

# PATHOLOGY FOCUS

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Medical Newsletter

## Featured articles:

- Calprotectin testing for the diagnosis and management of IBD
- New thyroglobulin antibody assay
- Reproductive genetic carrier screening
- Assessing allergy in clinical practice
- Providing your patients with the gold standard in cardiac health diagnostics

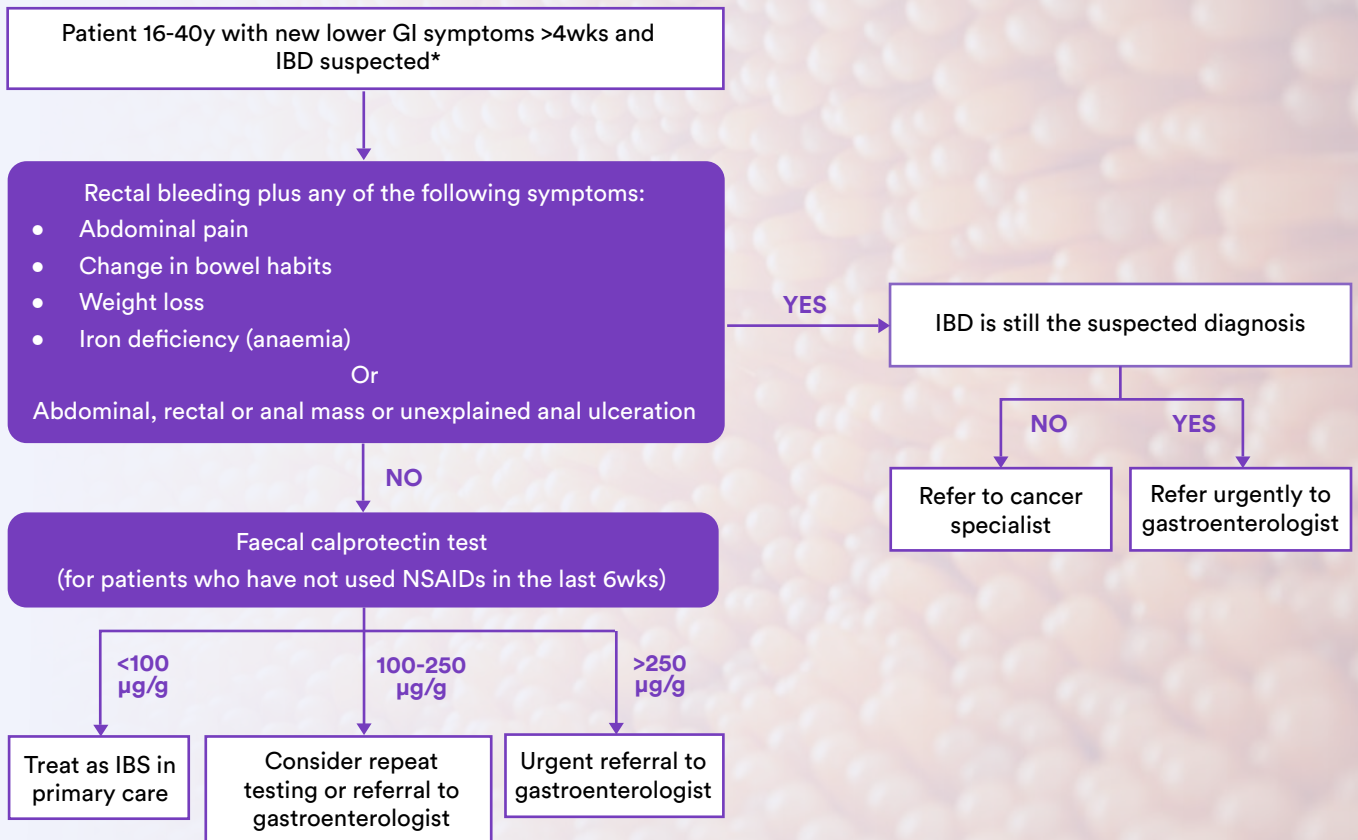
## Calprotectin testing for the diagnosis and management of IBD

By Associate Professor Louise Smyth

*Faecal calprotectin concentration 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. A large body of literature exists that supports the use of faecal calprotectin concentration in children, infants and neonates, as in adults. RIs in paediatric populations are poorly established but published faecal calprotectin concentrations, in health, are higher than in adults (see following pages).

**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 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
<ul style="list-style-type: none"> <li>Bacterial dysentery</li> <li>Giardia lamblia</li> <li>Helicobacter pylori gastritis</li> <li>Infectious diarrhea</li> <li>Viral gastroenteritis</li> </ul>	<ul style="list-style-type: none"> <li>Inflammatory bowel disease</li> <li>Autoimmune enteropathy</li> <li>Cirrhosis</li> <li>Cystic fibrosis</li> <li>Diverticulitis</li> <li>Eosinophilic colitis/enteritis</li> <li>Gastroesophageal reflux disease</li> <li>Juvenile polyp</li> <li>Microscopic colitis</li> <li>Peptic ulcer</li> <li>Untreated coeliac disease</li> </ul>
Neoplasms	
<ul style="list-style-type: none"> <li>Colonic and gastric polyps</li> <li>Colorectal cancer</li> <li>Gastric carcinoma</li> <li>Intestinal lymphoma</li> </ul>	
Drugs	Other
<ul style="list-style-type: none"> <li>NSAIDs</li> <li>PPIs</li> </ul>	<ul style="list-style-type: none"> <li>Age &lt;5y</li> <li>Untreated food allergy</li> </ul>

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

Although faecal calprotectin concentration 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, concomitant serum CRP.

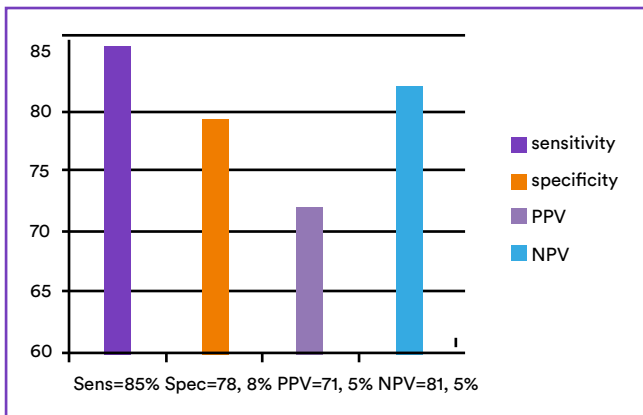


Figure 2. Pooled faecal calprotectin sensitivities, specificities, positive predictive value and negative predictive value of faecal calprotectin in discriminating between intestinal inflammation and functional disorders (Mumolo, et al., 2018). PPV: Positive predictive value; NPV: Negative predictive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

### Faecal calprotectin in paediatric populations

Several authors have established the usefulness of faecal calprotectin concentration in children. However, it is known that young children and infants have higher faecal calprotectin concentration 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 faecal calprotectin concentration responses, falling immediately after birth and then rising.

Li et al. (2015) have published faecal calprotectin levels from 288 healthy children 0-18 months. Their data is shown below (Figure 3).

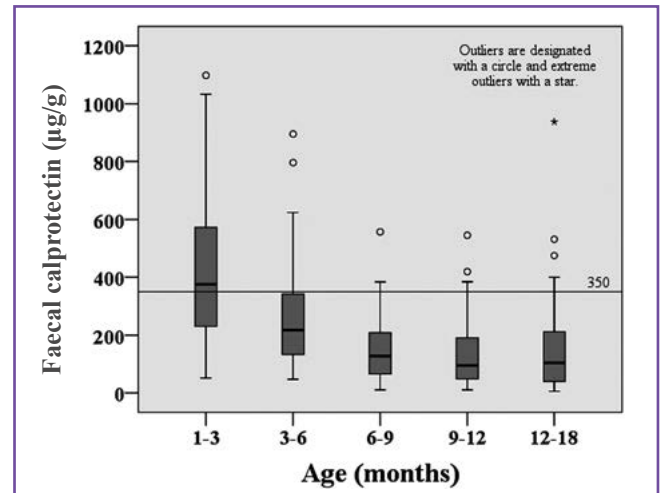


Figure 3. Faecal calprotectin concentrations in five age groups of healthy children (Li, et al., 2015).

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

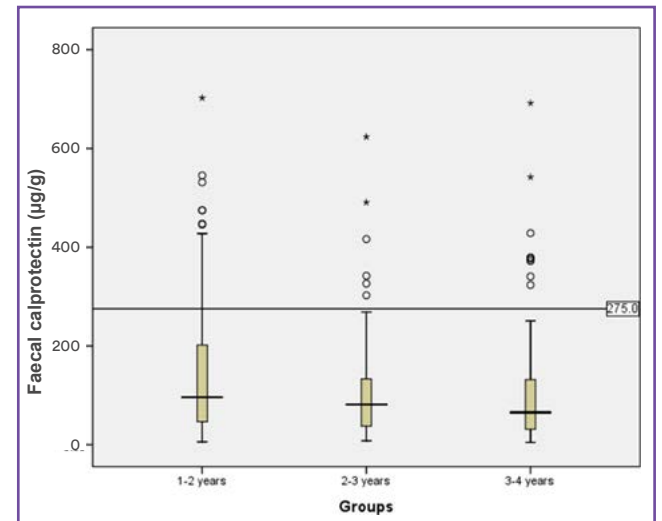


Figure 4. Faecal calprotectin concentrations in three age groups of healthy children (Zhu, Li, Wang, Shen, & Sheng, 2016).

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

## Laboratory analysis of faecal calprotectin

### Reference intervals

Most healthy adults will have a faecal calprotectin concentration <10 µg/g faeces. However, as the faecal calprotectin concentration 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 faecal calprotectin concentration 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

Faecal calprotectin concentration 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

The time between defaecation might affect the faecal calprotectin concentration; therefore, 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 must be received at the laboratory within 24hrs of collection. 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.

## Medicare Benefits Schedule Changes

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

## References

- Bressler, B., Panaccione, R., Fedorak, R. N., & Seidman, E. G. (2015). Clinicians' guide to the use of fecal calprotectin to identify and monitor disease activity in inflammatory bowel disease. *Can J Gastroenterol Hepatol*, 29(7), 369-372.
- Chang, S., Malter, L., & Hudesman, D. (2015, October 28). Disease monitoring in inflammatory bowel disease. *World J Gastroenterol*, 21(40), 11246-11259.
- Herrera, O. R., Christensen, M. L., & Helms, R. A. (2016). Calprotectin: Clinical Applications in Pediatrics. *J Pediatr Pharmacol Ther*, 21(4), 308-321.
- Johne, B., Fagerhol, M. K., Lyberg, T., Prydz, H., & Brandt, P. (1997). Functional and clinical aspects of the myelomonocyte protein calprotectin. *J Clin Pathol: Mol Pathol*, 50, 113-123.
- Lamb, C. A., Kennedy, N. A., Raine, T., & et al. (2019, September 27). British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut*, 68, s1-s106. Retrieved from <http://dx.doi.org/10.1136/gutjnl-2019-318484>
- Li, F., Ma, J., Geng, S., Wang, J., Liu, J., Zhang, J., et al. (2015). Fecal Calprotectin Concentrations in Healthy Children Aged 1-18 Months. *PLoS ONE*, 10(3).
- McKusick, V. A., & Converse, P. J. (2015, July 23). S100 CALCIUM-BINDING PROTEIN A8; S100A8. Retrieved December 8, 2016, from Online Mendelian Inheritance in Man: <https://www.omim.org/entry/123885?search=calprotectin&highlight=calprotectin>
- Mumolo, M.G., Bertani, L., Ceccarelli, L., Laino, G., Di Fluri, G., Albano, E., Tapete, G., Costa, F. (2018). From bench to bedside: Faecal calprotectin in inflammatory bowel diseases clinical setting. *World J Gastroenterology*, 24 (33):3681-3694.
- Roseth, A. G., Fagerhol MK, M. K., Aadland, E., & Schjonsby, H. (1992). Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. *Scand J Gastroenterol*, 27, 793-798.
- Thuijls, G., Derikx, J. P., van Wijck, K., Zimmermann, L. J., Degraeuwe, P. L., & Mulder, T. L. (2010). Non-invasive markers for early diagnosis and determination of the severity of necrotizing enterocolitis. *Annals of Surgery*, 251, 1174-1180.
- Zhu, Q., Li, F., Wang, J., Shen, L., & Sheng, X. (2016). Fecal Calprotectin in Healthy Children Aged 1-4 Years. *PLoS ONE*, 11(3).

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## Assoc. Prof. Louise Smyth

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Associate Professor Smyth is a graduate of the University of Western Australia and a Fellow of, and former state representative 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.



# New thyroglobulin antibody assay

By Dr David Deam



The thyroglobulin antibody test indicates the presence of circulating antibodies to thyroglobulin, which is a protein found in thyroid cells.

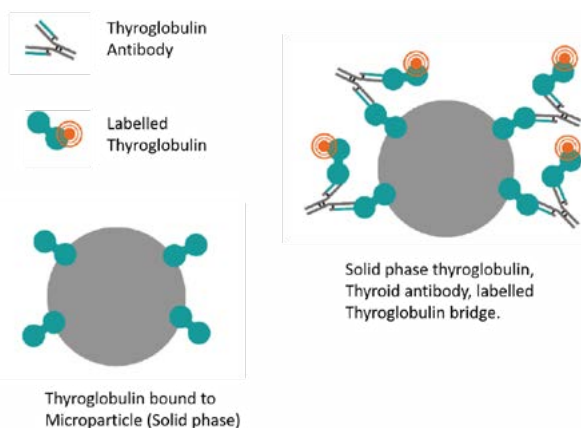
The antibody test is useful in two main situations:

1. As a marker of autoimmune thyroid disease.
2. In thyroid cancer patients where thyroglobulin is used as a tumour marker. The presence of thyroglobulin antibodies may interfere with the measurement of thyroglobulin.

## The new thyroglobulin antibody assay

The new Siemens a-TGII assay is a fully automated, one-step, analyte-bridging immunoassay using acridinium ester chemiluminescent technology. This assay uses human thyroglobulin in both the Lite Reagent and the Solid Phase. A direct relationship exists between the amount of anti-TG present in the patient sample and the response detected by the system.

The design of the new assay is shown below.



Repeatability and within-laboratory imprecision are less than 5%. The assay has a limit of quantitation (LoQ) of 1.8 IU/mL, with a linear measuring range up to 1000 IU/mL. Interference testing results showed there was no interference from Biotin. There was no hook effect even at very high levels of thyroglobulin antibody. A cut off value for autoimmune thyroid disease was determined to be 4.5 IU/mL.

The assay is standardised to the World Health Organization (WHO) International Reference Preparation, Anti-Thyroglobulin Serum, Human (NIBSC 65/093).

The advantages of the new assay are:

- Smaller sample volume
- Improved calibration stability
- Wider working range
- Directly standardised to WHO, with results reported in IU/mL rather than a manufacturer's proprietary unit
- Improved sensitivity
- Improved lot to lot variation

## Comparison with the old assay

The numerical correlation between the old and new assays is poor.

The concordance analysis shows a better picture with most negative results agreeing between the two says. A proportion of the patients who previously tested as positive will now be regarded as negative.

## Concordance analysis

		aTG (Old)		
		Negative	Positive	Total
a-TGII	Negative	289 (63%)	62 (14%)	351 (77%)
	Positive	25 (5%)	80 (18%)	105 (23%)
	Total	314 (69%)	142 (31%)	456 (100%)

The differences observed between these assays can be attributed to differences in standardisation, methodology and assay architecture. The wide heterogeneity of both exogenous antibodies and patient autoantibodies generally leads to poor correlation between different a-TG assays.

Our laboratories will be running both the old and new assay in parallel for several months. We will only be reporting the new assay; however, the old assay results can be obtained if required.

## Summary

- Siemens have introduced a new and improved thyroglobulin antibody assay (a-TGII), which will replace their existing assay shortly.
- The assay uses a different test methodology, standardisation and unit.
- The results will be numerically different from the old assay.
- There is variable correlation between the new and old results.
- We will perform both assays during the transition period but only report the new result.
- The new reference range is <4.5 IU/ml.

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# Reproductive genetic carrier screening

By Associate Professor Mirette Saad



Reproductive carrier screening is recommended to provide couples with options to reduce the risk of having a child with a severe genetic disorder. In line with current guidelines, Australian Clinical Labs is pleased to offer a wide range of genetic carrier screening options for your patients to identify individuals at risk, and can be performed in a timely and cost-effective manner. Our current offerings include “Gene Access” screen (three common conditions with serious impact - Cystic Fibrosis {CF}, Spinal Muscular Atrophy {SMA} and Fragile X Syndrome {FXS}), Comprehensive Carrier Screening (over 100 inherited diseases) and Ashkenazi Carrier Screening (eight conditions common in people of Ashkenazi Jewish ancestry).

## Genetic Counselling Services

Clinical Labs offer counselling services for positive cases of the tests we offer (no extra cost) upon your request and referral.

## Genetic carrier screening should be offered for every pregnancy

The Royal Australian and New Zealand College of

Obstetricians and Gynaecologists (RANZCOG) guidelines recommend that genetic carrier screening is offered for every woman and couples who are either planning or in the first stage of pregnancy, regardless of their risk factor. It's recommended that every woman be offered a genetic screening test for recessive conditions.

It is particularly important for the following groups:

- Currently pregnant or planning a pregnancy (including IVF)
- At increased risk for a specific disorder based on their ethnicity
- Have a family history of a genetic disorder
- Are planning to donate eggs, sperm or embryos

## When to test?

Genetic carrier screening should be offered to individuals or couples who are either planning or in the first stage of a pregnancy, with or without a family history. Ideally, screening is performed prior to conception to offer greater reproductive choice. Early detection is paramount as it allows more time for counselling and offers greater reproductive options for those at risk.

## Genetic carrier screening options available at Clinical Labs

	GENE ACCESS CARRIER SCREENING	COMPREHENSIVE CARRIER SCREENING	ASHKENAZI JEWISH CARRIER SCREENING
<b>Test information</b>	Gene Access gives patients information regarding their risk of having a child with cystic fibrosis (CF), fragile X syndrome (FXS) or spinal muscular atrophy (SMA). Tests for these conditions can be ordered individually or together as a group. <ul style="list-style-type: none"> <li>• One in 20 individuals are carriers of at least one of these conditions</li> <li>• 90% of carriers do not have a family history</li> <li>• One in 160 couples will be found to be at risk of having an affected child</li> </ul>	Comprehensive Carrier Screening screens up to 301 genes, by analysing up to 400 genetic mutations, to evaluate an individual's carrier status for more than 100 inherited diseases. <ul style="list-style-type: none"> <li>• 288 genes tested + an additional 13 optional</li> <li>• Enhanced SMA testing to help identify silent carriers</li> <li>• 21 X-linked disorders</li> <li>• For a full list of genes available, please see <a href="https://antenatal.clinicallabs.com.au/doctor/resources">antenatal.clinicallabs.com.au/doctor/resources</a></li> </ul>	Ashkenazi Jewish Carrier Screening can determine whether a patient is a carrier of any of the eight genetic conditions we test for that are more common in people of Ashkenazi Jewish ancestry.
<b>Population</b>	All ethnicities	All ethnicities	Ashkenazi Jewish population
<b>Genetic conditions</b>	CF, SMA & FXS	Comprehensive (100+ inherited diseases)	<ul style="list-style-type: none"> <li>• Tay-Sachs Disease</li> <li>• Canavan Disease</li> <li>• Niemann-Pick Disease</li> <li>• Bloom Syndrome</li> <li>• Cystic Fibrosis</li> <li>• Fanconi Anaemia</li> <li>• Familial Dysautonomia</li> <li>• Mucopolysaccharidosis IV</li> </ul>
<b>When to test?</b>	Ideally, prior to conception; otherwise, in early pregnancy.	Ideally, prior to conception; otherwise, in early pregnancy.	Ideally, prior to conception; otherwise, in early pregnancy.
<b>Cost</b>	CF: \$150, FXS: \$100, SMA: \$195 Total fee for all three: \$350. If patient is positive for a particular condition, partner can be tested for free - ONLY for this particular condition.	\$790 (Partner testing is \$700)	\$330
<b>Sample type</b>	Blood sample	Blood sample	Blood sample
<b>TAT</b>	7-10 business days	3-5 weeks	7-14 business days
<b>How to order?</b>	Fill out the Genetic Carrier Screening request form. You may request CF, FXS or SMA individually or as a group. The request form can be downloaded here: <a href="https://antenatal.clinicallabs.com.au/doctor/resources">antenatal.clinicallabs.com.au/doctor/resources</a>	Fill out the Comprehensive Carrier Screen request form. The request form can be downloaded here: <a href="https://antenatal.clinicallabs.com.au/doctor/resources">antenatal.clinicallabs.com.au/doctor/resources</a>	Fill out the Genetic Carrier Screening request form. The request form can be downloaded here: <a href="https://antenatal.clinicallabs.com.au/doctor/resources">antenatal.clinicallabs.com.au/doctor/resources</a>

## Reproductive Carrier Screening and New Medicare Benefits Schedule

A new MBS item is currently being considered to publicly fund the reproductive carrier screening for CF, FXS and SMA conditions for prospective parents.

For more information on genetic carrier screening services offered by Clinical Labs, please see the brochures available at [antenatal.clinicallabs.com.au/doctor/resources](https://antenatal.clinicallabs.com.au/doctor/resources).

If further information regarding testing is required, or you need to discuss a patient, please contact Associate Professor Mirette Saad as below.

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Associate Professor Mirette Saad is a Consultant Chemical Pathologist and the National Clinical Director of Molecular Genetics at Australian Clinical Labs. At Clinical Labs, A/Prof Mirette Saad leads the Molecular Genetic testing for non-invasive prenatal testing (NIPT), antenatal screening, personalised drug therapy and cancer. She is a Chair of the RCPA Chemical Pathology Advisory Committee, Member of the RCPA Genetic Advisory Committee, AACB and a Chair of the Precision Medicine Services at Australian Clinical Labs.

# Genetic carrier status and early pregnancy screening

By Dr Stuart Prosser



Dr Stuart Prosser is the Founder and Medical Director at One for Women and has extensive experience in General Practice, GP Obstetrics and Anaesthetics. He consistently advocates for a multi-disciplinary approach to health care, one that increases the quality of both the patient experience and outcomes.

At One for Women, we are increasingly seeing patients early in their pregnancy - prior to 10 weeks' gestation. This is fantastic as it allows us to undertake early pregnancy screening and education.

The initial consultation involves assessment for pre-eclampsia risk, cervical length shortening risk and importantly involves a discussion around genetic carrier screening and first trimester screening. While most patients are aware of first trimester screening with a large move towards NIPT and structural ultrasound over recent years, the understanding and knowledge of genetic carrier screening remains low despite a RANZCOG recommendation that all pregnant women should be offered this test.

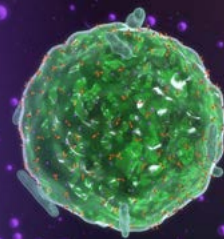
Ideally, we would like to discuss genetic carrier screening pre-conception to allow the prospective couple to know if they are carriers prior to considering having a baby together. If both partners are found to be carriers of an autosomal recessive condition, or if the female partner is a carrier of an X-linked condition, they have a 1 in 4 chance of each pregnancy being affected by the condition. They are then offered referral to a genetic counsellor to consider their reproductive options.

Once pregnant, options become more limited - diagnostic testing and the difficult decision of whether to continue with the pregnancy. Each of the genetic conditions causes significant morbidity and leads to shortened life expectancy.

In the last few years, I have seen an increased referral rate for genetic testing. Thankfully, I am starting to capture the patients prior to conception, allowing for screening to be undertaken and appropriate counselling about risk of inherited genetic conditions. In a similar way to NIPT testing, I believe that as the knowledge of the importance of genetic carrier screening increases, the rate of testing will continue to climb.

# Assessing allergy in clinical practice

By Associate Professor Louise Smyth



**Allergy is clinically, economically and socially important. It is common, may be life-threatening and results in lost school or work attendance. Furthermore, it can be intensely unpleasant for the patient.**

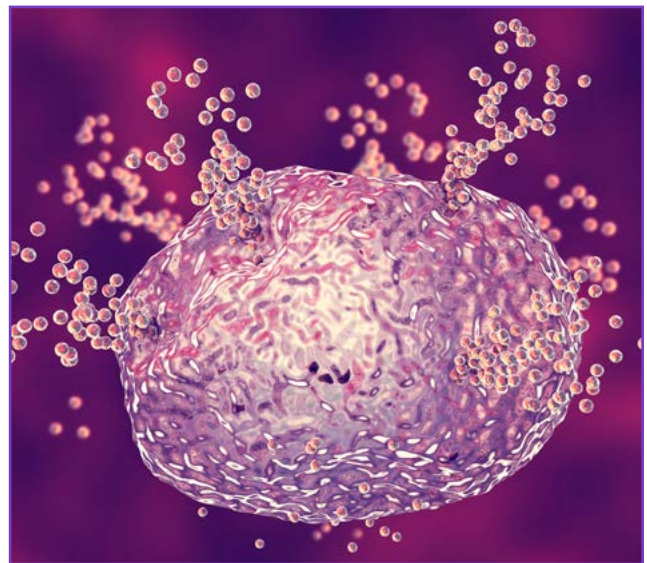
## Immune responses

Immune responses represent a continuum of protective strategies that have developed from very ancient needs. As multicellular, and then vertebrate, animals evolved, systems were required that could sustain, control and protect parts that are not directly contiguous with sources of energy production (nutrients, oxygen) nor able to directly remove wastes. All of these systems required a structural base and a rapid response mechanism. The outcome was a circulatory system, nervous and endocrine systems and the immune system. Each functioned through intimate, two-way interaction with the respiratory and gastrointestinal tracts, blood and genitourinary tract.

Protection of the complicated and expanded organism extended mechanisms used by single-celled organisms, biofilms and simple multicellular organisms, as well as some of the cellular interaction tools of invertebrates. Adaptive, or learned, immunity developed alongside the vertebrate nervous system and was supplementary to, augmenting, and dependent upon earlier mechanisms that largely comprise the innate immune responses.

Innate immunity continues to contribute barrier responses on skin and mucosal surfaces where the vertebrate organism's frontier is both exposed to danger and sources its supplies. It provides the earliest responses to colonisation of surfaces or invasion of tissues and it samples foreign material for assessment and response by the adaptive, lymphocyte-based, immune response. Critically, the acute inflammatory response of innate immunity both transports and presents foreign material to lymphocytes in secondary lymphoid tissue (lymph nodes, spleen, MALT, SALT), and carries accompanying coded messages that indicate the presence or absence of tissue damage. Thus, an adaptive immune response is generated by two distinct signals that define threatening non-self. Thirdly, the detailed milieu of the self/non-self-interaction generates differing chemical messaging via informative combinations of secreted cytokines that guide the direction of that response. These responses are predicated upon cells of innate immunity, in the form of *Antigen Presenting Cells (APCs)*, engaging those of adaptive immunity.

Adaptive immunity is characterised by its anamnestic nature. Not only is the cause of the response (antigen or allergen) remembered, but also the result of the first encounter. Following, and dependent upon, the innate response to damage, there is a combined T cell and B cell response whereby lymphocytes are engaged in maturation pathways that lead to the eradication of the threat.

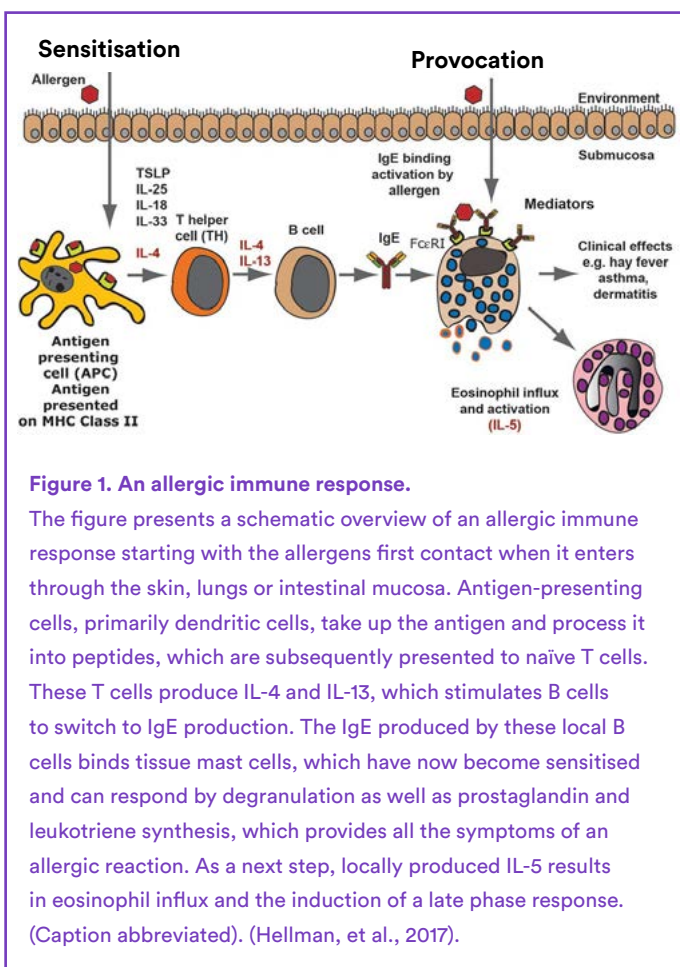


Antigens that can be engaged in the extracellular space can be dealt with by a coterie of soluble proteins, based upon the specificity of the B cell product: immunoglobulin. Which sort of immunoglobulin is directed by Helper T cells with differing profiles.

Intracellular pathogens require a response that kills the host cell. These pathogens require a cytotoxic T cell response.

Other pathogens exist primarily on the mucosal surfaces and are too big or unavailable for phagocytosis by cells of the innate response, e.g. helminths. For these, the adaptive immune response has developed an extracellular response dependent upon releasing pre-formed inflammatory (innate) mediators, directly onto the offending agent. This is driven by TH2 type T cell help directing B cell immunoglobulin class-switching to IgE production and is achieved by pre-engaging the responding cell (mast cells) by IgE bound to its membrane receptor FcεRI. Cross-linking of membrane bound specific IgE by its recognised (cognate) antigen results in mast cell degranulation, releasing packets of pro-inflammatory mediators. These cause local inflammation. Basophils and eosinophils are also involved in this response (Figure 1).



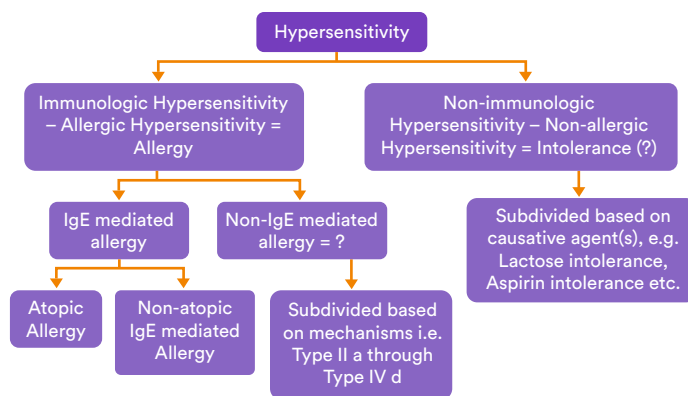


T cell help for B cells not only directs the subsequent immune response but also results in a small proportion of educated B cells converting to memory cells. These cells are on shortened pathways of specific recognition and response and are the basis of immune memory and avoidance of disease upon subsequent exposure, the secondary immune response. This response is further characterised by higher levels of class-switched antibody production and antigen receptor editing that results in increased affinity and avidity. Therefore, the secondary immune response resembles a clever Olympic athlete; it goes higher, stronger and faster and has an impeccable memory.

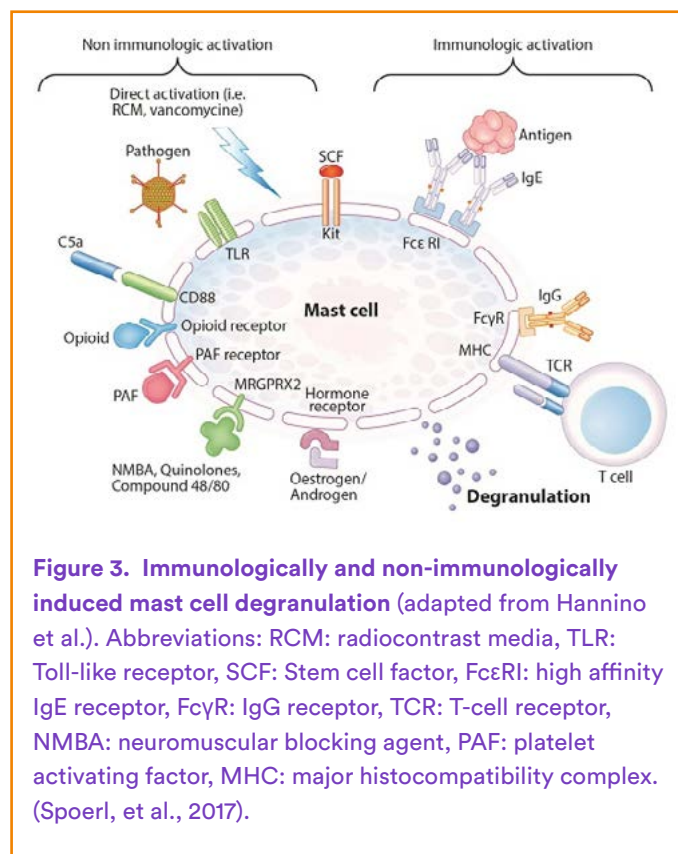
### Hypersensitivity, allergy and intolerance

Hypersensitivity reactions are those immune responses that result in tissue damage that is disproportionate to their defence function because they are excessive or inappropriate. In 1963, Gell and Coombs first published their seminal “Classification of allergic reactions underlying disease” that, although revised and modified by later authors, formed the basic approach to the pathophysiological mechanisms involved in both allergy and autoimmunity. The original classification designated IgE mediated, immediate hypersensitivity responses as Type I responses. These are the classical allergic responses. Since then, some drug and food allergies,

eczematous dermatitis and chronic hypersensitivity responses (such as chronic urticaria, asthma, chronic rhinitis, Eosinophilic Oesophagitis) have been shown to represent complicated and overlapping immune responses that may, or may not, be easily assessed in the routine laboratory (Figure 2). Importantly, more than one allergic disorder may exist in the same patient. Associated disorders, such as nasal polyps, are outside the scope of this article.



**Figure 2. Illustration of proposed minor changes of the food hypersensitivity nomenclature, using “intolerance” as short for “non-allergic hypersensitivity”/“non-immunological hypersensitivity”.** Dreborg, S. World Allergy Organization Journal (2015).



**Figure 3. Immunologically and non-immunologically induced mast cell degranulation** (adapted from Hannino et al.). Abbreviations: RCM: radiocontrast media, TLR: Toll-like receptor, SCF: Stem cell factor, FcεRI: high affinity IgE receptor, FcγR: IgG receptor, TCR: T-cell receptor, NMBA: neuromuscular blocking agent, PAF: platelet activating factor, MHC: major histocompatibility complex. (Spoerl, et al., 2017).

Furthermore, some clinical mimics or similarities may make assessment difficult (e.g. hereditary angioedema vs urticaria) and some clinical manifestations may have “allergic” and “non-allergic” aetiologies. Allergy, however, is perhaps the most deserving clinical state for the aphorism: listen to the patient for long enough and they’ll tell you the diagnosis. Intolerance should, strictly, be reserved for those clinical manifestations that do not have an immunologic pathogenesis, e.g. lactose intolerance due to decreased enzyme activity.

Therefore, symptoms and signs induced by a specific agent can be described as immune (allergic) or non-immune hypersensitivity responses and, immune hypersensitivity responses as Type I/Immediate/IgE mediated or non-IgE mediated allergic responses.

## Looking at allergy in routine clinical practice

All secondary immune responses are a response to previous exposure, including allergy, although that exposure may be difficult to identify (e.g. food allergens in infants/toddlers). Hence, the exposure history is usually centred on the clinical manifestation of allergic (or possible allergic) symptoms and signs. These may be obvious, particularly with food allergy where oral symptoms may arise within seconds to minutes following exposure (oral allergy syndrome – OAS), or they may require more, or less, detailed examination of the environment. Some simple approaches may be useful in persons with limited allergies: pollens are prominent outdoors in spring and summer, moulds – indoors especially in winter (but possibly perennial), house dust mite indoors and all year, animal dander following specific exposures, etc. It is also true that there are many “overlapping” allergies due to the fact that the allergen is often a highly conserved, shared peptide in related species, e.g. certain foods, pollens, insects, etc. This information is important in selecting laboratory testing of specific IgE, which can be used to manage, by avoidance or immunotherapy, severe or nuisance allergies. Other important tests may include eosinophil count (or activity) but testing should have a focus that is relevant to the symptoms produced after a timely exposure history.

Both skin prick testing (SPT) and serum specific IgE (previously RAST) are useful for identifying the presence of allergen-specific IgE in a given patient and are useful in assessment of *allergic rhinitis*. However the presence of antibody does not equal disease and results must be interpreted in the clinical context.

## Common respiratory allergens

- House dust mite
- Pollen
- Animal dander
- Mould

*Food allergens* present several diagnostic difficulties, partly because only certain foods/components are mandated in labelling by Australian food standards and partly because of “unseen contamination”, e.g. during food preparation. Furthermore, some non-food items (e.g. cosmetics) may contain food allergens. Food ingestion may result in a variety of clinical manifestations ranging from OAS through skin reactions to life-threatening anaphylaxis. Most food allergens are primarily managed by avoidance but diets can be restrictive and so it is important to correctly identify the relevant allergen. According to current information ASCIA states that food allergy occurs in around 2% of Australian and NZ adults with increased numbers in childhood, up to about 10% of infants. Again, SPT and specific IgE are useful for identifying the presence of allergen-specific IgE in a given patient, although SPT may be impractical in young children. Elimination diets and challenge may be required but should be conducted under specialist advice/supervision. A recent review by Eckman demonstrates the strong positive predictive values of serum specific IgE for certain food allergy diagnosis for atopic dermatitis in children (Table 1).

## Common food allergens

- Egg
- Cow’s milk
- Peanut
- Tree nuts
- Sesame
- Soy
- Fish
- Shellfish
- Wheat

Some less common food allergens, such as red meats, may be clinically important.

*Adverse drug reactions (ADRs)* are probably the most complex clinical event requiring consideration of an allergic response. ADRs are classified Type A if they are predictable due to the known properties of the drug and Type B if they are unpredictable or idiosyncratic. Type B reactions include allergic reactions that may be IgE mediated immediate responses, including anaphylaxis, or several cell-mediated immune responses. The clinical history is crucial in assessing drug reactions of all types. While serum specific IgE is available in the investigation of many potential drug allergies, specialist clinical assessment may be required. It is important to consider de-labelling of some patients who believe that they are penicillin-allergic, since potentially important therapeutic interventions may be unnecessarily curtailed.

*Insect* venoms are an important cause of allergy including anaphylaxis. Specific IgE is available for most clinically important insect venoms. Other insect allergens may

cause respiratory or skin symptoms and assessment can also be assisted by serum specific IgE.

Other in-vitro tests that may be used in the investigation of allergy include mast cell tryptase (obtained within 6hrs of possible anaphylaxis, preferably 1-2hrs) and serum complement. Allergic urticaria is usually of shorter duration than that associated with autoimmune disorders.

Some cases may be assisted by detection of serum specific IgE. Latex and contact allergies, such as nickel, are frequently assessed by clinical investigation.

Useful and updated guidelines for the clinical management of allergy are available from the ASCIA website ([www.allergy.org.au](http://www.allergy.org.au)).

**Table 1. Comparison of studies reviewing the positive predictive values of food specific IgE testing.**

Adapted from: Diagnostic evaluation of food-related allergic diseases (Eckman, et al., 2009).

Study	No. subjects	% Atopic dermatitis	Food	PPV value %/ Specific IgE level	Sens. for IgE level	Spec. for IgE level
Sampson HA	62	61%	Cow's milk	95%/15	57%	94%
Sampson HA and Ho DG	196	100%	Cow's milk	95%/32	51%	98%
Celik-Bilgili S et al	398	88%	Cow's milk	90%/88.8	*	*
Garcia-Ara C et al	170	23%	Cow's milk	95%/5	30%	99%
Osterballe M et al	56	100%	Egg white	100%/1.5	60%	100%
Boyano Martinez T et al.	81	43%	Egg white	94%/0.35	91%	77%
Celik-Bilgili S et al.	227	88%	Hen's egg	95%/12.6	*	*
Sampson HA and Ho DG	196	100%	Hen's egg	95%/6	72%	90%
Sampson HA	75	61%	Hen's egg	98%/7	61%	95%
Sampson HA	68	61%	Peanut	100%/14	57%	100%
Sampson HA and Ho DG	196	100%	Peanut	95%/15	73%	92%
Maloney JM et al	234	57%	Peanut	99%/13	60%	96%

#### How to order:

Complete a Clinical Labs General Pathology Request Form requesting serum specific IgE testing and clearly list the allergens to be tested. Please be as specific as possible with your selection, based on the detailed clinical history you have taken from the patient.

Note: Medicare will fund a maximum of four patient episodes per 12 month period. Each patient episode requires a new request form and specimen collection. A maximum of four single allergens or four multi panel mixes can be tested for each episode request, with the exception of ALATOP. ALATOP can be ordered with only one other single or multi panel mix. Any tests ordered in addition to this will be charged as an out of pocket cost to the patient.

#### References and further reading

- Gell, P. G. H. & Coombs, R. R. A. (1963). Clinical aspects of immunology. 1963 Blackwell Scientific. Oxford.
- Hellman Lars Torkel, Akula Srinivas, Thorpe Michael, Fu Zhirong. Frontiers in Immunology. 2017, Vol. 8, p1749. <https://doi.org/10.3389/fimmu.2017.01749>
- Spoerl D, Nigolian H, Czarnetzki C, Harr T. International Journal of Molecular Sciences. 2017, 18(6):1223. <https://doi.org/10.3390/ijms18061223>
- Eckman, J. Allergy, Asthma & Clinical Immunology 2009, 5:2 doi:10.1186/1710-1492-5-2 <http://www.aacijournal.com/content/5/1/2>
- Dreborg, S. World Allergy Organization Journal (2015) 8:37 <http://doi.org/10.1186/s40413-015-0088-6>
- Australian Society of Clinical Immunology and Allergy [www.allergy.org.au](http://www.allergy.org.au)

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# Providing your patients with the gold standard in cardiac health diagnostics



At Clinical Labs we offer superior and cost effective ambulatory blood pressure and Holter monitor services for your patients.

## Ambulatory blood pressure monitors

### Why choose ambulatory blood pressure monitoring (ABPM)?

Recognised as a gold standard test for blood pressure assessment, ABPM provides interval measurement over 24hrs for a full diagnostic assessment of blood pressure, while allowing for circadian rhythm and white coat or masked hypertension concerns. It is also effective in the assessment of drug effects, patient activity and work influence while providing better correlation with end-organ damage than isolated BP readings.

- Expert interpretation by cardiologist
- Rapid report turnaround

### How does ABPM work?

Automatic cuff inflation every 30 minutes during the day and every 1hr overnight, with ambulatory pulse waves, peripheral blood pressure, central haemodynamics, and arterial stiffness measured over a period of 24hrs to create a haemodynamic day/night profile. At the end of each test, comprehensive analysis is completed and a report is provided by experienced cardiologists.

### How to order:

Once your patient has their request form they will need to call a Clinical Labs Collection Centre offering this service to book a time to collect and be fitted with their ABPM. Patients in SA will need to call (08) 8205 5644.

### Turn-around time:

Your patient's ABPM report will be available 3 business days from the return of the ABPM to the Clinical Labs Collection Centre.

### Medicare Benefits Schedule Changes

There is currently an \$150 out of pocket cost for ABPM. From 1 November 2021, certain patients may be eligible for a Medicare rebate.

### Eligibility requirements:

- The item can only be claimed once in a 12-month period
- The patient must have an in-clinic BP of 140/90 to be eligible
- There is no requirement for the patient to be hypertensive to be eligible
- The item is only applicable to the diagnosis of hypertension and not the monitoring of hypertension

## Holter monitors

The resting 12-lead electrocardiograph (ECG) only gives a rhythm snapshot in time, whereas arrhythmias may be short-lived, paroxysmal and even asymptomatic, making documentation in many patients very difficult. Ambulatory ECG monitoring has been developed as a means of recording the ECG over a set period of time in patients suspected of having cardiac arrhythmias. A Holter monitor is preferable to all other monitoring as it provides a 24hr window of the heart rate changes and can be partnered to symptoms.

### The role of GPs in cardiac testing

Cardiologists prefer that patients presenting with the common warning signs, including palpitations, unexplained dizziness and slow heart rhythms, undertake Holter monitor testing from their GP before they are referred on.

### How to order:

Once your patient has their request form they will need to call a Clinical Labs Collection Centre offering this service to book a time to collect and be fitted with their Holter monitor. Patients in SA will need to call (08) 8205 5644.

### Cost:

Out of pocket cost of \$40 for Medicare card holders, bulk billed for pension card holders. \$185.50 out of pocket cost for each additional hour after first 24hrs.

### Turn-around time:

Your patient's Holter monitor report will be available within 3 business days from the return of the Holter monitor to the Clinical Labs Collection Centre.

## ECG

Since its introduction as a clinical investigative tool, the 12-lead ECG has been the gold standard for recognition of cardiac arrhythmias.

### How to order:

ECG is a walk in service, there is no need to book. Once your patient has their request form your patient can attend any Clinical Labs Collection Centre offering the ECG service.

### Cost:

Out of pocket cost of \$40 for Medicare card holders, bulk billed for pension card holders. (\$45 out of pocket for inpatients).

### Turn-around time:

Your patient's ECG report will be available 4hrs after their test at the Clinical Labs Collection Centre.

**Ambulatory blood pressure monitor, Holter monitor and ECG services are available at select Clinical Labs collection centres. To find the nearest collection centre offering these services, visit [clinicallabs.com.au/location](http://clinicallabs.com.au/location) and select the desired service from the 'All Facilities' drop down box. These services are not available through Clinical Labs collection centres located in WA or the NT.**



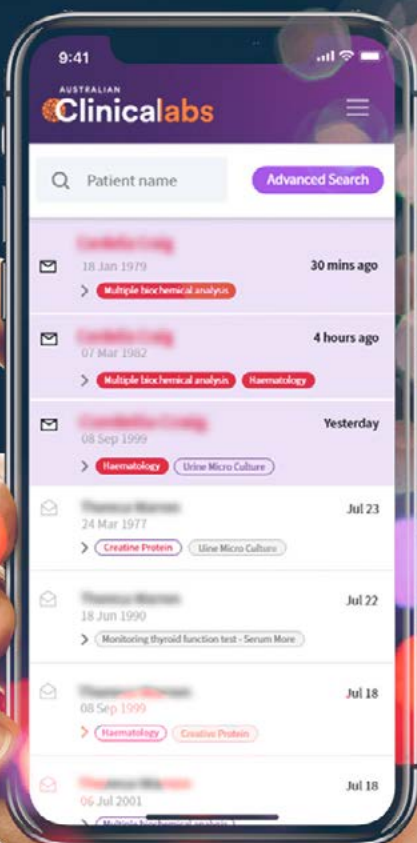
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