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Target, Technique, and Tissue: A Triple Diagnostic Lens on Chlamydia Trachomatis Detection

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ABSTRACT

Background: One of the most common sexually transmitted bacterial diseases, Chlamydia trachomatis (CT), is a major contributor to female infertility and reproductive tract infections, especially in low-resource environments. Controlling its spread requires accurate and easily available diagnostic tools, but because of the distinct intracellular biology of CT, many traditional approaches are ineffective. With an emphasis on target antigens, methodological comparison with immunofluorescence, and the impact of sampling locations on diagnostic accuracy, this review attempts to assess the diagnostic capability of immunohistochemistry (IHC) in detecting Chlamydia trachomatis. ***Methods:*** Using databases including PubMed, Scopus, Web of Science, ProQuest, Google Scholar, and Google, a narrative literature review was carried out. Initially, titles and abstracts were used to pick articles, and keywords like "Immunohistochemistry," "Chlamydia trachomatis," and "diagnostic" were used. Reference lists of pertinent studies were also manually searched. Experiments and studies involving animals were not included. Studies using immunohistochemical methods and their contributions to CT diagnosis were the main focus of the final inclusion. ***Results:*** Immunohistochemistry (IHC) overcomes diagnostic obstacles brought on by the bacterium's internal location and absence of obvious symptoms by enabling the direct imaging of CT antigens, especially the Major Outer Membrane Protein (MOMP), within the cytoplasm of infected epithelial cells. IHC is appropriate for formalin-fixed paraffin-embedded (FFPE) tissue samples and can be used in retrospective or resource-constrained contexts because it does not require living organisms or intact nucleic acids, in contrast to culture or nucleic acid amplification tests (NAATs). ***Conclusion:*** Many traditional diagnostic methods are limited in their usefulness by Chlamydia trachomatis's intracellular origin, immune evasion mechanisms, and asymptomatic persistence. An option that makes biological sense and is useful is immunohistochemistry, especially in places with limited resources. Future diagnostic frameworks have to give preference to techniques such as immunohistochemistry (IHC) that complement the distinct pathophysiology of the organism while fostering advancements in antibody synthesis and integration with reasonably priced molecular platforms.

Keywords: reproductive health; sexually transmitted disease; Chlamydia trachomatis; immunohistochemistry diagnosis.

INTRODUCTION

The most common bacterial sexually transmitted infection (STI) in the world is Chlamydia trachomatis. The World Health Organization (WHO) estimates that 129 million new cases of genital Chlamydia trachomatis infection occurred worldwide in 2020, making it a serious public health concern, especially for young, sexually active people between the ages of 15 and 24 [WHO, 2024]. The majority of infections, particularly in women, remain asymptomatic despite their widespread prevalence, which leads to underdiagnosis and raises the risk of significant reproductive consequences such ectopic pregnancy, tubal infertility, and pelvic inflammatory disease (PID) (Paavonen & Eggert-Kruse, 1999a)(Paavonen & Eggert-Kruse, 1999b). CT infection is very common, especially in young, sexually active women. The frequency in the US was found to be 4.7 overall and 13.5 among non-Hispanic Black women (Torrone et al., 2014; Torrone & Papp, 2015). A meta-analysis conducted globally revealed a frequency of 3.1 in women and 2.6 in males (Huai et al., 2020). Additionally, over 75 percent of

CT infections in women are asymptomatic, while only about 50 percent of those in men exhibit symptoms.

Possessing a distinct biphasic life cycle, the bacterium is an obligatory intracellular Gram-negative pathogen that has two distinct forms: the infectious, extracellular elementary body (EB) and the replicative, intracellular reticulate body (RB). Its intricate life cycle, capacity to elude host immune responses, and ability to silently remain in the genital canal make diagnosis extremely difficult (Cebral, Jua; Mut & Weir, Jane; Putman, 2011) (Wolf et al., 2001)(Olsen et al., 2021).

Once thought to be the gold standard, conventional diagnostic techniques like cell culture are now rarely employed because of their low sensitivity (50–80%), labor intensity, and requirement for specialist equipment (Chan & Cunningham, 1994; Rodrigues et al., 2024). Although molecular techniques such as nucleic acid amplification tests (NAATs) have a better sensitivity (up to 100%) (Lallemand et al., 2016), their availability in low-resource settings is limited due to their high equipment costs and highly controlled laboratory facilities. Because of persistent antibodies, serological techniques like immunofluorescence assays (IFA) and enzyme-linked immunosorbent assays (ELISA) are unable to differentiate between active and previous infections (Dietrich et al., 2010). Additionally, histochemical stains like Giemsa are not sensitive enough or specific enough, especially in patients who are asymptomatic (John R. Papp, PhD1, Julius Schachter, PhD2, Charlotte A. Gaydos, DrPH3, and Barbara Van Der Pol, 2012). On the contrary, immunology-based techniques like as immunohistochemistry (IHC) and IFA enable the direct identification of CT antigens in host cells, particularly the Major Outer Membrane Protein (MOMP) (Wang et al., 2006). IHC offers greater practicality because it may be carried out on formalin-fixed, paraffin-embedded (FFPE) tissue and permits spatial localization of antigen expression, whereas IFA necessitates fluorescence microscopy. The detection of CT in cervical, testicular, and rectal tissues has been accomplished with success using IHC (Arévalo et al., 2022; He et al., 2024). Through the use of clear and specific visual staining, IHC enables the direct detection of CT antigens in apkinsor smears. This style has been applied in a number of situations, such as sexually transmitted proctitis and CT detection of cervical kerchief (Arévalo et al., 2022) (Oumarou Hama et al., 2022a). The method is therefore a potentially useful diagnostic tool, particularly in settings without molecular diagnostic equipment. IHC is a very helpful individual choice in labs with restricted molecular structure, in addition to being realistic and reasonably priced.

THE UNIQUE BIOLOGICAL CHARACTERISTICS AND DIAGNOSTIC IMPLICATIONS OF CHLAMYDIA TRACHOMATIS

Mandatory Intracellular Parasite Disease

Chlamydia trachomatis is an obligate intracellular pathogen, meaning it can only replicate within the epithelial cells of its host. This strict intracellular lifestyle prevents the bacterium from growing on standard artificial media, unlike most free-living bacteria. As a result, conventional microbiological tools such as Gram staining or aerobic cultures on media like blood agar and MacConkey are ineffective for its detection. Specialized cell culture techniques using living host cells (e.g., McCoy or HeLa cell lines) are required for CT isolation, which restricts access to diagnostics to reference labs with sophisticated virology equipment (Land et al., 2009).

Biphasic Developmental Cycle

The distinctive biphasic growth cycle of Chlamydia trachomatis makes detection more challenging. Two morphologically and functionally different forms are involved in this cycle: The elementary body (EB) is a tiny, infectious, metabolically dormant species that has evolved for extracellular survival and host cell penetration. Reticulate Body (RB): A bigger, metabolically active, non-infectious form that multiplies inside the host cell's membrane-bound inclusion (Grossman, 1982; AbdelRahman & Belland, 2005).

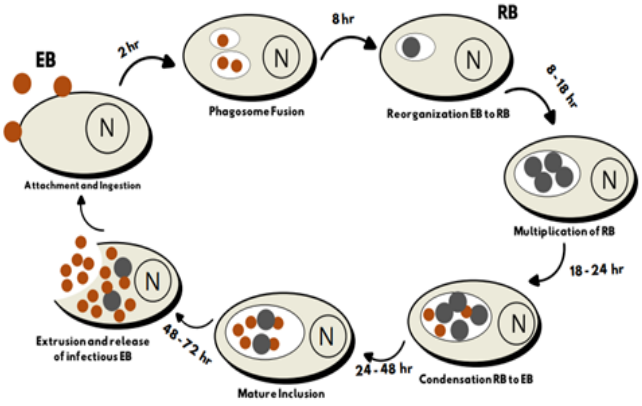

FIGURE 1: Life cycle of Chlamydia.
Within the first 2 h following internalization into cells, elementary bodies (EBs) fuse to form a nascent inclusion. Between 2 and 6 h postinternalization, EBs begin to differentiate into reticulate bodies (RBs). By 12 h postinfection (hpi), RBs can be observed dividing by binary fission, and by 18–24 hpi, they peak in numbers. Increasing numbers of RBs differentiate back to EBs around 24 hpi and continue differentiating until lysis or release occurs? 48–72 hpi depending on the chlamydial species.

The Major Outer Membrane Protein (MOMP), a species-specific and immunodominant antigen found in the EB's outer membrane, is the main target of diagnostic tests like IHC and IF (Luu et al., 2023) (Lundell et al., 1981). nevertheless, C. trachomatis frequently resists innate immune detection and recognition by traditional microbiological approaches because of its intracellular position, lack of peptidoglycan, and capacity to suppress host immune signals (Strauss & Strauss, 1994) (Wyrick, 2010). Accusation: These characteristics necessitate specific individual methods that either describe CT-specific facial antigens (like IHC or IFA) or nucleic acids (like NAATs). Therefore, when utilized to identify C. trachomatis, introduction staining (e.g., Giemsa) or

traditional culture-based styles sometimes have lower perceptivity and particularity.

Minimal Inflammatory Reaction of the Host

In inhibiting pro-inflammatory signaling and avoiding susceptible findings within addition bodies, C. trachomatis can evade strong vulnerable responses even when it causes long-term harm. Asymptomatic infections are often the result of the muted seditious hand (Wong et al., 2019). As a result, clinical judgment based solely on symptoms is incorrect, and serological indicators of acute infection (such as leukocytosis and C-reactive protein) might not be raised (Mouliou, 2023).

Absence of detectable antigens in the bloodstream

Chlamydia trachomatis is a hidden pathogen that often limits the expression of its antigen to intracellular spaces. During infection, it doesn't release a lot of antigens into the blood (Ahmed et al., 2016). This significantly lowers the range of blood-based antibody or antigen assays. False positives or negatives might result from antibody responses (such as IgG/IgM) that are often delayed, remain after infection ends, and exhibit cross-reactivity with different chlamydial species (Fox et al., 2022).

LIMITATIONS IN DIAGNOSTICS ORIGINATING IN CT'S BIOLOGY

Limitations of Culture Techniques

Conventional bacterial communities operate on synthetic, nutrient-rich medium. Nevertheless, Chlamydia trachomatis cannot develop outside of host cells due to its obligatory intracellular nature. (Bonnet et al., 2020). In fact, live epithelial host cells (such as McCoy or HeLa cells) are necessary for effective CT civilization in technical cell culture systems because they sustain the intracellular CT experimental cycle (Brown & Drexler, 2020). Specifically, designed settings for incubation, such as regulated temperature (35–37 °C), CO_2 monitoring, and antibiotic suppression of contaminating foliage. As the culture needs 48 to 72 hours and fluorescent antibody labeling to confirm additional bodies, professional laboratory technicians and longer reversal periods are required. Cell culture was once thought to be the gold standard, but it currently only exhibits moderate perceptivity (60–80), and because of its complexity and resource requirements, it is no longer feasible for routine opinion in the majority of clinical laboratories (Grossman, 1982).

High Resource Demands yet High Sensitivity in NAATs

NAATs are currently the gold standard for CT identification because, like Polymerase Chain Reaction (PCR) and Recap-intermediate Modification (TMA), they provide remarkable sensitivity and specificity by amplifying DNA or RNA sequences of Chlamydia trachomatis (CT) (Rahman et al., 2018). Even while this technique is far more delicate, it still has some significant drawbacks. The anatomical location of the infection in the towel is not shown by NAATs, but they do describe the presence of CT inheritable material. Because of this, opinions are sensitive in situations like gravidity or persistent seditious conditions (Coleman & Gaydos, 2018)(Ibanez-Escribano & Nogal-Ruiz, 2024). Perpetration of NAATs requires a laboratory with advanced molecular outfit similar as a thermocycler(PCR machine), high-quality reagents, and strict quality control procedures, which aren't available in numerous primary healthcare installations (Ibanez-Escribano & Nogal-Ruiz, 2024)(Rahman et al., 2018). In the molecular laboratory, cross-contamination is a common cause of false positive results. In the meantime, if the CT DNA is harmed or if there are obstructions in the case (such as blood, urine, or cervical mucus) that interfere with alteration, false negative results may occur. (Rodrigues et al., 2022).

Limitations of Conventional and Serological Staining Antibodies against CT are detected by serology (ELISA), which is ineffective for diagnosing ongoing infections since they can be elevated even after the infection has cleared up. Despite being inexpensive, Giemsa staining lacks specificity and is prone to false-negative results since CT elimination in asymptomatic individuals may be unclear or nonexistent (Aydin et al., 2025).

MOMP vs. Elementary Body (EB) as IHC Antibody Targets

The two primary targets of antibodies employed in immunohistochemistry (IHC) analysis to identify Chlamydia trachomatis (CT) are MOMP and EB. Monoclonal antibodies may readily recognize MOMP, a dominant antigen that reflects EB and RB and is stable in FFPE tissue. It offers high sensitivity and wide detection coverage across the course of the CT life cycle (Biologicals, 2016). For determining the sensitivity, specificity, and clinical significance of the diagnostic findings, the appropriate antigen target must be chosen (De Paschale, 2012; Safiia et al., 2024).

The primary structural protein in the cell wall of Chlamydia trachomatis is called Major Outer Membrane Protein (MOMP). It is expressed during both the Basic Body (EB) and Reticulate Body (RB) phases of the bacterial life cycle. MOMP is comparatively stable to formalin-fixed paraffin-embedded (FFPE) tissue fixation and processing, which makes it perfect for IHC analysis of clinical specimens (Jury et al., 2023). Benefits of Being an IHC Target (Redgrove & McLaughlin, 2014). The stability of it enables consistent findings on histopathology preparations, making it compatible with FFPE tissues. There are monoclonal antibodies that are both specific and efficient, enabling accurate result detection and replication between labs (Kokkat et al., 2013). Limitations: MOMP is less specific for active infections, even though it is extremely sensitive and can detect infections even when they are not active (Lalvani & Pareek, 2013; Tait et al., 2011). Features of the Elementary Body (EB): EB is the contagious type of C. trachomatis that manifests during the initial stages of infection. It is a particle that has the ability to adhere to and penetrate target epithelial cells. Is scarce and rapidly destroyed in FFPE tissue, particularly if the fixing duration is not

optimal (Baron., 1996)(Grygiel-Górniak & Folga, 2023). Benefits of Being an IHC Target: High specificity to active infection: The existence of a continuing infectious process is indicated by EB detection. Ideal for research on early pathophysiology or early infection transmission (Long, 2004). Restrictions: Low sensitivity: Antibodies to EB are frequently unable to identify infection, particularly in fixed tissues, because of their low levels and susceptibility to destruction. Unsuitable for regular screening: Their application in conventional diagnostic settings is limited by inconsistent detection rates (Tranchand-Bunel et al., 1999).

Technique Comparison: Immunofluorescence (IF) vs. Immunohistochemistry (IHC)

In identifying specific antigens, like Chlamydia trachomatis, two main methods are commonly used: immunohistochemistry (IHC) and immunofluorescence (IF) Oumarou Hama et al., 2022b). Despite sharing the fundamental idea of antibody binding to target antigens, there are notable distinctions between the two in terms of facility needs, sample type, sensitivity, and visibility (Myler et al., 2022). Basics of Visual Detection With IHC, an enzyme (such as horseradish peroxidase) initiates a chemical reaction that results in a brown hue (DAB) visible under a light microscope (Magaki et al., 2019). IF: Depends on fluorochromes, which can only be seen under a fluorescence microscope because they release fluorescent light when subjected to certain light (Cong et al., 2016). Staining Results' Stability IHC: Because staining is long-lasting and permanent, preparations can be inspected and stored without losing their visual quality (Magaki et al., 2019). IF: Fluorescence results are transient because they fade fast (photobleaching), particularly if improperly stored or exposed to too much light (Boudreau et al., 2016). Type of Sample and Preparedness IHC is appropriate for use on tissue that has been formalin-fixed paraffin-embedded (FFPE), a format frequently used in clinical pathology (Kokkat et al., 2013). IF: Offers the best results for fresh or cryosectioned samples because fluorescence may be hampered by fixing and embedding. Price and Availability of Facilities IHC: Can be carried out in many labs using common histologic pathology equipment, and is comparatively less expensive. IF: Only accessible at facilities with cutting-edge technology, it necessitates specific tools like fluorescence microscopes and dark rooms in addition to more costly dye ingredients (Liu et al., 2022). Potential Artifacts and Sensitivity IHC: Has great sensitivity, particularly when used in conjunction with protocol optimization techniques, including non-specific blocking and antigen retrieval (Magaki et al., 2019). IF: Usually more sensitive, but more likely to produce artifacts that might make interpretation more difficult, like autofluorescence and non-specific signals (Zhang & An, 2009). Clinical Use and Interpretation Simplicity IHC: Because it employs standard light microscopy and distinct contrast staining against the tissue background, it is simpler for pathologists to interpret (Gurcan et al., 2009). IF: Needs specific expertise because user experience and sensitivity to quickly fluctuating fluorescence levels are key factors in interpreting results (Grohmann et al., 2021).

TABLE 1: Comparison of IHC vs IF.

Aspects	Immunohistochemistry (IHC)	Immunofluorescence (IF)
Visual Detection	Chromogen dye (DAB), observed by light microscopy	Fluorochrome dyes, observed by fluorescence microscopy
Stability of Results	Stable and permanent	Fast fading (photobleaching)
Sample Type	Suitable for FFPE tissue	Optimal on fresh preparations/cryosection
Cost and Facilities	Cheaper, widely available	Expensive, limited and specialized facilities
Sensitivity	High (with optimization)	Very high, but prone to artifacts
Ease of Interpretation	Easy and commonly used in clinical practice	Difficult, requires expert training and interpretation

The diagnostic requirements, the resources at hand, and the examination's goal all influence the decision between IHC and IF. Because of its consistent results and simplicity of interpretation, IHC is recommended in standard clinical and pathological settings. On the other hand, IF is better suited for ultra-sensitive early infection diagnosis or study.

Comparing the Vagina/Urethra and Endocervix as Sample Sites

The choice of sampling site has a major influence on the precision of Chlamydia trachomatis (CT) detection, especially when using techniques like immunohistochemistry (IHC). Two often used sites are the endocervix and the urethra/vagina. Each has advantages and disadvantages depending on the tissue type, infection site, and infection phase (Bryan et al., 2019; L Falk 1, B-I Coble, P-A Mjörnberg & Affiliations, 2010).

Benefits of the Endocervix: Because the endocervical columnar epithelium is so vulnerable to pathogen invasion, it is a primary location of Chlamydia trachomatis tropism. Enables the diagnosis of current infections with excellent sensitivity, particularly in women with reproductive problems (Malhotra et al., 2013). In light of its target cell density and structural consistency, endocervical tissue is a good candidate for IHC analysis. Limitations include the intrusive nature of sample collection, the need for specific equipment (speculum), and the expertise of medical professionals. It is more challenging to conduct at facilities with limited resources and may cause discomfort for patients. (Screening and Treatment of Precancerous Lesions for Secondary Prevention of Cervical Cancer, n.d.).

Benefits of the Lateral Urethra and Vagina: less intrusive and more accessible, particularly in cases of external genital tract infections. Ideal for early illness detection or screening, even in groups with limited access to healthcare. Suitable for patients who decline cervical exams (Hickling et al., 2015). Limitations: Lower sensitivity compared to endocervical samples because they are less indicative of cervical or upper genital tract infections. Interpreting IHC results may be hampered by differences in squamous epithelial tissue and vaginal microbiota (Trifonova et al., 2014).

TABLE 2: Comparison Summary.

Location	Advantages	Disadvantages
Endocervix	CT main tropism (columnar epithelium), high sensitivity, suitable for IHC	Invasive, requires tools & skills
Urethra	Early detection, especially in external infection	Less representative of cervical infections

Particularly when assessing infertility issues, endocervical samples offer greater diagnostic accuracy and are better suited for IHC techniques. The sensitivity and representation of upper genital tract infections are compromised when using urethral /vaginal samples as a non-invasive substitute.

CONCLUSSION

The obligate intracellular pathogen Chlamydia trachomatis (CT) has distinct biological traits that make diagnosis more difficult, particularly because infections are frequently asymptomatic and invisible to standard techniques. According to this research, immunohistochemistry (IHC) is a clinically useful and physiologically sound diagnostic method, particularly in facilities with low funding.

The accuracy of the diagnosis is also greatly influenced by the selection of the target antibody (MOMP vs. Elementary Body), the detection method (IHC vs. IF), and the sampling location (endocervix vs. urethra/vagina). While EB is less stable and more specific for active infections, MOMP is more stable and sensitive for broad diagnosis. Compared to IF, which is more costly and complicated, IHC is better suited for normal practice. While urethral/vaginal samples can be a non-invasive option with poorer sensitivity, endocervical samples yield the best diagnostic results.

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