

PATHOLOGY FOCUS

December 2020 | Medical Newsletter 12

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- Inflammatory Bowel Disease:
A modern diagnostic perspective
- QuantiFERON-TB Gold Assay for
the Diagnosis of Latent Tuberculosis



SEASON'S
Greetings
and
HAPPY NEW YEAR!

Season's Greetings and Thank You for your Continued Support Throughout 2020

At the start of 2020, a new decade, never did we imagine we would be changing the way we worked to continue to provide healthcare during a global pandemic. We found ourselves in the same shoes as our patients, concerned about catching this new virus, as we worked quickly on processes for safely managing the health of Australians.

We saw the introduction of Telehealth and electronic pathology request forms, while strict safety protocols were put in place for face-to-face appointments, including social distancing in waiting rooms, regular cleaning, separate collection centres for COVID-19 testing and the wearing of masks/PPE. As we approach the new year, we need to continue to reassure patients that medical centres and collection centres are safe to visit.

In April this year, when the whole of Australia was faced with a stage 4 lockdown, we saw pathology testing numbers drop some 40%. This raised concerns from healthcare professionals on the future health costs of the pandemic, including increased cancer diagnoses. This is still of concern, with no end to the pandemic in sight, and we need to continue to communicate to patients the importance of attending their medical appointments and tests.

With the end of the year quickly approaching, we thank all our referrers for continuing to entrust Clinical Labs with the pathology testing for your patients. We wish you and yours an enjoyable holiday season and a safe and happy New Year. As we await a COVID vaccine, we are here to work together again in 2021, providing the best in healthcare and diagnostics for Australians.

Inflammatory Bowel Disease: A modern diagnostic perspective

By Associate Professor Louise Smyth

Chronic, diarrhoeal disease of unidentifiable aetiology is described as early as ancient Greek medical tracts and, in retrospect, both Fabry and Morgagni probably described cases prior to Matthew Baillie's 1793 report of lethality of an ulcerative colitis. Following that description, many of the chronic diarrhoeal disorders of unknown aetiology were described as ulcerative colitides. After the description by Sir Samuel Wilks, in 1859, of the autopsy changes found in a 42-year-old woman who died after several months of intractable diarrhoea and fever, the term gained prominence. When Sir William Hale White published a series of cases in 1888, "Ulcerative Colitis" (UC) entered the lexicon. However, a review of Wilks' case using modern diagnostic criteria, would prefer Crohn Disease (CD) but the seminal paper of Burrill Crohn, that differentiated his eponymous, idiopathic, chronic inflammatory disease of the bowel wall, with extra-enteric involvement, did not appear until the October, 1932, edition of JAMA, just 88 years ago, despite several earlier references to the differing patterns of disease and associated pathological findings. Nevertheless, the clinical presentation may not always be clear, especially during the first months of symptoms, and some cases may remain as undifferentiated colitis while others are otherwise resolved. The association between UC and colorectal carcinoma had already been reported, following the introduction of safe sigmoidoscopy, in 1909 by John Lockhart-Mummery. (Mulder, Noble, Justinich, & Duffin, 2014)

The mean Australian annual incidence rate for IBD reported to EpiCom (European Crohn's and Colitis Organization–Epidemiological Committee) in two independent cohorts from 2010 and 2011 was 30.3/100,000 (Vegh. et al. 2014). The prevalence in Australia is expected to increase by approximately 250% from 2010 to 2030. Furthermore, while still predominantly a disease affecting young adults, where the incidence may be reaching a plateau in Western countries (Weimers & Munkholm, 2018), both paediatric and elderly patients are increasingly being diagnosed (Gower-Rousseau, et al., 2013). In addition, these special groups and others may present with different disease phenotypes and require different treatment regimens. In this Northern French population, of similar size to that of Victoria, the incidence rate of CD increased from 5.3 to 7.6 per 10⁵ over the study period (1988-2008) while UC remained stable. Strikingly, the incidence of CD in patients under 20 years old nearly doubled, a finding replicated in Italy, Ireland, Scotland and Scandinavia. Importantly, the extent of disease and the inflammatory phenotype was shown to be more severe in younger patients. In fact, the Montreal criteria for the evaluation of adult IBD has been supplemented by the Paris criteria for paediatric disease.

Table 1 - Montreal and Paris Classifications for Crohn's Disease (CD)

	Montreal	Paris
Age at diagnosis	A1: < 17 y A2: 17-40 y A3: > 40 y	A1 a: < 10 y A1 b: 10-17 y A2: 17-40 y A3: > 40 y
Location	L1: Terminal ileal ± limited cecal disease L2: Colonic L3: Ileocolonic L4: Isolated upper disease*	L1: Distal 1/3 ileum ± limited cecal disease L2: Colonic L3: Ileocolonic L4 a: Upper disease proximal to Ligament of Treitz* L4 b: L4b: Upper disease distal to ligament of Treitz and proximal to distal 1/3 ileum*
Behaviour	B1: Non-stricturing, non-penetrating B2: Stricturing B3: Penetrating P: Perianal disease modifier	B1: Non-stricturing, non-penetrating B2: Stricturing B3: Penetrating B2B3: Both penetrating and stricturing disease, either at the same or different times P: Perianal disease modifier
Growth	n/a	G0: No evidence of growth delay G1: Growth delay

*In both the Montreal and Paris Classification systems, L4 and L4a/L4b may coexist with L1, L2, L3, respectively.

(After Levine et al., 2011)

Table 2 - Montreal and Paris Classifications for Ulcerative Colitis (UC)

	Montreal	Paris
Extent	E1: Ulcerative proctitis E2: Left-sided UC (distal to splenic flexure) E3: Extensive (proximal to splenic flexure)	E1: Ulcerative proctitis E2: Left-sided UC (distal to splenic flexure) E3: Extensive (hepatic flexure distally) E4: Pancolitis (proximal to hepatic flexure)
Severity	S0: Clinical remission S1: Mild UC S2: Moderate UC S3: Severe UC	S0: Never severe* S1: Ever severe*

*Severe defined by Pediatric Ulcerative Colitis Activity Index (PUCAI) ≥65.

(After Levine et al., 2011)

In this French, community-based study, the increased severity of early onset disease was evident despite greater use of immune suppression and biological anti-inflammatories, in this group.

“... clinical symptoms on diagnosis were more subtle in the elderly than in the younger age groups with less diarrhoea, abdominal pain, weight loss, fever and extra-intestinal manifestations (EIMs) in CD and less rectal bleeding and abdominal pain in UC. ... As for phenotypes, elderly onset CD was characterised by the predominance of pure colonic disease (L2) and inflammatory behaviour (B1) in accordance with the literature and in sharp contrast with the youngest age-group. On the other hand, UC location on diagnosis was more alike in the young-onset and elderly onset groups with more extensive disease than in the middle-age group.” (Gower-Rousseau, et al., 2013)

In the same study there was significantly more disease extension, stricturing and perforating behaviour in patients <17 years at diagnosis compared to those >60 years.

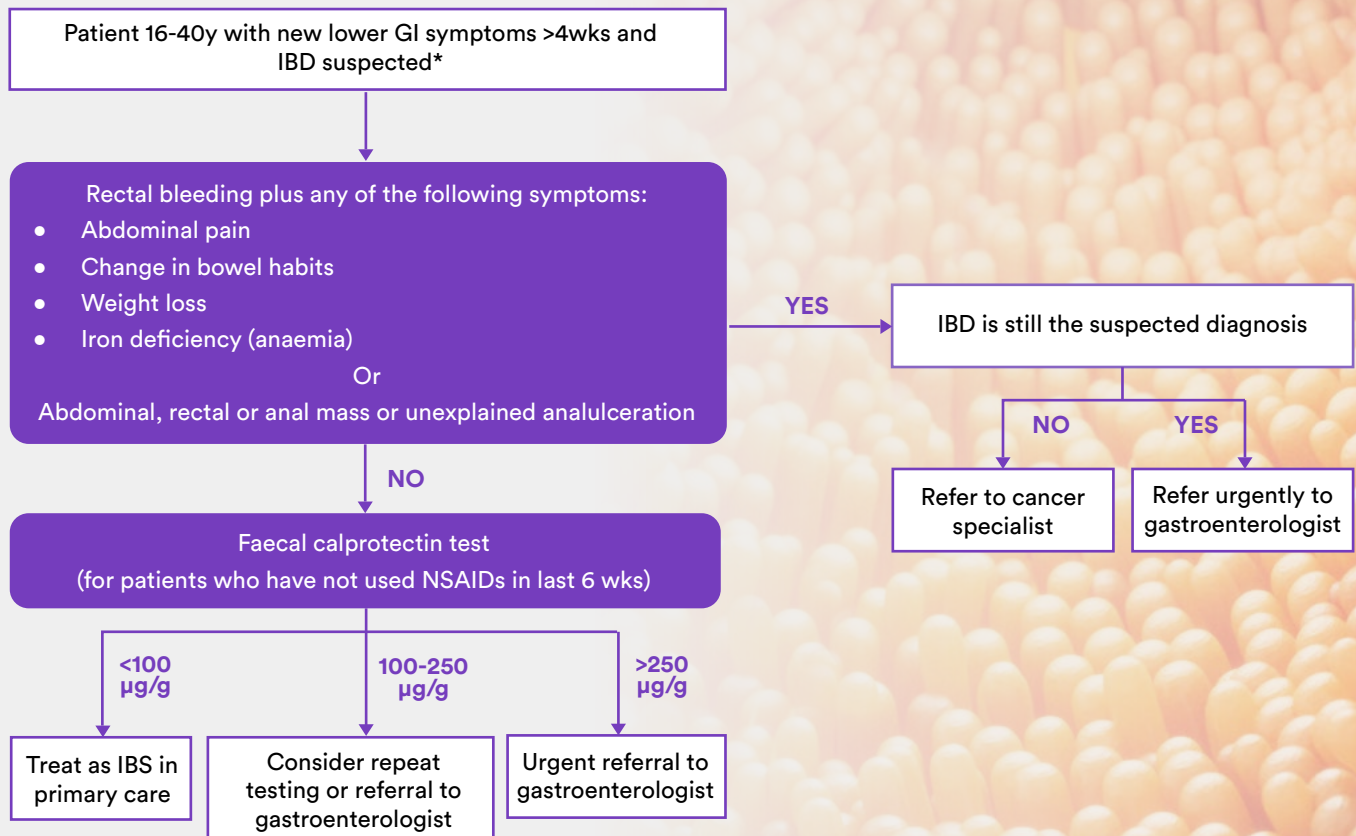
Elsewhere, an association with **Primary Immune Deficiency** (especially NOD2 gene & IL10 pathway) has been established in up to 20% of children with very early onset disease (<5 years) and these children should be assessed by a paediatric Clinical Immunologist (Kelsen & Sullivan, 2017). In all age groups, **intestinal infection should be excluded** as a cause of presenting symptoms, especially in high-risk groups but, infection is also an important complication of IBD (especially penetrating

CD) and presents a risk to patients who require immune suppression.

While the aetiology and pathogenesis of IBD remains unclear, it is ever more clear that they represent a complex interaction of genetic susceptibility and environmental factors resulting in abnormal immune responses directed against the gut microbiota of affected individuals. The changing epidemiology, detailed by Kaplan and Windsor (Kaplan & Windsor, 2020), underlines the association with increasing Westernisation/industrialisation. **Diagnosis is predicated upon endoscopy, imaging and histology** but features may change in serial small biopsies of bowel and a significant proportion of diagnoses are only secured after colectomy. **Selection of patients for diagnostic endoscopy or follow-up assessment of disease activity can be enhanced by the measurement of blood and faecal biomarkers, which may also favour one disease over the other, minimising expensive, invasive, higher-risk investigation.** It is especially useful to identify patients without evidence of bowel wall inflammation at presentation, who are more likely to have Irritable Bowel Syndrome (IBS). The usefulness of biomarkers has been established in adults (Kochhar & Lashner, 2017) and in children (Elitsur, Lawrence, & Tolaymat, 2005).

UK Guidelines suggest the following approach to differentiate patients presenting with symptoms that may be attributable to IBD or IBS.

Figure 1 – Use of Faecal Calprotectin Test in Primary Care



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

Table 3 - Usefulness of tests available at Clinical Labs for the assessment of possible or established IBD, other than biopsy.

Biomarker	Source	Clinical Use	Sensitivity	Specificity
Acute phase reactants				
CRP	Blood	General measure of systemic inflammation. CRP increased in most with active inflammation. CD > UC.	High	Low
ESR	Blood	General measure of systemic inflammation. ESR peaks and decreases slower than CRP. May be better at monitoring disease activity/response to treatment after the first 24 hours of onset while CRP may be more useful in the first 24 hours.	High	Low
Antibodies		Best used in combination.		
Anti-Saccharomyces cerevisiae antibodies (ASCA)	Blood	Adults and children with CD.	Low to moderate	Moderate to high
Anti-perinuclear antineutrophil cytoplasmic antibodies (p-ANCA)	Blood	Adults and children with UC.	Moderate to high	Low to moderate
ASCA+/pANCA-	Blood	Favour CD in clinical IBD.	Moderate	High
ASCA-/pANCA+	Blood	Favour UC in clinical IBD.	Moderate	High
Calprotectin	Faeces	IBD versus IBS. Activity of IBD & postoperative recurrence of CD.	High	Moderate. Measures granulocyte-mediated inflammation in the bowel wall. RI varies by age in children.

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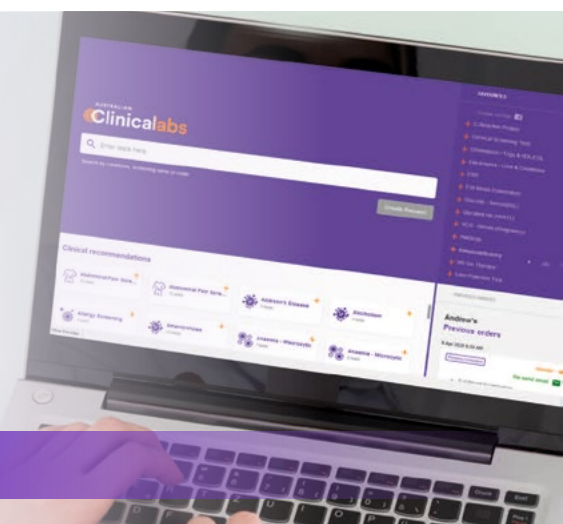


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Follow-up on Coeliac Disease (CD) Testing

Once a proband has been identified it is important to screen asymptomatic first-degree relatives (or other close relatives who may have symptoms). There are two schools of thought regarding the best screening of first degree relatives. Initial screening for susceptibility by HLA DQ2/8 identification has been shown to be cost-effective compared to repeated autoantibody testing and endoscopy for asymptomatic individuals. HLA DQ testing is done once and patients who have neither antigen should only be further investigated for CD under specialist management.



The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) has issued guidelines, updated in 2019. According to their recommendations:

1. If CD is suspected, measurement of total serum IgA and IgA-antibodies against transglutaminase 2 (anti-tTG) is superior to other combinations.
2. If total IgA is low/undetectable, an IgG based test is indicated. (ACL routinely tests for IgG antibodies against deamidated gliadin peptide for initial testing.)
3. If anti-tTG -IgA is ≥ 10 times the upper limit of normal and the family agrees, the no-biopsy diagnosis may be applied, provided endomysial antibodies (EMA-IgA) test positive in a second blood sample and patients with positive results have been referred to a paediatric gastroenterologist/specialist.
4. Patients with no/mild histological changes but confirmed autoimmunity should be followed closely.



In addition, the American College of Gastroenterology Clinical Guideline: Diagnosis and Management of Celiac Disease recommends that in children younger than 2 years of age for CD, the IgA tTG test should be combined with deamidated gliadin peptide.

Autoantibody levels should always be tested while patients are taking a gluten-containing diet as the concentration of antibodies has been shown to disappear or fall to near-negative levels within 12-18 months of strict adherence to a gluten-free diet. Although it is not possible to definitively correlate villus healing with the disappearance of serological markers, retesting of serology should occur at least at 6 and 12 months after diagnosis, and yearly thereafter, unless symptoms recur or there are complications.

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QuantiFERON-TB Gold Assay for the Diagnosis of Latent Tuberculosis

By Associate Professor Owen Harris



Interferon Gamma Release Assays (IGRA)s are in vitro blood tests of the cell-mediated immune system. The QuantiFERON-TB Gold (QFT) assay is an IGRA measuring T-cell release of IFN- γ following stimulation by antigens specific to Mycobacterium tuberculosis complex.

The test

The QFT assay consists of four tubes: the negative-control (nil) tube that measures background IFN, a positive control (mitogen) tube with a non-specific stimulant and two antigen tubes (TB1 and TB2) for diagnosis of latent M. tuberculosis infection.

TB1 contains peptides from the specific Mycobacterium tuberculosis antigens ESAT-6 and CFP-10 designed to elicit an immune response from CD4+ T-helper lymphocytes.

TB2 contains an additional set of peptides targeting a cell-mediated immune response from CD8+ cytotoxic T lymphocytes.

The four tubes are filled with a set amount of whole blood and incubated. During incubation, the antigens stimulate lymphocytes to produce interferon, which is measured by an enzyme immunoassay.



Performance

Since there is no gold standard for latent tuberculosis infection (LTBI), sensitivity and specificity are typically estimated using surrogate reference standards.

Sensitivity is estimated among culture-confirmed TB cases, while specificity is estimated among low-risk individuals with no known TB exposure in low-incidence settings.

Use in children

Due to differences in immune function in young children, (<5 years), QuantiFERON-TB Gold is unreliable and should not be used in this age group.

Interpretation of results

According to the manufacturer, the test is interpreted as positive when either antigen tube result is positive (≥ 0.35 u/l).

The medical literature, supported by case review experience at Clinical labs, indicates that in a low prevalence population, there is a high rate of false positive (non-specific) results when either TB1, TB2 or both are in the low positive range of 0.35 to 0.70 u/l.

Results in this range need to be carefully correlated with clinical findings and epidemiological risk before making a diagnosis of latent tuberculosis.



Calcified nodules in the left upper lobe, consistent with latent tuberculosis.

Indications for treatment of latent tuberculosis

The decision to treat latent TB, to prevent progression to active TB, depends on:-

The pre-test probability, in particular immigrants and expatriates, including health care workers, from countries with a high TB prevalence.

The risk of progression to active TB: 50% of the lifetime risk of progression occurs within the first 2 years of primary infection. Age-related risks are seen in infants (50%), children (12-25%) and adolescents (10-20%).

Groups with >5 times risk include patients with HIV infection, children <5 years, patients receiving TNF- α inhibitors, patients with a history of untreated or inadequately treated TB and patients with silicosis, chronic renal failure, leukaemia, lymphoma, or cancer of the upper or lower respiratory tract.

Groups with 1-5 times risk include patients post gastrectomy, with malnutrition, cigarette smokers, abusers of alcohol and drugs, patients with diabetes mellitus and immunosuppression with agents such as prednisolone (>15 mg daily).

Efficacy of treatment requires adherence to treatment for up to 9 months. The risk of side effects such as hepatitis (aggravated by age, pre-existing liver disease and alcohol), peripheral neuritis or allergy requires consideration.

A useful reference is Management of tuberculosis: a guide for clinicians, by Denholm JT, Eisen DP, Fox G, McBryde ES, Street A., published by Greenhill Publishing, 2017. ISBN 97890648137979.

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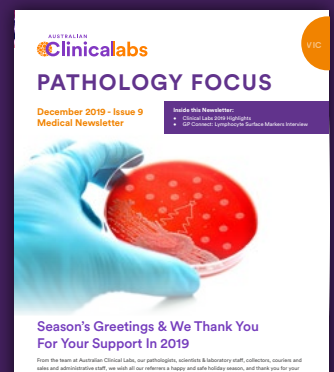
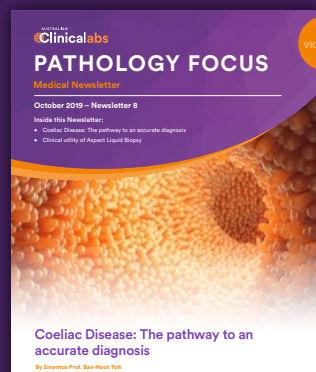
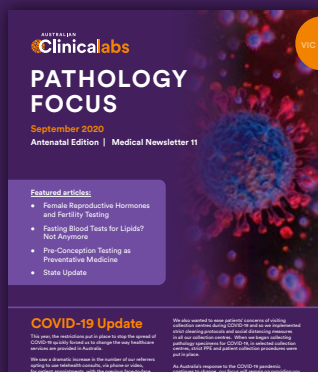
Associate Professor Harris is Clinical Director of Microbiology at Australian Clinical Labs. He graduated in Medicine from Monash University in 1971 and completed his training at Geelong Hospital, obtaining FRCPA in 1982. Associate Professor Harris worked in general pathology at Geelong Pathology followed by PathCare as Consulting Pathologist. For the past 20 years, he has been a Clinical Microbiologist, providing support for infection control and antimicrobial stewardship services at various hospitals. Associate Professor Harris has collaborated on many research projects with the Department of Infectious Diseases at Barwon Health and worked on Q fever outbreak investigations. He is involved in undergraduate and postgraduate teaching including supervision of infectious diseases/microbiology registrar training with Barwon Health. In 2015, Associate Professor Harris was appointed Senior Clinical Lecturer and Clinical Associate Professor at Deakin University.

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Some of our past articles not to be missed:

- Fertility Testing (September 2020)
- Melanoma (February 2020)
- Aspect Liquid Biopsy (October 2019)
- Vitamin D (September 2019)
- Urea Breath Testing (July 2019)
- STI Testing (October 2018)



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These tests are for asymptomatic travellers only. No Medicare rebate is available.

Travellers can order a test by:

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- ✔ After completing the test, their sample will be processed within 48 hours and they will be sent an SMS notification when their result is ready.
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If you have any questions about this testing, please contact CLWA.Covid@clinicallabs.com.au

eResults

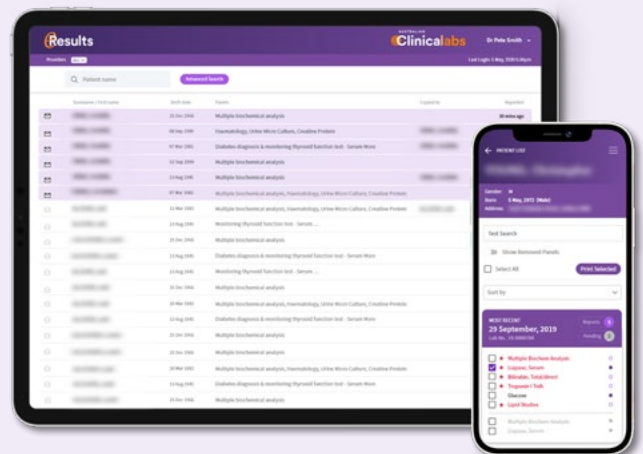
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