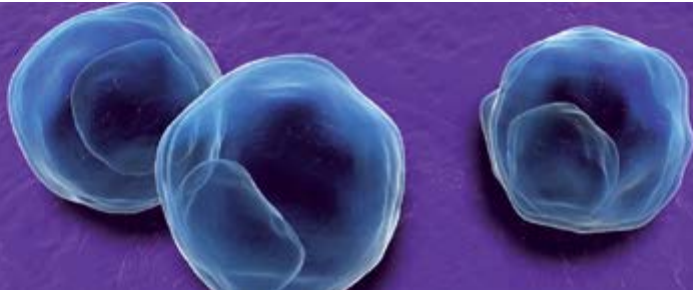


# ENZYME IMMUNOASSAYS FOR THE DIAGNOSIS OF CHLAMYDIA INFECTIONS

***Chlamydia* sp.**  
***Chlamydia pneumoniae***  
***Chlamydia trachomatis***

ELISA and IMMUNOBLOT kits are optimized and validated for detection of IgA, IgG and IgM antibodies in human serum and plasma

## Introduction



In terms of human health, the most important Chlamydia pathogens are *Chlamydia trachomatis* and *Chlamydia pneumoniae*. *Chlamydia psittaci* is primarily an animal pathogen, which can be transmitted to humans.

*Chlamydia trachomatis* is the most common sexually transmitted bacterial pathogen, causing venereal diseases in humans worldwide. The most vulnerable group is young people between 15 and 30 years of age. Urogenital chlamydia infections often occur in the form of “post-gonococcal inflammation”. Cervical chlamydia infection is currently considered to be one of the risk factors for uterine cervix carcinoma. *Chlamydia trachomatis* is also the most frequent cause of sterility in both men and women.

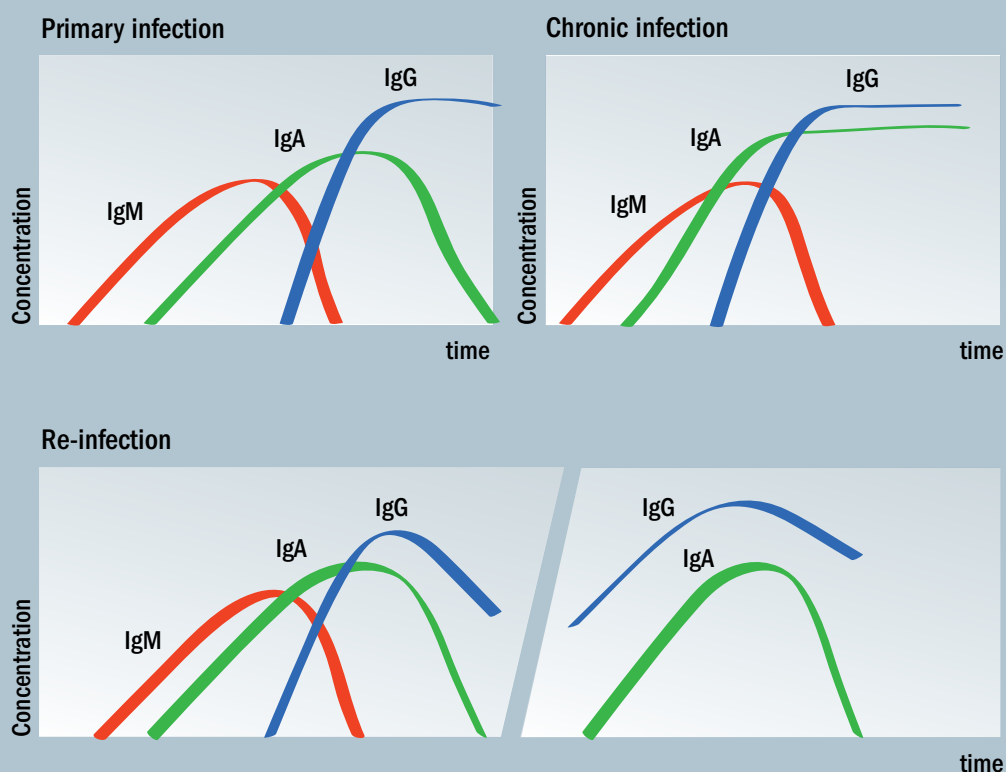
*Chlamydia pneumoniae* is the most widely spread *Chlamydiaceae* species in the human population. In recent years, the number of acute and chronic infections has increased. Primary infection generally occurs between 5 and 18 years of age. Major clinical symptoms include: rhinitis, sinusitis, otitis media, pharyngitis, bronchitis, atypical pneumonia with non-productive cough and indistinctive auscultatory findings.

*Chlamydia psittaci* can cause human diseases with atypical pneumonia-like (avian strains) or placentitis-like (mammal strains) manifestation.

## Antibody Response

The production of specific antibodies is delayed in the case of chlamydial infections. The IgM antibodies are produced in the 2<sup>nd</sup> and 3<sup>rd</sup> week after the outbreak of the disease; the production of IgA and IgG antibodies is slower (from the 6<sup>th</sup> to 8<sup>th</sup> week).

### Production of antibodies IgA, IgG and IgM



### IgM:

Occurrence of IgM antibodies without the IgA and/or IgG antibodies being present is the evidence of primary infection; IgM antibodies are generally not produced during re-infections.

### IgA:

These are produced later than IgM antibodies; their increase is typical during re-infections. IgA antibodies can be considered an indicator of active infection.

### IgG:

Isolated occurrence of IgG antibodies without clinical manifestations of the disease is characteristic of the post-infectious stage.

Detected seroconversion or quadruple increase of antibodies in pair sera (the first sample at the beginning of the illness, the second sample 2 to 3 weeks later) are a clear identification of active infection. Antibodies against Chlamydia can persist for a long time (months, or even years), yet it does not mean that it is an active infection.

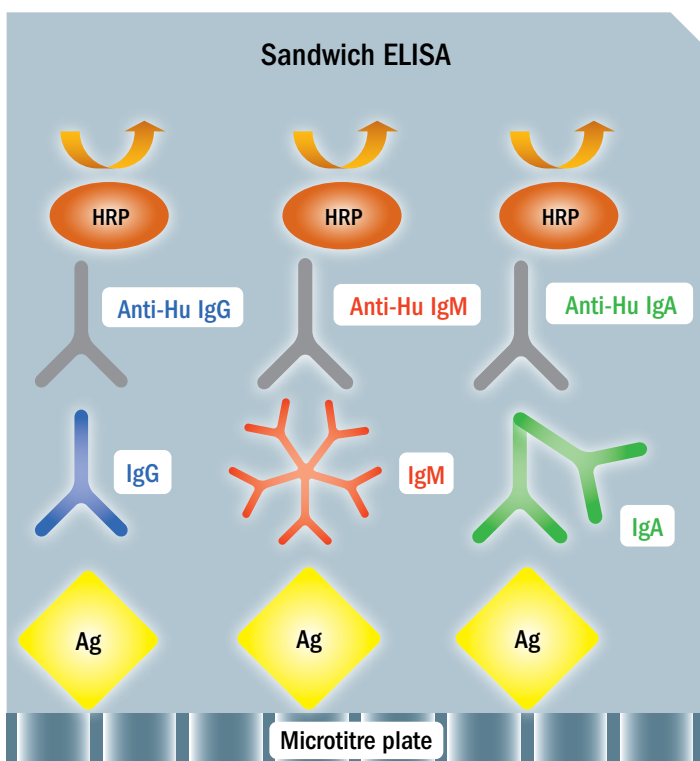
## ELISA

### Clinical Application

- ▶ Screening test for detection of human infection caused by the Chlamydia sp.
- ▶ Checking therapy results by using quantitative (semiquantitative) determination.

### Test Principle

The assays are based on a sandwich type of ELISA method.



### Antigens

#### EIA Chlamydia IgA, IgG, IgM

Inactivated and highly purified LPS antigen from *Chlamydia* sp. strains

#### EIA Chlamydia pneumoniae IgA, IgG, IgM

Inactivated and purified antigen from a strain of *Chlamydia pneumoniae*

#### EIA Chlamydia pneumoniae REC IgA, IgG

Mixture of highly specific recombinant antigens (MOMP, OMP2, OMP4, OMP5 and p54)












#### EIA Chlamydia trachomatis IgA, IgG, IgM

Mixture of highly specific recombinant antigens from a strain of *Chlamydia trachomatis* with high content of MOMP









### User Comfort

- ▶ Ready-to-use components
- ▶ Colour-coded components
- ▶ Interchangeable components
- ▶ Breakable colour-coded microplate strips
- ▶ CUT-OFF and calibrators included
- ▶ Semiquantitative evaluation (Index of Positivity) or quantitative evaluation (U/ml) of results
- ▶ Easy assay procedure

## Summary of EIA Protocol

Step No.	Test steps
1	 Dilute samples serum/plasma (1:101)
2	 Pipette controls and diluted samples 100 µl Blank = empty well
3	 Incubate 30 minutes at 37°C
4	 Aspirate and wash the wells 5 times
5	 Add 100 µl Conjugate Blank = empty well
6	 Incubate 30 minutes at 37°C
7	 Aspirate and wash the wells 5 times
8	 Add 100 µl Substrate (TMB-Complete) Including blank
9	 Incubate 30 minutes at 37°C
10	 Add 100 µl Stopping solution Including blank
11	 Read colour intensity at 450 nm

## Advantages

-  Identical assay procedure
-  High diagnostic specificity and sensitivity
-  High reproducibility
-  High dynamics of antibody response
-  Short total assay time
-  Ready for automation
-  Quantitative evaluation available
-  Customer support

## Results Interpretation

IgG	IgA	IgM	Interpretation
-	-	-	Negative result.
-	- / +	+	Eventual incipient infection. In order to confirm the results it is necessary to repeat the tests.
+	-	-	Persistent IgG antibodies after previous infection.
+	border line/low +	-	Previous infection. Beginning of reinfection.
+	++	-	On-going infection. (IgM not necessarily produced) Repeated infection. Chronic infection. (Chronicity confirmed by tests repeated after the 1st and 3rd months; occurrence of clinical symptoms)
+	+	+	On-going infection.

## Test Characteristics

ELISA	Diagnostic sensitivity	Diagnostic specificity
EIA Chlamydia IgA	98.8%	96.6%
EIA Chlamydia IgG	98.9%	98.9%
EIA Chlamydia IgM	95.9%	95.2%
EIA Chlamydia pneumoniae IgA	97.3%	95.3%
EIA Chlamydia pneumoniae IgG	97.7%	97.5%
EIA Chlamydia pneumoniae IgM	95.0%	97.9%
EIA Chlamydia pneumoniae REC IgA	96.6%	98.8%
EIA Chlamydia pneumoniae REC IgG	97.7 %	98.8%
EIA Chlamydia trachomatis IgA	97.2%	97.7%
EIA Chlamydia trachomatis IgG	97.9%	97.6%
EIA Chlamydia trachomatis IgM	96.3%	99.2%



# IMMUNOBLOT

## Clinical Application

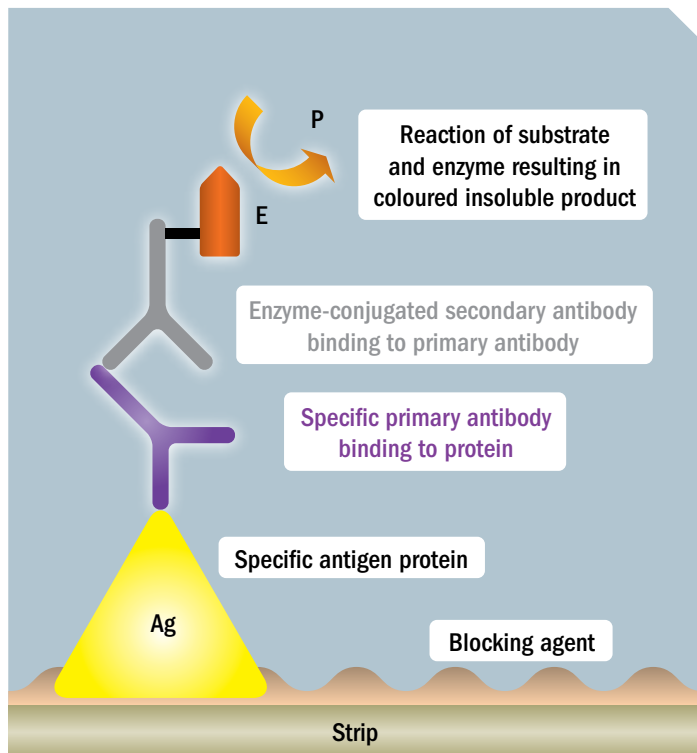
- ▶ Detailed determination for the presence of anti-Chlamydia specific antibodies
- ▶ Confirmation of ambiguous results
- ▶ Confirmation for ELISA tests

## User Comfort















- ▶ Ready-to-use components
- ▶ Colour-coded strips
- ▶ Positive and Negative controls
- ▶ Control of reaction course and Conjugate control are present on the strip
- ▶ Interchangeable components
- ▶ Easy assay procedure

## Test Principle

Recombinant antigens are transferred to a nitrocellulose membrane using a micro-dispensing method.



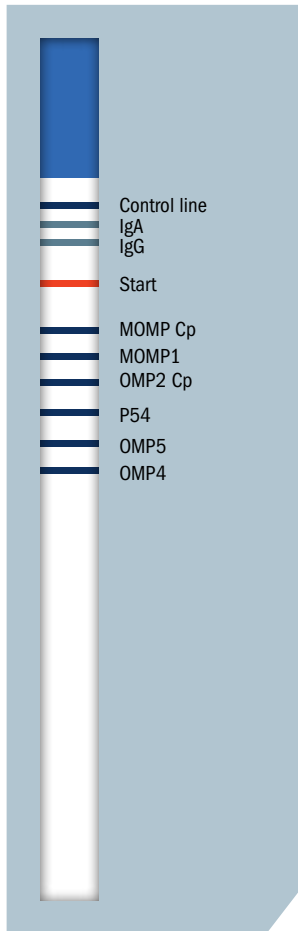
## Summary of Immunoblot Protocol

Step No.	Test steps
1	 Pipette Universal solution 2.5 ml
2	 Strips soaking 10 min. at room temperature Shaker
3	 Aspirate
4	 Dilute samples serum/plasma (30 µl+1.5 ml)
5	 Pipette Controls and diluted samples 1.5 ml
6	 Incubate 30 min. at room temperature Shaker
7	 Aspirate samples and wash strips with 1.5 ml of Universal solution 3-times for 5 min. Shaker
8	 Pipette Conjugate 1.5 ml
9	 Incubate 30 min. at room temperature Shaker
10	 Aspirate Conjugate and wash strips with 1.5 ml of Universal solution 3-times for 5 min. Shaker
11	 Pipette Substrate solution (BCIP/NBT) 1.5 ml
12	 Incubate 15 min. at room temperature Shaker
13	 Aspirate Substrate solution and wash strips with 2 ml of distilled water 2-times for 5 min. Shaker
14	 Sticking and evaluation of strips

## Antigens

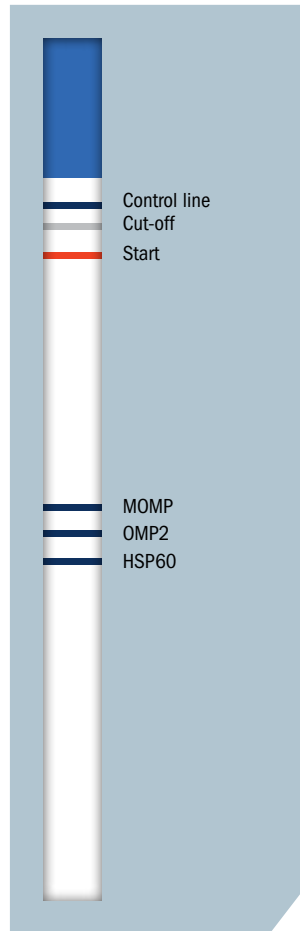
### BLOT-LINE

#### *Chlamydia pneumoniae*



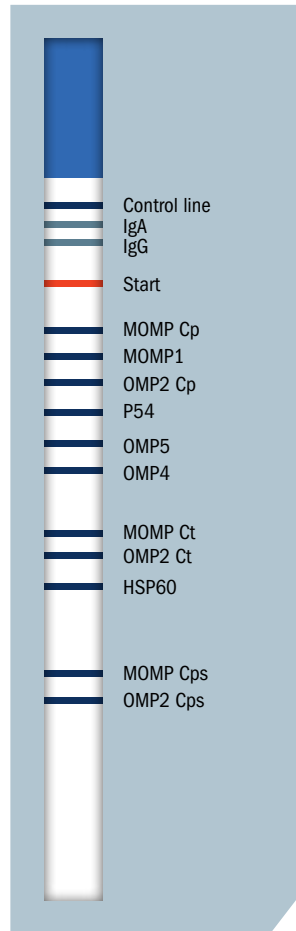
### BLOT-LINE

#### *Chlamydia trachomatis*



### BLOT-LINE

#### *Chlamydia sp.*



#### *Chlamydia pneumoniae*

**MOMP Cp** – dominant major outer membrane protein (species specific) – structural protein; metabolic function

**MOMP1** – isoform, produced by posttranslational modification

**OMP2 Cp** – outer membrane protein (species specific) – structural protein of *Chlamydia* outer membrane complex

**OMP4** – outer membrane protein

**OMP5** – outer membrane protein

**P54** – immunodominant outer antigen, highly specific to *Ch. pneumoniae* – sensitive marker for diagnosis of acute infection

#### *Chlamydia trachomatis*

**MOMP Ct** – dominant major outer membrane protein (species specific) – structural protein; metabolic function

**OMP2 Ct** – outer membrane protein (species specific) – structural protein of *Chlamydia* outer membrane complex

**HSP60** – heat shock protein (GroEL); marker of chronic infection

#### *Chlamydia psittaci*

**MOMP Cps** – dominant major outer membrane protein (species specific) – structural protein; metabolic function

**OMP2 Cps** – outer membrane protein (species specific) – structural protein of *Chlamydia* outer membrane complex

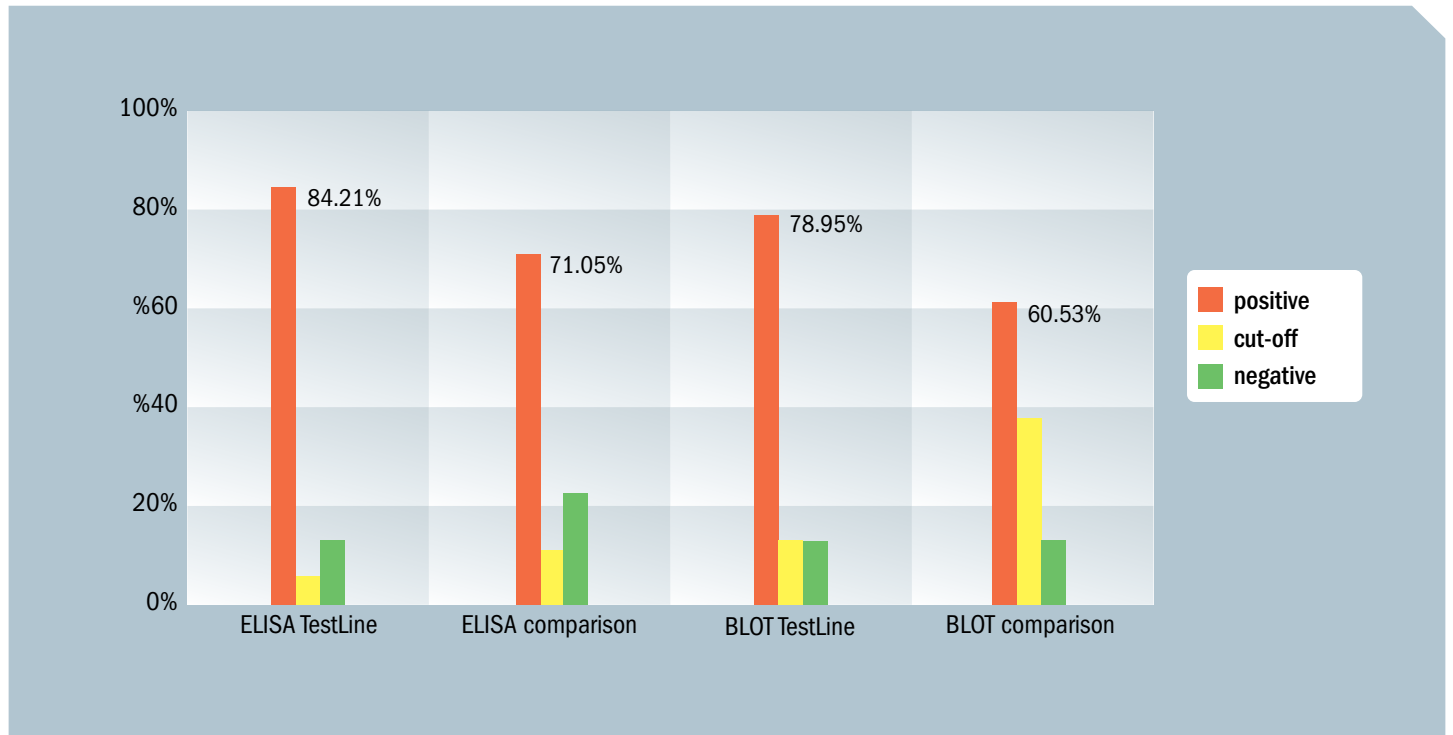
## Test Characteristics

Immunoblot	Diagnostic Sensitivity	Diagnostic Specificity
Chlamydia pneumoniae IgA	95.45%	93.55%
Chlamydia pneumoniae IgG	95.29%	94.29%
Chlamydia trachomatis IgA	97.44%	96.36%
Chlamydia trachomatis IgG	97.14%	98.04%
Chlamydia psittaci IgA	99.00%	99.00%
Chlamydia psittaci IgG	99.00%	99.00%

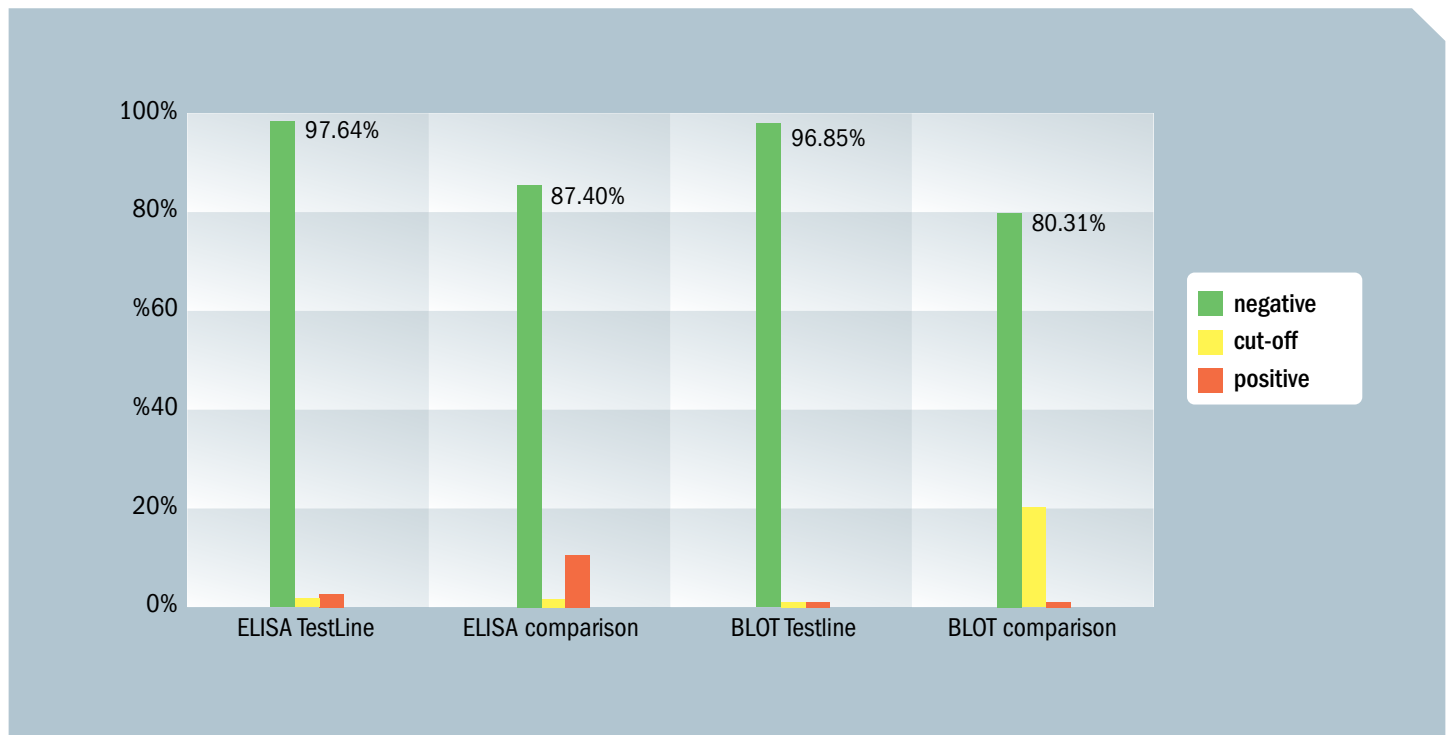
## Advantages

- Easy interpretation and reproducibility of results
- High diagnostic specificity and sensitivity
- Easy evaluation of the test
- Compatibility with all commercial immunoblot processing systems
- Customer support

## Reactivity of different diagnostic kits in a group of positive samples



## Reactivity of different diagnostic kits in a group of negative samples



# INFECTIOUS SEROLOGY

## Ordering Information

### ELISA

Cat. No.	Product	No. of Tests
ChA096	EIA Chlamydia IgA	96
ChG096	EIA Chlamydia IgG	96
ChM096	EIA Chlamydia IgM	96
ChpA96	EIA Chlamydia pneumoniae IgA	96
ChpG96	EIA Chlamydia pneumoniae IgG	96
ChpM96	EIA Chlamydia pneumoniae IgM	96
CpAR96	EIA Chlamydia pneumoniae REC IgA	96
CpGR96	EIA Chlamydia pneumoniae REC IgG	96
ChtA96	EIA Chlamydia trachomatis IgA	96
ChtG96	EIA Chlamydia trachomatis IgG	96
ChtM96	EIA Chlamydia trachomatis IgM	96

### IMMUNOBLOT

Cat. No.	Product	No. of Tests
CAL020	BLOT-LINE Chlamydia IgA	20
CGL020	BLOT-LINE Chlamydia IgG	20
CpAL20	BLOT-LINE Chlamydia pneumoniae IgA	20
CpGL20	BLOT-LINE Chlamydia pneumoniae IgG	20
CtAL20	BLOT-LINE Chlamydia trachomatis IgA	20
CtGL20	BLOT-LINE Chlamydia trachomatis IgG	20

## Contact

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Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.