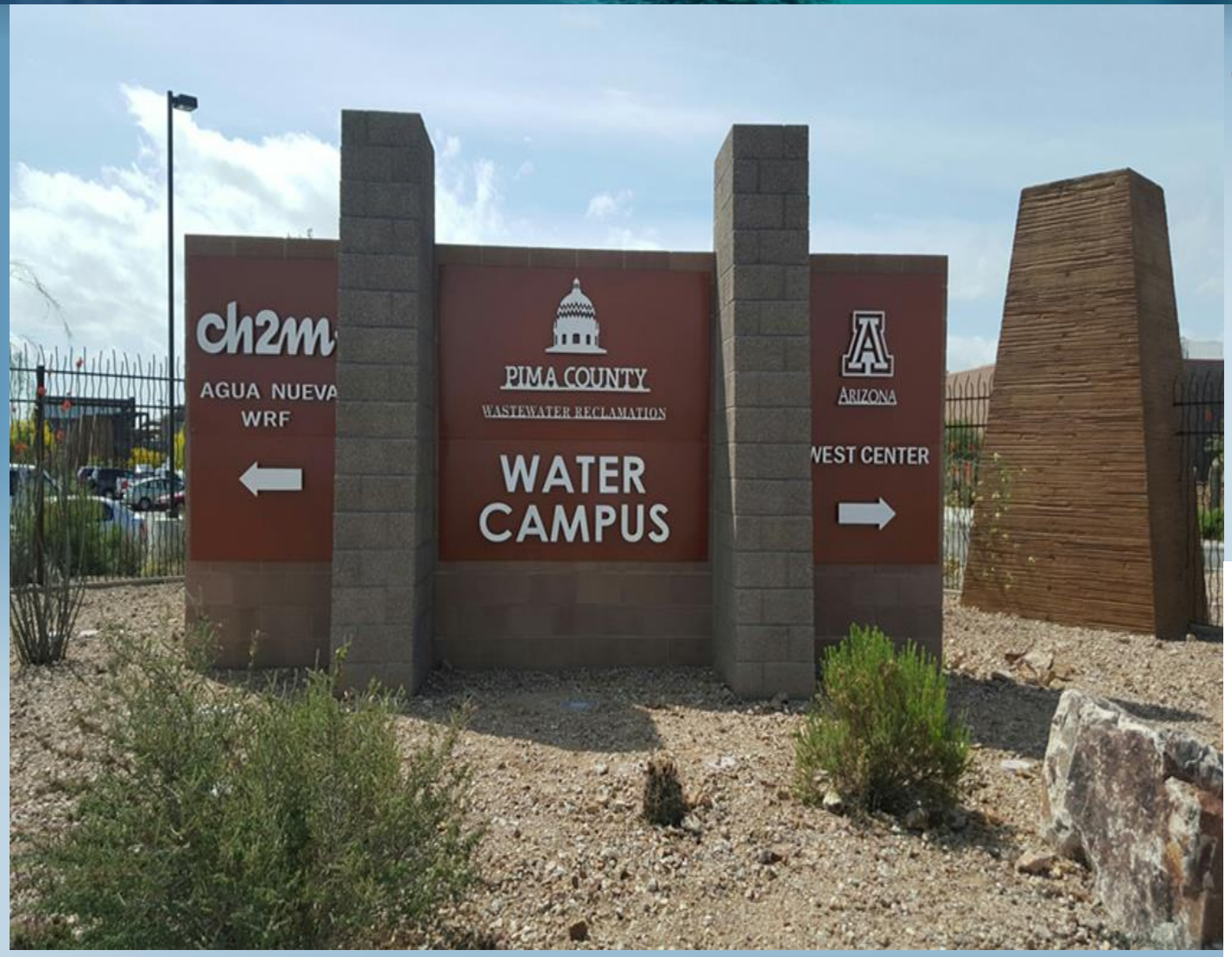
Microbes the Forever Contaminate



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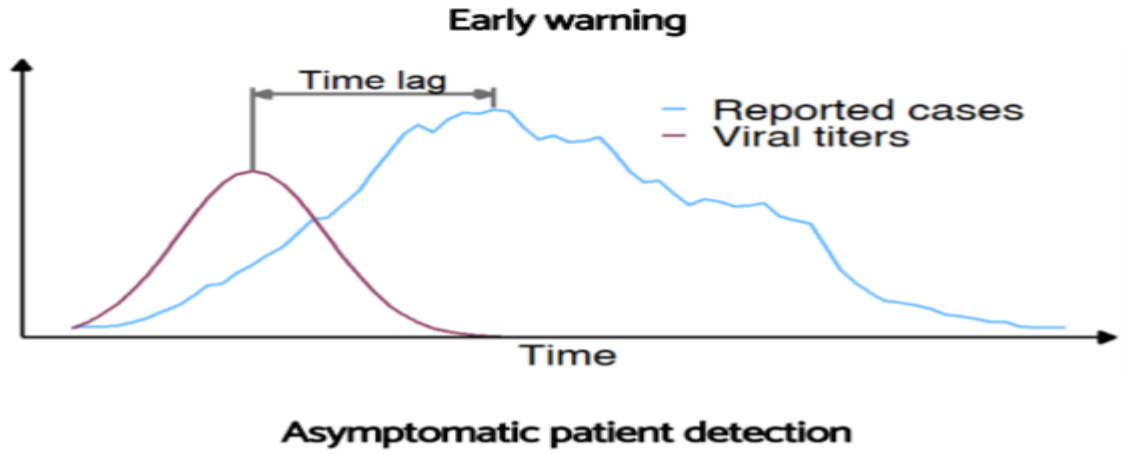
ARIZONA

THE UNIVERSITY OF ARIZONA

WEST CENTER

WATER & ENERGY SUSTAINABLE TECHNOLOGY



Wastewater based epidemiology

Water/Wastewater
Pilot Plant Studies

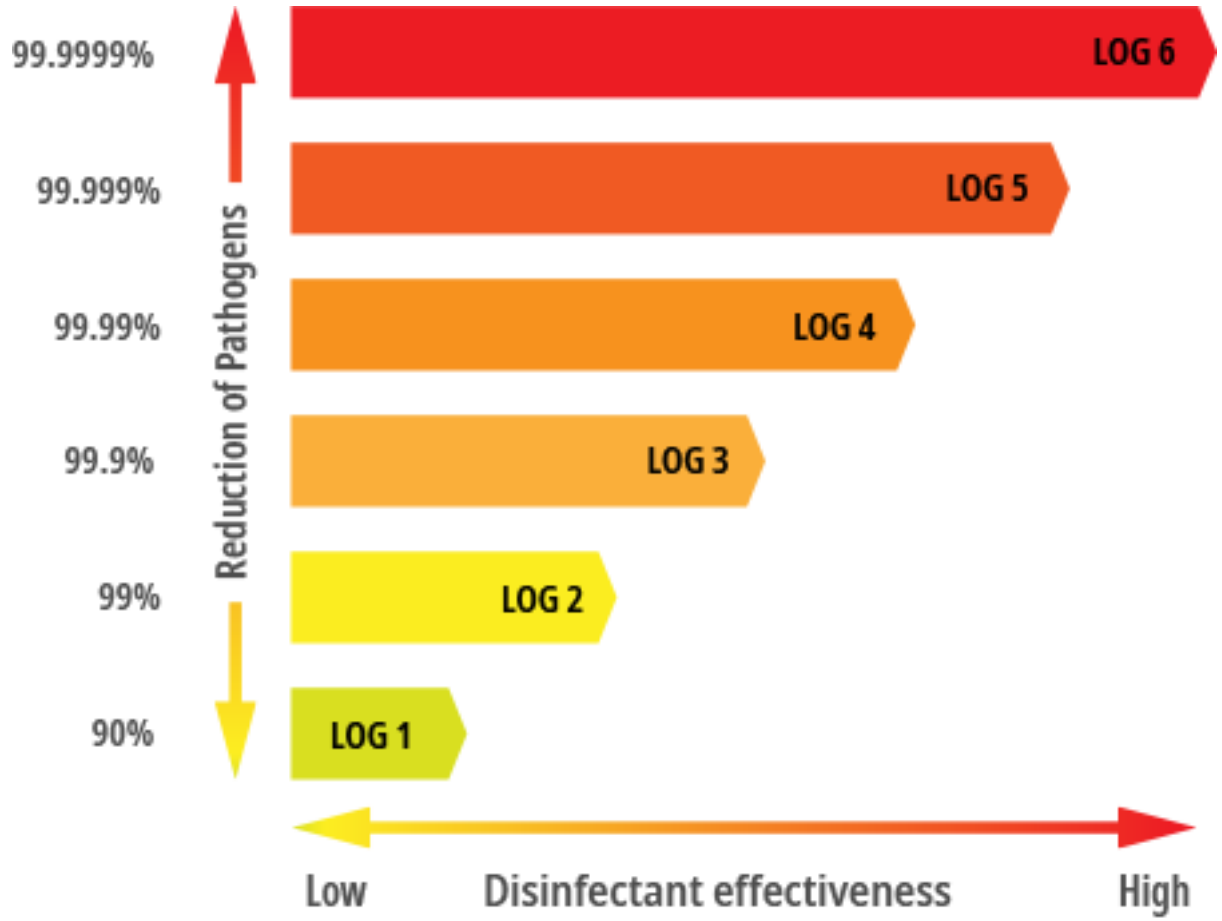
Quantitative microbial Risk Assessment

National Study on virus removal by wastewater treatment processes to establish log reduction credits

USEPA Sponsored Study on Virus Removal Credits @ WEST Center

- A Viral Pathogen and Surrogate Approaches For Assessing Treatment Performance in Water Reuse - 2021-2024 -WRF
- Determine virus removal to validate Log Reduction Values for viruses for water reuse treatment processes – 2022-2026 – EPA/WRF

What are Log Reduction Credits?



Multiple-barrier system

Primary treatment — 1 log

Secondary treatment — 2 log

Disinfection — 3 log

Pathogen log reduction credit in each treatment unit

Pathogen log reduction in the system = 6 log

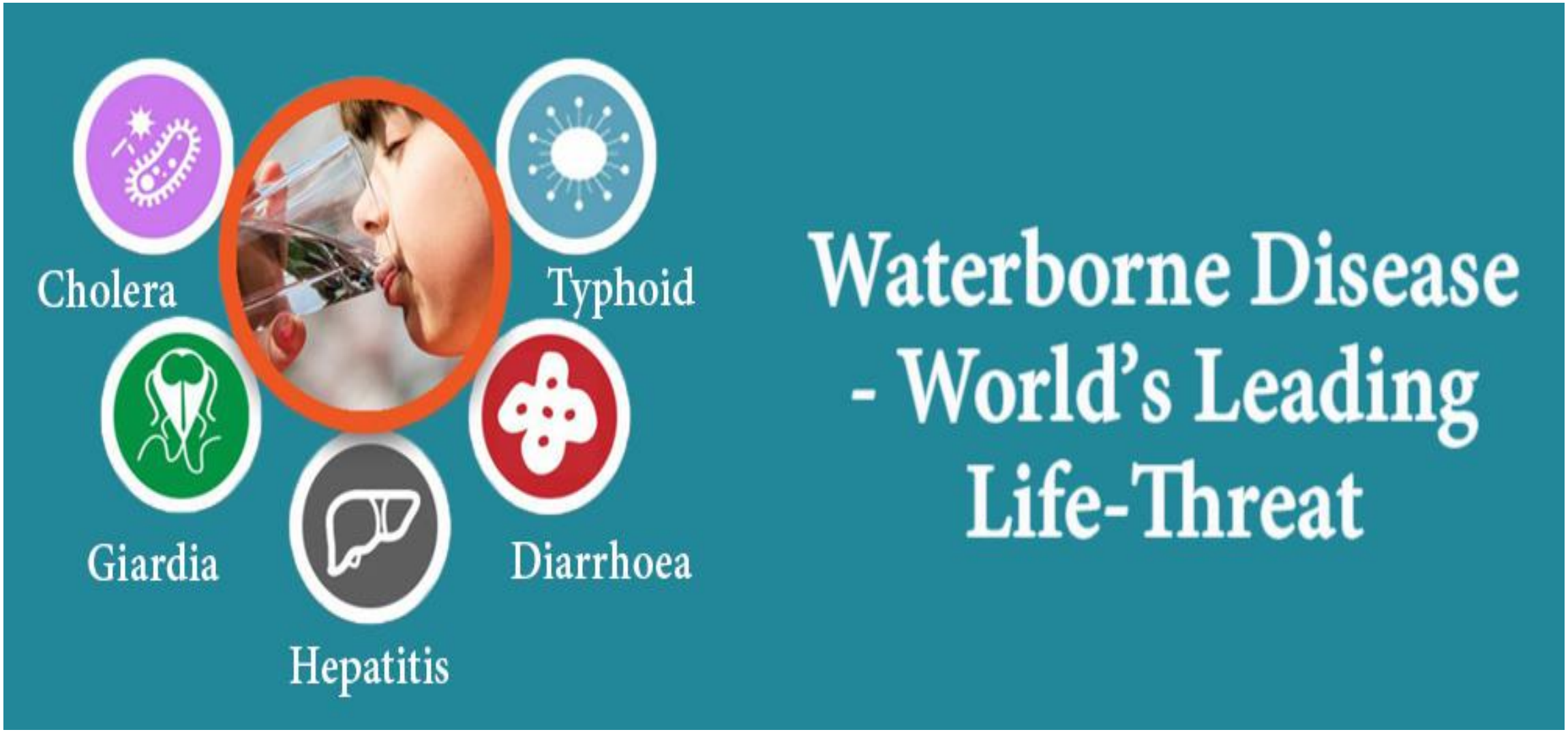
⇕

Performance target of pathogen log reduction with respect to the purpose of effluent usage

Why Log Reduction Credits for Pathogens?

- Pathogen testing
  - Costly $200 to $2,000 sample
  - Can take weeks for results
  - Requires specialized laboratories
  - Methods are not available for detection of all pathogens i.e., norovirus
  - More resistant to removal than bacterial indicators
  - Large volumes must be tested ~100,000 liters for some viruses

3,400,000 people die per year from waterborne diseases
80% of deaths per year in India



Approaches to Regulating Microbial Water Quality

- Monitoring of indicator microorganisms
  - Coliforms
  - Fecal coliforms
  - *Escherichia coli*
  - Coliphage
  - *Clostridium perfringens/ enterococcus*
- Disinfectant efficacy
  - measure of residual and/ or contact time or dose (UV light)
- Monitoring for pathogens

How do we determine LRV?

- USEPA Guideline – Drinking water must be treated to reduce the risk if infection for a virus to less than 1:10,000 per year or ~1:1,000,000 per day to 1:10,000,000
- In the case of rotavirus this = less than one virus in 100,000 liters of drinking water
- Log reduction depends on goal of reuse
  - Irrigation
  - Toilet flushing
  - Direct potable reuse

Calculating Risk

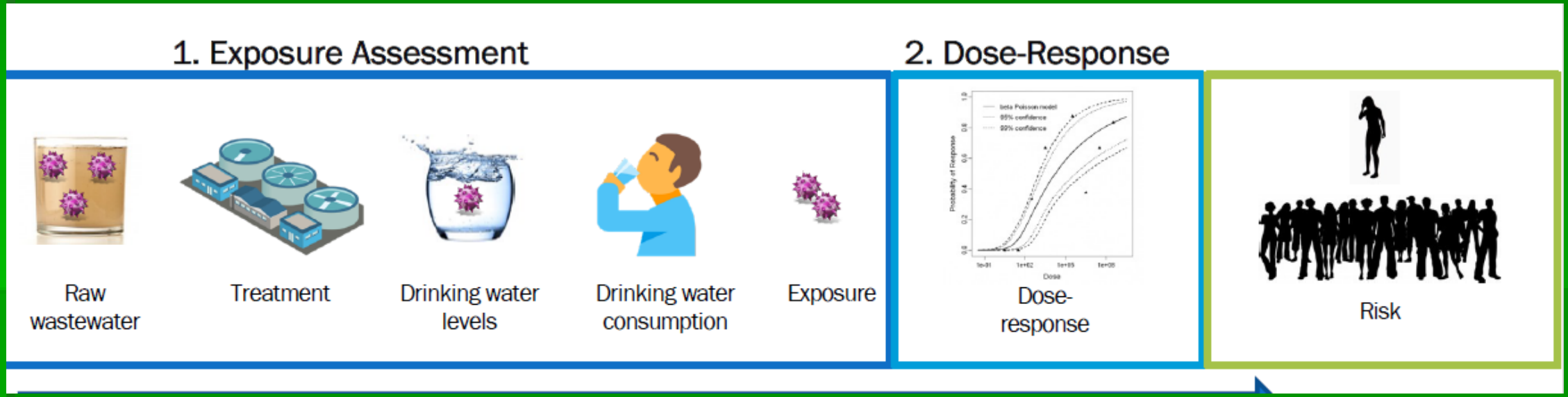


Table 3. Log Reduction Targets (LRTs) for Onsite Non-Potable Reuse Systems Based on 10^{-4} Risk Goa

Water Use Scenario	Enteric Viruses	Parasitic Protozoa	Enteric Bacteria
Domestic Wastewater/Blackwater			
Unrestricted irrigation	8.0	7.0	6.0
Indoor use[1]	8.5	7.0	6.0
Graywater			
Unrestricted irrigation	5.5	4.5	3.5
Indoor use	6.0	4.5	3.5
Stormwater (10% wastewater contribution[2])			
Unrestricted irrigation	5.0	4.5	4.0
Indoor use	5.5	5.5	5.0
Stormwater (0.1% wastewater contribution[2])			
Unrestricted irrigation	3.0	2.5	2.0
Indoor use	3.5	3.5	3.0
Roof runoff water			
Unrestricted irrigation	N/A	No data	3.5
Indoor use	N/A	No data	3.5

Onsite Non-Potable Water System: Guidance Manual (2020) Water Research Foundation

Table 1 Common end uses and LRV requirements

Use	LRV Requirement		
	Protozoa	Viruses	Bacteria
Commercial food crops	4.8	6.1	5.0
Dual reticulation	4.9	6.3	5.1
Fire fighting	5.1	6.5	5.3
Municipal use	4.0	5.2	4.0

Source: *NSW Guidance for RWMS Table 4*

North Carolina Criteria (type 2) Treatment Requirements

- 6 log reduction of *E. coli*
- 5 log reduction of Coliphage (surrogate for enteric viruses)
- 4 log reduction of *Clostridium perfringens (surrogate for Protozoan cysts/oocysts)*
- Provide dual disinfection with UV light

National Sanitation Foundation

Table 1

Log reduction targets for 10^{-4} per person per year benchmarks for ONWS using blackwater, graywater, or roof runoff

Water Use Scenario	Enteric Viruses	Parasitic Protozoa	Enteric Bacteria
Domestic Wastewater or Blackwater			
Unrestricted Irrigation	8.0	7.0	6.0
Indoor Use	8.5	7.0	6.0
Graywater			
Unrestricted Irrigation	5.5	4.5	3.5
Indoor Use	6.0	4.5	3.5
Roof runoff			
Unrestricted Irrigation	Not applicable[1]	No data[1]	3.5
Indoor Use	Not applicable[1]	No data[1]	3.5

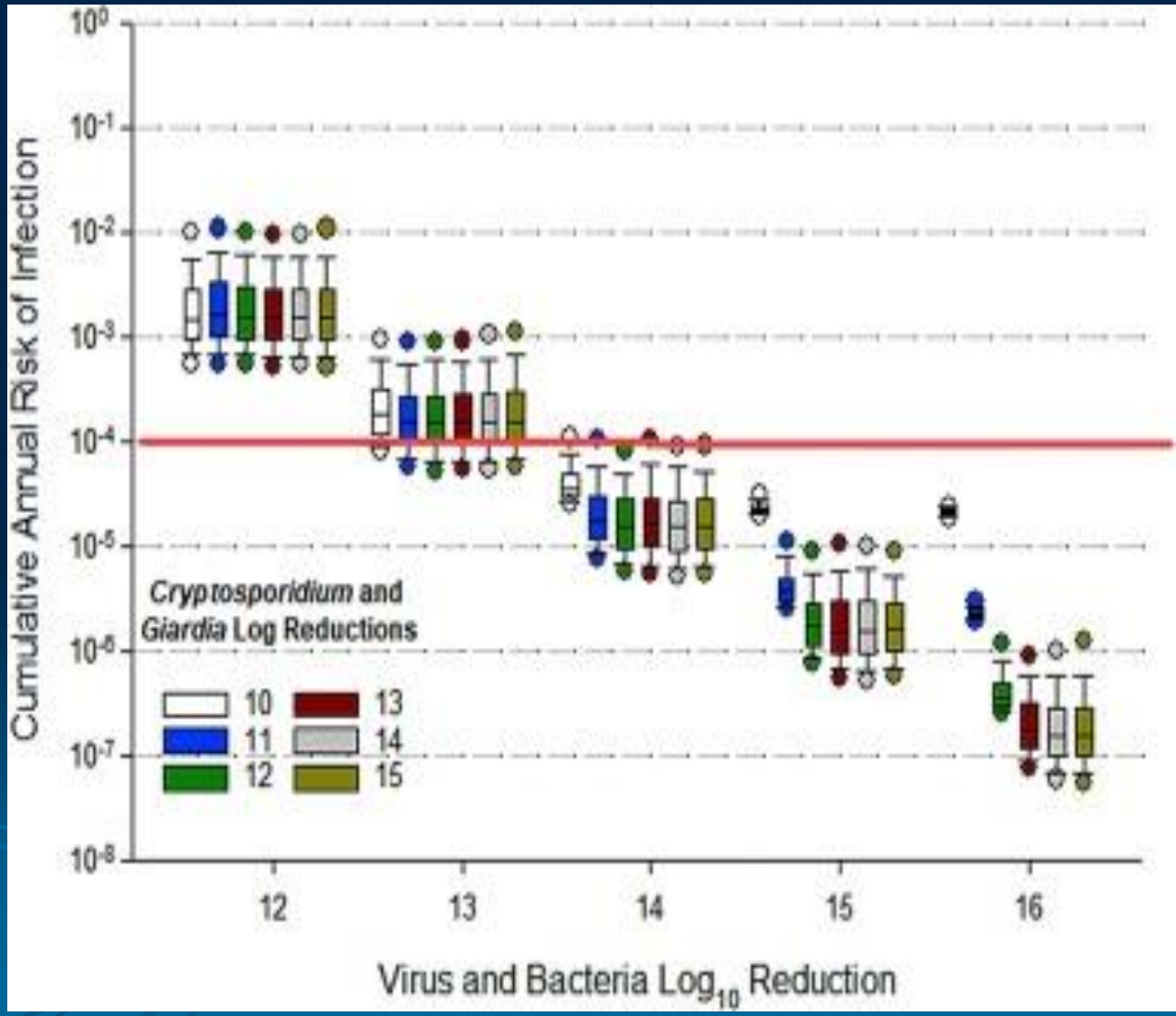
Notes:

1. States and/or local regulators can define the LRTs for virus and protozoa for roof runoff systems using one of the following suggested options:
   — Assign LRT values based on stormwater LRTs
   — Conduct research on the presence of virus and protozoa in roof runoff and assign LRT values based on research

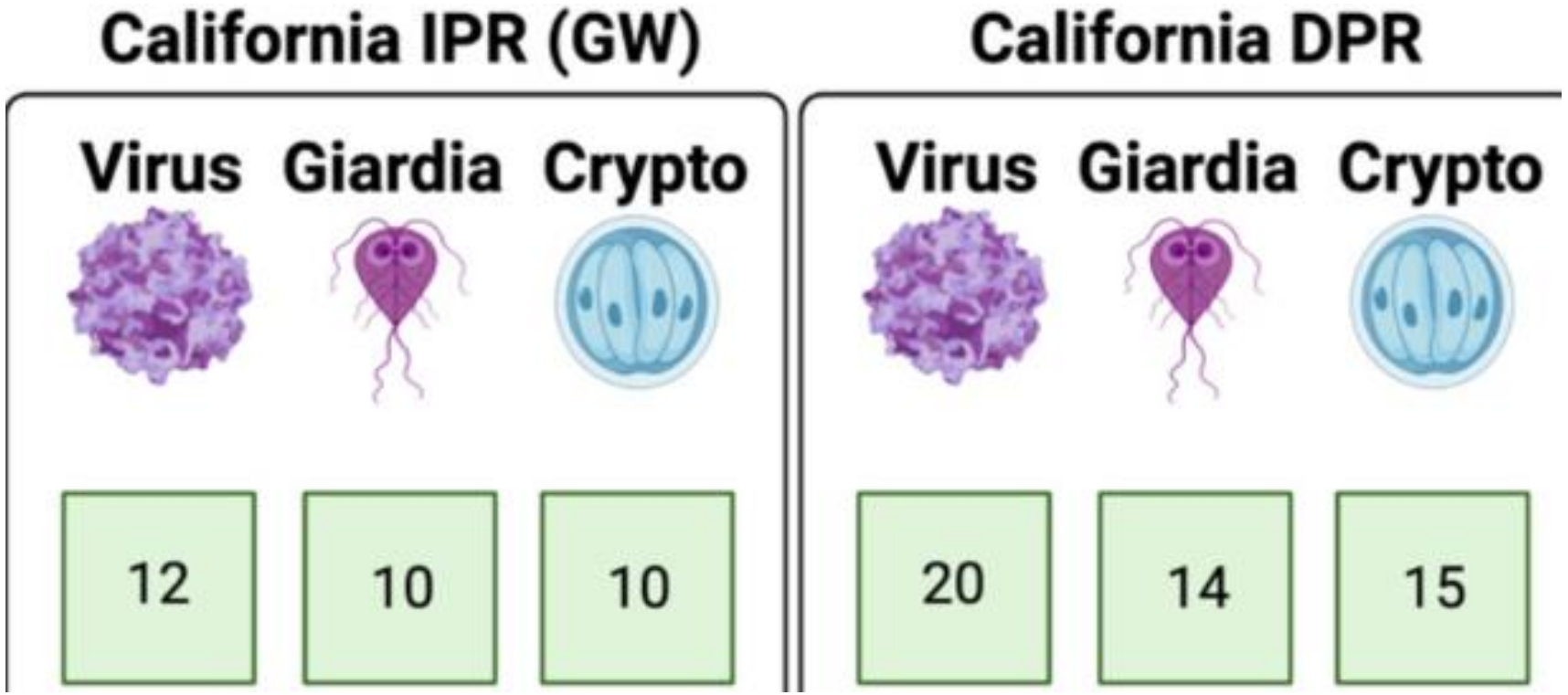
Source: Adapted from Sharvelle et al., 2017 (Table 3-3, page 26).

Greater Log removal of pathogens if you consider treatment variability (Sollor et al 2017)

- Cumulative annual risks are driven by days with highest wastewater pathogen loads
- Viruses need more than 14 logs reduction to achieve benchmark of 1/10,000 annual risk of infection



Log Removal Requirements
IPR = Indirect Portable Reuse
DPR = Direct Potable Reuse



Factors Creating Uncertainty in estimating virus Removal by Treatment

Factor	Uncertainty	Remarks
Disinfection	Large in application	Efficacy varies greatly dependent of the type and stain of virus and physical state (aggregates, association with particulate matter). Laboratory data may not reflect resistance of wild type strains.
Physical removal by membrane processes	0.1 log to 6.0 removal	Size, shape, hydrophobicity of the virus and membrane may affect removal; field scale operation conditions
Virus Concentration	Orders of magnitude	Varies greatly depending on the incidence of infection within a community

ISO 30500:2018E (2018 edition)

- Not intended for wash water or potable reuse

Non-sewered sanitation systems — Prefabricated integrated treatment units — General safety and performance requirements for design and testing

ISO 30500:2018E

Table 5 — Liquid effluent validation thresholds and log-reduction values (LRVs) for human health protection

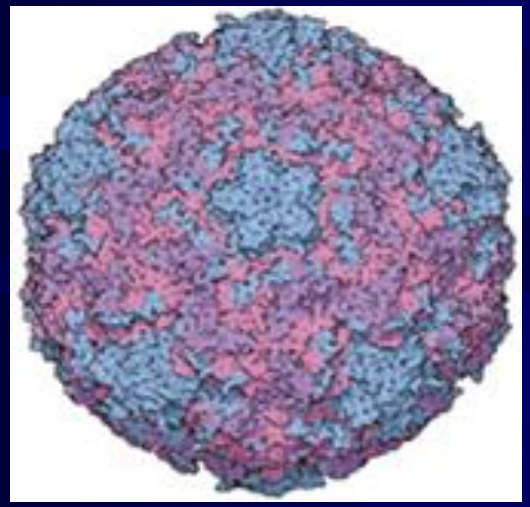
Parameter (Pathogen class)	Human enteric bacterial pathogens	Human enteric viruses	Human enteric Helminths	Human enteric Protozoa
Surrogate	using *E. coli*[b] as surrogate, measured in CFU or MPN	using MS2 Coliphage as surrogate, measured in PFU	using *Ascaris suum* viable ova as surrogate	using viable *Clostridium perfringens spores* as surrogate, measured in CFU
Max. concentration in liquids (number/l)	100	10	< 1	< 1
Overall LRV for liquid[a]	≥ 6	≥ 7	≥ 4	≥ 6

[a] Log-reduction values (LRVs) were derived from a quantitative microbial risk assessment (QMRA) as described by WHO 2016. For further information, see Reference [61] and Reference [72].

[b] *E. coli* strain KO11 (ATCC 55124) is used because it is chloramphenicol resistant. Therefore, this antibiotic may be added to the plating medium to suppress the growth of other, interfering bacteria.

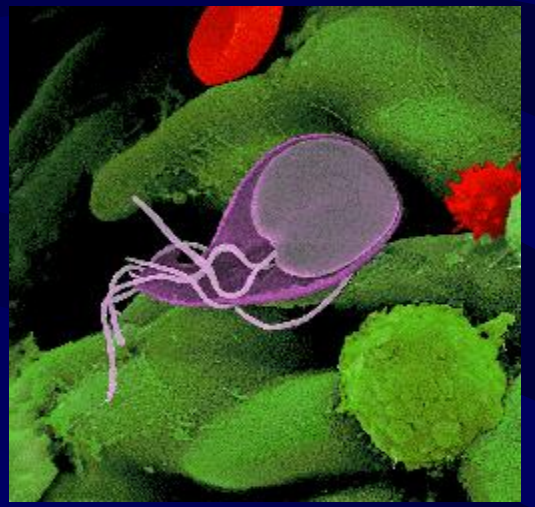
Types of Water borne/based Pathogens

Viruses



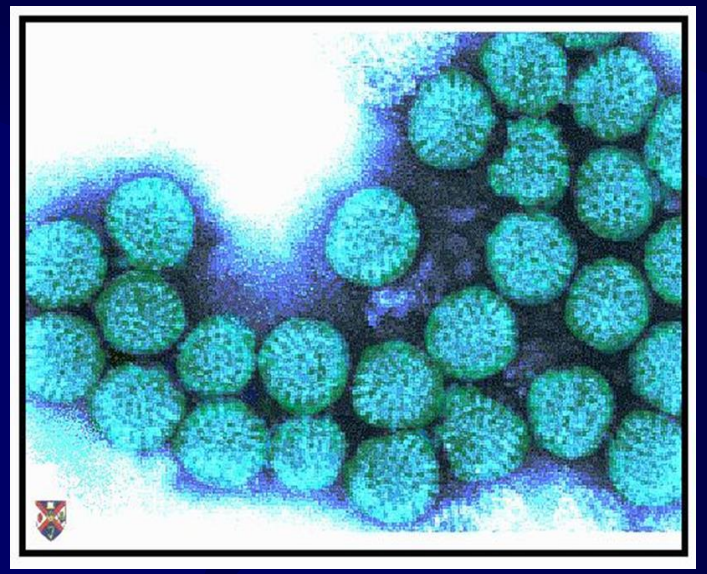
Bacteria

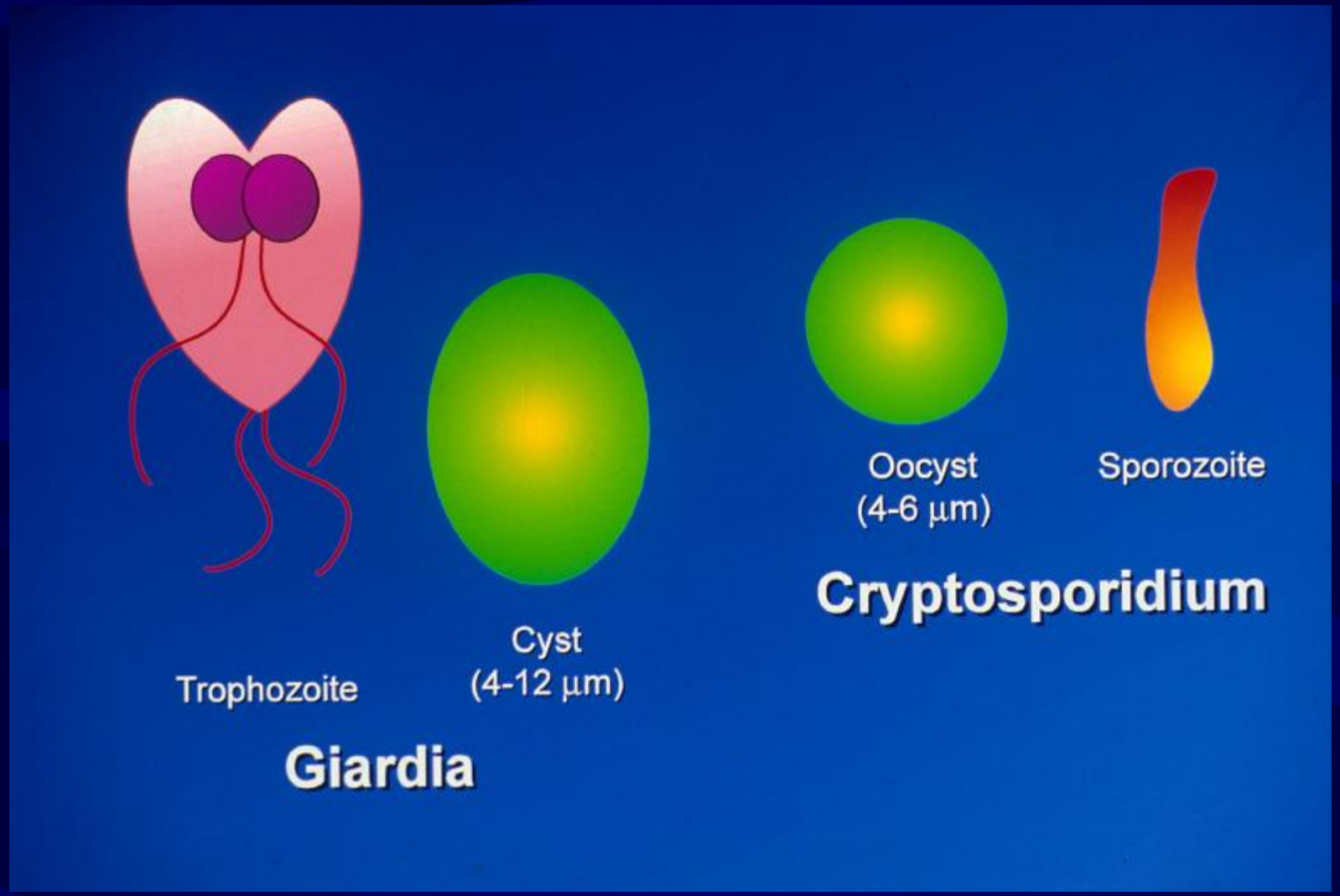
Parasites



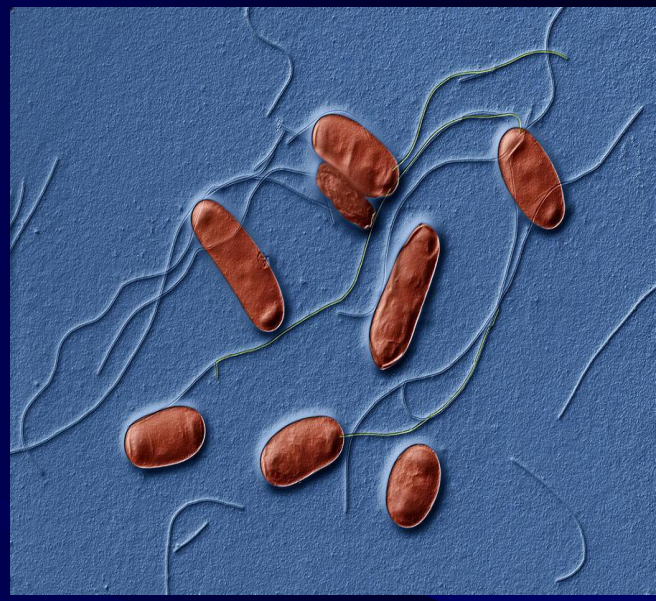
Illnesses Associated with waterborne viruses (new ones every year)

- Diarrhea
- Hepatitis A and E
- Fever and rash
- Meningitis
- Hand, foot and mouth disease
- Myocarditis
- Paralysis
- Mental disorders




Trophozoite
Cyst
(4-12 µm)
Giardia
Oocyst
(4-6 µm)
Sporozoite
Cryptosporidium

Waterbased Pathogens



- A pathogen which grows in the water
- Examples
  - *Legionella* (respiratory infection)- problem in showers heads
  - *Pseudomonas* – CDC study - most common cause of waterborne illness in the United States – contact lens infections, swimming ear infections
    - Eye, ear, skin and respiratory infections
  - *Mycobacterium spp.* – respiratory infections
    - Will grow in chlorinated distribution systems. Common in shower heads. Very resistant to chlorine and UV light.

Helicobacter pylori

Blue Green Algae toxins

→ Carcinogens

Toxoplasma

Coxsackievirus

→ Teratogens (Birth Defects)

Hepatitis A

Hepatitis E

→ Hepatogens (Liver Damage)

Campylobacter
Coxsackievirus
Echovirus
→ Nervous System Disorders

E. coli
Microsporidium
→ Renal Disease (Kidney Failure)

Coxsackievirus
Adenovirus
→ Heart Disease

Endocrine Disrupters

Coxsackie virus

- orchitis

Yersinia enterocolita

- Grave's Disease

Giardia lamblia

-hypothyroidism

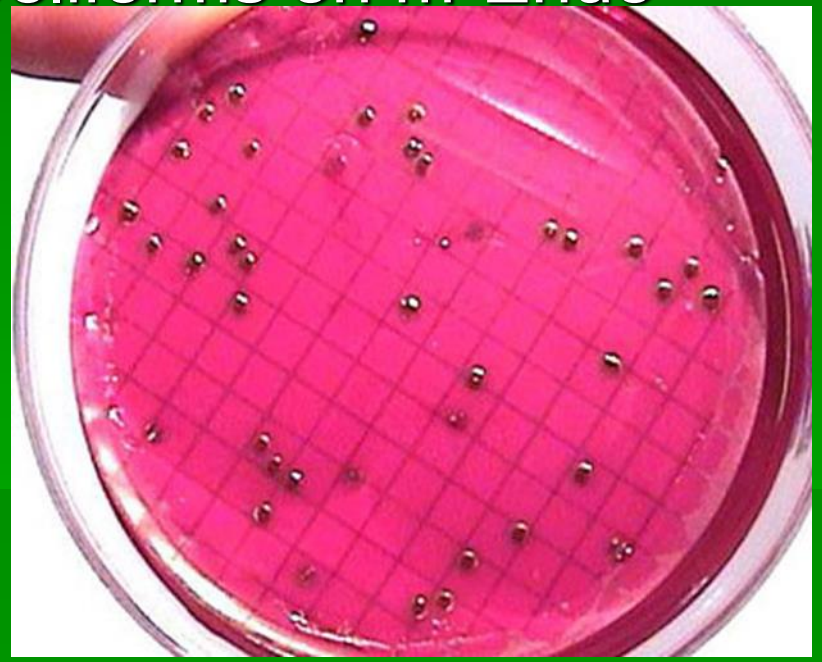
Heliobacter pylori

- atrophic thyroiditis (?)

Coliforms

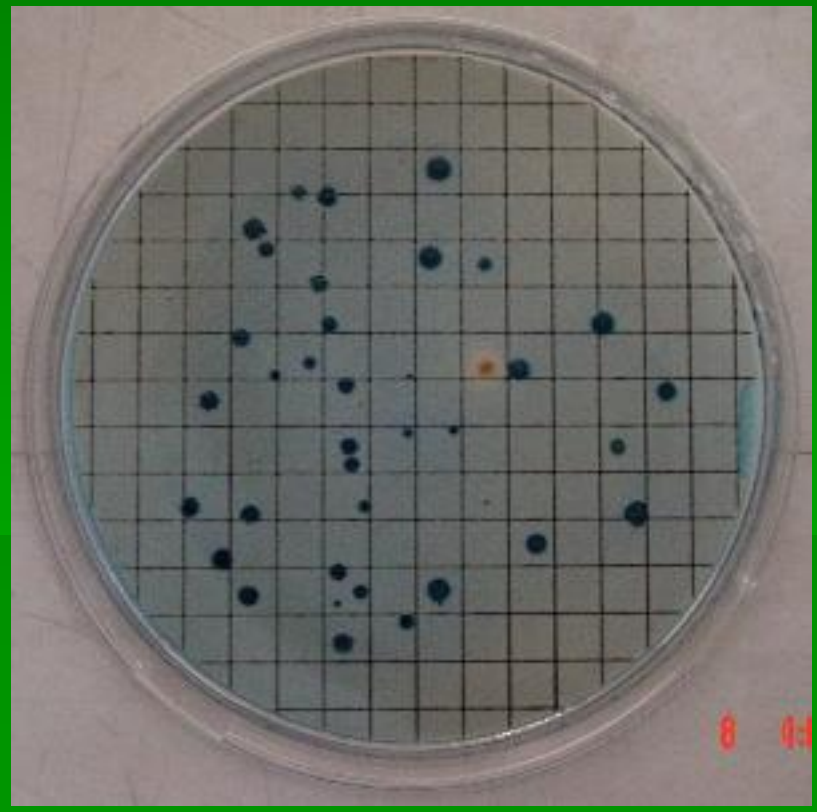
- Most used water microbial water quality indicator
- Can grow in stored wastewater
- Non-fecal sources
- Considered a conservative indicator for wastewater reuse
- Usually, 100 per 100 ml or less for standards

- Coliforms on m-Endo



Fecal Coliforms (mFC media)

- Considered more reflective of fecal contamination
- Used in pass for sewage discharges and recreation bathing standards

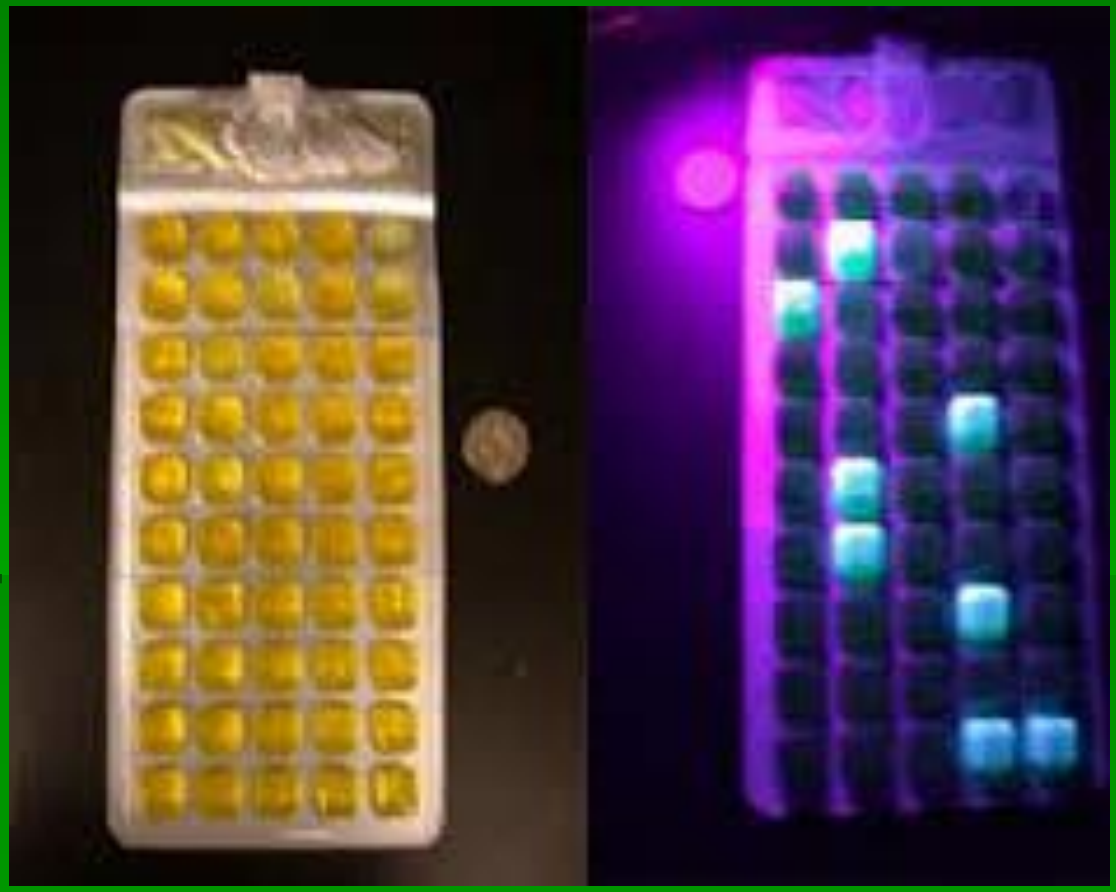


UV Light needed to Determine Presence of E. coli

- Visible light – yellow coliforms
- UV light blue fluorescence indicates *E. coli*

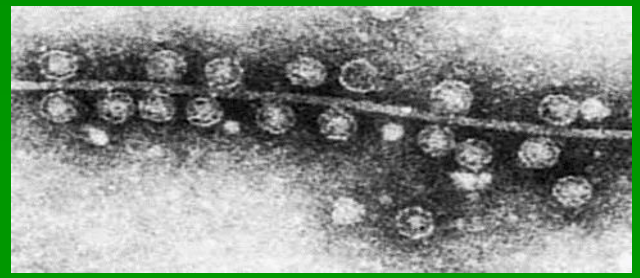
Escherichia coli

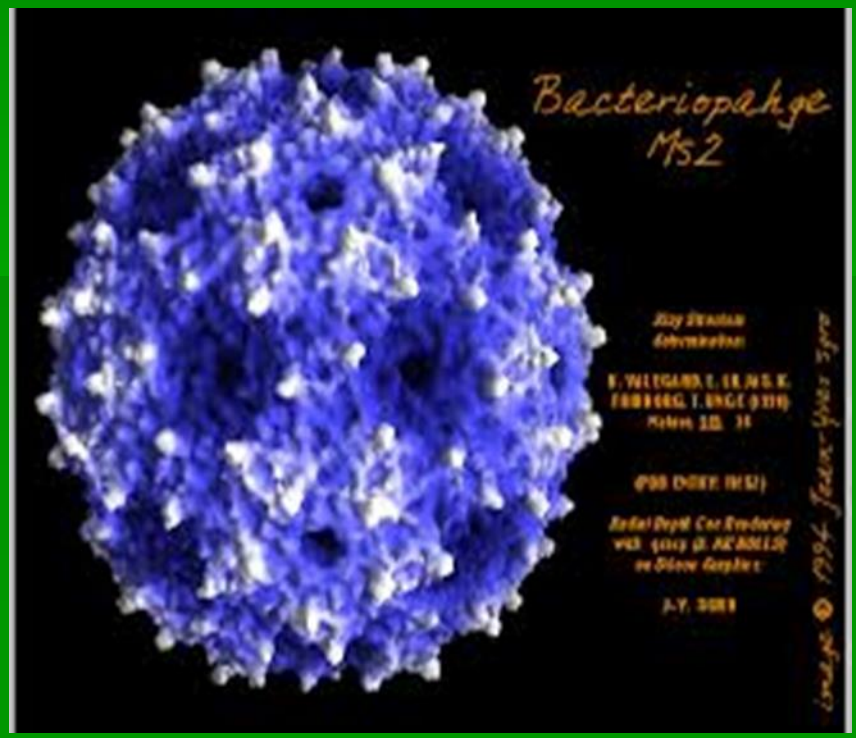
- Present in the feces of all warm-blooded animals
- Current indicator used for drinking water, produce irrigation and recreational standards- concentrations linked to illness in bathers



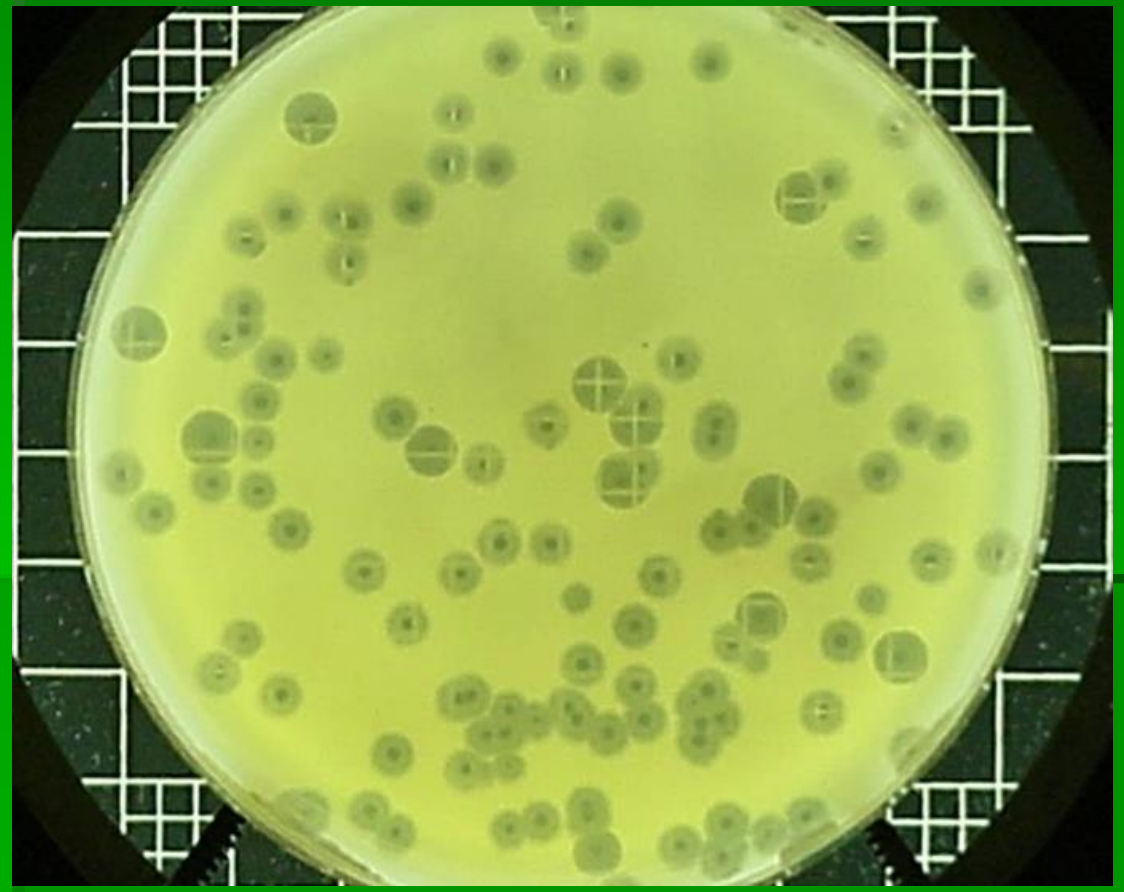
Coliphages (Bacterial Viruses)

- Bacterial viruses which infect coliform bacteria
- *E. coli* usually used as the host bacterium
- Two groups
  - Somatic coliphage
    - Attach to cell wall receptors
  - Male of F specific coliphage
    - Attach to the F^+ or sex pili





F-specific RNA Coliphage

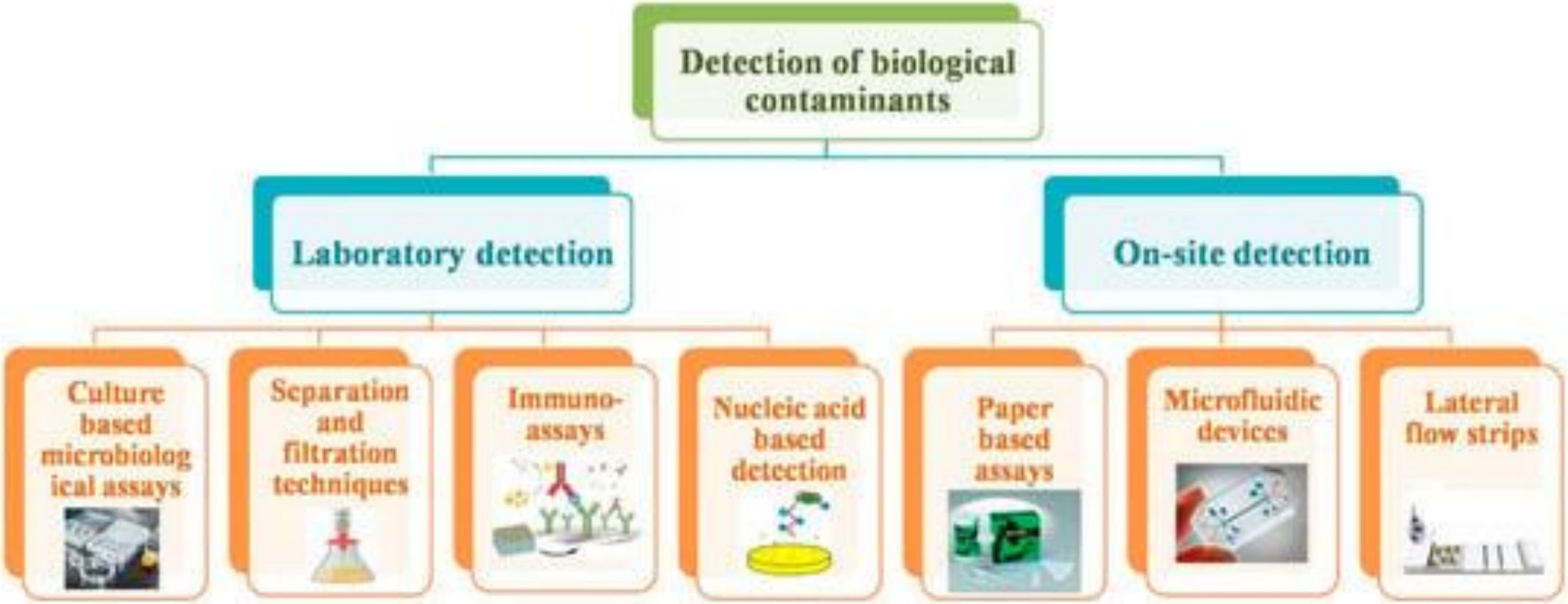


- Similar size and shape to most of the waterborne human enteric viruses
- Survival times in the environment and resistance to disinfectants are similar or greater compared to waterborne human enteric viruses

F-specific RNA Coliphage

- Used as an indicator of possible virus contamination in groundwater – Groundwater Treated Rule – USA
- Suggested as a possible standard for marine bathing waters in Europe
- USEPA considering standard for bathing waters in the United States

New Rapid Methods for Detection of Indicators and Pathogens



Test Kits now Available for Coliphage

BP1619

Bluephage Easy Kit for Enumeration of F-specific Coliphages

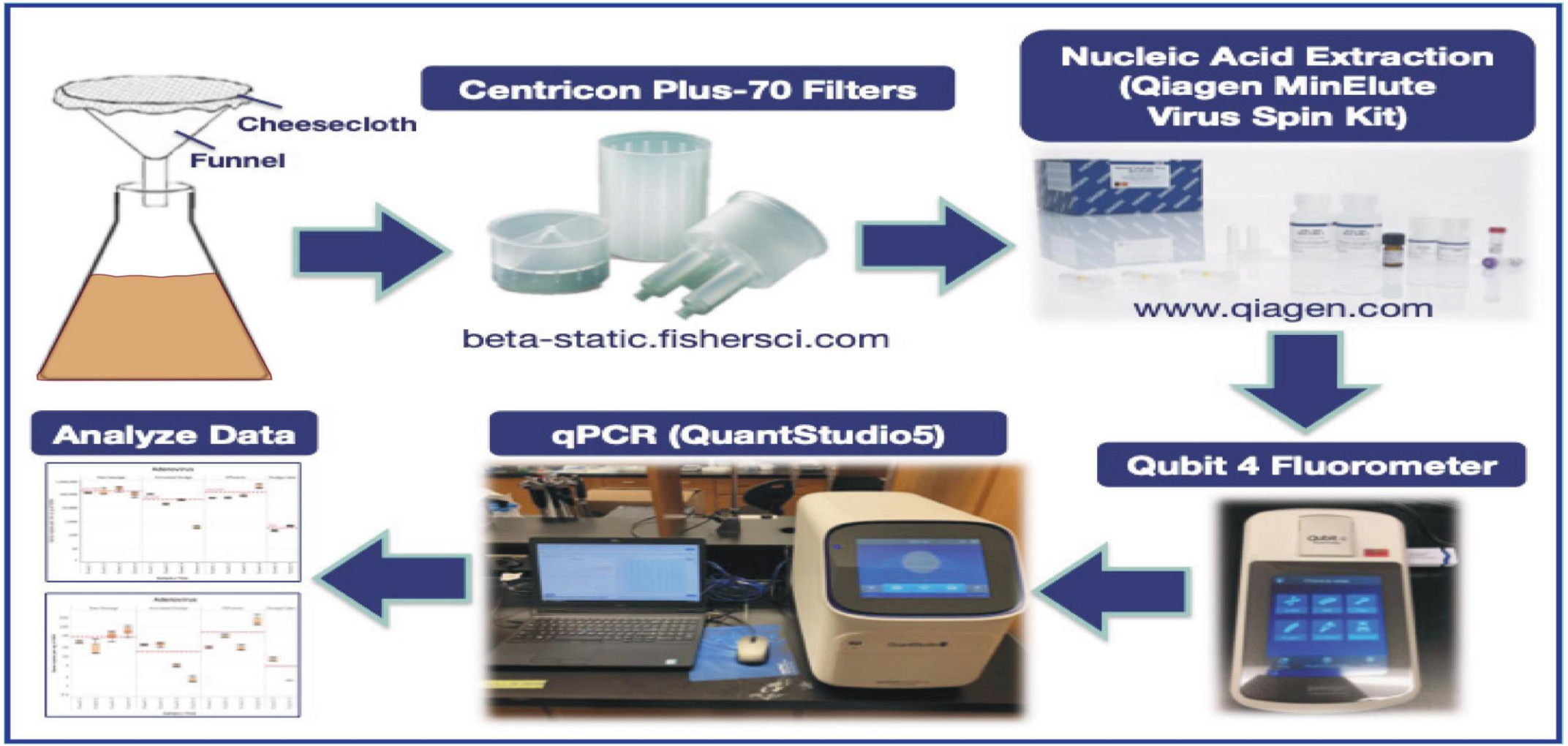
Bacteriophage: F-specific Coliphages
Method: US-EPA 1602, 1642 and 1643

Description
This detection and enumeration kit is based on US-EPA 1602, 1642 and 1643 methods. It contains all the consumables and biological material required to perform the analysis, including freeze-dried specific host-cells for the F-specific coliphage group, which are ready for use after 120 min of incubation.
TSA and TSB ready to use
Host strain (HS) and positive control included (MS2)

Additional material required and not provided in the Kit:
• Sterile Petri dishes

ColiMinder – Rapid automated remove control system for Monitoring Coliforms and *E. coli* Results in 15 minutes

New Test Kits for Virus Detection in Water by DNA/RNA Detection



Summary

Microbial Indicators

- Designed to insure proper operation of the treatment process

Waterbased Pathogens

- Need to be better assessed in Water Reuse Applications. Can grow in disinfected water distribution systems and devices (showerheads)

Log Reduction Values

- Designed to ensure treatment processes (system) control risks from bacterial, viral and protozoan pathogens