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Laboratory techniques for the diagnosis of chlamydial infections

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Abstract

Yolk-sac inoculation of embryonated eggs was superseded 25 years ago by the use of cell cultures (often McCoy) for the isolation of *Chlamydia trachomatis*. Centrifugation of specimens onto the cell monolayers was shown to increase sensitivity, but little of late has further improved sensitivity which is at least ten-fold greater than that of eggs. However, culture is slow and labour intensive so that non-cultural techniques without these drawbacks have come to dominate. Direct fluorescent antibody (DFA) tests are rapid and have sensitivities that range from 70% to 100% for men and 68% to 100% for women, and specificities that range from 87% to 99% for men and 82% to 100% for women; if the tests are read by competent observers the values are at the top end of the ranges. The detection rate may be enhanced even further by relatively low-speed centrifugation of specimens before staining. Skilled reading is not a feature of enzyme immunoassays (EIAs) which according to the literature have sensitivities that range from 62% to 97% for men and 64% to 100% for women, and specificities that range from 92% to 100% for men and 89% to 100% for women. However, comparison against poor reference tests is responsible for most of the higher values and the clinician should not be misled into believing that EIAs have excellent sensitivity; the lower values in the ranges are closer to reality. Furthermore, EIAs that are being designed for use by general practitioners should be regarded with the greatest caution since lack of sensitivity means that chlamydia-positive patients will go undetected. The polymerase chain reaction (PCR) is not bedevilled by insensitivity but it is no more sensitive than the most sensitive cell culture or DFA tests. PCR is unsuitable for routine diagnosis but has a place as a research tool. For men, examination of "first-catch" urine samples by the best of the non-cultural procedures provides an acceptable non-invasive approach to diagnosis; for women, the value of examining urine may be less, but needs to be thoroughly tested. However, there is little doubt that a Cytobrush used to obtain cervical specimens holds no practical advantage over a swab. Serological tests are reliant on the provision of paired sera for making a diagnosis; high antibody titres in single sera may be suggestive of an aetiological association in deep-seated chlamydial infections (epididymitis, arthritis, salpingitis, etc), but unequivocal interpretation is unusual, particularly in an individual case, since the distinction between a current and past infection is problematical.

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Certain serovars of *Chlamydia trachomatis*, as the name implies, cause trachoma. The implication of other serovars in causing genital-tract disease and non-blinding paratrachoma has been reviewed recently.[1] The need for services to diagnose chlamydial infections in patients attending sexually transmitted disease (STD) clinics, particularly women, and to screen non-STD clinic populations is not in dispute. However, the development of such services is not helped by the fact that chlamydiae, despite being bacteria, behave like viruses in that they require viable cells in which to replicate. Any need for the regular use of cell cultures imposes limitations on organising an efficient country-wide diagnostic service and has given impetus to the development of techniques that are independent of cell culture. These have burgeoned but, as outlined, bring with them their own problems. The advantages and disadvantages of some or all of the techniques have been discussed recently[1–4] and are presented in the table.

Collection, type and transport of specimens

It should not need stressing that the testing of a specimen that has been poorly taken and is

Table Advantages and disadvantages of techniques for detecting Chlamydia trachomatis

Factor considered	*DFA*	*Culture*	*ELISA*	*PCR*
Sites which may be tested	Any	Most	Limited by non-specific reactions	Any
Importance of well-taken specimen	Crucial	Crucial	Crucial	Crucial
Conditions during transport of specimen	Unimportant if fixed	Rapid or at low temp	Unimportant if in buffer	Rapid or at low temp; less crucial than for culture
Storage conditions	Short-term: 4°C. Long-term: −20°C if fixed	4°C overnight. Long-term: liquid N2	3–5 days: 4°C. Freezing may reduce sensitivity.	Short-term: 4°C. Long-term: −70°C. Unimportant if fixed
Assessment of adequacy of specimen	Examine smears for adequacy during test	Not practical	Not practical	Determine whether any DNA present
Special equipment needed	Fluorescence microscope	Centrifuge	Ranges from ELISA reader to a complete washing and reading system	Thermocycling machine and electrophoresis equipment
Processing of specimen	Simple	Tedious	Becoming easier for new tests	Lengthy; requires stringent precautions against contamination
Reading of test	Subjective/tedious	Subjective/moderately tedious	Objective/simple	Objective/simple
Time to result	30 min	12–72 h	3 h (becoming shorter for new tests)	12–24 h
Means of checking a result	Re-examine the slide	Re-examine the slide	Repeat the test	Use probe or endonuclease digestion
The result depends on	Expertise of the observer	Sensitivity of the cell culture	Inherent capacity of the test	Good controls and lack of contamination
Use as a test of cure	Limited	Recommended	Limited	Not practical
Ability to maintain strains	No	Yes	No	No

inadequate, or poorly transported or badly stored is a waste of time for all concerned. Tests of the distance a swab needs to be passed down the male urethra for optimal recovery of chlamydiae have not been undertaken, but 3–4 cm is often recommended to make it clear that deep urethral and not meatal swabbing is required however reluctant clinician and patient might be. Swabbing of conjunctivae after removal of excess exudate should be firm, as should that of the squamo-columnar junction of the cervix which also should first be wiped clean. Cotton-tipped swabs are superior to those of calcium alginate or Dacron and aluminium shafts superior to plastic or wood for the isolation of *C. trachomatis*.[5] Furthermore, swabs provided in commercial enzyme immunoassay kits may be toxic if used for collecting specimens for culture, a feature occasionally exhibited also by the Cytobrush in some hands,[6] and swabs other than those in kits may lead to non-specific immunoassay results. It is logical to believe that taking two or more swabs from patients rather than one will improve the detection rate and, indeed, the rate has been increased by pooling cervical and urethral specimens[7,8] and by taking three cervical specimens and culturing them individually.[9] A second endourethral swab from men has been said to improve recovery too[10]; whether the use of a meatal swab and an endourethral curette[11] is really superior to an endourethral swab taken after urination is difficult to assess from the experimental design. Of course, even if there is marginally superior detection which seems inherent in taking multiple specimens, it has to be weighed against the burden imposed on patient, clinician and laboratory staff.

Urine specimens

In view of the foregoing, the notion and, indeed, the evidence that it is sufficient to provide a "first-catch" urine specimen only is obviously attractive, particularly for the patient. Testing urine in cell cultures is too insensitive,[12,13] but the results of testing by enzyme immunoassays have been much more encouraging,[13–16] even though some[17,18] have not been over-enthusiastic about this non-invasive approach, particularly for asymptomatic men. Our results[19,20] leave no doubt that testing the centrifuged deposit from a first-catch urine sample is as sensitive as testing a urethral swab, if the same test is applied to each. Furthermore, it is more profitable to test a good urine sample, that is one that is cellular, than to test a poorly taken swab. It is unlikely that all immunoassays will provide results that are reliable (*vide infra*) but the results of examining a urine sample by IDEIA can be looked upon with considerable confidence by the clinician. It is obvious, of course, that this non-invasive approach may prove a valuable means of screening apparently healthy men, for whom urethral swabbing is likely to be out of the question.

Any suggestion that a urine sample from women could take the place of a cervical swab seems illogical, because it assumes that the urethra and cervix are infected concurrently. Early results[15] indicate that there is no advantage in testing urine, but the

proposition needs fuller evaluation. In the meanwhile, is there anything better than a cervical swab?

Value of a Cytobrush

The use of a Cytobrush to collect cervical material has attracted attention recently. An initial, poorly controlled evaluation[21] was inconclusive.[22] Subsequently, although most workers have not found the Cytobrush to be advantageous[6 23 24] or cost-effective if samples were tested by Chlamydiazyme,[25] a few[26 27] have concluded that its use was superior to swabbing for culture and direct fluorescent antibody tests. So what does one believe? Increasing the number of chlamydia-infected cells for examination, which the brush is likely to do, is intuitively a sound idea. Theoretically this might provide a positive result rather than a falsely negative one, particularly with an insensitive laboratory test. This is only likely to happen if the brush is used without causing bleeding and cells do not remain trapped within it, limitations which probably make it more trouble than it is worth in routine practice. The results indicate that this is the majority view. Discussion now turns to the laboratory tests.

Direct staining

Staining of cells in genital and ocular specimens with vital dyes was used first to detect chlamydial inclusions. However, the method is insensitive, notably with genital-tract specimens. This is certainly the case with Papanicolaou-stained cervical smears[28–30] but, despite the fact that cytologists should have abandoned this approach to the detection of chlamydiae years ago, there are still some[31 32] who toy with it. On the other hand, histiocytes in increased numbers and the presence of transformed lymphocytes in cervical specimens give some clue to the existence of a chlamydial infection,[33] one that can be confirmed by the use of specific tests.

Direct fluorescent antibody (DFA) tests

The use of fluorescein-conjugated monoclonal antibodies to detect chlamydial elementary bodies directly in cervical or other smears is a specific approach that has made a considerable impact on diagnosis in the last few years. MicroTrak, Pathfinder[34] and Monofluor[35] are some of the commercially available DFA tests in which monoclonal antibodies against the major outer membrane proteins of *C. trachomatis* produce brighter and less non-specific staining with either clinical smears or laboratory strains[36] than those against the lipopolysaccharide, for example, Imagen. A DFA test (Clonatec) combining both types of antibody has also been described but offered no advantage in sensitivity.[37] There is a wealth of information available for the MicroTrak test. This has been considered less sensitive than culture by some,[38–41] particularly for the detection of small numbers of organisms[42–44] or in a low-prevalence population.[45] However, others[46 47] found the method valuable in their low-prevalence populations and still others have found the method to be at least as sensitive as culture,[48–52] or almost so.[53–56]

Examination of the literature reveals that the specificities of DFA tests have ranged from 87% to 99% for men and from 82% to 100% for women and the sensitivities from 70% to 100% for men and from 68% to 100% for women. Although the sensitivity values vary, excellent sensitivity in the hands of some should not come as a surprise. Indeed, it is easy to appreciate that if, in a DFA test single elementary bodies can be detected with confidence, the sensitivity of other procedures by comparison may be found wanting. However, the success of the DFA tests does, of course, depend on the experience of the observers,[57] which includes the ability to detect small numbers of elementary bodies (<10) and to discriminate between specific and non-specific staining.[58 59] In this regard, the staining of *Gardnerella vaginalis* (GL Ridgway and G Mumtaz, personal communication) and parainfluenza 2 virus particles[60] by chlamydial antibody should be noted. However, even for the moderately experienced observer, the morphological difference between chlamydial elementary bodies and other bacteria should make discrimination easy. Direct immunofluorescence, as exemplified by MicroTrak, has some special attributes. Its use has enabled chlamydiae to be detected in the joints of patients with sexually acquired reactive arthritis[61] and, without question, it is more sensitive than culture for the detection of chlamydiae in endometrial or tubal specimens.[62 63] It has proved useful even for examining rectal specimens[64] and has the potential for detecting chlamydiae in semen samples.[65] It stands out as an easy way of testing single specimens and, together with other non-cultural techniques, has value in looking at specimens that have lost viable chlamydiae through prolonged transport[66] or sub-optimal storage. Specimens that are toxic in cell culture may be looked at in a DFA test[63] and, furthermore, the detection rate may be enhanced by relatively low-speed centrifugation of specimens before staining.[20] Direct immunofluorescence also may be used as a test of cure. This is exemplified by the finding that all of 30 chlamydia-positive non-gonococcal urethritis (NGU) patients had negative urethral tests 6–11 days after starting a course of tetracycline therapy.[67] However, a DFA test may remain positive after culture has suggested the disappearance of viable organisms. Thus, seven to ten days after 39 culture-positive women had completed doxycycline treatment (100 mg twice daily for seven days) and had become culture-negative, one of every five of them had a positive DFA test.[68] Of course, all this begs the question of whether tests of cure are worthwhile. In

cost-effective terms there seems no doubt that they are not.[4 69]

Detection by culture

The growth of chlamydiae in cultured cells 25 years ago revolutionised chlamydial research and had a major impact on their detection. Many cell lines are suitable for the growth of chlamydiae,[70] but the method of detection considered by many to be optimal involves the centrifugation of specimens onto McCoy cell monolayers which are treated with cycloheximide and subjected to monoclonal immunofluorescence staining after incubation; up to five blind passages have been said to increase sensitivity.[7] Even if this were plausible it is certainly impractical and comforting to know that some[71] consider one blind passage to be sufficient. It is tempting to speculate that the success of passage reflects inadequate reading of the primary cultures. The use of a DNA probe to detect inclusions in culture did not enhance sensitivity[72] and in recent years, apart from the treatment of cell cultures with mitomycin C[73] and the use of polyethylene glycol,[74] nothing has emerged to increase further the sensitivity of culture. Micromethods may be more convenient and rapid but no more sensitive[75] and prone to cross-contamination. Any diminished sensitivity they may have may be improved by sonicating and vortexing clinical specimens.[76]

In competent hands, what is detected by the cell culture technique and regarded as belonging to the *Chlamydia* species is highly likely to be so. This specificity forms the rationale for regarding cell culture as the "gold" standard against which non-cultural methods should be compared. However, competence is sometimes lacking and, in addition, the sensitivity of detection by cell culture has been estimated to be no more than 70–80% for women with cervical infection.[77] It depends on the sensitivity of the cells which can vary within a laboratory and unquestionably from one laboratory to the next. Thus, the "gold standard" is variable and it is easy to see why some regard it as a myth and in view of their expertise would rather place reliance on direct immunofluorescence as a comparator. This, of course, is not to denigrate the cell culture system which, because of its specificity, is still seen by some as the method of choice for chlamydial diagnosis. However, the culture of specimens in sensitive cell lines has never been undertaken by all laboratories in a position to do it[78] and is practised even less widely now since the advent of antigen detection systems.

Immunoassays

The use of various enzyme-linked immunosorbent assays (ELISAs) or enzyme immunoassays (EIAs) has been met with enthusiasm probably because, unlike the DFA tests, their reading is neither subjective nor laborious, although the test procedure itself may be complicated. For Chlamydiazyme (Abbott) and IDEIA (Novo Nordisk) the sensitivity values range from 62% to 97% for men and from 64% to 98% for women and the specificity values range from 92% to 100% for men and from 89% to 100% for women. Arguments about these values have been unremitting and hardly surprising. It must be expected that the results of studies which compare various assays done by different investigators will vary when they depend not only on the inherent capacity of the test under examination but also on how it has been read,[79–80] the sensitivity of the "gold-standard" with which it is compared and the capabilities of the investigators. Chlamydiazyme was the first commercial assay to come on the market and while some[81–89] have considered it to be "satisfactory" in terms of sensitivity and specificity, others[40 44 90–93] have been much more sceptical. Specificity can be jeopardised by cross-reactivity with other organisms.[90 94–97] It is desirable, therefore, to confirm a positive result by testing the remains of the transport medium by immunofluorescence with a MOMP-specific monoclonal antibody or by the use of a specific blocking antibody that has, for example, now been included as part of the Chlamydiazyme assay. The fact that it is implausible to check all negative results and, therefore, impossible to detect false negative ones, which lead to insensitivity, remains a problem. The IDEIA assay,[98] although considered unsuitable for examination of non-genital sites,[99] in particular the rectum,[94] has been considered by some to be more specific[100] and more sensitive[101 102] than Chlamydiazyme for testing genital specimens. Furthermore, it has been possible to increase the sensitivity of IDEIA without reducing its specificity by taking multiple swabs from the cervix, pooling them and so increasing the concentration of antigen tested.[100] However, while concentration was satisfactory in this situation, in another it was not. Thus, resuspension of urine deposits in 0·5 ml instead of 1·0 ml produced some falsely positive results, that is ones that could not be supported on checking by immunofluorescence (Hay PE, personal communication). Clearly, antigen concentration cannot be undertaken with impunity.

Overall, the results obtained for two other enzyme immunoassays (Pharmacia Chlamydia EIA and Ortho Diagnostic Systems Ltd) are little different from those described above, specificities being uniformly good and sensitivities ranging from 71–85% for specimens from men and 80–100% for those from women.[103–106] It remains to be seen whether the high sensitivity (97% for men) of the Syva MicroTrak EIA reported by one group[107] is a true reflection of its worth.

An immune dot blot technique with sensitivity and specificity values similar to those of culture has also

been used with success[108 109] but is not available commercially. The enzyme immunoassays mentioned are most of those now on the commercial market, their value assessed in a multitude of studies often with conflicting results. So what should clinicians believe? There is no doubt that assays have improved but it is important to understand that no assay, if it is to be specific, is likely to match the sensitivity of the best culture technique, or, indeed, that of a DFA test. The problem is that a zone exists between definite positive readings of optical density and definite negative readings, the so-called "grey-zone". To err on the safe side and always provide "definite" positives will be at the expense of sensitivity. Clinicians should be aware of the possibility of false results emanating from a laboratory and be prepared to question what seems unreasonable. False positive results should be few if positive results have been confirmed by a second laboratory test. Lack of sensitivity leading to false negative results is the most serious problem; negative results will not be doubly checked unless a special request is made. It is hard to predict but confirmation of results is probably even less likely to occur with the advent of membrane immunoassays, for example, TestPack Chlamydia[110 111] Clearview and Kodak Surecell.[112] These are simple to perform and, in effect, are "do-it-yourself" tests intended for clinicians to take up without resort to laboratory help. They may be useful in conjunctival infections[112] but, otherwise, clinicians will use them at their peril. The pitfalls inherent in any of the assays mentioned must apply to these too.

DNA probes and the polymerase chain reaction (PCR)

Palva *et al*[113] were probably the first of several investigators to use DNA probes. They used chromosomal DNA, from the L2 serovar of *C. trachomatis*, digested with the restriction enzyme Bam H1 and cloned into *E. coli* by means of the plasmid vector pBr322. The probe behaved specifically in preliminary sandwich hybridisation tests and in tests on genital-tract specimens good sensitivity and moderately acceptable specificity (85%) were achieved.[114] On the other hand, others[115] were less successful, particularly with specimens that were weakly positive in culture. Indeed, both false negative and false positive results were obtained. Since then impressions of the value of DNA probes have swayed back and forth. Dean *et al*,[116] screening a trachoma-endemic population, found that a probe based on a 7·0-kilobase cryptic plasmid from *C. trachomatis* had a sensitivity of 87% and a specificity of 91%, compared with culture; whether this is sufficiently sensitive is debatable. Certainly, others[117 118] found insufficient sensitivity to be a problem with the probes they used. A modification of the molecular probe is the use of luminescence.[119] However, the Probe Assay Chemiluminescence Enhanced (PACE, Gen-Probe Inc.) test needs more detailed evaluation. *In situ* DNA hybridisation is another approach to the detection of *C. trachomatis* and has been used with cervical scrapings and rectal biopsies, the results apparently being more or less comparable with those obtained by culture.[120–123]

The PCR is a new approach again, one that may leave other molecular tests behind. In allowing massive amplification of a DNA sequence, it has brought a hitherto unparalleled dimension to the problem of increasing sensitivity. The technique comprises repeated cycles of high temperature template denaturation, oligonucleotide primer annealing and polymerase-mediated extension. After 25 cycles, a hundred-thousand- fold increase in the DNA sequence under investigation may be achieved. Of several groups of workers now using this method to study and detect chlamydial DNA, Dutilh *et al*[124] found that a 129-base pair fragment of the major outer membrane protein of *C. trachomatis* was amplified in the 15 serovars of *C. trachomatis*; it was not amplified in different micro-organisms encountered in the genital tract so that the reaction seemed to be specific. Mahoney *et al*[125] noted that the PCR was a little more sensitive than an EIA but a little less sensitive than culture when used to test urethral and cervical specimens. Quinn *et al*[126] and Palmer *et al*[127] found that the PCR had high specificity and sensitivity; the latter workers, however, found that it was no more, and no less, sensitive than the MicroTrak DFA test when used to test specimens from men with NGU. This, coupled with the ease with which contamination with DNA can occur in the laboratory, unless the most stringent precautions are taken to prevent it, suggest that the PCR is unlikely to find widespread use in routine diagnosis. The occurrence of false positive results will remain a worry. Nevertheless, it would seem that the PCR has considerable potential as a research tool, for example as a means of confirming the existence or otherwise of chlamydiae in arthritic joints when other methods (enzyme immunoassay, culture) fail, and for examining specimens that are limited in quantity.

Detection of antibody

Various serological techniques have been used to study chlamydial infections. Complement fixation usually is not sufficiently sensitive to detect antibodies stimulated by uncomplicated genital infections, but has an acceptable place in the diagnosis of lymphogranuloma venereum infections and psittacosis.[128] Immunofluorescence (IMF) and enzyme immunoassays,[129 130] including a μ-capture ELISA for chlamydia-specific IgM,[131] are much more useful for all aspects of serology. Furthermore, immunoblotting has been used quite widely to

correlate structure with function, in other words to determine which chlamydial antigens stimulate antibody production.[132-134]

In considering the various clinical problems, several points emerge. Chlamydial antibody may not develop in about a fifth of men with acute NGU, the titres when measurable are usually quite low and it is rare to detect an antibody response. Indeed, there is no sense in attempting to make a diagnosis of chlamydial NGU on the basis of serology. The latter has been suggested as a complementary test[135] but its dubious value indicates that it should not be used even in this role.

In the case of epididymitis, patients who were culture positive (urethra and/or epididymal aspirate) in one study[136] always had IgG IMF antibody titres equal to or greater than 1:64, whereas those who were culture negative had lower antibody titres. While these data are more convincing than those presented by Kaneti *et al*,[137] diagnosing a current infection in an individual patient on the basis of a single antibody titre cannot be guaranteed. The contention by Kojima *et al*[138] that antibody in semen is diagnostic needs further support. In the case of patients with sexually acquired reactive arthritis (SARA) (usually men), chlamydial serum antibody titres tend to be higher than those in patients with uncomplicated NGU or other arthritides.[139] However, the titres overlap so that, again, a serological diagnosis of current infection on the basis of a single serum titre is only suggestive and not foolproof. The possibility that antibody titres in synovial fluids of SARA patients sometimes might be higher than those in the corresponding sera, indicating local production and another way of associating organism with disease, requires further exploration.

The occurrence of serum antibodies frequently in women in whom chlamydiae cannot be detected in the cervix or elsewhere illustrates the problems encountered in using serology for diagnostic purposes. Although the antibody titres tend to be higher in women with cervical infections than in men with NGU, it is rare to see a rising titre and, if nothing else, wasteful of resources to attempt to make a diagnosis of a cervical infection in this way or, indeed, on the presence of antibody in local secretions.[140] The detection of a rising antibody titre in pelvic inflammatory disease (PID) is uncommon but the titres tend to be higher in cases of PID than in uncomplicated cervical infections, and perhaps suggestive of an aetiological association if very high, an IMF titre of 1:$\geqslant$512 being used by Kristensen *et al*.[141] As in all single serum situations, what the "cut-off" should be is difficult to know and would probably vary from one laboratory to the next. Lower titres are used,[142] but unless supported by an IgM titre, there should be reluctance to make a firm diagnosis of chlamydial salpingitis in an individual case on the basis of a single serum antibody titre. In contrast, the occurrence of specific IgM antibody in the sera of babies who develop pneumonia in the first few months of life is pathognomonic of chlamydia-induced disease.[143]

In summary, the situation so far as serology is concerned is that, although a fourfold or greater antibody response should always be sought, this is rarely detected. A greatly elevated chlamydial antibody titre in a single serum may be diagnostically suggestive in the more deep-seated infections (epididymitis, SARA, PID), but caution should be exercised because seropositivity in itself is not highly predictive of active infection[144] and high titres do not always correlate with detection of chlamydiae.[145] High titres are more likely to be associated with chronic[146] or recurrent disease.[147] Since chlamydial infection in women leads in the short- or long-term to infertility, it comes as no surprise (indeed, it could not be any other way) that chlamydial antibody is found more often and in greater titre in infertile women with tubal abnormalities than in those without; there is considerable documentation to this effect.[1,148] What will not be clear in any individual is whether active infection still exists. In a negative sense it is helpful, perhaps, to know that the absence of antibody in women, determined by a sensitive serological test, probably excludes a chlamydial infection[149] either in the past or currently. It has been alleged that it is possible to distinguish between a past and a current infection by measuring IgA antibody in a single serum sample,[150] its presence purportedly denoting a current infection. However, the fact that such antibody has been seen to persist for several years in some patients who have had PID[151] is a clear indication of the impossibility of making such a distinction. Unfortunately, the practice of using IgA as a marker[152] seems slow to die.

Discussion

Several points need to be made and others re-emphasised. It is a sad fact that chlamydial diagnosis in the UK, and probably in other countries too, is made badly. To a large extent this stems from the widespread use of EIAs which generally are insensitive. It is true that EIAs have improved since first appearing, but clinicians should not be hoodwinked by extravagant claims in the literature where it is common to find that tests are regarded as performing excellently; this often comes from comparisons against tests which themselves are insensitive[81,89] or from the manipulation of data, unwittingly or otherwise, that leads to a false impression. It is unfortunate that many have come to use cell culture as the so-called "gold standard" for comparison; very often it is not gold, being inconsistent and insensitive in many laboratories, and it is certainly not standard.

Some have used the most sensitive test possible (identifying one elementary body in a DFA test) as the reference standard. Criticism that this is unrealistic because it is not what the majority do in practice, is misplaced; use of the most sensitive reference test determines the real value of the test under investigation. Occasionally, contrary to all the data that have hitherto accrued, a test is reported as grossly insensitive, for example a sensitivity of 40% for men by IDEIA[106]; common sense indicates that this is an aberration that should be ignored.

Opinion is divided on the question of whether the number of organisms shed by asymptomatic patients is smaller than by symptomatic ones.[42 54 55 70 93 153–161] The majority seem not to favour this view and few of those who do present evidence. This might suggest that chlamydial infection in the asymptomatic patient is no more difficult to diagnose than in the symptomatic one, but it remains a moot point. In contrast, there seems to be no dissention from the view that for a test of moderate sensitivity and relatively high specificity (which most EIAs have), the predictive value of a positive result will be acceptable in a high prevalence population but will become unacceptable in a low prevalence one. This means that in a low risk population there may be more false positive than true positive results. The notion, however, that a low risk population contains relatively more individuals with a small number of organisms is often expressed[39 162] but is questionable since it is supported only by weak evidence[154]; if infection occurs in a low risk group, there seems no reason why it should not run the same course and cause as much shedding as in a high risk group. It has to be understood, however, that even in a high risk group, small numbers of chlamydiae occur in about 40% of the population.[163] It is these that tend not to be detected by relatively insensitive methods, such as the EIAs, but only by the DFA tests and by the PCR. That is not to say that DFA tests do not have their problems. The number of elementary bodies used as the criterion of a positive result is still a contentious issue; the more that are used, the less sensitive the test. Furthermore, mis-reading may sometimes come to light only when the result seems quite unreasonable, as, for example, the claim of a high chlamydial prevalence rate for glue ear[164 165]; by the same token, a detection rate of 40% in NGU, by its mere reasonableness, might hide the fact that the wrong patients were being regarded as chlamydia-positive. Advances will be made only if DFA tests can be automated and the PCR is endowed with mechanisms that can be guaranteed to prevent DNA contamination. Such tests of high sensitivity that can be widely and easily used are needed urgently if all the chlamydia-positive patients in high risk groups are to be identified; at the moment many are not. Conversely, tests with exquisite specificity are needed for low risk groups to avoid large numbers of unnecessary and damaging false positive results.

It goes without saying that the most sensitive laboratory tests cannot overcome the deficit incurred by specimens being collected, transported or stored poorly. Assuming that this is not the case, chlamydial detection may be improved even further by taking multiple specimens; there are probably no microbiological situations where this does not apply. However, reason has to prevail, particularly when obtaining specimens from the male urethra; the fact that urine is proving to be as satisfactory as a swab is helpful in this regard. What the results of detection mean once obtained is probably more in the province of the clinician than the laboratory worker. However, it is worth saying that interpretation depends on the clinical situation and type of specimen. Thus, detection of chlamydiae in the cervix of a woman who complains of lower abdominal pain is likely to mean that the organisms have entered the upper genital tract, irrespective of whether laparoscopy reveals abnormal fallopian tubes.[63] On the other hand, the detection of chlamydiae in expressed prostatic secretion does not necessarily mean that the prostate is involved,[166] despite protestations to the contrary.[167]

Finally, it has become a ritual to say that serological procedures offer little in the way of making an accurate chlamydial diagnosis. Hopefully the day will come when antigens responsible for antibodies occurring in current and past infections will be cloned and expressed by molecular techniques and used to develop specific serology so that it does not remain enigmatic.

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