

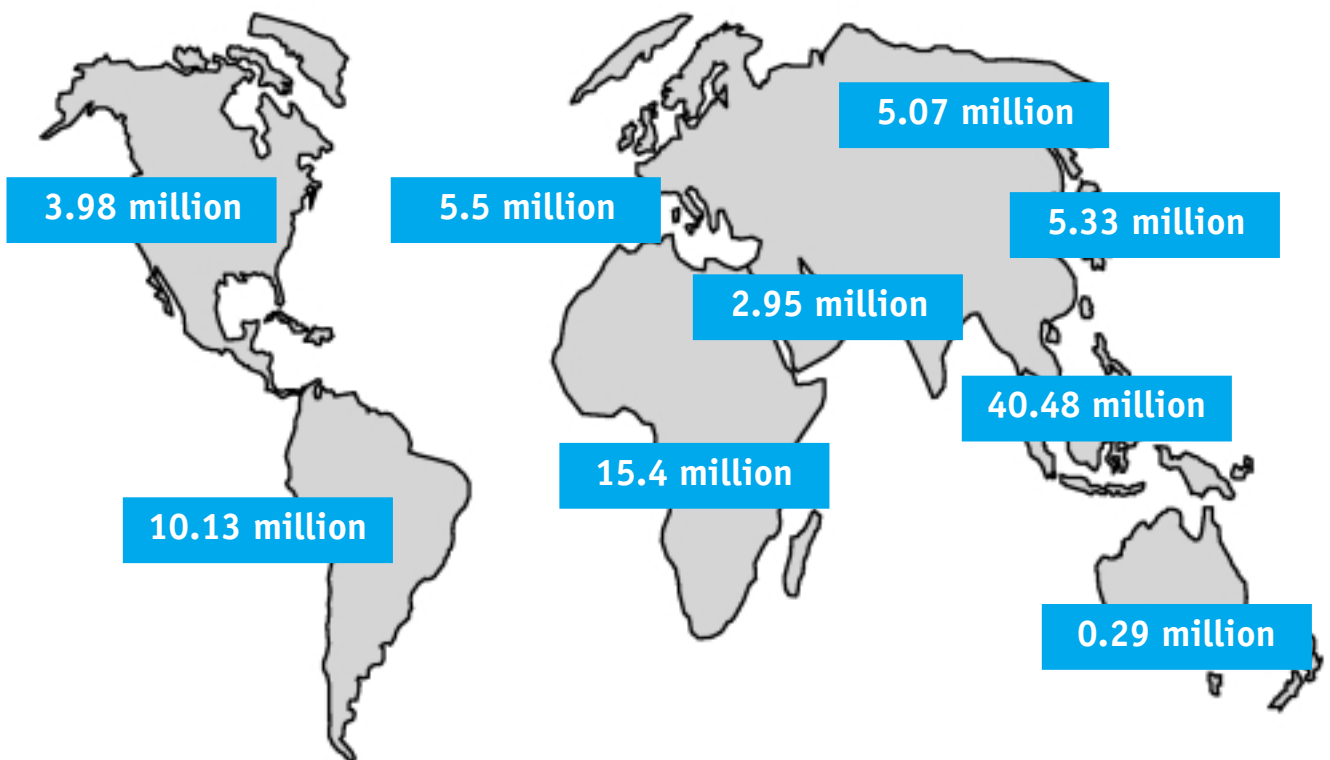
Chlamydia trachomatis



Information and Notes on Diagnosis

CHLAMYDIA TRACHOMATIS

worldwide



Estimated incidence worldwide for *C. trachomatis*
in 1995
(modified from Gerbase et al., 1998)

CHLAMYDIA TRACHOMATIS

General Characteristics

● Taxonomy

Gram negative bacteria:
Three human pathogenic species →
C. pneumoniae, *C. psittaci*, *C. trachomatis*

Very few differences in antigen structure

● Mode of life

Obligate energy-parasites:
Intracellular in mucosal epithelia, smooth muscle cells, endothelial cells and monocytes / macrophages

Protective mechanisms against intracellular lysis developed

● Reproduction

Long reproductive cycle (48 - 72 h):
Transition from the infectious, non-replicating form (elementary body) to the non-infectious, replicating form (reticulate body)

Susceptible to antibodies only in the replication stage

● Infection

Asymptomatic and oligosymptomatic infections:
Chronic disease frequent

Serious sequelae

● Treatment

Adequate drugs:
Tetracyclines, macrolides, quinolones

Cephalosporins and penicillins not effective

Duration of treatment will depend on the clinical picture

CHLAMYDIA TRACHOMATIS

Special Characteristics

● Dissemination

Worldwide

Control and prevention programs necessary

● Prevalence

700 million worldwide:

Prevalence is especially high in the developing countries.
Prevalence in Germany about 1.15 million

Estimated number of undetected cases substantially higher, because there are so many asymptomatic cases

● Incidence

90 million new cases worldwide per year:

Incidence especially high in developing countries.
Incidence in Germany about 0.3 million

Incidence of *C. trachomatis* infections

(from Gerbase et al., 1998).

| Regions | Males (million) | Females (million) |
|------------------------------------|--------------------|----------------------|
| North America | 1.64 | 2.34 |
| Western Europe | 2.30 | 3.20 |
| Australasia | 0.12 | 0.17 |
| Latin America and the Caribbean | 5.01 | 5.12 |
| Sub-Saharan Africa | 6.96 | 8.44 |
| North Africa and Middle East | 1.67 | 1.28 |
| Eastern Europe and Central Asia | 2.15 | 2.92 |
| East Asia and Pacific | 2.70 | 2.63 |
| South and South-East Asia | 20.20 | 20.28 |
| Total | 42.75 | 46.38 |

CHLAMYDIA TRACHOMATIS

Special Characteristics

● Clinical pictures

Different clinical pictures are associated with different serovars.

Existence of 18 serovars (A-L)

● Serovar A-C

Endemic trachoma:
An epidemic in developing countries

Millions of people become blind after infections lasting for years

● Serovar D-K

Urogenital infections / oculo-genital infections:

Clinical manifestations in both sexes:
Urethritis, reactive arthritis, proctitis, conjunctivitis

Special case: Neonatal pneumonia
During birth the baby is infected in the contaminated birth canal

Clinical manifestations in females and males:

| Female | Male |
|------------------|--------------|
| Cervicitis | Prostatitis |
| Endometritis | Epididymitis |
| Adnexitis / PID* | |
| Perihepatitis | |
| Perisplenitis | |
| Periappendicitis | |
| Peritonitis | |

Secondary sterility in women as a consequence of chronic adnexitis

Fertility problems in men due to chronic epididymitis (Witkin et al. 1995)

● Serovar L₁-L₃

Lymphogranuloma venereum (LGV):
A tropical sexually transmitted disease

Systemic disease with pronounced invasive characteristics

*Pelvic inflammatory disease

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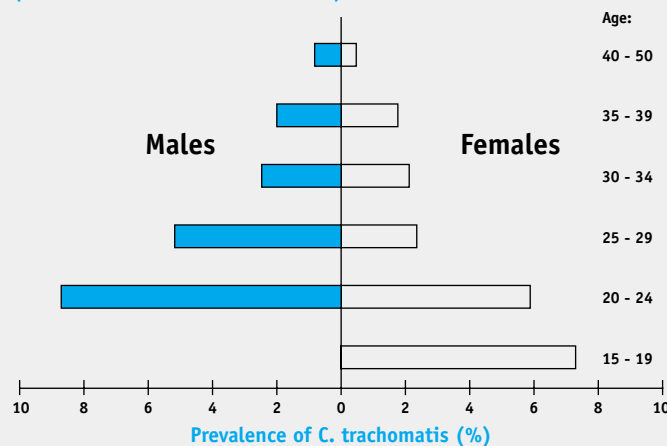
The Healthy Population

Asymptomatic females and males

Prevalence of antigen / agent

The prevalence of *C. trachomatis* in females and males is age-dependent

Investigations by ligase chain reaction (LCR) in urine samples in 1,581 males and 2,655 females (from Petersen et al., 1996).



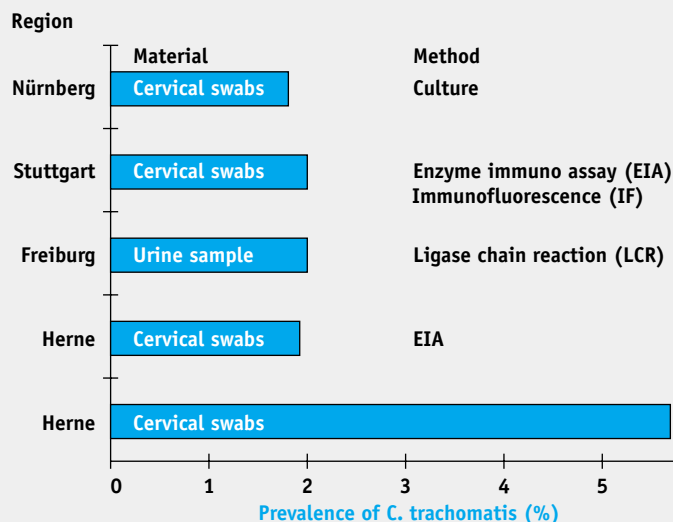
Low prevalence (age-dependent) of *C. trachomatis* in healthy women (average 2.0 %) and men (average 3.7 %)

Highest prevalence (women: 7.3 % vs. men: 8.7 %) at the beginning of a sexually active life style

Asymptomatic pregnant women

C. trachomatis prevalence in pregnant women

Investigations by various test methods in 14,319 pregnant women (from Hoyme and Spitzbart, 1996).



Mean age of pregnant women > 25 years

Highest *C. trachomatis* prevalence 5 - 10 years earlier

Low *C. trachomatis* prevalence (average 2 %) in pregnant women

Microorganisms that have already ascended are not detected (Petersen et al. 1998)

CHLAMYDIA TRACHOMATIS

The Healthy Population

● Asymptomatic females and males

Prevalence of antibodies

Antibodies against *C. trachomatis* in blood donors from Austria (from Hafner et al., 1998).

| Test | No. | IgA (%) | IgG (%) |
|---------------|-----|---------|---------|
| MIF | | | |
| MRL-MIF | 165 | 2.4 | 10.90 |
| SeroFIA | 165 | 1.8 | 6.13 |
| EIA | | | |
| C. trach. EIA | 165 | 18.8 | 26.10 |
| SeroCT | 165 | 11.5 | 15.25 |

(Median age distribution 32 years)

Antibodies against *C. trachomatis* in blood donors from Germany (unpublished)

Investigations in males (50 %) and females (50 %).

| Test | n | IgA (%) | IgG (%) |
|----------------------------|------------|-------------|-------------|
| pELISA (medac) | 299 | 11.0 | 16.1 |
| SeroCT (Savyon) | 299 | 17.8 | 14.3 |
| C. trach. EIA (Labsystems) | 299 | 17.7 | 21.0 |

(Median age distribution 40 years)

Antibody prevalence depends on the test used

IgA and IgG antibodies in healthy cohorts indicate asymptomatic, chronic infections

IgA prevalence (IgA half-life 6 days) indicates persisting agents

Extension of the diagnostic window by serology

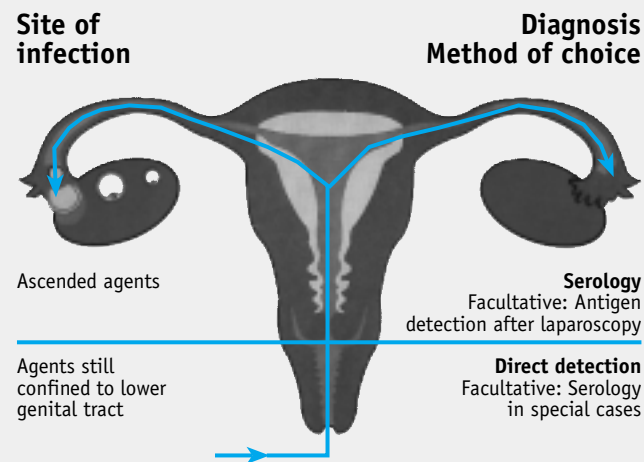
CHLAMYDIA TRACHOMATIS

Course of Infection and Diagnosis

● Adequate diagnosis

C. trachomatis first colonizes the mucosal epithelia of the lower genital tract. Symptoms, especially in women, are slight or even absent. The pathogens ascend to the uterine tubes and persist there for months or years.

The diagnostic procedure depends on the site of infection



The best method for *C. trachomatis* diagnosis depends on the site of current infection

Direct *C. trachomatis* detection: Method of choice in infections of the lower genital tract

C. trachomatis serology: Method of choice in infections of the upper genital tract

CHLAMYDIA TRACHOMATIS

Clinical Pictures

● Infections of the lower genital tract

Infections still confined to the lower genital tract

Such infections are diagnosed by direct examination. Sensitivity and specificity depend on the method employed. PCR and LCR show the highest sensitivity (> 90 %). Culture of Chlamydia is too insensitive (60 - 70 %). IFT and ELISA (sensitivity 60 - 75 %) can give false-negative and false-positive results.

False negative results may lead to omission of the treatment. The outcome will be chronic chlamydial disease

Urethritis

Up to 50 % of cases of non gonococcal urethritis are caused by *C. trachomatis*. The first symptoms appear 1 - 3 weeks after infection.

Parallel investigations of antigen and antibody to exclude *C. trachomatis* infection in doubtful cases

Cervicitis

The portal of entry of *C. trachomatis* in women is the cervix. Yet in 50 - 60 % of cases both cervix and urethra are infected. In 30 % the infection is exclusively cervical and in 5 - 30 % exclusively urethral (Black, 1997).

Invasion of the body by HIV-1/2 is facilitated by *C. trachomatis* infections

Proctitis

Infections of the rectum by *C. trachomatis* have been demonstrated in 4 - 8 % of homosexual cases (Black, 1997).

● Infections of the upper genital tract

Ascending infections

Chlamydiae ascend via the uterus to the adnexa, where they cause predominantly chronic, asymptomatic disease.

Chronic signs and symptoms with serious sequelae may be caused by reinfections and reactivations

PID

More than 50 % of cases of adnexitis are caused by *C. trachomatis* (Black, 1997).

Direct detection of Chlamydiae is only possible by laparoscopy.

CHLAMYDIA TRACHOMATIS

Chronic Manifestations

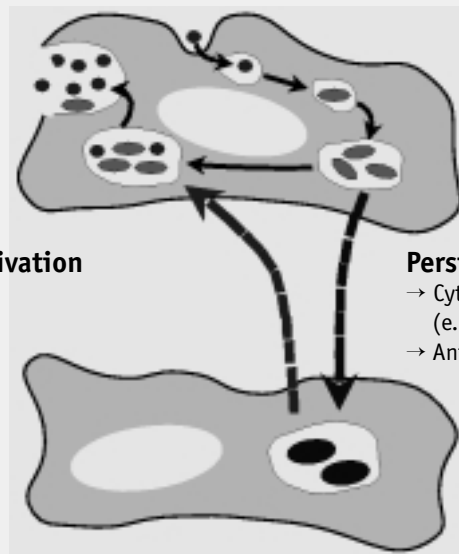
● Chlamydial infections

Asymptomatic chronic disease frequently develops after a Chlamydia infection. In the chronic stage there is a change from the active to the passive (persisting) metabolic phase, in which the profile of antigen expression also changes. During the persisting infection antigens are continuously formed. They stimulate the immune system and help to maintain the state of chronic inflammation.

The ability of Chlamydiae to change between different life stages with special morphological and metabolic characteristics handicaps the immune defence and impedes antibiotic therapy (Fig.).

Activity stages of Chlamydia in chronic cases

Chronic active infection



Chronic latent infection

Chronic disease may not become clinically apparent for years or decades until it is unmasked by late sequelae

Prevention by timely serological screening

Initiation and maintenance of persistent disease may be caused by various factors including cytokines or inadequate antibiotic therapy

CHLAMYDIA TRACHOMATIS

Genital Sequelae

● Premature birth

Almost every sexually transmitted disease, including syphilis, gonorrhoea and chlamydial infection, increases the risk of premature birth.

Decrease in the risk of preterm labor in problem cases by treatment with tetracyclines or macrolides

● Ectopic pregnancy

C. trachomatis infections endanger the course of pregnancy. Antibodies to C. trachomatis point to a fivefold increase in the risk of an ectopic pregnancy (Chernesky, 1998).

Parallel determinations of antigen and antibodies to exclude C. trachomatis infection in problem cases

● Infertility

1 in 7 marriages in Germany remain involuntarily childless. This is mainly due to secondary sterility. More than half of these cases are attributable to C. trachomatis infections.

Differentiation between patients with patent and occluded tubes by C. trachomatis antibody determinations

Prevalence of antibodies to C. trachomatis in women with fertility problems

| Author (Year) | Clinical Background | IgA (%) | IgG (%) | Method |
|----------------------------|-----------------------|-----------|-----------|--------------|
| Clad et al. (1994) | Tubes patent | 6 | 22 | MIF |
| Clad et al. (1994) | Tubes occluded | 20 | 84 | MIF |
| Patton et al. (1994) | TF*- Infertility | n.d.** | 71 | MIF |
| medac pELISA (1998) | Tubes occluded | 19 | 61 | ELISA |

* = Tubal Factor
** = not determined

Antibodies are detectable in more than 50 % of women with occluded tubes

Specific C. trachomatis serology to exclude chronic C. trachomatis infection in women with fertility problems

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Extragenital Sequelae

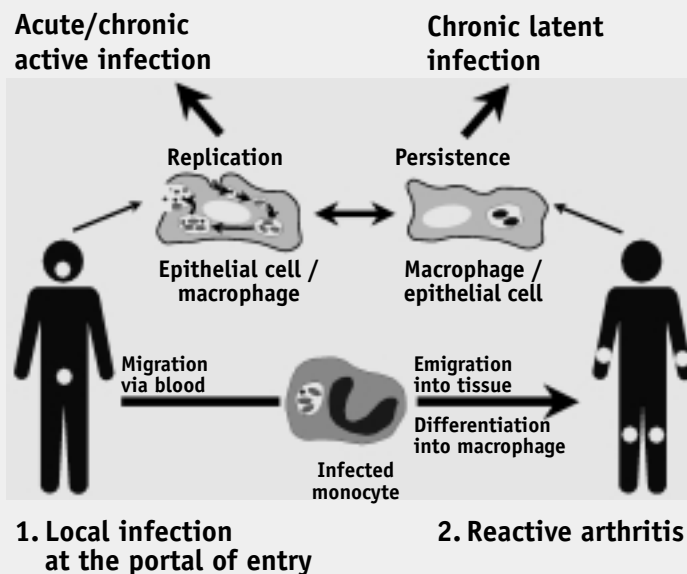
● Chlamydial induced arthritis (CIA)

CIA is a reactive arthritis and is triggered by an extra-articular infection. Weeks or months after a *C. trachomatis* urethritis / cervicitis / bartholinitis, reactive arthritis will develop in 1 - 3 % of cases (Köhler and Jendro, 1997).

According to recent findings *C. pneumoniae* is also associated with the development of reactive arthritis (Taylor-Robinson and Thomas, 1998).

Persisting agent forms predominate in CIA. Recurrent clinically symptomatic phases reflect the transition from the latent to the active stage (Fig.). Antigen formation continues in the non-replicating, persisting form. These antigens sustain the inflammatory reaction that leads to arthritis (Beatty et al., 1994; Köhler and Jendro, 1997).

Pathogenesis of chlamydial induced arthritis



Transport of the agents by monocytes into the joints

Detection of peripherally located Chlamydiae is seldom possible

Change from active to inactive agent forms in CIA

The cause of the arthritis can often be found by Chlamydia serology

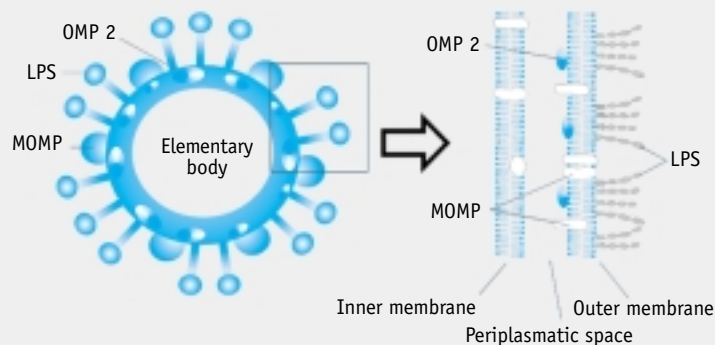
CHLAMYDIA TRACHOMATIS

Features of the C. trachomatis-pELISA

● Antigenic structures

The Chlamydia cell wall consists of both genus-specific and species-specific structures. The lipopolysaccharide (LPS) constitutes the component of the membrane common to all Chlamydia species (genus-specific). The membrane also contains various Outer Membrane Proteins (OMPs).

Structure of the Chlamydia cell wall



● Specific antigen

The Major Outer Membrane Protein (MOMP) contains within its variable domains the definitive species-specific epitopes for *C. trachomatis*. The constant regions of the MOMP also show cross-reacting, genus-specific epitopes. The specific *C. trachomatis* antigen used in pELISA has been selected to contain the definitive sequences within the variable domain IV.

Synthetic, highly specific *C. trachomatis* peptide, comprising all serovars

CHLAMYDIA TRACHOMATIS

Features of the C. trachomatis-pELISA

● Precision

Intra-assay variation

C. trachomatis-IgG-pELISA:

| Speci- men | Number of measurements | OD Mean value | Variation coefficient |
|---------------|---------------------------|------------------|--------------------------|
| 1 | 21 | 0.653 | 5.2 % |
| 2 | 21 | 1.789 | 3.4 % |
| 3 | 24 | 2.111 | 3.0 % |

C. trachomatis-IgA-pELISA

| Speci- men | Number of measurements | OD Mean value | Variation coefficient |
|---------------|---------------------------|------------------|--------------------------|
| 1 | 21 | 0.336 | 4.5 % |
| 2 | 24 | 0.818 | 6.1 % |
| 3 | 21 | 1.346 | 4.6 % |

Inter-assay variation

C. trachomatis-IgG-pELISA

| Speci- men | Number of measurements | OD Mean value | Variation coefficient |
|---------------|---------------------------|------------------|--------------------------|
| 1 | 13 | 0.345 | 12.9 % |
| 2 | 13 | 0.488 | 14.4 % |
| 3 | 13 | 1.395 | 6.7 % |
| 4 | 13 | 1.600 | 6.3 % |
| 5 | 13 | 1.830 | 5.9 % |

C. trachomatis-IgA-pELISA

| Speci- men | Number of measurements | OD Mean value | Variation coefficient |
|---------------|---------------------------|------------------|--------------------------|
| 1 | 13 | 0.104 | 8.8 % |
| 2 | 13 | 0.292 | 8.7 % |
| 3 | 13 | 0.403 | 9.1 % |
| 4 | 13 | 0.587 | 8.2 % |
| 5 | 13 | 1.172 | 5.9 % |

● Specification

Negative Control (NK) < OD 0.1

Positive Control (PK) > OD 0.8

Cut off = Mean value NC + Additive Term

CHLAMYDIA TRACHOMATIS

Features of the C. trachomatis-pELISA

● Specificity

Respiratory infections:

Sera from children aged 2 - 10 years

| | No. | Specificity |
|------------|-----|-------------|
| IgG | 100 | 99 % |
| IgA | 98 | 98 % |

High specificity for IgG and IgA antibodies to C. trachomatis

● Sensitivity

Acute peripheral infections:

Sera from STD-patients with proven Chlamydia urethritis / cervicitis

| | No. | C. trachomatis detection | Sensitivity % |
|------------|-----|--------------------------|---------------|
| IgG | 114 | CC* positive | 65 % |
| IgA | 114 | CC* positive | 33 % |

*CC = Cell culture

Usefulness of serology in acute peripheral infections influenced by delayed sero-conversion

Chronic persisting infections:

Sera from STD-patients with persisting genital symptoms were measured. In all sera IgG antibodies to C. trachomatis had already been detected by MIF.

| | No. | C. trachomatis-Serology | Sensitivit/Prevalence* % |
|-------------|-----|-------------------------|--------------------------|
| IgG | 41 | IgG-MIF positive | 98 % |
| IgA* | 41 | IgG-MIF positive | 41 % |

Highly sensitive for the detection of chronic persisting infections

● Prevalence

Patients with reactive arthritis:

| | No | Prevalence % |
|------------|----|--------------|
| IgG | 85 | 47 % |
| IgA | 85 | 40 % |

Active ongoing infections are indicated by concordant IgA and IgG responses

High risk groups (prostitutes):

| | No. | Prevalence % |
|----------------|-----|--------------|
| IgG | 85 | 75 % |
| IgA | 85 | 37 % |
| Antigen | 85 | 4 % |

Indication of past infections (seroscars) by an isolated IgG reaction

High antibody prevalences with only sporadically detectable antigen characterize high risk groups.

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