

# Chlamydial Heat Shock Proteins (cHSP)

## cHSP60-IgG-ELISA medac



medac

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## Definition

Heat shock proteins (HSP) are highly conserved intracellular proteins. Their amino acid composition has not changed very much throughout evolution and they are expressed in both procaryotes and eucaryotes with pronounced homology (1). The superfamily of the HSP includes various families which are classified according to their molecular weight.

## Features

Under physiological conditions HSP are continuously expressed throughout the whole life cycle at a constant level. They take care of well-ordered courses of anabolism and catabolism.

In response to physical and chemical stress stimuli of any kind the cells overexpress HSP in order to protect themselves from these adverse conditions (1,2).

The chlamydial HSP (cHSP) share an amino acid identity of about 50% with

## Chlamydia infections

Invasion of chlamydiae in the human causes in him stress situations; for the pathogens hostile surroundings arise, as the host induces an immune response to the presence of foreign-bodies.

During a primary infection the synthesis of cHSP is strongly upregulated which makes cHSP60 one of the predominant microbial proteins. The immunity to HSP that follows such primary infections is normally restricted to specific epitopes of the cHSP60 molecules.

## Auto-immunity


cHSP60 is mainly responsible for development and progression of various immunopathological processes which may lead to chronic inflammatory diseases. Not recognized or insufficiently treated chlamydia infections lead to persistence of the pathogens. Persistent or repeated exposition towards chlamydiae causes not only upregulated expression of cHSP60, but often also upregulated expression of hHSP60. As the corresponding immune response to cHSP60 is also directed towards the conserved epitopes of the HSP60 molecules, it comes to a cross-immunity against cHSP60 and hHSP60 with the result of autoimmunity (hypersensitivity) of the delayed type.

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cHSP60-induced autoimmune responses are associated with various clinical pictures. From many investigations it has been concluded that the detection of cHSP60 antibodies which reflect increased immunity to cHSP60 is of predictive value for the development of immunopathological processes and identifies already established autoimmunity.

**Antibody  
detection**

Heat shock proteins of the families 60, 70, and 90 belong to the first proteins which are expressed by the zygote after fertilization (4). During the first trimester of pregnancy they can also be detected in the decidua (5) and throughout the whole pregnancy in placental tissues (6). Autoimmunity to hHSP60 is not typically evident in women of reproductive age. During pregnancy an established sensitization to cHSP60 in connection with overexpression of hHSP60 may cause reactivation of HSP60 sensitized lymphocytes with subsequent induction of inflammatory processes.

**Pregnancy**


At this stage, such inflammatory processes disturb immunoregulatory mechanisms that are responsible for implantation (immunologically conditioned impairment of implantation), or the embryo itself is target of destructive autoimmune responses because of its pronounced HSP expression (5).

**Pre  
implan-  
tation  
period**

In both the early phase and during the further pregnancy course the maternal decidua and/or the embryo can be affected by HSP60-conditioned autoimmune reactions which may lead to an immunologically mediated rejection of the embryo (spontaneous abortion) (5,7).

**Post  
implan-  
tation  
period**

Fallopian tube pregnancy is the most frequent form of EP. Scarring, fibroses, and necroses, caused by persistent inflammatory processes (8), lead to extreme reduction of the tubal lumen and impair the function of the tubal ciliar epithelium. Overexpressed cHSP60 has a key function in the development of these immunopathological processes (2). The combined determination of cHSP60 and *C. trachomatis* antibodies enable clear identification of EP and are of high predictive value (9).

**Ectopic  
pregnancy  
(EP)**

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### Tubal factor infertility (TFI)



Infertility because of total occlusion of the tubal lumen (TFI) belongs to common sequelae of silent *C. trachomatis* infections in the upper genital tract (10) and the associated immunopathological/autoimmune processes induced by cHSP60 (11). Antibodies to cHSP60 are strongly associated with TFI (10) and are of high predictive value. The combined detection of cHSP60 and *C. trachomatis* antibodies even increases sensitivity and specificity (12).

### In vitro-fertilization (IVF)



Sensitization to hHSP60 and cHSP60 with corresponding autoimmune responses may negatively influence post-fertilization events (13,14). They include abnormal embryo development *in vitro*, adverse outcome of implantation after embryo transfer, or spontaneous abortion in early pregnancy stage.

### Reactive and rheumatoid arthritis



cHSP60 has been identified as an immunodominant antigen in synovial T cells. Additional involvement of structural chlamydial antigens (LPS, MOMP, OMP2) in immuno-pathological processes, to date, has been discussed controversially (15,16). Presently available results of autoimmune responses to only hHSP60 or of immunopathological processes caused by overexpression of hHSP60 and/or cHSP60 are controversial, too (17,18).

### Atherosclerosis



Independent from the classical risk factors, immune-inflammatory processes as possible pathogenetic factors for development and progression of atherosclerosis have been discussed. Besides the cHSP60-induced LDL oxidation to a highly atherogenic form (19,20) and the chlamydial LPS (cLPS) mediated foam cell formation in concert with oxidized LDL, elevated cLPS and cHSP60 antibody titers have been associated with atherosclerosis (21,22). As serum antibodies to cHSP60 cross-react with hHSP60, these immune responses in connection with the presence of cHSP60 and hHSP60 provide an ideal starting point for autoimmune responses.

To date, certain diagnostic approaches can be deduced from the existing findings. This is especially valid for identification of persistence and prediction of possible autoimmunity in the fields of gynecology, obstetrics, and reproductive medicine (5,7,8,10-12,13,14).

## Indications

## Diagnostic approaches

Indication	Detection of	Aim	Conclusion°
Spontaneous abortion	cHSP60 IgG C.tr. IgG/IgA in serum	Analysis of the cause	Indication to future abortions
Habitual abortions	cHSP60 IgG C.tr. IgG/IgA in serum	Analysis of the cause	Advise patient of possible further adverse pregnancy outcome
Ectopic pregnancy (EP)	cHSP60 IgG C.tr. IgG/IgA in serum	Prediction of EP	Supervise because of risk pregnancy
Infertility	cHSP60 IgG C.tr. IgG/IgA in serum	Non-invasive identification of TFI*	IVF
<i>In vitro</i> fertilization (IVF)	cHSP60 IgG C.tr. IgG/IgA in serum	Prediction of IVF failure	Advise patient of possible IVF failure
Rheumatic diseases	cHSP60 IgG C.tr. IgG/IgA in serum	Identification of autoimmunity	Consideration of immunotherapy

C.tr.: *Chlamydia trachomatis*;

\*:TFI= Tubal factor infertility; \*\*:CS= Cervical secretions; °:Deduced from literature

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### Precision



To establish intra-assay variance, six different sera were each tested in a 22-fold assay.

### Intra-assay variance

Serum	IgG	
	OD	CV (%)
1	0.053	(11)
2	0.683	(3)
3	1.324	(4)
4	0.067	(4)
5	0.653	(2)
6	1.305	(4)

To establish interassay variance, seven different sera were each tested by 11 independent test procedures.

### Interassay variance

Serum	IgG	
	OD	CV (%)
1	0.045	(19)
2	0.649	(5)
3	1.293	(6)
4	0.090	(23)
5	1.047	(8)
6	1.310	(9)
7	1.657	(5)

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In various cohorts the following cHSP60 IgG antibody prevalence was determined:

◀ **Prevalence**

Cohort	IgG prevalence
Blood donors	14% (n=100)
Children (Age 1 – 17 years)	8% (n=37)
Patients with fertility problems	59% (n=73)
Patients with rheumatic diseases	64% (n=44)

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### Literature

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