

EVALUATION OF ENZYME IMMUNOASSAY FOR THE DETECTION OF ANOGENITAL INFECTIONS CAUSED BY *CHLAMYDIA TRACHOMATIS*

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ABSTRACT

Background – Infection caused by *Chlamydia trachomatis* is now recognised as the most prevalent sexually transmitted disease in many parts of the world. Anorectal infections caused by *C. trachomatis* is not uncommon. Enzyme-linked immunoassay (EIA) detects an antigen lipopolysaccharide (LPS) of *C. trachomatis* directly in clinical specimens.

Objective – Our aim was to compare an enzyme immunoassay, Wellcozyme Chlamydia (WZ04) with cell culture for the diagnosis of chlamydial infection of the anogenital tract.

Method – Rectal swabs were taken from 100 prostitutes (80 females and 20 males) for chlamydia culture, WZ04 and direct immunofluorescence (DIF). In addition, endocervical specimens were obtained from the females for the above three tests.

Main Findings – All the positive rectal specimens were from females. Nine patients had a positive chlamydia culture from the rectum but negative WZ04 and DIF. Two patients had false positive results by WZ04 but negative culture and DIF. For cervical specimens, WZ04 identified 43% (3/7) of the culture positive cases. Specificity was 98.6%. WZ04 identified an additional specimen as positive which was also confirmed as positive by DIF.

Conclusion – Our study shows that in our hands enzyme-linked immunoassays such as Wellcozyme Chlamydia are neither sensitive nor specific in detecting *C. trachomatis* infection of the rectum. For cervical infections, the sensitivity of WZ04 was 43% and the specificity 98.6% as compared to culture, with a positive predictive value of 75% and a negative predictive value of 94.7%.

Keywords: anogenital infections, *C. trachomatis*, enzyme-linked immunoassay.

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INTRODUCTION

Infections caused by *C. trachomatis* are becoming increasingly important among the various sexually transmitted diseases (STD). Chlamydia infections are now the most prevalent and among the most potentially damaging STD in the United States and other developed countries⁽¹⁾. Furthermore, many patients are not detected clinically because the infection remains asymptomatic. Thus, early diagnosis and treatment are of major importance in the control of this infection.

The 'gold standard' for identification of *C. trachomatis* is

still tissue culture isolation of the agent⁽²⁾. It is estimated that culture has a sensitivity of 80%-90% and a specificity of 100%⁽³⁾. However, culture is time-consuming, labour-intensive and relatively costly. Direct antigen detection methods provide a rapid and less expensive alternative and to-date, two methods of antigen detection are available: (1) direct immunofluorescent-antibody assay (DIF), and (2) enzyme immunoassay (EIA). Compared with culture, the sensitivity of DIF is >90% and the specificity is >98%⁽⁴⁾. The sensitivity of available EIAs has varied from 67% to 90% and the specificity from 92% to 97%⁽⁵⁾, depending on the population studied and the sensitivity of the tissue culture system against which the EIA has been compared. These results were obtained with endocervical and urethral swab specimens. Thus, these tests are accepted as being specific, if not always sensitive, in high incidence populations⁽⁶⁾.

Anorectal infection caused by *C. trachomatis* is not uncommon. In a Seattle study, *C. trachomatis* was cultured from rectal specimens from 24 (8.3%) of 288 homosexual men and from 33 (21%) of 155 heterosexual women seen in an STD clinic⁽⁷⁾. In men, the infection is caused by anal intercourse. Women can acquire anorectal infections by anal intercourse⁽⁸⁾, but in most cases there is no history of anal intercourse and the infection is thought to have resulted from contiguous spread of infected secretions from the vagina.

To assess the efficacy of the enzyme immunoassay Wellcozyme Chlamydia (WZ04, Wellcome Diagnostics, England), we compared it with tissue culture to detect *C. trachomatis* in rectal and cervical specimens obtained from a group of prostitutes. Although WZ04 is intended primarily for the direct detection of *C. trachomatis* in endocervical and urethral swab specimens there have been no trials assessing the efficacy of WZ04 in detecting *C. trachomatis* infection of the rectum. If WZ04 proves to be useful for detecting rectal *C. trachomatis* infection, then its use can be extended to rectal swab specimens, in addition to endocervical and urethral swabs, thereby decreasing costs.

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MATERIALS AND METHODS

Rectal specimens were obtained from 100 prostitutes, either symptomatic or asymptomatic. They consist of 80 females and 20 males attending an STD clinic. In addition, cervical specimens were also obtained from the 80 females. The age group of the patients ranged from 20 to 58 years with the majority under 30 years old. The median age was 28 years. Three specimens were collected from each patient from the rectum, one for chlamydia culture, one for EIA using Wellcozyme Chlamydia (WZ04) and one for direct immunofluorescence (Chlamydia Direct IF, Bio-Merieux, France). For the females, an additional three specimens were obtained from the cervix, enabling comparisons of cell culture, WZ04 and DIF. The order of collection of specimens for the 3 tests was random. As tissue culture is less than 100% sensitive, we used a third test, DIF, to clarify discordant results between EIA and tissue culture.

Rectal specimens were obtained by passing a cotton-tipped swab on a plastic shaft 2 to 3 cm into the anal canal, using lateral pressure to avoid entering any faecal mass. Swabs were rotated on the rectal mucosa for 20 to 30 seconds. Any swab with gross faecal contamination was discarded and another specimen obtained.

The exocervix was cleaned of excess mucus and exudate. A cotton-tipped swab on a plastic shaft was then inserted into the endocervical canal and rotated for 10 to 30 seconds to ensure adequate sampling. The swab was withdrawn without touching the vaginal walls.

WZ04 is based on the microwell system which uses monoclonal antibodies directed against chlamydia LPS epitopes. The Chlamydia Direct IF is a direct fluorescent antibody technique using fluorescein-conjugated monoclonal antibodies which react with all 15 known human serovars of *C. trachomatis* and are capable of staining both elementary and reticulate bodies.

The swabs were transported with specimen and transport kits provided by the manufacturer. Both WZ04 and Chlamydia DIF were done according to manufacturers' directions. All positive WZ04 specimens were confirmed by the Wellcozyme Chlamydia Neutralisation Test (WZ05, Wellcome Diagnostics, England) for the presence of chlamydial antigen by selective antibody neutralisation.

Briefly, for the WZ04 procedure, one ml of the extraction buffer was added to each tube containing a specimen swab. The tubes were incubated at room temperature (about 23°C) for 10 minutes and then vortexed for 15 seconds to release the antigen prior to boiling. The positive control was vortexed for 15 seconds and 100 µl was added to 1 ml of extraction buffer. The sample tubes and positive control were placed in a boiling water bath for 4 minutes, then placed at room temperature for 2 minutes, cooled in a cold water bath and vortexed for 10 seconds before 150 µl each of the processed samples and positive and negative controls were added to the microwells. The negative control consists of the extraction buffer. 50 µl of the conjugate (containing alkaline phosphatase labelled mouse monoclonal antibody to chlamydial LPS antigen in a protein base) was then added to each well. The wells were incubated for 2 hours at 37°C. At the end of the incubation period, the plates were washed with the Wellcozyme Plate Washer. After washing, 50 µl of substrate containing nicotine adenine dinucleotide phosphate (NADP) was added to each well. The wells were incubated for 30 minutes at 37°C. 100 µl of amplifier (containing alcohol dehydrogenase and diaphorase) was then added to each well. The wells were incubated for 10 minutes at 25°C while colour develops. 50 µl stop solution (2M sulphuric acid) was added to each well. The absorbance of each well was read at 492 nm using the microwell plate reader spectrophotometer. A result was considered positive

if the optical density exceeded the mean of the negative control plus 0.1.

Briefly for Chlamydia Direct IF (Bio-Merieux), one drop of reagent 1 (containing murine monoclonal antibodies) was placed on each control slide and sample. The slides were incubated for 15 minutes at room temperature. The slides were next washed twice, 5 minutes each time in reagent 2 (containing phosphate buffered saline). One drop of reagent 3 (mounting medium for immunofluorescence) was added and the slides were read with a fluorescence microscope. Slides were recorded as positive if >10 distinct apple-green fluorescein-stained elementary bodies were observed.

Cotton-tipped swabs used for chlamydia tissue culture were placed in sucrose-phosphate transport medium (2-SP) and transported at 4°C to the laboratory within 24 hours. Specimens were inoculated onto cycloheximide-treated monolayers of McCoy cells on cover slips in glass vials within 24 hours of collection. The inoculated cells were centrifuged at 2500 to 3000 x g at 35°C for 30 minutes. The resultant lemon-yellow inclusions were stained with Giemsa 48 hours later and detected by darkfield microscopic examination. Culture was considered positive if the monolayers contained one or more chlamydial inclusions.

RESULTS

Comparisons of EIA with tissue culture and DIF for rectal and cervical specimens are shown in Tables I and II respectively. All the positive rectal specimens were from the female prostitutes. None of the male prostitutes had a positive rectal specimen for chlamydia. Nine out of eighty female patients (11%) had a positive chlamydia culture from the rectum but negative WZ04 and DIF. Two patients had a positive WZ04, but negative culture and negative DIF.

Table I – Comparison of EIA with tissue culture and DIF for rectal specimens (n=100).

	Rectal Culture Results		
		+	-
EIA	+	0 (0)	2 (0)
Results	-	9 (0)	91 (0)

() = DIF positive

Table II – Comparison of EIA with tissue culture and DIF for cervical specimens (n=80).

	Cervical Culture Results		
		+	-
EIA	+	3 (3)	1 (1)
Results	-	4 (0)	72 (0)

() = DIF positive

Of the 80 cervical specimens, there were 7 (9%) culture positive cases. WZ04 identified 3 of 7 of the culture positive cases. WZ04 identified an additional case as positive for *C. trachomatis* (which was culture negative) and this was subsequently confirmed positive by the DIF technique. As compared to culture, the sensitivity was 43% and the specificity was 98.6% (Table III). Predictive values are dependent on the prevalence of the condition as well as specificity and sensitivity of the test. Based on an overall prevalence of 9% as found on culture, the predictive value of a positive test was 75% and the predictive value of a negative test was 94.7%.

Table III – Sensitivity and specificity of WZ04 compared with culture for cervical specimens.

	Sensitivity % (no. of samples EIA positive and infected/total infected)	Specificity % (no. of samples EIA negative and not infected/ total not infected)	Predictive value (%) of positive test (no. of samples EIA positive and infected/ no. of samples EIA positive)	Predictive value (%) of negative test (no. of samples EIA negative and not infected/no. of samples EIA negative)
WZ04	43% (3/7)	98.6% (72/73)	75% (3/4)	94.7% (72/76)

DISCUSSION

Diagnosis of chlamydial proctitis is best made by isolation of *C. trachomatis* from the rectum. Nine patients (11%), all of whom were females, had chlamydia isolated from the rectum. All these patients were asymptomatic and only one patient gave a history of anal intercourse four days before the test. Three of these patients (excluding the patient with a history of anal intercourse) also had *C. trachomatis* isolated from cervix. This supports the idea that rectal infection could result from contiguous spread of infected secretions from the vagina and that it is usually asymptomatic.

In our hands EIA was insensitive in detecting chlamydial infection of the rectum, being unable to pick up any of the culture positive cases. Although the order of sampling of the specimens was randomised, we noted that 4 of the 9 positive chlamydia rectal culture specimens were sampled first before EIA and this may have accounted for the lowered yield from EIA. We had two false positive results with EIA, both being negative for culture and direct immunofluorescence. False-positive EIAs result from non-specific binding of test reagents or cross-reacting antigens in the clinical specimen⁽⁹⁾. Various bacteria, including *Acinetobacter sp*, *Klebsiella sp*, *Streptococcus sp*, are found in the rectum at concentrations of $>10^5$ /ml and can react in the Wellcozyme assay to yield false positive results⁽¹⁰⁾.

Direct immuno-fluorescent antibody staining for antigen in rectal secretions has been used to diagnose chlamydia proctitis⁽¹¹⁾. Our results were disappointing in that the DIF technique was unable to pick up any of the culture positive rectal swabs. This may be because DIF requires a more subjective assessment and is thus more operator dependent than other methods.

Compared with culture, the EIA (Wellcozyme) had a sensitivity of 43% in diagnosing cervical infections. Thus our results in terms of sensitivity was disappointing when compared with other studies. A number of factors may explain the discrepant sensitivities: small sample size, population characteristics, specimen collection techniques and laboratory standards.

Because tissue culture is <100% sensitive, evaluating non-culture diagnostic tests can be a problem⁽⁶⁾. The use of tissue culture as the reference method makes it difficult to establish conclusively which positive results by antigen detection are culture misses and which are true false-positive reactions. Thus,

one of our cervical specimens which was negative by culture but positive by both WZ04 and DIF may have reflected true infection. This patient had recent treatment with tetracycline for chlamydia cervicitis two weeks before. Several studies have shown that viable chlamydia disappear during the first few days of treatment but chlamydial antigen may persist for up to a week⁽¹²⁾. This may have accounted for the negative culture but positive EIA and DIF.

To our knowledge, there has been no trial to assess the efficacy of enzyme immunoassays for detecting rectal chlamydia infection. We conclude that in our hands Wellcozyme Chlamydia is not sensitive nor specific enough to detect chlamydia trachomatis infection of the rectum. For cervical infections, WZ04 had a relatively low sensitivity of 43% and a specificity of 98.6% with a positive predictive value of 75% and a negative predictive value of 94.7% in this study.

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