AUSTRALIAN Clinicallabs

Calprotectin Testing

Providing you with insight in diagnosing and managing Inflammatory Bowel Disease

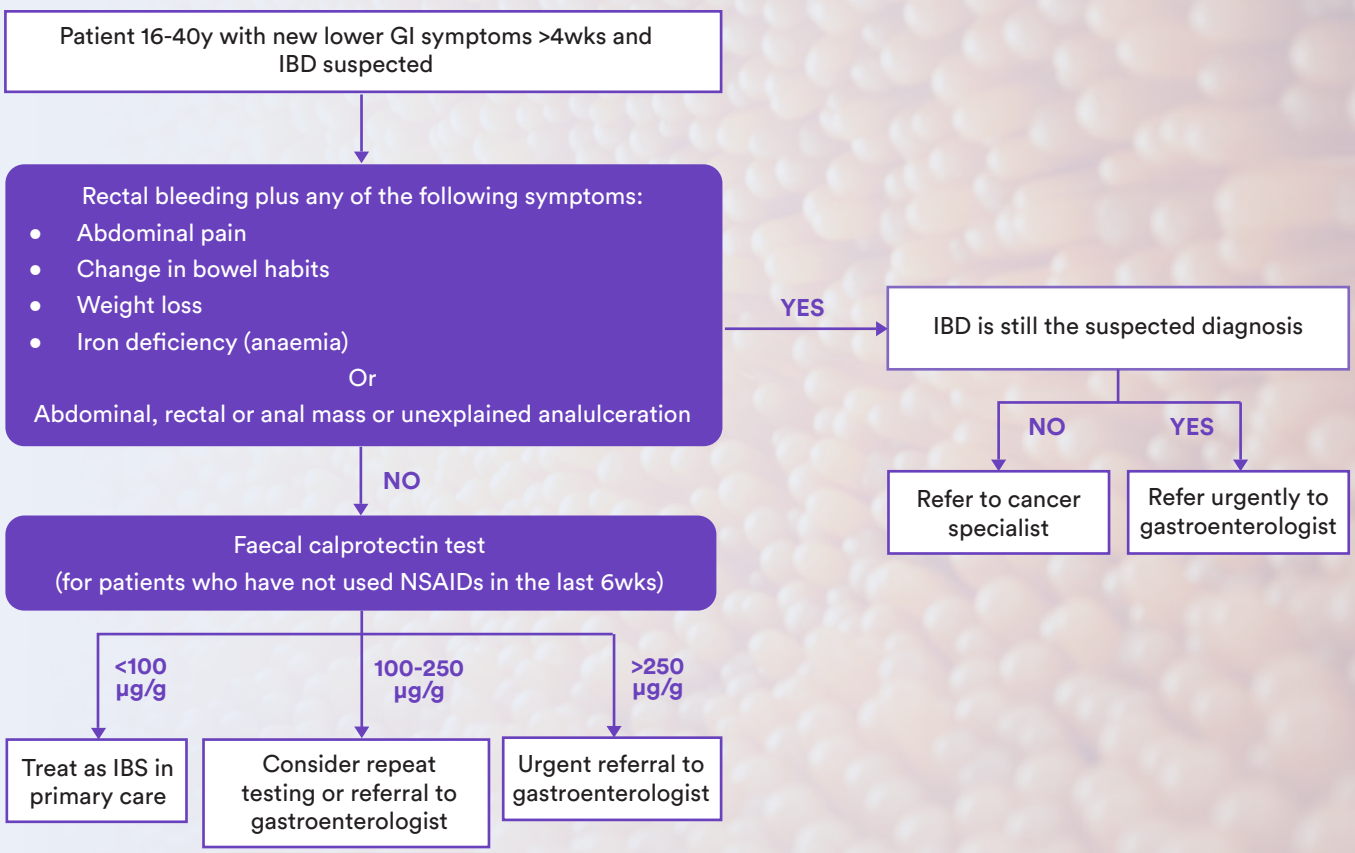
Included on the MBS as of 1st November 2021

Introduction to calprotectin testing

Faecal calprotectin concentration (FCC) is a useful, non-invasive method for distinguishing between organic and functional diarrhoeal disorders and for monitoring disease activity in established cases of gut wall inflammation.

Calprotectin concentrations are assayed on the DiaSorin Liaison XL instrument using chemiluminescent immunoassay technology. Reference intervals (RIs) are provided for adult patients. The assay in use at Clinical Labs has not been evaluated in a paediatric population, although a large body of literature exists that supports the use of FCC in children, infants and neonates, as in adults. RIs in paediatric populations are poorly established but published FCCs, in health, are higher than in adults (see below).

Figure 1. Use of faecal calprotectin test in primary care

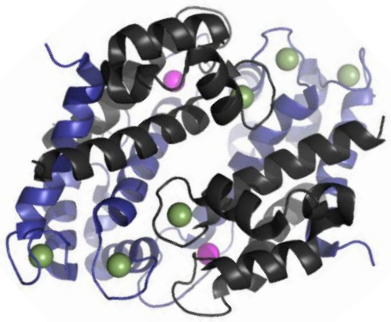


(After Lamb, Kennedy, Raine, et al., 2019)

Calprotectin

Calprotectin is a large protein with post-translational changes that include binding of each of zinc and calcium at different sites.

Although described in multiple cell types, the protein is generally used as a marker of neutrophilic inflammation. Calprotectin accounts for 60% of the protein content of the cytosol of neutrophils (Roseth, Fagerhol MK, Aadland, & Schjonsby, 1992). Actions of calprotectin include antimicrobial and anti-proliferative activity and an extracellular immunomodulatory effect. The name calprotectin was adopted because of its calcium binding properties and protective functions (Johne, Fagerhol, Lyberg, Prydz, & Brandt, 1997). As it is a member of the S100 protein family, it is officially designated S100 Calcium-Binding Protein A8 (HGNC Approved Gene Symbol: S100A8) (McKusick & Converse, 2015).



Clinical uses of calprotectin

Calprotectin concentrations have been shown to reflect pathology in many tissues and in associated body fluids, including plasma, saliva, urine and synovial fluid and there is an increasing literature referencing its assay in multiple clinical conditions.

Faecal calprotectin

Widespread clinical application of measurement of calprotectin levels has evolved for the non-invasive investigation of diarrhoeal gut disease, since the beginning of this century. Early faecal assays used stool volume vs. weight and the reported measurement units and RIs of these assays, therefore, differ from modern methods. Calprotectin in faeces has been shown to be a robust measure of neutrophilic inflammation of the intestinal mucosa. However, it is not specific for inflammatory bowel disease (IBD), being variably increased in other causes of gut wall inflammation and in various gastrointestinal malignancies and ingestion of some common drugs. Nevertheless, faecal calprotectin concentration (FCC) has been shown to vary with the degree of inflammation (Bressler, Panaccione, Fedorak, & Seidman, 2015) and by some authors to predict relapse in IBD (Chang, Malter, & Hudesman, 2015).

Table 1 - Factors and conditions associated with elevated faecal calprotectin levels

Infectious	Inflammatory conditions
• Bacterial dysentery • Giardia lamblia • *Helicobacter pylori* gastritis • Infectious diarrhea • Viral gastroenteritis	• Inflammatory bowel disease • Autoimmune enteropathy • Cirrhosis • Cystic fibrosis • Diverticulitis • Eosinophilic colitis/ enteritis • Gastroesophageal reflux disease • Juvenile polyp • Microscopic colitis • Peptic ulcer • Untreated celiac disease
Neoplasms	
• Colonic and gastric polyps • Colorectal cancer • Gastric carcinoma • Intestinal lymphoma	
Drugs	**Other**
• NSAIDs • PPIs	• Age <5y • Untreated food allergy

NSAIDs (Nonsteroidal anti-inflammatory drugs); PPIs (Proton pump inhibitors) (After Bressler, Panaccione, Fedorak, & Seidman, 2015)

Although FCC increases somewhat with age in adults it has been shown to be a superior non-invasive discriminatory test for the distinction between inflammatory and functional intestinal disease (Figure 2). PPV is increased by a raised, concomiant serum CRP.

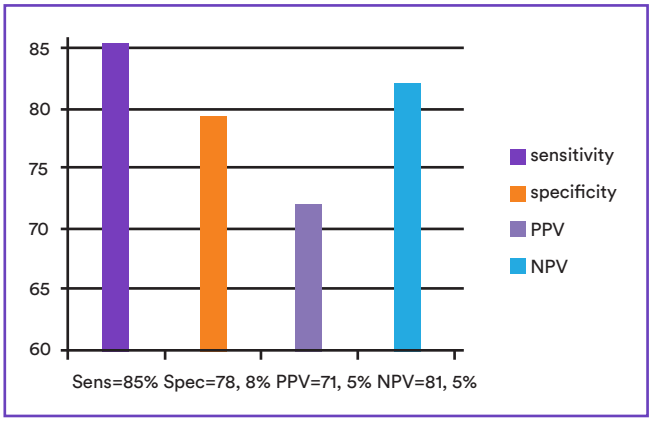


Figure 2. Pooled faecal calprotectin sensitivities, specificities, positive predictive value and negative predictive value of faecal calprotectin in discriminating between intestinal inflammation and functional disorders (Mumolo, et al., 2018). PPV: Positive predictive value; NPV: Negative predictive value.

Faecal calprotectin in paediatric populations

Several authors have established the usefulness of FCC in children. However, it is known that young children and infants have higher FCC than adults or older children. The literature regarding older children is less clear regarding RIs; however the longitudinal monitoring of individuals is clinically reliable (Herrera, Christensen, & Helms, 2016). Neonates and premature infants appear to have complicated FCC responses, falling immediately after birth and then rising.

Li et al have published FCC from 288 healthy children 0-18 months. Their data is shown below.

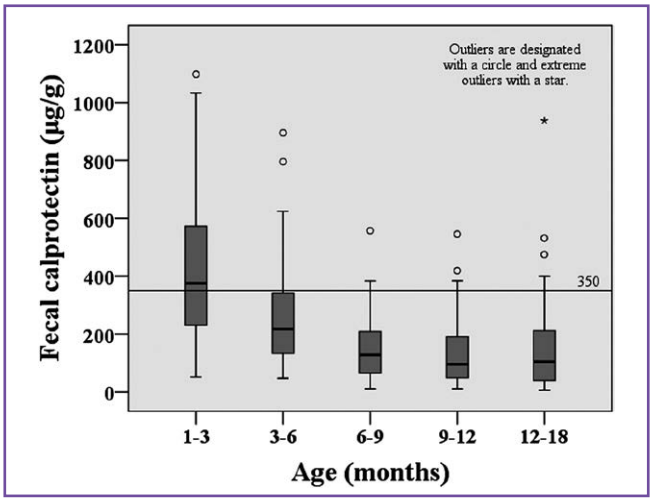


Figure 3. Faecal calprotectin concentrations in five age groups of healthy children (Li, et al., 2015).

The same group have also published equivalent data for 274 children 1 to 4 years.

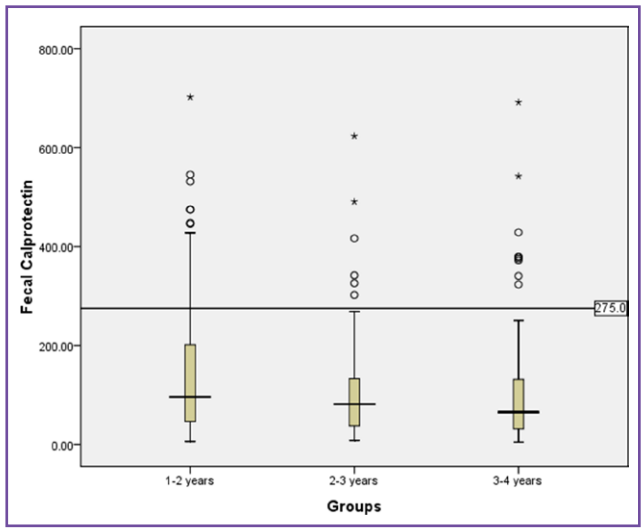


Figure 4. Faecal calprotectin concentrations in three age groups of healthy children (Zhu, Li, Wang, Shen, & Sheng, 2016).

Necrotising enterocolitis is associated with increased FCC. Wide ranging cut-offs are reported but Thuijls et al. (2010) have reported clinically relevant positive likelihood ratio (LR) of 12.29 and negative LR of 0.15 using a FCC cut-off of 286.2 µg/g faeces.

Laboratory analysis of faecal calprotectin

Reference intervals

Most healthy adults will have a FCC <10 µg/gram faeces. However, since the FCC is known to increase with age RIs are usually established that account for miscellaneous factors, including age. These RIs should not be used for longitudinal monitoring when the patient should become their own reference.

Based on the expected values from literature reports and the manufacturer's recommendation, Clinical Labs has adopted the following RIs for FCC in adult patients with clinically suspected IBD:

0-50 µg/gram	IBD unlikely but not excluded.
50-100 µg/gram	IBD likely; other inflammatory conditions, including but not limited to infection, Coeliac disease and Diverticular disease, cannot be excluded.
100 µg/gram	Almost exclusively IBD. Other severe inflammatory diseases not excluded.

Conclusion

FCC is a safe and reliable non-invasive test for inflammation of the bowel wall that can:

- Distinguish between patients with IBD and patients with IBS.
- Determine disease activity and risk of relapse in IBD patients, and assess the level of mucosal healing.
- Help to identify patients with abdominal symptoms who may require further investigative procedures and reduce the number of endoscopies performed for the diagnosis of diarrhoeal disease and monitoring of IBD.

Because it is not specific for IBD, it must be interpreted in the clinical context.

Specimen collection, transport and storage

As it is known that the time between defaecation may affect the FCC, the first stool of the day is recommended. Stool specimens should be collected into a clean, airtight container without preservative and stored at 2-8°C. The sample is required to be received and frozen at the laboratory within 24 hours of collection. Longer delay requires freezing at -20°C or below. Stool specimens that are liquid or very solid may be technically unsuitable.

Turn around time

Results are available within 3 to 4 business days of the specimen's arrival at our laboratory.

Cost

There is currently an out of pocket cost for Calprotectin testing. From 1st of November 2021, certain patients may be eligible for a Medicare Rebate.

About the Author

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Associate Professor Smyth is a graduate of the University of Western Australia and a Fellow of, and former state representative to the Board of Education of the RCPA. Associate Professor Smyth designed and implemented the Pathology programme for the School of Medicine at the University of Notre Dame Australia, Fremantle where she is a founding member of, and Associate Professor in the School of Medicine. She has a Graduate Certificate in University Teaching, qualifying her to supervise candidates for higher degrees as well as teaching undergraduate students. She is most interested in autoimmunity but has extensive experience, including autoimmunity, transplantation, immune deficiency and allergy. Her publications are predominantly in the field of bone marrow transplantation. Dr Smyth joined St John of God Pathology (now Australian Clinical Labs) in 2016.

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ACLMAR-BF-NAT-301.3 08/23