UK Standards for Microbiology Investigations

Cytomegalovirus Serology
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 https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

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.

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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.

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

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<tr>
<th>Amendment No/Date.</th>
<th>5/21.09.16</th>
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<td>Page 11.</td>
<td>Correction was made under subheading “Footnotes Relating to Immunocompetent Host Flowchart” in footnote (a) from myalgia of unknown origin to pyrexia of unknown origin.</td>
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<td>Hyperlinks updated to gov.uk.</td>
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<td>Updated logos added.</td>
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<tr>
<td>Whole document.</td>
<td>Whole document re-written and re-organised.</td>
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<tr>
<td>Scope.</td>
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<tr>
<td>Flowchart.</td>
<td>Flowchart separated into four flowcharts for serology in screening, immunocompetent host, pregnant women and congenital infection.</td>
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<tr>
<td>Footnotes.</td>
<td>Extensive footnotes included for each of the above flowcharts.</td>
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<td>References.</td>
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UK Standards for Microbiology Investigations#: Scope and Purpose

Users of SMI

- 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 SMI

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 https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories.

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.

UNDER REVIEW

1Microbiology 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 https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specified 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.
Scope of Document

Type of specimen
Blood, serum, plasma, urine, saliva, amniotic fluid

Scope
Cytomegalovirus (CMV) is a common infection that is usually harmless. It can cause serious disease in immunocompromised individuals and in babies who were infected in utero. The present SMI is composed of four algorithms that cover the investigation of CMV infection status in the following situations:

- screening of blood/organ donors, and of individuals at risk of CMV disease
- diagnosing CMV infection in symptomatic immunocompetent individuals (non-pregnant)
- diagnosing CMV infection in pregnant women
- diagnosing congenital infection

This 'CMV serology' SMI does not cover the diagnosis of CMV infection in immunocompromised individuals (including HIV-infected, graft recipient, immunosuppressive treatment). Molecular assays (e.g., p65 antigenemia) are preferred for diagnosis and monitoring of CMV infection and related disease in this patient type.

CMV belongs to the *Herpesviridae* family and persists in the host as a life-long latent infection. After primary infection, the endogenous virus may replicate de novo causing a reactivation. A new infection with an exogenous CMV can occur, referred to as reinfection. In all settings, the infection is usually asymptomatic in the immunocompetent host; however, some primary infections result in a mononucleosis syndrome or mild flu-like symptoms.

CMV is the most common cause of congenital infection, with approximately 0.5% of neonates infected. Most congenital infections are asymptomatic, with only 10 - 15% of neonates exhibiting clinical signs, such as intrauterine growth retardation, microcephaly, hepatosplenomegaly, petechiae and jaundice. Ninety percent of children with symptomatic infection, and 10 - 15% of those with asymptomatic infection, develop one or more long-term neurological sequelae such as mental retardation, psychomotor retardation, sensorineural hearing loss (SNHL), and eye abnormalities.

Definitions
For all antigen, antibody and NAATs testing the following definitions apply:

- **Reactive** – Initial internal-stage positive result pending confirmation.
- **Not reactive** – Initial internal-stage negative result.
- **Detected** – Report-stage confirmed reactive result.
- **Not detected** – Report-stage not reactive result.
Screening Flowchart

Please note: Equivocal results are not included in the above algorithm.

Footnotes Relating to Screening Flowchart

a) This includes blood/organ donors and individuals at risk of CMV disease. Screening of gametes/embryo donors is not a mandatory requirement.

b) Individuals at risk of CMV disease include future graft recipients and individuals receiving (or due to receive) immunosuppressive treatment. CMV IgG antibody is one of the markers required to evaluate the risk of CMV infection or reactivation, and to implement appropriate control measures and pre-emptive or preventive treatment.

c) Breast milk donors are no longer screened as there is evidence that pasteurisation and other processing techniques, including freezing, destroys contamination.

d) Be aware of possible passively acquired antibody. It is not recommended to screen for CMV IgG in patients who have recently received blood or blood products, including anti-D immunoglobulins. Passively acquired CMV IgG may lead to misinterpretation of the CMV infection status and to false seropositive or seroconversion results. Passively acquired immunoglobulins decrease over time, with a half-life of approximately 3 weeks. If this data is not available at the time of transplantation the worst case scenario must be considered in terms of preventing CMV infection.

e) Consider the use of two assays for transplant patients.

f) The detection of CMV IgG in blood and organ donors indicates potential infectivity of donations.
Immunocompetent Host Flowchart

Potential CMV-related illness in immunocompetent individuals

CMV IgM

Not reactive

Reactive

CMV IgG

Not reactive

REPORT: CMV IgM detected, CMV IgG not detected. Repeat CMV IgG testing in 1 to 3 weeks to further investigate possible CMV infection

REPORT: No evidence of recent CMV infection

REPORT: Evidence of recent primary CMV infection

REPORT: Suggestive of recent CMV infection

Report/notify case

Please note: Equivocal results are not included in the above algorithm.
Footnotes Relating to Immunocompetent Host Flowchart

a) Clinical mononucleosis, fever, hepatitis or pyrexia of unknown origin in immunocompetent individuals.

b) Immunocompetent women: where possible query pregnancy. If pregnant, refer to the algorithm for pregnant women.

c) The presence of CMV IgM may indicate one of the following:
   - primary infection
   - re-infection
   - reactivation
   - false-positive test result

Therefore the presence of CMV IgM cannot be used independently to diagnose primary CMV infection.

d) Consider excluding false positive CMV IgM due to acute EBV infection by testing for heterophile antibody or EBV VCA-IgM. Refer to V 26 - Epstein-Barr Virus Serology.

e) Consider testing CMV IgG and IgM on an earlier sample, if available, to aid interpretation.

f) Infants (<12 months): passively acquired maternal IgG may be present. Determine the maternal IgG status and, if positive, consider testing for CMV in the infant’s blood and/or urine. Refer to the algorithm for congenital infection if required.

g) Consider CMV NAAT on the existing serum or plasma sample. A positive CMV NAAT indicates primary CMV infection. If the CMV NAAT is negative, primary CMV infection is unlikely but cannot be excluded, and the CMV IgG test should be repeated within 1 to 3 weeks.

h) Review level of IgM reactivity and interpret results according to local assay experience.

i) Recent infection includes primary infection, reinfection and reactivation.

j) Consider IgG avidity testing on the existing serum sample, especially where timing of primary infection is important eg pregnancy (refer to the algorithm for pregnant women).

k) Where available, consider testing an earlier sample for IgG and IgM to differentiate between primary and secondary infection.
Pregnant Women Flowchart

Susicion of primary infection in a pregnant woman

CMV IgM

Not reactive

REPORT: No evidence of recent primary CMV infection

Reactive

CMV IgG

Not reactive

REPORT: CMV IgM detected, CMV IgG not detected. Repeat CMV IgG testing in 3 weeks to further investigate possible CMV infection

CMV IgG

REPORT: CMV IgG reactive

High

Intermediate

Low

Repeat IgG and IgM on the same sample to confirm seroconversion

REPORT: Consistent with recent primary CMV infection

Report/notify case

Refer to congenital infection algorithm

Repeat avidity in three weeks

Rise

Stable

REPORT: Recent CMV infection cannot be excluded

Repeat IgG and IgM on the same sample to confirm seroconversion

REPORT: No evidence of recent primary CMV infection in the past three months. Reactivation or re-infection cannot be excluded

REPORT: No evidence of recent primary CMV infection

Reactivation or re-infection cannot be excluded

REPORT: Evidence of recent primary CMV infection at the time of the earlier sample

REPORT: No evidence of recent primary CMV infection. Reactivation or re-infection cannot be excluded

CMV IgG

IgG Reactive

IgM Reactive

Reactive

Test earlier sample if available for CMV IgG and IgM

IgG Not reactive

IgM Not reactive

IgG Not reactive

IgM Reactive

Please note: Equivocal results are not included in the above algorithm
Footnotes Relating to Pregnant Women Flowchart

a) CMV infection should be suspected in symptomatic pregnant women presenting with clinical mononucleosis, fever, hepatitis or myalgia of unknown origin. If the woman is asymptomatic but concerns arise due to the foetus, refer to the congenital infection algorithm.

b) The presence of CMV IgM may indicate one of the following:
   - primary infection
   - re-infection
   - reactivation
   - false-positive test result

Therefore, the presence of CMV IgM cannot be used by itself to diagnose primary CMV infection.

c) Consider excluding false positive CMV IgM due to concurrent EBV acute infection by testing for heterophile antibody or EBV VCA-IgM.

d) Laboratories referring the sample for avidity testing may issue an interim report: ‘Suggestive of recent CMV infection. CMV IgG avidity result will follow. Please send another sample in 3 weeks’ time.

e) Seek alternative causes which present with similar illness in pregnancy.

f) Those laboratories not performing NAAT can ask for a repeat serology sample.

g) Consider NAAT on blood or urine sample.

h) Low avidity index is associated with high risk of congenital infection, whilst high avidity index detected in the first trimester of gestation is associated with low risk of vertical transmission17-20. If an earlier sample is available, test both samples (or the earlier sample only) for avidity. Increasing avidity results over time confirm acute infection around the time of the earlier sample; persistent low avidity results beyond 18 weeks (from the earliest sample tested) may be due to lack of specificity, and may require further confirmation with a different avidity assay. CMV IgG avidity results cannot exclude or confirm a reactivation or a re-infection.

i) Consider avidity testing on the earlier sample. Avidity is only recommended to be interpreted in the context of an IgM positive result; however, some experts may consider interpretation is possible where IgM is negative.

j) Risk of congenital infection is about 40%. Refer to fetal medicine. Congenital infection can be confirmed prenatally by detecting CMV in amniotic fluid. For optimal results amniocentesis must be performed at least 6 weeks after presumed time of maternal infection and after 21 weeks of gestation22-24.

k) Investigate CMV infection in the new born baby: perform CMV NAAT in urine or saliva within the first 3 weeks of life. Alternatively a positive blood CMV IgM can confirm the infection however the test lacks sensitivity and NAAT should be performed in case of negative result25. Refer to the algorithm for congenital infection8,9.
l) High avidity: no evidence of recent infection in the past 3 months\textsuperscript{12-16,26}.

m) Low avidity indicates recent infection of usually less than 3 months prior to sample date\textsuperscript{16}.

n) Interpretation of intermediate avidity is difficult and recent/relatively recent primary infection cannot be excluded, particularly in samples taken after the 1\textsuperscript{st} trimester\textsuperscript{16,17}. 
Congenital Infection Flowchart\textsuperscript{17,26}

Suspection of congenital infection

CMV IgG confirmed in maternal blood together with other evidence of CMV infection in the mother (refer to pregnant woman algorithm)\textsuperscript{a}

Antenatal diagnosis\textsuperscript{b, c}

CMV in amniotic fluid (NAAT)\textsuperscript{g}

Reactive\textsuperscript{h}

Not reactive

REPORT: Congenital infection highly unlikely\textsuperscript{i}

REPORT: Not reactive

Neonatal diagnosis ≤ 3 weeks of age\textsuperscript{c, d}

CMV in urine or saliva within 3 weeks after birth (Culture or NAAT)\textsuperscript{h}

Not reactive

Reactive

Confirm with detection of CMV in urine or saliva or Guthrie card\textsuperscript{h, i, k, l}

Repeat testing CMV in urine or saliva\textsuperscript{i, j, k, l}

CMV in Guthrie card\textsuperscript{l}

REPORT: Congenital CMV infection

REPORT: Not consistent with congenital infection

REPORT: Confirmed congenital CMV infection

REPORT: Not reactive

REPORT: Report/notify case

Late diagnosis > 3 weeks of age\textsuperscript{c, d}

>3 weeks but ≤ 12 months of age

CMV in urine or saliva (Culture or NAAT)\textsuperscript{h, i, k, l}

Not reactive

Reactive

Repeat testing CMV in urine or saliva\textsuperscript{i, j, k, l}

CMV in urine or saliva (Culture or NAAT)\textsuperscript{h, i, k, l}

Not reactive

Reactive

Not reactive

REPORT: Congenital CMV infection unlikely

>12 months of age

CMV IgG

Reactive

Not reactive

REPORT: Congenital CMV infection likely

Please note: Equivocal results are not included in the above algorithm.
Footnotes Relating to Congenital Infection Flowchart

a) Congenital CMV can be excluded if mother is CMV IgG negative. Congenital CMV infection can result from both primary and recurrent maternal infection. The risk of transmission is greater after primary infection (30-40%) than after recurrent infection (~1%)27. Not all congenitally infected babies are symptomatic at birth or develop sequelae (see Scope).

b) Antenatal diagnosis can be requested when there is suspicion of recent maternal infection or when there are ultrasound features such as intrauterine growth retardation, ventricular dilatation, intracranial calcification, microcephaly, ascites, hepatomegaly, abdominal calcification, thickened placenta.

c) Neonatal diagnosis is requested when clinical signs suggestive of congenital infection (such as intrauterine growth retardation, microcephaly, hepatosplenomegaly, petechiae and jaundice) are present at, or prior to, birth. It is also indicated for those infants born to a mother with documented recent infection, inconclusive results or with typical ultrasound abnormalities or when amniocentesis was declined.

d) Detection of CMV by NAAT (in urine or saliva) within the first 3 weeks of life is considered the gold standard method for the diagnosis of congenital CMV infection.

e) If a suitable sample for NAAT is not available, CMV IgM can be tested in the neonate’s blood. However, the test lacks sensitivity and NAAT should be performed if the CMV IgM result is negative25.

f) Late diagnosis is requested for infants and young children, usually asymptomatic at birth, who develop sequelae within a 5 to 7-year period such as sensorineural hearing loss, mental retardation, delay of psychomotor development and visual impairment28. The absence of CMV IgG in maternal blood after delivery excludes congenital CMV as the cause of hearing loss or other defect. The presence of CMV IgM in the booking blood supports, but does not confirm, the diagnosis of congenital CMV.

g) For optimal sensitivity, amniocentesis must be performed at a time that is at least 6 weeks after presumed time of maternal infection and after 21 weeks of gestation22-24.

h) Both urine and saliva of congenitally infected term neonates contain high levels of CMV and have equivalent sensitivity for diagnosis29. Real time PCR performed on dried saliva specimens was shown to be a highly sensitive and practical tool to diagnose congenital CMV5. Reactive CMV NAAT on samples taken after 3 weeks of age, cannot be distinguish congenital from postnatal or perinatal infection (refer to the ‘late diagnosis’ branch of the algorithm).

i) Viral excretion in urine and saliva lasts for several years with a steep decline after 5 years30. The median duration of urinary excretion assessed by culture was estimated to be 4.55 years in children born with asymptomatic and 2.97 years in symptomatic children31. Although there is some evidence to suggest that repeat testing should be carried out twice due to intermittent shedding, local policy may dictate that repeat testing once is acceptable30.
j) There is evidence to suggest that urine is superior to saliva when screening for postnatal CMV infections in preterm infants using NAAT.

k) If the result is discordant, investigate possible laboratory error and request a third sample to confirm.

l) CMV viral load is significantly lower in blood than in urine or saliva, and sensitivity of NAAT performed from a dried blood spot (Guthrie card) has been reported to be as low as 28%.

m) Investigate CMV infection in the neonate if pregnancy continues.

n) Negative predictive values of between 92.7% and 95.7% are reported for CMV NAAT assays of amniotic fluid were reported.

o) All babies and children with a confirmed congenital CMV infection must be followed up with regular paediatric examination and audiology assessment. Symptomatic neonates with CNS disease and/or focal organ disease may receive ganciclovir. Treatment of neonates with neurological symptoms can prevent developmental delays and hearing deterioration.
Notification to PHE\textsuperscript{38,39} or Equivalent in the Devolved Administrations\textsuperscript{40-43}

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health Protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’; it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland\textsuperscript{40,41}, Wales\textsuperscript{42} and Northern Ireland\textsuperscript{43}.
References


