U. S. Environmental Protection Agency, Office of Research and Development

Homeland Security Research Program

Field Protocols for Collection and Concentration of Water Samples

Detection of Pathogens and Bioterrorism Agents

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Outline

- Background. Need for effective sampling and sample processing methods for detecting pathogens in water
- A key step in sample processing: concentration of target organisms
- Evolution of the current concentration process
- Details of the field methods for concentration

Scenario: Suspected Bioterrorism Event

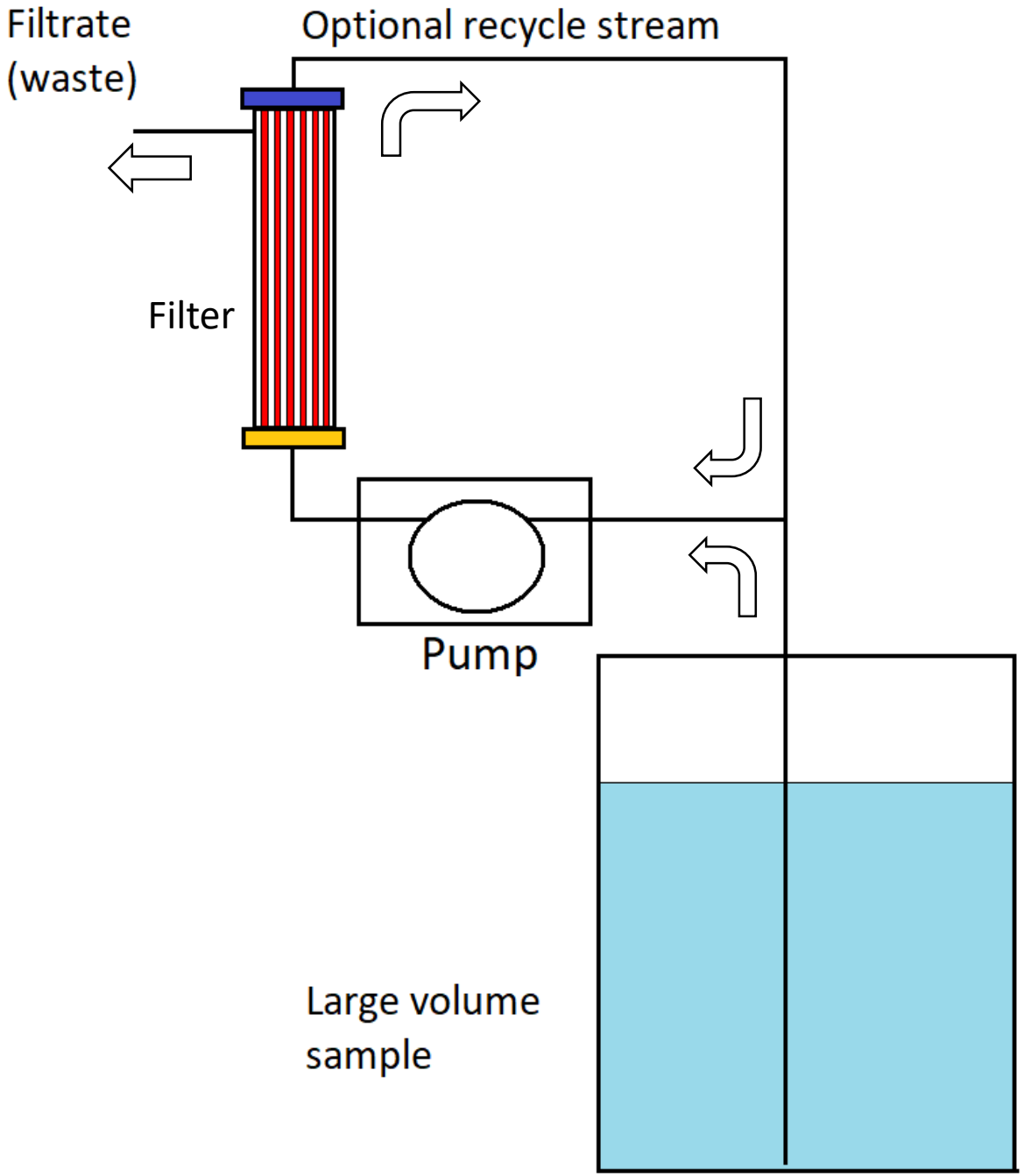
- Drinking, ground, or source water
- How to detect contamination?
  - Collect large volume water samples (40 –100 liters)
  - Concentrate microbes from a large volume down to a small volume (e.g. ,100 liters down to 0.5 liters)
  - Analyze concentrate in laboratory

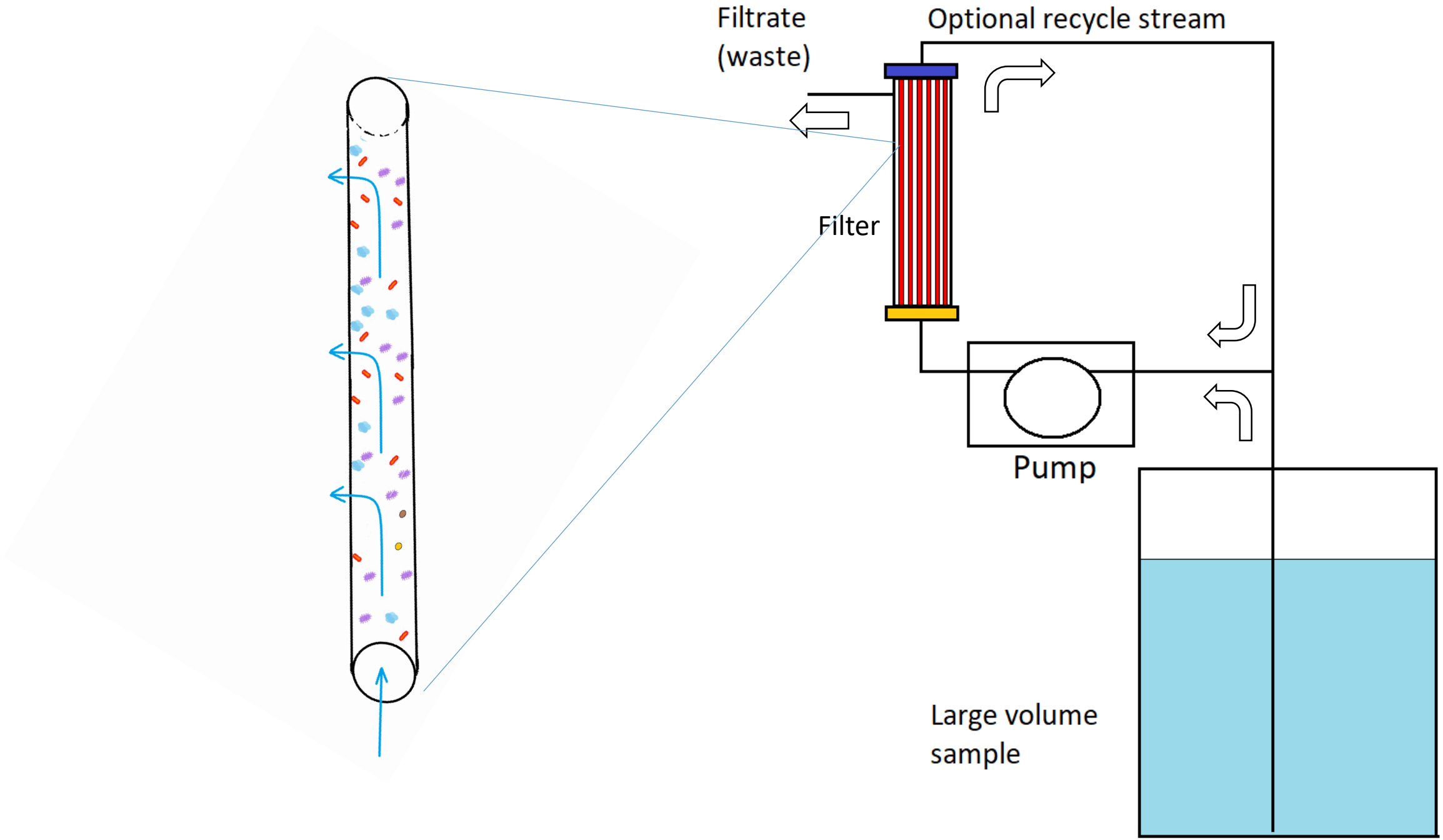
Scenario: Suspected Bioterrorism Event (Cont.)

- Where to sample?
  - Water main? Building plumbing? Drinking water plant? Reservoir?
- How to concentrate?
  - Specific for one type of organisms (e.g., spore)
  - Or one method for all types (virus, bacteria, protozoa)
- Concentrate in the field or lab?

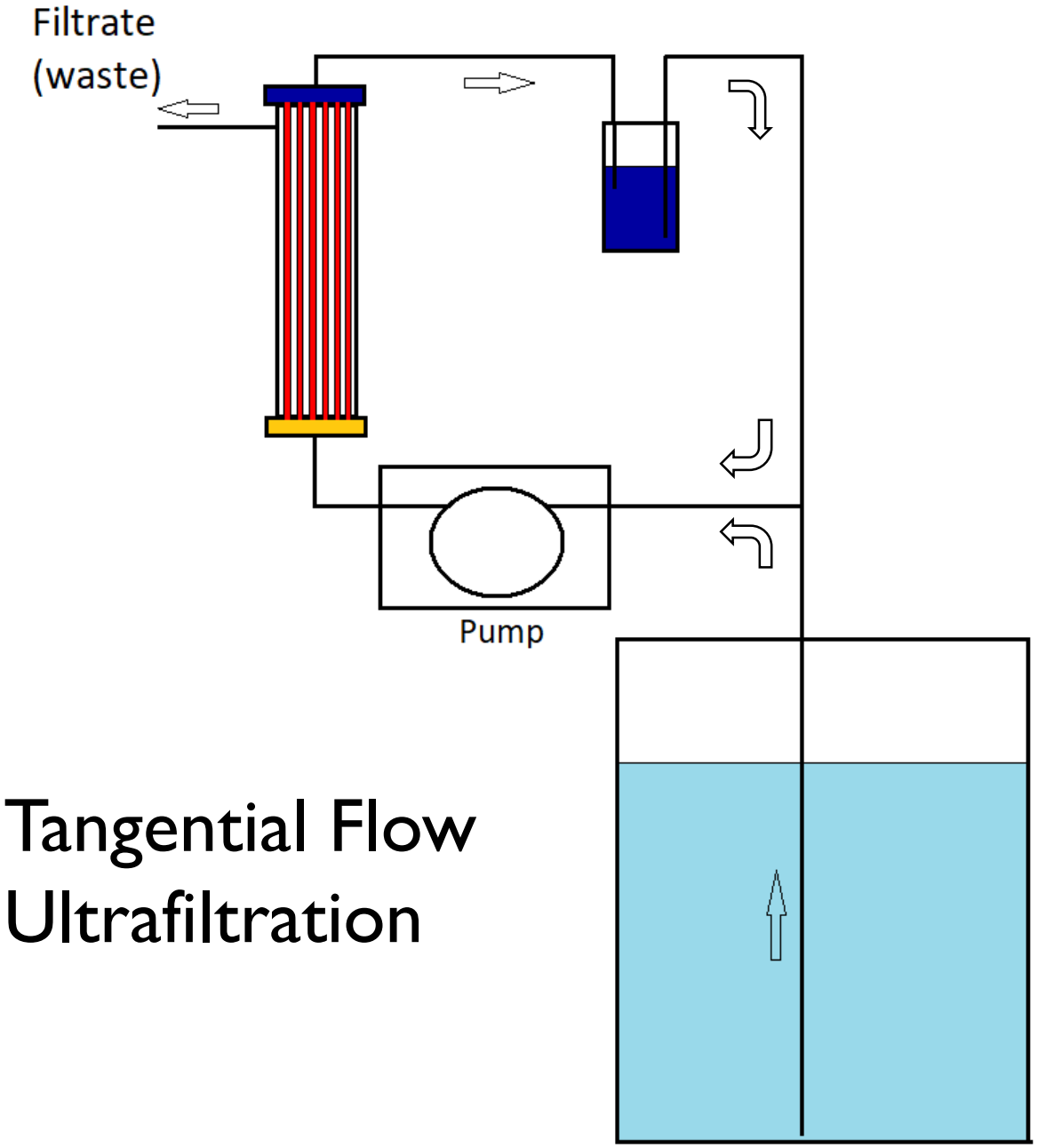
Recommended concentration Method:

- Hollow Fiber Ultrafiltration
  - For both biothreat agents
  - For routine monitoring of water borne pathogens



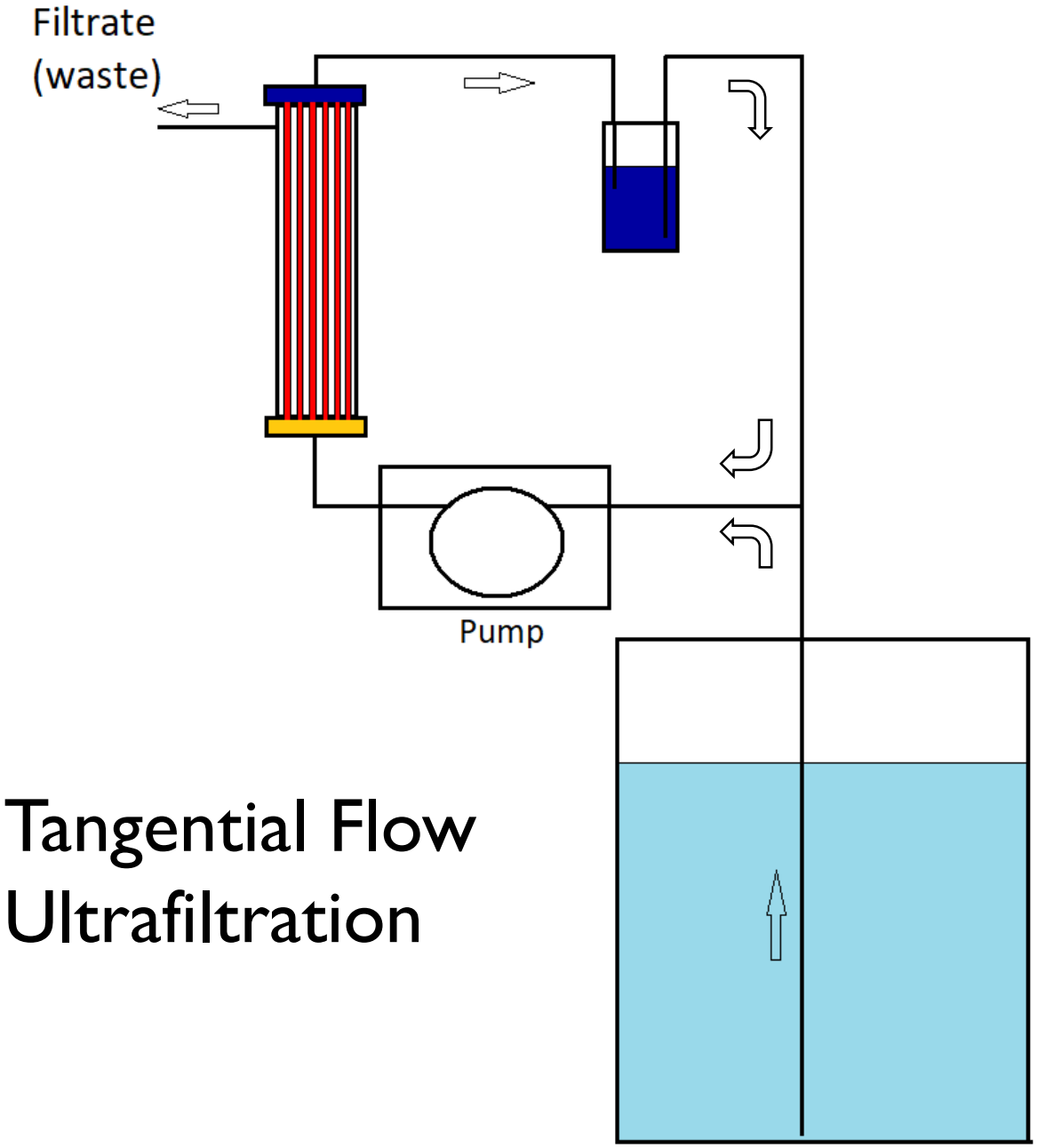

Filtrate
(waste)
Optional recycle stream
Filter
Pump
Large volume
sample

- Dialysis filters work well for this application
- Retains molecules with a molecular weight > 30,000 daltons
- Rough approximation: retains particles larger than 0.01microns
  - Captures viruses, bacteria, bacterial spores, protozoa
- 2.5 m^2 surface area for filtration (~15,000 fibers)
- Inexpensive (~$15 – 20) if used for non-medical purpose



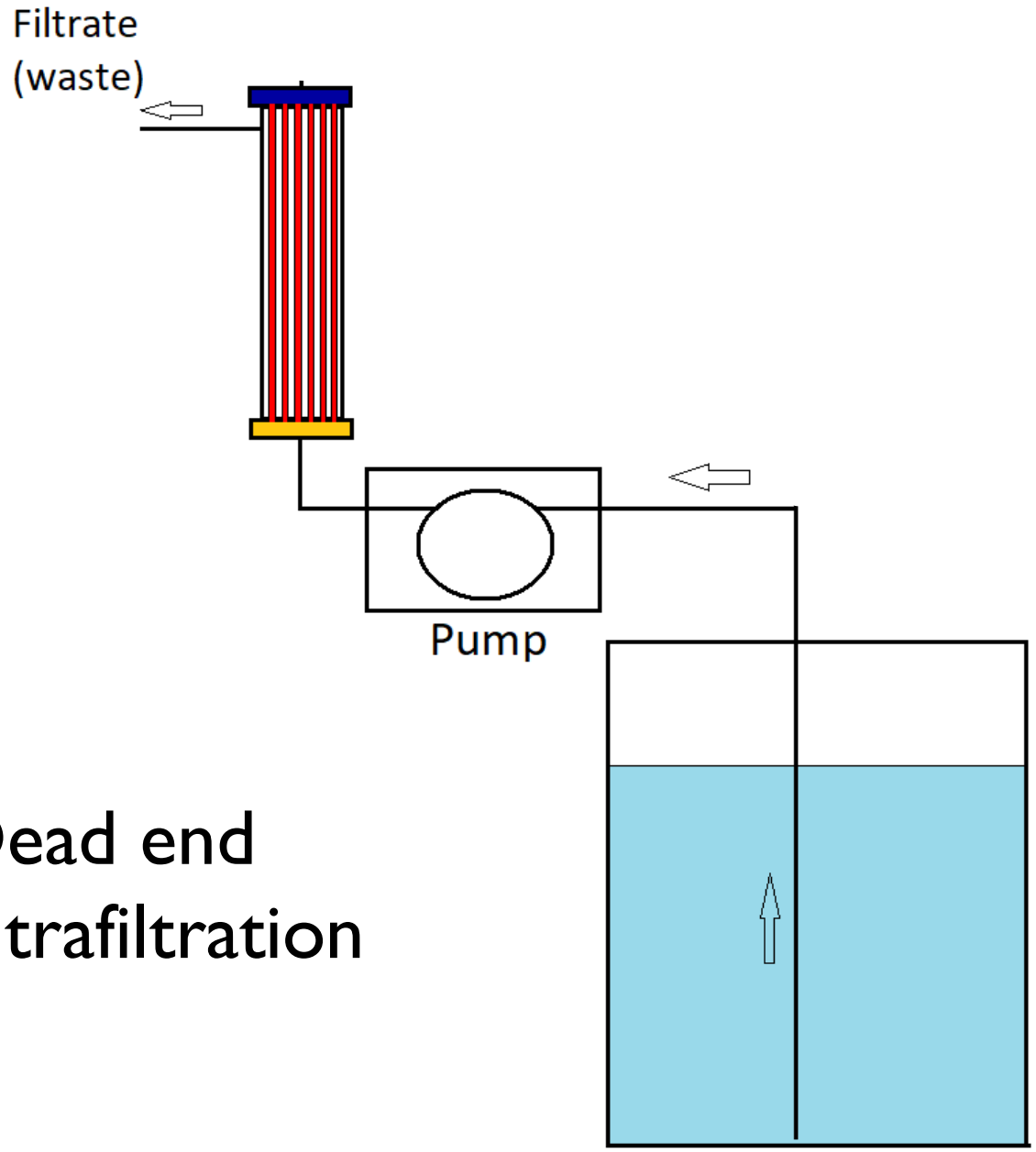
Tangential Flow Ultrafiltration

- Initial process was based on tangential flow filtration
- Lab based method developed
- Prototypes for field-based concentration built and patented and licensed
- Both methods highly effective in recovering organisms
- Challenges with both lab and field methods



Tangential Flow Ultrafiltration

- Challenges for both methods
- Lab based method
  - Required shipment of large volumes samples to laboratories.
  - Difficult for labs to process samples
- Field prototypes
  - Commercial partners: cost to produce devices outweighed the anticipated profits.



Dead end ultrafiltration

- Current recommendation: Concentrate in the field
- Use dead end filtration (no recycle stream)
  - Filter's large surface area minimizes clogging
  - Simpler to set up in field
- CDC and EPA collaborating in writing protocols for concentration in the field

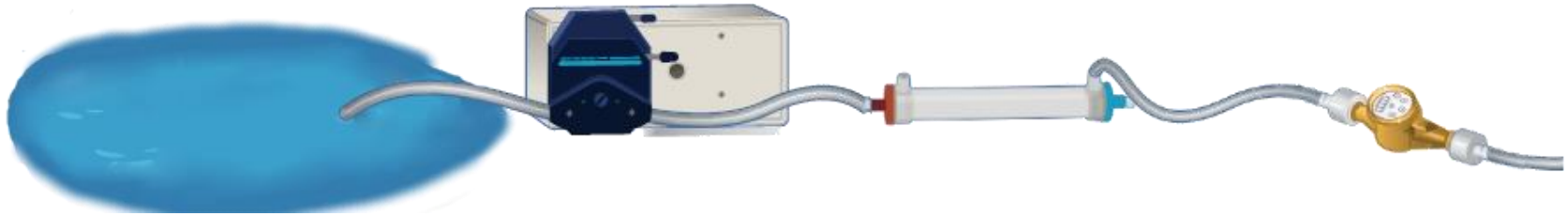
Field Protocols for Sample Collection and Concentration

- Three options
  1. Collect 1 liter grab samples for lab analysis (no field concentration)
  2. Connect filter directly to pressurized water source
     e.g., valve in water main, fire hydrant, hose bib in a building's premise plumbing
  3. Pump water from container/reservoir through filter
- Field protocols will give detailed instruction on each method
- Target audience:
  - Federal, State and local emergency responders
  - Waterborne outbreak environmental investigators

Concentration from a pressurized source

- Relatively simple to setup (no pump needed)
- Sample collection and concentration occur simultaneously
- Microbes collect on the filter
- After concentration filter is capped and sent to lab.
- Challenges:
  - Potentially high pressure
    - Pipe pressure can vary over time
  - Local water utility may require backflow prevention
  - May need to improvise plumbing connections

Concentration from a non-pressurized source



- Water either collected in a large container or drawn from a reservoir or other surface water
- Peristaltic pump forces water through filter
- Challenges:
  - Requires pump and source of electricity
  - Pump can over-pressurize filter if clogging occurs
  - Potential for exposure from sample water in large container (splashing)

Scope of field protocols

- Detailed instructions on preparing and conducting collection/concentration
- Recommendations for ensuring biosafety
  - Protocols designed so that operator can carry out in Level B personal protective equipment, if needed
- Sampling designs, packaging and shipping samples, and laboratory network capabilities
- May be supplemented with how to videos (e.g., YouTube)

Plans for a semi-automated concentration device

- Exploratory stages
- Prototypes had been built based on tangential flow filtration
- Build on past experience to design and build concentration device based on dead end filtration
- User friendly, computer controlled and encased, for less variability, more biosafety
- Design and build prototype that can be easily reproduced, "Maker friendly"
  - Low cost
  - Goal: help ensure technology can be more easily transferred to potential users
  - Not as dependent on a commercial partner to produce

Schedule for Completion of Field Protocols

- Anticipated that the protocols will be published in 2021.
- Will also be incorporated into EPA's Sampling and Analytical Document (ESAM)
  - https://www.epa.gov/esam

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Questions?

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