

Table 2: Comparison of microscopy and biochemical test strip (BTS) results for significant isolates.

Isolate	Total organisms	Organism detected		Leucocytes detected	
		Microscopy	BTS	Microscopy	BTS
<i>E. coli</i>	132	104	67	95	102
Coliforms	37	19	16	19	24
<i>Proteus</i> spp.	11	6	3	7	8
<i>Klebsiella</i> spp.	10	9	7	7	9
<i>Pseudomonas</i> spp.	7	2	2	4	6
<i>Enterococcus</i> spp.	19	11	4	7	9
<i>Staphylococcus</i> spp.	14	2	4	10	12
Other streptococci	8	3	1	3	3
Yeasts	4	4	0	2	3
Total	242	160	104	154	176

microscopy requests. Microscopy will remain important in the investigation of glomerular and tubular renal disease, localisation of bleeding within the urinary tract, and for other specific conditions, such as schistosomiasis. In these situations microscopy should be requested either directly by the clinician or by laboratory staff. We estimate microscopy to be necessary in only 8% of our total urine workload.

Within hospital laboratories urine microscopy places great demands on staff, yet for the vast majority of samples a prediction of positivity in a rapid turn-around time is all that is required. Biochemical test strips are a rapid alternative methodology for assessing such samples. Although microscopy cannot be replaced in all circumstances, the use of BTS on urine samples submitted for a simple infection screen is appropriate.

B.I.F. Batchelor\*, A.R. Hunt, I.C.J.  
Bowler, D.W.M. Crook

Oxford Public Health Laboratory, John Radcliffe Hospital,  
Headington, Oxford OX3 9DU, UK.

#### References

1. Lejeune B, Baron R, Guillois B, Mayeux D: Evaluation of a screening test for detecting urinary tract infection in newborns and infants. *Journal of Clinical Pathology* 1991, 44: 1029-1030.
2. Hiscoke C, Yoxall H, Greig D, Lightfoot NF: Validation of a method for the rapid diagnosis of urinary tract infection suitable for use in general practice. *British Journal of General Practice* 1990, 40: 403-405.

3. Flanagan PG, Davies EA, Rooney PG, Stout RW: Evaluation of four screening tests for bacteriuria in elderly people. *Lancet* 1989, i: 1117-1119.
4. Smith TK, Hudson AJ, Spencer RC: Evaluation of six screening methods for detecting significant bacteriuria. *Journal of Clinical Pathology* 1988, 41: 904-909.
5. Galen RS, Gambino SR: Beyond normality: the predictive value and efficiency of medical diagnoses. John Wiley and Sons, New York, 1975, p. 13.

#### Comparison of Direct and Indirect Immunofluorescence Assays for Rapid Detection of Cytomegalovirus in Shell-Vial Culture

Infections caused by cytomegalovirus (CMV) are one of the main causes of morbidity and mortality in immunocompromised patients (1, 2). Isolation in cell culture is one of the methods used for the diagnosis of infection by CMV. The use of conventional cell culture requires prolonged incubation and does not permit quantification of the viral load. In contrast, the shell-vial culture provides a specific diagnosis in 24 to 48 h, with a sensitivity equal to or greater than that of conventional culture. This method also permits quantification of the viral load (3, 4). The staining of the monolayers in shell-vial culture is generally carried out by means of an indirect immunofluorescence assay (I-IFA) with an anti-immediate early antigen (p72 kDa) monoclonal antibody and subsequent addition of a fluorescence-conjugated immunoglobulin. This assay requires two periods of incubation and a total of 1.5 h for completion (4, 5). The availability of a commercial direct immunofluorescence assay (D-IFA) for detection of CMV prompted us to carry out a prospective, par-

allel, and blinded comparison of the diagnostic efficacy of the I-IFA versus the D-IFA in the detection of CMV in shell-vial culture.

We performed a prospective study of 34 urine and 16 blood samples from immunocompromised patients with previous (< 24 h) cultures positive for CMV. After centrifugation at 1500 rpm for 10 min, the urine samples were inoculated on two shell vials (0.2 ml of the specimen) of the MRC-5 cell line (Vircell; Ingelheim Diagnostica, Spain). For blood samples we used the method of sedimentation in a dextran solution for polymorphonuclear leukocyte (PMNL) extraction, following the recommendations of Gerna et al. (6). The extracted PMNLs were inoculated in two MRC-5 shell vials (500 µl per vial). The vials were centrifuged at 700 x g for 45 min, incubated at 37°C for 18–24 h, and then fixed with a 50/50 mixture of acetone and methanol for D-IFA and with methanol for I-IFA for 10 min at 2–8°C. The vials were stained by an I-IFA with a monoclonal antibody against the p72 kDa immediate-early CMV antigen (clone E13; Argene Biosoft, France) and a D-IFA (clone E13-2; Chemicon International, USA) following the manufacturer's recommendations. Slides were examined by two independent observers for the number of infectious foci, the intensity of fluorescence, and background staining. All infectious foci present in the monolayers were then counted, establishing the total number present in the shell vial.

Statistical analysis was carried out using the Student's t test on paired data. All p values are two-tailed and considered significant if less than 0.05.

The I-IFA method detected all 50 of the positive samples, while the D-IFA detected only 38 (76%) ( $p = 0.001$ ). An analysis of the average value of the number of infectious foci observed in each of the vials showed that the I-IFA gave an average value of 36.3 versus 28.2 provided by the D-IFA ( $p = 0.04$ ). In general, the I-IFA detected between 3 and 66% more infectious foci than the D-IFA. In no case did the D-IFA detect more infectious

foci than the I-IFA. In the quantitative analysis of the two techniques, the D-IFA was found to be less sensitive than the I-IFA ( $p = 0.0001$ ) when the number of infectious foci in the shell vial was < 10 (Table 1). The diagnostic yield of the D-IFA differed according to the sample analyzed. Of the 34 urine samples studied, 4 (11.8%) were negative by the D-IFA, while of the blood samples, 8 (50%) were negative ( $p = 0.0001$ ). The same difference was observed when comparing the type of sample in the 12 D-IFA-negative/I-IFA-positive samples. Of these, 66% occurred in blood samples and 34% in urine ( $p = 0.0001$ ).

The majority of authors recommend the I-IFA method for the staining of cellular monolayers infected with CMV in the shell-vial culture (5–8). This method requires the use of a monoclonal antibody highly specific for CMV and subsequent reaction with a fluorescence-conjugated antimouse immunoglobulin. Each of these steps requires a 30 min incubation period, lengthening the staining process to a minimum of 60 to 90 min. It would seem logical to attempt to shorten the staining period using a D-IFA, which requires only one incubation period. However, the majority of D-IFAs used in the direct diagnosis of viral infections have displayed variable sensitivities when compared with the standard I-IFA method (7, 9).

The use of a second antibody marked with fluorescein-isothiocyanate in the I-IFA seems to amplify the fluorescent image produced by the infected cells (7, 10). The greater intensity of the I-IFA fluorescence makes it possible to carry out the study of the monolayer in a brief period of time, due to the ease with which infected cells are detected. In contrast, in the D-IFA studied, the majority of infected cells displayed a pale fluorescence and were difficult to observe without very thorough examination. As a result, the observation time required for the D-IFA was twice that of the I-IFA. The images obtained by the I-IFA exhibited better qualitative and quantitative results compared with the D-IFA. The poor quality of the fluorescence images would also explain the lower infectious foci count, since the weak fluores-

Table 1: Comparison of indirect and direct immunofluorescence assays in the detection of viral load in shell-vial culture.

No. of infectious foci	No. (%)		P value
	Indirect IFA	Direct IFA	
< 10	33 (100)	22 (66.6)	0.0001
11–50	10 (100)	9 (90)	NS
> 50	7 (100)	7 (100)	NS

IFA, immunofluorescence assay; NS, not significant.

cence displayed in the D-IFA would result in some infectious foci being overlooked. As a result, the samples more affected by the low sensitivity of the D-IFA were those that presented with the lowest viral load. In our study, detection sensitivity was lowest in the blood samples (50%). This is important, since positivity for CMV in a blood sample (viremia) has a high diagnostic value, while its presence in urine (viruria) has less clinical significance (1, 2).

J. Reina\*, M. Munar, I. Blanco

Virology Unit, Clinical Microbiology Service, University Hospital Son Dureta, Andrea Doria 55, 07014 Palma de Mallorca, Spain.

#### References

1. Rubin RH: Impact of cytomegalovirus infection on organ transplant recipients. *Reviews of Infectious Diseases* 1990, 12, Supplement: 754-766.
2. Gerna G, Parea M, Percivalle E, Zipeto D, Silini E, Bararini G, Milanesi G: Human cytomegalovirus viraemia in HIV-1-seropositive patients at various clinical stages of infection. *AIDS* 1990, 4: 1027-1031.
3. Gleaves CA, Smith TF, Shuster EA, Pearson GR: Comparison of standard tube and shell vial cell culture techniques for the detection of cytomegalovirus in clinical specimens. *Journal of Clinical Microbiology* 1985, 21: 217-221.
4. Arens M, Owen J, Hagerty CM, Reed CA, Storch GA: Optimizing recovery of cytomegalovirus in the shell vial culture procedure. *Diagnostic Microbiology and Infectious Disease* 1991, 14: 125-130.
5. Shuster EA, Beneke JS, Tegtmeyer GE, Pearson GR, Gleaves CA, Smith TF: Monoclonal antibody for rapid laboratory detection of cytomegalovirus infections: characterization and diagnostic application. *Mayo Clinic Proceedings* 1985, 60: 577-585.
6. Gerna G, Revello MG, Percivalle E, Morini F: Comparison of different immunostaining techniques and monoclonal antibodies to the lower matrix phosphoprotein (pp65) for optimal quantitation of human cytomegalovirus antigenemia. *Journal of Clinical Microbiology* 1992, 30: 1232-1237.
7. Gleaves CA, Lee CF, Kirsch L, Meyers JD: Evaluation of a direct fluorescein-conjugated monoclonal antibody for detection of cytomegalovirus in centrifugation culture. *Journal of Clinical Microbiology* 1987, 25: 1548-1550.
8. Sorbello AF, Elmendorf SL, McSharry JJ, Venezia RA, Echols RM: Rapid detection of cytomegalovirus by fluorescent monoclonal antibody staining and in situ DNA hybridization in a dram vial cell culture system. *Journal of Clinical Microbiology* 1988, 26: 1111-1114.
9. Johnston SLG, Siegel CS: Evaluation of direct immunofluorescence, enzyme immunoassay, centrifugation culture, and conventional culture for the detection of respiratory syncytial virus. *Journal of Clinical Microbiology* 1990, 28: 2394-2397.
10. Gardner PS: Immunofluorescence. In: *Specter S, Lancz G.J (ed): Clinical virology manual*. Elsevier, New York, 1986, p. 95-109.