Clinical Laboratory News

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Matrix Effect Experimental For Exogenous Analytes

- Collect six individual, drug free, native matrix samples
- Prepare to spike low and high concentration **test** samples for each of the six drug free native matrix samples using the standard sample preparation procedure in replicates of three; label 3 tubes for low and 3 tubes for high concentration aliquots for each **test** sample (n=36; 18 low and 18 high concentration samples)
- Prepare low and high concentration **control** samples (substituting an equivalent volume of water or solvent mixture, dependent on analyte solubility for native matrix) in replicates of three (consider the final volume to allow reinjection for comparison with test samples). Control samples are prepared using the standard sample preparation procedure; label 3 tubes for low and 3 tubes for high concentration aliquots for each **control** sample (n=6; 3 low and 3 high concentration samples). These samples are used as the reference – i.e. there should be no ion suppression or enhancement since no residual native matrix is present.
- Following sample preparation, **test** and **control** samples are spiked with anaylte(s) at two concentrations (low and high). Samples may be spiked using a low volume of solution containing analyte(s) or a small volume of the final preparation is removed (typically 5%) and the same volume of a solution containing analyte(s) added.
- Prepare singlicate calibrators and duplicate quality control samples using the standard sample preparation procedure
- Analyze the prepared samples following the test order described:
    - Calibrators, quality control set 1, solvent blank, low concentration test sample 1, low concentration control sample 1, low concentration test sample 2, low concentration control sample 2, low concentration test sample 3, low concentration control sample 3, high concentration test sample 1, high concentration control sample 1, high concentration test sample 2, high concentration control sample 2, high concentration test sample 3, high concentration control sample 3 ……. quality control set 2

- Process the data, establish that the calibration line and quality control samples meet acceptance criteria and determine analyte peak areas and internal standard peak areas for test and control samples
- Calculate the mean peak area, SD and %CV for test samples for each analyte
- Calculate the mean peak area, SD and %CV for control samples for each analyte
- Calculate the percent matrix effect for each analyte using the following equation:

$$\%\,\text{MatrixEffect} = \frac{\text{Mean peak area in test samples}}{\text{Mean peak area in controls samples}} x100$$

- Calculate the mean response for each analyte using the following equation:

$$\text{Response} = \frac{\text{Analyte peak area}}{\text{Internal standard peak area}}$$

- Additionally, calculate the normalized (internal standard adjusted) percent matrix effect for each analyte using the following equation:

$$\%\,\text{Normalized Matrix Effect} = \frac{\text{Mean response in test samples}}{\text{Mean response in controls samples}} x100$$

- The percent matrix effect is a quantitative measure of how much the matrix influences response
    - Values <100% indicate suppression
    - Values >100% indicate enhancement
- Percent matrix effect may differ between the six matrices tested, the response %CV provides a measure of variability and should be less than 15% (as stated in CLSI Guideline C62-A)
- Calculation of the normalized percent matrix effect should give confidence that the internal standard is compensating for matrix effects in the sample by giving a matrix effect value of close to 100%

Percent Matrix Effect Example Calculations

Analyte 1

No internal standard adjustment, low concentration:

Test Reference	Peak Area	Mean Peak Area	Test Peak Area % CV	Control Peak Area	Control Mean Peak Area	Control Peak Area % CV	% Matrix Effect
1	561	538	6.7	559	543	4.9	99
	500						
	554						
2	521	574					106
	607						
	595						
3	576	524		512			97
	500						
	496						
4	544	536					99
	531						
	533						
5	500	500		557			92
	492						
	508						
6	586	550					101
	532						
	533						

Mean Percent Matrix Effect = 99% (n=6, range 92-106%).

No internal standard adjustment, high concentration:

Test Reference	Peak Area	Mean Peak Area	Test Peak Area % CV	Control Peak Area	Control Mean Peak Area	Control Peak Area % CV	% Matrix Effect
1	29590	32848	6.9	29062	29249	0.7	112
	33180						
	35775						
2	29741	31694					108
	32801						
	32541						
3	33009	32445		29201			111
	35535						
	28792						
4	29074	28974					99
	28690						
	29157						
5	31138	31399		29484			107
	31264						
	31794						
6	29875	31735					108
	32771						
	32558						

Mean Percent Matrix Effect = 108% (n=6, range 99-112%)

Internal standard adjustment, low concentration:

Test Reference	Response	Mean Response	Test Response % CV	Control Response	Control Mean Response	Control Response % CV	% Matrix Effect
1	0.0858	0.0865	3.1	0.0836	0.0872	2.5	99
	0.0915						
	0.0821						
2	0.0879	0.0888					102
	0.0900						
	0.0886						
3	0.0859	0.0848		0.0879			97
	0.0838						
	0.0848						
4	0.0839	0.0864					99
	0.0882						
	0.0870						
5	0.0847	0.0867		0.0863			100
	0.0848						
	0.0907						
6	0.0873	0.0858					98
	0.0874						
	0.0826						

Mean Percent Normalized Matrix Effect = 99% (n=6, range 98-102%)

Internal standard adjustment, high concentration:

Test Reference	Response	Mean Response	Test Response % CV	Control Response	Control Mean Response	Control Response % CV	% Matrix Effect
1	5.46	5.47	2.8	5.58	5.63	1.5	97
	5.59						
	5.36						
2	5.36	5.34					95
	5.31						
	5.35						
3	5.52	5.55		5.79			98
	5.64						
	5.48						
4	5.84	5.59					99
	5.39						
	5.53						
5	5.80	5.59		5.52			99
	5.51						
	5.45						
6	5.60	5.64					100
	5.70						
	5.64						

Mean Percent Normalized Matrix Effect = 98% (n=6, range 95-100%)

In this example, there is little suggestion of a matrix effect, even without considering the internal standard, as matrix factors are in the region of 90-110%. Using internal standard adjusted matrix factor gives results close to 100%.

Analyte 2

No internal standard adjustment, low concentration:

Test Reference	Peak Area	Mean Peak Area	Test Peak Area % CV	Control Peak Area	Control Mean Peak Area	Control Peak Area % CV	% Matrix Effect
1	109	107	6.6	154	156	1.9	69
	107						
	106						
2	101	100					64
	96						
	102						
3	100	96		154			62
	101						
	88						
4	106	103					66
	111						
	93						
5	99	101		159			65
	111						
	93						
6	108	108					69
	109						
	107						

Mean Percent Matrix Effect = 66% (n=6, range 62-69%)

No internal standard adjustment, high concentration:

Test Reference	Peak Area	Mean Peak Area	Test Peak Area % CV	Control Peak Area	Control Mean Peak Area	Control Peak Area % CV	% Matrix Effect
1	3734	3749	20.5	5074	4989	4.3	75
	3641						
	3854						
2	3771	3669					74
	3730						
	3747						
3	3639	3406		4743			68
	3730						
	3638						
4	3408	3805					76
	3416						
	3393						
5	2586	2279		5149			46
	1841						
	2410						
6	1952	2564					51
	2581						
	3159						

Mean Percent Matrix Factor = 65% (n=6, range 46-76%)

Internal standard adjustment, low concentration:

Test Reference	Response	Mean Response	Test Response % CV	Control Response	Control Mean Response	Control Response % CV	% Matrix Effect
1	0.0343	0.0318	7.0	0.0350	0.0329	6.6	97
	0.0303						
	0.0309						
2	0.0343	0.0318					97
	0.0306						
	0.0305						
3	0.0335	0.0315		0.0323			96
	0.0324						
	0.0285						
4	0.0314	0.0309					94
	0.0335						
	0.0277						
5	0.0309	0.0313		0.0315			95
	0.0352						
	0.0278						
6	0.0326	0.0322					98
	0.0328						
	0.0312						

Mean Percent Normalized Matrix Effect = 96% (n=6, range 94-98%)

Internal standard adjustment, high concentration:

Test Reference	Response	Mean Response	Test Response % CV	Control Response	Control Mean Response	Control Response % CV	% Matrix Effect
1	1.20	1.22	4.7	1.17	1.16	3.2	105
	1.16						
	1.22						
2	1.17	1.19					103
	1.27						
	1.14						
3	1.15	1.19		1.15			103
	1.16						
	1.17						
4	1.22	1.16					100
	1.21						
	1.22						
5	1.22	1.22		1.10			105
	1.03						
	1.09						
6	1.17	1.11					96
	1.13						
	1.18						

Mean Percent Normalized Matrix Effect = 102% (n=6, range 96-105%)

In this example there is evidence of ion suppression, as the unadjusted matrix effects are well below 100%. However, using internal standard adjusted matrix effects gives results close to 100%.