

Improved sensitivity of an enzyme immunoassay IDEIA for detecting *Chlamydia trachomatis*

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SUMMARY In tests on 375 genital tract specimens a commercially available enzyme immunoassay for *Chlamydia trachomatis* (IDEIA; Boots-Celltech) was found to have sensitivity values of 62% for men and 74% for women, and a specificity of 97% for both groups, relative to the results obtained by a fluorescence assay (MicroTrak; Syva). The positive predictive value and the negative predictive value of the immunoassay were 91% and 87%, respectively. Collection of samples for IDEIA in transport medium in plastic phials, as opposed to glass phials recommended by the manufacturer, had no effect on these values. Tests of the sensitivity of IDEIA using laboratory strains of *C trachomatis* showed that the assay detected chlamydial elementary bodies only at dilutions at least 10-fold lower than those at which they could be detected by MicroTrak. Tests of the specificity of the assay with micro-organisms found in the genital tract, other than chlamydiae, showed that reactions occurred with a number of these. Testing three cervical swabs from the same patient, with the material taken into a single phial of transport medium, increased the sensitivity of IDEIA from 74% to 96%, without reducing the specificity which remained at 97%.

It is concluded that this approach enhances the value of the test in a sexually transmitted disease clinic population and may do so in a population with a low prevalence of chlamydiae.

Enzyme immunoassays for detecting *Chlamydia trachomatis* antigens are used widely.¹ We evaluated a commercially available enzyme immunoassay, Chlamydiazyme (Abbott), and found it to be insufficiently sensitive, specific, or reproducible for the routine testing of genital tract specimens from patients attending a sexually transmitted diseases clinic.² These findings, although disputed by others,³ were supported by the results of tests on specimens from female mice infected genitally with *C trachomatis*, a sensitivity of 62% and a specificity of 92% being recorded.⁴ In the same mouse system another enzyme immunoassay (IDEIA) had, by comparison, a sensitivity of 76% and a specificity of 94%.⁴ This suggested that the IDEIA might prove more sensitive than Chlamydiazyme in tests on human clinical specimens and, therefore, we undertook such an evaluation. Because this was not fully realised, as our observations show, we attempted to increase the sensitivity of the IDEIA by obtaining specimens that contained more antigen than usual as a result of multiple swabbing.⁵

Material and methods

Men seen at the Praed Street Clinic (St Mary's Hospital) with symptoms and signs of urethritis, but mainly those with untreated non-gonococcal urethritis (NGU), were studied. The latter was diagnosed if there were ≥ 5 polymorphonuclear leucocytes/high power microscope field ($\times 800$) in a Gram stained smear of urethral discharge, if diplococci were not seen, and if subsequent culture for gonococci was negative. Women studied were contacts of men with NGU or those who came within a clinical category considered to require testing for chlamydiae, such as cervicitis or pelvic inflammatory disease.

Material from the male urethra was obtained by inserting a nasopharyngeal swab (MW 142; Medical Wire and Equipment Co., Corsham, Wiltshire) 3-5 cm into the urethra and then rolling the swab on a MicroTrak slide. A second specimen was taken with a similar swab which was cut off into 1.0 ml of the specially supplied immunoassay transport medium (IDEIA) in plastic phials or into glass phials recommended by the manufacturer.

Cervical specimens were obtained by inserting a cotton tipped swab into the endocervical canal and

rotating it to remove epithelial cells. It was rolled on a MicroTrak slide and then cut off into the immunoassay transport medium. In one series three swabs taken consecutively were cut off into 1.0 ml of transport medium after the first of these swabs had also been rolled on a MicroTrak slide.

ENZYME IMMUNOASSAY

The amplified enzyme linked immunoassay (IDEIA; Boots-Celltech) is based on mouse antichlamydial monoclonal antibody linked to alkaline phosphatase, nicotinamide adenine dinucleotide phosphate substrate, and alcohol oxidoreductase-diaphorase amplification.⁶ Specimens were stored at 4°C for no longer than three days and then processed, or frozen at -70°C and processed subsequently. The specimens were tested and the results recorded exactly according to the manufacturer's instructions. As controls, a positive specimen supplied in the kit and negative specimens comprising transport medium alone, were included in each assay.

MICROTRAK IMMUNOFLUORESCENCE (IF) TEST

The processing of genital smears before and after treatment with the MicroTrak *C trachomatis* direct fluorescence antibody reagent was undertaken as described previously.⁷ The number of elementary bodies was recorded using the following scale: ± = 1-10; + = 11-100; ++ = 101-1000; +++ = > 1000 per whole smear.

Bacterial strains

To examine the specificity of the enzyme immunoassay, tests were undertaken with suspensions of various bacteria, some of which are found in the urogenital tract (table 1). Bacterial colonies from primary cultures were picked and subcultured to agar media. Colonies from these pure cultures were removed with a cotton tipped swab and 10-fold dilutions were prepared in phosphate buffered saline (PBS: pH 7.2). Volumes of 0.1 ml were added to the immunoassay transport medium, which was processed as described previously. The number of organisms in the suspensions was determined by inoculating 0.1 ml of the dilutions in PBS on to the appropriate agar medium. The media were incubated at 37°C and colonies counted when there was no further development. Titres are expressed as numbers of organisms/ml of transport medium.

C trachomatis strains

Strains of *C trachomatis*, which had been passaged in McCoy cells, were used to test the sensitivity of the enzyme immunoassay. These comprised strains of serovars D and K which had had multiple passages, and an infant ocular isolate (Boyd) and a cervical

Table 1 Reactivity of IDEIA with micro-organisms other than *C trachomatis*

Micro-organism	No of strains		No of organisms in test
	Tested	Reactive in IDEIA	
<i>Neisseria gonorrhoeae</i>	3	2	$3 \times 10^5 - 4 \times 10^6$
<i>Gardnerella vaginalis</i>	2	1	$4-6 \times 10^4$
Streptococcus (group A)	1	1	4×10^7
<i>Staphylococcus aureus</i>	1	1	5×10^7
Streptococcus (group B)	1	0	3×10^7
<i>Candida albicans</i>	1	0	4×10^7
<i>Escherichia coli</i>	1	0	8×10^8

isolate (59828), both of which had had only a single passage after primary isolation. Serial 10-fold dilutions were made in PBS and 0.1 ml of each was added to 1 ml of transport medium. In addition, 0.1 ml of each dilution was added to 1 ml of transport medium which was centrifuged and 10 µl of the deposit stained with MicroTrak to estimate the number of elementary bodies in each sample.

The following formulae were used. Sensitivity = IDEIA +, MicroTrak (IF) +/IF +; specificity = IDEIA -, IF -/IF -; positive predictive value (PPV) = IDEIA +, IF +/IDEIA +; negative predictive value (NPV) = IDEIA -, IF -/IDEIA -.

Results

In the first series of tests on 167 samples taken into glass phials, the overall sensitivity of IDEIA for samples from men and women combined was 66% and the overall specificity was 99%. The detailed results are recorded in table 2; the sensitivity of the test for samples from men (53%) was less than that for those from women (74%).

The nature of the phials made little difference to the results. In a second series, 208 samples taken into plastic phials were tested (table 3). The sensitivity of IDEIA for samples from women was the same as in the first series of tests, but it was greater (69%) than previously for samples from men. This accounted for the overall slightly greater sensitivity (71%) than that recorded in the first series.

The combined results of tests on samples from 131

Table 2 Performance of IDEIA (glass phials) using MicroTrak immunofluorescence as standard test

	Men No of specimens (46)	Women No of specimens (121)	Total No of specimens (167)
Sensitivity	10/19 (53)	26/35 (74)	36/54 (66)
Specificity	27/27 (100)	85/86 (99)	112/113 (99)
PPV	10/10 (100)	26/27 (96)	36/37 (97)
NPV	27/36 (75)	85/94 (90)	112/130 (86)

Table 3 Performance of IDEIA (plastic phials) using MicroTrak immunofluorescence as standard test

	Men No of specimens (85)	Women No of specimens (123)	Total No of specimens (208)
Sensitivity	18/26 (69)	30/41 (73)	48/67 (71)
Specificity	56/59 (95)	78/82 (95)	134/141 (95)
PPV	18/21 (86)	30/34 (88)	48/55 (87)
NPV	56/64 (88)	78/89 (88)	134/153 (88)

men and 244 women, a total of 375 in the first and second series, are shown in table 4. The sensitivity of IDEIA for samples from men was 62% and for samples from women was 74%, the specificity in each case being 97%. Elementary bodies were detected in 121 specimens by the MicroTrak technique, 48 (39%) of these containing fewer than 10 elementary bodies. IDEIA failed to detect chlamydial antigen in 29 (60%) of the 48 specimens: it failed to detect antigen in only eight (11%) of the 73 specimens that contained more than 10 elementary bodies.

Three cervical swabs were taken from each of 160 women and placed consecutively in the transport medium. The result of testing the aggregate samples by IDEIA, using MicroTrak as the standard, is shown in table 5. The sensitivity of IDEIA for these samples was 96% and the specificity 97%. In this series elementary bodies were detected in 23 of the specimens by the MicroTrak technique, seven (30%) containing fewer than 10 elementary bodies. IDEIA failed to detect chlamydial antigen in only one of the specimens—namely, one of those that contained fewer than 10 elementary bodies.

The results of testing four laboratory passaged strains of *C trachomatis* by IDEIA are shown in table 6. In each case elementary bodies were detected by IDEIA at a final dilution which was at least 10-fold lower than that at which they were detected by MicroTrak, or by cell culture.

Two strains of *Neisseria gonorrhoeae*, a strain of *Gardnerella vaginalis*, *Staphylococcus aureus*, and a Group A streptococcus reacted positively in the immunoassay (table 1). The numbers of some of these organisms which reacted are not in excess of the

Table 4 Performance of IDEIA in tests on 375 clinical specimens using MicroTrak immunofluorescence as standard test

	Men No of specimens (131)	Women No of specimens (244)	Total No of specimens (375)
Sensitivity	28/45 (62)	56/76 (74)	84/121 (69)
Specificity	83/86 (97)	163/168 (97)	246/254 (97)
PPV	28/31 (90)	56/61 (92)	84/92 (91)
NPV	83/100 (83)	163/183 (89)	246/283 (87)

Table 5 Performance of IDEIA (samples containing multiple swabs) using MicroTrak immunofluorescence as standard test

	No of samples (160)	All from women
Sensitivity	22/23 = 96%	
Specificity	133/137 = 97%	
PPV	22/26 = 85%	
NPV	133/134 = 99%	

numbers which might be expected in swabs from the genital tract.

Discussion

Results obtained previously by testing specimens from female mice infected genitally with *C trachomatis*⁴ suggested that IDEIA was a little more sensitive than the Chlamydiazyme immunoassay (76% compared with 62%), both assays in tests of this murine model having high specificity (94% and 92%, respectively) when each was compared with MicroTrak. Similarly, comparison of results of testing laboratory strains with IDEIA and with MicroTrak and culture shows that while IDEIA is 10- to 100-fold less sensitive than the two latter methods, the discrepancy is not as great as that observed when a similar comparison was made with Chlamydiazyme.² Such indications of the slightly superior sensitivity of IDEIA over Chlamydiazyme are consistent with our current results of tests on clinical specimens. Thus in comparison with the results we obtained previously with Chlamydiazyme² IDEIA has a marginally greater sensitivity in tests on samples

Table 6 Comparative sensitivity of IDEIA and MicroTrak immunofluorescence for detecting laboratory strains of *C trachomatis*

Strain	Highest dilution positive by:		Titre in cell culture	Fold difference in sensitivity*
	IDEIA	Immunofluorescence		
UW 31 (K)	10 ⁻⁵	10 ⁻⁷ 2 elementary bodies	9 × 10 ⁶ /ml	10 ²
Cal.8 (D)	10 ⁻⁶	10 ⁻⁷ 3 elementary bodies	1.6 × 10 ⁷ /ml	10 ¹
Boyd P ₁ (eye)	10 ⁻⁴	10 ⁻⁵ 4 elementary bodies	5 × 10 ⁶ /ml	10 ¹
59828 P ₂ (cervix)	10 ⁻⁴	10 ⁻⁶ 5 elementary bodies	6 × 10 ⁵ /ml	10 ²

*between IDEIA and other tests.

from men (62% compared with 58%) and women (74% compared with 67%). It is difficult to compare results obtained in different laboratories, but we note that such sensitivity is similar to that found by Tjiam *et al*⁸ but not as great as that recorded by some others.^{6,9,10} For those, like ourselves, who find the sensitivity of IDEIA unacceptably low, albeit in our hands a little greater than that of Chlamydiazyme, we wondered whether increasing the concentration of antigen in a sample by combining the material from three swabs from a woman would increase sensitivity more significantly. Although it can be argued that comparing the aggregate of three swabs by IDEIA with only one by MicroTrak is an inappropriate comparison, the latter use of one swab is standard procedure. On this basis, our results clearly indicated that increased sensitivity (from 74% to 96%) was achieved and that it was, indeed, a result of increasing the antigen concentration, as a similar proportion of samples from both the single and the multiple swab studies contained fewer than 10 elementary bodies by MicroTrak (39% and 30%, respectively). In other words increased sensitivity could not be attributed to testing a greater proportion of specimens containing a large number of elementary bodies. Increased sensitivity was also not at the expense of specificity, despite the fact that we noted that some bacterial strains provided positive results when tested by IDEIA. Whether some of these reactions might be due to components of the bacterial growth medium is uncertain.

The possibility of accomplishing a comparable increase in sensitivity without loss of specificity with Chlamydiazyme is debatable because this assay is based on a polyclonal antibody which reacts with an even greater variety of micro-organisms.² Furthermore, our previous tests on specimens from women² have indicated a lower specificity with Chlamydiazyme than with IDEIA. We do not force that the same approach of multiple swabbing could be used in attempting to increase the concentration of chlamydial antigen in specimens from men because this would probably be unacceptable on a routine basis. This is of less importance, however, than being able to provide adequate testing of specimens from women which we believe the multiple swab approach

with the IDEIA does. Increasing sensitivity without loss of specificity not only enhances the value of these tests in a sexually transmitted disease clinic population but should also do so in a population of low chlamydial prevalence. Whether, in fact, this is the case, needs to be tested.

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