UK Standards for Microbiology Investigations

Investigation of cytomegalovirus infection

This publication was created by Public Health England (PHE) in partnership with the NHS.

Issued by the Standards Unit, National Infection Service, PHE
Virology | V 28 | Issue no: 4 | Issue date: 26.06.19 | Page: 1 of 23

PHE publications gateway number: 2018381 © Crown copyright 2019
Contents

Amendment table ................................................................................................................... 3
1. General information ................................................................................................... 4
2. Scientific information ................................................................................................. 4
3. Scope of document .................................................................................................... 4
4. Safety considerations ................................................................................................ 4
5. Specimen processing and procedure ....................................................................... 4
6. Determination of CMV IgG status .............................................................................. 6
7. Diagnosing CMV infection in symptomatic non-pregnant immunocompetent individuals .................................................................................................................. 8
8. Diagnosing CMV infection in pregnant women ...................................................... 10
9. Diagnosing intrauterine and congenital infection .................................................. 13
10. Interpreting and reporting laboratory results ......................................................... 16
11. References ................................................................................................................ 21
Amendment table

Each UK 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.

<table>
<thead>
<tr>
<th>Amendment number/date</th>
<th>6/26.06.19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Issue number discarded</td>
<td>3.1</td>
</tr>
<tr>
<td>Insert issue number</td>
<td>4</td>
</tr>
<tr>
<td>Anticipated next review date*</td>
<td>26.06.22</td>
</tr>
</tbody>
</table>

**Section(s) involved**

<table>
<thead>
<tr>
<th>Amendment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole document.</td>
</tr>
<tr>
<td>Headings 1 and 2.</td>
</tr>
<tr>
<td>Document title.</td>
</tr>
<tr>
<td>References.</td>
</tr>
<tr>
<td>Flowcharts and footnotes.</td>
</tr>
<tr>
<td>Screening flowchart.</td>
</tr>
<tr>
<td>Immunocompetent host flowchart.</td>
</tr>
<tr>
<td>Pregnant women flowchart.</td>
</tr>
<tr>
<td>Congenital infection flowchart.</td>
</tr>
</tbody>
</table>

*Reviews can be extended up to five years subject to resources available.*
1. **General information**

View general information related to UK SMIs.

2. **Scientific information**

View scientific information related to UK SMIs.

3. **Scope of document**

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

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

This document does not cover CMV diagnosis in immunocompromised individuals (including human immunodeficiency virus (HIV) infected, graft recipient, immunosuppressive treatment). In these patients serological methods are of limited use and molecular assays are the preferred tools for diagnosis and monitoring of CMV infection and related disease. However, serological assays are used for pre-transplant assessment of the solid organ transplant donor and recipient and for screening donors of blood products to minimize risk of CMV infection in seronegative recipients.

Refer to Q 7 - Good practice when undertaking serology assays for infectious diseases for information regarding good laboratory practice in serological testing.

This UK SMI should be used in conjunction with other UK SMIs.

4. **Safety considerations**

This guidance should be supplemented with local COSHH and risk assessments. Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

5. **Specimen processing and procedure**

5.1 **Specimen type**

Blood, serum, plasma, urine, saliva, amniotic fluid.
5.2 Specimen transport and storage conditions

Specimens should be collected in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Specimens should be transported and processed according to manufacturer’s instructions or local validation data\(^4\).

Samples should be retained in accordance with The Royal College of Pathologists guidelines ‘The retention and storage of pathological records and specimens’\(^5\).
6. Determination of CMV IgG status

![Flowchart diagram]

**Report:**
- Reactive: CMV infection at some time or passively acquired antibody
- Not reactive: No evidence of past CMV infection

*Please note:* Equivocal results are not included in the above algorithm.
Footnotes relating to determination of CMV IgG status flowchart

a) This includes blood donors and organ donors and individuals at risk of CMV disease1.

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) Not all milk banks screen all potential breast milk donors as there may be effective treatment processes available that reduce or eliminate contamination6.

d) Passively acquired CMV IgG may be detectable in patients who have recently received blood or blood products, including anti-D immunoglobulins, leading to misinterpretation of the CMV infection status through false seropositive or seroconversion results3. 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) The detection of CMV IgG in blood and organ donors indicates potential infectivity of donations.
7. **Diagnosing CMV infection in symptomatic non-pregnant immunocompetent individuals**

- **Potential CMV-related illness in immunocompetent individuals**
  - CMV IgM
  - Not reactive
  - Reactive
    - CMV IgG
      - Not reactive
      - Reactive
        - CMV IgG
          - Not reactive
          - Reactive
            - CMV IgG
              - Not reactive
              - Reactive
                - **REPORT:** Evidence of recent primary CMV infection
                - **REPORT:** Suggestive of recent CMV infection
                - Report/notify case
            - **REPORT:** No evidence of recent CMV infection
        - **REPORT:** No evidence of recent CMV infection
    - **REPORT:** Evidence of recent primary CMV infection
    - **REPORT:** Suggestive of recent CMV infection

*Please note:* Equivocal results are not included in the above algorithm.
Footnotes relating to symptomatic non-pregnant immunocompetent individuals 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 or reinfection.

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.
8. Diagnosing CMV infection in pregnant women

**Suspicion of primary infection in a pregnant woman**

- **CMV IgM**
  - **Not reactive**
  - **Reactive**
    - **CMV IgG**
      - **Not reactive**
        - **REPORT: No evidence of recent primary CMV infection**
      - **Reactive**
        - **Reactive**
          - **CMV IgG avidity**
            - **(current sample)**
              - **REPORT: No evidence of recent primary CMV infection. Reactivation or re-infection cannot be excluded**
            - **High**
              - **Repeat avidity in three weeks**
            - **Low**
              - **Repeat avidity in three weeks**
            - **Intermediate**
              - **Repeat avidity in three weeks**
            - **REPORT: Recent CMV infection cannot be excluded**
          - **IgG Reactive IgM Not reactive**
            - **REPORT: Recent CMV infection cannot be excluded**
          - **IgG Not reactive IgM Not reactive**
            - **REPORT: No evidence of recent primary CMV infection. Reactivation or re-infection cannot be excluded**
            - **CMV IgG reacted**
              - **CMV IgG and IgM**
                - **Test earlier sample if available for CMV IgG and IgM**
            - **CMV IgG not detected. Repeat CMV IgG testing in 1-3 weeks to further investigate possible CMV infection**
          - **IgG Reactive IgM Reactive**
            - **REPORT: Consistent with recent primary CMV infection**
            - **Report/notify case**
            - **Refer to congenital infection algorithm**

**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 V 26 – Epstein-Barr virus serology.

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 1 to 3 weeks’ time.’

e) The presence of high-avidity CMV IgG antibodies before 16 weeks of gestation excludes primary infection in pregnancy; however, nonprimary infection is still a possibility. Indications for prenatal testing in nonprimary infections are less clear, and decisions should be made on a case-by-case basis when sonographic findings are suggestive of intrauterine infection. Therefore, seek alternative causes which present with similar illness in pregnancy.

f) Consider NAAT on blood or urine 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.

g) Low avidity index is associated with high risk of intrauterine infection, whilst high avidity index detected in the first trimester of gestation is associated with low risk of vertical transmission. If an earlier sample is available, test both samples (or the earlier sample only) for avidity. Increasing avidity results over time confirms 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.

h) 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.

i) Risk of intrauterine infection is about 32%. Intrauterine 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 gestation\textsuperscript{10,11,13}.

j) 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 result\textsuperscript{17-19}. Refer to the algorithm for congenital infection\textsuperscript{3,8}.

k) High avidity: no evidence of recent infection in the past 3 months\textsuperscript{9,10,14,20,21}.

l) Low avidity indicates recent infection of usually less than 3 months prior to sample date\textsuperscript{21}.

m) 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{14,21}. 
9. Diagnosing intrauterine and congenital infection

Suspected of congenital infection → CMV IgG confirmed in maternal blood together with other evidence of CMV infection in the mother (refer to pregnant woman algorithm)

Antenatal diagnosis ≤ 3 weeks of age

CMV in amniotic fluid (NAAT)

- Reactive
  - Repeat testing CMV in urine or saliva
  - Confirmed intrauterine CMV infection
  - Report/notify case

- Not reactive
  - REPORT: No evidence of intrauterine infection. Consider testing urine or saliva after birth

Neonatal diagnosis 3 ≤ 3 weeks after birth

CMV in urine or saliva within 3 weeks after birth (NAAT)

- Reactive
  - Repeat testing CMV in urine or saliva
  - CMV in Guthrie card (NAAT)
  - Confirmed congenital CMV infection
  - Report/notify case

- Not reactive
  - Not reactive
  - Not reactive
  - CMV IgG in urine or saliva
  - Not reactive
  - Repeat testing CMV in urine or saliva
  - Reports: Not consistent with congenital infection
  - Not reactive
  - Not reactive
  - Not reactive
  - CONGENITAL CMV infection unlikely

Late diagnosis >3 weeks of age

CMV in urine or saliva

- Reactive
  - Reactive
  - Reactive
  - Reactive
  - REPORT: Congenital CMV infection unlikely

- Not reactive
  - Repeat testing CMV in urine or saliva
  - REPORT: No evidence of congenital CMV infection

>12 months of age

CMV IgG

- Reactive
  - Reactive

REPORT: Confirmed intrauterine CMV infection

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

Virology | V 28 | Issue no: 4 | Issue date: 26.06.19 | Page: 13 of 23

UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England
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%). Not all congenitally infected babies are symptomatic at birth or develop sequelae.

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, echogenic bowel.

c) Neonatal diagnosis is requested when clinical signs suggestive of intrauterine infection (such as sensorineural hearing loss, intrauterine growth retardation, microcephaly, hepatosplenomegaly, petechiae, jaundice, intracranial calcification, brain white matter disease, polymicrogyria, retinitis/retinal scarring) 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 fetal ultrasound abnormalities or when amniocentesis for suspected intrauterine CMV infection 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. Note there is a risk of false positive detection of CMV by NAAT in the saliva of recently breast-fed infants; saliva swabs should be taken on two separate occasions, prior to a breast feed.

e) Late diagnosis is requested for infants and young children, usually asymptomatic at birth, who develop sequelae such as sensorineural hearing loss, mental retardation, delay of psychomotor development and visual impairment typically within a 5 to 7-year period but later in some cases.

f) 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 gestation; however, there may be clinical reasons to conduct this earlier.

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

h) Viral excretion in urine and saliva lasts for several years with a steep decline after 5 years. 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 acceptable.

i) If the result is discordant, investigate possible laboratory error and consider request of a third sample to confirm. Note there is a risk of false positive detection of CMV by NAAT in the saliva of recently breast-fed infants.
j) Sensitivity of NAAT performed from a dried blood spot (Guthrie card) has been reported to be between 70-80%\textsuperscript{29,30}.

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

l) Negative predictive values of between 92.7% and 95.7% are reported for CMV NAAT assays of amniotic fluid\textsuperscript{31}.

m) All babies and children with a confirmed congenital CMV infection must be followed up with regular paediatric examination and audiology assessment\textsuperscript{32}. Symptomatic neonates with CNS disease and/or focal organ disease may receive valganciclovir\textsuperscript{33}. Treatment of neonates with neurological symptoms can prevent developmental delays and hearing deterioration\textsuperscript{34,35}. 

## 10. Interpreting and reporting laboratory results

There are other combinations of results which have not been tabled but which do occur and require individual comments based upon profile and clinical setting, along with a further sample.

### 10.1 Immunocompetent host

<table>
<thead>
<tr>
<th></th>
<th>CMV IgM</th>
<th>CMV IgG</th>
<th>Interpretative Comment</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not reactive</td>
<td>Not reactive</td>
<td>No serological evidence of CMV infection at any time</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Not reactive</td>
<td>Reactive</td>
<td>Consistent with past CMV infection</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Reactive</td>
<td>Not reactive</td>
<td>CMV IgM detected, CMV IgG not detected. Repeat CMV IgG testing in 1 to 3 weeks to further investigate possible CMV infection</td>
<td>If IgG is reactive on the subsequent test this is evidence of primary infection. If IgG is not reactive on the subsequent test consider excluding false positive CMV IgM due to acute EBV infection by testing for heterophile antibody or EBV VCA-IgM</td>
</tr>
<tr>
<td>4</td>
<td>Reactive</td>
<td>Reactive</td>
<td>Suggestive of recent CMV infection</td>
<td>IgG avidity testing can be used to further investigate</td>
</tr>
</tbody>
</table>
### 10.2 Pregnant women, earlier antenatal serum sample not available

<table>
<thead>
<tr>
<th></th>
<th>CMV IgM</th>
<th>CMV IgG</th>
<th>CMV IgG avidity</th>
<th>Interpretative Comment</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not reactive</td>
<td>N/A</td>
<td>N/A</td>
<td>CMV IgM not detected. No evidence of recent primary CMV infection</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Reactive</td>
<td>Not reactive</td>
<td>N/A</td>
<td>CMV IgM detected. CMV IgG not detected. Repeat CMV IgG testing in 1 to 3 weeks to further investigate possible CMV infection</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Reactive</td>
<td>Reactive</td>
<td>High</td>
<td>No evidence of recent primary CMV infection in the past three months. Reactivation or reinfection cannot be excluded</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Reactive</td>
<td>Reactive</td>
<td>Intermediate</td>
<td>Recent primary CMV infection cannot be excluded. Repeat avidity in three weeks</td>
<td>A rise in avidity index on the repeat test is consistent with primary CMV infection. Recent primary CMV infection cannot be excluded if the repeat test is stable.</td>
</tr>
<tr>
<td>5</td>
<td>Reactive</td>
<td>Reactive</td>
<td>Low</td>
<td>Consistent with recent primary CMV infection within the preceding 3 months</td>
<td></td>
</tr>
</tbody>
</table>
### 10.3 Pregnant women, earlier antenatal serum sample available

<table>
<thead>
<tr>
<th></th>
<th>CMV IgM</th>
<th>CMV IgG (current sample)</th>
<th>Earlier CMV IgG</th>
<th>Earlier CMV IgM</th>
<th>Interpretative Comment</th>
<th>Notes</th>
</tr>
</thead>
</table>
| 1 | Reactive | Reactive | Reactive | Reactive | IgG avidity test results:  
High: No evidence of recent primary CMV infection in the past 3 months  
Low: Consistent with recent primary CMV infection  
Intermediate: Recent CMV infection cannot be excluded. Repeat avidity test:  
- Rise: interpret as low  
- Stable: Recent CMV infection cannot be excluded | IgG avidity test should be performed. Refer to serum sample not available reporting table |
| 2 | Reactive | Reactive | Reactive | Not reactive | No evidence of recent primary CMV infection. Reactivation or reinfection cannot be excluded |
| 3 | Reactive | Reactive | Not reactive | Reactive | Evidence of recent primary CMV infection before the time of the earlier sample |
| 4 | Reactive | Reactive | Not reactive | Not reactive | Consistent with recent primary CMV infection |
10.4 Intrauterine infection – antenatal diagnosis
The following reporting table is in the context of confirmed CMV IgG in maternal blood together with other evidence of CMV infection in the mother (refer to pregnant woman algorithm)

<table>
<thead>
<tr>
<th>CMV in amniotic fluid (NAAT)</th>
<th>Interpretive comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1     Detected</td>
<td>Confirmed intrauterine CMV infection</td>
</tr>
<tr>
<td>2     Not detected</td>
<td>No evidence of intrauterine infection</td>
</tr>
</tbody>
</table>

10.5 Congenital infection – neonatal diagnosis (within 3 weeks of birth)

<table>
<thead>
<tr>
<th>CMV in urine or saliva within 3 weeks of birth</th>
<th>Interpretive comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1     Detected</td>
<td>Confirmed congenital CMV infection</td>
</tr>
<tr>
<td>2     Not detected</td>
<td>Not consistent with congenital infection</td>
</tr>
</tbody>
</table>

10.6 Congenital infection – late diagnosis (between 3 weeks and 12 months of age)

<table>
<thead>
<tr>
<th>CMV in urine or saliva between 3 weeks and 12 months of age</th>
<th>CMV in Guthrie card</th>
<th>Interpretive comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1     Detected</td>
<td>Detected</td>
<td>Confirmed congenital CMV infection</td>
</tr>
<tr>
<td>2     Detected</td>
<td>Not detected</td>
<td>Congenital infection cannot be excluded</td>
</tr>
<tr>
<td>3     Not detected</td>
<td>N/A</td>
<td>Congenital CMV infection unlikely</td>
</tr>
</tbody>
</table>
### 10.7 Congenital infection – late diagnosis (over 12 months of age)

<table>
<thead>
<tr>
<th>CMV IgG after 12 months of age</th>
<th>CMV in Guthrie card</th>
<th>Interpretive comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reactive</td>
<td>Detected</td>
</tr>
<tr>
<td>2</td>
<td>Reactive</td>
<td>Not detected</td>
</tr>
<tr>
<td>3</td>
<td>Not reactive</td>
<td>N/A</td>
</tr>
</tbody>
</table>
11. References


Investigation of cytomegalovirus infection


23. NHS Screening Programmes. NHS Newborn Hearing Screening Programme 2009. A, VI


