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COMPARISON OF THE CONVENTIONAL BIOCHEMICAL TESTS RECOMMENDED FOR THE IDENTIFICATION OF *ESCHERICHIA COLI* IN WATER, IN REPORT 71, WITH THE API 20E SYSTEM

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Abstract

The API 20E technique for identification of *E.coli* was evaluated and the results compared with those obtained by the conventional biochemical tests recommeded in Report 71.

The results obtained showed very close correlation between the two methods. A total of 196 (81.6%) out of 240 isolates were identified as *E.coli* by API. system, while The enventional method ident : field only 194 (80.8%). Therefore the recommended tests in Report 71, which are based on very small number of data was found to be acceptable for the identification of *E.coli*.

The results also showed that, all the colonies showing the typical green metallic sheen on EMB agar are not always *E.coli*, although all the *E.coli* showed typical green metallic sheen colonies on EMB agar.

Introduction

The complete identification of *E.coli* requires a large number of biochemical tests, which are impractical in routine water examination. Report 71(3) recommends that *E.coli* should be differentiated from other members of the coliform group by the ability to ferment lactose with the production of gas and to produce indole in tryptophan water in 24 hours at $44C^0$ with IMVIC (++--) tests.

One of the several diagnostic kits available commercially for the identification of *Enterobacteriaceae* is the API 20E, which provides identification to species level, with an indication of the percentages biotype.

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The API system is a stanardized miniaturized version of conventional procedures for the identification of Enterobacteriaceae. The API 20E also can be used for the identification of other Gram-negative bacteria as well. The API 20E system is designed for 23 standard biochemical tests (1,2,3,4).

The aim of this study was to find out the adequacy of recommended tests in Report 71, for the identification of *E.coli* by comparison with the API 20E results.

Materials and methods

The API 20E system (Biomerieux), were used to test biochemical characteristics of cultured. The API 20E was used as follows:

1- The test isolate was plated out on nutrient agar and was incubated at 37°C for 24 hours.

2- A single colony was picked from the plate and emulsified into 5 ml of sterile distilled water.

3- The API gallery was inoculated according to the API procedure, the gallery was then incubated at 37°C for 24 hours.

4- At 24 hours the gallery was examined, then reagents were added where required.

5- The results of tests in the gallery were recorded and the possible identification of the test isolate was made with the aid of the API differential chart and the analytical profile recognition system. The full detail of procedures is provided with the API system.

The presence of *E.coli* among the isolates was tested by subculturing the isolates into LTB,MMGM,BGBB and tryptone water (TW) at 44°C for 24 hours. After the incubation period, positive gas and acid was recorded with LTB,MMGM,BGBB and the indole test was performed on tryptone water. The IMVIC tests of isolates were carried out according to procedures discribed in Report 71(4).

The results were interpreted as confirmatory for presence of *E.coli*, if the following criteria were observed.

(a) positive gas in LTB, indole in TW at 44°C , and IMViC (++--) tests.

(b) positive gas in BGBB, indole in TW at 44°C , and IMViC (++--) tests.

(c) positive gas in LTB,BGBB, indole in TW and IMViC (++--) tests.

For the colonial appearance of isolates, EMB plates were used. These plates were incubated at 37°C for 24 hours.

Results

The results of gas and acid production, together with colonical appearance of EMB agar plates are given in table 1. As is shown in table 1, all the bottles were positive for gas and acid at 37°C and 44°C. Of the 240 isolates, 204 isolates showed green metallic sheen colonies on EMB agar plates.

The biochemical characteristics of cultures are shown in table 2. Of 204 isolates that showed green metallic sheen colonies on EMB agar, eight isolates were not confirmed as *E.coli*. All of these isolates gave negative gas on LTB, MMGM, and BGBB.

Table 3 shows the comparison between the number of *E.coli* strains which were identified by the biochemical tests recommended in Report 71, and by the API system.

A total of 196 (81.6%) out of 240 isolates were idetified as *E.coli* by the API system, compared with 194 isolates (80.8%) by the biochemical tests. These reuslts shows a close agreement by both systems.

Discussion

The API 20E was found to be more convenient than the conventional biochemical test methods. However, some of the problems of the conventional biochemical methods also exist in the API system as well. A common problem encountered with the conventional biochemical test method is that some strains may be difficult and even impossible to indentify. This occurs because such strains do not give typical results in the test used for characterization. this problem ocurred with the API 20E in such a way that certain identification were not possible because the biotype number derived from the API 20E strip was not listed in the API profile index.

The assessment of test reactions were interpreted with the aid of a colour comparator provided by the API manufacturer. Weak reactions of certain isolates in some of the tests produced borderline results, which could effect the biotype number in the profile and cause incorrect identification. A known strain of *E.coli* was used as a control in each set of experiments and this was found to be useful for assessing borderline reactions of isolates identified as *E.coli* strains.

A strong correlation was found between the API system and conventional biochemical tests recommended in Report 71 for the classification

of *E.coli* strains. The results of this study indicate that the limited number of tests recommend in Report 71 are sufficient for the classification of *E.coli* strains.

It was also interesting to note that some of the isolated which produced typical green metallic sheen colonies on EMB plates were not identified as *E.coli* by both systems. On the other hand all the identified *E.coli* strains produced typical green metallic sheen colonies on EMB plates.

Table 1- The results of gas and acid production, together with morphology and colonial appearance on EMB plates of isolates from wastewater samples on tested media.

Isolation media	Incubation temp.	No. of Isolates	Morphology	Gram-reaction	A + G	Results from subculturing on EMB agar	
						(GM,NM)	NM
MMGM	37°C	60	all rod	all negative	all positive	45	15
MMGM	44°C	60	all rod	all negative	all positive	58	2
LTB	37°C	60	all rod	all negative	all positive	41	19
LTB	44°C	60	all rod	all negative	all positive	58	2

A = Acid (GM, NM) = Mixtures of green metallic sheen and non-green metallic sheen .

G = gas NM = non-green metallic sheen only.

Table 2 - The biochemical characteristics of cultures isolated on MMGM and LTB, from wastewater samples.

Isolation Media	No. of isolates	Incubation temp.	Growth on LTB at 44°C Positive (+)		Growth on MMGM at 44°C Positive (+)		Growth on BCIBB at 44°C Positive (+)		I 44°C (+)	I 37.5°C (+)	MR (+)	VP (-)	C (-)
			acid	gas	acid	gas	acid	gas					
MMGM	60	37 °C	45	45	44	45	43	43	43	44	48	44	49
MMGM	60	44 °C	57	57	57	57	55	55	58	58	60	60	60
LTB	60	37 °C	46	46	46	46	37	37	38	38	50	56	43
LTB	60	44 °C	60	60	59	59	57	57	58	58	59	59	59

Table 3 - Comparison between the number of *E.coli* strains which were identified by the biochemical test recommended in Report 71, and API system.

Isolation Media	Incubation temp.	No. of isolates	No. of isolates identified as *E.coli* by the API systems	No. of isolates identified as *E.coli* by the Report 71
MMGM	37 °C	60	42	41
MMGM	44 °C	60	58	57
LTB	37 °C	60	38	38
LTB	44 °C	60	58	58
Total		240	196	194

The table of 240 cultures were isolated from a wastewater sample.

Referees

1. Geiss, H.K. and Geiss , M. (1992): Evaluation of a new commerical system for the identification of *Enterobacteriaceae* and non - fermentative bacteria, European Journal of Clinical Microbiology 11, 610 - 616.
2. Nord, C.E. , Lindberg , A.A. and Kahlback (1974): Evalulation of five test kits - API , Auxotab , Enterotube , Path Tec and r/b for identification of *Enterobacteriae* , Med. Microbiol. Immunol. 159, 211-220.
3. Report 71 (1985): The bacteriological examinatioin of water supplies , HMSO, London.
4. Shayoganl , M., Mavpin , P.S. , Mc Glynn, D.M. (1978): Evaluation of the API 20E for identification of nonfermentative Gram-negative bacteria, Jornal of Clinical Microbiology 7, 539 - 545.