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Detection of calprotectin in clinical fecal samples: A COMPARATIVE STUDY OF ACTIM® CALPROTECTIN RAPID TEST AND BÜHLMANN FCAL ELISA

Kanto Laura[1], Reinman Mirka[1], Korvuo Armi[1], Juhila Juuso[1].

[1]Medix Biochemica, Klovinpellontie 3, FI-02180 Espoo, Finland

CALPROTECTIN – AN INFLAMMATORY BIOMARKER

Calprotectin (Figure 1) is a pro-inflammatory, calcium-binding protein complex primarily secreted by neutrophils at the site of inflammation.[1–3] Calprotectin is released and easily detected in the mucosa, where neutrophils accumulate. Analysis of fecal calprotectin is commonly used in clinical diagnostics and follow-up of inflammatory bowel diseases (IBD) – Crohn's disease and ulcerative colitis – that are characterized by pathological inflammation of the bowel.[4]

Fecal calprotectin is a non-invasive, stable and sensitive biomarker of IBD. While several assays for the detection of fecal calprotectin have been developed, both quantitative differences and low intra-assay agreement have been observed between different commercial calprotectin assays.[5,6] These differences may be caused by variable extraction devices and assay procedures.

In this study the performance of the semi-quantitative Actim® Calprotectin rapid test was compared with a quantitative Bühlmann fCAL ELISA reference test. Actim Calprotectin is a rapid, one-step dipstick test that reports fecal calprotectin concentration at three clinically relevant cut-offs (Table 1). Actim Calprotectin distinguishes patients with healthy mucosa or irritable bowel syndrome (IBS), from those with pathologic inflammation of the bowel.

TABLE 1. The clinical significance of fecal calprotectin concentration ranges.

Calprotectin concentration range	Clinical implication
<50 µg/g	No inflammation; possible IBS
50–200 µg/g	Mild inflammation
>200 µg/g	IBD

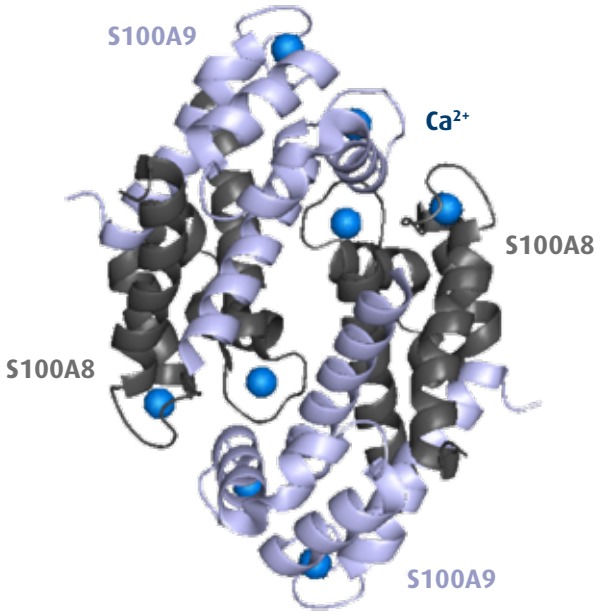

FIGURE 1. The complex of calprotectin heterodimers. Calprotectin subunits S100A8 (grey) and S100A9 (violet) each bind two calcium ions (blue). The model was generated from a publicly available calprotectin crystal structure (PDB ID: 1XK4) using PyMOL software.

QUANTIFICATION OF FECAL CALPROTECTIN

Actim Calprotectin is a hygienic and user-friendly dipstick test that can be performed without opening the test tube cap. The test includes a single activation step, and reports visually interpretable results in just 10 minutes. An Actim Calprotectin test result is reported as blue lines appearing on the test stick. Bühlmann fCAL ELISA is a 96-well plate-based test that requires serial washing and antibody binding steps, and necessitates the use of laboratory equipment (Figure 2).

In the present study, calprotectin concentration was quantified from 100 frozen clinical fecal samples. Fecal suspensions were prepared in sample buffer at the same time for both tests. The assays were then performed in parallel by Actim Calprotectin test (35032ETAC, Medix Biochemica) and Bühlmann fCAL ELISA (EK-CAL2-WEX, Bühlmann Laboratories AG) according to manufacturers' instructions.

The Actim Calprotectin rapid test has previously been calibrated to Bühlmann fCAL ELISA. Calprotectin concentration in the original fecal samples was also verified by Phadia Immunoassay Analyzer.

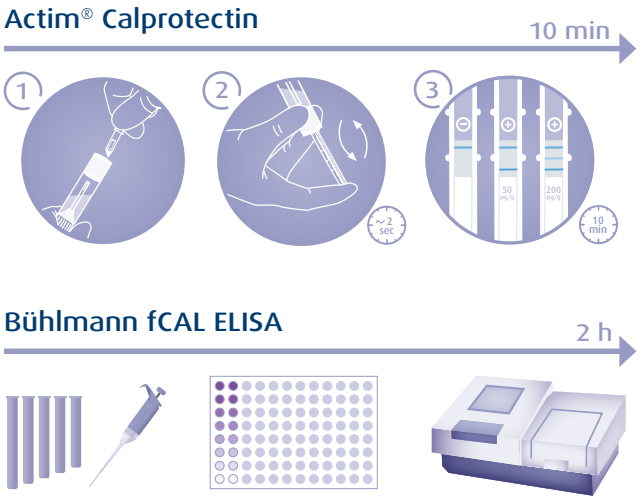

FIGURE 2. A schematic comparison of Actim Calprotectin and Bühlmann fCAL ELISA test procedures. Actim Calprotectin is a one-step dipstick test that requires a single activation step.

EXCELLENT AGREEMENT WITH QUANTITATIVE TEST

This comparative study showed that results from Actim Calprotectin were consistent with those of Bühlmann fCAL ELISA (Figure 3), with excellent agreement at all three clinically relevant concentration ranges (Table 2). Inter-assay disagreement was observed only for samples whose calprotectin concentration according to Bühlmann fCAL ELISA was near the cut-off thresholds of Actim Calprotectin test. Therefore, Actim Calprotectin provides a rapid and reliable alternative for IBD monitoring.

TABLE 2. Agreement between Actim Calprotectin and Bühlmann fCAL ELISA tests.

Calprotectin concentration	Agreement
0–50 µg/g	97%
50–200 µg/g	87%
≥200 µg/g	94%

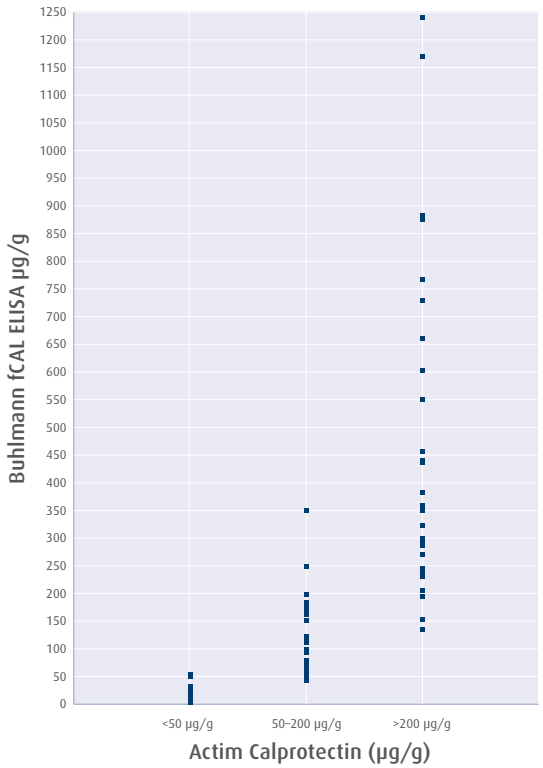

FIGURE 3. Agreement between Actim Calprotectin and Bühlmann fCAL ELISA tests in the quantification of fecal calprotectin concentration (µg/g) from clinical patient samples.

Acknowledgements

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