

**Case Study #3: Level 4 Statistical Flags and Results Appear Visually Nonlinear on Several Analytes**

**Initial Results:** A laboratory performed routine calibration verification / linearity testing using VALIDATE® TDM1. Level 4 results for all of the analytes tested were higher than the target, causing statistical flags. The following was the report generated for Digoxin using MSDRx®, the Maine Standards Data Reduction software:

TDM 1 DIGN  
published CLIA total allowable error is 0.2 ng/mL or 20%, whichever is greater

maine standards COMPANY LLC

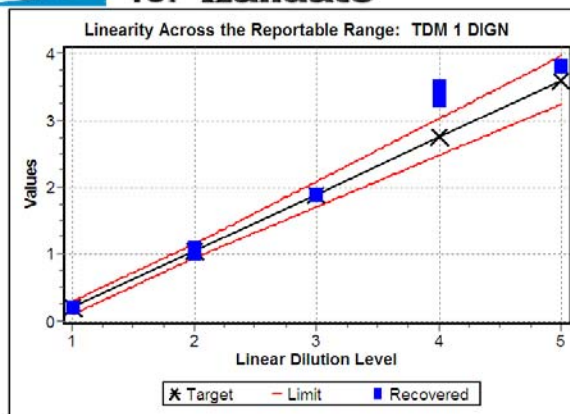
Accept  Comments

L	X	Rep 1	Rep 2	Rep 3
B	N/A			
1	1.0	0.2	0.2	0.2
2	2.0	1.1	1.0	1.05
3	3.0	1.9	1.9	1.9
4	4.0	3.3	3.5	3.4
5	5.0	3.8	3.8	3.8

Tested 0.20 to 3.80 ng/mL  
Validated \_\_\_\_\_ to \_\_\_\_\_ ng/mL

Mean versus Target Regression  
 $y = 1.124x - 0.065$

X	Target	Mean	+/- Diff	% Diff	+/- Limit	% Limit
1.0	0.200	0.200	0.000	0.0%	0.100	N/A
2.0	1.050	1.050	0.000	0.0%	0.105	10%
3.0	1.900	1.900	0.000	0.0%	0.190	10%
4.0	2.750	3.400	0.650	** 23.6%	0.275	10%
5.0	3.600	3.800	0.200	5.6%	0.360	10%



The laboratory contacted Maine Standards Company Technical Support. Technical Support advised the laboratory that the results were not consistent with Peers and that this curve was typical of product incompletely thawed and/or not thoroughly mixed. Frozen products need to be brought to room temperature and gently inverted several times before dispensing. Errors of this nature are most evident at higher concentrations.

**Troubleshooting:** The laboratory took the troubleshooting step of rerunning the calibration verification / linearity experiment using a new TDM1 kit. The updated MSDRx® report shows that all Levels are within the statistical limits. The laboratory accepted the updated results and determined that they had validated the linearity across the reportable range of the method.

TDM 1 DIGN  
published CLIA total allowable error is 0.2 ng/mL or 20%, whichever is greater

maine standards COMPANY LLC

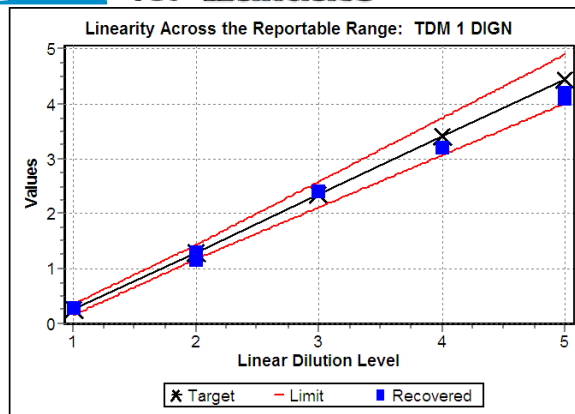
Accept  Comments

L	X	Rep 1	Rep 2	Rep 3
B	N/A			
1	1.0	0.3	0.3	0.3
2	2.0	1.2	1.3	1.15
3	3.0	2.4	2.4	2.4
4	4.0	3.2	3.2	3.2
5	5.0	4.1	4.2	4.15

Tested 0.30 to 4.15 ng/mL  
Validated 0.30 to 4.15 ng/mL

Mean versus Target Regression  
 $y = 0.922x + 0.081$

X	Target	Mean	+/- Diff	% Diff	+/- Limit	% Limit
1.0	0.256	0.300	0.044	17.2%	0.100	N/A
2.0	1.306	1.217	0.089	6.8%	0.131	10%
3.0	2.356	2.400	0.044	1.9%	0.236	10%
4.0	3.406	3.200	0.206	6.0%	0.341	10%
5.0	4.456	4.150	0.306	6.9%	0.446	10%



**Summary:** Maine Standards Company Technical Support advised that recovery was not consistent with Peers. In this case, inadequate thawing and/or mixing of the VALIDATE® product caused nonlinear response on the analyzer. Retesting the new material after properly thawing and mixing verified a linear response across the reportable range of the method.