J. Soc. Cosmet. Chem. **25** 307–323 (1974)

Relative merits of 'in use' and laboratory methods for the evaluation of antimicrobial products

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Presented on 13th November 1973 in Nottingham at the Symposium on 'Evaluation of Product Performance', organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—The relationship between the resistance of cultures grown in the laboratory and on the skin is examined in the light of factors known to influence RESISTANCE OF BACTERIA under laboratory culture. Examples are taken from tests designed to measure the efficacy of HAIR SHAMPOOS, DEODORANTS, BATH ADDITIVES and ANTISEPTICS to show that little correlation exists between activity measured in the laboratory and in practice. Both LABORATORY TESTS and 'IN-USE' TESTS should be carried out side by side as a means of identifying factors which influence the results. With this information design of more realistic tests should be possible.

INTRODUCTION

The methods used for the evaluation of the antimicrobial activity of a product can be divided into three groups.

Simple laboratory tests.

Simulated 'in-use' tests.

In-use tests.

The latter, by definition, refer solely to the evaluation of the product under conditions of usage and not for the description of other types of

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test (e.g. simulated 'in-use' tests). The distinction between the simple laboratory and simulated 'in-use' test is more difficult. It may be argued that any modification of a simple laboratory test, for instance by adjustment of pH, or by addition of body fluids, which bring it closer to the conditions of usage convert the test from a simple laboratory test to a simulated 'in-use' test. Equally, whilst a particular test may be classified as an 'in-use' test for one group of products the same test can only be regarded as a simulated 'in-use' test when applied to a totally different group of products designed for a different purpose. Thus the 'Glove Juice Test' as used by Lowbury, Lilly and Bull (1) is an 'in-use' test when applied to the evaluation of a surgical scrub. However, if the same test is applied to the evaluation of some other type of antimicrobial product, designed for instance as a deodorant, the test must be regarded as a simulated 'in-use' test since factors are present in the test which will not be present in the 'in-use' situation. For the purposes of the present paper simulated 'in-use' tests will be considered along with laboratory tests, and these will be compared with true 'in-use' tests.

The trend, in evaluating finished products making antibacterial claims, is to move away from the use of laboratory and simulated tests and to employ whenever possible 'in-use' tests. This follows the belief that only an 'in-use' test can positively evaluate the true antimicrobial activity of a product. Unfortunately, 'in-use' tests are more difficult to perform than laboratory tests or simulated 'in-use' tests. Moreover, they involve considerably more time and usually a panel of volunteers is required. Consequently, laboratory tests are still widely used during the development stages of a product. It may be argued, however, that if these tests fail to evaluate the final product satisfactorily, they may equally well fail to select the most suitable product during the development period.

The reason for the failure of laboratory tests in the evaluation of an antimicrobial product rests largely with a lack of knowledge of the factors governing performance in actual practice. Once these factors are established there is little doubt they can be applied to design of more realistic *in vitro* methods. At the same time more information is required of the factors influencing performance in *in vitro* methods. By this interplay it should be possible to design much more realistic laboratory tests, but in order to achieve this there is a need for both laboratory tests and 'in-use' methods to be carried out side by side. Illustrations from laboratory tests and 'in-use' tests being carried out by the author's laboratory are used to indicate the kind of factors which influence performance.

RESISTANCE AND SENSITIVITY OF BACTERIA

Simple laboratory tests are usually performed according to the rules of well-established protocols and strict attention is usually paid to details like the temperature at which bacteria are grown, the temperature at which the test is carried out, the age of the culture etc., but attention to this kind of detail is not sufficient.

Our knowledge of the relationship between the resistance of a culture of a particular organism grown in the laboratory and the same organism present on the skin is very scant. *Table I* illustrates the concentration of parachlorometaxylenol (PCMX) required to kill the same strain of *Pseudomonas aeruginosa* when grown in two different culture media. A factor of 10 is involved in the concentration effectively killing the culture yet the only difference between the two culture media resides in the concentration of magnesium ions. The media growing (*Table I*) the more resistant culture

Table I. Concentration of PCMX required to kill culture of *Ps aeruginosa* in medium containing either 4 ppm Mg^{2+} or 40 ppm Mg^{2+}

Organism	Culture medium contains	
	40 ppm Mg^{2+}	4 ppm Mg^{2+}
Ps aeruginosa (NCTC 1999)	2.4 mg g^{-1}	0.15 mg g^{-1}
Ps aeruginosa (recent isolate)	1.5 mg g^{-1}	0.12 mg g^{-1}

contains 36 ppm more Mg^{2+} ions than the media growing the sensitive culture. This is a very minor difference and unless one is aware of the problem it can be completely overlooked. Yet the penalites for doing so may be to reject a worthwhile antibacterial agent. This phenomenon has been repeated with every strain of *Ps aeruginosa* examined within the author's laboratory, including new isolates from patients suffering from *Pseudomonas* infections.

Whilst these results are obtained in a synthetic medium, in which careful control can be maintained over each of the ingredients, an identical situation can be achieved with the more complex nutrient media normally employed in bacteriological laboratories, simply by removing certain ions by the use of ion exchange columns and then replacing them at the required levels. Moreover, differences in the resistance of *Pseudomonas aeruginosa* cultures grown in different complex culture media (e.g. Oxoid nutrient

broth and Oxoid nutrient broth No. 2) to PCMX and other halogenated phenols can be explained in part by the magnesium content of the media. The effect, however, is more complex and phosphate ions have been shown to influence still further the resistance of the culture.

If *Pseudomonas aeruginosa* is transferred from a sensitive to a resistant type media, the culture becomes resistant within a few divisions and *vice versa*. The implications of these findings are important because they indicate that culture of this bacterium in the laboratory, even over a short period of time, can modify the resistance of the bacterium to such an extent that it cannot be related to the resistance of the organism in its natural state.

The influence of the growth medium on resistance is not confined to *Pseudomonas aeruginosa*. The incorporation of either glucose or glycerol into the medium used for the growth of *Staphylococcus aureus* also causes an increase in the resistance of the organism to a number of antibacterial agents which are lypophilic in nature (2, 3). This increase in resistance can be related to an increase in the lipid content of the outer surfaces of *S. aureus*. Whether staphylococci and micrococci, which are present on the skin, possess substantial quantities of lipid in their outer coat has not yet been established and so the question must remain unanswered as to whether glucose should or should not be added to the media used for the growth of micrococci and staphylococci for testing antimicrobial products.

Other, less well defined, factors also have a bearing on the resistance of staphylococci grown in the laboratory. Thus, filtering the growth media through filter paper during its preparation can have a profound effect on the subsequent culture dependent upon the type and grade of filter paper used *(Table II)*.

The difference in resistance probably arises from the absorption of certain minor components of the growth media on to the paper, but the difference highlights the influence that even minor components of a growth medium can exert on the resistance of a bacterium.

Table II. Sensitivity of *Staphylococcus aureus* to PCMX when grown in FDA Broth filtered through different types of filter paper

Filter paper	Concentration of PCMX required to kill culture in 10 min but not in 5 min
Green 803	0.45 mg g^{-1}
Whatmans No. 41	3.0 mg g^{-1}

The above observations are important for another reason also. The resistance or sensitivity shown by the bacterium is not universal to all antimicrobial agents, but is specific to certain types or groups of agent. Failure to recognize that such factors are imposing a possible unnatural resistance or sensitivity on a bacterial culture can lead to the selection of an agent which, whilst performing well in laboratory tests, can perform less satisfactorily in practice. Several factors have been highlighted here, but the question remains how many more, so far unrecognized, factors are influencing the resistance of the cultures used in the laboratory?

Age of culture

Once a factor influencing resistance is established steps can be taken to control it. For example, the age of the culture often has a bearing on the resistance of an organism, and it is generally reported that older cultures are more resistant than younger ones. However, this is by no means true and the reverse may be equally true for certain bacteria. Thus, the individual worker must decide, very often quite arbitrarily, whether to use a young or old culture. Either way he cannot be certain whether the resistance is related to the resistance existing in practice.

Numbers of bacteria used in test system

It is well known that the number of organisms used in a test may considerably affect the results obtained, particularly if the antibacterial agent is one which is highly active by virtue of a large proportion of the molecules binding to the active site. In laboratory testing less emphasis is usually placed on using realistic numbers of bacteria, more emphasis being placed on using a standard number, somewhat higher than those known to occur naturally. It is believed that the excess numbers may offset to some extent the exclusion from laboratory tests of other inactivating factors known to occur in practice. Eighteen to twenty-four hour cultures grown in a nutrient broth are often used on the assumption that the numbers of bacteria from such a culture remain fairly constant. This assumption is, however, only correct if the culture is treated in an identical manner each time it is grown. For instance, a 24-h static culture of a typical test bacterium grown for the testing of an antiseptic will produce approximately 2×10^8 organisms/g.

However, if this tube is removed from the incubator 3 or 4 h before it is used and gently shaken to examine the growth the numbers of bacteria may increase to 5×10^9 or greater by the time the culture is ready to use. As oxygen is often the rate limiting factor in a static culture containing 10 ml of broth in a tube of $\frac{3}{8}$–$\frac{1}{2}$ in diameter, the gentle shaking action has the effect of aerating the medium and permitting further growth to take place. Thus an unsuspecting operator may unwittingly alter the performance of a product merely by excessive keenness.

pH

Quite apart from the resistance of the bacterium other aspects may also play an important part in defining the activity of the product as measured in the laboratory.

The pH of conventional culture medium used for growth of bacteria usually ensures that testing is carried out around pH 7 or thereabouts unless the product is sufficiently buffered at either lower or higher pH to effect a change in the pH of the culture medium, or alternatively unless steps are taken to buffer the test medium to some alternative pH. A test carried out

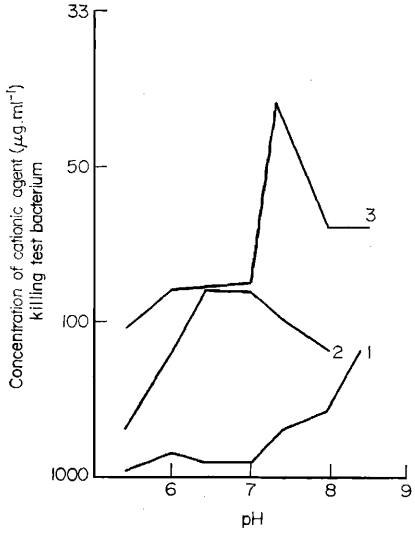

Fig. 1. The relationship between bactericidal activity of a cationic antimicrobial agent and the pH of the test medium. 1, *Staphylococcus aureus*. 2, *Pseudomonas aeruginosa*. 3, *Escherichia coli*.

at pH 7 may give a false impression of activity for cosmetics, the extent of which will depend on the pH profile of the antimicrobial agent incorporated into the product. A pH closer to that of the skin is to be preferred.

Cationic substances in particular show a marked diminution of activity at acid pH as may be seen in *Fig. 1* which shows the relationship between the pH and activity of a cationic antibacterial agent.

Between the range pH 5 and pH 8 this compound suffers as much as a four-fold reduction in activity when examined by a laboratory test.

A similar pH dependence may be seen if the compound is tested on skin which has been artificially infected with a series of test bacterium *(Table III)*.

Table III. Influence of pH on the performance of a cationic agent (500 μg ml^{-1}) when skin is artificially infected with a series of test bacterium

pH of skin	Mean percentage reduction in bacteria achieved 5 min after application of culture. Cationic agent and buffer applied to skin 1 h previous before addition of bacteria		
	S. aureus	*E. coli*	*Ps aeruginosa*
4.5	34	14	5
6.5–7.5	87	97	88

Even products which are designed to maintain the acid mantle of the skin may show, particularly upon dilution, a wide variation between the pH appertaining at the skin site and in the laboratory test tube as is indicated in *Table IV*. For those products whose activity is not materially affected by pH the difference of two units is probably insignificant, but with other products based upon antimicrobial agents which are pH sensitive a difference of two units may have a significant bearing on the results obtained.

Table IV. Influence of culture on pH of product

Product tested	pH of solution before addition of culture	pH of dilution after addition of culture	pH of dilution when applied to skin
Neat	5.7	5.7	5.9
1 : 3	6.0	6.3	5.7
1 : 10	6.2	6.3	5.4
1 : 100	6.7	7.0	5.0

pH of culture = 7.2. pH of skin = 5.0.

Effect of substances present on the skin

Currently laboratory evaluation of a product suffers from the disadvantage that it cannot take account of all the substances present on the skin which might influence the performance of the antimicrobial agent. Organic excretions from the skin can be demonstrated, in laboratory tests, to inactivate many antibacterial agents, yet these same antimicrobial agents when applied to the skin exert an antibacterial effect. The reasons for this are simple; they relate to the relative quantities of the excreted material. The quantity of substances such as sebum, fatty acids, proteins and soap, etc. present on the skin are relatively small, and because of this the antibacterial agent is able to act competitively between these substances and the bacterial cells. In most laboratory tests in which attempts are made to represent the situation appertaining on the skin, material representing organic excretions is usually used in excess and moreover is often added to the product prior to the addition of the bacterial culture. This latter action in itself can have a profound effect as may be seen from *Table V* in which PCMX is shown to be eight times more active when 10% blood is added along with the culture rather than prior to the addition of the culture.

Table V. Concentration of PCMX killing *Staphylococcus aureus* in the presence of 10% blood when the blood is added before the culture and with the culture

	Concentration of PCMX required to kill 0.5 ml 24 h culture added to 5 ml of PCMX solution plus 0.5 ml blood
Before the culture	4.8 mg ml^{-1}
With the culture	0.6 mg ml^{-1}

Although the quantity and quality of organic excretions on the skin may differ from person to person and in the individual on different sites and at different times it is necessary to establish these limits so that these limits may be applied in time to both the laboratory and simulated 'in-use' tests.

Design of tests

Design of test, be it a laboratory test, simulated 'in-use' test or 'in-use' test, is very important. However, it is often impossible to predict beforehand some of the factors which might be involved.

In designing antiseptics it is desirable that the working strength solution should give a 100% reduction in bacteria. Consequently, methods like the Rideal Walker Test, Chick-Martin Test and the A.O.A.C. Phenol Coefficient Tests (suitably modified to take account of organisms which are currently a problem) are used in the evaluation of the product. It is usual on the basis of these results to fix the 'in-use' concentration of the product, allowing, of course, a considerable safety margin and then to check this both in simulated and in 'in-use' tests.

In designing a hair shampoo for which claims can be made about the reduction of bacteria on the hair it would seem logical to carry out a similar type of test using a species likely to be representative of the most prominent bacterium to be encountered in practice.

A simple test was carried out on a number of possible formulations in which the extent to which the product could be diluted and yet still kill

Table VI. Comparison of performance between a formulation containing a number of different antibacterial agents (a) by a simple laboratory procedure and (b) by an 'in-use' test

Product	(a) Degree by which shampoo may be diluted and yet still kill *Staph. aureus* in 10 min but not in 5 min	(b) Average reduction in colony forming units from switches of hair measured before and immediately after shampooing	
		Week 1	Week 2
Shampoo base plus 0.2% *Bronopol**	1 : 30	98%	89%
Shampoo base plus zinc *Omadine*	1 : 200	93%	88%
Shampoo base plus 0.2% *Bronopol*+ 0.25% hexachlorophane B.P.	1 : 10	92%	87%
Shampoo base plus 0.2% *Bronopol*+ 0.25% *Fentichlor*†	Less than 1 : 5	80%	82%
Shampoo base plus 1.6% Iodophor	Less than 1 : 5	75%	81%
Shampoo base (no germicide)	Less than 1 : 5	35%	—
Water	—	5%	—

* *Bronopol*—The Boots Co. Limited, Nottingham.

† *Fentichlor*—The Cocker Chemical Co., Oswaldtwistle, Lancashire.

Staphylococcus aureus in 10 min but not in 5 min was determined. The results from this test are compared with the percentage reduction obtained from an actual 'in-use' trial in which the number of bacteria recovered from adjacent switches of hair immediately prior to and immediately after shampooing were determined *(Table VI)*.

There is little correlation between the laboratory test and performance in practice. From the laboratory test it would be assumed that the base containing zinc *omadine** would perform significantly better than the other products, but from the 'in-use' test this is not the case. The laboratory test is inadequate since it measures only the concentration of product giving approximately a 99.997% or greater kill at a time between 5 and 10 min under idealized conditions. It gives no information as to the activity of the 'in-use' dilution which is the important concentration.

Measurement of the antimicrobial activity of bath products by laboratory methods and in-use methods

The importance of working with the use dilution may be seen when comparing the activities of several possible bath additive products containing different antimicrobial agents.

By applying the simple laboratory method used above, to determine the extent to which the products can be diluted and yet kill *Staphylococcus albus* in 10 min but not in 5 min, it is assumed that the product based upon PCMX would perform better than either a product based upon cationic agents or upon *Vespedol*† *(Table III)*. The 'in-use' results, however, do not bear out these findings. As with the previous example, the use-dilution has not been studied and moreover only a percentage kill of the order of 99.997% or greater has been measured. There is need to know the relationship between a variety of dilutions and percentage kill (*Table VII)*.

When the percentage kill for each of the antimicrobial agents in the bath additive products is plotted as a function of the dilution a totally different picture emerges *(Fig. 2)*.

Vespedol, whilst requiring a relatively high concentration to give a 99.997% kill, reduces the number of bacteria to below 99% at a very low concentration, 'the slope of the graph between 99 and 100% being almost horizontal. The cationic substance shows a similar but less pronounced

* Olin Corporation.

† Registered Trade Mark, Reckitt & Colman.

Table VII. Comparison of antimicrobial activity of three bath additive products in a simple laboratory test and in an 'in-use' test

Product	Laboratory test	'In-use' test	
	Extent to which the product may be diluted yet kill *S. albus* in 10 min but not in 5 min	Percent reduction in numbers of bacteria recovered from skin and bath water after using product compared with soap and water control	
		Skin	Bath water
Bath additive with PCMX	1 : 1500	53 (26–72)	63 (62–65)
Bath additive with QAC	1 : 800	69 (51–89)	78 (55–90)
Bath additive with *Vespedol*	1 : 140	81 (76–87)	95 (90–99)

Figures in brackets represent maximum and minimum reduction.

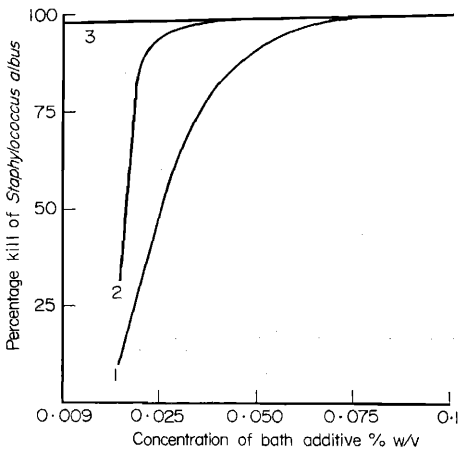

Figure 2. The relationship between concentration of bath additive and percentage kill of *Staphylococcus albus* at 40°C as determined by a simple laboratory procedure. 1, Bath additive with PCMX. 2, Bath additive with QAC. 3, Bath additive with *Vespedol*.

effect, whilst PCMX shows a curve more typical of a bactericidal agent. Abstraction of the percentage kills achieved by a dilution of 1 : 4400 (the concentration used in the 'in-use' test) indicate that the product based upon *Vespedol* is likely to be the most active, followed by the product based upon the cationic agent and in turn by the PCMX based product *(Table VIII)*.

Despite the good agreement with the 'in-use' results the laboratory test belies the 'in-use' performance to some extent, indicating a slightly greater activity for *Vespedol* and a considerably weaker activity for PCMX than occurs in practice. The slightly higher figure for *Vespedol* can probably be

Table VIII. Percentage kill given by bath additives at a dilution of 1 : 4400 as abstracted from results obtained in a laboratory test designed to measure percentage kills at different dilutions

Product	Percentage kill of *Staph. albus* at 1 : 4400
Bath additive with PCMX	25%
Bath additive with cationic substance	80%
Bath additive with *Vespedol*	99.97%

accounted for by the idealized situation exemplified by the laboratory test, but similar results would be expected for the other two products. This, however, is not the case and with PCMX it is difficult to account for the better performance in practice. Certainly, within the laboratory test, no account is taken of the effect of substances present on the skin, nor the accumulation, if any, of the antibacterial agent on the skin. Measurements indicate that this is very low and, moreover, such a situation would not account for the better performance exhibited by PCMX in the bath water as well as on the skin. It must be noted that the laboratory results are based upon a single strain of *Staphylococcus albus*, whereas the skin is host to a number of differing micrococcal species, as well as other bacterial species including diphtheroids.

The majority of normal skin flora when grown in the laboratory differ little in resistance to any one antimicrobial agent, although resistance to different antimicrobial agents varies considerably. However, it is not known how resistance of the various organisms actually on the skin differ in respect to an individual agent or how this resistance matches the resistance of laboratory cultures. Neither is the effect known of the influence of mixed populations on the overall resistance of the bacterial species present on the skin. There is some indication from laboratory tests that mixed populations are less resistant than the individual bacterial species making up the mixed population, but the information to date is rather scant. Such differences may well account for the discrepancy between the two sets of results.

Axillae odour and bacterial numbers

Axillae odour is according to Shelley, Hurley and Nichols (4) and other workers, the result of bacterial action on apocrine sweat. Consequently,

many deodorants make use of antibacterial agents as a means of reducing bacterial breakdown of the apocrine sweat with a view to reducing odour level.

In order to establish whether a significant reduction in axillary odour could be correlated with the antibacterial action of a product, a number of volunteers were asked to wash under the axilla with plain soap and water. The axilla were then covered with a pad which was removed 6 h later and assessed for odour by six assessors using a rating method on a 5-point scale. At the 6 h period the axilla were also sampled for bacterial flora by use of a modified glass cylinder method introduced by Story (5).

After 5 days each volunteer was supplied with a deodorant based upon hexachlorophane and asked to repeat the experiment, this time applying the deodorant to only one of the axilla after washing both axilla with plain soap and water. The remaining axilla served as a control. *Table IX* shows the results obtained for each subject. The log ratio of the bacterial count for the control axilla to that for the test axilla was taken as a measure of efficacy. The significance of this ratio was measured against the ratio for the control situation in which an antibacterial agent was applied to neither axilla. Similar criteria were applied to the odour level.

The results in *Table IX* indicate only a low degree of correlation. Although as yet the reasons for this have not been pinpointed they

Table IX. Correlation between antimicrobial activity of a hexachlorophane based deodorant and its effect in reducing axillary odour

Subject	Antibacterial assessment		Deodorant assessment		Degree of correlation
	Change in log ratio	*t* test	Change in odour	*t* test	
1	4.0	NS	−1.1	*	−
2	7.57	**	−0.5	NS	−
3	−0.70	NS	+0.1	NS	+
4	11.53	***	−0.5	NS	−
5	6.99	**	−1.3	*	+
6	5.70	**	−0·8	NS	−
7	3.79	NS	−0.9	NS	+
8	9.49	***	−1.4	**	+
9	8.97	***	−1.9	*	+

*** The difference is significant at 0.1% level.
** The difference is significant at 1% level.
* The difference is significant at 5%.
NS The difference is not significant.

undoubtedly lie with the antibacterial assessment. The number of bacteria present on the axilla at the time of sampling is relatively unimportant. What is important is the number of bacteria present which are capable of breaking down apocrine sweat. Since hexachlorophane is used as an antibacterial agent it is probable that a number of the bacteria isolated from the axilla are in a state of bacteriostasis and only begin metabolic activity, growth and division once transferred to the agar plate. In order to assess whether this is the case, fairly frequent sampling of the area is necessary to determine whether growth is taking place. However, the very nature of sampling serves to deplete the bacteria already present and to dilute and neutralize the action of the antibacterial agent. Quite clearly a very sophisticated system of measuring the antibacterial effect is necessary, preferably in which the metabolic state of the organisms can be measured on site. In the meantime until such methods are available, by far the easiest method of measuring the efficacy of this type of product is to determine its control in odour production.

Assessment of products intended as antiseptics

The assessment of the antibacterial activity of four products intended for use as antiseptics, by a laboratory test, a simulated 'in-use' test and an 'in-use' test highlights the problems facing anyone trying to design a test.

Measurement of activity within the laboratory by the simple technique of determining how far the product can be diluted and yet still retain activity (loop sampling method), indicates that three out of the four products meet the requirements of a good antiseptic. The fourth does not reach the necessary level *(Table X)*.

However, when the activity is measured by a simulated 'in-use' test in which *Staphylococcus aureus* is painted onto the skin and allowed to dry before application of the antiseptic, subsequent sampling 5 min later reveals that the product failing the loop sampling method reduces the level of bacteria by 100%. An iodophor preparation brings about the same reduction, but the other two products produce a smaller percentage reduction. The situation becomes more complex because the 'in-use' test reveals that none of the products reduce the level of bacteria by 100%, but that the product failing the loop sampling method reduces the level by a mere 28%. The iodophor preparation which performs best in the simple laboratory test performs relatively weakly in the 'in-use' situation, whilst the

Table X. Comparison of the activity of four antiseptic solutions as measured (a) by a laboratory test, (b) by a simulated 'in-use' test and (c) by an 'in-use' test

Antiseptic solution	In-use concentration	Ratio of killing dilution to recommended 'in-use' dilution	Percentage reduction in bacteria artificially applied to forearm (simulated 'in-use' test)	Percentage reduction in bacteria on skin ('in-use' test)
Chloroxylenol B.P.	0.12%	7.5	92	77
Chlorhexidine B.P.	0.05%	1.7	72	68
Iodophor Prep.	1% avI_2	100	100	60
Thiomersal B.P.	0.1%	Product does not kill when tested neat	100	28

other two products show a somewhat better performance. PCMX gives the best result despite its relatively weaker performance than the iodophor in the laboratory test. Without doubt bacteria painted onto the skin are exposed to a greater extent to the antimicrobial agent than are the natural flora, and organisms painted on the skin may be more akin to the transient flora and this may account for the results achieved with this test. The 'in-use' test, employing the method of Verdon (6), was performed on the perineum (7) of women patients in labour and the predominant organisms isolated were *Staphylococcus epidermis* and cutaneous corynebacterium, which is indicative that resident rather than transient flora were involved in the 'in-use' test. The poorer performance in the 'in-use' test of the iodophor can probably be accounted for in terms of the deactivation of the germicide by skin secretions such as protein. Iodophors tend to release iodine relatively slowly and this may be neutralized almost as fast as it is released. Mercurial compounds are not noted for their bactericidal action, but give good bacteriostatic activity. The reason for the good performance of thiomersal in the simulated 'in-use' test is not yet understood.

Test for adequate preservation

Preservation is an important aspect of all formulation work and the simplest type of test to check for 'adequate' preservation is an insult test in which the product is challenged with a number of likely bacteria

which are considered as possible contaminants. This challenge can either be made with single organisms or mixed cultures, and, moreover, may, if required, be repeated at intervals. This procedure can fail, however, primarily because the resistance of the organisms to a preservative may be lower when grown in the laboratory than the resistance which can occur in practice. Since resistance to antimicrobial agents is often variable in many organisms it is necessary to ensure during preservation tests that organisms of a suitable resistance are used.

This can be achieved by growing the organism in a suitable medium to which has been added either the preservative or the product in low concentration. The method of Basset, Stokes and Thomas (8) which uses the above principle has worked well in establishing whether working strength solutions of antimicrobial agents are likely to be contaminated. The results from practical experience correlating 100% with the predictions from the Basset *et al.* method (8).

Conclusions

In comparing the relative merits of 'in-use' and laboratory methods no attempt has been made to review the various methods available; neither has any attempt been made to review the various methods by which bacteria may be recovered from the skin. Such topics are covered in a number of reviews (9–11). Instead the purpose of this paper has been to demonstrate that sufficient data is not yet available to enable laboratory tests to be designed which can represent sufficiently closely, in most cases, the situation appertaining in practice. 'In-use' testing is necessary in order that an insight in the various factors affecting performance may be studied, but 'in-use' testing by itself is incapable of doing this without some interplay from the laboratory tests.

At the present time, therefore, wherever possible both 'in-use' and laboratory testing should be carried out, but if resources restrict both types of evaluation it is far safer to apply 'in-use' tests rather than laboratory tests. The 'in-use' tests have, however, to be very carefully designed, taking into consideration the various factors which may influence the final conclusions reached.

(*Received: 13th September 1973*)

REFERENCES

(1) Lowbury, E. J. L., Lilly, H. A. and Bull, J. P. *Brit. Med. J.* **1** 1251 (1963).
(2) Hugo, W. B. and Franklin, I. *J. Gen. Microbiol.* **42** 365 (1968).
(3) Cowen, R. A. Unpublished observations.
(4) Shelley, W. B., Hurley, H. J. and Nichols, A. C. *Arch. Dermatol. Syph.* **68** 430 (1963).
(5) Story, P. *Brit. Med. J.* **2** 1128 (1952).
(6) Verdon, P. E. *J. Clin. Pathol.* **14** 91 (1961).
(7) Byatt, M. E. and Henderson, A. Personal communication.
(8) Bassett, D. C. J., Stokes, K. J. and Thomas, W. R. G. *Lancet* **i** 1188 (1970).
(9) Lowbury, E. J. L. in Maubach, H. I. and Hildick-Smith, G. *Skin bacteria and their role in infections* (1965) (McGraw-Hill, London).
(10) Gucklhorn, I. R., *Mfg. Chem.* **40** (6) 23; (7) 38; (8) 71; (9) 33; (10) 33; (11) 35; (12) 38 (1969). **41** (1) 42; (2) 30; (3) 26; (4) 34; (6) 44; (7) 51; (8) 28; (9) 82; (10) 49; (11) 48; (12) 50 (1970). **42** (1) 34; (2) 35 (1971).
(11) Selwyn, S. and Ellis, H. *Brit. Med. J.* **1** 136 (1972).