Challenges in estimation of FSH for pharmacokinetic study by Architect i1000 (CMIA based clinical lab instrument) and commercial kits

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INTRODUCTION: The ARCHITECT FSH assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of follicle stimulating hormone (FSH) in human serum and plasma. This instrument is commonly used in clinical laboratories to determine FSH concentration in serum for clinical studies. It uses a two step immunoassay principle in which in the first step, sample and anti-β FSH coated paramagnetic microparticles are combined. FSH present in the sample binds to the anti-β FSH coated microparticles. After washing, anti-α FSH acridinium labeled conjugate is added in the second step. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of FSH in the sample and the RLUs detected by the ARCHITECT i optical system.

Selective modifications were made to the ARCHITECT FSH kit method to make sensitive, selective and specific analytical method for FSH to support pharmacokinetic (PK) studies.

INSTRUMENT AND SOFTWARE CHALLENGES:

In pursuit of developing FSH analytical method for PK study, specific modifications to Architect FSH method were made which were required for complete development and advanced validation.

- Kit/commercial calibrators were replaced with calibrators prepared using reference standard material and more levels were added in place of an in built calibration with two levels of commercial calibrators.
- Commercial controls were not used, instead in-house quality control (QC) samples were employed to validate and monitor assay performance.
- As FSH is an endogenous molecule, surrogate matrices were identified for the preparation of the curve. Fetal bovine serum (FBS) was used as it showed almost no FSH when quantified and could potentially be used as an alternative to human serum.

Instrument and Software challenges:

In the course of developing the method using this clinical grade instrument, certain software challenges related to sample processing, data acquisition and storage, security of the system and trail of activities etc. were identified and worked upon and resolved to comply with regulatory requirements.

Challenges identified and resolved:

i. **User access credentials could not be identified** - Prepared documentation (forms and logs) for instrument and software to track user access.

ii. **Data acquisition and storage**: Results of the samples were saved in local system of the computer as per software architecture - The results are printed and signed. Source data file in network drive which is transferred from CD-R/W is opened with excel sheet.

iii. **Data Processing File** opened with excel sheet is formatted as per the requirements of analyzing software (Soft Max Pro software) and fed into the software for analysis. The data is verified against raw data / print data.

iv. **No security features** were available with the instrument or software for the operator (Security privilege available for system administrator. However, no provision for password.) - Prepared precise and detailed document to track access of instrument and software

v. **Trail of activities** performed by instrument and software; Documentation procedure implemented to control, track and monitor all events of software / instrument to minimize risk. Specific log prepared to track access, operation, sample analysis, data acquisition and data transfer into network drive for back up.

MODIFICATION SUMMARY

Parameter	Commercial Architect FSH method	Modified FSH Method
Standards	Contains human FSH in liquid form	Lyophilized form, custom supplied reference material
Number of calibrators	Two levels calibrator; calibrators 1 and calibrators 2	Eight including zero calibrators
Quality controls	Commercial quality control samples	Six level QC including a novel QC 'Endogenous QC' (EQC)
Sample dilution	An automatic 1:5 dilution by Architect and reports result	Custom dilution of sample as per methods requirements
Sample analysis and data organization	Samples are analyzed in samples mode and data are stored only in local drive	Data are moved to network drive in well-suited formats and back up created

FSH METHOD SUMMARY:

Analyte : Recombinat Human FSH (Gonal-F)

Sample Analysis : Chemiluminescent Micro particle Immunoassay

Calibration curve range : 30 – 5000 pg/mL

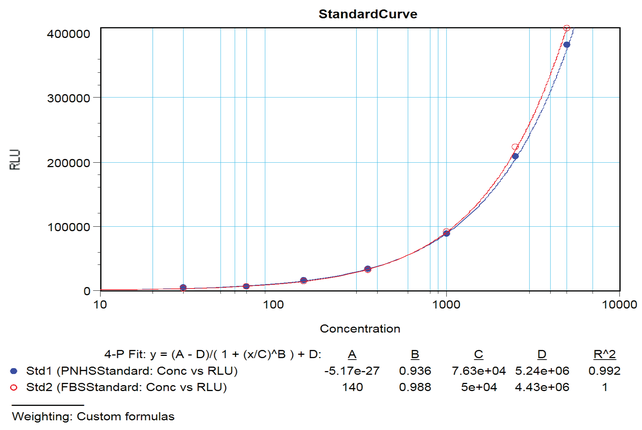
Quality Controls (pg/mL) : 30 (LLOQ), 90 (LQC), 1300 (MQC), 3750 (HQC) & 5000 (ULOQ)

Sensitivity : 30 pg/mL

CALIBRATION STANDARDS AND CURVE:

It was quite difficult to build a calibration curve in study matrix. As, human serum contains very high level of FSH. Hence, we had to look for surrogate matrices for the preparation of the curve. Fetal bovine serum (FBS) was one of them. FBS showed almost no FSH when quantified and could potentially be used as an alternative to human serum. As per regulatory requirements, we also assessed the response similarity of FSH calibrators between FBS and human serum.

Fig 1: *FSH curve comparison between surrogate matrix FBS and pooled human serum.*



Using the surrogate matrix the calibration curve was prepared. The FSH calibrators used to make the curve ranged from 30 to 5000 pg/mL. FSH calibrators were highly accurate as shown by % error.

Project: FSH

Parallelism Table: Curve comparison for recombinant FSH

Run #	Curve Id**	Intercept	Slope	Slope Difference (%)*
Parellelism I	Fetal Bovine Serum (FBS)	50000	0.988	5.6
	Neat Human serum	76300	0.936	

Note: * Slope Difference = ((Slope of Surrogate Matrix-Slope of Authentic Matrix)/Slope of Authentic Matrix))*100
** Curves were obtained from each respective matrix samples with the corresponding concentrations.

Acceptance criteria:
Comparison of Slope difference of each pairs of calculated curves must not be more than 15%.

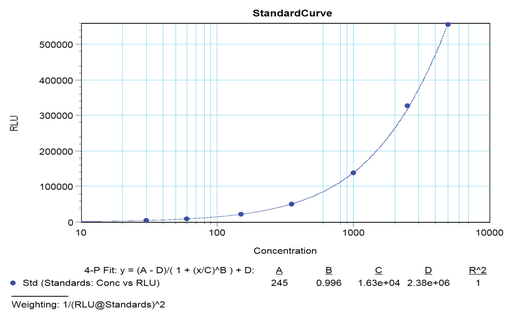


Fig 2a: *Calibration curve of FSH method using calibrators prepared in FBS*

Standards (pg/ml)

Sample	Position	Conc	RLU	Result	% Error
St01	A1	5000.000	555286	4922.983	-1.540
St02	B1	2500.000	327710	2575.489	3.020
St03	C1	1000.000	138818	994.019	-0.598
St04	D1	350.000	51202	350.220	0.063
St05	E1	150.000	22095	147.774	-1.484
St06	F1	60.000	9113	59.415	-0.975
St07	G1	30.000	4830	30.577	1.922
St08	H1	0.000	245	Range?	Range?

Fig 2b: *Accuracy of FSH calibrators in FBS as shown by the % error.*

QUALITY CONTROL:

Quality control (QC) samples were prepared in unaltered blank human serum as per regulatory requirements. Since, endogenous FSH was already present; the reference material was not directly spiked into human serum. The reference material was spiked into untreated serum in an additive manner. The endogenous FSH concentration was determined over several assays and taken into account in the final QC concentration.

Fig 3: *Accuracy of FSH quality control samples as shown by the % error.*

Sample	Conc	RLU	Result	% Error
ULOQ-1	5000	513265	4445.987	-11.080
ULOQ-2	5000	513050	4443.602	-11.128
ULOQ-3	5000	515710	4473.147	-10.537
HQC-01	3750	404324	3304.722	-11.874
HQC-02	3750	414522	3406.132	-9.170
HQC-03	3750	420677	3467.855	-7.524
MQC 2	1300	166128	1205.583	-7.263
MQC 3	1300	161660	1170.603	-9.954
MQC-1	1300	160994	1165.401	-10.354
LQC-1	90	12923	85.21	-5.323
LQC-2	90	12860	84.782	-5.798
LQC-3	90	12939	85.318	-5.202
LLOQ-1	30	4589	28.96	-3.468
LLOQ-2	30	4524	28.524	-4.921
LLOQ-3	30	4313	27.109	-9.637

ACCURACY AND PRECISION:

Intra-run QC samples

QC conc. (pg/mL)	Mean calculated conc. (pg/mL)	Std. deviation	% CV	% Bias	Mean Accuracy
5000	4443.339	13.452	0.3	-11.1	88.9
3750	3400.012	66.146	1.9	-9.3	90.7
1300	1255.759	4.409	0.4	-3.4	96.6
90	86.948	0.873	1	-3.4	96.6
30	28.302	1.029	3.6	-5.7	94.3

Inter-run QC samples

QC conc. (pg/mL)	Mean calculated conc. (pg/mL)	Std. deviation	% CV	%Bias	Mean Accuracy
5000	4448.792	14.691	0.3	-11	89
3750	3396.458	66.926	2	-9.4	90.6
1300	1218.144	43.551	3.6	-6.3	93.7
90	86.026	1.165	1.4	-4.4	95.6
30	28.25	0.895	3.2	-5.8	94.2

Calibration standards

Calibration std. conc. (pg/mL)	Mean calculated conc. (pg/mL)	Std. deviation	% CV	% Bias	Mean Accuracy
5000	5009.402	122.214	2.4	0.2	100
2500	2487.224	124.826	5	-0.5	99.5
1000	1008.6	20.62	2	0.9	101
350	350.092	0.181	0.1	0	100
150	150.949	4.489	3	0.6	101
60	58.914	0.709	1.2	-1.8	98.2
30	30.36	0.307	1	1.2	101

CONCLUSION: After overcoming software and commercial calibrator and QC challenges, specific, precise and sensitive method can be developed on clinical lab instruments using commercial kits. (Method for FSH was developed using Architect i1000 SR by CMIA in this case.)