Fecal Calprotectin Assay
(Reference — 2013.03.005)
Notice of Assessment

April 2014
1 GENERAL INFORMATION

1.1 Requestors
- HMR: EK-CAL automated assay
- Hôpital Saint-Luc, CHUM: EK-CAL manual assay
- CHUS: EliATM Calprotectin1 assay

1.2 Application for Review Submitted to MSSS:
- HMR: August 27, 2013
- Hôpital Saint-Luc, CHUM: September 27, 2013
- CHUS: July 7, 2013

1.3 Application Received by INESSS
   November 1, 2013

1.4 Notice Issued
   February 28, 2014

Note:
This notice is based on the scientific and commercial information submitted by the requestor and on a complementary review of the literature according to the data available at the time that this test was assessed by INESSS.

2 TECHNOLOGY, COMPANY AND LICENCE(S)

2.1 Name of the Technology
EK-CAL: Enzyme-linked immunosorbent assay (ELISA) with chromogenic substrate. Automated or manual.


2.2 Brief Description of the Technology, and Clinical and Technical Specifications
Calprotectin is a protein found in certain immune cells, principally neutrophils2 [Dale et al., 1985]. When the gastrointestinal tract becomes inflamed, neutrophils accumulate, and calprotectin can be detected in stool samples [Sherwood, 2012]. Fecal calprotectin is thus a marker of gastrointestinal inflammation [Abraham and Kane, 2012].

Before using either EK-CAL (automated or manual) or EliATM Calprotectin (automated), 50 to 100 mg of stool sample must be prepared and calprotectin extracted3 from the sample3 into a solution. Next, the assay itself involves successive steps of incubation4 and washing. Simply stated, the extraction solution is deposited in wells coated with monoclonal antibodies that capture the

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1 CHUS has submitted an application for designation for the EliATM Calprotectin test (written communication between the requestor and the Ministère de la Santé et des Services sociaux, July 10, 2013).
2 Neutrophils are the most abundant and prominent cells of the innate immune system. In their intracellular vesicles, neutrophils destroy a variety of microorganisms, primarily using enzymes [Murphy et al., 2008].
3 Calprotectin from a stool sample is extracted with a purpose-designed kit (e.g., Smart Prep, ScheBo® Quick Prep™ or EliA Stool Extraction Kit).
4 For the EK-CAL assay in particular, the 75 minute total incubation time is fairly short for an ELISA. (ALPCO. Calprotectin ELISA [website]. Available at http://www.alpco.com/products/Calprotectin_ELISA.aspx).
calprotectin. Other monoclonal antibodies that also recognize calprotectin and are conjugated to an enzyme (type of enzyme depends on the assay) are then added. Lastly, adding a substrate for this enzyme permits detection of the calprotectin-antibody complexes, either through a colour-generating reaction (EK-CAL), or through another reaction that excites fluorescence emission (EliA™ Calprotectin). The concentration of calprotectin is determined by reading either the absorbance (EK-CAL) or the fluorescence (EliA™ Calprotectin) with devices that compare sample values with standard values. The assay is quantitative.

2.3 **Company or Developer**
The EK-CAL assay is manufactured by Bühlmann Laboratories AG.
The EliA™ Calprotectin assay is manufactured by Phadia AB (now part of Thermo Fisher Scientific).

2.4 **Licence(s)**
Not applicable.

2.5 **Patent, If Any**
Not applicable.

2.6 **Approval Status (Health Canada, FDA)**

2.7 **Weighted Value**
EK-CAL automated: 31.80
EK-CAL manual: 34.25
EliA™ Calprotectin: 27.00
According to the requestors, the weighted value calculations include calprotectin extraction from stool samples.

3 **CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES**

3.1 **Targeted Patient Group**
According to information in the requests, fecal calprotectin testing in children and adults would be used for:
- Differential diagnosis between (1) inflammatory bowel disease (IBD), such as ulcerative colitis (UC) and Crohn disease (CD), and (2) irritable bowel syndrome (IBS)
- Monitoring the course of IBD
- Monitoring postoperative or treatment outcomes in IBD patients.

Regarding the differential diagnosis, a negative result would indicate IBS (no inflammation), whereas a positive result would support a diagnosis of intestinal inflammation [NICE, 2013]. An endoscopy would be indicated in the latter case to confirm IBD, infection, or cancer [Roche et al., 2013].
3.2 Targeted Disease(s)
Gastrointestinal symptoms (e.g., abdominal pain, diarrhea, etc.) are a relatively common reason for consulting a physician and may be due to IBD, colorectal cancer, infection, or IBS [Sherwood, 2012].

IBD cases are increasing in industrialized countries [Burri and Beglinger, 2011], with patients being mainly between 15 and 25 years of age [Higuchi and Bousvaros, 2013]. IBD symptoms can be atypical among children [Sidler et al., 2008]. IBD cases are idiopathic, lifelong, and characterized by flare-ups interspersed with periods of remission. The unforeseeable flare-ups can greatly affect a patient’s quality of life [Foell et al., 2009]. In the case of UC, the inflammation involves the colon; it commonly affects the rectum and extends more or less up to the cecum without reaching the small intestine [Roche et al., 2013]. Mortality is no higher for UC patients than for the rest of the population, but between 20% and 30% of patients will require excision of a part of the colon within 10 years after onset [NICE, 2013]. By contrast, the inflammation responsible for CD can be found anywhere in the GI tract, from mouth to perianal area [Peppercorn, 2013]. CD leads to intestinal complications (fistulas, colorectal cancer, etc.) [NICE, 2013] and slightly reduces life expectancy [Baumgart and Sandborn, 2012]. Lastly, IBD also generates extra-intestinal manifestations, some of which (e.g., arthritis) present more commonly with CD than with UC.

IBS is a troublesome functional disorder but is not associated with a poor prognosis [NICE, 2013]. The etiology of IBS is not established, and treatment is symptomatic. The syndrome affects approximately 6% to 22% of the general population, according to various studies [Drossman et al., 1997].

3.3 Number of Patients Targeted
Based on information provided in the requests:
- EK-CAL (automated): 2,000 tests annually
- EK-CAL (manual): 500 tests annually
- EliA™ Calprotectin: 1,700 to 2,500 tests annually

3.4 Medical Specialties and Other Professions Involved
General practitioners, family doctors, gastroenterologists, pediatricians.

3.5 Testing Procedure
Because of its calcium-binding properties, calprotectin remains stable in stools for at least 5 days at room temperature, such that a patient can collect stool samples at home using a non-sterile container and send them to the laboratory by mail [Kallel et al., 2011].

CHUS plans to conduct the EliA™ Calprotectin assay twice weekly; CHUM-Hôpital Saint-Luc anticipates a two-week response time for the EK-CAL manual assay; and HMR plans to conduct the EK-CAL automated assay every one week or two weeks.

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3 More specifically, in Western countries, ulcerative colitis incidence is 10-20 per 100,000, and prevalence is 100-200 per 100,000. Crohn disease incidence is 5-10 per 100,000, and prevalence is 50-100 per 100,000 [Waugh et al., 2013].

6 Although the etiology of inflammatory bowel diseases is unknown, it likely involves a genetic predisposition together with environmental factors and immune dysfunction [Ayling, 2012].
While an EK-CAL test kit is designed for a 96-well assay, the EliA™ test involves equipment and reagents sold separately in 48-well packages (4 sets of 12 wells).

According to information in the requests, the DSX™ system from DYNEX Technologies Inc. would be used for the EK-CAL automated assay; a plate washer and a plate reader would be used for the EK-CAL manual assay; and the Phadia 250 system would be used with the EliA™ Calprotectin assay (automated). The DSX™ system can process up to four 96-well plates simultaneously. The automated Phadia 250 system can perform both EliA™ and ImmunoCAP® tests at a rate of 60 tests per hour.

4 TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology

Fecal calprotectin testing may help to reduce the number of endoscopies performed, although endoscopy will continue to be needed for definitive diagnosis [Judd et al., 2011].

4.2 Brief Description of the Current Technological Context

Monitoring inflammation is very important in differentiating IBD from IBS and in evaluating IBD activity and treatment efficacy. To that end, clinicians normally use clinical examination, radiological imaging, and endoscopy with biopsy (gold standard) [Judd et al., 2011]. Another less common test is also available [Sherwood, 2012].

However, endoscopy is a relatively costly and invasive procedure, unpleasant for patients if not performed under general anesthesia, and not without risk [Roche et al., 2013]. It involves mobilizing considerable resources and requires an appreciable amount of preparation by the patient. Furthermore, several studies have shown that more than 60% of colonoscopies have normal results [Waugh et al., 2013]. Hence, a test that can differentiate IBD and IBS is important in speeding up diagnosis and reducing endoscopy waiting lists.

Calprotectin has thus become the most studied fecal marker of IBD [Kallel et al., 2011]. Moreover, the meta-analysis by Van Rheenen et al. [2010] accurately shows that fecal calprotectin is a useful screening tool for identifying patients most likely to need an endoscopy.

4.3 Brief Description of the Advantages Cited for the New Technology

Fecal calprotectin testing is not invasive and requires only a small quantity of fecal matter, in which, moreover, it is resistant to bacterial degradation, is evenly distributed, and is found in proportion to the degree of inflammation present [Abraham and Kane, 2012].

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2 Various ImmunoCAP® tests are available for measuring, inter alia, immunoglobulins and enzymes.
4 Treatment of IBD involves healthy diet and lifestyle, medications such as infliximab, an anti-tumour necrosis factor-alpha monoclonal antibody, and surgery to induce and maintain remission [NICE, 2013].
5 On endoscopy, lesions associated with Crohn disease are typically segmental and asymmetric, and healthy areas alternate with affected areas. Biopsies show loss of mucosa, an infiltration of immune cells and granulomas highly suggestive of IBD. The lesions associated with ulcerative colitis are fairly uniform and continuous from the anorectal junction, and stop rather abruptly. Histological analysis shows glandular distortions and mucus depletion related to lowered crypt density [Roche et al., 2013].
6 Another test for diagnosis of gastrointestinal inflammation — quantitative fecal excretion of indium-111-labelled granulocytes (111In) — is less common than endoscopy because it is impractical as a routine diagnostic test. Granulocytes are cells that contain cytoplasmic granules (i.e., neutrophils, eosinophils, and basophils) [Delves et al., 2011]. This test involves collecting blood from the patient, in vitro labelling of granulocytes with isotope 111In, returning blood to the patient, and measuring fecal isotope excretion over several days [Sherwood, 2012].
Fecal calprotectin testing is an marker of the condition of the entire digestive tract, whereas biopsies do not allow comprehensive coverage of the entire tract and cannot be performed in areas inaccessible by endoscopy. Fecal calprotectin is a more reliable marker than systemic, non-specific markers of inflammation such as C-reactive protein and erythrocyte sedimentation rate [Roche et al., 2013].

In a meta-analysis of 13 studies, Van Rheenen et al. [2010] showed that fecal calprotectin testing significantly reduced the number of patients requiring colonoscopy (by 67% for adults and 35% for children) and delayed diagnosis in less than 10% of the cases.

According to a US study by Yang et al. [2014], measuring fecal calprotectin for screening prior to endoscopy with biopsy for new patients (children and adults) presenting IBD symptoms is a valid, cost-effective strategy. In addition, a cutoff level of 50 µg/g reduces false negatives without substantially increasing costs.

A Swedish study shows that the estimated demand for colonoscopies was reduced by 50% with the use of fecal calprotectin tests (50 µg/g cutoff) to screen patients with suspected IBD, corresponding to a cost avoidance of approximately €1.57 million [Mindemark and Larsson, 2012].

**4.4 Cost of Technology and Options**

From April 1, 2012, to March 31, 2013, 35 fecal calprotectin tests were sent outside Quebec to Mayo Medical Laboratories (unit cost of $172 or $198, for a total $6,870\(^\text{13}\)).

**5 Evidence**

**5.1 Clinical Relevance**

**5.1.1 Other Tests Replaced**

The fecal calprotectin assay would not replace any test in the Index.

**5.1.2 Diagnostic Value**

For a recent report on fecal calprotectin testing for differential diagnoses [Waugh et al., 2013], the National Institute for Health Research (NIHR) conducted a systematic review of the scientific literature (see the results in section 5.2) and an economic evaluation.\(^\text{14}\) ELISA tests with chromogenic substrate and with fluorogenic substrate (EliA\(^\text{TM}\) Calprotectin assay) were both considered in the report, but no data was found in their regard. NIHR concluded, based on the original studies of clinical efficacy, that fecal calprotectin testing is a useful method of distinguishing between IBD and non-inflammatory disorders, and that no test method is preferable over another [Waugh et al., 2013]. The report’s authors also considered publications of other types (reports, systematic reviews, etc.), and the most recent publications\(^\text{15}\) also conclude that fecal calprotectin testing is a very useful diagnostic tool [Waugh et al., 2013].

\(^{13}\) Information provided by the Ministère de la Santé et des Services sociaux.

\(^{14}\) A key finding of this economic evaluation is that the ELISA assay of fecal calprotectin saves money. First, in primary care, the test results in average cost savings of £82 per patient (compared with general practice without fecal calprotectin testing), owing to the lower number of endoscopies. Second, in secondary care, the ELISA assay results in an average saving of £205 per patient (compared with referring all cases directly to the appropriate service for colonoscopy) [Waugh et al., 2013].

\(^{15}\) Report from the York Health Economics Consortium (YHEC) [2010] and systematic reviews by Jellema et al. [2011] and Henderson et al. [2013].
5.1.3 Prognostic and Therapeutic Value

Fecal calprotectin testing predicts the course of the disease over time and can facilitate treatment monitoring and patient treatment strategies (see results in section 5.2).

5.2 Clinical Validity

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>PRESENCE</th>
<th>ABSENCE</th>
<th>NOT APPLICABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>x</td>
<td></td>
<td></td>
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<tr>
<td>Specificity</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive predictive value (PPV)</td>
<td>x</td>
<td></td>
<td></td>
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<tr>
<td>Negative predictive value (NPV)</td>
<td>x</td>
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<tr>
<td>Likelihood ratio (LR)</td>
<td>x</td>
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<tr>
<td>Receiver operating characteristics (ROC) curve</td>
<td>x</td>
<td></td>
<td></td>
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<tr>
<td>Accuracy</td>
<td>x</td>
<td></td>
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</table>

In their systematic literature review, the authors of the NIHR collate the results of original studies based on comparisons of bowel disorders (IBD versus IBS, organic disease\textsuperscript{16} versus IBS, etc.) [Waugh et al., 2013]. Table 1 in the Appendix summarizes the results. The studies were combined within these comparison groups and thus provided sufficiently rich aggregate data (sensitivity, specificity and area under the curve). For each study considered in the systematic review, the authors also report PPV, NPV, LR and accuracy, but do not aggregate the data for these parameters. Interpretation of these data, which vary considerably, is therefore difficult. For a given parameter, the variance among the results can be explained by the differences between the studies, for example in the population (children or adults or both); the prevalence and classification of diseases (IBD, organic, etc.); or the subgroups created based on certain clinical manifestations (e.g., adenomas).

choepfer et al. [2010] have shown that, of several markers (including C-reactive protein), fecal calprotectin is the only one that can discriminate inactive CD from mild, moderate, or high CD activity on endoscopy. In a multi-centre prospective study, Jensen et al. [2011] show that fecal calprotectin testing with the EK-CAL kit is as sensitive in cases of small-bowel CD (92%) as in cases of colonic CD (94%) in a population of 83 patients aged 16 to 71 years.

In a brochure, the manufacturer of the EliA\textsuperscript{TM} Calprotectin assay indicates 97.7% sensitivity, 89.8% specificity, 96% PPV, 95% NPV, a positive LR of 9.58, and a negative LR of 0.03.\textsuperscript{17} The three studies identified that consider the prognostic or therapeutic value of the calprotectin assay are presented in Table 2 in the Appendix. The table shows that the fecal calprotectin assay predicted the course of disease over time and that it can facilitate monitoring and the determination of patient treatment strategies.

\textsuperscript{16} Organic disease is formally defined as a condition in which there is an observable and measurable disease process (for example, inflammation), and the term includes IBD [NICE, 2013].

\textsuperscript{17} Phadia AB [website]. Available at www.phadia.com/PageFiles/29347/Product%20information%20EliA%20Calprotectin.pdf.
5.3 Analytical (or Technical) Validity

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>PRESENCE</th>
<th>ABSENCE</th>
<th>NOT APPLICABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Reproducibility</td>
<td>x</td>
<td></td>
<td></td>
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<tr>
<td>Analytical sensitivity</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analytical specificity</td>
<td>x</td>
<td></td>
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<tr>
<td>Matrix effect</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Concordance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation between test and comparator</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other, depending on type of test</td>
<td></td>
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</table>

The three studies providing data on analytical validity are presented in Table 3 in the Appendix. All three used the ELISA with chromogenic substrate, and no study on EliA™ Calprotectin was identified. It is recognized that the fecal calprotectin assay correlates well in general with endoscopic and histological results for determining inflammation [Sipponen et al., 2008; Roseth et al., 1997], and the new results (Table 3) confirm this, in addition to showing that this assay has low day-to-day variability in patients with CD in remission. This test can therefore be used with confidence for making predictions and clinical decisions for this population. According to a study of three tests involving chromogenic substrate, the EK-CAL assay posted the best between-assay precision, while intra-assay precision is similar and adequate among all three. The authors conclude that assay standardization is required [Whitehead et al., 2013].

5.4 Recommendations from Other Organizations

In the United Kingdom, the most recent National Institute for Health and Care Excellence (NICE) guidelines on the topic, which are based on the NIHR report [Waugh et al., 2013], recommend, subject to certain conditions18, fecal calprotectin testing as an option to assist clinicians in the differential diagnosis of IBD and IBS in adults and children [NICE, 2013]. Moreover, fecal calprotectin testing in the United Kingdom is a cost-effective use of National Health System resources for distinguishing between IBD and IBS in adults in primary care [NICE, 2013].

6 ANTICIPATED OUTCOMES OF INTRODUCING THE TEST

6.1 Impact on Material and Human Resources
Not assessed.

6.2 Economic Consequences of Introducing Test Into Quebec’s Health Care and Social Services System
Not assessed.

6.3 Main Organizational, Ethical, and Other (Social, Legal, Political) Issues
Not assessed.

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18 Cancer is not suspected, local care pathways are identified, and appropriate quality assurance processes are in place for the testing [NICE, 2013].
7  IN BRIEF

7.1 Clinical Relevance and Clinical Validity

According to NIHR (on the basis of a systematic review of original studies), fecal calprotectin testing is a useful diagnostic method for differentiating IBDs from non-inflammatory disorders, and no assay method is preferable to another. Moreover, according to one study, fecal calprotectin is the only marker capable of discriminating inactive CD from mild, moderate, or high CD activity on endoscopy. In addition, according to three studies, this test can predict the course of the disease over time and can facilitate monitoring and the determination of patient treatment strategies. The test correlates well with the endoscopic and histological results. It can be used with confidence for making predictions and clinical decisions for patients with CD.

7.2 Analytical Validity

In one study, the EK-CAL kit had the best between-assay precision, while its intra-assay precision was adequate and similar to that of other assays. According to the authors of that study, however, assay standardization is needed (for enzyme assays using a chromogenic substrate).

7.3 Recommendations from Other Organizations

NICE recommends fecal calprotectin testing as an option to assist clinicians in the differential diagnosis of IBD or IBS in adults with recent-onset gastrointestinal symptoms for whom specialist assessment is being considered if cancer is not suspected. In children who have been referred for specialist assessment, fecal calprotectin testing is recommended as an option to assist clinicians in the differential diagnosis of IBD or non-IBD diagnoses (including IBS). For both children and adults, NICE recommends that appropriate quality assurance processes and care pathways be in place.
8 INESSS NOTICE IN BRIEF

Fecal Calprotectin Assay

<table>
<thead>
<tr>
<th>Status of the Diagnostic Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Established</td>
</tr>
<tr>
<td>√ Innovative</td>
</tr>
<tr>
<td>□ Experimental (for research purposes only)</td>
</tr>
<tr>
<td>□ Replacement for technology ____________, which becomes obsolete</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>INESSS Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>√ Include test in the Index conditionally upon:</td>
</tr>
<tr>
<td>- Development of an algorithm for clinical indications</td>
</tr>
<tr>
<td>- Close monitoring of prescriptions and outcomes</td>
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<tr>
<td>- Internal and external quality control</td>
</tr>
<tr>
<td>□ Do not include test in the Index</td>
</tr>
<tr>
<td>□ Reassess test</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additional Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Draw connection with listing of drugs, if companion test</td>
</tr>
<tr>
<td>□ Produce an optimal use manual</td>
</tr>
<tr>
<td>□ Identify indicators, when monitoring is required</td>
</tr>
</tbody>
</table>

NOTES:
There is little data available on the EliA Calprotectin technique. Caution regarding indiscriminate use of this test: This test should not be prescribed for adults for whom specialist assessment is not being considered, or for patients in whom cancer is a potential diagnosis, or for children with suspected inflammatory bowel disease who are not being referred to a specialist. MSSS must carry out monitoring to ensure that the use of this test serves to reduce the number of colonoscopies.
REFERENCES


Van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: Diagnostic meta-analysis. BMJ 2010;341:c3369.


APPENDIX A

Table A1: Diagnostic value. Results of the systematic review presented in Waugh et al.’s report [2013] on differential diagnoses made mainly in a context of secondary care and with an endoscopic method as comparator

<table>
<thead>
<tr>
<th>STUDY</th>
<th>SENSITIVITY (95% CI)</th>
<th>SPECIFICITY (95% CI)</th>
<th>AREA UNDER THE CURVE (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis: IBD versus IBS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 studies aggregated</td>
<td>0.93 (0.83 to 0.97)</td>
<td>0.94 (0.73 to 0.99)</td>
<td>0.97 (0.95 to 0.98)</td>
</tr>
<tr>
<td>Diagnosis: IBD versus non-IBD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 studies aggregated</td>
<td>0.99 (0.95 to 1.00)</td>
<td>0.74 (0.59 to 0.86)</td>
<td>0.99 (0.98 to 1.00)</td>
</tr>
<tr>
<td>Diagnosis: Organic disease versus IBS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 separate studies</td>
<td>Varies between 0.63 (0.44 to 0.80) and 0.90 (0.70 to 0.99)</td>
<td>Varies between 0.60 (0.50 to 0.70) and 0.93 (0.68 to 1.00)</td>
<td>NA</td>
</tr>
<tr>
<td>Diagnosis: Organic disease versus non-organic disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 separate studies</td>
<td>Varies between 0.43 (0.35 to 0.52) and 0.89 (0.85 to 0.92)</td>
<td>Varies between 0.47 (0.41 to 0.53) and 0.98 (0.96 to 1.00)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval; IBD = inflammatory bowel disease; n = number of patients in study; NA = not available.

Notes:
For the sensitivity and specificity indicated in the separate studies, the table shows the lowest and the highest values found in the studies.
The fecal calprotectin tests were performed using various ELISA assays with chromogenic substrate (including the EK-CAL assay). The results shown are for a 50 µg/g cutoff [as recommended by NIHR] [Waugh et al., 2013].
Table A2: Prognostic value. Results of studies on the use of fecal calprotectin testing as tool for predicting disease relapse

<table>
<thead>
<tr>
<th>STUDY</th>
<th>DESIGN</th>
<th>ASSAY TYPE</th>
<th>CUTOFF VALUE µg/g</th>
<th>NUMBER AND AGE</th>
<th>STAGE OF DISEASE</th>
<th>Se % (95% CI)</th>
<th>Sp % (95% CI)</th>
<th>PPV %</th>
<th>NPV %</th>
<th>LR</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lasson et al., 2013</td>
<td>Prospective study</td>
<td>Bühlmann Laboratories AG</td>
<td>262</td>
<td>n = 69 18 to 74 yrs</td>
<td>UC, after 3-month initial treatment</td>
<td>1 yr: 64.4 2 yrs: 51.1 3 yrs: 52.2</td>
<td>1 yr: 70.8 2 yrs: 81.8 3 yrs: 85.7</td>
<td>1 yr: 80.6 2 yrs: 85.2 3 yrs: 88.9</td>
<td>1 yr: 51.5 2 yrs: 45.0 3 yrs: 45.0</td>
<td>NA</td>
<td>1 yr: 0.69 (0.56 to 0.82) 3 yrs: 0.70 (0.57 to 0.83)</td>
</tr>
<tr>
<td>Mao et al., 2012</td>
<td>M-A of 6 prospective studies</td>
<td>Eurospital (Calprest assay) or in-house ELISA in 1 study</td>
<td>120-340</td>
<td>n = 672 (15 to 80 yrs)</td>
<td>IBD (UC and CD), in remission</td>
<td>IBD: 78 (72 to 83) UC: 77 (67 to 85) CD: 75 (64 to 84)</td>
<td>IBD: 73 (68 to 77) UC: 71 (64 to 77) CD: 71 (64 to 76)</td>
<td>NA</td>
<td>NA</td>
<td>Positive: IBD: 2.81 (2.09 to 3.78) UC: 2.47 (1.92 to 3.19) CD: 2.37 (1.56 to 3.61) Negative: IBD: 0.31 (0.20 to 0.47) UC: 0.36 (0.24 to 0.53) CD: 0.41 (0.27 to 0.61)</td>
<td>IBD: 0.83 ± 0.05 UC: 0.78 ± 0.04 CD: 0.79 ± 0.05</td>
</tr>
<tr>
<td>Molander et al., 2012</td>
<td>Prospective study</td>
<td>Calpro (PhiCal assay)</td>
<td>139</td>
<td>n = 60 (19 to 52 yrs)</td>
<td>IBD, under treatment</td>
<td>1 yr: 72</td>
<td>1 yr: 80</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1 yr: 0.838 (0.724 to 0.951)</td>
</tr>
</tbody>
</table>

Abbreviations: AUC = area under the curve; UC = ulcerative colitis; ELISA = enzyme-linked immunosorbent assay; CI = confidence interval; M-A = meta-analysis; CD = Crohn disease; IBD = inflammatory bowel disease; n = number of patients; NA = not available; LR = likelihood ratio; Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value.
Table A3: Analytical validity. Results of studies on Crohn disease

<table>
<thead>
<tr>
<th>STUDY</th>
<th>ASSAY TYPE</th>
<th>POPULATION</th>
<th>PRECISION</th>
<th>REPRODUCTIVITY</th>
<th>CORRELATION</th>
<th>RECOVERY</th>
<th>DILUTION LINEARITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NUMBER AGE STAGE</td>
<td>INTER-ASSAY (%)</td>
<td>INTRA-ASSAY (%)</td>
<td>95% CI</td>
<td>SPEARMAN</td>
<td>(mean % ± SD)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kappa coefficient</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naismith et al., 2013</td>
<td>Bühlmann Laboratories AG (EK-CAL assay)</td>
<td>n = 143 years</td>
<td>NA</td>
<td>NA</td>
<td>6.48</td>
<td>0.84</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(average)</td>
<td></td>
<td></td>
<td>(0.511 to 0.769)</td>
<td>(0.79 to 0.89)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In remission</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whitehead et al., 2013</td>
<td>Bühlmann Laboratories AG (EK-CAL automated assay)</td>
<td>NA</td>
<td>5.3-8.2</td>
<td>5.2-10.3</td>
<td>9.0</td>
<td>NA</td>
<td>96 ± 8</td>
</tr>
<tr>
<td></td>
<td>Immunodagnostik (PhiCal automated assay)</td>
<td>NA</td>
<td>7.0</td>
<td>8.1-11.6</td>
<td>3.0</td>
<td>NA</td>
<td>97 ± 16 + 17</td>
</tr>
<tr>
<td></td>
<td>Eurospital (Calprest® automated assay)</td>
<td>NA</td>
<td>7.1-8.2</td>
<td>4.8-8.6</td>
<td>2.0</td>
<td>NA</td>
<td>103 ± 6 + 12</td>
</tr>
<tr>
<td>Schoepfer et al., 2010</td>
<td>Calpro (PhiCal assay)</td>
<td>n = 165 years</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.75</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(19-83 years</td>
<td></td>
<td></td>
<td>r = 0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA, but diagnosis of over 3 months</td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ELISA = enzyme-linked immunosorbent assay; SD = standard deviation; n = number of patients in study; NA = not available; Se = sensitivity, i.e., the lowest detection limit; Sp = specificity, i.e., the sample blank detection limit.

* The intraclass correlation, calculated using log-transformed fecal calprotectin values, measures the proportion of the total variability (across all samples from all patients) that could be attributed to between-patient variation [Naismith et al., 2013].

Note: All studies were prospective and relative to a 50 µg/g cutoff. Control samples were the comparator, except in the study by Schoepfer et al. [2010], in which the comparator was endoscopy (Simple Endoscopic Score for Crohn Disease).