**Title:** *Microbial Limit Test of Finished Products* *Report Form*

**Customer Code: M0230030627002/01**
**Form Code: *F02-P96***

## *Identification*

| | | | |
|---|---|---|---|
| **Applicant** | Rasa salamat Arvand | | |
| **Address** | National Institute of Genetic Engineering and Biotechnology , Pajoohesh Blvd.,Iran-Tehran | | |
| **Product Name** | Destilled water | | |
| **Batch Number** | 14030625 | | |
| **Date of Receipt** | 1403.06.27 | **Test Duration** | 6 Days |
| **Date of Test** | 1403.06.27 | **Number of Received item** | 50 ml |
| **Date of Report** | 1403.07.02 | **Number of Tested item** | 20 ml |

## *Environmental conditions*

| | | | |
|---|---|---|---|
| **Temperature** | 23±3℃ | **Humidity** | NMT 50 %RH |

## *Procedure*

- The bioburden test was performed according to the United States Pharmacopeia 2024 (USP – NF 2024) <61> and <62> MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS.
- For microbial bioburden, the Membrane Filtration method or the Plate-Count Methods was used. The choice of a method was based on factors such as the nature of the product and the required limit of microorganisms. For determination of total aerobic microbial count (TAMC), the Soybean-Casein Digest Agar was used and the plates incubated at 30 to 35° C for less than 3 days. For total combined yeasts and molds count (TYMC), the Sabouraud Dextrose Agar were used, and the plates were incubated at 20 to 25°C for less than 5 days.
- For detection of specified microorganisms, the adequate amount of the examined product was used to inoculate a suitable amount of Soybean-Casein Digest Broth (TSB) or Sabouraud Dextrose Broth (SDB) as described in USP 2024, and incubated at 30 to 35°C for 18 to 24h and 3 to 5 days, respectively. For detection of each specified microorganism presence, apporporiate amount of incubated TSB/ SDB transferred to the appropriate culture medium as described in USP 2024. The possible presence of each specified microorganism was confirmed by identification tests. The product complies with the test if colonies of the types described are not present of if the confirmatory identification tests are negative.
- If the sample contained antimicrobial properties, the neutrilizing agents were added to the diluent or the media before sterilization.
- The Growth promotion and inhibitory properties of the media was performed according to USP 2024, and to verify testing conditions, a negative control was performed.
- Acceptance criteria for nonsterile pharmaceutical products based upon the total aerobic microbial count (TAMC) and the total combined yeasts and molds count (TYMC) are given in Tables 1 and 2. Acceptance criteria are based on individual results or on the average of replicate counts when replicate counts are performed.



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**Table 1. Acceptance Criteria for Microbiological Quality of Nonsterile Dosage Forms**

| Route of administration | TAMC (CFU/g or CFU/mL) | TYMC (CFU/g or CFU/mL) | Specified micro-organism(s) |
|---|---|---|---|
| **Non-aqueous preparations for oral use** | $10^3$ | $10^2$ | Absence of *Escherichia coli* (1 g or 1 ml) |
| **Aqueous preparations for oral use** | $10^2$ | $10^1$ | Absence of *Escherichia coli* (1 g or 1 ml) |
| **Rectal use** | $10^3$ | $10^2$ | - |
| **Oromucosal use** | $10^2$ | $10^1$ | Absence of *Staphylococcus aureus* (1 g or 1 ml) |
| | | | Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) |
| **Gingival use** | $10^2$ | $10^1$ | Absence of *Staphylococcus aureus* (1 g or 1 ml) |
| | | | Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) |
| **Cutaneous use** | $10^2$ | $10^1$ | Absence of *Staphylococcus aureus* (1 g or 1 ml) |
| | | | Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) |
| **Nasal use** | $10^2$ | $10^1$ | Absence of *Staphylococcus aureus* (1 g or 1 ml) |
| | | | Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) |
| **Auricular use** | $10^2$ | $10^1$ | Absence of *Staphylococcus aureus* (1 g or 1 ml) |
| | | | Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) |
| **Vaginal use** | $10^2$ | $10^1$ | Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) |
| | | | Absence of *Staphylococcus aureus* (1 g or 1 ml) |
| | | | Absence of *Candida albicans* (1 g or 1 ml) |
| **Transdermal patches (limits for one patch including adhesive layer and backing)** | $10^2$ | $10^1$ | Absence of *Staphylococcus aureus* (1 patch) |
| | | | Absence of *Pseudomonas aeruginosa* (1 patch) |
| **Inhalation use (special requirements apply to liquid preparations for nebulization)** | $10^2$ | $10^1$ | Absence of *Staphylococcus aureus* (1 g or 1 ml) |
| | | | Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) |
| | | | Absence of bile-tolerant gram-negative bacteria (1 g or 1 ml) |

**Table 2. Acceptance Criteria for Microbiological Quality of Nonsterile Substances for Pharmaceutical Use**

| | TAMC (cfu/g or cfu/mL) | TYMC (cfu/g or cfu/mL) |
|---|---|---|
| Substances for pharmaceutical use | $10^3$ | $10^2$ |

## ***Test Results***

| ***Description*** | ***Limits*** | ***Results*** |
|---|---|---|
| **Average TAMC (CFU/ml)** | $10^2$ | **<1** |
| **Average TYMC (CFU/ml)** | $10^1$ | **<1** |
| ***Escherichia coli*** | Absence | Absence |
| ***Staphylococcus aureus*** | - | - |
| ***Pseudomonas aeruginosa*** | - | - |
| ***Salmonella sp.*** | - | - |
| ***Candida albicans*** | - | - |
| ***Clostridia sp.*** | - | - |
| **Bile-Tolerant Gram-Negative Bacteria** | - | - |



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## Conclusion

The product to be examined complies with the test for USP 2024 YES ☒ NO ☐

## References

United States Pharmacopeia 2024 (USP-NF 2024) <61> MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: MICROBIAL ENUMERATION TESTS.
United States Pharmacopeia 2024 (USP -NF 2024) <62> MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: TESTS FOR SPECIFIED MICROORGANISMS.

**Description** For information

| **EXAMINER:** *EXAMINER A3* | **Lab. Manager:** *B. Moradi* | **CEO:** *M.Borjian* | **Nikopharmed laboratory sign** |
|---|---|---|---|
| Sign: | Sign: | Sign: | |
| A3 | | | |

- *The remaining samples after the test are kept in the laboratory for 3 months.*
- *Test results are only related to the tested products.*
- *Sampling has been done by the customer.*
- *Test results should not be replicated without the laboratory permission.*
- *If the tests were performed by the contractor, the name of the contractor is given in the description section.*



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