Microbial Pathogens and Fecal Indicator Bacteria in Reuse Water and other Venues: Caveats and Emerging Detection Technologies

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Background: Microbial Pathogens and Fecal Indicator Bacteria

Three-Pronged Approach for Reuse Water Safety

- Treatment to physically remove or kill pathogens (disease-causing microorganisms)
- Monitoring to assess effectiveness of treatment
- Limit direct exposure

Treatment and Monitoring Challenges

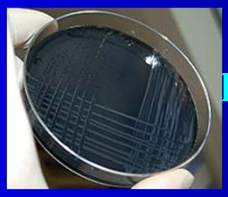
- Low numbers and sporadic occurrence of pathogens….
- but minimum infectious dose may be very low (e.g. *Cryptosporidium*, *E. coli* O157:H7),
- particularly for higher-risk populations, i.e. children, elderly, immunocompromised

Many Types of Pathogens with Varying Morphology, Physiology and Ecology

Bacterial pathogens

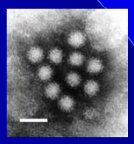
Salmonella

E. coli **O157:H7**



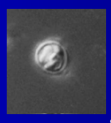
Campylobacter jejuni

Viral pathogens



Norovirus, Enterovirus

Protozoan pathogens



Cryptosporidium

Giardia

The Long-Standing Solution: Culturing Fecal Indicator Bacteria (Indicator Organisms)

- **Coliforms are Gram-negative, rod-shaped bacteria; fecal coliforms grow at 44.5°C**
- **Include genera such as *Escherichia (E. coli), Enterobacter, Citrobacter***
- **Closely related to *Salmonella, Shigella, Yersinia***
- **Coliforms are relatively fragile**

Characteristics of Ideal Indicator* *Mythical	Fecal or Total Coliforms	*E. coli*	Enterococci	*Clostridium perfringens*	Coliphage
Nonpathogenic	±	±	±	±	☺
Low cost and ease of measurement	👍	👍	👍	±	☹
Only source is fecal material	☹	±	☹	👍	±
Inactivation rate by treatment equivalent to pathogens	Depends	D	D	D	D
Does not multiply in environment	☹	☹	☹	👍	👍
Survival in environment (water) should be similar to pathogens	Depends	D	D	☹	☹
Always + when pathogens present; always – when pathogens absent	☹	☹	☹	☹	☹

The Less-than-Ideal Behavior of FIBs Has Consequences!

- FIBs may be absent when pathogens are present (false-negative)
- Consequence of false-negative: No warning; more people may get sick
- FIBs are frequently present when pathogens are absent (false-positive)
- Consequence of false-positive: "crying wolf"; hurts tourism, public confidence in water quality, ability to accurately assess health risk

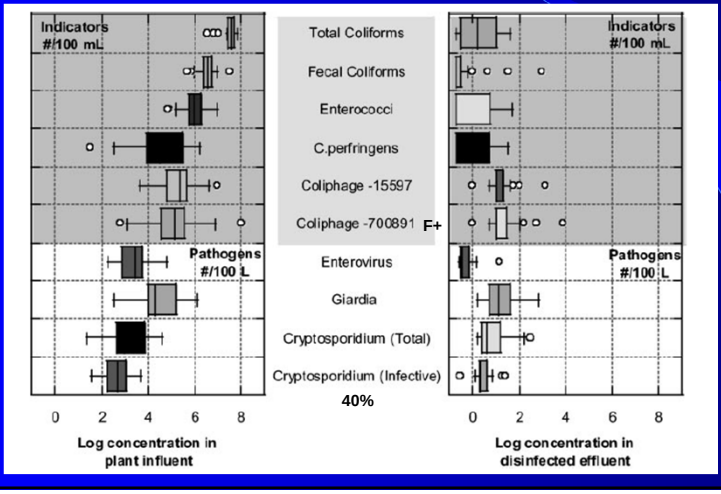
Data: Multi-Facility Study

Multi-Facility Study

- 6 reuse facilities (CA, FL, AZ)
- Capacity ranged from 1 – 25 mgd
- Sampled at least five times over a year
- Samples collected at peak flow times
- Sample points included influent, secondary effluent, filter effluent and post-disinfection
- Pathogens and various indicators measured

Rose, JB et al. 2005 WERF Report 00-PUM-2T
Harwood, VJ et al. 2005 Appl Env Microbiol 71:3163
Levine, AD et al. 2008 Water Environ Res 80:596

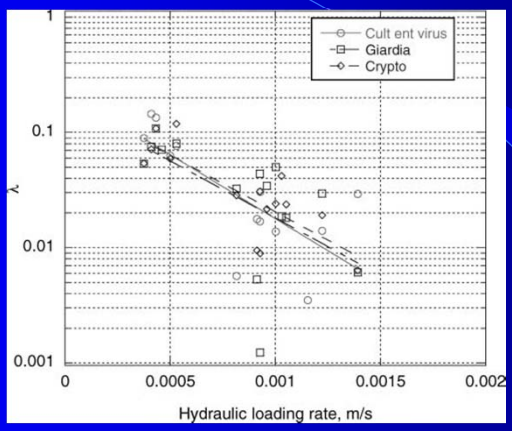
Microbial Concentrations in Influent vs. Disinfected Effluent (All Facilities)



Percent of Samples (All Facilities) Positive for Each Microbial Target at Each Treatment Stage

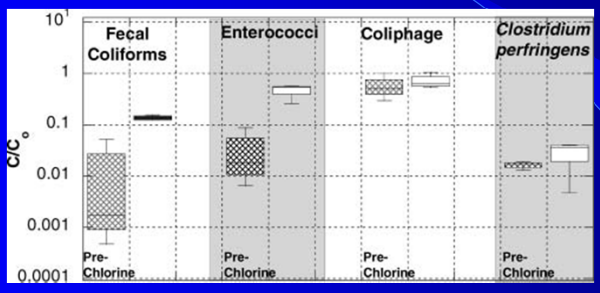
Indicator or pathogen	% of samples positive in each stage[a]			
	Influent	Biological treatment	Filter effluent	Disinfected effluent
Indicators				
Total coliforms	100	100	94	63
Fecal coliforms	100	97	65	27
Enterococci	100	94	84	27
Clostridium perfringens	93	86	79	61
Coliphages on 15597	100	97	83	38
Coliphages on 700891	100	93	80	45
Pathogens				
Enteric viruses	100	73	58	31
Giardia	100	94	88	80
Cryptosporidium				
Total oocysts	74	84	71	70
Infectious oocysts	32	19	19	20

Effect of Hydraulic Loading Rate on Filter Coefficient



- Data from 3 facilities using sand filters
- R^2 = 0.8 for enteric viruses; 0.6 for protozoa

Effect of Pre-Chlorination on FIB and Coliphage Removal by a Sand Filter

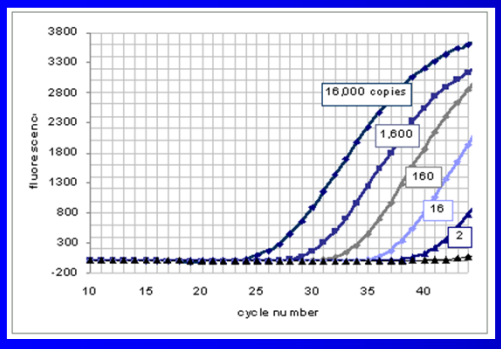


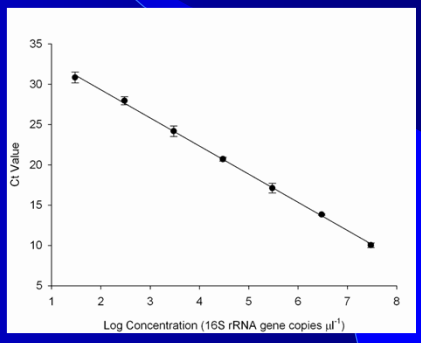
- Intensive sampling of secondary effluent over 10 hrs
- Grab samples collected every two hours
- 2 mg/L chlorine

More Observations from Multi-Facility Study

- Facility performance varied; good treatment for one type of microbe did not guarantee good treatment for others
- Ammonia reduced the efficacy of chlorination.
- UV disinfection (1 facility) was very effective at clearing infectious oocysts, but not as effective as chlorine for bacteria and coliphages
- Biological treatment with higher mixed liquor suspended solids (MLSS) and longer mean cell residence time (MCRT) gave better performance
- Larger sample sizes (300 ml) yielded better detection!

Emerging Technologies for FIB & Pathogen Detection– Is There a Better Way? Quantitative (Real-Time) PCR

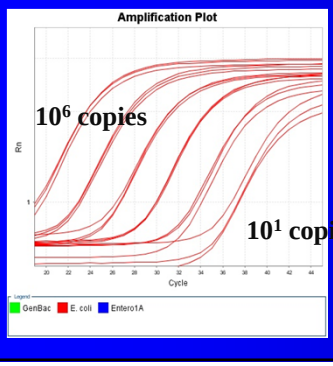




http://www.youtube.com/watch?v=QVeVIM1yRMU&feature=related

Advantages of qPCR Compared to Culture Methods

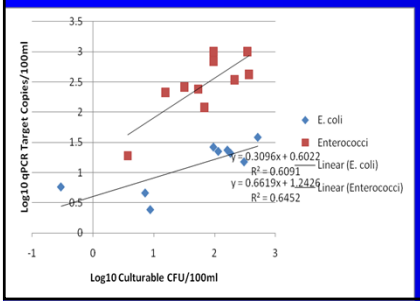
E. coli qPCR Assay



- Rapid! Half a day including prep time
- Specific – fewer false-positives (if the method is good)
- Versatile – essentially the same methodology for any organism; easy to expand lab's repertoire
- Can detect stressed cells, which may still be infectious

Some Disadvantages of qPCR

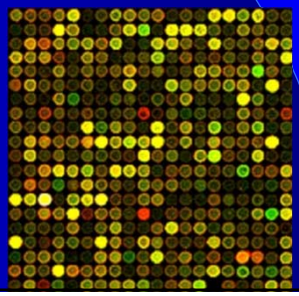
- Expensive equipment required.
- Molecular biology expertise for staff required.
- Detects viable and nonviable cells, therefore…
- the relationship of qPCR results to previous standards must be determined.

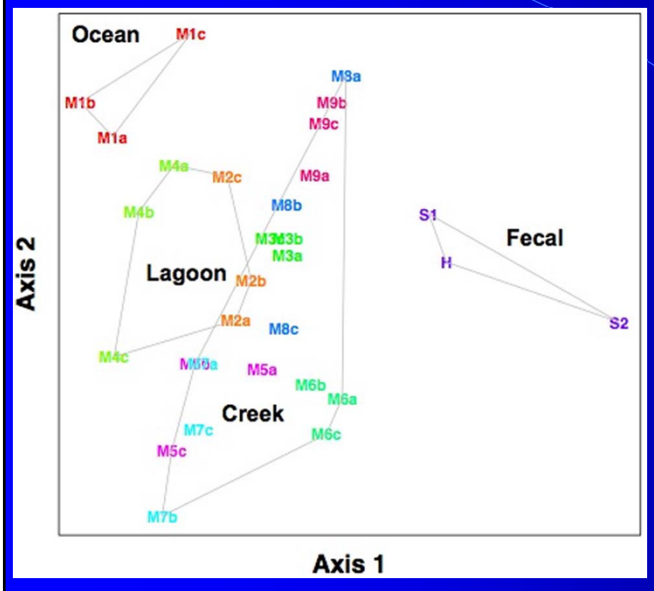


- Various compounds can inhibit the PCR reaction, therefore…
- Expertise in sample prep and testing for inhibition is required

Another Approach: PhyloChip (Phylogenetic Microarray)

- DNA for 8,741 bacterial and archaeal genes is affixed to support
- Developed by Lawrence Berkley National Laboratory





- Wu et al. 2010 Plos One: 5:311285
- Santa Barbara CA

Conclusions

- **FIBs have served us well for over 100 years, but their drawbacks mandate improvements.**
- **qPCR technology for environmental testing is close to the stage of development where it can replace and/or supplement FIB use**
- **This decade will probably see major changes in the way we monitor water quality, from beaches to potable water to reclaimed water.**

Questions?

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Characteristics of Six Reuse Facilities

TABLE 1. Comparison of wastewater reclamation facilities sampled for indicator organisms and pathogens in this study

Facility	Avg capacity ($m^3 \cdot s^{-1}$)	Biological treatment	Chemical use prior to filtration	Filter composition (depth, m)	Backwash frequency (h)	Type of disinfection
A	0.04	Activated sludge	None	Fabric (0.02)	24 to 72	Chloramines[a]
B	0.4	Activated sludge	Chlorine	Sand (0.3)	Automatic (daily)	Chloramines[a]
C	0.4	Activated sludge	Cationic polyelectrolyte	Anthracite (1.2)	48	Chloramines[a]
D	0.7	Activated sludge	None	Anthracite (0.8), sand (0.25)	48 to 168	Chloramines
E	0.08	Nitrification	None	Sand (1.2), upflow	Continuous	Ultraviolet light
F	0.13	Biological nutrient removal	None (alum added to secondary clarifier)	Anthracite (0.6), sand (1.2)	48 to 168	Chlorine

[a] Chloramines are formed due to the reaction of chlorine with residual ammonia.