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Catalase Test as an Aid to the Identification of *Enterobacteriaceae*

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Although the catalase test has been used for many years for rapid differentiation of the genera of gram-positive organisms, little has been said about its use in the family *Enterobacteriaceae*. It was further noted that a wide variety of methods exist for the execution of the catalase test, that there is no universally accepted strength specified for the hydrogen peroxide, and that no gradations for the vigor and speed of the reaction have been mentioned. Under the conditions of the clinical laboratory, we have developed a simple, rapid, and accurate method for the catalase test that has been of great value as an aid in the identification of the *Enterobacteriaceae*. With 3% H_2O_2, it was observed that *Serratia*, *Proteus*, and *Providencia* were vigorous catalase reactors. Only *Salmonella* and rare *Escherichia*, *Enterobacter*, and *Klebsiella* isolates were moderate catalase reactors. *Escherichia* and *Shigella* strains were mostly nonreactive, with less than one-third weakly (+) reactive, whereas most *Enterobacter* strains tended to be weakly reactive. *Klebsiella* strains were divided equally between nonreactive and weakly reactive. In practice, this test was also of great value in discerning nonpigmented *Serratia* cultured from the hospital environment and in detecting mixed flora containing nonspreading *Proteus*.

The importance of the catalase reactions, by which hydrogen peroxide is broken down to water and oxygen, is well known for its application to the differentiation of gram-positive organisms. For example, streptococci and *Diplococcus pneumoniae*, which are catalase-negative, are easily distinguished from staphylococci and micrococci, which are strongly catalase-positive. Similarly, catalase-positive anaerobic diphtheroids can be differentiated from *Actinomyces israeli*, which is catalase-negative, in spite of the nearly identical morphological appearance of these two organisms.

In principle, strict anaerobic organisms are generally thought to be catalase-negative, and aerobic or facultative organisms are considered to be catalase-positive (7, 10). That this is not absolutely true is shown by the failure of the streptococci to be catalase-positive. In like manner, members of the family *Enterobacteriaceae*, consisting of facultatively anaerobic organisms, exhibit great variations both as to genera and strains in their reactions to hydrogen peroxide. Under the conditions to be defined in this report, the catalase test suggests itself as a simple, rapid, and surprisingly accurate test amenable to the skills of the technologists in the clinical laboratory as an aid in the identification of certain genera of the *Enterobacteriaceae*.

MATERIALS AND METHODS

Quantitative responses to hydrogen peroxide were observed with concentrations of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0%. Fresh dilutions of H_2O_2 (technical grade, 30 to 35%) were made daily with deionized water. Several methods were used to observe the reactions. The first method entailed placing one drop of H_2O_2 from a dropper bottle onto a well-developed, isolated colony on an agar plate so that the reaction of one such clone could be observed. The second method was performed by dispensing drops of a visibly turbid culture in sensitivity broth (13 by 100 mm tubes containing 1 ml of Trypticase soy broth) onto microslides with a Pasteur pipette. After one drop of each concentration of peroxide was added to each drop of culture, a cover slip was doppped onto the mixtures. The frosted appearance of the bubbles elaborated under the cover slip indicated the speed of reaction. A third modification was that of dropping H_2O_2 into a turbid sensitivity broth tube and observing the vigor of the reaction. The last method involved dropping either a filter-paper disc or an antibiotic-susceptibility disc into a turbid broth culture prior to addition of a drop of peroxide and then noting the time lapse in seconds before the disc rose

to the surface as a measure of the strength of the reaction. The most sensitive method for detection of gas, however, is under a cover slip. When a weak or delayed catalase reaction occurred, a clone was placed onto a microslide, a drop of peroxide was added, and a cover slip was placed over the slide. Or, if the clone was well isolated, peroxide and cover slip were placed directly upon the colony. Any ambiguity as to weak or negative reactions is quickly dispelled by the absence or presence of trapped bubbles.

The organisms used in these tests were freshly isolated strains from clinical specimens, less than 24 hr old at the time of testing. More than half of the organisms shown in the tables were subjected to all four of the methods of determining catalase activity. Because it was found that the results obtained by any one method were compatible with those from the other methods, only the first method described above was used subsequently.

RESULTS

Table 1 compares the catalase reactions of the genera of *Enterobacteriaceae*. Among the *Escherichia* strains tested, 70% were totally nonreactive, and the remainder produced only weak evolution of gas. Whereas *Shigella* strains were similar to the *Escherichia* strains, *Salmonella* strains tended to produce moderate volumes of gas immediately. The tribe *Klebsielleae* proceeded from weakness to strength by genera; *Klebsiella* strains were equally divided between negative and weakly reactive, and *Enterobacter* strains displayed a reversal of the *Escherichia* results, with 68% weakly reactive. *Serratia* allied itself with *Proteus* and *Providencia*, being vigorously catalase-positive.

In Table 2, the effect of reducing the concentration of H_2O_2 is shown. The best comparison was seen between the concentrations 0.5 and 3.0%. At the lower concentration, *Escherichia*, *Klebsiella*, and *Enterobacter* were essentially reduced to catalase-negative, whereas *Serratia* remained almost always positive. It appears that the 0.5% concentration can be used to differentiate *Serratia* from the *Klebsiella* and *Enterobacter* strains, which are moderately reactive at the 3.0% level.

DISCUSSION

That the catalase test is simple, rapid, and inexpensive is evident. Its usefulness, however, within the family *Enterobacteriaceae* requires the establishment of two additional characteristics: applicability and accuracy. In the literature, statements concerning catalase production are frequently made as absolutes, without qualification as to weak or strong, slow or rapid evolution of gas. Only rarely is the method of testing stated, even in texts. A recent publication mentioned an organism as being catalase-positive; two pages later it was casually revealed that 35% H_2O_2 was used. Another author proposed that 20% peroxide containing methylene blue dye be used under a cover slip for greater visibility (9). Even the texts most often used for reference differ in the concentrations suggested. Many recommend 10% H_2O_2 poured over a slant culture (4–6), whereas others designate 3% (1–3, 8). By contrast, one of the most definitive of the early studies delineated the concentration, the method used, and the gradations of vigor of the reactions, and proposed a classification schema for the clinically important organisms based on the two criteria of catalase production and sensitivity to the killing effect of H_2O_2 (7). We found a high degree of agreement with those observations when tested with our own stock cultures. McLeod and Gordon, using 1.5% H_2O_2, recorded their reactions as nil, very slight, slight, strong, and very strong.

TABLE 1. *Comparison of the catalase activity of members of the family Enterobacteriaceae*

Genus	Catalase reaction to 3.0% H_2O_2				Total strains
	Negative	Weak/slow	Moderate	Vigorous	
Escherichia	145 (70.4)[a]	59 (28.6)	2	0	206
Shigella	15 (65.2)	8 (34.8)	0	0	23
Edwardsiella	0	2	0	0	2
Salmonella	1	7 (22.6)	23 (74.2)	0	31
Citrobacter	3	1	0	0	4
Klebsiella	37 (48.1)	38 (49.4)	2 (2.6)	0	77
Enterobacter	30 (28.6)	71 (67.6)	4 (3.8)	0	105
Serratia	0	0	0	93	93
Proteus	0	0	0	66	66
Providencia	0	0	0	4	4

[a] Number of strains (per cent).

Obviously it is presumptuous, then, to consider that everyone is using 3% concentrations. Our suggested protocol, which evolved while in actual use in the clinical laboratory for more than 1 year, represents the consensus of the technologists using the technique every day. No advantages accrued to using concentrations greater than 3%, so it was at this level that we proposed to standardize the technique. Gradations of negative (−), weak or delayed (+), moderate (++), and vigorous (+++) were found to be least susceptible to misinterpretation. Although these gradations are admittedly subjective, that should not imply that they are inaccurate. A few demonstrations with two or three organisms chosen from our daily plates were surprisingly effective in teaching this technique even to our students-in-training. The use of 0.5% H_2O_2 as confirmatory of the vigor of reaction is an aid in identifying colorless *Serratia* and *Pseudomonas aeruginosa* strains, because few other organisms are reactive at such a low concentration, except for staphylococci. At this level, it is better to observe reactions as they appear without using a cover slip. One last warning is to be sure that the colonies or the broth cultures are predominantly rapidly growing organisms. On selective media, many colonies are dead, or nearly so, as early as 48 hr, and some care is necessary before a negative test can be recorded. When in doubt, transfer a colony to sensitivity broth, and do the catalase test when visible turbidity has occurred.

Quality control is simple and practical. At the start of each week, 30 to 35% peroxide is freshly diluted 1:10 in sterile deionized water in small dropper bottles. The vigorous (+++) reaction is tested with *Serratia* strains, pseudomonads, *Proteus* strains, or staphylococci. The weak (+) reaction is observed on an uninoculated blood-agar plate. Because both red and white blood cells are catalase-positive (8), blood-agar and chocolate-agar can be used as controls. The latter, having no viable cells, yields no bubbles, for a negative control, but the bleaching action of the peroxide is further proof of its activity.

Because of the activity of the blood cells, it is advisable to remove colonies on blood-agar to a microslide for the catalase reaction. If this is not possible, an alternate method is to place one drop onto the clone and another drop onto an uninoculated portion of the same blood plate. The differing vigor of the two reactions can then be observed simultaneously. If they are both very weak, cover slips may be used on both areas for more sensitive determinations.

There are innumerable examples of the use of catalase for presumptive identification of organisms in the clinical laboratory. Colorless colonies on EMB, SS, or MacConkey agar plates which mimic stool pathogens are revealed to be *Serratia*, *Proteus*, *Providencia*, or *Pseudomonas* by vigorous catalase reactions, in contrast to salmonellae, which give weak to moderate reactions, or shigellae, which are negative. The test is accurate on Kligler's or citrate slant cultures, and anaerogenous *Serratia* strains, which mimic *Shigella* on Kligler's slants, are instantly detectable with peroxide. Similarly, citrate-positive *Klebsiella-Enterobacter* strains are distinguishable from *Serratia* with catalase reactions. Colony counts of urine specimens on EMB and blood-agar plates reveal mixtures in what may appear to be pure strains, as *Serratia* and *Proteus* strains react vigorously among the catalase-negative *Escherichia* strains. A rough estimate of the numbers of each in the mixed flora can also be made. On XLD plates, the vigorous catalase reaction allows quick detection of xylose-negative *Proteus*, *Providencia*, or *Pseudomonas* strains, which mimic shigellae. On Hektoen Enteric agar, H_2S-positive *Citrobacter* (−), *Salmonella* (++), and *Proteus mirabilis* (+++) strains are distinguishable, as are H_2S-negative *Proteus* strains, from shigellae, but slow lactose-fermenting *Escherichia* or *Alkalescens-dispar* strains will still mimic shigellae.

The catalase test simplifies epidemiology in the hospital sanitation and infection control

TABLE 2. *Effect of various concentrations of H_2O_2 on the catalase reaction*

Genus	0.5% H_2O_2				3.0% H_2O_2				Total strains
	−[a]	+	++	+++	−	+	++	+++	
Escherichia	66	1	0	0	46	17	4	0	67
Klebsiella	25	1	0	0	11	14	1	0	26
Enterobacter	24	2	0	0	5	17	4	0	26
Serratia	2	23	6	2	0	0	0	33	33

[a] Catalase reaction: − = no reaction; + = weak or delayed; ++ = moderate; +++ = vigorous.

schema, as it quickly points out the colorless *Serratia* and *Pseudomonas* strains that infest the hospital environment.

It has been observed since we have been using the catalase test that in many instances final reports have gone out 1 day earlier, because further tests which require overnight incubation, such as deoxyribonuclease plates or citrate slants, were not needed. The catalase test has earned its place in the laboratory along with the oxidase test, which it supplements admirably.

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