

Efficacy of Disinfectants on Common Laboratory Surface Microorganisms at R.S Mangaliso Hospital, NHLS Laboratory, South Africa

Pakiso Makhoahle^{1*}, Tshepiso Gaseitsiwe^{1,2}

¹Faculty of Health and Environmental Sciences, Central University of Technology-Free State, South Africa.

²Department of Microbiology, Robert Sebokwe Mangaliso Hospital, NHLS, Robert Sebokwe Mangaliso Hospital Kimberley.

Abstract

This study was conducted to ensure the safety of laboratory employees and minimize exposure to microorganisms or infections by using the right surface disinfectant after every shift. A sterility test was performed to detect the presence of potential microbial contaminants in the 5% BA, MCA, and saline. The common laboratory surface test microorganisms identified were *Acinetobacter lwoffii* and *Staphylococcus Lentus*. The efficacy of the disinfectants against each test organism at a 15-minute contact time was determined using a quantitative suspension test. Using the agar plate method, a 0.9 McFarland suspension of test organisms was inoculated on the surface bench, and swabs were taken before and after disinfection. Sodium hypochlorite (5%) was more potent than both 5% and 70% alcohol in the agar plate method and quantitative suspension test. *Acinetobacter lwoffii* (3.1 McF) and *Staphylococcus Lentus* were completely killed by 5% Sodium Hydrochloride within 5 min of contact. After 15 min of exposure to 5%, both test organisms survived and both tubes were still turbid when compared to the control tube (suspension of an organism without disinfectants). On the medium containing 3.4 McF and 3.1 McF suspension of test organisms with 1 ml 5%. In contrast, no growth was observed after an overnight incubation.

The outcome of this study further strengthened earlier studies and underscored the need to periodically assess the efficacy of disinfectants routinely supplied to the laboratory to ensure proper control of infections by using the right disinfectant at the right concentration for the right contact time.

Keywords: 5% sodium hypochlorite, 70% alcohol, 5% Distal- quaternary ammonium compound, Laboratory surface, Disinfectants, Contaminates

Corresponding author: Pakiso Makhoahle

E-mail ✉ pmakhoahle@cut.ac.za

How to Cite This Article: Makhoahle P, Gaseitsiwe T. Efficacy of Disinfectants on Common Laboratory Surface Microorganisms at R.S Mangaliso Hospital, NHLS Laboratory, South Africa. Bull Pioneer Res Med Clin Sci. 2022;2(1):1-12. <https://doi.org/10.51847/d5bXpXAtcl>

Introduction

Safety is mandatory for everyone working environment including the clinical laboratory especially when the environment is dealing with bacteria and other microbes. During the COVID-19 period, every household and even transport and shopping complexes were expected to disinfect to reduce the transmission of the infections. The

use of appropriate and reliable disinfectants on surfaces regularly is critical in preventing the transmission of colds, microbial infections, and other sicknesses among health workers [1-3].

In most clinical laboratories hazards such as chemicals, flames, infectious agents, and glassware are present and this inherently makes the working environment dangerous [4, 5]. Exposure to these potential hazards is possible

simply through direct contact with contaminated surfaces or through spillages and spattering of hazardous substances on the bench when PPE is not applied [5]. Cleaning plays a huge role in laboratories working with infectious agents or chemicals to minimize risks and improve the quality of results [6]. According to CLSI, a very essential method to ensure the safety of laboratory employees is to utilize good decontamination procedures with the use of effective disinfectants [5, 7, 8].

Decontamination is a process used in laboratories to reduce microbial contamination and minimize infection transmission [9]. Sterilization, disinfection, and antiseptic are types of decontamination. Sterilization and disinfection both remove pathogens, but sterilization differs from disinfectants because they completely kill all microorganisms including spores [9-11]. Procedures for sterilization include radiation, heating, steaming, and chemical sterilization; these methods are used to sterilize food, medicine, and surgical instruments [9, 11]. Antiseptics are substances used during surgery or other medical procedures to stop or slow down the growth of microorganisms [12, 13]. The difference between antiseptics and disinfection is that antiseptics are applied to the body whereas disinfectants are applied on non-living objects or surfaces such as cabinets, laboratory work areas, telephones, computer terminals, and equipment [5, 12, 13]. Disinfection is used mostly to decontaminate surfaces or air and for that reason, the purpose of this research will be to study the effectiveness of disinfectants used to decontaminate laboratory surfaces [13]. There are most common disinfectants used to decontaminate laboratory surfaces that include sodium hypochlorite (bleach/jik), alcohols, hydrogen peroxide, chlorine, aldehydes, peroxygenase, and quaternary ammonium compounds [13].

During the early 1960s, 3 categories (non-critical, semi-critical, and critical) of germicidal action to prevent risks of infection associated with the use of equipment or surfaces were suggested [14]. Environmental surfaces were considered noncritical items because they come in contact with intact skin and intact skin serves as a barrier to acquiring diseases or infection [14]. Therefore, when in contact with noncritical surfaces there is a low risk of transmitting pathogens to health workers. However, surfaces may become contaminated with infectious agents and may serve as a drive to initiate outbreaks for person-to-person transmission [14]. This controversy prompted the study of disinfectants and their effect on environmental surfaces to prevent transmission of microorganisms between surfaces and laboratory staff because they are more exposed to these microorganisms daily.

During the beginning of the 21st century, a study done in Canada tested nine liquid disinfectants (6% hydrogen peroxide, ammonium hydroxide windshield washer fluid, 70% ethanol, 37% methanol, 6% sodium hypochlorite,

70% isopropanol, and three commercial disinfectants) at room temperature (22 to 24 °C) for a period of 4, 13, and 33 min to examine their ability to reduce the infectivity of *Cryptosporidium parvum* oocysts (ATCC 87665) in cell culture [8]. Results of Susan *et al.* study indicated that 4 to 13 minutes exposure of to hydrogen peroxide and ammonium hydroxide reduced the infectivity of *Cryptosporidium parvum* oocysts while other disinfectants did not reduce the infectivity of the above organism after 33 minutes of exposure. According to the results, hydrogen peroxide and ammonium hydroxide disinfectants are suitable laboratory disinfectants against *Cryptosporidium parvum* oocysts [8].

Another study to assess the Microbiology quality and efficacy of two disinfectants (30% Jik and 2, 5% Lysol-hydrogen peroxide containing disinfectant) was conducted in the year 2017 in the indoor environments of the Medical Microbiology Laboratory Department of Babcock University Teaching Hospital, Ilisan-Remo, Ogun State, Nigeria [15]. According to the researcher's findings, the two disinfectants passed the sterility test as there was no significant growth of microbial contaminants. The bactericidal activity of the two disinfectants was also examined and only Lysol showed to be more effective than jik at the time of dilution and contact time testing [15]. However, the bactericidal activity of the two disinfectants was dependent on time, and therefore periodical assessment is required, factors such as temperature, higher concentration, and prolonged contact time which may influence the efficacy of these disinfectants had to be investigated [15].

Disinfectants are constituents of the disinfection process that destroys bacteria, viruses, fungi, and mould living on objects or surfaces, but they do not all remove endospores [3, 10, 16]. The antimicrobial activity of disinfectants occurs by inhibiting microbial growth e.g. bacteriostatic and fungistatic effects or through lethal activity e.g. sporicidal, bactericidal, fungicidal, and virucidal effects [16]. The active ingredients that are generally available are alcohols, chlorine, aldehydes, peroxygenase, and quaternary ammonium compounds [13]. Since there are many types of disinfectants on the market, it is important to understand the mode of action of each disinfectant, including its advantages and disadvantages to decide how to best disinfect and protect the work environment and its employees [3, 17].

Factors affecting the efficacy of disinfection

Disinfectant effectiveness depends on many factors, the following factors are explained:

Concentration of disinfectants

It is important to choose a suitable concentration of disinfectant that is best suited for each situation. To achieve the lethal effect of microorganisms, the

concentration of disinfectant must be increased to increase its efficacy and shorten the time for microbial killing [18, 19]. However, some disinfectants such as quaternary ammonium compounds and phenol are not similarly affected by concentration adjustments.

Physical and chemical factors

Temperature, pH of the environment, humidity, and water hardness are physical and chemical factors that influence the disinfectant procedure.

Temperature

Disinfectant efficacy increases when temperature increases but when the temperature is too high it may cause the disinfectant to decrease and cause potential health hazards [19].

pH

Influences the antimicrobial activity of disinfectants by changing their molecule or the cell surface [20]. An increase in pH can either increase some disinfectant's antimicrobial activity (such as glutaraldehyde and quaternary ammonium compounds) or decrease the antimicrobial activity of others, for example, phenols, hypochlorite, and iodine [19].

Humidity

Influences the activity of gaseous disinfectants such as chlorine dioxide and formaldehyde.

Water hardness

Reduces the rate of antimicrobial activity of certain disinfectants because cations such as magnesium and calcium in hard water interact with the disinfectant to form insoluble precipitates [21, 22].

Contact time

Sufficient time of contact must be allowed for the disinfectant to act efficiently on microbes. For different microorganisms, different time is needed; longer contact times are more effective than shorter contact times [19].

Types and number of microorganisms present

Most disinfectants except aldehyde are not effective against bacterial spores, knowing what type of microorganism is present, will help select a suitable and effective disinfectant [23, 24]. In addition, the greater the amount of microorganisms present, the more disinfectants are needed to kill the microbes [19].

Interfering substances (organic and inorganic) in the environment

Organic matter such as serum, blood, pus, fecal, or lubricant material can interfere with the antimicrobial activity of disinfectants. Interference occurs by a chemical reaction between the active ingredient and the organic matter resulting in a complex that is less effective to kill microorganisms [25, 26]. Sometimes organic matter acts as a physical barrier to protect microorganisms from being attacked by disinfectants [25, 26]. Chlorine and iodine are disinfectants interfered with by organic substances [25, 26]. Inorganic matter protects microorganisms from all disinfection processes resulting from occlusion in salt crystals [27, 28]. Hence it is important that cleaning should be done prior disinfection procedure [27].

Common microbes present on surfaces

Nosocomial pathogens are the most persistent microbes found on surfaces and increase the risks of healthcare-acquired infection [29]. That is why it's important to use disinfectants that have broad antimicrobial killing efficacy. **Table 1** shows the common pathogens and the survival period if left not decontaminated on the various surfaces [30].

Table 1. Survival of nosocomial and community-acquired pathogens on various surfaces [29, 30].

Organisms	Range of survival
<i>Acinetobacter</i> spp.	• 3 days to 1 year (in Vitro), 36 days within biofilm vs. 15 days for non-biofilm-forming strains
<i>Bordetella pertussis</i>	3 to >10 days; in pernasal swabs: >4 days
<i>Campylobacter jejuni</i>	>6 days, in water >60 days
<i>Clostridium difficile</i> spores	5 months
<i>C. difficile</i> , vegetative form	• 15 min (dry surface), 6 h (moist surface)
<i>Chlamydia pneumoniae</i>	≤96 h
<i>C. trachomatis</i>	<1 week
<i>Chlamydia psittaci</i>	15 days to months (environment)
<i>Corynebacterium diphtheriae</i>	7 days to 6 months
<i>Corynebacterium pseudotuberculosis</i>	1–8 days, up to several weeks (environment)
<i>Enterococcus</i> spp. including VRE	5 days up to 30 months

<i>Escherichia coli</i>	1.5 h to 16 months
<i>E. coli</i> O157:H7	27 days on spinach leaves, 179 days in soil, 98 days in water
<i>Haemophilus influenzae</i>	12 days
<i>Helicobacter pylori</i>	≤90 min; in water: 2–30 days
<i>Klebsiella</i> spp.	2 h to >30 months, ≤144 h in detergent solution
<i>Listeria</i> spp.	1 day–month, 141 days in water
<i>Mycobacterium bovis</i>	>2 months
<i>Mycobacterium tuberculosis</i>	1 day up to 4 months
<i>Neisseria gonorrhoeae</i>	1–3 days
<i>Neisseria meningitidis</i>	72 h
<i>Parachlamydia acanthamoebae</i>	<4 weeks, in the presence of blood <7 weeks
<i>Proteus vulgaris</i>	1–2 days
<i>Pseudomonas aeruginosa</i>	6 h up to 16 months; on the dry floor: 5 weeks; in aerosol: few hours
<i>Salmonella typhi</i>	6 h up to 4 weeks
<i>Salmonella typhimurium</i>	10 days up to 4.2 years
<i>Salmonella</i> pp.	1 day
Non-typhoid <i>Salmonella</i> spp.	336 days
<i>Salmonella enteritidis</i> (broiler farms)	1 year
<i>Salmonella enteritica</i> sv. <i>Tennessee</i>	30 days (dried in desiccated milk powder)
<i>Serratia marcescens</i>	3 days up to 2 months; on a dry floor: 5 weeks
<i>Shigella</i> spp.	• 2 days up to 5 months • 3–11 days in water
<i>Staphylococcus aureus</i> including MRSA and MSSA	• 7 days up to 1 year (in-vitro) • 9–12 days (plastic surfaces), 72 h (stainless steel) • 6 h (copper), ≤28 days (dry mops), ≤14 days (in water)
<i>Streptococcus pneumoniae</i>	1 day up to 30 months
<i>Streptococcus pyogenes</i>	3 days up to 6.5 months
<i>Vibrio cholerae</i>	1–7 days
<i>Yersinia enterocolitica</i>	Up to 64 weeks (in water)
<i>Yersinia pestis</i>	Up to 5 days

Selection of disinfectants

Selecting a disinfectant to best meet the needs of your facility is important to keep the working environment safe [3]. An ideal disinfectant should be broad-spectrum, non-irritating, non-toxic, non-corrosive, and inexpensive [14]. When choosing a disinfectant, consider the effectiveness against the potential pathogenic agent, safety to people, impact on equipment, environment, and expense [14]. Disinfectants have been also categorized into high-level, intermediate-level, and low-level disinfection according to the anti-microbial activity of the disinfectant [3, 14, 31]. Low-level disinfectant (0.4–1.6 % Quaternary Ammonium Compounds) is an agent that destroys all vegetative bacteria except tubercle bacilli, lipid viruses, some non-lipid viruses, and some fungi, but not bacterial spores. Intermediate-level disinfectants are an agent that destroys all vegetative bacteria, including tubercle bacilli, lipid-enveloped viruses, some non-lipid enveloped viruses, and fungus spores but not bacterial spores [3]. Examples of these agents are alcohol (ethyl, isopropyl) 70–95%,

iodophor compounds, and phenolic compounds (0.4–5 %). A chemical or physical agent or process that can kill some bacterial spores when used in sufficient concentration, temperature, and under suitable conditions are glutaraldehyde 2%, hydrogen peroxide 3–25 %, peracetic acid (variable), and chlorine dioxide [3].

Numerous scientific studies have provided evidence for the transmission of microorganisms between surfaces and health workers or patients. More research is required to gain a better insight into the role of surface disinfection in the laboratory to prevent laboratory-acquired infections [32]. Most importantly, research to decide on the safest and most effective disinfectant to use to protect the work environment and its employees has to be established [33]. The effect of disinfectant depends on the type of organism populations present on surfaces, the concentration of both organism populations, and the duration of exposure to disinfectant to kill or reduce these organisms [33, 34]. Understanding the proper types, concentrations, and contact times of the appropriate laboratory disinfectants is

a practice that needs to be implemented to reduce threatening pathogens and make the laboratory a safer place to work. The study of the quality and efficacy of laboratory disinfectants project was therefore an ideal opportunity to perform.

This study aimed to assess the effectiveness of 3 common laboratory surface Disinfectants (5% sodium hypochlorite, 70% alcohol, and 5% Distel (quaternary ammonium compounds)).

Objectives of the study were to:

- Test the sterility of the culture medium and saline.
- Identify test microorganisms present on the laboratory surface bench (before disinfection).
- Evaluate disinfectant efficacy against the test microorganism.

Materials and Methods

The observation and analytical study were carried out at Kimberley NHLS (Robert Mangaliso Sebukwe Hospital) in Northern Cape South Africa. The sampling and analysis were carried out for a period of 9 months (February – November 2020).

Contamination checks of agar plate and saline

Care and caution were exercised to ensure all consumables were not expired. A total of 17 half plates 5% blood and Macconkey agar of the same lot number was used for this project. One agar plate of the lot number in use which has not expired was incubated at 37°C in CO₂ for 2 days to ensure that the plates were not contaminated. A saline sterility test was done by placing 2 drops of saline of lot number in use throughout the entire 5% blood agar and the plate was incubated for 2 days at 37° C in CO₂. Five or more colonies on either plate indicate contamination. After 2 days of incubation, no growth was observed on the plates therefore there was no contamination on each product.

Sample collection

A sampling of the entire work-bench surfaces in the Microbiology (culture bench), Haematology (Rh bench), and chemistry (HBA1C bench) laboratory Department of Kimberley NHLS was collected in duplicate at the end of each shift (before disinfection) using sterile cotton swabs moistened with sterile saline.

Sample culture

Six half plates containing 5% Blood agar (BA) medium and MacConkey agar (MCA) medium were designated as

Micro-culture bench 1, Micro-culture bench 2, Haema-RH bench 1, Haema-RH bench 2, Chem-HBA1C bench 1 and Chem-HBA1C bench 2. Swab samples collected were inoculated onto the plates respectively. Using a wire loop working aseptically, the plates were streaked in four- the quadrant streak method as described in SOP TADM0155, and the plates were incubated at 37°C for in CO₂ 18-24 hours as described by Mokhtari *et al.* [35].

Identification of surface test organism isolates

After incubation, plates containing cultured samples were investigated. Colonies were identified by colonial morphology, Gram-stain, DensiCHEK, and Vitek 2 automated system.

Evaluation of disinfectant activity on each test Isolate

In-use disinfectants (5% sodium hypochlorite, 70% alcohol, and 5% Distel- quaternary ammonium compound) were obtained and placed into a 40ml specimen jar from the laboratory department. The efficacy of the disinfectants against the test organisms was evaluated using the agar plate method and the Quantitative Suspension Test.

• Agar plate method

This method test determines the effectiveness of disinfectants on laboratory bench surfaces. Three areas of the surface bench were designated as site 1, site 2, and site 3. Each surface was inoculated with 0.9McF suspension of the test organisms identified. To prepare 0.9 MCF suspension, a few colonies were emulsified in 3 ml saline in a plastic tube using a vortex mixer. The DensiCHEK™ Plus was used to measure the turbidity value, the ample containing tube was adjusted to give a 0.9 McF value. Before disinfection of each surface, swabs were taken on all 3 sites and cultured on half plates with 5% BA and MCA. Site 1 was disinfected with 70% alcohol for 15 minutes then a swab was taken and cultured. Site 2 was disinfected with 5% Distel for 15 minutes then a swab was taken and cultured. Site 3 was disinfected with 5% sodium hypochlorite for 15 minutes swab was taken and cultured. All the plates were incubated overnight at 37 °C in CO₂ then colonies were observed, counted, and compared and the results were recorded.

• Quantitative suspension test

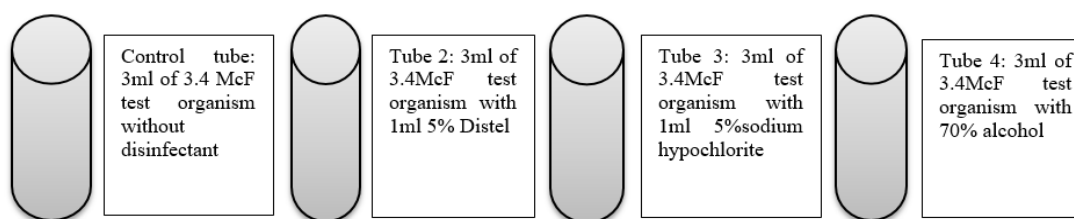


Figure 1. Illustration of quantitative suspension test

Eight plastic tubes were used for this analysis, the first 4 tubes contained 3 ml of 3.4 McF suspension for the test organism identified. The second 4 tubes contained 3.1 McF suspension of the other organism identified. The first tube of each 4 tubes was designated as the control tube which contained suspension of the test organism without disinfectant. The other 3 tubes contained the same suspension of the test organisms with 1ml of each of the 3 types of disinfectants that are being evaluated as explained in **Figure 1**.

In summary, 1 ml of the disinfectant being tested was pipetted using a plastic Pasteur pipette and added into a 3 ml standardized microbial suspension. This activity was performed at room temperature for contact times of 0, 5, 10, and 15 minutes. The timer was started when the test bacterial suspension and disinfectant were combined. At 0 times, McFarland readings were taken using the DensiCHEK™ plus instrument and at 5-minute intervals, each test

sample was re-suspended using a vortex mixture then turbidity readings were taken and recorded. After all, readings were taken and recorded, each test sample was cultured onto half plates with 5% BA and MCA then the plates were incubated overnight at 37°C in CO₂. Turbidity together with colonies on the plates where observed, disinfectant activity was compared, and the results were recorded.

Results and Discussion

The study assessed the efficacy of common laboratory surface disinfectants (70% alcohol, 5% sodium hypochlorite, and 5% Distel-quaternary ammonium compounds). Before the examination was carried out, a sterility check of the medium plate and saline was performed to ensure that the products were not contaminated. Identification of test organisms isolated from the Microbiology, Chemistry, and Haematology surface bench before disinfection is presented in **Table 2**.

Table 2. Identification of Test microorganisms

Surface bench	Colonial morphology	Gram stain results	Vitek 2 results
Microbiology culture bench	Grayish white, flat, and slightly mucoid colonies on BA, no growth on MCA	Gram positive bacilli	<i>Staphylococcus lentus</i> M155
Haematology RH bench	Grayish white, flat, and slightly mucoid colonies on BA, no growth on MCA.	Gram positive bacilli	<i>Staphylococcus lentus</i> M155
Chemistry HBA1C bench	Cream raised shiny and mucoid colonies on BA. Translucent colonies on MCA indicate that it is a non-lactose fermenter.	Gram negative bacilli	<i>Acinetobacter lwoffii</i> ATCC-15309

The organisms used in the study were identified and verified as summarised in **Table 2** before laboratory testing. *Staphylococcus lentus* M155 strain was isolated from the microbiology and hematology laboratories' surface benches before disinfection and on the chemistry surface bench, *Acinetobacter lwoffii* strain ATCC-15309 was isolated pre-disinfection. *Staphylococcus lentus* is a Gram-positive bacillus, oxidase-positive, coagulase-negative member of the bacterial genus *Staphylococcus* consisting of clustered cocci. *Staphylococcus lentus* is a common part of the normal flora of both humans and other animals. However, it can be pathogenic to humans and

cause endocarditic peritonitis, septic shock, infections of the urinary tract, a pelvic inflammatory disease most frequently, and wound infections. *Acinetobacter lwoffii* is a non-fermentative Gram-negative bacillus bacterium that is a member of the genus *Acinetobacter*. It is considered normal skin flora and can also inhabit the human oropharynx and perineum. It can cause infections in human hosts such as catheter-urinary infections in immunocompromised patients and gastroenteritis. *Acinetobacter lwoffii* and *Staphylococcus lentus* were the two test organisms used to examine the efficacy of the three surface disinfectants used at the laboratory. The

following are the results of the agar plate method using *Staphylococcus lentus* as a test organism:



Figure 2. Before disinfection



Figure 3. After disinfecting with 70% alcohol

The above figures show two mediums with growth before and after disinfection with 70% alcohol. **Figure 2** shows 2+ growth on both BA and MCA whereas **Figure 3** shows 1+ growth on BA and no growth on MCA. This indicates that the test organism survived and recovered from the BA plate even after disinfecting with 70% alcohol.



Figure 4. Before disinfection



Figure 5. After disinfection with 5% Distel

Figures 4 and 5 show two mediums of before and after disinfection with 5% Distel/ quaternary ammonium compounds. There was a 2+ growth of the test organism on both BA and MCA in **Figure 4**. In **Figure 5** there was 1+ growth of the test organism on BA but there is no growth on the MCA plate. This indicates that there was recovery and survival of the organism on BA but on MCA the organism was killed.

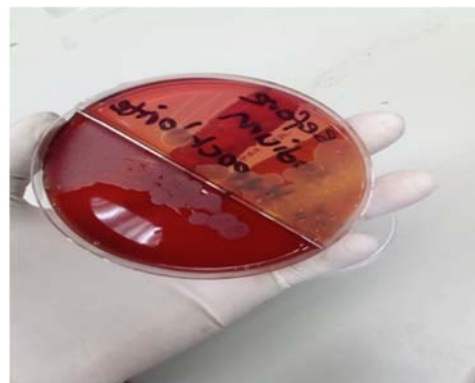


Figure 6. Before disinfection



Figure 7. After disinfection with 5% sodium hypochlorite

Figures 6 and 7 show two mediums of before and after disinfection with 5% sodium hypochlorite. There was 3+ growth of the test organism on both BA and MCA in **Figure 6**. In **Figure 7** there were less than 5 colonies on

BA and no growth on MCA, this indicates that the microorganism was completely killed after disinfection with 5% sodium hypochlorite.

The killing rate of disinfectants was also evaluated; the following line graph is a result of McFarland value versus time using *Staphylococcus lentus* as a test organism:

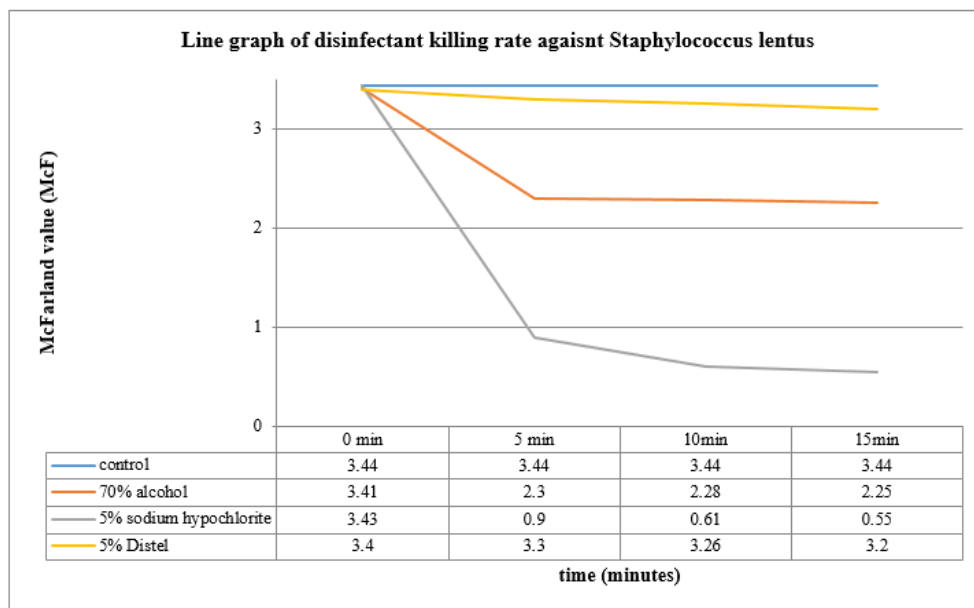


Figure 8. Bar graph showing McFarland value versus time using *Acinetobacter lwoffii* as a test organism:

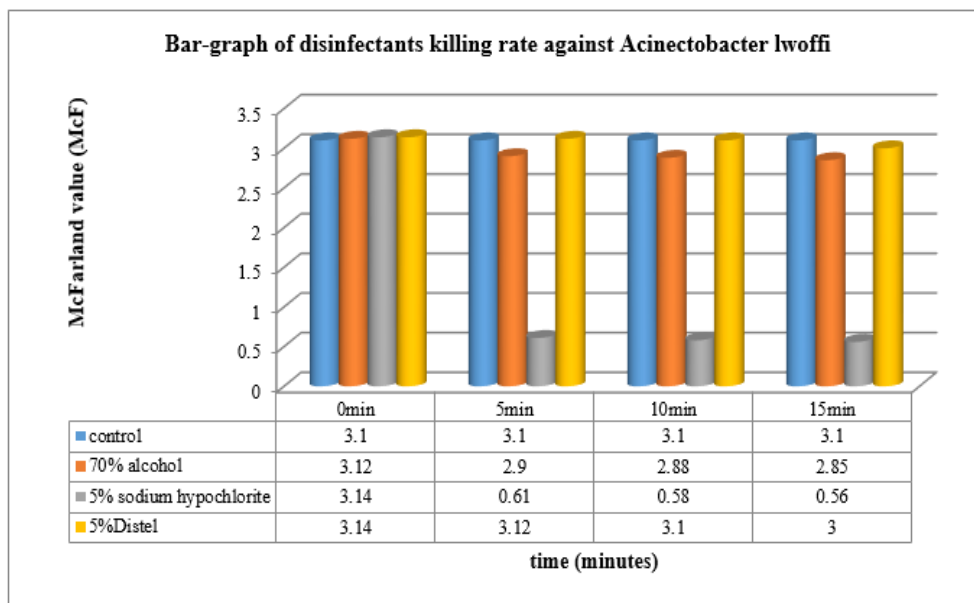


Figure 9. Bar-graph of disinfectants killing rate against *Acinetobacter lwoffii*

Analysis of both graphs shows that disinfection with 5% Distel after 15 minutes of contact time, did not make any major difference because the McF value remained at a range of 3McF. When looking at the killing rate of 70% alcohol, there was a slight decrease in McF value after a contact time of 15 minutes with the test organisms in **Figure 8**. Disinfection with 5% sodium hypochlorite showed a sharp decrease in McF value within 5 minutes of contact time with the test organism. Therefore 5% sodium

hypochlorite is a faster microbial killer than 5% distal and 70% alcohol (**Figures 8 and 9**). The outcome of this study shows the importance of monitoring and evaluating the disinfectants that we use. In a study by Enitan [15], it was reported that the bactericidal activity of 30% sodium hypochlorite was unsatisfactory in killing other bacteria after 10 minutes of contact time, it's also important to understand the organisms mostly isolated in your laboratory benches as the two are normal floras. This non-

correlation with our study could be attributed to the different concentrations of sodium hypochlorite and organisms of interest also as mentioned in the literature review, the concentration of disinfectant is one of the factors that affect the efficacy of disinfectants.

When examining the turbidity of the tubes containing a mixture of test organism suspension with 1ml of each disinfectant allocated in different tubes the following resulted:



Figure 10. Turbidity of tubes containing test organism suspension with different types of disinfectants.

Figure 10 shows the turbidity results of the quantitative suspension test. The first tube is the control tube which contains suspension of the test organism without disinfectant. The other 3 tubes contained the same suspension of the test organisms with 1ml each of 70% alcohol, 5% distal, and 5% sodium hypochlorite respectively. It is observed that the two tubes containing 70% alcohol and 5% distal remained turbid when compared to the control tubes whereas the tube containing 5% sodium hypochlorite was not turbid but it was translucent. According to the results, *Acinetobacter lwoffii* and *Staphylococcus lentus* still survived in the tube containing 70% alcohol and 5% distal after 15 minutes of exposure time but, in the tube containing 5% sodium hypochlorite, the two test organisms were completely killed after the 15 minutes exposure.

When looking at the plates cultured from the quantitative suspension test method the following results:



Figure 11. Control plate containing only suspension of *Staphylococcus lentus*



Figure 12. The plate contains a suspension of *Staphylococcus lentus* with 70% alcohol.

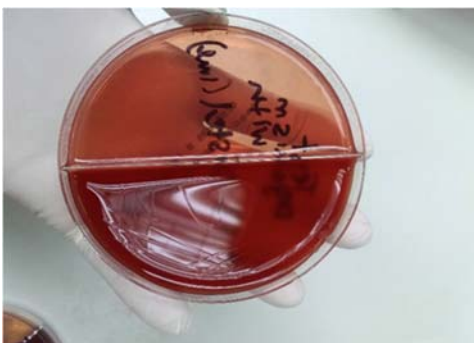


Figure 13. The plate containing a suspension of *Staphylococcus lentus* with 5% Distal



Figure 14. Plate containing a suspension of *Staphylococcus lentus* with 5% sodium hypochlorite.

The bactericidal effect (Logarithm reduction factor) of the disinfectants was determined by subtracting the logarithm of the survivors after disinfectant contact from the logarithm of the original inoculum in control plates, using the following formula:

$$\text{Logarithmic Reduction Factor (RF)} = \text{Log } N_c - \text{Log } N_d \quad (1)$$

Where:

N_c = Number of colonies from control plates (No disinfectant)

N_d = Number of colonies from test plates (after contact with disinfectant)

On the control plate in **Figure 11**, there are approximately 100 colonies, 6 colonies on a plate in **Figure 12**, and no colonies on both plates in **Figures 13 and 14**. Therefore the following was calculated:

$$\begin{aligned} \text{RF (70\% alcohol)} &= \text{Log } 100 - \text{Log } 6 \\ &= 2 - 0.778 \\ &= 1.22 \\ &= 1\text{Log} \end{aligned} \quad (2)$$

RF of 5% Distel and 5% sodium hypochlorite cannot be calculated because in mathematics log 0 is undefined as there is no growth (**Figures 13 and 14**). It's not a real number, you can never get zero by raising anything to the power of anything else. You can never reach zero, you can only approach it using an infinitely large and negative power. A summary of log reduction values is outlined in **Table 3**.

Table 3. Log reduction percentage

Log reduction	Percentage reduction
1Log reduction	90%
2Log reduction	99%
3Log reduction	99.9%
4Log reduction	99.99%
5Log reduction	99.999%

Table 3 shows the percentage growth reduction as converted from the RF calculation. In this case, 1-log reduction corresponds to a reduction of 90 percent from the original concentration; therefore 70% alcohol only killed 90% of the test organisms. The greater the log reduction the more effective the product is at killing bacteria and other pathogens that can cause infections.

The outcome of the study shows that in both the agar plate method and quantitative suspension test, 70% alcohol was not an effective surface disinfectant. These results do not correlate with studies of Woodman in 1998, Russo in 2000, and Hormann in 2000 which reported that 70%

alcohol was an effective disinfectant to fully eliminate bacteria. There is also a need to understand that surfaces might be contaminated by the different normal floras. The efficacy of 5% Distel quaternary ammonium compounds to eliminate surface microorganisms with the agar plate method was unsatisfactory but, with the quantitative suspension test method, the disinfectants showed to have completely inhibited the growth of *Staphylococcus lentus* after overnight exposure. This correlates with the study of Josephson (1997) and Gerba (2015) which reported that QACs reduce bacterial contamination on the surface and several infectious organisms in healthcare facilities [36, 37].

Conclusion

Five percent sodium hypochlorite proved to be the most effective disinfectant to use on laboratory surfaces as it completely inhibited the growth of the microorganisms within 5 minutes of exposure using both the agar plate method and quantitative suspension test. 5% Distel-quaternary ammonium compounds were also found to be effective disinfectants to use on laboratory surfaces but, it requires a prolonged contact time with the organism for the disinfectant to kill the microorganism. 70% alcohol has proven to be the most unsatisfactory disinfectant to use because all the test microorganisms recovered and survived after prolonged exposure to the disinfectant. Therefore 5% sodium hypochlorite is recommended to be used as laboratory surface disinfectant after every shift to control or prevent infection transmissions and to ensure that the laboratory employees working on benches are protected and the working environment is safe. The outcome of this study further strengthened earlier works and underscored the need to periodically assess the efficacy of disinfectants routinely supplied to the laboratory to ensure proper control of infections by using the right disinfectant in the right concentration for the right contact time.

Acknowledgments: The authors would like to acknowledge and thank the National Health Laboratory Services (NHLS) Academic affairs and research office for granting permission to do the study and for all the support from the Kimberley NHLS manager and Microbiology supervisor for making the study possible. Also, the Central University of Technology where the project was approved by the academic institution.

Conflict of interest: None

Financial support: None

Ethics statement: The study did not require approval from the Health Sciences Research Ethics Committee because the samples were not collected from humans,

animals, or plants but rather from non-living objects (work-surface bench). Due to the life-threatening pandemic that the world is currently facing, laboratory analysis was performed under strict conditions. Screening tests were done, and a face mask and PPE were applied to ensure the safety of everyone at the laboratory.

References

- Guimarães MA, Tibana A, Nunes MP, Santos KR. Disinfectant and antibiotic activities: A comparative analysis in Brazilian hospital bacterial isolates. *Braz J Microbiol.* 2000;31:192-8.
- Ferreira AM, Andrade DD, Rigotti MA, Ferreira MV. Condition of cleanliness of surfaces close to patients in an intensive care unit. *Rev Lat-Am Enfermagem.* 2011;19(3):557-64.
- Rutala WA. Selection of the ideal disinfectant. 2014. Available from: [http://disinfectionandsterilization.org/selection-of-the-ideal-disinfectant/February 21, 2014](http://disinfectionandsterilization.org/selection-of-the-ideal-disinfectant/February%2021,%202014). (Accessed February 21, 2016).
- Turgeon ML. Linne & ringsrud's clinical laboratory science: The basic and routine techniques, 6th ed. Mosby: Elsevier; 2012. Chapter 2. pp. 25-52.
- Scungio DJ, MT (ASCP), SLS, CQA (ASQ). Keeping the laboratory environment clean and safe. 2018. Available from: <https://www.mlo-online.com/continuing-education/article/13017006/keeping-the-laboratory-environment-clean-and-safe>
- Deepak D. Importance of cleanliness in laboratories. 2017. Available from: <https://lab-training.com/2017/08/21/importance-cleanliness-laboratories/>
- CLSI. Clinical laboratory safety; Approved guideline, 3rd ed. CLSI document GP17-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
- Weir SC, Pokorny NJ, Carreno RA, Trevors JT, Lee H. Efficacy of common laboratory disinfectants on the infectivity of *Cryptosporidium parvum* oocysts in cell culture. *Appl Environ Microbiol.* 2002;68(5):2576-9.
- Burgener J. Position paper on the use of ultraviolet lights in biological safety cabinets. *Appl Biosaf.* 2006;11(4):228-30.
- Yoo JH. Review of disinfection and sterilization—back to the basics. *Infect Chemother.* 2018;50(2):101-9.
- Gauri S, Poonam S, Pooja S, Nikhilesh J. Disinfect vs Sterilize. 2020. Available from: https://www.diffen.com/difference/Disinfect_vs_Sterilize
- McDonnell G, Russell AD. Antiseptics and disinfectants: Activity, action, and resistance. *Clin Microbiol Rev.* 1999;12(1):147-79.
- Abreu AC, Tavares RR, Borges A, Mergulhão F, Simões M. Current and emergent strategies for disinfection of hospital environments. *J Antimicrob Chemother.* 2013;68(12):2718-32.
- Spaulding EH. Chemical disinfection of medical and surgical materials. In: Lawrence C, Block SS, eds. *Disinfection, sterilization, and preservation*. Philadelphia: Lea & Febiger, 1968:517-31.
- Enitan SS, Okeleke OJ, Awharentomah DK, Akele YR, Nwankwo KJ, Arisi CP. Assessment of the microbiological quality and efficacy of two common disinfectants used in hospital laboratory. *Adv Biomed Sci.* 2017;2(6):31-43.
- Maris P. Modes of action of disinfectants. *Rev Sci Tech.* 1995;14(1):47-55.
- Ioannou CJ, Hanlon GW, Denyer SP. Action of disinfectant quaternary ammonium compounds against *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 2007;51(1):296-306.
- Russell AD, McDonnell G. Concentration: A major factor in studying biocidal action. *J Hosp Infect.* 2000;44(1):1-3.
- Russell AD. Factors influencing the efficacy of germicides. *Disinfection, sterilization and antiseptics: Principles, practices, challenges, and new research*. Washington DC: Association for Professionals in Infection Control and Epidemiology. 2004;2004:162-70.
- Attavar S. An overview of the antimicrobial effect of natural irrigants in disinfection of root canal system. *Pharmacophore.* 2022;13(1).
- Favero MS, Bond WW. Chemical disinfection of medical and surgical materials. In: Block SS, ed. *Disinfection, sterilization, and preservation*. Philadelphia: Lippincott Williams & Wilkins; 2001. pp.881-917.
- Rutala WA. Selection and use of disinfectants in health care. In: Mayhall, C. G, ed. *Infect. Control and Hosp. Epidemiol.* Philadelphia: Lippincott Williams & Wilkins; 1999. pp.1161-87.
- Gillis RJ, Schmidt WC. Scanning electron microscopy of spores on inoculated product surfaces. *MD.* 1983;1983:46-9.
- Rutala WA, Weber DJ. Healthcare Infection Control Practices Advisory Committee (HICPAC). Guideline for disinfection and sterilization in healthcare facilities. 2008;500:10-2. Available from: http://www.cdc.gov/hicpac/pdf/guidelines/Disinfection_Nov_2008.
- Lewis DL, Arens M. Resistance of microorganisms to disinfection in dental and medical devices. *Nat Med.* 1995;1(9):956-8.

26. Muscarella LF. Sterilizing dental equipment. *Nat Med*. 1995;1(12):1223-4.
27. Jacobs P, Wang JH, Gorham RA, Roberts CG. *Cleaning: Principles, methods and benefits. Disinfection, sterilization, and antisepsis in healthcare*. Champlain, NY: Polyscience Publications. 1998:165-81.
28. Gorham RA, Jacobs P, Roberts CG. Laboratory artifacts due to protein and salt crystals on the inactivation of *Bacillus stearothermophilus*. *J Hosp Infect*. 1998;40:9-2.
29. Kramer A, Guggenbichler P, Heldt P, Jünger M, Ladwig A, Thierbach H, et al. Hygienic relevance and risk assessment of antimicrobial-impregnated textiles. *Bio Text Skin*. 2006a;33:78-109.
30. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis*. 2006b;6:130-7.
31. World Health Organisation Laboratory Biosafety manual, 3rd ed. 2004. Available from: <http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf>
32. Daivasigamani S, Chidambaranathan AS, Balasubramaniam M. Colour stability of maxillofacial silicone materials after disinfection and aging procedures a systematic review. *Ann Dent Spec*. 2021;9(4):52.
33. Kengadaran S, Srisakthi D, Arumugham IM, Pradeepkumar R. Knowledge, attitude, and practice regarding dental unit waterline disinfection among dental practitioners of India. *J Adv Pharm Educ Res*. 2017;7(3).
34. Guideline for Disinfection and Sterilization in Healthcare Facilities. Factors Affecting the Efficacy of Disinfection and Sterilization. 2008. Available from: <https://www.cdc.gov/infectioncontrol/guidelines/disinfection/efficacy.html>
35. Mokhtari M, Zandi H, Jasmi Zad T, Sahl Abadi F, Montazari A. The evaluation of efficacy of common disinfectants on microorganisms isolated from different parts of Shahid Sadoughi Accidents Burns Hospital in Yazd in 2011. *Tolooebehdasht*. 2015;14(3):1-1.
36. Josephson KL, Rubino JR, Pepper IL. Characterization and quantification of bacterial pathogens and indicator organisms in household kitchens with and without the use of a disinfectant cleaner. *J Appl Microbiol*. 1997;83(6):737-50.
37. Gerba CP. Quaternary ammonium biocides: Efficacy in application. *Appl Environ Microbiol*. 2015;81(2):464-9. Available from: <https://aem.asm.org/content/81/2/464>