

Chlamydia

Enzyme immunoassays for the diagnostics of Chlamydia infection

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


IVD **CE** 2265

Diagnostic kits are intended for professional use in the laboratory.

 **TestLine**®

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 2nd and 3rd week after the outbreak of the disease; the production of IgA and IgG antibodies is slower (from the 6th to 8th week).

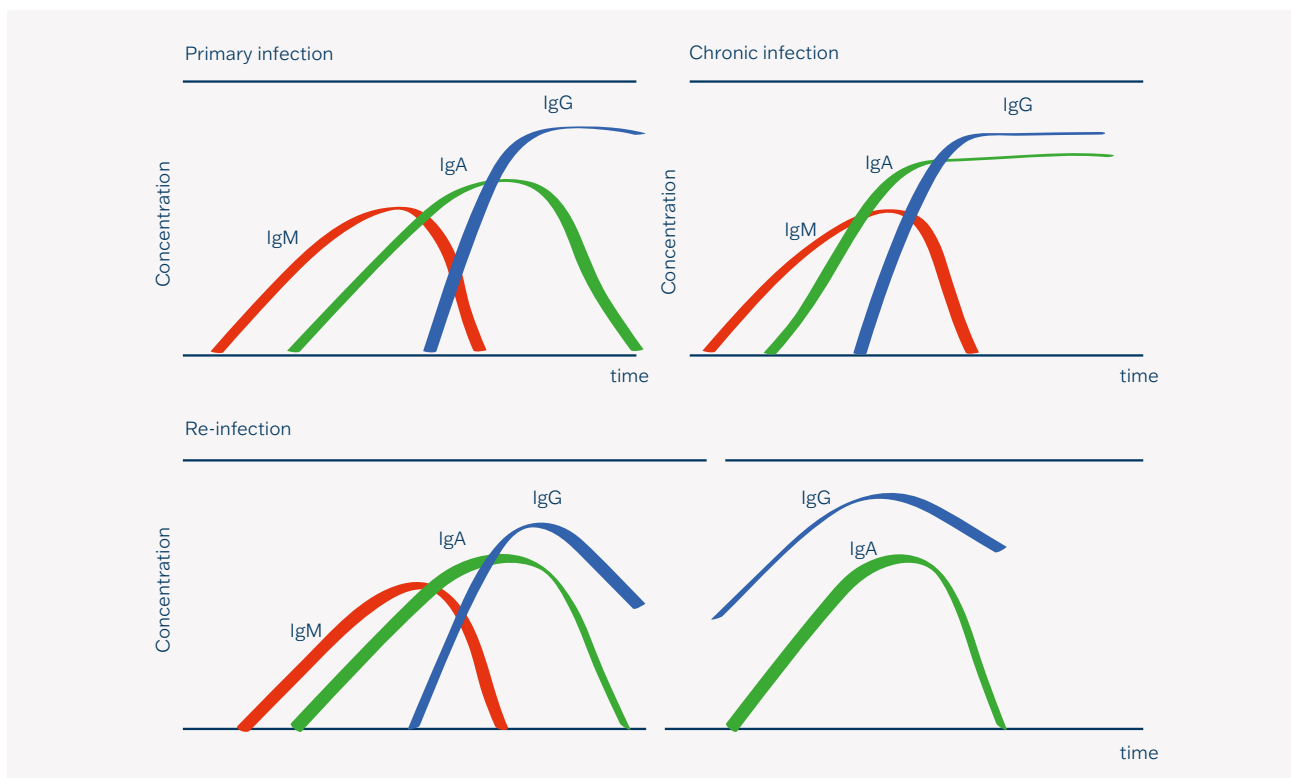
Production of antibodies IgA, IgG and IgM

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 as a marker 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.



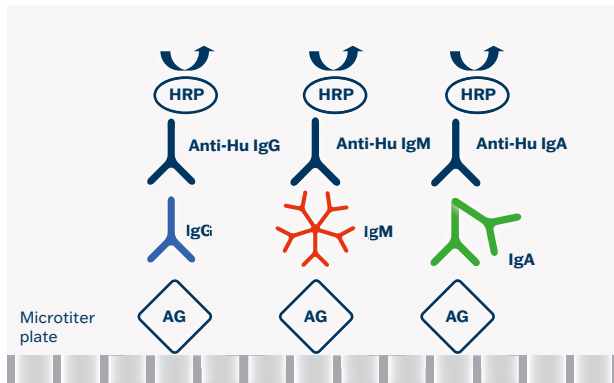
Results Interpretation

| IgG | IgA | IgM | Interpretation |
|------------|-------------------|------------|---|
| - | - | - | Negative result. |
| - | - / + | + | Eventual incipient infection. In order to confirm the results it is necessary to repeat the test |
| + | - | - | 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 1 st and 3 rd month; occurrence of clinical symptoms) |
| + | + | + | On-going infection. |

ELISA

Test Principle

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



Summary Protocol

| Step | Test steps |
|---|---|
|  | 1. Dilute samples – serum/plasma 1:101 (10 µl + 1 ml) |
|  | 2. Pipette controls and diluted samples 100 µl – blank = empty well |
|  | 3. Incubate 30 min. at 37 °C |
|  | 4. Aspirate and wash the wells 5 times |
|  | 5. Add 100 µl Conjugate – blank = empty well |
|  | 6. Incubate 30 min. at 37 °C |
|  | 7. Aspirate and wash the wells 5 times |
|  | 8. Add 100 µl Substrate (TMB-Complete) – Including blank |
|  | 9. Incubate 30 min. at 37 °C |
|  | 10. Add 100 µl Stopping solution – Including blank |
|  | 11. Read colour intensity at 450 nm |

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

Clinical Application

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

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

Advantages

- High diagnostic efficiency, good reproducibility and high dynamics of tests
- Identical assay procedure, total assay time 1.5 hours
- The possibility of independent verification using Certified control sera, complete customer support

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 | 98.8% | 99.0% |
| EIA Chlamydia pneumoniae IgG | 98.9% | 94.4% |
| EIA Chlamydia pneumoniae IgM | 94.7% | 99.9% |
| EIA Chlamydia pneumoniae REC IgA | 99.0% | 99.2% |
| EIA Chlamydia pneumoniae REC IgG | 96.6% | 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% |

Types of kits

SmartEIA kits are designed for automated processing using the Agility® analyser.

EIA



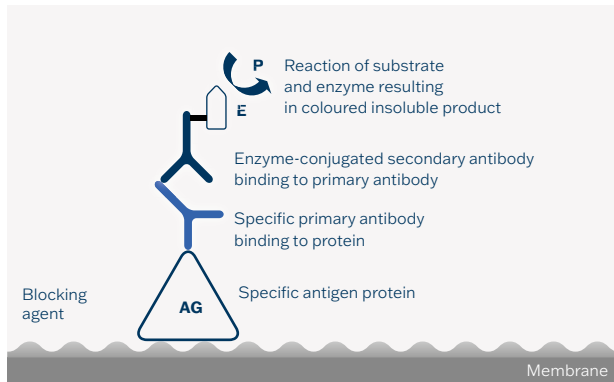
SmartEIA



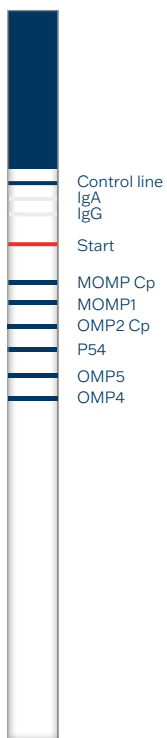
IMMUNOBLOT

Test Principle

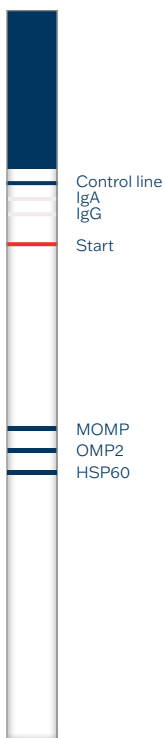
Recombinant antigens are transferred to a nitrocellulose membrane using a micro-dispensing method.



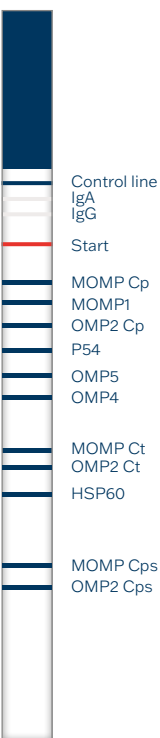
BLOT-LINE Chlamydia pneumoniae



BLOT-LINE Chlamydia trachomatis



BLOT-LINE Chlamydia



Clinical Application

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

Antigens

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

Summary Protocol

| Step | 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 1:51 (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 |

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
- Possibility of software evaluation

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

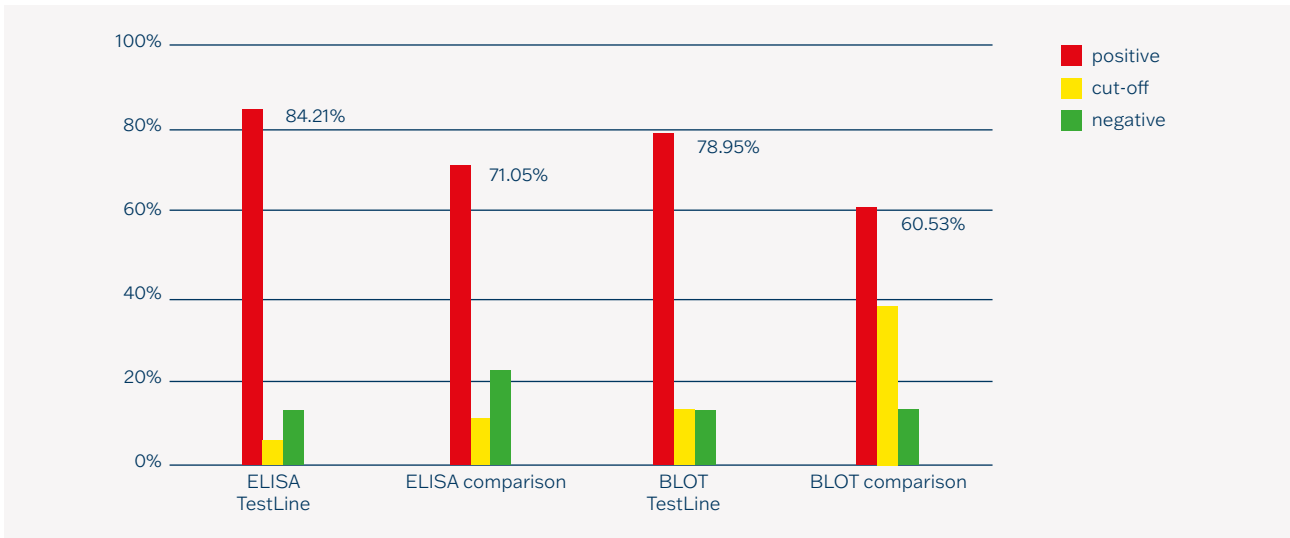


Test Characteristics

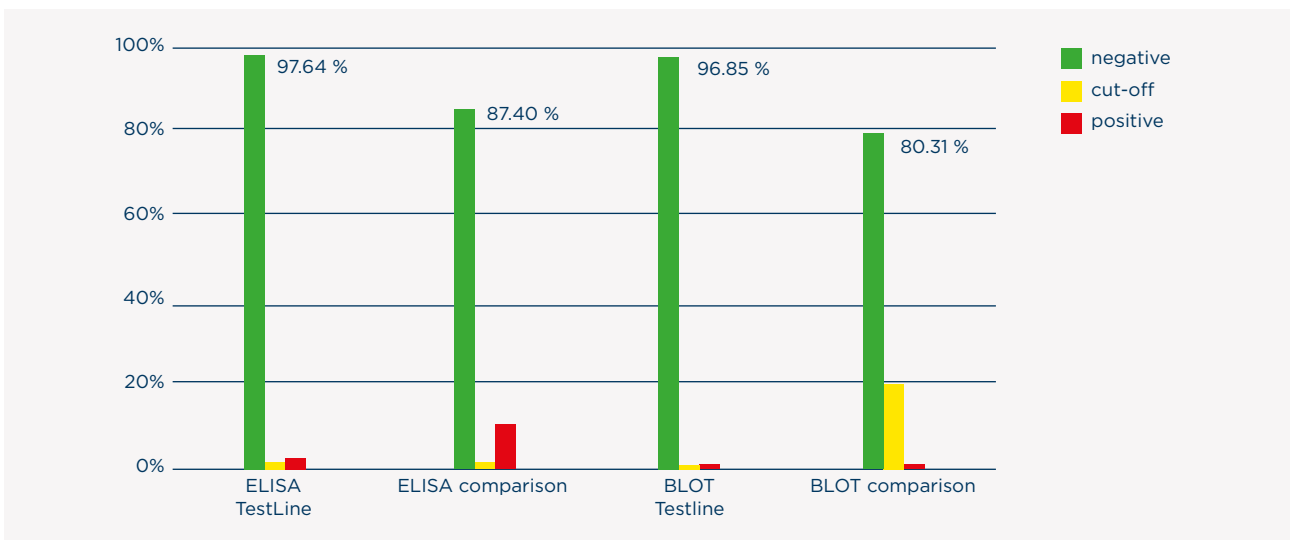
| Pathogen | Diagnostic Sensitivity | Diagnostic Specificity |
|---------------------------|------------------------|------------------------|
| Chlamydia pneumoniae IgA | 95.5% | 93.6% |
| Chlamydia pneumoniae IgG | 95.3% | 94.3% |
| Chlamydia pneumoniae IgM | 85.0% | 94.7% |
| Chlamydia trachomatis IgA | 97.4% | 96.4% |
| Chlamydia trachomatis IgG | 97.1% | 98.0% |
| Chlamydia psittaci IgA | 99.0% | 99.0% |
| Chlamydia psittaci IgG | 99.0% | 99.0% |

Comparative study

Reactivity of different diagnostic kits in a group of positive samples

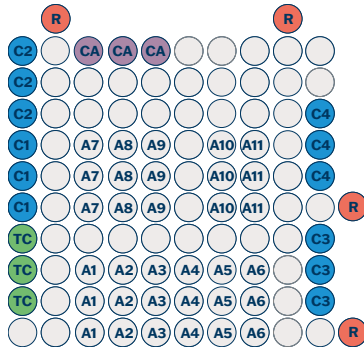


Reactivity of different diagnostic kits in a group of negative samples



MICROBLOT-ARRAY

Distribution of antigens and control spots















Description of antigens

- A1** – MOMP Cp
- A2** – MOMP1 Cp
- A3** – OMP2 Cp
- A4** – p54
- A5** – OMP5 Cp
- A6** – OMP4 Cp
- A7** – MOMP Ct
- A8** – OMP2 Ct
- A9** – HSP60
- A10** – MOMP Cps
- A11** – OMP2 Cps

Description of control spots

- R** – Reference
- TC** – Test control
- CA** – Conjugate control IgA
- CG** – Conjugate control IgG
- CM** – Conjugate control IgM
- C1** – Calibration 1
- C2** – Calibration 2
- C3** – Calibration 3
- C4** – Calibration 4

Protocol Summary

| Step | Test steps |
|---|--|
|  | 1. Pipette Universal solution 150 µl |
|  | 2. Strips soaking 10 min. at room temperature |
|  | 3. Aspirate |
|  | 4. Dilute samples – serum/plasma 1:51 (10 µl + 500 µl) |
|  | 5. Pipette Controls and diluted samples 100 µl |
|  | 6. Incubate 30 min. at room temperature |
|  | 7. Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min. |
|  | 8. Pipette Conjugate 100 µl |
|  | 9. Incubate 30 min. at room temperature |
|  | 10. Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min. |
|  | 11. Pipette Substrate solution (BCIP/NBT) 100 µl |
|  | 12. Incubate 15 min. at room temperature |
|  | 13. Aspirate Substrate solution and wash strips with 200 µl of distilled water 2-times for 5 min. |
|  | 14. Dry and evaluate strips |

User Comfort

- Low sample consumption
- Antigens spotted in triplicate – minimizing statistical variation
- Fully automatic assay processing and results evaluation
- Parallel testing of multiple markers simultaneously
- High sensitivity



Test Characteristics

| Pathogen | Diagnostic Sensitivity | Diagnostic Specificity |
|---------------------------|-------------------------------|-------------------------------|
| Chlamydia pneumoniae IgA | 94.4% | 94.3% |
| Chlamydia pneumoniae IgG | 94.6% | 96.0% |
| Chlamydia trachomatis IgA | 94.1% | 96.0% |
| Chlamydia trachomatis IgG | 92.7% | 98.3% |
| Chlamydia psittaci IgA | 100.0% | 100.0% |
| Chlamydia psittaci IgG | 80.0% | 99.0% |

Ordering Information

ELISA

| <u>Cat. No.</u> | <u>Product</u> | <u>No. of wells</u> |
|------------------------|---------------------------------------|----------------------------|
| 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 |
| SK-ChA096 | SmartEIA Chlamydia IgA | 96 |
| SK-ChG096 | SmartEIA Chlamydia IgG | 96 |
| SK-ChM096 | SmartEIA Chlamydia IgM | 96 |
| SK-ChpA96 | SmartEIA Chlamydia pneumoniae IgA | 96 |
| SK-ChpG96 | SmartEIA Chlamydia pneumoniae IgG | 96 |
| SK-ChpM96 | SmartEIA Chlamydia pneumoniae IgM | 96 |
| SK-CpAR96 | SmartEIA Chlamydia pneumoniae REC IgA | 96 |
| SK-CpGR96 | SmartEIA Chlamydia pneumoniae REC IgG | 96 |
| SK-ChtA96 | SmartEIA Chlamydia trachomatis IgA | 96 |
| SK-ChtG96 | SmartEIA Chlamydia trachomatis IgG | 96 |
| SK-ChtM96 | SmartEIA Chlamydia trachomatis IgM | 96 |

SmartEIA kits are designed for automated processing using the Agility® analyser



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Ordering Information

IMMUNOBLOT

| <u>Cat. No.</u> | <u>Product</u> | <u>No. of Tests</u> |
|-----------------|-------------------------------------|---------------------|
| 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 |
| CpML20 | BLOT-LINE Chlamydia pneumoniae IgM | 20 |
| CtAL20 | BLOT-LINE Chlamydia trachomatis IgA | 20 |
| CtGL20 | BLOT-LINE Chlamydia trachomatis IgG | 20 |
| BD-CAL024 | BlueBLOT-LINE Chlamydia IgA | 24 |
| BD-CGL024 | BlueBLOT-LINE Chlamydia IgG | 24 |

The BlueBLOT-LINE kits are designed for automatic processing using BlueDiver® analyser

Microblot-Array

| <u>Cat. No.</u> | <u>Product</u> | <u>No. of Tests</u> |
|-----------------|-------------------------------|---------------------|
| CAMA096 | Microblot-Array Chlamydia IgA | 96 |
| CGMA096 | Microblot-Array Chlamydia IgG | 96 |



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