PROTOCOL FOR THE VALIDATION OF DISINFECTION / SANITIZATION AGENTS WITH MINNCARE 2%, MINNCARE 3% and BACILLOCID 2%

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1 PROTOCOL APPROVAL

Approval of this protocol is joint responsibility of the following personnel:

	Name:	Role:	Department:	Sign/Date
Prepared By:				
Reviewed By:				
Approved By:				
Authorized By:				

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3 OBJECTIVE

The purpose of this protocol is to determine the efficacy of disinfectants used for routine disinfection/ sanitization of cleanrooms within Akorn India Pvt Ltd- Paonta Sahib premises. The scope of the protocol includes surface challenge testing as well as disinfectant neutralization and formulated disinfectant hold time validation.

The intent of this protocol is to that it shall be performed by an external contract testing laboratory. This protocol will serve as the guidance document for the contract testing laboratory. Contract testing laboratory shall transcript this procedure to its own protocol through its own document management system. The contract testing laboratory should record all data and pertinent information associated with the testing and materials in a controlled laboratory notebook or other GDP compliant manner, and supplies the resultant data to Akorn upon completion of the study. Testing of the sanitization agents should be performed in the following order:

1) Minncare 2 % Composition: Hydrogen Peroxide (22.0%) and Peroxyacetic acid (4.5%)
2) Minncare 3 % Composition: Hydrogen Peroxide (22.0%) and Peroxyacetic acid (4.5%)
3) Bacillocid 2 %

In the event that the agents are required for use prior to the completion of this protocol, an interim report shall be written for those agents completed in order to analyze the data and determine if the agents met the acceptance criteria specified for use.

All agents will be tested against all substrates and isolates, with the following exceptions:

4 BACKGROUND

The USFDA Guidance for Industry “Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice” document identifies in Section X.(Laboratory Control), subsection-A (Environmental Monitoring), Subsection-3 (Disinfection Efficacy), “…The suitability, efficacy, and limitations of disinfecting agents and procedures should be assessed. The effectiveness of these disinfectants and procedures should be measured by their ability to ensure that potential contaminants are adequately removed from surfaces…” Further information on the details of test methods for performing such an assessment are found in USP 39 NF 34 General Chapter <1072>.

Per USP 39 NF 34 General Chapter <1072>, a sound cleaning and sanitization program is needed for controlled environment used in the manufacture of sterile Pharmacopeial drugs to prevent microbial contamination into drugs. 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.

USP <1072> offers a three-tiered approach to performing disinfectant challenge testing, consisting of the following items:

- Use Dilution Testing
- Surface Challenge Tests
- Statistical Comparison

Review of the use dilution testing step provides an indication that the test is most applicable to those sanitization agents not previously studied for determination if the agent is effective on bacteria applied to stainless steel cylinders and immersed in the agent under review. The sanitization agents proposed for use as part of this protocol are generally accepted as effective for their given use, and have a significant acceptance with both industry and regulatory bodies as being appropriate for use in the cleanroom environment due to the rigor required of the manufacturer of the agent to make efficacy label claims. Furthermore, the testing method proposed for use dilution testing is generally not representative of cleaning of a modern aseptic core, where various surfaces require sanitization those are not able to be immersed in a sanitization agent.

In lieu of the use dilution testing, Akorn chose to focus on the Surface Challenge Tests, which are much more representative of cleaning that will be performed in a controlled environment and provide “worse case” conditions to challenge the selection of sanitization agents. USP <1072> recognizes that: “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 (see Microbiological Control and Monitoring of Aseptic Processing Environments, USP<1116>); 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.”

The review of Environment Monitoring (EM) data as recommended in USP <1072> following a sanitization agent change is also of limited value, especially for new construction where there is no previous monitoring data available. In lieu of performing this type of review of data, Akorn proposes an “In-Situ Sanitization” study to directly evaluate the efficacy of the sanitization agents as they are applied and used in the production areas themselves. This method has no provision for deliberate introduction of microbes into the

controlled areas, but instead relies on the natural flora that are present following shutdowns or other times when the controlled areas are declassified. In this manner the agents can be evaluated for efficacy under actual use conditions in order to provide evidence of effectiveness in-situ.

Based upon these points and references, Akorn selected a combination of surface challenge testing and in-situ efficacy testing to best provide evidence that the sanitization agents chosen as formulated, applied, and used in the controlled areas are capable of reliably reducing any bioburden present.

5 SCOPE

The scope of this protocol is limited to the following combinations of Disinfectant/Sanitizer and Substrate:

Table- 1

Disinfectant	Stainless steel	Panel	Epoxy	Glass	Acrylic	Poly Vinyl Chloride	Silicone	Door
Minncare 2%	X	X	X	X	X	X	X	X
Minncare 3%	X	X	X	X	X	X	X	X
Bacilloid 2%	X	X	X	X	X	X	X	X

X = Applicable

6 RESPONSIBILITY

ROLE	RESPONSIBILITIES
Microbiology Department	• Preparation, review and approval of qualification protocol. • Monitoring of protocol completeness, accuracy, technical excellence and applicability. • Review and Approval of Data Analysis and Final Summary Report
Testing Laboratory	• Perform the testing per the requirements of this protocol. • Review the protocol and transcript transcribe to its own Protocol. • Ensure all personnel performing the testing have been properly trained. • Document all pertinent data associated with testing, including lot numbers, expiry dates, manufacturers, and other relevant information for all materials used in the protocol. • Record the data in a controlled logbook using GDP. • Trained (certified) in accordance with contract laboratory data verification SOP to witness in real time all protocol entries made by the executor as part of the protocol execution. • The verifier role will be present during the entire execution of the protocol along with the Executor (Performer). • The Verifier will document their verification of protocol activities and verify that all entries made by the executor are accurate, correct, and recorded in real time (contemporaneously) • Data compilation and review. • Validation reports preparation, review, approval and recommendation thereafter (if required). • To generate deviations observed during execution of protocol.
Engineering	• To provide surface coupons representative of the surfaces in the cleanrooms to which the sanitization agents under validation will be applied to.
Reviewer	• The Reviewer will ensure the completed protocol or individual test cases have met all acceptance criteria specified in the protocol • Verify that exception or deviations to the protocol have been successfully resolved
QA	• Final Authorization of Protocol and Report.

7 DEFINITIONS

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.

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.

Sanitizing Agent — An agent for reducing, on inanimate surfaces, the number of all forms of microbial life including fungi, viruses, and bacteria.

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.

Contract laboratory: An organization or company or facility that tests products, materials, etc. according to agreed requirements. The test organization can be affiliated with the government or can be an independent testing laboratory. They are independent because they are not affiliated with the producer nor the user of the item being tested.

8 DISINFECTANT VALIDATION PROGRAM DETAIL

8.1 SELECTION OF CHALLENGE MICROORGANISMS

Micro-organisms have a wide diversity in their classification on the basis of Morphology, Cellular structure, Gram Stain Character, resistance property to various chemical agents. Hence every disinfectant must be challenged for efficacy against representative micro-organism of a group. Commonly this includes reference cultures as mentioned in various pharmacopoeias and the Environment Isolates which are isolated from air or water system of the facility.

- Bacterial Spores – *Bacillus subtilis* ATCC 6633
- Fungal Spores – *Aspergillus brasiliensis* ATCC 16404

- Yeast – *Candida* albicans ATCC 10231
- Vegetative Gram Negative – *Pseudomonas aeruginosa* ATCC 9027
  Eschereschia coli ATCC 8739
- Vegetative Gram Positive – *Staphylococcus aureus* ATCC 6538

Note: Environmental isolates are not included in this protocol as the facility is newly refurbished and the microbiological flora of the plant has not yet been established. Plant sourced isolates will be determined once the EM program is operational and subsequent sanitizer validation activities will occur at that juncture in time.

8.2 SELECTION OF SURFACES

The surfaces chosen to be tested as part of this protocol represent the various materials of construction of the manufacturing and sterility test area cleanrooms which undergo routine sanitization as part of normal production operations. The list of surfaces present in the controlled areas is compiled as follows:

- Stainless Steel (SS304 as worse case)
- Panel (Powder Coated Steel)
- Epoxy Resin
- Glass
- Acrylic
- Grade A Filling Curtains (Polyvinyl Chloride PVC)
- Silicone
- Door (Powder Coated Steel)

Each of these surface types will be obtained where possible from “aged” materials in order to best represent the current conditions of the surfaces as installed in the cleanrooms. Furthermore, “In-Situ” studies to perform evaluation of cleaning efficacy on the actual cleanroom surfaces with the normal microflora present at the facility will be performed to further demonstrate the efficacy of the selected agents under as used conditions.

8.3 SELECION OF EXPOSURE TIME:

The exposure times for sanitization/disinfecting agent on the selected surfaces in section 8.2 will be conducted as per following table in order to obtain baseline data to demonstrate that the agents are effective:

Table - 2

S. No.	Disinfectant	Contact time
1	Bacillocid 2 %	2 min., 4 min., 6 min.
2	Minncare 2 %	2 min., 4 min., 6 min.
3	Minncare 3 %	2 min., 4 min., 6 min.

Process optimization, where the variable of exposure time will be evaluated, will be performed in the subsequent validations using plant sourced isolates once the facility and EM programs come online. At this point in time, additional studies will be performed to evaluate the efficacy of the sanitization agents against the in house isolates at various exposure times in order to increase the efficiency of the process and better simulate real world conditions by using the natural flora of the facility.

8.4 ROTATION POLICY

The development of microbial resistance to antibiotics is a well-described phenomenon. The development of microbial resistance to disinfectants is less likely, as disinfectants are more powerful biocidal agents than antibiotics and are 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.

USP 39 NF 34 General Chapter <1072> states: “Because it is theoretically possible that the selective pressure of the continuous use of a single disinfectant could result in the presence of disinfectant-resistant microorganisms in a manufacturing area, in some quarters the rotation of disinfectants has been advocated. However, the literature supports the belief that the exposure of low numbers of microorganisms on facility and equipment surfaces within a clean room where they are not actively proliferating will not result in the selective pressure that may be seen with the antibiotics. 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. Other disinfection rotation schemes may be supported on the basis of a review of the historical environmental monitoring data.”

With reference to this, bactericidal disinfectant will be rotated weekly while Sporicidal disinfectant will be used weekly twice or as defined in SOP. The time periods will be monitored through the data collected as part of the Environmental Monitoring program.

8.5 REVIEW OF MICROBIOLOGICAL ENVIRONMENT MONITORING DATA

After the commencement and approval of disinfectant validation report, disinfectants will be used as per decided rotation and an in-situ sanitizer study performed. The purpose of this study is to evaluate the microbiological condition of the cleanroom surfaces prior to and immediately following the application of a validated sanitizer application. The study is typically commenced as a facility comes out of shutdown to simulate worst case conditions and maximize the inherent bioburden present in the area prior to sanitization.

This type of study also takes into account the variables that are unable to be simulated as part of the USP <1072> guidance, including the effect of mechanical application of sanitizer and if present, worn conditions of the surfaces in the cleanroom. The trending of data prior and subsequent to the change in a sanitizing agent recommended in USP <1072> is captured as part of the routine EM trending program, and will be reviewed per the current procedures for identification of trends.

9 MATERIALS AND EQUIPMENT USED IN THE STUDY

9.1 Surface Coupons to be Tested

- Stainless Steel (SS304 as worse case)
- Panel (Powder Coated Steel)
- Epoxy Resin
- Glass
- Acrylic
- Grade A filling curtains (Polyvinyl Chloride PVC)
- Silicone
- Door (Powder Coated Steel)

9.2 Disinfection/Sanitization Agents to be Tested

- Minncare, 2%
- Minncare 3%
- Bacillocid, 2%

9.3 Microorganisms to be Tested (ATCC cultures)

- *Bacillus subtilis* ATCC 6633
- *Staphylococcus aureus* ATCC 6538
- *Pseudomonas aeruginosa* ATCC 9027
- *Escherechia coli* ATCC 8539
- *Candida albicans* ATCC 10231

- *Aspergillus brasiliensis* ATCC 16404

9.4 Media and Reagents Required for Testing

- Dey Engley Broth
- Dey Engley Agar
- 70% Isopropanol
- Soybean Casein Digest Medium (SCDM)
- Soybean Casein Digest Agar (SCDA)
- 0.9% Saline

9.5 Equipment/Accessories Required for Testing

- Hand Gloves
- Inoculating Loop
- Bunsen Burner
- Test tubes
- Nose Mask
- Sterilized Wipes and Tips
- Biosafety Cabinet
- Autoclave
- Incubator 20-25 ^{0}C
- Incubator 30-35 ^{0}C

10 PREREQUISITES

- Equipment and instruments used to execute the testing in this protocol must be within calibration for the duration of the testing. The status of each instrument used will be documented within the report.
- Media used in the execution of this protocol shall be tested for its growth promoting properties as part of the positive controls performed in conjunction with the testing.
- SOP Verification: All the SOPs for equipment and instruments being used shall be verified to be in place and valid for the time period of execution.
- Training shall be provided to all personnel involved in the execution of this validation protocol and associated SOPs to be used. Training shall be recorded in a training attendance sheet as mentioned in contract testing laboratory training SOP and attached to this protocol.

11 DISINFECTANT/SANITIZER VALIDATION METHOD

11.1 FORMULATION OF SANITIZATION AGENTS

The details of formulation of the disinfection/sanitization agents should be documented on an Annexure.

11.1.1 Minncare, 2 %

Add 20 mL Minncare concentrate to 980 mL purified water to produce 1000 mL of Minncare 2% solution.

11.1.2 Minncare, 3 %

Add 30 mL Minncare concentrate to 970 mL purified water to produce 1000 mL of Minncare 3% solution.

11.1.3 Bacillocid, 2 %

Add 20 mL Bacillocid concentrate to 980 mL purified water to produce 1000 mL of Bacillocid 2 % solution.

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11.2 PREPARATION OF STOCK CULTURE SUSPENSION

11.2.1 The stock culture suspension is maintained by the contract testing laboratory providing the validation services. The history and details of the cultures used, with supporting documentation, shall be included in the final report of this protocol as an attachment and evaluated as part of the final report.

11.3 VALIDATION OF NEUTRALIZATION:

11.3.1 **Working suspension preparation:**

Based on the stock suspension count, on the day of the test, prepare serial dilutions in 0.9% saline solution from the stock suspension to yield a working suspension of 100 to 1000 CFU/mL. This suspension serves as an inoculum for Neutralizer efficacy test and Toxicity test.

Note: Contract testing laboratory can use ready to use culture suspension having 100 to 1000 CFU/mL as per its availability.

11.3.2 **Following micro-organisms are used for the validation of neutralizer.**

- *Bacillus subtilis* ATCC 6633
- *Staphylococcus aureus* ATCC 6538
- *Pseudomonas aeruginos*a ATCC 9027
- *Escherechia coli* ATCC 8739
- *Candida albicans* ATCC 10231
- *Aspergillus brasiliensis* ATCC 16404

11.3.3 **Test for Neutralizer efficacy:**

11.3.3.1 Dispense into six test tubes 1mL of disinfectant solution then add 8 mL of neutralizing solution and mix. Allow it to stand for 15 minutes.

11.3.3.2 At the conclusion of the 15-minute waiting period, inoculate each test tube separately with 1.0 mL of working suspension (100 to 1000 CFU/mL) of *B. subtilis, P. aeruginosa, E. coli, S aureus, C. albicans* and *A. brasiliensis* respectively.

11.3.3.3 Vortex for 30 seconds and allow the tubes to stand for 5 minutes. Transfer the contents of each tube into an individual sterile filtration assembly. Filter the solution through pre-sterile 0.45µ membrane filter followed by rinsing with 50mL sterile normal saline and place the membrane filter on to pre-sterilized and solidified DE petri plate.

11.3.3.4 Repeat this procedure for all test tubes.

11.3.3.5 Incubate the plates in inverted position at 30°C to 35°C for 48 to 72 hours for bacterial culture and 20°C to 25°C for 3 to 5 days for yeast/mold cultures.

11.3.3.6 After incubation count the numbers of colonies formed and document the results.

11.3.3.7 Using the data obtained from the testing, fill in the appropriate Annexure documents to calculate the percent recovery from the efficacy study and compare to the acceptance criteria.

11.3.3.8 Ensure that all relevant information, including lot numbers, expiry dates, manufacturer, and other useful data is recorded for each material used in the study in the laboratory logbook. Ensure that all process steps are documented in the logbook as proof of execution of the required steps.

11.3.4 **Positive control test:**

11.3.4.1 Prepare 6 test tubes and label as positive control test.

11.3.4.2 Dispense into each test tube 9.0 mL of sterile saline solution and then inoculate each test tube separately with 1.0 mL of working suspension (100 to 1000 CFU/mL) of *B. subtilis, P. aeruginosa, E. coli, S aureus, C. albicans* and *A. brasiliensis* respectively.

11.3.4.3 Mix and allow the tubes to stand for 15 minutes. Transfer the solution in sterile filtration assembly. Filter the solution through pre-sterilized 0.45µm membrane filter followed by rinsing with 50mL sterile normal saline and place the membrane filter on to pre-sterilized and solidified DE petri plate.

11.3.4.4 Incubate the plates in inverted position at 30°C to 35°C for 48 to 72 hours for bacterial culture and 20°C to 25°C for 3 to 5 days for yeast/mold cultures.

11.3.4.5 After incubation count the numbers of colonies formed and record the results.

11.3.4.6 Ensure that all relevant information, including lot numbers, expiry dates, manufacturer, and other useful data is recorded for each material used in the study in the laboratory logbook. Ensure that all process steps are documented in the logbook as proof of execution of the required steps.

11.3.5 **Negative Control Test**

11.3.5.1 Prepare one test tube and label as negative control test.

11.3.5.2 Dispense into the test tube 9.0 mL of DE Broth and 1mL of sterile saline.

11.3.5.3 Allow the DE Broth to stand for 15 minutes. Transfer the solution in sterile filtration assembly. Filter the solution through pre-sterilized 0.45µm membrane filter followed by rinsing with 50mL sterile normal saline and place the membrane filter on to pre-sterilized and solidified DE petri plate.

11.3.5.4 Incubate the plate in an inverted position at 30°C to 35°C for 48 to 72 hours.

11.3.5.5 After incubation determine if there is growth on the negative control and record the result. Ensure that all relevant information, including lot numbers, expiry dates, manufacturer, and other useful data is recorded for each material used in the study in the laboratory logbook. Ensure that all process steps are documented in the logbook as proof of execution of the required steps.

11.3.5.6 Ensure that all relevant information, including lot numbers, expiry dates, manufacturer, and other useful data is recorded for each material used in the study in the laboratory logbook. Ensure that all process steps are documented in the logbook as proof of execution of the required steps.

Note: The pour plate method can be used as an alternative to the filtration method and can be conducted as per contract testing laboratory procedure.

11.4 PREPARATION OF SURFACE CHALLENGE COUPONS

11.4.1 Perform moist heat sterilization on the heat stable coupons needed to perform the study. A minimum of 13 coupons should be sterilized for each of the surface material types per run being performed:

- **Stainless Steel**
- **Glass**
- **Door**
- **Panel**

11.4.2 Perform a chemical sterilization of the non-heat stable coupons needed to perform the study, including:

- **Epoxy**
- **Grade A filling curtain (Polyvinyl Chloride)**
- **Silicone**

11.4.2.1 A chemical sterilization can be performed by the use of a cold sterilant such as Spor-Klenz RTU or other suitable approved agent, which recommends that the substrate be immersed for 30 minutes at 20ºC.

11.4.2.2 The immersion in the sterilant should be followed by submersion in isopropanol and placed in a laminar flow hood to dry completely prior to use.

11.4.2.3 Following this treatment, the coupons may be used immediately or placed into autoclave bags (inside the LAF) for use at a future date.

Note: Contract Testing Laboratory can follow its own procedure for chemical sterilization and same shall be documented.

11.5 PREPARATION PROCEDURE FOR CULTURE SUSPENSION:

11.5.1 Based on the stock suspension count, on the day of the test, prepare serial dilutions in 0.9% saline solution from the stock suspension to yield a working suspension of $>10^4$ CFU/mL. This suspension serves as an inoculum for Surface Challenge Test.

Note: Contract testing laboratory can use ready to use culture suspension having $>10^4$ CFU/mL

11.6 SURFACE CHALLENGE TEST: TO DETERMINE THE EFFECTIVENESS OF USE CONCENTRATION IN REDUCING THE BIOBURDEN ON THE SURFACE:

11.6.1 Following micro-organisms are used for the surface challenge test.

- *Bacillus subtilis* ATCC 6633
- *Staphylococcus aureus* ATCC 6538
- *Pseudomonas aeruginos*a ATCC 9027
- *Escherechia coli* ATCC 8739
- *Candida albicans* ATCC 10231
- *Aspergillus brasiliensis* ATCC 16404

11.6.2 **Wipe Method**

11.6.2.1 Assign two coupons for each test organism and mark one as test and the other as the positive control.

11.6.2.2 Transfer separately 1.0mL of each working culture suspension on the surface of designated coupons and spread the suspension on the surface using sterile L – shaped spreader. Air dry the coupons under LAF.

11.6.2.3 Obtain approximately 500 mL of disinfectant solution of the concentration specified, that has been allowed to sit undisturbed for a minimum of 24 hours subsequent to formulation. The purpose of this rest period is to establish the ability of the sanitizers to maintain efficacy following formulation for a period of time.

11.6.2.4 Using cotton soaked in the disinfectant solution; wipe the coupons with dried culture suspension, marked as “test”.

NOTE: DO NOT APPLY DISINFECTANT ON POSITIVE CONTROL COUPON.

11.6.2.5 Allow the test coupons to remain wetted with the disinfecting/sanitization agent for the maximum exposure times provided in the Table - 2, to simulate the requirements specified during sanitization of the facility. If necessary, re-wet the surfaces in order to prevent them from drying during the all exposure periods.

11.6.2.6 Leave the Test coupon and positive control coupons under LAF/ Biosafety cabinet for drying.

11.6.2.7 After the required exposure time, collect swab samples using pre moistened sterile cotton swab from both the coupons marked as “test” and “positive

control" and transfer the swab in to 10 mL neutralizing fluid DE Broth (10^{-1} dilution). Allow to stand for 15 minutes.

11.6.2.8 . Vortex for 30 seconds and allow the tubes to stand for 5 minutes

11.6.2.9 For positive controls carry out dilution as mentioned in section 11.6.5

11.6.2.10 For the test coupons transfer the solution in sterile filtration assembly. Filter solution through pre-sterile 0.45µ membrane filter followed by rinsing with 50mL sterile purified water or normal saline and place the membrane filter on to pre-sterilized and solidified DE agar petri plate. If required, dilution shall be carried out as described in section 11.6.4

11.6.3 **Spray Method (Applicable for Minncare 2% & 3% for SS surface)**

11.6.3.1 Assign two coupons for each test organism and mark one as Test and the other as Positive Control.

11.6.3.2 Transfer separately 1.0mL of each working culture suspension on the surface of designated coupons and spread the suspension on the surface using sterile L – shaped spreader. Air dry the coupons under LAF.

11.6.3.3 Obtain approximately 500 mL of disinfectant solution of the concentration specified, that has been allowed to sit undisturbed for a minimum of 24 hours subsequent to formulation. The purpose of this rest period is to establish the ability of the sanitizers to maintain efficacy following formulation for a period of time.

11.6.3.4 Spray the coupon (containing dried culture suspension marked as test) with the disinfectant or sanitizer. Ensure that the entire surface of the coupon is wetted.

NOTE: DO NOT APPLY DISINFECTANT ON POSITIVE CONTROL COUPONS

11.6.3.5 Allow the test coupons to remain wetted with the disinfecting/sanitization agent for the maximum exposure times provided in the Table - 2 , to simulate the requirements specified during sanitization of the facility. If necessary, re-wet the surfaces in order to prevent them from drying during the all exposure periods.

11.6.3.6 Leave the Test and Positive Control coupons under LAF/ Biosafety cabinet for drying.

11.6.3.7 After the required exposure times, collect swab samples using pre moistened sterile cotton swab from both the coupons marked as "test" and "control" and transfer the swab in to 10 mL neutralizing fluid DE Broth (10^{-1} dilution). Allow to stand for 15 minutes.

11.6.3.8 Vortex for 30 seconds and allow the tubes to stand for 5 minutes

11.6.3.9 For positive controls, carry out dilutions as mentioned in section 11.6.5.

11.6.3.10 For the test coupons, transfer the solution in sterile filtration assembly. Filter the solution through pre-sterile 0.45µ membrane filter followed by rinsing with 50mL sterile purified water or normal saline and place the membrane filter on to pre-sterilized and solidified DE agar petri plate. If required, dilution of sample shall be carried out as mentioned in section 11.6.4.

Note: The pour plate method can be used as an alternative to the filtration method and can be conducted as per contract testing laboratory procedure.

11.6.4 Test Article Dilutions

11.6.4.1 For the test articles, perform serial dilutions to 10^{-4} dilution.

11.6.4.2 Set up 3 test tubes for each control to be tested and label accordingly.

11.6.4.3 To each test tube add 9 mL of DE Broth.

11.6.4.4 Add 1 mL of the swab rinse solution to the first tube. This is the 10^{-2} dilution.

11.6.4.5 Repeat this process to create the 10^{-3} and 10^{-4} dilutions.

11.6.4.6 Filter each test tube of solution through a pre-sterilized 0.45µm membrane filter and rinse the filter with 50mL of sterile saline.

11.6.4.7 Using aseptic technique, transfer the rinsed membrane filter to a pre-sterilized and solidified DE agar plate.

11.6.4.8 Label the plates with the appropriate dilution and volume of solution filtered and place into the incubation required for the organism being tested as described above.

11.6.4.9 After incubation count the number of colonies formed and record the results in the logbook with the dilution and volume for each result.

11.6.4.10 Ensure that all relevant information, including lot numbers, expiry dates, manufacturer, and other useful data is recorded for each material used in the study in the laboratory logbook. Ensure that all process steps are documented in the logbook as proof of execution of the required steps.

11.6.5 Positive Control Dilutions

11.6.5.1 For the positive controls, perform a serial dilution to 10^{-4} dilution.

11.6.5.2 Set up 3 test tubes for each control to be tested and label accordingly.

11.6.5.3 To each test tube add 9 mL of DE Broth.

11.6.5.4 Add 1 mL of the swab rinse solution to the first tube. This is the 10^{-2} dilution.

11.6.5.5 Repeat this process to create the 10^{-3} and 10^{-4} dilutions.

11.6.5.6 Filter each test tube of solution through a pre-sterilized 0.45µm membrane filter and rinse the filter with 50mL of sterile saline.

11.6.5.7 Using aseptic technique, transfer the rinsed membrane filter to a pre-sterilized and solidified DE agar plate.

11.6.5.8 Label the plates with the appropriate dilution and volume of solution filtered and place into the incubation required for the organism being tested as described above.

11.6.5.9 After incubation count the number of colonies formed and records the results in the logbook with the dilution and volume for each result.

11.6.5.10 Ensure that all relevant information, including lot numbers, expiry dates, manufacturer, and other useful data is recorded for each material used in the study in the laboratory logbook. Ensure that all process steps are documented in the logbook as proof of execution of the required steps.

Note: Analysis shall be carried out in triplicate for each coupon/organism/exposure time along with positive control.

For swab sampling method, contract testing laboratory shall follow its own respective SOP.

11.7 Log Reduction Calculation:

Using the results of testing as record in the controlled laboratory logbook, populate the data required per contract laboratory forms/annexure in order to calculate the efficacy of the sanitizer solutions on the various combinations of substrate and organism for each sanitizer tested.

Log Reduction = Log N_0 (Microbial Population Recovered From Positive Control) – Log N (Number of Organisms Recovered After Treatment).

For example, if 12,500 CFU of microorganisms are recovered from the positive control and 5 CFU recovered after disinfectant application, the calculation shall be:

= log (12500) – log (5)

= 4.09 – 0.69

= 3.94 log reduction obtained.

Ensure that copies of all logbook pages associated with this protocol have been copied and included in the final report as the source data for the study.

11.8 Prepared Disinfectant Hold Time Study:

The hold time study, which entails holding the disinfection agents for 24 hours subsequent to formulation but prior to use, will be performed as part of the validation, and will not require a separate study. The times of formulation and of subsequent use are tracked on the sheets respective to the activity that is occurring.

12 ACCEPTANCE CRITERIA

The following acceptance criteria are specified for the validation of sanitization agents:

- The neutralization study should demonstrate a recovery of 70% - 130% for all combinations of organism and sanitization agent tested.
- In case of surface challenge test positive control should yield 50 to 200% recovery of the microorganism in comparison to inoculums added.
- Agents should demonstrate a 3 log reduction of vegetative test organisms and mold spores.
- Agents (excluding Isopropanol) should demonstrate a 2 log reduction of Bacillus spore organisms.
- An agent may meet one acceptance criteria and not meet another and still be acceptable for use. For example, if a compound were to meet the 3 log reduction of vegetative test organisms and mold spores, but fail to meet the 2 log reduction of spores, the agent may still be certified for routine sanitization, but not as “sporicidal”.

13 DEVIATIONS

Deviations within the testing process at contract laboratory shall be handled under the contract laboratory quality system. If the acceptance criteria are not met, contract laboratory shall conduct an investigation as per its investigation procedure. If it is determined that the root cause is not the result of error than AIPL shall recommend additional testing of disinfectants/sanitizer with either increased disinfectant/sanitizer contact time or use concentration.

14 TRAINING REQUIREMENTS

Training shall be provided to concerned personnel from Contract Laboratory Supervisor or designee. If required, AIPL shall arrange the training session to the contract laboratory personnel. All the training shall be documented.

15 LIST OF ANNEXURES

Contract laboratory shall prepare the respective annexures as per their own document management system to record the analytical activities.

16 REFERENCES

16.1 USFDA Guidance for Industry "Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice".

16.2 USP 39 NF 34 General Chapter <1072>

16.3 USP 39 NF 34 General Chapter <71>

16.4 USP 39 NF 34 General Chapter <62>

17 ABBREVIATIONS

SOP	Standard Operating Procedure
NA	Not Applicable
AIPL	Akorn India Private Limited
IPA	Isopropanol or Isopropyl alcohol
%	Percent
No.	Number
DE	Dey Engley
CFU	Colony Forming Unit
LAF	Laminar Air Flow
mL	Milliliter
PVC	Polyvenyl chloride
GDP	Good Documentation Practices
EM	Environmental Monitoring
ºC	Degree centigrade
ATCC	American Type Culture Collection

18 CERTIFICATION REPORT

After the compilation of all analytical results and related data contract testing laboratory shall provide the certification report which includes the analysis summary, analysis output and conclusion regarding the disinfectant/sanitizer are meeting the acceptance criteria or not.

On the basis of contract testing laboratory certification report, comments and recommendation shall be made by AIPL :

Comments:

Recommendation:

19 REPORT APPROVAL

	Name:	Role:	Department:	Sign/Date
Prepared/ Compiled By:				
Reviewed By:				
Approved By:				
Authorized By:				

20 REVISION HISTORY

Revision No.	CCR/DCR No.	Reason for Change	Changes requested by (Department)
00		New protocol prepared	Microbiology