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# The laboratory evaluation of iodophor disinfectants with yeast suspensions

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## SUMMARY

The report investigates the variation in results noticed when testing iodophor disinfectants in the presence of a 5% (w/v) yeast suspension.

It was found that these variations were not related to individual iodophor formulations but bore a direct relationship with the storage time of the prepared yeast suspension.

## INTRODUCTION

It is commonly felt that laboratory tests to evaluate disinfectants should simulate natural conditions as much as possible, and to this end the disinfectants should come into contact with some form of organic contaminant.

Various materials have been used, but for some time a 5% (w/v) yeast suspension has been generally accepted, following the work of Garrod (1935). He found a yeast suspension easy to prepare giving both particulate and dissolved organic material. It could be made up in bulk and held in cold storage without any appreciable change in properties, consistent results being obtained with its use. However, with the advent of iodophors which are noticeably affected by organic matter, variability in results have become apparent.

As official tests for the approval of disinfectants incorporate the use of a yeast suspension, the test for the evaluation of General Purpose Disinfectants under The Diseases of Animals (Approved Disinfectants) Act (Ministry of Agriculture, Fisheries and Food) was chosen to investigate whether the variability originated within the disinfectants or within the tests.

## MATERIALS AND METHODS

### *Test organisms*

*Salmonella choleraesuis* (N.C.T.C. 10653 or N.C.I.B. 10383) was used after culture in 10 ml. of nutrient broth for 24 hr. at $37 \pm 1°$ C. Subcultures from the 4th to the 14th were used in the test.

### *Media*

Nutrient broth (Oxoid CM67) prepared according to the manufacturer's instructions was dispensed as (i) 9·5 ml. broth + 0·5 ml. horse serum (Oxoid SR35) in 1 oz. bottles, or (ii) 10 ml. broth in $6 \times \frac{5}{8}$ in. capped test tubes.

Table 1. *Percentage (w/v) titratable iodine in the iodophors*

| Iodophor | Titratable iodine (%, w/v) |
|---|---|
| I | 2·34 |
| II | 3·20 |
| III | 2·81 |

*Hard water*

This was prepared by the W.H.O. method (World Health Organization, 1973) giving a water with a hardness of 342 parts per million and a pH of 5·6. It was sterilized at 120° C. for 15 min.

*Yeast suspension*

This was prepared by the method described in BS 808 (Chick-Martin Test) (1938), but with W.H.O. hard water in place of the distilled water. Blocks of 1 lb. of compressed moist yeast were obtained from Distillers Company (Yeast) Ltd, being marketed as 'Yeast for BSI C10 test'.

*Disinfectants*

Three iodophors (I, II and III) with available iodine content as shown in Table 1 were diluted with W.H.O. hard water. One hundred ml. of the initial dilution were prepared and the four further working dilutions were subsequently obtained by 10 % serial reductions throughout the test procedure.

*Sodium thiosulphate solution*

A 0·392 % (w/v) sodium thiosulphate solution was prepared in deionized water. This was used to check the titratable iodine content of the initial and final test dilutions by a modification of the method of Vogel (1961). The end-point was taken as loss of colour of the iodophor, starch being omitted as an indicator because of its ineffectiveness in the presence of surfactant. By this means it was found that all the dilutions were accurate.

*Basic method*

Using standard aseptic techniques the test for General Purpose Disinfectants was performed as described by M.A.F.F.,* as follows. A 4 % (v/v) dilution of the salmonella culture (viable organism count not less than $10^8$ organisms per ml.) was prepared with the yeast suspension, dispensed in 2·5 ml. vols. in five $6 \times \frac{3}{4}$ in. capped test tubes and placed in a water bath at 4° C. An equal volume of one of the disinfectant dilutions was added to each of these tubes, thoroughly mixed immediately and at intervals throughout the test. After a contact time of 30 min,

* This test has not been published, but an explanatory note setting out the Ministry's test procedures may be obtained from The Animal Health Division 1, Ministry of Agriculture, Fisheries and Food, Government Buildings, Block B, Hook Rise South, Tolworth, Surbiton, Surrey.

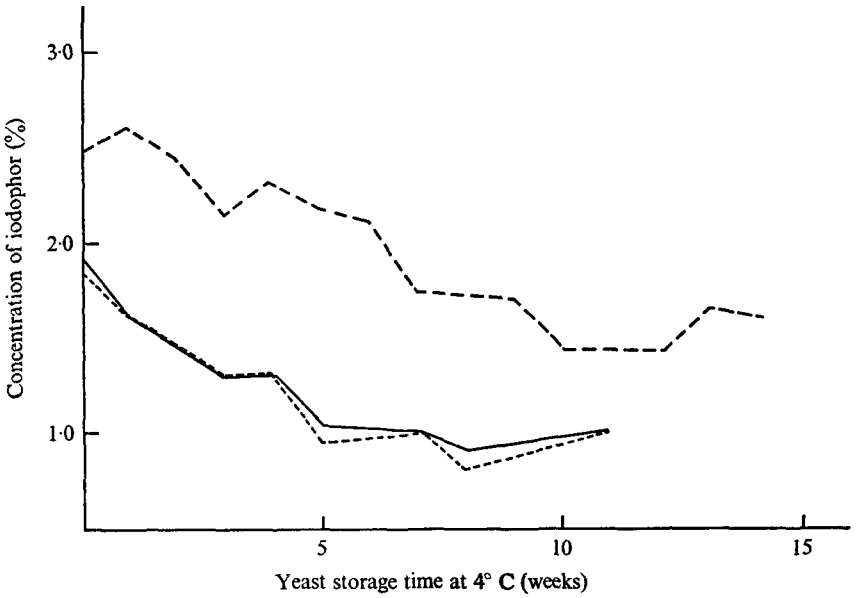


Fig. 1. Results obtained for the three iodophors with the same yeast suspension.

0·1 ml. of each test mixture was transferred to 10 ml. of the nutrient broth/horse serum to inactivate any residual disinfectant. After thorough shaking these were further dispensed into corresponding groups of five test tubes of nutrient broth (1 ml. per test tube). The subculture tubes were incubated for 48 hr. at 37° C. and then examined for growth. No growth in two or more of the five tubes was classed as a pass dilution.

The same production batch of each of the three iodophors was used for the following modifications:

*Modification I.* The basic method was performed on the iodophors I, II and III using a single yeast suspension which was stored at 4° C. in 100 ml. vols. in screw-capped bottles. One bottle was removed each week and used in the tests.

*Modification II.* The basic method was performed using iodophor I and three batches of yeast, A, B and C.

One block of yeast was obtained each week for 3 weeks and the suspension from each was dispensed in 25 ml. amounts and labelled A, B and C respectively. Four samples from each batch were randomly selected and used in applying the test to the disinfectant. This was carried out at weekly intervals for approximately 14 weeks.

## RESULTS AND DISCUSSION

Results obtained using modification I are shown graphically in Fig. 1. It can be seen that the variations in weekly results follow a similar pattern for each of the three iodophors irrespective of composition. From this it can be deduced that these variations come from within the test and are not due to the individual formulations. There is an indication that the longer the storage time of the yeast suspension, the lower is the concentration of iodophor passing the test.

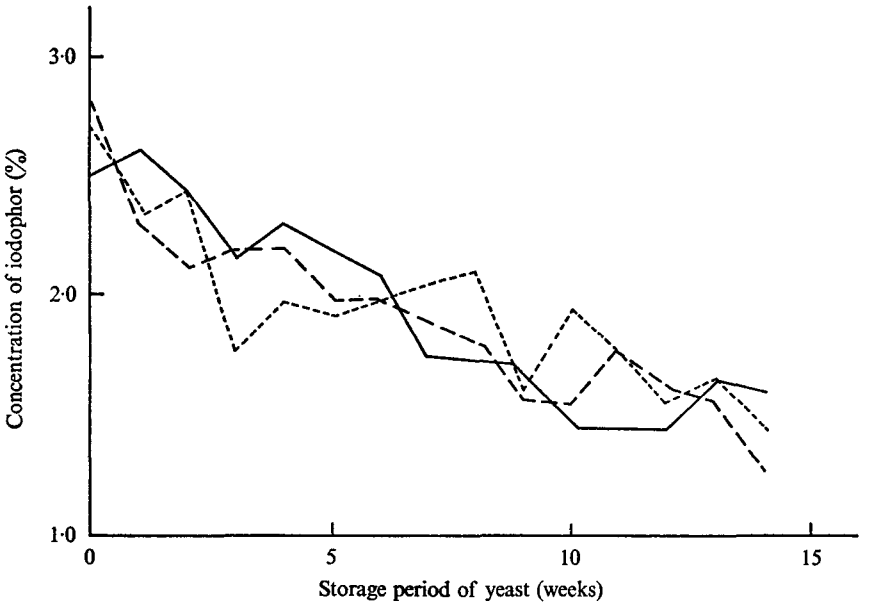


Fig. 2. Results obtained with yeasts A, B and C for iodophor I.

Table 2. *Two-way analysis of variance for the results obtained with yeast batches A, B and C*

| Source | Sum of squares | D.F. | Variance estimate | *F* |
|---|---|---|---|---|
| Between weeks | 5·39003 | 11 | 0·49 | 16·89 |
| Between batches | 0·015267 | 2 | 0·007634 | 0·26 |
| Residual | 0·6382 | 22 | 0·029 | — |
| Total | 6·0435 | 35 | — | — |

The results obtained in modification II are shown in Fig. 2. A two-way analysis of variance was performed on the mean results from the four random samples from each batch to determine whether there was a significant difference between:

(*a*) The results obtained for each batch.

(*b*) The results obtained each week.

This analysis indicated that there was no significant difference between the batches but that the results obtained when the yeast had been stored for varying lengths of time differed significantly as shown in Table 2.

Because of this finding the results for the three yeast batches for each week were pooled and the means plotted in Fig. 3. This graph confirms that the pass dilution alters with time. In fact, the concentration needed to give a pass result with a 14-week-old yeast suspension is half that required with a 1-week-old yeast suspension.

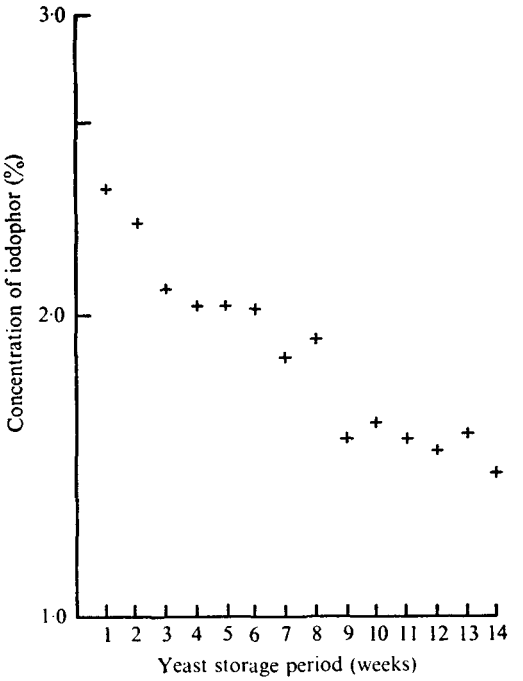


Fig. 3. Combined results with yeasts A, B and C for iodophor I.

## CONCLUSION

This work has not yet been duplicated with other types of disinfectants. However, it would seem that when a yeast suspension is used in evaluating a disinfectant some variation in results may be experienced depending on the length of time the prepared yeast has been stored before the test is performed.

As tests incorporating yeast suspensions are widely used commercially, the authors think that it is sufficient at this time to have shown that this variation occurs rather than to attempt to explain the phenomenon. It would appear therefore that there is need for investigation into alternative substances to simulate organic contamination giving reproducible results.

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