

## **Evidence review**

# **Value of calprotectin in screening out irritable bowel syndrome**

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## The product

Calprotectin is a protein originating from neutrophils (a type of white blood cell) which can be used as an indicator of inflammation in the bowel. Measurement in faecal samples can be made quantitatively by enzyme linked immunosorbent assay (ELISA) or semi-quantitatively/qualitatively by lateral flow chromatographic immunoassay.

## Field of use

Faecal calprotectin concentrations may be of value in the diagnostic assessment of patients presenting to their GP with gastrointestinal symptoms or in further assessment of patients referred to secondary care gastroenterology clinics. The test is intended to distinguish between organic and functional bowel disease, minimising unnecessary referrals for endoscopy.

## National guidance

The National Institute for Health and Clinical Excellence (NICE) has published guidelines on irritable bowel syndrome in adults, including the tests useful in diagnosis.

The British Society for Gastroenterology has published guidelines on inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Both documents include advice on appropriate tests for diagnosis.

The Department of Health's 18 week pathway for change in bowel habit describes what testing should be available to the patient along the diagnostic pathway.

## Evidence reviewed

Evidence on the following topics was reviewed systematically:

- clinical performance of faecal calprotectin as a biomarker for organic bowel disease
- clinical performance of faecal calprotectin assays compared with other diagnostic tests
- technical performance of available methods of measuring faecal calprotectin
- economic evidence on the cost of making a diagnosis in patients with gastrointestinal symptoms.

Reviews, prospective studies and case-controlled studies were included in the evidence assessed.

## CEP's verdict

Faecal calprotectin measurement performs well clinically in distinguishing organic bowel disease from functional bowel disease (IBS). The majority of studies show it to have a sensitivity and specificity over 80% where the cutoff is most frequently 50 µg/g of faeces. Predictive values were not calculated as often, but most positive predictive values (PPV) were 70-90% and most negative predictive values (NPV) were also 70-90%. NPVs of 70-90% should be sufficient to make a diagnosis of IBS from a normal faecal calprotectin result in those with appropriate symptoms. However, likelihood ratios are below the limits defining a high quality diagnostic test. Further studies on a primary care population may be needed to support use of faecal calprotectin measurement in primary care.

Faecal calprotectin gave a better clinical performance than other diagnostic tests. These included erythrocyte sedimentation rate (ESR), C reactive protein (CRP), several serological markers, other neutrophil product biomarkers, labelled white cell tests, intestinal permeability tests and M2-pyruvate kinase.

Faecal calprotectin measurement is much simpler than labelled white cell studies previously used for the investigation and assessment of IBD and carries no added risk to the patient. Faecal lactoferrin performed clinically as well as or almost as well as calprotectin in all studies but calprotectin performance was slightly better overall. Faecal calprotectin results may be combined with those of other markers or with the matching of symptoms to Rome criteria to enhance the clinical performance.

There are good ELISA tests available for reliable measurement of faecal calprotectin in a laboratory setting. There are also some point of care devices available which appear to be promising in their ability to rule out IBD, but there is little published evidence available at present.

No economic analysis of the use of faecal calprotectin measurement was found in the literature. CEP consequently commissioned the York Health Economics Consortium to undertake such an analysis, which is reported separately [1] and summarised in this evidence review. This analysis indicated that faecal calprotectin measurement offers the potential for substantial cost savings.

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## Background and objectives of the review

### Definition of irritable bowel syndrome

Irritable bowel syndrome is a functional bowel disorder in which abdominal pain or discomfort is associated with defecation or a change in bowel habit, and with some features of disordered defecation [2].

### Prevalence

IBS is thought to affect 10-20% of the general population and is twice as common in women as in men. IBS most often affects 20-30 year olds but also significant numbers of older people. The costing report to the NICE guidance on IBS states an estimated prevalence of IBS in the UK as 2.34 million (1.61 million female and 0.73 million male) with about 78,000 new cases every year [3].

It is thought that up to 70% of IBS sufferers may not seek medical help but IBS may still be responsible for 12% of primary care consultations and 28% of referrals to gastroenterologists [4, 5]. Of those referred to secondary care for invasive tests (approximately 3% of sufferers) about 70% do not have any severe abnormality on endoscopy [6].

### Symptoms

The symptoms of irritable bowel syndrome (IBS), chronic abdominal pain/discomfort, bloating, excessive flatus or change in bowel habit, can be very similar to those of inflammatory bowel disease (IBD). It is thought that a number of factors are involved in causing IBS, including genetic, environmental and immune [7], however research is revealing that some patients with IBS do have biochemical signs of gastrointestinal inflammation [2]. IBS may comprise several heterogeneous subgroups. For example, it is suggested that 4-26% of IBS patients initially developed the disorder following gastroenteritis. The persisting inflammation after the acute infection may play a role in post-infection IBS [8]. The appropriate treatments for IBS and IBD are very different and therefore correct diagnosis is important.

Symptoms are the only positive tests for IBS. These can be assessed for meeting the Rome criteria for IBS, but confirmation of IBS has to involve ruling out other disorders with similar symptoms [9]. Following the Rome I and II criteria, new Rome III diagnostic criteria for IBS have been published (table 1) [2, 10].

**Table 1. Rome III criteria****Recurrent abdominal pain or discomfort at least 3 days per month in the last 3 months associated with 2 or more of the following features:**

Improvement with defecation

Onset associated with a change in frequency or stool

Onset associated with a change in appearance of stool

**Symptoms that cumulatively support the diagnosis of IBS**

Abnormal stool frequency (&gt;3 bowel movements /day or &lt; 3/week)

Abnormal stool appearance (lumpy/hard or loose/watery)

Abnormal stool passage (straining, urgency or feeling of incomplete evacuation)

Passage of mucus

Bloating

Rome III criteria are reported to be an improvement on earlier versions which were thought to be useful in research but were not easy to use in routine medical care [5]. The symptom frequency required to meet the new criteria has reduced symptomatic time from 25% to 10%. The prevalence of IBS has increased as a result but there is less of a bias towards more serious disease which has often included a greater number of patients with psychological disorders. These new criteria have helped to make the case for IBS diagnosis by inclusion (positive criteria) if no 'red flag' alarm symptoms are present [11]. The alternative, diagnosis by exclusion, can potentially result in many tests being carried out to rule out other differential diagnoses. The majority of these additional tests may be unnecessary. Important conditions to exclude have been food allergies, thyroid dysfunction, coeliac disease and IBD, but a review of IBS diagnosis quotes a study in which a full diagnostic assessment was given to 196 symptomatic patients and the results of this assessment were negative for 194 of the patients (99%). These 194 patients were given a final diagnosis of IBS. For patients with IBS who suffer from significant bouts of diarrhoea, testing for coeliac disease is advisable due to the significant prevalence of coeliac disease in the UK. It has been acknowledged that some testing might be necessary in IBS diagnosis for reassurance purposes, especially for those suffering from anxiety or depression [10, 12, 13].

The new criteria include assessment of mental health implications for IBS patients and allow more focus on the average IBS patient who may respond better to diet and drug therapy early in the course of their disease, giving better patient satisfaction and fewer specialist referrals. This in turn should result in a decrease in healthcare requirements and surgical rates; both of which will reduce costs. A study from 20 years ago found that early investigations, reassurance and simple treatment resulted in 85% of IBS patients being largely symptom free at 6 months [10, 14].

## Investigations

The key point in the diagnostic process is deciding which patients should receive endoscopic and/or radiological investigation. There must be a balance between abuse of expensive invasive tests and the under-diagnosis of potentially harmful diseases. There is a need to address the economic and social costs of intestinal disease [15].

Colonoscopy is the gold standard procedure for identifying IBD but it is invasive, not without risk, limited in availability and expensive. Alternatively, radiological tests may be used but these are also costly, entail a certain degree of risk from the use of ionising radiation and may be subject to long waiting times due to the high demand placed upon radiology services. *In vitro* diagnostic tests such as faecal calprotectin offer the possibility of safer and more rapid diagnosis of the absence of IBD, minimising patient anxiety, inconvenience, and exposure to unnecessary prolonged investigation. Availability of such a test in primary care might further reduce the time to diagnosis.

The major differential diagnoses and investigations to be considered in patients with gastrointestinal symptoms are:

- coeliac disease – endomysial antibodies and anti-tissue transglutaminase
- food intolerance – food challenge test
- disaccharide intolerance – breath test, disaccharide challenge test
- IBD/IBS – faecal calprotectin and other biomarkers
- infection or bacterial overgrowth – bacterial culture of faeces, breath test
- diverticular disease of the colon
- colorectal carcinoma – faecal occult blood test (FOBT), colonoscopy
- bile acid induced diarrhoea – liver function, medicosurgical history.

The most important diseases to distinguish will vary with the age of the patient [6]. Colonoscopy should come later in the diagnostic process when other non-invasive tests have indicated that it is necessary. It may be required to differentiate between the two main types of IBD, Crohn's disease (CD) and ulcerative colitis (UC).

The excretion of <sup>111</sup>Indium labelled leucocytes has been used to assess the level of activity in IBD but is no longer used due to patient exposure to ionising radiation and the requirement to collect faeces over four days. However, the test did suggest that leucocytes migrate into the intestinal mucosa in conditions of inflammation, resulting in the formation of abscesses in the crypts of the gut wall. This makes it likely that there might be raised concentrations of proteins originating from these cells in the faeces. In some instances elevated blood concentrations of inflammatory biomarker concentrations may be detected, but these are a less direct reflection of inflammation in the bowel than are faecal biomarker concentrations [7, 16].

Non-invasive, reliable, relatively inexpensive tests are needed to rule out IBS and avoid unnecessary endoscopies. Negative results for an inflammation biomarker such as faecal calprotectin may be able to do this.

The test would need a high negative predictive value (NPV) for IBD to minimise the risk of IBD being misdiagnosed as IBS. A reduction in the number of patients referred to secondary care for investigation might improve the investigation response time for patients with IBD [17].

## Scope of the review

This review covers the use of laboratory and point-of-care tests for faecal calprotectin and other inflammatory markers in distinguishing IBS from IBD. The use of these tests in assessing disease activity or monitoring progress/response to treatment is not covered for IBD or IBS. All types of published evidence are examined including primary research, reviews and manufacturers' information. Clinical, technical, operational and economic evidence is presented, and the impact on patient pathways of using calprotectin to screen out IBS is evaluated.

## Details of calprotectin measurement

Calprotectin is an abundant calcium and zinc binding protein found mainly in neutrophils and to a lesser extent in monocytes and reactive macrophages. It is not present in T or B lymphocytes [18]. Calprotectin accounts for about 60% of the total proteins in the cytosol (non-nuclear) fraction of neutrophils. It has antimicrobial activity and is resistant to enzymatic degradation both *in vivo* and *in vitro*. It can also inhibit proliferation of normal and malignant cells. Removal of zinc may be the mechanism of action for all these activities [19-22].

Calprotectin can be measured by laboratory and point of care (POC) tests:

- Laboratory tests
  - Enzyme linked immunosorbent assay (ELISA)
  - Time resolved fluorimetric immunoassay (TRFIA)
- Point of care tests
  - Lateral flow immunochromatographic tests

## Laboratory based quantitative tests – principles of operation

### **ELISA**

Most laboratory tests for calprotectin are ELISAs carried out in microtitre plates. The calprotectin within the standard material or from a patient sample extract is attached onto the surface of the plate by means of an antibody to calprotectin already attached to the surface. The plate is washed to remove remaining sample and the detection

antibody to calprotectin is then added to the wells. This antibody may be monoclonal or polyclonal and is linked to an enzyme. The final step following further washing to remove excess reagent is to add enzyme substrate and detect the enzyme product either by colorimetry or fluorimetry depending on the substrate. The amount of enzyme product is a measure of the calprotectin present in the standard or patient sample extract.

## **TRFIA**

A capture antibody is adsorbed onto the surface of the microtitre plate wells. The sequence of adding sample, washing and adding a detection antibody is similar to that for an ELISA method. TRFIA has a final addition of enhancement solution in place of the enzyme substrate in ELISAs. Fluorescence is then measured during a defined time window. Fluorescence is a measure of the calprotectin present in the standard or sample [23]. TRFIA was an in-house method and will not be considered further as it is not commercially available.

## **Point of care semi-quantitative/qualitative tests – principles of operation**

Most semi-quantitative and qualitative methods are based on chromatographic immunoassay involving lateral flow of sample and reagents along a membrane strip encased within a plastic palette which contains a sample well and an elongated window for result viewing. The expected position of the lines developed during the test are usually imprinted on the plastic at the side of the window. The test lines (T) contain anti-calprotectin antibodies and the control line (C) contains anti-immunoglobulin antibodies, both of which have been dried onto the membrane strip. Antibodies to calprotectin bound to a detection molecule, eg a gold conjugate, are dried onto the conjugate pad in the sample well. When sample is added to the sample well the labelled antibodies bind to calprotectin in the sample and the complex migrates along the membrane by capillary action. The calprotectin/labelled antibody complexes are bound to the test line by the immobilised antibodies to a different part of the calprotectin molecule. The unbound labelled antibody moves on to be bound by the immobilized immunoglobulin antibodies in the control line. A visible developed control line is essential to show that the test has run properly [24]. Where there is more than one test line they indicate different concentrations of calprotectin present.

## **Accessories**

At least one of the commercially available tests can be used with an electronic reader which will eliminate the subjective nature of reading the result by eye.

## **Technical limitations**

The point of care tests will be much quicker to run (minutes) than an ELISA test (hours) but will only provide a single result from a single device. In contrast an ELISA

test using a full microtitre plate could provide about 40 test results in duplicate, depending on the number of standards included.

## National guidance

### National Institute for Health and Clinical Excellence (NICE)

Guidance from NICE [11] states that patients presenting with the symptoms of abdominal pain/discomfort, bloating or change in bowel habit should be asked if they have any 'red flag' indicators:

- unexplained weight loss
- rectal bleeding without cause
- family history of bowel or ovarian cancer
- change in bowel habit lasting more than 6 weeks in someone over 60 years.

They should also be assessed and clinically examined for other 'red flag' indicators:

- anaemia
- abdominal or rectal masses
- inflammatory biomarkers for IBD. Calprotectin is not mentioned specifically.

Presence of 'red flag' indicators will result in patient referral to secondary care for further investigation including endoscopy. Results from these further tests will identify patients with organic bowel disease enabling appropriate treatment to be started.

IBS should only be considered a diagnosis if the abdominal pain is relieved by defecation or is associated with altered bowel habit and two of the following four symptoms (based on the Rome criteria for IBS [2, 6, 10]):

- altered stool passage
- abdominal bloating
- symptoms made worse by eating
- passage of mucus.

Other features such as lethargy, nausea, backache and bladder symptoms are common in people with IBS, and may be used to support the diagnosis.

Patients who have symptoms meeting the IBS diagnostic criteria should have the following tests performed to exclude other diagnoses:

- full blood count (FBC)
- erythrocyte sedimentation rate (ESR)
- C-Reactive protein (CRP)
- Antibody testing for coeliac disease (endomysial antibodies (EMA) and tissue transglutaminase (TTG)).

The guideline states that ultrasound, endoscopy, thyroid function tests, parasite tests, FOBT and hydrogen breath tests are not needed to confirm a diagnosis of IBS [11].

The NICE guideline CG61 on irritable bowel syndrome in adults does not mention calprotectin, but the NICE guidance for a particular treatment for Crohn's disease, currently for use in research, does include reduction of inflammatory markers such as faecal calprotectin in the key effectiveness outcomes for this procedure [25].

## **British Society for Gastroenterology**

Guidelines on IBD state that IBD is confirmed by a combination of biochemical, endoscopic, radiological, histological or nuclear medicine based tests. The biochemical tests include markers of inflammation such as whole blood ESR and serum CRP. Faecal calprotectin is not mentioned [26].

IBS guidelines state that initial investigation of patients with the symptoms of IBS should include FBC, ESR and CRP [27]. Again, calprotectin is not mentioned. The UK incidence of coeliac disease is sufficiently high to make it cost effective to measure EMA and TTG antibodies. Patient management for coeliac disease is very different from that for IBS.

## **Current standard approaches to care**

### **Current 18 week pathway for change in bowel habit**

The aim of the 18 week patient pathway is to take no more than 18 weeks from the time the patient is first referred to the start of treatment, on pathways that do or might involve consultant led care. Care should be delivered where the patient requires it.

The 18 week pathway for change in bowel habit is the non-cancer pathway for change of bowel habit. If cancer is suspected, eg in those having 'red flag symptoms', the cancer 2 week pathway applies. Patients will be referred to secondary care endoscopic investigation to rule out the presence of polyps, adenomas or bowel cancer. Colonoscopy is the gold standard procedure, but CT colonography is increasingly the first choice radiological test since it is less invasive and provides an image of the whole colon and extra-colonic structures [28].

In the absence of 'red flag symptoms' current primary care assessment should include application of the ROME III criteria [2]. Initial diagnostic tests include:

- blood tests (blood count, thyroid/liver function tests, EMA or TTG and CRP)
- stool microbiology and culture for those working with the sick and in catering
- flexible sigmoidoscopy.

Local primary care commissioning should be reviewed to ensure that current service design permits best use of resources [28].

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## Sources

The following databases were searched for clinical and technical information on calprotectin and other biomarkers for inflammatory bowel disease or irritable bowel syndrome:

- Web of Science
- PubMed
- MEDLINE\*
- Excerpta Medica Database (EMBASE)\*
- British Nursing Index (BNI)\*
- CINAHL\*

\* via the National Library for Health website.

Some additional databases were searched for economic information on the use of calprotectin measurement to rule out irritable bowel syndrome:

- Bandolier\*
- Cochrane Library Database of Systematic Reviews\*
- Database of Abstracts and Reviews of Effects (DARE)\* †
- NHS Economic Evaluation Database (NHS EED)\* †
- Health Technology Assessment Database (HTA)\* †

\* via the National Library for Health website

† via the York Centre for Reviews and Dissemination (CRD) website.

## Search terms

Search terms used:

- calprotectin
- irritable bowel syndrome
- inflammatory bowel disease.

In order to search for other biomarkers the following terms were added to the above terms and the searches were also carried out for the other biomarkers, but without calprotectin:

- lactoferrin
- M2-Pyruvate kinase (M2-PK)
- polymorphonuclear neutrophil elastase (PMN-e)
- ESR
- CRP

- cytokines
- ANCA
- ASCA
- S100A12
- intestinal permeability
- Rome III criteria.

An additional selection of papers was made for calprotectin measurement which would only be used for technical details, not clinical information, and therefore it was not necessary to exclude cancer. Search terms for these papers were:

- calprotectin
- measurement.

The search terms for papers on the economic aspects of use of calprotectin measurement in the investigation of bowel disease were:

- calprotectin
- inflammatory markers
- inflammatory bowel disease
- irritable bowel syndrome
- economics
- cost effectiveness
- cost
- quality adjusted life years (QALY).

## **Inclusion and exclusion criteria**

Inclusion criteria:

- English language
- published within the last 10 years
- human not animal studies.

Exclusion criteria:

- cancer.

No evidence was found on the value of faecal calprotectin measurement in monitoring of IBS.

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## Assessment of quality of papers found

Assessment was based on the Oxford Centre for Evidence-based Medicine levels of evidence.

**Table 2. Hierarchy of evidence**

Level	Type of evidence
1	Systematic reviews of Randomised Controlled Trials (RCT), meta-analysis, individual RCTs
2	Systematic reviews of cohort studies, individual cohort studies
3	Systematic reviews of case-controlled studies, individual case-controlled studies
4	Case series, poor quality cohort, case-control studies
5	Expert opinion without explicit critical appraisal

The majority of information included in the report came from level 1-3 publications.

## Calprotectin

It is important to be able to distinguish patients with IBD or IBS in order to avoid subjecting those with IBS to unnecessary investigations. Previously, Rome criteria for IBS may have been fulfilled by 25-33% of patients with organic disease, making misdiagnosis by positive criteria a real possibility [5, 29]. A clinical diagnosis of IBS was made largely by exclusion of other disorders [29]. However, some of the available laboratory tests lack specificity for gastrointestinal disorders and other procedures are very invasive and expensive.

A specific direct non-invasive test is needed to rule out the presence of organic disease and avoid unnecessary tests. A faecal marker will provide a more direct indication of bowel inflammation than markers measured in blood. Some gastroenterologists have felt that collection of faeces would be unpopular with patients and therefore be a limiting factor in using faecal biomarkers to assess patients with bowel symptoms [30], but others have found good compliance with faecal sample collection [31, 32].

It is important to make a correct diagnosis of IBD in a reasonable period of time. It has been known to take 4 years to come to a diagnosis of Crohn's disease, with increased risk that complications might develop [33]. Delayed diagnosis in children may additionally affect growth and sexual maturation [34].

Plasma calprotectin concentrations increase 5-40 fold in infectious and inflammatory conditions. In healthy people faecal calprotectin concentration is about 6 times that of plasma calprotectin [16, 19, 29]. Elevated concentrations of faecal calprotectin have been found in the faeces of both adults and children with IBD [20, 35-37]. It should not be thought of as a marker of organic disease, but as a marker of 'neutrophilic intestinal inflammation'[19].

Faecal calprotectin measurements correlated well with the most sensitive investigations for assessing IBD such as intestinal permeability, <sup>111</sup>Indium labelled granulocyte excretion and endoscopic grading. These sensitive tests are more invasive and expensive and possibly not well tolerated by the patient [18, 29]. The current gold standard for assessing intestinal inflammation is endoscopy [19] but calprotectin concentrations correlate more closely with histological than macroscopic/endoscopic findings, suggesting that calprotectin measurement is the better test to assess IBD activity [38]. It has also been stated that faecal calprotectin measurement can identify inflammation in the small bowel, an area which cannot be examined fully by standard endoscopy. However, overall calprotectin may be better in detecting lesions in the large bowel than in the small bowel [18, 39]. In a study of children referred for investigation of bowel symptoms, faecal calprotectin concentrations correlated significantly with <sup>99</sup>Tc labelled white cell scores (a means of

locating and assessing bowel inflammation) for small bowel and colonic inflammation[38].

Comparison of the clinical performance of quantitative or qualitative tests should be undertaken with caution unless in the same study. The way in which patients in a study are selected will affect the sensitivity and specificity given by a test and the prevalence of disease may differ which will affect any positive and negative predictive values calculated.

The following statistical abbreviations are used in table 3 below:

- PPV – positive predictive value
- NPV – negative predictive value
- LR+ – positive likelihood ratio
- LR- – negative likelihood ratio
- OR – odds ratio
- DA – diagnostic accuracy.

See appendix 1 for definitions of these terms.

**Table 3. Clinical performance of calprotectin in bowel disease**

Reference	Calprotectin ELISA assays	Patient source	No. of patients	Identify OD*/IBD	Cutoff (µg/g)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-	OR	DA
Adults													
[29]	In-house Roseth 1992	CD <sup>†</sup>	116	CD <sup>†</sup>	50	97							
[29]	In-house Roseth 1992	GIC <sup>‡</sup>	220	IBD	150	100	97						
[5]	In-house Roseth 1992	GIC <sup>‡</sup>	602	OD*	50	89	79	76	89			28	
[20]	Calprest	GIC <sup>‡</sup>	205	OD*	50	83	82	90	71				82
[36]	PhiCal	GIS §	30+116	IBD	50	79							
[35]	Calprest	GIC <sup>‡</sup>	70	OD*	50	64	80	70	74				
[35]	Calprest	GIC <sup>‡</sup>	10	IBD	170	100	95						
[23]	Calprest	GIC <sup>‡</sup>	203	IBD	100	81-87	100						
[23]	Calprest	GIC <sup>‡</sup>	203	IBD	40	89-90	87						
[37]	Calprest	GIC <sup>‡</sup>	50	IBD	90	85	100						
[4]	Calprest	GIC <sup>‡</sup>	73	OD*	60	100	79	60	100				
[40]	Calprest	GIC <sup>‡</sup>	120	IBD	18.6	61							
[31]	PhiCal	GIC <sup>‡</sup>	74	IBD	50	83	100	100	77				89
[41]	Immunodiagnostik	GIC <sup>‡</sup>	139	IBD	30	100	37						
[42]	Immunodiagnostik	GIC <sup>‡</sup>	88	IBD	15	93	100	100	91				

Reference	Calprotectin ELISA assays	Patient source	No. of patients	Identify OD*/IBD	Cutoff (µg/g)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-	OR	DA
[43]	In house Roseth1992	GIC †	148	OD*	25	80	74	87	65	14			
[44]	Calprest	CR **	144	IBD	80	78	83	86	80				
[45]	Calprest	GIC †	83	IBD	50	63	86	90	51	4.5	2.3	9.9	
[46]	PhiCal	GIC †	136	IBD	50	83	100	100	74				89
[24]	Buhlmann Laboratories	CR **	140	OD*	50	77	79	75	79				
[47]	PhiCal	CR**	65	IBD	70	74	84						
[47]	Buhlmann Laboratories	CR**	65	IBD	70	79	87						
Children													
[35]	Calprest	GIC †	50	OD*	50	70	93	96	56				
[35]	Calprest	GIC †	8	IBD	50	70	93						
[34]	Calprest	GIC †	45	IBD	95	93	89	93	89				
[48]	PhiCal	GIC †	61	IBD	50	100	67	75	100				

\* organic disease

† Crohns disease

‡ Gastro intestinal clinic

§ Gastrointestinal symptoms

\*\* Colonoscopy referral

Symptomatic patients with Crohn's disease, seen in a gastroenterology clinic, had significantly higher faecal calprotectin concentrations than those with IBS ( $p < 0.0001$ ) [29].

Later studies confirmed the finding of significantly higher faecal calprotectin concentrations in organic bowel disease than IBS ( $p < 0.0001$ ), but PPVs and NPVs varied (table 3) [5, 20]. In a small study of patients with Crohn's disease, faecal calprotectin concentrations were statistically significantly different from those of healthy controls and patients with IBS ( $p < 0.001$ ) [37].

A study of patients having a small bowel barium follow through (BaFT) procedure found that faecal calprotectin measurement was a good indicator of the presence of organic bowel disease. The authors suggested that a result of  $< 60 \mu\text{g/g}$  on a single sample would make a small bowel BaFT unnecessary [4].

A study comparing the diagnostic accuracy of faecal calprotectin measurement in adults and children concluded that it was a good marker for IBD in both groups, with a higher diagnostic accuracy in children than adults. The low NPV of 56% for children and the lower NPV for adults compared with Tibble [5] may have been influenced by the greater number of cases of coeliac disease included. The NPV of 56% indicates that faecal calprotectin cannot be used to exclude the presence of organic disease in children [35].

The majority of individual studies reporting PPV and NPV have found NPVs greater than 70%, with 4 of the 11 having NPVs greater than 80%. One possible explanation for the low result of 65% was the presence of inactive IBD in some patients, but no cause was suggested for the NPV of 51%. NPVs for children also varied, as stated above. This was supported by the findings of one of the reviews quoted in table 8 [19]. The majority of the studies with NPVs greater than 70% used patients presenting in secondary care with gastrointestinal symptoms and referral for colonoscopy. A few included known cases of IBD. The incidence of bowel disease in this population may be higher than that in the population presenting to their GP with gastrointestinal symptoms. The GP may have referred patients on the basis of results from some initial tests with poor sensitivity and specificity for bowel disease.

In one study of patients referred for colonoscopy, the majority (85%) of the subgroup who had normal colonoscopies also had normal faecal calprotectin concentrations. However, the median concentration for this subset was  $25 \mu\text{g/g}$  which was significantly different from the  $11.5 \mu\text{g/g}$  median concentration of a group of healthy controls in the same study [49]. This slight elevation of faecal calprotectin concentration in patients thought to have IBS has been seen in other studies, but the difference between the two groups has not always been significant (table 4) [4, 20, 29, 37, 50].

**Table 4. Comparison of results in healthy controls and IBS patients**

Healthy control	IBS	Significance of difference	Cutoff ( $\mu\text{g/g}$ )	Reference
11.5*	25*	p=0.01	50	[49]
10*	20*	p=0.0004	50	[29]
10	19	p=0.04	60	[4]
11	22	Not stated	50	[20]
9.3	20.5	p=0.06	50	[37]
22	26	Not stated	50	[51]
70%Neg <15	30% 62% Neg 38% <15	p=0.312	Semi-quantitative <15, <60	[50]

\* Reported as mg/L converted to  $\mu\text{g/g}$

### Cutoff point

Cutoff point should be determined on the basis of a 'gold standard', requiring full gastrointestinal investigation, taking account of how the test is to be used. If faecal calprotectin measurement is used to rule out IBS, thus avoiding endoscopy, a low cutoff is best and high sensitivity is very important. False positive results are preferable to false negative results [20]. A high negative predictive value is needed to provide clinicians with the reassurance that referral for secondary care investigations is not required.

Comparison of data at different cutoff values is only relevant where the same method has been used for all measurements [4]. Cutoff point concentration for calprotectin in some published studies varies from 18.6 to 250  $\mu\text{g/g}$  of faeces. The differences in the design of studies and the characteristics of the patients included may influence the optimum cutoff found [37, 52]. The upper reference limit for absence of disease is suggested as 50  $\mu\text{g/g}$ , but it is also suggested that different cutoff values are used for patients with known inflammatory disease (higher) and for screening purposes (lower) [16]. A study of children with IBD used a cutoff of 100  $\mu\text{g/g}$  to identify active IBD but a cutoff of 50  $\mu\text{g/g}$  to exclude active IBD [53].

Choice of the correct cutoff is essential for efficient and cost effective use of faecal calprotectin measurements in the investigation of symptomatic patients.

### Semi-quantitative and qualitative faecal calprotectin measurements

The Prevent ID CalDetect rapid test for calprotectin appears to be as proficient as the PhiCal ELISA test in distinguishing IBD (table 5). The authors of the study concluded that the rapid test was suitable for use in primary care, but highlighted the fact that its cost effectiveness in this situation should be assessed [54].

**Table 5. Comparison of Prevent ID and PhiCal in distinguishing IBD from IBS [54]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
Prevent ID CalDetect cutoff 15 µg/g	100	95	82	100	18.2	0
Prevent ID CalDetect cutoff 60 µg/g	61	98	88	91	27.2	0.4
Calprotectin ELISA cutoff 60 µg/g	96	87	65	99	7.3	0.5

Similar results were obtained by others (table 6). They concluded that using a cutoff of <15 µg/g, it was a useful screening test to exclude gastrointestinal inflammation, but that samples giving concentrations greater than 15 µg/g should be analysed by a quantitative method. The rapid test could therefore be useful in an outpatient setting [55].

**Table 6. Prevent ID parameters in identifying IBD in patient with PhiCal result > 50µg/g [55]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Prevent ID CalDetect cutoff 15 µg/g	96	70	79	94
Prevent ID CalDetect cutoff 60 µg/g	66	100	100	72

Another study (table 7) concluded that the Prevista rapid test was a cost-efficient method for the evaluation of patients with gastrointestinal symptoms in an outpatient department; no financial data were provided. The test was not found to be a good marker for diverticulosis (the condition of having diverticula).

**Table 7. Comparison of Prevista and Bulhmann calprotectin ELISA in identifying active IBD [24]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC of ROC*
Prevista calprotectin cutoff 50 µg/g	89	80	59	96	0.896
Bulhmann calprotectin ELISA cutoff 50 µg/g	100	79	60	100	0.955

\*AUC of ROC: Area under the curve of receiver-operator characteristics curve analysis

CalDetect however, was shown to distinguish between acute diverticulitis and IBS ( $p < 0.005$ ) and between symptomatic diverticular disease and IBS ( $p < 0.005$ ) [50].

### Conclusion on POC tests

The studies including point of care tests for faecal calprotectin found that they were satisfactory in identifying IBD or discriminating between IBD and IBS. Data were not

provided to back up statements of their cost effectiveness. A reliable study into cost effectiveness of POC devices for faecal calprotectin measurement is required, which should take into account the savings made from gaining a result early and possibly in the primary care setting.

## Information from reviews

**Table 8. Calprotectin performance in identifying IBD in adults and children**

Reference	No. of studies	Adult/Child	No. of patients	Identification of organic disease/IBD	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
[19]	4	Adult	70-602	organic/functional	50 µg/g*	63-82	79-83	63-77	74-93	3.17-4.83	0.14-0.46
[19]	3	Child	22-50	organic/functional	50 µg/g*	69-100	86-93	80-96	56-100	7.00-13.36	0.00-0.34
[52]	9	Adult + child	1267	IBD/no IBD (M) <sup>†</sup>	50 µg/g	89	81				
[16]	16 (11A+5C)	Adult + child	754	IBD/IBS (M) <sup>†</sup>	Not given	80	76				
[16]	14 (10A+4C)	Adult + child	2475	organic/functional (M) <sup>†</sup>	Not given	83	84				

\* where known

<sup>†</sup> (M) meta-analysis

A number of the studies cited in table 3 are included in the reviews in table 8

A review on the role of calprotectin as a marker of intestinal inflammation has shown slightly higher sensitivities and PPVs in children than in adults (table 8). NPVs were quite variable, partly due to the number of children with coeliac disease but normal faecal calprotectin concentrations. For these seven studies the LR+ and LR- values varied from 3.17-13.36 and 0-0.46 respectively, across the limits marking a good diagnostic test (>10 and <0.1 respectively). The LR values for children were slightly better than those for adults. Some of the variation in the performance figures would have been due to the different cutoffs used in these studies [19].

A meta-analysis of nine studies of patients with IBD compared with patients with no IBD found that faecal calprotectin measurements gave a good pooled sensitivity and specificity for distinguishing these two conditions (table 8). However, the studies combined differed significantly and involved adults and children [52].

A recent review of calprotectin as a marker of IBD categorised studies into two groups; those aimed at distinguishing organic from functional intestinal diseases, and those attempting to distinguish patients with IBD from those with a similar clinical history but no IBD (table 8) [16]. The findings are similar to those of Von Roon *et al* [52].

As NPVs for faecal calprotectin are generally high, a negative faecal calprotectin result (below the cutoff) should be an indication that organic disease is very improbable in symptomatic patients, thus distinguishing patients with IBS [16, 36]. Calprotectin measurement could therefore be used to identify those patients for whom an endoscopy is unnecessary. Conversely a high faecal calprotectin concentration is a strong indicator to perform a colonoscopy to confirm or rule out IBD or other organic pathologies [16, 21]. The overall conclusion of the review was that biological markers in general should currently be a complement to and not a substitute for radiology, endoscopy and histology and should never replace good clinical judgement [16].

## **Conclusion on faecal calprotectin measurement**

Faecal calprotectin concentrations have been shown to correlate with recognised 'gold standard' tests for IBD and may also have a role in detection of small bowel disease as well as that in the large bowel. They have been shown to be significantly higher in patients with organic bowel disease than in those with IBS. Faecal calprotectin was thought to be a good marker for organic bowel disease in symptomatic adults and children.

Faecal calprotectin concentrations are higher in patients with IBS than healthy controls, but still well below a cutoff of 50 µg/g.

The most common cutoff point for a normal result is 50µg/g (table 3). As stated above, a low cutoff is good in screening to rule out IBS. The study using a cutoff of 25 µg/g gave an LR+ of 13.95 indicating that the patients with a raised faecal calprotectin result were highly likely to have IBD and not to have IBS.

The sensitivities and specificities reported in tables 3 and 8 were generally high, with the studies showing the highest sensitivities having the lowest specificities, as expected, thus increasing the risk of false positive results.

The majority of individual studies reporting PPV and NPV have found NPVs greater than 70%, with some greater than 80%. The majority of these studies had selected patients suffering from gastrointestinal symptoms.

Few of the studies in table 3 reported LR+ and LR- values, but one review reported LR+ values of mostly >4 and LR- values of mostly <0.4; however, these fall short of the definitions for a very good diagnostic test (LR+ >10 or LR- <0.1). A further large study of faecal calprotectin measurements in patients with symptoms of bowel disease might provide more definite conclusions.

The reviews were all of the opinion that faecal calprotectin measurement could distinguish organic from functional bowel disease, but there was variation in the views on the reliability with which it can do this.

Faecal calprotectin concentrations are raised in inflammation and cancer of the bowel. The test is not useful in differential diagnosis of organic bowel disorders; thus it cannot be used as a screening tool for IBD in patients with bowel disease [16, 36, 52]. Its best use is to distinguish organic from functional disease, helping to rule out IBS, and thereby reducing the number of unnecessary follow-up investigations. A raised faecal calprotectin concentration may lead to an early colonoscopy and a quicker start of appropriate treatment [18].

## Faecal calprotectin in relation to other biomarkers

As stated above, comparison of clinical performance of quantitative or qualitative test results should be made cautiously unless results were obtained in the same study.

### ESR and CRP in blood

Faecal calprotectin concentrations correlate with the degree of gastrointestinal mucosal inflammation [20] and are a more direct measure of this than CRP and ESR measurements. In symptomatic patients, faecal calprotectin, ESR and CRP concentrations were significantly higher in those with Crohn's disease than in those with IBS  $p < 0.0001$  for each marker, but faecal calprotectin performed best in identifying bowel disease [29].

A study of various markers of inflammation concluded that faecal calprotectin, intestinal permeability and positive Rome I criteria provided a good non-invasive way of differentiating between organic and non-organic intestinal disease. These tests out-performed both ESR and CRP (table 9). Using a panel of tests improved the likelihood of excluding IBS from the differential diagnosis. The study concluded that faecal calprotectin measurement could be included in the algorithm for assessment of suspected non-organic intestinal disease following specialist referral [5].

**Table 9. Laboratory test parameters for bowel disease [5]**

Test	PPV (%)	NPV (%)	OR
<b>Organic bowel disease</b>			
Faecal calprotectin >50 µg/g	76	89	27.8
Permeability ratio >0.05	56	89	8.9
CRP >5 mg/L	67	68	4.2
ESR >10 mm/hr	62	69	3.2
Calprotectin >50 µg/g + permeability ratio >0.05 (small intestinal disease)	70	74	15.0
Calprotectin >50 µg/g + permeability ratio <0.05 (colonic disease)	56	89	13.3
<b>Functional bowel disease</b>			
Positive Rome criteria	86	69	13.3
Calprotectin < 50 µg/g + permeability ratio <0.05 (non-organic disease)	92	70	25.0
Calprotectin < 50 µg/g + permeability ratio <0.05 + positive Rome criteria (non-organic disease)	97	61	46.0

Carroccio *et al* also found faecal calprotectin measurements more useful than CRP and ESR in distinguishing organic bowel disease from IBS (table 10) [35].

**Table 10. Laboratory test parameters for organic bowel disease [35]**

Test	Sensitivity (%)	Specificity (%)
Faecal calprotectin < 50 µg/g	66	84
CRP < 1 mg/L	40	90
ESR <12 mm/h	66	63

Faecal calprotectin was better than ESR, CRP or ESR and CRP in detecting the presence of organic disease in patients with diarrhoea (table 11). It was suggested that the NPV (100%) indicated that a single normal faecal calprotectin concentration removed the need for radiological investigation, effectively excluding organic intestinal disease [4]. This does not agree with the later review on faecal calprotectin in IBD which included a large number of studies [16].

**Table 11. Laboratory test parameters for organic bowel disease [4]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Faecal calprotectin > 60 µg/g	100	79	60	100
CRP >6 mg/L	77	70	42	91
ESR >10 mm/h	79	67	42	91
CRP >6 mg/L + ESR >10 mm/h	50	84	50	84

Measurements of faecal calprotectin, serum anti-*Saccharomyces cerevisiae* antibody (ASCA), serum anti-neutrophilic cytoplasmic antibody with perinuclear staining (pANCA), and bowel wall ultrasonography in children with gastrointestinal symptoms all distinguished IBD from non-IBD better than serum CRP or ESR (table 12) [34].

**Table 12. Laboratory test parameters for inflammatory bowel disease in children [34]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Faecal calprotectin >95 µg/g	93	89	93	89
ASCA/pANCA	78	89	91	73
BWUS *	74	78	83	67
CRP	41	78	73	53
ESR	52	78	78	48
FC + ASCA/pANCA + BWUS	43	100	100	56
FC or ASCA/pANCA or BWUS	100	67	82	100

\* BWUS Bowel wall ultrasound

Calprotectin gave the best single test performance. If all three tests were positive the probability of the presence of IBD was calculated to be 99.47% and if all three tests were negative/normal then the probability of the presence of IBD was 0.69%. A wider population needs to be studied to confirm these results [34].

### Serological markers in blood

Tests for ASCA (an antimicrobial antibody), pANCA (an autoantibody), outer membrane porin C of *Escherichia coli* (Omp C) and CBir 1 flagellin from motile bacterial flagellae are commercially available [56].

A review of laboratory evaluations of IBD in children concluded that there is no single screening test for IBD. Routine biochemical markers (FBC, CRP and ESR) are effective for moderate-to-severe disease, but not for mild disease. In comparison, serological tests such as ASCA, pANCA and the newer OmpC and anti-c Bir1 are more expensive, less sensitive and may have unacceptable false positive rates. Faecal tests were thought to be promising, but compliance with collection of faeces was a possible issue [30, 57].

A recent review on calprotectin as a marker of IBD concluded that faecal calprotectin provided better results than ESR, CRP, ANCA or ASCA [16].

Flagellin is the main structural component of bacterial flagella and is known to activate the immune system. Serum concentrations of antibodies against the A4-Fla2 and Fla-X flagellins were measured in patients referred for gastroenterological investigation (table 13). All those diagnosed with IBS had fulfilled the Rome II criteria.

**Table 13. Mean calprotectin and frequency of antibodies in different patient groups [51]**

Test	HC*	IBS	CD	UC
Calprotectin µg/g	22	26	257	183
ASCA % present	2	6	59	0
p-ANCA % present	0	0	10	52
A4-Fla2 % present	7	29	48	8
Fla-X % present	7	26	52	10

\* HC Healthy controls

Antibodies against the A4-Fla2 and FI-X flagellins, similar to ASCA, were raised more often in cases of CD than UC. In contrast to ASCA and pANCA, flagellin antibodies were found significantly more frequently in those with IBS, especially post infectious IBS, compared with healthy controls. It may be useful in terms of future treatment to

be able to identify this subgroup of patients with IBS, but further evaluation is needed [51].

### **Information on serological markers without comparison with calprotectin**

In considering the performance of ASCA, pANCA, OmpC, anti-12 (from *Pseudomonas fluorescens*) and CBir 1, but not faecal calprotectin, Bruining and Loftus concluded that the single most useful serology test for inflammation in the gastrointestinal tract was ASCA. However, it was not recommended for widespread screening because its sensitivity was low [58, 59]. Use of additional antibodies (eg. OmpC, anti-12 and CBir1 flagellin) may improve the identification of CD, but the performance of this panel of antibodies needs further independent assessment [60]. Similar conclusions were reached by a study of children [61].

It is recognised that ASCA and pANCA concentrations can help differentiate between CD and UC, ASCA being raised more commonly in CD and pANCA in UC [58].

### **Conclusions on biomarkers measured in blood**

Faecal calprotectin measurements discriminate between IBD and IBS better than ESR or CRP measurements.

Measurement of a variety of antibodies in serum has indicated that the best test is ASCA, but that even this test does not perform as well as faecal calprotectin in detecting organic bowel disease and would not rule out IBS reliably. ASCA and pANCA may have a role to play in distinguishing between CD and UC.

### **Measurement of polymorphonuclear neutrophil (PMN) products in faeces**

The drive to find reliable non-invasive markers for IBD came initially from the need to diagnose children with minimal use of invasive tests. Ultrasonography and imaging of autologous leucocytes are non-invasive methods, but they require specialist equipment and skills and are therefore expensive to perform [62]. PMN products (table 14) are biomarkers of neutrophilic intestinal inflammation. The absence of elevated concentrations of these can rule out the presence of IBD [63].

**Table 14. Polymorphonuclear neutrophil (PMN) products**

Product	Source cell types	Intracellular location	Properties/activities
Calprotectin	neutrophils, monocytes	cytoplasm	antimicrobial resistant to enzymic degradation
Lactoferrin	neutrophils	secondary granules	antibacterial
PMN-elastase	neutrophils	primary granules	proteinase
Myeloperoxidase	neutrophils	primary granules	antimicrobial
Lysozyme	neutrophils	granules	attacks bacterial cell walls
S100A12	neutrophils	cytoplasm	pro-inflammatory

### Multiple markers

A review of neutrophil products stated that they were the most promising class of faecal biomarkers for IBD [19]. Performance of PMN-elastase (PMN-e), calprotectin, myeloperoxidase (MPO), lysozyme and lactoferrin was compared in differentiating IBD from IBS (table 15). PMN-e and calprotectin were the best markers and correlated with severity of inflammation [40]. The performance of lactoferrin was not as good as that reported by others, where lactoferrin sensitivity and specificity for distinguishing the two conditions was 78% and 90% respectively. Calprotectin was not measured in this latter study so there was no comparison of performance [62].

**Table 15. Laboratory test ROC analysis [40]**

Test	Area under curve	Significance of difference to PMN-e
PMN-e	0.92	
Calprotectin	0.87	p = 0.33 Not significant
Myeloperoxidase	0.75	p<0.001
Lysozyme	0.73	p<0,0001
Lactoferrin	0.69	p<0.0001

A study in 2007 measuring faecal biomarkers calprotectin, lactoferrin and PMN-e concluded that faecal calprotectin was the most accurate marker of these in discriminating IBD from IBS (table 16). There was a slight rise in sensitivity and NPV when both calprotectin and PMN-e measurements were used, but addition of lactoferrin did not improve performance [42].

**Table 16. Laboratory test performance in inflammatory bowel disease [42]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Calprotectin (>15 µg/g)	93	100	100	91
Lactoferrin (>7.3 µg/g)	82	100	100	80
PMN-e (>62ng/g)	84	87	91	79
Calprotectin + PMN-e	96	100	100	94
Calprotectin + Lactoferrin + PMN-e	96	100	100	94

A review of faecal biomarkers in IBD concluded that currently faecal calprotectin and lactoferrin are the best biomarkers to distinguish IBD from IBS [64]. Reports differ on the relative performance of these markers [40, 44], but they performed better than a variety of other biomarkers such as <sup>111</sup>Indium labelled granulocyte excretion, lysozyme, PMN-e, human neutrophil lipocalin, myeloperoxidase, eosinophil-derived proteins and  $\alpha$ 1-antitrypsin [64].

### Lactoferrin

A study of patients referred for a colonoscopy found that measurements of faecal calprotectin and faecal lactoferrin were equally good as markers of inflammatory intestinal disease and correlated well with each other (Cohen' kappa  $\kappa=0.63$ ) (table 17). Faecal calprotectin and lactoferrin results correlated with endoscopic and histological findings in UC but not in CD. Patients with IBS had normal faecal calprotectin concentration and all but one had normal faecal lactoferrin concentrations, the exception being only marginally raised [44].

**Table 17. Laboratory test overall performance in inflammatory bowel disease [44]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	Diagnostic accuracy (%)
Calprotectin	78	83	86	80
Lactoferrin	80	85	87	81

In another study including faecal biomarkers lactoferrin, calprotectin and haemoglobin, lactoferrin measured by ELISA gave better discrimination between IBD and IBS than calprotectin ELISA results, lactoferrin rapid test (Leuko-Test), haemoglobin by Hexagon OBTI, CRP and blood leucocytes (table 18). Faecal markers are non-specific in the inflammation they detect and therefore cannot replace endoscopy in those patients with raised faecal calprotectin and lactoferrin concentrations. The authors thought the absence of a raised faecal calprotectin and lactoferrin should be included in the definition of patients with IBS [31]. However, this might not apply to children. A study found faecal calprotectin concentration was raised relative to a control group in children with functional abdominal pain ( $p=0.01$ ) [32].

**Table 18. Laboratory and POC test performance in inflammatory bowel disease [31]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Calprotectin ELISA	83	100	100	77
Lactoferrin ELISA	86	100	100	80
Lactoferrin LEUKO TEST (FRT*)	69	100	100	69
FOB Hexagon OBTI	64	100	100	61
CRP	64	85	88	57
Blood leucocytes	47	90	89	49

\* FRT: faecal rapid test

A further recent study by the same group used the same markers with the addition of ASCA and p-ANCA. The best tests for discriminating between IBD and IBS were confirmed as the quantitative measurement of faecal lactoferrin and calprotectin (table 19). The IBD antibodies ASCA and ANCA added only marginal additional diagnostic value (further 1-2%) to the faecal leucocyte biomarkers and therefore were not recommended for distinguishing IBD from IBS. Both faecal calprotectin and lactoferrin correlated with endoscopic assessment of severity in moderate to severe CD and faecal calprotectin, but not lactoferrin, in moderate to severe UC [46].

**Table 19. Overall accuracy of laboratory and rapid tests distinguishing IBD from IBS [46]**

Test	Accuracy (%)
Calprotectin ELISA	89
Lactoferrin ELISA	90
Lactoferrin LEUKO TEST (FRT)	78
FOB Hexagon OBTI	74
CRP	73
Blood leucocytes	63
CD markers	55
UC markers	49
Calprotectin + CD/UC markers	91/90
Lactoferrin + CD/UC markers	92/91

A study of patients with IBD found that concentrations of faecal biomarkers lactoferrin, calprotectin and PMN-e were higher in patients with IBD than in those with IBS. None of the markers was consistently better than the other two in its ability to reflect endoscopic inflammation. For all three markers, diagnostic accuracy was better (80%, 80% and 74% respectively) than that of CRP (64%) [41].

Investigation of the ability of rapid tests to discriminate between IBD and IBS found that faecal calprotectin and lactoferrin concentrations correlated well whether measured by ELISA or rapid test ( $\kappa= 0.67- 0.76$ ). Both rapid tests were able to distinguish inflammatory from non-inflammatory bowel disease (table 20). Calprotectin was slightly better than lactoferrin at ruling out IBD. The authors recommended that a study of the accuracy and cost effectiveness of calprotectin be carried out in a primary care population rather than the selected secondary care population in the published study [54].

**Table 20. Laboratory and rapid test parameters for distinguishing IBD from IBS [54]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
Calprotectin Prevent ID (FRT) (cutoff 15 $\mu\text{g/g}$ )	100	94.5	82.1	100	18.2	0
Lactoferrin IBD EZ VUE (FRT)	78	99.0	94.7	94.7	75.1	0.2
Calprotectin ELISA	95.7	86.8	64.7	98.8	7.3	0.5
Lactoferrin ELISA	78.3	90.1	66.7	94.3	7.9	0.2

### S100A12

S100A12 has similar protein properties to calprotectin and is also secreted by neutrophils. S100A12 may have pro-inflammatory activity. Histological staining showed extracellular S100A12 around the S100A12 positive cells, indicating secretion, in gut biopsies with considerable inflammation, but not in biopsy samples from patients with IBS, all of whom fulfilled the Rome II criteria [45].

Faecal S100A12 was found to be better than faecal calprotectin in distinguishing IBD from IBS in a group of patients with gastrointestinal symptoms. Concentrations of faecal S100A12 were significantly raised in IBD patients compared with IBS patients. The concentrations in the latter group did not differ significantly from those in healthy controls. The performance of faecal S100A12 in distinguishing IBD from IBS was better than that of faecal calprotectin (table 21). However, S100A12 is raised in bacterial, but not viral, enteritis so the latter must be ruled out in patients with raised faecal S100A12 concentrations. The authors concluded that S100A12 is more specific than calprotectin for the presence of infiltrating neutrophils and is a useful addition to the non-invasive biomarkers of intestinal inflammation [45].

**Table 21. Test performance in distinguishing IBD from IBS [45]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-	OR
Faecal S100A12	86	96	98	76	21.5	6.9	13.5
Faecal calprotectin	63	86	90	51	4.5	2.3	9.9
CRP	58	79	76	53	2.8	1.9	8.2
ESR	56	75	74	42	2.2	1.7	2.9

Support for this was provided from a study of biomarker release from tissue *in vitro*. Although calprotectin is present at much higher concentrations than S100A12, the increased release from inflamed tissue compared to non-inflamed tissue was 28 fold for S100A12 and 8 fold for calprotectin [65].

Faecal S100A12 concentrations have distinguished children with IBD from healthy controls with a sensitivity and specificity of 96% and 92% respectively at a cutoff of 10 mg/kg (10 µg/g) [66]. More recently S100A12 showed higher specificity than calprotectin in distinguishing children with IBD from those without IBD using the cutoff values of 10 mg/kg and 50 mg/kg respectively. ROC curve analysis highlighted this better performance of faecal S100A12 against the other markers investigated (table 22). The authors concluded that S100A12 was a good non-invasive screening test for selection of children requiring further invasive tests [48].

**Table 22. Test performance in identifying IBD [48]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC of ROC
Faecal S100A12	97	97	97	97	99
Faecal calprotectin	100	67	75	100	95
Blood S100A12	22	81	50	54	52
Blood ESR	74	93	92	77	91
Blood CRP	81	79	81	79	89

### Information on faecal neutrophil products without comparison with calprotectin

Faecal lactoferrin concentrations were significantly higher in patients with IBD than in patients with IBS. Concentrations were very similar in IBS and healthy controls. The qualitative test performed well in distinguishing IBD from IBS at a cutoff of 4 µg/g. A raised faecal lactoferrin result was 100% specific for ruling out IBD (table 23) [62].

**Table 23. Quantitative and qualitative lactoferrin test parameters in distinguishing IBD from IBS [62]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+
Lactoferrin (quantitative)	78	90			7.8
Lactoferrin (qualitative)	86	100	100	87	

This conclusion was supported by other studies, using quantitative measurements, including one on children where faecal lactoferrin concentrations distinguished IBD from those with IBS with a sensitivity of 97% and specificity of 100% [7, 67].

### Conclusion

Neutrophil product performance in distinguishing IBD from IBS is variable. Most work has been done on calprotectin. In general calprotectin's performance is similar to that of lactoferrin, PMN-e and S100A12 and better than that of MPO and lysozyme. There is a significant amount of published evidence on lactoferrin and calprotectin but little on the other four biomarkers. Calprotectin performed better than lactoferrin in three of the eight studies above, equal to lactoferrin in four and worse than lactoferrin in one.

The newer marker S100A12 performed similarly to calprotectin in one study and much better in another. In this latter study calprotectin performance was worse than in the majority of other studies.

Several studies have shown that a panel of biomarkers may provide the best discrimination between IBD and IBS. Further studies in patients with gastrointestinal symptoms, including children, are needed to validate the range of faecal biomarkers fully before their clinical use expands [64].

### Luminal nitric oxide

Nitric oxide (NO) production in the gut mucosa maintains good perfusion, regulates microvascular and epithelial permeability and the immune response [68]. The source of NO gas is probably epithelial cells rather than neutrophils. Rectal NO concentrations are increased in adults and children with gastrointestinal but not systemic inflammation, but NO is not specific for IBD. A review of novel markers of IBD showed that luminal NO is greatly increased in microscopic colitis which may be overlooked on histological examination. Faecal calprotectin and lactoferrin concentrations and luminal NO concentrations distinguished between IBD and IBS and could be useful as a screening test in primary care. Low concentrations of these biomarkers in the presence of inflammatory bowel symptoms would almost certainly rule out IBD [17, 68].

A study in 2007 found a significant increase in both luminal NO and faecal calprotectin in patients with IBD compared with healthy controls ( $p < 0.001$ ). However, there was no correlation between the NO and calprotectin results, suggesting that they may reflect different parts of the inflammatory process [68].

## Conclusion

Currently there is insufficient evidence available to consider luminal NO for reliable discrimination between IBD and IBS, but it might be useful in cases of microscopic colitis.

## White cell scans and faecal excretion

These procedures expose the patient to radiation and are expensive. They involve the return of  $^{111}\text{Indium}$  or  $^{99}\text{Technetium}$  ( $^{99}\text{Tc}$ ) labelled autologous white cells to the patient where the labelled white cells migrate to areas of inflammation, and can be located by scanning or detected in the faeces. Faecal  $^{99}\text{Tc}$  measurement does not provide quantitative information on bowel inflammation as the label dissociates from the white cells after about 4 hours and is excreted in faeces independently of white cells. However,  $^{99}\text{Tc}$  does give superior abdominal scanning results.

## Labelled granulocyte excretion

$^{111}\text{Indium}$  labelled granulocyte scanning/excretion is an established measure of intestinal inflammation providing an assessment of intestinal function rather than structure. The required four day faecal collection is demanding and unpleasant. Faecal calprotectin concentration in a single sample correlated well with four day excretion of  $^{111}\text{Indium}$  labelled granulocytes ( $r = 0.70$ ) and with four day calprotectin excretion ( $r = 0.85$ ). Faecal calprotectin measurement in a single sample is a relatively cheap, easy test to perform without added risk to the patient [29].

## White cell scanning

In a study involving children suffering from small or large bowel inflammation, faecal calprotectin concentrations showed a significant positive correlation with  $^{99}\text{TC}$  labelled white cell scan scores ( $r = 0.73-0.80$ ,  $p < 0.01-0.001$ ). It was concluded that measurement of faecal calprotectin could identify children with active IBD but it could not replace the use of invasive tests to investigate complications and identify disease distribution [38].

## Conclusion

Faecal calprotectin is as good at identifying IBD as tests involving radioactive labels, but is not able to provide information on disease distribution. As a biomarker it has the obvious advantage of avoiding exposure of patients to radioactivity.

## Intestinal permeability

Intestinal permeability is another established test of intestinal function. A ratio of the excretion of a disaccharide to a monosaccharide, specifically ingested for the test, is calculated. The test is time consuming and lacking in specificity; results can be abnormal in a variety of small intestinal diseases [69]. Faecal calprotectin concentration was a better marker for organic intestinal disease than intestinal permeability ratio, but a combination of positive Rome criteria, normal intestinal permeability and faecal calprotectin results increased the OR for identifying patients with IBS from 13.3 to 46 (table 9) [5].

A study using  $^{51}\text{CrEDTA}$  to measure intestinal permeability found good correlation between intestinal permeability and gut lavage calprotectin concentrations ( $r=0.79$   $p<0.0001$ ). Both tests gave higher results in IBD than functional conditions. Increased intestinal permeability might be partly a consequence of increased transepithelial migration of neutrophils [70].

Proximal gastrointestinal and colonic permeability and faecal calprotectin were increased in children with functional abdominal pain and irritable bowel syndrome (FAP/IBS). The calprotectin concentrations correlated significantly with the 'pain interfering with activity' rating [32].

In a study looking at diarrhoea and constipation, predominant subgroups of IBS patients, there was an increase in small bowel permeability only in the diarrhoea predominant group. Faecal calprotectin concentrations were not significantly different in these groups and there was no correlation between faecal calprotectin concentrations and intestinal permeability [71].

### **Information on intestinal permeability without comparison with calprotectin**

Gut permeability changes in CD, UC and IBS. Reviews have concluded the change in gut permeability in CD may be important in the initiation of the disease. It has not been determined whether increased gut permeability is involved in the mechanism of IBS [72-74].

Some patients (5-30%) with acute gastroenteritis progress to having chronic gastrointestinal symptoms when free from the infectious agent and are classified as suffering from post infection IBS (PI IBS). The risk of developing PI IBS may increase with the severity of the gastroenteritis, but there may also be a subset of IBS patients who are more susceptible to inflammatory stimuli. Increase in intestinal permeability may result in low level neuromuscular inflammation causing disturbed gut sensations and motility [75, 76]. However, a large study in a local population following a waterborne outbreak of acute gastroenteritis showed that symptoms of IBS were

associated with a small increase in intestinal permeability irrespective of previous attack of gastroenteritis [76].

A variety of inflammatory conditions and drugs can alter gut permeability. Intestinal permeability measurements cannot distinguish IBS from healthy controls nor post infection from non-post infection IBS. However, they may be helpful in indicating the site of intestinal inflammation [5, 71, 74].

### Conclusion

Intestinal permeability testing is not suitable for ruling out the presence of IBS routinely. Measurement of faecal calprotectin is less complex and provides more definitive results. Combination of both tests with Rome criteria gave good IBS identification.

### Faecal M2-pyruvate kinase

Pyruvate kinase is a key enzyme in glycolysis and is present in all cells. M2-pyruvate kinase (M2-PK) is a form of the enzyme present in rapidly dividing cells. It has been measured in serum and faeces and is raised in patients with various different cancers. In a study comparing measurements of faecal M2-PK and calprotectin in patients with lower gastrointestinal symptoms, calprotectin performed better in the detection of organic bowel disease (table 24), but it should be noted that the faecal calprotectin cutoff was lower than the usual 50 µg/g cutoff. The authors suggested that faecal M2-PK may have a role as part of a panel of biomarkers used in the evaluation of patients with bowel symptoms [43]. M2-pyruvate kinase measurements in children were able to distinguish IBD from healthy controls. At a cutoff of 5 U/g there were 2.9% false positives in the control group and 70.8% of the IBD patients were positive [77].

**Table 24. Test parameters for organic gastrointestinal disease [43]**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+
Faecal M2-PK >3.7 U/mL	73	74	89	57	4.57
Faecal calprotectin >25 µg/g	80	74	87	65	13.97

### Conclusion

There is insufficient evidence published on the ability of M2-PK to distinguish between organic and functional bowel disease to fully assess its performance. Currently it appears that calprotectin performs better than M2-PK as a faecal biomarker.

## Breath tests

These are used to detect bacterial overgrowth or the presence of *Helicobacter pylori* but they lack accuracy and are not recommended for routine clinical practice [15].

## Anti-endomysial and anti-transglutaminase antibodies

These are recognised tests for the presence of coeliac disease. Four percent of patients with IBS have coeliac disease; therefore these tests should be performed to identify such patients and start the appropriate treatment [15].

## Biomarkers currently playing a minor role in IBD/IBS discrimination

Studies into the following biomarkers in blood have largely focussed on understanding the pathogenesis of IBD and/or IBS, but in due course some of them may become useful markers of either disease for use in non-invasive tests:

- allele and genotype frequencies for interleukin 10 [78]
- tumour necrosis factor alpha (TNF $\alpha$ ) [79]
- transforming growth factor  $\beta_1$  (TGF- $\beta_1$ ) [80]
- human defensin  $\beta$ -2 (HBD-2) [81]
- faecal serine-protease activity [82].

## Operational issues

### Biological variability

There is a subgroup of people who have labile faecal calprotectin concentrations with greater day to day variation, sometimes exceeding the recommended cutoff concentration. This makes interpretation of a single calprotectin measurement less certain if the result is close to the cutoff point [83].

### Distribution in faeces

Studies of the faecal calprotectin concentrations from multiple spot samples taken from one faecal sample concluded that the variability in calprotectin distribution in the faeces had an insignificant effect on the clinical utility of the test [4, 84-86]. Calprotectin concentrations from single faecal samples have been shown to correlate with those from corresponding four day faecal collections with only a slight compromise in sensitivity and reproducibility. The single spot collection makes the test more acceptable for widespread routine use [29, 87].

## Faecal matrix

Hard stools had significantly higher calprotectin concentrations than soft stools in a randomly selected population of 50-70 year olds without known IBD. However, all mean concentrations were below the normal cutoff of 50 µg/g [88].

In one study two small or aqueous samples gave very different faecal calprotectin results by ELISA and rapid test methods which was possibly due to a variation in the amount of sample normally collected for the rapid test. It was suggested that all small or aqueous samples should be measured by ELISA as these results were not dependent on the faecal consistency [55].

## Interference

No dietary restrictions are necessary prior to collecting a sample for faecal calprotectin measurement by the PhiCal test since no interferants in the assay were identified from the most likely substances [85]. However, taking non-steroidal anti-inflammatory drugs can result in enteropathy and thus raised faecal calprotectin concentrations can be present in patients on NSAIDs who may have a normal colonoscopy [89]. It has been recommended to avoid NSAIDs for a period of several weeks before measuring faecal calprotectin [35].

## Blood loss

Blood loss into the faeces could theoretically result in raised concentrations of faecal calprotectin without the presence of inflammation. It has been calculated that blood losses of 50-100 mL/day could provide faecal calprotectin concentrations of 25-30 µg/g [23, 90]. Others have estimated that it would require loss of 300 mL blood/day to result in a median faecal calprotectin of 175 µg/g [49].

## Stability

Calprotectin is resistant to proteolytic degradation in faeces; therefore faecal samples for calprotectin measurement can be stored at room temperature. The period of storage without loss of calprotectin varied from 3 to 7 days in different studies [4, 18, 91, 92]. Other biomarkers (PMN-e, MPO and lactoferrin) may be stable in faeces for up to 4 days [40], but there has been some disagreement on stability at room temperature [24]. S100A12 was stable in faeces for 7 days, similar to calprotectin [66].

## Compliance with sample collection

Some doubt has been expressed as to whether patients would be willing to collect faecal samples. However, collection of three samples for FOB estimation had an average compliance of 63% (range 35-92%); the compliance for a single sample collection required for faecal calprotectin may be better than this [49]. Outpatient compliance with faecal collection was found to be good, 95% of patients found the

collection straightforward and were willing to make further collections [31]. There was 100% compliance in faecal sample collection in a group of children suffering from symptoms of bowel inflammation [38].

### Analysis time

Analysis time varies between quantitative and semi-quantitative methods. An ELISA may take 3-4 hours, but multiple results may be obtained; a single microtitre plates can provide about 40 patient results. In contrast, the rapid tests may take only 3-10 minutes, but only a single result is provided by each device; however, this might suit a GP surgery or gastroenterology outpatient clinic situation very well [24, 55].

### Normal ranges

The range of calprotectin concentrations is similar in healthy adults and children, but infants in their first year of life have higher faecal calprotectin concentrations. Gathered data gave a range of median faecal calprotectin concentrations for adults, children and infants (table 25). The ranges of results around these medians can be quite large [19, 93]. Normal ranges in children and adults need to be properly established for the most effective use of the test [7].

**Table 25. Faecal calprotectin concentrations in healthy controls [19]**

No. of studies	No. of healthy controls	Age	Medians ( $\mu\text{g/g}$ )	Limits of ranges ( $\mu\text{g/g}$ )
14	9-320	Adults	9.3-58	4-897
7	15-77	Children	11-49	6-176
6	11-69	Infants (1-12 weeks)	150-278	22-2880

In adults, faecal calprotectin concentrations have been shown to increase with age. However, the increase seen in cases of IBD is much greater than that seen with increasing age alone [68, 88].

### Operational/technical advantages and disadvantages of faecal calprotectin measurement as a marker for inflammation

Advantages:

- it has good stability in faeces at room temperature
- it can be quantified by an inexpensive ELISA test
- only 5 mg faeces is required, so the patient can collect and send the sample to the laboratory where it can be stored frozen
- variability in calprotectin distribution in faeces does not affect the clinical usefulness of the test

- there are rapid tests available requiring no special equipment to perform which can be used outside the laboratory.

Potential disadvantages:

- NSAIDs should not be taken for several weeks before calprotectin measurement
- blood loss into the gut could increase faecal calprotectin concentration. Sample should not be collected during menstruation or around the time of a nose bleed to avoid contamination of the faecal sample.
- unacceptability of collecting faecal samples, but this may be declining since recent studies have shown good compliance.

## Technical performance of laboratory and point of care tests

The ideal test should be:

- simple
- highly sensitive and specific
- reliable
- inexpensive and cost effective
- safe
- non-invasive
- convenient
- acceptable to the patient
- objective
- easy to implement
- amenable to serial measurements [38, 94].

Calprotectin is stable in faeces, directly linked with inflammation and easy to measure, making it a good biomarker for inflammation. Calprotectin measurement is non-invasive, safe, gives good sensitivity and specificity and can be measured by laboratory and POC tests.

### Laboratory based tests - quantitative enzyme linked immunosorbent assays

- Calprest (formerly PhiCal)
- Bulhmann ELISA
- Immunodiagnostik ELISA

### Point of care tests – semi-quantitative/qualitative lateral flow immunochromatographic tests

- PreventID CalDetect, Alpha Laboratories

- Quantum blue calprotectin rapid test (has an electronic reader), Buhlmann Laboratories AG and Alpha Laboratories
- Semi-quantitative rapid test, Prevista GmbH & Co KG.

### Quantitative methods

The PhiCal calprotectin method uses a polyclonal initial antibody and measures enzyme product by optical density reading at 405 nm [84, 85]. The Buhlmann assay uses a monoclonal initial antibody and measures enzyme product by optical density reading at 450 nm [47]. The advantage of a monoclonal initial antibody is that it will always bind one specific part of the calprotectin molecule and controlled production is possible from a specific clone of cells.

### Manufacturers' performance data

Table 26. Manufacturer's performance data for quantitative tests

Assay	Intra-batch CV (%)	Inter-batch CV (%)	Spiking recovery (%)	Dilution linearity recovery (%)	Limit of detection
Buhlmann calprotectin	2.7-8.1	3.0-4.6	97-103	88-124	
PhiCal calprotectin	7.6-10.8	7.5-10.3	99.9-100	90.4-135	2.8 ng/mL
Immunodiagnostik calprotectin	4.2-9.8	6.0-16.6	103-118	93-109	2.9 ng/mL

Calprotectin assays from different manufacturers have similar performance (table 26).

### Performance data from published literature

A study in 2008 compared two ELISAs (PhiCal and Buhlmann calprotectin ELISA) on samples from a group of patients with UC or CD and a group of healthy controls. Both tests showed adequate accuracy and similar imprecision (table 27).

Table 27. Comparison of Bulmann and PhiCal ELISAs for calprotectin [47]

Intra-assay CV	Manufacturer	Positive sample	Negative sample
	Buhlmann	6.6	5.4
	PhiCal	4.1	6.9
Inter-assay CV	Manufacturer	Positive sample	Negative sample
	Buhlmann	9.3	1.7
	PhiCal	8.9	3.6

The Buhlmann assay had better imprecision than PhiCal at low calprotectin concentrations, but the reverse was true at higher calprotectin concentrations. The

Buhlmann assay had better linearity than the PhiCal assay especially at higher calprotectin concentrations [47].

A method based on that of Roseth gave an intra- and inter-assay variation of 2% and 15% respectively at a cutoff of 10mg/L (equivalent to 50 µg/g) (table 28) [5, 29, 92].

The limit of detection (LOD) found by Wassell [37] was lower than the 15.6 µg/g quoted by the manufacturer, but no information was provided by the manufacturer on how this figure was obtained. A second study by the same group reported slightly higher imprecision, but a lower LOD (table 28) [4].

**Table 28. Published performance of calprotectin assays**

Assay	Intra-batch CV (%)	Inter-batch CV (%)	LOD* (µg/g)	Correlation (other method)	Reference
Roseth method ELISA	2	15			[5], [29]
TRFIA	2.1-8.3	7.0-12.1		r = 0.96 (Calprest)	[23]
PhiCal		<10	20		[55]
Calprest	1.4-4.5 <sup>†</sup>	11.2-21.2 <sup>†</sup>	6.5		[37]
Calprest	2.8-6.9 <sup>†</sup>	14-19 <sup>†</sup>	4.4		[4]
Calprest	3.1*	10*			[35]
Calprest	6	7			[44]
Calprest	1.9	14.8			[88]

\* LOD was calculated as the mean of the zero standard + 3 x SD of repeat measurements

<sup>†</sup> Coefficients of variation (CVs) were calculated at calprotectin concentrations of 24-76 µg/g

The results in table 28 agree with the statement in a review that previous and present calprotectin tests have intra-assay CVs of <5% and inter-assay variation of 10-40% [19].

For comparison D'Inca *et al* found an intra-batch CV of 8% and inter-batch CV of 10% for a lactoferrin ELISA [44] and de Jong found S100A12 imprecision to be a little higher: intra-batch CV 9.6% and interbatch CV 29.1% by an in-house method [66].

### Conclusion

Different methods have similar imprecision performance and generally agree with that stated by their manufacturers. The same method in different hands also has a fairly consistent performance indicating a reasonably robust test. Two other neutrophil product biomarkers gave slightly higher intra-batch CVs and one gave a

much higher inter-batch CV. Performance appears to compare well with imprecision found with ELISAs for other analytes.

## **Semi-quantitative and qualitative methods**

### ***Manufacturers' performance data***

Bulhmann Quantum Blue rapid test correlates with the Bulhmann calprotectin ELISA ( $r=0.93$ ). No performance data are provided on the Prevent ID CalDetect as it is a semi-quantitative test. No data were found on the Prevista test.

### ***Performance data from published literature***

Preventis Prevent ID CalDetect semi-quantitative rapid test results for faecal calprotectin correlated well with PhiCal quantitative ELISAs results ( $\kappa=0.69$ ). The rapid test had comparable diagnostic performance to the quantitative more expensive and time consuming ELISA test and should be suitable for use in primary care provided adequate instruction is provided (table 5) [54].

Another study of Prevent ID performance showed considerable imprecision in the measurement of three samples repeatedly over five days, but the samples all had ELISA results greater than  $60 \mu\text{g/g}$  which was a poor sample for testing the four result categories (negative,  $<15 \mu\text{g/g}$ ,  $15\text{-}60 \mu\text{g/g}$  and  $60\mu\text{g/g}$ ) of this semi-quantitative test [55].

A different qualitative faecal calprotectin rapid test (Prevista GmbH) was compared with an ELISA (Bulhmann Laboratories); the results of the two methods correlated significantly ( $r=0.862$ ,  $p<0.001$ ). The rapid test was said to be a suitable alternative for the ELISA. Both tests used a cutoff of  $50 \mu\text{g/g}$  [24].

## **Conclusion**

The results from available rapid tests appear to correlate quite well with ELISA results and the tests have been proposed as suitable for use in primary care.

## **Patient pathway**

A patient suffering from persistent gastrointestinal symptoms will first visit their GP. In the absence of 'red flag symptoms', the ideal response for the patient would be that the GP could instigate the necessary tests to enable a diagnosis of IBS or organic bowel disease to be made.

Organic bowel disease would need further investigation in secondary care and would be governed by the change in bowel habit 18 week pathway. The patient would undergo various additional tests some of which might be invasive and not without risk.

In contrast, a diagnosis of IBS made reliably from the tests requested in primary care could result in the patient being started on appropriate treatment as soon as the initial test results clarified the diagnosis. The only reason for referral to secondary care would be for treatment such as psychological therapies or dietary advice. Such rapid and reassuring local support could benefit the patient significantly and may reduce the duration of their disease and therefore the attendant healthcare costs.

Removing cases of IBS from the 18 week pathway for change in bowel habit should improve access to appropriate diagnostic investigations for patients with IBD and minimise the number of unnecessary investigations carried out in secondary care, particularly endoscopy which is relatively expensive.

## Cost information

In the USA it is estimated that IBS patients cost about \$25 billion annually due to the numerous investigations performed and days off work [17]. In 1992 the excess cost of diagnosing IBS in the USA was estimated as \$8 billion.

If measurement of faecal calprotectin is to be used as a screening tool it needs to be practical and cost-efficient. In one study children had high compliance with non-invasive tests. The results were available within 24 hours. The cost of measuring faecal calprotectin, serum ASCA, serum pANCA and bowel wall ultrasonography was about \$150 (US) which compared favourably with invasive investigations [34].

A study of patients with lower gastrointestinal symptoms suggested that use of a combination of non-invasive faecal biomarkers and Rome criteria could identify a significant proportion of patients as having inactive IBD or IBS and thus avoid them undergoing endoscopy. Endoscopy should be reserved, it was thought, for those with raised faecal biomarkers. This would be cost beneficial for endoscopy services as up to 40% of new gastroenterology referrals are for suspected IBS. At the 2007 costs of about £40 for a faecal biomarker test and £550 for a colonoscopy the faecal biomarker would be economically advantageous if it could reliably rule out the need for a colonoscopy [43].

The costing report on implementing NICE guidance on IBS highlights that treatment and therefore some of the cost of the disorder occurs within primary care. The assumption is made that the patient may be referred to secondary care if the symptoms are atypical. The authors of the costing report have calculated an increase in spend of £225,000 if all patients are tested for FBC, ESR, CRP and EMA/TTG (the antibodies specific to coeliac disease). However, they have also calculated that £6,970,000 can be saved from a great reduction in the number of imaging procedures, thyroid, parasite, FOBT and breath tests, down from 4-36% to 1-13% of the incidence population. The overall saving would therefore be £6,744,000 [3]. If the ESR (cost £3.04) and CRP (cost £1.60) tests were replaced by for example

calprotectin (approximate costs, kit only: laboratory £10, POCT £15), the direct savings would be reduced (maximum £6,376,000), but the diagnosis and start of appropriate treatment might be quicker, with clear benefits to the patient.

A study on the use of inflammatory markers and their cost effectiveness which looked at ESR, white blood cell count (WBC), CRP, sialic acid and protein fractionation in patients with inflammation-related symptoms, not including IBD, concluded that the single most cost effective test was CRP. However, this was only effective in 53% of patients; inclusion of sialic acid or WBC increased this to 60% or 72% respectively. If all three of the above tests were used they were effective in 78% of patients [3, 95].

In relation to IBD these findings suggest that calprotectin might be a good marker to measure as it has been shown to perform better than CRP, ESR and FBC, but data on average calprotectin cost per test are needed to make this assessment. The cost of a faecal calprotectin test is currently about £10-12 per patient for the reagents alone whereas the unit costs for FBC, ESR and CRP are £3.04, £3.04 and £1.60 respectively [3]. A study on the cost effectiveness of faecal calprotectin measurement is needed.

An economic modelling study assessed different diagnostic pathways for a hypothetical cohort of patients with symptoms suggestive of IBD in a decision analysis model. Blood concentrations of ASCA and pANCA and the 'gold standard' invasive endoscopy tests (barium upper gastrointestinal tract series, small bowel follow through and colonoscopy) were included. Primary (high NPV) and confirmatory (high PPV) blood tests were used which had different cutoff concentrations. The authors found that the largest cost savings (\$550/patient) occurred with the use of sequential blood tests, primary test followed by confirmatory measurements for positive samples from the primary test. These savings were due to a 39% reduction in the number of standard invasive tests (average \$2188/test) needed. Sensitivity and threshold analysis demonstrated the robustness of these results as the sequential blood testing remained the most cost effective method until the invasive test costs decreased by 80%, the IBD prevalence was greater than 83% or the incidence of patients with non-IBD returning with persistent symptoms was >89%. The authors stated that the assumptions of this model needed to be tested in a clinical prospective trial which included the viability of performing the blood tests in primary care [96].

It is harder to know if faecal calprotectin measurement could replace ASCA and pANCA in this study and the conclusions remain unchanged. Calprotectin has been shown to perform better in screening those with IBD symptoms but it is recognised that its specificity for IBD is not as high as that for these two antibodies, which are good at distinguishing CD and UC.

Another economic modelling study on the cost effectiveness of endoscopy in the diagnosis of IBS concluded that these costly procedures should be scheduled at the end of the diagnostic workup pathway as they can be responsible for 50-75% of the total diagnostic costs. The diagnosis of IBS can be established with a relatively high probability (80%) with just 4 relatively inexpensive, non-invasive tests: history and physical examination, laboratory tests, hydrogen breath test and small bowel follow through. The laboratory tests included FOB, ESR, general laboratory panel with FBC and stool investigations for infections and infestations but not faecal calprotectin. Endoscopy should be used when serious organic disease is reasonably likely and needs to be ruled out [9]. These conclusions are similar to those of Dubinsky [96] that the simpler cheaper tests should be used initially to screen out those who do not need endoscopic investigation. Calprotectin could have a role as such a screening out test and might replace some of those undertaken by Suleiman and Sonnenburg [9].

Suppliers of one calprotectin ELISA have calculated that use of faecal calprotectin measurement as a primary screen to distinguish IBD and IBS in those with gastrointestinal symptoms could save the average district general hospital £364,000/yr (2008 costs) if a negative calprotectin rate of 40% is assumed [97].

## **Conclusion**

Costs of diagnosis of a prevalent condition such as IBS can be high as are the costs of the continuing disease. It is therefore important to diagnose IBS as cost effectively and quickly as possible and start patients on the most effective treatment. The cost of faecal biomarker testing compares very favourably with that of colonoscopy.

Savings can be made in the diagnostic assessment of IBS/IBD. There is agreement that it is best to use cheaper tests initially to reduce the number of colonoscopies required in the final stages of diagnosis of IBD. Measurement of faecal calprotectin, with or without other biomarkers, could be useful at this initial stage as it provides better discrimination between IBS and IBD than the tests (ESR and CRP) mentioned in the published costing and economic studies and might therefore further reduce the number of colonoscopies required.

## Objectives

The economic analysis aimed to evaluate the cost-effectiveness of faecal calprotectin in comparison with other diagnostic tests for the detection of inflammation in patients who present with IBS in primary care. The analysis included clinical and economic data in order to compare the costs and effectiveness of different diagnostic tests.

## Methods

The economic analysis used a decision tree approach in order to model the diagnostic pathway for a cohort of 1,000 hypothetical patients who present with suspected IBS. The model was developed in Microsoft Excel, and generates the expected outcomes and costs for the patient cohort, by taking into account the number of patients on each of the pathways in the decision tree.

The comparisons made in the analysis are shown in table 29. The baseline analysis compares calprotectin alone with ESR and CRP, *ie* the tests currently used to detect inflammation.

**Table 29. Comparisons for analysis**

Comparison	Strategy A	Strategy B
1	Calprotectin alone	ESR + CRP
2	Laboratory calprotectin	Point-of-care calprotectin

The analysis was conducted from the perspective of the payer, *ie* the NHS. The time horizon of the model comprised the time taken to reach a confirmed diagnosis of either IBD or IBS. Due to the short time horizon, discounting of costs and benefits was not undertaken.

The patient population considered in the model comprised patients presenting to the GP in primary care with lower gastrointestinal symptoms, suggestive of IBS, as indicated by Rome criteria. ‘Red flag’ alarm symptoms of more serious bowel disease were considered to be absent, and patients of 45 years and above were excluded, since such patients would follow a different pathway from those specified in the model.

The model structure was validated by clinical experts to ensure that current clinical practice was accurately represented. The diagnostic pathway for patients presenting with IBS symptoms involves diagnostic tests to determine whether they have inflammation of the bowel. The tests for inflammation will return either positive or negative results:

- for the patients who have a positive test result, indicating inflammation, the model assumes that this would be investigated further by endoscopy, results of which will then confirm inflammation (IBD true positives) or not (IBD false positives)
- patients who have a negative test result (ie normal results) are managed for IBS. The first level of IBS management involves following dietary and lifestyle advice. For those who find this unsuccessful for adequately controlling their symptoms, a second level of IBS management is provided using medication. Different paths can be taken by the patients, dependent on whether they are able to achieve adequate control of symptoms through the different forms of IBS management. Where symptoms are inadequately controlled, further investigations are carried out. Hence the associated costs and outcomes differ for the true negative and false negative patients followed in the model.

Full details of the model structure can be seen in figure 1. A separate decision tree was developed for each of the test strategies, that is, for calprotectin, ESR plus CRP, laboratory calprotectin and point-of-care calprotectin.

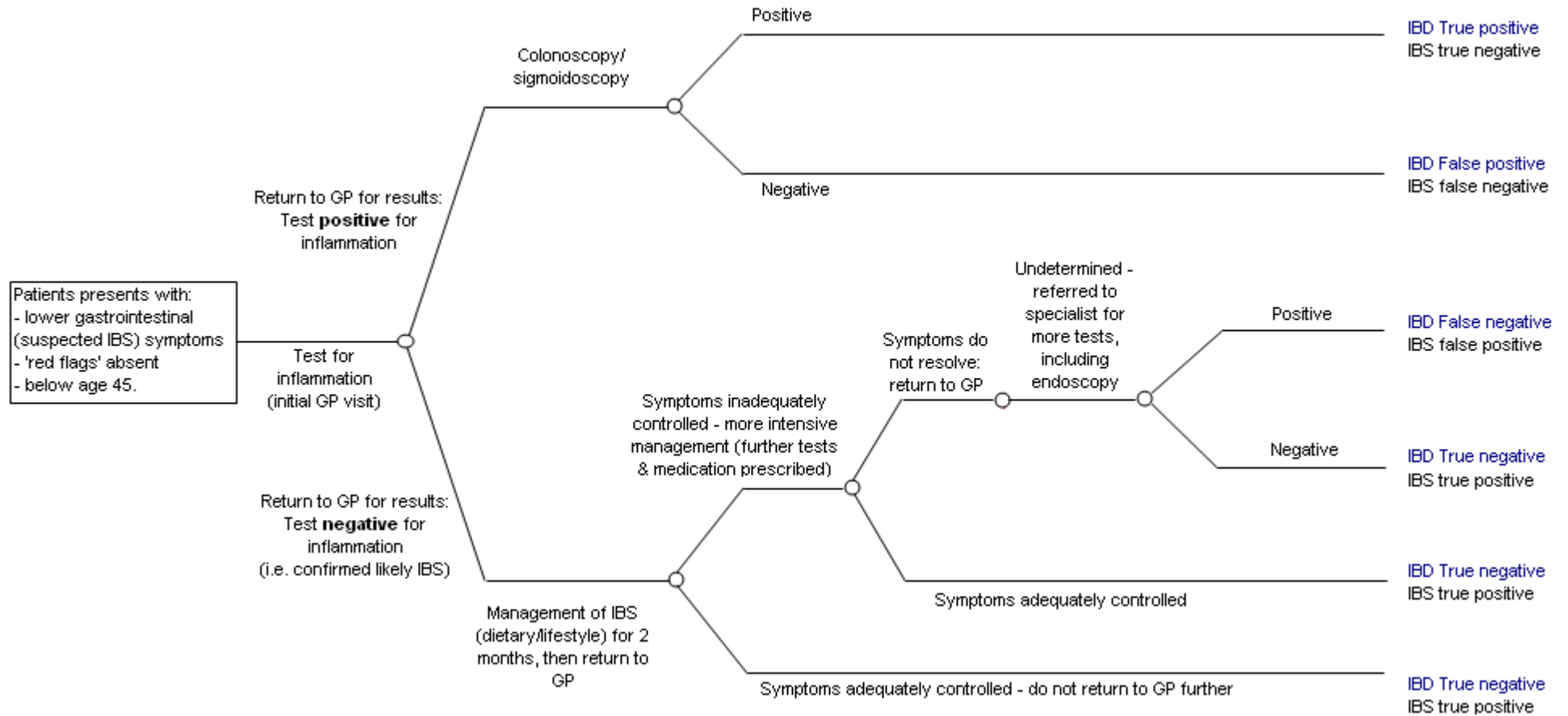
## Costs

Since the analysis was taken from the perspective of the NHS, the costs included in the model were limited to direct costs. All costs were evaluated in 2008/09 pounds (£). For each test strategy (eg ESR + CRP), total costs were calculated by assigning costs to the corresponding numbers of patients who were at the end of each pathway in the model. The relevant costs comprised the costs of the tests and endoscopic procedures, medication and the associated resource use, such as GP visits. The total costs include the costs associated with the different pathways followed by patients in order to reach a correct diagnosis, using endoscopy as necessary.

## Outcomes

The outcomes included in the analysis comprise the number of correctly diagnosed cases of IBS and the numbers of correctly diagnosed cases of IBD from the initial use of each diagnostic method. For each comparison, the total numbers of correctly diagnosed IBS and IBD cases were calculated for both strategies under consideration and compared to demonstrate the associated incrementals. As the model followed each patient through to a final correct diagnosis (assuming endoscopy to be the gold standard) the initial success rates act as a proxy for the clinical benefit of faster diagnosis and institution of therapy.

Figure 1. Model structure



## Economic evaluation

Cost-effectiveness models are used to assess the relative benefits of a given intervention or strategy using patient outcomes and the costs incurred in achieving those outcomes. The additional cost per unit of benefit gained is of key interest to policy and decision makers. This is known as incremental analysis; results are presented as incremental cost-effectiveness ratios (ICERs).

The ICER for comparing two testing strategies can be calculated using the formula below, which divides the difference in the costs of the two strategies by the difference in the effectiveness of the strategies:

$$ICER = \frac{Cost_{Strategy1} - Cost_{Strategy2}}{Effect_{Strategy1} - Effect_{Strategy2}}$$

The model generates the total expected costs and effectiveness in order to estimate incremental cost-effectiveness ratios using the number of correctly identified IBS patients and the number of correctly identified IBD patients, ie the incremental cost per correctly diagnosed IBS patient and the incremental cost per correctly diagnosed IBD patient.

## Data

The data used in the model were sourced from published studies where possible, identified from the literature review. Expert clinical opinion was sought in order to validate the model structure and to provide estimates where the necessary data were absent. The final model structure therefore reflects the views of clinical experts and the availability of good quality data.

## Accuracy of tests

For each comparison, the accuracy values for both strategies were drawn from the same study (table 30). This was to ensure that the specificities and sensitivities were calculated using the same patient population and study conditions in order to minimise bias which would arise from combining diagnostic accuracy values from different studies. The model assumed that the endoscopic procedures were 100% accurate.

**Table 30. Diagnostic accuracy values used in model**

Test	Sensitivity (%)	Specificity (%)	Reference
Calprotectin	90	80	Tibble <i>et al</i> 2002 <sup>a</sup> [5]
ESR + CRP	35	73	Tibble <i>et al</i> 2002 <sup>a</sup> [5]
Calprotectin (Lab)	96	87	Otten <i>et al</i> 2008 [54]
Calprotectin (POC)	61	98	Otten <i>et al</i> 2008 [54]

<sup>a</sup> For a subset of patients who were positive for Rome criteria and negative for 'red flags'.

### **Resource use pathway data**

The literature helped to guide the pathways and resource use included in the model. More detailed information on current resource use patterns in the NHS was sought from clinical experts, such as the proportion of patients who would have adequate control of symptoms following dietary and lifestyle IBS management.

### **Cost data**

Cost data were obtained from standard national sources, such as the NHS National Tariff [98] and the British National Formulary [99], in the first instance. Table 31 provides a summary of the cost data used in the model.

**Table 31. Costs used in the model**

Test/Resource	Cost	Reference
Calprotectin (Lab) <sup>a</sup>	£25.00	KingsPath (Buhlmann ELISA) [100]
Calprotectin (POC) <sup>a</sup>	£27.68	Alpha Labs (CalDetect kit) [101]; PSSRU Unit Costs (staff costs <sup>b</sup> ) [103]
CRP	£1.60	Indicative tariff [102]
ESR	£3.04	Indicative tariff [102]
Colonoscopy	£544.45	National tariff [98]
Sigmoidoscopy	£365.59	National tariff [98]
GP visit	£36.00	PSSRU Unit Costs [103]
Specialist visit	£188.81	National tariff [98]
IBS medication (mebeverine, 100-tablet pack)	£9.43	British National Formulary [99]

<sup>a</sup> The calprotectin test cost comprises the test kit, labour and overheads; <sup>b</sup> nurse consultation cost of £11 was included.

### **Prevalence data**

Data on the prevalence of IBS and IBD patients in primary care have not been identified. The majority of studies relating to such patients are based in secondary care, where the patients will tend to have more severe symptoms than those of patients presenting in primary care. Therefore, prevalence data were derived from

clinical expert opinion; 90% of the patient population had IBS and the remaining 10% had IBD.

## Results

### Base case analysis (comparison 1)

For the comparison of calprotectin alone against ESR plus CRP, calprotectin was found to be the dominant diagnostic strategy for all outcomes explored in the analysis. The use of calprotectin compared with ESR plus CRP resulted in an additional 63 correctly diagnosed IBS cases and an additional 55 correctly diagnosed IBD cases, at cost saving of £13,464 for the hypothetical cohort of 1,000 patients (tables 32 and 33). The calprotectin strategy is therefore considered to be cost-saving.

**Table 32. Results of calprotectin versus ESR + CRP**

Test strategy	Correctly diagnosed IBS cases	Correctly diagnosed IBD cases	Total costs
Calprotectin alone	720	90	£312,143
ESR + CRP	657	35	£325,606

**Table 33. Incremental analysis: calprotectin versus ESR + CRP**

Incremental cost	Additional correctly diagnosed IBS cases	Cost per correctly diagnosed IBS case	Additional correctly diagnosed IBD cases	Cost per correctly diagnosed IBD case
-£13,464	63	Dominant	55	Dominant

### Comparison 2

The use of laboratory-based calprotectin tests generated additional costs for the patient cohort compared with the use of point-of-care calprotectin tests. The laboratory-based calprotectin test generated additional correctly diagnosed IBD cases but resulted in fewer correctly diagnosed IBS cases, and hence additional unnecessary endoscopies. In terms of identifying IBS, point-of-care calprotectin testing was therefore the dominant strategy. The corresponding results are shown in tables 34 and 35.

**Table 34. Results of calprotectin (Lab) versus calprotectin (POC)**

Test strategy	Correctly diagnosed IBS cases	Correctly diagnosed IBD cases	Total costs
Calprotectin (Lab)	781	96	£281,663

Calprotectin (POC)	880	61	£245,496
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**Table 35. Incremental analysis: calprotectin (Lab) versus calprotectin (POC)**

Incremental cost	Additional correctly diagnosed IBS cases	Cost per correctly diagnosed IBS case	Additional correctly diagnosed IBD cases	Cost per correctly diagnosed IBD case
£36,167	-99	No benefit	35	£1,030

## Sensitivity Analysis

Sensitivity analysis was undertaken for the following parameters: diagnostic accuracy of the tests, cost of the tests, endoscopy cost and the prevalence of IBD/IBS. The base case results were shown to be most sensitive to the cost of endoscopy, the cost of calprotectin and the specificity and sensitivity of the tests. As the sensitivity of calprotectin was lowered and specificity was increased, additional cost savings were demonstrated. Where sensitivity was increased, at the expense of a loss in specificity, incremental costs were incurred.

## Conclusions

The analysis has evaluated the benefit of using calprotectin in terms of its potential for screening out more cases of IBS in primary care than other tests for inflammation, and therefore reducing the number of unnecessary referrals to secondary care for endoscopy.

The model has been developed to represent real clinical practice as far as is possible given the data that are available. Due to information being absent relating to certain points in the diagnostic pathway, assumptions have been made which may simplify the situation that occurs in reality. These have been highlighted throughout the economic report and efforts have been made to take a conservative approach wherever possible. It must be remembered that any economic model should be regarded as a simplified representation of the real world, which aims to inform decision-makers about resource allocation by providing estimates of expected costs and outcomes for different strategies under evaluation, based on currently available data.

This analysis has demonstrated that faecal calprotectin is less costly and more effective, in terms of diagnostic accuracy, than the standard tests currently used for distinguishing IBS from IBD. The calprotectin testing strategy led to additional patients receiving the correct diagnosis for IBS and IBD, with fewer unnecessary endoscopies being undertaken. When comparing laboratory-based calprotectin with point-of-care calprotectin, point-of-care calprotectin was the dominant strategy for

identifying IBS, due to correctly identifying more IBS patients, avoiding unnecessary endoscopies and having lower total costs for the cohort.

The use of calprotectin for the detection of inflammation of the bowel would enable improved management of IBS patients in primary care due to it being possible for GPs to be more confident about their diagnosis by excluding the possibility of inflammation. This would reduce the need for referral to secondary care and therefore reduce health care resource utilisation and ultimately result in cost savings.

Measurement of faecal calprotectin has the potential to aid discrimination between IBD and IBS significantly. No published information on the cost effectiveness of faecal calprotectin measurement was found, therefore an economic analysis was carried out separately.

Faecal calprotectin is recognised as an informative biomarker, alone or in combination with others, in discriminating between organic and functional bowel disease. The optimum cutoff used in patients with gastrointestinal symptoms is 50 µg/g faeces. Views vary on the adequacy of the reported clinical performance of faecal calprotectin measurement for acceptable screening out of IBS.

Few studies have calculated likelihood ratios for faecal calprotectin measurements and only a minority of the available data indicate a high quality test for ruling in IBD or ruling out IBS. The test appears to provide clearer information in children than in adults. A further clinical study may be needed to clarify this.

The clinical performance of faecal calprotectin measurement is better than that of other tests reviewed. A normal faecal calprotectin result may be sufficient to make a diagnosis of IBS. If it is included in a panel of biomarkers to improve distinction of IBS from IBD, ideally all tests should be accessible from primary care. Negative results may be sufficient to rule out IBD and avoid colonoscopy, but positive results should always be investigated further, probably involving referral to secondary care.

## Key clinical findings

- Correlates with 'gold standard' tests for IBD, but with less risk to the patient.
- Concentration raised in organic, but not functional bowel disease.
- Most published NPVs are 71%-91% for patients referred with gastrointestinal symptoms.
- Most published LR data fail to designate the test as a good diagnostic tool.
- Semi-quantitative rapid tests can be used to rule out IBD, but confirmation of IBD requires quantitative measurement.
- Better indicator of bowel inflammation than blood tests
- Similar performance to faecal lactoferrin, PMN-e and S100A12.
- Inclusion in a panel of biomarkers and Rome criteria may improve the discrimination between IBS and IBD.

## Key technical findings

- Published ELISA performance matches manufacturers' quoted performance.
- Semi-quantitative rapid test results may be useful in primary care.
- Good sample stability at room temperature.

## **Key patient pathway effects**

- Availability of faecal calprotectin results should enable a diagnosis of IBS to be made in primary care.
- Use of POC tests in primary care could shorten the time to diagnosis, but this needs further studies.

## **Key costing findings**

- Diagnostic and social costs of IBS can be high.

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## Definition of statistical terms

### Sensitivity or true positive

The proportion of patients with the disease who are correctly identified by a positive test ( $a / a+c$ ).

### Specificity or true negative

The proportion of individuals without the disease who are correctly identified by a negative test ( $d / b+d$ ).

### Positive predictive value (PPV)

The proportion of patients with a positive test who are correctly diagnosed as having the disease or the probability that a patient with a positive test has the disease ( $a / a+b$ ).

### Negative predictive value (NPV)

The proportion of patients with a negative test who are correctly diagnosed as not having the disease or the probability that the patient is completely disease free ( $d / c+d$ ).

Positive and negative predictive values are influenced by the prevalence of the disease. A low prevalence of disease tends to lower the PPV and raise the NPV.

		Condition		
		Positive	Negative	
Test outcome	Positive	a True positive	b False positive	Positive predictive value
	Negative	c False negative	d True negative	Negative predictive value
		Sensitivity	Specificity	

### Likelihood ratio

The likelihood ratio combines sensitivity and specificity values. It is the probability of a given test result among people with a disease divided by the probability of that test result among people without the disease.

### Positive likelihood ratio (LR+)

The positive likelihood ratio indicates how much more likely a positive test result is in those with the target disease than in those without the target disease.

Positive likelihood ratio = sensitivity / (1- specificity).

LR+ >10 is a good rule-in test for diagnosis.

### **Negative likelihood ratio (LR-)**

The negative likelihood ratio indicates how much less likely a negative test result is in those with the target disease than in those without the target disease.

Negative likelihood ratio = (1-sensitivity)/specificity.

LR-< 0.1 is a good rule-out test for diagnosis.

### **Odds ratio**

The odds of having a disease are calculated, for a given population, as the number of patients with the disease divided by the number of patients without the disease.

An odds ratio is calculated by dividing the odds in the diseased group by the odds in the disease free (control) group. A result > 1 indicates that a positive test result is more likely in the disease group than in the control group,

### **Receiver operating characteristic (ROC) analysis**

The ROC curve can be used to select the best test for a particular diagnostic purpose. Sensitivity (true positive) is plotted against 1 – specificity (false positives) for a range of cutoff values. Tests with good performance curve upward and leftward giving a large area under the curve, whereas tests with poor performance tend towards the 45° diagonal, giving a small area under the curve. The optimum cutoff value is the point which falls closest to the top left hand corner of the graph (representing 100 % sensitivity and a false positive rate of zero). The advantage of the ROC curve is that performance is displayed across the whole range of potential diagnostic cutoffs.

### **Diagnostic accuracy**

This is characterized by the sensitivity and the specificity of the test.

Diagnostic accuracy = true positive + true negative / total outcomes

$$\text{Diagnostic accuracy} = \frac{a + d}{a + b + c + d}$$

**Cohen's kappa**

This measures the agreement between two means of assessment of the same thing. It could be two methods measuring the same analyte or two analyte results assessing the same disease state.

The equation for  $\kappa$  is:

$$\kappa = \frac{\text{Pr}(\tilde{a}) - \text{Pr}(e)}{1 - \text{Pr}(e)}$$

where 'Pr(a)' is the relative observed agreement between the two means of assessment and Pr(e) is the hypothetical probability of chance agreement.

If there is complete agreement then  $\kappa = 1$ , if there is no agreement then  $\kappa$  is  $< 0$ .

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
<b>Calprotectin only</b>					
Costa <i>et al</i> 2003 [20]	Prospective study. Calprotectin measured by ELISA (Calprest) in faecal samples from healthy controls and patients attending gastroenterology outpatient clinic; IBD (CD and UC), intestinal neoplasia, IBS disease activity was also assessed.	205	Calprotectin	Faecal calprotectin is a direct and sensitive biomarker for intestinal inflammation. The measurement is easy to perform, reliable and therefore suitable for use in initial discrimination between organic and functional disease.	None
Summerton <i>et al</i> 2002 [36]	Prospective study. Calprotectin measured by ELISA (PhiCal) in faecal samples from healthy controls and patients attending for routine endoscopy; upper gastrointestinal lesions, IBD, IBS, colonic disorders. Some pre and post endoscopy results; most were just post endoscopy.	30+116	Calprotectin	Faecal calprotectin concentration was raised in inflammation and cancer, but not in other gastrointestinal diseases studied including IBS. The test could be useful as a general screen for gastroenterology patients to identify IBD and cancer, but it cannot distinguish between these two diseases.	None
Tursi <i>et al</i> 2002 [50]	Case-control study. Calprotectin measured by semi-quantitative method (Caldetect) in faecal samples from healthy controls and patients with a new endoscopic diagnosis of diverticular disease or IBS. Comparison made with histological assessment.	48	Calprotectin	Faecal calprotectin test may be useful in distinguishing diverticular inflammation from IBS. Concentrations may be related to degree of diverticular disease and decrease after treatment.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Van der Sluijs Veer <i>et al</i> 2006 [23]	Prospective study. ELISA (Calprest) and in-house time resolved fluorimetric immunoassay (TRFIA) methods used on random faecal samples from healthy controls and patients with IBD, IBS and other intestinal diseases.	203	Calprotectin	TRFIA and Calprest identical in their ability to discriminate between IBD and non-organic gastrointestinal disorders. The extended measuring range of TRFIA reduced the number of samples with results outside the measuring range from 30% to 4%.	Minimal
Wassell <i>et al</i> 2004 [37]	Prospective study. Calprest measurement in random stool samples from healthy controls and patients with a diagnosis of CD or IBS.	50	Calprotectin	A single calprotectin measurement may assist in the differential diagnosis of CD and IBS. Its use could reduce the number of invasive investigations undertaken in assessing patients with possible IBS.	None
Husebye <i>et al</i> 2001 [83]	Prospective study. PhiCal measurement of 110 faecal samples from patients scheduled for colonoscopy, from 8 or 6 different days per patient. 5 patients were found to have an abnormality at colonoscopy.	14	Calprotectin	The biological day to day variation of faecal calprotectin concentration is marked in patients referred for colonoscopy without inflammation or cancer. This must be considered when interpreting a single result as an indicator of the presence of organic bowel disease.	None
<b>ESR CRP</b>					
Carroccio <i>et al</i> 2003 [35]	Prospective study. Calprest measurement of faecal samples from healthy controls and patients diagnosed with IBD or undergoing full assessment to identify the cause of chronic diarrhoea.	70 adult 50 child	Calprotectin ESR CRP	Faecal calprotectin is an accurate marker of IBD in children and adults with higher diagnostic accuracy in children. False negative results occurred in those with coeliac disease and false positives in patients with liver cirrhosis or users of NSAIDs.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Dolwani <i>et al</i> 2004 [4]	Prospective study. Calprest measurement of single faecal samples from healthy controls and patients undergoing barium follow through for investigation of diarrhoea and or abdominal pain.	73	Calprotectin ESR CRP	A single faecal calprotectin result of <60 µg/g would exclude the presence of organic gastrointestinal disease and therefore remove the need for a barium follow through test. Faecal calprotectin is a more accurate measure of intestinal inflammation than ESR or CRP.	None
Eder <i>et al</i> 2008 [33]	Prospective study. Immunodiagnostik calprotectin ELISA measurement of single samples from controls who had had a diagnosis of organic bowel disease excluded and patients with confirmed CD.	31	Calprotectin ESR CRP	Faecal calprotectin measurement can be useful in the diagnosis of CD and may provide differentiation from IBS. Further studies are needed.	None
Tibble <i>et al</i> 2002 [5]	Prospective study. Calprotectin measured by Roseth method and other blood biomarkers in single faecal and blood samples respectively from patients with gastrointestinal symptoms. All patients had a small intestinal permeability ratio test performed by the saccharide method and were assessed against Rome criteria.	602	Calprotectin ESR CRP FBC Small intestinal permeability	Faecal calprotectin is a non-invasive, safe and effective test for detecting organic gastrointestinal disease. The greatest distinction between organic disease and IBS came with the combination of the faecal calprotectin result with those of the permeability ratio and Rome criteria assessment.	None
<b>Lactoferrin</b>					
D'Inca <i>et al</i> 2007 [44]	Prospective study. Calprest measurement of single faecal samples from patients undergoing colonoscopy for gastrointestinal symptoms.	144	Calprotectin Lactoferrin	Faecal calprotectin and lactoferrin are easily measured and both tests are useful in detecting bowel inflammation in symptomatic patients. The tests have similar diagnostic accuracy. Results seem to reflect endoscopic and histological disease activity in UC, but not in CD.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Langhorst <i>et al</i> 2008 [41]	Prospective study. Biomarkers were measured in faecal and blood samples from patients with IBD or IBS undergoing routine diagnostic ileocolonoscopy. Disease activity indices were assessed. Calprotectin was measured by the Immunodiagnostik ELISA.	139	Calprotectin Lactoferrin PMN-e CRP	Faecal calprotectin, lactoferrin and PMN-e can distinguish IBD from IBS and all show a better diagnostic accuracy than CRP. The three faecal markers are equal in their reflection of endoscopic results. Combining results increased the diagnostic accuracy with respect to inflammation seen on endoscopy in UC.	None
Otten <i>et al</i> 2008 [54]	Prospective study. Calprotectin (PhiCal) and lactoferrin measured by ELISA and rapid test in faecal samples from patients referred for endoscopy and found to have IBD or IBS.	114	Calprotectin Lactoferrin	The rapid test for both calprotectin and lactoferrin performed as well as their respective ELISAs in indicating the presence of bowel inflammation. The rapid tests are suitable for primary care to exclude the presence of IBD.	Cost effectiveness unknown.
Schoepfer <i>et al</i> 2007 [31]	Prospective study. Biomarkers were measured in faecal and blood samples from out-patients and in-patients undergoing a diagnostic assessment for gastrointestinal symptoms. A set of 3 faecal samples was collected by each patient. Feasibility of outpatient faecal collection was assessed. Calprotectin measured by the PhiCal method.	74	Calprotectin Lactoferrin FOB CRP Leucocytes	Quantitative measurement of calprotectin and lactoferrin gave the best accuracy for the detection of inflammation. Endoscopy is required to determine the cause of the inflammation. The absence of elevated faecal calprotectin or lactoferrin should be included in the definition of patients with IBS. Faecal sampling feasibility was high in the studied group of outpatients.	None
Schoepfer <i>et al</i> 2008 [46]	Prospective study. Biomarkers were measured in faecal and blood samples from patients undergoing a diagnostic assessment for abdominal pain, altered bowel habit and or ano-rectal bleeding. A set of 3 faecal samples was collected by each patient. Calprotectin was measured by the PhiCal method.	136	Calprotectin Lactoferrin FOB CRP Leucocytes ASCA pANCA	Quantitative measurement of faecal calprotectin and lactoferrin are very accurate in distinguishing IBD from IBS. Addition of ASCA and pANCA results only increases the diagnostic accuracy marginally. Endoscopy is required to determine the cause of the inflammation. ASCA and pANCA may be useful in distinguishing between CD and UC. Absence of raised faecal leucocyte markers is needed for a diagnosis of IBS.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Schroder <i>et al</i> 2007 [42]	Prospective study. Biomarkers were measured in faecal samples from patients referred for chronic diarrhoea. Calprotectin was measured by the Immunodiagnostik method.	88	Calprotectin Lactoferrin PMN-e	All three biomarkers provided a reliable, non-invasive distinction between IBD and IBS, but calprotectin provided the best accuracy. Combined results of more than one marker does not improve the diagnostic accuracy.	None
Silberer <i>et al</i> 2005 [40]	Prospective study. Biomarkers were measured in samples of 3 consecutive faecal collections in healthy controls and patients undergoing an ileocolonoscopy. Calprotectin was measured by the Calprest method.	79	Calprotectin Lactoferrin Lysozyme MPO PMN-e	Calprotectin and PMN-e gave the best differentiation of active IBD from IBS. They reflected the severity of IBD.	None
<b><sup>111</sup> Indium</b>					
Tibble <i>et al</i> 2000 [29]	Patients underwent the four day faecal excretion of <sup>111</sup> Indium white cells test.	22	Calprotectin ESR	Faecal calprotectin concentrations correlated with <sup>111</sup> Indium test results, providing an alternative test. Single samples can be used.	None
	Cross-sectional study. Faecal calprotectin was measured in healthy controls and patients with confirmed CD, by Roseth method.	116	CRP	Faecal calprotectin was able to distinguish between healthy controls and CD with a sensitivity of 96%.	
	Prospective study. Faecal samples from patients referred for differentiation of potential IBD or IBS were measured.	220		Faecal calprotectin also performed well in discriminating between CD and IBS. It may be useful in screening for bowel inflammation.	
<b>Intestinal permeability</b>					
Berstad <i>et al</i> 2000 [70]	Prospective study. Intestinal permeability (IP) measured by <sup>51</sup> Cr EDTA method. PhiCal measurements made on gut lavage samples collected from patients with known or suspected IBD.	38	Calprotectin	IP and gut lavage calprotectin concentrations are raised in IBD and discriminate between organic and functional bowel disease. Increased neutrophil passage across the gut epithelium may influence increased IP.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Shulman <i>et al</i> 2008 [32]	Prospective study in children. IP measured by the saccharide method. Calprotectin measured in faecal samples from healthy controls and patients with functional abdominal pain and IBS (FAP/IBS), by an ELISA method marketed by Genova Diagnostics.	109	Calprotectin	Children with FAP/IBS have increased gastrointestinal permeability. Faecal calprotectin concentrations are raised just above the cutoff indicating the presence of low grade inflammation. Correlation of calprotectin with pain interference with activities demonstrates a relationship between these factors.	None
<b>M2-Pyruvate kinase</b>					
Chung-Faye <i>et al</i> 2007 [43]	Prospective study. Biomarkers measured by ELISAs in single faecal samples from patients with lower gastrointestinal symptoms or known IBD. Calprotectin was measured by a method based on that of Roseth.	148	Calprotectin M2-PK	Similar to faecal calprotectin, M2-PK is raised in IBD and in colorectal cancer. In this study calprotectin performed better in identifying organic disease. Both tests appear to be markers for disease activity in IBD.	Cost of biomarker measurement compares favourably with the cost of invasive investigations
<b>Serum antibodies to flagellins</b>					
Schoepfer <i>et al</i> 2008 [51]	Prospective study. Antibody measurement in serum samples and PhiCal measurement of faecal samples from healthy controls and patients with known or suspected IBD or IBS.		Calprotectin A4-Fla2 Fla-X ASCA pANCA	Antibodies to flagellins were found significantly more often in IBS than in healthy controls. Incidence of these antibodies was greater in the post infectious IBS (PI IBS) group than the non-PI-IBS group suggesting that immune reactivity to flagellin antigens may have a role in the development of PI-IBS. Faecal calprotectin was normal in IBS and raised in IBD.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
<b>Faecal S100A12</b>					
Foell <i>et al</i> 2008 [65]	Prospective study. Biomarkers were measured in supernatants from biopsy material taken from patients with known IBD or IBS and those without inflammation. Calprotectin was measured by a method developed by Foell.	120	Calprotectin S100A12	The direct release of these biomarkers from inflamed tissue to a much greater extent than non-inflamed tissue may reflect secretion from neutrophils which have migrated to the tissue. S100A12 release was lower than calprotectin, but the difference between inflamed and non-inflamed tissue much greater. These biomarkers in turn have an inflammatory effect on the tissue which would explain the correlation of faecal biomarkers with disease activity in IBD.	None
Kaiser <i>et al</i> 2008 [45]	Prospective study. S100A12 and PhiCal measurement of faecal samples from healthy controls and patients with gastrointestinal symptoms or known IBD.	171	Calprotectin S100A12	Faecal S100A12 performs well in distinguishing IBD from IBS or healthy controls. It reflects inflammatory activity in chronic IBD, but it is also raised in bacterial enteritis so this must be ruled out before interpreting S100A12 results. In this study S100A12 performed better than calprotectin, but the latter performed worse than in many other studies.	None
de Jong <i>et al</i> 2006 [66]	Prospective study in children. S100A12 measured with in-house method in faecal samples from healthy controls and patients with newly diagnosed IBD.	23	S100A12 ESR CRP	Faecal S100A12 performs well in discriminating between IBD in children and healthy child controls. Concentrations correlate with standard serum inflammatory markers. S100A12 is a suitable marker for assessing bowel inflammation. Further evaluation is required.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Sidler <i>et al</i> 2008 [48]	Prospective study in children. In-house S100A12 and PhiCal measurements were made in faecal samples from healthy controls and patients with gastrointestinal symptoms suggestive of an organic bowel disease.	61	S100A12 Calprotectin ESR CRP	Both faecal tests performed well and better than the standard blood based inflammatory tests. S100A12 gave a better specificity for IBD, but calprotectin a higher NPV for ruling out IBD. S100A12 appears to be suitable to select children for more invasive investigations. Further studies required.	None
<b>Calprotectin measurement</b>					
Ton <i>et al</i> 2000 [85]	Methodologic study. Calprotectin measured by new Phical method in 59 healthy controls and 30 hospitalized patients. Faecal calprotectin extraction yield, distribution in samples, assay interference and comparison with Roseth method were assessed.	30	Calprotectin	Measurement of calprotectin in faecal samples was accurate and reproducible. No interference found with relevant foods, drugs or nutritional treatments therefore no dietary restrictions are needed by the patient.	None
Vestergaard <i>et al</i> 2008 [55]	A methodologic study. Calprotectin concentrations measured by quantitative Phical ELISA and Prevent ID Caldetect semi-quantitative rapid test in 5 faecal samples from healthy subjects and 95 samples from patients with IBD, chronic diarrhoea, abdominal pain or other reasons.	82	Calprotectin	Prevent ID Caldetect performs well as a screening test for excluding gastrointestinal inflammation when the 15 µg/g cutoff is used. When the faecal calprotectin concentration is > 15 µg/g the rapid test result should be confirmed by a quantitative method to avoid false negatives. The rapid test cannot be used to monitor disease activity as quantitative results are required..	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Damms and Bischoff 2008 [24]	A case control trial. Calprotectin concentrations measured by quantitative Buhlmann ELISA and Prevista semi-quantitative rapid test in single faecal samples from patients referred for colonoscopy with lower gastrointestinal symptoms.	140	Calprotectin	Both quantitative and semi-quantitative tests accurately identify active IBD and colorectal cancer (CRC), but cannot distinguish adenomas from CRC or controls. The rapid test may provide more useful results than the FOB test; further study is needed.	None
Canini <i>et al</i> 2006 [34]	A prospective study in children. Calprest measurements of faecal samples and blood based biomarkers in blood samples from patients being assessed for suspected IBD. IP ratio measured by the cellobiose/mannitol method and bowel wall thickness ultrasound measurement was also performed on all patients.	45	Calprotectin ESR CRP ASCA pANCA	The inclusion of non-invasive tests in the initial diagnostic assessment for IBD in children may avoid unnecessary invasive procedures for some patients. These tests may also be useful when the diagnosis is initially uncertain by other non-invasive tests. Further studies are needed in a less selected population.	Cost of biomarker measurement compare favourably with cost of invasive investigations.
Denis <i>et al</i> 2007 [39]	Cohort study study with retrospective disease activity scores. Blood based biomarkers measured on blood samples and calprotectin measured by an ELISA marketed by NovaTech Diagnostica on faecal samples from patients with clinically active CD.	28	Calprotectin ESR CRP Fibrinogen $\alpha$ -1 glycoprotein	Patients with clinically active CD, but normal CRP, had active mucosal lesions at endoscopy, although mild. Faecal calprotectin was mildly to moderately raised, but similar to the other biomarkers there was no correlation with the intensity of the endoscopic score.	None
Gacia Sanchez <i>et al</i> 2006 [89]	Prospective study. Calprest measurement of faecal samples from patients undergoing colonoscopy.	190	Calprotectin	Raised faecal calprotectin found in IBD and CRC. The test is a non-invasive inexpensive reliable means of selecting from patients with gastrointestinal symptoms those who should have a colonoscopy. NSAIDs can raise faecal calprotectin concentrations in patients with normal colonoscopies.	Minimal

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Gaya <i>et al</i> 2005 [94]	Cross-sectional comparative study. Calprest measurement of faecal samples from patients with known CD and symptoms of a relapse. All patients underwent a white cell scanning test.	35	Calprotectin	A single faecal calprotectin measurement is reliable in detecting the presence of inflammation in CD compared to white cell scanning results. The test is much simpler and less risk to the patient than white cell scanning.	None
Kristinsson <i>et al</i> 2001 [84]	Prospective study. PhiCal measurement of two spot samples taken from the same faecal sample on two consecutive days from patients diagnosed with CRC.	155	Calprotectin	Faecal calprotectin concentration was raised in CRC, but this is not specific for CRC as it is also raised in IBD. Further investigation of the specificity of the test is needed. A single spot sample is sufficient for reliable measurement of the calprotectin concentration.	None
Tibble <i>et al</i> 1999 [104]	Prospective study. Calprotectin measured by in-house method based on Roseth 1992 in faecal samples from patients being treated with NSAIDs. <sup>111</sup> Indium white cell labelled test was performed on all patients.	47	Calprotectin	Measurement of faecal calprotectin can detect NSAID enteropathy, 20% of concentrations were comparable to those seen in cases of IBD. The test provides the same information as the <sup>111</sup> Indium white cell labelled test, but is much simpler.	None
Kristinsson <i>et al</i> 1998 [90]	Prospective study. Calprotectin measured by in-house method based on Roseth 1992 in faecal samples from patients newly diagnosed with CRC.	119	Calprotectin CEA CRP	Faecal calprotectin concentrations were raised in CRC, but it is unlikely to be able to identify specific colonic disorders because it is also raised in IBD.	None
Poullis <i>et al</i> 2004 [88]	Prospective study. Calprest measurement of samples from a random population from whom cases of IBD had been excluded.	320	Calprotectin Lactoferrin CRP $\alpha$ -1 anti-trypsin	Faecal calprotectin concentrations are associated with lifestyle risk factors for CRC. Elevated calprotectin concentrations in hard stools.	None
Tibble <i>et al</i> 2000 [87]	Prospective study. Calprotectin measured in faecal samples from patients previously diagnosed with CD or UC but then in remission, by a method based on that of Roseth. The saccharide small intestinal permeability ratio test was also carried out on all patients.	80	Calprotectin	Faecal calprotectin predicts clinical relapse of disease activity in patients with CD and UC. Small intestinal permeability test is useful in predicting relapse in patients with small intestinal CD. Single spot faecal samples can be used for faecal calprotectin measurement.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Bunn <i>et al</i> 2001 [38]	Prospective study in children. Calprotectin was measured in faecal samples from patients who were referred for white cell scanning or colonoscopy, by a method based on that of Roseth..	36	Calprotectin	Faecal calprotectin results correlated well with the more invasive measures of bowel inflammation in children with IBD. Use of this test might reduce the use of invasive tests in such patients.	None
Tibble <i>et al</i> 2001 [49]	Prospective study. Biomarkers were measured in faecal samples from patients diagnosed with CRC or undergoing colonoscopy for investigation or follow up of bowel disorders. Calprotectin was measured by a method based on that of Roseth.	295	Calprotectin FOB	Faecal calprotectin is a more sensitive marker for CRC than FOB, but is less specific. Faecal calprotectin concentrations were higher than control values in patients with IBS, but within the normal range.	None
Kolho <i>et al</i> 2006 [53]	Prospective study in children undergoing colonoscopy. PhiCal measurements on faecal samples from patients with no abnormalities, those with CD and those with non-IBD disease. Children put on glucocorticoid treatment were followed up.	57	Calprotectin	Faecal calprotectin is a sensitive marker for chronic colitis. Treatment with glucocorticoids results in decreasing faecal concentrations, but not usually to normal concentrations suggesting some ongoing inflammation in clinically silent disease. Use of a higher cutoff is best for identification of IBD, but exclusion of IBD requires the use of a lower cutoff.	None
Malickova <i>et al</i> 2008 [47]	Methodologic study. Calprotectin was measured by Buhlmann and PhiCal ELISAs in faecal samples from healthy controls and patients with CD and UC.	65	Calprotectin	Both methods performed well. The Buhlmann method using a monoclonal antibody against calprotectin showed higher sensitivity and specificity compared to the PhiCal method using polyclonal antibodies. Technical performance was acceptable.	None
Reinders <i>et al</i> 2007 [68]	Prospective study. PhiCal, nitrate and nitrite measurements were made in faecal samples from healthy control and patients with known IBD. Nitric oxide (NO) was measured in samples of rectal gas.	32	Calprotectin Rectal nitric oxide Nitrate and nitrite	Faecal calprotectin and rectal NO were raised during bowel inflammation, but no correlation was found between the two measurements. This suggests that they may reflect different parts of the inflammatory process.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
<b>Economic studies</b>					
Takemura <i>et al</i> 2003 [95]	Cost effectiveness study. The yield and cost of the biomarkers were analysed in primary care outpatients with inflammation related symptoms. The result was considered useful if it influenced the doctor's diagnosis or decision making. Costs were based on single or simultaneous measurements.	177	CRP White blood cell count ESR Sialic acid Protein fractionation	The combination of CRP and white blood cell count (WBC) gave the best cost effectiveness compared to CRP testing alone. Additional measurement of sialic acid gave better cost effectiveness than ESR or protein fractionation. Optimum use of these inflammatory markers should involve consideration of cost-effectiveness.	Whole study
Dubinsky <i>et al</i> 2002 [96]	Decision model study. Decision analysis compared the cost effectiveness of initial serological screening followed by standard invasive testing with the latter alone. A hypothetical cohort of patients with gastrointestinal symptoms who did not meet the criteria for IBS were analysed.	N/A	ASCA pANCA	Initial serological testing may be a cost effective alternative to standard invasive testing. The economic benefits seem to be achieved by avoiding invasive evaluations in patients without IBD. Testing of the model in a prospective comparative study was recommended.	Whole study
Suleiman and Sonnenburg 2001 [9]	Cost effectiveness study using a decision model. The Bayes formula was used to calculate the increase in certainty for consecutive tests in the diagnostic work up for IBS. Incremental cost-effectiveness ratio was also calculated.	N/A	FOB ESR General laboratory panel	Inexpensive and non-invasive tests should be used first in the diagnosis of IBS, to rule out other diagnoses. Even though they have high incremental cost ratios, flexible sigmoidoscopy and colonoscopy should be carried out when serious organic disease is indicated and needs to be ruled out.	Whole study
<b>Papers not including calprotectin measurement</b>					
<b>ASCA and pANCA</b>					
Elitsur <i>et al</i> 2005 [61]	Retrospective study of charts of children diagnosed with IBD. Diagnosis was by clinical examination, radiology and endoscopy. Serum marker accuracy to diagnose CD or UC was assessed.	101	ASCA pANCA Anti-OmpC	ASCA and p-ANCA antibodies had insufficient sensitivity and specificity for children with UC or CD, nor were they useful in cases of indeterminate colitis. Anti-OmpC has poor sensitivity for these diseases.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Kaila <i>et al</i> 2005 [59]	Retrospective study including adults and children. ASCA was measured on samples randomly selected from a serum bank established for studies of inflammation and inflammatory markers.	381	ASCA	A positive ASCA result makes highly likely that IBD is present and that it is probably CD. In symptomatic patients a positive test should encourage further investigation.	None
<b>Lactoferrin</b>					
Dai <i>et al</i> 2007 [67]	Prospective study. IBD-SCAN measurement in faecal samples from healthy controls and patients having 3 or more bowel movements/day for 2 weeks.	121	Lactoferrin	Faecal lactoferrin is a sensitive and specific marker for IBD and can discriminate between inflammatory and non-inflammatory bowel disease. Raised faecal lactoferrin can exclude IBS in clinical practice.	None
Kane <i>et al</i> 2003 [62]	Prospective study. Quantitative measurement of lactoferrin in faecal samples from patients with a history of CD, UC or IBS. Qualitative measurements were carried out on a subgroup of these patients.	215	Lactoferrin	Faecal lactoferrin is a sensitive and specific marker for IBD and a good test to rule out IBS.	None
Walker <i>et al</i> 2007 [7]	Prospective study in children and young adults. Biomarkers were measured and faecal and blood samples from patients with known or suspected IBD and IBS.		Lactoferrin ESR	Faecal lactoferrin is a sensitive and specific biomarker for detecting inflammation in children. Concentrations correlate well with ESR results and disease activity indices. Further studies are needed.	None
<b>Intestinal permeability</b>					
Dunlop <i>et al</i> 2006 [71]	Prospective study. IP measured in patients with post-infectious IBS with constipation or diarrhoea and in patients with post-infectious IBS and non-post-infectious IBS. IP measured by <sup>51</sup> Cr – EDTA method.	60		Small intestinal permeability is often abnormal in diarrhoea predominant IBS. Patients without a history of previous gastrointestinal infection appear to have a more marked alteration in IP.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Marshall <i>et al</i> 2004 [76]	Prospective study. IP was measured in patients with Rome 1 IBS and in non-IBS controls by the lactulose/mannitol method.	132		IBS symptoms are associated with a subtle increase in IP irrespective of previous gastroenteritis.	None
<b>M2-Pyruvate kinase</b>					
Czub <i>et al</i> 2007 [77]	Prospective study in children. Pyruvate kinase was measured in faecal samples from patients with known IBD.	107	M2-pyruvate kinase	Raised faecal M2-PK concentrations could be a useful indicator of IBD activity in children, but further studies comparing it with other tests are needed.	None
<b>Minor markers</b>					
Gonsalkorale <i>et al</i> 2003 [78]	Allele and genotype frequencies for the biomarkers were determined from DNA extracted from peripheral blood leucocytes	2304	Interleukin 10 Transforming Growth Factor- $\beta_1$ (TGF- $\beta_1$ )	Some IBS patients may be genetically predisposed to produce lower concentrations of anti-inflammatory cytokine interleukin 10, supporting the existence of an IBS subgroup with an inflammatory component to their disease.	None
Wiercinska-Drapalo <i>et al</i> 2001 [80]	Prospective study. TGF- $\beta_1$ measured by enzyme immunoassay in plasma from healthy controls and patients with endoscopically confirmed UC.	45	TGF- $\beta_1$	Increased TGF- $\beta_1$ production can be related to inflammatory activity in UC and can be considered to be a biomarker for the disease.	None
Langhorst <i>et al</i> 2007 [81]	Prospective study. Human defensin $\beta$ -2 (HBD-2) measured in faecal samples from healthy controls and patients with known UC or IBS.	46	HBD-2	Contrary to expectations HBD -2 concentrations were raised in IBS (though less than in UC) supporting the involvement of inflammation in IBS.	None

Ref.	Design	No of patients	Biomarkers	Conclusion	Economics
Roka <i>et al</i> 2007 [82]	Prospective study. Serine protease activity measured in faecal samples from patients with Rome II IBS or UC.	53	Serine protease activity Mast cell tryptase Pancreatic elastase	Faecal serine protease activity is increased in diarrhoea predominant IBS, but there are no changes in mast cell tryptase or pancreatic elastase. Serine protease activity may therefore be a factor in development of diarrhoea predominant IBS.	None

## **Evidence review: Value of calprotectin in screening out irritable bowel syndrome**

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