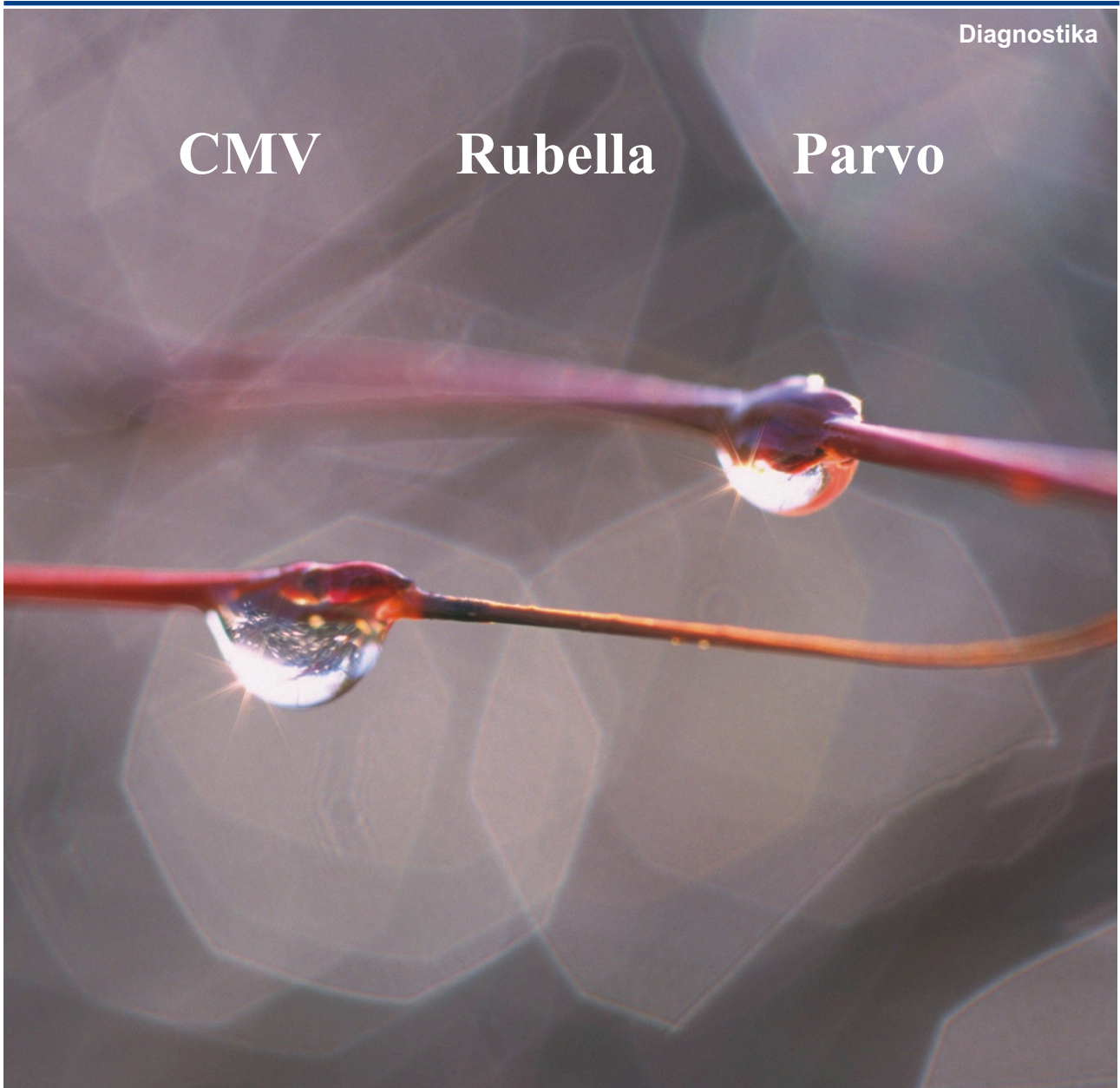


CMV

Rubella

Parvo



Antenatal Care Protects Mother and Child

CMV-, Rubella- and Parvovirus B19 antibody Diagnostic Testing from medac

medac



Cytomegalovirus

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medac tests

CMV-IgM-ELA test PKS medac	110/PKS	96 (96x1)
CMV-IgA-ELA test PKS medac	112/PKS	96 (96x1)
CMV-IgG-ELA test PKS medac	115/PKS	96 (96x1)
CMV-IgG-ELA test PKS medac	116/PKS	5x96 (96x1)
CMV-IgG-ELA test medac	115/12	96 (12x8)
CMV-IgG-ELA standard kit medac	117D	
Rubella-IgM-ELA test PKS medac	135/PKS	96 (96x1)
Rubella-IgG-ELISA PKS medac	136/PKS	96 (96x1)
Parvo B19-IgM-ELISA medac	190	96 (12x8)
Parvo B19-IgG-ELISA medac	195	96 (12x8)

Introduction

CYTOMEGALOVIRUS

Cytomegalovirus, rubella and parvovirus B19 infections are among the principal blood borne transplacentally transmitted virus infections in pregnancy.

In pregnant women primary and secondary infections are usually symptomless or inconspicuous, but they can cause serious fetal damage. Even in children who have shown no abnormalities at birth, late lesions cannot be ruled out.

Routine antenatal care should therefore include serological testing to check the immune status of the woman and to diagnose any fresh virus infections that may arise.

Cytomegalovirus is a human pathogen belonging to the Herpesviridae family, which is of considerable importance in medicine. One typical feature of these viruses is that after a primary infection they remain latent within the body and in certain circumstances can be reactivated.

In immunocompetent persons CMV infectious usually run their course without conspicuous symptoms or signs. People whose immunity is impaired, however, may develop serious clinical illness.

CMV is of special significance as the cause of prenatal infections. About 1% of all live-born children are affected by CMV infection. Of all congenital virus infections, it is the most frequent. Maternal antibodies do not protect the fetus from infection. However, it seems certain that infection of the mother, especially a primary infection, will cause clinically relevant infection of the fetus.

Diagnostic investigation of primary infection in pregnancy is therefore of great importance. Should a congenital infection be detected, paediatric monitoring is essential so that any damage to the child can be recognised and treated. A serological check at the beginning of pregnancy is useful to ascertain the immune status of the mother. Women who are CMV seronegative should be tested again during the second trimester.

Characteristics

Symptoms and signs

Significance

Diagnosis

Frequency, symptoms and signs and sequelae of prenatal CMV infections

Prevalence

Symptoms and signs

Sequelae

Pregnant women

Nonimmune (seronegative)

Immune (seropositive)

Primary infection in 1-5% of pregnant women

Secondary infection in 10-20% of pregnant women



in 30-40% of these women → infection of fetus

in 0,5-1,5% of fetuses → infections during a recurrence



in 10% of infected Fetuses → serious postnatal conditions:

- hepatosplenomegaly
- thrombocytopenia
- hyperbilirubinaemia
- CNS lesions
- pneumonia
- seizures
- blindness
- deafness
- pareses

in ≤1% of children → clinical abnormalities e.g. hearing loss, psychomotor disorders

Late sequelae:
in 90% of clinically affected patients and in 5-15% of children with inapparent infections:

- hearing loss
- intellectual retardation
- postural and motor disabilities
- dental defects
- learning difficulties

CMV-diagnostic testing

CYTOMEGALOVIRUS

- ◆ **CMV serology is currently the only practicable means of distinguishing between primary and secondary infection.**
- ◆ The only proof of a primary infection is the appearance of CMV antibodies for the first time. For this event to be identified, the previous immune status must be known to have been seronegative.
- ◆ A positive IgM test points to an acute infection. In secondary infections (endogenous reactivation or exogenous reinfection), IgM levels are not measurable in about 50% of cases.
- ◆ Reactivation of CMV is frequently accompanied by a rise in IgA antibodies. IgA determinations can therefore be of great value in differential diagnosis, especially when there is no IgM response.
- ◆ Determination of IgG titer and significant rises in titer on subsequent measurements offer another resource for improving the diagnosis of CMV infections and reactivations.

**Primary
infection**

**Secondary
infection**

Recommended diagnostic procedure for CMV infections during pregnancy

Immune status known

Pregnant women whose serological status before pregnancy is known



seropositive (IgG)

↓
no further tests

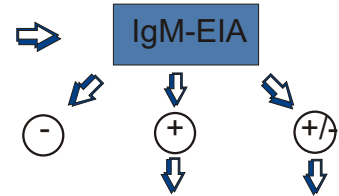
↓
seronegative

↓
For identifying a potential primary infection:

1. Detection of CMV-specific IgM
2. CMV IgG seroconversion (two tests in the second and fourth months of pregnancy)
3. If the patient is persistently seronegative → follow-up

Immune status unknown

Pregnant women whose serological status before pregnancy is unknown



Western blot+ / avidity

primary infection
WB +/AI <30%

secondary infection
WB +/AI >60%

↓
Prenatal diagnosis (22nd week)

↓
amniotic fluid

virus isolation	pos	neg	neg
PCR	pos	pos	neg
Probability of transmission	100%	40%	0%

Introduction

RUBELLA

The causative agent of rubella, the rubella virus, belongs to the family of Togaviridae.

Postnatal infections run a relatively harmless course with little or no fever; in children the disease is usually inapparent.

In contrast, however, primary rubella infections in the early stages of pregnancy carry a high risk of fetal damage or multiple defects.

During the viraemic phase the infection rate is 80-90% for the placenta and 60-70% for the embryo. The number of cases of rubella embryopathy is about 100 per 800,000 live births.

Rubella embryopathies and their clinical features

<u>Syndrome</u>	<u>Organ</u>	<u>Clinical features</u>
Gregg's syndrome	heart	- patent ductus arteriosus - aortic stenosis
	eyes	- cataract - glaucoma - retinopathy
	ears	- inner ear defects
Congenital rubella syndrome		- intellectual retardation - low birth weight - growth retardation, osteopathy - encephalitis - hepatosplenomegaly - pneumonia - thrombocytopenia - purpura
Late rubella syndrome		- chronic rash - growth arrest - interstitial pneumonia - IgG and IgA hypogammaglobulinaemia - persistence of IgM
Late manifestations		- hearing loss - diabetes mellitus - progressive panencephalitis - seizures

Characteristic

Significance

Clinical features

Rubella diagnostic testing

Recommendations

The rubella reference centre of the Robert Koch Institute has published clear recommendations for determining immune status, and detecting or elucidating primary and reinfections, as also for prenatal diagnosis.

Investigation of acute cases

The serological diagnosis of acute rubella infections is nowadays usually based on the detection of rubella-specific antibodies (IgG and IgM). The IgG tests used for this purpose should be quantifiable and calibrated against the WHO standard.

Assessment of immune status

Quantitative rubella IgG determinations are assuming an increasingly important place in the ascertainment of immune status. IgM antibodies are evaluated by means of index readings.

Diagnostic investigation during pregnancy

Because of the serious consequences of a primary infection occurring in a seronegative woman, rubella testing is an essential element of routine antenatal care.

The interpretation of serological findings when rubella infection in early pregnancy is suspected has as its main purpose the identification of primary infections, because they will demand appropriate precautionary measures or therapeutic inter-vention.

Characteristic rubella test patterns

RUBELLA

Immunity

from previous immunisation

from previous infection

- * rubella IgG positive (>35 IU/ml, HAHT >1:32)
- * highly avid rubella IgG antibodies (AI >60%)
- * rubella IgM negative
- * presence of E2-IgG conformation antibodies

Primary infection

characteristic clinical features

- * 2 independent rubella IgM tests positive
- * low avidity rubella IgG antibodies (AI <30%)
- * absence or seroconversion of IgG
- * absence of E2-IgG conformation antibodies

Immunisation

check antibody response not less than 6-8 weeks after immunisation

- * post-immunisation titer is 1-2 steps lower than after wild virus infection
- * HAHT 1:16 should always be combined with IgG determinations (>15 IU/ml assessed as positive)
- * E2-IgG conformation antibodies emerge after some delay

Reinfection

by family contacts

from temporary immunosuppression

inability to produce protective antibodies owing to a partial T-cell defect

Immunity

and

immunisation

Primary infection

and

reinfection

Recommended diagnostic procedures for investigating rubella infections

Basic and extended Basic investigations

Basic investigations

Determination of immune status

- * HAHT
- * HIG
- * *Rubella-IgG-EIA*

Extended basic investigations

If HAHT 1:16 and IgG-EIA are borderline

- * 2. serum sample after 4 months
- * Immunoblot

Ricognition of primary infections

- * HAHT from paired sera
- * *Rubella-IgM-EIA* (especially when HAHT is positive and HIG is negative)

Elucidation of primary/reinfection or persistent IgM

- * *alternative IgM test*
- * IgA testing
- * *measure avidity of IgG antibodies*
- * Immunoblot
- * peptide response

Special diagnostic investigations

Special diagnostic investigations

Early antenatal diagnostic investigations

- * cell culture and PCR from relevant material

Later antenatal investigations

(from the 22nd week of pregnancy)

- * *test for IgM* in fetal blood
- * peptide response and immunoblot (compare maternal and fetal)
- * PCR from fetal blood

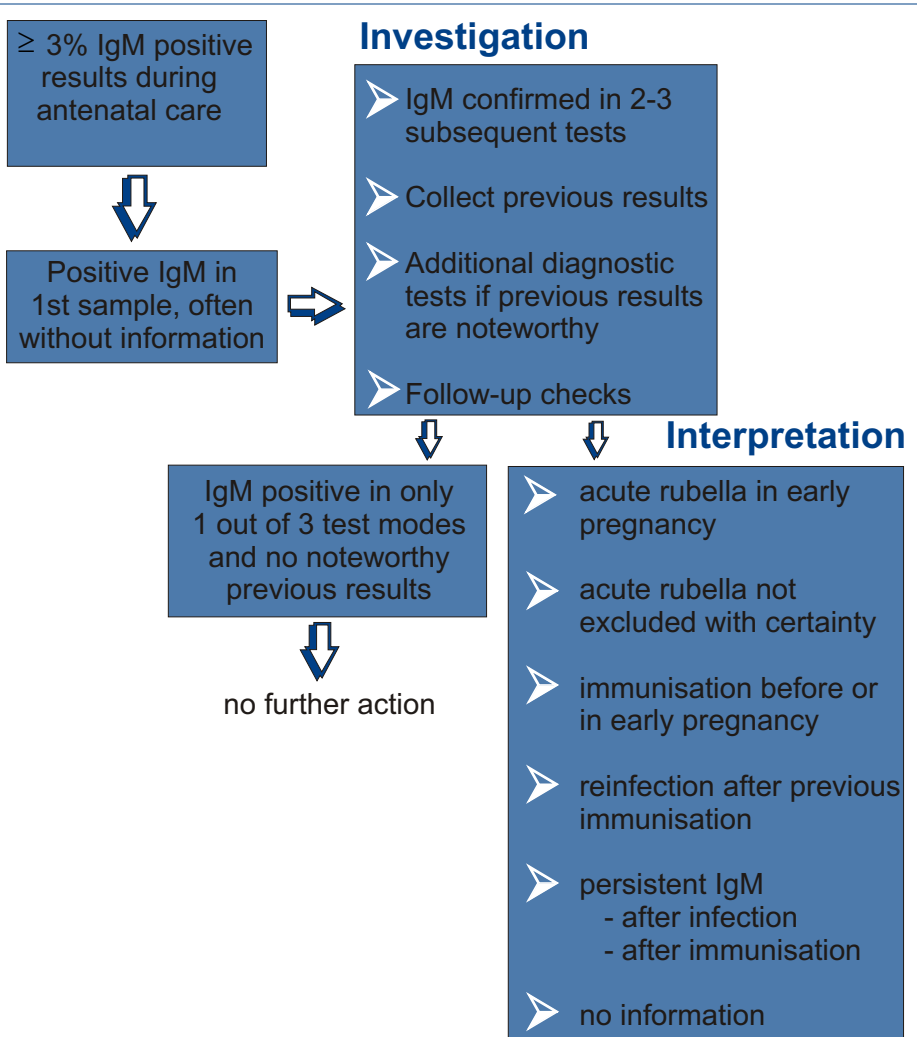
Testing for congenital infection

(as transmitted maternal immunity fades)

- * *test* the baby for *IgM*
- * test the urine for virus
- * test for E2-IgG conformation antibodies
- * PCR

Diagnostic investigation of serological findings when rubella infection in early pregnancy is suspected

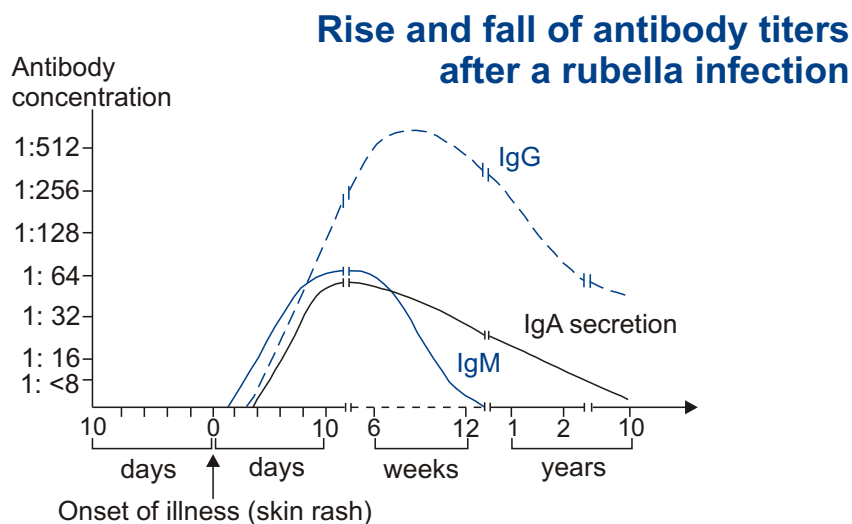
RUBELLA



Investigation

Investigation

Antibody kinetics



Introduction

Characteristics

The human-pathogenic Parvovirus B19 was discovered in 1975 by Cosshardt et al. in the plasma of healthy blood donors and has been assigned to the family of Parvoviridae.

Its prevalence among the population is stated as 40-70%, depending on age and sex.

Clinical features

B19 infections run a course similar to that of most systemic virus infections. Postnatal infections are relatively harmless, and in children usually asymptomatic. There is frequently a typical skin eruption, known as erythema infectiosum. This rash, like the polyarthralgia or polyarthritis which commonly occurs at the same time, is thought to be an immune-mediated reaction. A characteristic feature of these B19 infections is pronounced anaemia with a prompt rise in antibodies.

Significance

Parvovirus B19 displays a pronounced tropism for erythropoietic bone marrow precursor cells and for the fetal liver, in which it causes lysis as it multiplies. When the fetus is infected, the consequent inhibition of erythropoiesis can lead to fetal anaemia followed by hydrops fetalis and/or spontaneous abortion.

Because the symptoms and signs are usually inconspicuous B19 infection in pregnancy is often overlooked. There is as yet no evidence that B19 infection causes embryopathies.

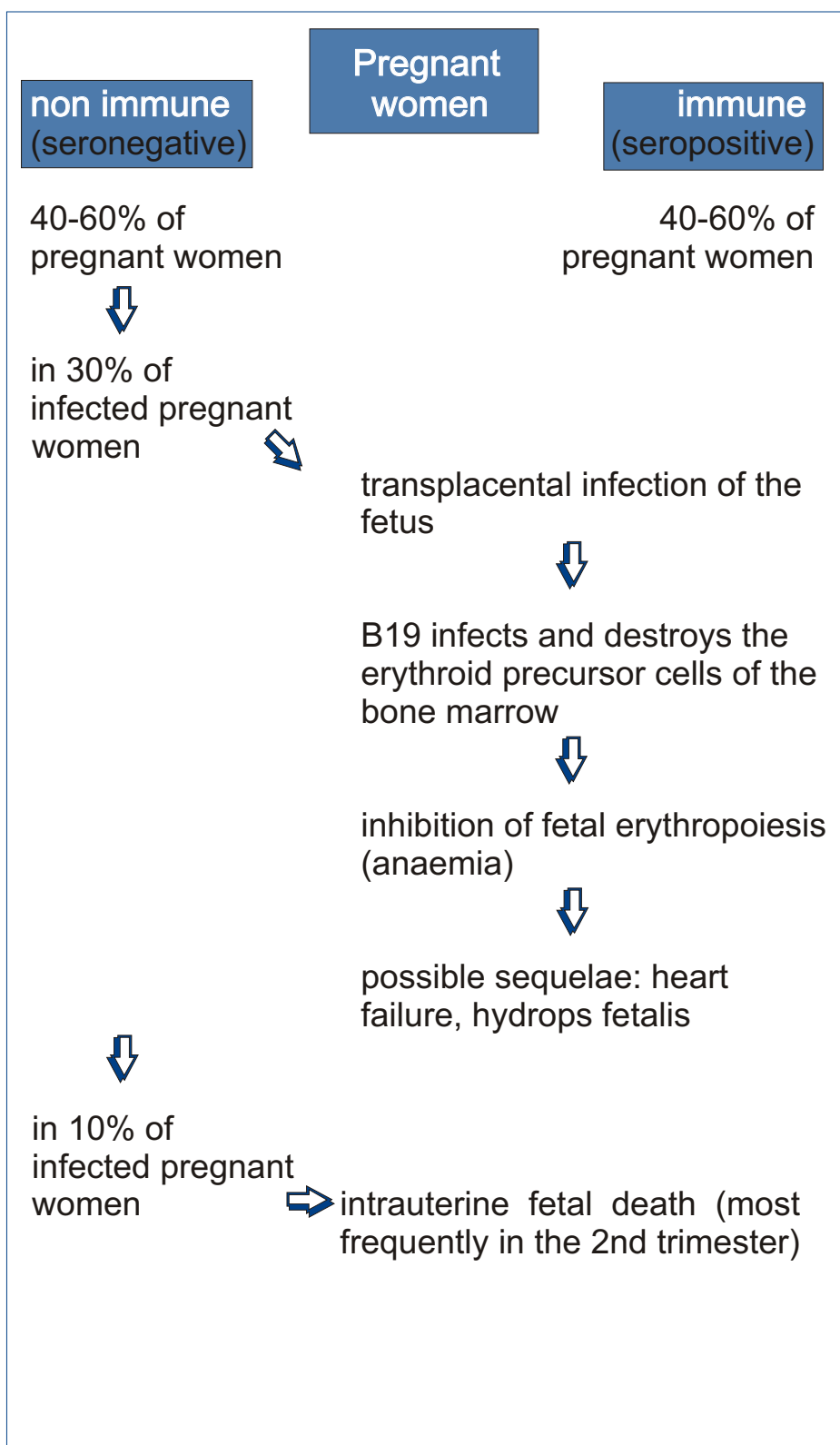
Diagnostic investigations

Because of the hazards to the fetus described above, diagnostic investigation of primary infection during pregnancy is worthwhile. Commercial test systems are available for measurement of B19 antibodies (ELISA, IFT, Immunoblot).

According to recent reports, the use of recombinant empty virus particles (VP1 and VP2 structural protein) from the baculovirus expression system guarantees maximum sensitivity and specificity, because the antigen produced in this way is closely similar to the native virus. A large proportion of the antibodies formed during the course of the infection is directed against VP1 protein. These are claimed to be the actual protective antibodies. VP2 proteins have proved useful mainly for the detection of antibodies against conformational epitopes.

Prevalence, clinical features and sequelae of prenatal B19 infections

PARVOVIRUS B19



Prevalence

Sequelae

Recommended procedure in pregnant women when B19 infection is suspected

Immediate determination of B19 immune status in pregnant women (IgG and IgM)

IgG positive/
IgM negative

Previous infection ⇒ no further action

IgG negative/
IgM negative

No evidence of acute infection at present
to exclude seroconversion ⇒ follow-up check after 1-2 weeks

IgG positive
or negative
but
IgM positive

Suspect acute infection ⇒ immediate investigation necessary
(ultrasound every 8-10 days, alpha-fetoprotein, to detect any signs of hydrops fetalis)



If there is ultrasound evidence of hydrops fetalis
test for antibodies in umbilical cord blood (fetal B19 IgM is not always detectable) and test for B19-DNA by the PCR (chorionic villus biopsy, ascites, umbilical cord blood)

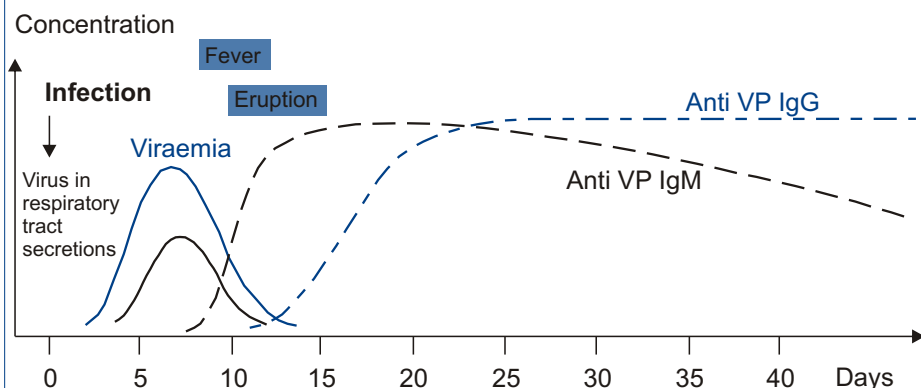


If infection of the fetus has been proved
intrauterine transfusion with erythrocyte concentrate

Unexplained
serological
findings

Supplementary investigations (Immunoblot, IFT, PCR) to confirm or to exclude an acute infection

Virus multiplication and antibody formation during an acute Parvovirus B19 infection



Antibody
kinetics

The advantages of the medac virus serology range

VIRUS RANGE

- ◆ By the correct choice of tests the diagnostic laboratory can meet the following demands:
 - high sensitivity and specificity
 - early reactivity during the course of the infection
 - easy handling in the laboratory
 - reproducibility
 - quantifiability (for IgG)
 - reasonable costs
- ◆ The high sensitivity and specificity of the medac tests has been documented by extensive diagnostic evaluations and independent reports from scientific experts.
- ◆ The utilisation of the μ -capture and α -capture principle for IgM and IgA tests guarantees a high degree of diagnostic reliability:
 - ⇒ no nonspecific reactions or false-positive results due to rheumatoid factors.
 - ⇒ no blocking of IgM/IgA antibodies by high IgG antibody titers.
- ◆ The IgG tests are based on the principle of single point quantification and are calibrated against WHO and international standards and reference methods.
- ◆ Breakable wells ensure economical test kit usage.
- ◆ A pipetting control system (PKS) creates colour changes which allow visual checking of each stage in the pipetting operation.
- ◆ Uniformity of reagents and incubation stages make possible automated processing on open ELISA analytical systems.
- ◆ All tests which require licensing have been registered at the Paul Ehrlich Institute.

Requirements
of diagnostic
testing

Advantages of
the medac tests

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