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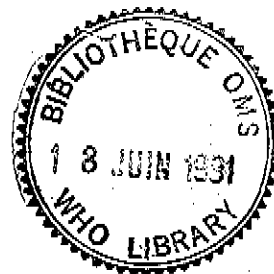
ENGLISH ONLY

PRACTICAL GUIDE ON RATIONALE  
AND  
TESTING PROCEDURES FOR  
DISINFECTION OF HANDS

by

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### Preface

Handwashing or disinfection is one of the main methods for preventing the spread of infection. However, there are variations in the use of disinfectants for this purpose and in the testing methods used in different countries.

Handwashing, with or without a disinfectant, is considered adequate for most clinical procedures in many countries. In others, disinfection with an alcoholic solution is considered necessary at least for procedures that include real or suspected contamination of hands after contact with body secretions, excretions or microbiological material. In contrast to handwashing, "hygienic hand disinfection", a term first introduced in German speaking countries, has always referred there, exclusively to the killing of transient organisms on the hands by use of an antimicrobial agent. Since, however, this term has been introduced into other countries, there is no general agreement as to whether (mechanical) "removal" of transients is acceptable in the definition. This is reflected in the text and further discussed; but it is agreed that it is important to wash or disinfect the hands thoroughly and at the right time.

The differences in requirements influence the test methods and particularly their interpretation. The Vienna and Birmingham tests are both included in this report since there are individual preferences for one or other of these techniques, but both provide similar information on the order of effectiveness of agents. The main difference is in the interpretation. Agents significantly less effective than a standard alcohol preparation will fail the Vienna test, whereas agents significantly more effective than soap and water will pass the Birmingham test.

The decision to use a washing technique, with or without a disinfectant, or an application of alcohol for a specific task, or in a particular area, must be decided by individual infection control workers since little clinical evidence is available from clinical trials.

It is generally agreed that a disinfectant is necessary for the hands of surgical staff before carrying out an operation. However, alcoholic preparations are preferred in some countries and are used as a control in test methods. Antiseptic detergents are also widely used and are compared with non-medicated soap in test methods.

## 1. Introduction

The hands are one of the main routes of spread of infection (for evidence see ref. 36). They are frequently in close contact with the surrounding environment and can readily acquire undesirable microorganisms, subsequently transferring them to critical sites such as wounds, instruments and food. This mode of spread is particularly important in hospitals where susceptible individuals are located close to potentially hazardous sources. The hands may transfer organisms, which are present on the skin but are not multiplying (transients), or may act as a source of infection, by releasing organisms which are multiplying on the skin (residents) or are present in septic lesions. The distinction between these groups is important when considering hand disinfection. Transients are readily removed by washing or disinfection, whereas 'residents' are more difficult to kill or remove.

## 2. The microbial flora of the hands

The surface of the hands consists of creases, hair-follicles, sebaceous areas, sweat glands and nails, which support varying numbers of organisms.

### 2.1. Resident flora

The 'resident' flora consists mainly of coagulase-negative staphylococci and micrococci, diphtheroids and Propionibacterium acnes. Other organisms such as Staphylococcus aureus are occasionally residents but are usually present as transient contaminants. The distinction between residents and transients is not always clear since occasionally organisms such as Gram-negative bacilli, e.g. Klebsiella spp., may grow on the hands for periods varying from several hours to many weeks, particularly in units where contamination is likely to be heavy, e.g. burns and dermatology units (17). However, the only Gram-negative bacillus which may be considered a consistent resident is Acinetobacter calcoaceticus var. anitratus. The true residents (e.g. coagulase-negative staphylococci) are generally of low pathogenicity and cause infection mainly in association with foreign bodies

or invasive procedures e.g. implant surgery, i.v. and dialysis (CAPD) sites, also in neonates and immunosuppressed patients. As previously stated they are difficult to remove by washing and are less easily eradicated by disinfectants than transients and about 20% remain in the deeper parts of the skin after thorough disinfection procedures (80).

### 2.2. Transient flora

The skin is an inhospitable site for most organisms due to its dryness, and organisms acquired by the skin compete poorly with the resident flora, which produce antagonistic substances such as bacteriocines and fatty acids.

Transient organisms are acquired from other patients, equipment and the environment and are potentially more hazardous than the residents. Examples are Klebsiella spp., Escherichia coli, Pseudomonas aeruginosa, Salmonella or Shigella spp. and Staphylococcus aureus which may occasionally be a resident.

Transients remain on the outer surface of the skin and can be removed by washing or are killed by most disinfectants. However, the numbers on the hands may be large, e.g.  $10^6$  after touching a contaminated dressing, or emptying a bag containing infected urine, and inadequate washing or disinfection may leave large numbers behind.

### 2.3. Skin flora in lesions

Damaged skin may allow the growth of a wide range of organisms, especially Staphylococcus aureus, which frequently colonises dermatitic lesions. Increased numbers of residents and potential pathogens are also more likely to be present on skin damaged by frequent washing with a detergent or by scrubbing (56). Organisms present on damaged skin, or in infected lesions, are particularly difficult to remove by washing or disinfection and tend to remain until the lesion is healed.

### 3. Strategies to prevent microbial transfer via hands

The appropriate strategy depends on the activity to be undertaken or the procedure which has been carried out and on the condition of the hands. A different washing or disinfection procedure is required if the purpose is to prevent the transfer of transients from that of a surgeon preparing his hands for a surgical operation. Other techniques are used for treating carriers of Staphylococcus aureus or hospital personnel with septic lesions on their hands. These differing strategies will be considered in relation to the various "schools".

#### 3.1. Hands as a vehicle of transient flora

##### 3.1.1. Protection of hands

If hands are clean, the principle should be observed that avoiding contamination is safer than the subsequent elimination of microorganisms. This may be achieved by non-touch techniques such as the use of forceps or gloves.

Although microorganisms are washed off latex or plastic gloves more easily than skin (46), it is preferable to change gloves between patients since a high proportion will still be contaminated after washing (19). In addition, handwashing is strongly encouraged after use and removal of gloves by some authors (19) since up to 50% of hands were found to be contaminated in spite of this protective measure.

##### 3.1.2. Degerming procedures

If hands are contaminated, appropriate measures should be used to eliminate potential pathogens or to reduce them to a safe level. Washing with non-medicated soap will be adequate in most circumstances, but in some situations disinfection with alcoholic solutions is regarded necessary by some workers especially in German speaking countries.

###### 3.1.2.1. Hand washing

Washing alone will remove over 99% of transient organisms in 30 - 60 s (52) but does not kill them. This procedure may contaminate the sink and its surroundings, but in most instances this hazard appears to be of minor significance

in the spread of infection. The average hand-wash takes only 15 - 20 s (8, 83).

This may be sufficient in "normal" patient-care procedures provided the technique is thorough and all parts of the hands are covered including the tips of the fingers and thumbs.

#### 3.1.2.2. Hygienic hand disinfection

This is a more effective process than washing and reduces the release of transient organisms by 4 to 5 logs (often) depending on the method and agent used (tab.1).

It involves the use of a disinfectant to eliminate all, or a substantial part, of the transient flora. In German speaking countries disinfection implies the killing, rather than the removal, of transient organisms on the surface of the hand, and - based on test results - it is believed that this can best be achieved by using alcoholic solutions although it has been shown that also an aqueous solution of povidone-iodine is equally effective (69). There, the following indications are thought to require a hygienic hand disinfection: every known or suspected hand contact with patients' blood, excretions or secretions, even when hands are gloved; after patient-care under precautions of source isolation; after working in the microbiological laboratory.

Infection control staff in other countries, however, believe that antiseptic detergents are acceptable if significantly more effective than non-medicated soap (6), and in other countries washing alone, either with or without disinfectant, is regarded as sufficient for most clinical procedures (22). Disinfection with an antiseptic detergent or alcoholic solution rather than washing with non-medicated soap is often recommended in special units such as intensive care, premature baby units, burns, isolation, and also during outbreaks of infection, before aseptic procedures and after contact with particularly hazardous organisms. In the absence of clinical confirmation of the value of aqueous and alcoholic formulations, countries or individual hospitals must decide on their own definitions and policy.

### 3.1.2.3. Agents

#### Antibacterial effect:

Antiseptic-detergents usually contain chlorhexidine, povidone-iodine, triclosan or hexachlorophane (4). Most of these products are not (69) or only slightly more effective than non-medicated soap, but results of tests performed by different workers are variable. Usually the difference between non-medicated soap and an antiseptic detergent does not exceed 1 log (6, 69). Examples giving an idea of the range of order of their efficacy can be seen in table 1. Antiseptic detergents are usually less effective against Gram-negative bacilli than Gram-positive cocci (see 70). Alcoholic solutions of these agents have little, if any, additional immediate effect than the alcohol alone for hygienic hand disinfection (6). The value of a residual effect on transient organisms is uncertain (6), but may be of value during outbreaks e.g. staphylococcal cross-infection in a premature baby unit.

Alcoholic solutions are more effective than aqueous solutions and are a requirement for hygienic hand disinfection in some European countries. The most effective agents are short-chain primary aliphatic alcohols (6, 69), in particular n-propanol, which is very effective but not always available. Equivalent concentrations of alcohols (by volume) are ethanol 70-80%, isopropanol 60% and n-propanol 40% (71). Alcoholic hand disinfectants should always contain a suitable emollient. They are convenient to use in the absence, or lack of close proximity, of a hand washbasin. They are also convenient for between patient procedures where return to a handwash basin is impractical, and do not contaminate the environment. However, hands must be physically clean before application. Possible disadvantages include drying of the skin in the absence of a suitable emollient, inflammability and lack of activity against some viruses.

#### Antiviral effect:

Alcohols are generally effective against enveloped (lipophilic) viruses such as herpes simplex and HIV, also adeno- and rotaviruses (14, 27, 35), but not rabies virus (26). However, alcohols are less effective against hydrophilic viruses e.g.

enteroviruses (27, 35). Whereas ethanol seems to be active in high concentrations (27, 35) iso- and n-propanol are not. This is also true for cationic compounds such as quaternary ammonium compounds and chlorhexidine, ampholytes, iodophores and phenolic compounds. Chlorine releasing agents such as hypochlorites or Chloramin T may be an effective alternative and are the only agents for virus hand disinfection officially accepted by the Federal Office of Health in the FRG. Hepatitis B virus may also be inactivated by high-percentage ethanol (28) or isopropanol (11) and peracetic acid 0,2% in combination with moderate concentrations of alcohols (33% v/v) such as ethanol or isopropanol (81) or emulsifiers (33). In view of the uncertainty of action of disinfectants against some viruses particularly over short time periods, e.g. 15 - 30 secs, it is advisable to wash hands with soap or detergent before a disinfectant application if a 'risk' of viral transfer, particularly an enterovirus, is likely.

#### 3.1.2.4. Choice of Product

In addition to the microbiological efficacy, acceptability by the user is a major consideration. Before general introduction of a product into a hospital, a preliminary trial of staff acceptability should be carried out. The assessment should include irritancy as well as cosmetic acceptability. A product which is not acceptable by staff will not be used, or if an irritant product is used, damage to the skin could increase bacterial colonisation.

### 3.1.2.5. Technique of hand washing or using an antiseptic detergent

The hands should be lathered with soap or antiseptic detergent applied with minimal dilution with water for the required time, e.g. 10 - 30 s ensuring the whole surface of the hands are covered without unnecessary splashing. The hands should be rinsed and dried thoroughly with a clean towel, which is then used to turn off the taps (unless they are elbow or foot-operated).

Minimal requirements for hand washing areas are:

- conveniently located wash-basins without plugs (6, 22, 66);
- running hot and cold water preferably from a mixer tap;
- single use towels, e.g. disposable paper towel dispenser and a container for used towels;

Dispensers for liquid soap and/or an alcoholic disinfectant (and possibly a cosmetic hand lotion) may also be required, depending on existing policy.

Bar soap has the disadvantage of possible contamination with Gram-negative bacilli, particularly if stored in a wet dish, but it is transportable, cheap and is as effective as liquid soap. If used, it should be kept dry, and the hands should be thoroughly rinsed after use. Liquid soap should contain preservatives if used in refillable dispensers. These should never be topped up but replaced or properly cleaned and then refilled with fresh soap (41). If, however, soap is distributed in non-refillable single-use containers, such as plastic bags, preservatives are not necessary. This possibility is preferable as preservatives may be allergenic and are not always effective, but the system may be more costly.

### 3.1.2.6. Hand disinfection with alcohol

The rub-in technique is the only acceptable method for using an alcoholic solution. Another method of application is immersion in a bowl of alcohol. This has a number of disadvantages, e.g. a possible source of contamination, rapid evaporation, fire-hazard, excessive amounts used etc. Spraying also has deficiencies e.g. uncertain dosing, inadequate coverage, and inhalation risks.

### 3.2. Hands as a source of resident flora

The resident flora of the hands may cause infections during a surgical operation if a glove is punctured or broken. The removal of all the readily detachable transient flora and as many of the resident flora as possible is therefore desirable. This is particularly important in operations involving prosthetic surgery where the resident flora of the skin is more likely to cause infection. In general surgery, the usual resident flora of the hands rarely cause infection, but Staphylococcus aureus may occasionally be a resident and requires removal or destruction. Since the constitution of the flora of the hands of the surgeon is rarely known, a technique for eliminating as many residents as possible including Staphylococcus aureus is usually advised as a routine.

Most surgical operations are completed in an hour or two, but some occasionally extend to 6 h or more. The moist conditions under a glove may allow organisms to proliferate during this period and, even during short operations, organisms may reach the skin surface from deeper layers. An antiseptic agent with continuing activity is therefore desirable. Chlorhexidine, triclosan or hexachlorophane preparations all show a continuing effect due to residual activity. Povidone-iodine has no proven sustained effect (67, 73). Alcohols are more penetrative and therefore exhibit a much stronger immediate effect. Consequently, their activity is also long lasting. They would appear to be appropriate for operations up to 3 h (44, 67). Some antiseptic detergents e.g. those containing chlorhexidine show an increasing effect on repeated application (3, 37, 40). This cumulative effect is, however, of limited usefulness as the effectiveness of the agent is less during the first few operations than on those carried out later in the day.

Most detergents, without the addition of antimicrobial agents, do not reduce the number of residents sufficiently, even if applied for several minutes (61, 67).

The optimal time of a application of disinfectant for surgical hand preparation is uncertain, as epidemiological data in support of a specific reduction in resident flora are not available. In German speaking countries the effect of n-propanol

60% v/v, rubbed into the hands for 5 min was arbitrarily chosen as a standard. If with another preparation, the same effect can be achieved in the same or shorter time it is accepted as sufficient (13, 58). One of the authors (G.A.J.A.), however, is of the opinion that if they exert a significantly greater effect than non-medicated soap over a shorter period, e.g. 2 min they may be acceptable in other countries:

### 3.2.1. Techniques of eliminating residents:

There are three techniques for reducing the resident skin flora by use of an antiseptic preparation:

Surgical scrub, surgical hand disinfection with alcohol or combinations of both.

#### 3.2.1.1. Surgical scrub

The hands are moistened and an appropriate amount of the antiseptic detergent is thoroughly rubbed onto all areas of the hands and wrists using a defined technique. Finger-nails are scraped or cleaned with a brush. Then, the hands and wrists are rinsed and the process is repeated. The forearms may be included if required. After a final rinse, the hands are dried with a sterile linen or paper towel. The hands should be held above the wrists during washing to avoid contamination from untreated skin. Except for the finger nails brushing should generally be avoided as the hands may become damaged so increasing the risk of bacterial proliferation. Brushing should also be restricted to the first operation of the day. Nails should be kept short to facilitate cleaning. The duration of the scrub depends on the antimicrobial effectiveness of the agent but should not take longer than 5 min.

#### 3.2.1.2. Technique of surgical hand disinfection with alcohol

After a social handwash with non-medicated soap and the necessary nailcare, hands are dried with a clean, but not necessarily sterile, towel. Then, approximately 5 ml of an alcoholic solution is poured onto the cupped hands and rubbed into the whole surface of the hands and wrists using a defined technique. The

forearms may be included, if required. When dry this is repeated. It is particularly important to ensure that all areas of the hands are covered when using an alcoholic solution (83). The hands should be kept wet with such a preparation for the necessary agreed time requirement e.g. 2-5 min. The addition of agents with residual effect (e.g. chlorhexidine gluconate) to alcoholic solutions may prolong the antiseptic effect of a surgical hand disinfection (67).

The alcoholic method has the advantage that an undiluted agent is applied to the skin, there is no environmental contamination, and subsequent drying of the hands with a towel is unnecessary. It is often also the method of choice between operations, when hands need not be cleaned but must be disinfected.

3.2.1.3. Combined techniques: Combined techniques also exist which take advantage of the rapid action of alcoholic preparations and the sustained effect of other antimicrobial agents such as chlorhexidine. If such a combined technique is used hand disinfection with an alcohol should be performed after the surgical scrub with an antiseptic detergent (40). If a chlorhexidine-containing detergent is used, instead of unmedicated soap, before the alcohol, a significantly greater reduction of the bacterial release from the hands is achieved (68).

A comparison of the effectiveness of these techniques and various agents is shown in Table 2.

### 3.3. Colonized or infected hands of staff of operating team

Staff should not operate if a septic lesion is present on the hands. A minor lesion may sometimes be covered by a waterproof dressing, but generally exclusion from the operating room until the lesion is healed is preferable. Hands may be colonized by Staphylococcus aureus particularly if the skin is damaged by excessive washing in detergent or if eczematous lesions are present. The member of staff should again be excluded from the operating theatre until the lesions have healed or until the hands are free from Staphylococcus aureus. Treatment with antiseptic preparations is unlikely to be effective and may cause deterioration of the skin.

If a surgeon, or a member of the support team, in the operation theatre has caused infections of surgical wounds from an organism which he/she is carrying, he/she is probably a disperser. If lesions are present on the skin the subject should be excluded as already described, but if a healthy carrier, he/she should be treated. The carriage site is likely to be the nose or perineum, but the hands are also likely to be contaminated or, less commonly, colonized. The nose should be treated with an appropriate nasal antiseptic cream, e.g. neomycin and chlorhexidine, or neomycin and bacitracin or mupirocin, three or more times a day, and the subject should bath with an antiseptic detergent daily for 1 week in the first instance (41). The treatment should be repeated if not initially effective. Less commonly, only the hands are colonized and, if they appear healthy, these can be treated with an antiseptic-detergent for all washings, or an alcoholic disinfectant can be applied four or more times a day.

#### **4. Testing of disinfection procedures**

The effectiveness of hygienic hand disinfection in the prevention of spread of infection was convincingly demonstrated in Vienna by Semmelweis when he succeeded in lowering the maternal mortality rate from an average of 13.7% in 1846 to 1.3% in 1848 by introducing a regimen of hand disinfection using chlorinated lime as an antimicrobial agent. His success demonstrated the role of the hands of medical staff in transmitting hospital infection at a time when bacteriology was in its infancy. Semmelweis's findings are still well accepted, and hand hygiene is included in most medical and nursing procedures involving direct and indirect patient contact. However, the opportunities for repeating such experiments are no longer available.

The effect of any disinfection procedure, used as a preventive measure against infection, can be assessed either, directly, by determining its influence on the frequency of infection or, indirectly, by testing its effect on the microbial flora involved. The former method, namely, assessing the effect of a disinfection

procedure by epidemiological analysis is probably the most meaningful approach, as the ultimate aim of hand disinfection is to reduce the number of hand transmitted infections (48). Moreover, this way of testing could generate additional information on other important features of a disinfection procedure, such as side effects, acceptability and consequently handwashing compliance. It must be realized, however, that testing of the antimicrobial effectiveness must be the primary aim as without this preliminary assessment other positive features are meaningless. Furthermore, clinical studies and epidemiological analyses are tedious and time consuming. If hygienic hand disinfection is taken as an example, it can be estimated by power analysis that about 2500 patients would have to be included in each of 2 groups in order to demonstrate a significant difference in infection rates when one group is nursed by personnel using disinfectant A and the other by personnel employing a better method B (76). These calculations were based on the following theoretical estimates: infections due to hand transmission are 2% of patients when nursed by personnel using method A and 1% for the more efficient method B. The desired level of significance is 5% and the power of the statistical test is 90%.

This example illustrates the difficulty of demonstrating a significant difference between two disinfection procedures even when the difference between their clinical effects is unrealistically high. Thus, the direct assessment of the influence of an agent on the infection rate cannot be the first choice in routine testing of degerming procedures. Instead, tests will have to concentrate on the measurement of the disinfection process. Valid statements on the efficacy of a procedure are only possible if assessed according to a strict scientific test protocol. It is, therefore, important that as many conditions as possible are controlled by the investigator. This is much more difficult to achieve in in-use tests than in the laboratory. However, a compromise is possible by using a test model simulating in use conditions. The results are then more meaningful than those obtained from suspension or similar tests. For this reason, only tests on hands

will be dealt with here. It must be admitted, however, that tests are only of assistance in providing information on the rank order of effectiveness, since a statistically significant difference in a laboratory test does not imply clinical significance i.e. a reduced incidence of infection. In vitro tests are often recommended to establish preliminary information on the antimicrobial spectrum, on working concentrations, neutralizing agents and, later, further testing on side effects and acceptability may be necessary (54, 56).

#### 4.1. Evaluation of Procedures for Hygienic Hand Disinfection

##### Principles

Since hygienic hand disinfection is concerned with the inactivation of transient microorganisms only, a situation must be created in the laboratory similar to that which would occur under working conditions. This is achieved by employing the model of artificially contaminated hands (8, 13, 18, 20, 39, 46, 49, 55, 57, 64). However, it must be realized that even this model is to some extent artificial. The mode of contamination has been varied by investigators in many ways (see 70). However, the disinfection effect seems to be influenced very little by this (30, 39) if one excludes the removal effect which depends very much on the adherence of the test organism to the skin (39).

The choice of the test organism is limited by the possible infectious hazard to the volunteers, the resistance of the organism to environmental influences, such as drying, and to the ease of its isolation and identification on culture media in the presence of skin flora. Many microorganisms have been tried and Escherichia coli is now commonly used (see 39). There are some arguments against its use, e.g. it dies rapidly on drying and is not representative of Gram-positive organisms. There are, however, also arguments in its favour: it is the most frequent cause of nosocomial infections although not of cross-infection: it is more resistant than Staphylococcus aureus to agents like hexachlorophane, quaternary ammonium compounds, ampholytic detergents, and chlorhexidine, which are common additions to hand disinfectant preparations; it is easy to

distinguish from skin bacteria on bacteriological media; quantitative cultures of E. coli avoid the problem of breaking up of aggregated cocci, giving variable results such as may occur with staphylococci. It is also less dangerous to volunteers than Staphylococcus aureus which has occasionally been described as a cause of septic lesions in volunteers (8, 24, 53). It has been suggested that other Gram-negative bacilli such as Klebsiella or Serratia marcescens which are frequently prevalent as hospital acquired pathogens pose a more realistic challenge (19, 20). Although we suggest Escherichia coli because it gives satisfactory results under the controlled conditions of the test, there is a sound argument for using a Gram-positive coccus as well. A suitable non-pathogenic strain of S. aureus is at present being tested (Ayliffe - personal communication).

Another important aspect of the test model is the method of sampling for surviving test organisms removed from the hands. Here it should be clearly emphasized that most sampling techniques determine how many test organisms are released or recovered from the skin surface and not how many are actually there. In most tests the efficacy of a disinfection procedure is defined as the reduction of the release, or recovery of test organisms and not just by their reduction.

A variety of sampling methods have been described: direct impression or finger streaks onto a solid culture medium, indirect impression techniques using an imprinting surface such as a moist fabric, sticky tapes, rinsing techniques, using beads or brushes, rubbing the skin of the hands on moist objects, or with dry aids such as sand, brushes or scrapers, and examination (microscopical or cultural) of skin sections (see 70).

The principle employed in most models is the rinsing technique. The finger-tips ('finger-tip method') may be used by kneading and/or rubbing them in a suitable recovery medium. This is usually more effective if they are rubbed against the bottom of a petri-dish or on small glass-beads (8, 18, 42). It is also possible to sample the whole hand in a basin (9, 10, 16, 32, 60, 61, 62, 63, 84), in a plastic bag (23), or surgical glove (1, 20, 25, 50), each of which are filled with a suitable

sampling fluid. The glass cylinder method (15) and its variations (59, 82, 87) allow sampling from any even area of the skin surface. Each of these methods has its advantages and drawbacks. If one allows for the different volumes of sampling fluid and subsequent dilution the relative yield of test organisms from the plastic bag, basin method, glove or finger-tip may be considered equally good (49).

The microbial reduction due to a disinfection procedure may be established by measuring the recovery of test organisms before and after the procedure by means of one of the accepted sampling methods. The ratio of the resulting 'PRE-' and 'POST' values in an individual person is a direct measure of the efficacy of the disinfection procedure. The disinfection effect should be expressed as the fraction (= reduction factor, RF, 77) of the PRE/POST values or the difference between the logarithms ( $\log \text{PRE} - \log \text{POST} = \log \text{RF}$ ) of the bacterial counts rather than calculating percentages, which may be misleading, (77). A 99% reduction represents a 2 log RF. The best estimate of the efficacy of a disinfection procedure in a given population of volunteers is the geometric mean of the individual RFs or the arithmetic mean of their logarithms (= log RF).

The interpretation of this mean (log) RF, however, is difficult for several reasons:

- i) There are no epidemiological data indicating how effective a disinfection procedure should be in order to prevent hand transmitted infection.
- ii) As the efficacy of a disinfection procedure varies with individuals the average efficacy depends on the composition of the population of test persons (77). For this reason, variable results may be obtained in different laboratories or in repeated experiments with different test persons in the same laboratory. Thus, comparison of the mean test result with a pre-determined, hypothetical value (e.g.  $10^5$  reduction) is justified only if the sample size (= number of test persons) is sufficiently large to compensate for these variations.
- iii) In spite of attempts to standardize the test models, external influences, that cannot always be identified, have also been shown to cause significant

differences of the mean (log) RFs.

The best way to compensate for this variation is to compare the reduction factors obtained with the test agent with those obtained with a standard disinfection procedure or handwash performed by the same volunteers, on the same day and under similar environmental conditions (31, 72). Each volunteer is then acting as his (her) own control and extraneous influences are mainly nullified. Using this procedure, test results have been shown to become comparable between different laboratories (76). If the efficacy of the standard disinfection procedure has been chosen such that it can also serve as a standard for effectiveness as in the "Vienna" test model (see 5.1.1.), an acceptable disinfectant should not be significantly less active than the standard. If non-medicated soap is used as a standard as in the "Birmingham" test (see 5.1.2.) the test should be significantly more effective. The two test models for assessing the efficacy of procedures for hygienic hand disinfection are presented in detail in the Appendix.

## 4.2. Evaluation of Procedures for Surgical Scrub or Hand Disinfection

### Principle

The effect of a disinfectant on the normal (= resident) flora of the hands is assessed. The recovery or release of skin bacteria from both hands is measured before disinfection, immediately after disinfection from one hand, and one to the several hours after disinfection from the other hand which is gloved. In German speaking countries the log RF values obtained for the immediate and sustained effects are compared with those obtained with n-propanol 60% v/v, which is used in a standard disinfection procedure (ST) on the same test persons (13, 58). This is done to compensate for extraneous influences stemming mostly from the volunteers. Some models measure also the cumulative effect observable after multiple disinfection procedures (20, 40, 50).

There are several other workable test models, such as that suggested by Lowbury et al (e.g. 44), that by Michaud et al (50) or that published by the FDA (20). None of them, however, contains a requirement for the extent of reduction of bacterial release. Therefore, two models are presented here which include such requirements. In the Appendix a detailed description of the official test method of the Societies for Hygiene and Microbiology in Austria and the FRG (13, 18, 58) and a method used in Birmingham are presented.

## 5. A P P E N D I X

### 5.1. Hygienic hand disinfection

#### 5.1.1. Vienna test model (77)

The following model, which was first described in 1974 (77) and proposed as an official test in 1977 (31, 72) contains all the aforementioned requirements. Except for very minor details it has been adopted by the Austrian and German Societies for Hygiene and Microbiology and by the Federal Office of Health of the FRG as the official test method (13, 57). In comparative studies in other laboratories it has proved workable, and has produced comparable and reproducible results (76). If the basic principles are followed, i.e. use of artificially contaminated hands, assessment of release of test organisms before and after disinfection, and comparison of the reduction factors to those obtained with a parallel standard disinfection procedure, then the technical details as given below are not too important and could be altered to answer specific questions or for experimental purposes. An example would be the choice of test organism and the methods of sampling and culturing. In the interest of comparability between laboratories, however, it is preferable to follow one reference method or at least to include it in the investigation of new hand disinfectants and/or procedures.

##### 5.1.1.1. Definitions

A procedure for hygienic hand disinfection consists of several contributory factors involved in reducing the release of the test organism from the hands, e.g. the efficacy of the disinfectant in destroying test organisms, its concentration, and the duration and method of application. The procedure under test (P) is the disinfection procedure to be evaluated. The standard disinfection procedure (ST) is always tested in parallel on the same volunteers, on the same day and under similar environmental conditions (temperature and relative humidity of air).

#### 5.1.1.2. Prerequisites for evaluation

Before performing practical tests with volunteers, the following information must be obtained in laboratory tests:

- a) Antimicrobial spectrum of the test agent (bactericidal action in suspension tests against a range of agreed microorganisms, e.g. Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 11229, Proteus mirabilis ATCC 14153, Pseudomonas aeruginosa ATCC 15442, Candida albicans ATCC 10231, Mycobacterium tuberculosis ATCC 25618. (For further details see ref. (13).
- b) Appropriate neutralizers of the disinfectant (see table 3 and ref. 65).
- c) The disinfection time suggested by the manufacturer must not exceed 1 min.
- d) Procedures requiring the addition of water before or during the disinfection procedure (as, for instance, with disinfectant detergents) are not suitable for hygienic hand disinfection as explained before. If, however, still required, this model can also be used to compare the effectiveness of a washing procedure to that of the standard procedure.

#### 5.1.1.3. Principles of evaluation

The release of test organisms from the finger tips of artificially contaminated hands is measured before and after disinfection. The ratio of these values is a direct measure of the disinfecting activity of the procedure. It is an expression of the sum of all factors involved in a procedure, e.g. microbicidal as well as mechanical action. In order to compensate for extraneous influences, which mostly stem from the volunteers, the test results are compared with those of a standard disinfection (ST), which is run in parallel with the disinfection procedure under test (P).

The principle of the test procedure is shown in Fig 1.

#### 5.1.1.4. Experimental design

On theoretical considerations of experimental techniques a cross-over technique as described below is advised. Systematic experiments, however, have shown that this is not a critical requirement with artificially contaminated hands as

the outcome of an evaluation has been shown not to be influenced by the sequence of P and ST even with long-acting and strongly bacteriostatic agents such as chlorhexidine (29).

By means of some randomization technique, the volunteers are allotted into 2 groups of about the same size. In the first run, members of group 1 use P and those of group 2 use ST. In a second run, after a hand-wash of 2 min, the experiment is repeated but with changed roles.

#### 5.1.1.5. Volunteers

For valid evaluation, data from at least 12 volunteers must be available. But, to ensure comparability of interpretation between different laboratories or experiments, results of not more than 15 persons should be processed as the chance for a difference between means to become statistically significant increases with the sample size!

Persons with obvious hyperkeratosis, septic skin lesions on the hands, or with excessively long finger-nails should not be included as volunteers.

#### 5.1.1.6. Test organism

The standard organism is Escherichia coli ATCC 11229. Other organisms may be included, if required to answer specific questions. It has been shown, however, that the choice of the test organism does not significantly alter the outcome of an experiment for the evaluation (5, 79) unless there is a significant deficiency in the antimicrobial spectrum of the test agent. This, however, should be detected in the preceding in vitro experiments.

The test organism is used in the stationary phase, i.e. the culture is approximately 24 hours old, and the suspension in CSB used for artificial contamination must not contain less than  $10^8$  cfu/ml.

#### 5.1.1.7. Nutrient media and sampling fluids

Casein-Soy broth (CSB) serves as a liquid culture medium, as a sampling fluid and as a diluent. Casein-Soy agar with added sodium-desoxycholate 0,05% w/v is used for quantitative (surface) cultures. Sodium-desoxycholate inhibits the

growth of skin staphylococci.

Suitable disinfectant-neutralizers (Table 3 and see ref. 65) must be included in the sampling fluids and diluents, but not in the plates used for counting as some of the neutralizers may inhibit growth of the test organism. These neutralizers should be present in all sampling fluids and diluents, e.g. in those for the assessment of PRE- as well as of POST-values in both test and standard disinfection procedures. The efficacy of the neutralizers is established in preceding laboratory tests.

#### 5.1.1.8. Artificial contamination

The hands are washed with non-medicated soap and water for 2 min, and dried with paper towels. They are, then, immersed for 5 s up to the mid-metacarpals in 2 litres of a CSB-culture of the test organism. After having carefully allowed surplus liquid to drain back into the container, the hands are left to dry in the air for exactly 3 min. They are held in a horizontal position with the fingers spread out and rotated to and fro to avoid the formation of droplets.

#### 5.1.1.9. Assessment of PRE-values

Immediately after drying, the release of test bacteria before disinfection is assessed by rubbing all five finger-tips for 1 min on the base of a petri-dish (9 cm) containing 10 ml of sampling fluid. A separate dish is used for each hand. From dilutions  $10^{-3}$  and  $10^{-4}$  of these sampling fluids in CSB, volumes of 0,1 ml are spread over the surface of CSA-plates.

A recontamination of hands with the test organism after assessment of PRE-values is not necessary as this does not influence the ultimate outcome of the test (79).

#### 5.1.1.10. Disinfection procedures

Standard disinfection (ST): Immediately after sampling for the PRE-values, 3 ml of iso-propanol 60% (v/v) are poured into the cupped hands and rubbed on to the skin up to the wrists; after 30 s this procedure is repeated for another 30s.

Disinfection procedure under test (P): this is always performed according to

the instruction of the manufacturer but must not take longer than 60 s. (For general requirements to evaluate P see also Diagram 1).

#### 5.1.1.11. Assessment of POST-values

The same sampling procedure is used as described for the PRE-values, but volumes of 1,0 and 0,1 ml of the undiluted sampling fluid and 0,1 of a dilution  $10^{-1}$  are plated out for subsequent counting. For testing antiseptic-detergents, which may be not as effective as alcohols, a further dilution step may be necessary. Before incubation the surface of the plates containing 1,0 ml sampling fluid must be dried.

#### 5.1.1.12. Incubation

All plates are incubated aerobically at  $36 \pm 1^{\circ}\text{C}$  for 24 h. With some compounds such as chlorhexidine a longer incubation period may be necessary.

#### 5.1.1.13. Calculations

These calculations can easily be performed with any programmable pocket-calculator. Examples are given in references 13 and 57. Viable counts per ml of sampling fluid should be obtained whenever possible from plates showing 30-300 colonies. If 2 adjacent dilution steps both yield suitable counts (for instance, 299 colonies from  $10^{-1}$  and 31 from  $10^{-2}$ ), the weighted arithmetic mean of both is used (in this example:  $(299 + 31) : 1,1 \times 10^1 = 3000$ ). If the colony counts are grossly disproportional to the dilution steps from which they stem, insufficient neutralization of the disinfectant must be suspected.

The viable counts per ml sampling fluid are transformed to logarithms ( $\log_{10}$ ). For computational reasons, values of 0 have to be set at 1, which gives 0 as  $\log_{10}$ . Since 0 should only occur with the POST-values and here only with the best disinfectants, this adjustment can be accepted for routine test purposes. The log counts from right and left hands of each volunteer are averaged separately for PRE- and POST-values of both runs (ST and P).

The means of the averaged log PREs of all volunteers in both ST and P must not be lower than 5,00. Otherwise the experiment must be repeated (see Diagram 1).

From the differences between the PRE- and POST-values the log reduction factors are established for each volunteer for ST and P. With the ST, not more than 20% of the volunteers (= 3 of 15, 2 of 12-14) are allowed to produce values of less than 3,00 (see Diagram 1). The arithmetic means of the log RFs of all the volunteers are calculated for ST and P. That for ST must be at least 3,9; otherwise the mean of P should not be evaluated and the experiment repeated. (This latter requirement is not contained in the German guidelines).

#### 5.1.1.14. Evaluation of P

The data obtained may be used for evaluation of P only if the experiment can be regarded as acceptable according to the above quality criteria (see Diagram 1). If this is the case, procedure P may be regarded as suitable for hygienic hand disinfection if its mean log RF is not significantly smaller than that of ST (Diagram 2). Thus, with a mean log RF equal or larger than that of ST no further calculation is necessary. If, however, the mean log RF of P is smaller than that of ST, a statistical procedure must be performed to test for statistical significance of this difference. For this, the parameterfree WILCOXON matched-pairs signed-ranks test should be employed rather than the parametric paired t-test since the quality of the data do not meet those of an interval scale (34). If the difference is significant at a level of  $p = 0,1$  (directional testing) P must be rejected.

By power-analysis at the above settings and on the assumption of s.d. = 0,85 and at a desired power of the statistical test of 95% it has been shown that the discriminative power of this test model is 0,55-0,65 log steps (76) or that disinfection methods under test will be recognized as significantly inferior if their mean log RF is smaller than that of the standard by at least this difference.

## 5.1.2. Birmingham test

### 5.1.2.1. Introduction

A technique for assessing the efficacy of various antiseptic detergents and alcohols is described. The finger tips of volunteers are inoculated with a suspension of the test organism which is then recovered and the organism is re-applied; the hands are then treated with a standard disinfectant and surviving organisms recovered. The relationship between the numbers of bacteria applied and recovered indicates the effectiveness of the standard preparation. The procedure is then repeated using the product under test and a comparison is made with the standard. Escherichia coli is commonly used as a test organism, although the use of other organisms such as Staphylococcus aureus, or combinations of test organisms, is desirable (4).

### 5.1.2.2. Preparation of organisms

Broth cultures are prepared after plating nutrient agar slopes of the test organism on blood agar. After incubation, five colonies are transferred to 20 ml of Nutrient Broth (Oxoid No 2) and incubated for 18 h at 37°C. The test organisms usually selected are Escherichia coli ATCC 11229 or Staphylococcus aureus NCTC 4163. Both these test organisms have been widely used for hand disinfection studies for many years, no adverse effects have been reported.

### 5.1.2.3. Application of test organisms

The subjects taking part in the tests must have intact skin and should have short finger nails. During the preceding week, and test period, subjects should be prohibited from using other disinfectants and detergents which may influence the outcome of the tests. They should also be excluded if they are receiving antibiotics or other forms of antimicrobial therapy.

Hands are washed initially with non-medicated soap to remove natural transients, and are then dried thoroughly on paper towels. The palmar surfaces of the finger tips and thumbs of both hands are inoculated with 0.02 ml of the standard broth culture of the test organisms, e.g. Escherichia coli. Opposing fingers and thumbs

are rubbed together for 40 s and dried in the air for a further 80 s.

#### 5.1.2.4. Recovery of test organisms

The tips of fingers and thumbs are immersed in a bowl containing 100 ml of nutrient broth containing appropriate neutralizers (table 4) for the disinfectants under test, and rubbed vigorously on glass beads (3-5 mm in diameter) for 1 min. With a surface dropping technique, 2 x 0.5 ml of the neat broth and 5 x 0.02 ml of 10 fold dilutions, where appropriate, are transferred to the surface of well dried agar plates containing medium suitable for the growth of the test organism (e.g. blood agar for Escherichia coli and Nutrient agar, Oxoid No 2 with 1% serum for Staphylococcus aureus). Incubate for 18 h at 37°C (Escherichia coli) and an additional 24 h at room temperature (Staphylococcus aureus). Where possible counts are made on plates showing 30 - 300 cfu.

After sampling, the finger tips are rinsed under running water and thoroughly dried using paper towels.

#### 5.1.2.5. Application of standard skin disinfectant

Test bacteria are re-applied to the finger tips and after 2 min the standard formulation i.e. bar soap, or 5 ml alcohol, is applied to the hands using a standard technique (see Fig.2), comprising of five stroke backwards and forwards; palm to palm, right palm over left dorsum, left palm over right dorsum, palm to palm with fingers interlaced, backs of fingers to opposing palm with fingers interlocked, rotational rubbing of right thumb clasped in left palm and left thumb clasped in right palm, rotational rubbing with clasped fingers of right hand in palm of left hand and clasped fingers of left hand in palm of right hand. The hands and wrists are rubbed in this way until the end of the 30 s period. Finally, the hands are either sluiced under running water and dried on two paper towels (soaps and detergents) or allowed to air dry (alcohols) for a further 30s. Surviving bacteria are recovered in the manner already described.

#### 5.1.2.6. Application of test skin disinfectant

The whole procedure is then repeated using the product under test. Antiseptic detergents are assessed by moistening the hands and applying 5 ml of the test formulation using the technique described and alcoholic formulations by applying 5 ml to the cupped hands and rubbing in for 30 s also using the same technique.

#### 5.1.2.7. Calculation of results

The method of calculating results is summarized in Diagram 3. It is conventional, in this type of study, to work in  $\log_{10}$  units. The mean counts are thus geometric rather than arithmetic. Plates showing viable cfu of test organisms between 30 - 300 are counted. These counts are transposed to  $\log_{10}$  values and post counts are subtracted from pre counts for both standard and test, to give log reduction factors. The mean  $\log_{10}$  reduction factors can then be calculated. Statistical comparisons between test and standard are made using the students 't' test between paired samples. Differences are regarded as significant if  $p < (0.05)$ .

## 5.2. Surgical Scrub or Surgical Hand Disinfection

### 5.2.1. 'DGHM-model' (13, 57)

The test described is the official test method of the societies for Hygiene and Microbiology in Austria and the FRG. This is the latest version (DGHM 1981; ÖGHMP 1981) but the test principles have been followed since 1969. Therefore much experience exists with this test in German speaking countries.

#### 5.2.1.1. Definitions

A procedure for surgical hand disinfection consists of several contributory factors involved in reducing the release of transient and resident skin bacteria from the hands, e.g. the efficacy of the disinfectant or antiseptic detergent in destroying or removing this flora, its concentration, and the duration and method of application. The procedure under test (P) is the disinfection procedure to be evaluated. The standard disinfection procedure (ST) is always tested in parallel on the same volunteers.

#### 5.2.1.2. Prerequisites for evaluation

The time of the procedure under test (P) must not take longer than 5 min.

#### 5.2.1.3. Experimental design (Fig. 3)

A cross-over design is necessary: By means of some randomization technique, the volunteers are allotted to 2 groups of about the same size. In a first run, members of group 1 use P and those of group 2 use ST. In a second run, after at least one week, the experiment is repeated but with changed roles. Similarly, the right hand of a person is randomly allotted for assessment of the immediate or sustained effect. This allocation remains constant in both runs.

#### 5.2.1.4. Volunteers

For valid evaluation, data from at least 18 volunteers must be available. But to ensure comparability of results between different laboratories or experiments concerning the power of statistical tests the results of not more than 20 persons should be evaluated.

Persons with obvious hyperkeratosis, septic skin lesions of the hands, or with excessively long finger-nails should not be included as volunteers. In addition,

volunteers must not use hand disinfectants or medicated soaps for at least 3 days prior to the experiment.

#### 5.2.1.5. Nutrient media and sampling fluids

Casein-Soy agar (CSA) without selective agents is used as a solid nutrient medium.

Casein-Soy broth (CSB) serves as a sampling fluid and as a diluent.

Suitable disinfectant-neutralizers (see table 3 and ref. 65) must be included in the sampling fluids and diluents, but not in the counting plates as some of them may inhibit growth. These neutralizers should be present in all sampling fluids and diluents, e.g. in those for the assessment of PRE- as well of POST-values for both, test and standard disinfection procedures. The efficacy of the neutralizers is established in preceding laboratory tests.

#### 5.2.1.6. Assessment of PRE-values

Hands are washed with non-medicated soap and running water for 30s to remove transient organisms, and dried with paper towels. Then, the hands are sampled as described for hygienic hand disinfection (5.1.1.9.).

Dilutions  $10^{-1}$  and  $10^{-2}$  are made from the sampling fluids and 0,1 ml are plated from the undiluted and diluted liquids.

#### 5.2.1.7. Disinfection procedures

After sampling the finger tips are rinsed to remove the sampling fluid and dried on a paper or sterile towel.

Standard disinfection (ST): Immediately after sampling for the PRE-values, 3 ml of n-propanol 60% (v/v) are poured into the cupped hands of one half of the volunteers and rubbed well into the hands and wrists until nearly dry. This procedure is repeated as often as necessary to keep the hands moist during 5 min. Usually, a volume of 9-18 ml is necessary for this. This standard disinfection procedure has been chosen arbitrarily on basis of its excellent performance with the intention to integrate also the surgeon's hands into the concept of asepsis in surgery.

Disinfection procedure under test (P): The other half of volunteers employs P according to the instructions of the producer but not longer than for 5 min.

If the disinfectant is not an alcoholic solution hands must be rinsed well after disinfection.

#### 5.2.1.8. Assessment of POST-values

Immediate effect: Immediately after disinfection the hand, which was designated by randomization for the assessment of the immediate effect, is sampled as described under 5.2.1.6.

Sustained effect: After completion of disinfection the other hand is airdried and covered with a sterile surgical glove, be worn for 3 hours. Thereafter, this hand is sampled as described.

From undiluted sampling fluids volumes of 1,0 and 0,1 ml and from a  $10^{-1}$  dilution 0,1 ml are plated on CSA.

#### 5.2.1.9. Incubation

All cultures should be incubated at  $36 \pm 1^{\circ}\text{C}$  for 48 hours.

#### 5.2.1.10. Calculations

The necessary calculations can easily be performed with any programmable pocket-calculator. Examples are given in references 13 and 58. The details are the same as for hygienic hand disinfection (5.1.1.13) excepting that the values of right and left hand are not averaged but are used for calculation of log RFs of either the immediate (one hand) or the sustained effects (other hand).

#### 5.2.1.11. Evaluation of P (Diagram 4)

The data may be used for evaluation of P only if values from at least 18 persons are available and if each of the 4 mean log PRE-values (immediate and sustained effect of both ST and P) amounts to at least 3,5. Otherwise the whole test has to be repeated.

A procedure for surgical hand disinfection is accepted if the mean log RFs assessed for immediate as well as for sustained effects are not significantly lower than those of ST. If, therefore, both means of P are equal to or larger than those of ST, the procedure P is accepted.

If, however, one or both effects (immediate and/or sustained) of P are smaller than those of ST, a test on the statistical significance of this finding has to be performed, wherever necessary. For this, the WILCOXON matched-pairs signed-ranks test is used. If the difference on one of the two means (immediate or sustained action) is significant at a level of  $p = 0,1$  (unidirectional testing) P must be rejected. By power-analysis it can be calculated that at the above settings and on the assumption of s.d. = 1,0 and at a desired power of the statistical test of 95% the discriminative power of this test model is 0,8 - 0,9 logs. This signifies that procedures for surgical scrub or disinfection will be recognized as significantly inferior to the standard if their mean log RFs for immediate or sustained effect are smaller than those of the standard by at least this difference.

### 5.2.2. Birmingham test

#### 5.2.2.1. Introduction

A technique for assessing the immediate and prolonged efficacy of various antiseptic detergents and alcohols is described. This can be established by comparing the logarithmic or percentage reductions in the number of resident bacteria recovered from the hands before and after an initial treatment and/or repeated applications (42). The prolonged (residual) effect after wearing the gloves for 3 hours may also be assessed (44).

The resident skin flora are recovered using a glove sampling technique first described by Michaud, McGrath and Goss (50).

#### 5.2.2.2. Restrictions on Volunteers

The subjects taking part in the tests must have intact skin and should have short finger nails. During the preceding week, and test period, subjects should be prohibited from using other disinfectants and detergents which may influence the outcome of the tests. They should also be excluded if they are receiving antibiotics or other forms of antimicrobial therapy. Non-medicated soaps are provided for volunteers to use throughout the test and they are issued with rubber gloves to be worn during their daily routine should they come into contact with detergents, acids, bases or solvents. On the days of the study where sampling occurs rubber gloves are not worn before sampling as this may increase the resident flora. Thus, on these days the volunteers must not handle the above mentioned substances until sampling has been performed. Hand creams should not be used on any of the test days.

#### 5.2.2.3. Procedure for determining baseline counts

Baseline counts can be obtained from all volunteers by performing a standard procedure and sampling both hands individually on three separate occasions with at least 48 hours between each test.

The procedure for determining baseline counts is as follows.

The hands and forearms, to approximately 3 inches above the wrist, are washed with non-medicated soap for 30 s. The nails are cleaned under

a running tap with a manicure stick, the hands rinsed well and dried on disposable paper towels. The hands are then sampled for baseline resident skin flora.

#### 5.2.2.4. Recovery of resident skin flora

A computer generated random scheme may be used to determine whether the right or left hand is used for immediate or prolonged effect. Immediate effect measurements are allocated to the same hand for each preparation, if more than one are to be tested in the same experiment. The hands are sampled by donning loose fitting sterile, unpowdered, surgical gloves containing 50 ml of neutraliser/sampling solution (e.g. nutrient or tryptone soya broth containing appropriate neutralizers for the products under test).

The gloves of each hand are sealed at the wrist with an adjustable strap and massaged for 1 min using a standard sampling procedure:

For the purpose of sampling, the hand is divided into 4 areas which are each massaged 5 times applying firm but even pressure. Starting with the finger tips followed by the fingers, fingerwebs, the palm and the back of the hands. This procedure is repeated 3 times in 1 min. The hands are opened and closed 3 times to help mix the sampling fluid.

The strap is removed and a volume of fluid is transferred by pipette to a sterile bottle. The gloves are then removed and the hands and wrists rinsed free of residual sampling fluid under a running tap and dried. The number of viable resident flora are counted using a surface drop counting technique. The arithmetical means of the three samples for each hand are transposed to log values and these are used as the starting counts.

#### 5.2.2.5. Application of hand wash formulations

The standard or test scrub procedure of treatment is carried out not less than 48 hours after the last baseline sample. Immediate and prolonged effects may be assessed using the following method of application: -

Hands are washed for 30 s with soap and water, and nails cleaned under a running tap with a manicure stick. Hands are rinsed and excess water shaken off. Five ml of aqueous formulations are applied and spread over the hands and forearms to the elbow washing for 15 s. Using a sterile nail brush, the nails of each hand are scrubbed for 15 s and the hands and forearms washed for a further 15 s. Hands and arms are rinsed well under a running tap for 15 s with the fingers uppermost ensuring that the water flows from the finger tips towards the elbow. The handwash application is repeated and hands are washed in the standard manner for a further 2 min. Hands and arms are rinsed for 15 s as described above, and dried thoroughly using one disposable paper towel to dry the fingers and hands and a separate one to dry each arm starting with the wrist and working towards the elbow.

To assess the efficacy of alcoholic hand rubs, the hands are washed for 30 s with soap and water, the nails cleaned under a running tap with a manicure stick, rinsed, excess water shaken off and dried on paper towels. Five ml of the alcoholic standard test formulation are applied using the same technique as that described for aqueous formulations. If a two min application period is chosen, four aliquots of the alcoholic formulation will be required.

#### 5.2.2.6. Handwashing technique

To ensure total coverage of the hand surfaces after applying and spreading the handwash formulations the hands are washed using the method described by Ayliffe 1978 (4) shown in Fig. 2.

#### 5.2.2.7. Post treatment sampling: Immediate effect

Immediately after the washing procedure, the designated hand is sampled using the technique previously described.

#### 5.2.2.8. Post treatment sampling: Prolonged effect

The prolonged or residual effect may be measured by wearing a sterile, unpowdered surgical glove on the designated (other) hand for three hours, during which time the volunteer will be free to perform normal duties.

The gloved hands are sampled in the manner previously described for baseline counts. Dilutions are prepared in suitable media such as Ringer's solution, TSB or nutrient broth. Special neutralizers for the products under test may also be incorporated provided they are not inhibitory.

Ten fold dilutions are prepared from all glove rinse samples and these are plated out for enumeration using a surface dropping technique. 0.5 ml of the undiluted sample is plated out in addition to 0.1 ml of the dilutions so that as few as 2 cfu per ml of sampling fluid can be detected.

The neutralization systems used must be validated before carrying out the assessment.

#### 5.2.2.9. Calculation of results

The criterion for evaluating the antimicrobial effects of the test formulations is the reduction of the viable count of the resident microflora of the hand. Immediate and prolonged effects are calculated separately for each volunteer by subtracting the count after washing from the appropriate mean baseline count. The mean immediate reduction and the mean prolonged reduction effects of each formulation can then be determined. It is conventional, in this type of study, to work in  $\log_{10}$  units. The mean counts are thus geometric rather than arithmetic. The number of viable bacteria on sample plates in the range of 30 - 300 are counted and, after making the necessary allowance for the step in the serial dilution, the figure is converted to a  $\log_{10}$  value. Post counts are subtracted from pre counts for both immediate and prolonged effect to give log reduction factors. The mean  $\log_{10}$  reduction factors can then be calculated for standard and test formulations. Statistical comparisons between test and standard are made using the students 't' test between paired sampled. Differences are regarded as significant if  $p < 0.05$ .

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TABLE 1.

Examples for the efficacy of various degerming procedures to reduce the release of transient flora from hands (according to 69)

Procedures (all 1 minute)	Conc %	Log Reduction
<i>Rubbing-in:</i>		
<i>n</i> -propanol	60 v/v	5.5
	50 v/v	4.9
	40 v/v	4.3
isopropanol	60 v/v	4.2
ethanol	80 v/v	4.5
	70 v/v	4.0*
	60 v/v	3.8
povidone-iodine solution	1 g/v	4.0
<i>Washing with:</i>		
povidone-iodine soap	0.75 g/v	3.2*
chlorhexidine soap	4.0 g/v	2.9*
phenolic soap	mixed	2.9*
liquid soap OAB 9	20 v/v	3.0*

\* Significantly less effective than standard.

Values derived from different experiments but from one laboratory with the Vienna model (see Fig. 1); the mean log reduction by standard disinfection always being between 4.1 and 4.3

**Table 2**

Examples for the efficacy of various degerming procedures to reduce the release of resident flora from the hands (according to various investigators (see 70))

Procedures	Conc. (%)	Time (min)	log Reduction after disinfection		Ref.
			immediate	3 hours	
<b>Surgical scrub</b>					
<u>Washing with</u>					
- soap + water (for comparison)		5	0,4	n.v.	61
		5	0,4*	-0,1*	75
- povidone-iodine liqu.soap	0,8 w/v	5	0,9-1,0*	0,2*	73,75
		2	0,5	n.v.	38
- chlorhexidine liqu. soap	4,0 w/v	5	0,9*	0,9*	75
		3	0,8*	0,8-1,0*	73,75
		2	0,9	1,6	45
- hexachlorophene liqu. sp.	3,0 w/v	4	0,3	1,0	51
- benzethonium chloride	10,0 w/v	6	1,3	n.v.	21
		3	1,0	n.v.	21
		2	0,9	n.v.	21
- cetrimide	1,0 w/v	2	0,4	n.v.	38
<b>Surgical hand disinfection</b>					
<u>Rubbing in</u>					
- n-propanol	60 v/v	5	2,9-3,0*	1,6-2,4*	75
- iso-propanol	70 v/v	5	2,4*	2,1*	86
	70 v/v	2	1,2	0,8	44
	60 v/v	5	1,7*	1,0*	73
- iso-propanol + chlorhexidine gluc.	70 v/v 0,5 w/v	5	2,5*	2,7*	86
- ethanol	95 v/v	2	2,1	n.v.	40
	80 v/v	5	2,5	n.v.	12
	80 v/v	2	1,5	n.v.	2
	70 v/v	2	1,0	0,6	44
	70 v/v	2	0,6	n.v.	21
- povidone-iodine watery solution	1 w/v	5	1,9*	0,8*	74
		2	0,4	n.v.	47
<b>Combined techniques</b>					
<u>Washing with/followed by</u>					
<u>Rubbing-in</u>					
- unmedicated liquid soap/ followed by iso-propanol + chlorhexidine gluc.		3 + 4	1,7*	1,1*	68
	70 v/v 0,5 w/v				
- chlorhexid.gluc.liqu.soap/ followed by iso-propanol + chlorhexidine gluc.	4 w/v	3 + 4	2,5*	1,7*	68
	70 v/v 0,5 w/v				

\* These values were assessed by using the same test method (58)

n.v. = no values available

Table 3. Examples of effective disinfectant-neutralizers

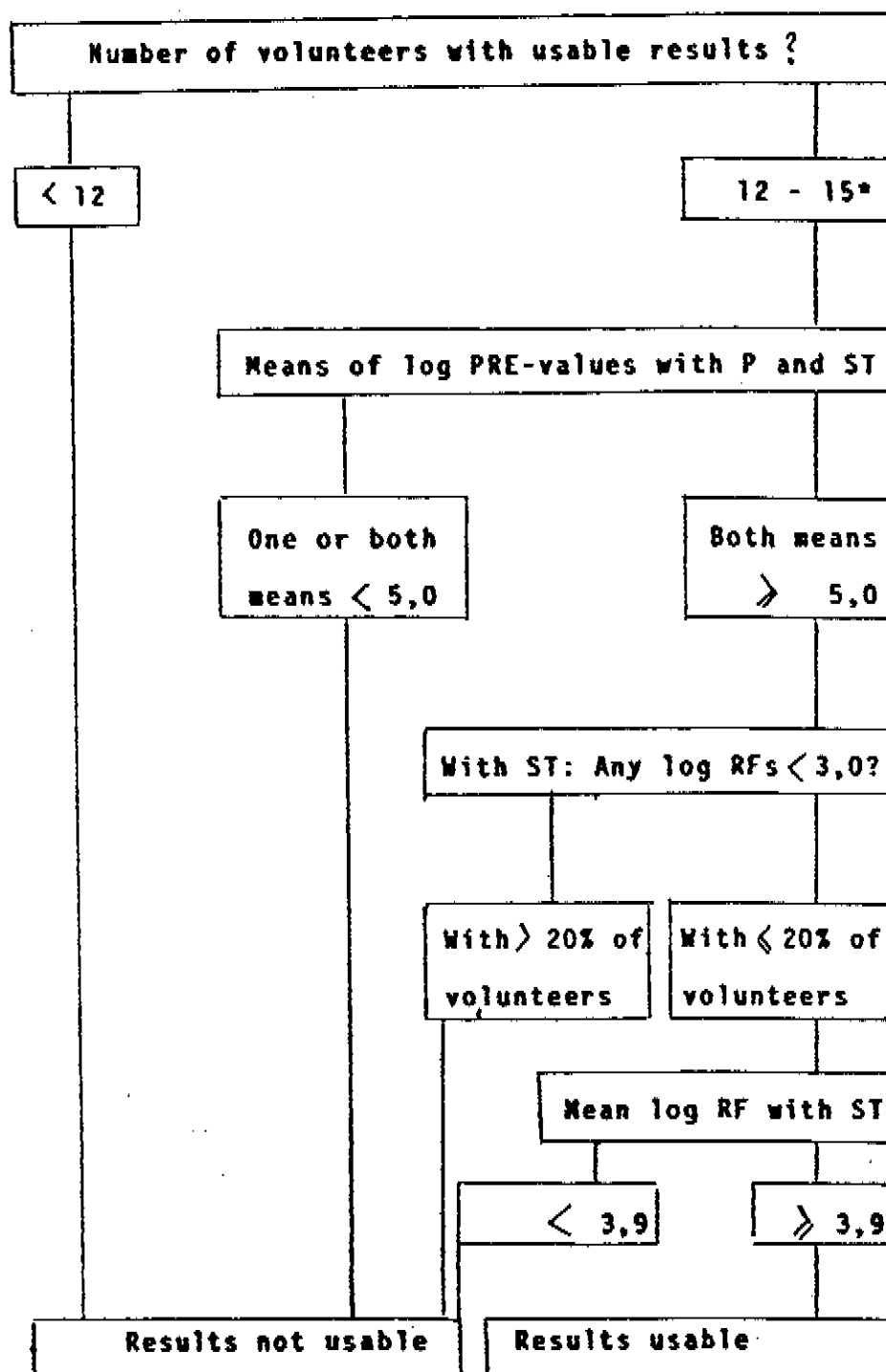
Disinfectant	Neutralizer (concentration per litre sampling fluid or diluent)
Chlorhexidine gluconate	Tween 80 (30ml) + lecithin from eggs (3 g) + histidine (3 g) (higher concentrations, up to 5 times, may be necessary!)
Povidone iodine soap	Tween 80 (30 ml)
Chlorine compounds	+ lecithin from eggs (3 g) + histidine (1 g) + sodium thiosulphate (5 g) + bovine serum albumin lyophilized (1 g)
Phenolic compounds	Tween 80 (30 ml) + lecithin from eggs (3 g) + Histidine (1 g) + sodium thiosulfate (5 g)
Quaternary ammonium	Tween (30 ml) + saponin (30 g) + histidine (1 g) + cysteine (1 g)

TABLE 4.

NEUTRALISERS USED IN THE BIRMINGHAM TEST

Antiseptic	Neutraliser	Concentration	Used in recovery broth	dilutions	plating out
Chlorhexidine (0.5% - 4%)	Lecithin Tween 80 (50g Tween 5g lecithin) mixture	0.75% of the mixture	YES	NO	NO
Povidine-iodine (7.5%)	sodium thiosulphate	1%	YES	NO	NO
Triclosan (0.5%-2%)	Lecithin Tween 80	1% 3%	YES	NO	YES (concentrations as for chlorhex- idine no inhibition found)
Alcohols	Nil	if alcohol is used as the standard the same recovery media is used as for the test formulation			
Unmedicated soap	Nil	if unmedicated soap is used as the standard the same recovery media is used as for the test formulation			

Diagram 1. Flowdiagram for quality control of experiments for evaluation of procedures for Hygienic hand disinfection (Vienna model 57)



\* Do not use results of more than 15 volunteers for evaluation

P = procedure under test    ST = standard disinfection with  
iso-propanol 60 v/v 1 min

Diagram 2. Flowdiagram for decision on acceptance or rejection of a procedure under test (P) for hygienic hand disinfection in the Vienna model (57)

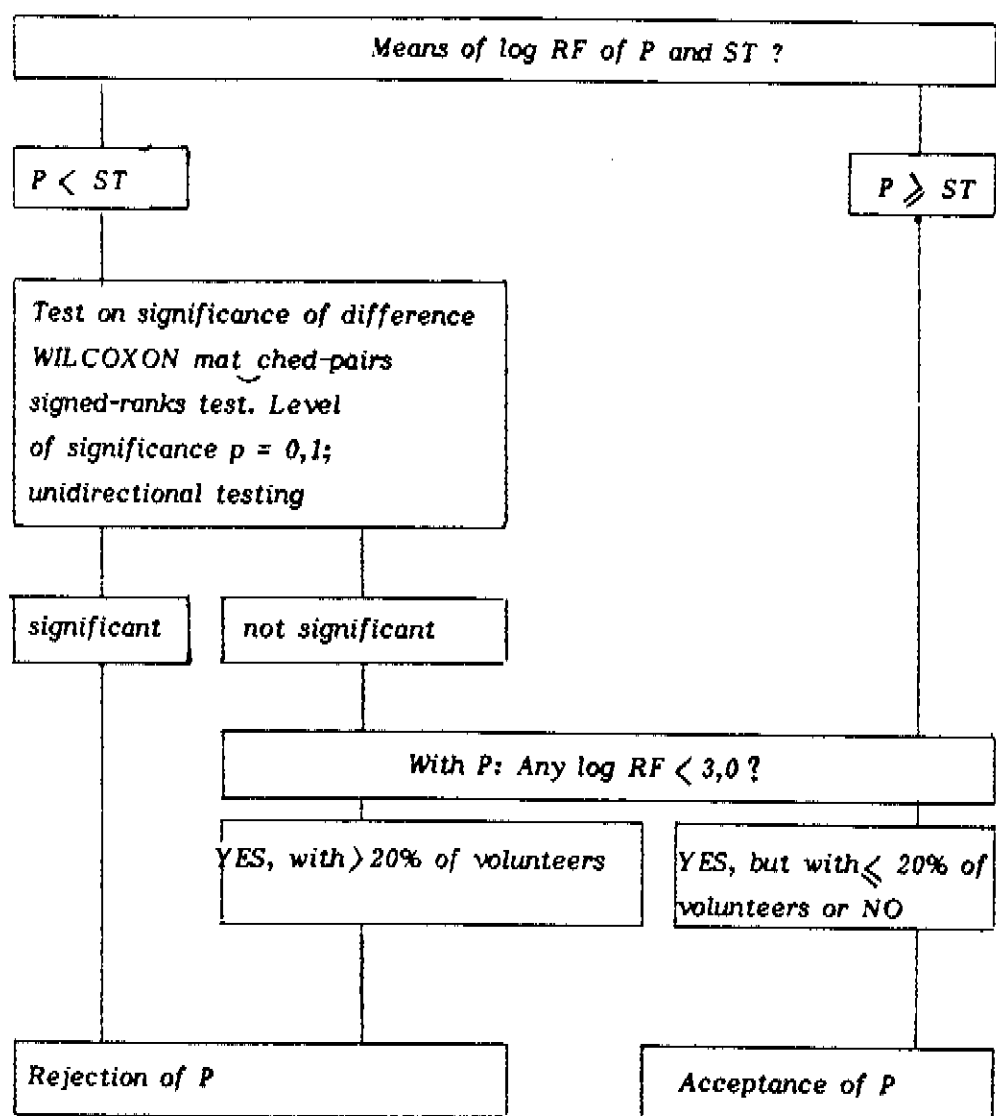


Diagram 3: Hygienic hand disinfection procedure: calculation of efficacy of test and standard skin disinfectant in the Birmingham test

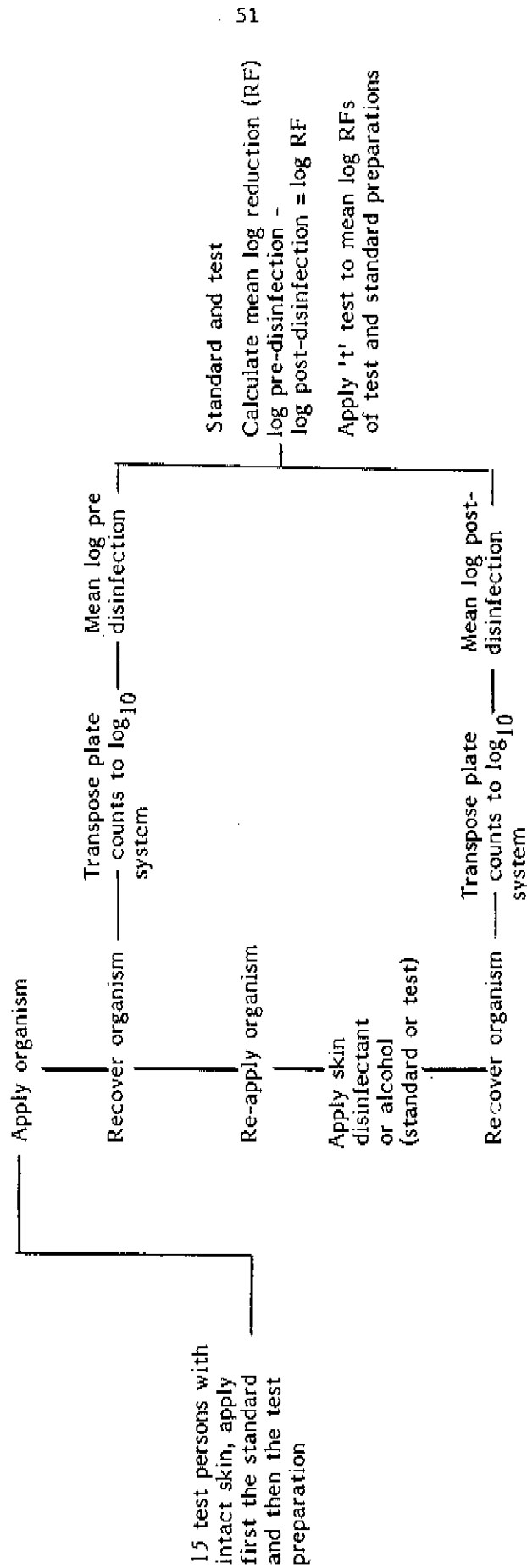
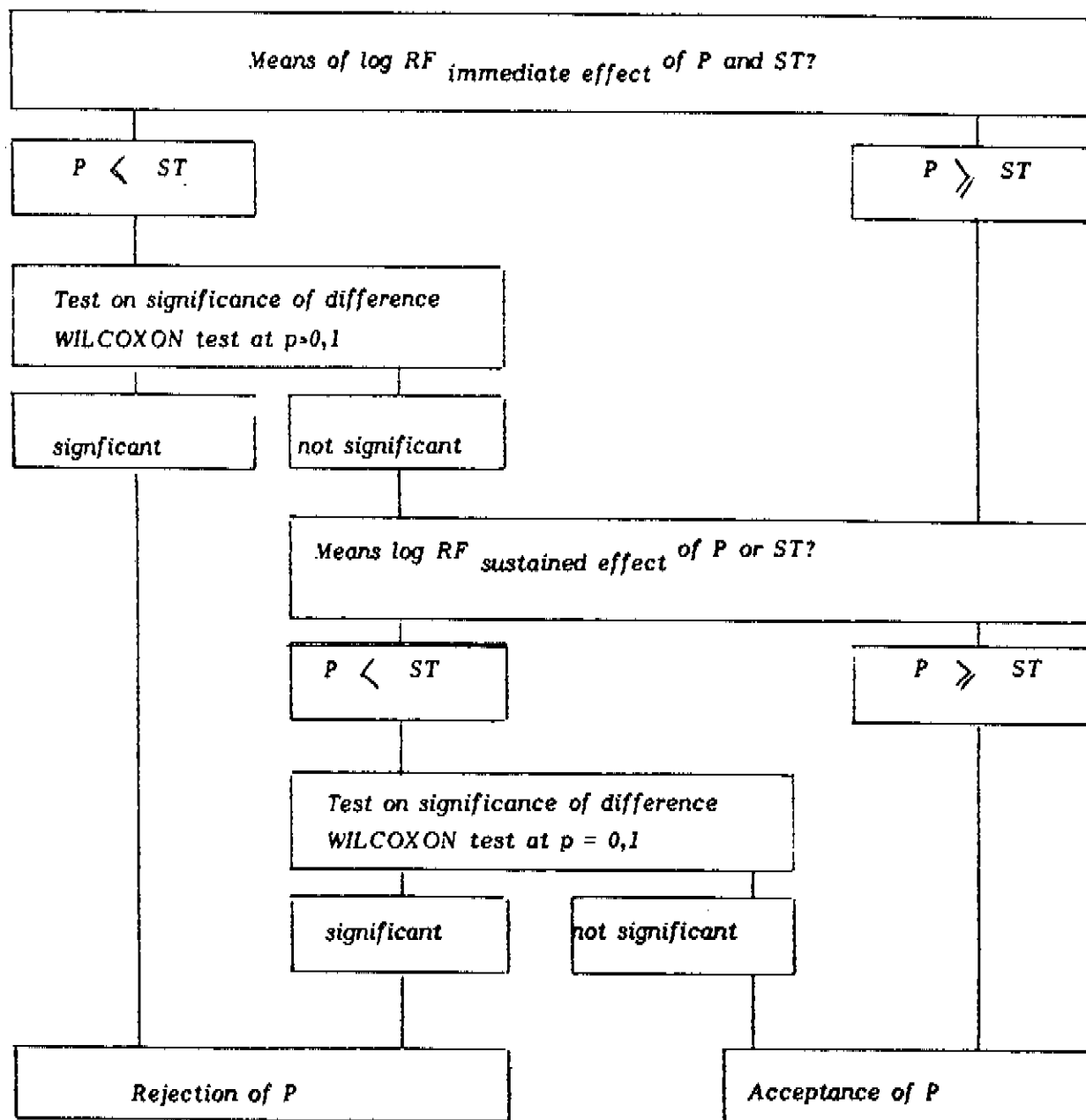
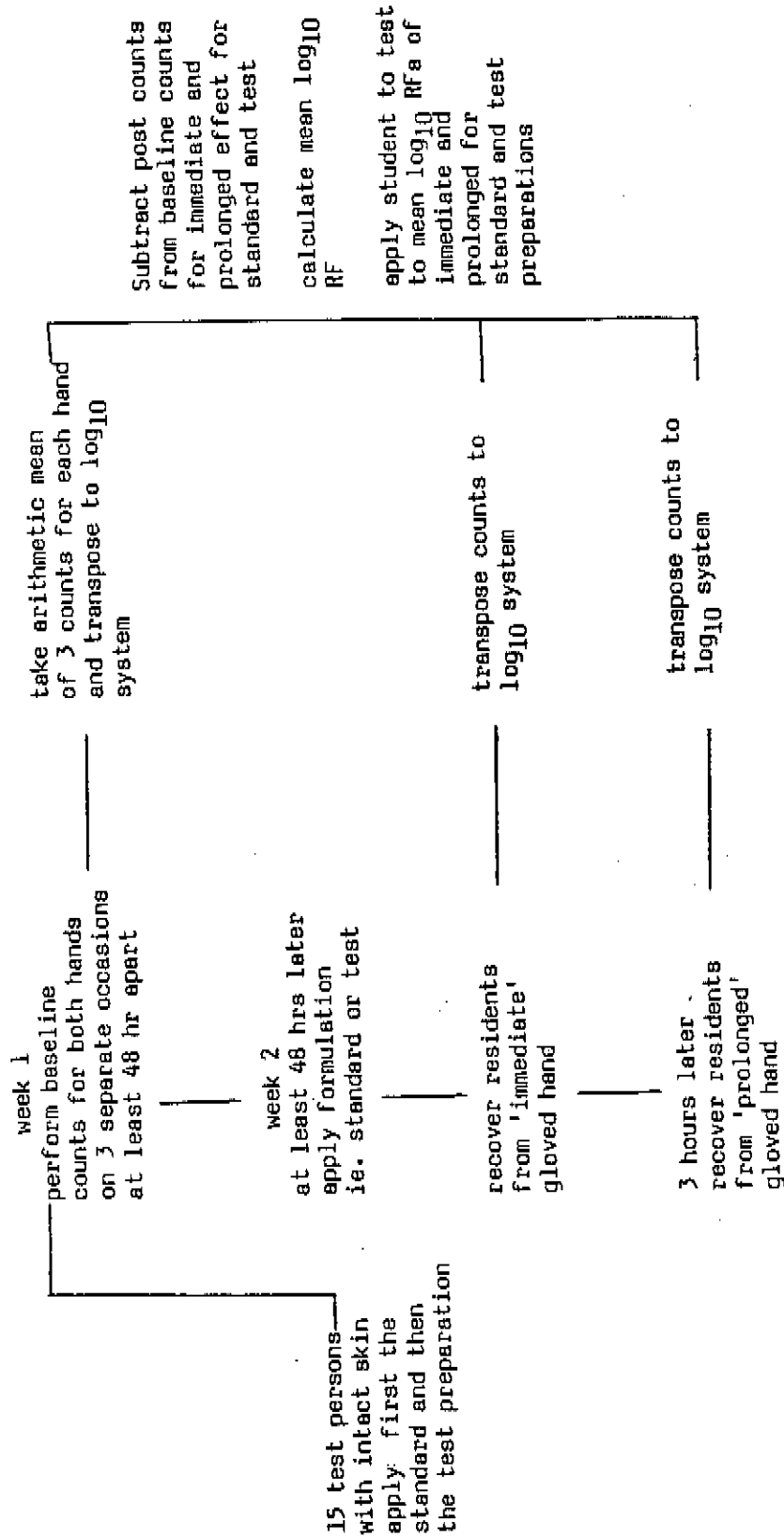


Diagram 4. Flowdiagram for decision on acceptance or rejection of a procedure under test (P) for surgical hand scrub or disinfection in the DGHM model (18, 58)



ST = standard disinfection with n-propanol 60% v/v, 5 min

Diagram 5: Surgical hand disinfection procedure: calculation of efficacy of test and standard skin disinfectant in the Birmingham test



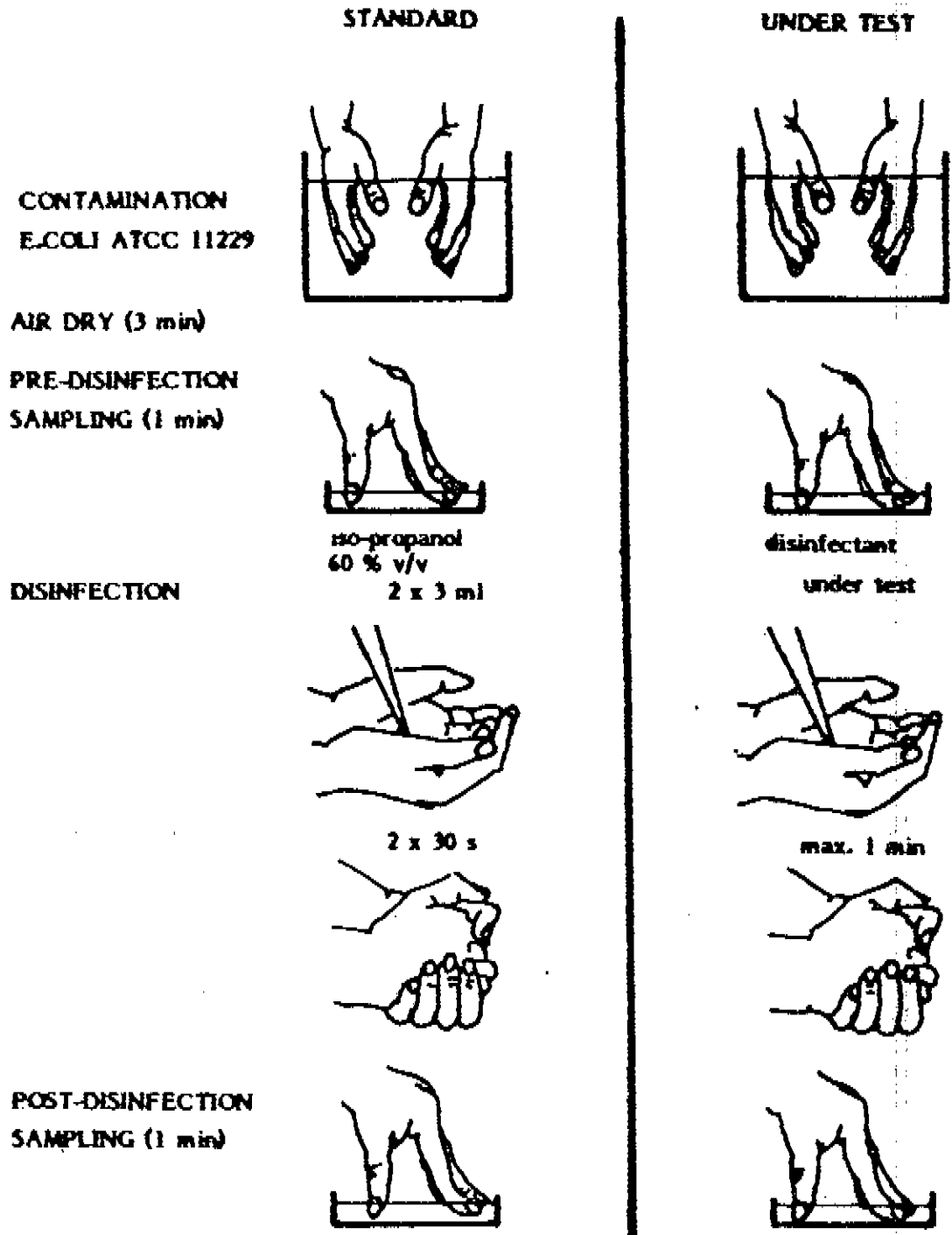
Legends

Fig. 1: Principles of the Vienna model for testing procedures for hygienic hand disinfection  
(18, 57)

Fig. 2: Handwashing technique for the Birmingham test

Fig. 3: Principles of the model for testing procedures for surgical hand scrub or disinfection  
(18, 58)

Figure 1. TESTING PROCEDURES FOR HYGIENIC HAND DISINFECTION ("VIENNA MODEL")



AFTER STANDARD, PROCEDURE UNDER TEST IS TESTED BY THE SAME VOLUNTEERS

Figure 2. Handwashing Technique  
in the Birmingham Model

Aqueous formulations - wet hands and wrists apply 5 ml of the test or standard formulation to the cupped hands and wash hands using the following procedure, each step consisting of five strokes backwards and forwards

Alcoholic formulations - apply 5 ml of the test or standard formulation to the cupped hands and rub in also using the following procedure



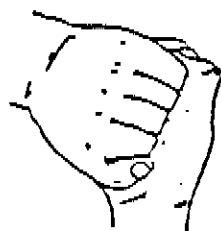
1. Palm to Palm



2. Right palm over left dorsum and left palm over right dorsum



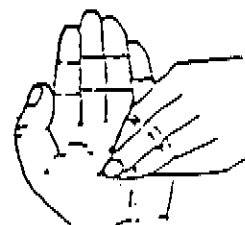
3. Palm to palm fingers interlaced



4. Backs of fingers to opposing palms with fingers interlocked



5. Rotational rubbing of right thumb clasped in left palm and vice versa



6. Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa

Continue washing hands and wrists until 30 secs have elapsed. In the case of aqueous formulations, rinse hands and dry thoroughly.

ONE HALF OF VOLUNTEERS TESTING STANDARD

OTHER HALF OF VOLUNTEERS TESTING PROCEDURE UNDER TEST

PRE-DISINFECTION SAMPLING, 1 min (both hands)



DISINFECTION

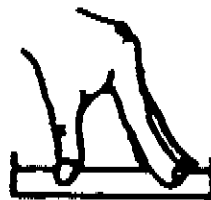


n-propanol 60% v/v; keep hands wet for 5 min

disinfectant under test; procedure according to instruction of producer



1st POST-DISINFECTION SAMPLING, 1 min FOR IMMEDIATE EFFECT



ONE HAND

ONE HAND

3 hours wearing gloves

3 hours wearing gloves

2nd POST-DISINFECTION SAMPLING, 1 min FOR SUSTAINED EFFECT



OTHER HAND



OTHER HAND

AFTER ONE WEEK: SAME VOLUNTEERS PERFORM TEST WITH CHANGED ROLES

