Testing a New Alcohol-Free Hand Sanitizer to Combat Infection

Following universal precautions is an integral part of OR staff members’ responsibilities in perioperative patient care. The precautions mandate routine hand washing with soap and water before and after all patient contact, and especially before invasive procedures. Although intended to reduce the postoperative risk of infection of healing incisions and wounds, universal precautions, including hand washing, are followed only 55% of the time in the nation’s hospitals.¹

The contributing factors behind this insufficient hand washing are manifold; however, identified principal causes include the following.

- Direct patient caregivers are handling an excessive patient load. Conservative estimates indicate that physicians attend to 20 to 30 patients per day, and other health care personnel (eg, nurses, physical therapists, respiratory therapists) may have as many as 200 patient contacts per day.
- The repeated hand washing required for that volume of patients causes dryness and subsequent microabrasions of the skin.²
- The skin on the hands has a short period of time to recover between washings.

These factors, among others, have led to a great increase in the use of rinse-free instant hand sanitizers as a supplement to proper hand washing with soap and water.

The most widely used hand sanitizers are gels and foams that rely on alcohol as the main antimicrobial ingredient. Alcohol, however, solubilizes and strips away sebum and lipids that guard against bacterial infections of the skin.³ Extensive use of alcohol-containing hand sanitizers actually increases the skin’s susceptibility to infection by transient disease-causing bacteria. This situation can increase the chances of spreading disease-causing microorganisms among patients.

The threat of spreading disease could be avoided by using alcohol-free hand sanitizers that complement, rather than compromise, the natural barrier functions of the skin. An acceptable alcohol-free formula would require an antimicrobial agent that kills a wide variety of disease-causing microorganisms, including gram-positive and gram-negative bacteria, fungus, and molds. This formula also would need to allow the active ingredient to penetrate the skin while minimizing skin irritation.

ABSTRACT

Universal precautions require that perioperative health care personnel wash their hands before and after all patient contact. Time constraints, however, can make adhering to universal precautions, including proper hand washing, difficult. Some perioperative health care workers, therefore, routinely use rinse-free hand sanitizers to supplement normal hand washing. This study evaluated immediate and persistent antimicrobial effectiveness of two alcohol-containing hand sanitizers and a novel surfactant, allantoin, benzalkonium chloride (SAB) hand sanitizer using a federally approved effectiveness protocol. Results indicate that all three products were equally effective after a single application. After repeated use, the alcohol-containing sanitizers did not meet federal performance standards, and the alcohol-free sanitizer did. These properties and others illustrated in this article indicate that the nonflammable, alcohol-free SAB hand sanitizer is the most favorable of the rinse-free hand sanitizer formulas for normal hand washing. AORN J 68 (August 1998) 239-251.

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Such a formula is obtained by combining certain surfactants and allantoin with the widely used antimicrobial agent, benzalkonium chloride. This formula is known as a surfactant, allantoin, and benzalkonium chloride (SAB) sanitizer.

ESTABLISHING THE HYPOTHESIS

Based on this information, researchers at Woodward Laboratories, Inc., Los Alamitos, Calif., hypothesized that the prolonged use of alcohol-containing hand sanitizers would be less effective at degerming the skin than an alcohol-free SAB sanitizer. To test this, they used a protocol validated by the US Food and Drug Administration (FDA) for the performance of health care personnel antiseptic hand washes. This protocol was developed specifically to test the degerming effectiveness of handwash preparations with extended use and is accepted as a national clinical standard for such performance testing.

LITERATURE REVIEW

The literature review for this study indicated that rinse-free hand sanitizers are, by definition, intended for degerming skin without the aid of rinsing with water. This type of product has steadily gained popularity in professional circles as a supplement to hand washing with soap and water. The types of rinse-free hand sanitizers generally are grouped into two broad categories:

- alcohol-based products
- alcohol-free products.

The need for immediate and persistent protection. The FDA clearly seeks both an immediate and persistent degerming activity in antiseptic preparation by its definition of a personnel disinfectant:

a non-irritating, antimicrobial-containing preparation designed for frequent use and which will reduce the number of transient microorganisms to a baseline level after adequate washing, rinsing, and drying. Such preparations also are expected to have a broad antimicrobial spectrum, be fast-acting, and persistent.

A hand sterilizer’s immediate antimicrobial effectiveness is based on the physical removal and immediate inactivation (ie, within 60 to 180 seconds of exposure to the antimicrobial agent) of microorganisms residing on the hands. The persistent antimicrobial effectiveness of a hand sanitizer is defined as its microbiocidal activity after up to six hours of the product’s application.

Alcohol-based products. These products vary greatly in composition, ranging from 54% isopropanol to 70% ethanol. The choice of this type of rinse-free antimicrobial product often is subjective and mainly based on factors such as cost, presence of emollients in the formula, fragrances, delivery vehicle (eg, gel, foam), size, and marketing. Selection is less often based on the product’s effectiveness at eliminating bacteria after a single application.

Although alcohol-based formulas that comply with federal composition standards generally are considered effective, alcohol-based antiseptic handwash preparations are flammable and do not demonstrate persistent antimicrobial activity. Also, repeated use often can cause drying and irritation of the skin. Alcohol strips the skin of essential oils and sebum, which act as a natural protective barrier against bacterial infection and precipitate protein. When applied to wounds or raw surfaces, therefore, it not only increases the risk of injury, but also forms a coagulum under which bacteria may subsequently thrive. It is, therefore, not useful for the disinfection of open lesions or abraded, inflamed skin. Together, these and other adverse properties greatly limit the alcohol-based antimicrobial product’s immediate effectiveness and increase the chances for the spread of infection.

Chlorhexidine and hexachlorophane. The persistent antimicrobial activity sought by the FDA has been demonstrated by using the alcohol-free compounds of chlorhexidine and hexachlorophane with a water rinse. These compounds, however, have not been extensively used in rinse-free hand antiseptic application, in part because they are neither absorbed nor dissipated quickly enough to be convenient or user-friendly, and in part because they have aesthetically displeasing side effects such as odor. Additional limitations include a relatively narrow antimicrobial spectrum of certain compounds, such as triclosan.

Benzalkonium chloride. Benzalkonium chloride (BAC) is an alcohol-free, antimicrobial compound that has been widely used in the health care industry for more than 60 years in formulas for preservatives, surface cleansers, sterilizing agents, and topical antiseptic sprays. The chemical properties of BAC

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make it a good candidate for persistent antimicrobial activity in mammalian tissue. Extensive exposure to certain nonalcohol antimicrobial agents, including some surfactants, however, can make it have a detrimental effect on the skin unless the active ingredient is formulated with compounds that mitigate this effect.

A unique balance of penetration and nonirritation is attained when BAC is combined with surfactants and allantoin. This type of alcohol-free sanitizer formula is absorbed rapidly into the skin with little impact on the skin’s natural barrier function and is predicted to be more useful and effective as a rinse-free hand sanitizer than alcohol-containing formulas.

THEORETICAL AND CONCEPTUAL FRAMEWORK

Purpose of the study. The goal of the study was to provide information about the effectiveness of rinse-free hand sanitizers when used as a supplement to normal hand washing. The study was designed to evaluate the immediate and persistent antimicrobial properties of two types of alcohol-containing, rinse-free hand sanitizers (ie, 62% ethanol, 70% ethanol) and an alcohol-free SAB hand sanitizer (ie, 0.133% BAC, 0.5% allantoin).

Study design. An FDA-mandated protocol was used to measure the effectiveness of sanitizer products on hands that have been heavily contaminated with Serratia marcescens bacteria, a pathogen common in hospital-acquired infections. The test is useful for identifying formulas that are effective, first-line defenses against massive personal contamination. The FDA protocol recommends a water rinse; however, the formulas were intended for use without a water rinse. Antimicrobial performance thus was determined both with a water rinse and without a water rinse in separate sets of experiments.

The bacteria Serratia marcescens used in this study grows in red-colored colonies, allowing researchers to track only the fate of bacteria introduced on the hands for the purposes of the test. Before testing, all Serratia stocks were found to be susceptible to gentamicin, according to National Center of Clinical Laboratory Standards. The experiments were conducted in an environmentally controlled clinical research laboratory, and data was gathered from February to September 1997.

Test solutions. The antimicrobial hand-wash preparations were two commercially available alcohol-based formulas and one alcohol-free SAB formula (Table 1). The nonantimicrobial control handwash formula that was used for the initial baseline wash—establishing the mechanical reduction of bacteria—was the commercially available Ivory hand cleanser. Although Ivory soap was used as a representative of nonantimicrobial hand cleansers, other nonantimicrobial cleansers would have served as an adequate control because the principal deglazing action of any cleanser that lacks an antimicrobially active ingredient occurs through a mechanical removal of bacteria and not by a direct impact on bacterial viability.

Table 1

<table>
<thead>
<tr>
<th>HAND SANITIZERS TESTED</th>
</tr>
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<tbody>
<tr>
<td><strong>Alcohol-based hand washes</strong></td>
</tr>
<tr>
<td><em>Solution 1.</em> Active ingredient: Ethyl alcohol (ie, 62% vol/vol). Other ingredients: Isopropyl alcohol, water, emollients, and thickener.</td>
</tr>
<tr>
<td><em>Solution 2.</em> Active ingredient: Ethyl alcohol (ie, 70% vol/vol). Other ingredients: Emulsifying wax, methyl gluceth 20, polyoxyethylene, stearyl ether, and cetyltrimethylamine.</td>
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<tr>
<td><strong>SAB hand wash</strong></td>
</tr>
<tr>
<td><em>Solution 3.</em> Active ingredient: Benzalkonium chloride (ie, 0.13% vol/vol). Other ingredients: Water, hydroxypropylmethyl cellulose, propylene glycol, cocamidopropyl betaine, cocamidopropylamine oxide, cetyltrimethyl ammonium chloride, quaternium-12, imidazolidinyl urea, quaternium-15, allantoin, methyl paraben, propyl paraben, eucalyptol, methyl salicylate, and triethanolamine.</td>
</tr>
<tr>
<td><strong>Control soap</strong></td>
</tr>
<tr>
<td>Ivory hand cleanser. Ingredients: Water, sodium laureth sulfate, sodium lauryl sulfate, cocamidopropyl betaine, and fragrance.</td>
</tr>
</tbody>
</table>

Table 2

| DISTRIBUTION OF SUBJECTS AND CONDITIONS |
|-------------------------------|-----------------|------------------|
| **Formula** | **Condition** | **Number of subjects** |
| SAB solution | with rinse | 21 |
| | without rinse | 14 |
| 62% ethanol | with rinse | 17 |
| | without rinse | 16 |
| 70% ethanol | with rinse | 5 |
| | without rinse | 5 |
Table 3

**HAND SANITIZER EFFECTIVENESS PROTOCOL**

1. Cultures of *Serratia marcescens* (ie, easy-to-count, red-colored bacteria) are prepared to a concentration of approximately 10⁶ bacteria per milliliter of inoculum, and effectiveness is established through a series of contamination and washing cycles.

2. Subjects wash their hands using the control soap.

3. A baseline is determined by inoculation of the subjects’ hands followed by immediate sampling using the glove juice method, which is used for each appropriate contamination and wash cycle.

4. A control value for the mechanical degerming activity is sent through a contamination and wash cycle using the control soap.

5. The test subjects proceed through a series of 10 wash cycles with the test solution. Ten minutes pass between each contamination and wash cycle, and the entire series is accomplished in approximately two hours.

6. Glove juice samples to establish antiseptic effectiveness are taken after the first, third, seventh, and 10th contamination and wash cycles, as required by the US Food and Drug Administration.

7. A similar procedure is used for both the rinse and nonrinse protocols.

**Data collection.** The study began with a one-week pretest conditioning period during which subjects were not allowed to use medicated soaps, strong acids or bases, and other antimicrobial products. The antimicrobial effectiveness of the hand sanitizers was judged by a series of hand contaminations that were followed by washes with either a control, nonantimicrobial soap or the test formulas (Table 3).

**Glove juice sampling.** Researchers used the FDA-approved glove juice sampling technique for bacteria collection.

1. Subjects removed all jewelry and adornments from hands.

2. Five mL of *Serratia marcescens* inoculum were spread over subjects’ hands for 45 seconds.

3. Hands were allowed to air dry for two minutes.

4. Polyethylene gloves containing 50 mL of collection fluid each were placed on the subjects’ hands and secured above the wrists with rubber bands.

5. Collection fluid was spread over the subjects’ hands and massaged for one minute in a standardized manner to ensure uniform recovery of the collection sample.

6. Collection samples from the hands were pooled and immediately plated onto tripticate soy agar (TSA) mediums with both neat samples and serial dilution cultures to guarantee accurate colony counts.

The procedure allowed for a complete sampling of the surface area of each hand below the wrist.

**Testing.** After the pretest conditioning week, subjects’ hands were contaminated as described and then sampled. The number of bacteria recovered from the unwashed hands represented the baseline, which was representative of the maximum bacterial contamination that the unprotected skin could retain. After this, hands were recontaminated and then washed with 5 grams of a nonantimicrobial soap as a control for mechanical degerming action alone. The bacteria remaining on the hands were sampled and plated. After this, subjects’ hands were contaminated, washed with 5 grams of the appropriate test sanitizer, and sampled. This last step (ie, contaminate, wash, sample) was repeated 10 times, with five minutes elapsing before the start of the next contamination, wash, and sample cycle. This resulted in a 10-minute recovery period between the subjects’ actual washing with the test soap.
In the second series of tests, the 30-second rinse step was omitted, and sampling was performed immediately after washing to test the sanitizers' effectiveness without a water rinse. In each type of test (ie, with or without a water rinse), bacteria remaining on the hands was sampled and plated after the first, third, seventh, and 10th washes.

**Collecting cultures.** Cultures of FDA-mandated *Serratia marcescens* were prepared according to the method stated in the FDA protocol. Stock bacteria were grown to a concentration of approximately $1 \times 10^8$ viable bacteria per milliliter of growth medium (ie, Trypsin soy broth). Cultures were agitated before use. No neutralizers were used in the collection fluid; this prevented the buildup in the subjects' skin of neutralizer that would skew the results. The collection fluid had a pH of 7.8 and consisted of
- $0.04\%$ KH$_2$PO$_4$,
- $1.0\%$ K$_2$HPO$_4$, and
- $0.1\%$ Triton X-100.

Within three minutes of acquisition, samples from both the alcohol-based and SAB antiseptic hand sanitizers were diluted using the collection fluid that contained the appropriate neutralizers and were plated for growth on the TSA medium. Cultures were grown overnight at $37^\circ$ C ($98.6^\circ$ F) before counting. Washing and rinsing, when applicable, were conducted under running tap water that contained less than one viable bacterium per milliliter.

**STATISTICAL ANALYSES AND CALCULATIONS**

Statistical analyses were conducted using the Student's $t$ test with the aid of Statview statistical analysis software. Data presented in this document represent the mean and the standard error of the mean for the number of subjects in each test group.

All of the raw data (ie, bacterial colony counts) were converted to a log$_{10}$ scale to be compatible with the calculation model used. Briefly, the log$_{10}$ scale deals with exponents such that the log$_{10}$ of 100 (ie, $10^2$) is 2. For example, if 1,000 bacteria are counted, that number could be expressed in a power

![Figure 1](image1.png)

**Figure 1 • Hand sanitizer effectiveness performance with a water rinse.**

![Figure 2](image2.png)

**Figure 2 • Hand sanitizer effectiveness performance without a water rinse.**
The SAB hand sanitizer’s
degerming effectiveness
increased throughout the
hand contamination protocol.

of 10, as $1 \times 10^3$; thus, the $\log_{10}$ value of 1,000 is 3. This method of converting bacteria counts to log values effectively reduces the statistical variations in bacteria counts from person to person and is useful in comparative studies.

In these experiments, researchers calculated a value called a reduction factor (RF), which is one way to measure how well a test solution decreases the amount of bacteria on subjects’ hands. It is calculated as

$$RF = \log_{10} \left( \frac{\text{baseline bacterial count}}{\text{postwash bacterial count}} \right)$$

If 10,000 bacteria, therefore, were recovered from the hands for the baseline, and only 100 were recovered after the wash with the test solution, the RF value would be 2. Another way to look at RF is if the RF is 2, then 99% of all bacteria have been killed; if the RF is 3, 99.9% have been killed, and so on.

A small value for RF means that there was only a small reduction in the number of bacteria on the hands after washing with the test formula. Most antimicrobial soaps and sanitizers will give an RF of approximately 2 in this type of test. In contrast, a large value for RF means that there was a large reduction in the number of bacteria on the hands. The FDA-approved protocol used for this study requires a minimum RF of 2 after the first hand wash, and a minimum RF of 3 after the 10th hand wash.

RESULTS

The first series of experiments was performed to compare the effectiveness of the SAB hand sanitizer formula to a commercially available ethanol-based formula (ie, 62% vol/vol) with the inclusion of a 30-second water rinse. The results show that, after a single hand wash, both the SAB hand sanitizer formula and the 62% ethanol-based hand sanitizer formula had a degenerating activity that was approximately 20% greater than the degenerating activity of the control nonantimicrobial hand wash (Figure 1). Both the alcohol-based and SAB hand-wash formulas demonstrated an RF value of 2.8 ± 0.2.

The degenerating efficacy of the alcohol-based hand wash decreased during the remainder of the hand contamination and wash cycles, falling to a level that was below the minimum acceptable FDA standard of RF = 3. In contrast, the degenerating effectiveness of the novel SAB hand sanitizer formula increased over the course of the hand contamination and wash cycles required by the protocol.

Rinse-free testing. Both of the hand-wash formulas examined are intended for use without rinsing with water; thus, the above protocol was modified so that the 30-second water rinse was omitted. The results showed that the ethanol-containing hand wash had a moderate degenerating action compared to the control nonantimicrobial hand wash after the first hand wash (Figure 2). The degenerating effectiveness of the remaining hand contamination and decontamination cycles was markedly decreased for the 62% alcohol-based sanitizer. The degenerating activity of the SAB hand sanitizer formula paralleled the results obtained with the rinsing protocol and showed a steady increase in germicidal activity throughout the course of the experiment, exceeding the FDA minimum standard.

Greater ethanol concentration testing. The most antimicrobially active ethanol concentrations lie in the range of 50% to 70% vol/vol in water; thus, researchers were curious to know whether an increased ethanol concentration in an ethanol-based sanitizer would improve antimicrobial performance. The researchers, therefore, examined the effectiveness of a different, commercially available hand sanitizer that also contained emollients, but had an ethanol concentration of 70% (ie, vol/vol).

The results show that, although the 70% ethanol formula initially performed better than the 62% formula, antimicrobial effectiveness decreased as before with successive washes in both the rinse and nonrinse protocols. Although the initial wash with the SAB sanitizer produced approximately the same RF as the 70% ethanol-containing formula in the rinse protocol, subsequent washes with the SAB formula produced bacterial reductions greater than
the 70% ethanol formula. Likewise, in the nonrinse protocol, the SAB formula's effectiveness was approximately the same after the first wash, but was significantly greater than the 70% ethanol formula for subsequent contamination and recontamination cycles.

**Subjective testing.** In addition to these objective results, subjects were asked to subjectively evaluate the condition of their hands after the completion of the formula tests. A significant number of subjects (i.e., 47%) who had completed the test protocol with the alcohol-based hand sanitizer formulas—either in the rinse or nonrinse protocol—reported palmar pain or discomfort. After visual inspection, these subjects were found to have pronounced swelling that was, in some instances, accompanied by erythema of the palmar tissues. Also, the group that used ethanol-containing products tended to indicate some discomfort in palmar surfaces for one to several days after the test. In contrast, none of the subjects that used the SAB hand sanitizer formula reported any pain or discomfort of their hands after completing either the rinse or the nonrinse protocol.

**DISCUSSION**

The Centers for Disease Control and Prevention (CDC) has stated that hand washing is the single most important factor in the prevention of disease and the spread of infections. Officials at the CDC estimate that one-third of all hospital-acquired infections are avoidable and are caused by a lack of adherence to established infection control practices such as hand washing.16

This insufficient hand washing has led to a great increase in the use of waterless hand sanitizers by health care personnel. This study evaluated the effectiveness of two ethanol-containing hand sanitizers and a novel SAB, ethanol-free hand sanitizer using an FDA-approved protocol.

After a single application, the alcohol-free SAB sanitizer and both alcohol-based formulas reduced bacteria more than a control nonantimicrobial handwash formula. When the protocol was repeated omitting the water rinse, similar results were achieved. This illustrated that the first time either of these types of products is used on any given day, degeming activity results that exceeds the federal requirements for antiseptic hand washes.

To be of any value in a health care setting, however, a hand antiseptic should give persistent antimicrobial activity with repeated use. Accordingly, the alcohol-free SAB sanitizer, with or without the water rinse, produced increased antimicrobial effectiveness over time with no adverse effects. In contrast to this, repeated use of the alcohol-based sanitizers produced a decrease in antimicrobial effectiveness over time and was accompanied by swelling, erythema, and discomfort of the palmar surface of subjects' hands. Importantly, by the completion of both the rinsing and nonrinsing protocols, antimicrobial persistence of the SAB formula was so pronounced that its performance exceeded federal requirements for antiseptic hand washes by at least 50%. The tested alcohol-based hand sanitizers, however, failed to meet this federal standard in both the rinse and nonrinse protocols.

In summary, the study showed:

- the SAB hand sanitizer formula had a greater sustained degemming activity than the alcohol-containing hand sanitizer formula,
- the alcohol-containing hand sanitizer became less effective with repeated use and irritated the hands of subjects, and
- the SAB hand sanitizer formula became more effective without irritation after repeated use.

**LIMITATIONS AND DIRECTIONS FOR FUTURE STUDY**

A potential limitation to this study is that it was carried out in the controlled environment of a clinical research laboratory on model pathogens artificially introduced onto the hands of subjects according to a federally approved test protocol. Future research, therefore, would need to include studies of the impact on nosocomially derived infections in clinics, in which either an alcohol-containing or an alcohol-free hand sanitizer was routinely used to supplement normal hand washing.
Also, the interval between washes for each of the sanitizers tested in this study was 10 minutes—an amount of time chosen to model the effects of frequent, acute use, as might occur in a clinical environment that requires 10 to 12 patient contacts per hour. It would be informational, however, to perform the tests described in this document allowing a greater period of time between consecutive washes.

In the same way, the federally mandated time for the actual hand-washing procedure was two minutes, although in vitro data indicate that the formulas are effective in a nonskin environment after as little as 10 to 15 seconds. A second investigational parameter for future work, therefore, could include varying the hand wash duration, as well.

RECOMMENDATIONS FOR CLINICAL PRACTICE

Nurses in the OR face a situation that is particularly challenging in terms of maintaining hand sanitization. For example, nurses may at one moment be required to open storage drawers or handle and move equipment, such as lights and foot stools, and at the next moment be required to assist in wound dressing. In cases in which nurses must make the transition between equipment handling and assisting directly with the patient, universal precautions, such as hand washing and the wearing and changing of gloves, should take precedence. In situations in which hands should be sanitized before donning new gloves (eg, inadvertent contamination because of glove tearing) where soap and water are not immediately available, however, this study’s results indicate that the alcohol-free SAB formula would be more effective with continued use than the alcohol-based formula at hand sanitization.

It is recommended that perioperative health care personnel who have frequently been using alcohol-based instant hand sanitizers to supplement normal hand washing consider the benefits of using an effective alcohol-free instant hand sanitizer, such as the SAB sanitizer. This formula is quick-acting, does not require a water rinse, and, unlike alcohol-based hand sanitizers, is not flammable—a quality particularly important for perioperative safety in general.

RECOMMENDATIONS FOR EDUCATION

The point for clinical education that may be gained from this study is that, although alcohol-based instant hand sanitizers are widely used in professional and nonprofessional circles, alcohol also is an effective organic solvent. As such, it readily strips away the natural chemical components of the skin (eg, sebum, lipids) that impede water loss and bacterial infection. Frequent and prolonged use of alcohol-containing hand sanitizer products, therefore, can be counterproductive to hand sanitization and can damage the skin.

The results of this study are presented to help perioperative health care professionals choose an appropriate product for rinse-free hand sanitization as a supplement to normal hand washing, not to undermine the fundamental importance of proper hand washing. This study further serves to educate professionals about the limitations of alcohol-containing hand sanitizers and the advantages of alcohol-free hand sanitizers in both a perioperative and general clinical setting.

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NOTES
3. S C Harvey, “Antiseptics and disinfectants: fungicides; ectoparasiticides,” in Goodman and


