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Advances and Innovations to Achieve Microbially Safe and Sustainable Water: Detection, Treatment, and Risk Management

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Abstract

In the late 1800s and early twentieth century, the emergence of microbiology science and advances in engineered water and wastewater treatment ushered in a sanitary revolution that improved the microbial quality of water and wastewater and reduced the risks of waterborne disease. Yet, by the mid-twentieth century, many unknowns still remained about human enteric virus pathogens in water and fecal wastes and their risks of causing waterborne diseases, such as poliomyelitis, infectious hepatitis, and gastroenteritis.

With advances in the ability to culture human enteric viruses in mammalian cells in the 1950s and 1960s, it became possible for scientists and engineers to investigate and quantify the occurrence and risks from human enteric viruses in water and wastewater. The first international conference on "Transmission of Viruses by the Water Route," held in 1965, catalyzed the creation of the field of environmental virology. At that time, I entered this emerging field as a graduate student and began to research, along with others, the many questions about human enteric viruses in water, wastewater, and the environment.

Since then, major advances have been made in the following areas:

- Methods to concentrate and detect human enteric virus pathogens in water and wastewater.
- Determining the survival and transport of viruses in water and other environmental media.
- Removal and inactivation of viruses by water and wastewater treatment processes.
- Determining the effectiveness of coliphages (i.e., bacterial viruses of *E. coli*) as fecal indicator viruses to predict the presence and risks of enteric virus pathogens.

These advances were facilitated by the development of quantitative microbial risk assessment (QMRA) in the 1990s and its incorporation into a new, integrated, holistic, health risk-based framework, called The Stockholm Framework, for safe water and sanitation developed by the World Health Organization.

The creation of this framework for safe water and sanitation coincided with the United Nations Millennium Development Goals (MDGs) that included, by 2015, reducing the number of people worldwide lacking access to safe water and sanitation. While the MDGs were influential, many people today still lack access to microbially safe water and effective sanitation, especially in the developing world. With the launch of the new 2015 Sustainable Development Goals, including a goal to achieve sustained access to microbially safe water and adequate sanitation for all by 2030, we now have an opportunity to further contribute new and improved innovations and advances in science and engineering to achieve this much needed goal. There is still much to do, and we can all contribute in our own ways.

1. Introduction

1.1 Role of Scientific and Technical Innovations in Microbiology to Advance Water, Sanitation, and Hygiene

The need for clean water and effective sanitation and hygiene arose from concerns about communicable diseases that had been known, but not understood, for centuries. It was not until the mid- to late-1800s that scientific and technical evidence began to link certain recognized diseases (e.g., cholera, typhoid fever, dysentery, and hepatitis) to fecal contamination of water, inadequate sanitation, and poor personal hygiene. During this time, the following occurred:

- The germ theory of infectious diseases was developed.
- The seminal epidemiological investigations of John Snow linked a cholera epidemic in London in 1854 to a fecally contaminated water pump.
- An inquisitive physician named Ignaz Semmelweis documented the protective effects of hand washing with bleaching powder (i.e., calcium hypochlorite solution) to reduce the risk of disease to hospital patients.

In the late 1800s, a new era dawned in the science of microbiology. Practical innovations and new tools to culture, observe, isolate, and identify the bacteria, protozoan parasites, and viruses that caused infectious diseases made it possible to link them to fecal wastes and contaminated water. These innovations included:

- Improved microscopes.
- Microporous (bacteriological) ceramic filters.
- Techniques to stain cells with chemicals.
- Observation and quantification of bacteria growth with gas production in different liquid culture media.
- Observation and enumeration of individual bacteria as discrete colonies on solidified (gelled) culture media.

The bacterial agents that caused typhoid fever *(Salmonella typhi)*, cholera *(Vibrio cholera)*, and bacterial dysentery *(Shigella dynsenteriae)* were all discovered before 1900 (Exner, 2015). *Escherichia coli (E. coli)* and related coliform bacteria also were discovered at this time and became recognized as common inhabitants of the intestinal tract that are shed in high concentrations in human and animal feces.

Through the use of the simple broth culture and solid media culture techniques that had been developed, *E. coli* and other coliforms soon became widely used as convenient indicator bacteria to detect and quantify the fecal contamination of drinking water and other environmental media. Bacterial water quality criteria and quantitative standards were established and came into use in the early twentieth century. These bacteria (along with their culture and quantification methods and historical water quality criteria) remain in use today.

Despite the availability and widespread use of these simple, inexpensive culture-based methods to routinely detect and quantify *E. coli* and other fecal indicator bacteria in water and wastewater, there are many places worldwide where drinking water, recreational water, and crop irrigation water, as well as treated and untreated wastewater discharges to the environment, are rarely or never tested for

microbial quality. Too many people suffer and die from waterborne diseases because the water is not protected from, treated against, or tested for fecal microbes.

In this lecture, some major barriers to microbially safe water will be considered to highlight the progress made over the course of my 50 years in this field in the following areas:

- Access to simple, practical tools for anyone to test the microbial quality of their water anywhere at any time.
- Simple, sensible, user-oriented, and holistic systems to assess and manage the microbial risks of water from its source to the consumer.
- Addressing the range of pathogens in water and wastewater, especially human enteric viruses

1.2 Microbial Water Testing and the Ability to Assess Drinking Water Quality to Determine Safe Water Access

The lack of regularly available and strategic microbial water quality testing for water and wastewater systems at critical control points remains an unmet need in many parts of the world, contributing to the ongoing lack of access to safe water, sanitation, and hygiene. Quantifying the microbial quality of water and wastes at the right times, in the right places, and for the right reasons makes it possible to distinguish between safe and unsafe water, fecal wastes, and their residuals. Microbial monitoring can trigger actions and support efforts to improve and maintain the quality of water and wastes through technical measures and behavior changes for improved water quality and effective sanitation.

2. Water Quality Monitoring to Support the Sustainable Development Goals

2.1 Access to Microbially Safe Water

Beginning in the late 1990s, the United Nations mounted a global initiative using Millennium Development Goals (MDGs) to promote and track increased access to safe water. Unfortunately, this decades-long effort gave misleading results because the metric was the *type of water source used* (classified as either "improved" or "unimproved") rather than its *measured microbial quality*.

At the time, microbial water quality testing was considered unfeasible on a global scale; however, a number of studies from this period, including those in developing nations such as the Dominican Republic and Vietnam, showed that many "improved" drinking waters were in fact microbially unsafe, especially at point-of-use (POU) in households (Baum et al., 2014, Brown et al., 2013). Although this "water source classification" metric led to the conclusion that more people had gained access to safe water over the last 20 years, many of these same people still drank microbially unsafe water and remained at risk of exposure to pathogens and waterborne disease.

With the United Nation's new 2015 Sustainable Development Goals (SDGs) (Table 1), access to safe water (listed as Goal 6) will be based on measuring microbial quality. The goal is to bring microbially safe water to all by 2030. To achieve this goal, reliable and accessible methods are needed to measure the concentrations of *E. coli* and other fecal coliforms in drinking water. What microbial testing methods are available and accessible to do so worldwide? Until recently, not many.

Table 1. The United Nations Sustainable Development Goals of 2015

No.	Global Goal
1)	No poverty
2)	Zero hunger
3)	Good health and well being
4)	Quality education
5)	Gender equality
6)	Clean water and sanitation
7)	Affordable and clean energy
8)	Decent work and economic growth
9)	Industry, innovation, and infrastructure
10)	Reduced inequalities
11)	Sustainable cities and communities
12)	Responsible consumption and production
13)	Climate action
14)	Life below water
15)	Life on land
16)	Peace and justice strong institutions
17)	Partnerships for the goals

Adapted from www.globalgoals.org and sustainabledevelopment.un.org.

2.2 Innovation to Improve Access to and Encourage the Widespread Use of Testing for *E. Coli*

A major reason for the lack of monitoring the microbial quality of water globally has been the absence of simple, portable, low-cost bacteriological tests that do not require additional hardware, electricity, sterilization, or analysts with advanced skills. Not many communities can analyze microbes in drinking water. When they do, usually it is infrequent, poorly done, and conducted for the wrong reasons.

The inability to test drinking water in the field in low-resource settings and, especially, in developing countries led me to develop an alternative, simple, self-contained, portable, disposable, and affordable quantal (Most Probable Number, or "MPN") test to quantify *E. coli* in a standard 100 milliliter (mL) volume of water. Going back to first principles, the Compartment Bag Test (CBT) is similar to the multiple volume tube tests that have been used for over 100 years, but with improvements. As shown in Figure 1, the test involves a sterile clear plastic bag with five internal compartments of different volumes to which is added a 100-mL water sample supplemented with culture medium for *E. coli*. The bag is incubated overnight at ambient temperatures that can range from 25 to 44.5°C, allowing *E. coli* bacteria to grow. Then each bag compartment is observed visually for the presence or absence of a distinctive blue or blue-green color change indicating the growth of *E. coli*. Based on the numbers of compartments that are positive or negative for *E. coli*, the concentration of *E. coli* is determined using a table provided with the test. After testing, the compartment bag and its contents are decontaminated by adding chlorine tablets to kill and safely dispose of the bacteria. The CBT is a practical tool that can be used by anybody for water management, decision-making, and supporting Goal 6 of the Sustainable Development Goals.

Figure 1. Steps of the Compartment Bag Test for E. coli.

3. Designing, Operating, and Managing Water and Wastewater Systems to Address Microbial Risks

Globally, many water and wastewater systems have difficulty maintaining the microbial quality of drinking water and discharged wastewater. A key reason may be the lack of a sound rationale and basis for microbial water quality testing. In many locations, regulations require water to be tested for microbial quality and other quality parameters, but the reasons often are unclear or unknown. Hence, such testing is rarely conducted or not conducted at all, and the data are collected without clear, actionable purposes. Access to this data may not always be available immediately or at all to those responsible for water management.

Traditionally, water quality testing has focused primarily on the microbial quality of the end product. In the United States, this practice goes back to the inception of drinking water quality regulations for water in interstate commerce in 1914. It was not until the 1970s that municipal drinking water supplies and wastewater treatment systems became regulated nationally under both the Safe Drinking Water Act and Clean Water Act of the U.S. Environmental Protection Agency (USEPA); however, the historical focus remained on end-product microbial quality of water, wastewater effluents, and residuals, which undermines the need to address water and wastewater management using a systems-based, holistic, integrated approach that is health-risk based and goes beyond dealing with the engineering aspects of water and wastewater systems by utilities or other providers.

In developed nations, policies, practices, regulations, and standards for drinking water quality and sanitation management have existed almost since modern engineered drinking water and sanitation systems were first implemented in the late 1800s (Baker, 1948). For drinking water supplies, the approach has been based largely on applying engineering technologies to:

- Acquire, develop, and maintain clean sources of water, like protected aquifers and surface water reservoirs.
- Treat water by one or more alternative physical, chemical, and (sometimes) biological processes to improve quality.
- Convey water to consumers using closed and protected distribution and storage systems to maintain quality.

For more than 100 years, best practices for water treatment included:

- Slow sand (biological) filtration and, later, rapid granular media (physical-chemical) filtration.
- Chlorine disinfection and, later, ultraviolet disinfection.
- Chemical coagulation-flocculation of turbid waters with inorganic aluminum and iron salts.

The health-related benefits of developing and using improved water treatment practices were recognized in the early twentieth century as the numbers of cases of typhoid fever and dysentery decreased dramatically with the implementation of engineered water filtration treatment and the chlorination of drinking water systems. It was a tremendous achievement in sanitary engineering and public health. But despite these advances, many drinking water supplies in the United States and abroad remain vulnerable to microbial risks due to fecal contamination from point and non-point sources, especially in the developing world.

It was not until the 1990s that the following two developments occurred to better assess and address the microbial health risks of unsafe water and sanitation at the level of the user community (including the household level):

- Developing and applying quantitative microbial risk assessment as an integrated, quantitative, health risk-based approach.
- Identifying, characterizing, accepting, and formalizing POU water treatment and safe storage as valid, science-based, and practical methods of providing safe water to consumers.

4. Quantitative Microbial Risk Assessment for Safe Water

By the 1980s, Quantitative Risk Assessment (QRA) was an established science and tool for environmental and health-related decision making in other fields; however, it was only when water industry experts were mobilized in the early 1990s that QRA principles were applied to microbes of concern in drinking water and, later, wastewater. At a workshop sponsored by the American Water Works Association in 1991, a team of experts created the basic framework for quantitative microbial risk assessment (QMRA), which was later published in *Journal AWWA* (Sobsey et al., 1993). The QMRA system elements of hazard identification, exposure assessment, health effects assessment, and risk characterization make it possible to provide data that informs utility managers and others of potential health risks from pathogens in water and wastes (Figure 2). This framework led to the growing use of QMRA to inform drinking water quality assessments and management systems.

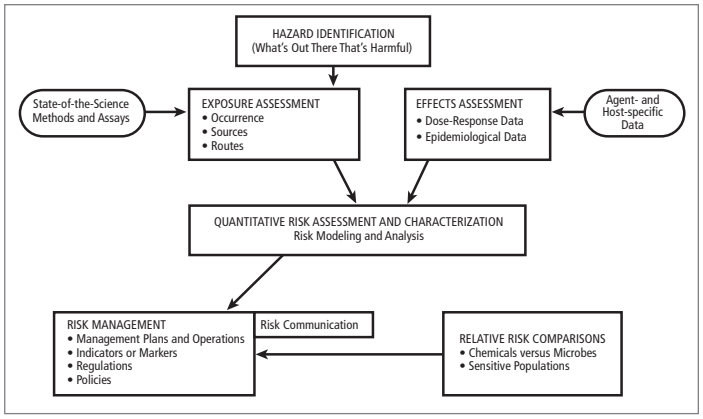

Figure 2. Conceptual framework and elements of quantitative microbial risk assessment (QMRA).

5. World Health Organization's Stockholm Framework for Water and Sanitation, and the Development of Water and Sanitation Safety Plans

Building on the development of QMRA as a system and resource to identify, estimate, and take action on microbial risks from drinking water, the World Health Organization (WHO) called on global experts in water, sanitation, and hygiene science and engineering in the late 1990s to participate in the development of a holistic, integrated health-risk based approach incorporating QMRA to manage microbial and other health risks in water and wastes. This system, called the Stockholm Framework (Figure 3), employed data on public health status (such as from disease surveillance and QMRA) to determine acceptable risks for pathogens of concern and set health-based targets that inform risk management plans for water or wastewater (Bartram et al., 2001). It addressed water quality management for

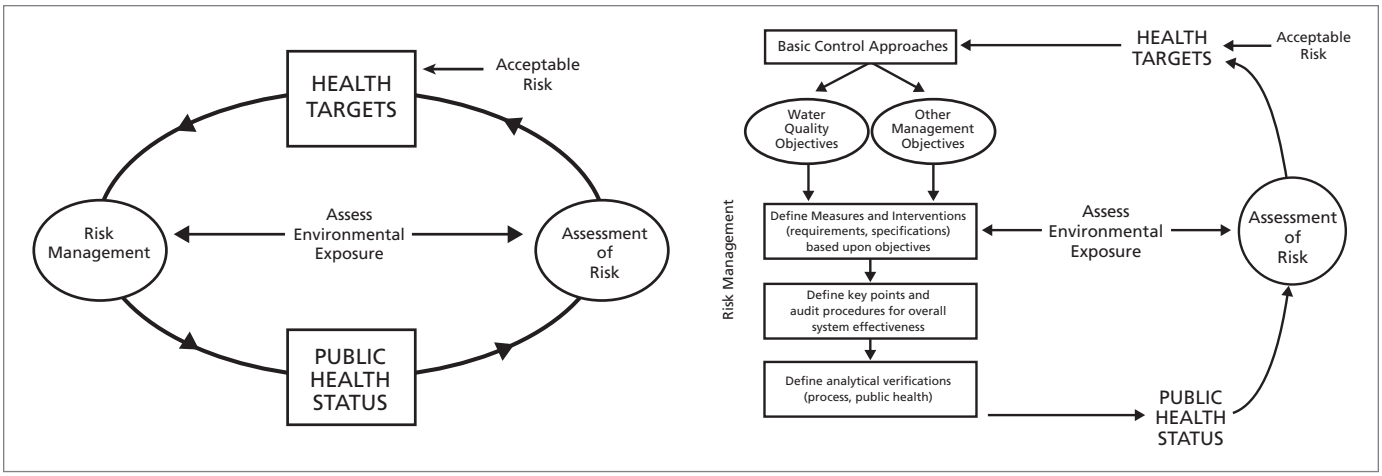

Figure 3. World Health Organization's Stockholm Framework for Integrated Management of Water and Sanitation Health Hazards.

both drinking water and recreational water, as well as the agricultural and aquaculture use of wastewater and excreta, and its principals were incorporated in the Third Edition of the *WHO Guidelines for Drinking Water Quality* (WHO, 2004).

These new health risk-based frameworks and their guidelines were transformational, leading to the development of comprehensive, practical Water Safety Plans and Sanitation Safety Plans as management systems that are adaptable to water supplies and wastewater systems of any type and scale, regardless of resources or capacities. These Safety Plans and their emphasis on systems assessments – with ongoing operational monitoring of critical control points across all elements of the system (including the use of strategic monitoring) – provide a basis for proactive management and continued improvement over time; therefore, we now have reason to be optimistic that the Water and Sanitation Safety Plans will provide a sustainable basis to achieve and maintain access to safe water and sanitation in support of the Sustainable Development Goals through the United Nations' Joint Monitoring Program led by WHO and the United Nations International Children's Emergency Fund (UNICEF).

6. Achieving Access to Microbially Safe Water through Household Water Treatment and Safe Storage

A new era began in the 1990s with the recognition and promotion of technological innovations and evidence-based science for POU household water treatment and safe storage (HWTS) by scientists, engineers, water service implementers, policymakers, and regulators to address unsafe water at the household level in the developing world (Figure 4). Until then, little attention had been paid to HWTS for developing nations (Chaudhuri and Sattar, 1990). Although managing and treating water at POU (especially in households) was an ancient and well-known practice (typically, by such methods as boiling, settling, and filtering), approaches and technologies differed greatly between the developed and developing world.

In the developed world, many POU treatment options were available commercially. In 1987, the USEPA developed performance evaluation targets and protocols as guidelines to evaluate the efficacy of POU treatment technologies, such as filters, ultraviolet radiation units, membrane filters, adsorbents, and chemical disinfectants. POU performance was based on

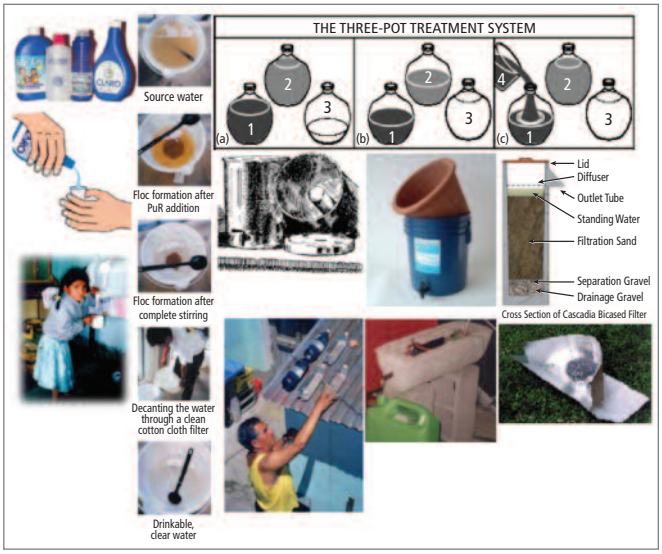

Figure 4: Point-of-use household water treatment technologies.

order-of-magnitude (log_{10}) reduction targets for bacteria, viruses, and protozoan parasites (6, 4, and 3 log_{10}, respectively), as determined by carefully controlled, independent laboratory challenge studies for treatment performance efficacy over time using waters of specified quality to which specific test microbes were added. The National Sanitation Foundation (now NSF International), an independent entity providing technical support and performance specifications to assist the water technology industry, also had its own system to evaluate and certify POU water treatment devices, based on the same or similar performance targets and protocols of the USEPA. Later, the systems used by both the USEPA and NSF International were combined and remained so until 2013.

The developing world, however, lacked many of the POU or household treatment technologies available to those in developed nations, and there was no other international guidance to identify effective POU technologies or an independent uniform system by which performance could be evaluated based on agreed-upon criteria. Many people drank water treated only by boiling, settling, or filtering. Until the Third Edition of the *WHO Guidelines for Drinking Water Quality* (WHO, 2004), POU household water treatment was not mentioned or supported by WHO guidance and performance targets, presumably because of the lack of technical- and health-based evidence that documented performance efficacy, lack of agreed-upon targets for performance, and lack of a known independent system and protocol to assess performance.

In the early 1990s, new evidence emerged from both lab and field studies to document the performance of several POU water treatment technologies used in the developing world to reduce concentrations of microbes in water. POU water treatment, by adding free chlorine or by exposing water in clear plastic bottles to sunlight for a day (called solar disinfection), began to be tested for efficacy in the lab and field (Mintz et al., 2001). Soon, such treatments were being promoted by non-governmental organizations and others. Also, the appearance of a new pandemic strain of *Vibrio cholera* that rapidly spread a cholera epidemic throughout Peru and other Latin American countries in the early 1990s served as a catalyst for POU household water treatment. A vigorous response to the cholera epidemic by the Pan American Health Organization (PAHO), U.S. Centers for Disease Control and Prevention (CDC), national governments, and others included the promotion of drinking water chlorination at POU in households to prevent the waterborne spread of cholera.

Encouraging the chlorination of water in community supplies that had chlorinators, contact basins, and access to chlorine was possible and soon implemented; however, no system existed to provide drinking water chlorination to communities and households without centralized water treatment, storage, and piped distribution systems. PAHO and the CDC collaborated with other stakeholders to develop a simple system by which household members could chlorinate water in a designated water collection and storage container (such as a 5-gallon jerry can fitted with a spigot to dispense water) by adding a capful of concentrated free chlorine solution to deliver a chlorine dose of a few milligrams per liter to the water (Quick et al., 1996; Mintz et al., 2001). The concentrated chlorine solution was produced onsite by electrolyzing a brine solution that was then dispensed into bottles and distributed with promotional materials. This so-called “safe water system” was promoted and implemented in many countries during the cholera epidemic. It continues to be promoted and used worldwide today.

Initially, some stakeholders were skeptical that the electrolyzed brine solution contained free chlorine and would kill *V. cholerae*. To address this skepticism, my lab evaluated the disinfection efficacy of the solutions produced by the electrolytic generators. In batch lab-scale disinfection kinetics experiments, we showed that the electrolyzed solutions indeed contained free chlorine at the intended concentration and killed *V. cholerae*, as well as several other enteric microbes (Venczel et al., 2004). PAHO and the CDC soon conducted the first randomized controlled field trials to show that household water chlorination and safe storage not only reduced microbes in water, but also significantly reduced the risks of diarrheal disease (Quick et al., 1999). Subsequent field trials in different countries by my group and others further documented that household water chlorination and other POU treatments (such as solar disinfection) improved water quality and reduced the risk of diarrheal disease (Sobsey et al., 2003).

Based on growing evidence that HWTS was both feasible and effective, a comprehensive critical review monograph was prepared at the request of the leadership of the WHO Water, Sanitation, and Hygiene program to describe and critique the technologies available for HWTS and the evidence that such treatments reduce diarrheal disease (Sobsey, 2002). This report became a key source of scientific evidence to support the inclusion of HWTS in the Third Edition of the *WHO Guidelines for Drinking Water Quality* (WHO, 2004).

In 2003, WHO created an International Network to Promote Households Water Treatment and Safe Storage,[1] and the University of North Carolina became a founding member. HWTS soon became recognized and accepted globally as a practical and effective way to increase access to safe water at POU. In further research, other HWTS technologies were found to be effective in producing microbially safe water that reduced the risk of diarrheal disease. Additional lab and field research through the 2000s documented that microporous filters (e.g., porous ceramic pots and candle filters; intermittent flow slow sand filters called biosand filters) also improved household water quality and significantly reduced the risk of diarrheal disease. We then analyzed HWTS technology options based on objective measures of performance and available performance data and concluded that POU filters like ceramic and biosand filters were the most effective of the simpler, low-cost technology options because they improved water quality, reduced the risk of diarrheal disease, were easy to use effectively and continuously, and did not require ongoing consumables like chemical additives (Sobsey et al., 2008). HWTS is now recognized as a key approach to achieve safe water at the POU for those who do not have access to community-piped water or for those whose community-piped water is unsafe or provided only intermittently.

7. Advances and Innovations to Address Viruses in Water and Wastes: A 50-Year Research Journey

7.1 Background on Environmental Virology

It was not until the mid-twentieth century that the scientific community began to recognize and address the health risks to drinking water and recreational water of disease-causing viruses in human fecal wastes. Viruses were first discovered in the late 1800s as unique, non-cellular pathogens that infected and killed host cells (e.g., bacteria, plants, and humans and other mammals); however, it was not until the late 1940s that it became possible to culture and assay human viruses reliably and conveniently in mammalian host cell (or tissue) cultures. A virus disease of great concern in the early twentieth century was poliomyelitis, for which transmission by fecally contaminated water had been suggested as early as the late 1800s. But it was not until the late 1930s and early 1940s that polioviruses were first detected in fecally contaminated water samples by infecting monkeys and observing the paralytic diseases they caused.

In the 1950s and 1960s, the newly developed mammalian cell culture methods to propagate and assay viruses were first applied to detect polioviruses and other culturable viruses in sewage and fecally contaminated water. At this point, the emerging field of environmental virology began to progress scientifically and technically. A major limitation at the time was the inability to directly study some of the most important waterborne viruses because they had neither been isolated and identified nor propagated in mammalian cell cultures. The viruses causing infectious hepatitis and so-called "non-bacterial gastroenteritis" still were unknown, uncharacterized, and not directly detectable, despite epidemiological evidence of waterborne outbreaks of disease being caused by them.

In December 1965, the first international conference in this nascent field of environmental virology, attended by its earliest and foremost pioneers, was held at the Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio. At that time, I was a new Masters graduate student beginning to study environmental virology. My faculty research adviser, Alberto Wachs of at the University of Pittsburgh, handed me all the conference manuscripts as a reading and learning assignment (they were eventually published in a book titled *Transmission of Viruses by the Water Route* [Berg, 1967]). I read every conference paper, took a course in medical virology, and quickly became hooked on this emerging field.

7.2 Advances in Methods to Concentrate Human Enteric Viruses from Large Volumes of Water

In the 1960s, there were no established and approved methods to reliably recover and detect either pathogenic or fecal indicator viruses from drinking water or other environmental waters, and there were no allowable limits for them in water and wastewater, as had already been established for fecal bacteria such as *E. coli* and the other coliforms. Without reliable methods to recover and detect viruses in water, it was not possible to directly quantify, assess, and manage their exposure risks from drinking water and other fecally contaminated environmental media. Reliable methods to detect and quantify viruses in water, wastes, and other environmental samples were much

[1] http://www.who.int/household_water/en/

needed to understand their occurrence, survival, transport, fate, and response to treatment processes, and such methods were being actively researched at the time.

Indeed, by the early 1970s, most of the candidate methods still used today to recover and concentrate human enteric viruses from water and other environmental samples had already been identified and were being further developed and applied to virus detection in field samples; however, the many methods available to choose from had not been compiled and critically reviewed. With encouragement from my post-doctoral mentors, Joseph Melnick and Craig Wallis at Baylor College of Medicine, I wrote a comprehensive and critical review of the methods to detect enteric viruses in water and wastewater, which was presented at an "International Conference on Viruses in Water" in Mexico City in 1974 and later published in a book entitled, *Viruses in Water* (Sobsey, 1976).

A major methodologic limitation for human enteric virus detection in water was that virus concentrations often were very low, requiring their initial concentration from large volumes for their detection. The viruses in these environmental samples also had to be purified to separate them from contaminating cellular microorganisms (particularly bacteria and fungi) and from other sample constituents that would contaminate or kill the mammalian cell cultures in which the viruses had to be grown to detect them. The efficiency of virus recovery and detection in the samples was unknown; therefore, the concentrations of viruses in these samples were uncertain.

My own research on methods to recover and concentrate enteric viruses in water and wastes began in 1971 in the Wallis and Melnick lab. Pioneering efforts were being made to concentrate human enteric viruses from large sample volumes (i.e., tens to hundreds of liters) of water and sewage using a field portable device called the portable virus concentrator (Figure 5). The portable virus concentration contained pumps, water and reagent vessels, a series of microporous cartridge filters for either the removal of unwanted particles or the adsorption of viruses, and a pH meter, all mounted on a wheeled dolly. It weighed so much and was so complicated that it took two people to move and use it, along with a water sample collection pump and gasoline-powered electrical generator. Our research focused on further improving the performance of this state-of-the-art device (Wallis et al., 1972; Homma et al., 1973; Sobsey et al., 1973). Surely, there was a simpler, easier, and lighter way to concentrate human enteric viruses from water.

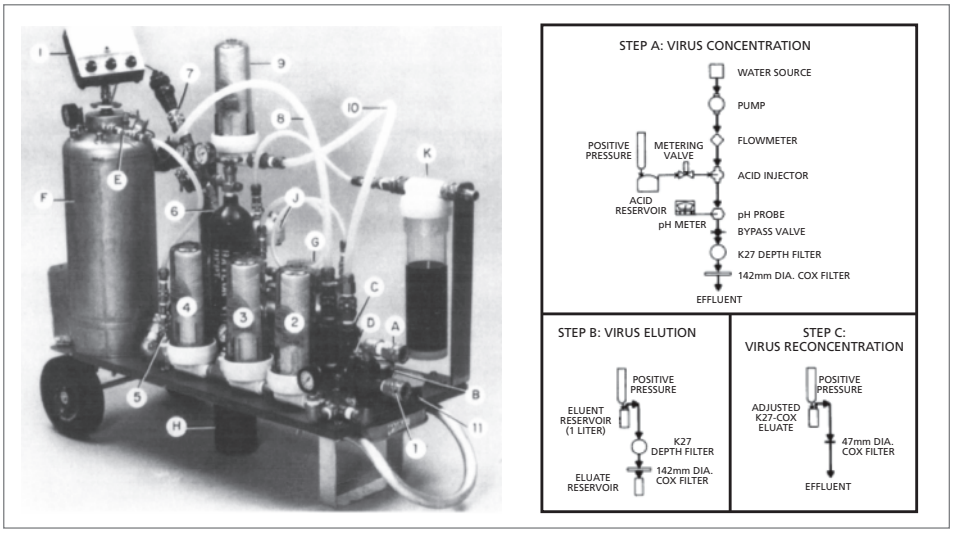

Figure 5: Portable virus concentrator developed at Baylor College of Medicine.

At that time, the microporous filters used to concentrate viruses from water and sewage by electrostatic adsorption were negatively charged near neutral pH, as were the viruses. To make viruses in water and other samples adsorb to these filters electrostatically, the pH of the water had to be lowered and multivalent cation salts of magnesium or aluminum had to be added, which required additional equipment and reagents. The viruses that adsorbed to the filter were eluted using a high pH aqueous buffer. The eluted viruses were inoculated directly into cell cultures for virus assay or were further concentrated if the sample volume was still too large. As a second step, a smaller filter or alternative concentration and purification method was used to concentrate and purify the viruses prior to their detection in mammalian cell cultures.

Upon joining the University of North Carolina in 1974, new research was undertaken to simplify and improve the recovery and concentrationof viruses from large volumes of water using positively charged microporous adsorbent filters. The hypothesis was that negatively charged viruses would adsorb directly to positively charged filters at typical pH levels in water near neutrality, without the

addition of multivalent cation salts and pH adjustment. Indeed, it was found that electropositive microporous filters adsorbed viruses directly from water at pH 8 or lower, and the adsorbed viruses could then be eluted with small volumes of alkaline organic buffer solutions for subsequent virus infectivity assay (Sobsey and Jones, 1979; Sobsey and Glass, 1980), as shown in Figure 6. As a result, an electropositive filter was all one needed to capture viruses from water. No pH adjustment, no multivalent cation addition, and no pumps or meters.

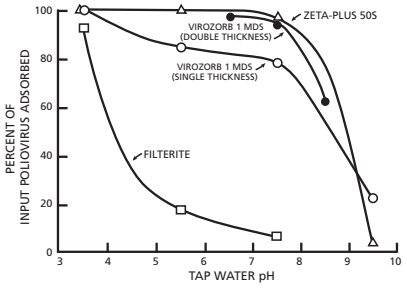

Figure 6 (Left): Effect of tap water pH on poliovirus adsorption efficiencies for AMF Cuno and Filterite 0.45-µm-porosity filters. AMF Cuno: Poliovirus input, 10^4 PFU/ml; tap water volume, 3.8 liters; filter disk diameter, 47 mm; adsorption flow rate, 1.5 ml/min per cm^2 of filter surface. Filterite: Poliovirus input 10^5 PFU/ml; tap water volume, 0.5 liter; filter disk diameter, 47mm; adsorption flow rate, 10 ml/min per cm^2 of filter surface; data from Sobsey and Jones (25).

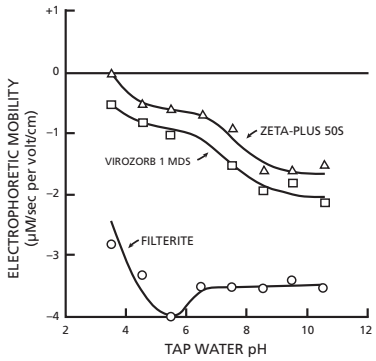

Figure 6 (Right): Effect of tap water pH on EM values of AMF Cuno and Filterite filter particles. Filter disks disrupted by low-speed blending in tap water; pH adjustment with 0.05 N Hcl or NaOH; particles electrophoresed in 300-V field.

Figure 6: Left: Poliovirus adsorption is greater to an electropositive rather than electronegative filter. Right: Surface charges of Filterite and two charge-modified filters over pH 3.5 to 11.

This finding led to the commercial development of the first customized electropositive microporous adsorbent filter to concentrate viruses directly from large volumes of water, which was manufactured and sold commercially beginning around 1980. Used worldwide, the electropositive cartridge filter became the USEPA-approved virus concentration filter to monitor viruses for the USEPA's Information Collection Rule in support of the Safe Drinking Water Act. This filter remains widely used today.

8. Hepatitis A Virus as the Cause of Waterborne Infectious Hepatitis: Advances in Environmental Virology Research to Inform Water Quality Management

8.1 Hepatitis A Virus in Water

Evidence that the acute liver disease, infectious hepatitis, was caused by viruses was first reported by Findlay, Dunlop, and Brown in 1931, and experimental evidence for its transmission in human volunteers was first documented in the early 1940s by several different investigators. Epidemiological evidence for waterborne transmission was reported by Neefe and Stokes in 1945; however, it was not until the late 1970s that HAV was first cultured in mammalian host cells, which created opportunities to detect it in water and sewage and study both its survival in the environment and response to water treatment processes, including disinfection.

Initially, HAV grew in primate cell cultures without causing visible host cell damage (i.e., cytopathogenic effects), making it difficult to detect. Its detection and quantification required tedious immunofluorescent assays that were impractical for applied environmental studies; however, a radioimmunofocus assay using HAV antibodies labeled with radioactive iodine soon was developed that made it possible to visibly see and enumerate the individual foci of HAV infection in host cell layers, analogous to virus plaques (Figure 7) (Lemon et al., 1983). This advancement facilitated applied and environmental research on HAV.

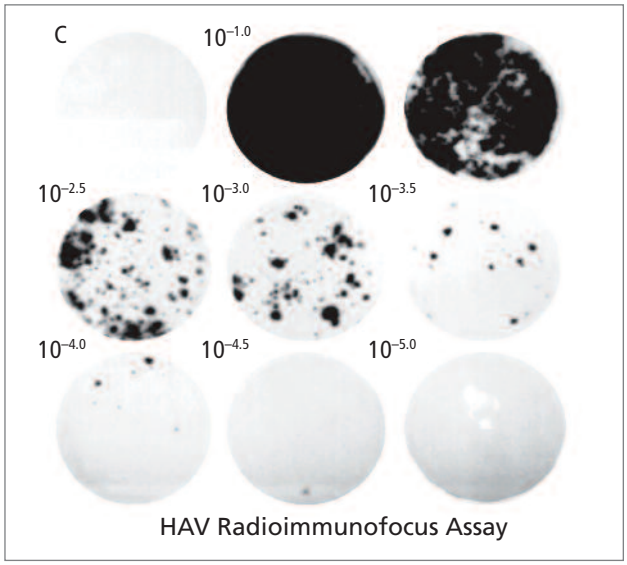

Figure 7. Radioimmunofocus assay for enumerating Hepatitis A virus in cell cultures.

The quantitative infectivity assay was used to investigate HAV survival and

transport in water and other environmental media, its response to water and waste treatment processes, and its ability to be recovered and concentrated from water by the methods used for other human enteric viruses. A suspected community-wide waterborne outbreak of HAV in rural Western Maryland in 1981 provided the opportunity to recover and detect HAV from the untreated groundwater serving as the community's drinking water. The new electropositive cartridge filter was used for HAV recovery and concentration, and detection was by both primate infection and quantitative infectivity assay in monkey kidney cell cultures (Sobsey et al., 1984). It was the first waterborne outbreak of HAV investigated using such methods to recover, isolate, and detect the infectious virus in the drinking water incriminated in the outbreak. Such methods to investigate outbreaks of waterborne viral disease now are more widely available and used regularly by the USEPA and CDC, among others.

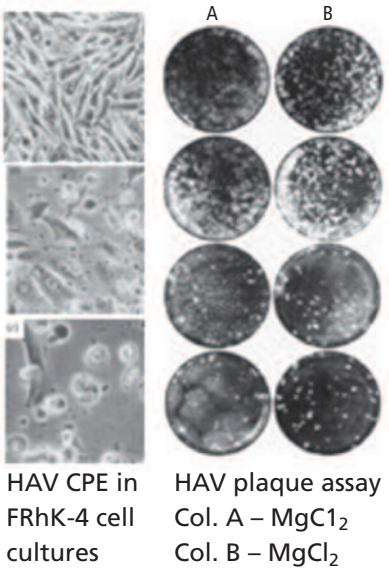

Figure 8. Cytopathogenic strain for a plaque assay of the infectivity of Hepatitis A virus.

Within a few years after the initial isolation of HAV in primate cell cultures, we were successful in selecting a rapidly replicating cytopathogenic variant of the virus that could be assayed by a standard enumerative plaque assay method (Figure 8) (Cromeans et al., 1987). This cytopathogenic strain of HAV was used for additional studies in applied and environmental virology. For example, lab-scale batch disinfection experiments were conducted to determine the inactivation kinetics of HAV in water by chemical disinfectants, such as free and combined chlorine, ozone, and iodine, and by UV radiation with low-pressure monochromatic mercury lamps (Sobsey et al., 1988; 1991; Battigelli et al., 1993; Hall and Sobsey, 1993). Much of this HAV disinfection data was used by the USEPA to develop CT values (i.e., disinfectant concentration times contact time) for the USEPA's *Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources* (USEPA, 1991).

8.2 Further Advances and Future Directions in Assessing and Managing the Risks of Waterborne Human Enteric Viruses

Over the last 30 years, further advances have been made in the detection of human enteric viruses in water and other environmental samples to inform risk assessment and facilitate the management of drinking water, reclaimed water, and other environmental exposure media. An important analytical breakthrough in the 1990s was the development of practical methods to amplify *in vitro* the numbers of copies of specific genes in human enteric viruses, other microbes, and other living creatures, within as little as several hours. These methods included the polymerase chain reaction (PCR) method and other related molecular biological methods for *in vitro* nucleic acid amplification and detection.

The ability to amplify many-fold the numbers of copies of a specific nucleic acid of an organism within a test tube using nucleic acid building blocks, enzymes, primers (i.e., specific nucleic acid templates corresponding to the virus gene of interest), and other simple reagents led to the ability to detect low numbers of human enteric viruses in water and other environmental samples. Nucleic acid methods have made it possible to detect human enteric viruses that are still not culturable in cell cultures, such as the noroviruses that are the major cause of viral gastroenteritis globally, including from drinking water exposures.These methods have greatly facilitated and advanced quantitative risk assessments of specific human enteric viruses in water, wastewater, and other fecally contaminated exposure media, such as foods.

Despite the development and effective use of both cell culture and nucleic acid based methods to detect and quantify specific human enteric virus pathogens in water and wastewater to inform QMRAs and support risk management, it is not likely these detection methods will come into widespread use to manage the risks of waterborne human enteric viruses on a practical and routine basis in the near future. Too many different human enteric viruses of health concern are shed fecally and are potentially present in water and other fecally contaminated environmental media. It makes the direct detection and quantification of all these viruses too impractical and

complicated for routine use. Furthermore, direct detection by nucleic acid methods also detects the nucleic acids of viruses that are not infectious (due to die-off and disinfection) and are no longer a human health risk, thereby giving a potentially false positive result (Sobsey et al., 1998). Instead, we need to consider the availability and use of a more practical fecal indicator virus system for all the human enteric viruses to predict their possible presences. We already do this to manage the risks of enteric bacterial pathogens by the use of *E. coli* and other fecal indicator bacteria. Is there an analogous fecal indicator for viruses? Yes, there is. It is most likely to be coliphages, the group of viruses that infect *E. coli* bacteria.

8.3 Innovations in the Detection and Use of Coliphages as Fecal Indicator Viruses

To better address the risks of viruses in water and wastewater through improved management systems (like Water and Sanitation Safety Plans), we should consider using fecal indicator viruses that are easy to detect and quantify rapidly and affordably, like the coliphages. Viruses that infect bacteria (called bacteriophages) were discovered independently by Frederick Twort in England in 1915 and Félix d'Hérelle in France in 1917. A wide range of bacteriophages of different host bacteria were discovered and isolated soon thereafter (including those infecting *E. coli* and termed "coliphages"). The presence of coliphages and other bacteriophages in human feces and sewage was well known in the 1920s using *E. coli* and other host bacteria to isolate and quantify them (d'Herelle, 1926). By the 1950s, efforts were made to detect and quantify coliphages in wastewater and water as fecal indicator viruses by either of the two culture-based infectivity methods first discovered by the bacteriophage pioneers (Scarpino, 1975), as follows:

- Propagating them in enrichment broth cultures on *E. coli* hosts, followed by spotting some of the enriched broth onto *E. coli* hosts in ager media plates to detect the lysis of the *E. coli* by the infectious coliphages.
- Direct plaque assay on agar media containing confluent lawns of *E. coli* host bacteria to observe and count the individual, clear circular areas (plaques) of coliphage infection of *E. coli* bacteria.

Despite their potential as fecal indicator viruses, criteria and standards for the use of coliphage in managing water quality were not developed until decades later and still have only limited applications.

The USEPA became interested in coliphages as indicator viruses with the development of the Ground Water Regulation in the 1990s and the need for reliable methods to detect them in large volumes of groundwater as evidence of vulnerability. A team of investigators developed and evaluated candidate methods to detect and quantify coliphages in groundwater by culture-based methods for the USEPA (Sobsey et al., 2004). Despite efforts to improve coliphage detection methods by modifications like membrane filtration and the molecular detection of coliphage nucleic acids, the original broth enrichment culture-spot plate method and a single agar layer plaque assay method (as developed decades earlier by the bacteriophage pioneers) were the most effective and easiest to use. These modified classical methods were adopted and approved by the USEPA to support the 2006 Ground Water Rule as USEPA Methods 1601 and 1602 (USEPA 2001a; 2001b; 2008).

To document the effectiveness of coliphages as fecal indicator viruses, they were compared to human enteric viruses for presence, survival, and fate in water, wastewater, and soils, and for response to water and wastewater treatment processes. It was found that male-specific coliphage (MS2) and naturally occurring F+/male-specific coliphages survived similar to HAV, Norwalk Virus, and poliovirus in fecal wastes and in various soil types suspended in treated wastewater, although HAV survived longer in seawater (Gray et al., 1993; Callahan et al., 1995; Chung and Sobsey, 1993; Meschke and Sobsey, 1998; 2003). In columns of various unsaturated soils dosed with water or wastewater, the removal of coliphage MS2 was less than or similar to the removals of poliovirus and HAV, but somewhat greater than echovirus 1 (an enterovirus) in some soil types (Sobsey et al., 1995).

More recently, the USEPA has embarked on new efforts to evaluate and develop criteria and standards for coliphages as fecal indicator viruses for municipal wastewater discharges and recreational water quality (USEPA, 2015). As a result, coliphage detection methods are now being revisited for further improvements, such as reducing the time to obtain results by monitoring. A promising approach is to

briefly enrich coliphages in broth culture on *E. coli* host bacteria for 2 to 3 hours. Then, the coliphages grown in the broth are detected rapidly by a 1-minute particle agglutination method that uses synthetic beads coated with coliphage antibodies. This method, summarized in Figure 9, works in principle, but needs further improvement to detect coliphages in less than 3 hours (Love and Sobsey, 2007).

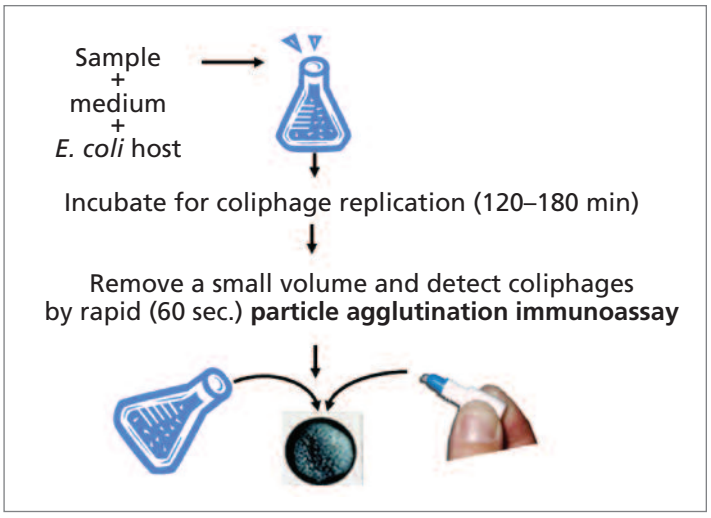

Figure 9. Coliphage Latex Agglutination Test: A simple, rapid method to detect coliphages in water.

In field studies, it has been possible to show that the presence and concentrations of coliphages in recreational waters are predictive of the risks of gastrointestinal illness in people who bathed in these waters (Colford et al., 2007). In other field studies, the concentrations of coliphages, human enteric viruses, and other pathogens in raw sewage, treated sewage effluent, reclaimed water, and drinking water sources have been assessed to determine quantitatively if the presence and concentrations of coliphages in such samples and their reductions by wastewater treatment and water reclamation processes are predictive of those for human enteric viruses. Evidence to date suggests that coliphages are predictive of human enteric viruses in these respects, although more and better data are needed to document these relationships.

Nevertheless, we need to ask why it has taken so long to examine and consider more carefully the relationships of coliphages to fecal indicator viruses. We have been able to detect and quantify coliphages for the last 100 years; therefore, it is time to look more carefully at coliphages as fecal indicator viruses and at their ability to predict human enteric virus levels and waterborne disease risks from human exposures to fecally contaminated water and fecal wastes.

9. Future Actions to Address Enteric Pathogens in Water and Wastes in the Era of the Sustainable Development Goals

In summary, tremendous advances, innovations, and insights have been achieved over the past 50 years in the field of water microbiology and health, especially to address the risks of human enteric viruses. Effective methods have been developed to detect and quantify human enteric viruses and fecal indicator viruses in water, wastewater, and other environmental media.

We now can detect all known excreta-borne and waterborne pathogens, although perhaps not easily, routinely, or cost-effectively. We can develop methods to rapidly detect new pathogens that may emerge, particularly by using nucleic acid-based molecular methods that are specific, sensitive, and rapid. Easily detectable fecal indicator bacteria and viruses now are available to better address concerns about enteric bacterial, viral, and (perhaps even) protozoan parasite pathogens. We can continue to conduct careful quantitative lab and field studies to determine pathogen and fecal indicator occurrence, concentrations, survival, fate in the environment, and responses to water and wastewater treatment processes. Together with our epidemiology colleagues, we can detect and quantify waterborne and excreta-borne disease risks from pathogens and link them to various exposure pathways and media (such as through drinking water and recreational water exposures) in which we can detect and quantify these pathogens and/or indicators for them. With the now well-established tools of QMRA, we also can make reliable, quantitative predictions of the risks of infection and illness caused by exposures to pathogens from water, wastewater, and other environmental transmission pathways. For more effective management of water and wastewater systems, we now have integrated, systems-based Water and Sanitation Safety Plans that are health-risk based, comprehensive, proactive, adaptable to different water and waste systems, and supported by new and expanding institutional resources and practical tools.

Despite these advances, we live in a world where too many people lack access to safe water and proper sanitation. More effort is needed to bring our existing resources, systems, and tools to these people, their communities, and institutions. With the new systems that have been developed through Water and Sanitation Safety Plans and the various analytical tools used to support them, such as QMRA and improved microbial detection methods, we must ask why more has not been done to achieve universal access to safe water and sanitation if the goal is access to all by 2030? It is only 14 years from now! What has to be done to transform the vision of Sustainable Development Goals for safe water and sanitation into an achieved reality? What must be done that has not been done before, and what can we do better?

We need the will and/or ability to reach out and engage with stakeholders to deliver the various existing resources needed to achieve the Sustainable Development Goal of safe water and effective sanitation for all. There are major technological, societal, behavioral, and economic challenges to overcome to achieve this goal. For example, practical, affordable, and acceptable technologies are needed to control fecal pathogens at their human and animal sources by effective containment and treatment processes. In places where community-scale infrastructure is lacking and/or infeasible, onsite sanitation systems are needed that are more effective, user friendly, acceptable, safe, and sustainable than latrines and septic-tank/soil absorption systems.

Another challenge is the inability of entrenched or ineffective bureaucracies to make changes in policies, practices, and regulations in a timely manner. These problems are beyond our expertise as water scientists, engineers, and managers; therefore, other stakeholders with different skill sets must be included in a team approach to implement and support the Sustainable Development Goal of access to safe water and sanitation. We must reach out to engage with and mobilize them.

We also need to reach out and connect with consumers and users. If the public is not included in these efforts, progress will be slow or not occur at all. Consider previous water and sanitation implementation situations that went wrong or were halted because consumers, customers, and constituents were not included in the process. We know from experience that when the public is included, progress in water and sanitation can be achieved.

As leaders and practitioners in water and wastewater science and technology and environmental health, we can make a difference in the efforts to achieve the Sustainable Development Goal of safe water and proper sanitation for all. I have some ideas as to what I am capable of doing to further contribute to this effort. I expect many of you have ideas about what you can do as well.

Here is my list of actions to take in microbial water science and technology to support the Sustainable Development Goal for water and sanitation:

1. Continue to develop new, better, and more accessible methods to test water for health-related microbes anytime, anywhere, and by anyone. Try to create new and improved tests for direct and simple culture-based detection and quantification of pathogens for which such methods are lacking, such as *Vibrio cholerae* (the bacterium that causes cholera).

2. Continue to develop new, improved, and innovative methods to reduce virus and protozoan parasite pathogens in water. For viruses and cellular microbes, we are exploring chitosans – chemical derivatives of chitin from the shells of crustaceans – to improve and make more "green" and environmentally friendly the coagulation and flocculation of water and wastewater to remove health-related microbes, rather than using alum and iron salts. We have shown in the lab that chitosan coagulation-flocculation efficiently removes viruses and bacteria from water and greatly improves their further removal by microporous filtration processes over a wide range of coagulant doses and pH levels. Now is the time to further consider such improved chemical coagulation-flocculation technologies for water and wastewater treatment to bring such treatment sustainably to both the developed and developing world.

3. Document the performance of and encourage the use of fecal indicators that address not only bacteria, but also enteric viruses and protozoans. Coliphages continue to show promise as fecal indicator viruses, and the spores of the bacterium *Clostridium perfringens* have shown promise as protozoan parasite indicators in some samples and settings. We should encourage the further evaluation and use of a suite of fecal indicators, such as *E. coli*, coliphages, and *Clostridium perfringens*, to monitor water and wastewater quality and the reduction of microbes by water and wastewater treatment and reclamation processes. In North Carolina, these three fecal indicators have been incorporated into wastewater regulations for reclaimed waters used for both nonpotable and potable purposes. Treating wastewater to meet only bacteriological effluent quality requirements may give unreliable information on the reductions of viruses and protozoan parasites, unless these other fecal indicator microbes also are analyzed.

4. Evaluate and encourage the use of fecal indicators to monitor access to safe sanitation. Currently, microbial monitoring of treated fecal wastes is not being considered as a sanitation target for Goal 6 of the Sustainable Development Goals. Yet methods exist to detect and quantify fecal bacteria in wastes that are as easy as or easier to use than those for drinking or recreational water and treated wastewater. How will we know that sanitation treatment processes and systems are effective in reducing pathogens to low levels in human wastes returned to the environment without a microbial analysis of those treated wastes? You cannot tell if fecal wastes have been rendered low in fecal microbes by just looking at the treated wastes or knowing the processes used to treat them. In the United States, we already require monitoring for fecal indicator bacteria in sewage effluent discharges; therefore, the metrics for access to safe sanitation need to include the microbial analysis of fecal wastes, just as they are included in the metrics for access to safe drinking water.

5. Search for key antimicrobial resistant enteric bacteria in wastewater and water to determine their presence, antimicrobial resistance properties, and potential to cause infection, as well as colonization and adverse health effects from environmental exposures. Antimicrobial resistance of microbes is now one of the greatest concerns of the United Nations as a global health threat. Working with WHO and the European Union, we are beginning to address the environmental aspects of the risks of these antimicrobial resistant bacteria, including how to detect and quantify key ones in water and wastes, determine the impacts of interventions to reduce their presence and concentrations in fecal wastes and water, and assess human health risks from environmental exposures. We soon may live in a world in which the bacterial infections we once treated with antimicrobials to prevent and control human illness and death are no longer treatable due to profound antimicrobial resistance. If we return to the world of the pre-antibiotic era that ended in the mid-twentieth century, we face a scary and uncertain future of infectious disease risks that we endured 70 years ago. Let's not go back to that era.

In conclusion, there is much microbiologists and other water scientists, engineers, and managers can do to contribute to the goal of safe water and sanitation for all. I have my "to do" list. Do you have yours?

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The 2016 Clarke Prize Honoree

Mark D. Sobsey, Ph.D.

Kenan Distinguished Professor of Environmental Sciences and Engineering
Gillings School of Global Public Health
University of North Carolina at Chapel Hill

Mark D. Sobsey was selected as the 2016 recipient of the NWRI Athalie Richardson Irvine Clarke Prize for his outstanding leadership and contributions to the fields of environmental health microbiology, virology, and water sanitation and hygiene. His research has resulted in tremendous advancements in the water industry, particularly in minimizing the risks of exposure to waterborne disease.

A microbiologist and environmental health scientist by training, Dr. Sobsey has worked nationally and globally for 45 years to improve water quality and protect public health. He has led groundbreaking efforts to understand, detect, and control waterborne viruses (such as norovirus and Hepatitis A and E viruses), bacteria, and parasites, and his work has directly influenced the development of guidance and policies by prominent public health safety organizations like the U.S. Environmental Protection Agency (USEPA), Centers for Disease Control and Prevention, and World Health Organization.

Among his most notable achievements, Sobsey's work on methods to concentrate and examine viruses (including fecal indicator viruses) in groundwater has become the standard for the water industry. For example, he developed an innovative filtration technique – known as the MDS filter – that was more practical and effective than conventional filters and, ultimately, helped develop a better understanding of the occurrence, concentration, and public health significance of viruses in the environment. His work in this area informed the analytical method used for viruses in the USEPA's Ground Water Rule, which standardized practices in the United States to detect and control the presence of microbial pathogens (particularly viruses) in drinking water wells. In addition, his efforts to develop improved methods to detect and control numerous waterborne viruses influenced the Surface Water Treatment Rule under the USEPA's Safe Drinking Water Act.

Dr. Sobsey received a B.S. in Biology and an M.S. in Hygiene from the University of Pittsburgh, and a Ph.D. in Environmental Health Sciences from the University of California, Berkeley.

The

ATHALIE RICHARDSON IRVINE

Clarke Prize

for Outstanding Achievement

in Water Science and Technology

The 2016 Clarke Prize Lecture, *Advances and Innovations to Achieve Microbially Safe and Sustainable Water: Detection, Treatment, and Risk Management,* was prepared by Mark D. Sobsey, Ph.D., the Kenan Distinguished Professor of Environmental Sciences and Engineering of the Gillings School of Global Public Health at the University of North Carolina at Chapel Hill. He presented the Lecture on Thursday, October 3, 2016, at the Twenty-Third Annual Clarke Prize Award Ceremony and Lecture, held at the Newport Beach Marriott Hotel and Spa in Newport Beach, California.

The National Water Research Institute (NWRI) of Fountain Valley, California, established the Clarke Prize in 1993 to recognize research accomplishments that solve real-world water problems and to highlight the importance of and need to continue funding this type of research. Dr. Sobsey was the twenty-third recipient of the prize, which includes a medallion and $50,000 award.

The Clarke Prize was named after NWRI's co-founder, the late Athalie Richardson Irvine Clarke, who was a dedicated advocate of the careful stewardship and development of our water resources. The Joan Irvine Smith & Athalie R. Clarke Foundation provide funding for this award.

More information about the Clarke Prize can be found at www.CLARKEPRIZE.com.

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