Use of Dilutional Linearity as a Screening Tool in Selection of a Commercial Kit for Biomarker Quantification: A case study

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OBJECTIVE

Monocyte chemotactic protein-1 (MCP-1) is a potential prognostic biomarker of acute coronary syndromes. Several commercial kits are available for quantification of MCP-1. We setout to identify, select and qualify a commercial kit for reliable quantification of MCP-1 in human plasma.

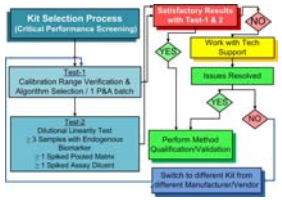
INTRODUCTION

Research Use Only (RUO) kits are widely available for a variety of biomarkers and can be adapted for biomarker bioanalytical studies. However, it is important to understand that not all RUO kits perform as claimed; therefore one should be diligent in selecting a reliable kit before undertaking the analytical method qualification or validation. We have developed an effective kit screening process that we routinely use. In this case study, efficient application in selection of a MCP-1 kit that performed well in analytical method qualification is presented.

METHOD

- Assays were performed essentially as described in the kit insert with minor modifications.
- Kit screen process consists of two sets of tests (Figure 1).
  1. Calibration Range Verification and Algorithm Selection: Consisted of one precision & accuracy (P&A) experiment.
  2. Dilutional Linearity Test: Consisting of three samples with an endogenous biomarker (parallelism), one spiked pool matrix (matrix effect on recovery) and one spiked assay diluent (control for dilutional linearity in defined matrix).
  3. If results are unsatisfactory and issue is not resolved even with manufacturer's technical support, switch to a different kit from another vendor that satisfies the kit screen process.

Fig. 1: Kit Screen Process



RESULTS

Fig. 2: Calibration Range Verification & Algorithm Selection (**Test-1**)

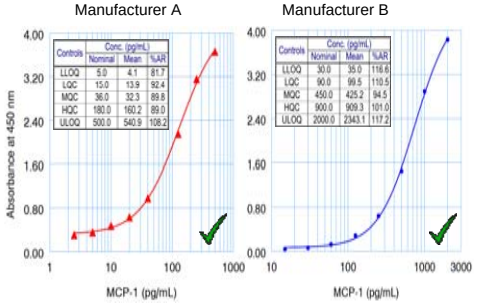


Table 1: Dilutional Linearity Screen (**Test-2**)

Manufacturer A		Manufacturer B	
Dilution	Adjusted Conc. (pg/mL)	Dilution	Adjusted Conc. (pg/mL)
Neat	39.3	Neat	698.8
2	77.2	2.5	705.5
4	106.3	5	682.2
6	113.6	10	663.0
8	116.6	20	700.6
Mean	90.6	Mean	690.0
%CV	36.1 ✗	%CV	2.5 ✓

- Human plasma samples containing endogenous MCP-1 were serially diluted in assay diluent and analyzed.
- Kit from Manufacturer A passed Test-1 but failed Test-2.
- Kit from Manufacturer B passed both tests and selected for analytical method qualification.

Table 2: Dynamic Range of Quantification

Statistics	Nominal Standard Concentrations (pg/mL)							
	2,000	1,000	500	250	125	60	30	15*
Mean	1911.3	1035.4	484.6	247.5	129.7	65.4	31.8	11.0
n	3	3	3	3	3	3	3	3
SD	14.46	10.91	5.87	1.36	0.17	1.26	1.00	1.02
%CV	0.8	1.1	1.2	0.6	0.1	1.9	3.2	9.3
%AR	95.6	103.5	96.9	99.0	103.8	108.9	106.0	73.1

* Anchor Point

RESULTS (CONT.)

Table 3: Precision of Measurements (Assay Controls)

Parameters	Statistics	Nominal Concentrations (pg/mL)				
		30	90	450	900	2,000
		LLOQ	Low	Medium	High	ULOQ
# of Observations	# Results	9	9	9	9	9
	# Runs	3	3	3	3	3
Accuracy	Mean	32.8	97.5	426.2	908.7	2269.3
	Mean Bias (%RE)	9.4	8.3	5.3	1.0	13.5
	% Analytical Recovery	109.4	108.3	94.7	101.0	113.5
Precision	Intra-Assay (%CV)	≤ 5.6	≤ 1.1	≤ 3.3	≤ 2.0	≤ 6.9
	Inter-Assay (%CV)	7.1	2.1	2.3	1.7	6.3
Total Error	%CV+%RE	16.4	10.4	7.6	2.6	19.7

Table 4: Precision of Measurements (Plasma Controls)

Plasma Controls	No. of Runs	No. of Results	Conc. (pg/mL)	Precision (%CV)	
				Intra-Assay	Inter-Assay
Control-1	4.0	12	146.3	≤ 3.2	3.2
Control-2	4.0	12	1708.1	≤ 3.8	3.9

- Plasma control samples showed excellent Inter- and Intra assay precision of measurements.
- Concentrations determined in plasma control samples are used as nominal concentration when these samples are used for stability evaluation experiments.

Table 5: Assay Specificity

MCP-1 QC	Baseline Conc.	MCP-1 Conc. (pg/mL)							
		+ sCD-14				+ MCP-3			
		Mean	n	%CV	% AR	Mean	n	%CV	% AR
LQC	97.7	101.1	3	1.0	103.5	98.6	3	0.8	100.9
HQC	919.2	926.9	3	0.5	100.8	913.3	3	1.2	99.4

- LQC and HQC samples were assayed in the absence (**Baseline**) and presence of Test Compounds (sCD-14 and MCP-3) at conc. of 180 and 1800 pg/mL, respectively.

Table 6: Short-Term Stability in Human Plasma

Stability	Control-1 (146.3 pg/mL)			Control-2 (1708.1 pg/mL)			Result
	% Nom.	n	%CV	% Nom.	n	%CV	
Fresh	98.5	3	1.0	100.6	3	1.6	
Bench-Top (18 hrs, RT)	101.7	3	2.8	103.3	3	1.6	Pass
Refrigerator (43 hrs, RT)	100.2	3	0.8	103.2	3	0.1	Pass
Freeze/Thaw 3 Cycles	104.1	3	0.5	106.7	3	0.5	Pass

RESULTS (CONT.)

Table 7: Assay Selectivity (Addition Recovery)

Plasma Sample	Measured Conc. (pg/mL)		AR%
	Unspiked	Spiked	
1	125.0	1674.2	96.0
3	119.3	1721.8	99.3
5	115.7	1670.4	96.4
7	131.8	1717.8	98.3
9	130.3	1835.1	105.7
11	110.1	1828.3	106.5
13	136.2	1808.4	103.7
15	130.7	1768.8	101.6
17	108.7	1651.3	95.6
19	224.3	1965.8	108.0
Mean Conc. in Buffer		1613.1	100.0

$$\%AR = \frac{\text{Spiked} - \text{Unspiked Plasma}}{\text{Mean Conc. in Spiked Buffer}} \times 100$$

Table 8: Dilutional Linearity (Spiked Human Plasma)

Dilution Factor	MCP-1 Conc. (pg/mL)		%AR (% Nominal)
	Measured	Dilution Adjusted	
4	2048.6	8194.6	80.8
8	1367.6	10940.9	107.8
16	615.3	9845.2	97.0
80	129.4	10348.8	102.0
160	68.6	10980.1	108.2
Mean		10061.9	99.2
n		6	6
SD		1143.68	11.27
CV%		11.4	11.4

Nominal Conc. (pg/mL) = 10146.3

CONCLUSION

- Evaluation of dilutional linearity using biological matrix samples can be effectively used in selection of a reliable commercial kits for biomarker quantification.
- The selected kit (from Manufacturer B) showed method performance characteristics acceptable for the intended use.
- Performance screening of commercial kits provides a cost effective tool that can save time and resources in bioanalysis of biomarker.

REFERENCES

- Khan, M., Adaptation of fit-for-purpose biomarker assay validation using commercial kits: A CRO perspective. Drug Research 11/12: 33-34, (2010).
- Lee, JW *et. al.*, Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurements. Pharm Research 23 (2): 312-28 (2006).