



# Bioclone Australia Pty. Limited.

## ELEGANCE *Chlamydia pneumoniae* IgM ELISA

### Intended Use

The ELEGANCE *Chlamydia pneumoniae* IgM ELISA has been designed for the *in vitro* diagnostic measurement of anti-*C pneumoniae* IgM in the screening of human serum.

### Principles of the ELEGANCE *Chlamydia pneumoniae* IgM ELISA

The *Chlamydia pneumoniae* IgM ELISA measures anti-*Chlamydia pneumoniae* IgM in human serum by an enzyme-linked immunosorbent assay method (ELISA). Highly purified *Chlamydia pneumoniae*-specific outer membrane protein complex, bound to the microwells, reacts with the anti-*Chlamydia pneumoniae* IgM in the sample. After a wash, any bound IgM is further reacted with anti-human IgM polyclonal antibody labelled with alkaline phosphatase. A substrate solution, containing p-nitrophenol phosphate (p-NPP), reacts with alkaline phosphatase to produce colour correlating with the presence of anti-*Chlamydia pneumoniae* IgM in the sample. The result is determined by calculating an index value from optical density (OD) values relative to control material.

### Clinical Significance

Until recent years, it was thought that *Chlamydia pneumoniae* was a subspecies of *Chlamydia psittaci*, but in 1989 the existence of this third *Chlamydia* species was established.<sup>1</sup> Depending on the host, *Chlamydia pneumoniae* can bring about acute chronic upper respiratory infection, bronchitis, and pneumonia-based lung disease. Depending on the site of inflammation, these can lead to very serious complications.<sup>2,3,4</sup> Research results are also suggesting the involvement of *Chlamydia pneumoniae* in asthma, lung infections and other chronic diseases.

### Sample Preparation

Immediately before use, bring samples to room temperature and mix thoroughly by vortexing. Using a sample dilution plate, pipette:

- a. 10  $\mu$ L of sample and add 200  $\mu$ L of Latex Solution\*. Shake plate (for IgM). Incubate for 10 minutes at room temperature.

This is a final dilution factor of 1 in 21 (IgM), to be used in the assay. Do not dilute control samples.

### Protocol (for IgM)

All reagents at room temperature & gently mixed before use.

1. Pipette 100  $\mu$ L of:
  - a. Wash Solution\*\* (1 well - 'blank')
  - b. *Chlamydia pneumoniae* Negative Control (2 wells)
  - c. *Chlamydia pneumoniae* IgM Positive Control (2 wells) Note: Do not dilute control solutions.
  - d. Diluted sample (into remaining wells).
2. Cover the plate with the lid and incubate at 37°C for 60 minutes.

3. After incubation, aspirate the liquid and rinse each well three times with 300  $\mu$ L of wash solution\*\*. After the final wash, invert the plate and tap firmly on an absorbent lint-free tissue to remove any remaining wash solution.
4. Pipette 100  $\mu$ L of *Chlamydia pneumoniae* IgM Antibody Reagent into each well.
5. Cover the plate with the lid and incubate at 37°C for 60 minutes.
6. After incubation, aspirate the liquid and rinse each well three times with 300  $\mu$ L of wash solution\*\*. After the final wash, invert the plate and tap firmly on an absorbent lint-free tissue to remove any remaining wash solution.
7. Pipette 100  $\mu$ L of substrate solution to each well.
8. Cover the plate with the lid and incubate at room temperature (20-25°C) for 10 minutes.
9. After the 10 minutes, pipette 25  $\mu$ L of *Chlamydia* Stop Solution into each well.
10. Mix gently with a plate shaker for 10 seconds.
11. Using a microplate reader, measure the OD at 405 nm. Correct for 'blank' (subtract OD of microwell to which 100  $\mu$ L of Wash Solution was pipetted).

\* This is a 8-fold dilution of a concentrate.

\*\* This is a 10-fold dilution of wash concentrate in water.

### *C pneumoniae* IgM ELISA Kit Components

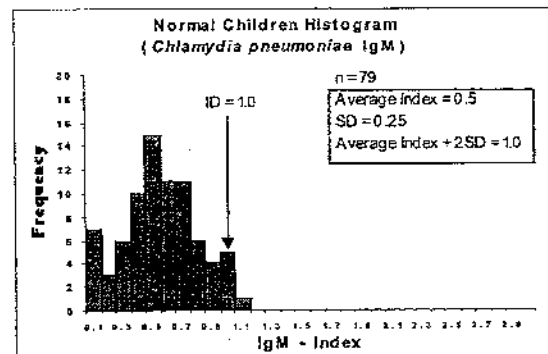
(Store at 4°C)

#### Kit Components

|                                   | Cat#               |
|-----------------------------------|--------------------|
| <i>C pneumoniae</i> IgM ELISA Kit | 96 Wells 40CPM0096 |
| Coated Microwells                 | 1 plate CPA96      |
| Latex Solution                    | 2.6ml CPML96       |
| IgM Antibody Reagent              | 10.5ml CPMB96      |
| Negative Control                  | 1.4ml CN1          |
| IgM Positive Control              | 1.4ml CPMP1        |
| Wash Concentrate†                 | 50ml CW96          |
| Substrate Solution                | 10ml CSL96         |
| Stop Solution                     | 5ml CSOH96         |

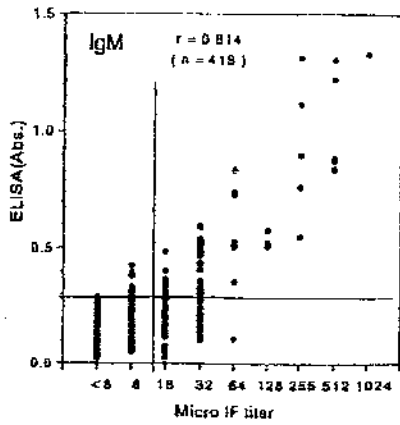
† If crystallized warm to 37°C before dilution.

### Sample Ranges (Children)



### Comparison to Micro-IF

Acute Respiratory Patients  
*Chlamydia pneumoniae* IgM



### Intraassay / Interassay Coefficient of Variation

Intraassay coefficient of variation is typically <math>< 10\%</math> for the IgM ELISA.

Interassay coefficient of variation is typically <math>< 10\%</math> for the IgM ELISA.

#### Interassay

| Sample     | 1     | 2     | 3     |
|------------|-------|-------|-------|
| n =        | 10    | 10    | 10    |
| Average OD | 0.364 | 0.602 | 1.000 |
| SD         | 0.032 | 0.053 | 0.058 |
| %CV        | 8.8   | 8.7   | 5.8   |

### Comparison to Micro-IF Method

(Acute Respiratory Patients (n=418))

| IgM      |   | Micro-IF Method |             | Total       |
|----------|---|-----------------|-------------|-------------|
|          |   | +               | -           |             |
| ELEGANCE | + | 49              | 12          | 61 (14.6%)  |
|          | - | 44              | 313         | 357 (85.4%) |
| Total    |   | 93 (22.2%)      | 325 (77.8%) | 418 (100%)  |

Positive Rate: 49 / 93 = 52.7%  
 Negative Rate: 313 / 325 = 96.3%  
 Total Rate: 362 / 418 = 86.6%

(Antigen Positive Patients (n=51))

| IgM      |   | Micro-IF Method |            | Total      |
|----------|---|-----------------|------------|------------|
|          |   | +               | -          |            |
| ELEGANCE | + | 23              | 1          | 24 (47.1%) |
|          | - | 0               | 19         | 27 (52.9%) |
| Total    |   | 31 (60.8%)      | 20 (39.2%) | 51 (100%)  |

Positive Rate: 23 / 31 = 74.2%  
 Negative Rate: 19 / 20 = 95.0%  
 Total Rate: 42 / 51 = 82.4%

| IgM                      | Western Blot Method (analysis of above) |            |       |
|--------------------------|---|------------|-------|
|                          | +                                       | -          | Total |
| ELEGANCE +<br>Micro-IF - | 6 (50.0%)                               | 6 (50.0%)  | 12    |
| ELEGANCE -<br>Micro-IF + | 7 (15.9%)                               | 37 (84.1%) | 44    |

| IgM                      | Western Blot Method (analysis of above) |           |       |
|--------------------------|---|-----------|-------|
|                          | +                                       | -         | Total |
| ELEGANCE +<br>Micro-IF - | 1 (100%)                                | 0 (0.0%)  | 1     |
| ELEGANCE -<br>Micro-IF + | 1 (12.5%)                               | 7 (87.5%) | 8     |

### REFERENCES

1. Grayston, JT, Kuo, C-C, Campbell, LA, Wang, S-P. *Int. J. Sys. Bacteriol.* 39: 88-90, 1989.
2. Grayston, JT, Campbell, LA, Kuo, C-C, Mordhorst, CH, Saikku, P, Thom, DH, Wang, S-P. *J. Infect. Dis.* 161: 618-625, 1990.
3. Grayston, JT. *Dis. 15: 757-763, 1992.*
4. Kishimoto, H. *J. Infect. Dis.* 64: 986-992, 1990

#### Technical Service / Ordering Information

The ELEGANCE *Chlamydia pneumoniae* IgM ELISA kit is manufactured by:  
 Biocline Australia Pty Limited, 71-73 Railway Parade, Murrumbidgee NSW, 2204, Australia.  
 Orders and full technical service are available by contacting Biocline on:  
 Telephone: +61 (0)2 8517 1966  
 Facsimile: +61 (0)2 8517 2990  
 Freecall: 1800 251 138 (within Australia)

Email: [technical@biocline.com.au](mailto:technical@biocline.com.au) or [sales@biocline.com.au](mailto:sales@biocline.com.au)  
 Web: <http://www.biocline.com.au>