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The use of inactivators in the evaluation of disinfectants

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SUMMARY

Since the earliest days of disinfectant testing bacteriostatic effect has misled many workers in this field. This problem has long been appreciated by some and a considerable battery of neutralizers has been employed, often with great success. Other anti-bacteriostatic measures, used without control, fail to revive damaged though viable organisms.

This paper sets out to describe some of the problems that are encountered in this confused field and the means whereby they may be overcome.

INTRODUCTION

Many pitfalls beset the complex and much fought over subject of chemical disinfectant testing. Not the least of these is the omission, incorrect choice or misuse of an inactivator to neutralize bacteriostatic effect. Much of the basic information on this subject has been available for a long time but it has remained unappreciated by some workers. It therefore seems timely to draw attention, once again, to these matters.

Extravagant claims have been made and much misplaced enthusiasm shown for the disinfectant virtues of particular products when the unwary have mistaken bacteriostasis for a true kill. The error may be exaggerated when an incorrect or insufficient inactivator is employed. Too powerful an inactivator may complete the work the disinfectant has often failed to do.

Misconceptions linger to this day, often influenced by earlier and equally misconceived reports. Many publications on disinfectant testing still fail to recognize the misleading effect of inhibition by the disinfectant or by the so-called inactivator. However there is a growing appreciation of the need for controlled inactivation.

The viability of an organism is most commonly demonstrated by its ability to reproduce. For nearly a century, however, it has been recognized that organisms which have been treated with a disinfectant and which fail to grow *in vitro* and are thus presumed dead, may, in fact, still retain their pathogenicity (Geppert, 1889).

THE PROBLEMS

Bacteriostasis will arise as a result of disinfectant carried over into the recovery medium. It will also occur when disinfectant molecules become attached to the

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cell wall of the organism by weak chemical bonds. Washing the organism, or voluminous dilution, with a non-inactivating medium may overcome the former but is quite ineffective against the latter (Chiori, Hambleton & Rigby, 1965).

The realization that many types of disinfectant are bacteriostatic at vastly greater dilution than those at which they are quickly bactericidal has led to the use of a wide range of chemical and, occasionally, physical neutralizers. The choice of an inactivator should be strictly limited by certain criteria. It must, by definition, neutralize the disinfectant it is used against. It should not give rise to any inhibiting effect, either of its own or as a result of any products formed when it is combined with the disinfectant. Its action should also be fairly rapid; slow neutralization allows continued bactericidal effect long after any timed period has ended.

In reality few, if any, of the commonly used inactivators are completely non-inhibitory and great care must be taken in the use of higher concentrations. Inactivators are employed either as an intermediate stage for diluting or neutralizing any disinfectant carried over before transfer to a growth medium or they may be included in the growth medium itself, be it liquid or solid. Both systems have their disadvantages. In the first, small numbers of survivors may become less apparent simply through dilution. In the second, the inactivator's own inhibitory power tends to have greater effect.

Checking the performance and suitability of an inactivator is no easy process.

First, it must be appreciated that organisms that have been physically or chemically damaged may require growth conditions very different from those needed by undamaged organisms. Low concentrations of disinfectants have been employed to bring about the mutation of organisms, resulting in much altered nutritional requirements (Englesberg, 1952). Selection of a suitable medium for recovery can be done only on the evidence of damaged organisms, a point strongly emphasized by Harris (1963). As a result of damage to the organism the inactivator may exert a different effect (Valko & Dubois, 1944).

No hard and fast rules can be laid down regarding inactivators – the great variety of organisms and test methods prevents this; but general guidelines, before specific checking, may be of some value.

No individual test can supply all the information regarding the inactivator's performance. A modification of the British Standard 3286: 1960 method of checking inactivators may act as a guide. But when recovering damaged organisms only the quantitative comparison of a variety of inactivators and conditions of use will lead to the most advantageous choice at that time.

It will be seen from a study of published work that this procedure is not normally carried out. Strong evidence, especially from non-commercial bodies, is now available to demonstrate the necessity of checking inactivators before and during use. The blind assumption that any concentration of inactivator will work all the time is not good enough.

INACTIVATORS IN COMMON USE

Many different inactivators are in vogue, most often, it seems, as a result of tradition rather than proved necessity. Detailed reading will bring to light countless others that have been superseded by more 'impressive' products. Those in current use include the thioglycollate, thiosulphate and bisulphite salts of sodium, the surfactants Lubrol W, Tween 80 and Lissapol N and various organic compounds of animal origin such as lecithin, cholesterol and serum.

Sodium thioglycollate

Geppert used ammonium disulphide as an inactivator for mercuric chloride. It was later found that the thiols, usually in the form of sodium thioglycollate, would perform the same function (Marshall, Gunnison & Luxen, 1940). So apparently successful was thioglycollate broth, both as an inactivator and in its designed purpose as an anaerobic medium (Brewer, 1940), that its use for sterility testing was made official (U.S.P. XVI 1960). The wisdom of this is to be doubted. Examination of work by McClung (1940) shows that for small numbers of organisms – a condition likely to be met in disinfectant testing – recovery was better in media containing no thioglycollate. Engley & Dey (1970) have reported a toxic effect of thioglycollate against, in particular, *Staphylococcus aureus*. A similar result was obtained in this laboratory against *Pseudomonas aeruginosa* and other organisms.

The status of thioglycollate broth as the official medium for sterility testing in the United States has led to its misuse as a recovery agent against such diverse products as buffered glutaraldehyde and chlorine dioxide (commercial literature). Though undoubtedly effective in neutralizing mercurial compounds, its toxic nature must give rise to concern.

Sodium thiosulphate

Another much misused chemical is sodium thiosulphate. It was employed initially, at very low concentrations (0·1 ml. of 3 % solution per 170 ml. sample) to neutralize chlorine residues in water samples (Mallman & Cary, 1933). For this application the results showed the need for such inclusion. A slightly higher concentration of 0·5 ml. of 1 % solution, per agar plate, was used by Mudge & Smith (1935). Their results showed that, even at this still comparatively low concentration, in 6/18 samples growth was seriously diminished by the presence of the thiosulphate. In a further 2/18 samples growth was not improved by its use.

It can be seen that even this small amount of thiosulphate, when included in the growth medium, has a profound effect on the growth of survivors. Concentrations varying between 0·1 % and 5 % have been used, mostly without any apparent form of control.

Reports on the toxic nature of sodium thiosulphate against, in particular, staphylococci (Kayser & van der Ploeg, 1965; Gross, Cofone & Huff, 1973) agree with unpublished results from this laboratory. The degree of inhibition will vary in different basal media. Other damaged organisms which have shown themselves to be affected include the more chemically resistant pseudomonads and mycobacteria.

Thiosulphate has also proved popular for use against the iodophors. Unfortunately sodium thiosulphate reacts with iodine to form sodium tetrathionate. This compound is itself an inhibitor and as such is used as a selective agent for the isolation of salmonellas.

Elemental iodine is sufficiently inactivated by the organic material in nutrient media (Gershenfeld & Witlin, 1950) as are chlorine and hypochlorite. This makes the use of sodium thiosulphate unnecessary. The inclusion of more organic material in the form of serum may be of some value where higher concentrations of these disinfectant ions are countered.

Sodium bisulphite

Sodium bisulphite is recommended as an inactivator for glutaraldehyde and occasionally against formaldehyde (Rubbo & Gardner, 1965). Though it is often included in growth media, it should only be used as an intermediate diluting stage.

Engley & Dey (1970) have reported the inhibitory nature of bisulphite at low concentrations. Bergen & Lystad (1972) found it a better bactericide than glutaraldehyde alone.

Sodium bisulphite is not used in this laboratory. Nutrient broth has given the best recovery results, provided the concentration of aldehyde in the medium is kept sufficiently low.

Further study possibly based on the work of Nash & Hirsch (1954), may eventually provide a more effective inactivator.

Surfactants

Ever since the introduction of the quaternary ammonium compounds, diguanides and ampholytes as disinfectants, they have been recognized as being bacteriostatic at very high dilutions. Normal or enriched recovery media, though partially effective in reducing the activity of these compounds, are insufficient as inactivators.

Various detergent surfactants have been quoted as enhancing the recovery of inhibited organisms.

Anionic and cationic surfactants often prove themselves very effective inactivators when used in conjunction with undamaged organisms. However they are toxic (Weber & Black, 1948) and should not be used for the recovery of damaged organisms, especially those that are Gram positive.

Nonionic surfactants such as Lubrol W, Tween 80 and Lissapol N have only low toxicity levels against most organisms, with the result that high concentrations may be used, e.g. 7 % Lubrol W (Bergen & Lystad, 1972).

Tests performed in this laboratory show that all three compounds will work well in a given situation. Variations of the disinfectant or the test organism may require the use of a different inactivator. For example, Lubrol W at concentrations of 0·1 % and above is reported as being inhibitory to streptococci. Tween 80 should be used instead (Imperial Chemical Industries, 1973).

Phenol and the phenolics require inactivation (Flett, Haring, Guiteras & Shapiro, 1945) for which Tween 80 appears to be suitable (J. F. Gardner, personal communication).

Table 1

Disinfectant	Inactivator
Aldehydes	Enriched nutrient medium or see Nash & Hirch (1954)
Phenolics Pine Fluids	A non-ionic surfactant, Tween 80 should be the first choice
Ampholytes Diguanides Quaternary ammonium compounds	A non-ionic surfactant, possibly with lecithin
Mercurials	Sodium thioglycollate at a very low concentration
Elemental iodine Bromine Chlorine Hypochlorites Hypobromites	Nutrient medium possibly with sodium thiosulphate at a low concentration
Iodophors Hypochlorites + detergents Hypobromites + detergents	A non-ionic surfactant, Tween 80 should be the first choice

Modern iodophor preparations require inactivation not, as is commonly assumed, by sodium thiosulphate but by a nonionic surfactant. Again Tween 80 produces much improved recovery results.

Surfactants cause lysis of blood. Lower colony counts may occur when these compounds are used as diluents when transfer is to blood agar plates (Davies, 1949). Another form of enriched agar should be used in such cases.

Animal products

Lecithin, cholesterol and serum have been variously described as inactivators. Lecithin is popularly employed with Lubrol W or Tween 80 (Letheen broth).

The use of cholesterol was advocated by McCulloch, Hauge & Migaki (1949) as it was an important constituent of brain and egg yolk, both of which had proved effective inactivators for quaternary ammonium compounds. Its insolubility makes it difficult to use and, as with lecithin, recovery results are very variable.

Serum has little value as an inactivator except against halide ions. It is, on the other hand, of undoubted value as an enrichment for damaged organisms, a subject not covered by this paper.

SUGGESTIONS FOR IMPROVED INACTIVATION

A list of potential inactivators is shown in Table 1. A suggested method of checking them may be of some assistance.

The initial selection of any inactivator may be made using a modification of the British Standard 3286: 1960 method. The modification consists of incubating the containers of inactivator and of inactivator plus disinfectant when the normal test is completed. Growth should occur in both containers. As this test is performed with undamaged organisms it is an insufficient guide on its own.

Final selection of the inactivator and the concentration to be used is probably best made by the following procedure.

1. The organism is subjected to the normal disinfectant test.
2. Similar samples of the disinfectant/organism mixture are placed in a variety of possible inactivators and incubated.
3. The inactivator which allows growth in the greatest number of samples, or, which gives the most prolific growth should be considered the most suitable.
4. Once a particular inactivator has been selected the test should be repeated using a range of concentration of that inactivator.

This procedure must be repeated whenever any variation, such as a change of organism, is introduced into the test.

The same principle may be applied in the selection of the recovery medium.

In every case the concentration of disinfectant in the recovery medium should be kept very low.

Inactivators may be included either in solid or liquid media. Recovery results are often much poorer on agar. Unpublished work in this laboratory shows that wherever possible a liquid inactivator medium should be used.

Damaged organisms invariably have an extended lag phase. Incubation periods of seven days or more should be considered normal. The commonly accepted 48 hr. incubation period is often insufficient.

CONCLUSION

The need for an inactivator step in disinfectant testing must be quite obvious. The necessity of controlling this step is less well appreciated.

Variations between organisms, the damaging agent and the degree of damage will all serve to alter the recovery requirements.

Most commonly used inactivators are inhibitory to some damaged organisms though this may not be apparent from experimental results based on undamaged organisms. Only by the comparison of a variety of inactivator media and conditions, while recovering damaged organisms, will a more suitable choice be made.

The ultimate truth regarding the best recovery conditions may never be known though every effort should be made to enhance the growth of the damaged organisms.

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