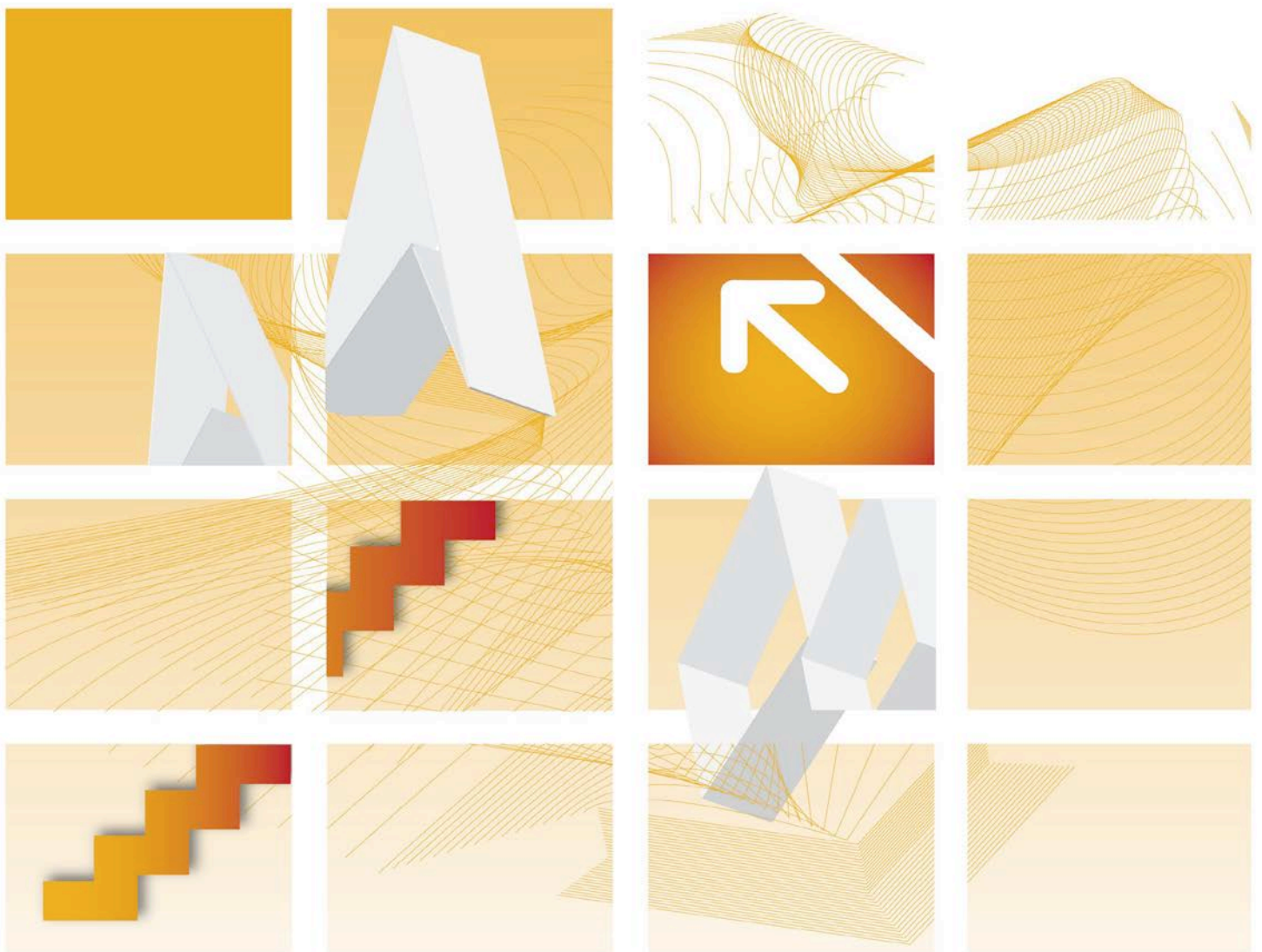




# UK Standards for Microbiology Investigations

## Microbiological Investigation of Patients with Acute Lymphadenopathy and Fever



## Acknowledgments

---

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

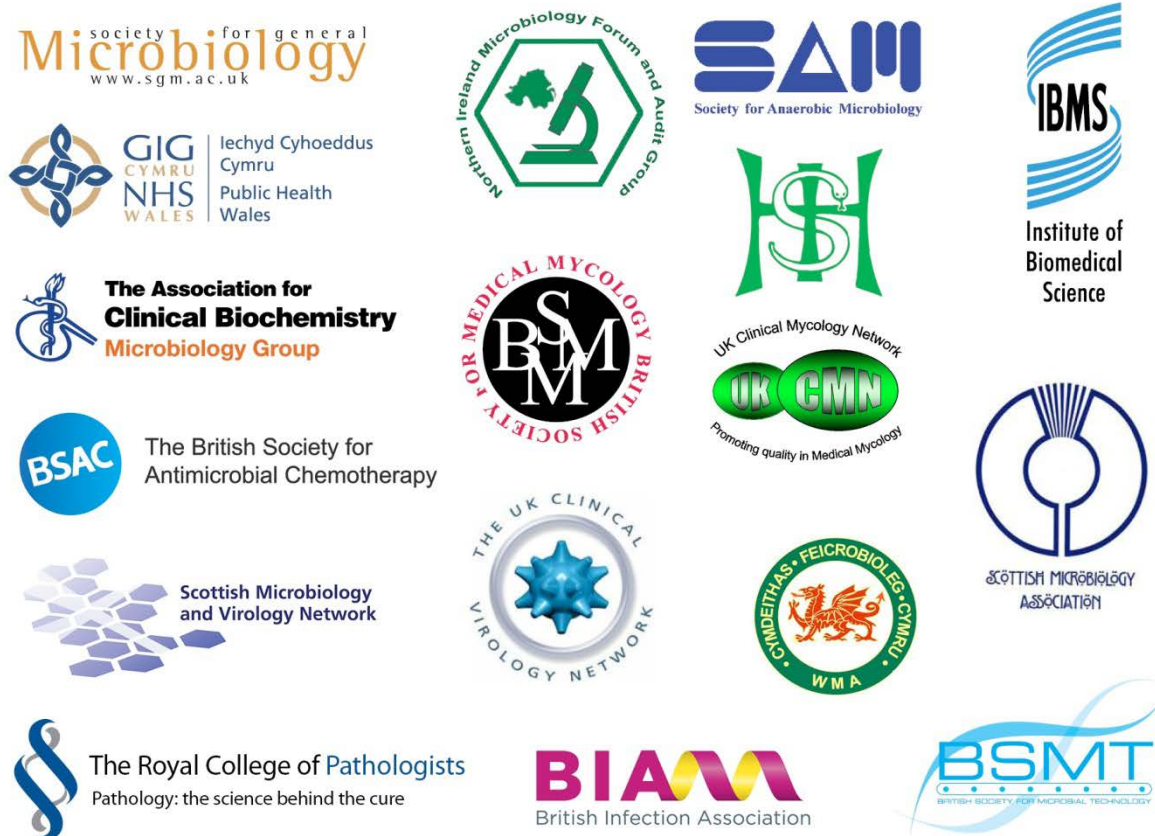
The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

For further information please contact us at:

Standards Unit  
Microbiology Services  
Public Health England  
61 Colindale Avenue  
London NW9 5EQ  
E-mail: [standards@phe.gov.uk](mailto:standards@phe.gov.uk)

Website: <http://www.hpa.org.uk/SMI>

UK Standards for Microbiology Investigations are produced in association with:



## Contents

---

ACKNOWLEDGMENTS .....	2
CONTENTS .....	3
AMENDMENT TABLE .....	4
UK STANDARDS FOR MICROBIOLOGY INVESTIGATIONS: SCOPE AND PURPOSE.....	5
SCOPE OF DOCUMENT .....	8
INTRODUCTION .....	8
1 EPSTEIN BARR VIRUS .....	9
2 CYTOMEGALOVIRUS .....	11
3 <i>TOXOPLASMA GONDII</i> .....	12
4 HUMAN IMMUNODEFICIENCY VIRUS .....	13
5 RUBELLA .....	14
6 SYPHILIS .....	15
7 LYMPHOGRANULOMA VENEREUM (LGV) .....	15
REFERENCES .....	17



NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

## Amendment Table

---

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@phe.gov.uk](mailto:standards@phe.gov.uk).

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	6/21.10.13
Issue no. discarded.	3.1
Insert Issue no.	3.2
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p>

Amendment No/Date.	5/29.01.13
Issue no. discarded.	3
Insert Issue no.	3.1
<b>Section(s) involved</b>	<b>Amendment</b>
Contents page.	Accreditation logo added.
Whole document.	Minor formatting amendments.

## UK Standards for Microbiology Investigations<sup>#</sup>: Scope and Purpose

---

### Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

### Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

### Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at <http://www.hpa.org.uk/SMI/Partnerships>. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

---

<sup>#</sup> Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.



## Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

## Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

## Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives [http://www.hpa.org.uk/webc/HPAwebFile/HPAweb\\_C/1317133470313](http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313). The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

## Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

### **Suggested Citation for this Document**

Public Health England. (2013). Microbiological Investigation of Patients with Acute Lymphadenopathy and Fever. UK Standards for Microbiology Investigations. G 2 Issue 3.2. <http://www.hpa.org.uk/SMI/pdf>.

## Scope of Document

---

### Type of Specimen

Serum samples, PCR samples – urine and throat swab, genital swabs in cases of syphilis and LGV (others as directed)

### Scope

This SMI describes the microbiological investigation of patients with acute lymphadenopathy and fever.

This SMI should be used in conjunction with other SMIs.

## Introduction

---

The following infections will be covered:

- Epstein-Barr Virus (EBV)
- Cytomegalovirus (CMV)
- *Toxoplasma gondii* (*T. gondii*)
- Human Immunodeficiency Virus (HIV)
- Rubella
- Syphilis
- Lymphogranuloma venereum (LGV)

This group of agents are major causes of acute lymphadenopathy and fever that are often diagnosed by virology laboratories. Other agents of significance diagnosed in bacteriology laboratories are:

Streptococci (see [ID 4 - Identification of \*Streptococcus\* species, \*Enterococcus\* species and Morphologically Similar Organisms](#))

Typical and atypical Mycobacteria (see [B 40 - Investigation of Specimens for \*Mycobacterium\* species](#))

Bartonella (see [ID 1 - Introduction to the Preliminary Identification of Medically Important Bacteria](#) and [B 37 - Investigation of Blood Cultures \(for Organisms other than \*Mycobacterium\* species\)](#))

*Actinomyces* species (see [ID 15 – Identification of Anaerobic \*Actinomyces\* species](#) and [ID 10 – Identification of Aerobic \*Actinomycetes\* species](#))

The physician also needs to be aware of non-infectious causes of acute lymphadenopathy and fever eg lymphoproliferative and neoplastic disorders.



# 1 Epstein Barr Virus<sup>1</sup>

## 1.1 Epidemiology

Transmission often occurs before 3 years of age and is largely asymptomatic. In developed countries, primary infection often occurs during adolescence, presenting as Infectious Mononucleosis (IM). By age 5 seroprevalence in the UK and USA is 50% and by adulthood it is 95%.

## 1.2 Clinical Features

IM can range from a mild to a severe debilitating illness. Typical features are lymphadenopathy (seen in 94% of cases), general malaise and pharyngitis (84%). Atypical lymphocytosis is a characteristic feature of the first two weeks of illness, due to a vast increase in absolute numbers of CD8+ T lymphocytes. More than 20% of white cells may be large “atypical” activated cells, this may also be seen in CMV mononucleosis. The higher the lymphocytosis the greater the specificity for infectious mononucleosis<sup>2</sup>. Smaller percentages of white cells may be “atypical” cells during infections other than EBV. Absolute numbers of circulating B-lymphocytes are normal or slightly raised. Lymphadenopathy is largely cervical. Fever occurs in about 80% of patients and rash in 5%. Jaundice occurs in 9% of cases, although hepatocellular enzymes are raised in 80 to 90% of cases. Splenomegaly occurs in about 50% of cases. Most IM cases resolve spontaneously over a 2 to 3 week period. Malaise may occur for weeks or months in some patients.

## 1.3 Complications

Autoimmune haemolytic anaemia occurs in about 2% of patients with IM, and mild thrombocytopenia is common. Neurological symptoms occur in less than 1% of cases as an acute, severe, progressive disease although complete recovery is the norm. Stridor and respiratory arrest may be seen in association with massive enlargement of lymphoid tissue in the pharynx. Rarely primary EBV in a person who is immunocompetent may be complicated by haemophagocytosis caused by activation of the monophagocytic system in multiple organs. Administration of ampicillin or amoxicillin produces a pruritic, maculopapular rash in about 95% of patients. Splenic rupture is uncommon but life-threatening; advice to avoid contact sports for three to four weeks is usually given<sup>2</sup>. Infection in boys with X-linked lymphoproliferative syndrome progresses to overwhelming primary EBV infection that frequently results in acute liver failure and haemophagocytic syndrome. Individuals with an immunodeficiency or who are immunocompromised are at risk of lymphoproliferative disease or lymphoma. EBV is associated with almost 100% of cases of endemic Burkitt's lymphoma and 25% of sporadic cases. EBV is associated with other malignant diseases including some cases of Hodgkin's lymphoma and nasopharyngeal carcinoma.

## 1.4 Laboratory Diagnosis<sup>3</sup>

This section should be read in conjunction with [V 26 - Epstein-Barr Virus Serology](#).

### Heterophile Antibodies<sup>4</sup>:

Appear in the serum early in IM.

The classic test used a doubling dilution titration of serum and sheep red cells (use of horse red cells increases sensitivity). Prior absorption of the serum with guinea-pig kidney emulsion increases specificity, by eliminating agglutination seen in normal serum due to "Forssman antibody". Specificity can be further improved by absorption of reactive samples with ox cell stroma, which specifically removes heterophile antibody seen in IM. Classic tests are usually known as Paul-Bunnell tests.

Commercial IM screening kits (often based on latex particle slide agglutination) developed from these classical techniques are now more widely employed; these are widely known as monospot tests.

Monospot tests are 84 to 100% sensitive in IM (compared to EBV VCA IgM IF tests). False negatives are seen particularly in cases aged less than 14 years. Specificity for IM is 89 to 100%, but the Monospot may remain positive for many months after resolution of symptoms and may be positive in asymptomatic primary EBV infection.

### **Antibodies to Viral Capsid Antigen (VCA):**

IgG, IgM and IgA are all typically detectable in the serum of patients with IM by the time of onset of symptoms. A number of assays are available but are not well standardised.

IgG has often reached a high titre by presentation, making the demonstration of a rising titre of IgG difficult.

IgA and IgM decline to undetectable levels during convalescence, within 3 to 6 months.

IgM detection by Indirect Immunofluorescence (IF) is a highly sensitive test for IM. Complexes of EBV IgG and IgM class rheumatoid factor can cause false positive results. EBV IgG can cause false negatives by competing with EBV IgM. Serum samples should be pre-treated to remove IgG before the IF test. Non-specific anti-cell fluorescence may be seen but should be readily differentiated from specific fluorescence by an experienced observer.

ELISA tests for EBV IgM are reliable and more widely used.

Weak cross-reactions may cause false positive EBV VCA IgM results, particularly during primary CMV infection.

Reactivation of EBV can produce similar patterns of reactivity to primary infection; differentiation from primary infection may require avidity testing or heterophile antibody testing<sup>5,6</sup>.

### **Antibodies to EBNA-1:**

Anti-EBNA IgG is not usually detectable until the convalescent period. Its detection excludes recent or current IM. Commercial EBNA-1 IgG ELISAs are available and widely used.

### **EBV DNA tests<sup>7-10</sup>**

Quantitative PCR assays are used in the monitoring of patients who are immunocompromised and at risk of EBV associated lymphoproliferative disease, where very high levels may be observed. Lower levels are seen in uncomplicated IM. A role for EBV DNA quantitation in the diagnosis and monitoring of IM and its complications has been suggested. Levels are higher in IM than in healthy carriers

and higher still in complicated IM. However 11% of IM cases are EBV DNA negative at presentation. EBV DNA quantification has not yet been standardised with an accepted international standard and there is no consensus on whether plasma, whole blood or cells should be tested. EBV quantitation in clinical practice is used in diagnosis and monitoring of EBV lymphoproliferative disease and complicated IM but not usually in the diagnosis of uncomplicated IM except to assist diagnosis where serology is difficult to interpret.

## 2 Cytomegalovirus<sup>11,12</sup>

---

### 2.1 Epidemiology<sup>12</sup>

Infection is widespread and is usually inapparent. In the UK the seroprevalence in adolescents is around 40% and increases at about 1% per annum thereafter. Seroprevalence ranges from 40 to 100% depending on ethnicity and social class and sexual risk factors. Congenital infection occurs in about 0.5% of all live births. Perinatal transmission can occur at birth or by breastfeeding. Infection can also occur in the early years of childhood, following infection at nurseries or day-care centres, as young children can excrete the virus in respiratory secretions and urine for many months. Sexual transmission to seronegative individuals can occur at the onset of sexual activity. Transmission may also occur through blood products. Average age of patients with CMV mononucleosis is higher than IM cases due to EBV.

### 2.2 Clinical Features

Mononucleosis caused by CMV is difficult to distinguish clinically from the cases that are due to EBV infection. Enlargement of lymph nodes and spleen is not striking but may occur. Tonsillitis or pharyngitis is rare. Severe hepatitis or jaundice is unusual although slightly raised hepatocellular enzymes are common. The most common features are malaise (67% of cases), fever (46%), and sweats (46%). In individuals who are immunocompetent the majority of cases resolve spontaneously.

### 2.3 Complications

A variety of severe infections can occur especially in a patient who is immunocompromised, including pneumonitis, retinitis, hepatitis and gastrointestinal disease. If untreated these are associated with severe morbidity or mortality. Severe congenital disease is more likely in cases resulting from maternal primary infection<sup>13</sup>. Active cytomegalovirus infection in a woman of child bearing age should prompt the virologist to confirm or exclude pregnancy and inform the patient's midwife or obstetrician accordingly. Infection of immunocompetent adults may trigger Guillain-Barré syndrome<sup>12</sup>.

### 2.4 Laboratory Diagnosis

Detection of CMV IgM by commercial ELISA is the most widespread method in use for confirming a diagnosis of primary CMV infection in a person who is immunocompetent with an IM-like syndrome. Cross reaction is a common problem with patients reacting to both CMV and EBV IgM tests when they have a primary infection, 44% of patients with a primary CMV infection will initially also react in EBV IgM tests. CMV can be confirmed by PCR in about 60% of IM-like patients because immunocompetent patients may only be viraemic for the first few days of their illness. IgM antibody titres against EBV are lower and rapidly decline during the 3–12 month follow-up, while a

progressive rise of cytomegalovirus IgG antibody titre is observed over time, in the absence of high-avidity cytomegalovirus antibodies<sup>14</sup>. CMV IgG avidity is a well described serological technique for confirming recent infection<sup>15</sup>. Heterophile antibody tests are usually negative. CMV PCR on blood is not a widely used first investigation in this setting, it is reserved as a monitoring, diagnostic and management tool in the immunocompromised and in pregnancy and congenital infection and in the confirmation of IgM test results. PCR of dried blood spots has become an important technique in evaluating congenital CMV.

## 3 *Toxoplasma gondii*

---

### 3.1 Epidemiology<sup>16-18</sup>

Infection occurs as a worldwide zoonosis involving a wide range of mammals, with cats being particularly important vectors of transmission. The incidence varies between countries. Infection usually occurs following ingestion of oocysts in raw or under-cooked meats and vegetables contaminated with animal excreta. Seroprevalence in women of childbearing age in the UK has declined in recent decades to about 9% overall and it increases with age.

### 3.2 Clinical Features<sup>17</sup>

Only 10 to 20% of infections are symptomatic in adults and the clinical presentation is not distinctive. When it occurs it is usually cervical lymphadenopathy but any or all lymph nodes may be enlarged. If symptomatic, fever, malaise, myalgia, sore throat, maculopapular rash and hepatosplenomegaly may be present. *T. gondii* is estimated to cause 3 to 7% of clinically significant lymphadenopathy. *T. gondii* is estimated to cause 1% of mononucleosis syndromes. Infection is usually benign and self-limited. Symptoms, if present, usually resolve within a few months and rarely persist beyond 12 months.

### 3.3 Complications<sup>17</sup>

Severe disease can occur in patients who are immunocompromised usually following reactivation of latent infection manifesting as encephalitis, pneumonitis or chorioretinitis. Primary infection during pregnancy can result in congenital infection with damage, particularly in the first trimester.

### 3.4 Laboratory Diagnosis<sup>19,20</sup>

Commercial ELISA and latex agglutination systems are available to screen for total *T. gondii* antibodies and *T. gondii* IgM. These are sensitive enough to detect all but the lowest levels of antibody detected on reference tests such as the dye test and ISAGA. Acute toxoplasmosis in individuals who are immunocompetent can, therefore, be effectively excluded by negative screening test results<sup>21</sup>. The use of highly sensitive and specific assays for recent infection such as ISAGA, ELIFA and IgG avidity is recommended to confirm acute infection, particularly in pregnancy, and will require the involvement of a Reference Unit<sup>22</sup> (see [P 5 - Investigation of Toxoplasma Infection in Pregnancy](#)).

## 4 Human Immunodeficiency Virus

### 4.1 Epidemiology<sup>23,24</sup>

A pandemic is occurring with 33 million people estimated in the UNAIDS 2010 Global Report to be living with HIV/AIDS, including 2.6 million newly infected in 2009. About 68% of infected people are in sub-Saharan Africa. The annual number of new HIV infections has been steadily declining since the late 1990s and there are fewer AIDS-related deaths due to the significant scale up of antiretroviral therapy over the past few years. Although the number of new infections has been falling, levels of new infections overall are still high, and with significant reductions in mortality the number of people living with HIV worldwide has increased. Worldwide, transmission is predominantly heterosexual. By the end of 2009 there were an estimated 86,500 people living with HIV in the UK of whom 22,200 (26%) were unaware of their infection. During 2009, 6,630 persons were newly diagnosed with HIV in the UK. One in six men who have sex with men (MSMs), and one in sixteen heterosexuals newly diagnosed with HIV in the UK had acquired their infection within the previous 4-5 months before diagnosis. New AIDS diagnoses in infected persons decreased from a peak of 947 cases in 2003 to 516 in 2009.

### 4.2 Clinical Features<sup>25</sup>

A mononucleosis-like syndrome often occurs during primary infection, about 3 to 4 weeks post-exposure. Fever is present in 80 to 90% of cases. Lymphadenopathy occurs in 40 to 70% of cases, often involving several extra-inguinal sites and can on occasions persist for at least 3 months. Rash and pharyngitis both occur in 40 to 70% of cases. Some signs are more a feature of acute HIV than other causes of mononucleosis, including the maculopapular (or morbilliform) rash, diarrhoea, meningoencephalitis and mucocutaneous ulceration. This syndrome can be misdiagnosed and may be under diagnosed.

### 4.3 Laboratory Diagnosis

Tests for HIV antibody first become positive 22 to 27 days after acute infection<sup>25</sup>.

The detection of high titre viral RNA or viral p24 antigen in a patient with a negative test for HIV-1 antibodies establishes the diagnosis of acute HIV-1 infection. Viral RNA testing is the more sensitive assay and detects HIV-1 infection 3 to 5 days earlier than p24 antigen tests and 1 to 3 weeks earlier than antibody assays. Levels of RNA are greater than 50,000 copies/mL in acute HIV infection<sup>25</sup>. Most assays for RNA are licensed only for monitoring of chronic HIV and not diagnosis of acute infection, but they are widely used in this context. Subsequent documentation of seroconversion is essential to confirm HIV infection, though prompt intervention with antiretrovirals may blunt the expected antibody response.

Fourth generation HIV antibody assays that will simultaneously detect HIV-1 p24 antigen are now the standard of care and data suggest that they will detect HIV-1 infection 2 to 18 days earlier than third generation assays (for antibody alone)<sup>26-29</sup>. However, there are reports of two window periods being observed with this assay, presumably because of detection of the antigen peak and then the antibody rise with a period of non-reactivity between<sup>30</sup>. Point of care tests (POCT) for HIV are becoming widely used and high sensitivity and specificity can be achieved however there can be



discrepancies between laboratory and field evaluations of performance with false negative results being reported<sup>31-35</sup>. Fourth generation POCT are available.

## 5 Rubella

---

### 5.1 Epidemiology<sup>24,36,37</sup>

The epidemiology of rubella has changed considerably since the introduction of MMR in 1988 and outbreaks are now rare in the UK. Confirmed cases of rubella have fallen from 3922 in 1996 to 12 in 2010. Until recently, the seroprevalence of antibody in children and adults was 97 to 98%. However in the mid-1990s a fall in vaccine uptake in young children occurred as a result of suggestions of complications to MMR use that have since been refuted, to the extent that rubella epidemics are once more a possibility. People arriving in the UK from less developed countries, lacking rubella vaccination programmes, may be susceptible and at risk of infection.

### 5.2 Clinical Features<sup>38</sup>

Rubella presents as a mild, febrile illness with a transient erythematous rash and lymphadenopathy affecting the post-auricular, sub-occipital and posterior cervical glands. Arthralgia and arthritis sometimes occur in adults. Children often have few clinical symptoms. In all cases, symptoms resolve spontaneously. Clinical diagnosis alone is unreliable, and CMV and parvovirus B19 infection should be considered in the differential diagnosis.

### 5.3 Complications

Congenital rubella can follow primary infection in pregnant women. Severe foetal damage can occur if infection occurs in the first 16 weeks of gestation<sup>39</sup>. Reinfection when it occurs is usually sub-clinical and there is a low but definite risk to the foetus following maternal infection in the first 16 weeks of gestation<sup>40</sup>.

### 5.4 Laboratory Diagnosis<sup>38,40</sup>

The serological diagnosis of rubella is well established. Commercial EIAs usually make it possible to detect specific IgM within 4 days of onset of rash and for 4 to 8 weeks thereafter. IgM assays can be of high specificity, but the low prevalence of rubella in the UK means that the predictive value of a positive IgM assay alone is now low. It is particularly important in pregnant women to establish the diagnosis with certainty. The collection of multiple blood samples to establish a rising titre of IgG or the presence of low avidity antibody is indicated to confirm a diagnosis of acute rubella. A significant rise in antibody titre can be detected by a quantitative EIA, HAI or LA titration. Seroconversion can be detected by SRH. Although HAI antibodies may develop 1 to 2 days after onset of symptoms antibodies detected by EIA, LA or SRH may be delayed until 7 to 8 days. Rubella specific IgG and IgM antibodies may be detected in saliva using antibody capture immunoassays and results correlate well with serum antibodies.

Rubella virus may be detected in clinical samples by isolation in cell culture or by reverse transcription PCR. In rubella acquired postnatally virus excretion is of greatest duration from the pharynx, typically commencing before the onset of clinical symptoms and continuing after the rash has faded but usually ceasing before the lymphadenopathy has settled.



## 6 Syphilis

---

### 6.1 Epidemiology<sup>24,41</sup>

There has been a substantial increase in numbers of primary, secondary and early latent cases of syphilis diagnosed in the UK. During 2007, 3762 diagnoses of infectious syphilis were made, more than in any other year since 1950. MSM account for 73% of infectious syphilis and HIV co-infection is common in those diagnosed with syphilis (27%), reflecting the close relationships between the epidemics. The increased number of syphilis cases in women of reproductive age has resulted in an increase in cases of congenital infection. This increase has been punctuated by a series of outbreaks, the first of which occurred in 1997 amongst heterosexuals in Bristol. Although outbreaks have occurred in many UK cities, most diagnoses have been reported from Manchester, London and Brighton. The largest outbreak began in London in 2001.

### 6.2 Clinical Features<sup>42</sup>

The vast majority of patients with secondary syphilis have a rash and most have lymphadenopathy. All other signs, including fever, chancre still being present, condylomata lata, mucous patches and alopecia are less frequent.

### 6.3 Complications<sup>42,43</sup>

Patients with secondary syphilis may suffer hepatitis, meningitis, peripheral neuropathy, perceptive deafness, splenomegaly, periostitis, arthritis or iridocyclitis. None of these is seen in more than 2% of patients.

### 6.4 Laboratory Diagnosis<sup>43</sup>

Diagnosis is most commonly confirmed serologically using a combination of reagin tests (VDRL and RPR) and specific anti-treponemal assays such as TPHA or TPPA, and IgG and IgM by EIA or IF. Combined treponemal IgG and IgM detection EIAs are preferred for screening. False negative reagin tests may occur due to prozone effects. All the specific tests are almost invariably positive in secondary syphilis, delayed serological response is rare - even with HIV co-infection. Serological tests cannot differentiate from other treponemal infections, such as yaws. PCR for *T. pallidum* will be useful in the minority of secondary syphilis cases in which the primary chancre is still observed at diagnosis. Rapid tests for syphilis are available but their main role is likely to be in field conditions in developing countries and potentially in outreach work, as their performance can be lower than conventional laboratory tests<sup>44</sup>.

## 7 Lymphogranuloma venereum (LGV)

---

### 7.1 Epidemiology<sup>41</sup>

Prior to 2004, LGV was predominantly a tropical disease, rarely seen in the UK. Following the emergence of this disease in the Netherlands and other parts of Europe an epidemic is now well established in the UK. Similar to the ongoing outbreaks of LGV in Western Europe and America, the epidemic is concentrated in the MSM population. As initially little was known about the transmission and symptoms of this disease, enhanced surveillance was set up in the UK to collect information on demographics, transmission and clinical presentation.

Epidemics of LGV are continuing especially among MSM who are known to be HIV infected. MSM account for 99% of LGV cases. HIV co-infection is common in those diagnosed with LGV (74%) reflecting the close relationships between the epidemics. Eight hundred and forty nine cases of LGV were diagnosed between 2003 and 2008, the majority of whom had symptoms of proctitis (rectal pain, discharge, bloody stools and constipation).

## 7.2 Clinical Features<sup>45,46</sup>

Most LGV cases (82%) present because of symptoms, others present as contacts (5%), through referral (4%), or are detected during routine examination (5%) and screening for STI or HIV. Proctitis remains the commonest presentation, almost all with systemic symptoms (fever and malaise) also had proctitis. Symptoms include rectal pain, discharge, tenesmus, and other signs of lower gastrointestinal inflammation including constipation and abdominal pain. Genital and inguinal symptoms are rare with only a small minority presenting with inguinal lymphadenopathy. The primary ulcer usually goes unnoticed. The incubation period is extremely variable (range 3-30 days) from time of sexual contact with an infected individual; the primary lesion is transient and often imperceptible, in the form of a painless papule or pustule or shallow erosion. Extra-genital lesions have been reported such as in the oral cavity (tonsil) and extra-genital lymph nodes.

## 7.3 Complications<sup>47</sup>

Patients with acute proctitis related to LGV usually respond well to antibiotic therapy. Left untreated, chronic inflammation may lead to stricture and fistula formation as well as local lymphatic obstruction and lymphoedema. Patients with chronic infection including abscess, fistulas, and strictures often require surgical intervention.

## 7.4 Laboratory Diagnosis<sup>48</sup>

The case definition used by PHE is confirmation of *C. trachomatis* (see [V 37 – Chlamydia trachomatis Infection - Testing by Nucleic Acid Amplification Tests \(NAATs\)](#)) and presence of an LGV serovar, L1, L2, or L3, by genotyping.

Reference services will test rectal specimens from patients with symptoms of proctitis or urethral specimens from patients with inguinal lymphadenopathy or in contact with LGV that are known to be positive for *C. trachomatis* DNA. Serology for *C. trachomatis* has been used in Europe and can suggest the possibility of LGV, but does not confirm cases because of a lack of specificity, and has not been used in England as part of the case definition.

## References

---

1. Johannsen EC, Kaye KM. Epstein Barr Virus (Infectious Mononucleosis, Epstein-Barr Virus-Associated Malignant Diseases, and Other Diseases). In: Mandell GL, Bennett JE, Dolin R, editors. Douglas and Bennett's Principles and Practice of Infectious Diseases. 6th ed. Vol 2. Edinburgh: Churchill Livingstone; 2009. p. 1989-2010.
2. Ebell MH. Epstein-Barr virus infectious mononucleosis. *Am Fam Physician* 2004;70:1279-87.
3. Haque T, Crawford DH. Epstein Barr virus. In: Zuckerman AJ, Banatvala JE, Schoub BD, editors. Principles and practice of Clinical Virology. 6th ed. Chichester: John Wiley & Sons Ltd; 2009. p. 199-222.
4. Medical Devices Agency. Fourteen commercial IM screening kits. MDA. 98/63 ed. Norwich: HMSO; 1998. p. 5-51.
5. Nystad TW, Myrmel H. Prevalence of primary versus reactivated Epstein-Barr virus infection in patients with VCA IgG-, VCA IgM- and EBNA-1-antibodies and suspected infectious mononucleosis. *J Clin Virol* 2007;38:292-7.
6. Robertson P, Beynon S, Whybin R, Brennan C, Vollmer-Conna U, Hickie I, et al. Measurement of EBV-IgG anti-VCA avidity aids the early and reliable diagnosis of primary EBV infection. *J Med Virol* 2003;70:617-23.
7. van Laar JA, Buysse CM, Vossen AC, Hjalmarsson B, van Den BB, van Lom K. Epstein Barr viral load assessment in immunocompetent patients with fulminant infectious mononucleosis. *Arch Intern Med* 2002;162:837-9.
8. Leung E, Shenton BK, Jackson G, Gould FK, Yap C, Talbot D. Use of real time PCR to measure Epstein Barr virus genomes in whole blood. *J Immunol Methods* 2002;270:259-67.
9. Berger C, Day P, Meier G, Zingg W, Bossart W, Nadal D. Dynamics of Epstein Barr virus DNA levels in serum during EBV associated disease. *J Med Virol* 2001;64:505-12.
10. Stevens SJ, Pronk I, Middeldorp JM. Toward standardization of Epstein Barr virus DNA load monitoring: unfractionated whole blood as preferred clinical specimen. *J Clin Microbiol* 2001;39:1211-6.
11. Crumpacker CS, Zhang JL. Cytomegalovirus. In: Mandell GL, Bennett JE, Dolin R, editors. Douglas and Bennett's Principles and practice of Infectious Diseases. 6th ed. Vol 2. Edinburgh: Churchill Livingstone; 2009. p. 1786-801.
12. Griffiths PD. Cytomegalovirus. In: Zuckerman AJ, Banatvala JE, Griffiths P, Schoub B, Mortimer P, editors. Principles and Practice of Clinical Virology. 6th ed. Chichester: John Wiley & Sons Ltd; 2009. p. 161-98.
13. Fowler KB, Stagno S, Pass RF, Britt WJ, Boll TJ, Alford CA. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. *N Engl J Med* 1992;326:663-7.
14. Manfredi R, Calza L, Chiodo F. Primary Cytomegalovirus Infection in Otherwise Healthy Adults with Fever of Unknown Origin: A 3-Year Prospective Survey. *Infection* 2006;34:87-90.
15. Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. *Clin Microbiol Rev* 2002;15:680-715.

16. Ryan M, Hall SM, Barrett NJ, Balfour AH, Holliman RE, Joynson DH. Toxoplasmosis in England and Wales 1981 to 1992. *Commun Dis Rep CDR Rev* 1995;5:R13-R21.
17. Montoya JG, Boothroyd JC, Kovacs JA. *Toxoplasma gondii*. In: Mandell GL, Bennett JE, Dolin R, editors. *Douglas and Bennett's Principles and Practice of infectious Diseases*. 6th ed. Vol 2. Edinburgh: Churchill Livingstone; 2009. p. 3170-98.
18. Nash JQ, Chissel S, Jones J, Warburton F, Verlander NQ. Risk factors for toxoplasmosis in pregnant women in Kent, United Kingdom. *Epidemiol Infect* 2005;133:475-83.
19. Roberts A, Hedman K, Luyasu V, Zufferey J, Bessieres MH, Blatz RM, et al. Multicenter evaluation of strategies for serodiagnosis of primary infection with *Toxoplasma gondii*. *Eur J Clin Microbiol Infect Dis* 2001;20:467-74.
20. Petersen E, Borobio MV, Guy E, Liesenfeld O, Meroni V, Naessens A, et al. European multicenter study of the LIAISON automated diagnostic system for determination of *Toxoplasma gondii*-specific immunoglobulin G (IgG) and IgM and the IgG avidity index. *J Clin Microbiol* 2005;43:1570-4.
21. Medical Devices Agency. Biomerieux Vidas Immunoassay System for Chlamydia, Cytomegalovirus and *Toxoplasma* Assay. 94/57. HMSO. Norwich. 1994. p. 47-76
22. Pinon JM, Dumon H, Chemla C, Franck J, Peterson E, Lebech M. Strategy for diagnosis of congenital toxoplasmosis: evaluation of methods comparing mothers and newborns and standard methods for postnatal detection of immunoglobulin G, M and A antibodies. *J Clin Microbiol* 2001;39:2267-71.
23. The Joint United Nations Programme on HIV/AIDs. Report on the Global AIDS epidemic. 2010.
24. Health Protection Agency. HIV in the United Kingdom: 2010 report. 4(47). 2010.
25. Kahn JO, Walker BD. Acute human immunodeficiency virus type 1 infection. *N Engl J Med* 1998;339:33-9.
26. Ly TD, Laperche S, Courouce AM. Early detection of human immunodeficiency virus infection using third and fourth generation screening assays. *Eur J Clin Microbiol Infect Dis* 2001;20:104-10.
27. Brust S, Duttman H, Feldner J, Gurtler L, Thorstensson R, Simon F. Shortening of the diagnostic window with a new combined HIV p24 antigen and anti-HIV1/2/0 screening test. *J Virol Methods* 2000;90:153-65.
28. van Binsbergen J, Keur W, Siebelink A, van de DM, Jacobs A, de Rijk D. Strongly enhanced sensitivity of a direct anti HIV-1/-2 assay in seroconversion by incorporation of HIV p24 ag detection: a new generation vironostika HIV Uni Form II. *J Virol Methods* 1998;76:59-71.
29. Gurtler L, Muhlbacher A, Michl U, Hofmann H, Paggi GG, Bossi V. Reduction of the diagnostic window with a new combined p24 antigen and human immunodeficiency virus antibody screening assay. *J Virol Methods* 1998;75:27-38.
30. Meier T, Knoll E, Henkes M, Enders G, Braun R. Evidence for a diagnostic window in fourth generation assays for HIV. *J Clin Virol* 2001;23:113-6.
31. Black V, von Mollendorf CE, Moyes JA, Scott LE, Puren A, Stevens WS. Poor sensitivity of field rapid HIV testing: implications for mother-to-child transmission programme. *BJOG* 2009;116:1805-8.
32. Brown P, Merline JR, Levine D, Mince LR. Repeatedly false-negative rapid HIV test results in a patient with undiagnosed advanced AIDS. *Ann Intern Med* 2008;149:71-2.

## Microbiological Investigation of Patients with Acute Lymphadenopathy and Fever

33. World Health Organization Department of Essential Health Technologies. WHO Department of Essential Health Technologies. HIV Assays: Operational Characteristics (Phase 1). Report 12: Simple/Rapid tests, Whole blood specimens. 2002. p. 1-30.
34. Dewsnap CH, Mcowan A. A review of HIV point-of-care tests. *Int J STD AIDS* 2006;17:357-9.
35. Shafran SD, Conway B, Prasad E, Greer J, Vincelette J, Ellis CE, et al. Field evaluation of the Merlin immediate HIV-1 and -2 test for point-of-care detection of human immunodeficiency virus antibodies. *Clin Infect Dis* 2002;34:658-61.
36. Health Protection Agency. Confirmed cases of Measles, Mumps and Rubella 1996-2010. 2010.
37. Demicheli V, Jefferson T, Rivetti A, Price D. Vaccines for measles, mumps and rubella in children. *Cochrane Database Syst Rev* 2005;CD004407.
38. Best JM, Icenogle JP, Brown DW. Rubella. In: Zuckerman AJ, Banatvala JE, Griffiths P, Schoub B, Mortimer P, editors. *Principles and Practice of Clinical Virology*. 6th ed. Chichester: John Wiley & Sons Ltd; 2009. p. 561-92.
39. Munro ND, Sheppard S, Smithells RW, Holzel H, Jones G. Temporal relations between maternal rubella and congenital defects. *Lancet* 1987;2:201-4.
40. Morgan-Capner P, Crowcroft N. Guidelines on the management of, and exposure to, rash illness in pregnancy (including consideration of relevant antibody screening programmes in pregnancy). *Commun Dis Public Health* 2002;5:59-71.
41. Health Protection Agency. Syphilis and Lymphogranulom Venereum: Resurgent Sexually Transmitted infections in the UK: 2009 Report. 2009.
42. Mindel A, Tovey SJ, Timmins DJ, Williams P. Primary and secondary syphilis, 20 years' experience. *Genitourin Med* 1989;65:1-3.
43. Kingston M, French P, Goh B, Goold P, Higgins S, Sukthankar A, et al. UK National Guidelines on the Management of Syphilis 2008. *Int J STD AIDS* 2008;19:729-40.
44. Shrestha S, McIntyre PG. Evaluation of two commercial point-of-care assays for antibodies to *Treponema pallidum*. *J Infect* 2007;55:571-2.
45. Jebbari H, Alexander S, Ward H, Evans B, Solomou M, Thornton A, et al. Update on lymphogranuloma venereum in the United Kingdom. *Sex Transm Infect* 2007;83:324-6.
46. Ward H, Martin I, Macdonald N, Alexander S, Simms I, Fenton K, et al. Lymphogranuloma venereum in the United Kingdom. *Clin Infect Dis* 2007;44:26-32.
47. British Association for Sexual Health and HIV. National guideline for the management of lymphogranuloma venereum. Clinical Effectiveness Group (Association of Genitourinary Medicine and the Medical Society for the Study of Venereal Diseases). 2006.
48. Health Protection Agency. Enhanced Surveillance of LGV. 2010.