# Diagnostic Accuracy of Two Faecal Calprotectin Immunoassays

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# Inflammatory Bowel Disease (IBD)

- an idiopathic disease
- probably involves an immune reaction of the body to its own intestinal tract/ gut microflora

- the 2 major types of IBD are **ulcerative colitis** (UC) and **Crohn‘s disease** (CD)

# Inflammatory Bowel Disease (IBD)

- Incidence of IBD is continuously rising, especially in developed countries

- No latest data about incidence and prevalence of CD and UC in  are available; however, values are near to north-west Europe data, with
  - an **incidence** of **3** to **5/100,000** inhabitants and
  - a **prevalence** of up to **150/100,000** inhabitants

Lukas, Dig Dis, 2010; Pozler, Pediatr Gastroenterol Nutr, 2006 ; Lakatos, Inflamm Bowel Dis, 2011.

# Inflammatory Bowel Disease (IBD)

- In parallel with the rising amount of information about immunopathological processes in IBD, a question about the usefulness of different immunological laboratory analyses in diagnostics, differential diagnostics, and the monitoring and prognosing of IBD arises.

- Diagnostics of IBD are currently based on **endoscopy, imaging**, and **biopsy** evaluation – all the mentioned procedures are invasive, and time and money consuming.

Mowat, Gut, 2011

# Inflammatory Bowel Disease (IBD)

- Routine laboratory tests contribute to the IBD diagnostics; however, they often have limited sensitivity and specificity.

- This is due to the fact that systemic inflammatory markers such as serum **C-reactive protein**, other serum acute phase proteins, **erythrocyte sedimentation rate**, **blood count**, or **autoantibodies** can have normal or almost normal values, even during the acute IBD flare.

Vermeire, Gut 2006

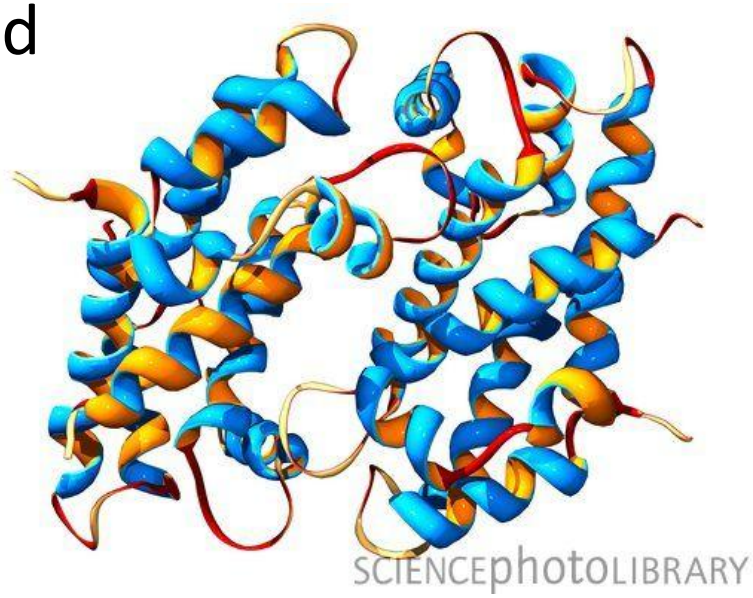
# Calprotectin

- Therefore, what's needed is a **non-invasive**, **simple**, **time saving**, and **inexpensive** method which would be able to
  - detect inflammatory changes in intestinal mucosa exactly,
  - distinguish functional disorders of the gut from organic disorders,
  - anticipate imminent relapse in known IBD, and
  - act as a tool for self-monitoring of the disease.
- **Faecal calprotectin** was identified two decades ago to be such a revolutionary marker.

Røseth, Scand J Gastroenterol, 1992

# Calprotectin

- synonyms: calgranulin, MRP8/14
- the main antigenic glycoprotein in neutrophil granulocytes cytoplasm
- 24 kDa heterodimer, composed of a light and heavy chain



# Calprotectin

- belongs to the S100 protein calcium binding superfamily
- has important antimicrobial properties
- It is released after neutrophil activation, has a strong bacteriostatic effect, and participates in cytokine cascade.

# Calprotectin

Calprotectin has excellent stability in a stool, so that it could serve as a suitable marker for routine laboratory diagnostics.

# Calprotectin

- fecal calprotectin levels correlate significantly with **endoscopic disease activity** in IBD

  (d'Haens, Inflam Bowel Dis 2012)

- the use of faecal calprotectin as a screening test in suspicion to IBD **substantially reduces the number of invasive measurements** necessary in the diagnostic work-up

  (Mindemark, Clin Biochem 2012)

# Calprotectin

- In UC, faecal calprotectin is related to disease **activity** and **extent** of disease

  (Ricanek, Scand J Gastroenterol, 2011; Xiang, World J Gastroenterol, 2008)

- In **CD**, the fecal calprotectin concentration is a reliable marker of **mucosal affection**, but probably not for systemic disease activity

  (Ricanek, Scand J Gastroenterol, 2011; Sidler, Inflamm Bowel Dis, 2007)

- fecal calprotectin was found to be a useful marker for predicting **relapse** in patients with IBD

  (García-Sánchez, J Crohns Colitis, 2010)

# Starting point of the study

Immunoanalytic assays using **monoclonal** as well as **polyclonal** capture antibodies, which are specific to the calprotectin heterodimeric and polymeric complexes, are commercially available.

Comprehensive information on the diagnostic characteristics of different assays is lacking, and it is not known whether the different assays yield comparable results.

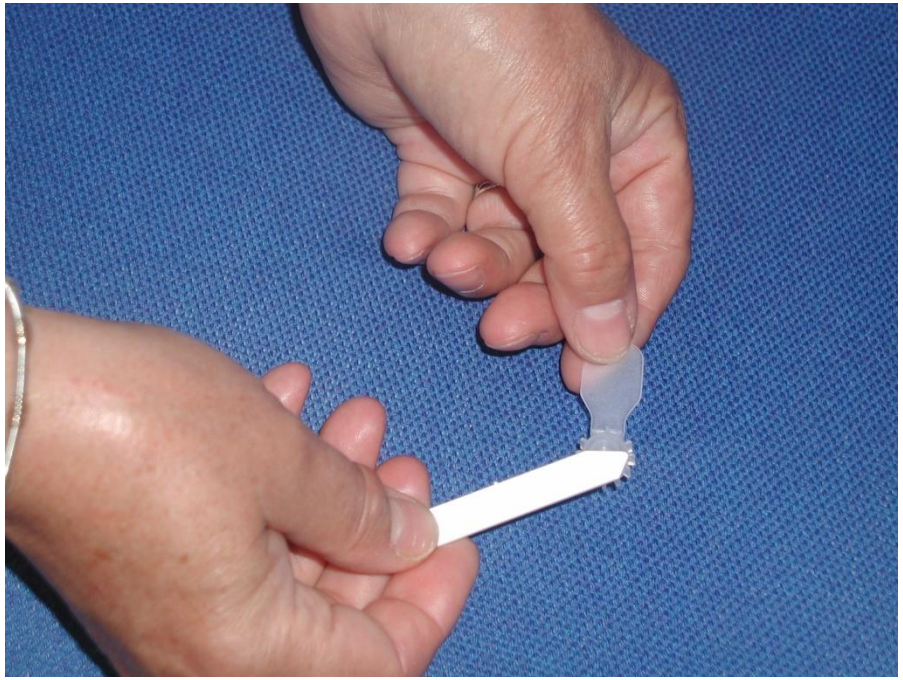
# Aim of the study

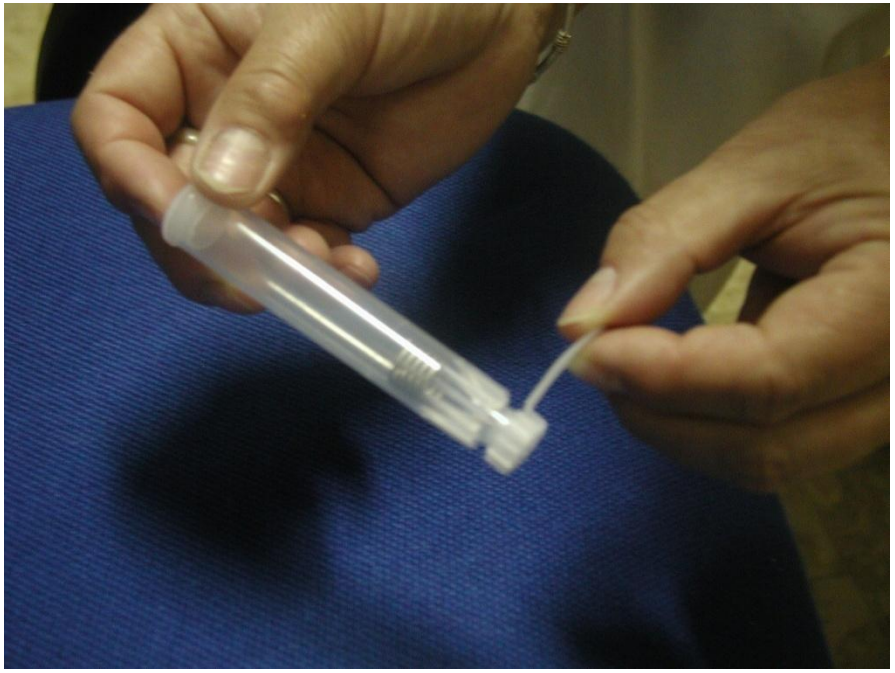
- to evaluate two different ELISA tests for the diagnosis and follow-up of IBD
  - technical performance
    - linearity
    - Imprecision
    - agreement
  - diagnostic accuracy
    - sensitivity
    - specificity
    - PPV
    - NPV
    - AUC

# Materials

- 163 stool samples
  - 36 patients with biopsy-confirmed Crohn‘s disease
  - 29 patients with proven ulcerative colitis
  - 98 healthy persons

# Materials





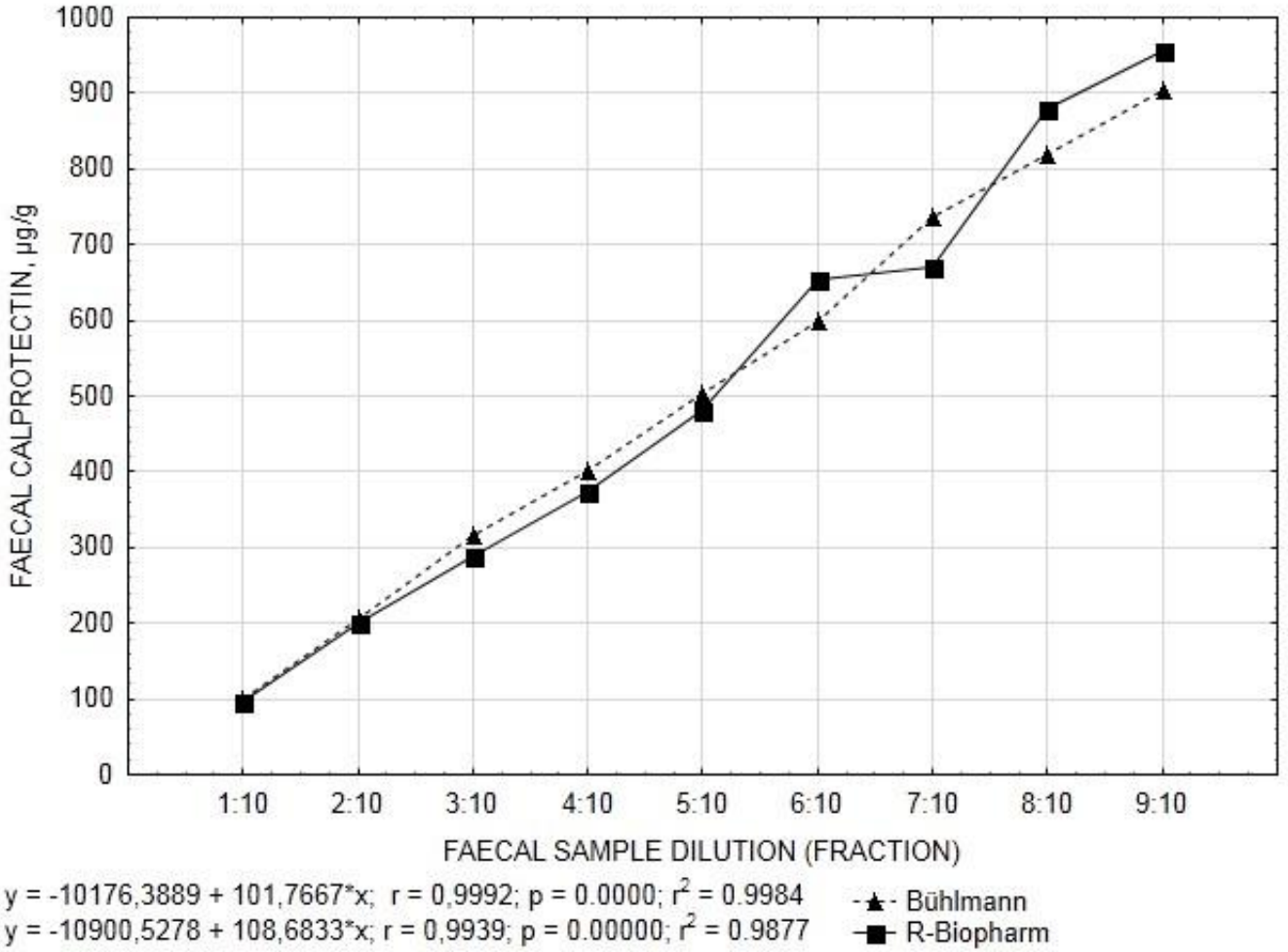
The same patients were tested in both assays, and a faecal sample from each individual originated from the same stool sampling.

# Methods

| | BŰHLMANN | R-BIOPHARM |
|---|---|---|
| Sample type | human faeces | human faeces |
| Capture antibody towards calprotectin | **Monoclonal** | **Polyclonal** |
| Sample size | 20 µl (1:50) | 20 µl (1:50) |
| Incubation times (min) | | |
| -stool eluate | 30‘ | 45’ |
| -conjugate | 30‘ | 45’ |
| -substrate | 15‘ | 30’ |
| Total incubation time | 75‘ | 120’ |
| Number of calibrators | 5 | 8 |
| Conjugate label | horse-radish peroxidase | horse-radish peroxidase |
| Substrate | tetramethylbenzidine | tetramethylbenzidine |
| Reference value | < 50 µg/g | < 50 µg/g |
| Measurement range | 10-600 µg/g | 7.8 - 1000 µg/g |
| ELISA reader wavelength | 450 nm | 450 nm |

# Linearity

Assay linearity was determined by diluting a faecal sample containing a high concentration of calprotectin with increasing amounts (from 10% to 90%) with a faecal sample that did not contain calprotectin, in increments of 10%, i.e. from 1:10 to 9:10.



# Linearity

| Dilution | Observed values, µg/g | | Recovery, % | |
|---|---|---|---|---|
| | BÜHLMANN | R-BIOPHARM | BÜHLMANN | R-BIOPHARM |
| **neat value** | 550 | 531 | - | - |
| **1 : 2** | 561 | 518 | 102 | 98 |
| **1 : 4** | 569 | 604 | 103 | 114 |
| **1 : 8** | 581 | 612 | 106 | 115 |

Both ELISAs showed good linearity. A better result was achieved by Bühlmann, because the linearity for the R-Biopharm ELISA was not perfect at high values.

*NOTE: Sample dilutions use the colon to denote a fraction (e.g. 1:2 means 1 part diluted to the final volume of 2 parts) .*

# Methods comparison

| | | Median µg/g | Mean µg/g | SD | Lower quartile µg/g | Upper quartile µg/g | p-value |
|---|---|---|---|---|---|---|---|
| CD | **BÜHLMANN** | 202.34 | 266.86 | 63.29 | 81.05 | 261.87 | 0.382 NS |
| | **R-BIOPHARM** | 250.45 | 302.54 | 45.87 | 70.98 | 287.55 | |
| UC | **BÜHLMANN** | 350.47 | 398.35 | 36.07 | 110.93 | 337.65 | 0.408 NS |
| | **R-BIOPHARM** | 394.76 | 356.09 | 54.74 | 95.41 | 324.87 | |
| CTRL | **BÜHLMANN** | 31.87 | 22.88 | 15.60 | 11.23 | 51.30 | 0.547 NS |
| | **R-BIOPHARM** | 25.04 | 19.77 | 14.34 | 4.57 | 43.1 | |

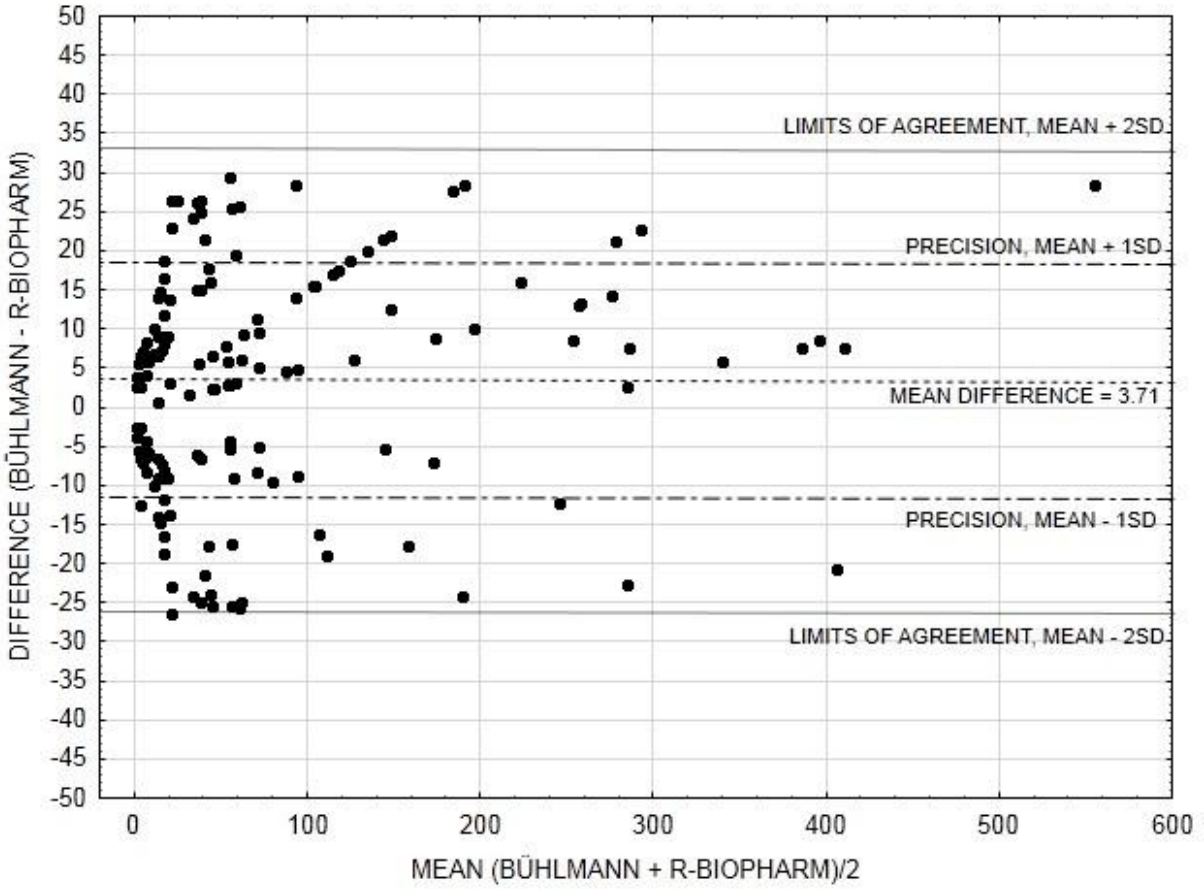
# Methods comparison

Using the Spearman's rank correlation coefficient, we assessed correlation among two assays with the correlation coefficient *r* of **0.931**.

However, a high correlation does not mean that two methods have a high degree of agreement.

# Methods comparison

Therefore, a Bland-Altman analysis, which calculates the mean difference between two methods of measurement, and a 95% limit of agreement as the mean difference (2 SD), were performed.



# Diagnostic performance of the faecal calprotectin ELISAs

| | | Sensitivity % | Specificity % | PPV % | NPV % | ROC AUC (95% CI) |
|---|---|---|---|---|---|---|
| cut-off 50 µg/g | **Bühlmann** | 98 | 92 | 84 | 96 | 0.945 (0.909;0.0.978) |
| | **R-Biopharm** | 94 | 99 | 94 | 88 | 0.912 (0-885; 0.939) |
| cut-off 70 µg/g | **Bühlmann** | 100 | 91 | 95 | 87 | 0.985 (0.952; 0.995) |
| | **R-Biopharm** | 98 | 100 | 94 | 95 | 0.965 (0.913; 0.977) |

# Conclusions

- Both ELISAs demonstrated good technical performance.
- Linearity, imprecision, and drift were good.
- A high correlation among the methods from two manufacturers was found.
- An inter-assay agreement was satisfactory, which means that the result of one ELISA method can be comparable by the result of another.

# Conclusions

- The diagnostic performance of both assays evaluated was excellent, and the areas under the ROC curves were high and were not statistically different among the assays evaluated.
- Both assays have high sensitivities and specificities for the diagnosis of IBD.

# Conclusions

- These findings are of clinical importance, as they encourage the use of this simple and easily available biomarker in the diagnostic approach to patients with abdominal discomfort.
- Due to excellent concordance of the assays evaluated here, monoclonal as well as polyclonal-based imunoassays could be useful laboratory tools.

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# Thank you for your attention.

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