

Evaluation of a Novel Solid-Phase Immunoassay, Clearview Chlamydia, for the Rapid Detection of *Chlamydia trachomatis*

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Clearview Chlamydia (Unipath Limited, Bedford, United Kingdom) is a rapid immunoassay for the direct detection of *Chlamydia trachomatis* antigen. This assay was evaluated against the tissue culture method by using 376 paired endocervical specimens. The Clearview assay had a sensitivity of 93.5% and a specificity of 99% when it was compared with the tissue culture method. This assay does not require specialized equipment or trained personnel and yields results within 30 min from the time that a specimen is collected.

In recent years there has been an increasing realization of the importance of *Chlamydia trachomatis* as a genital tract pathogen (6). The traditional method for detecting *C. trachomatis* is tissue culture on McCoy cells, which is expensive, laborious, and time-consuming. Recently easier, less expensive, rapid methods which detect the chlamydial antigen have become available; these include direct fluorescent antibody systems and enzyme immunoassays. However, these methods still require specialized laboratory experience and equipment. In this study we assessed a novel, rapid, solid-phase immunoassay, Clearview Chlamydia (Unipath Limited, Bedford, United Kingdom), which is currently marketed in the United Kingdom and the United States and requires no specialized laboratory equipment, and compared it with the tissue culture method.

The Clearview assay detects chlamydial antigen by using a solid-phase sandwich immunoassay with a colored indicator system. Heat-extracted chlamydial antigen is applied to an absorbent pad on the sample window of a Clearview test device, where the antigen combines with latex-labeled monoclonal antibody to produce a complex. The complex is carried on a membrane strip to a band of immobilized monoclonal anti-chlamydia antibody, which captures the complex to form a sandwich, resulting in a clearly visible blue line. Some antibody-sensitized latex is carried onward to the control zone, where it is bound to a band of immobilized anti-mouse antibody, forming a control line which indicates that the test has been completed correctly.

Endocervical swabs were obtained from 376 new and re-registered consecutive female patients who attended the Department of Genitourinary Medicine, Birmingham, United Kingdom, from October 1989 to January 1990. Patients who had received antimicrobial agent chemotherapy during the previous 6 weeks were excluded.

The cervix of each patient was wiped with a dry cotton wool ball. A sterile cotton-tipped plastic swab was then inserted into the endocervical canal, rotated against the wall several times, and placed in an appropriate container. Two swabs were collected from each patient and were marked first and second to indicate the order of collection. Swabs for culture were placed into 2 ml of the transport medium and stored at 4°C for less than 24 h before inoculation. The transport medium was Eagle minimum essential medium

supplemented with Earle salts, vitamins, nonessential amino acids, and 10% fetal calf serum. The immunoassay samples were stored at 4°C and were tested within 5 days of collection by us in the Genitourinary Clinic. Swabs were randomized to ensure that for each method we used equal numbers of first- and second-collection swabs.

Two 12-mm cover slip cultures of McCoy tissue culture monolayers, pretreated with 5-iodo-2-deoxyuridine, were inoculated with 0.5 ml of vortex-mixed tissue culture transport medium. The cover slip cultures were centrifuged at $2,500 \times g$ for 1 h and incubated at 35°C for 48 to 72 h. One cover slip culture was fixed, while the other was passaged into fresh McCoy cells and incubated for an additional 48 to 72 h before fixation. Fixed cover slips were stained for the presence of *C. trachomatis* inclusion bodies by immunofluorescence (Syva Chlamydia Culture confirmation), and the number of inclusion-forming units was counted for the positive cultures. All of the original specimens were retained at -20°C. When discrepant results occurred, the centrifuge deposit of the remaining transport medium was examined for chlamydial elementary bodies by the immunofluorescence (MicroTrak) method. The presence of five or more elementary bodies was considered to indicate a positive result.

An extraction tube was filled with 0.6 ml of the extraction reagent. The specimen swab was immersed in the reagent and stirred. The tube containing the swab was then placed into a heating device and left at 80°C for 10 min. After removal from the heater the swab was stirred in the tube and gently removed. During this process the rim of the extraction tube was pinched between thumb and finger to remove any liquid from the swab. The resultant extracted liquid was then allowed to cool for 5 min. After this 5 drops of the extract was added to the sample window of the test unit, which had been placed on a level surface. One positive control (supplied with the kit) and one negative control (an unused swab) were included with every run. A line appearing in the result window within 15 min from the addition of the extract was taken to indicate a positive result for chlamydial antigen.

C. trachomatis was isolated by tissue culture from 17.5% (66 of 376) of the endocervical samples. Of these, six became positive only after passage. Sixty-five samples were positive as determined by the Clearview assay (Table 1). There were seven discordant results. Two of the three tissue culture-negative but Clearview-positive samples had chlamydial elementary bodies as demonstrated by direct immunofluorescence. Of the four samples that were positive as deter-

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TABLE 1. Analysis of the results obtained by using the Clearview assay and tissue culture of specimens from 376 patients

Clearview assay result	No. of culture results			Total
	Positive		Negative	
	Initially	After passage		
Positive	57	5	3 ^a	65
Negative	3	1	307	311
Total	60	6	310	376

^a Two of these results were positive as determined by the direct immunofluorescence test.

mined by tissue culture and negative as determined by the Clearview assay, three had fewer than 30 inclusion-forming units, while the other was detected after passage. The levels of sensitivity and specificity for the Clearview test when its results were compared with the tissue culture results were 93.5 and 99%, respectively. The positive predictive value was 95.4%, and the negative predictive value was 98.7%.

The high levels of sensitivity and specificity for the Clearview Chlamydia test found in our study compare very favorably with the best values obtained with other antigen detection assays (4, 8). The level of sensitivity obtained with the Clearview assay was comparable to that obtained with the Surecell Chlamydia Test (Kodak Clinical Products) (3), but was higher than the level of sensitivity reported for the TestPack Chlamydia test (Abbott Laboratories) (1). Of the four false-negative results, all of the specimens contained only small numbers of organisms. This is comparable to the false-negative results obtained with other antigen assays (2, 7). The Clearview assay was positive for five patients from whom chlamydia was isolated only after passage. Passage is both expensive and time-consuming and is not routine in most laboratories which utilize tissue culture for the diagnosis of chlamydial infections.

In two tissue culture-negative but Clearview-positive patients, chlamydia elementary bodies were demonstrated in the culture transport medium by using direct immunofluorescence, and these patients were therefore true-positive

patients. This phenomenon has been demonstrated with other antigen assays (5) and is probably due to the presence of nonviable organisms.

The Clearview Chlamydia assay does not require expensive laboratory equipment or trained personnel. It takes only 30 min to perform and can be used within a clinic setting, where rapid diagnosis of chlamydial infections enables patients to be treated immediately and followed up effectively. The performance of this immunoassay in a low-prevalence general practice population is currently being evaluated. The results of our study show that this assay compares very favorably with tissue culture for diagnosis of female genital chlamydial infections.

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