ANALYTICAL PERFORMANCE ASPECTS OF F-POINT FIBRINOGEN IVD ASSAY

SUMMARY & KEY NOTES

DUBLIN, 01NOV2019

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

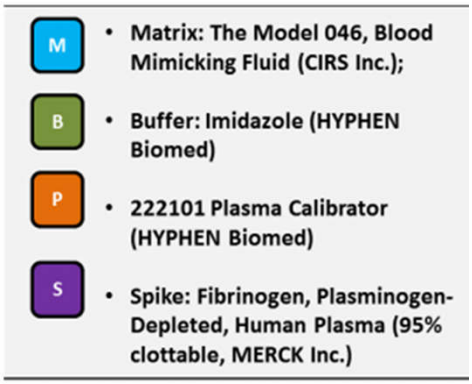
"Guideline on bioanalytical method validation". EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2. – European Medicines Agency, 2011.

ANALYTICAL SAMPLES:

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

(M:B:P)+S

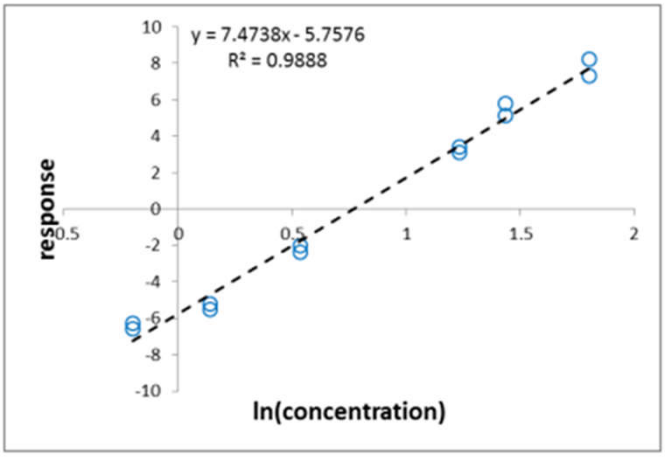
where M, B, P are the volumes of corresponding fluids and S is amount of final fibrinogen concentration spike (g/L)



CALIBRATION PROCEDURE

6 Calibration Standards (CS) x 2 Runs-> F-Point Response Fitting ->
R-Square Calculation -> Back-calc. Concentrations ->
Calibration Quality Assessment by mean difference with nominal values

CS levels	Prep Protocol {M:B:P} (v/v) + S (g/L)	Actual FIB (g/L)
CS1	{0.25:0.57:0.18} + 0	0.82
CS2	{0.25:0.51:0.24} + 0	1.15
CS3	{0.25:0.39:0.36} + 0	1.71
CS4	{0.25:0.03:0.72} + 0	3.44
CS5	{0.25:0.03:0.72} + 1	4.20
CS6 (ULOQ)	{0.3:0.7} + 3.6	6.06



Response = A1*ln (Concentration) + A2

Fit Parameters	value	se	pr >|t|
A1: slope	7.474	0.251	7.301E-12
A2: intercept	-5.758	0.275	3.242E-10

R-SQUARE: **Excellent**

Two parameters of the best fit are introduced back to the system to get the back-calculated concentrations:

Concentration* = exp((Response-A2) / A1)

CALIBRATION QUALITY ASSESSMENT

Calibration Standard		conc X	resp Y	back-calculated X	mean % difference	acceptance criteria, +/- %
CS I	Replicate1	0.82	-6.3	0.930	11.2	20.0
	Replicate2	0.82	-6.6	0.893		
CS 2	Replicate1	1.15	-5.2	1.077	-8.2	15.0
	Replicate2	1.15	-5.5	1.035		
CS 3	Replicate1	1.71	-2	1.653	-5.8	15.0
	Replicate2	1.71	-2.4	1.567		
CS 4	Replicate1	3.44	3.1	3.271	-3.0	15.0
	Replicate2	3.44	3.4	3.405		
CS 5	Replicate1	4.2	5.8	4.695	6.8	15.0
	Replicate2	4.2	5.1	4.275		
CS ULOQ	Replicate1	6.06	7.3	5.738	0.7	15.0
	Replicate2	6.06	8.2	6.472		

CALIBRATION QUALITY ASSESSMENT: **Passed**

QUALITY CONTROL PROCEDURE

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

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

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				

BETWEEN-RUN QUALITY CONTROL: **Passed**

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				

WITHIN-RUN QUALITY CONTROL: **Passed**

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:
0.29 g/L

LLOQ

*3 Candidates: LoD, 2*LoD, lowest QC -> F-Point*
-> Assessment of Accuracy & Precision
-> LLoQ = min conc where acceptance criteria met

Candidate Sample	conc x	back-calc. value,g/L		mean% diff	CV%	Acceptance criteria%, +/-
LoD	0.29	within-run	5 runs	31.0	29.4	20
		between-run	3 days	26.1	11.7	20
2*LoD	0.59	within-run	5 runs	15.3	21.1	20
		between-run	3 days	19.1	10.5	20
Lowest QC	0.81	within-run	5 runs	16.0	15.9	20
		between-run	3 days	19.3	9.80	20

LLOQ:
0.81 g/L