Senior Project -

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Microbial Challenge Testing

of

Soft Contact Lens Solutions

by

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Introduction

This paper presents a single trial run in which soft contact lens saline solutions were innoculated with low level microbial loads. The solutions used were Lens Plus, Unisol 4, Sensitive Eyes, Boil n Soak, and Opti-Soft. Each solution was fresh, unopened and used straight from its container. The microorganisms used were S.epidermidis, P. aeruginosa, S. marcescens, C. albicans and S. aureus. Each was obtained from serial dilution with isotonic saline of pure broth culture. The bacteriostatic/cidal properties of each was determined for each microorganism.

Background

I. Microorganisms

This selection of challenge organisms was based on the FDA's "Testing Guidelines for Class III Soft (Hydrophilic) Contact Lens Solutions."[5] These microorganisms are given in Table I. This selection was modified due to availability and culture capability at Ferris State University. These microorganisms are given in Table II. Thus, although S. epidermidis, P. aeruginosa, S. marcessans, and C. albicans were selected, availability dictated diferent ATCC numbers. S. aureus was added to the list. Since A. fumigatus was not available at the time of the experiment (the stock culture died), it was deleted from the list. Since Ferris State University did not have the capability of live tissue culture, herpes simplex was deleted.

S. epidermidis, a gram positive cocci, is an opportunistic pathogen. Its relative frequency in the conjunctival flora is 75-90%.[2] S.aureus, a gram positive cocci, is the most common cause of both acute and chronic conjunctivitis as well as bacterial keratitis.[2] Its relative frequency in the conjunctival flora is 25-40%.[2] S. aureus tends to produce oval, yellow-white, densely opaque stromal suppuration surrounded by relatively clear cornea.[3] P. aeruginosa and S.marcessens, gram negative rods, are also opportunistic pathogens. The relative frequency of gram negative rods in conjunctival flora is 0-5%.[2] P. aeruginosa rarely causes conjunctivitis except in the immunocompromised host, after prolonged hospitalization or in the presence of a cosmetic shell. P. aeruginosa has been recovered from unopened "distilled" water and has been reported to replicate in eye drops, weak antiseptics, irrigating solutions and eye cosmetics.[4] P. aeruginosa usually causes irregularly sharp ulceration, thick mucopurulent exudate, diffuse liquefactive necrosis, and semi-opaque "ground glass" appearance of adjacent stroma. The infection may progress rapidly and result in corneal perforation within 48 hrs.[3] S. marcessens is more rarely a cause of both acute or chronic bacterial conjunctivitis. Yet, S.marcessens has increasingly been reported as a contaminant of contact lens care products.[4] S. marcessens causes a shallow ulceration, gray-white pleomorphic suppuration and diffuse stromal opalescence. Endotoxins present in gram negative bacteria may induce ring-shaped corneal infiltrates.[3] Although a rare infection, C. albicans is the most common cause of yeast keratitis. It most frequently affects a compromised host. A typical lesion has a yellow-white color and is associated with dense suppuration similar to bacterial keratitis.[3]

II. Solutions

The solutions tested are given in Tables III & IV. Table III presents a description of the solution which includes the manufacturer, buffer and preservative. Table IV presents solution data which includes lot number, expiration date. pH and background count.

Sorbic Acid, the preservative in Sensitive Eyes, is the "weakest" agent considered. Sorbic acid's primary advantage is the very low incidence of allergic or toxic reactions associated with its use. Thimerosal, the preservative in Boil n Soak, is an organic mercury compound. Although very effective, 20-50% of patients can be expected to develop sensitivity to the compound. Polyquad, the preservative in Opti-Soft, is a surfactant. It has been approved for chemical disinfection for contact lens with a water content of 45% or less. EDTA is a chelating agent used in each of the preserved salines. Although not an effective antimicrobial agent when used alone, it acts a synergist by binding metal ions required for cell metabolism.

Apparatus

The aparatus used for this paper is shown in Figure I. It consists of a filter assembly, a filter manifold, a side-arm vacuum flask used as a filtrate trap, and a vacuum pump. The filter assembly consists of a pyrex bowl clamped to a porous porcelain septum. This assembly is sealed on both ends with aluminum foil during steam sterilization and subsequent cooling. A Gelman GN-6 Sterilized Membrane is then inserted between the bowl and septum. This membrane has a 0.45 micron absolute retention and prevents the passage of viable microorganisms. A grid is preprinted on the membrane to facilitate colony counting. Solution is filtered through the membrane. The membrane is removed from the assembly and inserted onto culture media in a petri dish. Brain Heart Infusion (BHI) Agar is used to culture bacteria; Sabouraud Dextrose (SD) Agar is used to culture yeast. Incubation is at 35 C.

Procedure

I. Determination of Background Count

The background microbial count for each solution was determined. Although each solution is marketed as sterile and was used prior to its expiration date, sterility was verified. Fifty (50) ml of each solution was filtered onto the Gelman membrane, the membrane incubated on BHI agar for 24 hrs, and the cultures counted.

II. Determination of Dilution Level

In order to obtain a countable innoculum for each solution sample, dilution levels were established for each organism. Serial dilutions of broth culture for each organism were made with isotonic saline. These were filtered onto the Gelman membranes, the membranes incubated on appropriate agar (BHI agar for bacteria; SD agar for yeast) for 24 hrs., and the cultures counted. The dilution for S. epidermidis, S. marcescens, C.albicans and S.aureus was 10^{-5}. The dilution for P. aeruginosa was 10^{-6}.

III. Determination of Innoculum Count

Isotonic saline was prepared by dissolving reagent grade sodium chloride in distilled water. This solution was autoclaved to achieve sterility. Five 50 ml samples were measured into sterilized flasks. Each microorganism at the pre-determined dilution was charged to one of the 50ml samples of sterile isotonic saline. Each innoculum was filtered immediately, incubated on agar for 24 hrs., and the colonies counted. The number of colonies represented the innoculum count and are given in Table V.

IV. Solution Innoculation and Incubation

Solution innoculums were prepared at the same time as innoculum counts were being determined. However, these solutions were incubated for 24 hrs prior to filtration, culture and counting. Five 50 ml samples of prepared sterile isotonic saline were innoculated, incubated, filtered, cultured and counted at 24 and 48 hrs. These provided the controls. Fifty 50 ml samples of each contact lens solution were similarly treated. Thus, each contact lens solution was tested in duplicate with each of five microorganisms.

Results

The background count of each solution is given in Table IV. The solutions are sterile. The single colonies recovered from Lens Plus and Unisol 4 are not significant and probably represent low level contamination during transfer of the filter paper to the culture medium.

The innoculum count for each microorganism (Table V) demonstrates that viable organism was charged to each contact lens sample and control.
Each of the 5 controls and 50 contact lens solutions provided no countable colonies after 24 and 48 hr agar culture. Thus, all the microorganisms died during incubation in solution.

Discussion

Based on the preservatives used, Lens Plus, Unisol 4 and the controls were expected to show an increased microbial count; Sensitive Eyes was expected to maintain a static count; and both Boil n Soak and Opti-Soft was expected to show a reduced count. These results were expected within an individual variation for each microorganism. That no viable organisms were recovered was completely unexpected. One possible explanation is that none of the solutions contain a nutrient content. Since none of the microorganisms is a spore former, lacking a nutrient source, none was able to survive.

The FDA protocol for disinfection testing of contact lens specifies an "organic soil" load consisting of a killed yeast, Saccharomyces cerevisiae, suspended in complement inactivated bovine or horse serum.[5] This, howewver, is "included in the test to mimic deposits on lenses from eye secretions." While this organic soil load is realistic for contact lens testing which are contaminated with protein and lipid deposits, it is not realistic for solutions. The attempt here was to simulate a low level contamination, such as, touching the spout of container, or leaving the container uncapped. In both cases, the innoculum level is low, and there is a negligible introduction of organic soil.

Suggestions for Future Research

The "no nutrient" hypothesis can be tested by repeating this experiment with solutions artificially contaminated with an organic soil load. Such a load can be that specified by the FDA or, more simply, by sterile BHI or SD broth. Finally, this hypothesis needs to be tested for high level contamination, as would occur with undiluted broth culture. This would require an unsoiled and soiled testing series.

Fig. I

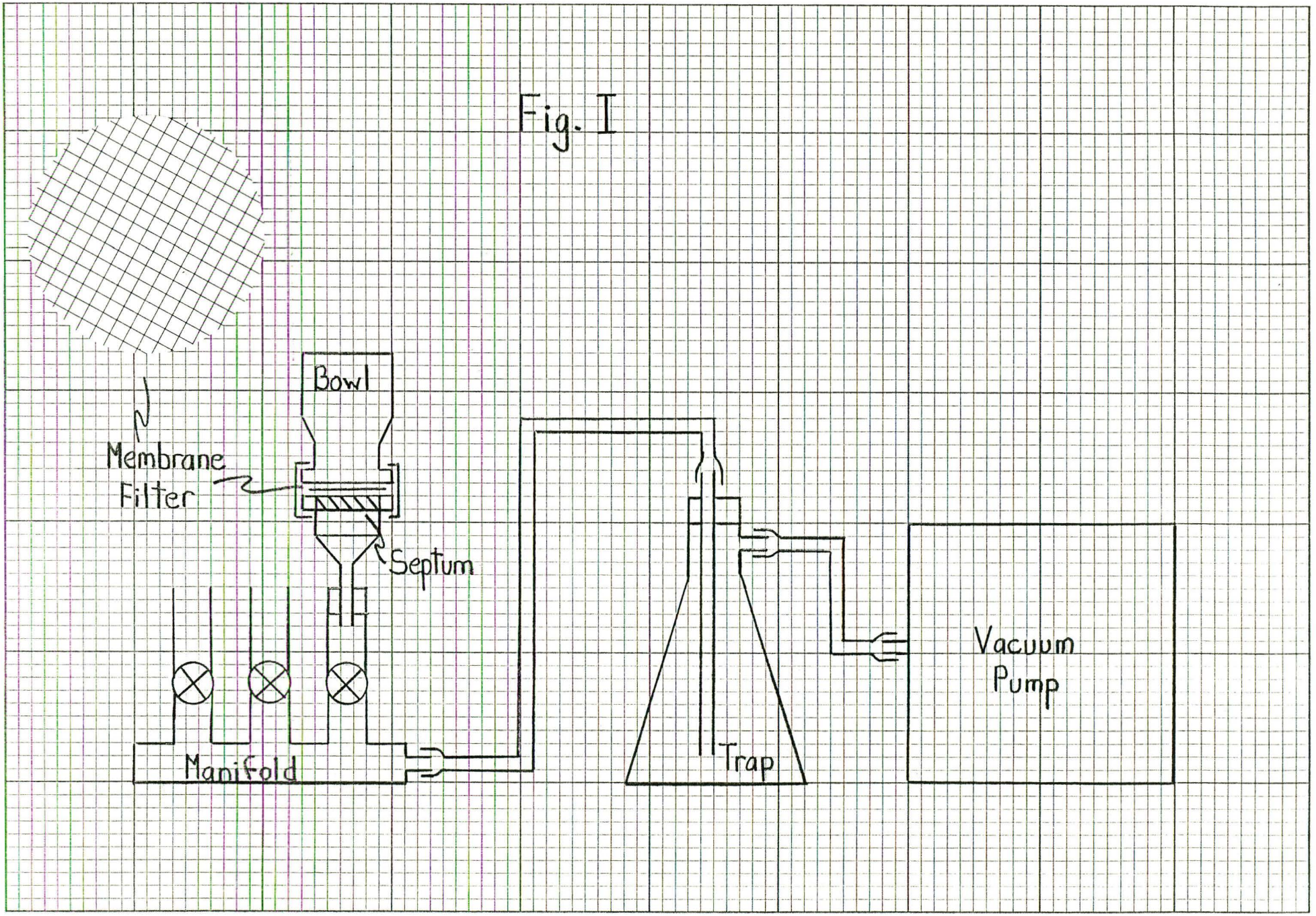

Table I

FDA Challenge Organisms for Chemical Disinfection (1)

Organisms		
Staphylococcus epidermidis	ATCC	17917
Pseudomonas aeruginosa	ATCC	15442
Serratia marcescens	ATCC	14041 (8 UK)
Candida albicans	ATCC	10231
Aspergillus Fumigatus	ATCC	10894
Herpes Simplex	ATCC	VR260

(1) Testing Guidelines for Class III Soft (Hydrophillic) Contact Lens Solutions, pg 48,1985 FDA Draft

Table II

Challenge Organisms Used (Based on Availability)

Organism		
Staphylococcus epidermidis	ATCC	12228
Pseudomonas aeruginosa	ATCC	9027
Serratia marcescens	Patient Isolate (Reed City Hospital)	
Candida albicans	ATCC	1114
Staphyloccus aureus	ATCC	25923

Table III

Description of Isotonic Saline Solutions

Name	Manufacturer	Buffer	Preservatives
Lens Plus*	Allergan	None	None
Unisol 4	CoperVision	Borate	None
Sensitive Eyes	Bausch&Lomb	Borate	EDTA; 0.1% Sorbic Acid
Boil n Soak	Alcon	Borate	0.1% EDTA; 0.001% Thimerosal
Opti-Soft	Alcon	Borate	0.1% EDTA; 0.001% Polyquad

*aerosol-N_2

Table IV

Solution Data

Name	Lot No.	Exp.Date	pH	Background Count
Lens Plus	J2592	5/89	6.17	1 colony
Unisol 4	CJ6031	10/89	7.43	1 colony
SensitiveEyes	GK664	10/88	7.35	None
Boil n Soak	HKBO	3/88	7.59	None
Opti-Soft	GFFG	12/87	7.51	None

Table V

Innoculum Count

Microorganism	Number
Staphylococcus epidermidis	1395
Pseudomonas aeruginosa	91
Serratia marcescens	428
Candida albicans	48
Staphylococcus aureus	670

References

(1) Bartlet, J.D. and Jaanus, S.D., Clinical Ocular Pharmacology, Butterworths, 1984, pp 330-331.

(2) Jones, D.B., Liesegang, T.J. and Robinson, N.M., "Laboratory Diagnosis of Ocular Infections", Cumitech 13, American Society for Microbiology, 1981, pg 11.

(3) Kanski, J.J., Clinical Ophthalmology, Butterworths, 1984, pp 5.7&5.13.

(4) Ward, M.A. and Miller, M.J., "The Microbiology of Contact Lens Wear", Contact Lens Forum, February 1988, pp 25-29.

(5) "Testing Guidelines for Class III Soft (Hydrophillic) Contact Lens Solutions", 1985 Draft, Appendix H, pp 45-48.

(6) Package Inserts for (1) Lens Plus, (2) Unisol 4, (3) Sensitive Eyes, (4) Boil n Soak, (5) Opti-Soft.