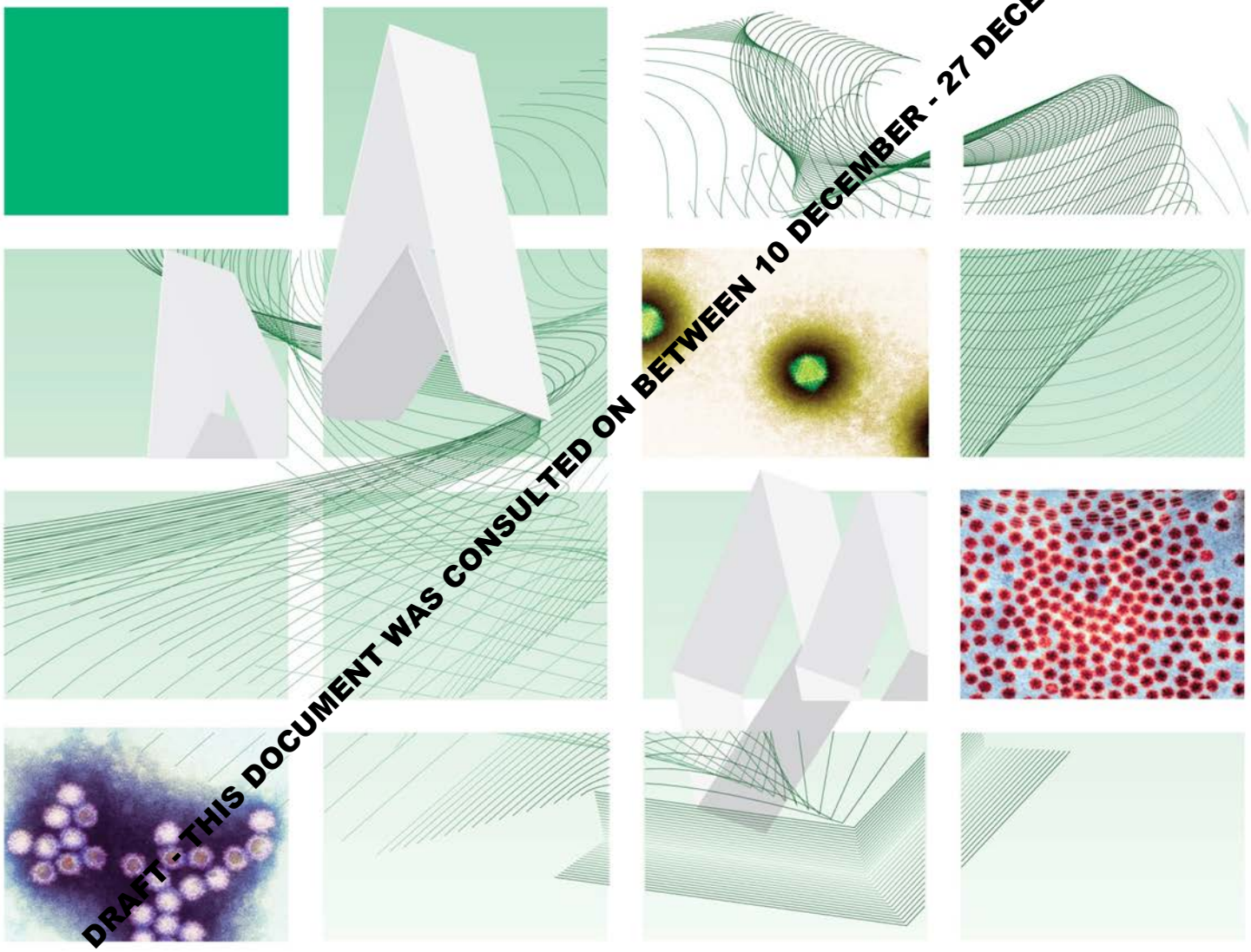
Public Health England

NHS

UK Standards for Microbiology Investigations

Investigation of cytomegalovirus infection



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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.

Amendment number/date	New amendment number/dd.mm.yy <tab+enter>
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Anticipated next review date*	
Section(s) involved	**Amendment**

*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) 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[1,2]
- diagnosing CMV infection in symptomatic immunocompetent individuals (non-pregnant)
- diagnosing CMV infection in pregnant women
- diagnosing congenital infection

This document does not cover CMV diagnosis in immunocompromised individuals (including HIV-infected, graft recipient, immunosuppressive treatment). In these patients serological methods are of limited usefulness and molecular assays (PCR or pp65 antigenemia) are the preferred tools for diagnosis and monitoring of CMV infection and related disease[3]. 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[3].

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.

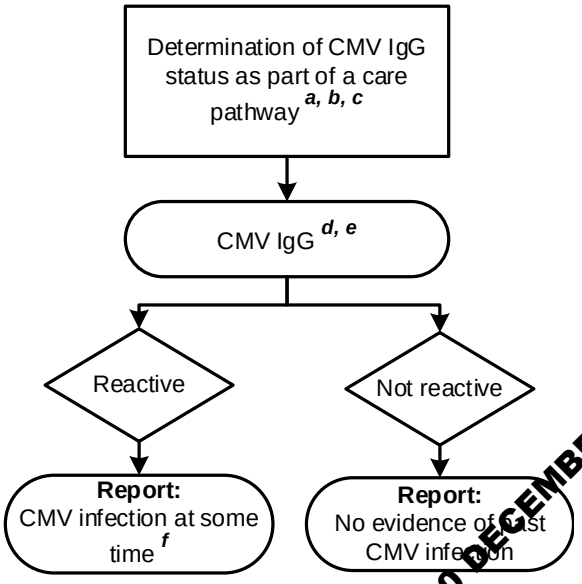
5.2 Specimen transport and storage conditions

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

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. Screening of blood/organ donors, and of individuals at risk of CMV disease



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[1]. Screening of gametes/embryo donors is not a mandatory requirement but may be carried out in certain circumstances depending on the patient's travel and exposure history, and the characteristics of the cells donated[6].

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.

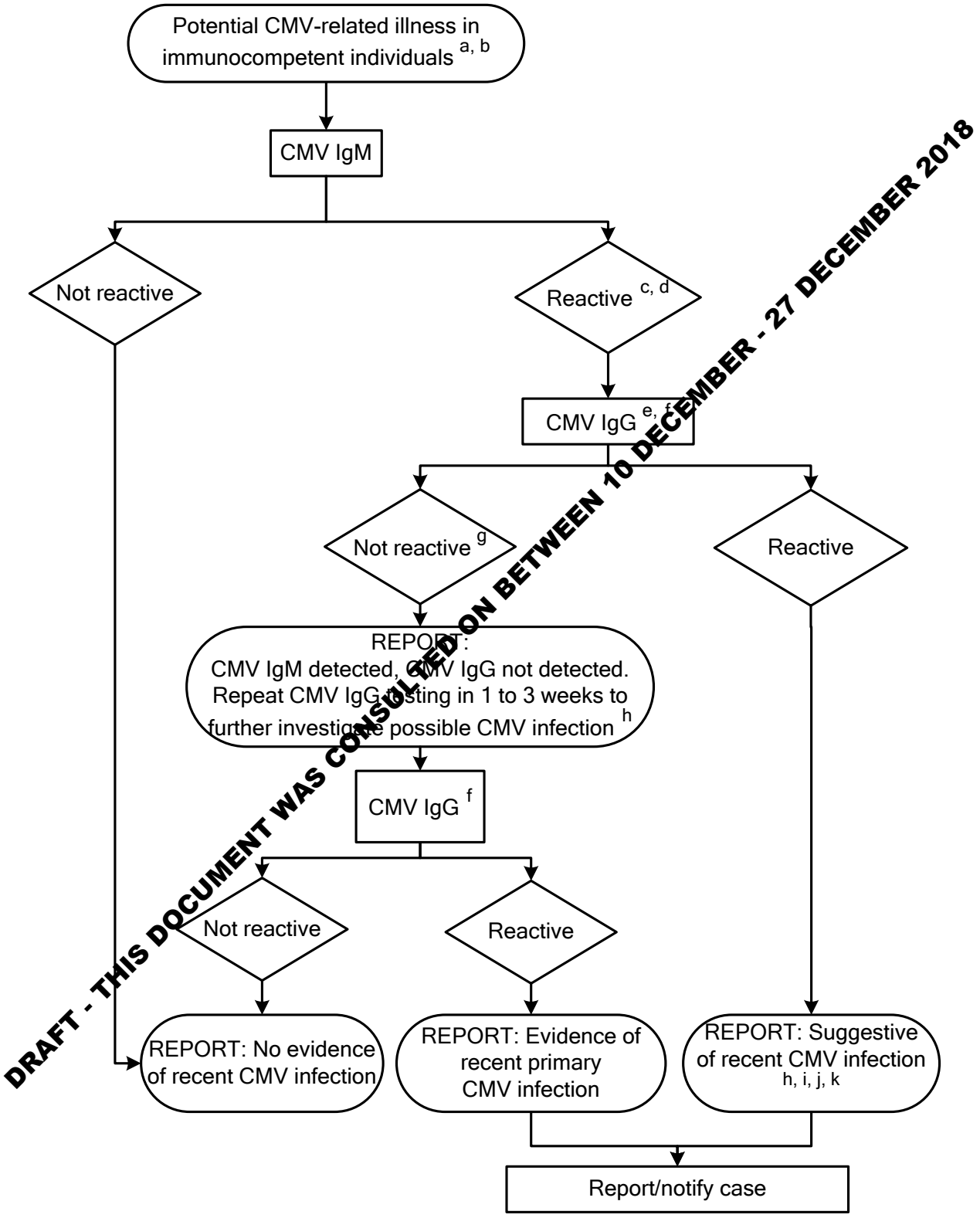
c) Breast milk donors are no longer screened as there is evidence that pasteurisation and other processing techniques, including freezing destroys contamination[7].

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[3]. 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[1].

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

7. Diagnosing CMV infection in symptomatic immunocompetent individuals



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[3,8].

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.

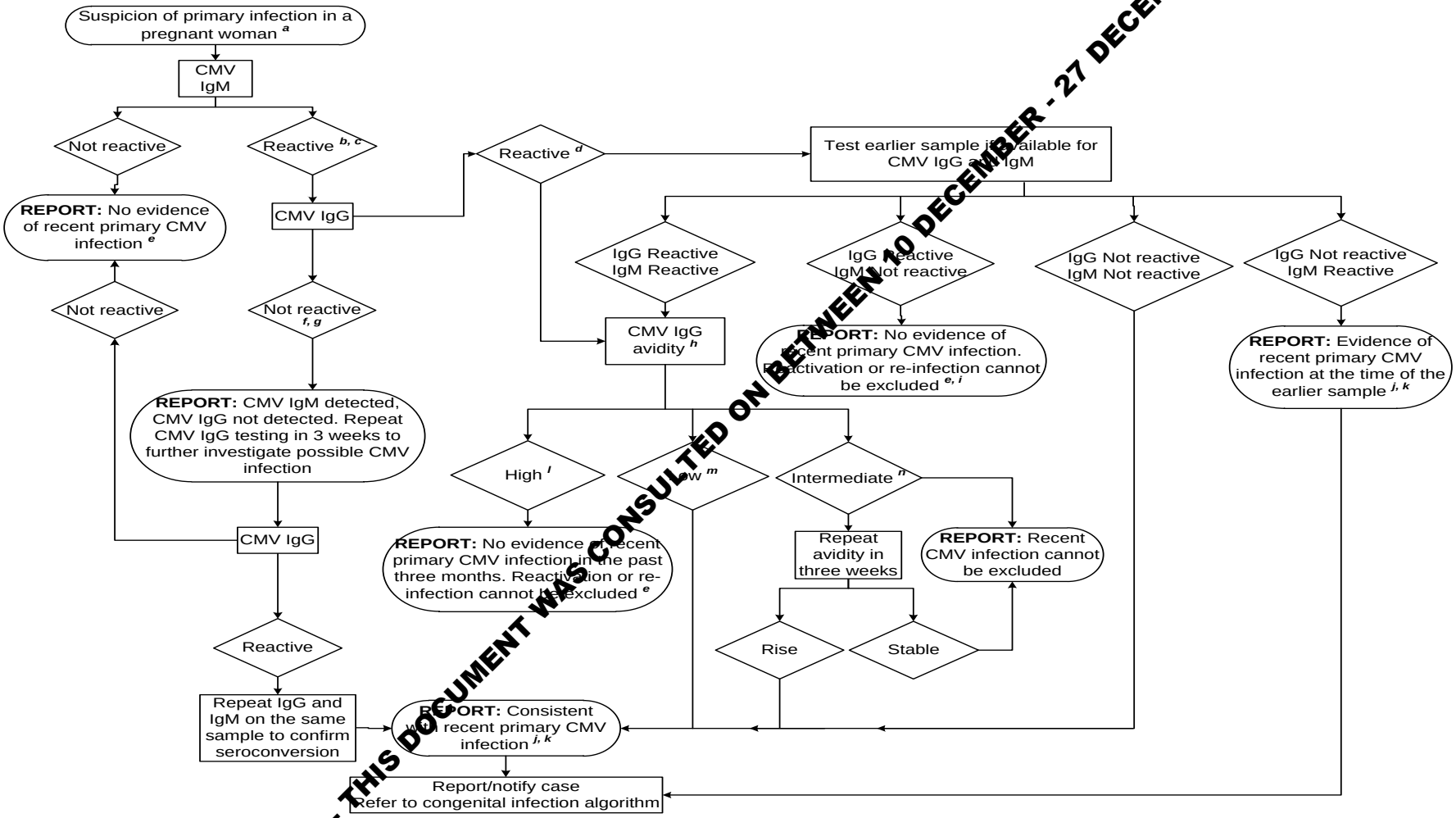
h) Review level of IgM reactivity and interpret results according to local assay experience.

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.

8. Diagnosing CMV infection in pregnant women



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 3 weeks' time.'

e) The presence of high-avidity CMV IgG antibodies before 16 weeks of gestation excludes primary infection; however non-primary infection is still a possibility. Indications for prenatal testing in non-primary infections are less clear, and deisions should be made on a case-by-case basis when sonographic findings are suggestive of congenital infection[8]. 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 transmission[9-12]. 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[13]. 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 gestation[14-16].

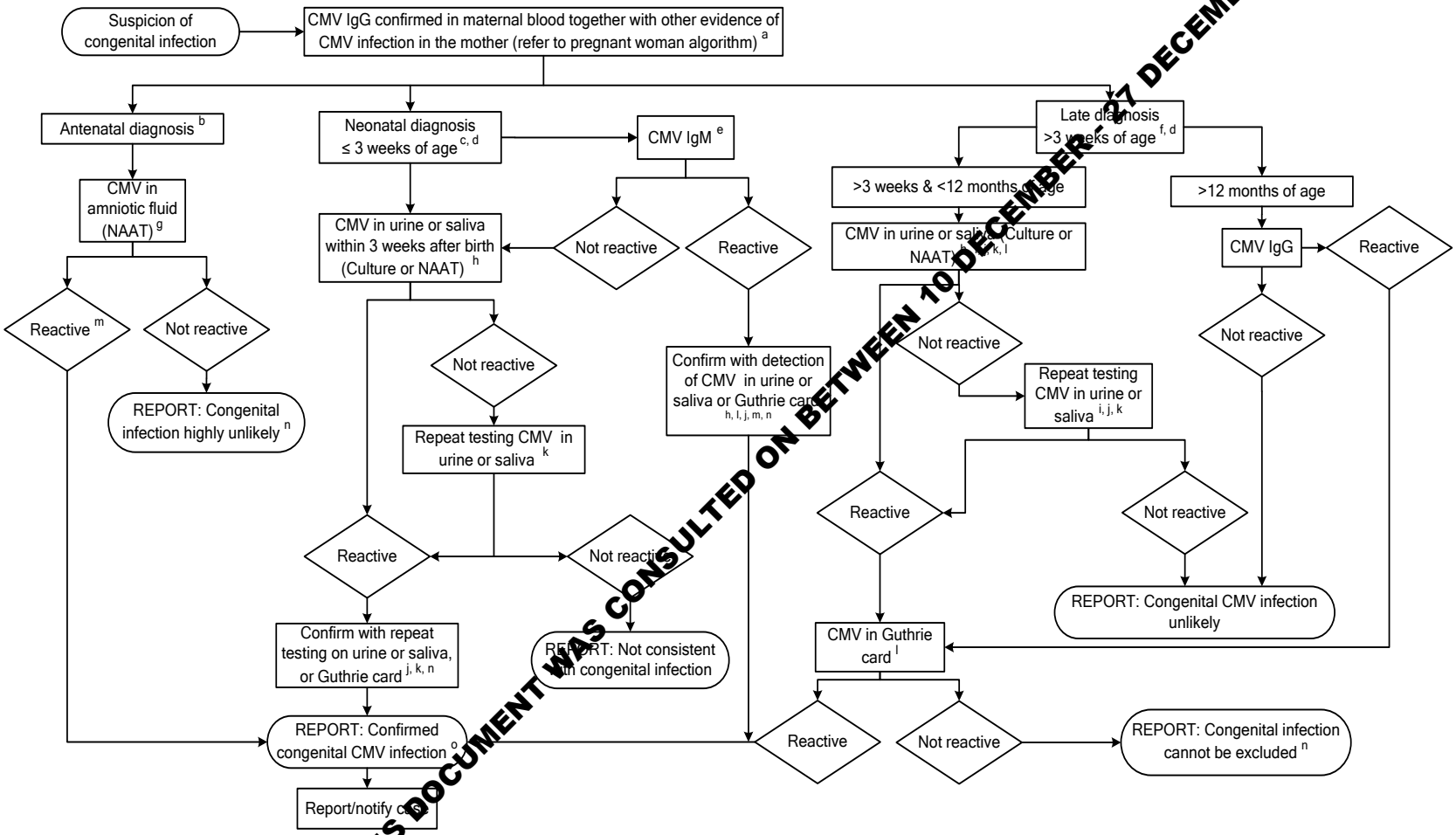
k) Investigate CMV infection in the new born baby: perform CMV NAAT in urine or saliva within the first 3 weeks of life. Alternatively a positive blood CMV IgM can confirm the infection however the test lacks sensitivity and NAAT should be performed in case of negative result[17]. Refer to the algorithm for congenital infection[3,8].

l) High avidity: no evidence of recent infection in the past 3 months[18-23].

m) Low avidity indicates recent infection of usually less than 3 months prior to sample date[23].

n) Interpretation of intermediate avidity is difficult and recent/relatively recent primary infection cannot be excluded, particularly in samples taken after the 1st trimester[12,23].

9. Diagnosing congenital infection



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%)[24]. 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 negative[17].

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 impairment[25]. 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 gestation[14-16].

h) Both urine and saliva of congenitally infected term neonates contain high levels of CMV and have equivalent sensitivity for diagnosis[26]. Real time PCR performed on dried saliva specimens was shown to be a highly sensitive and practical tool to diagnose congenital CMV[27]. 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).

i) Viral excretion in urine and saliva lasts for several years with a steep decline after 5 years[28]. 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 children[29]. 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[28].

j) There is evidence to suggest that urine is superior to saliva when screening for postnatal CMV infections in preterm infants using NAAT[30].

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%[31,32].

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[33].

o) All babies and children with a confirmed congenital CMV infection must be followed up with regular paediatric examination and audiology assessment[34]. Symptomatic neonates with CNS disease and/or focal organ disease may receive ganciclovir[34]. Treatment of neonates with neurological symptoms can prevent developmental delays and hearing deterioration.[35,36]

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

	CMV IgM	CMV IgG	Interpretative Comment	Notes
1	Not reactive	Not reactive	No serological evidence of CMV infection at any time	
2	Not reactive	Reactive	Consistent with past CMV infection	
3	Reactive	Not reactive	CMV IgM detected, CMV IgG not detected. Repeat CMV IgG testing in 1 to 3 weeks to further investigate possible CMV infection	If IgG is reactive on the subsequent test this is evidence of primary infection
4	Reactive	Reactive	Suggestive of recent CMV infection	IgG avidity testing can be used to further investigate

10.2 Pregnant women, earlier antenatal serum sample not available

	CMV IgM	CMV IgG	CMV IgG avidity	Interpretative Comment	Notes
1	Not reactive	Not reactive	N/A	CMV IgM not detected. CMV IgG not detected. No evidence of recent primary CMV infection	
2	Reactive	Not reactive	N/A	CMV IgM detected. CMV IgG not detected. Repeat CMV IgG testing in 3 weeks to further investigate possible CMV infection	
3	Reactive	Reactive	High	No evidence of recent primary CMV infection in the past three months. Reactivation or reinfection cannot be excluded	
4	Reactive	Reactive	Intermediate	CMV infection cannot be excluded. Repeat avidity in three weeks	A rise on the repeat test is consistent with primary CMV infection. CMV infection cannot be excluded if the repeat test is stable.
5	Reactive	Reactive	Low	Consistent with recent primary CMV infection	

10.3 Pregnant women, earlier antenatal serum sample available

	CMV IgM & IgM	Earlier CMV IgM	Earlier CMV IgG	Interpretative Comment	Notes
1	Reactive	Reactive	Reactive	See notes	IgG avidity test should be performed. Refer to serum sample not available reporting table
2	Reactive	Reactive	Not reactive	Evidence of recent primary CMV infection at the time of the earlier sample	
3	Reactive	Not reactive	Reactive	No evidence of recent primary CMV infection. Reactivation or reinfection cannot be excluded	
4	Reactive	Not reactive	Not reactive	Consistent with recent primary CMV infection	

10.4 Congenital 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)

	CMV in amniotic fluid	Interpretive comment
1	Reactive	Confirmed congenital CMV infection
2	Not reactive	Congenital infection highly unlikely

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

	CMV in urine or saliva within 3 weeks of birth	CMV IgM within 3 weeks of birth	Confirmed by repeat test of urine or saliva	Interpretive comment
1	Reactive	N/A	Reactive	Confirmed congenital CMV infection
2	Not reactive	N/A	Reactive	Confirmed congenital CMV infection
3	Not reactive	N/A	Not reactive	Not consistent with congenital infection
4	Reactive	Reactive	N/A	Confirmed congenital CMV infection

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

	CMV in urine or saliva between 3 weeks and 12 months of age	Confirmed by repeat test of urine or saliva	CMV in Guthrie card	Interpretive comment
1	Reactive	N/A	Reactive	Confirmed congenital CMV infection
2	Reactive	N/A	Not reactive	Congenital infection cannot be excluded
3	Not reactive	Reactive	Reactive	Confirmed congenital CMV infection
4	Not reactive	Reactive	Not reactive	Congenital infection cannot be excluded
5	Not reactive	Not reactive	N/A	Congenital CMV infection unlikely

10.7 Congenital infection – late diagnosis (over 12 months of age)

	CMV IgG after 12 months of age	CMV in Guthrie card	Interpretive comment
1	Reactive	Reactive	Confirmed congenital CMV infection
2	Reactive	Not reactive	Congenital infection cannot be excluded
3	Not reactive	N/A	Congenital CMV infection unlikely

11. References

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