



# Immunoserology – infectious mononucleosis

Insight – December 2015

Infectious mononucleosis (IM) is a syndrome most commonly caused by infection with Epstein-Barr virus (EBV), a member of the Herpes virus family.

Approximately 50 percent of the population will be infected with EBV within the first decade of life, with the majority of later infections occurring from the age of 10 – 20 years. It is in this latter group that the IM syndrome is most often seen, though infections in other ages can occur.

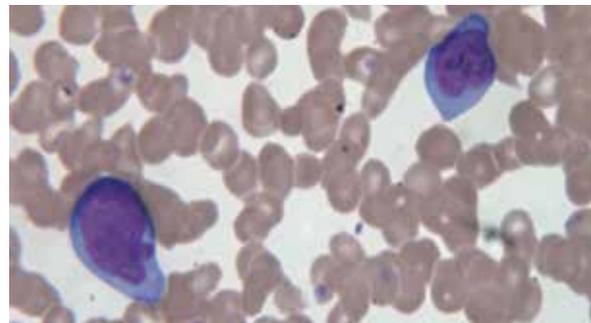
## Transmission and clinical picture

The spread of EBV is primarily through infected saliva, most commonly by kissing, as high titres of the virus are found in saliva soon after infection. Shedding from the oral cavity can continue for months, and as with other Herpes viruses, EBV can establish latency in B cells. The incubation period following exposure is 1 – 2 months.

The typical syndrome is varied, and signs and symptoms include (in descending order of prevalence):

- Asymptomatic
- Fatigue
- Malaise
- Lymphadenopathy
- Sore throat
- Nausea
- Fever
- Hepatosplenomegaly
- Myalgia

The classic triad of signs and symptoms in EBV IM is fever, pharyngitis and lymphadenopathy. In contrast, streptococcal pharyngitis (caused by *Streptococcus pyogenes*) is classically associated with an abrupt onset of high fever, headache, exudative pharyngitis, anterior cervical lymphadenopathy and a scarlatiniform rash.



**Figure 1. Atypical, viral-type activated lymphocytes of infectious mononucleosis**

## Associated laboratory abnormalities and haematologic complications

A high percentage (>10%) of atypical lymphocytes on the blood film has a moderate sensitivity and high specificity for EBV<sup>1</sup>. Associated cytopenias are not uncommon in EBV infection, particularly neutropenia and thrombocytopenia. The mechanism is likely a combination of marrow suppression and immune clearance.

The production of antibodies to red cells may lead to an autoimmune haemolysis caused by IgG antibodies, or cause red cell agglutination with or without haemolysis, secondary to IgM and complement.

Infection with EBV causes a typically mild hepatitis, associated with abnormalities in the liver enzymes. The picture may be mixed, however the elevation is typically highest in the hepatocellular enzymes, ALT and AST.

The presence of haemolysis may lead to an increase in unconjugated bilirubin, while increased lymphocyte turnover can produce a marked increase in LDH<sup>2</sup>.

## Collecting the specimen/sample

If EBV is strongly suspected, request serology, an FBE and LFT (see 'Recommendations'). It is not advised to obtain a throat swab for bacterial pathogens. Group A streptococci can be resident in the oropharynx without causing disease and isolation of this organism may cloud the clinical picture.



## Immunoserology – infectious mononucleosis

### Differential diagnosis

Other causes of an infectious mononucleosis-like syndrome are cytomegalovirus (CMV), toxoplasmosis and HIV seroconversion. These differentials are of increased importance during pregnancy and should be considered.

### Laboratory investigation

#### Heterophile antibodies

IM caused by EBV was traditionally diagnosed through demonstration of the presence of heterophile antibodies. The Monospot test is a commercial assay that exploits the ability of these antibodies to agglutinate red cells from other animal species. However, a significant disadvantage of the Monospot test is that it is a manual assay with a subjective visual endpoint, and is therefore vulnerable to operator error. In addition, heterophile antibodies may not be present in the first or second week of illness, or may persist for a long period in some patients, and therefore their presence can be misleading in the setting of another intercurrent illness.

The test is not of value in pre-school age children, in whom it is rarely positive.

False negative results may occur in patients with defective humoral immunity. Although the specificity is high in the setting of a **high clinical pre-test probability**, rare false positive results may occur in some other viral illnesses or lymphoma.

More sensitive and specific serologic assays, targeting antibodies to specific viral antigens, are now the standard diagnosis.

#### Serological testing

Melbourne Pathology performs three antibody tests for determination of EBV infection: EBV viral capsid antigen (VCA), IgM and IgG and EBV nuclear antigen (EBNA) IgG. Figure 2 illustrates the structures these antibodies are directed against.

VCA IgM and IgG antibodies are performed as a matter of routine when EBV serology is requested. EBNA IgG is added to the panel when IgM antibodies are detected. IgM antibodies can be non-specific or cross-reactive, and the EBNA antibodies help determine the timing of infection, as EBNA antibodies do not reach detectable levels until 2 – 4 months after the onset of illness. Figure 3 illustrates the progression of viral replication and antibody development throughout the course of infection.

#### EBV PCR

Throat swabs for EBV PCR should not be collected for diagnosis of IM as a matter of routine. The virus may be present without clinical disease, so the results may not be helpful. In addition, this test is non-rebatable by Medicare and will lead to out-of-pocket fees for the patient.

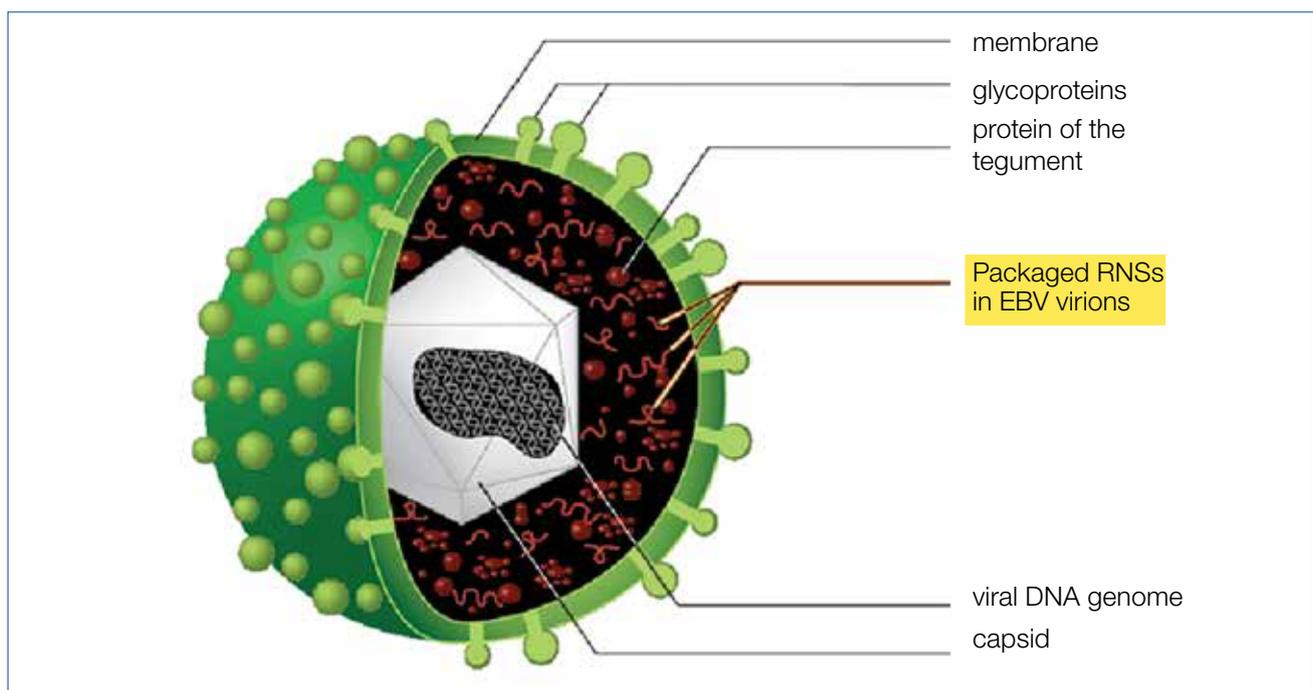


Figure 2. Source: German Research Centre for Environmental Health, 2015

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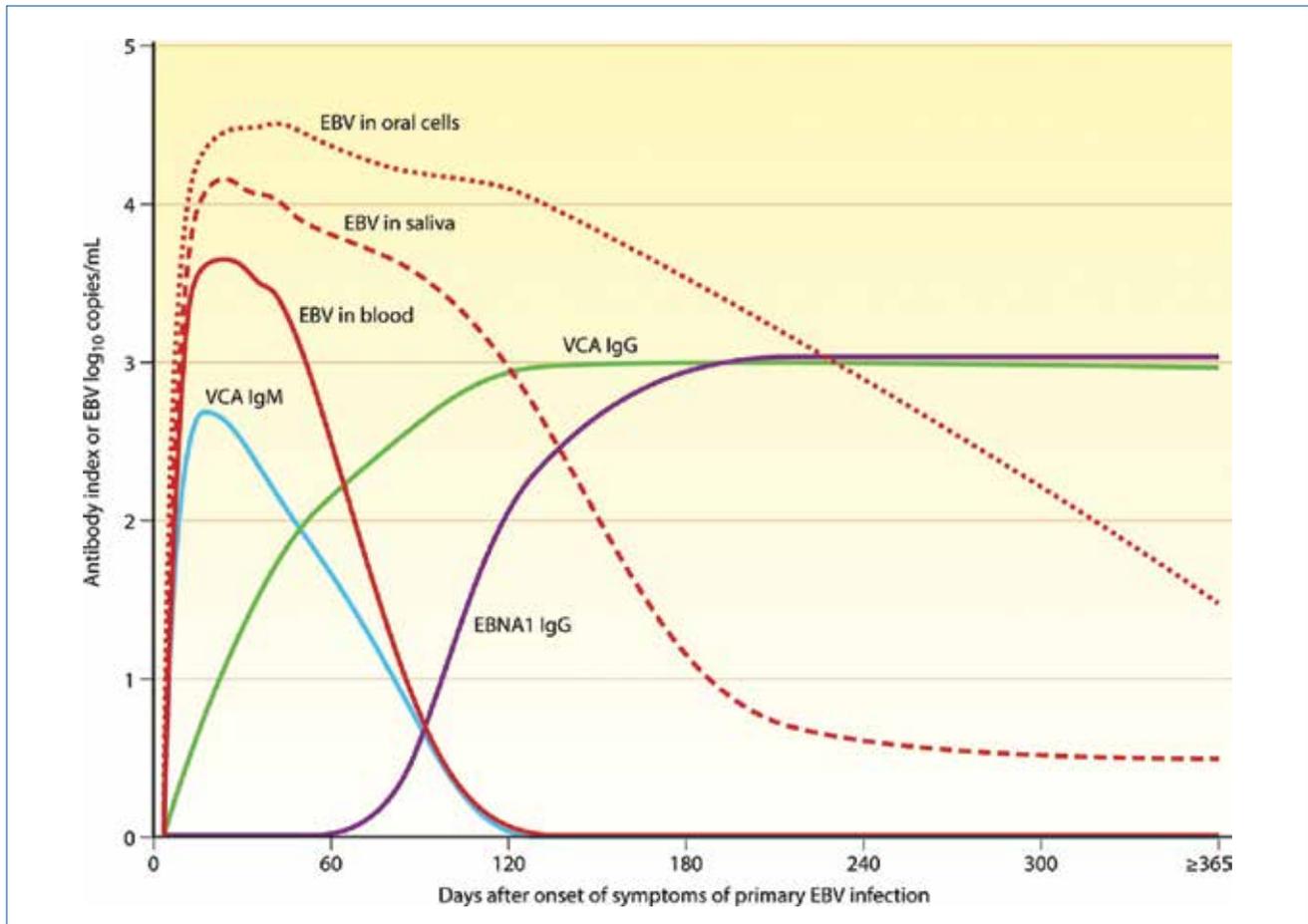


Figure 3. Source: Clinical Microbiology Reviews, January 2011

	VCA IgM	VCA IgG	EBNA IgG
Seronegative	-	-	-
Primary infection	+	+	-
Past infection	-	+	+

Table 1. VCA: viral capsid antigen; EBNA: Epstein-Barr nucleic acid

### Recommendations

If you suspect a patient has an infectious mononucleosis syndrome, the following tests should be considered:

- Full blood examination (atypical lymphocytes)
- Liver function tests (elevated transaminases)
- EBV serology (Monospot no longer required)
- CMV serology. Other infectious serology as per patient risk factors
- When haemolysis is suspected, a Coombs test, reticulocyte count, haptoglobin and LDH are of value.

### References

1. Ebell MH. Epstein-Barr virus infectious mononucleosis. Am Fam Physician. 2004 Oct 1;70(7):1279 – 1287
2. Coates P. Liver function tests. RCPA Common Sense Pathology. June 2009.



**Dr Gemma Robertson**

MBBS

**Microbiology Registrar**

Dr Robertson graduated from the University of Queensland in 2008 and is in her fourth year of microbiology training with the RCPA. She has previously worked in Brisbane and Townsville, where she cultivated

an interest in tropical medicine and parasitology, and is due to complete a Master of Public Health and Tropical Medicine this year.

Dr Robertson is currently undertaking research into faecal parasites, particularly Strongyloides, in collaboration with the University of Melbourne. She joined Melbourne Pathology as a Microbiology Registrar in February 2015.



**Dr Lyn Waring**

BSc, MBBS, FRCPA

**Director of Microbiology**

Dr Waring graduated from the University of Western Australia in 1985. She then completed two years of paediatric training, trained in Medical Microbiology at the Fairfield Infectious Diseases

and Royal Children's Hospitals in Melbourne, and went on to complete an Infectious Diseases Fellowship at Stanford University Medical School in the United States.

Dr Waring received her FRCPA in Medical Microbiology in 1995. She worked as a Medical Microbiologist at Dorevitch Pathology for the next seven years. She then moved to California where she worked for Chiron on the development of inhalational antibiotics, and a meningococcal Gp B vaccine, then for Roche Molecular Systems as Director of Clinical Research and Medical Affairs.

Dr Waring returned to Australia in 2008 and worked as a Medical Microbiologist at the Princess Margaret Hospital for Children and at the King Edward Memorial Hospital for Women in Perth. She returned to Melbourne in 2010 to join Melbourne Pathology as the Director of Microbiology and Immunoserology. Her special interests include infection control, antibiotic resistance, antenatal infections, mycology and parasitology.



**Dr Ellen Maxwell**

MBBS, FRACP, FRCPA

**Medical Director &  
Director of Haematology**

Dr Maxwell is a University of Melbourne graduate who completed combined fellowships with the College of Physicians and the College of Pathologists in 1997.

She trained initially at the Austin and Repatriation Medical Centres and later the Alfred Hospital where she developed a keen interest in coagulation and transfusion medicine.

Dr Maxwell is a current member of the Victorian Blood User Group, the National Blood Transfusion Committee, The Australian Red Cross Blood Service Advisory Committee and the Serious Transfusion Incident Reporting Working Group (DHS Victoria). She has been an active member of many committees for the RCPA and ANZSBT.

Dr Maxwell was appointed Medical Director at Melbourne Pathology in September 2009.

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