“Life is an endless series of experiments.”
MATERIAL & METHODS:

A total of 223 patients of Acute Coronary syndrome (ACS) were taken up for the study. Out of them, 18 patients were not considered because of their incomplete data. Therefore the study population consisted of 205 patients of ACS.

All these patients were admitted in Intensive Coronary Care Unit of Surat Municipal Institute of Medical Education and Research (SMIMER) and Maskati Hospital, Surat. Recruitment of patients began in January-2002, and the study was completed in June 2006.
Fig.14 Showing Trial profile

Trial Profile:

223 Patients enrolled
18 - patients Data incomplete
205 - study patients

151
ST elevation
Myocardial Infarction

54
Unstable Angina
&
Non ST elevation
Myocardial Infarction

64
(42.4%)
Seronegative

87
(57.6%)
Seropositive

33
(61.0%)
Seronegative

21
(39.0%)
Seropositive

108
Seropositive

100
Seropositive

60 Azithromycin
40 Placebo

8 Withdrawn
(Need for antibiotics 3
CABG 1
PTCA 2
Stroke 2)

Fig.14 reveals profile of patients and design of the present study.
These 205 patients comprised of two groups of patients.

I. The acute ST elevation acute myocardial infarction (AMI) group consisted of 151 patients; admitted because of AMI.

II. The non-ST elevation myocardial infarction group consisted of 54 patients of Unstable Angina (UA) or Non-Q wave myocardial infarction.

**Inclusion criteria in ST elevation AMI group:**

- Presence of typical clinical manifestations of myocardial infarction.
- Onset of symptoms within 36 hours before admission.
- Verification of AMI by typical changes in ECG eg.
  - ST elevation of ≥ 0.2 mV in leads V1-V3 or 0.1 mV in other leads.
  - ST segment depression consistent with posterior MI.
  - Established MI defined as presence of Q waves of ≥ 0.3 sec. in leads V1-V6 or II, aVL, aVF.
- Raised levels of CPK-MB, Troponin T or Troponin I.

**Inclusion criteria in non – ST elevation myocardial infarction group:**

Patients of unstable angina met the criteria for type III b of Braunwald’s classification for unstable angina. In addition to the absence of clear cut electrocardiographic and cardiac enzyme changes diagnostic of a myocardial infarction, these patients had one
or more episodes of acute onset angina at rest, lasting at least for 10 minutes within
the preceding 48 hours.

**Exclusion criteria in both groups:**

- NYHA functional class III or IV congestive heart failure.
- Left bundle branch block.
- Hepatic or Renal failure.
- Chronic bronchitis
- Chronic macrolide or tetracycline or quinolone use.
- Contraindication to macrolide therapy.

Additionally, 90 healthy relatives of routine patients attending Maskati Hospital served
as Control group. These subjects were age and sex matched to the patients in the two
study groups. None of the controls had any symptoms or signs of coronary heart
disease (CHD) as judged by a negative history and a normal resting ECG. All subjects
were free of any chronic disease.

Baseline history and physical examination were completed in all patients. Venous
blood samples were drawn for Chlamydia pneumoniae serological tests. Fasting
venous blood samples were also drawn for measurement of plasma fibrinogen, C-
reactive protein (CRP); glucose; and complete lipid profile (serum cholesterol,
triglycerides, HDL, LDL and VLDL). Total leukocyte count was also estimated.

All 205 patients and 90 controls were screened for serum IgG antibodies against
C.pneumoniae by ELISA. Basis of antigen preparation were HEP-2 cells infected with
the "CDC / CWL – 029 " strain of C. pneumoniae. The ELISA test kit manufactured by M/S EUROIMMUN AG, Germany was used in the present study.

Additionally, 100 patients in the study group were simultaneously also subjected to Indirect Immunofluorescence test to determine IgG antibody titers against C. pneumoniae. Cells (EU 38) infected with C. pneumoniae were the standard substrate as basis of antigen. The kit for Indirect Immunofluorescence Test manufactured by M/S EUROIMMUN AG, Germany was used in the present study.

The sensitivity, specifically, positive predictive value, negative predictive value and the diagnostic accuracy of ELISA as compared to Indirect Immunofluorescence Test were compared in these 100 patients, in whom IgG antibodies were estimated using both the tests.

Based on the results of serological tests for C. pneumoniae, these 205 patients were stratified into two groups: Seropositive and Seronegative.

Anti-Chlamydia pneumoniae antibody titers were remeasured after 3 months in seropositive group to confirm stable elevation. Seropositive patients were entered in placebo controlled study to assess the effects of azithromycin antibiotic therapy on anti C. pneumoniae antibody titer.

Starting from first day of admission onwards, azithromycin, a specific antibiotic against C. pneumoniae, was administered as 500 mg in a single dose, one hour before or two
hours after meals, for 3 days. The anti C.pneumoniae antibody titers were again tested after 6 months.

Only those patients who were seropositive initially, and who showed stable elevation of antibody titer at the end of 3 months were finally considered for evaluation of the effect of azithromycin in seropositive patients.

Each patient in the study group, whether seropositive or seronegative, was followed up regularly for 6 months.

Adverse cardiovascular events like cardiovascular death, resuscitated cardiac arrest, nonfatal myocardial infarction or stroke unstable angina requiring hospitalization, and unplanned urgent coronary intervention in the form of coronary angioplasty or coronary artery bypass graft surgery, were closely monitored for 6 months from the original C.pneumoniae titer determination. The information was obtained from patients hospital visits, telephone inquiries, postal communication, personal follow up visits, case notes and hospital records. The occurrence of such adverse events was compared between

1) Seropositive and seronegative groups.

2) Azithromycin and Placebo treated patients.

SEROLOGICAL TESTS FOR C.pneumoniae:

[A] ELISA

All 205 patients and 90 controls were screened for serum IgG antibodies against C.pneumoniae by ELISA. Basis of antigen preparation were HEP - 2 cells infected
with the 'CDC / CWL – 029' strain of C.pneumoniae. The ELISA test kit manufactured by M/s EUROIMMUN Ag Germany was used in the present study. The test was carried out as per the details and instruction supplied by the manufacturers.

**Principles of the Test**

The ELISA test kit provides a quantitative in vitro assay for human antibodies of the IgG class against Chlamydia pneumoniae in serum or plasma. The test kit contains microtiter strips each with eight break-off reagent wells coated with C.pneumoniae antigens. In the first reaction step, diluted patient samples are incubated with the wells. In the case of positive samples, specific IgG antibodies (also IgA & IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme – labeled anti – human IgG (enzyme conjugate), which is capable of promoting a colour reaction.

**Contents of the test kit**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Component</th>
<th>Colour</th>
<th>Format</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Microplate wells</strong></td>
<td></td>
<td>12 X 8</td>
</tr>
<tr>
<td></td>
<td>Coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><strong>Calibrator – 1</strong></td>
<td>dark red</td>
<td>1X2.0ml</td>
</tr>
<tr>
<td></td>
<td>200 RU / ml (IgG, human), ready for use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><strong>Calibrator – 2</strong></td>
<td>red</td>
<td>1X2.0ml</td>
</tr>
<tr>
<td></td>
<td>20 RU / ml (IgG, human), ready for use</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Component</td>
<td>Color</td>
<td>Volume</td>
</tr>
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<td>---</td>
<td>----------------------------------------</td>
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</tr>
<tr>
<td>4</td>
<td>Calibrator - 3</td>
<td>light red</td>
<td>1X2.0ml</td>
</tr>
<tr>
<td></td>
<td>2 RU / ml (IgG, human), ready for use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Positive control</td>
<td>blue</td>
<td>1X2.0ml</td>
</tr>
<tr>
<td></td>
<td>(IgG, human) ready for use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Negative control</td>
<td>green</td>
<td>1X2.0ml</td>
</tr>
<tr>
<td></td>
<td>(IgG, human) ready for use</td>
<td></td>
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<tr>
<td>7</td>
<td>Enzyme conjugate</td>
<td>green</td>
<td>1X12ml</td>
</tr>
<tr>
<td></td>
<td>Peroxidase - labelled anti – human IgG (rabbit), ready for use</td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>Sample buffer</td>
<td>light blue</td>
<td>1X100ml</td>
</tr>
<tr>
<td></td>
<td>ready for use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Wash buffer</td>
<td>colourless</td>
<td>1X100ml</td>
</tr>
<tr>
<td></td>
<td>10x concentrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Chromogen / substrate solution</td>
<td>colourless</td>
<td>1X12ml</td>
</tr>
<tr>
<td></td>
<td>TMB / H₂O₂, ready for use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Stop solution</td>
<td>colourless</td>
<td>1X12ml</td>
</tr>
<tr>
<td></td>
<td>0.5 M sulfuric acid, ready for use</td>
<td></td>
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</tbody>
</table>

**Storage and Stability:**

The test kit has to be stored at a temperature between +2° C to +8° C, do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

**Preparation and Stability of the Reagents:**

All reagents must be brought to room temperature(+18° C to +25° C) around 30 min. before use. After first use, the reagents are stable until the indicated expiry date if stored at +2° C to +8° C, and protected from contamination.
Coated wells | Ready for use.
---|---
Calibrators & Controls | Ready for use. Reagents must be mixed thoroughly before use.
Enzyme conjugate | Ready for use. Must be mixed thoroughly before use.
Sample buffer | Ready for use.
Wash buffer | Wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to 37°C & mix well before diluting. The quantity required should be removed from the bottle using a clean pipette, and dilute with deionized or distilled water (1 part reagent plus 9 parts distilled water). The ready to use diluted wash buffer is stable for 1 month when stored at +2°C to +8°C and handled properly.
Chromogen / substrate solution | Ready for use. Close the bottle after use immediately, as the contents are sensitive to light. The chromogen / substrate solution must be clear on use. Do not use the solution if it is blue coloured.
Stop solution | Ready for use.

Preparation and Stability of the serum or plasma samples:

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted 1: 101 in sample buffer. Mix well by vortexing.
Calibrators and controls are pre-diluted and ready for use, do not dilute them.

**Incubation:**

**Sample incubation:**

Transfer 100 μl calibrators, positive & negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for 30 min. at room temperature (+18° C to +25° C).

**Washing:**

Empty the wells and subsequently wash 3 times using 300 μl of working strength wash buffer for each wash. Leave the wash buffer in each well for 30 – 60 sec. per washing cycle, then empty the wells. After washing thoroughly dispose of all liquid from the micro plate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

**Conjugate incubation:**

Pipette 100 μl of enzyme conjugate (peroxidase-labeled anti-human IgG) into each of the microplate wells. Incubate for 30 min. at room temperature (+18° C to +25° C).

**Washing:**

Empty the wells. Wash as described earlier

**Substrate incubation:**

Pipette 100 μl of chromogen / substrate solution into each of the microplate wells. Incubate for 15 min. at
room temperature (+18° C to +25° C)(Protect from direct sunlight).

Stopping the reaction: Pipette 100 μl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen / substrate solution was introduced.

Measurement: Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength of between 620 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring slightly shake the microplate to ensure a homogenous distribution of the solution.

Fig. 15 showing pipetting protocol for microtiter strips:

<table>
<thead>
<tr>
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<th>12</th>
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<tbody>
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<td>A</td>
<td>C</td>
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<td>P</td>
<td>4</td>
<td>P</td>
<td>12</td>
<td>P</td>
<td>20</td>
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<tr>
<td>B</td>
<td>C</td>
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<td>P</td>
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<tr>
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<td>C</td>
<td>3</td>
<td>P</td>
<td>6</td>
<td>P</td>
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<td>P</td>
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<tr>
<td>D</td>
<td></td>
<td>Pos.</td>
<td>P</td>
<td>7</td>
<td>P</td>
<td>15</td>
<td>P</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>E</td>
<td>Neg.</td>
<td>P</td>
<td>8</td>
<td>P</td>
<td>16</td>
<td>P</td>
<td>24</td>
<td></td>
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<tr>
<td>F</td>
<td>P</td>
<td>1</td>
<td>P</td>
<td>9</td>
<td>P</td>
<td>17</td>
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<tr>
<td>G</td>
<td>P</td>
<td>2</td>
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<td>P</td>
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<td>H</td>
<td>P</td>
<td>3</td>
<td>P</td>
<td>11</td>
<td>P</td>
<td>19</td>
<td></td>
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<td></td>
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</tbody>
</table>
Fig. 15 shows the pipetting protocol for microtiter strips for quantitative analysis of 24 patients samples (P1 to P24).

**Calculation of results:**

**Quantitative:**

The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point to point plotting of the extinction values measured for the 3 calibrators against the corresponding units (linear / linear). Use 'point to point' plotting for calculation of the standard curve by computer. The following plot (Fig. 16) is an example of a typical calibrator curve.

Fig. 16 showing typical calibrator curve.

If the extinction of a serum sample lies above the value of calibrator 1 (200 RU / ml), the result should be given as > 200 RU / ml.
The upper limit of the reference range of non-infected persons (cut-off) recommended by EUROIMMUN is 20 relative units (RU / ml).

**Test characteristics:**

**Calibration:**

As no international reference serum exists for antibodies against Chlamydia pneumoniae, the calibration is performed in relative units (RU).

For every group of tests performed, the extinction values of the calibrators and the relative units and / or ratio determined for the positive and negative controls must lie within the limits state for the relevant test kit lot.

The activity of the enzyme used is temperature dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature during substrate incubation, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

**Antigen:**

Basis of antigen preparation were HEp – 2 cells infected with the ‘CDC / CWL – 029’ strain of C.pneumoniae. Elementary bodies purified from cell lysates were treated with sodium dodecylsulfate. The used sodium contains all relevant antigens localized in the outer membranes of the elementary bodies. The outer membrane is composed of
lipopolysaccharides (LPS) and numerous proteins (outer membrane proteins, OMPs). The main portion is provided by the MOMP antigen (major outer membrane protein).

**Linearity:**

The linearity of the test was investigated by assaying serial dilution of patient sample, with high antibody concentrations. The Anti – Chlamydia pneumoniae ELISA (IgG) is linear in the measurement range of 2 – 200 RU / ml.

**Detection limit:**

The detection limit is defined as a value of three times the standard deviation of an analyte – free sample and is the smallest detectable antibody titre. The detection limit of the Anti – Chlamydia pneumoniae ELISA (IgG) is approximately 1 RU / ml.

**Cross reactivity:**

The three Chalmydia species C.trachomatis, C.psittaci and C.pneumoniae are very similar. For this reason, antibodies against C.trachomatis and C.psittaci almost always show cross reaction with the LPS as well as the MOMP antigen of C.pneumoniae. Thus, an exclusive determination of species antibodies against C.pneumoniae is not possible with the currently available test systems. No cross reactions with other antibodies have been found.
Reproducibility:

The manufacturer has investigated reproducibility of the test. The reproducibility of the test was investigated by determining the intra and inter assay coefficient of variation (CARDIOVASCULAR) using 3 sera with values at different points on the calibration curve. The intra assay CVs are based on 20 determinations and the inter assay CVs on 4 determinations performed on 6 different days (Table 17).

Table 17 showing Intra-Assay-Variation and Inter-Assay Variation.

<table>
<thead>
<tr>
<th>Intra - Assay - Variation, n = 20</th>
<th>Inter - Assay - Variation, n = 4 X 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Mean value (RU / ml)</td>
</tr>
<tr>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>113</td>
</tr>
</tbody>
</table>

Specificity and Sensitivity:

Samples from 54 patients (origin: Europe) were investigated using the EUROIMMN anti Chlamydia pneumoniae ELISA (IgG) and a microimmunofluorescence test (IF) as a reference method. 30 from 35 sera found positive in MIF and 15 from 15 sera found negative in M1 correspond with ELISA results.

Reference range:

The levels of the anti Chlamydia pneumoniae antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 300 healthy blood donors by the manufacturer. With a cut-off of 20 RU / ml, 65.8% of the blood donors were anti-Chlamydia pneumoniae positive (IgG) which reflects the known percentage of infections in adults.
INDIRECT IMMUNOFLUORESCENCE TEST:

The test was carried out as per details and instructions supplied by the manufacturers.

Test principle:

The indirect immunofluorescence test is a standardized in vitro assay for the determination of specific antibodies against C.pneumoniae. Cells infected with the bacterium are fixed as BIOCHIPs into the reaction fields of a microscope slide. With EUROIMMUN BIOCHIP Mosaics, different substrates can be positioned next to each other in one reaction field and incubated with one serum sample, allowing a detailed patient antibody profile which can be established with a single test.

Antigen:

Cells (EU 38) infected with Chlamydia pneumoniae are the standard substrate used for the determination of antibodies to Chlamydia pneumoniae. The infected cells are mixed with non-infected cells to enable a direct comparison between positive and negative reactions.

Test procedure:

EUROIMMUN BIOCHIP slides are incubated using the proprietary TITERPLANE Technique (Fig. 17 ), which enables multiple samples to be incubated next to each other and simultaneously under identical conditions. Results are evaluated by fluroscence microscopy. Incubation of the substrate with the positive and negative control sera provided in each kit verifies correct performance of the test and aids evaluation.
Fig. 17 showing TITERPLANE Technique

Pipettor:
- 10 µl per field (3 x 2 mm)
- 25 µl per field (5 x 5 mm)
- 70 µl per field (7 x 5 mm)

Incubate: 30 min

Wash:
- 1 s flush
- 5 min cuvette

Diffused samples
Samples are applied to the reaction fields of a reagent tray. The BIOCHIPs slides are then placed in to the recesses of the reagent tray, where all BIOCHIPs of the slides come into contact with the fluids, and the individual reactions commence simultaneously. Position and height of the droplets are exactly defined by the geometry of the system. As the fluids are confined to a closed space, there is no need to use a conventional "humidity chamber".
Preparation of reagents, serum and plasma samples:

The slides, Fluorescein-labeled secondary antibody, positive and negative controls and embedding medium have been supplied ready for use. As far as PBS – Tween is concerned; one pack of "Salt for PBS" should be dissolved in 1 litre of distilled water and mixed with 2 ml of Tween 20. The prepared PBS-Tween can be stored at +2 °C to +8 °C, generally for 1 week. PBS-Tween should not be used if the solution becomes cloudy or contamination appears. Reaction fields of the reagent tray must be hydrophilic and surrounding area hydrophobic.

Qualitative evaluation:

The sample to be investigated is diluted 1:100 in PBS – Tween. For example. Dilute 10.1 μl sample in 1000 μl PBS – Tween and mix thoroughly, e.g. vortex for 4 seconds.

Apply 25 μl of diluted sample to each reaction field of the reagent tray, avoiding air bubbles. Transfer all samples to be tested before starting the incubation. Use a polystyrene pipetting template.

Start reactions by fitting the BIOCHIP Slides in to the corresponding recesses of the reagent tray. Ensure that each sample makes contact with its BIOCHIP and that the individual samples do not come in to contact with each other. Incubate for 30 min at room temperature (+18 °C to +25 °C).

Rinse the BIOCHIP Slides with a flush of PBS-Tween using a beaker and immerse them immediately afterwards in a cuvette containing PBS-Tween for at least 5 min. Shake with a rotary shaker.
Apply 20 μl of Fluorescein-labelled anti-human globulin to each reaction field of a clean reagent tray. Add all droplets before continuing incubation. Use a stepper pipette. The labeled anti-human serum should be mixed with a pipette before use. To save time, conjugate can be pipetted onto separate reagent trays during the incubation with the diluted sample.

Remove one BIOCHIP Slide from cuvette. Within 5 seconds blot only the back and the long sides with a paper towel and immediately put the BIOCHIP Slide into the recesses of the reagent tray. Do not dry the areas between the reaction fields. Check for correct contact between the BIOCHIPs and liquids. Then continue with the next BIOCHIP Slide. From now on, protect the slides from direct sunlight. **Incubate for 30 minutes** at room temperature (+18°C to +25°C).

Rinse the BIOCHIP Slides with a flush of PBS-Tween using a beaker and put them into a cuvette with PBS-Tween for at least 5 minutes. Shake with a rotary shaker. **10 drops** of Evans Blue for each 150 ml phosphate buffer can be added for counterstaining.

**Place embedding medium** onto a cover glass – drops of 10 μl per reaction field. Use a polystyrene embedding template. Remove one BIOCHIP Slide from PBS-Tween and dry the back, all four sides, as well as the surface around, but not between the reaction fields with a paper towel. Put the BIOCHIP Slide, with the BIOCHIPs facing downwards, onto the prepared cover glass. Check immediately that the cover glass is properly fitted into the recesses of the slide. Correct the position if necessary.
Immunofluorescence patterns:

Positive reaction: Only the infected cells (30-50% of the total cells) fluoresce, while non-infected cells are negative. Fluorescence is observed in the inclusion bodies in the cytoplasm. These contain elementary bodies (infectious form of Chlamydia, 300 nm in diameter) and reticular bodies (non-infectious form of Chlamydia, 1000 nm in diameter). Free elementary and reticular bodies lying between the cells can also react. (Fig. 18)

Fig. 18 showing positive immunofluorescence reaction

If a patient's sample contains antibodies against Chlamydia pneumoniae, the same pattern is essentially obtained as for the positive control serum.

Negative reaction: No specific fluorescence is observed in the cells. (Fig. 19)
Antibodies against autoantigens:
If all cells in view are clearly fluorescing, this can indicate the presence of autoantibodies against components of the cell nucleus (ANA) or cytoplasm. The fluorescence pattern of antibodies against C. pneumoniae must be differentiated from these patterns:

Autoantibodies against cell nuclei (ANA):
Cell nuclei of all infected and non-infected cells fluoresce. A homogeneous pattern is seen. (Fig. 20)
Fig. 20 showing autoantibodies against cell nuclei (ANA)

Autoantibodies against mitochondria (AMA): Granular fluorescence in the cytoplasm of all infected and non-infected cells is observed. (Fig. 21)
If the positive control shows no specific fluorescence, pattern or the negative control shows a clear specific fluorescence the results are not to be used and the test is to be repeated.

**Inter lot variation:**

Slides from at least 10 different anti-Chlamydia pneumoniae IIFT kit lots were incubated with 10 different positive and negative control sera. The deviation amounted to no more than ± 1 fluorescence intensity level.
**Reference range:**
Sera from 200 healthy blood donors were investigated for anti-Chlamydia pneumoniae antibodies using the EUROIMMUN IIFT. With a cut-off titer of 1:100,111 (55.5%) of the blood donors were positive (IgG), in agreement with the known level of immunity in adults.

**Analytical specificity:**
No cross reactions were observed on investigation of various substrates (viruses, other bacterial species, autoantigens). There was no interference from haemolytic or lipaemic sera, or sera from patients with jaundice. The lipopolysaccharide coats of all three Chlamydia species are very similar. Therefore, antibodies against C.psittaci and C.trachomatis often show reactions with C.pneumoniae.

**Evaluation data:**
Sera from 46 patients with a suspected C. pneumoniae infection, 36 patients with a diagnosed C.trachomatis infection and 18 healthy blood donors were investigated for antibodies against C. pneumoniae using the EUROIMMUN indirect immunofluorescence test (IIFT) and a reference microimmunofluorescence test (MIFT) by the manufacturers. The sensitivity of the EUROIMMUN IIFT with respect to the reference MIFT amounted to 98%, and the positive predictive value (PPV) was 99%. Investigation of a panel of blood donors (n=99) yielded a specificity of 100% for the EUROIMMUN IIFT (Table 18).
Table 18 showing comparison between IIFT and MIFT.

<table>
<thead>
<tr>
<th>N =100</th>
<th>Reference test system Anti-C.pneu. MIFT (IgG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mixed panel)</td>
<td>positive</td>
</tr>
<tr>
<td>EUROIMMUN Anti-C.pneu. IIFT (IgG)</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>negative</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N =99</th>
<th>Reference test system Anti-C.pneu. MIFT (IgG)</th>
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</thead>
<tbody>
<tr>
<td>(blood donors)</td>
<td>positive</td>
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<tr>
<td>EUROIMMUN Anti-C.pneu. IIFT (IgG)</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>negative</td>
</tr>
</tbody>
</table>

Fibrinogen concentrations were measures by Clauss clotting assay using FibriPrest Kit, which was manufactured by Diaqnostica Stag.Co.

C-reactive protein (RP) was measured on the principle of the latex agglutination assay described by Singer and Plotz. The major advantage of this method is the rapid two minute reaction time. The TRANS-SERO kit employed for CRP estimation in the present study was manufactured by Transgeniks C.

**Statistical considerations:**

Statistical analysis was done through SPSS software version (10.0)

Descriptive analysis was calculated by comparing mean + SD for continuous variables and percentages for categorical variables.
Bivariate and multivariate analysis were conducted by using logistic method through Wald statistics, Chi square and also OR, to compare individual variable with outcome variable. Those variables at P value 0.1 are included in the multivariate model.

The value of \( P < 0.05 \) was considered as significant.

For comparison of ELISA and indirect immunofluorescence tests, agreement analysis was done using Kappa statistics.
<table>
<thead>
<tr>
<th><strong>PROFORMA</strong></th>
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<td><strong>Name</strong></td>
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**Complications**
- Cardiovascular death
- Myocardial Infarction
- Hospitalization for unstable angina
- Recurrent angina
- PTCA
- CABG
- Stroke
- Respiratory infection
- Urinary tract infection