



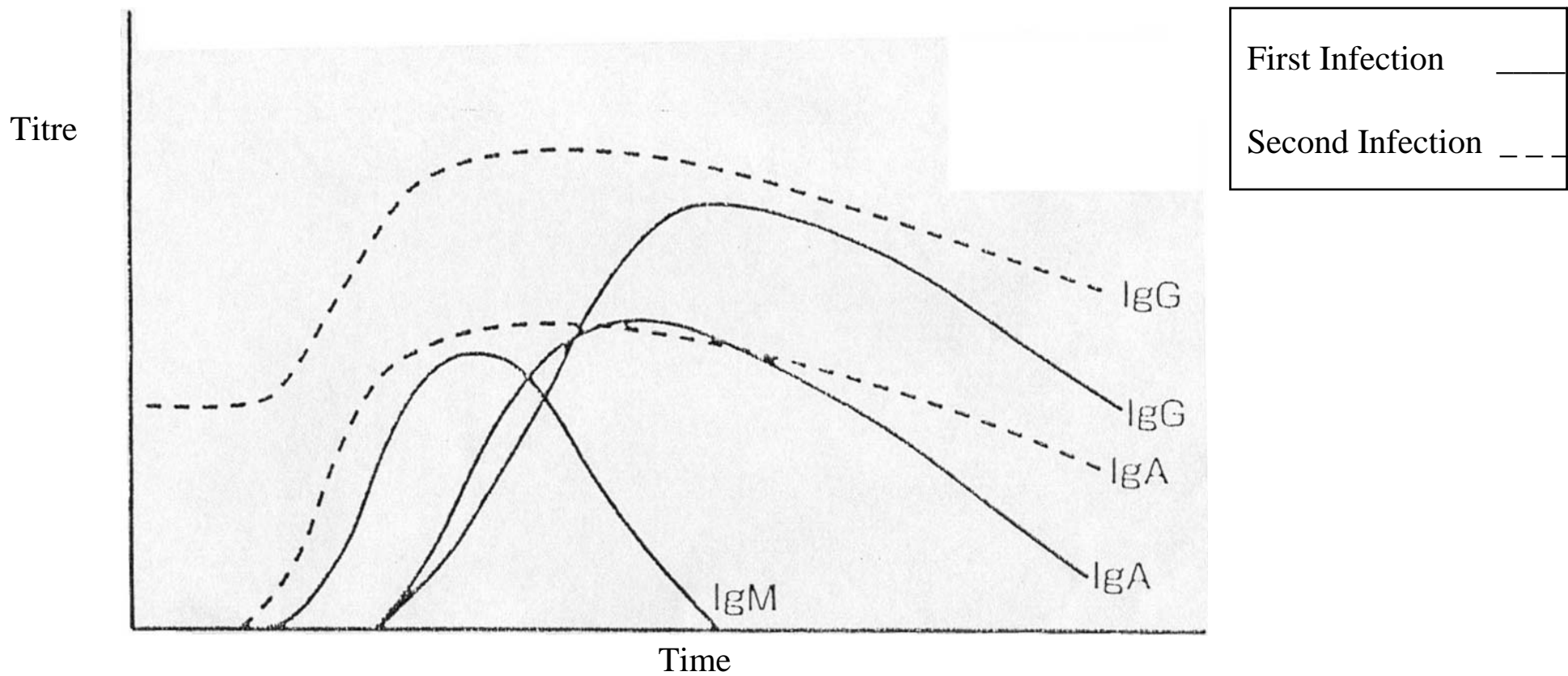
ELEGANCE
Chlamydia pneumoniae
IgG, IgA & IgM ELISAs

For the determination of
Anti-Chlamydia pneumoniae IgG, IgA & IgM in human serum



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Antibody Response Patterns for *Chlamydia pneumoniae* infection



This figure indicates antibody response patterns for *Chlamydia pneumoniae* infection. The unbroken line shows the results at initial infection, while the dotted line indicates the re-infection pattern.

Chlamydia pneumoniae diagnosis

To avoid chronic diseases

To decide on proper antibiotics

To decide on correct medication period

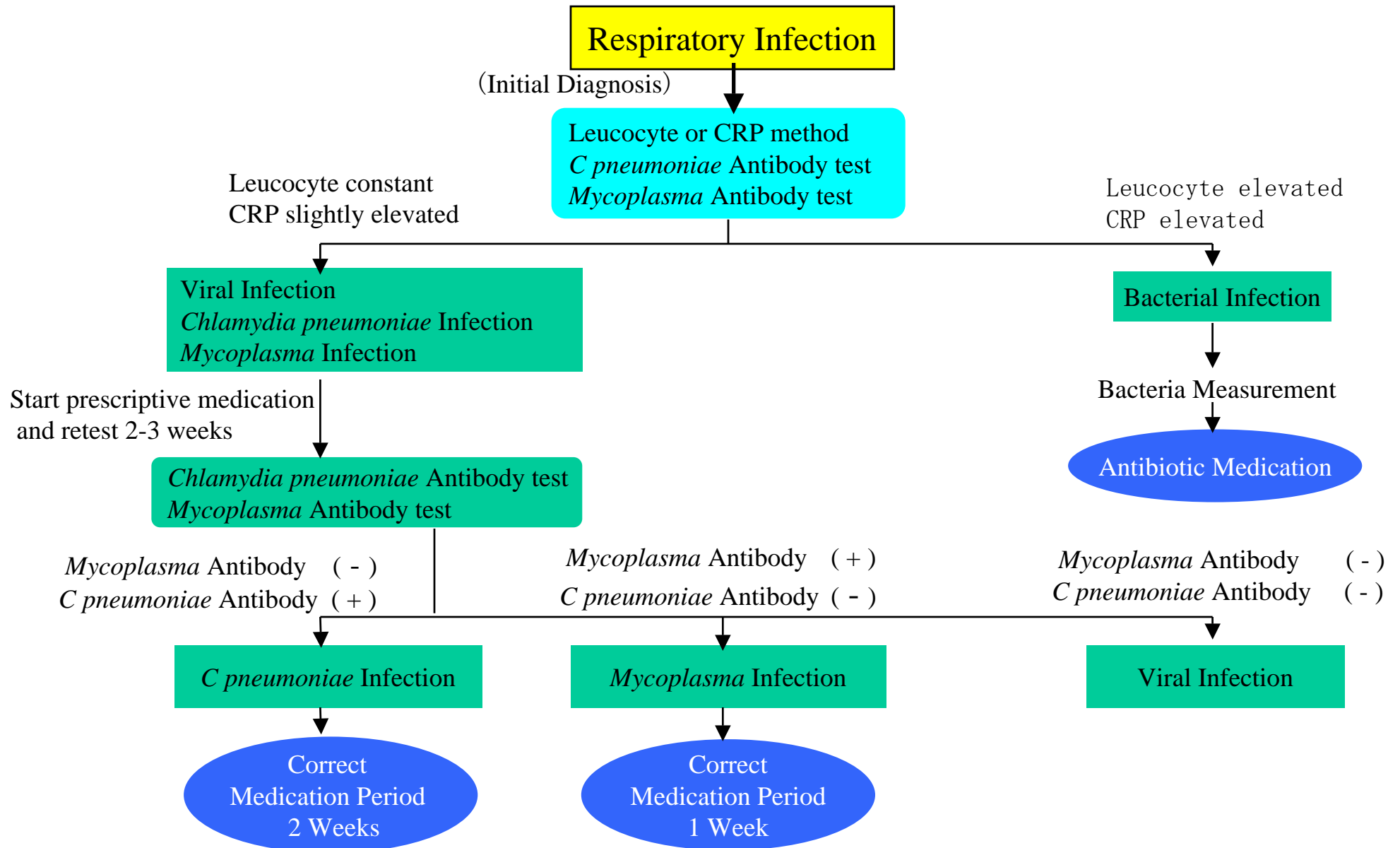
It is necessary to distinguish between *C pneumoniae* and *Mycoplasma*

Correct diagnosis of *C pneumoniae* is essential

Comparison between *Mycoplasma* and *C pneumoniae* infections

	<i>Mycoplasma</i>	<i>Chlamydia pneumoniae</i>
Infection	<ul style="list-style-type: none">• Infection may last around 2 weeks• High fever	<p>Infection period is longer than <i>Mycoplasma</i> - may last as long as 3-4 weeks</p> <ul style="list-style-type: none">• Mid-range fever
Patients	Mainly elderly	Infects all age groups
Medication period	1 week	2 weeks

Respiratory Infection Examination Flowchart

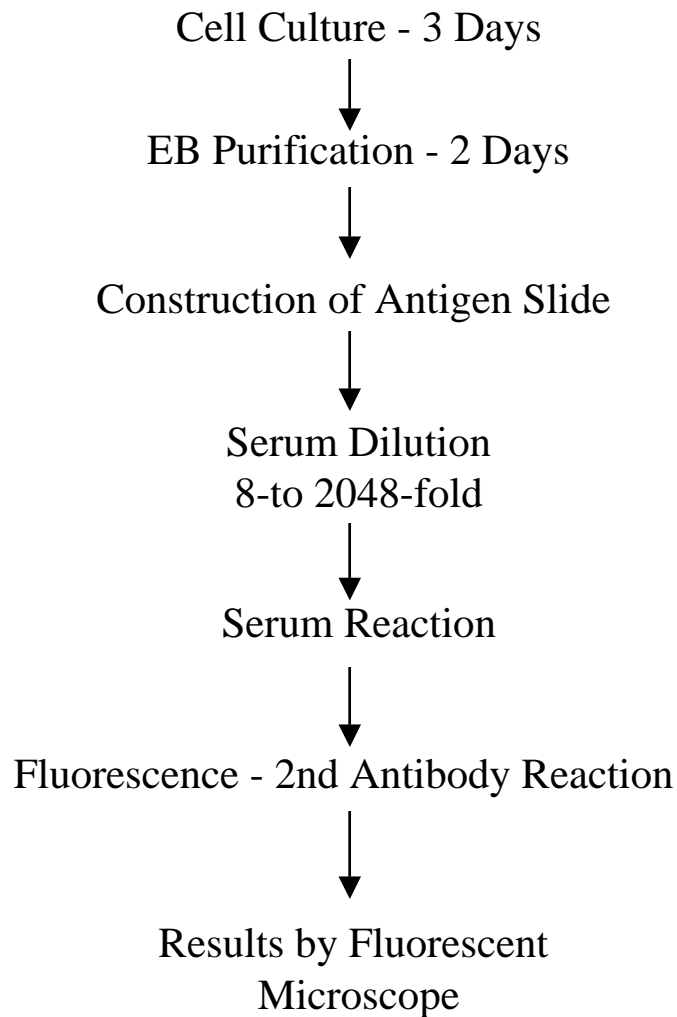


Detection Methods for *Chlamydia pneumoniae*

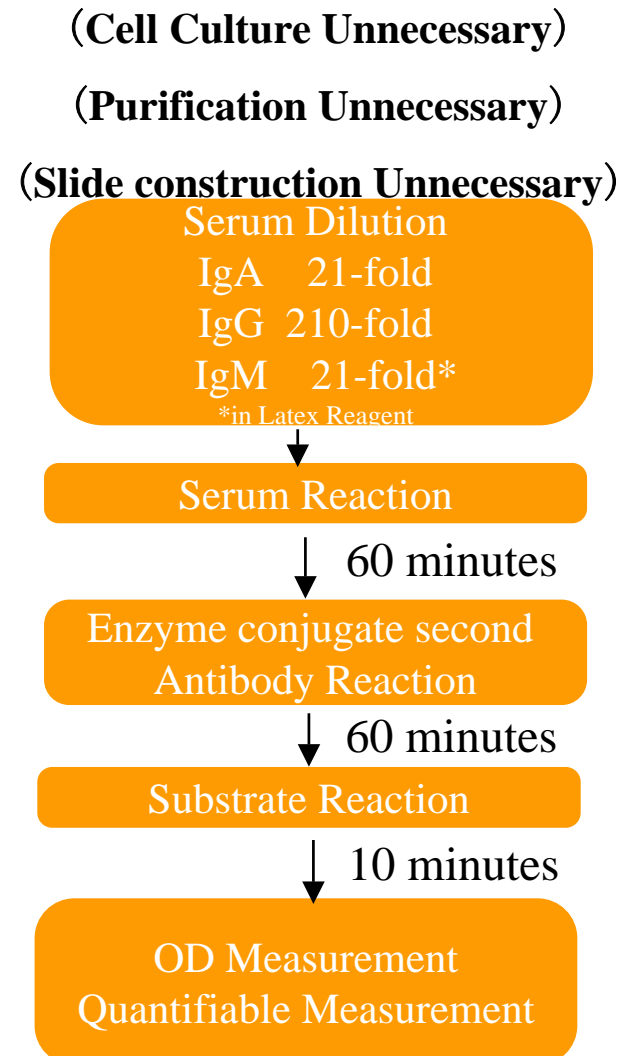
	Method	Sample Number	Results	Handling	Positive Features	Negative Features
Antibody Detection Method	<i>ELEGANCE</i> <i>C pneumoniae</i> ELISA	Able to measure large quantities	Measurement of OD	Easy	<ul style="list-style-type: none"> •No need to prepare antigen •High Specificity •Objective judgement 	<ul style="list-style-type: none"> · Samples need dilution
	Micro IF Method	Small Quantities	Fluorescent Microscope (By eye)	Some Difficulties	<ul style="list-style-type: none"> •Measurement of specific species •Measurement of serological antibodies for <i>C trachomatis</i> 	<ul style="list-style-type: none"> · Need cell culture and treatment of antigen · Laborious procedure of serum preparation · Some non-specific reaction · High level of skill required
	MFA Method	Small Quantities	Fluorescent Microscope (By eye)	Difficult	<ul style="list-style-type: none"> •No need for purification of antigen •Measurement of serological antibodies for <i>C trachomatis</i> 	<ul style="list-style-type: none"> · Need cell culture · Laborious procedure for serum dilution · Some non-specific reaction · Highly skilled judgement
	Western Blot Method	Small Quantities	Observe Colour (By eye)	Difficult	<ul style="list-style-type: none"> •High Sensitivity 	<ul style="list-style-type: none"> · Need treatment of antigen · Procedure is extremely laborious · Non-specific reactions may occur
Antigen Detection Method	PCR Method	Small Quantities	Chemiluminescence (By eye)	Difficult	<ul style="list-style-type: none"> •High Sensitivity •Final diagnostic method for infection 	<ul style="list-style-type: none"> · Sampling may be troublesome · Procedure of assay troublesome · Highly skilled judgement · Some non-specific reaction
	All Culture Method	Small Quantities	Fluorescent Microscope (By eye)	Difficult	<ul style="list-style-type: none"> •Final diagnostic method for infection 	<ul style="list-style-type: none"> · Sampling may be troublesome · Time taken to culture cells
	Direct Fluorescent Antibody Method	Small Quantities	Fluorescent Microscope (By eye)	Some Difficulties	<ul style="list-style-type: none"> •Final diagnostic method for infection 	<ul style="list-style-type: none"> · Sampling may be troublesome · Low sensitivity · Skill required for accurate analysis

Comparison between Micro-IF and *ELEGANCE* methods

Micro-IF



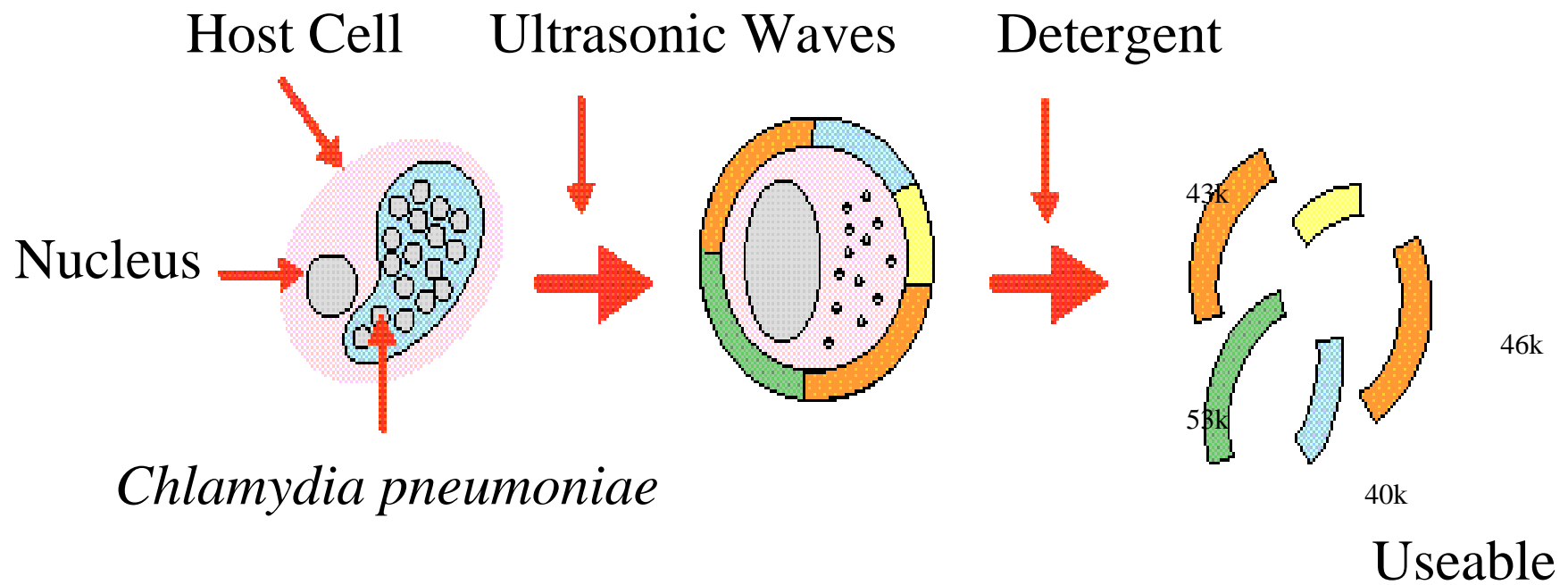
ELEGANCE



Characteristics of *ELEGANCE Chlamydia pneumoniae* ELISAs

- Using a *C pneumoniae*-specific highly purified outer membrane protein complex, *ELEGANCE* offers superior specificity.
- *ELEGANCE* is easy to use and ensures that large numbers of tests may be completed simultaneously, quickly and efficiently.
- Serum samples are easy to collect for testing.
- Where the antigen is difficult to collect, *ELEGANCE* offers the most convenient diagnostic method.
- Automatable ELISA method offers absolute objectivity in results and meets assay sensitivity requirements.
- Measurement of IgG, IgA and IgM is possible.

Chlamydia pneumoniae Antigen Preparation - Overview



Antigen クラミジア感染細胞

Kit Components Provided - IgG ELISA

- *Chlamydia pneumoniae* Coated Microwells 96 Wells
- *Chlamydia pneumoniae* IgG Antibody Reagent 10.5 mL
- *Chlamydia* Negative Control 1.4 mL
- *Chlamydia pneumoniae* IgG Positive Control 1.4 mL
- *Chlamydia* Wash Concentrate 50 mL
- *Chlamydia* Substrate Solution 10.5 mL
- *Chlamydia* Stop Solution 5 ml

Kit Components Provided - IgA ELISA

- *Chlamydia pneumoniae* Coated Microwells 96 Wells
- *Chlamydia pneumoniae* IgA Antibody Reagent 10.5 mL
- *Chlamydia* Negative Control 1.4 mL
- *Chlamydia pneumoniae* IgA Positive Control 1.4 mL
- *Chlamydia* Wash Concentrate 50 mL
- *Chlamydia* Substrate Solution 10.5 mL
- *Chlamydia* Stop Solution 5 mL

Kit Components Provided - IgM ELISA

- *Chlamydia pneumoniae* IgM Coated Microwells 96 Wells
- *Chlamydia* Latex Concentrate 2.6 mL
- *Chlamydia pneumoniae* IgM Antibody Reagent 10.5 mL
- *Chlamydia* Negative Control 1.4 mL
- *Chlamydia pneumoniae* IgM Positive Control 1.4 mL
- *Chlamydia* Wash Concentrate 50 mL
- *Chlamydia* Substrate Solution 10.5 mL
- *Chlamydia* Stop Solution 5 mL

Overview of assay procedure

1st incubation

Sample Preparation
IgA: 21 x dilution
IgG: 210 x dilution
IgM: 21 x dilution*
* in Latex Reagent

Sample Dispensing
100µl
37°C 60 minutes

2nd incubation

Wash
Wash solution: 300µl x 3

Enzyme-labelled anti-IgA
Antibody conjugate: 100µl
or

Enzyme labelled anti-IgG
Antibody conjugate: 100µl
or

Enzyme labelled anti-IgM
Antibody conjugate: 100µl
37°C 60 minutes

Colour

Wash
Wash solution: 300µl x 3

Substrate Reaction
Substrate solution : 100µl
20–25°C 10 minutes

Stop Solution Reaction
Stop solution: 25µl

OD
405nm

Chlamydia Antibody: Calculation of results

Calculation of Correction Coefficient

$$\text{Correction Coefficient (K)} = \frac{\text{Positive Control Reference Value}}{\text{Positive Control (Mean OD)}}$$

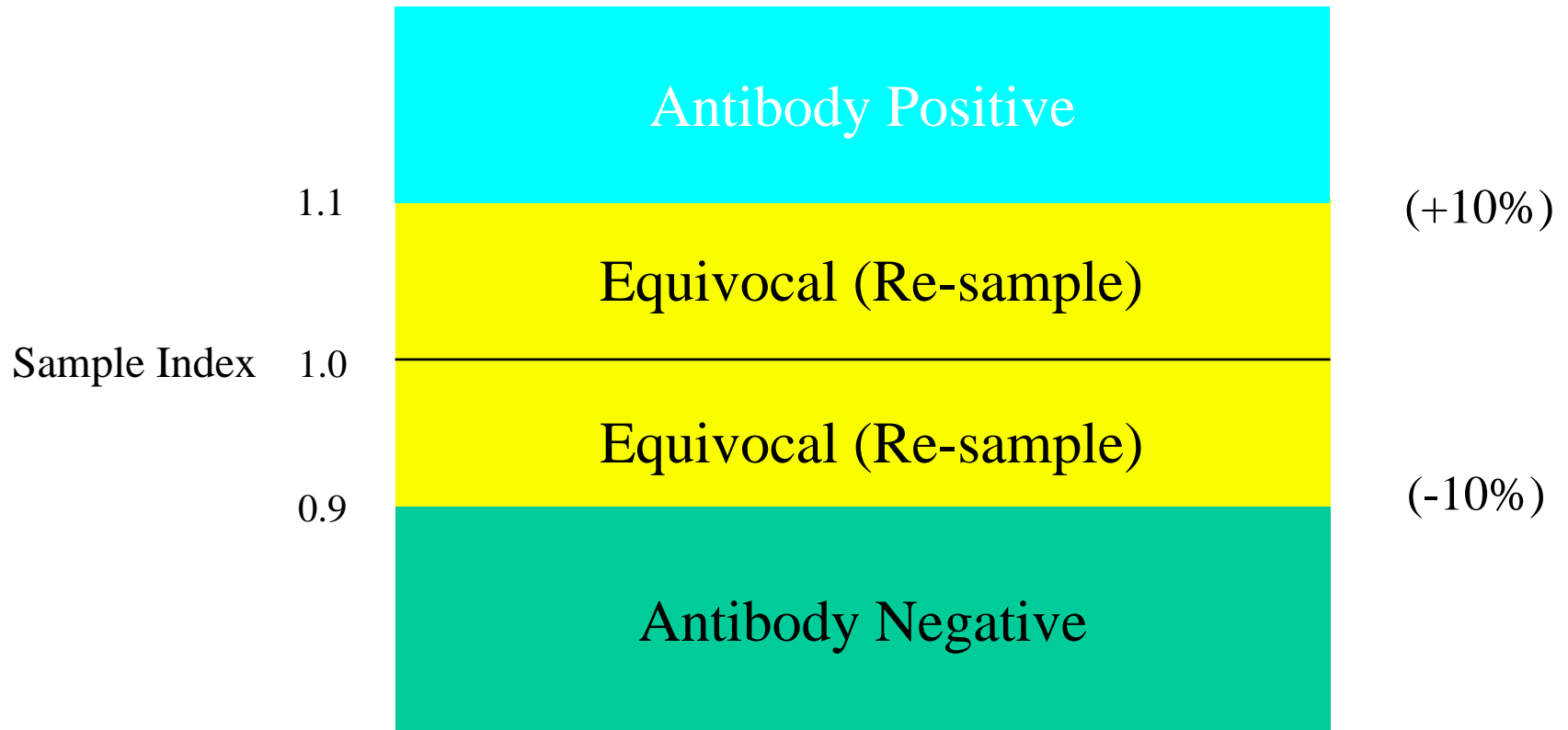
Calculation of Cut-Off Value

$$\text{Cut-Off Value} = (\text{Mean Negative Control OD}) \times K + 0.20 \text{ (0.3 for IgM)}$$

Calculation of Corrected Mean Sample OD

$$\text{Corrected Mean Sample OD} = \text{Mean Sample OD} \times K$$

Chlamydia Antibody: Interpretation of Sample Measurement



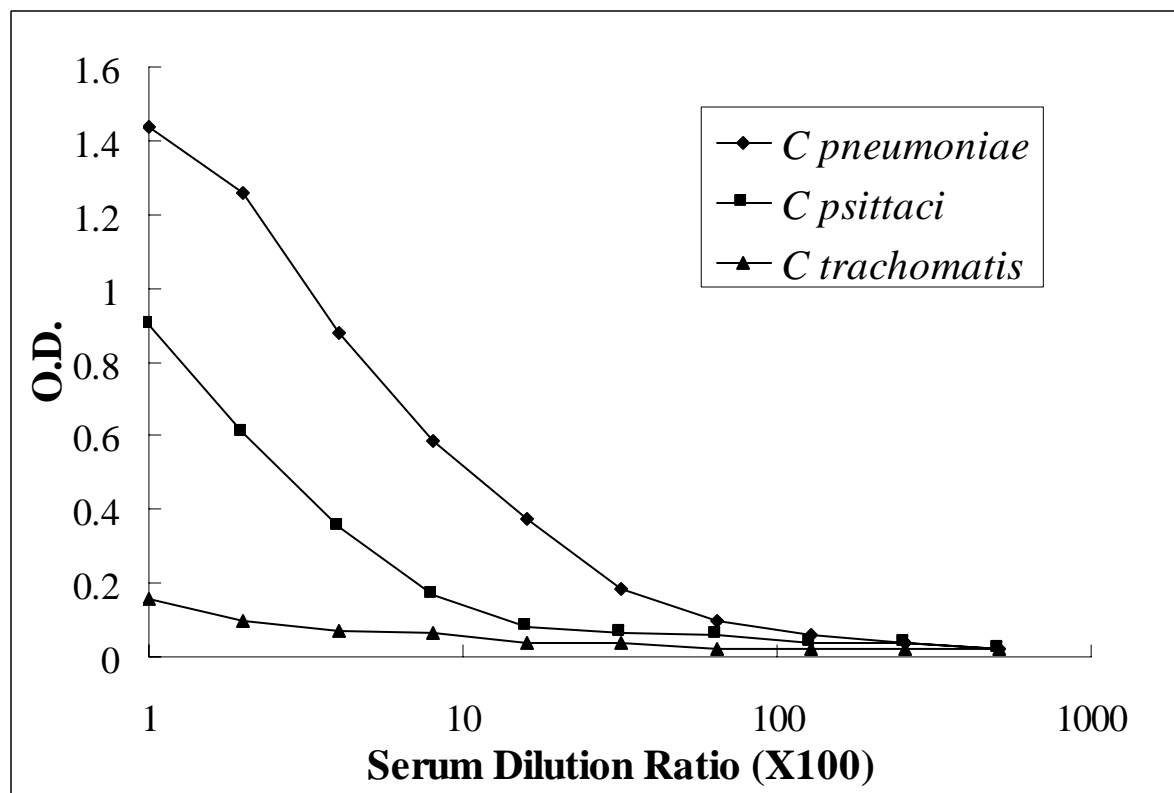
$$\text{Sample Index} = \frac{\text{Corrected Mean Sample OD}}{\text{Cut-Off Value}}$$

Potential *C trachomatis* crossreactivity (*C pneumoniae* IgG comparison)

Sample ID	Micro-IF Method			<i>ELEGANCE</i>
	<i>C. pneumoniae</i>	<i>C. psittaci</i>	<i>C. trachomatis</i>	OD
1	<8	<8	16	0.143 (-)
2	<8	<8	8	0.115 (-)
3	<8	<8	16	0.106 (-)
4	<8	<8	16	0.070 (-)
5	8	<8	16	0.148 (-)
6	<8	<8	16	0.088 (-)
7	8	8	16	0.089 (-)
8	<8	<8	64	0.121 (-)
9	<8	<8	64	0.124 (-)
10	<8	16	16	0.117 (-)
11	<8	<8	128	0.117 (-)
12	<8	<8	128	0.120 (-)
13	<8	<8	64	0.063 (-)

Where the Micro-IF methods showed negatives to *C pneumoniae* and positives to *C trachomatis*, *ELEGANCE C pneumoniae* method also showed samples to be negative.

C pneumoniae IgG ELISA: Crossreactivity Determination (Mouse model)



Cross-
reactivity

C psittaci: Approx. 25%

C trachomatis: Approx. 3%