Detection of toxin production by *Corynebacterium diphtheriae*: results of a trial organised as part of the United Kingdom National External Microbiological Quality Assessment Scheme

JJS SNELL, JV DEMELLO, PS GARDNER, W KWANTES,* R BROOKS*

From the Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, Colindale, London NW9 5HT, and the *Public Health Laboratory, Cockett Road, Swansea, SA2 0FA

SUMMARY Four strains of *Corynebacterium diphtheriae* were sent to UK participants in the UK National External Microbiological Quality Assessment Scheme, who were asked to examine the strains for toxin production by in vitro methods. Laboratories achieved 162/176 (92%) and 160/175 (91%) correct results with two rapid toxin producers and 145/175 (82%) with a slow toxin producer. With a non-toxigenic strain 26/175 (15%) laboratories reported toxin production. Of the 173 laboratories reporting on all four strains, only 120 (69%) achieved the correct result for all. There was no significant association between the use of various methods and results, with the exception that laboratories using a full set of positive, weak positive, and negative controls made fewer errors than those not using controls. A number of unsatisfactory practices were revealed by the trial, however, and recommendations on preparation of inoculum, media, peptones, animal sera, and use of controls are made.

The United Kingdom National External Microbiological Quality Assessment Scheme has been described previously.¹ As part of the bacteriology portion of this scheme simulated specimens containing various pathogens are distributed to participants. Although most of these pathogens occur commonly in the UK, rarer but important pathogens are occasionally distributed for educational purposes. *C. diphtheriae* has been distributed on a number of occasions with an average success rate in isolation and identification of 85%. No systematic information had been sought on toxigenicity testing of these strains, and in view of the continued occurrence of this species in the UK² the Public Health Laboratory Service thought it would be useful to investigate the ability of laboratories to demonstrate toxin production in the event of another outbreak.

Material and methods

LABORATORIES PARTICIPATING

All 408 UK laboratories participating in the bac-

teriology portion of the UK National Microbiological Quality Assessment Scheme were included in the trial. Most participants are hospital and public health laboratories.

ORGANISMS

Four strains of *C. diphtheriae* were distributed to participants as freeze dried cultures. The strains were: specimen 793 (NCTC 10648) and 795 (clinical isolate), both rapid toxin producers; specimen 794 (NCTC 3984), a slow toxin producer; and specimen 796 (NCTC 10356), non-toxigenic. The strains were fully characterised and tested for toxigenicity in the Division of Microbiological Reagents and Quality Control (DMRQC) by the method described by Davies³ before dispatch to participants.

QUESTIONNAIRE AND REQUEST FORMS

Participants were asked to test the strains for toxigenicity only if they normally performed this test in their laboratory. Tests for toxigenicity by each laboratory's routine in vitro method were requested and participants were asked to complete a questionnaire giving details of the methods used.

Accepted for publication 28 March 1984

Results

PARTICIPANTS' REPORTS

Reports were received in time for analysis from 177 laboratories. An additional 152 laboratories indicated that they did not perform toxigenicity tests. Reports were not returned from 71 laboratories, and eight returned them too late for analysis. Of the laboratories returning reports in time for analysis, 48 reports were received in DMRQC between seven and 10 days after the distribution, 96 between 11 and 15 days, 24 between 16 and 20 days, and nine between 21 and 23 days. Although 177 reports were received, a few results on individual strains were missing owing to loss of the cultures or similar laboratory mishaps. With the two rapid toxin producers, strains 793 and 795, 162/176 (92%) and 160/175 (91%) laboratories obtained positive results. Of the laboratories reporting negative results for the toxigenic strains, seven recognised their results as invalid owing to negative results in controls. With the slow toxin producer 145/175 (82%) laboratories obtained positive results. With the non-toxigenic strain 26/175 (15%) laboratories reported toxin production. Of the 173 laboratories reporting on all four strains, only 120 (69%) achieved the correct result for all.

PREINCUBATION OF CULTURES

Participants were asked to treat growth obtained from the freeze dried material as if it was that obtained from primary plating of clinical material. Preincubation following subculture on to a suitable medium before performing the toxigenicity test was therefore required. The number of laboratories using various preincubation media were: Loeffler's serum slopes, 70; blood agar, 44; various tellurite containing media, 24; Columbia agar, 14; nutrient agar, 1; Dorset egg, 1; more than one medium, 22; media not stated, 1. The periods of preincubation used by laboratories were: 4 to 7 hours, 57; 18 hours or "overnight," 38; 24 hours, 41; 36 hours, 2; 48 hours, 29; 72 hours, 2; tested at more than one time, 6; period not stated, 2.

METHOD USED

Jameson's method⁴ was used by 65 laboratories, Elek's⁵ by 99, Elek's plus Jameson's by six, and Elek's "modified" in an unspecified manner by seven.

MEDIA USED

Table 1 shows the number of laboratories using various media for the toxigenicity test plate.

Table 1 *Media used*

Medium	No of laboratories using medium
Elek, Southern Group Laboratories	51
Virulence agar, Difco Laboratories Ltd	13
Elek or Elek type, home made	94
Columbia agar	6
More than one medium	1
Various others*	11
Not stated	1

*Includes GC agar base, blood agar base, Elek with glucose substituted for maltose, and agar base with lysed blood and tellurite.

Table 2 *Peptones used*

Peptone	No of laboratories using peptone
Difco proteose No 3	68
Oxoid proteose	9
Difco proteose	53
Oxoid special	4
Evans	11
Oxoid neutralised	2
Oxoid bacteriological	6
Oxoid unspecified	3
BDH unspecified	2
Oxoid tryptone	1
Gibco "columbia mixture"	2
Lab M balanced	1
Lab M "columbia"	1
More than one	2
Not stated	12

Table 3 *Antitoxins used*

Antitoxin	No of laboratories using antitoxin
Berna, Swiss Serum and Vaccine Institute, Bern	65
Lister Institute	63
Difco Laboratories Ltd (paper strips)	12
Wellcome Research Laboratories	11
Imunoloski Zavod, Zagreb	4
Connaught Laboratories, Canada	1
More than one used	2
Not stated	19

PEPTONES USED

Table 2 shows the number of laboratories using various peptones in the test media.

ANTITOXINS

Table 3 shows the number of laboratories using antitoxin produced by various manufacturers.

Laboratories obtained their antitoxin from various sources including hospital pharmacies, 49 laboratories; manufacturer, 47; Department of Health and Social Security, 4; laboratory supplier, 6; other hospitals or public health laboratories, 11; Public Health Laboratory Service Supplies Department, 20; not stated 40. Of the individual antitoxins in use, 83 had not reached their expiry date, two were one year older than the expiry date, 39 were

Table 4 Use of control cultures

Combination of controls	No (%) of laboratories using controls
No controls used	11 (6%)
Rapid positive + slow positive + negative	85 (48%)
Rapid positive + negative	49 (27%)
Rapid positive	17 (10%)
Slow positive + negative	5 (3%)
Slow positive	3 (2%)
Rapid positive + slow positive	5 (3%)
Negative	2 (1%)

two years older, and 19 were three years or more older. Expiry dates were not available from 34 laboratories.

USE OF CONTROL CULTURES

The various combinations of control cultures used are shown in Table 4. Only 85/177 (48%) laboratories used a full set of rapid positive, slow positive, and negative controls and these reported fewer false negative or false positive results (37 of 338) than the 11 laboratories using no controls (14 of 44, $\chi^2 = 14.66$, $p < 0.001$).

Discussion

The results of this trial revealed serious weaknesses in the testing of *C diphtheriae* for toxigenicity by UK laboratories, with only 69% of laboratories achieving correct results with all the specimens. As proficiency testing specimens measure the best that laboratories can do, rather than the average,⁶ this standard of performance can hardly be considered satisfactory. Although an association between various techniques and numbers of correct results obtained was not shown in most instances, many of the practices were contrary to good practice as described in various reviews of in vitro toxigenicity testing.^{3,7,8} Various media were used for preincubation of the cultures before inoculation on to the test media. Although information is lacking on the efficiency of some of these media, freshly prepared Loeffler's serum slopes have been recommended for this purpose. Dorset egg slopes or a good nutrient agar base with added blood or serum either as slopes or as poured plates have also been used successfully at the Public Health Laboratory, Swansea. The period of preincubation appears greatly excessive in some laboratories. Rapidly growing organisms are required for the test and sufficient growth for inoculation can normally be obtained in 6 hours on the culture media recommended above. Indeed, if the test is to have any practical value it is essential to use a medium which promotes rapid growth so that a young subculture is available to inoculate the test

plate and thus obtain an early result. Several media not specifically designed for in vitro toxigenicity testing were used for the test plate. Unless evidence is available that these media work reliably, it is safer to use media specifically designed for the purpose. If other media are used it is essential to ascertain that they are suitable, either by assurance from the manufacturer that they have been tested for the purpose or preferably by testing in the user's laboratory. Although one manufacturer states in its catalogue that columbia agar may be used for toxigenicity testing, the medium is not specifically quality controlled for this property by the manufacturer.

A wide variety of peptones were in use and it is known that peptones vary considerably in their suitability for Elek's test.^{8,9} The use of Evans peptone (now unavailable) or Difco proteose peptone has been recommended.³ Although the catalogues of manufacturers of peptones should give an indication as to which peptones are suitable for toxigenicity tests, it is our experience that batch variation may occur. The onus is on the user to determine the suitability of each batch for the test system in use. Information on the animal sera used to supplement the test medium was not sought in this trial. Sera may vary considerably in their suitability for Elek's test. Traditionally, horse serum is used, but this is subject to batch variation. Newborn calf serum, usually readily available in virus laboratories and easily obtained from commercial sources, has been found to be eminently suitable at the Public Health Laboratory, Swansea, and batch variation has so far not been encountered. However, the suitability of each batch must be tested by the user.

It is disappointing that only 48% of laboratories were using adequate controls. It cannot be emphasised too strongly that controls are an essential part of any microbiological test, especially so with one as technically demanding as Elek's. The use of a rapid toxin producer, a slow toxin producer, and a non-toxigenic strain (all obtainable from the National Collection of Type Cultures, Central Public Health Laboratory, Colindale) as controls must be considered mandatory for this test. The "positive" controls confirm the ability of the system to detect the presence of toxin and, combined with the "negative" control, enable the toxin "line" to be identified.

A number of laboratories complained of difficulties in obtaining antitoxin. The Lister product is no longer available and there has been a supply problem in the past. Antitoxin is now readily available from the Swiss Serum and Vaccine Institute, Bern, and is imported by Regent Laboratories Ltd, Cunard Road, London NW10 6PN. Antitoxin is also stocked by some hospital pharmacies. Antitoxin

normally needs to be diluted for use in in vitro toxigenicity tests and must be pretested to determine the correct concentration to be used. This normally is from 500 to 1000 units/ml.

As with other areas of microbiology surveyed as part of the UK National Quality Assessment Scheme, a large number of variations on a single method are in use. Variations introduced as a result of experiment by workers experienced in a technique may be beneficial, but we question the competence of many laboratories to evaluate fully the effects of the variations revealed by this trial. Unless laboratories are expert in this test there is much to be said for adopting a standard method such as that described by Davies.³ Even if the technique is standardised, the test is technically demanding and needs to be rigidly quality controlled. Although the effect of postal delays cannot be excluded, the long delays in returning reports, with 19% of laboratories returning reports more than two weeks after the distribution, is possibly a reflection of some of the difficulties encountered in performing a non-routine test.

The large amount of quality control needed to ensure reliable results with this test renders it unsuitable for the casual user. Laboratories intending to test clinical isolates must maintain control strains and reagents tested and ready for use to ensure that there will be no delay in reporting.

In view of the large amount of effort needed to ensure satisfactory results with this test and the

infrequency of testing clinical strains, a policy of referral to selected centres would have much to commend it.

References

- ¹ Snell JJS, de Mello JV, Gardner PS. The United Kingdom national microbiological quality assessment scheme. *J Clin Pathol* 1982;**35**:82-93.
- ² Anonymous. Communicable Disease Report United Kingdom July to September 1982. *Community Med* 1983;**5**:63-6.
- ³ Davies J. Elek's test for the toxigenicity of corynebacteria. In: Willis AT, Collins CH, eds. *Laboratory methods 1*, Public Health Laboratory Service Monograph series no 5. London: Her Majesty's Stationery Office, 1974;1-7.
- ⁴ Jameson JE. A modified Elek test for toxigenic *Corynebacterium diphtheriae*. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* 1965;**24**:55-7.
- ⁵ Elek SD. The plate virulence test for diphtheria. *J Clin Pathol* 1949;**2**:250-8.
- ⁶ Lamotte LC, Guerrant GO, Lewis DS, Hall CT. Comparison of laboratory performance with blind and mail-distributed proficiency testing samples. *Public Health Rep Washington* 1977;**92**:554-60.
- ⁷ Brooks R. Guidelines for the laboratory diagnosis of diphtheria. Geneva: World Health Organization, 1974.
- ⁸ Maniar AC, Fox JG. Techniques of an "in vitro" method for determining toxigenicity of *Corynebacterium diphtheriae* strains. *Can J Public Health* 1968;**59**:297-301.
- ⁹ Frobisher M, King EO, Parsons EI. A test in vitro for virulence of *Corynebacterium diphtheriae*. *Am J Clin Pathol* 1951;**21**:282-5.

Requests for reprints to: Dr PS Gardner, Director, Central Public Health Laboratory, Division of Microbiological Reagents and Quality Control, 175 Colindale Ave, London NW9 5HT, England.