

PATHOLOGY FOCUS

June 2021 - Issue 14
Medical Newsletter

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- Enhanced role of Respiratory Pathogen PCR: New player disrupts established seasonal patterns
- Syphilis: An old enemy returns
- The issue of iron deficiency in women
- The end of winter is the ideal time to test patients for vitamin D deficiency

Enhanced role of Respiratory Pathogen PCR: New player disrupts established seasonal patterns

By Dr Sudha Pottumarthy-Boddu

The current COVID-19 pandemic due to the SARS-CoV-2 virus, started in late 2019 and required unprecedented public health measures to control its spread. Physical distancing, improved respiratory hygiene, and international travel and border restrictions impacted the activity of influenza and other respiratory viruses in the 2020 season (see following page for findings).

COVID-19's impact on seasonal respiratory viruses

- During 2020, the number of GP influenza-like illness (ILI) consultations were four times less than the five year average for the same period (1.6 versus 8.1 per 100 consultations)¹.
- Through the 2020 season, laboratory-confirmed influenza notifications were almost 8 times less than the 5 year average (21,266 versus 163,015)¹.
- Respiratory syncytial virus (RSV) activity also remained low in winter 2020.

In contrast, a marked change in RSV trends² was noted in early 2021, with:

- interseasonal resurgence of RSV in Australian children and a rise in case numbers;
- higher median patient age, 18.4 months versus a range of 7.3 to 12.5 months (P < 0.001; 2012–19).

This change has been attributed to an expanded cohort of RSV-naïve patients coupled with waning population immunity².

The unpredictable nature of the trends of respiratory infections in the upcoming winter 2021 season places renewed emphasis on testing for a broad range of respiratory pathogens.

Clinical Labs Respiratory PCR Panel Diagnoses Jan–April 2019 vs. Jan–April 2021 in Victoria

Virus type	Number of positive diagnoses	
	2019	2021
Influenza A & B	2956	60
RSV (A&B)	905	3545
Parainfluenza 1, 2, 3, & 4	545	18
Human Metapneumovirus	242	82
Human Adenovirus	372	218
Human Rhinovirus	1402	2484

Victorian data prepared by Associate Professor Owen Harris.

Why test for respiratory viruses?

The multiplex PCR to diagnose influenza and respiratory viral infections allows the clinician to have a rapid and accurate diagnosis. This will enable the clinician to initiate targeted treatment early, avoiding inappropriate antibiotic therapy.

Respiratory Pathogen PCR Testing at Clinical Labs

Turnaround Time	< 24hrs	24hrs	> 24hrs
Tests included	<ul style="list-style-type: none"> • SARS-CoV-2 (COVID-19) 	<ul style="list-style-type: none"> • Influenza A & B • RSV (A&B) • Parainfluenza 1, 2, 3, & 4 • Human Metapneumovirus • Human Adenovirus • Human Rhinovirus 	<ul style="list-style-type: none"> • Influenza A & B • RSV (A&B) • Parainfluenza 1, 2, 3, & 4 • Human Metapneumovirus • Human Adenovirus • Human Enterovirus/Rhinovirus • Mycoplasma pneumoniae • Bordetella pertussis/Bordetella parapertussis
What to request	SARS-CoV-2 (COVID-19)	Respiratory Viral Screen/Multiplex PCR	Extended Multiplex PCR
Sample required	Nose/throat or nasopharyngeal swab (must use dry flocked swab)		
Cost	Medicare bulk billing available, subject to Medicare guidelines and criteria		

Additional clinical tests recommended based on relevant symptoms

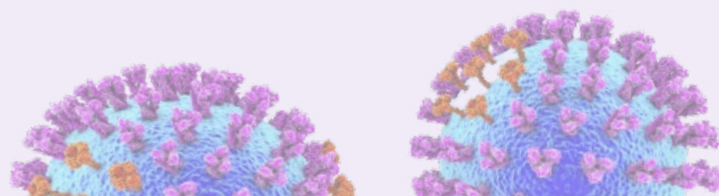
- If you suspect a lower respiratory infection, the appropriate sample is sputum for MCS
- If the patient presents with pharyngitis symptoms, obtain a swab from the throat for culture

How to order PCR tests

- To assist the laboratory during flu outbreaks, please limit testing to suspected pathogens to ensure rapid result delivery (see table above)

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Dr Sudha Pottumarthy-Boddu comes to us from Houston, Texas, where she was Assistant Professor in the Department of Pathology and Laboratory Medicine at the University of Texas, School of Medicine. She was also the Technical Director of the Clinical Laboratory Services at the Houston Department of Health and Human Services. After graduating from medical school in India, Dr Pottumarthy-Boddu migrated to New Zealand and completed her Pathology/Microbiology Fellowship training with the Royal College of Pathologists of Australasia. She is a recipient of various awards and scholarships, including the Neil Prentice Memorial Prize of RCPA. She is also a Diplomate of the American Board of Medical Microbiology. Over the last 10 years she gained experience in various hospital, research, and public health laboratories in the US, publishing over 30 articles in peer-reviewed journals and presenting at various national and international conferences. Detection of the first USA isolate of *Enterobacter* spp., with NmcA carbapenem hydrolyzing enzyme and establishing clinical significance of *Nocardia verterana* are noteworthy. Dr Pottumarthy-Boddu's main research interests are antimicrobial susceptibility trends and molecular methods in the diagnosis of infectious diseases.

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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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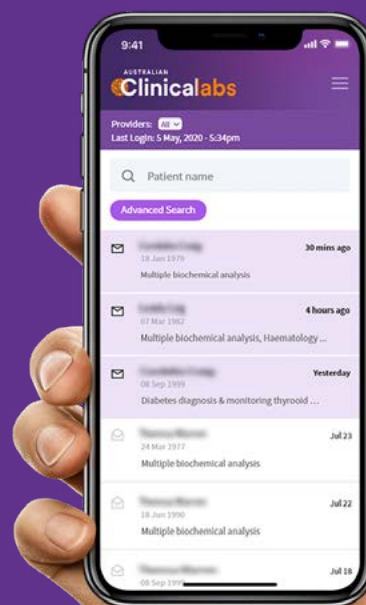
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Syphilis: An old enemy returns

By Dr Linda Dreyer

Sexually transmitted infections (STIs) have burdened humans from the beginning of time, although we have all the means to manage and control STIs we are not keeping them at bay. In recent times we have seen a rise in infection rates, and even the re-emergence of some.

In countries where testing and antibiotics were readily available, very low levels of syphilis were detected in the 20th century. In 2010, only five new infections per 100,000 people were reported in Australia.

Unfortunately, rates of syphilis are increasing in high-income countries across the globe. Data from the Kirby Institute showed that in 2018, there were 5,078 infectious syphilis notifications, 3,015 more notifications than in 2014 – an increase of 146%. A multi-jurisdictional outbreak of syphilis began in northwest Queensland in January 2011; from there it spread to the Northern Territory, then to Western Australia, and recently to South Australia.

2017. In South Australia, seven cases of infectious syphilis have been detected in pregnant Aboriginal women since November 2016, and two cases of congenital syphilis were reported. Queensland had 20 cases of congenital syphilis notified from 2010 to 2020. In 2020, a total of 19 cases of congenital syphilis were reported in Australia; this number includes stillbirths.

Why is this a problem?

A baby can contract congenital syphilis through transplacental transmission from its mother. The transmission rate is highest (60% to 90%) during untreated primary and secondary syphilis. This decreases to approximately 40% in early latent syphilis and <10% in late latent syphilis.

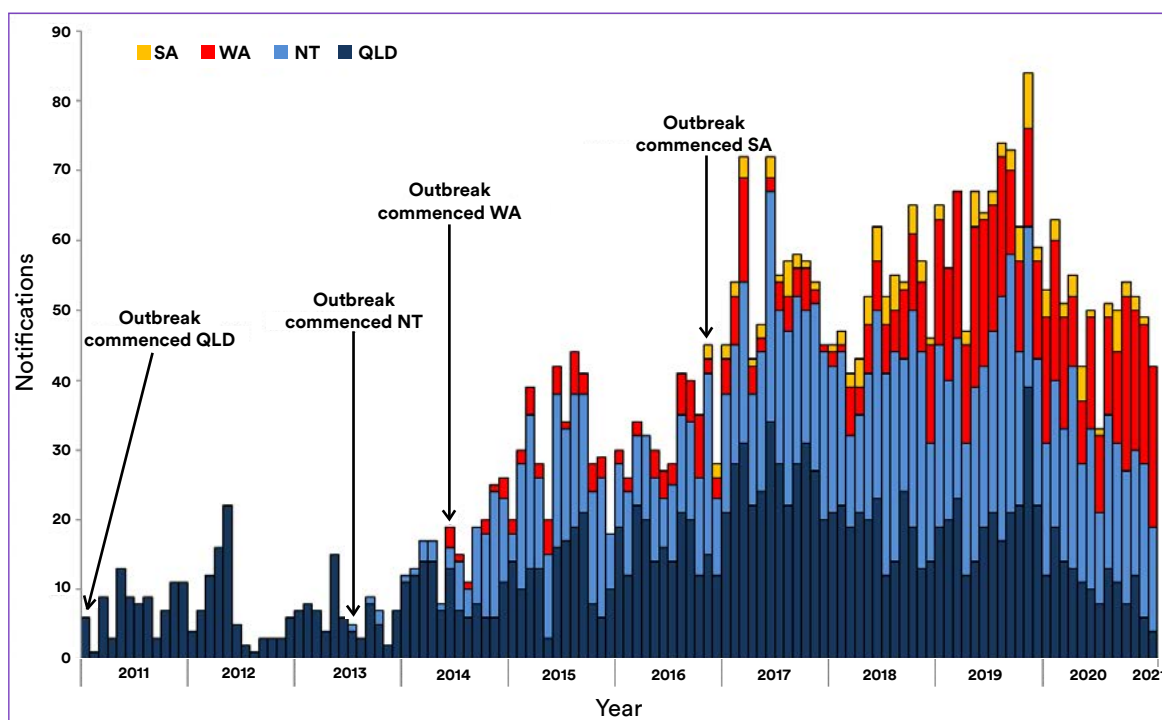


Figure 1 - Infectious syphilis outbreak cases notified in Aboriginal and Torres Strait Islander people from commencement of the outbreak to 31 January 2021

In Victoria, the reported cases of syphilis increased from 635 cases in 2014 to 1,676 cases in 2019. The reduced testing during the COVID pandemic in 2020 was responsible for the small decrease in numbers with only 1,444 cases notified. This has also led to an increase in congenital syphilis, which is of great concern. This is caused by mother-to-child transmission of infection, and has re-emerged in Victoria with 10 cases reported since

Congenital syphilis can result in stillbirth, prematurity, low birth weight, or neonatal death. A baby with congenital syphilis may present with early or late onset disease. They may appear normal at birth, but at 2 months will develop a range of clinical manifestations that include a variety of symptoms from skin lesions to organomegaly and bone, blood, and brain abnormalities.

Late onset disease is seen after 2 years of age, and this includes sight and hearing problems, bone and teeth malformations, and central nervous system abnormalities.

What can we do?

- All pregnant women should get a syphilis test during their first antenatal visit, as well as all pregnant patients who never received antenatal care and present for the first time late in pregnancy.
- All women presenting at any stage of pregnancy with clinical signs of any other sexually transmissible infection should get tested for syphilis.
- Repeat syphilis tests at 28 to 32 weeks of pregnancy, and at delivery, in all women at high risk of sexually transmissible infections.
- Repeat syphilis testing in all women from communities experiencing an outbreak of syphilis.
- If patient presents with a genital lesion, a swab for syphilis PCR as well as serology is recommended.
- Syphilis should be excluded in all sexually active patients presenting with a rash.



Image shows secondary stage syphilis “palmar” lesions on the palms of the hand.

Treatment of cases and contacts

Prompt treatment of a pregnant woman diagnosed with syphilis with long acting (benzathine) penicillin is recommended. Short acting formulations such as benzylpenicillin should not be used, because they are ineffective.

Make sure the patient is not lost to follow-up. Sexual contacts of women diagnosed with syphilis during pregnancy should be tested and treated. All babies born to mothers diagnosed with syphilis in pregnancy will require follow-up by a specialist paediatric clinic.

Conclusion

Syphilis is still a challenging disease to diagnose, and true to the name the “Great Imitator” may masquerade as a wide range of other medical conditions. Physicians must be aware of the increase in congenital infections and actively screen pregnant patients, not only at the first antenatal visit but more frequently when risk factors are present. To eliminate congenital syphilis, early case detection, appropriate treatment, and adequate follow-up of infected women, their babies, and their sexual partners is required.

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specialists. She also sat on the Infection Control Committee and the Antimicrobial Stewardship Committee of the Pretoria Academic Hospital. She came to Melbourne and joined Australian Clinical Labs (formerly Healthscope Pathology) in 2008 as a Senior Registrar and obtained Fellowship of The Royal College of Pathologists of Australasia (FRCPA) in 2010. Dr Dreyer has special interests in the appropriate use of antimicrobials, infection control, and molecular diagnostic assays in contemporary clinical microbiology.

Dr Linda Dreyer completed her undergraduate studies in 1996, receiving a Bachelor's degree in Medicine and Surgery (MBChB) from the Faculty of Health Sciences, University of Pretoria, South Africa. Following four years of clinical practice, as Medical Officer in the Department of Family Medicine she commenced specialisation in 2000. She was appointed as Registrar in Clinical Virology at the University of Pretoria/ Gauteng Province, where she worked for two years, and in 2003 she was appointed as Senior Registrar in Microbiology. Dr Dreyer received her Master's degree in Clinical Microbiology (MMed (Path)) from the University of Pretoria in 2006. She worked as a consultant for the National Health Laboratory Services (NHLS) in Pretoria until January 2008. During her time at NHLS she was involved in teaching medical students and microbiology registrars and gave lectures to nursing staff, medical students, and

The issue of iron deficiency in women

By Associate Professor Chris Barnes

Iron deficiency is the most common cause for anaemia across all populations and is estimated to affect up to 2 billion worldwide¹. Patients presenting with iron deficiency is a common diagnostic and management problem, and iron deficiency in females is particularly common due to the increased iron requirements associated with menstruation and pregnancy.

Symptoms of iron deficiency

Iron is essential to several biological functions, including energy production via mitochondrial metabolism, enzymatic processes involving neurotransmitters, and skeletal and cardiac muscle, and immune-based processes. This is in addition to haemoglobin mediated delivery of oxygen. This explains the common and varied symptoms associated with iron deficiency including lethargy, fatigue, brain fog (occasionally described misogynistically as “mummy brain”), restless legs, pica, and hair loss. These symptoms of iron deficiency occur prior to the development of iron deficiency anaemia, because iron stores are depleted initially from the liver and then from other iron enzymes and proteins in order to preserve erythropoiesis.

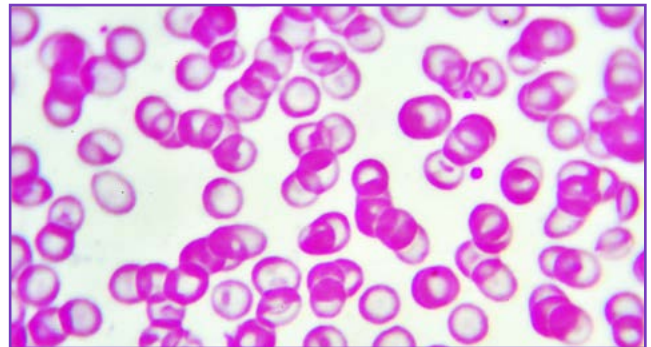
Iron deficiency anaemia will develop gradually over time, and patients may present with marked anaemia as low as 30 g/L. Whilst physiological compensation may occur, patients with marked iron deficiency anaemia may be at risk of cardiovascular instability including heart failure.



Healthy nails versus abnormal nails: an iron deficiency (anaemia) symptom.

The impact of menorrhagia on iron levels

The most common risk factor associated with iron deficiency in females is menorrhagia. Whilst the average age at menarche is 13.8 years, menarche as early as 9 years may be considered normal and is influenced by both genetic and nutritional factors. The volume of menstrual blood loss may be difficult to accurately assess. Functional measures including asking about the passing of large clots, the need for double menstrual products to avoid flooding, the need to change products overnight, or concern about flooding during the day can all be used to assess for the presence of menorrhagia².



Abnormal red blood cells in a patient with anaemia

Benefits of iron studies in diagnosing iron deficiency

Laboratory tests to screen for iron deficiency in female patients can be helpful as a part of good clinical practice even in the absence of symptoms of menorrhagia. Anaemia secondary to iron deficiency can be identified in up to 18% of otherwise healthy women, whereas iron deficiency (in the absence of anaemia) was present in 48% of 271 “asymptomatic” women participating in a community running event³.

A complete assessment of iron studies is recommended as an important first step in the investigation of patients with potential iron deficiency. Whilst an isolated low serum ferritin test is an adequate test if the patient is otherwise well, it is well known that ferritin is an acute phase reactant and may be falsely elevated in acute and chronic inflammation, fatty liver, raised BMI, or in the setting of OCP use. Additional laboratory markers available when ordering iron studies may be helpful in the assessment of iron deficiency; a low transferrin saturation (<20%) supports a diagnosis of iron deficiency in patients with concomitant inflammation or systemic illnesses (even in the presence of a normal serum ferritin).

Below is a table to assist in the interpretation of iron studies in females presenting with risk factors or symptoms suggestive of iron deficiency.

	Iron	Transferrin saturation	Ferritin	Soluble transferrin receptor
Iron deficiency	Decreased	Decreased	Decreased	Increased
Iron deficiency + acute phase response	Decreased	Normal or decreased	“Normal” <100µg/L	Increased
Acute phase response	Decreased	Decreased	Increased	Normal
Iron overload	Increased	Increased	Increased	Decreased

Clinical Labs iron studies reference ranges

The table below details the reference ranges for Clinical Labs iron studies testing for female patients.

TEST	GENDER	AGE	LOWER LIMIT	UPPER LIMIT
IRON SERUM	F & M	All	10	30
TRANSFERRIN	F & M	All	2.1	3.8
IRON SATURATION	F	All	15	45
FERRITIN SERUM	F & M	6m – 15 years	20	140
FERRITIN SERUM	F	15 – 50 years	30	200
FERRITIN SERUM	F	>50 years	30	300

Treatment

Treatment of iron deficiency should focus on the underlying cause including consideration of any sources of bleeding including menorrhagia, polymenorrhoea, or occult gastrointestinal blood loss. Iron supplementation in either oral or parenteral form is required after sources of blood loss have been addressed.

Oral iron supplementation may be associated with a high risk of gastrointestinal symptoms including nausea and abdominal pain. Second daily oral iron supplementation may reduce the incidence of the effects and may be associated with improvement in hepcidin-mediated iron absorption⁴.

Parenteral iron therapy has the potential to rapidly increase iron stores and with the availability of parenteral preparations that are associated with less risk of acute reactions, are an acceptable and attractive alternative to consider in females presenting with iron deficiency⁵.



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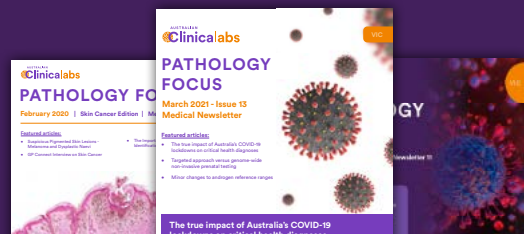
Associate Professor Chris Barnes is the National Director of Haematology and provides strategic direction nationally for haematology at Clinical Labs. He is a clinical and laboratory trained haematologist who has been part of Melbourne Haematology and has worked with Clinical Labs (and previously Healthscope) for several years. A/Prof Barnes also works at the Royal Children's Hospital and is director of the Haemophilia Treatment Centre. He has experience in both management and leadership positions. A/Prof Barnes has an active clinical research interest and is also director of Melbourne Haematology (Clinical) and Melbourne Paediatric Specialists.

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Some of our past articles not to be missed:

- Targeted NIPT (March 2021)
- Bowel Disease (December 2020)
- Melanoma (February 2020)



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The end of winter is the ideal time to test patients for vitamin D deficiency

By Dr Wessel Jenner

Vitamin D levels fluctuate significantly depending on the season. At the end of winter, approximately 36% of Australians are vitamin D deficient, in comparison to 14% at the end of summer. This makes the end of winter the best time to test patients for vitamin D deficiency.

Target vitamin D levels

The international recommendations for adequate vitamin D levels vary. However, based on a review of current literature and recently published recommendations^{1,2}, Clinical Labs suggests that an adequate vitamin D status is a serum level equal to or greater than 50 nmol/L at the end of winter. This level should be 10-20 nmol/L higher at the end of summer to allow for seasonal decrease.



This figure is based on the level below which parathyroid hormone (PTH) concentrations begin to rise and the risk of fractures increases.

As for all tests, Australian Clinical Labs' pathologists and scientists will continue to evaluate current literature, and our target range may change as new evidence emerges.

Who to test

Vitamin D testing should be ordered for patients at risk of vitamin D deficiency, including:

- Housebound people - including the sick and disabled
- Elderly in high care
- People with darker skin
- People who cover their skin due to religious or cultural reasons
- People who regularly avoid the sun
- People who work indoors
- Vegetarians

Also patients with:

- Signs, symptoms, and/or planned treatment of osteoporosis or osteomalacia
- Proximal muscle weakness

- Increased alkaline phosphatase (ALP) with otherwise normal LFTs
- Hyperparathyroidism, hypo- or hypercalcaemia, or hypophosphataemia
- Malabsorption (i.e., CF, IBD, coeliac, etc.)
- Medications known to decrease vitamin D levels (e.g., anticonvulsants)
- CRF and transplant recipients

Further testing

When ordering a vitamin D test for a patient, also ordering a serum calcium assessment and parathyroid hormone test, will assist in placing the vitamin D level within the context of overall calcium homeostasis. If osteoporosis is present, fasting blood crosslaps (CTX) will provide a way of monitoring bone turnover in response to therapy.

Pregnancy

Routine testing is only recommended in pregnant women at increased risk of vitamin D deficiency.

If testing is performed:

Supplementation is advised for women with vitamin D levels <50 nmol/L.

If testing is not performed:

Consider supplementing with 400 IU only in women at higher risk of deficiency.

Treatment

If patients are unable, for a variety of reasons, to gain the required amount of sun exposure for vitamin D production, supplementation may be required.

A maintenance dose of up to 1000 IU/day may be adequate, however some individuals will require higher doses. Severe vitamin D deficiency (serum level <20 nmol/L) may require 3,000-5,000 IU/day for 6 to 12 weeks.

Serum 25-OHD should be retested no earlier than 3 months following commencement of supplementation with vitamin D or a change in dose. Once a desirable target has been achieved, especially at the end of winter, no further testing is required unless risk factors change².

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