3. Kelly CP, Pothoulakis C, LaMont JT: The authors reply. New England Journal of Medicine 1994, 330: 1755.
4. Peterson LR, Kelly PJ: The role of the clinical microbiology laboratory in the management of *Clostridium difficile*-associated diarrhea. Infectious Disease Clinics of North America 1993, 7: 277–293.
5. Arthur M, Molinas C, Depardieu F, Courvalin P: Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. Journal of Bacteriology 1993, 175: 117–127.
6. Mundy LS, Shanholtzer CJ, Willard KE, Gerding DN, Peterson LR: Laboratory detection of *Clostridium difficile*: a comparison of media and incubation systems. American Journal of Clinical Pathology 1995, 103: 52–56.
7. George WL, Sutter VL, Citron D, Finegold SM: Selective and differential medium for isolation of *Clostridium difficile*. Journal of Clinical Microbiology 1979, 9: 214–219.
8. Peterson LR, Olson MM, Shanholtzer CJ, Gerding DN: Results of a prospective eighteen-month clinical evaluation of culture, cytotoxin testing, and Culturette brand latex testing in the diagnosis of *Clostridium difficile*-associated diarrhea. Diagnostic Microbiology and Infectious Disease 1988, 10: 85–91.
9. Shanholtzer CJ, Willard KE, Holter JJ, Olson MM, Gerding DN, Peterson LR: Comparison of VIDAS *C. difficile* toxin A immunoassay (CDA) with *C. difficile* culture, cytotoxin, and latex test. Journal of Clinical Microbiology 1992, 30: 1837–1840.
10. Walker RC, Ruane PJ, Rosenblatt JE, Lyerly DM, Gleaves CA, Smith TF, Pierce Jr. PF, Wilkins TD: Comparison of culture, cytotoxic assays, and enzyme-linked immunosorbent assay for toxin A and Toxin B in the diagnosis of *Clostridium difficile*-related enteric disease. Diagnostic Microbiology and Infectious Disease 1986, 5: 61–69.
11. Barbut F, Kajzer C, Planas N, Petit J-C: Comparison of three enzyme immunoassays, a cytotoxicity assay, and toxigenic culture for diagnosis of *Clostridium difficile*-associated diarrhea. Journal of Clinical Microbiology 1993, 31: 963–967.
12. Bond F, Payne G, Borriello SP, Humphreys H: Usefulness of culture in the diagnosis of *Clostridium difficile* infection. European Journal of Clinical Microbiology & Infectious Diseases 1995, 14: 223–226.
13. Willey SH, Bartlett JG: Cultures for *Clostridium difficile* in stools containing a cytotoxin neutralized by *Clostridium sordellii* antitoxin. Journal of Clinical Microbiology 1979, 10: 880–884.
14. Levett PN: Effect of antibiotic concentration in a selective medium on the isolation of *Clostridium difficile* from faecal specimens. Journal of Clinical Pathology 1985, 38: 233–234.
15. Samore MH, DeGirolami PC, Tlucko A, Lichtenberg DA, Melvin ZA, Karchmer AW: *Clostridium difficile* colonization and diarrhea at a tertiary care hospital. Clinical Infectious Diseases 1994, 18: 181–187.
16. Gerding DN, Johnson S, Peterson LR, Mulligan ME, Silva J Jr: *Clostridium difficile*-associated diarrhea and colitis: SHEA position paper. Infection Control and Hospital Epidemiology 1995, 16: 459–488.

Comparison of an Automated Enzyme Immunoassay with a Direct Fluorescent Antibody Test and Polymerase Chain Reaction for the Detection of *Chlamydia trachomatis* in Diagnostic Specimens from Male Patients

C.Y.W. Tong*, C. Valentine, O.P. Arya

Endourethral swabs and first-pass urine (FPU) samples from 148 male patients were tested for *Chlamydia trachomatis* by an automated enzyme immunoassay (EIA) (Vidas; bioMérieux, France), a direct fluorescent antibody (DFA) test (MicroTrak; Syva, USA) and two polymerase chain reaction (PCR) methods. *Chlamydia trachomatis* was considered present if a specimen was positive by at least two methods. This expanded criterion identified 27 patients (18%) as truly infected. One of the PCR methods was most sensitive for both types of specimen. When the recommended cut-off value of Vidas was reduced by 50%, its sensitivity on endourethral swabs was comparable to that of the DFA test, but the DFA test performed better with FPU. In general, FPU was suitable only for PCR.

Chlamydia trachomatis is an important cause of sexually transmitted disease. For many years cell culture was the gold standard in the laboratory diagnosis of *Chlamydia trachomatis* infection (1). However, it is increasingly evident that some of the newer molecular nonculture methods are more sensitive than cell culture (2–5). As a result, many researchers have tried to introduce expanded gold standards in the evaluation of tests for *Chlamydia trachomatis* (2, 3, 5). Here, we describe a prospective comparison of three different

Department of Medical Microbiology and Genito-Urinary Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK.

nonculture methods: an automated enzyme immunoassay (EIA) (Vidas; bioMérieux, France), a direct fluorescent antibody test (DFA) (MicroTrak Direct Specimen; Syva, USA) and a semi-nested PCR using primers HP1, HP2, and HP3 (6). These three methods utilise different principles and detect different targets, viz. lipopolysaccharide antigen (Vidas), major outer membrane protein antigen (MOMP) (DFA test) and a fragment of the MOMP gene (semi-nested PCR). An expanded positive criterion was used to define true positive specimens.

Materials and Methods. One hundred forty-eight sequential male patients attending the genitourinary medicine clinic of the Royal Liverpool University Hospital for any reason and who had not had any antibiotic in the preceding three weeks were enrolled into this prospective study. An endourethral swab was taken from each patient using a Vidas specimen collection kit. At least 6 ml of first-pass urine (FPU) was then obtained from each patient. When received in the laboratory, 2 ml of Vidas sample treatment reagent was added to each endourethral swab collection tube. Two ml of FPU was centrifuged at 12,000 x g for 30 min and the pellet resuspended in 350 µl of Vidas sample treatment reagent. Three hundred fifty µl from each of the two prepared specimens was then tested by Vidas according to the manufacturer's protocol. Residual material from each endourethral swab tube and another 2 ml of FPU were centrifuged at 12,000 x g for 30 min and the pellets were resuspended in 75 µl of phosphate-buffered saline, 25 µl of which was put on a slide, air dried and acetone fixed, and stained with DFA reagents according to the manufacturer's recommendations. Semi-nested PCR was performed on 12.5 µl of the resuspended material as described elsewhere (6). All tests were performed blindly and by separate technicians to avoid possible bias in the reading of results.

Although the Vidas protocol recommends a test value of ≥ 80 (obtained by subtracting the fluorescent reading of a standard from that of the sample) as a positive result, we lowered the cut-off value by 50% to 40 to determine whether this improved the sensitivity. As described previously by others (7), we considered the presence of one or more elementary bodies (EBs) in the DFA test as positive. A positive semi-nested PCR result was indicated by the presence of a target band of 380 base pairs (bp) in agarose gel electrophoresis. Doubtful products were confirmed by restriction digestion with *Eco*RI. This gave two fragments of 236 bp and 144 bp in samples that contained the target PCR product (6). Cell culture was not attempted because the Vidas transport medium was incompatible with culture, and urine is unsuitable for culture of chlamydiae (8, 9). A separate swab for culture was not appropriate because one of the purposes of the project was to compare the performance of the three tests on identical samples.

Table 1: Analysis of positive results by a combination of Vidas EIA (revised cut-off), DFA test and semi-nested PCR (SN-PCR) on the two types of specimens. Parentheses indicate number confirmed positive.

Method	Endourethral swabs (ES)	FPU specimens	Combined ES and FPU specimens
All three methods	19	9	21
DFA test + SN-PCR	0	3	1
Vidas + SN-PCR	1	2	1
Vidas + DFA test	1	4	1
Vidas only	0	0	0
DFA test only	3 (1[a])	2 (0)	3 (0)
SN-PCR only	7 (1[a], 2[b])	12 (6[a], 2[b])	9 (3[b])
True positive results	25	26	27

[a] Confirmed by examining the results of the corresponding paired sample.
[b] Confirmed by KL-PCR.
FPU, first-pass urine.

None of the three methods used in this study was considered sufficient on its own as a confirmatory test. A specimen was regarded as truly positive only if it was positive by at least two of the three methods. Specimens positive by one method were correlated to their corresponding paired samples and were considered truly positive if the combination of paired endourethral swabs and FPU gave positive results by at least two methods. Specimens positive by only one method after this exercise were further evaluated by another PCR test (KL-PCR using KL1 and KL2 primers) that amplifies the plasmid DNA of *Chlamydia trachomatis* (4, 10). Such specimens were then considered truly positive if they were positive by the KL-PCR method. Because false-positive results associated with a method could occur in both samples from a patient, patients with results that were positive by one method only and not confirmed by KL-PCR were not considered truly infected. Sensitivity and specificity of each method on the two types of specimens were calculated using the above expanded positive criterion.

Results and Discussion. Using the cut-off level recommended by the manufacturer, the Vidas EIA identified 19 endourethral swabs and 14

Table 2: Percent sensitivity and specificity of Vidas EIA, DFA test, and semi-nested PCR according to the expanded positive criterion.

Method	Endourethral swabs		FPU specimens	
	Sensitivity	Specificity	Sensitivity	Specificity
Vidas				
Cut-off = 80	76	100	54	100
Cut-off = 40	84	100	58	100
DFA test	84	98	62	98
SN-PCR	92	97	85	97

FPU, first-pass urine.

FPU specimens as positive, all of which fulfilled our defined criteria of true positive. By reducing the cut-off value to 40, 21 endourethral swabs and 15 FPU specimens were positive, and all were confirmed as true positive. The DFA test identified 23 endourethral swabs and 18 FPU specimens as positive, of which 21 endourethral swabs and 16 FPU specimens were confirmed as true positive. Semi-nested PCR identified 27 endourethral swabs and 26 FPU specimens as positive, of which 23 endourethral swabs and 22 FPU specimens were confirmed as true positive. One-third of the positive semi-nested PCR products and two-thirds of the unconfirmable ones were subjected to restriction digestion, and all showed the expected digestion pattern, indicating a good specificity of direct agarose gel visualisation.

Ten endourethral swabs were positive by only one method, two of which were confirmed positive by correlating the results from FPU specimens and two by correlating the results of KL-PCR. Fourteen FPU specimens were positive by only one method, six of which were confirmed positive by correlating the results from endourethral swabs and two by correlating the results of KL-PCR. Thus, overall, 25 endourethral swabs and 26 FPU specimens were identified as truly positive (Table 1). The paired specimens of two endourethral swabs and one FPU specimen identified as positive were negative by all three methods. Seventy-six percent (19/25) of endourethral swabs identified as positive were positive by all three methods, while only 35% (9/26) of positive FPU specimens were positive by all three methods. Overall, 27 of 148 patients (18%) were identified as truly infected. Although the study population was nonselective, this relatively high prevalence was not totally unexpected, since most patients attending the clinic had risk factors for chlamydial infection and many were symptomatic.

First-pass urine gave far fewer concordant positive results in all three tests than endourethral swabs (Table 1). This suggests that generally there were fewer EBs in the FPU specimen. Comparison of quantitative DFA test results for positive samples from both endourethral swabs and FPU specimens confirmed this. This possibility was further reinforced by the poor outcome of testing FPU by both Vidas and the DFA test, although satisfactory sensitivity was achieved by the semi-nested PCR. The quantitative results for endourethral swabs and FPU specimens that were both positive by Vidas, however, yielded more variable results, with a number of FPU specimens giving a higher reading than the endourethral swabs. Since Vidas detected lipopolysaccharide antigen rather than EBs, it is possible that some urine samples contained a substantial amount of lipopolysaccharide antigen but few EBs.

The volume of FPU used in this study should be adequate, as another group found no difference between 1 ml and 6–7 ml of urine if high speed centrifugation was used (11). The sequence of sampling swabs and urine, however, may be important, although another group found no effect of changing the sequence in symptomatic patients (8). Nevertheless, because two endourethral swabs were negative by all three methods while the corresponding FPU specimens were positive, FPU could be used as a supplementary specimen to endourethral swabs in diagnosis.

By lowering the cut-off value of the Vidas EIA, the sensitivity of the assay for endourethral swabs was raised from 76 to 84% and that for FPU from 54 to 58%, with no effect on its specificity, which remained at 100% (Table 2). The sensitivity with FPU was much lower than that previously reported, although that with endourethral swabs was similar (12). This is probably the result of testing different patient populations and using different gold standards. The use of an expanded criterion probably had a greater adverse effect on the sensitivity of the assay for FPU than on that for endourethral swabs, which generally con-

tained more targets. These differences in patient populations and gold standards could also explain the improved specificity in this study compared to that reported in a previous study (12).

The DFA test had a sensitivity of 84 and 62%, respectively, for endourethral swabs and FPU specimens. All samples positive only by the DFA test had either one or two EBs visualised. Because the DFA test is a subjective test, the meaning of these unconfirmable low-level positive results is uncertain. Some workers consider any visualisation of EBs as true positive (7). In this study, however, we defined true positive as that which was positive by two methods. Hence, the specificity of the DFA test was lower at 98% for both endourethral swabs and FPU specimens. The effective test volume used for endourethral swabs was comparable in the Vidas EIA and the DFA test, and both tests appeared to have similar sensitivity. However, the DFA test used only a third of the volume of FPU used by Vidas. Despite this, the DFA test had a better sensitivity with FPU. This suggests that the DFA test is better suited for FPU than the Vidas EIA.

Semi-nested PCR was the most sensitive of the three tests evaluated, with a sensitivity of 92% with endourethral swabs and 85% with FPU specimens. The specificity was 97% with both specimen types. In our hands, semi-nested PCR was slightly more sensitive than KL-PCR. Although there are many copies of plasmid DNA and only one MOMP gene per EB, semi-nested PCR had another round of amplification, which may explain its slightly higher sensitivity. However, KL-PCR was used as the arbitrating PCR in this study, as it was well studied, widely used, and known to produce specific results (4, 10).

Examination of clinical notes revealed that 42 patients had urethral discharge. One of these patients had a positive *Neisseria gonorrhoeae* culture and the other 41 were diagnosed with nongonococcal urethritis. Seventeen (40.5%) of these 42 patients, including the patient with gonorrhoea, were positive for *Chlamydia trachomatis* by our expanded criterion. Five of the six patients positive only by semi-nested PCR and one of the three patients positive only by the DFA test were symptomatic. It is therefore possible that samples positive only by semi-nested PCR or the DFA test were, nevertheless, true positive samples.

To conclude, the performance of the Vidas EIA on endourethral swabs from male patients could be improved, without impairing the test's high specificity, by lowering the cut-off value by 50%. Semi-nested PCR was the most sensitive of the three assays. However, the expanded standard used in this study is not yet perfected, and further work is warranted to address the implication of samples that are positive only by the DFA test and semi-nested PCR. Although FPU samples are much easier to obtain than endourethral swabs, recognition of *Chlamydia trachomatis* in them by conventional antigen detection methods appears unsatisfactory. With the availability of standardised commercial amplification methods such as Amplicor (Roche, Switzerland) and Ligase Chain Reaction (Abbott, USA), the use of FPU and its relative advantage over the use of endourethral swabs should be further evaluated.

References

1. Smith TF: Chlamydia. In: Schmidt NJ, Emmons RW (ed): Diagnostic procedures for viral, rickettsial and chlamydial infections. American Public Health Association, Washington DC, 1989, p. 1165–1198.
2. Bassiri M, Hu HY, Domeika MA, Burczak J, Sevensson L-O, Lee HH, Mardh P-A: Detection of *Chlamydia trachomatis* in urine specimens from women by ligase chain reaction. Journal of Clinical Microbiology 1995, 33: 898–900.
3. Chernesky MA, Lee H, Schachter J, Burczak JD, Stamm WE, McCormack WM, Quinn TC: Diagnosis of *Chlamydia trachomatis* urethral infection in symptomatic and asymptomatic men by testing first-void urine in a ligase chain reaction assay. Journal of Infectious Diseases 1994, 170: 1308–1311.
4. Mahony JB, Luinstra KE, Jang D, Sellors J, Chernesky MA: *Chlamydia trachomatis* confirmatory testing of PCR-positive genitourinary specimens using a second set of plasmid primers. Molecular and Cellular Probes 1992, 6: 381–388.
5. Thejls H, Gnarpe J, Gnarpe H, Larsson P-G, Platz-Christensen J-J, Ostergaard L, Victor A: Expanded gold standard in the diagnosis of *Chlamydia trachomatis* in a low prevalence population: diagnostic efficacy of tissue culture, direct immunofluorescence, enzyme immunoassay, PCR and serology. Genitourinary Medicine 1994, 70: 300–303.
6. Palmer HM, Gilroy CB, Thomas BJ, Hay PE, Gilchrist C, Taylor-Robinson D: Detection of *Chlamydia trachomatis* by the polymerase chain reaction in swabs and urine from men with non-gonococcal urethritis. Journal of Clinical Pathology 1991, 44: 321–325.
7. Thomas BJ, Evans RT, Hawkins DA, Taylor-Robinson D: The sensitivity of detecting *Chlamydia trachomatis* elementary bodies in smears by use of a fluorescein-labelled monoclonal antibody: comparison with a conventional chlamydial isolation. Journal of Clinical Pathology 1984, 37: 812–816.

8. Chernesky MA, Castriciano S, Sellors J, Stewart I, Cunningham I, Landis S, Seidelman W, Grant L, Devlin C, Mahony J: Detection of *Chlamydia trachomatis* antigens in urine as an alternative to swabs and cultures. Journal of Infectious Diseases 1990, 161: 124–126.
9. Smith TF, Weed LA: Comparison of urethral swabs, urine and urinary sediment for the isolation of *Chlamydia*. Journal of Clinical Microbiology 1975, 2: 134–135.
10. Mahony JB, Luinstra KE, Sellors JW, Jang D, Chernesky MA: Confirmatory polymerase chain reaction testing for *Chlamydia trachomatis* in first-void urine from asymptomatic and symptomatic men. Journal of Clinical Microbiology 1992, 30: 2241–2245.
11. Thomas BJ, Gilchrist C, Hay PE, Taylor-Robinson D: Simplification of procedures used to test urine samples for *Chlamydia trachomatis*. Journal of Clinical Pathology 1991, 44: 374–375.
12. Steingrimmson O, Olafsson JH, Sigvaldadottir E, Palsdottir R: Clinical evaluation of an automated enzyme-linked fluorescent assay for the detection of chlamydial antigen in specimens from high risk patients. Diagnostic Microbiology and Infectious Disease 1994, 18: 101–104.

Types of Methicillin-Resistant *Staphylococcus aureus* Associated with High Mortality in Patients with Bacteremia

T. Nada[1], S. Ichiyama[1,2*], Y. Iinuma[2], K. Inuzuka[4], H. Washida[5], M. Ohta[3], K. Shimokata[2], N. Kato[3], N. Nakashima[1]

Forty-seven strains of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from 47 patients with bacteremia were analyzed by chromosomal DNA digestion pattern using pulsed-field gel electrophoresis and evaluated for serological coagulase type, enterotoxin type, and toxic shock syndrome toxin-1 production. The mortality rate was significantly higher in the older patients (≥ 51 years of age) than in the younger patients (≤ 50 years of age) (50% vs. 4%, p=0.0007). Methicillin-resistant *Staphylococcus aureus* strains of serological coagulase type II were more likely to be associated with mortality in older patients than were strains of the other types (p=0.037).

[1]Department of Clinical Laboratory Medicine, Nagoya University Hospital, [2]First Department of Internal Medicine, and [3]Department of Bacteriology, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan.
[4]Department of Clinical Laboratory Medicine, and [5]Department of Urology, Anjo Kosei Hospital, Aichi Prefecture, Japan.

Methicillin-resistant *Staphylococcus aureus* (MRSA) sometimes causes life-threatening infections in patients with severe underlying conditions or in postoperative patients (1–4). Among these infections, MRSA bacteremia is one of the most serious infections, with a high mortality rate (2–8). The severity of MRSA infections, including MRSA bacteremia, depends on both the clinical characteristics of the patients and the microbiological characteristics of the causative bacteria (2, 3). We previously reported the usefulness of pulsed-field gel electrophoresis (PFGE) in comparing genomic DNA fingerprints among MRSA isolates for epidemiologic studies (9). In the present study we analyzed both the clinical characteristics of the patients and the microbiological characteristics of MRSA isolates associated with the mortality rates of MRSA bacteremia.

Patients and Methods. A total of 47 hospitalized patients with MRSA bacteremia were evaluated: 18 from Nagoya University Hospital from February 1991 to October 1992 (with the exception of 1 strain isolated in June 1989) and 29 from Anjo Kosei Hospital from January 1988 to August 1992. Nagoya University Hospital is a 900-bed, tertiary-care, university-affiliated hospital, with an ICU and a neonatal ICU (NICU). Anjo Kosei Hospital is a 680-bed hospital with an ICU and an NICU. Both are located in Aichi Prefecture, Japan. Patients were considered to have MRSA bacteremia when two or more blood cultures were positive for MRSA, or when one blood culture was positive for MRSA and the clinical course was consistent with systemic staphylococcal infection.

In both hospitals during the study period, blood cultures were performed using blood culture bottles (BCB System; F. Hoffmann-La Roche, Switzerland). All of the 47 MRSA isolates from the patients with bacteremia were stored at −80°C in skim milk until use. Susceptibility testing for the strains was performed by the Kirby-Bauer method (10) using an oxacillin disk.