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Evaluation of a new assay for the quantitative determination of calprotectin in human feces (CALiaGold®)

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Introduction

The objective of this study is to evaluate the analytical and clinical performances of a new assay for the quantitative determination of calprotectin in human feces (CALiaGold®) on SENTiFIT® 270 Analyzer. The presence of calprotectin in human stool specimens is intended as an aid in the assessment of intestinal mucosal inflammation. The assay results can be useful in distinguishing organic, inflammatory disease of the gastrointestinal tract (inflammatory bowel disease, IBD, e.g. Crohn's disease or ulcerative colitis, UC) from functional disease (irritable bowel syndrome, IBS), in patients with chronic abdominal pain, and as an aid to IBD monitoring.

Aims and Methods

The CALiaGold® test is a particle enhanced turbidimetric immunoassay (PETIA) and allows quantification of calprotectin in fecal extracts. Fecal samples are dissolved in the extraction buffer using the CALiaGold® Tube device. The extracts are incubated with reaction buffer and mixed with polystyrene nanoparticles coated with calprotectin-specific antibodies (immunoparticles). Calprotectin in the sample mediates immunoparticles agglutination. Sample turbidity, measured by light absorbance, increases with calprotectin-immunoparticle complex formation and is proportional to the calprotectin concentration. The detected light absorbance allows quantification of calprotectin concentration via interpolation on an established calibration curve.

Results

The Limit of Blank (LOB) (1 saline sample x 20 replicates x 3 runs) was 12.2 µg/g. The Limit of Detection (LOD) (1 sample at 4-fold LOB concentration x 20 replicates x 3 runs) was 18.3 µg/g. The Limit of Quantitation (LOQ) (8 dilution levels starting from sample at concentration 100 µg/g x 10 replicates x 1 run) was 21.3 µg/g at %CV lower than 20%. The Intra Assay (3 runs x 3 samples x 20 replicates) gave the following results:

	Sample 1	Sample 2	Sample 3
n	60	60	60
µg/g	50.7	115.5	1576.4
SD	3.4	4.9	105.1
%CV	6.6%	4.3%	6.7%

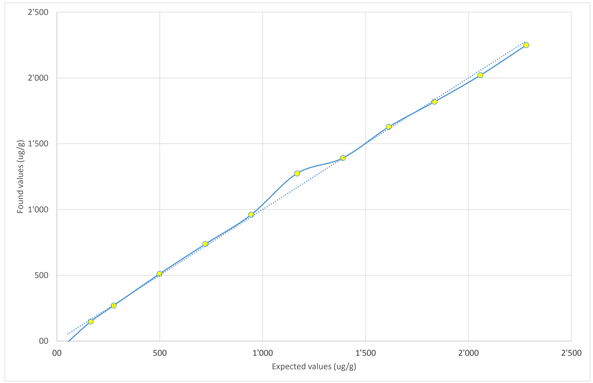
Total imprecision study (44 testing days x 2 runs x 2 observations x 4 level samples – during total time of 66 days) gave the following results:

	Lev 1	Lev 2	Lev 3	Lev 4
n	176	176	176	176
µg/g	59.1	161.3	509.9	1435.2
SD	4.0	5.3	10.1	33
%CV	6.7%	3.3%	2.0%	2.3%

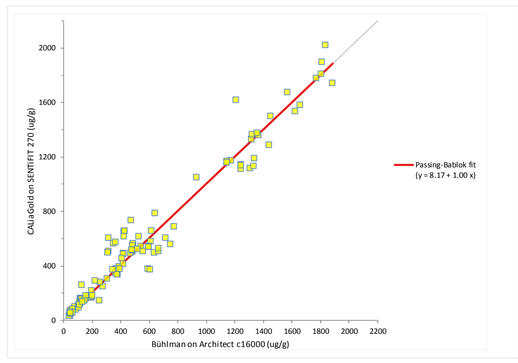
Reagent on board stability was up to 66 days, obtaining % bias versus Time 0 (mean value):

	Lev 1	Lev 2	Lev 3	Lev 4
µg/g	60.0	161.4	509.5	1433.2
% bias	-4.06%	0.0%	-0.6%	-0.2%

The test was linear up to 2249.5 µg/g, considering the % bias versus theoretical concentration lower than ±10% for each dilution point of linearity curve.



This test (y) was compared with Bühlmann fCAL turbo on Architect c16000 (x) that uses the same methodology and gave the following results: Y = 8.17 + 1.00; correlation coefficient (r) = 0.979; number of samples = 114.



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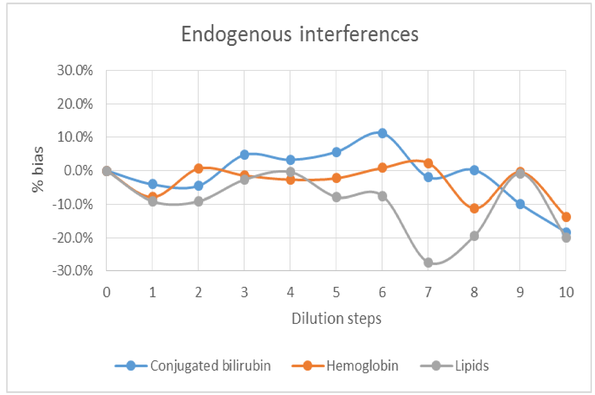
N	114	
	Minimum	Maximum
Bühlman on Architect c16000	40.4	1885.9
CALiaGold on SENTiFIT 270	31.0	2017.5
Correlation - r	0.979	

Equation: CALiaGold on SENTiFIT 270 = 8.17 + 1.00 Bühlman on Architect c16000

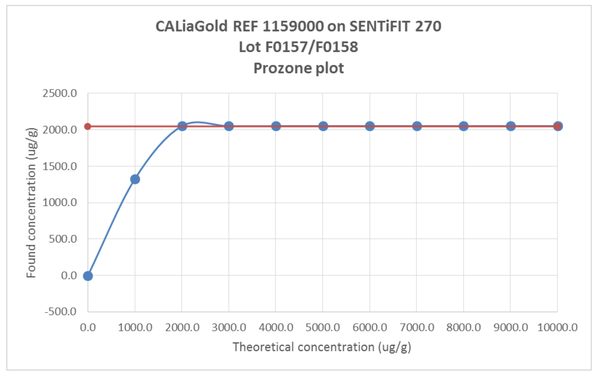
Parameter	Estimate	Bootstrap 95% CI
Intercept	8.17	0.3434 to 15.33
Slope	1.00	0.9522 to 1.023

CI based on 999 bootstrap samples.

The test is not affected by the presence of conjugated bilirubin up to 0.5 mg/L, hemoglobin up to 0.07 g/L, lipids up to 1.2 g/L.



There is not Hook effect/antigen excess up to 1323.9 µg/g. High concentration samples (up to 10000 µg/g) were flagged by the instrument (no false negative results).



Conclusions

Analytical and clinical performances of CALiaGold® assay on SENTiFIT® 270 Analyzer meet the requirements for its use as quantitative determination of calprotectin in human feces. Specificity and precision make this assay very suitable for routine measurement of this analyte.