

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				\checkmark
		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				V
		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		
		1	mean :	0.066		
		S	tdev :	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 accepted conc

Candidate	conc x			mean%	CV%	Acceptance
Sample		value,g/L		diff		criteria%, +/-
LoD	0.3	within-run	5 runs	31.0	29.4	20 x
		between-run	3 days	26.1	11.7	20 x
1.5*LoD	0.45	within-run	5 runs	24.4	22.7	20 x
		between-run	3 days	23.3	11.0	20 x
2*LoD	0.6	within-run	5 runs	15.3	21.1	20 x
		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**