Comparison of detection procedures for *Chlamydia trachomatis*, including enzyme immunoassays, in a mouse model of genital infection

D. TAYLOR-ROBINSON and M. TUFFREY

Division of Sexually Transmitted Diseases, Clinical Research Centre, Watford Road, Harrow, Middlesex, HA1 3UJ

Summary. Two chlamydial enzyme immunoassays, Chlamydiazyme® and IDEIA®, were evaluated in a mouse model of chlamydial genital-tract infection. The Chlamydiazyme assay and the IDEIA were assessed on specimens from 10 and 11 mice, respectively. The animals were infected with *Chlamydia trachomatis*, strain SA₂f, and the results obtained by these methods on vaginal specimens taken on 4 or 5 occasions during 41–42 days were compared with those obtained in cell culture and to a less extent by the MicroTrak® direct immunofluorescence test. In comparison with culture, the Chlamydiazyme assay had a sensitivity of 62% and a specificity of 92%; IDEIA had a sensitivity of 76% and a specificity of 94%. These assays sometimes did not detect chlamydiae in specimens taken immediately before specimens which proved positive by culture and the immunoassays were less sensitive if swabs were taken after those for culture. The IDEIA also failed to detect chlamydiae in the late stage of the murine infection when chlamydial elementary bodies were seen by immunofluorescence. The implications of the observations for investigations in the human field as well as for further studies in the mouse are discussed.

Introduction

Methods available for the detection of *Chlamydia trachomatis* organisms (chlamydiae) comprise cultural procedures, staining of elementary bodies with fluorescent monoclonal antibodies and, more recently, enzyme immunoassays. The methods have been evaluated by testing specimens from patients who are then usually treated, precluding the possibility of following infection by taking sequential specimens. Animal models of chlamydial infection, however, are not restricted in this way. Thus, female mice given inocula of *C. trachomatis* in the genital tract sustain an infection which, although self-limited, persists for several weeks (Tuffrey *et al.*, 1982). This enables vaginal specimens from numerous animals to be collected easily over a prolonged period in a sequential fashion, providing an outstanding opportunity to compare the sensitivity and specificity of the various chlamydial detection methods. In this way, we have evaluated two commercially available enzyme immunoassays (Chlamydiazyme® and IDEIA®), comparing their performance with that of a cultural procedure and also IDEIA to some extent with a

direct fluorescent antibody technique (MicroTrak®).

Materials and methods

Mice

Specific pathogen-free outbred TO female mice, 9 weeks old, from the Pathogen-free Unit at the Clinical Research Centre (CRC) were maintained in the Infectious Suite at the CRC.

Infection of mice

The mice were treated with progesterone (Depo-Provera; Upjohn) and were given inocula of 1×10^6 inclusion-forming units of the LGV-2 serovar SA₂f of *C. trachomatis* suspended in sucrose-phosphate medium via the right uterine horn, as described previously (Tuffrey and Taylor-Robinson, 1981). Each mouse was ear-tagged for identification.

Detection procedures for chlamydiae

Culture. A sterile cotton-wool nasopharyngeal swab (Medical Wire and Equipment Co. Ltd; MW142) was inserted and rotated in the vagina, and then agitated vigorously and expressed in 1.0 ml of cold sucrose-

phosphate transport medium (2SP) containing 10% heat-inactivated fetal calf serum. Specimens were stored in liquid nitrogen until inoculated into cycloheximide-treated McCoy-cell cultures and the inclusions were stained with Giemsa, as described previously (Thomas *et al.*, 1977). Two monolayers of cells were used for each specimen and the results are shown as the number of chlamydial inclusions per monolayer.

Monoclonal fluorescent antibody direct test (MicroTrak; Syva). Nasopharyngeal swabs were inserted in the vagina, rotated and then rolled on the marked area of the slide. The fixing of these smears, and their examination after treatment with the 'MicroTrak *Chlamydia trachomatis* direct fluorescent antibody reagent', were undertaken as described previously (Thomas *et al.*, 1984), as was the counting and recording of the numbers of elementary bodies (EBs) in the smears.

Enzyme-linked immunosorbent assay (Chlamydiazyme; Abbott). This assay is based on a rabbit antichlamydial polyclonal antibody and a peroxidase substrate. Specimens were taken with the 'Chlamydiazyme' swab provided and were kept at 4°C for no longer than 3 days. The assay was performed according to the manufacturer's instructions and exactly as described previously (Taylor-Robinson *et al.*, 1987) with a multichannel automatic washing device (the Abbott Proquantum system). As controls, a positive specimen and negative specimens supplied in the kit were included in each assay and its successful performance was based on the results for these specimens fulfilling the manufacturer's stated values.

Amplified enzyme-linked immunoassay (IDEIA; Boots Celltech). This assay is based on a mouse antichlamydial monoclonal antibody and an alkaline phosphatase substrate with an alcohol oxidoreductase-diaphorase amplification (Pugh *et al.*, 1985). Vaginal specimens were taken as previously (Tuffrey and Taylor-Robinson, 1981) with nasopharyngeal swabs which were cut off into 1.0 ml of the specially supplied transport medium in 1-ml glass vials. These specimens were stored at 4°C for no longer than 3 days and were processed and the results recorded exactly according to the manufacturer's instructions. As controls, a positive specimen and negative specimens supplied in the kit were included in each assay and the results were obtained as a print-out from a standard ELISA reader.

Experimental procedures

Experiment 1. Ten mice were inoculated with chlamydiae and together with eight uninoculated mice, serving as controls, were used to assess the value of the Chlamydiazyme assay in comparison with chlamydial culture. On days 5, 19, 27, 35 and 42 after inoculation, swabs were taken separately for each test without the mice being anaesthetised. To minimise sampling error, swabs for the Chlamydiazyme assay were taken first, and those for culture second, from odd-numbered mice on days 5, 27 and 42 and from even-numbered mice on days 19 and 35. The swabs were taken in the reverse order on the other days. To determine whether swabs that were

taken first held an advantage, two swabs taken consecutively on day 32 were tested in cell culture.

Experiment 2. Eleven mice inoculated with chlamydiae and seven uninoculated controls were used to assess the value of the IDEIA in comparison with chlamydial culture and the MicroTrak test. Each mouse was swabbed separately for the IDEIA and culture on days 6, 11, 19, 25 and 41 after inoculation, the swabs for the IDEIA being taken first, and those for culture second, only on the days indicated in table II. Swabs for the MicroTrak test were taken on days 15, 22 and 41 after inoculation.

Assessment of the value of immunoassays in comparison with culture

The following formulae were used:

$$\text{Sensitivity} = \frac{\text{number of specimens positive by both assay and culture}}{\text{number of specimens positive by culture}}$$

$$\text{Specificity} = \frac{\text{number of specimens negative by both assay and culture}}{\text{number of specimens negative by culture}}$$

Positive predictive value (PPV)

$$= \frac{\text{number of specimens positive by both assay and culture}}{\text{number of specimens positive by assay}}$$

Negative predictive value (NPV)

$$= \frac{\text{number of specimens negative by both assay and culture}}{\text{number of specimens negative by assay}}$$

Results

Chlamydiazyme assay compared with culture

The results of the first experiment are shown in table I. Chlamydiae were isolated from all of the 10 mice 5 days after inoculation and then from a progressively smaller number so that only three mice were culture-positive 42 days after inoculation. The Chlamydiazyme assay was also positive most often 5 days after inoculation, but the number of positive results diminished more rapidly. Overall, of the 29 occasions on which chlamydiae were isolated, the Chlamydiazyme assay was positive on only 18 occasions. Chlamydiae were not isolated from any of the eight uninoculated mice on the single occasion on which they were tested and none of the 40 specimens taken from these control animals during the course of the experiment gave positive results in the assay. Thus, the Chlamydiazyme assay, in comparison with culture, had a sensitivity of 62%, a specificity of 92%, a PPV of 90%, and a NPV of 69%.

The results of testing by culture two consecutive

Table I. Results of attempts to detect chlamydial strain SA₂f by Chlamydiazyme and in culture

Mouse no.	Result of Chlamydiazyme (Ch.) assay and number of inclusions in culture (Cult.) produced by specimens taken on indicated day after mouse inoculation										
	5		19		27		32	35		42	
	Ch.	Cult.	Ch.	Cult.	Ch.	Cult.	Cult.	Ch.	Cult.	Ch.	Cult.
1	+	324	—	4	—	0	0,0	—	0	—	NT
2	+	1	+	0	—	0	0,0	—	0	—	NT
3	+	175	—	0	—	NT	0,0	—	30	—	3
4	—	69	+	3	—	168	2,4	—	0	—	0
5	+	5	—	4	—	93	208,67	—	73	+	> 250
6	+	> 250	+	0	—	11	0,0	—	0	—	0
7	+	207	—	116	+	71	> 250,31	+	3	—	0
8	+	85	+	85	+	3	0,0	—	0	—	0
9	+	63	—	313	+	5	> 250,32	+	> 250	+	8
10	+	3	—	0	—	0	0,0	—	0	—	0
Controls*											
11–18	—	0	—		—		—	—		—	

NT = no test (cell monolayers destroyed).

* Controls not swabbed for culture of chlamydiae after day 5.

vaginal swabs taken on day 32 (table I) showed that the isolation rate was not diminished by a second swabbing but, generally, fewer inclusions were demonstrable. Testing of the swab taken second rather than first also reduced the sensitivity of the Chlamydiazyme assay. Thus, in the main comparative tests on the other 5 days, the overall sensitivity of the assay was reduced from 84·6% for the swabs taken first to 44% for those taken second. Despite inferior sensitivity, the assay was positive on two occasions when chlamydiae were not recovered in culture. One of these results (mouse no. 6; day 19) was probably a true positive because chlamydiae were isolated from the same mouse one week later. The other (mouse no. 2; day 19) may have been a false positive result, but, as noted previously, such results were not seen in the control group.

Amplified immunoassay (IDEIA) compared with culture and with MicroTrak

The results of the second experiment, except for those by MicroTrak, are shown in table II. Of the 38 occasions on which chlamydiae were isolated from the 11 inoculated mice, the IDEIA was positive on 29 occasions; in three instances when attempts to culture failed because of disruption of the cell monolayers, the IDEIA was positive and in two cases subsequent tests indicated that the culture results were falsely negative. Chlamydiae were not isolated from any of the seven uninoculated mice

on the two occasions on which they were tested and none of 28 tests on these control mice by the IDEIA proved positive. Overall, IDEIA in comparison with culture had a sensitivity of 76%, a specificity of 94%, a PPV of 97% and a NPV of 64%. However, the sensitivity of the IDEIA was 84% if the swabs for this test were taken first, and 68% if they were taken second.

The MicroTrak test was positive on specimens from all mice on days 15 and 22 after inoculation. On day 41 after inoculation, chlamydiae were seen usually in small numbers (< 10 EBs) in smears from six of the 11 mice but were not detected by the IDEIA in any of them.

Discussion

The order in which swabs are taken is always an important issue in comparative tests. There were five instances in the mouse experiments where a swab taken first for one or other of the immunoassays gave a negative result whereas that taken second for culture gave a positive result, and only one instance where the reverse was true. It is not surprising, therefore, that taking a specimen from the mouse vagina for the immunoassays immediately after a specimen for culture proved less sensitive than taking it first. Of course, a swab taken second is inevitably at a disadvantage compared with that taken first and legitimate assessment of sensitivity must, therefore, be based, as presented here, on the results of all swabbings in

Table II. Results of attempts to detect chlamydial strain SA₂f by IDEIA and in culture

Mouse no.	Results of IDEIA and number of inclusions in culture (Cult.) produced by specimens taken on indicated day after mouse inoculation							
	7		11		19		25	
	IDEIA	Cult.	IDEIA	Cult.	IDEIA	Cult.	IDEIA	Cult.
1	—	1	—*	0	+	0	+	1
2	+	1	+	3	—*	94	+	> 250
3	+	222	+	296	+	> 500	+	NT
4	+	1	+	NT	—	> 250	+	38
5	+	2	—	49	+	13	—	89
6	+	243	+	3	+	31	+	> 250
7	+	2	+	2	—*	> 500	+	37
8	+	3	+	4	—	319	+	> 250
9	+	6	+	3	+	98	+	3
10	+	75	+	125	—	30	+	68
11	—	0	+	NT	—*	72	+	100
Controls†								
12–18	—	0	—	0	—	—	—	—

NT = no test (cell monolayers destroyed).

* Swab for IDEIA taken first.

† Controls not swabbed for culture on days 19 and 25.

which those taken first alternate with those taken second. However, no such difference was seen in the human situation between first and second genital swabs tested in the Chlamydiazyme assay (Taylor-Robinson *et al.*, 1987), possibly because of the larger mucosal surface swabbed and the availability of more material. Nevertheless, the observation in the mice does indicate the importance of randomising the order of swabbing in evaluating tests in the human clinical field.

Our finding that the Chlamydiazyme assay was less sensitive than culture for detecting chlamydiae in the mouse vagina was in agreement with the results of tests on human genital specimens in which the same assay was less sensitive than either the MicroTrak test or culture and was considered unsatisfactory for testing such specimens (Taylor-Robinson *et al.*, 1987). Use of the IDEIA rather than the Chlamydiazyme assay enhanced somewhat the ability to detect chlamydial antigen in the mice. However, the magnitude of the increased sensitivity does not suggest that the IDEIA, despite its amplification system, is likely to be the complete answer to the successful testing of human specimens for chlamydiae, despite some encouraging reports (Caul and Paul, 1985; Pugh *et al.*, 1985; Sherwood, 1986). The Chlamydiazyme assay was not compared with the MicroTrak test on specimens from the mice but the IDEIA failed to detect chlamydiae in the late stage of the murine infection when EBs

were seen by immunofluorescence. This again points to the potential problem that may exist in testing human specimens.

The specificity of the Chlamydiazyme assay was superior to its sensitivity in the mouse model, a result again in agreement with that obtained with human clinical specimens (Taylor-Robinson *et al.*, 1987). The specificity of tests on mice may be attributable to the use of specific pathogen-free animals. It is difficult to assess whether the specificity of the IDEIA might prove greater than that of Chlamydiazyme for testing human specimens on the basis of our findings in the mouse model since the mouse is free of human genital-tract bacteria that are known to be cross-reactive (Taylor-Robinson *et al.*, 1987). However, the fact that the IDEIA is based on a monoclonal rather than a polyclonal antibody may be to its advantage.

These observations on the mouse model have implications not only for the human field, as discussed, but also for further studies on the model. In both situations, cultural procedures are labour-intensive and time-consuming and detection of chlamydiae directly by means of fluorescent antibodies is fatiguing over long periods. However, because both the Chlamydiazyme assay and IDEIA are relatively specific, at least in the mouse model, and longitudinal studies allow occasional discrepancies to be identified, a reasonable strategy for mouse studies might be as follows. The first of two

consecutive swabs could be tested by an enzyme immunoassay and the second stored and tested subsequently by culture, or immunofluorescence, only if the assay result proved to be negative. If the first experiment described here is taken as an example, this strategy would mean that only half of

the specimens would need to be tested by culture or immunofluorescence.

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