

## A Rapid Field Test for Quaternary Ammonium Salts Used as Germicides\*

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THE increasing use of various types of quaternary ammonium salts as germicides has indicated the need for a rapid, simple test for these materials which may be applied to routine determinations of concentration in the field. In connection with studies on various quaternary ammonium compounds to determine the degree of specificity of their germicidal action, a method has been developed which indicates promise as a routine quantitative field test.

Auerbach (1943, 1944) has shown that in alkaline solutions an anionic indicator combines with cationic quaternary ammonium salts to form a colored complex. The color, however, is similar to that of the anionic indicator, and the results must be determined in degrees of color intensity. The difficulty of accurately gauging such differences under poor lighting conditions, together with the necessary manipulations and attention to detail required by Auerbach's method, indicate that this procedure is not too well adapted to field use.

In a further study of this principle, it has been found that certain anionic indicators in acid solution, when added in excess to a mixture of quaternary salt and ethylene dichloride, produce a yellow-green color in the ethylene dichloride layer of the mixture which contrasts sharply with the blue-violet color in the aqueous layer, thereby rendering the end-point of the

test much more easily visible. An added advantage of the test proposed here is the requirement of only two solutions to perform the test, as against the three required by the other method.

The proposed routine procedure for testing the concentration of bactericidal quaternary salt solutions may be carried out as follows:

- (1) One ml. of the quaternary ammonium salt solution is placed in a small-bore test tube.
- (2) Approximately 1 ml. of ethylene dichloride is added and mixed thoroughly by covering the end of the tube with the thumb and inverting several times. This method of mixing is preferable to shaking, since the layers tend to separate more rapidly after mixing.
- (3) Brom phenol blue indicator solution, buffered at pH 4.5 to 4.8 with McIlvaine's standard citrate-phosphate buffer, is added dropwise (one drop to approximate 0.05 ml.), the contents of the tube being well mixed by inverting after each addition of indicator and the layers allowed to separate before adding the next drop. The concentration of the indicator solution may be varied somewhat, depending on the relative emphasis placed by the user on accuracy and speed. The test was developed on the basis of 0.04 percent brom phenol blue, which gives good sensitivity (Table 1) and excellent reproductibility of results. However,

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TABLE 1

RESULTS OF TESTING VARIOUS QUATERNARY AMMONIUM GERMICIDE SOLUTIONS BY THE PROPOSED METHOD

Quaternary Ammonium Salt

Number of drops of 0.04% brom phenol blue indicator required to give endpoint with germicide concentration:

Trade name	Chemical name	1:14,000	1:12,000	1:10,000	1:8,000	1:6,000	1:4,000
Hyamine 10X	p-tert-octyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride	7	8	10	12	15	21
Hyamine 1622, Polymine D, Phemerol	p-tert-octyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride	7	8	10	12	15	21
Isothan Q4	lauryl pyridinium bromide	7	8	10	12	15	21
Isothan Q15	lauryl isoquinolinium bromide	7	8	10	12	15	21
BTC, Roccal, Zephiran	alkyl (C <sub>8</sub> H <sub>17</sub> -C <sub>18</sub> H <sub>37</sub> ) dimethyl benzyl ammonium chlorides	7	8	10	12	15	21
Tetrosan	alkyl (C <sub>8</sub> H <sub>17</sub> -C <sub>18</sub> H <sub>37</sub> ) dimethyl 3, 4-dichlorobenzyl ammonium chlorides	7	8	10	12	15	21
Quartol, Onyxide	oleyl dimethyl ethyl ammonium bromide	7	8	10	12	15	21
Ceepryn	cetyl pyridinium chloride	7	8	10	13	15	21

in practise it may be considered preferable to sacrifice somewhat on sensitivity in favor of speed, in which case an indicator concentration of 0.16 percent is recommended (Table 2). A

TABLE 2  
ENDPOINTS FOR RAPID TEST, USING STRONG INDICATOR SOLUTION

Germicide concentration in solution tested	Number of drops of 0.16% brom phenol blue required to give endpoint
1:9,000 .....	3
1:7,000 .....	5
1:5,000 .....	7
1:3,000 .....	10

large number of test kits have been assembled and used in the field, incorporating the stronger indicator solution, and have proved very satisfactory. The indicator solution is prepared double strength, according to Clark and Lubs' standards, and diluted with an equal volume of buffer as noted above.

- (4) The number of drops of indicator required to produce a permanent yellow-green color in the ethylene dichloride (lower) layer, contrasted with a blue-violet color in the aqueous (upper) layer, indicates the concentration of quaternary ammonium salt in the germicidal solution (Tables 1 and 2).

It will be noted (Table 1) that all the quaternary germicides available for

testing gave identical results by this method. While it is true that use of an acid medium destroys the specificity of the test, in that amines will give the same reaction, it is felt that the contrasting colors developed are a sufficient improvement in readability under poor lighting conditions to warrant use of the method for routine check tests on rinse solutions known to contain quaternary ammonium salts as the active ingredient.

Preliminary tests have shown that concentrations of 1 percent protein, fat, or starch in the germicide solution reduce the amount of indicator required to give the end-point by about 1 drop when 0.04 percent indicator is used. This may be considered to indicate the proportion of germicide bound by the contaminating material and thus unavailable for action against bacteria.

It is felt that this procedure indicates promise as a routine field test where a rapid and reasonably simple procedure is required for checking quaternary ammonium solutions. It is not claimed nor intended to be an accurate quantitative analytical method. Where such data are desired, techniques such as that of Auerbach or chemical analysis should be employed.

#### REFERENCES

- Auerbach, M. E. Germicidal Quaternary Ammonium Salts in Dilute Solution. A Colorimetric Assay Method. *Ind. Engin. Chem., Anal. Ed.*, 15, 492 (1943).  
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