1. **Objective:**

To verify the total microbial count and pathogen in temozolomide by pour plate method has been carried out as per the protocol no.: ON/QM/GS/P-052. These report represents and discuss the results obtained in the validation study.

1. **Qualification Procedure:**

As a part of the study the following cultures were used and the tests were performed as per the General studies protocol and observations made are as follows.

1. *Pseudomonas aeruginosa*

2. *Staphylococcus aureus*

3. *Candida albicans*

4. *Aspergillus brasiliensis*

5. *Bacillus subtilis*

6. *Escherichia coli*

7*. Salmonella*

8. *Clostridia*

**Allotment** **of IND number and preparation of culture suspension:**

* IND number was allotted with dilution to the above cultures and reconstituted with 8.8mL of saline as per the SOP no.:JEQM021.
* For the results refer Annexure-IV.

**Medias used for validation study and details given in the table**.

| **S.No** | **Name of the media** | **Lot no.** | **Mfg date/**  **Preparation date** | **Exp date/Use before date** | **GPT status** |
| --- | --- | --- | --- | --- | --- |
|  | SCDA Plates | BG20i26 | 19-09-20 | 03-2021 | Pass |
|  | SDA Plates | ONMD20-505 | 26/11/20 | 11/12/20 | Pass |
|  | Saline | ONMD20-0482  ONMD20-0509 | 12/11/20  28/11/20 | 12/12/20  28/12/20 | NA |
|  | SCDM (Soya bean casein digest medium) | ONMD20-0490 | 17/11/20 | 17/12/20 | Pass |
|  | SCDA Molted bottles | BG20K14 | 2020-11 | May-2021 | Pass |
|  | SDA Molted bottles | BG20J21 | 2020-10 | April-2021 | Pass |
|  | MB (Macconkey Broth) | ONMD20-0495 | 21/11/20 | 21/12/20 | Pass |
|  | MA (Macconkey Agar) | ONMD20-0501 | 16/11/20 | 11/12/20 | Pass |
|  | RVSEB | LQI690 | 2020-07 | 2022-01 | Pass |
|  | XLDA | ONMD20-503 | 26/11/20 | 11/12/20 | Pass |
|  | EE Broth | LQI871 | 2020-09 | 2021-04 | Pass |
|  | RCM | LQI115 | 2020-01 | 2021-02 | Pass |
|  | VRBGA | ONMD20-504 | 26/11/20 | 11/12/20 | Pass |
|  | SDB | LQZ016 | 2020-02 | 2021-08 | Pass |
|  | Columbia agar | BG20F07 | 2020-06 | 2020-12 | Pass |
|  | CA | ONMD20-500 | 26/11/20 | 11/12/20 | Pass |
|  | MSA | ONMD20-502 | 26/11/20 | 11/12/20 | Pass |

**Testing method:**

* Dissolved 2gm of Temozolomide in 20mL of SCDM (0.5%Soyalecithin and 2% Tween 80) – considered it as **Solution “A”.**
* Note: For salmonella, dissolved 1gm of Temozolomide in 10mL of SCDM (0.5%Soyalecithin and 2% Tween 80) – considered it as **Solution “A1”.**

**TAMC:**

* Transferred 1mL of solution-A aseptically into two Petri plates and added 20-30 mL of sterile Soybean Casein Digest Agar maintained at not more than 45°C and gently swirled the plates for equal distribution of the sample.

**TYMC:**

* Transferred 1mL of Solution-A aseptically into two Petri plates and added 20-30 mL of sterile Sabouraud Dextrose Agar maintained at not more than 45°C and gently swirled the plates for equal distribution of the sample.

**Recovery:**

* **Product Positive Control:** Transferred 1mL of Solution A into 5 different test tubes and added known amount of culture suspension which is having NMT 100cfu.Considered it as **Solution B**.
* After vortexing transferred 0.1mL of solution-B into two Petri plates and added 20-30 mL of sterile Soybean Casein Digest Agar maintained at not more than 45°C and gently swirled the plates for equal distribution of the sample, in case of Bacteria and SDA in case of fungal cultures.
* **Positive Control:** Taken 1mL of SCDM into 5 separate test tubes and added the culture suspension which is having NMT 100cfu.Considered it as **Solution C.**
* After vortexing transferred 0.1mL of known culture into two petri plates and added 20-30 mL of sterile Soybean Casein Digest Agar maintained at not more than 45°C and gently swirled the plates for equal distribution of the sample, in case of Bacteria and SDA in case of fungal cultures.
* **Pathogens:** Transferred 1mL of Solution A in 10 mL of SCDM (0.5%Soyalecithin and 2% Tween 80) and Incubated at 30-35°C for 18-24 hrs. Considered it as Product Control.
* Taken 10mL of SCDM, added 1mL of solution A, then added NMT 100cfu and incubated at 30-35°C for 18-24 hrs. Considered as Positive Product Control.
* Taken 10mL of SCDM and added NMT 100cfu of culture suspension and incubated at 30-35°C for 18-24 hrs. Considered as Positive Control.
* After incubation period taken 0.1mL of PPC, PC and Product control and added to 10mL of macconkey broth.
* Then incubated at 42-44°C for 24 to 48hrs.
* After 24 hrs of incubation, inoculated loop full of macconkey broth on to Macconkey agar and incubated at 30-35°C for 18-72 hrs.

**Note:** The details of culture suspension used for the study mentioned in the below table.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name of the Organism** | **Dilution taken**  **for study** | **No of cfu in**  **Per 1mL**  **solution** | **No of cfu in**  **per 0.1mL**  **solution** | **Final inoculum concentration in Solution B** |
| *Staphylococcus aureus,* | 10-4 | 70000 | 7000 | For 1mL of SCDM 10uL of inoculum added |
| *Pseudomonas aeruginosa* | 10-2 | 72000 | 7200 | For 1mL of SCDM 10uL of inoculum added |
| *Bacillus subtilis* | 10-3 | 60000 | 6000 | For 1mL of SCDM 10uL of inoculum added |
| *Candida albicans* | 10-1 | 44000 | 4400 | For 1mL of SCDM 10uL of inoculum added |
| *A.brasiliensis* | 10-3 | 38000 | 3800 | For 1mL of SCDM 10uL of inoculum added |

pH of the sample verified. Find below table for pH.

|  |  |
| --- | --- |
| **B.No** | **pH** |
| ONR200101 | 7.18 |
| ONR200102 | 7.16 |

**TEST RESULTS:**

**Product control (Total Aerobic Microbial Count) (TAMC):**

(Petri plate 1 + Petri plate 2)

% of recovery : CFU per gm = ---------------------------------------------- x Dilution factor

2

**Total aerobic microbial count (TAMC)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Batch No.** | **Dilution plated** | **Plate 1** | **Plate 2** | **Average** | **Total cfu per gm** |
| ONR200101 | 1:100 | Nil | Nil | NA | < 10 cfu/gm. |
| ONR200102 | 1:100 | Nil | Nil | NA | < 10 cfu/gm |

**Total Yeast and Moulds Count (TYMC):**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Batch No.** | **Dilution plated** | **Plate 1** | **Plate 2** | **Average** | **Total cfu per gm** |
| ONR200101 | 1:100 | Nil | Nil | NA | < 10 cfu/gm |
| ONR200102 | 1:100 | Nil | Nil | NA | < 10 cfu/gm |

**Positive control and Product positive controls**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Batch No.** | **Test Organism in cfu** | | | | | | | | | | |
| ***Staph.aureus*** | | ***Pseudomonas aeruginosa*** | | | ***Bacillus subtilis*** | | ***Candia albicans*** | | ***A.brasiliensis*** | |
| **Media** | | | | | | | | | | |
| **SCDA** | | **SCDA** | | **SCDA** | | | **SDA** | | **SDA** | |
| **PPC** | **PC** | **PPC** | **PC** | **PPC** | | **PC** | **PPC** | **PC** | **PPC** | **PC** |
| ONR200101 | 48 | 67 | 47 | 65 | 44 | | 55 | 44 | 46 | 34 | 37 |
| ONR200102 | 55 | 50 | 47 | | 42 | 36 |

**RECOVERY**

**Batch No.** ONR200101

|  |  |
| --- | --- |
| **Name of the organism** | **% of Recovery** |
| *Staph.aureus* | 48/67X100 : 72% |
| *Pseudomonas aeruginosa* | 47/65X100 : 72% |
| *Bacillus subtilis* | 44/55X100 : 80% |
| *Candida albicans* | 44/46X100 : 96% |
| *A.brasiliensis* | 34/37X100 : 92% |

**Batch No.** ONR200102

|  |  |
| --- | --- |
| **Name of the organism** | **% of Recovery** |
| *Staph.aureus* | 55/67X100 : 82% |
| *Pseudomonas aeruginosa* | 50/65X100 : 77% |
| *Bacillus subtilis* | 47/55X100 : 85% |
| *Candida albicans* | 42/46X100 : 91% |
| *A.brasiliensis* | 36/37X100 : 97% |

**Note:** Final population: As determined from Product Positive control

Initial population: As determined from Positive control

**PATHOGENS TESTING:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Batch No.** | ONR200101, ONR200102 | | | |
| **Name of the specified organism** | **Product control** | **Product Positive control** | **Positive control** | **Negative control** |
| *Escherichia coli* | Absent | Present | Present | No growth |
| *Pseudomonas aeruginosa* | Absent | Present | Present | No growth |
| *Staphylococcus aureus* | Absent | Present | Present | No growth |
| *Salmonella* | Absent | Present | Present | No growth |
| *Candida albicans* | Absent | Present | Present | No growth |
| *Bile tolerant gram negative bacteria* | Absent | Present | Present | No growth |
| *Clostridia* | Absent | Present | Present | No growth |

1. **Data review:**

All the test results and attachments were reviewed and found satisfactory.

1. **Exception/deviation/Discrepancy:**

No deviation/discrepancy was observed during the study of verification of Protocol for

Microbial limit test (MLT) Method validation to determine the total microbial counts

and Pathogens in Temozolomide.

1. **Conclusion:**

After reviewing the results, it can be concluded that the validated test

Parameters TAMC,TYMC and pathogens were within the acceptance criteria.

In Product Positivecontrol (PPC) recovery is more than 70 % when compared

with Positive control. Based on the results it can be concluded that the

Temozolomide is not interfering with the microbial growth.

1. **Recommendations:**

Based upon the conclusion described in interim summary report, the method

is suitable and recommended for the regular analysis of Temozolomide.

The final summary report shall be prepared after the analysis of the 3rd batch.

1. **Validated parameters:**

Not Applicable.

**7.0 Certification**

|  |  |  |  |
| --- | --- | --- | --- |
| **Department** | **Name** | **Designation** | **Signature /Date** |
| **Prepared by** | | | |
| Quality Control (Microbiology) |  |  |  |
| **Reviewed by** | | | |
| QA-Validation |  |  |  |
| Quality Control (Microbiology) |  |  |  |
| **Approved by** | | | |
| Quality Assurance |  |  |  |