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Technical Note

Membrane Filter Sterility Test of Antihemophilic Factor Concentrate

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INTRODUCTION

Concentrates of human antihemophilic factor, USP (F-VIII), often are required as intravenous therapy by patients with severe hemophilia A (1,2). Sterility testing of the concentrates, prior to release, is done by direct inoculation of the reconstituted product into growth medium. The membrane filter sterility test is preferred over direct inoculation because there are fewer false-positive results with the membrane method (3). The membrane method is more sensitive to very low levels of microbial contamination (4) and is less expensive than direct inoculation (5). However, F-VIII contains substantial amounts of fibrinogen and fibronectin and is sufficiently viscous that it cannot be filtered through a 47-mm-diameter disk filter rated at a pore diameter of 0.45 μm, whether under negative pressure or positive pressure to 2.1 kg/cm^2. This work indicates that F-VIII can be membrane filter sterility tested if the product is reconstituted in dilute sterile trypsin and that microbial contaminants, if present, are not adversely affected by the trypsin.

MATERIALS AND METHODS

Sterile, freeze-dried porcine trypsin (Catalog No. 610-5095, GIBCO, Grand Island, N.Y.) was reconstituted to 98 mg% with sterile water and mixed. The GIBCO product (a crude preparation containing chymotrypsin, amylase, and other enzymes in addition to trypsin) is the same product that is used at 100 mg% for the dispersion of mammalian cells. Freeze-dried F-VIII (Hemofil®, Catalog No. 060737, Hyland Therapeutics Division, Travenol Labs, Los Angeles) was reconstituted to at least 22.5 IU/ml with water for injection or the 98 mg% trypsin, injected through sterile disposable 0.2-μm-rated pore diameter membrane filters (Catalog No. SLGV 025 LS, Millipore Corp., Bedford, Mass.). The pH of the reconstituted F-VIII was about 7.3–7.4 and was buffered only by constituent proteins. The pH optimum for trypsin activity is 7.8–8. The F-VIII, in distilled water or dilute trypsin, was incubated for 0.5–1 hr at 20–22°C and then the entire contents were transferred to a presterilized, closed canister, membrane filter sterility test system (Catalog No. XX26 147 91, Millipore; or Catalog No. SM 265 42, Sartorius Filters, Hayward, Calif.) for a conventional sterility test. Testing for microbial growth inhibition was done by adding, to some final containers reconstituted with water or dilute trypsin, about 100 colony-forming units of an organism/ml of F-VIII solution. One-milliliter samples were then taken, in triplicate, for standard plate counts (incubated under CO_2 for *Clostridium sporogenes*) at various times over 24 hr. The organisms were *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Cl. sporogenes* ATCC 11437, *Enterobacter aerogenes* ATCC 13048, *Pseudomonas cepacia* ATCC 35254, and *Staphylococcus aureus* ATCC 12598. All organisms were from the American Type Culture Collection, Rockville, Md.

RESULTS AND DISCUSSION

Invariably, we found that (a) 98 mg% trypsin for 0.5–1 hr at 20–22°C made the F-VIII readily filterable through sterility test membrane filters; (b) short-term (<3-hr) exposure to F-VIII, with or without trypsin, did not alter significantly the added colony-forming units of any of the organisms; (c) long-term (12-hr) exposure to F-VIII reduced colony-forming units (Tables I and II); but (d) colony-forming units were slightly higher in trypsin-treated than in trypsin-free F-VIII. In one test with *E. aerogenes* and another with *S. aureus*, regrowth of the organisms commenced between 12 and 24 hr only in trypsinized F-VIII.

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Table I. Colony-Forming Units per Milliliter in Various Media at the Indicated Times After the Addition of *B. subtilis* Spores; Temperature, 20–22°C

Time (hr)	Medium			
	Saline	SCD[a]	F-VIII[b]	F-VIII + trypsin
0	61[c]	57	52	62
1	68	73	55	54
3	73	72	50	53
5	60	100	NR	52
8	NR[d]	100	35	39
12	NR	1400	21	37
24	66	7×10^6	7	17

[a] Soybean–casein digest broth.
[b] Antihemophilic factor, USP.
[c] Average of three plates, rounded to the nearest integer.
[d] Not readable due to spreading of the organism.

Table II. Colony-Forming Units per Milliliter in Various Media at the Indicated Times After the Addition of *P. cepacia;* Temperature, 20–22°C

Time (hr)	Medium			
	Saline	SCD[a]	F-VIII[b]	F-VIII + trypsin
0	80[c]	86	127	108
1	75	100	125	98
3	77	111	120	102
5	82	120	82	97
8	89	123	59	74
12	76	460	46	55
24	78	TNTC[d]	30	33

[a] Soybean–casein digest broth.
[b] Antihemophilic factor, USP.
[c] Average of three plates, rounded to the nearest integer.
[d] Too numerous to count.

Sterile trypsin has been used for many years in the dispersion of mammalian cells for tissue culture (6,7). We speculated that mammalian cells are more labile than bacteria and yeasts and that the enzyme was unlikely to do harm. Membrane filter sterilized solutions of penicillinase are used routinely in the sterility testing of penicillin (8,9), and preparations containing carboxymethylcellulose are incubated with cellulase so that they can be sterility tested by the membrane method (10).

We are unaware of any previous publication on the bacteriostatic properties of F-VIII. The slow die-off may reflect the unavailability of a key nutrient such as iron. Haptoglobin kills bacteria by making iron unavailable (11), and some haptoglobin is present in F-VIII.

After we had developed and validated the typsin method, unpublished work at Hyland by T. K. Hazlet and R. DeVreker, dated August 1973, came to our attention. They added sterile-filtered streptokinase to F-VIII. Streptokinase activates plasmin (a protease), which hydrolyzes fibrinogen. A filterable digest was obtained when 1 to 30 units was added per ml of F-VIII and the mixture incubated at 37°C for 30 min. The effects, if any, on microbial viability were not studied.

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