**CURRENT METHOD ON LABORATORY DIAGNOSIS OF CHLAMYDIA TRACHOMATIS INFECTION**

**SEMINAR**

**(MLS 501)**

**BY**

**OKOH AMARACHUKWU CYNTHIA**

**2018030185932**

**DEPARTMENT OF MEDICAL LABORATORY SCIENCE**

**ENUGU STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY**

**NOVEMBER, 2024.**

**CURRENT TREND ON LABORATORY DIAGNOSIS OF CHLAMYDIA TRACHOMATIS INFECTION**

**OKOH AMARACHUKWU CYNTHIA**

**PROF. NWOBODO H.A**

**(Supervisor)**

**DEPARTMENT OF MEDICAL LABORATORY SCIENCE, ENUGU STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY**

**A SEMINAR SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, ENUGU STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY**

**IN PARTIAL FUFILMENT FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE (B.MLS) DEGREE IN MEDICAL LABORATORY SCIENCE**

**NOVEMBER, 2024.**

**DECLARATION**

I, Okoh Amarachukwu Cynthia hereby declare that the seminar titled ‘Current Method on Laboratory Diagnosis Of Chlamydia Trachomatis Infection**’** is based on the original work carried out by me under the supervision of Prof. Nwobodo H.A in partial fulfillment of the requirements for the award of the Bachelor of Medical Laboratory Science degree at Enugu State University of Science and Technology.

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Okoh Amarachukwu Cynthia Date

**CERTIFICATION**

I hereby certify that the work recorded in this seminar emanated from the research carried out by Okoh Amarachukwu Cynthia of the department of Medical Laboratory Science, Enugu state University of Science and Technology and supervised by me.

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Prof Nwobodo H.A Date: November 2024

(Supervisor)

**DEDICATION**

This seminar is dedicated to my parents, whose unwavering support and love have been my guiding light. Your sacrifices have laid the groundwork for my success. To my siblings, thank you for your encouragement, your presence has made every challenge easier. Lastly, to my biggest supporter, your faith in me inspires me to strive for greatness every day.

**ACKNOWLEDGEMENT**

I want to extend my sincere gratitude to everyone who has been part of this project. Thank you to my supervisor Prof. Nwobodo H.A for his guidance and support. I am also thankful to my classmates for their collaboration. . A big thank you to my friends and my well-wishers your guidance and support. Thank you all for making this seminar a success.

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 **SUMMARY**

Lower genital tract infections with Chlamydia trachomatis are predominantly asymptomatic in men and women. Diagnostic technology has provided several approaches to the diagnosis of C trachomatis. Outside of cells, Chlamydia can die or degrade without optimal storage and transportation. Because some of the other assays perform better on certain specimen types, it is important for laboratories to recognize these differences and provide advice to physicians and nurses collecting patient specimens, with the objective of diagnosing lower genital tract infections to prevent transmission and upper tract damage. Most invasive specimens, such as cervical or urethral swabs, may be collected for culture, antigen or nucleic acid detection. Noninvasive samples such as first-void urine and vaginal swabs can be easily collected by the patient; these samples must be tested by more sensitive nucleic acid amplification tests. These newer investigative strategies should enable implementation of screening programs to identify and treat partners. Serology has not been particularly useful for the diagnosis of acute C trachomatis infections in adults. Presently, it appears that antibiotic-resistant C trachomatis is not a clinical problem. Laboratories providing C trachomatis diagnosis require participation in continuous quality improvement programs.

 **CHAPTER ONE**

 **INTRODUCTION**

**1.1 BACKGROUND OF STUDY**

*Chlamydia trachomatis* is a gram-negative obligate intracellular bacterium and is the leading cause of bacterial sexually transmitted infections (STIs) worldwide. It affects both men and women and is often asymptomatic, particularly in women, contributing significantly to widespread transmission. Because of its often silent nature, infected individuals often do not seek treatment, allowing the disease to spread unchecked. The World Health Organization (WHO) reports that more than 130 million new cases of *C. trachomatis* infection occur each year, primarily affecting adolescents and young adults under the age of 25, who are the largest demographic group at increased risk of transmission (World.Health Organization, 2024; Torrone *et al*., 2021).

 This widespread prevalence highlights the urgent need for effective surveillance and rapid diagnosis to manage and prevent further transmission. The bacteria are divided into several different serotypes, each associated with specific clinical manifestations. Serotypes D-K are responsible for the majority of urogenital infections in sexually active individuals, resulting in conditions such as cervicitis, urethritis, and proctitis. Serotypes A-C, associated with trachoma, are commonly found in areas with poor sanitation and limited access to health care, where they are a major cause of preventable blindness. Finally, L1-L3 serotypes are associated with lymphogranuloma venereum (LGV), a more serious STI that primarily affects men who have sex with men (MSM) in high-risk populations and has been increasingly recognized in Western countries in recent years (Centers for Disease Control and Prevention, 2023; Geisler, 2022).

 The clinical significance of *C.trachomatis* infection extends beyond immediate health impacts and extends to long-term reproductive complications. In women, untreated *C.trachomatis* infection can lead to serious consequences, including pelvic inflammatory disease (PID), chronic pelvic pain, infertility, and ectopic pregnancy, posing a significant burden to individual and public health (Brunham *et al.,* 2023; Haggerty *et al*., 2020). Studies indicate that up to 30% of *C.trachomatis* infections are untreated.In women, *C.trachomatis* can progress to pelvic inflammatory disease, which significantly increases the risk of infertility and other reproductive complications (Taylor *et al.,* 2021). In men, although less common, complications can include epididymitis and prostatitis, which can affect fertility and quality of life.

 Additionally, *C.trachomatis* infection can increase the risk of HIV acquisition, highlighting the urgent need for early intervention and comprehensive STI management strategies (CDC, 2023; Johnson *et al.,* 2019).

Laboratory diagnosis is essential to identify *C.trachomatis* infection, particularly because asymptomatic cases contribute to increased transmission rates. Accurate and rapid diagnosis is essential to prevent serious health consequences associated with untreated infection. Traditional diagnostic techniques, such as cell culture and antigen detection, have previously been used to test for *C.trachomatis*. However, these methods often lack the sensitivity to detect asymptomatic cases, leading to delayed diagnosis and subsequent transmission (WHO, 2024; Schachter *et al*., 2018).

 To address these limitations, more advanced diagnostic methods, including nucleic acid amplification tests (NAATs), have been developed and are now considered the gold standard. NAATs can detect *C.trachomatis* with high sensitivity and specificity by identifying the bacterial genetic material, making them very effective for early detection, even in asymptomatic individuals. These tests provide more accurate, faster, and less invasive diagnostic options, facilitating timely treatment and helping to reduce transmission rates. In addition, advances in point-of-care testing are making diagnosis accessible in resource-limited settings, supporting earlier detection and treatment in a variety of populations (Torrone *et al*., 2021; Van Der Pol *et al.,* 2021).

This seminar will explore current trends in the laboratory diagnosis of Chlamydia trachomatis, reviewing traditional and modern diagnostic techniques to better understand their role in the management and control of this common infection. By assessing advances in diagnosis, we can appreciate the importance of accurate laboratory testing in addressing the global burden of *C.trachomatis*.

**MAGNITUDE OF PROBLEM**

Globally, Chlamydia trachomatis is responsible for millions of new infections annually. The World Health Organization estimates that approximately 129 million new cases occur each year, with a high incidence among young adults and adolescents. Given that up to 70% of cases in women and around 50% in men can be asymptomatic, the need for effective diagnostic methods is essential to improve health outcomes and reduce the disease burden.

**AIM**

The aim of this seminar is to explore the laboratory diagnostic methods available for the detection of Chlamydia trachomatis

 **CHAPTER TWO**

**2.1 Epidemiology**

*Chlamydia trachomatis* infection is the most common bacterial STI worldwide, with approximately 129 million new cases yearly across various age groups and regions (Newman *et al.,* 2015). It primarily affects sexually active individuals aged 15 to 24 years, with the highest rates seen in adolescents and young adults due to biological and behavioral factors. Infection rates are particularly high in areas with limited access to sexual health services, highlighting disparities inhealthcare access and the need for large-scale screening programs (CDC, 2023). In North America and Europe, *C.trachomatis* is the leading cause of sexually transmitted bacterial infections. However, global data show alarming rates in sub-Saharan Africa, South Asia, and parts of Latin America, areas with limited resources for routine testing and treatment (Rowley *et al.,* 2019). Epidemiological studies have also shown gender differences in infection rates, with women having higher rates of infection due to asymptomatic progression and sociocultural barriers to accessing sexual health services.

 **2.1 LABORATORY DIAGNOSIS**

**2.1.1 Sample Collection**

 Effective diagnosis of Chlamydia trachomatis begins with appropriate sample collection, as the accuracy and sensitivity of diagnostic methods are highly dependent on the quality of the sample collected. Cervical and urethral swabs are commonly used, although other sampling sites such as rectal and pharyngeal may also be appropriate, particularly in sexually active patients. Recently, urine samples have emerged as a preferred non-invasive alternative, particularly in asymptomatic individuals and in settings where invasive sampling may preclude testing (Poljak *j*., 2023). Proper handling and transport of specimens to the laboratory is essential tomaintain the viability of *C.trachomatis*, especially when using culture methods, which can yield false-negative results if the sample is degraded. Training healthcare providers in optimal sampling techniques also helps prevent contamination and ensures greater diagnostic accuracy (Chernesky, 2020).

**2.2 Diagnostic Techniques for Chlamydia trachomatis**

Chlamydia testing is indicated in patients with urogenital, anorectal, and ocular symptoms, patients with STIs other than chlamydia, sexual contacts of people with STIs, and those referred for chlamydia screening [7].Diagnostic procedures for detecting CT infection include direct and indirect methods. In general, localized infections are tested by direct pathogen detection tests, type cultures, antigen tests (EIA, directfluorescent antibody (DFA), hybridization tests, and nucleic acid amplification tests. Indirect methods rely on the detection of antibodies against C. trachomatis, which can be applied to the diagnostic evaluation of chronic/invasive infections (PID, LGV) and post infectious complications, such as sexually acquired reactive arthritis (SARA). Under these conditions, the pathogen has passed through the epithelium and may no longer be detected in the swab. On the other hand, serology is not suitable for diagnosing acute infections of the lower genital tract and anus, since antibody responses are not detected until several weeks or months later and are often not usually prominent

**2.3 TRADITIONAL DIAGNOSTIC METHODS**

**2.3.1 CELL CULTURE**

Cell culture was considered the “gold standard” for detecting C.trachomatis prior to the development of NAAT17.The culture method was once considered the "gold standard" due to its high specificity, as it only identifies viable organisms, ensuring accurate identification of active infections. (Chernesky, 2020). During culture, a clinical sample (usually collected through swabs) is inoculated onto a monolayer of host cells, such as McCoy or HeLa cells and incubating for 48 to 72 hours, when infected cells developed characteristic intra-cytoplasmic inclusions containing primary and reticulosomes of C.trachomatis. These inclusions were detected by staining with a fluorescently conjugated monoclonal antibody specific for the major outer membrane protein (MOMP) of C.trachomatis17, 18. Cell culture has a specificity close to 100%. However, it is not recommended for routine use due to its lack of sensitivity, technical complexity, long turnaround time, requirement for sample transportation and storage, and limited availability of suitable samples [31,32]. Because only viable organisms can be detected, it remains the method of choice in forensic and for anti-biotic susceptibility testing [33].

**2.3.2 Direct Fluorescent Antibody (DFA) test:**

DFA testing involves staining smears of samples with fluorescently labeled antibodies that specifically bind to *C.trachomatis* basal bodies. The presence of fluorescent particles under fluorescence microscopy indicates infection. DFA testing has the advantage of being relatively rapid but requires highly trained personnel and specialized microscopy equipment. Although DFA testing can be effective, especially in symptomatic patients, its sensitivity is often lower than that of nucleic acid-based testing, which contributes to its limited utility in routine diagnostics (Palmer *et al*., 2019).

**2.3.3 Enzyme-linked immunosorbent assay (ELISA) Method**:

 The ELISA detects C.trachomatis antigens using specific antibody-antigen interactions, resulting in a spectrophotometrically measurable color change.The intensity of the color correlates with the amount of antigen present in the sample, providing an estimate of the bacterial load. The ELISA is relatively rapid, cost-effective, and suitable for high-throughput screening, which is particularly useful in resource-limited settings where more advanced techniques may not be available. The method also allows laboratories to process multiple samples simultaneously, simplifying workflow (Land *et al.,* 2012).The sensitivity and specificity of ELISA are moderate and can lead to false-positive results, often due to cross-reactivity with other bacterial antigens. Additionally, ELISA cannot distinguish between active and past infection, limiting its use in clinical diagnosis.

**Serological Testing**

Serological tests detect antibodies (IgG and IgM) against C. trachomatis in a blood sample. The presence of IgM usually indicates recent infection, while IgG suggests past exposure. Serological tests can provide epidemiological data, which are useful for understanding the spread of C. trachomatis in the community, especially in areas where more direct diagnostic methods are not available. Serological tests are not ideal for diagnosing active infections because the presence of antibodies can persist long after the initial infection, leading to potential misinterpretation. Individual variability in immune responses further complicates the accuracy of the test in detecting active infections (Gomes *et al.,* 2011).

**2.4 Current /emerging Diagnosis of *Chlamydia trachomatis***

**2.4.1 Nucleic acid amplification tests (NAATs):**

NAATs detect *C.trachomatis* DNA or RNA in samples by amplifying specific genetic sequences, usually by polymerase chain reaction (PCR). This approach allows for extremely low bacterial counts, making it very effective in detecting asymptomatic cases. NAATs are the most sensitive and specific diagnostic methods available, with the ability to detect active infections even at low bacterial counts. NAATs can be performed on invasive (swab) and noninvasive (urine) samples, making them versatile and accessible (Poljak *et al.,* 2023). Nucleic acid amplification testing (NAAT) requires specialized laboratory equipment, which can be expensive and difficult to access in resource-poor settings. Additionally, although NAATs can confirm the presence of *C.trachomatis* DNA or RNA, they cannot differentiate between viable and nonviable organisms, meaning that a positive result may not confirm an active infection (Land *et al.,* 2020).

**2.4.2 Point-of-Care (POC) Diagnostics**:

Point-of-care diagnostics are tests that can be performed at or near the patient's point of care, such as a clinic, hospital, or community health facility. They are designed to provide immediate results, allowing for rapid clinical decision-making. Point-of-care tests are typically performed by healthcare professionals. They can include a variety of tests, such as blood sugar tests, rapid antigen tests for infections (such as chlamydia or HIV), and other tests that do not require complex laboratory equipment. For example, Point-of-care testing for *Chlamydia trachomatis* may include a rapid antigen detection test or a nucleic acid amplification test that can provide results in a short period.

Self-testing kits are designed for individuals to perform their tests, often in the privacy of their own home. These kits are user-friendly and include all necessary documentation and clear instructions for use (Horner *et al.*, 2018). Self-testing allows individuals to test without the involvement of a healthcare professional, which can improve confidentiality and reduce the stigma often associated with STI testing. For example, Chlamydia trachomatis self-testing kits may include urine or swab tests that the user collects and sends to a laboratory for analysis, or use instant-result tests that provide rapid feedback (Garcia *et al.*, 2016).

**2.4.3 Next-generation sequencing (NGS)**

Thi**s** is an innovative method that promises to be a future for *C.trachomatis* research and diagnosis. NGS enables comprehensive genomic analysis of the bacteria, providing information on genetic diversity and potential antibiotic resistance mechanisms. Although NGS is not yet a standard diagnostic tool in clinical practice, it is valuable for understanding the epidemiology of chlamydial infections and monitoring the emergence of resistant strains (Horner *et al.,* 2018). The ability to sequence multiple samples simultaneously could improve our understanding of transmission dynamics in communities and facilitate outbreak investigations. As costs decrease and the technology becomes more accessible, NGS could eventually play a major role in routine diagnostics, especially in the context of public health surveillance.

 **CRISPR-based diagnostics**:

 CRISPR technology is at the forefront of advanced diagnostic approaches for infectious diseases, including *Chlamydia trachomatis*. CRISPR-based diagnostics exploit the specificity of the CRISPR-Cas system to detect the pathogen's target nucleic acid. This method allows for highly accurate detection of *C.trachomatis* in clinical samples, even at low concentrations. A notable advantage of CRISPR-based diagnostics is the potential for rapid testing and point-of-care applications. These systems can provide real-time results, which is essential for effective patient management and infection control. Additionally, the modular nature of CRISPR technology allows for customization to target specific strains or resistance markers, making it a versatile tool for future diagnostic development (Chen *et al*., 2019).

**CONCLUSION**

The laboratory diagnosis of *Chlamydia trachomatis* infection is a critical aspect of public health, enabling timely treatment and the prevention of serious complications such as infertility and pelvic inflammatory disease, especially among women. Over the years, various diagnostic methods have been employed, including traditional techniques such as culture, direct fluorescence antibody (DFA) testing, and enzyme immunoassays (EIAs), as well as modern methods like nucleic acid amplification tests (NAATs). Of these, NAATs have emerged as the gold standard due to their exceptional sensitivity and specificity in detecting *Chlamydia trachomatis*.

While NAATs are highly accurate, they require specialized equipment and trained personnel, making them less accessible in resource-limited settings. In contrast, rapid tests and EIAs, while quicker and easier to perform, have limitations in terms of sensitivity, potentially leading to false-negative results. Despite these challenges, rapid diagnostic tests remain valuable in certain contexts, particularly for initial screenings.

The increasing availability of advanced technologies, such as Next-Generation Sequencing (NGS) and CRISPR-based diagnostics, offers exciting prospects for the future of *Chlamydia trachomatis* detection. NGS allows for comprehensive analysis and detection of genetic variations, while CRISPR-based diagnostics offer high specificity and rapid results, potentially enabling point-of-care testing in resource-constrained environments. These innovations could significantly enhance diagnostic capabilities, providing a more accessible and accurate approach to detecting *Chlamydia trachomatis* globally.

In conclusion, while current diagnostic methods provide valuable tools for the detection of *Chlamydia trachomatis*, the integration of newer technologies and improvements in accessibility will be key to enhancing global diagnostic capacity, reducing transmission rates, and ultimately improving public health outcomes.

**RECOMMENDATION**

 **Wider Use of NAATs:** Expand the use of NAATs in clinical settings, especially for asymptomatic individuals and high-risk groups, while reducing costs and improving accessibility.

 **Incorporation of NGS and CRISPR:** Integrate Next-Generation Sequencing (NGS) and CRISPR-based diagnostic tools for more accurate, rapid, and cost-effective detection, particularly in research and specialized clinical settings.

 **Training and Capacity Building:** Provide training for healthcare workers and laboratory personnel to ensure accurate use of diagnostic methods and improve detection of asymptomatic cases.

**Routine Screening and Public Awareness:** Promote regular screening, particularly among sexually active individuals, and increase public awareness of available diagnostic methods to reduce transmission.

 **Ongoing Research and Innovation:** Support continued research into affordable diagnostic technologies, including point-of-care testing and new molecular tools, to improve global access to accurate diagnostics.

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