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Analytical Precision and on-board stability Evaluation of the EMIT Methotrexate and Amikacin and EMIT 2000 Vancomycin and Digoxin Assays on the Atellica DT 250 Analyzer

Daughtry, J., Griffin, K., Glover, J., Johnson, N., Israel, B.
Siemens Healthcare Diagnostics Inc., Newark, DE, U.S.

Background

Methotrexate (a folic acid analog used for chemotherapy and as an immunosuppressant),[1] Amikacin (an aminoglycoside antibiotic),[2] Vancomycin (a glycopeptide antibiotic),[3] and Digoxin (a cardiac glycoside)[4] are life-saving drugs requiring careful monitoring to ensure correct therapeutic dosing while avoiding or minimizing severe adverse events and toxicity. Quantitative accuracy and precision is paramount to patient care.

The EMIT Methotrexate (MTX), and Amikacin (AMIK) assays and the EMIT 2000 Vancomycin (VANC) and Digoxin (DIG) assays are homogeneous assays for the quantitative analysis of their respective analytes in human serum or plasma used for therapeutic drug monitoring and drug overdose diagnosis. Protocols for these assays have been developed for use on the new Atellica DT 250 Analyzer and the data here demonstrate their performance and on-instrument stability.

Methods

Precision was evaluated using low, medium, and high controls per the CLSI EP05-A3 guideline:

- For all assays: 1 reagent lot, 2 runs/day for 20 days, ≥2 hours between runs

Recovery was determined by spiking purified analyte into negative serum targeting 8–9 levels across the assay range and calculating % difference between the mean result and mean target for 5 replicates

Method Comparisons were conducted per CLSI EP09c guidelines. Only sample results within each assay's range were included in the final analysis.

- 1 reagent lot, individual patient samples (native or contrived) comprising the assay range, 2 replicates/sample/instrument.
- Only results within assay range and first replicates were used for statistical analyses.

On-board instrument stability (OBS) was evaluated per the CLSI EP25 ED2:2023 guideline.

- 1 reagent lot loaded on Day 0 and stored onboard for the duration of the study
- Reagent bottles capped when not in use.
- Calibration at Day 0, periodic recalibration (using semi-quantitative calibration curves)
- Drift of mean recovery (≥ 5 replicates/sample) evaluated over duration of study

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Results

Assay precision

Precision evaluation indicated repeatability CVs ranged from 1.6–3.0% for MTX, 2.4 to 4.6% for AMIK, 1.2 to 2.2% for VANC, and 4.1 to 7.5% for DIG. For within-lab precision, CVs ranged from 1.9–4.0% for MTX, 2.8–5.6% for AMIK, 4.2–5.9% for VANC, and 5.8–11.6% for DIG.

Table 1. Repeatability and within-lab precision for all assays (n = 80) on the Atellica DT 250

Assay	Control	Mean (µmol/L)	Repeatability		Within-lab precision	
			SD (µmol/L)	CV (%)	SD (µmol/L)	CV (%)
EMIT MTX	Low	0.42	0.013	3.0	0.017	4.0
	Medium	1.46	0.023	1.6	0.028	1.9
	High	9.09[a]	0.165	1.8	0.188	2.1
EMIT AMIK	Low	6.9	0.17	2.4	0.20	2.8
	Medium	18.1	0.48	2.6	0.73	4.0
	High	30.4	1.39	4.6	1.69	5.6
EMIT 2000 VANC	Low	7.4	0.16	2.2	0.44	5.9
	Medium	18.5	0.23	1.2	0.77	4.2
	High	7.4	0.16	2.2	0.44	5.9
EMIT 2000 DIG	Low	0.64	0.048	7.5	0.074	11.6
	Medium	1.07	0.047	4.4	0.073	6.8
	High	2.65	0.109	4.1	0.154	5.8

[a]*Sample was diluted for testing*

Assay range/recovery

All three assays tested met predefined criteria, demonstrating acceptable sample recovery.

Table 2. MTX recovery.

Spiked Sample (µg/mL)	Mean (µg/mL)	% Difference	% Recovery
0.30	0.30	7	107
0.53	0.51	-2	98
0.76	0.74	-3	97
0.98	1.00	0	100
1.21	1.19	-4	96
1.44	1.52	3	97
1.67	1.76	2	98
1.89	1.94	-1	99
2.12	2.12	-4	96

Table 3. AMIK recovery.

Spiked Sample (µg/mL)	Mean (µg/mL)	% Difference	% Recovery
3.0	3.2	7	107
5.0	4.7	-6	94
10.0	10.3	3	103
20.0	18.4	-8	92
25.0	25.7	3	103
30.0	32.7	9	109
42.0	40.1	-5	95
45.0	43.4	-4	96

Table 4. VANC recovery.

Spiked Sample (µg/mL)	Mean (µg/mL)	% Difference	% Recovery
5.0	5.0	0	100
10.0	9.3	-7	93
15.0	13.8	-8	92
20.0	18.4	-8	92
25.0	23.8	-5	95
30.0	27.4	-9	91
35.0	32.1	-8	92
40.0	37.3	-7	93
45.0	42.5	-6	94

Table 5. DIG recovery.

Spiked Sample (µg/mL)	Mean (µg/mL)	% Difference	% Recovery
0.30	0.29	-3	97
0.50	0.60	20	120
0.80	0.69	-14	86
1.00	0.94	-6	94
2.00	2.07	3	103
2.50	2.56	2	102
3.50	3.70	6	106
4.50	4.92	9	111

Method comparison

Method Comparison testing between the Atellica DT 250 analyzer and the Viva-E/Viva-ProE systems for each assay demonstrated strong correlations for AMIK, MTX, VANC and DIG. See Table 6 and Figure 1.

Table 6. Regression statistics for all for assays.

Assay	Regression Type	n	Slope	95% CI (Slope)	Intercept	Corr. Coeff. (r)	Range
AMIK	Ordinary Least Square	50	0.97	0.94–0.99	0.0	.995	0.3–2.0 µg/mL
MTX	Weighted Least Square	50	0.95	0.91–0.98	–0.2	.975	3.1–42.9 µmol/L
VANC	Weighted Deming	40	1.06	1.01–1.10	–0.1	.993	5.8–45.1 µg/mL
DIG	Ordinary Least Square	60	1.06	1.01–1.10	–0.09	.998	0.34–4.27 ng/mL

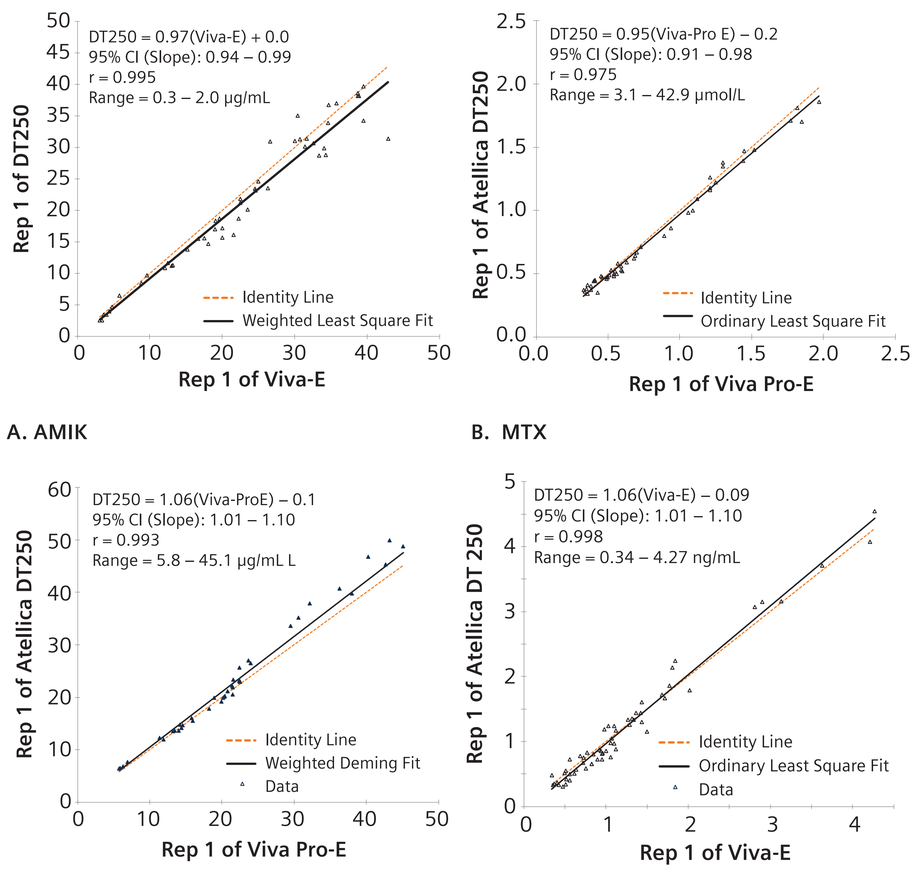

Figure 1. Regression analysis results for A. AMIK; B.MTX; C. VANC; D. DIG.

On-board stability

On-board stability on the Atellica DT 250 Analyzer was found to be up to 8 weeks for all assays.

Conclusion

The EMIT Methotrexate, Vancomycin, Amikacin, and Digoxin assays on the new Atellica DT 250 Analyzer demonstrated low CVs, strong correlation, and precise, robust performance across clinically relevant concentrations. In addition, the analyzer supported up to eight weeks of onboard reagent stability for all assays.

References

1. *Methotrexate. https://www.pdr.net/drug-summary/?drugLabelId=1797*
2. *Amikacin. https://www.pdr.net/drug-summary/?drugLabelId=676*
3. *Vancomycin. https://www.pdr.net/drug-summary/?drugLabelId=802*
4. *Digoxion. https://www.pdr.net/drug-summary/?drugLabelId=724*

All CLSI Guidelines can be found on the CLSI website: https://clsi.org

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