DISINFECTANTS AND ANTISEPTICS

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Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Comments received after the last date will not be considered by the IPC before finalizing the general chapter.

Please send any comments you may have on this draft document to lab.ipc@gov.in before the last date for comments.

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Disinfectants and Antiseptics

Note: - The following chapter is for information and is not an official requirement

1.0 Introduction

A sound cleaning and sanitization program is needed for controlled environments used in the manufacture of pharmaceutical articles to prevent the microbial contamination of these articles. Sterile drug products may be contaminated via their pharmaceutical ingredients, process water, packaging components, manufacturing environment, processing equipment, and manufacturing operators. Current Good Manufacturing Practices (cGMPs) emphasize the size, design, construction, and location of buildings and construction materials, and the appropriate material flow to facilitate cleaning, maintenance, and proper operations for the manufacture of drug products. All GMP areas should be subject to cleaning; disinfection must also be performed in areas where it is necessary to control environmental bioburden, for example: where starting and primary packaging materials, intermediate or bulk products are exposed to the environment, and in sterile production facilities. When disinfectants are used in a manufacturing environment, care should be taken to prevent the drug product from becoming contaminated with chemical disinfectants as a result of the inherent toxicity of the disinfectants. The requirements for aseptic processing include readily cleanable floors, walls, and ceilings that have smooth and nonporous surfaces; particulate, temperature, and humidity controls; and cleaning and disinfecting procedures to produce and maintain aseptic conditions. The cleaning and sanitization program should achieve specified cleanliness standards, control microbial contamination of products, and be designed to prevent the chemical contamination of pharmaceutical ingredients, product-contact surfaces and/or equipment, packaging materials, and ultimately the drug products. These principles also apply to nonsterile dosage forms where the microbial contamination is controlled by the selection of appropriate pharmaceutical ingredients, utilities, manufacturing environments, sound equipment cleaning procedures, products especially formulated to control water activity, inclusion of suitable preservatives, and product packaging design.

In addition to disinfectants, antiseptics are used to decontaminate human skin and exposed tissue and may be used by personnel prior to entering the manufacturing area. Chemical sterilants may be used to decontaminate surfaces in manufacturing and sterility testing areas. Furthermore, sterilants may be used for the sterilization of pharmaceutical articles, and UV irradiation may be used as a surface sanitizer, when appropriately validated.

This general information chapter will discuss the selection of suitable chemical disinfectants, antiseptics and sterilants; the demonstration of their bactericidal, fungicidal, and sporicidal efficacy; the application of disinfectants in the sterile and non-sterile pharmaceutical manufacturing area; and regulation and safety considerations.

2.0 Definitions

Antiseptic- An agent that inhibits or destroys microorganisms on living tissue including skin, oral cavities, and open wounds.

Chemical Disinfectant- A chemical agent used on inanimate surfaces and objects to destroy

infectious fungi, viruses, and bacteria, but not necessarily their spores. Sporicidal and antiviral agents may be considered a special class of disinfectants. Disinfectants are often categorized as high-level, intermediate-level, and low-level by medically oriented groups based upon their efficacy against various microorganisms.

Cleaning Agent- An agent for the removal from facility and equipment surfaces of product residues that may inactivate sanitizing agents or harbor microorganisms.

Decontamination- The removal of microorganisms by disinfection or sterilization.

Disinfectant- A chemical or physical agent that destroys or removes vegetative forms of harmful microorganisms when applied to a surface. Disinfectants are purposed to achieve an appropriate and specified level of reduction in microorganisms.

Sanitizing Agent- An agent for reducing, on inanimate surfaces, the number of all forms of microbial life including fungi, viruses, and bacteria. Sanitizing agent are purposed to achieve an appropriate and specified level of reduction in microorganisms.

Sporicidal Agent- An agent that destroys bacterial and fungal spores when used in sufficient concentration for a specified contact time. It is expected to kill all vegetative microorganisms.

Sterilant- An agent that destroys all forms of microbial life including fungi, viruses, and all forms of bacteria and their spores. Sterilants are liquid or vapor-phase agents.

3.0 Classification of Disinfectant

Chemical disinfectants are classified by their chemical type. This includes aldehydes, alcohols, halogens, peroxides, quaternary ammonium compounds, and phenolic compounds (Table 1).

The effectiveness of a disinfectant depends on its intrinsic biocidal activity, the concentration of the disinfectant, the contact time, the nature of the surface disinfected, the hardness of water used to dilute the disinfectant, the amount of organic materials present on the surface, and the type and the number of microorganisms present.

Table 1. Classification of Antiseptics, Disinfectants and Sporicidal Agents

Chemical Entity	Classification	Example
Aldehydes	Sporicidal agent	2 per cent Glutaraldehyde
Alcohols	General purpose disinfectant, antiseptic, antiviral agent	70 per cent Isopropyl alcohol, 70 per cent alcohol
Chlorine and sodium hypochlorite	Sporicidal agent	0.5 per cent Sodium hypochlorite
Phenolics	General purpose disinfectant	500 µg per g Chlorocresol, 500 µg per g chloroxylenol
Ozone	Sporicidal agent	8 per cent Gas by weight
Hydrogen peroxide	Vapor phase sterilant, liquid sporicidal agent, antiseptic	4 µg per g H_2O_2 vapor, 10 per cent – 25 per cent solution, 3 per cent solution

Substituted biguanides	Antiseptic agent	0.5 per cent Chlorhexidine gluconate
Peracetic acid	Liquid sterilant, vapor phase sterilant	0.2 per cent Peracetic acid, 1 µg per g peracetic acid
Ethylene oxide	Vapor-phase sterilant	600 µg per g Ethylene oxide
Quaternary ammonium compounds	General purpose disinfectant, antiseptic	Concentration dependent on application, Benzalkonium chloride
β-Propiolactone	Sporicidal agent	100 µg per g β-Propiolactone

Under the Drugs and Cosmetics Act 1940 and the rules made there under, the Regulatory Authorities registers chemical disinfectants and sterilants marketed in India and requires manufacturers to comply with the standards prescribed therein. Certain liquid chemical sterilizers intended for use on critical or semi critical medical devices are defined and regulated by Food and Drug Administration (FDA).

4.0 Selection of an Antiseptic for Hand and Surgical Site Disinfection

Hands and surgical sites are disinfected in a hospital setting to reduce the resident flora and to remove transient flora (e.g., *Streptococcus pyogenes*) and methicillin-resistant *Staphylococcus. aureus* and *Pseudomonas aeruginosa* that have been implicated in hospital-associated infection. Use of antiseptics to disinfect hands has been shown to be more effective than soap and water in reducing the counts of bacteria on the skin; repeated antiseptic use further reduces these counts. These principles may be applied to clean-room operators in the pharmaceutical industry.

Common antiseptics include 4 per cent chlorhexidine, 10 per cent povidone–iodine, 3 per cent hexachlorophene, 70 per cent isopropyl alcohol and 0.5 per cent chlorhexidine in 95 per cent alcohol.

5.0 Selection of a Disinfectant for Use in a Pharmaceutical Manufacturing Environment

When selecting a disinfectant for use in a pharmaceutical manufacturing area, the following points should be considered:

- the number and types of microorganisms to be controlled;
- the spectrum of activity of commercially available disinfectants;
- the disinfectants microbicidal claims;
- the disinfectant or sanitizer supported by the ~~IS Standard/ Indian regulatory Authority~~ Regulatory Authorities.
- the concentration, application method, and contact time of the disinfectant;
- the nature of the surface material being disinfected and its compatibility with the disinfectant;
- the amount of organic compounds on the surface that may inactivate a disinfectant;
- the possible need to maintain a residual bactericidal activity of the disinfectant on the surface;

- the corrosiveness of the disinfectant to equipment with repeated application;
- the safety considerations for operators applying the disinfectant and working in the disinfected area; the compatibility of the disinfectant with cleaning agents and other disinfectants;
- the planned disinfectant rotation done in order to prevent resistance of the microorganism to the disinfectant; and
- the steps that need to be taken to avoid the contamination of pharmaceutical products by a disinfectant.

6.0 Theoretical Discussion of Disinfectant Activity

Plots of the log of the number of microorganisms per ml surviving in a disinfectant solution indicate that first-order kinetics can be applied as a gross approximation to the reduction in microbial count with respect to time. In practice, the plots show a more sigmoid curve with a slower initial reduction in numbers followed by an increasing rate with respect to time.

The rate constant, K, for the disinfection process can be calculated by the formula:

$$(1/t)\ (\log N_0/N)$$

Where, t = time, in minutes, for the microbial count to be reduced from N_0 to N;

N_0 = initial number of microorganisms in cfu per ml;

N = final number of microorganisms in cfu per ml.

As with a first-order chemical reaction, the same concentration of disinfectant reduces the number of organisms more rapidly at elevated temperatures. This can be expressed as a temperature, T, coefficient per 10° rise in temperature, Q_{10}, calculated by the formula:

$$\text{Time to decontamination at T°/Time to decontamination at T}$$

Where, T = T° – 10.

Further evidence that a first-order reaction is an inadequate description of disinfection is that the Q_{10} values for chemical and enzyme reactions are 2 to 3, while the common disinfectants phenol and alcohol have a Q_{10} of 4 and 45, respectively.

Critical to the successful employment of disinfectants is an understanding of the effect of disinfectant concentration on microbial reduction. A plot of the log of the time to reduce the microbial population in a standard inoculum to zero against the log of the disinfectant concentration is a straight line with the slope of the line termed the concentration exponent, n.

The relationship can be expressed as follows:

$n = (\log \text{ of the kill time at concentration } C_2) - (\log \text{ of the kill time at concentration } C_1)/(\log C_1 - \log C_2)$

Where, C_1 and C_2 are the higher and lower disinfectant concentrations, respectively.

The wide differences in concentration exponents, n, have practical consequences in picking the use dilution of different disinfectants and in using dilution to neutralize a disinfectant in disinfectant-effectiveness testing and routine microbial monitoring of the manufacturing environment.

For example, mercuric chloride has a concentration exponent of 1, so a 3-fold dilution will reduce the disinfectant activity by 3^1 (or by one-third), while phenol with a concentration exponent of 6 will have a 3^6 (or a 729-fold) reduction in disinfectant activity. Disinfectants with a larger concentration exponent or dilution coefficient rapidly lose activity when diluted.

The concentration exponents for some disinfectants are mentioned in Table 2.

Table 2. Concentration Exponents of Common Antiseptics, Disinfectants and Sterilant

Disinfectants	Concentration Exponents
Hydrogen peroxide	0.5
Sodium hypochlorite	0.5
Mercuric chloride	1
Chlorhexidine	2
Formaldehyde	1
Alcohol	9
Phenol	6
Quaternary ammonium compounds	0.8 to 2.5
Aliphatic alcohols	6.0 to 12.7
Phenolic compounds	4 to 9.9

Another important consideration may be the pH of the disinfectant. Many disinfectants are more active in the ionized form, while others are more active in the nonionized form. The degree of ionization will depend on the pK_a of the agent and the pH of the disinfection environment. For example, phenol, with a pK_a of 10, will be more effective at a pH below 7 where it is nonionized.

7.0 Mechanism of Disinfectant Activity

The sites and modes of action of some representative disinfectants are mentioned in Table 3.

Table 3. Mechanism of Disinfectant Activity Against Microbial Cell

Target	Disinfectant
Cell wall	Formaldehyde, hypochlorite, and glutaraldehyde
Cytoplasmic membrane, action on membrane potential	Anilides and hexachlorophene
Membrane enzymes, action on electron-transport chain	Hexachlorophene
Action on ATP	Chlorhexidine and ethylene oxide
Action on enzymes with −SH groups	Ethylene oxide, glutaraldehyde, hydrogen peroxide, hypochlorite, and iodine

Action on general membrane permeability	Alcohols, chlorhexidine, and quaternary ammonium compounds
Cell contents, general coagulation	Chlorhexidine, aldehydes, and quaternary ammonium compounds
Ribosomes	Hydrogen peroxide
Nucleic acids	Hypochlorites
Thiol groups	Ethylene oxide, glutaraldehyde, hydrogen peroxide, and hypochlorite
Amino groups	Ethylene oxide, glutaraldehyde, and hypochlorite
General oxidation	Hypochlorite

8.0 Microbial Resistance to Disinfectants

The development of microbial resistance to antibiotics is a well-described phenomenon. The development of microbial resistance to disinfectants is less likely to occur at significant levels, as disinfectants are more powerful biocidal agents than antibiotics. In addition, they are normally applied in high concentrations against low populations of microorganisms usually not growing actively, so the selective pressure for the development of resistance is less profound. However, the most frequently isolated microorganisms from an environmental monitoring program may be periodically subjected to use-dilution testing with the agents used in the disinfection program to confirm their susceptibility, as there are real differences among different species in resistance to the lethal effects of different sanitizers.

Microorganisms differ greatly in their resistance to disinfection agents. The order of resistance of clinically significant microorganisms to chemical disinfectants from most to least resistant is mentioned in Table 4.

Table 4. The Resistance of Some Clinically Important Microorganisms to Chemical Disinfectants (Listed in Order of Decreasing Resistance)

Type of Microorganisms	Examples
Bacterial spores	*Bacillus subtilis* and *Clostridium sporogenes*
Mycobacteria	*Mycobacterium tuberculosis*
Nonlipid-coated viruses	Poliovirus and rhinovirus
Fungal spores and vegetative molds and yeast	*Trichophyton*, *Cryptococcus*, and *Candida* spp.
Vegetative bacteria	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella* spp.
Lipid-coated viruses	Herpes simplex virus, hepatitis B virus, and human immunodeficiency virus

9.0 Disinfectant Challenge Testing

Under the Drugs and Cosmetics Act 1940 and the rules made there under, Indian regulatory bodies require companies that register public health antimicrobial agents including disinfectants, antiseptics, sanitization agents, sporicidal agents, and sterilants to ensure the safety and effectiveness of their products before they are sold or distributed. Companies registering these products must address the chemical composition of their product, include toxicology data to document that their product is safe if used as directed on the label, include efficacy data to document their claims of effectiveness against specific organisms and to support the directions for use provided in the labeling, and provide labeling that reflects the required elements for safe and effective use. While these label directions provide valuable information, they may not be helpful in terms of the products' use as disinfectants in a manufacturing environment.

The official disinfectant testing methods, Phenol-Coefficient Test are published in the Schedule O of Drug and Cosmetics Act 1940 and Rules 1945; IS 1061:2017 and in Association of Official Analytical Collaboration (AOAC).

To demonstrate the efficacy of a disinfectant within a pharmaceutical manufacturing environment, it may be deemed necessary to conduct the following tests:

(1) use-dilution tests (screening disinfectants for their efficacy at various concentrations and contact times against a wide range of standard test organisms and environmental isolates);

(2) surface challenge tests (using standard test microorganisms and microorganisms that are typical environmental isolates, applying disinfectants to surfaces at the selected use concentration with a specified contact time, and determining the log reduction of the challenge microorganisms); and

(3) a statistical comparison of the frequency of isolation and numbers of microorganisms isolated prior to and after the implementation of a new disinfectant.

This is considered necessary because critical process steps like disinfection of aseptic processing areas, as required by GMP regulations, need to be validated.

The disinfectant efficacy test must have realistic acceptance criteria. In practice, sufficient organisms need to be inoculated on a 2-inch × 2-inch square of all the surfaces like stainless steel, glass, plastic vinyl, epoxy etc in the facility being decontaminated, i.e., a coupon, to demonstrate at least a 2 (for bacterial spores) to 3 (for vegetative bacteria) log reduction during a predetermined contact time (i.e., 10 minutes over and above the recovery observed with a control disinfectant application). The efficacy of the neutralizers and their ability to recover inoculated microorganisms from the material should be demonstrated during the use-dilution or surface-challenge studies. Points to remember are that

- disinfectants are less effective against the higher numbers of microorganisms used in laboratory challenge tests than they are against the numbers that are found in clean rooms;
- that inocula from the log growth phase that are typically employed in laboratory tests are more resistant, with the exception of spores formed during the static phase, than those from a static or dying culture or stressed organisms in the environment; and
- that microorganisms may be physically removed during actual disinfectant application in the manufacturing area.

Although not all inclusive, typical challenge organisms that may be employed are mentioned in Table 5.

Table 5. Typical Challenge Organisms

Microorganisms	**Typical Environmental Isolates***
Bactericide	
E. coli, ATCC 11229; *S. aureus,* ATCC 6538; *P. aeruginosa,* ATCC 15442	*M. luteus, S. epidermidis, Coynebacterium jeikeium, P. vesicularis*
Fungicide	
C. albicans, ATCC 10231 or 2091; *Penicillium chrysogenum,* ATCC 11709; *A. brasiliensis,* ATCC 16404	*P. chrysogenum, A. brasiliensis*
Sporicide	
B. subtilis, ATCC 19659	*B. sphaericus, B. thuringiensis*
** Typical environmental isolates should be selected based on the environment flora of the facility*	

Because a wide range of different materials of construction are used in clean rooms and other controlled areas, each material needs to be evaluated separately to validate the efficacy of a given disinfectant. Table 6 contains a list of common materials used in clean room construction.

Table 6. Typical Surfaces to be Decontaminated by Disinfectants in a Pharmaceutical Manufacturing Area

Material	**Application**
Stainless steel 304L and 316L grades	Work surfaces, Filling Equipment and Tanks
Glass	Windows and Vessels
Plastic, Vinyl	Curtains
Plastic, Polycarbonate	Insulation Coating
Lexan® (plexiglass)	Shields
Epoxyl-coated Gypsum	Walls and Ceilings
Fiberglass-reinforced Plastic	Wall Paneling
Tyvek®	Equipment Wraps
Terrazzo Tiles/ Epoxy under flooring	Floors

10.0 Determination of Bactericidal, Fungicidal and Yeasticidal Activity of Antiseptic Medicinal Products

This section describes a test that can be used for the determination of antimicrobial activity in antiseptic medicinal products that are miscible with water and intended for administration by direct contact with the skin or mucous membranes. The extent of testing is dependent on the declared

antimicrobial activity of the product.

The test determines whether a product exhibits bactericidal, fungicidal or yeasticidal activity and complies with an established specification for such activity. This test cannot replace or confirm the assessment of the clinical efficacy of such preparations.

10.1 Principle

Antimicrobial activity is determined by adding test suspensions of micro-organisms (bacteria, fungi or yeasts, separately) to the sample antiseptic product. The mixture is maintained at 33±1° for contact times of 5 min for bactericidal activity and 15 min for fungicidal or yeasticidal activity. Additional contact times may be chosen, according to the stated use of the antiseptic medicinal product. At the end of the contact time, an aliquot is taken and the antimicrobial activity in this aliquot is immediately stopped by a validated method. Two methods are available: dilution-neutralisation and membrane filtration. The procedure is validated to verify its ability to demonstrate the required reduction in the count of viable micro-organisms by the use of appropriate controls.

10.2 Test Organisms and Growth Conditions

Prepare standardised stable suspensions of test strains as mentioned in section 9.2.1. In order to prevent any phenotypic changes in the strains used, the organisms used in the test should not be more than 5 passages from the original culture. One passage is defined as inoculation and growth of the organisms from existing culture to a fresh medium. Grow each of the microbial test strains separately as mentioned in Table 7.

Table7. Test Organisms and Growth Conditions

Strains for Bactericidal activity testing	
Microorganisms	**Growth Conditions**
Bacillus subtilis ATCC 6633 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541 *Escherichia coli* NCTC 10538 *Pseudomonas aeruginosa* ATCC 15442	Grow each of the bacterial species separately in Casein soyabean digest agar or Casein soyabean digest broth. After incubation, harvest the growth and obtain a microbial count of 1-5×10^8 CFU per ml. - For Preparation of test strains incubate at 30° to 35° for 18 to 24 hours and subculture at least twice. - For testing of product and validation of test incubate at 30° to 35° for ≤3 days.
Strains for Yeasticidal activity testing	
Candida albicans ATCC 10231	Grow in Sabouraud dextrose agar or Sabouraud dextrose broth. After incubation, harvest the growth and obtain a microbial count of 1-5×10^7 CFU per ml. - For Preparation of test strains incubate at 20° to 25° for 48 to 72 hours and subculture at least twice. - For testing of product and validation of test incubate at 20° to 25° for ≤5 days.
Strains for Fungicidal activity testing	

Candida albicans ATCC 10231 *Aspergillus brasiliensis* ATCC 16404	Grow in Sabouraud dextrose agar or Sabouraud dextrose broth. After incubation, harvest the growth and obtain a microbial count of 1-5×10^7 CFU per ml. - For Preparation of test suspension of *C. albicans* incubate at 20° to 25° for 48 to 72 hours. - For Preparation of test suspension of *A. brasiliensis* spores incubate at 20° to 25° for 5 to 7 days or until good sporulation. - For testing of product and validation of test with *C. albicans* and *A. brasiliensis* incubate at 20° to 25° for ≤5 days.

The recommended solutions and media are described in general chapter 2.2.9. Purified water is used. All reagents are sterile prior to use.

The test for bactericidal, fungicidal or yeasticidal activity is performed with the designated strains as mentioned in Table 7. In addition to these micro-organisms, it may be necessary to add other bacterial or fungal strains that represent the indications of the antiseptic medicinal product tested. Single-strain challenges are used. The counts are performed in duplicate and the arithmetic mean of the results is calculated and expressed in CFU per ml.

10.2.1 Preparation of Test Strain

After incubation, harvest the growth and resuspend each of the organisms separately in sterile saline to obtain a microbial count mentioned in Table 7. To suspend spores of *Aspergillus brasiliensis*, 0.05 per cent polysorbate 80 may be added to the saline. Use bacterial and yeast suspension within 2-4 hours. The suspension may be stored at 2° to 8° for a validated period of time. A stable spore suspension stored in suitable preserving medium for validated period of time.

10.2.2 Preparation of Antiseptic Product Test Solution

The concentration of the antiseptic product test solution shall be, if possible, 1.25 times the in-use test concentration because it is diluted to 80 per cent during the test and the method validation.

10.2.3 Neutralising Agents

Neutralising agents are used to neutralize the antimicrobial activity of the antiseptic product. The common neutralizing agents are listed in Table 1 of general chapter 2.2.9. Microbial Contamination in Nonsterile Products. The neutralisation time is not less than 10 second and not more than 60 second.

10.3 Methods

Prior to testing, equilibrate the temperature of all reagents to 33° ± 1°.

10.3.1 Dilution Neutralisation Method

Transfer 1.0 ml of a 0.3 percent w/v solution of *bovine albumin* into a tube, add 1.0 ml of the test suspension and maintain at 33° ± 1° for 2 minutes. Add 8.0 ml of the antiseptic product test solution and maintain at 33° ± 1° for the chosen contact time. Then, take a 1.0 ml sample of the test mixture and transfer into a tube containing 1.0 ml of *water* and 8.0 ml of the neutralizing agent and maintain

at 33° ± 1° for the appropriate neutralisation time. Take 1.0 ml of the neutralized test mixture, in duplicate, and inoculate using the pour-plate or surface-spread method. Incubate the plates according to the conditions mentioned in Table 7. After incubation, perform the count.

10.3.1.1 Suitability of Test/Control

For all methods, prepare a validation suspension containing 100-1000 CFU of the test micro-organisms per milliliter.

10.3.1.1.1 Experimental conditions control

Transfer 1.0 ml of a 0.3 percent w/v solution of *bovine albumin* into a tube, add 1.0 ml of the validation suspension and maintain at 33° ± 1° for 2 minutes. Add 8.0 ml of *water* and maintain at 33° ± 1° for the chosen contact time. Take 1.0 ml of this mixture, in duplicate, and inoculate using the pour-plate or surface-spread method. Incubate the plates according to the conditions mentioned in Table 7. After incubation, perform the count. The number of CFU recovered following incubation is not less than 0.5 × (number of CFU in the validation suspension)/10.

10.3.1.1.2 Neutralising agent control

Transfer 1.0 ml of a 0.3 percent w/v solution of *bovine albumin* into a tube, add 1.0 ml of the validation suspension and 8.0 ml of the neutralising agent used in the test and maintain at 33° ± 1° for the appropriate neutralisation time. Take 1.0 ml of this mixture, in duplicate, and inoculate using the pour-plate or surface-spread method. Incubate the plates according to the conditions mentioned in Table 7. After incubation, perform the count. The number of CFU recovered following incubation is not less than 0.5 × (number of CFU in the validation suspension)/10.

10.3.1.1.3 Dilution-neutralisation method control

Transfer 1.0 ml of a 0.3 percent w/v solution of *bovine albumin* into a tube, add 1.0 ml of a saline solution and 8.0 ml of the product test solution and maintain at 33° ± 1° for the chosen contact time. Transfer 1.0 ml of this mixture into a tube containing 8.0 ml of the neutralising agent and maintain at 33° ± 1° for the appropriate neutralisation time. Then add 1.0 ml of the validation suspension and mix. After 30 minutes, take a sample of 1.0 ml of the mixture, in duplicate, and inoculate using the pour-plate or surface-spread method. Incubate the plates according to the conditions mentioned in Table 7. After incubation, perform the count. The number of CFU recovered following incubation is not less than 0.5 × (number of CFU in the validation suspension)/10.

10.3.2 Membrane Filtration Method

Proceed as described in section 9.3.1, carrying out immediately the filtration step in place of the neutralisation step. Use membrane filters having a nominal pore size not greater than 0.45µm. The type of filter material is chosen such that the microbe-retaining efficiency is not affected by the components of the sample to be investigated. For each of the micro-organisms listed, a single membrane filter is used. Appropriately dilute 0.1 ml of the test solution and immediately filter the total volume, then rinse the membrane filter with an appropriate volume of the diluent. Perform the test in duplicate. Incubation conditions mentioned in Table 7. After incubation, perform the count.

10.3.2.1 Verification of the selected experimental conditions and of the membrane filtration method

10.3.2.1.1 Experimental Conditions Control

Proceed as described in section 9.3.1.1.1, except at the end of the contact time, take the sample in duplicate, and transfer into a separate membrane filtration apparatus. Filter immediately and then transfers each of the membrane filters to the surface of separate plates. Incubate the plates according to the conditions mentioned in Table 7. After incubation, perform the count. The number of CFU recovered following incubation is not less than 0.5 × (number of CFU in the validation suspension)/10.

10.3.2.1.2 Membrane Filtration Method Control

Proceed as described in section 9.3.1.1.3, except at the end of the chosen contact time, take that sample in duplicate, and transfer into a separate membrane filtration apparatus. Filter and rinse as described in section 9.3.2, then cover the membranes with rinsing liquid and add a sample of the validation suspension. Filter again and transfer each of the membrane filters to the surface of separate plates. Incubate the plates according to the conditions mentioned in Table 7. After incubation, perform the count. The number of CFU recovered following incubation is not less than 0.5 × (number of CFU in the validation suspension)/10.

10.4 Acceptance Criteria

Unless otherwise justified and authorised, the preparation has a:

- bactericidal activity if the defined number of CFU is reduced by at least 5 $\log_{10}$;
- fungicidal activity if the defined number of CFU is reduced by at least 4 $\log_{10}$;
- yeasticidal activity if the defined number of CFU is reduced by at least 4 $\log_{10}$.

11.0 Disinfectants in a Cleaning and Sanitization Program

The selection of suitable disinfectants and the verification of their effectiveness in surface challenge testing is critical in the development of a cleaning and sanitization program.

Issues associated with the successful implementation of such a program are the development of written procedures, staff training, decisions on disinfectant rotation, institution of application methods and contact times, environmental monitoring to demonstrate efficacy, and personnel safety.

The cGMP, *Equipment Cleaning and Maintenance,* details the requirements for written procedures for cleaning, maintenance, and sanitization of pharmaceutical manufacturing equipment. These procedures should address the assignment of responsibility, establishment of schedules, details of cleaning operations, protection of clean equipment prior to use, inspection for cleanliness immediately prior to use, and maintenance of cleaning and sanitization records.

Staff involved in disinfection require training in microbiology, industry practices for cleaning and sanitization, safe handling of concentrated disinfectants as per manufacturer instruction or material safety data sheet provided by supplier, the preparation and disposal of disinfectants, and appropriate application methods. It should be emphasized that the preparation of the correct dilutions is critical because many disinfectant failures can be attributed to use of disinfectant

solutions that are too dilute. Typically, disinfectants used in aseptic processing and filling areas are diluted with Sterile Purified Water or water for injection, and are prepared aseptically. Alternately, the disinfectant may be diluted with Purified Water or Water for Injection, and then sterile filtered to eliminate microorganisms that may potentially persist in a disinfectant. Diluted disinfectants must have an assigned expiration dating justified by effectiveness studies.

The rotation of an effective disinfectant with a sporicide is encouraged. It is prudent to augment the daily use of a bactericidal disinfectant with weekly (or monthly) use of a sporicidal agent. The daily application of sporicidal agents is not generally favored because of their tendency to corrode equipment and because of the potential safety issues with chronic operator exposure, except in isolator systems where an automated sporicidal application is required/recommended. Other disinfection rotation schemes may be supported on the basis of a review of the historical environmental monitoring data. The requirement to remove disinfectant and cleaning agent residues from surfaces should be considered, where appropriate, as a precaution against: inactivation any disinfectant subsequently applied; damage to facility surfaces; interference in recovery of microorganisms during surface monitoring and potential product contamination.

The greatest safety concerns are in the handling of concentrated disinfectants and the mixing of incompatible disinfectants. For example, concentrated sodium hypochlorite solutions (at a concentration of more than 5%) are strong oxidants and will decompose on heating, on contact with acids, and under the influence of light, producing toxic and corrosive gases including chlorine. In contrast, dilute solutions (at a concentration of less than 0.5%) are not considered as hazardous. Under no circumstances should disinfectants of different concentrations be mixed. Material Safety Data Sheets for all the disinfectants used in a manufacturing area should be available to personnel handling these agents. Appropriate safety equipment such as face shields, safety glasses, gloves, and uniforms must be issued to personnel handling the disinfectant preparation, and personnel must be trained in the proper use of this equipment. Safety showers and eye wash stations must be situated in the work area where disinfectant solutions are prepared.