Sampling and Detection Methods for *Cryptosporidium* and Fellow Travelers

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Susan Boutros, Ph.D.

President and Laboratory Director

Environmental Associates Ltd., 24 Oak Brook Drive,
Ithaca NY 14850

(607)272-8902

Overview

- Water Uses
- Sources of Contamination
- Fluctuations
- *Cryptosporidium* &Fellow Travelers
- Sampling & Analysis
- Strategy

Water Uses

- Drinking Water
  - Surface Sources
  - Groundwater
- Recreational Water
- Non-potable
- Reuse
- Industrial

Water uses impact planning for protection.

Water Uses

- Drinking Water
  - Surface Sources
  - Groundwater
    - EPA Symposium on *Cryptosporidium* occurrence in groundwater and removal by subsurface passage
      - (http://es.epa.gov/ncer/publication/meetings/9-9-2003/agenda.html)
- Recreational Water
- Non-potable
- Reuse
- Industrial

Sources Enteric pathogens

- Human enteric pathogens - Human wastes
- Zoonotic transmission - Domestic/ Agricultural, Companion, Wild

The challenge is to identify, track, abate & treat.

Fluctuations in Pathogen Levels

- Year to year
  - Host Levels
- Seasonal
  - Marked differences related to human and animal activity through year
- Rainfall
  - May increase or dilute
- Other Events
  - Treatment failures upstream
  - Intentional

Cryptosporidium &Fellow Travelers

- Protozoa
- Bacteria
- Viruses

Size and surface chemistry will affect transport.

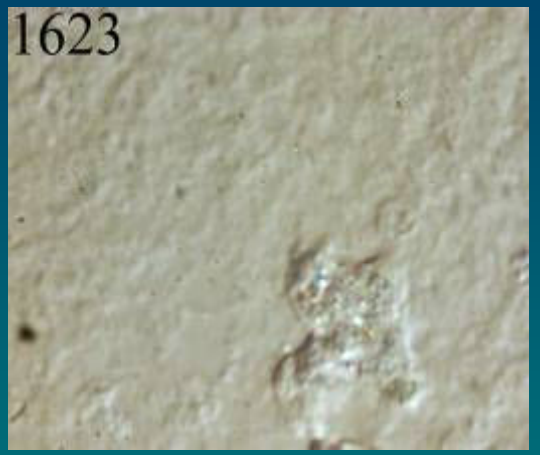
Cryptosporidium & Fellow Travelers

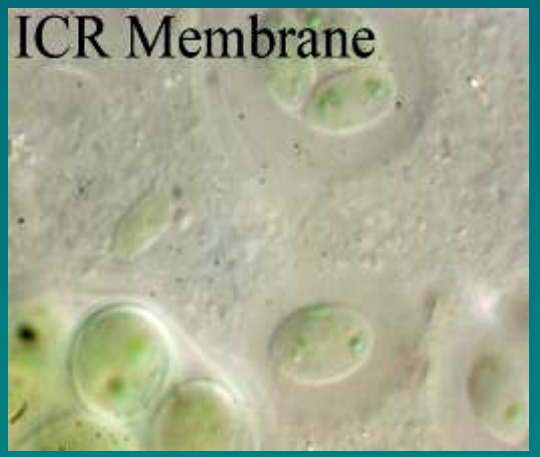
- Protozoa (um= one millionth of a meter)
  - Giardia 6 -18 um
  - Cryptosporidium 4-7 um
- Bacteria 1-10 um
- Viruses 20-80nm (nm=one millionth of a mm)

Transport patterns are not always the same.

Method 1623 and beyond

- IMS separation
  - Reduced microscope time
- Improved Filters
- Can add different determinative procedures
  - PCR to determine species, etc.
  - FISH; Fluorescent *in situ* hybridization
  - Focal Detection Method (FDM)





Sample Collection

Envirochek & Envirochek HV

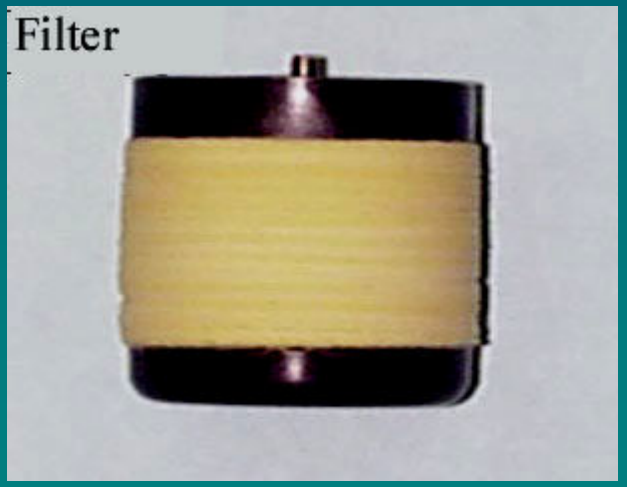
Advantage
Integrated Filter Holder

Disadvantage
More expensive than competing product

Sample Collection

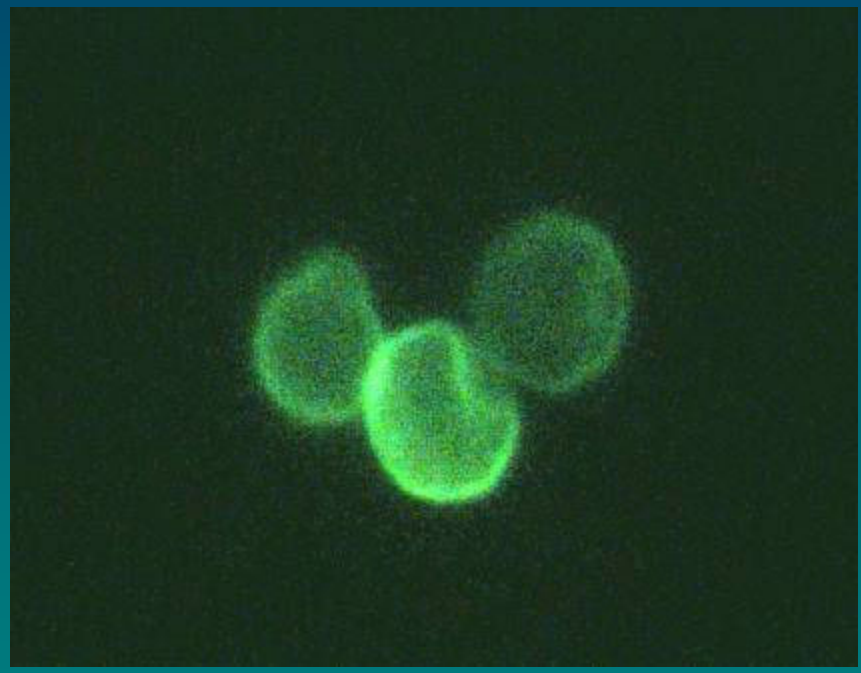
IDEXX FiltaMax

- **Advantage**
  Less Expensive
  Tolerant to pressure
- **Disadvantage**
  More complicated processing



Method 1623: Microscopic Exam

- Cryptosporidium
  - IFA
    - Size 4-7 um
    - Apple green Fluorescence
  - DAPI
  - DIC



Method 1623: Microscopic Exam

- Cryptosporidium
  - IFA
  - DAPI
    - Up to 4 sky blue nuclei
  - DIC

Method 1623: Microscopic Exam

- Cryptosporidium
  - IFA
  - DAPI
  - DIC
    - "Empty"
    - "With amorphous structure"
    - "With internal structure" one to four sporozoites

This is not a viability assay!

Method 1623 & Cell Culture Focal Detection Method (FDM) for Viability

- Sample extract is put on cells for several days. After incubation, cells are examined for evidence of oocyst infection and proliferation (Foci).
- EPA Alternate Test Procedure (ATP) limited use with special project.
  - ATP Application, 1623 and sample preparation EAL / FDM Dr. George DiGiovanni, Texas A&M (Miami Dade Water and Sewer District / Brown and Caldwell / Environmental Associates Ltd.)

Cryptosporidium & Fellow Travelers

- Protozoa
- Bacteria
- Viruses
  - Virus Methods
    - Plaque Assay vs ICR MPN
    - Initial Cell Culture / PCR
    - Direct PCR
      - Limited volume and viability determination
      - Only method for non-culturable viruses e.g. HepA, Human Calici / Norovirus

Human Enteric Viruses

Virus Group	No. of Types	
Enteroviruses Poliovirus Coxsackievirus Echoviruses Enteroviruses (types 64 -71)	59	
Hepatitis A virus	1	
Reoviruses	3	
Rotaviruses	8	
Adenoviruses	49	
Astroviruses	7	
Hepatitis E virus	1	
Calicviruses (norovirus; saporovirus)	2	

The 15 Most Commonly Reported Nonpolio Enterovirus Serotypes, by rank 2000 - 2001 (n=1,862) CDC data

Rank	Serotype	%
1	echo 18	22.0
2	echo13	20.8
3	coxsackie B5	11.9
4	coxsackie B2	6.3
5	echo 6	6.1
6	echo 11	4.5
7	coxsackie A9	4.0
8	echo 9	3.3
9	coxsackie B4	3.2
10	echo 4	3.1
11	coxsackie B3	2.4
12	coxsackie B1	2.0
13	echo 30	1.8
14	echo 25	1.2
15	enterovirus 71	1.1

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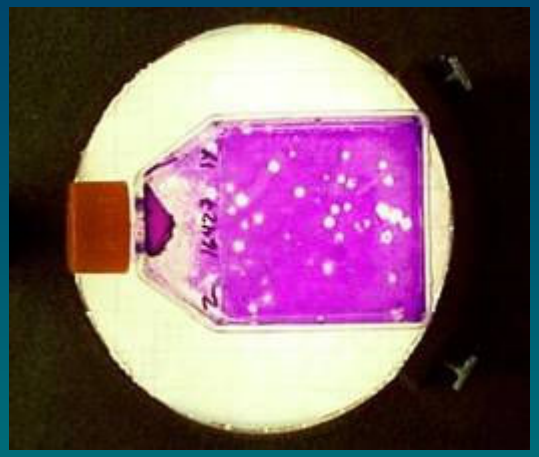
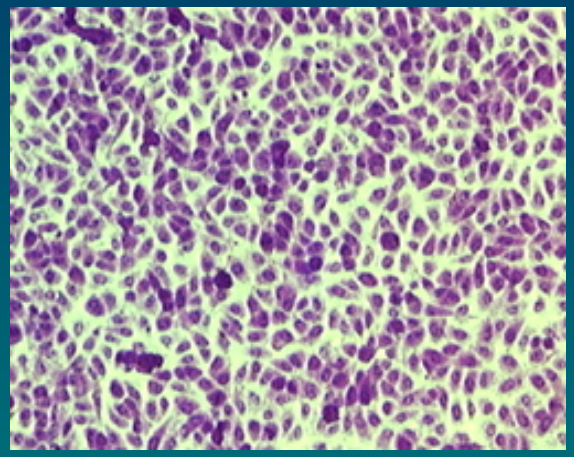
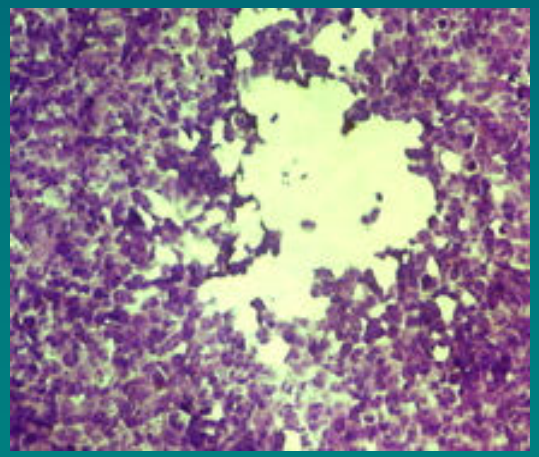
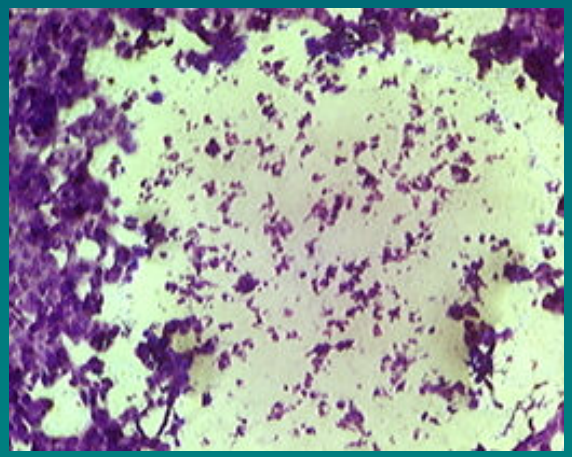
Total = 93.5

503 Regulations: Plaque Assay

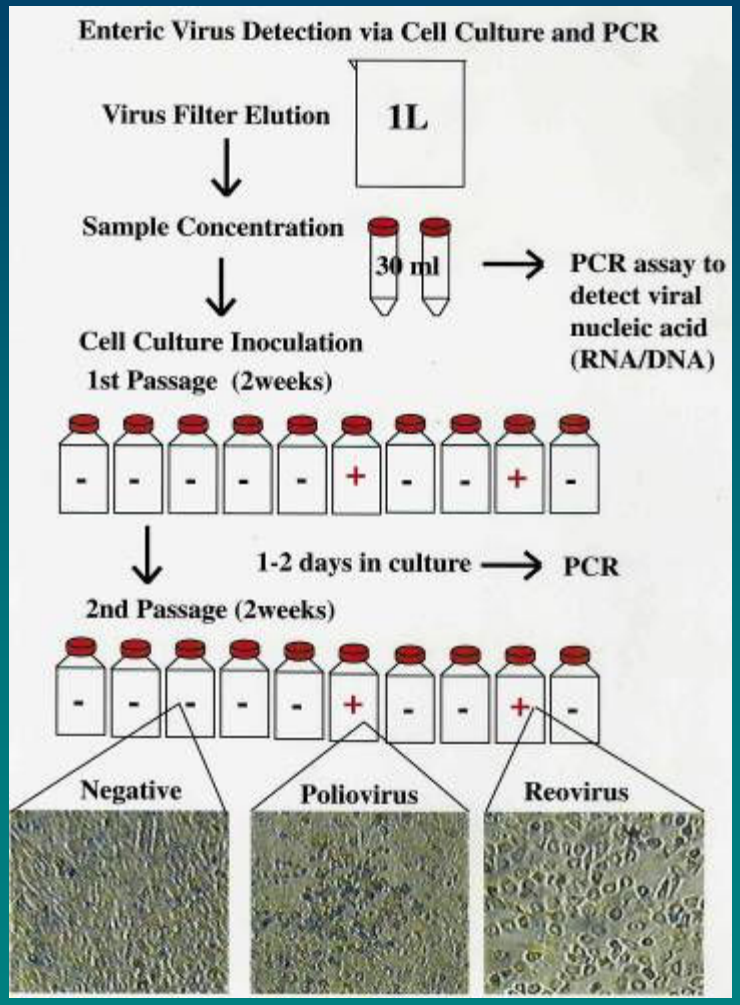






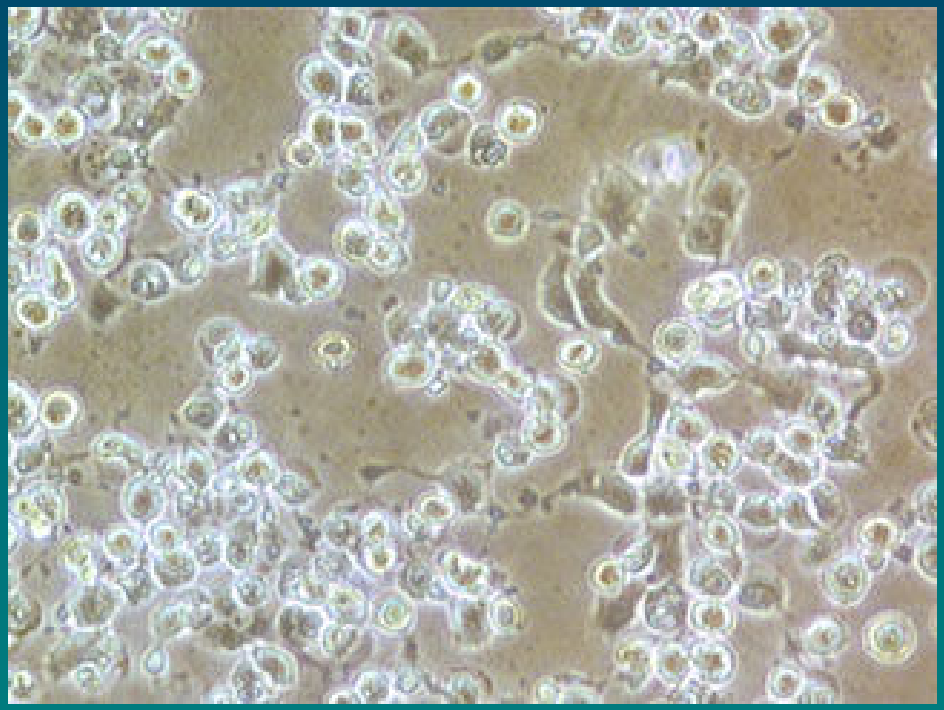


ICR Method: Cytopathic Effect (CPE)



ICR Method: Cytopathic Effect (CPE)

- Virus testing
- MPN Assay
  - 20 flask MPN, two passages, 4 wks min.
  - Better ways to reduce toxicity
  - More sensitive
  - Cytopathic effect (CPE)



Virus Testing: Initial Cell Culture / PCR

- Follow initial cell culture with extraction and PCR
  - Cell lines: BGM, CaCo, MA-104
  - Primer selection
- Primer selection
  - Pan-Entero Primers
    - Echo
    - Coxsackie
    - Entero
  - Reo

Virus Testing: Initial Cell Culture / PCR

- Assay Capabilities
  - Viability
  - Qualitative vs Semi-quantitative
    - Presence / Absence
    - Modified MPN
      - Low / Medium / High
  - Shorten assay time from weeks to days
- Assay Limitation: Viruses that can be cultured.

Strategy: Method Decisions

- Analytes : Protozoa, bacteria, viruses
- Volume: e.g Crypt.: 10 liters to 50 liters
- Frequency: 1-2x a month / rainfall events
- Methods: e.g Crypto.
  - Total counts/ DAPI+/ Internal Structure
  - PCR: Species / Viability?
  - Cell culture: Focal Detection Method (FDM)
- Method Validation: Beyond 1623 & ICR virus

Strategy: Sampling Decisions

- Select Key Points
  - Sewage Discharge
  - Selected Tributaries
  - Spaced Points
- Monitor by Calendar vs Rain Events
  - Seasonal Changes
  - Rainfall
- Determine Species at Selected Sites
- Protozoa, Viruses, Bacterial Surrogates

Strategy: Summary

- Sources & Fluctuations
- Analytes & Methods
- Data Quality / Quality Plan / Study Objectives
- Costs / Time Frame / Short and Long Range Plans

This River Basin in 5, 50 or 150 years…