Evaluation of a Novel Point of Care Testing Device for Rapid Measurement of Clozapine in Whole Blood

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Background

- Positive clinical response is associated with threshold clozapine blood levels[1-7]
- For monitoring drug levels and adherence practice guidelines, clinical support tools, and expert consensus recommendations[8-12] recommend measuring clozapine in blood
- Measurement of clozapine blood levels can provide clinicians objective evidence to help avoid treatment failures.
- Knowledge of nonadherence to atypical antipsychotics, including clozapine, may help clinical decision making.[13]

Point of Care (POC) testing of clozapine blood levels offers:

- immediacy of results for clinicians and patients,[9]
- capillary sampling that is preferred by both clinicians and patients,[14] and
- improved utilization of clozapine, which is the only effective drug for treatment-resistant schizophrenia, where monitoring levels is important for effective use.[15]

The aim of this study was to validate the POC MyCare Insite Clozapine Test for use in clinical practice.

Methods

Reagents for CE marked MyCare® Psychiatry Clozapine Assay Kit, an automated homogenous immunoassay to measure clozapine levels in serum and plasma, were modified for testing clozapine levels in whole blood (WB) with a POCT analyser, the MyCare Insite. This small, portable device generated clozapine levels in under 7 minutes for near patient testing.

As reported in the literature, WB contains ~20% less clozapine than serum or plasma prepared from WB.[21,22] Clozapine monitoring recommendations are based on studies on serum/plasma levels; the WB results were standardized to give results comparable to serum measured by liquid chromatography - tandem mass spectrometry (LC-MS/MS). Reassignment of calibrator concentrations was performed with a "training set" of 95 clozapine blood samples collected from 21 patients treated at the University of Maryland, School of Medicine, Treatment Research Program. Capillary WB and EDTA plasma were simultaneously collected and clozapine was measured on the MyCare Insite, and by LC-MS/MS.

Performance validation was done according to Clinical Laboratory Standards Institute Guidelines for precision, interference, linearity, detection capability and method comparison. Each study was conducted with at least five operators and fifteen analysers.

For the validation study method comparison a total of 313 individual samples were collected during patients' regular visits at two sites of the South London and Maudsley NHS Foundation Trust. Capillary whole blood samples were tested immediately using the MyCare Insite Clozapine Test by two operators using four analysers. Matching venous plasma samples were tested by a validated LC-MS/MS method.

Results

Assay bias associated with endogenous interferents and an extreme range of hematocrits was < 10% (Table 1).

The specificity of the antibody has previously been characterized: no interference from over 140 small molecules was found.[16] The assay is specific for clozapine and does not cross-react with metabolites or structurally related antipsychotic medications (Table 2).

Results are reported from 0 to 1600 ng/mL. Sensitivity (Table 3) was determined according to CLSI Guideline EP17-A2.

Table 1: Interferents and Hematocrit

Interferent	Level	
Rheumatoid Factor	510 IU/mL	
Total Protein	6.1 g/dL	61 g/L
Icteris	44 mg/dL	753 µmol/L
Lipemia	510 mg/dL	5.7 mmol/L
Hemolysate	1050 mg/dL	
Hematocrit	35% - 50%	

Table 2: Selectivity

Compound	Cross-Reactivity
Norclozapine	5.5%
Clozapine N-oxide	5.3%
Quetiapine	< 0.5%
Olanzapine	6.6%

Table 3: Sensitivity

Limit of	ng/mL
Blank (LoB)	34
Detection (LoD)	126
Quantitation (LoQ)	175

The agreement between Insite and LC-MS/MS observed for the calibrator assignment training set (Chart 1) was reproduced in the validation method comparison (Chart 2) with 304 patient samples. Four samples > 1600 ng/mL on the MyCare Insite were eliminated from the analysis, as were five discrepant samples to be retested. The CLSI EP6 linearity was demonstrated with 10 levels from 122 to 1390 ng/mL, measured n = 5 (Chart 3). Precision was evaluated over 20 days according to CLSI guideline EP05-A3; the average results from 3 lots generated by 6 different operators using 22 analysers are shown (Chart 4). Repeatability with patient samples (Table 4) showed that replicate two differed from replicate one by no more than 10%.

Chart 1: Method Comparison for Calibrator Assignment,
n = 95, R = 0.9, slope = 1.02, intercept = -23

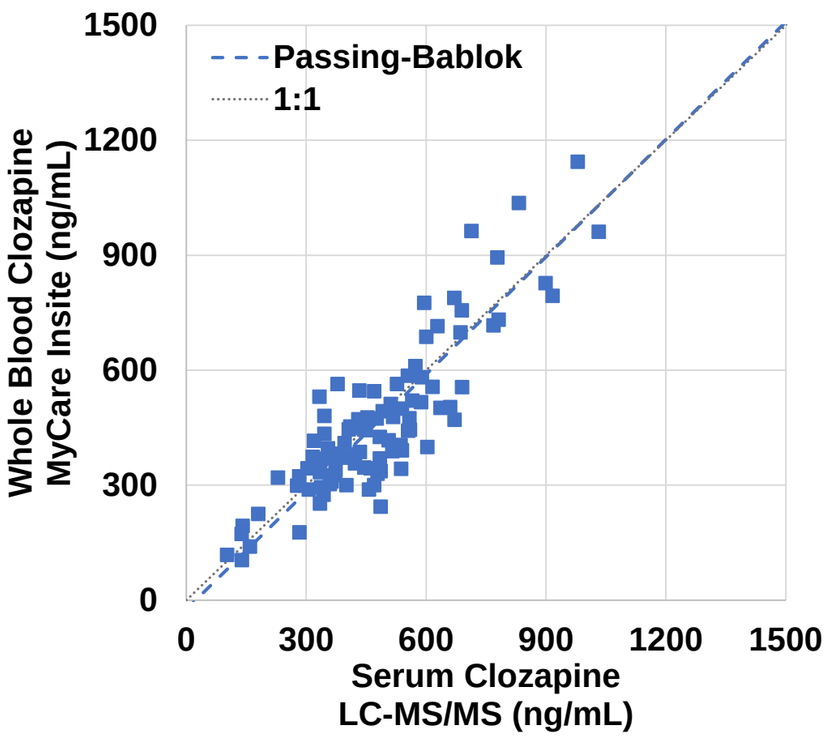


Chart 2: Method Comparison for Validation,
n = 304, R = 0.9, slope = 0.971, intercept = -21.2

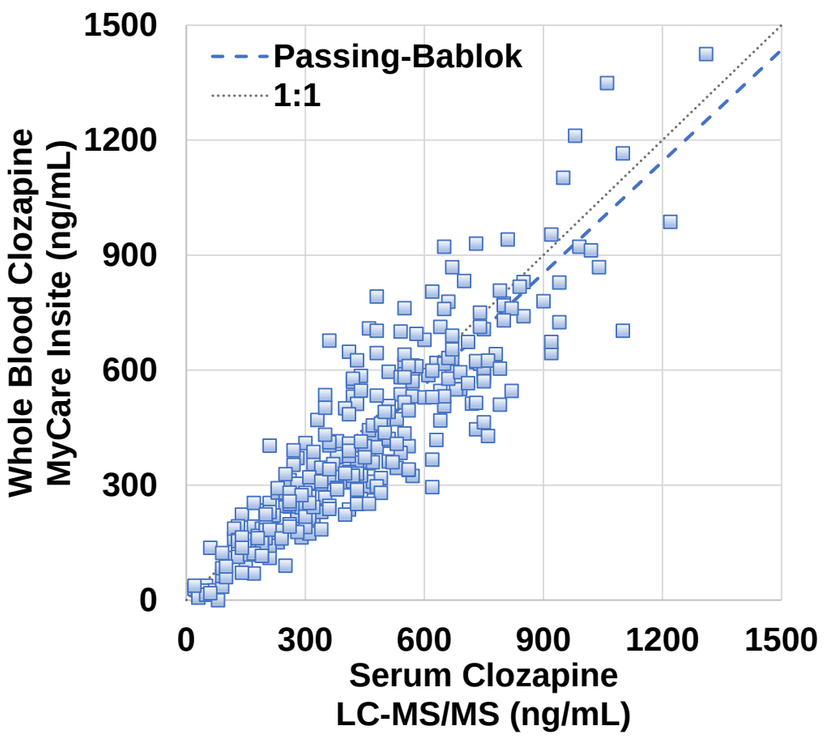


Chart 3: Linearity
0% deviation from linearity; ≤ 10% CV above LoQ

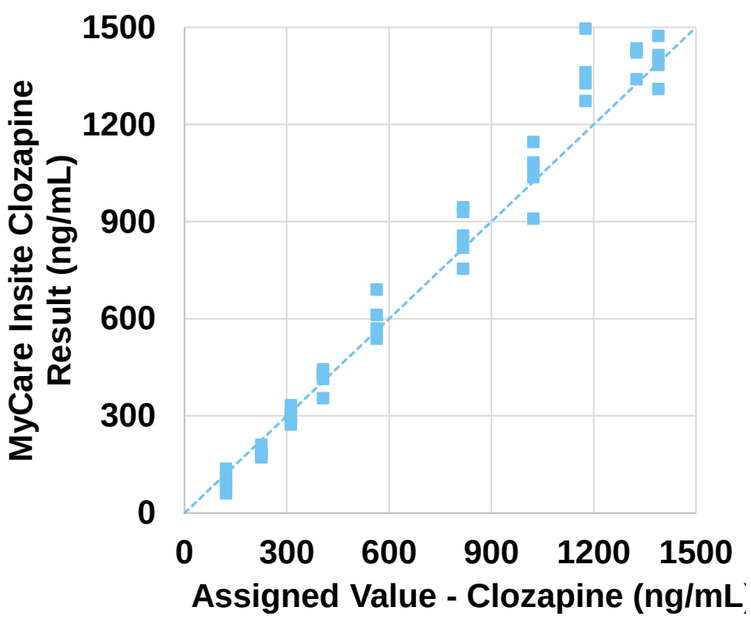
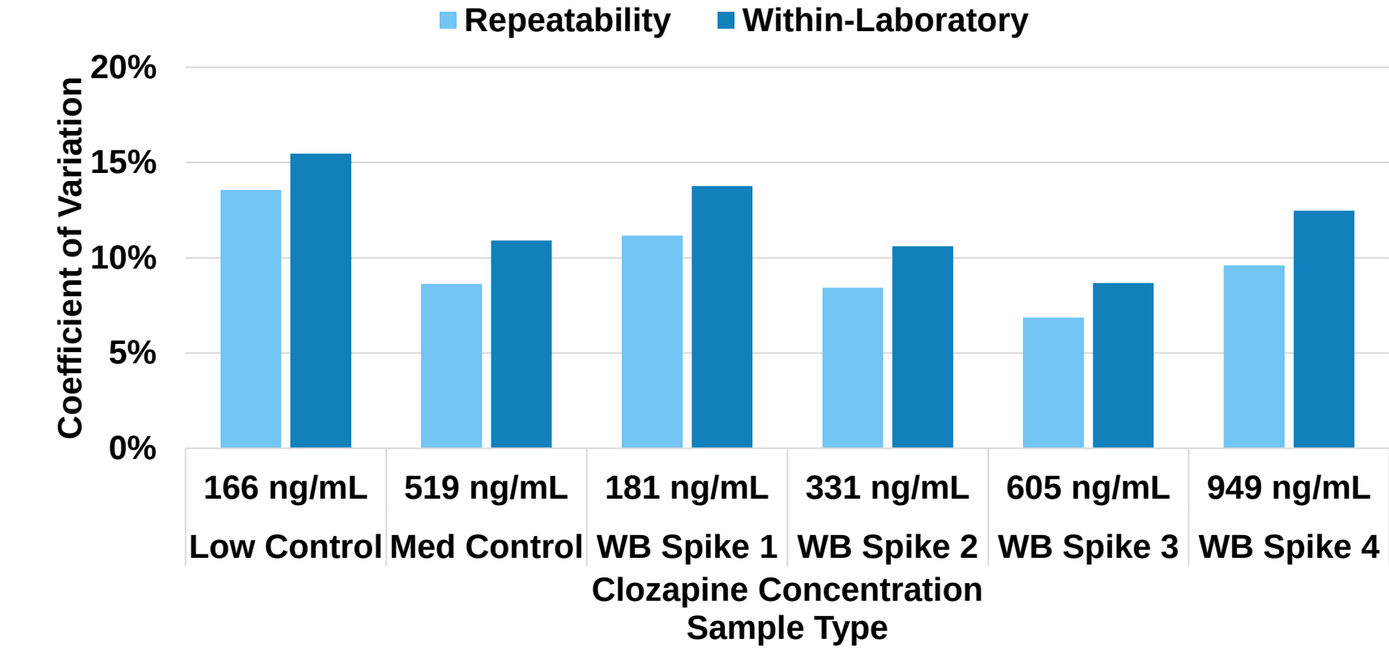


Table 4: Patient Sample Repeatability

ng/mL (mean)	Difference
453	-1%
382	-10%
329	-2%
199	1%

Chart 4: Precision
repeatability < 12% on whole blood samples; within-laboratory < 16%



Conclusions

The MyCare Insite Clozapine Test:

- Is the first point of care method developed to test patients on clozapine
- Provides accurate and reliable point-of-care results for clozapine
- Could be used to meet an unmet clinical need by providing psychiatrists with close to patient testing and immediate results for clozapine levels.

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