

## Achieving rapid and reliable results with CALiaGold® a new robust fCal assay on Atellica CH 930

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### Background

Fecal calprotectin (fCal) measurements are generally recommended for gastrointestinal disorders to differentiate between inflammatory bowel disease and irritable bowel syndrome. For a long period of time only microtiter-ELISA based assays were available. Problems with such assays are long test-turnaround times and that they are labor demanding. Over the last years, improvements in sampling tubes and sample pretreatment have enabled fCal measurements to be performed on automated clinical chemistry platforms. The objective of this study was to evaluate the analytical performances of CALiaGold®, a new robust fCal assay from Sentinel Diagnostics, in comparison to an existing fCal assay from Bühlmann, BÜHLMANN® fCAL turbo. Also, the feasibility to use this type of sample material on an automated chemistry analyzer (the Atellica CH 930, Siemens Healthineers) was evaluated.

### Methods

The two particle enhanced turbidimetric immunoassays CALiaGold® (Sentinel Diagnostics) and BÜHLMANN® fCAL turbo (BÜHLMANN), that both quantifies calprotectin in fecal extracts, were evaluated on an Atellica CH 930 analyzer (Siemens Healthineers). The reagent manufacturers provided assay parameters and open channels were used on the Atellica CH 930 analyzer. No extra washing steps were programmed on the Atellica CH 930 analyzer before, during or after testing and routine samples were processed as normal during the testing. The 97 fecal patient samples were extracted for both assays according to the manufacturers instructions for use (IFU). All 194 extractions were done by the same person in the laboratory. After extraction, the tubes were stored at 4°C over night and tested in duplicate the following day on the Atellica CH 930 analyzer. According to the Bühlmann IFU the tubes were centrifuged for 10 minutes prior to testing whereas the Sentinel tubes didn't have to be centrifuged according to the Sentinel IFU. Samples above the measuring range were diluted 1:10 for each assay in accordance with the specific IFU. Intra assay (20 replicates x 2 levels) and total imprecision (9 days x 5 replicates x 2 levels) for both assays was tested using CALiaGold® Control Set from Sentinel Diagnostics. For total imprecision testing the QC storage was used onboard the Atellica CH 930 analyzer. Thus, QC material was on board for 9 days and completely handled by the Atellica CH 930 analyzer, all QC runs were programmed and automatically performed.

### Results

No observed interference to or from other samples or assays on the Atellica CH 930 analyzer was observed during the test period. Also, the laboratory in Falun, over the past years, has done more than 20000 fCal tests on their Atellica CH 930 analyzer with no observed interferences. Both assays showed good analytical performances, both with a total imprecision below 3.5%. As seen in Table A, intra assay and total imprecision were comparable between the two assays. Onboard stability of the QC material was satisfactory for the entire testing period of 9 days.

	Intra assay				Total imprecision			
	Sentinel QC1	Sentinel QC2	Bühlmann QC1	Bühlmann QC2	Sentinel QC1	Sentinel QC2	Bühlmann QC1	Bühlmann QC2
n	20	20	20	20	45	45	45	45
Mean µg/g	94.4	372.1	249.9	1027.1	103.6	373.4	243.4	1017.7
SD	1.8	5.2	6.3	14.7	3.2	6.9	3.7	8.2
CV%	1.9	1.4	2.5	1.4	3.1	1.9	1.5	0.8

Table A. Intra assay and total imprecision for both fCal assays shows comparable CV.

No hook effect was observed for the Sentinel CALiaGold® assay up to the highest tested sample of 20075 µg/g. On the other hand, the Bühlmann fCAL assay showed a clear hook effect on this sample with an initial value of 1472 µg/g and after manual dilution 1:50 it became 65000 µg/g indicative of an initial hook effect. Out of the 97 patient samples, 9 were below the measuring range for either or both methods and excluded from the correlation data, 9 samples were above the measuring range for both methods and these were diluted 1:10. As mentioned above, one of these samples was excluded from the correlation data since it showed a hook effect for Bühlmann fCal. Both assays were in agreement on values, both below and above the measuring range. Hence, 87 patient samples were tested in duplicate for both assays to investigate the correlation and regression between the assays, Figure 1 and 2. The data shows good correlation between the two assays over the entire measuring range (Figure 1). If values up to 400 µg/g (for the Bühlmann assay) are considered (Figure 2), the correlation is still good and the Sentinel CALiaGold® assay gives approximately 40% higher values. These results are in good agreement with the given grey zones of each manufacturer, Bühlmann has a gray zone between 80 – 160 µg/g whereas Sentinel gives a gray zone between 50 – 200 µg/g.

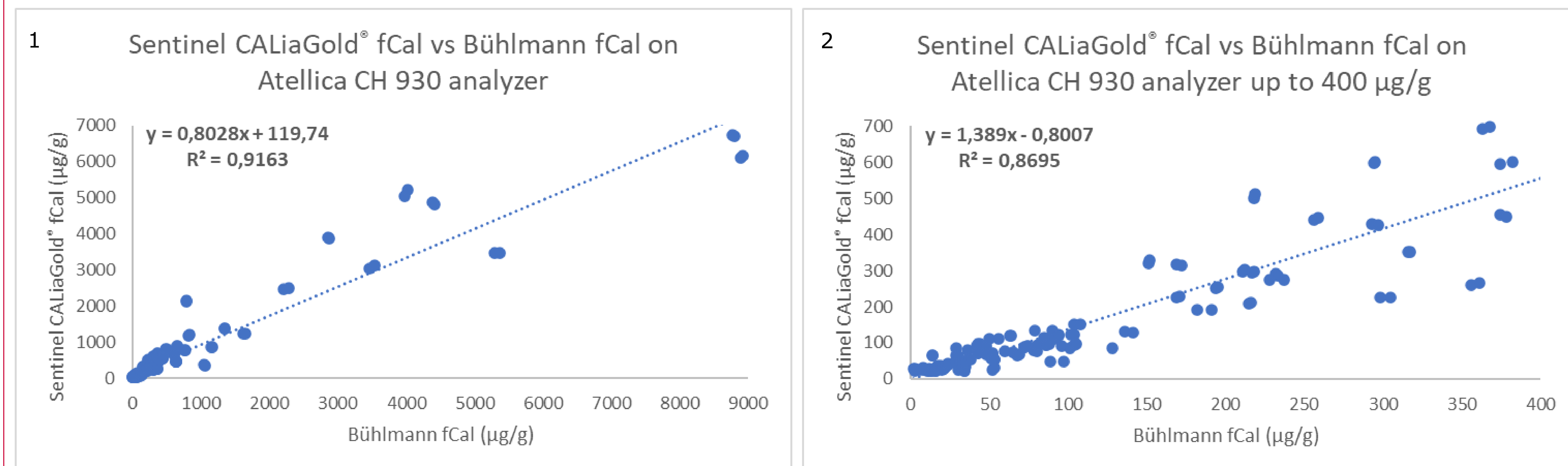


Figure 1 and 2. Correlation data between the fCal assays CALiaGold® and BÜHLMANN® fCAL turbo when measured on an Atellica CH 930 analyzer.

### Conclusions

The combination of CALiaGold® kit and Atellica CH 930 analyzer with its capacity of 1200 photometric tests per hour, on-board QC handling and automated maintenance can be recommended to measure fCal in a simple, fast, safe way and with high quality fashion. The overall analytical performances of the two assays are good and comparable. An observed analytical advantage for the CALiaGold® fCal assay is that it seems to be less sensitive to hook effect compared to the BÜHLMANN® fCAL turbo assay. From a workflow perspective CALiaGold® is considered the preferred assay since it doesn't have to centrifuge 10 minutes before testing as in the case with BÜHLMANN® fCAL turbo. From a laboratory perspective this concept will decrease the workload from the increasing number of fCal tests and improve the availability of the testing without compromising on safety when considering sample or assay interference. In conclusion, particle enhanced turbidimetric immunoassay for fCal such as the CALiaGold® assay reduces test-turnaround times and permit more laboratories to perform the measurements.