

Protocol for Propofol Microbial Growth Retardation Studies

TITLE:

Protocol to Study the Microbial Growth Retardation in Propofol Intravenous Fat Emulsion.

OBJECTIVE:

The purpose of this study is to determine the microbial growth retardation in propofol intravenous fat emulsion formulations containing preservative additives, when challenged with *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231, and *Pseudomonas aeruginosa* ATCC 9027.

EXPERIMENTAL:*Test Samples*

Vial Size	Test Product A	Test Product B
	Propofol Injectable Emulsion	Diprivan® (Propofol Injectable Emulsion) (Manufacturer: Astra Zeneca)
20 mL Vial	1-3 Lots	1-3 Lots
50 mL Vial	1-3 Lots	1-3 Lots
100 mL Vial	1-3 Lots	1-3 Lots

Note:

- ❖ Each lot will be tested in triplicate.
- ❖ Product will be labeled as follows: A1, A2 or A3 if more than one manufacturer product is tested during the study. Otherwise identify as product A if a single manufacturer product sample is tested during a study. Ensure the identity of product A1, A2, A3, etc. is properly documented in the laboratory records and in the final report, i.e. Lot # and manufacturer.
- ❖ Test Product B will be tested as required, either stand alone or in comparison to Test Product A.
- ❖ Number of lots tested will be based on the statistical significance required for the study conducted, with a minimum of 1 up to a maximum of 3 lots per manufacturer/ presentation.

Microorganisms

Pseudomonas aeruginosa ATCC 9027
Escherichia coli ATCC 8739
Staphylococcus aureus ATCC 6538
Candida albicans ATCC 10231

Procedure:

Microbial Growth Retardation Test Procedure (Attachment 1).

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(ATTACHMENT 1)

Microbial Growth Retardation Test Procedure

I. Organism Inocula

Organism suspensions will be prepared from 18 to 24-hour slant cultures for bacteria and 48-hour slants for *C. albicans*. The growth will be harvested from the slants with physiological saline (0.9%). The saline suspensions will be adjusted using the % Transmittance (T) readings on a spectrophotometer set at 550 nm wavelength. *P. aeruginosa* and *E. coli* will be adjusted to approximately 68-72%T, *S. aureus* to approximately 60 %T, and *C. albicans* to 1-2 %T. The %T readings may be altered if organism counts are expected to change based on previous data, subject changes should be explained and justified in the corresponding report. A 10^{-4} dilution of these organism suspensions will then be prepared in saline so that the final concentration of the inoculated samples is approximately 100CFU/mL. The inoculum concentration required will be contained in a volume of the stock microorganism suspension that is between 0.5 % and 1.0 % of the volume of the product. Each organism concentration will be confirmed by using duplicate pour plates prepared at the time of inoculation. The inocula verification plates will be incubated as outlined below. Purity of the cultures used will be evaluated visually from the inocula verification plates. No additional confirmation or organism identification will be performed unless evidence suggests potential contamination of the product aliquots.

II. Sample Preparation, Storage and Analysis

The Propofol Intravenous Fat Emulsion samples for testing will be prepared and tested as outlined below.

1. The finished product sample containers will be aseptically opened.
2. Aliquots of each test sample will be transferred to a sterile container for each microorganism to be tested. Approximately 20 mL of product will be required for each container for inoculation.
3. One container of each product will be inoculated with the specified organisms (approximately 2.0×10^3 CFU/0.1 mL) to 20 mL of product to obtain approximately 100 CFU/mL of finished product.
4. The samples will be plated at 0, 24 and 48-hour time points. The test products should be mixed well immediately following inoculation, and before serial dilution at 0, 24 and 48 hours. At each time point, duplicate 1 mL aliquots of all sample containers and its diluted samples (see sample dilution schemes below) will be plated using a standard pour plate method. The bacteria will be plated using Tryptic Soy Agar (TSA). Yeast platings will be performed using Sabouraud Dextrose Agar (SDA). All transfers, dilutions, inoculations, and platings will be performed in a biological safety cabinet to reduce the risk of contamination of the product during testing.
5. The bacterial isolation plates will be incubated inverted at 30° C to 35°C for 3 days. Agar plates for isolation of yeast will be incubated inverted at 20°C to 25°C for 5 to 7 days.
6. After the initial time point (time zero), the inoculated sample containers for each organism will be stored at 20°C to 25°C.
7. Following incubation, examine the plates for growth, count the number of colonies, and express the average for the two plates in terms of the number of microorganisms per mL of test sample.

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III. Test Controls

Agar sterility controls will be prepared during plating to evaluate the media used. The agar sterility controls will be incubated with sample plates at the appropriate conditions.

For control purposes, 20 mL of physiological saline will be inoculated with each of the test organisms, serially diluted, plated in duplicate, incubated and enumerated at 0, 24 and 48 hours in a similar manner to that of the samples. Physiological saline without any inoculation will be plated in duplicate without further dilution, incubated and enumerated at 0, 24 and 48 hours to serve as the negative control for aseptic handling.

IV. Sample Dilution Schemes

Time Point	Test Product A	Test Product B	Control
0	Undiluted	Undiluted	Undiluted
24 & 48 Hrs.	1. Undiluted 2. 1:10,000 3. 1:100,000	1. Undiluted 2. 1:10,000 3. 1:100,000	1. Undiluted 2. 1:100

Note: Dilution will be prepared in TAT broth containing 4.0% (V/V) polysorbate 20 (40 mL/L of broth) as the neutralizer

V. Evaluation of Results

Following incubation of the plates, counts will be obtained for each test and control samples. The estimated number of organisms present per mL of the product will be calculated based on the amount plated and any dilutions performed. Where no growth is observed from the plates, the result will be calculated to reflect the minimum detectable organism level.