CHAPTER 1

INTRODUCTION

Chlamydia are Gram-negative, obligate intracellular bacteria in the family Chlamydiaceae, genus Chlamydia of which current classification provides for only 3 species, namely C. trachomatis, C. psittaci (1, 2, 3, 4, 5) and the new, C. pneumoniae (6) (Diagram 1 : Taxonomy)

Diagram 1 Taxonomy of Chlamydia

Order : Chlamydiales
Family : Chlamydiaceae
Genus : Chlamydia
Species : Chlamydia trachomatis
C. psittci
C. pneumoniae

Chlamydia trachomatis ranks as one of the major cause of bacterial infection afflicting mankind, an estimate of 500 million to 1 billion individuals being affected worldwide (7, 8). Strains of C. trachomatis are serotyped by micro-immunofluorescence into 15 serotypes. Serotypes A, B, Ba, C are trachoma serotypes, usually associated with endemic trachoma causing blindness (2). Serotype D, E, F, G, H, I, J, K are oculogenital serotypes, causing genital tract infections known as sexually transmitted diseases of which the clinical syndromes are similar to those caused by N. gonorrheae. Moreover, these types can cause a variety of other significant complications, for example, epididymitis and infertility in man, cervicitis, salpingitis,

pelvic inflamatory disease (PID) and infertility in woman (9, 10, 11, 12, 13, 14, 15, 16). In addition, infants from cervical infected mothers may develop inclusion conjunctivitis or pneumonia (17, 18, 19, 20, 21, 22, 23). Serotypes L_1, L_2, L_3 are causative agent of lymphogranuloma venereum diseases. (1, 2,24, 25). Clinical signigicance of various C. trachomatis serotypes were summarized in Table 4 p 15.

Chlamydia trachomatis is now recognized as the most prevalent and are among the most damaging agents of all sexually transmitted diseases (STD) in the United State and Westhern countries (7, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35). An estimate of 3-4 million Americans suffered from a chlamydial infection each year (36). Current studies showed that up to 50% of non gonococcal urethritis (NGU) were attributed to C. trachomatis (26, 37, 38, 39). Men with NGU or non-specific urethritis (NSU) from such infection will be very important spreaders of C. trachomatis to other person especially their partners.

One of the most important and pressing problems of C. trachomatis infection in sexually transmitted disease are ascribed to the serious complications in untreated patients (36, 40). Nevertheless, many patients remain untreated (41) since asymptomatic infection occuring frequently in both sexes may remain unknown for months (15, 18, 42). Thus early detection and prompt treatment are the crucial factors for controlling the diseases. Heretofore efforts to control the spread of C. trachomatis have not succeeded because of the diagnostic problem. Several laboratory tests have been set up to aid the diagnosis but avilable method are laborious, expensive and time comsuming (32, 41, 43).

The definitive and most reliable diagnosis of chlamydial infection is the cultivation of the organisms in cell culture.

This is the standard method of proving chlamydial infection with high specificity (7, 15, 18, 27, 44). However, it is expensive, time-consuming, difficult to perform and requires special laboratory equipment and trained personnel (4, 7, 28).

An alternative method for definitive diagnosis is the direct demonstration of specific antigens in inflamatory exudate using monoclonal antibody staining. It is a method of high sensitivity and specificity nevertheless requires expensive reagents and equipment, for instance, monoclonal antibody, fluorescent microscope and trained specialist (7, 15, 18, 26, 27, 28, 43, 44, 45, 46)

The detection of specific antibodies in patients' sera can be indirectly used to support the diagnosis of infection (47), and there had been reports that chlamydial IgG antibodies at titer of 1:32 or above was significantly correlated with isolation results (48). Nevertheless, the standard procedure of micro-immunofluorescent test for IgG, IgM and IgA antibodies detections both in serum and local secretion, is likewise a very complex method requiring antigen preparation, a fluorescent microscope and well-trained workers (49, 50, 51).

As an alternative to these methods, much effort has been devoted to the development of a rapid, simple and inexpensive test for the diagnosis of infections caused by this organism (4, 32). Recently, rapid immunoperoxidase assay, a simple method for specific IgG, IgM or IgA antibody detection, has been reported by Gerna et al. (52, 53, 54, 55) and was subsequently used to identify C. trachomatis specific antibodies both in serum and secretion with sensitivity and specificity comparable to the standard method of micro-immuno-fluorescent test (29, 30, 55, 56, 57, 58).

In addition, several studies suggested that the presence of secretory IgA antibody was closely related to chlamydial infections (47, 58, 106). Terho and Meurman suggested that the absence of local IgA antibodies was a strong indication against actual chlamydial infection (37). This was supported by Darougar who provided evidence suggesting that the presence of specific IgG or IgA antibody at any level in local discharges were useful indications for the provisional diagnosis of infection (47).

In Thailand, C. trachomatis was found to be the most prevalent causing agent of non-specific urethritis (48, 60). While culture technique is time-consuming, complicated and is only available in some laboratories, serology may be helpful in diagnosis for infection. The rapid immunoperoxidase in some laboratories still has not been evaluated. It is our purpose to examine this technique for identifying secretory IgA antibody and its application for diagnosis in non-specific urethritis patients.

Research objectives.

1. To evaluate the rapid immunoperoxidase assay for detecting Chlamydia trachomatis antibodies as compare with the standard method, micro-immunofluorescence test.
2. To find the relationship of specific secretory IgA antibody by rapid immunoperoxidase with chlamydial infection.
3. To investigate the incidence of serum and secretory chlamydial antibodies in non-specific urethritis patients.

LITERATURE REVIEW

History of C. trachomatis.

Chlamydia trachomatis inclusions were first described

in conjunctival smears from patients suffering from trachoma in 1907 by Harberstaedter and Prowazek (61). In 1911, Linder et al. (62) demonstrated similar inclusions in ophthalmia of the newborns and in the urethral and cervical material from their parents. Later on, Bedson et al (63) had made the first isolation of chlamydial agent from patients with psittacosis in 1930.

In 1946, Wall (64) reported that C. trachomatis could be grown in the yolk sac of hen's embryonated eggs. This agent was first isolated in 1957 from the eye of hyperendemic trachoma by Tang et al. (65) and subseguently in 1959 from the genital tract and the eye in paratrachoma, by Jones et al (66). Accordingly etiological relationships of the organisms were proved and the organisms were referred to as "TRIC agent" to indicate their origin from either trachoma (TR) or paratrachoma including inclusion conjunctivitis (IC).

McCoy cells were originally used as normal replicating cells for isolation of C. trachomatis by Gordon et al in 1963 (67). It was originally shown that irradiation of McCoy cells leading to nonreplication improved the efficiency of chlamyidal isolation (68). Since then the original protocol has been modified. The growth of C. trachomatis in cycloheximide treated McCoy cells becomes one of the standard tissue culture methods for C. trachomatis isolation and the most reliable means of diagnosing infection with this organism (69).

The serological methods have been developed to detect antibodies to C. trachomatis. In 1935, Bedson (70) reported the first use of a complement fixation test for detecting antibody in psittacosis patient. The test was useful for diagnosing LGV but of little value for other oculogenital C. trachomatis infections because only a small proportion of the patients

developed CF titers and these titers are usually low (47, 64, 71).

In 1970 Wang and Grayston (72) reported the use of micro-immunofluorescent test (m-IF) which permitted quantitation of antibodies directed against the specific antigenic type. The elementary bodies of C. trachomatis was used as antigen in this sensitive and specific method (71, 73, 74). Later, in 1971, enzyme-linked immunosorbent assay (ELISA), originally described by Engvall and Prelmann (75) and Van Weeman and Schuurs (76) has been used to detect antibodies to chlamydia. In 1980 enzyme linked fluorescence immunuassay (ELFA) using a fluorogenic substrate has also been used in detecting chlamydial antibodies (71).

BIOLOGY, CLASSITICATON AND CLINICAL FEATURES

1. Characteristics of Chlamydia.

Chlamydia are gram negative bacteria that lack the ability to synthesize high energy compounds such as adenosine triphosphste (ATP) and guanosine triphosphate (GTP). These compounds, essential for metabolism and respiration, must be provided for by the infected host cells and were thus called "energy parasites" (77). This is also attributed to their lack of cytochromes and other components of the respiratory electron chain (78).

Accordingly Chlamydia were obligate intracellular bacteria, with unique developing cycle. They replicate by binary-fission within the cytoplasm of host cells, forming characteristic intra-cellular inclusions under a light microscope (2). Although Chlamydia are classified as bacteria, they share properties with viruses and bacteria. For instance, Chlamydia grow only intracellularly like viruses and the cultivation of C.

trachomatis can only be done by cell culture. However, like bacteria, they contain both DNA and RNA, divide by binary fission, possess cell wall similar to those of gram negative bacteria and are susceptible to many-broad spectrum antibiotics (2, 36, 78).

The compared properties of Chlamydia and other organisms was shown in Table 1 (79, 80, 81).

Table 1 Comparison of characteristics of C. trachomatis with other microorganisms*

Properties	Bacteria	Mycoplasma	Chlamydiae	Viruses
Cell wall	rigid wall muramic acid	membrane lipid	wall muramic acid	– (protein)
Free living	+	+	–	–
Size	0.2–0.5 u	0.25–0.5 u	0.3–0.7 u	23–300 nm
Nucleic acid	DNA+RNA	DNA+RNA	DNA+RNA	DNA or RNA
Reproduction	binary fission	budding	binary fission	eclipse
Cultivation	artificial media	artificial media	cell	cell
Sensitive to interferon	–	–	+	+
Sensitive to antibiotics	+	+	+	–

* Adapted from (79, 80, 81)

2. Cellular morphology and structure

Chlamydia are spherical non motile gram negative bacteria, 0.2–2 micron in diameter depending on their stage of development in a unique obligatory intracellular growth cycle. (78, 82)

The structure of chlamydia is unique. They posses no peptidoglycan layer therefore their regidity does not depend on the presence of muramic acid. It appears that a single protein, the major outer membrane protein, is important in maintaining structural rigidity (83). This specific protein makes up ~30-40% of the weight of the organism and approximately 60% of the weight of the cell membrane. It is ~39,000-45,000 Kdal and contains antigens of species, serogroup and serovar specificity (84).

3. Antigens of Chlamydia trachomatis

Chlamydia is classified as a gram negative bacteria, containing both an inner and an outer membrane, with a periplasmic space. It differs from gram negative bacteria in the absence of peptidoglycan (83). The rigidity of the chlamydial cell wall is possibly attributed to the intramembrane disulfide bridging rather than to the convalent bonding of the cell wall to the peptidoglycan layer (83).

Chlamydial lipopolysaccharide (LPS) is similar to the LPS of gram negative bacteria (85) and is found associated with the outer membrane (86).

Various antigens are present on the surface of C. trachomatis as follows (87, 88).

3.1 Genus or group specific antigens

These are lipopolysaccharide and glycolipid antigens sharing determinants common to all chlamydia and present on both elementary bodies and reticulate bodies.

3.2 Species-specific antigens

These are heat-labile proteins expressed on the outer membrane with molecular weight ranged from 40 Kdal to

approximately 155 Kdal. These antigens differentiate C. trachomatis from C. psittaci by the reactivity of antiserum against these antigens of all 15 serotypes of C. trachomatis without reactivity with C. psittaci. These species-specific protein antigens constitute almost 10% of the outer membrane hence it was called the major outer membrane protein (MOMP).(83)

3.3 Type specific antigens

These are proteins associated with the MOMP range in molecular weight from 30 Kdal to 40 Kdal. They are heat-stable, trypsin sensitive, non lipid components (89). With the use of micro-immunofluorescence devised by Wang and Grayston (49) as an immunotyping test, 15 serotypes of C. trachomatis has been identified.

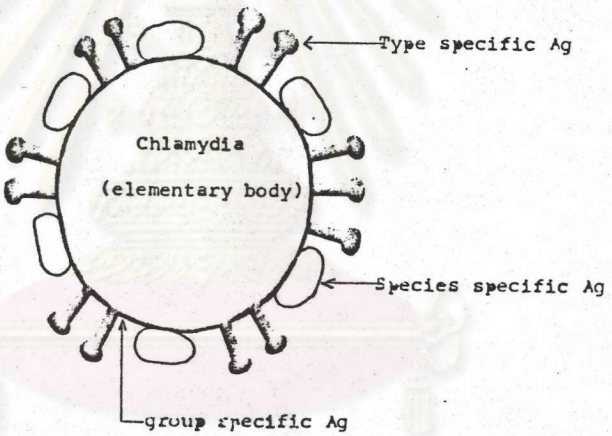

Figure 1 Antigens of C. trachomatis (90)

4. Genome

Chlamydia contain both DNA and RNA, the DNA of which is circular with an estimated molecular mass of 660×10^6 daltons (660 Kdal) (91, 92, 93). The genome consists of $6-11 \times 10^5$ base pairs which is approximately half the size of Neisseria or Rickettsia

DNA and less than one fourth of Escherichia coli DNA. On the other hand it is comparable to Mycoplasma, and it is larger than viral DNA (3-5 times that of the Pox virus or bacteriophage T_4) (79, 94)

The intraspecies DNA reassociation homology amoung different C. trachomatis strains was estimated to be 96-97% (95). In addition, C. trachomatis has been reported to contain a plasmid with a molecular mass of 4.4 MDal (96, 97). This dense DNA core is surrounded by a trilaminar cytoplasmic membrane, external to which is a trilaminar outer envelope some 9-10 nm thick (78).

5. Taxonomy

Taxonomy of Chlamydia was shown in diagram 1.

Although the intraspecies DNA homology among different C. trachomatis strains was high, the homology between the two species of C. trachomatis and C. psittaci is only 10% as determined by DNA hybridization (95). The G-C content (G+C)% of C. trachomatis and C. psittaci are 44.4% and 41.2% respectively (98).

C. trachomatis and C. psittaci are related by their common developmental cycle, common antigens and similar biological and metabolic activity, justifying their inclusion within the same genus (78). However, there are differences, for example, sensitivity to sulfonamide and production of an iodine-staining glycogen like material within the inclusion vacuole in C. trachomatis (2).

Summary of their characteristic differences are presented in table 2.

Table 2 Features distinguishing between C. trachomatis and C. psittaci (78, 99)

Features	C. trachomatis	C. psittaci
Principal host (S)	Humans	Nonprimate vertibrate
Inclusions	Compact glycogen - containing, stain with iodine.	Diffuse non-glycogen-containing, Do not stain with iodine.
Laboratory growth	With exception of LGV, agents require centrifugation on to special prepared tissue culture cells	Grow readily in tissue cluture without centrifuga-tion or special cell treatment
(Guanine+Cytosine)% of DNA	44	41
Sulfonamide susceptibility	Sensitive	Resistant

6. Growth cycle

As mentioned above, Chlamydia are obligatory intracellular bacteria because of their inability to synthesize high energy compounds such as adenosine triphosphate (ATP) and guanosene triphosphate (GTP) essential for metabolism and respiration. These compounds must be provided for by the infected host cells. Survival is ensured by a unique development cycle (1, 8, 100) in which two morphologically distinct forms are involved.

6.1 Elementary body (EB) is a small, dense, spherical bacteria particle (0.25-0.35 micron in diameter) (2, 8). It is relatively resistant to environmental factors, being the form transmitted from cell to cell or from host to host and is not

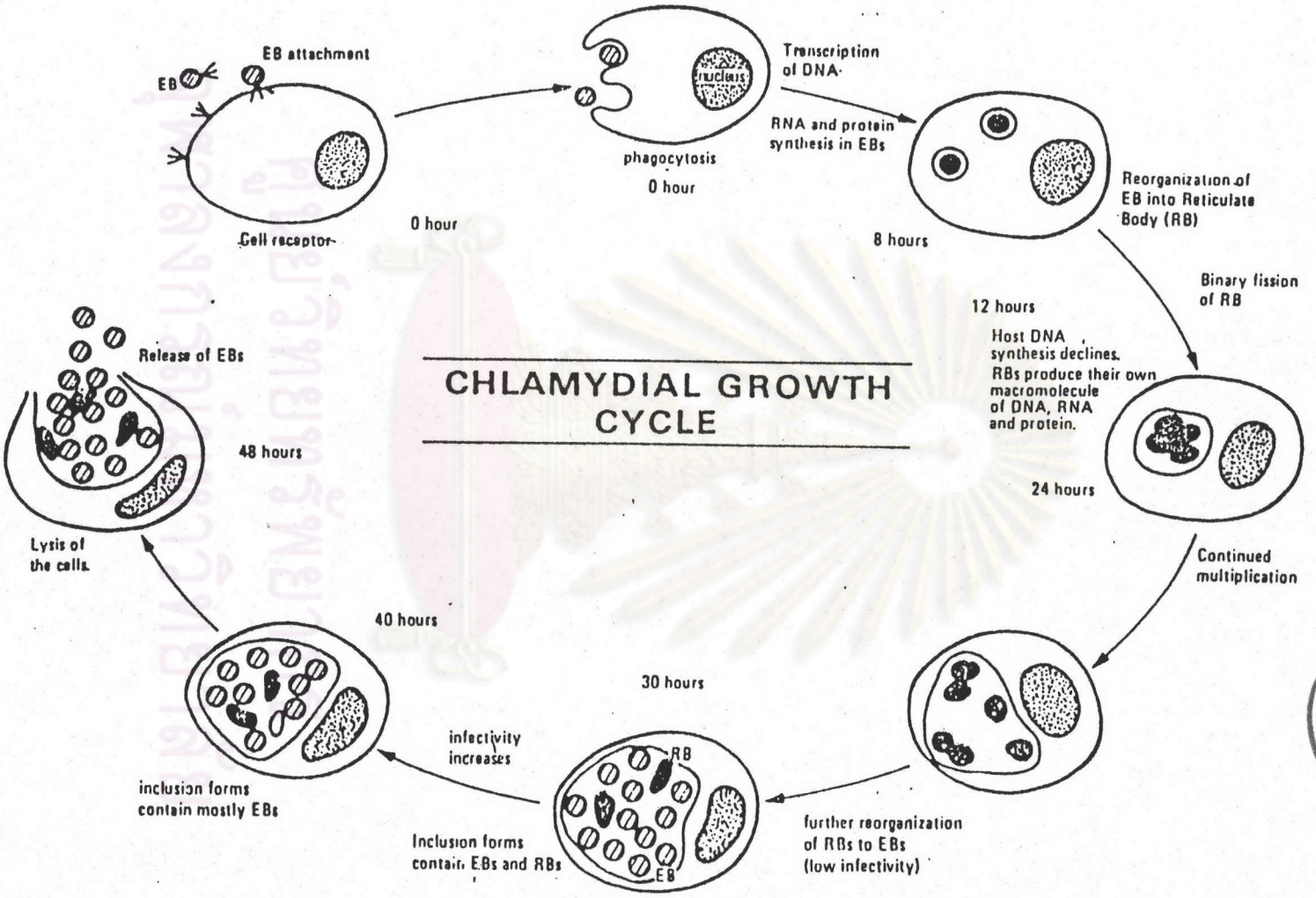

Figure 2 Chlamydial growth cycle. EB,elementary body RB,reticulate body. (105)

metabolically active (1). It is surrounded by a rigid trilaminar cell envelope similar in composition to those of other gram-negative bacteria but is highly specialized for its prime purpose of protecting the EB from the extracellular environment during transmission from cell to cell making the EB an extracellular infectious particle (78, 101).

6.2 Reticulate body (RB) or initial body is an intracellular form which is relatively fragile but is much larger and with RNA richer than EB (0.5-1 micron) (2). The RB form is metabolically active and divides by binary fission (1), it is concerned solely with the multiplication of the chlamydial population within the infected cell (78).

The RB is surrounded by a trilaminar cell envelope that is so fragile and flexible that pleomorphism results (101). The main features of the structure of EB and RB are summarized in table 3.

Table 3 Characteristics of Elementary body (EB) and Reticulate body (RB) (78, 101)

Characteristics	EB	RB
Morphology	Small, electron-dense core, rigid	Large, pleomorphic gram-negative
Size	0.2-0.3 u	1.0 u
Metabolic activity	Inactive	Active
Infectivity	+	-
Toxicity	+	-
Sonication	Resistant	Sensitive
Effect of trypsin	Resistant	Sensitive

Table 3 (continue)

Characteristics	EB	RB
RNA:DNA	1:1	3:1
Hemagglutinin	Present in some strains	Absent
Permeability	Slightly	Marked
Envelope subunit	Present in some strains	Absent

Once infection occurs, the EB adheres closely to the host cell membrane, perhaps to specific sites and then it is ingested by a phagocytic process which seem to be induced by the EB itself making the normally nonphagocytic host cell phagocytic (98). The organism enters the phagosome of a cells and can remain there for the entire growth cycle (103). It appears to be capable of inhibiting phagosomal fusion, enabling it to avoid normal defense mechanisms of the host cell (1).

Within the first hours of infection, the EB undergoes a morphologic change, becoming a large RB particle that is RNA richer and is metabolically active (1, 2).

After approximately 8 hours, the initial body begins dividing by binary fission. The host cell pool of precursors were utilized to synthesize chlamydial RNA, DNA and protein (104).

Approximately 18 to 24 hours after infection, the RB undergo a condensation process and reorganization, during which they become EB (1, 2), the number of EB increases and appears to predominate. Both EB and RB are found in the inclusion.

Multiplication continues until 48-72 hours after

infection. The mature inclusion may contain up to 10,000 chlamydial particles, which were then release outside to initiate another cycle of infection via cell lysis (8, 78)

7. Clinical Significance of C. trachomatis

The clinical significance of C. trachomatis was listed in table 4.

Table 4 Serotypes and clinical spectrum of C. trachomatis infection (36, 24, 25, 105)

Serotypes	Host	Infection	Complications
L_1, L_2, L_3	Women, Men	Lymphogranuloma venereum	Vulva/rectal carcinoma
A, B, Ba, C	Men, Women, Children	Trachoma (Endemic blinding trachoma)	Blindness
D, E, F, G, H, I J, K	Men	non specific Urethritis, Postgonococcal urethritis, Subclinical conjunctivitis	Epididymitis, Prostatitis, Reiter's syndrome, sterility
D, E, F, G, H, I J, K	Women	Cervicitis, non specific Urethritis, Subclinical conjunctivitis	Salpingitis, Perihepatitis, Sterility, Dysplasia Postpartum endometritis, Prematurity, Still birth, Neonatal death

Table 4 (continue)

Serotypes	Host	Infection	Complication
D,E,F,G,H,I J,K	Infants	Oculogenital infection (inclusion conjunctivitis), Pneumonia, Asymptomatic gastrointestinal tract carriage, Otitis media	

8. Pathologic Features and Epidemiology

Chlamydia trachomatis is essentially a pathogen of mucosal surface, infecting and replication within epithelial cells. Chlamydial infection transmitted to a new host cell is not limited to the lower genital tract but may occur at other anatomic site, such as the eyes or respiratory tract (eg. in neonates born to mother with cervical infections), oropharynx or rectum (24, 25, 106)

9. C. trachomatis infection in Non-specific urethritis patients.

Chlamydial urethritis in men is generally mild and asymptomatic infections are common (47, 106) with an incubation period from one to three weeks. After contacted the disease, patients notice urethral irritation, dysuria and mucoid or clear discharge with occassional mucopurulent discharge (106).

One of the most serious problems, other than

complication listed above is that untreated asymtomatic patients will become the reservoirs of the organism for transmission to contact partners. Harrison et al indicated that the prevalence of infection in pregnant women has ranged from 2% to 25% (23, 107). Moreover, many studies revealed that infants born through an infected birth canal are likely to become infected (22) among which the risk of inclusion conjunctivitis range from 18% to 50%, and pneumonia from 11% to 18% (108, 109). Overall 30-50% of infants exposed to infected maternal secretions will develop symptoms (21).

10. Immune response to C. trachomatis infection in the genital tract.

The immune system of the host becomes stimulated after the organisms propagate within and numerous cells were infected. Polymorphonuclear cells would infuse into the infected area (87). Since infections occur at the mucosal surface, secretory antibodies (local antibodies) as well as serum antibodies to C. trachomatis are produced. In addition the cell-mediated response would also occur (80, 87, 110, 111).

Chlamydial infection in experimental animals are usually self-limiting indicating that host immune response can eliminate chlamydia from the genital tract (106). In 1982, Rank et al (112) revealed that treatment of guinea pig inclusion conjunctivitis with estradiol increased the duration and intensity of disease. Since estradiol suppressed local antibody response without effecting the serum antibodies nor cell mediated immune response. This strongly indicates that local antibody response was an important element in the resolution of the diseases. On the other hand, in infected animals treated with antithymocyte serum suppressing cell-mediated immunity, the disease persisted despite the presence of antibodies in

both genital tract secretion and serum (13). These results show that elimination of the guinea pig inclusion conjunctivitis agent from the genital tract requires both a humoral and cellular immune response.

11. Treatment of Chlamydial infections

Tetracycline and erythromycin are routinely used in treatment against C. trachomatis. As for tetracycline, 250 mg 6 hourly for 2 weeks has been suggested. Dosage for erythromycin base is 250 mg 6 hourly, or 500 mg of erythromycin stearate twice daily, for two weeks (106).

12. Laboratory Diagnosis

The diagnosis of C. trachomatis infection is difficult in asymptomatic patients or those with non specific symptoms (7, 47, 106). Accurate defining of infected patients is therefore essential. It helps clinicians to make accurate diagnosis and institute proper treatment and follow up (39). It provides health service such as informed counseling and encouraged treatment of contacts since this may be the best method to prevent complications (39).

Consequently laboratory diagnosis of chlamydial infection becomes necessary for treatment, prevention and control of this disease. Heretofore, two main approaches have been developed ie isolation of infectious agents, antigens detection and specific antibodies tests.

I. Isolation of infectious organisms or antigens detections:
I.1 Cultivation of Chlamydia (114)

This is the definite laboratory diagnosis for chlamydial infection, (7, 15, 18, 27, 36, 44), the most popular technique being inoculation of clinical specimens onto cycloheximide-

treated McCoy cells (114). The basic principle involves centrifugation of the inoculum onto the monolayer cell at 300 g for 1 hours. incubation at 37° C for 48 to 72 hrs followed by staining with iodine to detect the glycogen-positive (red-brown stained) inclusion bodies.

McCoy cells are originally human synovial cells previously used by Gordon as normal replicating cells for growth of chlamydia (115). The cells were later treated either to reduce the cellular metabolism or alter the surface charge (116). McCoy cell line generally used nowadays in laboratories over the world are mouse fibroblast (117). Other cell lines, for instance, Hela 229 cells originated from human cervical cancer (118) and BHK-21 cells from baby hamster kidney (119) can also be used (36, 116).

The major advantage of culture is its high specificity (100%) (36), the sensitivity being approximately 80% (36). However there are certain disadvantages such as high cost, complexity of laboratory equipment, special methods required for collection, transportation and maintenance of specimens, time consumption (2-6 days) and problem of contamination (7, 15, 18, 27, 36, 44, 58).

I.2 Direct detection of chlamydial in smears or clinical specimens.

I.2.1 Giemsa staining (116)

This is the most popular method of cytologic identification of chlamydial infections from epithelial cell scrapings (eg. conjunctival, cervical, urethral). Giemsa staining is very useful for the diagnosis of ocular infection (116) which is identified by visualizing characteristic intracytoplasmic inclusions (2, 36). Generally the cell nuclei stains red, the cytoplasm blue, while an inclusion body being adjacent to the

cell nucleus, consists of blue-stained RB and purple-stained EB (116).

Giemsa staining is low cost and simple but it is time consuming and requires an experienced observer (116). In addition, its specificity and sensitivity are low (15-50%) (21, 36, 58), therefore this technique should be used only in laboratories in which culture are not available (21).

I.2.2 Direct immunofluorescence (DFA) (120)

This method has been widely used to detect chlamydiae because of its higher sensitivity over Giemsa staining and culture, the sensitivity and specificity being about 90% to 100% and 80% to 99% respectively (7, 15, 18, 27, 29, 40, 45, 100, (100, 120). It is easy and rapid but requires the use of expensive fluorescein-conjugated monoclonal antibodies, a fluorescent microscope, and trained microscopist for interpretation of the result (2, 58).q

I.2.3 Enzyme immuno assay (EIA) (64)

This method has been developed using anti-chlamydial antibody conjugated with horseradish peroxidase. The procedure is simple, rapid and the results determined objectively with a sensitivity and specificity of 67-90% and 92-98% respectively (4, 36, 41, 46, 64).

I.3 Nucleic acid spot hybridization (28, 91)

This method is recently developed in 1985 by Hyypia et al, utilizing a radiolabelled probe to detect C. trachomatis DNA from clinical specimen. The sensitivity and specificity of this method are very high nevertheless the technique is costly, complex and require special laboratory equipments.

II. Detection of chlamydial antibody.

II.1 Complement fixation test (CF) (70)

This test was first developed to detect antibodies against chlamydia in psittacosis (70) and later lymphogranuloma venereum desease. The test is sufficiently sensitive to identify antibodies in systemic infections such as LGV and psittacosis but the sensitivity is limited in localized chlamydial infections (121) such as ocular and genital infection (2, 47).

The CF test detects antibodies to a heat-stable, acid, polysaccharide antigen common to all chlamydia namely 2-keto-3 deoxyoctanoic acid (89). The serologic proof of diagnosis is based on the demonstration of a fourfold or greater rise in antibody titers between acute and convalescent phase in psittacosis. For LGV patients, the titer above 1:16 is considered significant while titer above 1:64 highly supports the clinical diagnosis. (2, 47)

II.2 Micro-immunofluorescence (m-IF) (50, 72)

This test was first developed by Wang and Grayston in 1970 (72) to type for strains of C. trachomatis but was soon adapted to detect chlamydial antibodies in 1974 in which purified chlamydial EB are used as antigens. Originally the whole range of C.trachomatis serotypes designated A, B, Ba, C, D, E, F, G, H, I, J, K, LGV_1, LGV_2 and LGV_3 was included (50). Later on the test has been simplified by pooling antigens for practical routine use. This could be done on the basis of serologic and epidemiologic or pathologic similarity as listed in table 5.

Table 5 Antigen pool for m-IF test of chlamydia

	Authors	Classification on	Antigen pools
1.	Wang and Grayoton, 1975 (122)	Serologic similarities	CJ, A, B, ED, L_1L_2, LL_3, GF, H, I
2.	Treharne et al, 1977 (73)	Epidemiologic, pathologic similarities	$Pool_1$ A,B,C $Pool_2$ D,E,F,G,H,I,J,K $Pool_3$ L_1,L_2,L_3 $Pool_4$ C. psittaci

The m-IF test detects type-specific chlamydial antibodies in whole blood, serum and local secretion or discharge. It is much more sensitive than CF and can identify specific IgG, IgM or IgA antibodies (2, 47, 50, 111). Unlike the CF, m-IF appears to be able to differentiate between infections by C. trachomatis and C. psittaci and can provide a serological diagnosis for the stage of disease (50). This has been widely used in epidemiologic and clinical studies of chlamydial infections such as LGV, trachoma and oculogenital infection. In addition it is also used for provisional diagnosis in some laboratories (47).

II.3 Enzyme-linked immunosorbent assay (111)

ELISA was first used to detect chlamydial antibodies by Lewis et al in 1976 (111). It detects group-specific IgG or IgM antichlamydail antibodies against whole EB (123) or extracted group-specific soluble antigens (124) but the methods are usually less sensitive than the m-IF test especially in measuring IgM antibody (2).

II.4 Radioimmunoassay (RIA) (37)

This test detects group-specific antibodies using C. trachomatis serotype L_2 as antigen (37). Its high sensitivity is comparable to that of the ELISA (47, 111), but the workers must be careful during the use of radioactive agent. Moreover the radioisotope has limited shelflife and the reagent was expensive.