STANDARD OPERATING PROCEDURE		
SOP NO.: GLP-DA-06		Page No.: 1 of 13
Title: **AUDITING ANTIMICROBIAL EFFICACY STUDIES**		
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1. **PURPOSE**

To provide guidance and a standard procedure for auditing laboratory produced raw data on antimicrobial efficacy studies (tests), and to verify the integrity and accuracy of such data when accepted by the Agency as presumptive evidence of performance of antimicrobial products registered for public health use To assure compliance with FIFRA Good Laboratory Practice Standards regulations

2. **SCOPE**

This standard operating procedure (SOP) will be used in auditing tests and studies intended to demonstrate the performance (efficacy) of such products as sanitizers, disinfectants, tuberculocides, sterilants. This listing is not inclusive or limited to the antimicrobials noted. For the purpose of this discussion, the term "studies" includes tests, inasmuch as a study may be an aggregation of tests. The auditor should determine if the laboratory tests were performed according to the appropriate standard operating procedures, protocols, or a pre-approved study design, and that the raw data agree completely and accurately with the efficacy data submitted to the Agency. Additionally, this SOP should assist in reviewing and identifying laboratory practices that may negatively impact on the validity of those data and may be used as an adjunct to SOP GLP C-01 for guidance when conducting a facility GLP compliance review.

Some of the suggested questions offered in this SOP are general in nature, applicable to any or all of the bacteriological and fungicidal tests that may be reviewed (e.g., cultivation of microorganisms, proper dilution of test material, etc.). Others may have direct relevance to specific Association of Official Analytical Chemists (AOAC) tests and are included under named test heading.

Although relatively simple in concept, germicidal tests are very detailed and are performance sensitive. Variations in performance result when test details are altered, amended, or ignored. It is therefore

advisable that the auditor have a good working knowledge of the test procedure that is being renewed.

3. OUTLINE OF PROCEDURE

- Test Method evaluation
- Audit Procedure
  - General Considerations
  - Test Substance
  - Data Recording
  - Test Conditions
  - Laboratory Equipment and Materials
  - Culture Media Preparation
- Specific Tests
  - Use Dilution/Glass Slide Spray Tests
  - Fungicidal Test
  - Sanitizer Test
  - Chlorine Germicidal Equivalent Test
  - Sporicidal Test
  - Tuberculocidal Test
  - Re-Use Tests
  - Virucidal Studies
- Organization and Personnel

4. REFERENCES

4.1 USEPA OPPTS Test Guidelines Series 810, Office of Prevention, Pesticides, and Toxic Subtances (OPPTS), Washington, DC.

4.2 DIS/TSS Enclosures, OPP registration Division, Antimicrobial Program Branch, Washington, DC

4.3 Official Methods of Analysis, Association of Official Analytical Chemists (AOAC), Arlington, VA

4.4 Standard Operating Procedures, LDIB

GLP-C-01 GLP Compliance Inspection
GLP-S-02 Evidence Gathering
GLP-S-04 Full Report Format
GLP-S-05 Glossary of Terms

5. SPECIFIC PROCEDURES

5.1 TEST METHOD EVALUATION

The efficacy test data submitted to the agency for review and evaluation are largely developed by agency-approved methods or procedures. Judgement as to the appropriateness of a cited method or test used ultimately rests with the Registration Division reviewer. Efficacy data on antimicrobials are largely developed by procedures outlined in the USEPA OPPTS Test Guidelines, Series 810, Product Performance; Registration Division's DIS/TSS enclosures; and EPA's Good Laboratory Practices Standards (40 CFR 160). Most of the bacteriological and fungicidal efficacy data submitted on public health-use antimicrobial products are developed using standard test methods spelled out in the manual published by the AOAC (Ref. 4.3). Some of these tests are modified or amended to meet the Agency's requirements. The inclusion of hard water and/or organic soil load into the test system are modifications that are frequently made. Other modifications that may be made to the standard method include changes in contact time, contact temperature, and the testing against micro-organisms other than those listed. Test reports submitted to the Agency and study protocols must reflect all such changes, modifications, or amendments.

Agency pre-approval of test protocols is not normally required except in virucidal studies involving human immuno-deficiency virus (HIV) and Hepatitis virus. Virucidal studies, some tuberculocidal and some chemosterilant tests employ procedures that may not be considered standard tests by the agency but follow the principles described in the agency's DIS/TSS enclosures or other material supplied by the agency.

The auditor should review raw data for those studies with particular care because of the critical medical uses of those products.

5.2 AUDIT PROCEDURES

The audit should consist of an examination of the raw data on the specific tests or studies selected for review. All notebooks, worksheets, scratch-sheets, notes, computer printouts, calculations, graphs and tables pertaining to the given test or study should be considered raw data ant be subject to examination. The auditor should interview the Study Director, the technicians who performed the test(s) or were otherwise involved in the study. The following series of questions, devised to cover a spectrum of laboratory activities, should help in eliciting information on general laboratory practices, as well as on specific points on the test(s) under review. The auditor is encouraged to augment these questions with his/her own, or expand on them, especially where the test or study is within his/her area of expertise or experience.

5.2.1 General Considerations

- **!** Does the facility specialize in efficacy testing of antimicrobial products?
- **!** Were test procedures properly identified by title (if tests are standard tests), or a reference?
- **!** If AOAC tests were involved, which edition of the Official Methods of Analysis was cited?
- **!** Did the laboratory (testing facility) have an effective Quality Assurance (QA) unit or QA officer entirely separate from and independent of the personnel engaged in the direction and conduct of that study?
- **!** Were all raw data and test reports signed and dated by each individual scientist who conducted the testing?

5.2.2 Test Substance

- **!** Was the chemical composition of the test substance completely identified?
- **!** Was the test substance (including additives or activators) properly identified by a lot/batch number and date of manufacture?

- Was there any verification (such as chemical analysis) showing that the test substance was truly representative of the marketed product?

- Was the test substance a production sample, pilot sample, a marketplace sample, or a laboratory sample prepared specifically for the test?

5.2.3 Data Recording

- Was a study plan or protocol prepared?
- Was the protocol and all changes approved by the study director?
- Were test start and completion dates recorded in the raw data?
- Were observations and raw data recorded at the time of observation?
- Were the raw data legibly recorded in ink in bound notebooks, or worksheets, or by direct computer entry?
- If pre-printed worksheets were used, were test elements of AOAC tests that may be subject to variability, such as contact time, culture media, temperature, etc., preprinted on the worksheets?
- Were there any erasures on the raw data or were any original data obscured by changes in entries?
- Were apparent data corrections explained, initialed, and dated?
- Were any pages missing from bound notebooks?
- Was there any evidence that additional tests were performed on the same test substance but not reported
- Were calculations, if any, clearly shown? Were they accurate?

5.2.4 Test Conditions

- Were the thermometers used in testing traceable to National Institute of Standards and Technology (NIST), formerly National Bureau of Standards (NBS), or otherwise properly calibrated?

- Were waterbath and incubator temperatures closely monitored and recorded?
- How was the water that was used in diluting the test substance purified?
- Are water still and ion exchange column(s)properly maintained and serviced? How old are they? Was the date of installation of ion exchange columns recorded?
- Were dilution schemes recorded?
- Did the data show that test sample dilution were accurately made?
- How often were (are) stock solution retitrated? When was (is) the last date of preparation and titration?
- Was the source of the test culture identified?
- Was the identity/purity of the test culture confirmed? (Confirm the date when this was done.)
- How were (are) test cultures maintained?
- Was test organism(s) resistance checked against phenol?
- Were the phenol resistance tests run concurrently with the definitive study?
- If synthetic hard water was used, was its preparation recorded (including calculations) in the raw data?
- Was the hard water assayed for ppm of Calcium Carbonate ($CaCO_3$) at the time of its use in the study?

5.2.5 Laboratory Equipment and Materials

- How were (are) the accuracies of the incubator and waterbath temperature(s) determined?
- Were (are) the operating temperature and steam pressure of the autoclave checked with calibrated instruments or devices? How often was (is) this done? Confirm when it was last done prior to the audited study.
- How often was (is) the pH meter calibrated? What standards are used and how old are they? Was the pH meter calibrated prior to the study?

- How frequently was (is) the spectrophotometer checked for accuracy and how was this done prior to the study?
- When was the burette used in titrating hard water calibrated?
- How often were (are) analytical and other balances checked for accuracy? Is this done in house, or by a balance service person?
- Did (does) the laboratory maintain a log book or record of all equipment and instrument calibrations and service?
- If pre-sterilized equipment or materials (e.g., Petri dishes, pipettes, syringes, reagents, serum, enrichments) were (are) used, how was (is) the sterility verified?

5.2.6 Culture Media preparation

- Was the culture medium used for growing the bacterium fully described (beyond the terms "nutrient broth" and “nutrient agar")?
- Was information relative to source, lot number expiration date, and storage practices for the culture media recorded?
- Was the description of the preparation and pH of the media documented?
- What methods were (are) used to detect growth in media not inoculated with the test bacteria?
- What kind of container was (is) the beef extract in? If in squeeze tube, what is done to it before it is used?

5.3 SPECIFIC TESTS

5.3.1 Use-Dilution/Glass Slide Spray Tests

- Were all test cultures in daily transfer for at least 4 days prior to use?
- Were cultures transferred daily through weekends and holidays?
- After how many daily transfers would the culture be started anew from the stock slant?

- When were the cultures last tested for smoothness/roughness prior to the study? What were the results?
- Was Anatone or another peptone used in the culture media? Was the source, lot number, and date of purchase recorded?
- Was the organic soil, if used, identified as to source and date purchased?
- When and how were contaminated carriers prepared?
- Was the temperature and drying time for contaminated carriers recorded?
- Was a determination of the surviving micro-organisms on the test carriers made?
- Were carriers screened for pitting, scratching, and other imperfections? Were Use-Dilution test carriers biologically screened?
- Was the method of removing the pellicle from the Pseudomonas aeruginosa broth culture identified?
- When recovery medium was used, was it recorded? (When made; how was it stored?)
- What method(s) was used to check for neutralization of the test material? Was it recorded?
- In 60-carrier use dilution tests, were all tests done on the same day using the same test bacteria and diluted product of the same lot?
- Was the growth from positive tubes examined by Gram stain and/or subcultured for identification?
- If sterile-packaged materials, supplies, and equipment were used, how was sterility checked in the laboratory?
- What kind of serum, if any, was used? What brand? When and where was it obtained?
- What was the serum concentration, if used in the test? Was this recorded?
- What neutralization procedure was done? Was it documented?

5.3.2 Fungicidal Test

- Was the date of preparation of the conidiaspore suspension indicated?
- Was the conidial challenge of $5x10^6$ conidia/mL used?
- What was the date of preparation of the conidial suspension?
- What kind of serum, if any, was used? What brand? From where and when obtained?
- How was the serum, if used, added to the test system? What was its final concentration?

5.3.3 Sanitizer Test

- Was the preparation of the synthetic hard water adequately described? Briefly, described how hard water was prepared. Were such elements as burette readings and calculations recorded?
- As a test control check, was the accuracy of the hard water tolerance verified using known quaternary ammonium chloride standards of known hard water tolerance? Was the name of the quaternary ammonium chloride spelled out and its concentration recorded?

5.3.4 Chlorine Germicidal Equivalent Test

- How was the sodium hypochlorite (NaClO) titrated?
- How old was the NaClO stock solution? Where was it stored?
- What material was the container made of?
- Was the NaClO stock container protected from light?

5.3.5 Sporicidal Test

- What culture medium was used in growing bacterial cultures?
- How were the test carriers prepared? Stored?
- How were spores checked for the required resistance?
- If gaseous compounds were tested, were spores hydrated?
- Was spore viability demonstrated?
- Were the test tubes containing recovered bacteria heat shocked? (What temperature; how long?)
- What was the source of suture loop material?

- ! Did the germicidal solution affect the suture loops way?
- ! Were suture loops extracted with chloroform ($CHCl_3$)?
- ! Are the carriers (porcelain cylinders and suture loops) reused?

5.3.6 Tuberculocidal Test

- ! What Mycobacterium bovis strain was used? When and where was it obtained?
- ! Was the culture medium and recovery media used in the test adequately identified?
- ! What was the age of the dry media and components used to prepare the media?
- ! What was the age of the prepared media?
- ! Were lot numbers of the recovery media and enrichments available (recorded)? How old were the media/enrichments?
- ! Were any sterility checks made on serum and enrichments (such as a control tube in the test)?
- ! What neutralizer was used?
- ! What procedure(s) was carried out to measure neutralization before the test was carried out?
- ! What was the percent light transmittance of the culture suspension? Was it recorded?
- ! Were phenol control tests done at the same time as the tuberculocidal test?
  - In the quantitative suspension tuberculocidal tests, was the test culture started from a lyophilized culture?
  - How long did it take to reach the numbers of the TB bacteria needed to run the tests after the culture was obtained from outside sources?
  - Was the preparation of the stock culture (from lyophilized culture) and subsequent transfers recorded for each step? Were incubation periods for each step recorded?
  - What was the light absorbance reading when the culture was harvested? Were plate counts done to verify the cell concentration?
  - Was the type of culture medium used for each step recorded? Were the components identified?

- Was the stock culture supply stored at a low (-60 CE) temperature?
- What were the viable test organism counts of the challenge test culture?
- Were plate counts, calculations, and survival curves recorded?
- Were the survival curves an average of at least four separate studies?

5.3.7 Re-Use Tests

! Was a re-use test protocol developed and approved? Was it followed accurately?
! Was the complete set of inhalation equipment (two sections of corrugated rubber tubing, rebreathing bag, face mask, endotracheal tube, “Y” tube) used in the test?
! Were plate counts to determine the bioburden to the test system made? What were they?
! Were determinations of the active ingredient(s) and pH of the test solution made during the simulated use study?
! Were the optional hard water and/or blood serum used in the test system?

5.3.8 Virucidal Studies

! Was (is) the facility equipped with appropriate equipment (e.g., biohazard hood, egg incubator, ultra-centrifuge, low-temperature freezer, filtration equipment for cold sterilization, CO_2 incubator, microscope)? List additional equipment not named.
! Did (does) the facility contain an animal isolation room?
! What kind and percent efficiency was (is) the air filtration system?
! How often were (are) air filters cleaned/replaced?
! Did (does) the facility prepare its own cell culture?
! Were study protocols available?

- Were they followed accurately?
- Was the individual conducting the test experienced in that procedure?
- Where changes in study protocols approved by the study director?
- What was the source of the virus culture used?
- Was the virus culture checked for contamination with bacteria or other viruses?
- Were cell lines specific for the virus used in the test?
- Was the titer of the test virus determined?
- Was the growth medium for propagating the cell line identified?
- How was the sterility of the growth medium maintained or checked during the testing?
- Was the method of calculating virus titer indicated?
- Was the cytotoxic effect of the test material considered and pretested?
- Were all observations and test results recorded and calculated?
- If embryonated eggs were used, were their age (days old) indicated?
- What checks were made to determine if the egg was not contaminated with a bacterium or other virus?
- What was the source of the eggs?
- What were the egg incubator temperature and relative humidity for replicating the virus?
- What was the egg inoculation route? Was it recorded?
- Was the virus titration end-point determination recorded?
- What animals were used in virucidal tests? Was the name the animal supplier recorded?
- How was their health checked? How often?
- Were they appropriate test animals?
- Was the route of inoculation indicated/recorded?
- How was the titration end-point determined?
- What quality control measures were taken to insure the health of the animal(s)?

5.4 ORGANIZATION AND PERSONNEL

- Was the educational background, training, and experience of each individual involved in the testing available?
- Were CVs on each individual available?
- Are the scientists members of the AOAC, ASTM, SIM, CSMA, ASM, or of other scientific organizations oriented toward antimicrobial testing?

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