**MICROBIAL LIMIT TEST (MLT) METHOD SUITABILITY PROTOCOL TO DETERMINE THE TOTAL MICROBIAL COUNT IN ABIRATERONE ACETATE TABLETS 500MG**

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# OBJECTIVE:

The objective of this protocol is to verify the total microbial count and pathogens in Abiraterone acetate tablets 500mg by pour plate method by using harmonized method at Oncology block of Jodas Expoim Private Limited, plot No: 55, phase-3, Biotech Park, Karkapatla village. This study shall be perform at Quality Control microbiology department.

Validation of three batches shall be performed at manufacturing site.

Vendor Name:

# EXECUTION TEAM:

1. **Training to the executors:**

* The executors shall be trained before the execution of protocol. Fill the details in training Format as per the Training SOP No. JEQA019 and attach the training record.

1. **List of Executors Involved in Qualification Study:**

* Record the details of the executors involved in the Qualification activity like name, department, designation and training details with signature and date in the Annexure-1.

# PERSONNEL RESPONSIBILITIES:

Responsibilities of individual department / personnel while execution of protocol is as under:

**Microbiology**

* Responsible for preparation and review of protocol.
* Provision of training to all concerned persons on protocol prior to execution
* Execution of the protocol.
* Assist in the investigation of variances if any.
* Prepare the summary and conclusion of the activity.
* Head / Designee shall be responsible for review, approve of the protocol,

Summary & conclusion and certification of the protocol.

**QA-Validation**

* Responsible for review of protocol.
* Assist in the investigation of variances if any.
* Verifies that the test requirements described in protocol are meeting criteria for the performed tests and properly documented.

**Quality Assurance**

* Quality Assurance Head / Designee shall be responsible for review, approve

Of the protocol, summary & conclusion and certification of the protocol.

# QUALIFICATION PRE REQUISITES:

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| --- | --- | --- | --- | --- |
| **Sr. No.** | **Description** | **Yes/No/NA** | **Checked by(QM)** | **Verified**  **by (QM)** |
|  | Training is imparted to personnel responsible to execute the study. |  |  |  |
|  | Others if any \_\_\_\_\_\_\_\_\_\_\_\_\_. |  |  |  |

**Note:** The above pre-requisites should be verified and if complete general study protocol activity shall be carried out.

**Comments:**

**Reviewed by (QA): Date:**

# QUALIFICATION PROCEDURE:

* 1. **Materials and equipments:**
* Sabouraud dextrose agar (SDA) molted media bottles
* Soya bean casein digest agar (SCDA) molted media bottles
* Soya bean casein digest agar (SCDA) plates
* Soya bean casein digest medium
* Macconkey broth
* Macconkey Agar
* Empty petriplates
* 0.9% Saline
* Micropipette tips100μL and 1000μL.
* Sterile 70% Isopropyl alcohol
* Test tubes
* Product sample.
* Incubators, temperature 30 to 35°C
* Incubators, temperature 20 to 25°C
* Incubators, temperature 42 to 44°C
* Bio safety Cabinet
* Micropipettes
* Water bath
* Inoculation loops
* Spreaders
* Vortex mixer

NOTE: Document the Equipment’s used for the test in Annexure-2.

* 1. **Cultures to be used for the study:**

|  |  |
| --- | --- |
| **Name of the Strains** | **ATCC No. (or) Equivalent Numbers** |
| **For recovery** | |
| *Pseudomonas aeruginosa* | ATCC 9027 |
| *Staphylococcus aureus* | *ATCC 6538* |
| *Candida albicans* | *ATCC 10231* |
| *Aspergillus brasiliensis* | *ATCC 16404* |
| *Bacillus subtilis* | *ATCC 6633* |
| **For pathogens** | |
| *E.coli* | ATCC 8739 |

* 1. **Inoculum preparation from Cryovial:**
* Prepare SCDA, SDA Slants/plates as per SOP JEQM018 “Preparation, storage and control of prepared media” otherwise use readymade media slants or plates.
* Take out the cryovial from deep freezer.
* Mark the Prepared or ready to use slant/plate with details of date of sub culturing and name of the organism.
* Thawing shall be perform by placing the cryovial at room temperature.
* Take one loop of culture with sterile loop and streak the bacterial culture in SCDA and incubate in 30 – 35ºC for ≤72 hours, SDA for fungal culture in 20 - 25ºC for ≥72 hours .The inoculation shall be performed under Biosafety cabinet.
* Note: All working cultures shall be prepare in duplicate.
* Documentation shall be done in “Subculture Record of cryovial” Annexure-3
  1. **Preparation of culture suspension:**
* From a working slant/plate transfer a loopful of the culture to 10 mL of sterile saline solution and vortex the saline solution to mix the culture completely or until to get uniform suspension.
* Take 8 tubes for each culture containing 9 mL sterile 0.9% saline solution and label them with the name of the organism and name of the dilution (10-1 to 10-8) and date.
* Prepare tenfold serial dilutions of the suspension up to the 10-8 dilution.
* Label the plates with name of the organism, name of the dilution and date of inoculation.
* After serial dilutions pipette out 0.1mL of culture suspension from 10-1 to 10-8 tubes on to the surface of agar media plates and spread the culture suspension with a sterilized spreader.
* Perform the activity in duplicates and keep one plate as negative control.
* Use SCDA plates for bacterial cultures, SDA for fungal cultures.
* Incubate the SCDA plates in inverted position in the incubator having the temperature 30-35°C for ≤72hrs.
* Incubate the SDA plates in inverted position in the incubator having the temperature 20-25°C for ≥72hrs
* After completion of incubation take out the petriplates from the incubators and Observe for the results.
* Count the number of colonies under colony counter observed in each dilution, and enter the results in Annexure -4.
* Enter the details of quantity of suspension prepared in Annexure-5.
* Use the dilution which is having NMT 100cfu for the test.
  1. **Sample preparation:**
* Take10 gms of Abiraterone acetate tablets 500mg in 100mL of Soya bean Casein digest broth (0.5% Lecithin and 2% polysorbate 80) (Solution A) 1:10Dilution. Consider it as product control.
* From the Solution A prepare 5 aliquots of 10 mL (solution B). In these aliquots inoculate each organism separately to get NMT 100cfu/1ml of media. Consider it as Product positive control.
* Prepare 5 aliquots of 10 mL of SCDM (0.5% Lecithin and 2% polysorbate 80)
* In these aliquots inoculate separately each organism to get NMT 100cfu/1ml of media (solution C).Consider it as positive control.
* 100 ml Soyabean Casein digest medium (0.5% Lecithin and 2% polysorbate 80) keep as a Negative control.
* Record the details in Annexure-6.
  1. **Test for Total Aerobic Microbial Count and Total Yeasts and Molds count:**

**Product control:**

* Inoculate 1mL of solution – A in duplicate into empty sterile petriplates, then add 20-30 mL of Soybean casein digest Agar medium in case of bacterial cultures and Sabouraud agar medium in case of fungal cultures.
* Rotate the plates in clockwise and anti-clock wise direction, after solidification incubate the SCDA plates at 30 – 35°C for 3-5 days, and SDA plates at 20 – 25°C for 5-7 days
  1. **Recovery test for total Aerobic microbial count (positive product control)**

1. **Test for *Bacillus Subtilis:***

* Add 1mL of the Solution – B (Positive Product Control) in duplicate in sterile Petri plates.
* Add 20-30mL of Soya bean casein digest agar into each plate. Incubate at 30-35°C for 24-72hrs.
* Record the details in Annexure-6.

1. **Test for *Pseudomonas aeruginosa*.**

* Add 1mL of the Solution – B (Positive Product Control) in duplicate in sterile Petri plates.
* Add 20-30mL of Soybean casein digest agar into each plate. Incubate at 30-35°C for 24-72hrs.
* Record the details in Annexure-6.

1. **Test for *S.aureus:***

* Add 1mL of the Solution – B (Positive Product Control) in duplicate in sterile Petri plates.
* Add 20-30mL of Soybean casein digest agar into each plate. Incubate at 30-35°C for 24-72hrs.
* Record the details in Annexure -6.

1. **Test for *Candida albicans:***

* Add 1mL of Solution-B (Positive Product Control) suspensions in duplicate in sterile Petri plates. Add 20-30mL of SDA into each plate. Incubate at 20-25°C for 3-5days.
* Record the details in Annexure-6

1. **Test for *Aspergillus brasiliensis:***

* Add 1mL of Solution-B (Positive Product Control) suspensions in duplicate in sterile Petri plates. Add 20-30mL of SDA into each plate. Incubate at 20-25°C for 3-5days
* Record the details in Annexure-6
  1. **Positive control:**
* Inoculate 1mL of solution-C in duplicate into empty sterile petriplates, then add 20-30 mL of Soybean casein digest Agar medium in case of bacterial cultures and Sabouraud dextrose agar in case of fungal cultures.
* Rotate the plates in clockwise and anti-clock wise direction, after solidification incubate the SCDA plates at 30 – 35°C for 24 -72hrs and SDA plates at 20 – 25°C for 3-5days.
* Record the details in Annexure-6.
  1. **TEST FOR SPECIFIED MICRO – ORGANISAMS:**

1. ***E.coli***

**Product control**:

* Take 10 mL of Solution-A, add in 100 mL of SCDM and incubated under Laboratory incubator for18-24 hrs at 30-350C.

**Positive product control:**

* Take 100mL of SCDM, add 10ml of solution “A” and add NMT 100cfu of *E.coli.*
* Incubate the tube at 18-24 hrs at 30-350C.

**Positive control:**

* Take 100mL of SCDM, add NMT 100cfu of *E.coli*.
* Incubate the tube at 18-24 hrs at 30-350C.
* After 18hrs inoculate a portion of 1 mL from product positive control and positive control to 100mL of Macconkey broth.
* Incubate at 42°C to 44°C for 24 to 48hrs.
* After 18hrs inoculate a loop full from product positive control and positive control on Macconkey agar.
* Incubate at 30°C to 35°C for 18 to 72hrs.
* After 18hrs check the plates for characteristic growth of *E.coli* on product positive control and positive control.
* If growth of colonies indicates the possible presence of *E.coli*.

**Product and Negative control:**

* Keep one SCDM 100mL as negative control, Incubate the bottle at 18-24 hrs at 30-350C.
* After 24hrs inoculate a portion of 1mL from Negative control and Product control in 100mL of macconkey broth.
* Incubate at 42°C to 44°C for 24 to 48hrs.
* After 48hrs inoculate a loop full from product control and negative control on Macconkey agar.
* Incubate at 30°C to 35°C for 18 to 72hrs.
* After 72hrs check the plates for characteristic growth of *E.coli* on product control and negative control.
* Record the results in Annexure-7.

**Acceptance criteria:**

* The colony characteristics observed on product positive control, and positive control should be similar.
* No colonies shall be observed in product control and negative control..
  1. **Acceptance Criteria**
* Negative controls shall not show any growth.
* The method stands validated when a count of any of the test organisms recovery should be not less than 70% from the calculated value of the inoculums.
* After completion of validation activity a consolidated report shall be prepared with

the inclusion of results.

* 1. **Re-verification of the method suitability**
* The suitability of the method shall be re-verified in case of any change in the testing performance, or the product, which may affect the outcome of the test results.
  1. **Recommendation In Case of Recovery Failure**
* If in spite of the incorporation of suitable inactivating agents and dilution if it is not

Possible to recover the viable cultures and where the article is not suitable for employment of pour plate method, it can be assumed that the failure to isolate the

Inoculated organism is attributed to the bactericidal / fungicidal activity of the sample

* This information shall serve to indicate that the sample is not likely to be contaminated with the given species of microorganisms.
* However, monitoring should be continued in order to establish the spectrum of

Inhibition and bactericidal / fungicidal activity of the article at a dilution where nearest recovery is obtained.

* 1. **Frequency**
* Any major process change in the existing product.
  1. **Report And Conclusion**
* Report and conclusion shall be made after completion of validation activity.

# INCIDENTS/DISCREPANCIES RECORD:

* List the Incidents/discrepancies observed if any during the execution of the protocol and evaluate the Incident/discrepancy, and implement the corrective actions for the same and attach the Incidents/discrepancy record.

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| **S. No.** | **Description of the Incident/Discrepancy** |
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# ABBREVIATIONS:

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Abbreviations** | **Description** |
|  | SOP | Standard Operating Procedure |
|  | VA | Validation |
|  | QA | Quality Assurance |
|  | QM | Quality control (Microbiology) |
|  | cfu | Colony Forming Units |
|  | NLT | Not Less Than |
|  | NMT | Not More Than |
|  | °C | Degree centigrade |
|  | % | Percentage |
|  | SCDA | Soya bean Casein digest Agar |
|  | SDA | Sabouraud Dextrose Agar |
|  | mL | millilitre |
|  | USP | United States pharmacopeia |
|  | GTP | General Test procedure |
|  | PPC | Product Positive Control |
|  | NC | Negative Control |
|  | PC | Positive Control |
|  | MA | Macconkey Agar |
|  | MB | Macconkey Broth |
|  | SCDM | Soya bean casein digest medium |
|  | Ph.Eur | European pharmacopeia |

# ATTACHMENTS:

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| S.No | Attachments |
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# REFERENCE DOCUMENTS:

* USP42: ˂1227˃: General information- Validation of microbial recovery from pharmacopeial articles.
* USP <61>Microbiological examination of non-sterile products: Test for microbial enumeration tests.
* USP <62>Microbiological examination of non-sterile products: Test for specified microorganisms.
* Ph.Eur< 2.6.12> Microbiological examination of non-sterile products: Microbial enumeration tests.

# ANNEXURES:

|  |  |
| --- | --- |
| Annexure-1 | Details of the executors |
| Annexure-2 | Equipments used |
| Annexure-3 | Subculture record of Cryovial |
| Annexure-4 | Inoculum determination |
| Annexure-5 | Format for culture suspension. |
| Annexure-6 | Recovery of the product and results |
| Annexure-7 | Test for specified Microorganisms |

# SUMMARY

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| --- | --- | --- | --- |
| **Description** | **Yes/No/NA** | **Checked by (QM)** | **Verified by(QM)** |
| All acceptance criteria set forth in the protocol were met |  |  |  |
| All applicable data sheets were signed as checked and verified. |  |  |  |
| Incidents if any |  |  |  |
| Whether acceptable |  |  |  |
| If not acceptable, action taken |  |  |  |

**END OF THE DOCUMENT**