Evaluation of hemolysis in assay validation and the impact on the analysis of study samples

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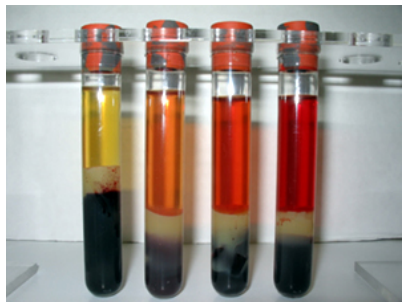
Defining the problem



- Recent validation guidelines from EMA and ANVISA call for an evaluation of the matrix effect in hemolyzed plasma samples
- In our lab this is currently tested with plasma spiked with 2 % of hemolyzed blood
- QCs (in 6-fold at low and high level) are prepared in this matrix and quantified with a plasma calibration curve; 4/6/15 acceptance criterion at each level
- Study samples on the other hand are only visually identified as hemolyzed based on the color and no test is performed to determine the degree of hemolysis

Example from a recent dog TOX study

- During assay validation 2% hemolyzed plamsa was tested and found acceptable
- Large number of study samples appeared to be clearly hemolyzed (pink to dark red)
- The appearance of some samples suggested a degree of hemolysis higher than 2%
- Not clear what to do with the hemolyzed samples

Defining the problem

If you demonstrated that 2% hemolysis does not affect accuracy of your assay but have no idea of the degree of hemolysis in your study samples, how can you be assured that your assay can accurately quantify the analyte in any “red” study sample?

How would you tackle this?

Example from a recent dog TOX study: approach taken

- An additional method validation run was done with QCs in 2% hemolyzed plasma and QCs in hemolyzed pure blood
- Bias and precision for QCs in 2% hemolyzed plasma and hemolyzed whole blood were all within 15%

Results from hemolyzed plasma & hemolyzed blood experiment

	20.0 µg/mL		10000 µg/mL	
	2% hemolyzed blood in plasma	hemolyzed whole blood	2% hemolyzed blood in plasma	hemolyzed whole blood
%Bias	8,0	-3,5	11,0	10,0
	-4,5	-8,0	11,0	11,0
	-6,0	-8,5	10,0	8,0
	-4,0	-4,5	10,0	9,0
	-4,0	-5,5	8,0	12,0
	-8,5	-4,5	9,0	12,0
Intrarun Mean	18,9	19,4	11000	11000
Intrarun %C.V.	2,2	5,9	1,1	1,5
Intrarun %Bias	-5,5	-3,0	10,0	10,0

Discussion

- Through this experiment we demonstrated that the assay allows accurate quantification in samples with a degree of hemolysis ranging from 2 – 100 %
- If the assay works with 100% hemolyzed blood there is no need to determine the degree of hemolysis in study samples
- All study samples were analyzed and results reported
- Important side note: it remains important to flag hemolyzed samples to the PK analist. Even though results may be accurate, for drugs that bind to RBCs levels could be different from those found in non-hemolyzed plasma samples
- This approach will not work for every assay.
- Not a proposal to do this on a routine basis. Should be decided case by case based on study samples.

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