

Infectivity Assays For Chlamydia Trachomatis

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Citation

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Abstract

Chlamydia trachomatis (Ct) is an obligate intracellular bacteria that is leading cause of sexually transmitted infections and preventable blindness in the world. Many quantification and infectivity assay for Ct are used for research and clinical purposes—the oldest being inclusion counting at 24 hours post infection. With changing time and evolution in technology direct fluorescence microscopy and its modifications have been used in addition to flow cytometric analysis. Nucleic acid amplification is used for clinical detection and quantification of Ct. Electron microscopy can also be used for titration of Ct stocks. In the review we overview all the available methods for Ct infectivity assays and discuss their advantages and disadvantages.

INTRODUCTION

Chlamydia trachomatis (Ct) is an obligate intracellular prokaryotic parasite of eukaryotic cells. It is the leading cause of sexually transmitted infections and preventable blindness in the world^[1,2]. Due to their intracellular character, determining the pattern and quantification of infection is a complex challenge. In the biological study of Chlamydia, it is very important to quantify and know the titer (IFU/ml) of *C. trachomatis* for the study of the organism.

Quantification and titer of *C. trachomatis* is generally performed by infectivity titration in cell cultures or by physical counting techniques that rely on microscopic visualization of the *C. trachomatis* inclusion. The Elementary Body is the infectious stage of the bacterium ^[3] and, thus, relevant for both research and clinical laboratory settings.

REVIEW OF METHODS

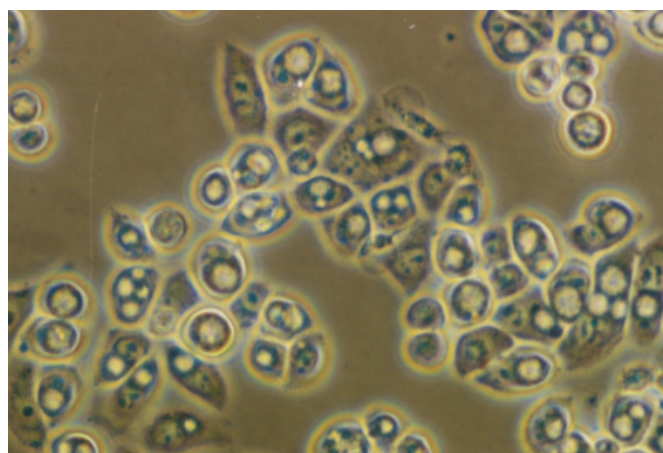
LIGHT MICROSCOPY

For many years researchers have exploited the ability to see Ct inclusions under light microscope for infectivity assay. The number of inclusions formed in host cells can be calculated with the naked eye under a microscope (Figure 1). Chlamydia EB stocks can be used to infect epithelial cells lines. As there is one-to-one relationship between EB and inclusion at low MOIs, the numbers of inclusions formed in the host cells are counted under light microscope in a given area. If the number of host cells is known, then the percentage of infection and infectivity titer of Chlamydia can

be calculated using serial dilutions. The precision and accuracy of this method, however, depends on the number of inclusions per field and the number of fields counted. ^[4]

Figure 1

Figure 1: Digital photographs taken with light microscopy. Hela Cells infected with Ct at 24 hours post infection



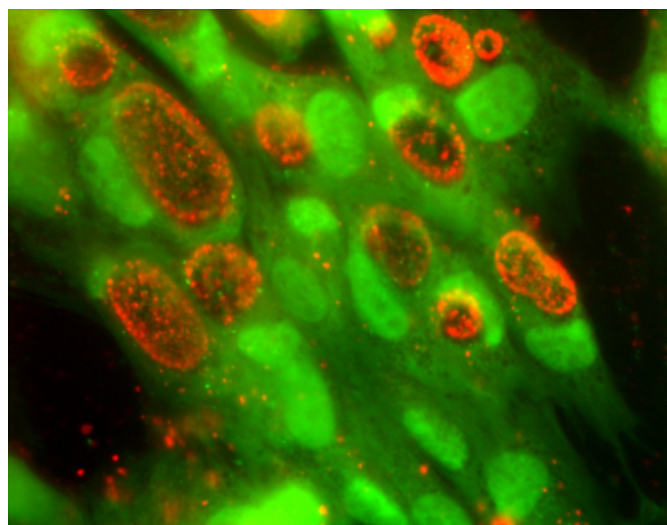
The main limitation of this method is that it can only be used for Chlamydia serovars which have visible inclusions under light microscope e.g Ct serovar E, so this method cannot be used for serovars which make smaller inclusions e.g. Serovar D. Moreover, inclusion counting cannot be carried out at earlier time points (<20 hours post infection) as the inclusions are too small to be seen under light microscope. Also sometime inclusions can be confused with host cell vacuoles leading to over estimation of infectivity titer. Misidentification of inclusions can result in significant discrepancies in the final result.

DIRECT IMMUNO FLUORESCENCE (DIF) ANTIBODY LABELING

Chlamydia specific FITC labeled Anti Lps can also be carried out to determine the number of cells infected with Ct. This method that has been used to quantify chlamydial infection since the early 70s. The inclusions can be visualized under fluorescent microscope and can be quantified and infectivity can be calculated by percentage of host cells infected in a given number of cells. [5] Figure 2

Figure 2

Figure 2 : DIF antibody labeling. Red indicates Ct , while in green are HeLa Cells



This method eliminates the major drawbacks of light microscopy; Ct with smaller inclusions can be assayed and vacuoles can be distinguished from inclusions. Non-specific fluorescence, background fluorescence and longer experiment time are major drawbacks of this method. In addition the precision and accuracy of this method, however, depends on the number of inclusions per field and the number of fields counted- similar to under light microscopy.

AUTOMATION OF DIF

To overcome the problem of limitation of the visual counting under light microscope and Fluorescent microscope Hori et al. in 2000 devised a simple and rapid method for counting a large number of inclusions using an image analysis system and proposed a sampling method based on a statistical analysis of the data obtained with 84 microscopic fields [4]. This technique also defines sampling methods for different MOIs.

DIRECT ENUMERATION BY ELECTRON

MICROSCOPY(EM)

In this method EB suspensions is mixed with equal volumes of latex nanospheres of known concentration. Samples were fixed to copper grids, stained with uranyl acetate, and examined under EM. Latex spheres and EBs were counted in several fields and the EB concentrations were calculated from the ratio of EBs to latex spheres. Percentage of infection can also be determined [6]. EM is very accurate method, but has potential limited availability. The processing of sample for EM is time consuming and expensive.

FLOW CYTOMETRIC ANALYSIS

Another tried and tested method is using cytometry for Ct infectivity assay and titration[7]. HeLa cells plated in monolayers are infected with serial dilutions of EBs and at 24 hours post infection, cells can be trypsinized and fixed, permeabilized and stained using FITC conjugated Ct specific anti LPS antibody Flow cytometry analysis gives a objective quantification of the infection based on fluorescent emitted by FITC conjugated antibody.

The main advantage of flow cytometric counting is that it is quantitative test and there is no user bias. Like any other technique, user-training can be important. A larger number of cells are needed for FACS analysis in addition to expensive equipment , technical for and high running costs.

NUCLEIC ACID AMPLIFICATION METHODS

Ligase chain reactions (LCR) have been recently reported for the quantification of Ct infection[8]. LCR conditions routinely used for C. trachomatis detection are designed to maximize sensitivity and do not provide quantitative information about the concentration of C. trachomatis. This method can be used for direct quantification from clinical specimen too. Variation in copy number for the plasmid containing the LCR target is also a potential source of error in this assay. Similarly Real Time-PCR can also be used for quantification and discrimination of Ct serovars[9]. Nucleic acid based quantification has primary application for clinical purpose- as the infected specimen can be directly used for LCR and for discriminating between serovars for epidemiological purposes.

DISCUSSION

The techniques discussed above are used for Chlamydia infectivity assay and titration. Each of them has their advantages and disadvantages. Depending on equipment, time and material availability, user can choose the method of

titration. Based on our experience, we suggest that light microscopy at 24 hours is fast and quick method for Ct serovars visible under light microscope for routine purposes. But it is not the most accurate method as inclusions can be missed or vacuoles can be counted as inclusions. Some Ct serovars have too tiny inclusions to be seen under light microscope. Methods using FITC labeled antibody are more specific and have definite advantage over the light microscopy. It has high specificity and sensitivity. Nucleic acid amplification is not often used in research, but is promising for clinical detection and quantification of infection. We found that flow cytometry has comparable accuracy to direct florescent microscopy and under light microscope, but still there can be variation depending upon Ct serovars. We suggest that this method should be supplemented with Flow cytometry for Ct infectivity assay and titration.

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