AQUINO™ Rehydration Fluid for GPT LQ254IX

Rehydration fluid for GPT is recommended as a diluting fluid for performing Growth Promotion Test with longer stability.

Schematic representation:

Note: Target dilution is a dilution which contains 10-100 CFU/0.1mL

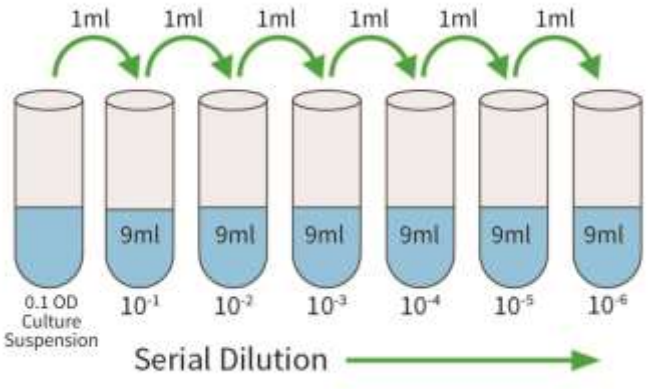
Detailed Procedure with an Example:

If one has to store *Escherichia coli* ATCC 8739 for GPT then follow steps mentioned below.

Recommended medium as per pharmacopoeia: Soyabean Casein Digest Agar, MacConkey Agar
Other Industry: As desired

Day 1:

1. Prepare 0.1 OD culture suspension from 18 hour old grown *Escherichia coli* ATCC 8739.
2. Prepare serial dilution till 10^{-6} to get 10-100 CFU/0.1 ml.



3. If one has to spread 10 plates of 100 µl each per day then aliquot 1.1 ml (10 plates X 100µl = 1000µl and 100µl extra) of $10^{-5}$and/or 10^{-6} in cryovials and store aliquot of these dilutions at -20°C or below upto -80°C.
4. Plate out 100µl of $10^{-5}$and/or 10^{-6} on Soyabean Casein Digest Agar (MH290) to determine the count.
5. Incubate plate at 30-35°C for 18-24 hours.

Day 2: Selection of dilution

1. Record the results and select the desired dilution giving 10 – 100 CFU/0.1ml.
2. If 10^{-6} is giving expected count then aliquots of this dilution can be used for further studies.
3. If 10^{-6} dilution is having 80 CFU/0.1 ml on 2nd day, then according to factor of 2 acceptance criteria range for *Escherichia coli* ATCC 8739 of this set will be 40-160 CFU/0.1 ml.
4. The lot must be compared with previously approved lot for growth promoting and indicative properties.

GPT till 20th day:

1. Henceforth 10^{-6} dilution of *Escherichia coli* ATCC 8739 can be used till 20th day for GPT.
2. For further use, as and when required remove one cryo vial of 10^{-6} dilution and allow it to thaw.
3. Plate out 100µl of 10^{-6} of *Escherichia coli* ATCC 8739 on test media.
4. Incubate plate at 30-35°C for 18-24 hours.
5. Observed count should be within 40-160 CFU/0.1 ml as per factor of 2 criteria.

Principle and interpretation:

The rehydration fluid is recommended for preparation of stable test strain suspension for use in growth promotion testing (GPT). The growth promotion test is used to determine the ability of solid or liquid media to support growth of an array of microorganisms. The standardized stable suspensions are used so that the suitability of this test to detect microorganism in presence of product can be established.

The GPT should be performed on each lot of purchased ready-prepared medium, each lot of dehydrated medium or medium prepared from components in the laboratory to determine the suitability of the media to support or inhibit growth as well as to determine the indicative properties

The basic requirements for the GPT are as follows:

1. The new batch of medium must be inoculated with a small number of microorganisms.

2. The laboratory should test the medium with the microorganisms required by the pharmacopeias.

3. The microorganisms must not be more than five passages removed from Reference Culture

In order for the new lot of medium to be approved for use, growth on the new lot of medium must be comparable to growth obtained on a lot of medium previously approved by the laboratory.

The rehydration fluid for GPT serves longer stability to test suspension for upto 20 days, which reduces overall time taken for the inoculum preparation.

Advantages:

1. Growth Promotion Tests (GPT), described in various pharmacopoeias (USP, EP, BP, JP, IP) is carried out to determine suitability of test medium for growth of specified microorganisms.
2. Dilution methods for GPT are tedious while commercial cfu preparations are expensive.
3. Culture preparations in this fluid can be stored upto 20 days.
4. Reduces overall time taken for inoculum preparation.
5. Overcomes tedious dilutions preparations.
6. Cost effective.

Type of Specimen:

Pure bacterial isolate

Specimen Collection and Handling:

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines. (1, 2, 3,4,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:

1. The fluid is applicable for growth promotion testing of bacterial cultures.
2. Certain fastidious organisms may not show stability as recommended.
3. It is recommended to store aliquot at -20°C or below.
4. Repeated freeze thaw of a vial is not recommended.

Performance and Evaluation:

Performance of the medium is expected when used as per the direction on the label within the expiry period, when stored at recommended temperature.

Cultural response:

The count obtained with rehydration fluid upto 20 days must be within a factor of 2 of the initial count obtained. (Serially diluted so as to obtain 10-100 CFU/0.1ml) on non-selective media.

Cultural Response:

Organism	Inoculum (CFU)	Recovery on 0 day	Recovery after 5 days	Recovery after 10 days	Recovery after 15 days	Recovery after 20 days
Escherichia coli ATCC 8739 (00012*)	10-100	10-100	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2
Escherichia coli ATCC 25922 (00013)*	10-100	10-100	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	10-100	10-100	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2
Staphylococcus aureus ATCC 25923 (00034)*	10-100	10-100	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2
Pseudomonas aeruginosa ATCC 9027 (00026*)	10-100	10-100	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2
Pseudomonas aeruginosa ATCC 27853 (00025)*	10-100	10-100	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2
Bacillus subtilis subsp. *Spizizennii* ATCC 6633 (00003*)	10-100	10-100	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2
Salmonella Typhimurium ATCC 14028 (00031*)	10-100	10-100	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2
Salmonella Abony NCTC 6017 (00029*)	10-100	10-100	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2	Must be within factor of 2

Acceptance Criteria:

Solid Media:

The average number of colonies on the plates from the new batch of the medium must be within a factor of 2 of the average number of colonies from the previously approved batch of the medium.
For example, if the average number of colonies on the previously approved media is 60.
Then the average number of colonies on the plates from the new batch of medium must be between 30 and 120.

Liquid Media:

Turbidity of the new batch of media should be comparable with the previously approved batch of media.

Storage and Shelf Life:

On receipt store between 2-8°C. Use before expiry date on the label.

Disposal:

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials must be decontaminated and disposed of in accordance with current laboratory techniques (6, 7).

References:

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3. European Pharmacopoeia, 2017, European Dept. for the quality of Medicines.
4. Japanese Pharmacopoeia, 2014.
5. Indian Pharmacopoeia, 2018, Govt. of India, the controller of Publication, Delhi, India.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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