WORLDLAB · EUROMEDLAB ROMA 2023

P0152

SENTINEL DIAGNOSTICS

EVALUATION OF CALIAGOLD® ASSAY FOR THE QUANTIFICATION OF FECAL CALPROTECTIN WITH THE NEW SENTiFIT® 800 HIGH-THROUGHPUT ANALYZER

C. Paparella, A. Barazzutti, R. Lucini, S. Brambilla
Sentinel CH. SpA, Via Robert Koch 2, 20152 Milano, Italy

Background
The objective of this study is to evaluate the analytical performances of the CALiaGold® assay (Sentinel Diagnostics) for the quantitative determination of Calprotectin in human feces on the high-throughput, fully automated SENTiFIT® 800 Analyzer (Sentinel Diagnostics). The presence of Calprotectin in human stool specimens is intended as an aid in the assessment of intestinal mucosal inflammation. The assay's results can be used as an aid to diagnosis in distinguishing organic, inflammatory disease of the gastrointestinal tract from functional disease, in patients with chronic abdominal pain and, furthermore, as an aid in IBD disease monitoring.

Methods
The CALiaGold® test is a Particle Enhanced Turbidimetric ImmunoAssay (PETIA) and allows quantification of Calprotectin in fecal extracts. Calprotectin is extracted from fecal samples using the extraction buffer contained in the CALiaGold® pierceTube device (Sentinel Diagnostics). Calprotectin available in the sample mediates immunoparticles agglutination. The tests were conducted following CLSI[1] (Clinical and Laboratory Standards Institute) Guidelines and Microsoft Excel statistical tool Analyse-it was used. Performances evaluation included Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantitation (LoQ), intra-assay imprecision, total imprecision, linearity, on board reagent stability, instruments correlation, Hook effect.

Results

Test	Results
Limit of Blank (LoB)	Lot A 1.25 µg/g Lot B 2.31 µg/g
Limit of Detection (LoD)	Lot A 4.5 µg/g **(Figure 1.)** Lot B 3.6 µg/g
Limit of Quantitation (LoQ)	Lot A 14.8 µg/g Lot B 15.7 µg/g **(Figure 2.)**
Intra Assay Imprecision	Sample 1 (38.6 µg/g): %CV 2.6% Sample 2 (104.7 µg/g): %CV 1.9% Sample 3 (513.1 µg/g): %CV 3.9% Sample 4 (1042.8 µg/g): %CV 1.5% Sample 5 (1883.7 µg/g): %CV 1.8%
Total Imprecision (during 20 testing days up to reagent age of 60 days)	Sample 1 (39.4 µg/g): %CV 5.7% Sample 2 (113.0 µg/g): %CV 4.3% Sample 3 (524.3 µg/g): %CV 2.4% Sample 4 (1081.4 µg/g): %CV 2.7% Sample 5 (1955.6 µg/g): %CV 3.7%
On board reagent stability (up to 33 days) **(Figure 3.)**	Sample 1 (36.5 µg/g): min % bias: -3.9% max % bias: 2.9% Sample 2 (107.5 µg/g): min % bias: -8.5% max % bias: 4.1% Sample 3 (507.3 µg/g): min % bias: -3.1% max % bias: 1.3% Sample 4 (1041.4 µg/g): min % bias: -0.9% max % bias: 2.1% Sample 5 (1855.4 µg/g): min % bias: -3.6% max % bias: 0.0%
Linearity **(Figure 4.)**	Up to 2200 µg/g
Instrument correlation (vs. SENTiFIT® 270) **(Figure 5.)**	Passing-Bablok fit: y = -3.97 + 0.98 x r = 0.996
Hook effect	Not detectable up to 6000 µg/g

Figure 1. Limit of Detection (LoD)
Lot A results, worst of the two lots.

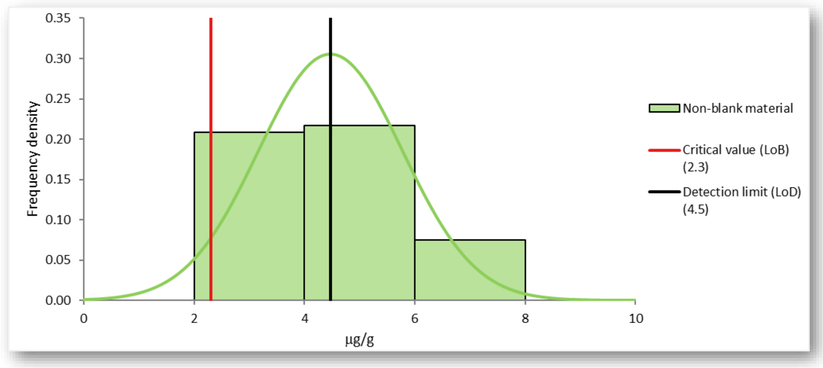
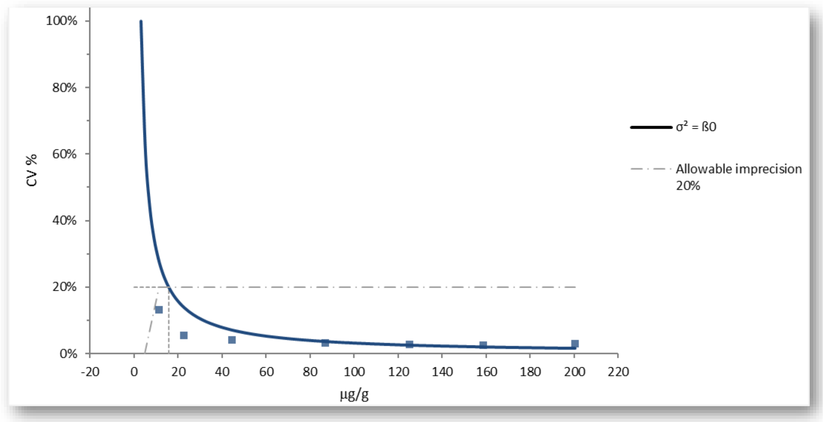


Figure 2. Limit of Quantitation (LoQ)
Lot B results, worst of the two lots.



Model	Constant variance function
Equation	σ² = ß0

Parameter	
β_0	9.89773
-LogLikelihood	167.46
$U_{minimum}$	-1.5
$U_{maximum}$	200.5

Inverse Prediction

CV	U
20.0%	15.7

Figure 3. On board reagent stability
Calculated as % bias vs. Time 0.

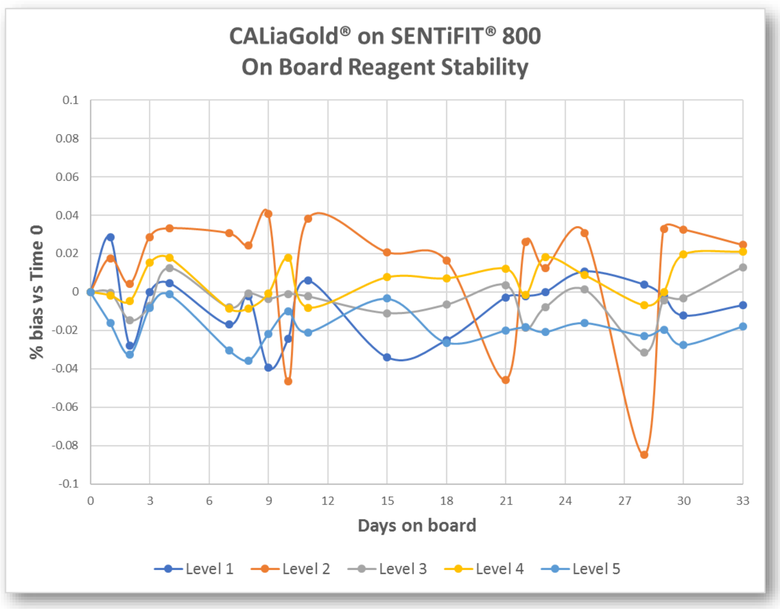


Figure 4. Linearity
Bias vs. predicted value.

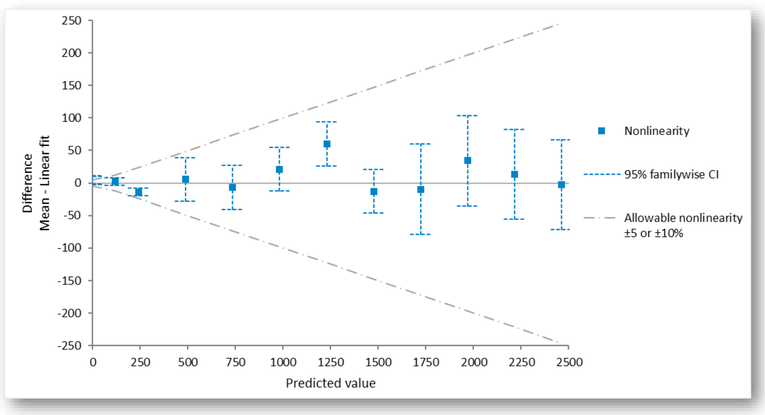
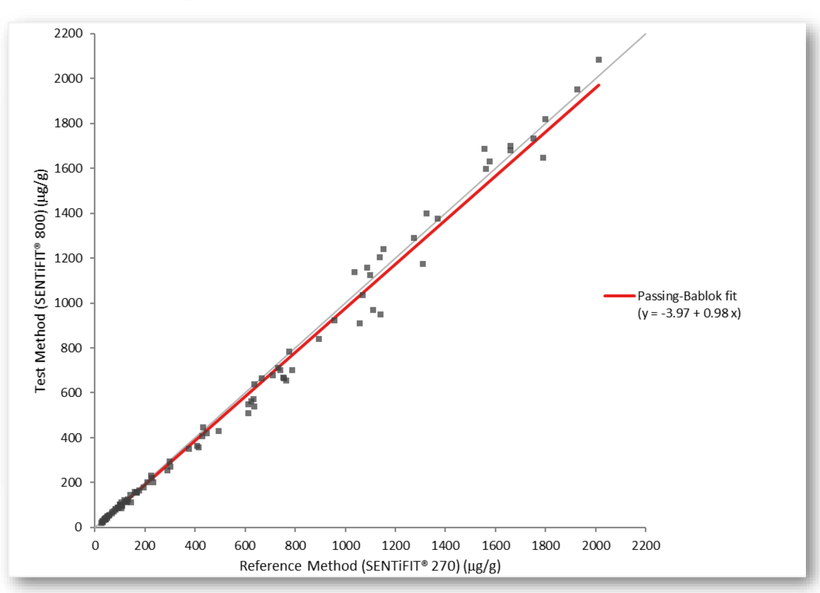


Figure 5. Instruments correlation
With Passing-Bablok fit: y = -3.97 + 0.98 x r = 0.996



Conclusions
Analytical performances of CALiaGold® assay on the high-throughput, fully automated SENTiFIT® 800 Analyzer meet the requirements for its use as quantitative determination of Calprotectin in human feces and make this assay very suitable for the routine measurement of this protein.

References
1) CLSI. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures (EP17-A2); Evaluation of Linearity of Quantitative Measurement Procedures (EP06); Evaluation of Precision of Quantitative Measurement Procedures (EP05-A3); Molecular Diagnostics Methods for Infectious Diseases (MM03); Quantitative Molecular Methods for Infectious Diseases (MM06-A2)