Method Suitability Report
Membrane Filtration Sterility Test with QTMicro Apparatus

Wellness Complete - preserved with Benzyl Alcohol 2%

This report provides details on the specifics of a Membrane Filtration Sterility Test using the QTMicro apparatus. The method suitability study was performed as part of protocol MNI-2013-001, where this sample had the sample ID MNI-2013-001-050 and the title "Wellness Complete - preserved with Benzyl Alcohol 2%".

The qualified formulation[1] (normalized to 100 mL) is:

Choline Chloride FCC - 10.8157
Chromic Chloride 6H2O USP - 0.21793 g
Dexpanthenol USP - 0.318
Inositol Purified Powder - 8.0714
Leucine (L) FCC - 0.40357 g
Methionine (DL) FCC - 4.03571 g
Methylcobalamine Powder - 0.0807 g
Niacinamide USP Powder - 4.03571 g
Pyridoxal-5-Phosphate Monohydrate Powder - 0.0161 g
Riboflavin-5-Phosphate Sodium USP - 0.43101 g
Thiamine HCl USP - 4.03571 g
Lidocaine HCl USP - 0.05165 g
Benzyl Alcohol NF Liquid - 3.2286 mL
Sterile Water for Injection

This formulation was qualified for a USP <71> Sterility Test at a maximum volume[2] of 35 mL with the QT Micro filtration apparatus.

This method suitability report applies only to this specific formulation at a maximum volume per membrane as listed above using the QTMicro. The basic method suitability procedure and the qualified Sterility Test are described below.

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[1] This study is valid only for the CSP formulation submitted. Changing preservatives (or preservative concentration), active(s) concentration or suspending solution components renders this study invalid. Also note that this formulation description is not sufficient for compounding instructions and is not presented as such but only to list the ingredients and their concentrations.

[2] The volume of material used in a USP <71> Sterility Test is determined by a combination of the unit fill volume and the number of units made during a single run (a "batch") as described in Tables 2 and 3 of this chapter. A specific situation may require a larger volume of CSP for a sterility test than was qualified in this study. In this case, it may be necessary to use more than one QTMicro per recovery medium, or to use a different sterility test method consistent with USP <71>.

Method Suitability Procedure

The purpose of the Method Suitability Test is to demonstrate that the CSP does not inherently (by composition) inhibit detection of low levels of viable bacteria (Method Suitability) after appropriate rinsing in the QTMicro.

It is possible that antimicrobial residues of the CSP could adhere to the membrane in the QTMicro, inhibiting the growth of contaminating microorganisms. This test employs the maximum volume of CSP qualified, assuming that lesser volumes should leave less antimicrobial residue on the filter to be neutralized.

The basic outline of the Method Suitability Study is as follows:

1. Filter the volume of CSP determined by USP <71> through each of 7 QTMicro Devices. The volume of CSP filtered is determined by the category of CSP (antibiotic, ophthalmic or other) and by details provided in USP <71>:
    a) Table 2 – Minimum quantity per each container based on unit fill volume
    b) Table 3 – Minimum number of containers based on batch size
2. Leave the QT Micro filter moist
3. Filter a 20 mL aliquot of Diluting Fluid D through each QTMicro – leave the filter moist Diluting Fluid D (as per USP <71>)
4. Filter a second 20 mL aliquot of Diluting Fluid D through each QTMicro – leave the filter moist
5. The third 20 mL aliquot of Diluting Fluid D is then inoculated with <100 CFU of each challenge organism individually (see Table 1). Two QTMicro will not be inoculated and serve as the "Microbial Purity Controls"
6. The inoculated rinse is filtered through each QTMicro – leave the filter moist
7. Depending on the challenge organism, fill the QTMicro with either Soybean Casein Digest Broth (a.k.a. SCDB, Trypticase Soy Broth, or TSB) or Fluid Thioglycollate Medium (a.k.a. FTM) (see Table 1). One of the "Microbial Purity Control" QTMicro samples will be filled with SCDB and the other with FTM.
8. Test tubes containing at least 10 mL the different growth media (SCDM or FTM) (a.k.a. "Media Blanks") will be inoculated with <100 CFU of the challenge organisms and serve as growth promotion controls (Positive Controls).
9. Two additional QTMicro devices will be filled with either SCDM or FTM and serve as "Media Sterility Controls" (Negative Controls)
10. The test samples and controls will be incubated as described in Table 1
11. A passing Method Suitability Test will meet all of the acceptance criteria in Table 2.

Table 1. Challenge Organisms and Growth Conditions

Name	Gram/type	ATCC Number[3]	Media	Incubation Temperature	Incubation Time
Staphylococcus aureus (*S. aureus*)	Gram Positive Coccus (round)	6538	FTM	30-35°C	3 days
Pseudomonas aeruginosa (*P. aeru*)	Gram Negative Bacillus (rod)	9027	FTM	30-35°C	3 days
Bacillus subtilis (*B. subtilis*)	Gram Positive Bacillus, Spore former	6633	SCD	20-25°C	5 days
Clostridium sporogenes (*C. sporo*)	Gram Positive Bacillus, spore former, anaerobic	19404 or 11437	FTM	30-35°C	3 days
Candida albicans (*C. albicans*)	Yeast	10231	SCD	20-25°C	5 days
Aspergillus brasiliensis (*A. brasiliensis*)	Mold	16404	SCD	20-25°C	5 days

[3] American Type Culture Collection identification number

Table 2. Method Suitability Acceptance Criteria

Media	Organism	Result
Method Suitability Challenge Samples Fluid Thioglycollate Medium (FTM)	*C. sporo, P. aeru, S. aureus*	Growth expected in this **Test Sample** in not more than 3 days
CSP Microbial Purity Control Fluid Thioglycollate Medium (FTM)	None	Growth not expected in this **Microbial Purity Control** (product negative control) after 14 days
Positive Control Fluid Thioglycollate Medium (FTM)	*C. sporo, P. aeru, S. aureus*	Growth expected in this **Positive Control** in not more than 3 days
Negative Control Fluid Thioglycollate Medium (FTM)	None	Growth not expected in this **Negative Control** after a minimum of 5 days
Method Suitability Challenge Samples Soybean Casein Digest Medium (SCD)	*B. subtilis, A. brasiliensis, C. albicans*	Growth expected in this **Test Sample** in not more than 3 days (bacteria) or 5 days (yeast and mold)
CSP Microbial Purity Control Soybean Casein Digest Medium (SCD)	None	Growth not expected in this **Microbial Purity Control** in 5 days
Positive Control Soybean Casein Digest Medium (SCD)	*B. subtilis, A. brasiliensis, C. albicans*	Growth expected in this **Positive Control** in 5 days
Negative Control Soybean Casein Digest Medium (SCD)	None	Growth not expected in this **Negative Control** after a minimum of 5 days

Sterility Test Method Qualified

The USP <71> Sterility Test is designed to check a specific number and volume of samples for sterility in both an aerobic recovery medium and an anaerobic recovery medium. It is necessary that the correct number of samples and volumes (according to Tables 2 and 3 in USP <71> are used in this test. The Method Suitability test establishes the ability of the recovery method to neutralize antimicrobial residues from this amount of CSP. Therefore, the compliant Sterility Test Method Suitability Test must have used at least the volume of CSP to be tested. The exact conditions of the test (volume of diluting rinses, number of diluting rinses and formulation of diluent) must be established in the Method Suitability Study.

Conditions of a compliant USP Sterility test for the specific formulation and total volume of CSP described above are escribed below:

1. Determine the amount of product needed for a compliant Sterility Test using Tables 2 and 3 of USP <71>
   a) If this volume of material to be tested is less than or equal to the volume qualified, proceed
   b) If this volume of material to be tested exceeds the volume qualified:
      i. Perform an additional method suitability test to evaluate the larger volume
      ii. Adjust the fill volume and number of units for this fill to bring the volume to be testing in line with the qualified parameters
      iii Use different equipment. *NOTE: All compliant Sterility Tests must have a relevant method suitability test.*
2. The formulation cited above is passed through a QTMicro apparatus for TSB, and a second QTMicro for FTM recovery media
3. Three 20 mL volumes of Diluting Fluid D (see USP <71>) are passed through each QTMicro apparatus to rinse the filter.
   Note: The diluting fluid volumes should be completely filtered (without drying the membrane) before the next volume is filtered.
4. One QTMicro is filled with TSB, the other with FTM.
   a) The TSB-filled container is incubated at 22.5°C (± 2.5°C) for 14 days
   b) The FTM-filled container is incubated at 32.5°C (± 2.5°C) for 14 days
5. The incubated, media-filled QTMicro are checked regularly through the 14 days and at the end of the incubation period (if necessary) for growth. The Sterility Test fails if either microbial media shows evidence of growth.

Scott Sutton, Ph.D. — 12/8/14 Date