J Clin Pathol 1980;33:571-574

An evaluation of the Replireader in the identification of Enterobacteriaceae isolated from urine and in the recording of sensitivity tests performed by an agar dilution method

PAMELA M WATERWORTH

From the Department of Microbiology, University College Hospital, London WC1E 6AU, UK

SUMMARY In the Replireader system for identifying Enterobacteriaceae, plates of biochemical media are inoculated with a replicator and the results are put into a computer. The machine correctly identified 92·2% of 734 strains of Gram-negative bacilli isolated from urine; it was incorrect in 0·8% and failed to recognise 7%. The Replireader was also used to record the results of sensitivity tests using a plate dilution method in which the drugs were provided by impregnated filter papers (Adapads).

The introduction of replica plating devices has made it possible to inoculate large numbers of plates with 30 or more organisms very quickly. This not only greatly facilitated the determination of minimum inhibitory concentrations (MIC) but also led subsequently to the use of agar plates containing selected 'break-point' concentrations of antibiotics for routine sensitivity testing.

Dilution methods are claimed to have some advantages over the disc test, the most important of which are said to be that they are much less affected by the size of the inoculum, and that they are easier to read and interpret. One of the main disadvantages of the method is that plates inoculated with spots of inoculum are not only extremely boring to read, they are also very easy to misread when only a small number of strains have, or have not, grown. When MIC are being read, the misidentification of one spot is very often apparent when the next plate is read, but this is seldom the case when break-points are used.

The Repliscan (Cathra) was developed not only to reduce the tedium of reading this type of sensitivity test, but also to perform another equally repetitive and time-consuming task in the clinical laboratory, namely, the identification of Enterobacteriaceae. The Replireader (Cathra) is a smaller machine using the same memory bank and performing the same functions as the Repliscan; as this both costs less and requires less space, it seemed more suitable for small to medium sized laboratories. The present study was carried out to assess the value of the Replireader in the identification of Gram-negative bacilli isolated from urine and in the interpretation of sensitivity tests done on these organisms by the break-point method.

Received for publication 22 November 1979

Material and methods

THE REPLIREADER (CATHRA)

In this system 16 plates containing different biochemical media, and up to 23 containing antibiotics, are inoculated with the Replicator (Cathra); up to 36 strains can be tested on 85 mm plates. After overnight incubation each plate is placed in turn on a viewing box. A spot light illuminates the first spot of growth (from below) to permit visual reading of the result, which is then recorded electronically and fed into a computer. The light then moves to the next spot and the process continues throughout the plate. When all the plates have been read a print-out is obtained giving the identification of the organism followed by the results of the sensitivity tests.

The biochemical plates were provided by the manufacturers and the sensitivity plates were prepared in this department.

ORGANISMS TESTED

A total of 734 strains of Gram-negative bacilli were tested. Of these, the majority of strains of

providencia, serratia, and indole-positive proteus were stock cultures; the remaining organisms had recently been isolated from urine. The inoculum was prepared by adding a 2 mm loopful of either an overnight broth culture to 5 ml sterile water, or, more frequently, of a comparable suspension prepared in a small volume of broth from growth on an agar slope. About 0·5 ml of each diluted culture was placed in a well in the Replicator.

SENSITIVITY TESTS

Drug-containing plates were prepared by placing 83 mm filter-papers impregnated with the appropriate amount of drug in 85 mm Petri dishes and covering them with 17 ml Isosensitest agar (Oxoid), to which a further 0·8% Oxoid No. 1 agar had been added, melted, and cooled to 50°C. All plates were refrigerated overnight to permit diffusion. These papers, now commercially available as Adapads, were kindly supplied by Mast Laboratories.

Eight drugs were tested. Two concentrations were used for six of these. If an organism was inhibited by both concentrations it was classed as sensitive, if only by the higher as moderately sensitive, and if not inhibited by either as resistant. Only one concentration of nitrofurantoin and nalidixic acid was used, and the results are given as sensitive or resistant to this. The drugs tested and the concentrations used are given in Table 1.

Table 1 *Drug concentrations used in agar plates*

Drug	*mg/ml in agar*		*Disc content*
Ampicillin	8	64	25
Carbenicillin	64	256	100
Gentamicin	1	4	10
Nalidixic acid	32		30
Nitrofurantoin	32		200
Sulphamethoxazole	16	64	100
Tetracycline	16	64	30
Trimethoprim	2	8	1·25

The results obtained with the Replireader were compared to those that had previously been reported in the diagnostic laboratory. These organisms had been identified according to the classification of Cowan and Steel using home-made biochemical media. Where the results did not agree, further tests were done using the API 20 E system. Sensitivity tests had been done by the Stokes disc method,[1] usually on primary cultures, on Isosensitest agar (Oxoid). The disc contents used are given in Table 1. Where there was a 'major' discrepancy between the results (ie, sensitive by one method and resistant by the other) the disc test was repeated. MIC were also determined for 98 strains.

Results

IDENTIFICATION OF STRAINS

The Replireader correctly identified 92·2% of the 745 organisms tested; six strains (0·8%) were incorrectly identified and 52 (7%) were reported as 'not on file'. The identity of the organisms is given in Table 2. As would be expected in a collection of strains mainly from urinary tract infections, 86% were *Escherichia coli*, *Klebsiella aerogenes*, or *Proteus mirabilis*, and the failure rate among these was only 4% despite the relatively high number of *P. mirabilis* that were not identified. The Replireader does not identify non-fermentative organisms, and the result 'affermentative' was accepted as indicating *Pseudomonas* spp. Although acinetobacter was said to be included in the computer data, the machine failed to recognise any of nine strains tested. Citrobacter and enterobacter have been classed together in the Table because of the complexity of the results. There was complete agreement between API and the Replireader with only six strains; a further eight were said to be citrobacter by both methods, but not the same species, and the remainder were citrobacter by one and enterobacter by the other. Some strains of proteus evidently present problems to the API system as well and are classed simply as *Proteus* sp; two of five such organisms were said to be *P. vulgaris* by the Replireader, as were three strains said to be *P. mirabilis* by API.

Table 2 *Identity of organisms tested with the Replireader*

Species	*No. tested*	*No. NOF*	*%*
E. coli	467	11	2·4
P. mirabilis	72	10	13·9
Kl. pneumoniae	102	5	4·9
P. morgani	11	0	
P. vulgaris	12	1	8·3
Serratia	9	2	22·2
Providencia	11	2	18·2
P. rettgeri	4	0	
'*Proteus* spp'	5	3	60·0
Acinetobacter	9	9	100·0
Pseudomonas spp	13	3	23·0
Citrobacter / Enterobacter	24	6	25·0
Total	739	52	7·0

A further six strains (0·8%) were incorrectly identified by the Replireader.
Total number of strains tested: 745.
NOF—not on file.

The laboratory's identification was wrong on eight (1·1%) occasions, but there is no means of knowing whether the error arose from mis-identification or possibly from other causes.

Table 3 *Number of discrepancies between the results of the break-point and the disc tests occurring with each drug (omitting laboratory errors)*

Drug	*No. of discrepancies (%)*	
	Major	*Minor*
Ampicillin	5 (0·8)	28 (4·3)
Carbenicillin	0	15 (2·3)
Gentamicin	0	3 (0·5)
Nalidixic acid	1 (0·15)	2 (0·3)
Nitrofurantoin	13 (2·0)	36 (5·5)
Sulphamethoxazole	16 (2·5)	61 (9·3)
Tetracycline	7 (1·0)	34 (5·2)
Trimethoprim	11 (1·7)	30 (4·6)
Total	53 (1·0)	209 (4·0)

SENSITIVITY TESTS

Sensitivity tests were done on 673 organisms; eight drugs were tested, making a total of 5384 tests. There were 89 major discrepancies, and in 36 of these, involving 20 organisms, the laboratory report was clearly incorrect; in some of these it seemed unlikely that the organism tested with the Replireader was in fact that referred to in the laboratory report. The distribution of the remaining 52 discrepancies (1% of the total tests) between the eight drugs is given in Table 3, together with that of 209 minor discrepancies. A considerable number of the discrepancies arose with strains of *P. mirabilis*; 20 strains found resistant to the single concentration of nitrofurantoin used with the Replireader had been reported as moderately sensitive by the disc test method. Similarly, nine strains reported as resistant to trimethoprim by the disc were inhibited by 2 mg/l in the plate. All but one of the discrepancies seen with carbenicillin, 10 of those with ampicillin, and 14 with nitrofurantoin were found with klebsiella, and in nearly all these the organism appeared more sensitive by the disc test. With tetracycline the variation was nearly always between moderate by the Replireader and resistant by the disc, and it was found that the MIC of such organisms was frequently 32 or 64 mg/l.

The inoculum used for tests with the Replireader was heavier than ideal when testing sulphonamides, and it was sometimes difficult to decide whether a film of growth was significant. With one exception, all the organisms showing discrepancies appeared more sensitive by the disc method, 41 of them being the difference between sensitive and moderate.

Discussion

The purpose of this study has been to assess the use of the Replireader as a tool in the routine work of a diagnostic laboratory examining large numbers of urine specimens and wishing to identify the organisms isolated. The failure of the machine to recognise 7% of the isolates is the same as that reported by Brown and Washington,[2] who examined a much larger number of organisms with the Repliscan; they considered these results to be similar to those obtained with other identification systems. It is understood that the data bank for these machines has recently been enlarged and the number of failures may therefore be reduced. The problems of identifying enterobacter, citrobacter, providencia, serratia, and some proteus, which were also experienced by Brown and Washington, may remain, but the commercial availability of other systems, such as the API 20 E, make it feasible to maintain a back-up service with little trouble.

On 33 occasions organisms were not identified the first time. This was sometimes due to the culture being mixed, which was usually easily recognised; it can also be due to lack of experience. The citrate and the ornithine and lysine decarboxylase plates were not always easy to read but improvement comes with practice.

The results of the sensitivity test obviously depend largely on the break-point concentrations chosen and on the Adapads used to produce these; these will be considered in another paper. The impressions of the Replireader must therefore be largely subjective. The addition of 0·8% agar to the medium was very satisfactory. *P. mirabilis* still swarmed a little but this was never sufficient to interfere with reading the plate. The spots of growth are often less easy to see on plates containing filter papers, particularly when proteus is partially inhibited by nitrofurantoin or tetracycline. On the other hand, sulphonamide tests are perhaps easier because a thin film of growth is not so readily seen. The inoculum used was heavy; it was chosen so as to avoid having to make extra dilutions of the cultures, which is undesirable in routine work. Apart from some problems with sulphamethoxazole there was no evidence of error arising from this.

Although the initial cost of the machine is substantial, it does not subsequently require any special equipment, and the potential saving of both time and money is considerable. This is obviously greater when large numbers of strains are tested, but Brown and Washington[2] considered the Replireader to be an economic proposition for the identification of as few as 10 strains a day. Similarly, if large numbers of sensitivity tests are done, the amount of medium required will be much less than is needed for a similar number of disc tests.

If this machine is to be used at all it would make sense to use it for both purposes. A decision to change from disc tests to a dilution method for

sensitivity testing will be influenced by various factors, but there can be no doubt that the Repli-reader makes reading plates which have been inoculated with a multiple replicator both easier and quicker.

References

[1] Stokes EJ. *Clinical Bacteriology* 4th ed. London: Arnold, 1975:216.

[2] Brown SD, Washington JA II. Evaluation of the Repliscan system for identification of *Enterobacteriaceae*. *J Clin Microbiol* 1978;**8**:695-9.

Requests for reprints to: Pamela M Waterworth, Department of Microbiology, University College Hospital, London WC1E 6AU, UK.