EPA
United States
Environmental Protection
Agency

Water Sample Filtration Methods Using VIRADEL Procedures

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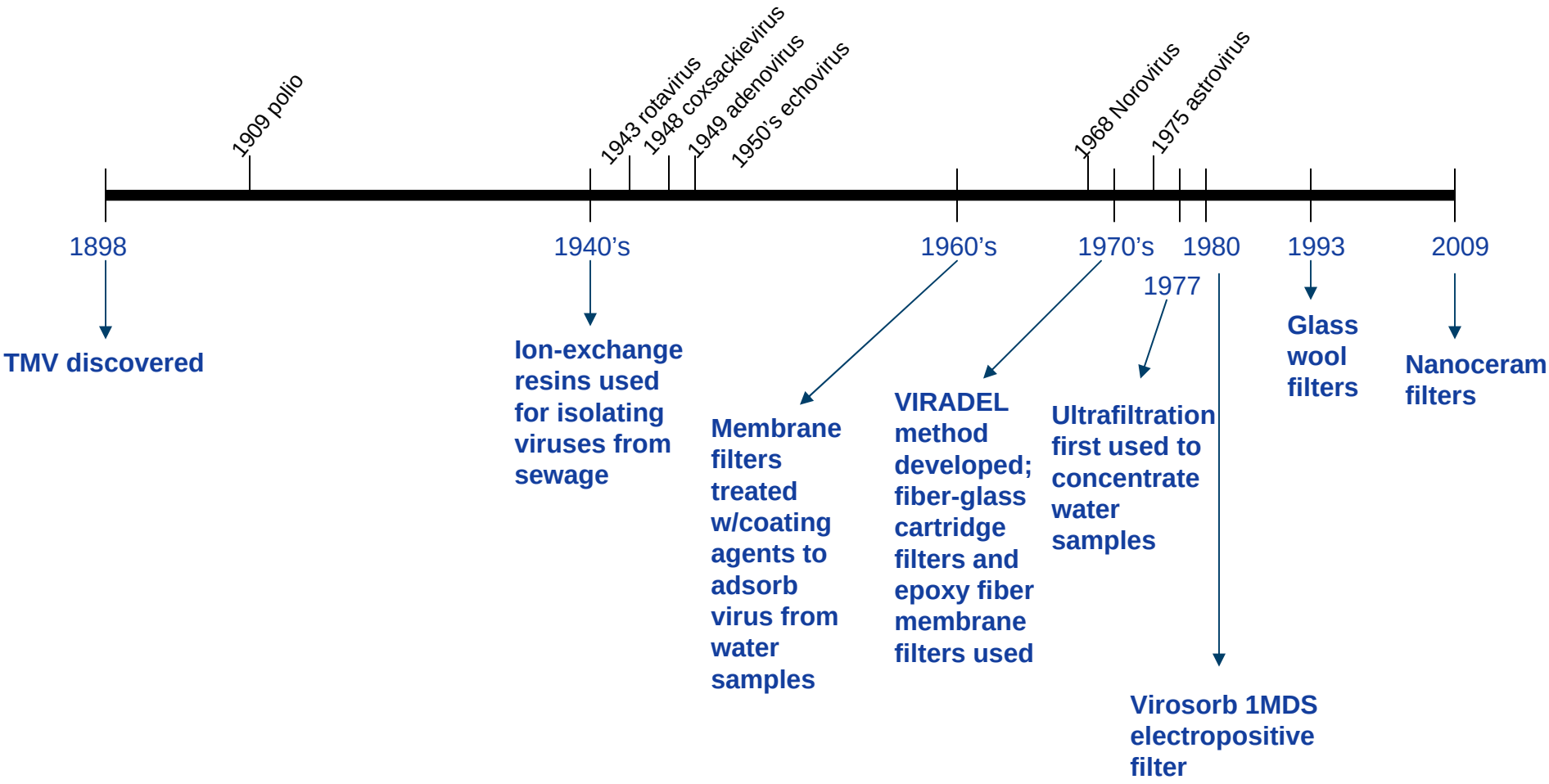
- Background/History of Viral Filtration
- VIRADEL Method using the Virosorb 1MDS Electropositive Filter
- Variations in the Method
- Future Directions

Background/History



Background/History

- 1965 Symposium on the Transmission of Viruses by the Water Route.
    - Focus was on methods for recovering viruses from water.
    - Resulted in a growing interest in methods research.
- 1970 U.S. Environmental Protection Agency was created.

Background/History

- 1975 WHO Working Group on Bacteriological and Virological Examination of Water
  - Several methods were selected for promulgation, including the VIRADEL technique.
- 1981 Standard Methods for the Examination of Water and Wastewater, 15th edition.
- 1984 U.S. EPA Manual of Methods for Virology EPA/600/4-84/013 was published.
- 1988 ASTM published methods on large-volume virus sampling.

Background/History

- From July 1997 through December 1998, the U.S. EPA Information Collection Rule (ICR) was enacted which required the EPA to collect data as part of a national research project to support development of national drinking water standards which protect public health.
    - For virus sampling, the Virosorb 1MDS filter was utilized with the VIRADEL technique.

VIRADEL Method Using Virosorb 1MDS Filter-ICR Protocol

Filter water sample through Virosorb 10 inch cartridge filter.

Elute with 1L of 1.5% beef extract, 0.05M glycine, pH 9.5.

Adjust pH to 3.5 and mix for 30 minutes (organic flocculation).

Centrifuge at 2500 X g for 15 minutes at 4ºC.

Resuspend pellet in 30ml of 0.15M sodium phosphate, pH 9, until dissolved.

Centrifuge at 4000 to 10,000 X g for 10 minutes at 4ºC.

Pour off supernatant; discard pellet. Adjust the pH to 7-7.5. Filter sterilize. Freeze for analysis.

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Centrifugation

Recovery Data from ICR PE Samples

Sample size	828
Mean Recovery (%)	56
Standard Deviation (%)	51

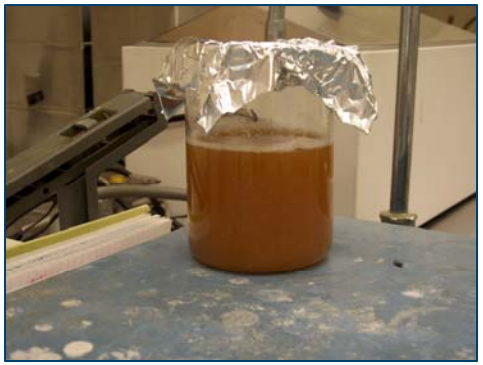
Inter-laboratory	Low Seed	Medium Seed	High Seed
Sample size	299	202	327
Mean (%)	71	54	44
Standard Deviation (%)	70	37	31

The intralaboratory mean recoveries ranged from 36 to 85%.
Analyst mean recoveries ranged from 33-98%.

Variations in the Method

- Beef extract type and concentration
  - ICR-Beef V, now discontinued
  - Alternatives: Difco dessicated beef, BBL beef extract
  - 1.5% to 3%, with and without 0.05M glycine
  - 1.6L of beef extract vs. 1L of beef (ICR method)
- Secondary concentration
  - Organic flocculation
  - Celite concentration
  - PEG

Variations in the Method



Recoveries range from 60-90% for enteroviruses.

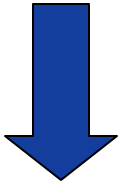
Celite Method

1. Add 1.5g of celite to the eluent.
2. Drop the pH to 4 and mix for 10-30 minutes.
3. Collect celite on a sterile pre-filter.
4. Elute virus from celite with 40-80 ml of 0.15M sodium phosphate.
5. Adjust pH to 7-7.5. Filter sterilize. Freeze for analysis.

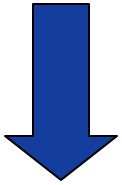
Future Direction-A Complete Virus Method

Filtration/Concentration:

1MDS, Nanoceram, Ultrafiltration, CFC, Glass Wool



Second-Step Concentration



Detection

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Questions?

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.