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Evaluation of procedures for hygienic hand-disinfection: controlled parallel experiments on the Vienna test model

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SUMMARY

Controlled parallel experiments were performed on the Vienna test model for the evaluation of procedures for hygienic hand-disinfection in three laboratories (Vienna, Mainz, Birmingham). The degerming activity of four procedures, each taking 1 min, was assessed repeatedly and compared with that of a standard disinfection procedure (ST) using isopropanol 60 % (v/v). The mean log reductions (mean log RF) for each procedure were as follows: *n*-propanol 50 % (v/v) 4·85 and 5·14 in Vienna (V) and Mainz (M) respectively, ethanol 70 % (v/v) + chlorhexidine-gluconate 0·5 % (w/v), 4·01 (V), 3·76 (M) and 4·00 in Birmingham (B). Washing procedures were less effective, mean log RF 's of 3·19 (V), 3·49 (M) and 3·04 (B) were obtained with povidone-iodine soap, and 2·91 (V), 3·37 (M) and 3·27 (B) with a liquid phenolic soap. Analysis of variance on the data from Vienna and Mainz revealed significant differences of means not only between procedures (' preparations ') but also on repeat testing. To compensate for the influence of variables such as test subjects, laboratory and day, the Vienna test model provides a method of standardization by testing a ST in parallel with the test procedure (P).

Standardization of the results was obtained by pair-wise substraction, log RF_{P_i}-log RF_{ST_i}. Analysis of variance on the resulting values demonstrated that comparability of the results between laboratories and on repeat testing was achieved. The relative variation of the measurements within the laboratories ranged from 0·9 to 4·2 %. As assessed by power-analysis, a disinfection procedure will be detected as significantly ($P = 0{\cdot}1$) inferior to the standard processes in 95 of 100 experiments if it produces a mean log RF that is at least 0·55–0·65 log units smaller than that of the standard.

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INTRODUCTION

In 1974 a test model for the evaluation of procedures for hygienic hand-disinfection was proposed by Rotter and co-workers (1974) and, with slight modification, this was adopted as the official test method by the Austrian and German societies for hygiene and microbiology, and by the Federal Office of Health (Bundesgesundheitsamt), Berlin (Deutsche Gesellschaft für Hygiene und Mikrobiologie, 1981; Österreichische Gesellschaft für Hygiene, Mikrobiologie und Präventivmedizin, 1981). In principle, the reduction of test bacteria from the artificially contaminated hand, obtained with the disinfection procedure under test (P), is assessed and compared with that of a standard disinfection procedure (ST). Both tests must be performed using the same volunteers and on the same day. Surviving test bacteria are recovered from the hands using a finger-tip method, and the reduction is calculated as the difference between the logarithms ($\log_{10}$) of the viable counts before (pre-values) and after (post-values) disinfection. These differences are called log reduction factors (RF). The mean of the log RF 's of all individual test persons is a measure of the efficacy of the degerming procedure. According to official requirements, a disinfection procedure must not be significantly less efficacious than the ST.

This investigation was designed to produce data on the accuracy and reproducibility of the test model. In 1977 and 1978 multiple parallel experiments were undertaken under controlled conditions in two laboratories with the partial participation of a third laboratory and in collaboration with the European Committee on Standardization of Disinfectants. Four disinfection procedures were investigated, together with the aforementioned ST. The results of these experiments were also intended to produce data for power-analysis that until now has been based on the results of a few experiments only. Also, it was intended to recalculate the sensitivity (discriminative power) of the method described in the official guidelines.

METHODS AND MATERIALS

Laboratories

The full protocol was followed by the two hygiene institutes of the Universities of Vienna and Mainz. The Hospital Infection Research Laboratory, Birmingham, participated with three tests under the agreed conditions. Each laboratory recruited 15 volunteers who were prepared to participate in all experiments. Their hands were examined and those with damaged skin, i.e. wounds, eczema or signs of dermatitis, were excluded. Fingernails were not allowed to be too long. For computing purposes the identification numbers of the volunteers were the same for all experiments.

Disinfection procedures

The four procedures included the following disinfectants and modes of application:

(1) Three ml *n*-propanol 50% (v/v) was applied to the cupped hands and vigorously rubbed over the entire surface including wrists; special attention was

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paid to finger-tips, subungual spaces and interdigital areas. After 30 s another portion of 3 ml was similarly applied for a further 30 s.

(2) Ethanol 70 % (v/v) + chlorhexidine-gluconate 0·5 % (w/v) was used in the same way.

(3) A specially prepared povidone-iodine (PVP-I) liquid soap containing PVP-I 10·0 g, sodium-laurylethersulphate 14·0 g, diethanolamid of laurinic acid 3·6 g, in 100 ml distilled water was used by the laboratories in Vienna and Mainz. As PVP-I preparations cannot be stored for long periods and as the Birmingham laboratory was asked to participate later in the studies, the above preparation was not used and was replaced by Betadine surgical scrub (Napp Laboratories) containing 7·5 % PVP-I (0·75 % available iodine). Five ml of the solution was applied to the hands and after 10 s about 3 ml of tap-water was added to produce a lather. Hands were then washed for 50 s and rinsed under a running tap for a further 15 s.

(4) Similarly, a specially prepared phenolic liquid soap containing hexachlorophane 0·5 g, 2-phenylphenol 1·0 g, 4-chlor-2-benzylphenol 2·0 g, disodiumlauryl-ethanol-polyglycol-ether-sulphosuccinate 17·0 g, sodiumalkylsulphonate 3·0 g, isopropanol 20·0 g, citric acid 0·9 g and disodiumhydrogenphosphate (12 H_2O) 1·5 g in 100 ml of distilled water was used. This disinfectant was applied to the hands in a similar manner to PVP-I.

Isopropanol 60 % (v/v) was used as the ST, which was performed in parallel with the test P and applied as the other alcoholic solutions. With the exception of the isopropanol standard and Betadine surgical scrub a single batch of disinfectant was prepared for comparative tests in all three test centres.

To prevent any residual antibacterial effect after completion of the disinfection procedures, neutralizers were incorporated into sampling fluids (pre- and post-disinfection samples) used for the recovery of test bacteria and into the diluents of these suspensions, but not into the medium for plate counts. Neutralizers were varied according to the disinfectant and were as follows (concentrations per litre): ethanol/chlorhexidine – 30 ml Tween 80, 30 g saponin, 1 g histidine, 1 g l-cysteine; PVP-I liquid soap – 30 ml Tween 80, 3 g lecithin, 1 g histidine, 5 g sodium thiosulphate, 1 g albumin (bovine, lyophilized, 92 %); phenolic liquid soap – 30 ml Tween 80, 3 g lecithin, 1 g histidine and 5 g sodium thiosulphate.

For tests with *n*-propanol no neutralizer was used. The sampling and dilution fluids used in the ST with isopropanol contained the same neutralizer as those of the disinfection procedure tested in parallel. Casein-soy broth (CSB) served as a liquid medium for culturing test bacteria and, with or without neutralizer, as the recovery and dilution medium. It was also used for preparation of the bacterial suspension in which the volunteers immersed their hands. Casein-soy agar (CSA) with 0·05 % added sodium-desoxylcholate was used as the medium for plate counts.

The test bacterium *Escherichia coli* ATCC 11229 was cultured on CSA for 18 h at 35 °C and a dense suspension prepared in 5 ml of CSB. This was subsequently added to 2 l of CSB and the suspension incubated overnight. The resulting bacterial suspension, containing at least 10^8 c.f.u./ml, was used for contamination of hands. In each experiment the actual count was established using a surface-plating technique.

The ST always preceded the P and both were performed on the same day using the same volunteers.

In all experiments the volunteers washed their hands with a proven non-antimicrobial soap for 2 min before starting the experiment, and dried them on a paper towel. The hands were then immersed, up to the middle of the palms, in the bacterial suspension for 5 s. After careful draining, the hands were allowed to dry in the air for 3 min. For this, the hands were kept in a horizontal position with fingers slightly spread and slowly turned (pronation and supination) in order to avoid accumulation of the suspension at the finger-tips. After exactly 3 min surviving test bacteria were recovered by rubbing and kneading the finger-tips for 60 s against the bottom of two 9 cm diameter Petri dishes (one for each hand) containing 10 ml of sampling fluid.

This was immediately followed by the appropriate disinfection procedure. After washing with either PVP-I liquid soap or the phenolic disinfectant–detergent, remaining water was shaken off carefully with finger-tips uppermost. This is necessary to avoid recontamination of the sample area by water running down from other previously contaminated areas such as the wrists. The finger-tips were then sampled for assessment of post-values as described above.

To establish the number of surviving organisms, 0·1 ml of the sampling fluids and of appropriate dilutions were spread on the surface of CSA plates. The dilution procedures included the use of a fresh pipette and careful mixing at each dilution-step. All plates were incubated for 20 ± 2 h at 36 ± 1°C.

In Vienna and Mainz, each preparation was tested five times. In Vienna, all four preparations were tested once within a week – two preparations on Tuesdays, the other two on Thursdays. The test with one preparation was always preceded by a parallel test with the ST. Thus, two complete experiments, each comprising two tests, were performed on each of these days.

In Mainz, the full series of four preparations was usually tested within one experimental day, two preparations in the morning and two in the afternoon, but the standard disinfection test was performed only once in the morning and once in the afternoon. Therefore, one standard served as a means of comparison for two preparations tested on the same half-day. However, some experiments with only one preparation (plus standard) had to be performed separately.

In Birmingham, ethanol/chlorhexidine, PVP-I liquid soap and the phenolic soap were tested in separate weeks, but always together with the standard.

Viable counts per millilitre of sampling fluid were obtained, if possible, from plates showing 30–300 colonies. Mean log values of the counts from the right and left hands of each volunteer were calculated for pre- and post-values, and the difference, i.e. log RF, established. The mean of the log RF 's of all volunteers was used to measure the efficacy of the procedure.

Mean log RF 's obtained with the various preparations were tested for significance of differences between them and the standard using Wilcoxon's matched-pairs signed-rank test (Siegel, 1956). The one-sided test for significance was used at $P = 0{\cdot}1$, as laid down in the Austrian and German guidelines.

All other statistical comparisons of means were done either by the t test, for independent samples, or by analysis of variance using a mixed model for the latter. This was necessary as the factor 'repetition' is dependent on the test person, whereas the factors 'preparation' and 'place' are independent variables. These latter calculations were made by the computer centre, University of Vienna.

As a measure of precision the relative variability (V_r) within the results of one laboratory were calculated as:

$$V_r = \frac{V}{\sqrt{(n-1)}} \times 100(\%),$$

where V is

$$V = \frac{\mathrm{SD}}{\bar{X}},$$

V, coefficient of variation; SD, standard deviation of sample; $\bar{X}$, sample mean.

In order to compare results between the different places a standardization procedure was applied by subtracting each individual log RF obtained using the ST from the appropriate value as measured with the P. In one experiment the mean of the individual differences of all volunteers gives the average deviation from the standard; thus per procedure, place and repetition:

$$\text{average deviation} = \frac{\Sigma_{i=1}^{n}(\mathrm{P}_i - \mathrm{ST}_i)}{n},$$

where P_i = log RF of preparation under test with volunteer i ($i = 1, \ldots, n$), ST_i = log RF of standard tested in the same experiment with the same volunteers i ($i = 1, \ldots, n$) and n = number of volunteers.

All calculations concerning statistical power-analysis were done as described by Cohen (1977).

RESULTS

The mean log RF 's obtained with each of the four preparations under test and the standard are shown in Fig. 1. These show, on average, a clear range of order of efficacy, i.e. *n*-propanol ⩾ isopropanol > ethanol + chlorhexidine > PVP-I soap ≏ phenolic soap. In Birmingham, however, the PVP-I soap was less effective (log RF: 3·04), although insignificantly, than the phenolic soap (log RF: 3·27), but this was also observed once in Vienna and twice in Mainz.

As revealed by analysis of variance on the log RF 's obtained in Vienna and Mainz, significant differences exist between the means of the four preparations under test and, less so, between those of the two laboratories, but not between repeat experiments. If, however, variation contributed by the factor 'place' is eliminated by analysing the results of one place only (i.e. Vienna), then the differences between repeat experiments are found to be significant as well.

The precision within a laboratory, expressed as the relative variation among the mean log RF 's of five repeat experiments with each of the four preparations at Vienna and Mainz, ranged from 0·9 to 4·2% (Table 1).

With the ST (isopropanol 60%, v/v), mean log RF 's between 3·76 and 5·20 were measured (Fig. 1), with an overall mean of 4·37. On average, the results from Vienna and Mainz were very similar, those reported from Birmingham were lower but well within the range.

In contrast to the insignificant contribution of the factor 'place' to total variation, highly significant differences were observed between repeat experiments in Vienna and Mainz. As revealed by further analysis, these differences are entirely attributable to significant variation of the results obtained in different weeks but not to variation of results within the same day or the same week. This has been established by analysis of variance for both places separately.

Table 1. *Relative variations* (V_r) *of the mean log reduction factors between repeats at three places*

Preparation:	No. of repetitions	Relative variation (%) Vienna	Mainz	Birmingham
n-Propanol	5	2·8	2·6	—
Ethanol/chlorhexidine	5	0·9	2·0	—
PVP-I soap	5	3·8	3·6	—
Phenolic soap	5	2·2	4·2	—
Iso-propanol (ST)	20/16/3	1·6	2·5	2·4

—, No repetitions done.

The relative variations between the repeats of the standard procedure at the three places ranged from 1·6 to 2·5% (Table 1).

When the results obtained with the four preparations under test were standardized by subtracting from the individual log RF 's, the appropriate values as measured with the ST, the mean deviations (Fig. 2) from the standard appear as positive figures if the P was more effective, and as negative if it was less effective than the ST. In addition, each of these values was identified if this deviation was found to be statistically significant. This was the case with *n*-propanol four times, with ethanol + chlorhexidine twice, and with PVP-I soap five times at each of the two laboratories (Vienna and Mainz). In 11 experiments with phenolic soap the difference was not significant, apart from one occasion (Mainz). With ethanol/chlorhexidine, positive but insignificant deviations resulted once in Vienna and Mainz.

On average, the mean deviations (Fig. 2) from the standard indicate the following range of order with respect to the efficacy of the four preparations under test: *n*-propanol $\gg$ ethanol + chlorhexidine $>$ PVP-I soap $\simeq$ phenolic soap.

Analysis of variance of these values revealed that significant differences exist between the preparations but not between the means of 'places' and 'repeat tests'. If one eliminates variation contributed by the factor 'place' by analysing the standardized results of one place alone (i.e. Vienna), repeat testing still remains a factor without significance for reproducibility at one place. This is in contrast to the findings above with non-standardized results.

From the above results the following basic facts for power-analysis were derived: for the purposes of computation, the log RF 's obtained from 15 volunteers in any experiment may be assumed to be normally distributed.

The population variance (SD) in experiments with alcoholic solutions (A) may be taken as $SD_A = 0{\cdot}85$, with disinfectant-detergents (D) $SD_D = 0{\cdot}55$. As the two groups of disinfectants produce results of obviously different variance, the following principles were applied: for statistical comparison of an alcoholic solution with the (alcoholic) standard the common variance (SD_C) may be assumed, $SD_C = 0{\cdot}85$ as SD is equal for both populations. For statistical comparison of a disinfectant–detergent with the (alcoholic) standard as common variance a value of $SD_C = 0{\cdot}72$ was assessed by:

$$SD_C = \sqrt{\frac{(SD_A)^2 + (SD_D)^2}{2}}.$$

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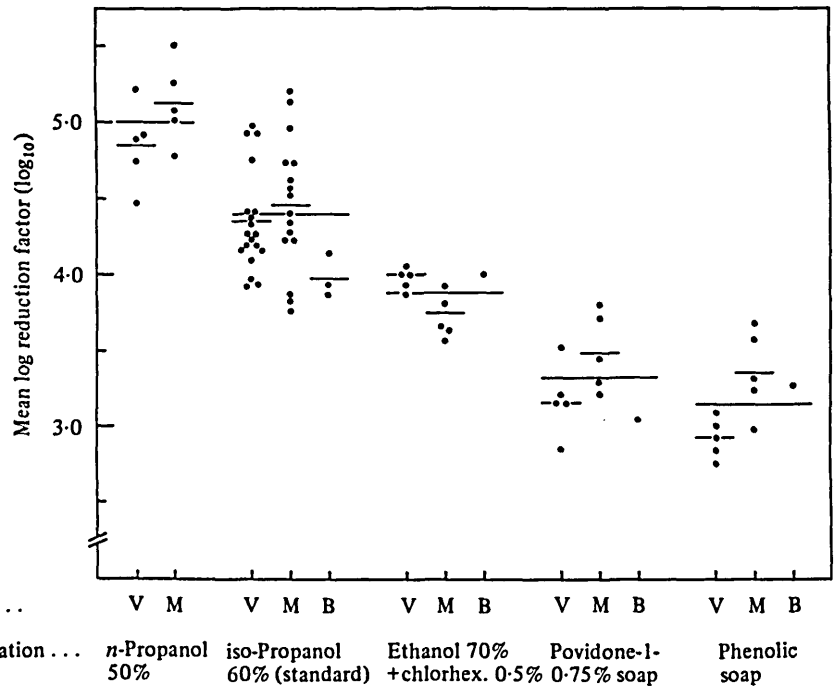

Fig. 1. Mean log reduction of release of test bacteria (*E. coli* ATCC 11229) from the finger-tips of artificially contaminated hands by disinfection for 1 min with five different preparations in repetitive experiments at Vienna (V), Mainz (M) and Birmingham (B). —, group; ——, total.

Since the results for both (the P and the ST) are derived from the same volunteers on the same day and under comparable environmental conditions, statistical tests may be applied which test for differences between paired observations.

Furthermore, in comparisons of alcoholic solutions a product–moment correlation of at least $R = 0{\cdot}6$ was found. Therefore this value was also introduced as additional information in the power-analysis of such cases. However, as in the experiments with disinfectant-detergents the correlation with the results of the parallel standard disinfection was variable, no correlation was assumed in power-analysis of experiments with disinfectant-detergents.

In the Vienna model significance tests are required only if P are less effective than the standard (otherwise, the procedure is accepted as sufficiently active). Therefore, only directional testing had to be considered for power-analysis.

With the prerequisites already mentioned, and with 15 volunteers, a difference of the means can be expected to be significant in a paired *t*-test with $P = 5$ % (10 %) in 90 (95) of 100 experiments if this difference exceeds 0·55–0·58 log units. For the slightly less powerful non-parametric matched pairs signed rank test (Wilcoxon Test) which was proposed in the official guidelines in Austria and the FRG, this difference amounts to 0·61–0·65.

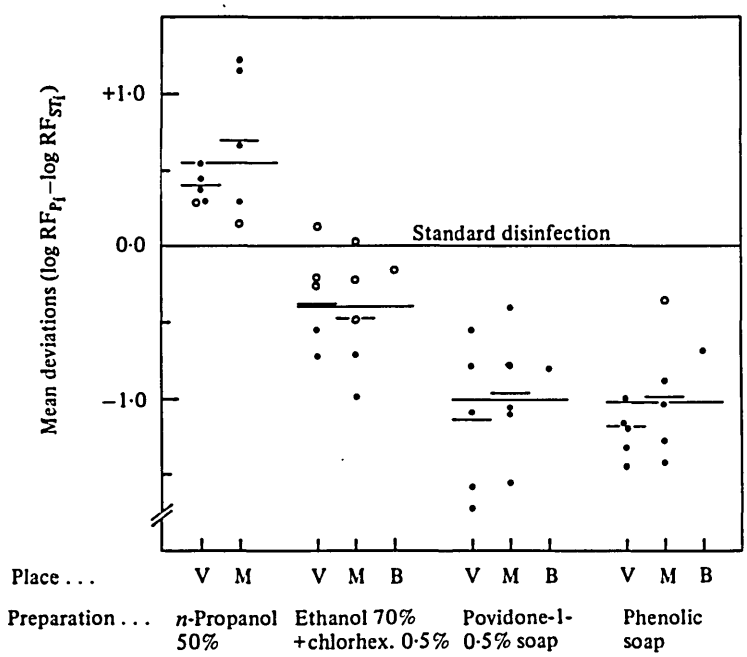

Fig. 2. Standardized results; mean deviations of results as obtained with preparations under test from results with standard disinfection procedure tested in parallel at Vienna (V), Mainz (M) and Birmingham (B). —, group; ——, total; ●, mean deviation significant at $P = 0{\cdot}1$; ○, mean deviation not significant at $P = 0{\cdot}1$.

DISCUSSION

The purpose of this investigation was to evaluate the Vienna test model for hygienic hand-disinfection. This was done by studying the following criteria: specificity, sensitivity, reproducibility and accuracy.

Specificity does not appear to be a special problem as the object of measurement, i.e. the reduction in test bacteria by a disinfection procedure, is under the control of the investigator, who establishes the release of an easily recognizable test bacterium before and after the disinfection procedure.

Sensitivity may be defined by the 'discriminative power', namely the power to discriminate between the mean log RF of the P and that of the ST. This power has been established as 95% with $P = 10\%$ if the difference of the means is in the range of 0·55–0·65 units of the logarithmic scale. In other words, a disinfection method will be recognized as significantly inferior if the standard is, at the minimum, 3·5–4·5 times more efficacious. It is of importance to realize that any experimental design not matching the results of an experiment (e.g. testing a procedure and the standard in different weeks or with different volunteers) would require many more volunteers; for the above assumptions 35 instead of 15 would be necessary! This is also true for another procedure which compares the test result with a fixed theoretical value (Kundi *et al.* 1975). As an additional drawback, such a design would not allow for the influence of the test population. Thus, the Vienna test model offers the advantage of high discriminative power with a comparatively

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small sample size. Fig. 2 demonstrates that mean deviations from the standard were found significant only when they exceeded the calculated critical difference, i.e. 0·55 log units.

The accuracy of a new method is usually assessed by testing a standard specimen in parallel with the unknown or by using the procedure at the same time as a reference method. Neither are possible here because each group of volunteers would be expected to produce, within certain limits, its own results with a particular disinfection procedure (Rotter, Mittermayer & Kundi, 1974) and a reference method does not exist. Also, the results of this investigation have shown that values obtained from different groups of test persons and even between those of the same groups in different weeks can, indeed, differ significantly. To compensate for this the Vienna test model uses a comparative evaluation in parallel with a ST that is performed with the same volunteers and on the same day, thereby abolishing differences between 'places' and 'repeats'. Hence, the mean deviations from the standard may be taken as a measure of the efficacy of a disinfection procedure. This is very important as compensation of such mean differences leads to comparability of test results obtained in different laboratories and at different times.

With this fact in mind, the standardization results may be analysed in accordance with the findings in the three laboratories. As already found in previous investigations (Rotter, Koller & Kundi, 1977), *n*-propanol (50%, v/v) was consistently the most effective preparation, exceeding the disinfection power of the standard in all ten experiments.

On average, ethanol (70%, v/v) + chlorhexidine (0·5%, w/v) proved slightly less effective than the standard (−0·33, −0·48, −0·15), but only in two of the experiments (Vienna and Mainz) was this difference significant. On the other hand, the difference was not significant in two other experiments, one at each place, and on one occasion this was reversed. The chance of obtaining these results was distributed identically in Vienna and Mainz. This finding is typical for borderline cases and means that any of these possibilities may occur in routine tests with a chance of 40:40:20. Apart from the interest of the disinfectant manufacturer, this is of no great relevance as the hazard to the patient is unlikely to be affected if such a preparation is used in hospitals as the preparation is nearly as good as the standard. If, however, the manufacturer protests against accidental rejection of his product and wishes to prove his point, he would have to demonstrate, with more (e.g. 30) volunteers, that the mean deviation from the standard is smaller than the difference detectable by the test model (i.e. 0·55–0·65).

Results from all three laboratories showed that PVP-I and phenolic soap were virtually identical and significantly inferior to the standard in 21 of the 22 experiments. With PVP-I inferior activity is not unexpected as this has been observed with commercially available preparations in other investigations as well (Ayliffe, Babb & Quoraishi, 1978).

The variation among the results of several experiments within laboratories ranged between 0·9 and 4·2% (Table 1). This is higher than that usually found in chemical tests but this precision is still reasonably good for a biological test method. No information was obtained on the variation of results between laboratories as their number was too small.

The Vienna model, or a similar test (Ayliffe, Babb & Quoraishi, 1978) allows for the influence of volunteers by incorporating a standardized parallel procedure for comparison. Although the anti-microbial efficacy of the standard agent is not important, there are advantages in using a standard which is known to be acceptable for hand-disinfection. However, a product with no anti-microbial activity, e.g. non-medicated soap, might also be used to determine whether the test agent is more active than a negative control. The choice of *E. coli* as the single test organism might also be criticized since it dies rapidly on drying and, although a common cause of hospital infection, does not spread readily between patients. Nevertheless, it is easily identified when mixed with normal resident flora on the skin and is an acceptable non-virulent organism for use on volunteers. An additional test organism might be considered desirable, particularly a Gram-positive coccus (Ayliffe & Babb, 1979).

Some problems with hygienic hand-disinfection remain. Is an agent producing a log 5 reduction likely to be more effective in reducing cross-infection via the hands than one giving a log 4 or even log 3 reduction? The power of this test distinguishes a log 0·5 difference between agents as significant. Although this would seem reasonable, there is no indication that this has any clinical relevance. The other area of controversy is whether removal by washing away the organisms can be included as part of the disinfection process. Nevertheless, in spite of these problems, the test method gives reasonable reproducible and repeatable results, and until more data are available its interpretation must be left to the individual judgement of infection control staff.

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