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State-of-the-Science Review: Evidence for Pathogen Removal in Managed Aquifer Recharge Systems

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State-of-the-Science Review: Evidence for Pathogen Removal in Managed Aquifer Recharge Systems

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Abstract and Benefits

Abstract:
Managed aquifer recharge (MAR) systems, such as induced bank filtration (IBF), soil aquifer treatment (SAT), aquifer storage and recovery (ASR), and aquifer storage transfer and recovery (ASTR) are widely used in the process of drinking water production, water reuse, or subsurface water storage. Potential water sources for MAR (e.g., recycled water, surface water, and stormwater) can contain a wide range of enteric pathogens that pose a risk to human health. Long-term experiences operating full-scale MAR processes confirm significant pathogen reduction. MAR processes can be very cost effective relative to other pathogen barriers, while not sacrificing water recovery. Thus, in terms of energy, sustainability, and longevity, it may be one of the most robust treatment processes for our use.

For groundwater recharge with recycled water leading to potable reuse, regulations in the U.S. typically require assigning log reduction credits for pathogens and microbial indicators, making this a critical component of the overall design and permitting of a MAR project. However, despite decades-long experience of high-quality treatment performance demonstrated at various MAR systems for pathogen removal, regulators, designers, and system operators continue to be challenged with assigning and demonstrating appropriate treatment credits for pathogen reduction.

This study fills the need to document and disseminate the state of knowledge of pathogen reduction through MAR processes using wastewater or wastewater-influenced surface water and compare and assess the benefits, limitations, and challenges of different national and international regulatory approaches for microbial disease protection. The results of this study provide guidance on options for determining defensible pathogen log removal credits in MAR systems. This study fulfills three key objectives:

Evidence Summary. A state-of-the-art compendium on the current national and international knowledge regarding removal of pathogens in groundwater systems.

Regulatory Guidance. Summarize and evaluate different national and international approaches for assuring microbial health protection of MAR systems.

Research Roadmap. Identify insufficient or missing information to develop research suggestions for further consideration by the Water Research Foundation (WRF).

Keywords: Managed aquifer recharge, pathogen, virus, oocyst, fate and transport, water reuse, regulation, microbial risk assessment, log removal.

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Acronyms and Abbreviations

1-D	One-dimensional
3-D	Three-dimensional
ADEQ	Arizona Department of Environmental Quality
ASR	Aquifer storage and recovery
ASTR	Aquifer storage transfer and recovery
ATP	Adenosine triphosphate
BAF	Biological aerated filters
BDOC	Biodegradable dissolved organic carbon
CDPHE	Colorado Department of Public Health and the Environment
DDW	(California) Division of Drinking Water
SWRCB	(California) State Water Resources Control Board
CCR	California Code of Regulations
CDPH	California Department of Public Health
cfu	Colony forming units
COMSOL	Multiphysics software package
COVID-19	Coronavirus disease
CrAssphage	Bacteriophage
DALY	Disability adjusted life years
DAPI	Staining technique using 4’,6-diamidino-2-phenylindole
°C	Degrees Celsius
DEQ	Department of Environmental Quality
DGSnp	DNA-labeled, glycoprotein-coated silica nanoparticles
DNA	Deoxyribonucleic Acid
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DWD	Drinking Water Directive
DWR	Department of Water Resources
EU	European Union
EV	Environmental value
F&T	Fate and transport
FDEP	Florida Department of Environmental Protection
FeO(OH)	Ferric oxyhydroxide
F-RNA	F-Specific ribonucleic acid bacteriophages
FRNAPH	F-specific RNA bacteriophages
ft	feet
GRS	Groundwater replenishment system
GW	Groundwater

GWR	Ground Water Rule
GWS	Groundwater system
GWUDI	Groundwater under direct influence
HACCP	Hazard Analysis and Critical Control Point
IBF	Induced bank filtration
Idaho DEQ	Idaho Department of Environmental Quality
IESWTR	1998 Interim Enhanced SWTR
IFA	Immunofluorescence assay
IGRAC	International Groundwater Resources Assessment Centre
IPR	Indirect potable reuse
ISMAR	International Symposium on Managed Aquifer Recharge
ISSM	Information System and System Management
L	Liters
LRV	Log removal value
LT1ESWTR	Long-term1 SWTR
LT2ESWTR	Long-Term 2 Enhanced Surface Water Treatment Rule
MAR	Managed aquifer recharge
mg/L	Milligrams per liter
mL	Milliliters
MODFLOW	U.S. Geological Survey modular finite-difference flow model
MPA	Microscopic particulate analysis
MS2	Bacteriophage
NAC	Nevada Administrative Code
NPDES	National Pollutant Discharge Elimination System
NTU	Nephelometric turbidity units
O3	Ozone
PCR	Polymerase chain reaction
PDF	Probability distribution function
Pfu	Plaque forming units
pfu/L	Plaque-forming unit/Liter
PMMoV	Pepper mild mottle virus
ppb	Parts per billion
pppy	per person per year
ppt	Parts per trillion
PRD-1	Bacteriophage
PWS	Public water system
QMRA	Quantitative microbial risk assessment
QMRAcatch	Online calculator model
qPCR	Molecular methods

RBF	Induced riverbank filtration
RIB	Rapid infiltration basin
RMZ	Recharge management zone
RNA	Ribonucleic acid
RO	Reverse osmosis
RTCR	Revised Total Coliform Rule
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SAT	Soil aquifer treatment
SWTR	Surface Water Treatment Rule
TDS	Total dissolved solids
Texas CEQ	Texas Commission on Environmental Quality
TN	Total nitrogen
TOC	Total organic carbon
TTC	Thermotolerant coliforms
UIC	Underground Injection Control
USDW	Underground source of drinking water
USEPA	U.S. Environmental Protection Agency
UV	Ultraviolet
UVA	Ultraviolet absorbance
Virginia DEQ	Virginia Department of Environmental Quality
Virginia HSRD	Virginia Hampton Road Sanitation District
VPA	Virginia pollution abatement
VPDES	Virginia Pollutant Discharge Elimination System
WAC	Washington Administrative Code
WSDH	Washington State Department of Health
WHO	World Health Organization
WRF	The Water Research Foundation
WW	Wastewater
ΦX-174	Coliphage

Executive Summary

ES.1 Background

Managed aquifer recharge (MAR) systems are broadly used in the process of drinking water production, water reuse, or subsurface water storage. MAR systems include induced bank filtration (IBF), such as induced riverbank filtration (RBF); soil aquifer treatment (SAT); rapid infiltration basins (RIBs); aquifer storage and recovery (ASR); and aquifer storage transfer and recovery (ASTR).

The presence of human health pathogens in water produced from MAR systems is a key risk for groundwater (GW) quality and drinking water safety that must be managed. Regulations in the United States that apply to microbial risk protection in MAR systems were mostly developed over a decade ago and were based on the best scientific understanding available at the time on pathogen occurrence, indicators and surrogates, and fate and transport in the subsurface.

However, our scientific understanding on these issues has quickly advanced in recent years with less expensive and highly sensitive molecular and other analytical detection methods as well as our understanding of pathogen occurrence, indicators, and surrogates. Progress in deterministic and probabilistic modeling has also led to new tools that can better predict fate and transport and quantify the risk of pathogen breakthrough into drinking water production wells.

Regulators in the United States are now tasked with enforcing regulatory and permitting approaches that assure public health protection, which remains challenging. Although prescriptive regulatory approaches specifying minimum subsurface retention times and maximum log removal credits are simple and consistent, they sometimes do not capture the complexities of site-specific water quality and hydrogeological MAR conditions. As a result, existing regulatory approaches enforced by some state agencies for microbial risk protection might be overly conservative, imposing unnecessary costs for MAR operation and needlessly limiting MAR water storage and production capacity in some cases.

Some monitoring requirements set by permitting agencies are not always effective in discovering and preventing microbial contamination of drinking water wells leading to outbreaks in rare cases. In some instances, microbial risk assessments are challenging because biological tracers used to inform risk may be present at too low concentrations in source waters and without detection at the drinking water production well uncertainty on log removal achieved remains for public health risk assessments.

Regulations governing pathogens in drinking water are generally based on endemic rather than epidemic disease (outbreaks). Randomized controlled trials used to measure endemic disease are complex and expensive because they require enrollment of large numbers of willing volunteers.

This study documents and disseminates the current state of pathogen reduction through MAR processes using wastewater or wastewater-influenced surface water and compares and assesses the benefits, limitations, and challenges of different national and international regulatory approaches for microbial disease protection. We focus specifically on MAR systems using treated wastewater or wastewater-influenced surface water and refer to other publications for MAR systems using stormwater or surface water. Unless otherwise specified, when MAR systems are described in this report, it pertains to those systems that are planned MAR systems that utilize wastewater or wastewater-influenced surface water as source water.

The results of this study are intended to provide planners and regulators with more nuanced guidance and options for policy and regulatory approaches to assure groundwater quality and effective public health protection for MAR systems.

ES.2 Study Objectives

This report is an outcome of a research project through The Water Research Foundation (WRF), *Compiling Evidence of Pathogen Reduction through Managed Aquifer Recharge and Recovery* (#4957). This research study has three key objectives:

1. **Evidence Summary.** This report provides a state-of-the-art compendium on the current national and international knowledge of the occurrence and quantitative removal of pathogens in managed aquifer recharge systems using wastewater or wastewater-influenced surface water.
2. **Regulatory Guidance.** This report summarizes, compares, and evaluates different national and international approaches for assuring microbial health protection of MAR systems. Methods for permitted log removal credit determinations, data collection and analysis, policy justifications, past experiences, and challenges are summarized to develop general guidance and recommendations on how regulations could be improved in the future to stay practical, effective, and defensible.
3. **Research Roadmap.** This report identifies insufficient or missing information relevant to better understanding pathogen removal, monitoring and testing approaches for site-specific demonstration studies, and regulatory implementation.

ES.3 Status and Development of MAR Systems in the United States

Today, MAR systems operate in at least 23 states, and the number is growing. Many IBF, SAT, and ASR systems have been in operation for several decades and have been thoroughly studied to characterize and quantify the removal of microbial pathogens and other source water contaminants. In recent years, MAR systems are being integrated more regularly into new or expanded treatment trains for potable reuse and drinking water production. The main drivers for this development are:

- **Increased water demand.** Continued population growth in the West, South, and parts of the Midwest require additional water sources where drinking water resources are already limited. In response, there is a need to expand existing MAR system capacity and planning for new systems for indirect potable reuse.
- **Water resilience planning.** Climate change and severe drought impacts expand beyond the

South and Western U.S. and have motivated water resource planners to diversify drinking water source portfolios. MAR is regarded as a cost-effective (pre-) treatment option that also allows for subsurface storage to strengthen supply resiliency.
- **Regulatory shift from unintentional to planned recharge.** Some facilities that have historically operated *de facto* recharge systems are now subject to greater public and regulatory scrutiny. To assure public safety, previously grandfathered systems now may require permits and monitoring programs.

Other drivers and benefits include environmental benefits related to groundwater-dependent ecosystems and over drafted aquifers, depleted streams, as well as greenhouse gas, energy, and/or cost reduction for drinking water production.

ES.4 Pathogens of Concern in MAR Systems

ES.4.1 Pathogen Occurrence

Today, the estimated list of potential pathogens in wastewater or surface water comprises over 200 pathogens that can be transmitted by water. This list is growing as molecular methods and metagenomic screening becomes more widely used and previously unknown pathogens are easier to detect. This applies specifically to viruses. Among all pathogens present in municipal wastewater, viral pathogens are typically present in the highest numbers, followed by bacteria and protozoan parasites. Due to the variety and unique properties of new viruses detected in recent years (smaller size, nucleic acid structure, etc.), this group of pathogens makes it particularly challenging to extrapolate their environmental behavior from one group of viruses to another.

This report summarizes characteristics of most common pathogens, indicators, and surrogates that influence occurrence, fate, and transport in the subsurface. This includes typical source water concentration ranges, size, and surface characteristics. Microbial removal in MAR systems is driven by various factors, including pathogen diversity; evolution and natural selection; treatment process performance; and its variability at full scale. Understanding these factors is helpful in selecting appropriate indicators and surrogates for pathogens in MAR systems and in selecting informed assessments of anticipated fate and transport behavior in subsurface systems.

ES.4.2 Analytical Methods

A wide variety of methods have been employed to detect microorganisms in water. In the last decade, traditional cultural methods that come with limitations on detection limits have been advanced given progress in analytical instrumentation and antibody and nucleic acid assays. Today, we can detect a much wider variety of organisms for which cultural methods do not exist (e.g., norovirus) using polymerase chain reaction (PCR) methods. Although PCR methods do not reveal whether detected pathogens are infectious, they have broadened our toolbox for quantifying treatment performance, preventative risk monitoring, and detection of useful indicators and surrogates under field conditions. Low concentrations of pathogens in treated effluent and MAR systems coupled with the very low infectivity of certain pathogens have driven the need for methods several orders of magnitude more sensitive than even state-of-the-art chemical tracer detection.

But analytical advancements over the past decades are not limited exclusively to molecular methods. The U.S. Environmental Protection Agency (USEPA) has developed standard methods for detecting and quantifying infectious, male-specific, and somatic coliphages in water that can help expand today's monitoring practice, which primarily (and often exclusively) focuses on fecal coliforms testing.

ES.4.3 Role of (Pre-Recharge) Treatment

The degree of wastewater treatment prior to recharge impacts the level of infectious pathogens and indicators in the MAR source water. When deriving an overall risk calculation, regulators include pathogen removal during treatment between raw sewage and the final product quality. During secondary activated sludge treatment, an estimated 1 to 2 logs of pathogens are removed, while tertiary treatment involving filtration and chlorine disinfection can further reduce infectious organisms by an estimated 3 to 5 logs total. Even higher removal rates can be achieved using advanced treatment systems such as membranes and advance oxidation.

Just as source concentrations of pathogens can vary by location and over time, the efficiency of pretreatment may vary based on hydraulic conditions or the individual treatment process performance. For this reason, some countries have implemented routine monitoring requirements that use surrogates specifically selected to indicate conservative, inadequate performance for the critical treatment processes barriers in question.

ES.4.4 Indicator and Surrogate Selection

Indicators have traditionally been used to determine whether a water source has fecal contamination and whether enteric pathogens might be present. Surrogates are organisms, particles, or substances used in the field and laboratory to study the fate, transport, and removal of pathogens in a specific environment. U.S. regulations of MAR systems have focused on monitoring fecal coliforms or *E. coli* as bacterial indicators for pathogen presence since they are inexpensive and easy to measure. While there is no ideal organism that can relate exactly to the risk of infection from exposure to water, indicators and surrogates can be used to judge the performance of groups of pathogens in response to treatment processes. However, this makes it challenging to agree on the best indicators and surrogates for conservative (but not too conservative) use to inform pathogen risk assessments. Given the diversity of MAR systems, it is scientifically clear that site-specific conditions need to drive the selection of the most suitable indicators and surrogates. Factors influencing this selection include source water pretreatment, quality, and occurrence frequency, as well as MAR system characteristics (e.g., type of MAR system, geological material, vadose vs. saturated conditions, residence time, and dynamic hydrological conditions) and the post-treatment process. These and other factors determine the risk of different pathogen types that a specific MAR system is reasonably susceptible to (i.e., viruses, protozoa, and bacteria). For example, in MAR systems with long residence times, travel distances, and fine-grained aquifer material, viruses may pose the largest health risk. Furthermore, aerobic spores are suitable surrogates for *Cryptosporidium* oocysts that pose a risk in IBF systems with short residence times.

Using multiple indicators and surrogates can be appropriate for an overall risk assessment of the system and for individual treatment process barriers within this system. Organisms that can be detected only with molecular methods may have value as appropriately conservative surrogates, but they are overly conservative as indicators. The report discusses considerations for selecting commonly and less commonly used indicators and surrogates in more detail for recharge, infiltration, and induced bank filtration systems. Chemical tracers are generally not recommended as useful surrogates, since pathogens can transport more quickly than chemical solutes in the subsurface due to size exclusion from small pore spaces with lower or stagnant flow conditions.

ES.5 Current Understanding of Pathogen Occurrence and Fate and Transport

ES.5.1 Factors Influencing Pathogen Fate and Transport

Over the past two decades, advances in analytical methods, monitoring, modeling, and research under controlled pilot and field conditions have contributed to a better understanding of the factors and quantitative relationships impacting the fate and transport of pathogens in MAR systems. Generally, pathogen removal in subsurface systems is a combination of die-off (decay or inactivation), predation, and physical attenuation (e.g., attachment and detachment). Die-off or decay rates for various pathogens and common surrogates are also summarized in this report and are specific to organisms, water quality, and MAR systems. Biologically acclimated laboratory column tests have proven useful for assessing the immediate and fast removal of pathogens in the schmutzdecke and upper first few feet (ft) of soil infiltration due to decay, predation, and adsorption. However, laboratory simulations have shown that decay rates during *longer* retention time studies repeatedly overestimate observed decay rates under field conditions. Proper design of the lab simulations can reduce this discrepancy to some extent for example, through temperature control and use of native pathogens in the experiments. *In situ* cells placed into monitoring or production wells allow for estimating decay rates in ambient groundwater conditions. Results of this method may be too conservative if interpreted as overall removal rates since retardation and predation are not measured this way.

Decay rates represent the basis for log removal credits granted by U.S. regulations for virus removal in MAR systems. When these regulations were developed, sufficient information on other removal mechanisms was not available. Fate and transport studies have demonstrated that irreversible adsorption is an important removal mechanism for viruses. Today, advances in deterministic modeling of virus transport allow for better estimating adsorption and desorption of target viruses under different hydrogeological conditions.

Chapter 4 of this report discusses relevant factors driving pathogen transport and removal in MAR systems. Some of these factors can be easily quantified and cause-effect relationships sufficiently understood to make reasonable removal predictions for various groups of pathogens (e.g., temperature, decay rates). For other factors, cause-effect relationships are qualitatively understood, but quantitative predictions on removal rates require empirical data collection at the lab and/or field-scale (e.g., the role of organic carbon for adsorption and degradation of pathogens during short- and long-term MAR treatment). Lastly, some factors are

known to influence pathogen removal, although mechanism are not completely understood, and it is unclear whether it is appropriate to transfer findings between different MAR field sites (e.g., the impact of suboxic redox conditions on the fate of pathogens).

Equally relevant for public health assessments is to assess our remaining limitations for predicting pathogen removal and the levels of uncertainty of our predictions. Unknown site conditions, unexpected treatment failures, and the dynamic nature of MAR systems leave an inherent risk. Several methods are available to characterize remaining unknowns and risks and to develop appropriate safeguards. Unknown site conditions include heterogeneities in the subsurface that we may not be able to fully characterize. Field tracer tests using chemicals *and* viral surrogates informed through modeling can help identify preferential flow paths and unexpected hydrogeological conditions and inform where to strategically place monitoring wells to recognize early changes in water quality. Unexpected treatment failures could be caused by variable source concentrations of pathogens or variable degrees in treatment efficiencies. Identifying critical control points and associated appropriate surrogates can reduce uncertainties and lower required MAR safety factors. Climate change, variable pumping rates, and dynamic water flow regimes due to weather events can vary hydrological conditions, significantly impacting the transport of pathogens. Event-specific sampling combined with modeling can help identify potentially vulnerable conditions and appropriate mitigation measures.

ES.5.2 Methods for Predicting Pathogen Removal in MAR Systems

The authors and other researchers agree that a site-specific combination of some of these steps is appropriate for describing pathogen removal in MAR systems: literature review, laboratory-scale, *in situ* tests, field data collection, and modeling.

A literature review is an appropriate first step when attempting to better understand pathogen removal at specific MAR site. A review of observed inactivation rates for the pathogen(s) of interest (see Section 4.2 and Tables 4-2 through 4-4) can help establish the potential range of removal. Documented performance from laboratory or field studies carried out under similar conditions (e.g., temperature) to the MAR site provide may further provide insight and help determine if default credits can be adjusted to reflect site specific conditions without additional study.

Laboratory-scale studies can help simulate pathogen removal in the upper infiltration zone, where the highest log removal rates are commonly observed. However, these results should not be directly extrapolated to field conditions, since solution and solid-phase chemistry, microbial activity change, and subsurface heterogeneities play a larger role with distance. For best results, laboratory experiments should be conducted with native source water and surrogates, since their size, shape, net charge, and survival can differ from more homogenous spiked species. Laboratory studies are further useful to identify which physical and chemical processes are most active in a particular soil or aquifer medium.

In situ tests have been proposed to measure decay rates under ambient conditions to demonstrate to regulators their potential as alternatives to default log removal values. Scientists still consider the rates developed from these tests overly conservative for reasons

discussed in the report. However, they are proposed as defensible (conservative) approaches for demonstrating alternative log removal performance to regulatory agencies.

Full-scale field studies use tracer tests to determine groundwater flow direction, preferential flow paths, dilution ratios with native groundwater, residence times, and log removal values for pathogens. Combining hydraulic modeling with tracer tests can inform the design of sampling campaigns and locations to yield reasonably accurate mass balance results and conclusions for pathogen fate and transport. If chemical tracers are used, applying tracer test results directly to infer pathogen transport can be misleading. Conservative tracers and reactive pathogens will exhibit very different transport pathways, and concentrations may not be correlated. For example, chemical tracers travel in structure and matrix pathways, whereas pathogens and surrogates are removed in the matrix and are primarily transported through high-velocity (structure) pathways.

Monitoring networks in the field often do not accurately quantify pathogen arrival time, location, and concentrations. Here deterministic (mechanistic) models provide a useful complimentary tool to guide and interpret sampling results, especially when mass balances are not achieved even with conservative tracers or when surrogate concentrations are too low for quantification and sampling locations are too infrequent to provide a complete hydrological picture. These models help identify factors that control the fate and transport of pathogens in the subsurface and improve our mechanistic system understanding. Geophysical techniques are increasingly being employed to better characterize subsurface heterogeneity. In addition, stochastic modeling approaches can be used to quantitatively investigate the influence of subsurface heterogeneity on the mean and variance of concentrations.

Only a small fraction of facilities operating planned or *de-facto* MAR systems see the need for or have the resources to take advantage of these options. For this reason, it is reasonable to develop regulatory approaches structured in tiers to provide permitting flexibility. This should reflect the different types of MAR systems that are operated in various environmental settings. For example, sites with little characterization could be required to meet stricter regulatory standards, while sites with a higher level of characterization would be allowed to demonstrate that a lesser level of regulatory standards would still be adequate to assure public health protection. (There is an inherent inequity in allowing poorly characterized sites to meet the same regulatory standard as well characterized sites. Current practices that seem to permit this inequity will inevitably result in highly protective, perhaps overly conservative, regulations for all sites).

ES.6 Current U.S. Regulatory Practices and Their Challenges

This report summarizes relevant U.S. and international regulations for groundwater and protection against pathogens in drinking water. Justifications for certain regulatory requirements, where relevant and accessible, are described to highlight scientific evidence considered when the regulations were developed, in contrast to scientific progress made through today.

In the United States, state agencies typically regulate and permit recharge, injection, IBF, and storage and recovery systems. USEPA oversees four key regulatory programs that also relate to the protection of microbial pathogens in groundwater and well water: the Underground Injection Control Program, the Revised Total Coliform Rule, the Groundwater Rule, and the Groundwater Under Direct Influence provisions of the Surface Water Treatment Rule. These federal regulations set general requirements for well operators and define indicator organisms for monitoring to assess the risk of microbial contamination.

Several states have developed more stringent requirements for operators of MAR and Groundwater under direct influence (GWUDI) systems fed using treated wastewater effluents or surface water impacted by treated wastewater. However, not all state agencies have pertinent regulations in place, and some are in the process of adopting regulations as the first MAR systems in their states are proposed. This report summarizes current programs and describes selected state programs in more detail for California, Oregon, Florida, Washington, and Colorado.

Regulatory approaches in the United States typically emphasize a multi-barrier approach to protecting public health against pathogen-contaminated ground water used as source water for public water supply systems. Enforceable drinking water standards and treatment techniques are codified in three drinking water regulations:

- **Revised Total Coliform Rule**: For undisinfected ground water, total coliform detection in the distribution system under the Revised Total Coliform Rule (RTCR) triggers source water monitoring for *E. coli* (or enterococci or coliphage).
- **Ground Water Rule**: Detection of *E. coli* (or enterococci or coliphage) in undisinfected source ground water can result in corrective action. The execution of this rule still relies strongly on fecal coliforms as the prime indicator used in groundwater monitoring programs. USEPA has developed analytical tools for enteric virus monitoring but as of today, the regulatory language remains optional, and many states do not enforce virus monitoring in permit renewals.
- **Surface Water Treatment Rule**: The groundwater under direct influence (GWUDI) provisions of the Surface Water Treatment Rule (SWTR) require regulation of GWUDI systems as surface water rather than groundwater. All surface water systems are required to be disinfected (unlike ground water systems) so *Cryptosporidium* is the primary target pathogen because it is resistant to inactivation by disinfectants other than UV. GWUDI regulations allow for site-specific demonstration studies to prove log removal performance by subsurface passage for the target pathogens *Cryptosporidium* and *Giardia*. Scientific and regulatory consensus is emerging that aerobic spores are suitable surrogates that yield conservative estimates for oocyst log removal values. Not all states require using spores in demonstration studies, although notable state regulation exceptions exist (such as Colorado). Viruses are not included in GWUDI assessments since they are generally assumed to be adequately inactivated as the well water is chlorinated prior to distribution. This approach may warrant rethinking in the future as more viruses show signs of resistance to disinfection.

Current regulatory practices for MAR systems by state agencies result in several challenges, which were identified in this project:

- **California recycled water regulations for surface and subsurface applications.** These regulations were originally developed as draft criteria more than thirty years ago, progressively evolving through their formal adoption for groundwater in 2014. They set detailed, prescriptive requirements for design and log removal credits in MAR systems. Potable reuse regulations in California currently rely on "travel time" as the only surrogate to give log removal credits. A broader regulatory framework is needed to determine proper treatment and log removal determinations beyond travel time. However, the biggest criticism is that these regulations do not provide flexibility for site-specific demonstration studies or guidance to utilities on how these studies should be conducted so they are acceptable to regulators. Several regulatory requirements, which are understandable given the scientific knowledge available at the time, are considered overly conservative and not scientifically defensible for some MAR systems. Further, these regulations focus on *Cryptosporidium* oocyst and virus removal for public health protection, and utilities receive little guidance on how to select appropriate surrogate organisms for log removal demonstration studies.
- **Uncertainty in risk assessments.** It is not yet well established whether the applied regulatory standards at some sites are overly strict because the biological pathogen surrogates used are not detectable somewhere in the subsurface. The lack of surrogate breakthrough at the well limits risk calculations and leaves uncertainty.
- **Regulatory consistency among states.** State regulatory approaches are generally inconsistent for MAR systems, such as those related to minimum set back distances, minimum subsurface retention times, pre-recharge treatment requirements, log removal requirements, and monitoring needs and frequencies. Several systems operating as *de facto* MAR systems are not categorized as such, due to historical categorizations under different permitting programs.

ES.7 Regulatory Approaches in Other Countries

This report describes the programs in Australia, The Netherlands, Germany, and Canada in more detail to highlight alternative regulatory frameworks to the United States for protecting public health from microbial contamination in MAR systems.

The Australian Guidelines for Water Recycling emphasize a systematic, comprehensive risk management approach in which MAR operators are required to conduct a hazard analysis and critical control point (HACCP) analysis and define preventative measures to guarantee public health protection. Compared to some statewide approaches in the United States, Australian guidelines are less prescriptive and instead emphasize the risk assessment process to develop performance-based outcomes for public-health protection.

The Netherlands are unique in that its hydrogeology is characterized by homogenous, finely grained sand deposits for which microbial fate and transport in IBF systems have been studied in detail for decades and are now well understood. In The Netherlands, minimum setback distances are determined for individual field site using fate and transport models developed for

these conditions. Modeling and monitoring emphasis is on viruses and viral surrogates, since they are most persistent in the aquifers there, and well water after recovery is typically not disinfected in The Netherlands prior to distribution.

In 2015, the German EPA suggested a new procedure for drinking water utilities to assess microbial risk in IBF and *de facto* MAR systems. This procedure recommends a quantitative microbiological risk assessment that some utilities are currently testing. The process attributes a relevant risk that triggers the need for this risk assessment for any drinking water wells fed by surface waters that contain coliphage, *E. coli,* and Enterococci above defined threshold concentrations (100 colony forming units [cfu]/100 milliliters [mL], respectively). Regular sampling for these indicators and other surrogates is required, and risk mitigation must be identified in the catchment area and through post-treatment in case threshold concentrations are exceeded.

Canada lacks federal regulations for MAR systems and instead allows individual provinces to develop their own. MAR systems in Canada apply primarily to GWUDI systems using bank filtration. Ontario has developed a regulatory paradigm for GWUDI systems that uses a different approach than US GWUDI and MAR regulations. Specifically, Ontario does not prescribe requirements for treatment credits for pathogen removal and log removal targets based on subsurface travel time. Instead, the regulatory focus is on direct monitoring of key water quality parameters for public health protection in the well water.

Note that here monitoring programs focus on possible changes of baseline water quality in wells, setting stringent alert levels to draw attention to changes from baseline water quality conditions. If a MAR system has been operating successfully without issues, an important component of any regulatory framework should be to ensure a monitoring program is in-place that would indicate any significant changes or disturbances to the source water quality, watershed conditions, etc.

ES.8 Research and Knowledge Gaps

This study identified the several knowledge gaps related to the following areas:

1. **Suitability of surrogates and indicators.**
   - Benefit of metadata-analysis of indicators and surrogates on understanding log removal.
   - Value of increased consideration to use of aerobic spores and enteric viruses as surrogates in GWUDI and MAR systems.
2. **Monitoring of pathogen and indicator removal by MAR in near or real time.**
   - Automated concentration and detection systems by digital droplet PCR (ddPCR) are becoming more readily available to the water and wastewater industry.
3. **New indicators and surrogate opportunities.**
   - Novel approaches for assessing fate and transport characteristics of target pathogens deserve evaluation, including silica beads with virus-specific proteins, online flow cytometry, plant-based surrogates such as algae or viruses (e.g., pepper mild mottle virus [PMMoV]), and the relevance of antibiotic or disinfection resistant pathogens.
4. ***In situ* approaches for developing fate parameters for pathogens.**

- Methods such as diffusion chambers used to measure *in situ* inactivation rates.

5. **Aquifer recharge systems not covered in this study.**
   - Several aquifer recharge systems are not explicitly covered in this study. These include dry wells, stormwater infiltration, combined stormwater/recycled water infiltration basins (common in California), or effluent disposal through land application.
6. **Fate and transport modeling.**
   - Fate and transport models do not yet adequately reflect the dynamics of IBF systems, such as release pulses, attachment, and detachment process. Some of these models are currently being developed, but they are not yet published or available for utilities.
7. **Testing of procedures for demonstration studies for alternative log removal value (LRV) at MAR sites.**
   - This study developed recommendations for site-specific demonstration studies for alternative LRV to permit agencies. These recommendations should be tested in pilot studies to vet the proposed guidelines and best management practices for regulatory consideration by state agencies.

ES.9 Conclusions and Recommendations

This section summarizes the main recommendations from this study relative to regulatory limitations and considerations for selecting surrogates and indicators for viruses, bacteria, and oocysts based on their respective opportunities and limitations.

Utilities that consider regulatory default log removal credits too conservative can ask for a well-defined tiered workflow to assess site-specific conditions and MAR performance based on science and acceptable to regulators.

The following process is proposed as a tiered workflow to demonstrate less conservative removal values to regulators as utilities progress through the tiers recognizing site-specific conditions.

If a set of default regulatory credits are acceptable to MAR system owners/operators these would be adopted, and further site characterization is recommended but not a regulatory requirement. In case regulatory credits would be significantly inconsistent with existing literature (i.e., far more conservative than scientific publications would suggest), a variance request could be made to update the regulatory defaults with site specific credits based on updated literature. This step may be sufficient in some cases.

Additional steps may be required to demonstrate and support variance request from default regulatory credits or other requirements as indicated in Figure ES-1. The 10-step process is not always required in its entirety, rather relevant site-specific portions of this process should be considered by MAR operators in coordination with regulatory agencies that most appropriately and cost-effectively will demonstrate whether alternative regulatory requirements can be justified while adequately protecting public health. Completion of steps 6, 8, 9 or 10 may provide suitable “off-ramps” within this process to sufficiently justify site specific variance requests.

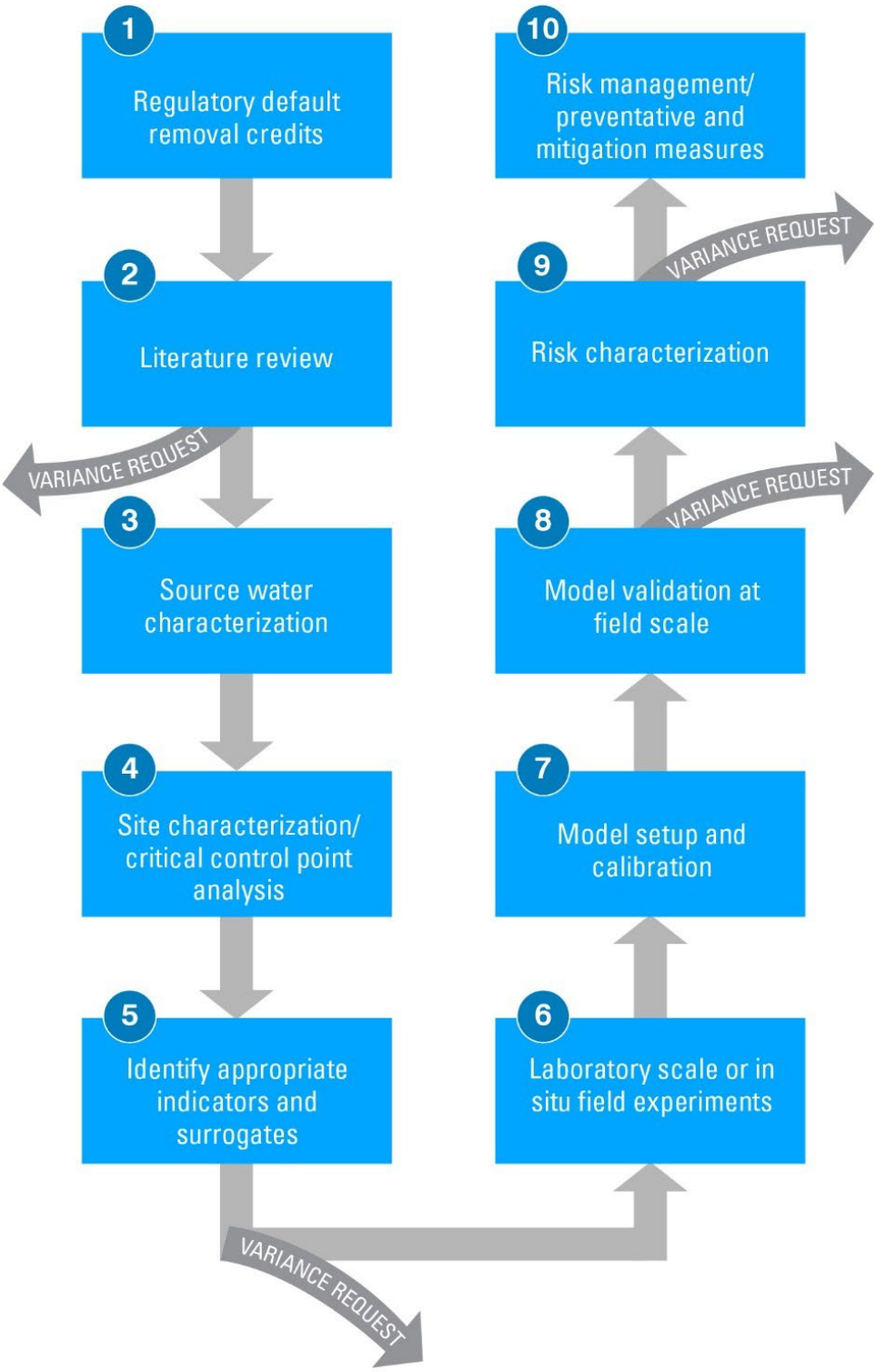

Figure ES-1. Proposed Tiered Approach for Site-Specific Pathogen Removal Credit Demonstrations in Wastewater or Wastewater-Influenced Source Water MAR Systems.

ES.10 Related WRF Research

- Geochemical Considerations for Managed Aquifer Recharge (MAR) Implementation in Potable Reuse (5051)
- Pathogen Monitoring in Untreated Wastewater (4989)

CHAPTER 1

Background

1.1 Introduction

Managed aquifer recharge (MAR) systems are widely used in drinking water production, water reuse, or subsurface water storage (Dillon 2005; Maeng et al., 2011; Regnery et al., 2017). MAR systems include various treatment systems, such as induced riverbank filtration (RBF), soil aquifer treatment (SAT), rapid infiltration basins (RIBs), aquifer storage and recovery (ASR), and aquifer storage transfer and recovery (ASTR). While ASR systems inject water into an aquifer and recovery water from the same well (e.g., for water storage in winter and recovery in summer months), ASTR systems have a dedicated well for water injection into aquifers for storage, and a separate well for recovery of stored groundwater (GW) (e.g., when continuous injection and/or recovery is desirable). MAR systems are employed for various purposes, such as water storage in the subsurface, water treatment and purification during infiltration, treated effluent disposal, and/or salinity barriers.

Potential water sources for MAR include recycled water, surface water, and stormwater, or a combination of these water sources. These sources can contain a wide range of enteric pathogens that pose a potential risk to human health. This study focuses on MAR systems that use recycled water (treated wastewater effluents) or wastewater-impacted surface water. This report does not cover pathogen occurrence and removal in MAR systems fed specifically with stormwater, and any readers interested in that topic should read key studies that address it (e.g., NRC, 2016). Unless otherwise specified, when MAR system are described in this report, it pertains to those systems that are planned MAR systems that utilize wastewater or wastewater influenced surface water as source water.

MAR systems are intentionally planned, designed, and operated to augment groundwater supplies. Dillon et al. (2009) defined MAR as the purposeful recharge of water to aquifers for subsequent recovery or environmental benefits. As such, MAR systems are subject to regulatory performance and monitoring requirements. This study focuses on *planned* MAR systems and summarizes the current state-of-the-science understanding of the occurrence, fate, and transport of pathogens in these systems. Note, however, that *de facto* aquifer recharge sites are a reality at various locations in the United States. (Maliva, 2020). *De facto* recharge refers to systems that infiltrate water into the subsurface for disposal of unwanted water without the intent of reuse. Unplanned recharge can also happen independent of unwanted effluent (land) disposal, such as in cases where treated effluent enters or becomes part of drinking water source supplies in lakes, rivers, or groundwater systems. Typically, these systems do not receive significant engineering, permitting, or regulatory attention. *De facto* recharge can be a heritage of historically developed site conditions. Sites with historically used aquifer infiltration to treat effluent disposal due to a lack of surface water discharge options might also fall into this category. In the United States, these systems may not be subject to the same regulatory requirements as a MAR system due to their regulatory categorization, age, or grandfathered status. Even though the focus of this study is on MAR systems, pathogen-related

information on fate and transport is applicable to *de facto* aquifer recharge sites, which typically have less available and accessible data.

The presence of human health pathogens in water produced from MAR systems is a key risk for groundwater quality and drinking water safety that system operators and regulators must manage. Long-term operation of full-scale MAR processes confirms significant pathogen reduction during soil/aquifer infiltration and groundwater transport. However, the science surrounding pathogen detection and identification is continuously developing. A key question that this study seeks to help answer is how microbial risk in MAR systems can be adequately regulated and managed without overly conservative restrictions or insufficient safety contingencies.

Assigning log reduction credits for pathogens and microbial indicators is a critical component to the design and permitting process of groundwater recharge systems that use recycled or surface water for potable reuse. Despite decades-long experience of high-quality treatment performance demonstrated at various MAR systems for pathogen removal, scientists, MAR operators, and regulators still have little consensus on appropriate treatment credits for pathogen reduction assigned to given project sites. This remains a challenge for several reasons:

1. **No common accepted approaches.** No common nationally or internationally accepted procedures or guidelines are established for determining and assigning pathogen removal treatment credits for MAR systems.
2. **Full-scale data frequently not available.** In some cases, regulators need to determine and permit treatment credits before full-scale MAR operation and production of drinking water commences. Therefore, the actual *in situ* pathogen removal performance at the site in question may not be able to be demonstrated *a priori in situ*.
3. **Direct log removal measurements are challenging.** Pathogens of human health concern in the feed water and/or the product water often have concentrations too low to be quantified in MAR systems with cost-effective sampling and detection methods. Therefore, extrapolation based on indicators or surrogates is needed to develop scientific estimates for log removal performance.
4. **Microbial agents vary from chemical constituents.** The fate, transport, and modeling of microbial agents (e.g., viruses) differ from that of chemicals. Microbial agents can have varying surface characteristics and behavior in the same population, and the subsurface may differ among field sites.
5. **Site specific conditions.** The hydrogeological and environmental conditions of MAR sites, log removal performance, and detention times are site specific and can differ significantly in the same state and among different U.S. regions. This variability must be considered in assigning removal credits.

Regulations governing pathogens in drinking water are based on endemic rather than epidemic disease (outbreaks). Randomized controlled trials used to measure endemic disease are complex and expensive because they require enrollment of large numbers of willing volunteers. One randomized controlled study, conducted at a bank filtration site (Colford et al., 2009)

found evidence of an attributable risk to drinking water. Additional randomized controlled studies are needed to confirm these findings.

Considering these challenges, some regulatory approaches in the United States have adopted constant log removal credits for MAR systems regardless of site-specific conditions. The Long-Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) assigns up to 1-log removal credit for reducing *Cryptosporidium oocyst* for all riverbank filtration wells that meet certain basic design criteria (U.S. EPA, 2006). California's regulations for indirect potable reuse allow 1-log virus reduction for each month retained underground (SWRCB 2018). However, these approaches, while easy to administer and permit, can be problematic, since the categorical definition of removal credit, regardless of site conditions, may be overly conservative or too optimistic depending on the specific MAR site conditions. Alternative regulatory approaches have allowed utilities in some states to conduct site specific demonstration studies for indirect potable reuse or groundwater under direct influence (GWUDI) regulations for RBF to claim site-specific log removal credits. Regulatory guidance on how such demonstration studies are best performed is not standardized among states, and guidance for subsurface infiltration or injection for groundwater recharge generally does not exist in the United States.

Pathogen attenuation during MAR processes varies widely throughout the world, though the removals associated with GWUDI systems are potentially more widely demonstrated and accepted. Over the past years, new research developments have resulted in more sensitive pathogen analysis and detection, improved fate and transport modeling, quantitative microbial risk assessments, and more sensitive detection of emerging pathogens, and they have contributed to a better understanding of the overall microbial risks in MAR systems. These developments can help planners, operators, and regulators of MAR systems answer common questions that this study also seeks to help answer, such as these:

1. How many log treatment credits will a full-scale MAR system provide?
2. How can site-specific demonstrations be most cost effectively conducted to quantify anticipated MAR water quality improvements?
3. Which suitable surrogates and indicators can confirm performance at full-scale?
4. How can column studies be designed to simulate subsurface processes as closely as possible, and how do the results from column studies transfer to future full-scale operation?
5. How can fate and transport modeling best be used to support site-specific investigations?
6. What monitoring should be performed for a system and how frequently? What are peak events for a given system and how important is it to capture them?
7. Can risk assessments properly inform how much pre- or post-treatment is required or advisable to best protect public health?

This study fills the need to document and disseminate the state-of-knowledge of pathogen reduction through MAR processes and compares and assesses the benefits, limitations, and challenges of different national and international regulatory approaches for microbial disease protection. The results of this study will give planners and regulators more nuanced guidance on ways to determine defensible pathogen log removal credits in MAR systems.

1.2 Study Objectives

This report is an outcome of the WRF research project, *Compiling Evidence of Pathogen Reduction through Managed Aquifer Recharge and Recovery* (#4957). This research study has three key objectives:

1. **Evidence Summary.** This report provides a state-of-the-art compendium on the current national and international knowledge of the quantitative removal of pathogens in managed aquifer recharge systems. This review identifies key parameters that determine the efficacy of pathogen attenuation during MAR, including the role of source water quality, recharge operations, residence time in the subsurface, aquifer characteristics, prevalent redox conditions in the subsurface, and temperature.
2. **Regulatory Guidance.** This report summarizes, compares, and evaluates different national and international approaches for assuring microbial health protection of MAR systems. The report summarizes methods for permitted log removal credit determinations, data collection and analysis, policy justifications, past experiences, and challenges to develop general guidance and recommendations for how to develop and implement defensible and practical regulations in the future.
3. **Research Roadmap.** Based on the state-of-the-art compendium, this report further identifies insufficient or missing information relevant to better understanding pathogen removal, monitoring, and testing approaches for site-specific demonstration studies and regulatory implementation.

1.3 Key Drivers and Questions for This Report

This report is broken down into 8 chapters. Table 1-1 provides an overview of each chapter's organization, key drivers, and focus.

Table 1-1. Organization of the State-of-the-Science Report.

Chapter	Focus	Key Drivers and Questions
Chapter 1	Project Introduction	Chapter 1 introduces the study motivation and objectives and provides an overview of the report organization.
Chapter 2	Status and Development of MAR Systems in the U.S.	Chapter 2 gives an overview of MAR systems evaluated in this study and summarizes the current MAR status and developments in the United States.
Chapter 3	Pathogens of Concerns in MAR Systems	Chapter 3 summarizes the role of source water on pathogen occurrence in MAR systems. It also discusses various recycled water qualities (disinfected tertiary treated effluent vs. RO permeate and the impact of nutrient removal).
Chapter 4	Factors Influencing Pathogen Fate and Transport and Pathogen Removal, Inactivation rates, Field Measurements, and Methods for Predicting Pathogen Removal	Chapter 4 discusses our current understanding of pathogen occurrence, fate, and transport related to the impact of various site-specific factors on pathogen removal, including source water quality characteristics, operational MAR system parameters, hydrogeological conditions, and the fate and transport modeling of pathogens. The chapter summarizes selected field measurements to bracket pathogen removal in MAR sites and discusses laboratory, pilot, and field scale methods and modeling approaches available for predicting pathogen removal in MAR systems.
Chapter 5	Current U.S. Regulatory Practices and their Challenges	Chapter 5 compares and discusses the regulatory approaches of various state jurisdictions in the United States for microbial risk protection in different MAR systems with international regulatory approaches.
Chapter 6	Managing Microbial Risk	Chapter 6 discusses the pathogen removal goals set by regulators to protect human health risk and discusses the justification behind them.
Chapter 7	Critical Knowledge Gaps	Chapter 8 identifies key knowledge gaps and recommends future areas of continued research.
Chapter 8	Conclusions and Recommendations	Chapter 9 highlights relevant conclusions and recommendations for selecting indicators and surrogates; systematic, defensible approaches for site specific demonstration testing; and recommended alternative regulatory approaches.

The information summarized in this report is based on a comprehensive literature review, a national and international expert workshop, and interviews with selected U.S. utilities summarizing information from both the United States (in particular California, Arizona, Colorado, and Florida) and other countries (including Australia, Germany, Spain, The Netherlands, United Kingdom, and Canada).

1.4 Review Sources

Standard searches of peer-reviewed literature were conducted using Web of Science, Google Scholar, PubMed, expert workshops, and conference proceedings (e.g., Information System and System Management [ISSM]; International Symposium on Managed Aquifer Recharge (ISMAR)) and research reports. Reviewed material included peer-reviewed journal articles, books, professional meeting proceedings, published reports, and web-based tools to estimate pathogen inactivation during MAR (Umwelt Bundesamt. n.d. Schijven et al. 2017). In addition, unpublished information was collected from utilities as available and accessible.

CHAPTER 2

Status and Development of MAR Systems in the United States

2.1 MAR Systems Overview

Groundwater recharge systems have been in operation in some parts of the United States for over a century, and many have been operational for several decades (NRC 2008). MAR systems for water storage in the subsurface differentiate between how water is applied to the system. Water may be applied via spreading basins (SAT or RIB) or directly recharged into the subsurface, either into the vadose zone using dry wells or galleries or by injection into the saturated zone of an aquifer (ASR or ASTR). In RBF systems, groundwater wells near a river, stream, lake, or reservoir induce a hydraulic gradient to infiltrate surface water for recovery. A detailed overview of different MAR systems is provided by Maliva (2020).

Water traveling through the vadose and saturated zone of an aquifer undergoes treatment through filtration, adsorption, volatilization, and biochemical processes depending on the predominant redox conditions (e.g., oxic, suboxic, anoxic, or fully anaerobic) (Stuyfzand, 1998; Regnery et al., 2015a). Pathogenic inactivation also occurs through physicochemical reactions and predation by bacteria and other living organisms specifically in the top infiltration layer, which is called the schmutzdecke, the biological layer that develops on the soil surface of the aquifer infiltration zone. “Soil” refers to the thin, organic-rich material near the surface above geological materials and deposits.

SAT systems are prevalent in the Southwestern United States where infiltration basins have been used for large-scale infiltration of reclaimed water in urban areas, at times in combination with local or imported surface water and stormwater. Infiltrated water is typically of secondary or tertiary treated quality and receives additional purification during treatment in the vadose zone. Recharged water is protected from evaporation and recontamination (such as from aerosol deposits, algae growth, or bird excrements). The longest operating SAT systems in the United States are located in California and Arizona.

ASR systems are employed by municipalities that may lack sufficient land area or have hydrogeological conditions not conducive to surface infiltration. ASR systems recharge groundwater aquifers when water is available to store and be recovered when needed. This study focuses on ASR systems that are fed with recycled water. Other water sources include groundwater recharge with treated drinking water or surface water for which certain states (e.g., California) have developed specific requirements.

ASR systems typically require a higher level of treatment of reclaimed water prior to injection. While California currently requires advanced treatment using reverse osmosis (RO) membranes prior to direct injection, Arizona allows vadose zone injection using Class A+ Reclaimed Water (secondary treatment, filtration, nitrogen removal, and disinfection), and Nevada allows injection of treated wastewater of Reuse category A+ after three separate treatment processes

for pathogen removal, which does not need to include RO treatment. Advanced engineered treatment is necessary for ASR and ASTR systems to minimize well head fouling due to physical and biological clogging over time (Jeong et al. 2018), and to adequately protect the quality of the aquifer the water may be directly injected into from physical and biological contamination.

Saline-water intrusion barriers find application in some coastal areas in the eastern and western United States. Another example is the Llobregat Delta in Barcelona, Spain. At that Llobregat Delta, pumping or infiltration of surface or reclaimed water on the inland side creates a hydraulic gradient that keeps salt water from intruding into potable aquifers.

RIBs are similar to SAT systems in that reclaimed water is infiltrated into the subsurface through basins. RIBs have historically been used to dispose of treated effluent in areas without suitable surface water discharge alternatives. The infiltration to the vadose zone is designed to occur relatively quickly. Water quality improvements during infiltration have recently become more relevant for consideration in RIB system, since water reuse for potable supplies has greater interest in the Southeast in states like Florida.

Riverbank filtration, which is more appropriately referred to as induced bank filtration (IBF), refers to systems where surface water is infiltrated through the bank of rivers or lakes through a hydraulic gradient induced through aquifer wells close by. Aquifer wells may consist of horizontal or vertical well types or extraction galleries. IBF takes advantage of the natural water quality improvements occurring during infiltration. The biological and physico-chemical purification processes for organic and inorganic contaminants and microbial pathogens in IBF systems are similar to SAT or ASR systems. However, the changing nature of flowing streams and rivers causes noticeable differences in process kinetics, subsurface residence times, and overall system performance dynamics (Regnery et al. 2015b). IBF systems have operated along river systems throughout the United States for many decades, primarily in the midwestern states and on the west coast. Many more alluvial well systems are in operation throughout the country in hydraulic connection with nearby surface waters influenced to various degrees by treated effluent discharges. Few of these wells are designed and officially categorized as IBF systems, however. In the United States and Canada, IBF wells are subject to GWUDI assessments to determine the risk for pathogen breakthrough and the level of post-treatment required for recovery well water. Wells categorized as GWUDI require post-treatment equivalent to surface water treatment.

Examples of unintentional aquifer recharge systems found throughout the United States include stormwater retention basins, surface water reservoirs, impoundments that leak, leakage from sewer mains, on-site or septic system infiltration, and disposal of treated effluent by infiltration in areas without suitable surface water discharge. These systems are either not regulated at all or are not regulated for pathogen risk management. While the scientific principles of pathogen occurrence, fate and transport, and other topics discussed in this report apply to these systems, this report does not specifically develop recommendations for these types of unintentional aquifer recharge systems.

Figure 2-1 provides a schematic of the prevalent MAR systems discussed in this study.

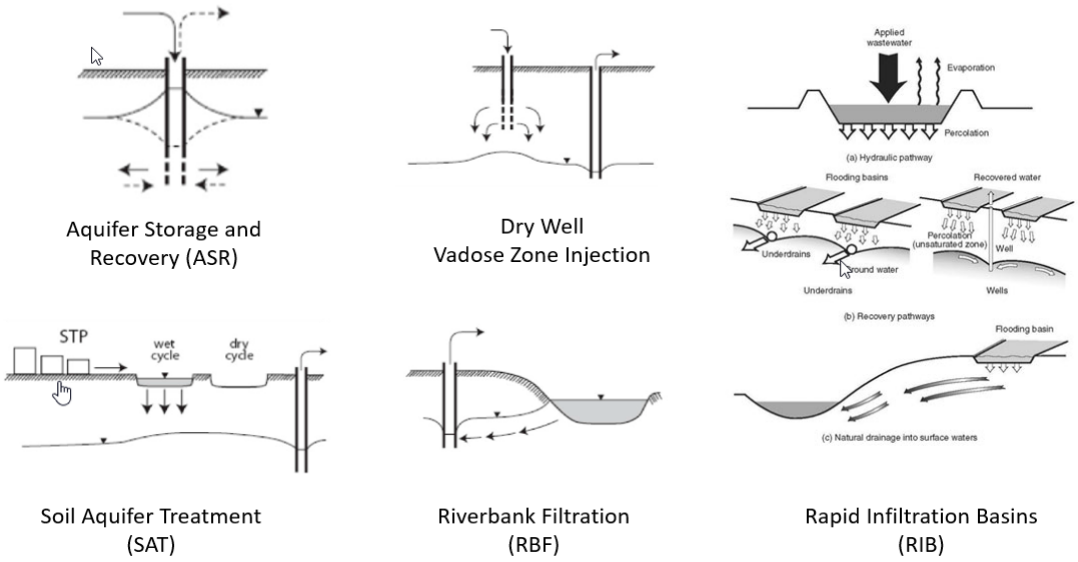

Figure 2-1. Simplified Representation of Different MAR System Types.
Source: Adapted from Page et al. 2018 and Mousavinezhad et al. 2015.

For drinking and recycled water treatment, concern for human health exposure focuses primarily on viruses and protozoa, and less on bacteria. This is for several reasons. One, greater removals of viruses and protozoa are required because significantly fewer numbers of viruses and protozoa need to be ingested to cause infection compared to bacteria (Haas et al., 2014; Gerba et al., 2017). Furthermore, viruses are of major concern during MAR treatment because they are small and can persist in groundwater long-term. Bacteria and parasites are larger than viruses and are better removed by filtration processes in subsurface systems (Betancourt et al., 2014; Regnery et al., 2017). Even some of the smaller, newly recognized protozoan pathogens such as microsporidia are removed to a greater degree than viruses under the same conditions (Brusseau et al., 2005).

2.2 MAR System Status and Development in the United States

MAR systems in the United States and those in other parts of the world using surface water or reclaimed water have a long history dating back to the turn of the twentieth century (NRC, 2008). Beginning in 1911, Orange County began recharging water from the Santa Ana River, which is currently largely made up of upstream wastewater discharges, to supplement local groundwater (California Regional Water Quality Control Board, 1995). Long Island, New York, and Southern California started scientific studies on artificial recharge for groundwater storage beginning around the 1930s. In 1962, the Sanitation Districts of Los Angeles County implemented the first ever large-scale planned operation of groundwater recharge in the United States that used municipal wastewater, using secondary effluent as source water, and recharging via recharge basins (NRC, 1994). This project was later upgraded to include tertiary filtration and disinfection. Artificial recharge in the fast-growing State of Arizona did not begin at a large scale until the Granite Reef Underground Storage Project was permitted in 1994. The project was owned by the Salt River Project, Chandler, Gilbert, Mesa, Phoenix, Scottsdale, and Tempe.

The International Groundwater Resources Assessment Centre (igrac) maintains an up-to-date inventory of worldwide MAR systems (Igrac, 2020). Today, MAR systems in the United States are operated in at least 23 states, and the number is growing. Infiltration ponds and basins are primarily used in Washington, California, and Arizona. ASR and ASTR systems are common in many states, including Washington, Oregon, Idaho, Utah, California, Texas, Colorado, Florida, South Carolina, Virginia, and New Jersey. Where aquifer conditions under rivers, lakes or reservoirs allow recovery of infiltrated water through wells, IBF systems can be a suitable MAR option. IBF systems are operated in Ohio, Indiana, Kentucky, Missouri, Nebraska, California, Colorado, and Oregon.

Where hydrogeological conditions allow, MAR processes can be more cost effective than other pathogen and contaminant barriers, direct surface water treatment, or direct potable reuse. In terms of energy, sustainability, and longevity, MAR systems can be one of the most robust treatment processes for drinking water sources. Water storage via MAR is possible in a wide variety of confined and unconfined aquifers consisting of unconsolidated alluvial deposits, limestone, and fractured and/or porous rocks.

Direct injection is necessary for confined aquifers and in locations where suitable land is limited for surface recharge. Surface recharge or ASR is suitable for shallow or deeper aquifers that allow water to be easily recovered.

Many of the United States, IBF, SAT, and ASR systems have been in operation for several decades and have been thoroughly studied to characterize and quantify the removal of microbial pathogens and other source water contaminants. In recent years, however, U.S. utilities have become more and more interested in formally integrating MAR systems into new or expanded treatment trains for potable reuse and drinking water production. A number of factors contributed to the proliferation of MAR systems in different parts of the United States:

1. **Increased water demand.** Recent population dynamics have resulted in significant growth in states such as California, Nevada, Idaho, Arizona, Colorado, Texas, and Florida. Additional drinking water sources are needed, leading to an expansion of existing MAR systems, or planning for new MAR systems for indirect potable reuse.
2. **Water resilience planning.** Climate change and severe drought impacts expand beyond the south and western U.S., and in recent years have motivated water resource planners to diversify drinking water source portfolios. Potable reuse projects are now also seen in states that have traditionally had little need for water reuse (e.g., Virginia, Idaho, and Oregon). Raw water supply planning has become more challenging given climate uncertainties and increasingly declining raw water source qualities. MAR is regarded as a cost-effective treatment option that also allows for subsurface storage to strengthen supply resiliency in areas experiencing repeated drought conditions.
3. **Regulatory shift from unintentional to planned recharge.** Some facilities that have historically operated *de facto* recharge systems have now become subject of a higher public and regulatory scrutiny. In order to assure public safety, previously grandfathered systems may require now permits and monitoring programs in some regions.

4. **Capacity expansion of existing MAR system.** Continued population growth in urban areas requires an expansion of available drinking water sources. As the water supply in many western states is already overcommitted the expansion of water reuse becomes a feasible strategy. This can be achieved by either expanding the MAR system or demonstrating to regulators that the existing MAR system is safe even under higher loading rates and short residence times.
5. Other drivers and benefits include environmental benefits related to groundwater-dependent ecosystems and over drafted aquifers, depleted streams, greenhouse gas, energy, and/or cost reduction for drinking water production.

Given the increased interest in MAR system implementation and expansion across the U.S. a review of the current state-of-the science understanding of pathogen fate and transport in the subsurface is critical as it can help inform system design and operation, as well as regulatory approaches to adequately protect groundwater quality and human health from pathogen contamination.

CHAPTER 3

Pathogens of Concern in MAR Systems

This chapter introduces pathogens, surrogates, and indicators that are relevant to MAR systems along with their characteristics that influence their fate and transport behavior in MAR systems. The chapter also discusses the rapidly changing role of analytical methods in the detection of microbial agents in MAR systems. The mechanisms and factors influencing fate and transport of pathogens, indicators, and surrogates in MAR systems are further discussed in Chapter 4.

3.1 Pathogens in MAR Systems

3.1.1 Pathogen Occurrence in Wastewater

The list of potential pathogens in wastewater or surface water is large (Table 3-1). More than 200 pathogens have been identified that can be transmitted by water. The type and concentration of pathogens in domestic wastewater depends on a number of factors, including the occurrence of the pathogens in the community, time of year (many pathogens are seasonal), social economic factors (higher rates of infection in certain social-economic groups), and water use per capita. The type of pathogens and the concentration in surface waters varies greatly depending upon region (incidence of infections in a community), time of year, the degree of wastewater treatment before discharge, the type of disinfectant before discharge, runoff during rainfall events, stormwater overflows from combined wastewater systems, impact of septic tanks, agriculture drainage, recreational use (bathers), etc. (Pepper et al., 2015).

Table 3-1. Size Ranges and Concentrations of Major Pathogens of Concern Detected in Raw Domestic Wastewater Influents and Viral Indicators and Surrogates.

Pathogen	Size	Number per Liter[1]
Protozoa	**Length x Width (µm)**	
Cryptosporidium spp.	4.0-5.5	10^2 - 10^4
Cyclospora cayetanensis	7.5-10	<10^2-10^4
Entamoeba histolytica	10-20	2-893
Giardia lamblia	12-15 x 5-9	10^2-10^5
Microsporidia	1-4	2-10^3
Toxoplasma gondii	5-50	0 to rare*
Bacteria	**Length x Width (µm)**	
Campylobacter spp.	0.2-0.5 x 0.5-5.0	10^2-10^5
Listeria spp.	0.5-4 x 0.5-2.0	10^5
Pathogenic *E. coli*	1.0-2.0 x 0.5	Fecal Coliform: 10^7 – 10^8
Salmonella spp.	0.7-1.5 x 2.0-5.0	10^3 – 10^5
Shigella spp.	0.3-1.0 x 1.0-6.0	10^2-10^7
Vibrio spp.	1.5-3.0 x 0.5	101-10^5
Yersinia	1.0-3.0 x 0.5-0.8	10^5
Viruses	**Diameter (nm)**	Enteric virus (cell culture assays): 10^4 – 10^6
Adenovirus	70	10^5- 10^{11}
Achi viruses	23	10^4 - 10^7
Astrovirus	28-35	10^2 - 10^7
Bocavirus	20	10^3-10^4
Circoviruses	15-22	
Enteroviruses	23-30	10^3-10^6
Hepatitis A and E	27-34	<10^2 - 10^7
Noroviruses	23-40	10^3 – 10^6
Sapovirus	40-46	
Parvoviruses	18-23	10^7
Rotavirus	60-80	10^7
Indicator / Surrogate Viruses	**Diameter (nm)**	**10^3-10^8**
MS2 (Escherichia virus, Bacteriophage)	27	N/A**
Qβ (Escherichia virus, Bacteriophage)	28	N/A**
ΦX-174 (Escherichia virus, bacteriophage)	32	N/A**
Pepper Mild Mottle Virus	17 x 312 (rod-shaped)	10^6 to 10^{10}
Somatic coliphages	Variable	10^3 - 10^4
Male specific ribonucleic acid (RNA) coliphages (FRNAPH)	Variable	10^3-10^9
CrAssphage	Variable	10^5-10^{12}
Bacteroides phage	Variable	10^1-10^6

Note:
1.Table values adapted from: Pepper et al., 2015; Kitajima and Gerba, 2015; Gerba et al., 2017; Kitajima et al., 2014; Wang et al., 2018; McCall et. al., 2020; Farkas et al., 2020; Global Water Pathogen Project, 2020)
*Felines are the most common source.
**Not applicable. Concentration data for a specific bacteriophage in sewage is not available as hosts are non-specific to various virus types that are present in wastewater.

Table 3-1 lists typical concentration ranges of selected pathogens and indicators or surrogates in domestic wastewater influents. The concentrations of pathogens in surface water are very watershed dependent and can vary greatly depending on the type of wastewater discharges and human /agricultural activity, and rapidly changing environment events (rainfall). Thus, generic statements on concentrations in surface waters are not possible.

The number of types, species, phenotypes, and genotypes of enteric pathogens increase every year and could potentially be present in domestic wastewater (Gerba et al., 2017). Some of the new viruses may have unique properties (smaller size, nucleic acid structure), making it challenging to extrapolate data from one group of viruses to another. Also, some viruses, particularly RNA viruses, can mutate very rapidly, resulting in new types that can become more infectious to humans and cause pandemics, as seen with the recent evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Of all pathogens, viruses pose specific challenges for human health protection. As demonstrated by Gerba et al., 2017, we estimate today that the concentration of viruses present in untreated wastewater is about 100 times greater than previously estimated. Due to major advances in molecular biology and medical diagnostics, we currently know of more than 50 new viral pathogens potentially present in wastewater than we did 20 years ago (Regnery et al., 2017). Some of these newly discovered human pathogenic viruses are as small as 15 nm (i.e., circo viruses). Regardless, many viruses are still unknown, and it is difficult to use one type or group of viruses to represent the behavior of all enteric viruses. Roughly 260 animal-borne viruses are known to infect humans, and this is estimated to account for less than 0.01 percent of the total number of zoonotic viruses that humans could potentially contract (Carroll et al., 2018). Data from past pandemics suggests there may be close to 1.67 million unknown viruses, and somewhere between 631,000 and 827,000 of these could potentially infect humans.

3.1.2 Pathogen Removal during Wastewater Treatment

In municipal wastewater, overall viral pathogens are typically present in the highest numbers of all pathogens, followed by bacteria, protozoan parasites (Pepper et al., 2015; Gerba et al., 2017) (Table 3-1), and helminths (worm eggs). Helminths are present at low numbers in untreated wastewater in developed countries (Pepper et al., 2015) because they are large enough to be readily removed during primary and secondary settling in conventional treatment. The settling also reduces protozoa, which also have a large size that limits their ability to transport through soil, geological materials, and aquifers compared to bacteria and viruses. The protozoa *Giardia* and *Cryptosporidium* are waterborne pathogens of primary concern in conventional drinking water treatment, since they possess an environmentally resistant stage (i.e., cysts, oocysts) and are more resistant to chlorine, chlorine dioxide, and ozone than viruses and bacteria (Pepper et al., 2015).

Pathogenic bacteria are significantly reduced after conventional wastewater treatment and disinfection and generally have a more limited survival time in the environment than protozoan cysts/oocysts and viruses (Pepper et al., 2015). Under nutrient-rich and certain environmental conditions, they may experience regrowth in the environment (Sadowsky and Whitman, 2011). Viruses are generally removed to a lesser degree during wastewater treatment than other pathogens because of their small size (Pepper et al., 2015). Table 3-1 presents the typical size range of pathogens, surrogate, and indicators detected or used in MAR studies along with typical concentration ranges in raw wastewater.

Viruses also may mutate and evolve to become more resistant to disinfectants. For example, enteric virus resistance to chlorine disinfection has been found to vary 10-fold between the

same strain of virus (Meister, et al., 2018). In the presence of free chlorine, virus numbers can be reduced, although this requires prolonged contact times. Different viruses exhibit different resistances to disinfectants. For example, adenoviruses are very resistant to UV light disinfection, while reoviruses appear to be more resistant to oxidizing disinfectants (such as chlorine) than other enteric viruses (Pepper et al., 2015; Betancourt and Gerba, 2016). These two viruses are also most commonly isolated and detected downstream of wastewater disinfection systems (Betancourt and Gerba, 2016; Rodriguez et al., 2008; Qiu et al., 2018).

3.1.3 Relevant Pathogen Characteristics for Fate and Transport in MAR Systems

Besides size and shape, fate and transport of pathogens are determined by charge density, isoelectric point (the pH value at which the net surface charge switches its sign), and hydrophobicity. These characteristics are specifically relevant for viruses due to their small size at which surface interactions with subsurface materials dominate retardation behavior compared to bacteria or oocysts. The isoelectric point of viruses dictates charge, ionic strength, and adsorption potential of pathogens (US EPA 2015) as further discussed in Section 4.1.1. Isoelectric points can vary widely within a single virus species (see Table 3-2. The isoelectric point distribution of virus strains as a whole follows a bell-shaped curve, with most viruses falling between an isoelectric point range of 4.5 and 5, and an overall range for all viruses between 2 to 8 (Michen & Graule, 2010). Data for charge density and hydrophobicity are not listed herein, since available for only a few viruses.

Table 3-2. Isoelectric Points of Various Viruses.
Data source: Michen & Graule 2010

Virus	Isoelectric Point
Adenovirus (Human Adeno C)	4.5
Hepatitis A	2.8
Enteroviruses	4.00 - 6.75
Norwalk virus	5.5 – 6.0
Poliovirus - 1	4.0 – 8.3
Rotavirus	8.0
Indicator / Surrogate Viruses	
MS2 (Escherichia virus, Bacteriophage)	2.2 – 4.0
PRD-1 (Bacteriophage)	3.8 - 4.2
ΦX-174 (Escherichia virus, bacteriophage)	2.6 – 7.4

Because each individual pathogen is unique, the greater the population size, the more likely that some individuals are expected to have traits that aid their survival or removal by transport through the soil and geological materials. For viruses, this likely explains why removal is often non-linear (Pang 2009). Furthermore, microbial pathogens evolve, and traits that affect their survival, persistence, and concentration in wastewater may change over time (Table 3-3).

Table 3-3. Factors Affecting Microbial Removal by Treatment Processes.

Factors	Pathogen Removal Impact
Pathogen Biodiversity	Resistance to disinfectants and processes that involve surface interactions vary with species, geno- and phenotype, and population size. The larger the population (number of individuals), the more likely some members of the population will not be removed to the same degree. Small differences in the proteins or nucleic acid or interaction with particulates or chemicals can result in greater resistance to removal by treatment processes (Dowd et al., 1998; Zhong et al., 2017).
Evolution and Natural Selection	Virus exposure to higher temperatures in the environment make them more resistant to disinfectants, and future generations are more resistant when exposed to higher temperatures. (For example, groundwater temperatures in Arizona are as high as 32 °C, and storage of water in ponds prior to or during infiltration increases the temperature.) Viruses that tend to aggregate (Gerba and Betancourt 2017) will be selected over time because of greater resistance to disinfection due to shielding. (Which viruses form aggregates and under which water quality and environmental conditions is not yet fully understood.) Treatment, specifically disinfection, has also been suggested to place selective pressure on the evolution of certain viruses, leading to greater resistance (Rachmadi et al., 2018).
Variability of the Treatment Processes Performance	The efficiency of treatment processes is variable, affected by the quality of the influent stream and changes in sub-optimal performance for a specific process. For MAR, this may be changes in the depth to groundwater over time, rainfall events, use of waters of different quality (e.g., stormwater, algal blooms, etc.) Short duration sub-optimal events (below specification) of duration, for example, disinfection interruptions as short as 15 minutes, can drastically increase MAR source concentrations of pathogens and the annual risks from waterborne pathogens (Soller et al., 2018b; Haas and Trussell, 1998).
Importance of Process Scale for Removal Efficiency	Full-scale treatment processes may not operate ideally or as well as laboratory- or pilot-scale systems. For example, short circuiting has been observed to occur (e.g., in some full-scale disinfectant contact tanks or in heterogeneous subsurface environments) (Pang, 2009; Morrison et al., 2020a).

3.2 Analytical Methods and Detection Sensitivity

The analytical methods employed for microbes drive our ability to detect and quantify pathogens to assess environmental removal and persistence. A wide variety of methods have been employed for detecting microorganisms in water. Table 3-4 lists some of the more common methods along with respective advantages and limitations.

Traditionally, cultural methods (detection of growth in laboratory media) have been used, but advances in analytical instrumentation and antibody and nucleic acid assays have led to the development of methods that can detect a much wider variety of organisms for which no culture methods exist (e.g., norovirus). Using molecular methods, groups of related viruses or other organisms can be detected, e.g., all of the adenoviruses can be detected with one assay. Although these methods have limitations, they do provide a toolbox for answering specific questions about routine monitoring or assessing treatment processes.

Table 3-4. Methods for the Detection of Microorganisms in Water and Their Advantages.

Method	Microscopic	Cultural	Physiological	Antibody Methods	Nucleic Acid
Method Specifics	•Visible light •Fluorescence •Flow cytometer •Imaging	•Growth on media •Cell culture •Direct counts	•Substrate utilization •Carbon respiration •Radiolabeled tracers •Adenylate energy change •Enzymatic assays •Stable isotope probing	•Immunoassays •Immuno-sensors	•Hybridization-based assays (probes) •Polymerase chain reaction (PCR) •Deoxyribonucleic Acid (DNA)-fingerprinting •Recombinant DNA techniques •Sequence analysis •Metagenomics
Advantages	•Useful for protozoan detection •Does not require growth of the organism	•Can determine infectivity	•Does not require growth media or animal cells for detection	•Does not require growth of the organism for detection	•Can detect low levels of organisms •Does not require cultivation of the organisms •Approaches available to determine viability in some cases •Can detect entire genera of viruses, families, or classes of microorganisms with one primer set
Limitations	•Only small volumes can be assayed •Need specific antibodies for each species •Cannot determine viability •Not as sensitive as molecular methods	•Will not detect non-culturable organisms i.e., norovirus •Requires specific cell lines for different viruses and protozoa •Underestimates number of infectious viruses (cell culture methods may only detect 1:10,000 of all the infectious viruses)	•Mostly useful for bacteria and protozoan •Provide general information on bacterial populations rather than specific detection of an organism •Enzymatic assays often require specific antibodies for detection and are not as sensitive as molecular methods •May not be able to determine viability	•Need a specific antibody for each target organism •Cannot determine viability of the organism •Not as sensitive as molecular methods	•Small volumes can only be assayed - although concentration of samples is possible •Interfering substances may be present in samples

3.2.1 Microscopic Methods

Microscope observation has been primarily used for protozoa detection and identification. This usually requires the use of specific antibodies labeled with a fluorescence dye. Training is required for observation of internal bodies in the cyst or oocyst. While a rough assessment of viability of protozoan cysts or oocysts can be determined by looking at internal bodies, it is not an absolute measure of viability. A limitation of these methods is the small volume that can be processed and the and the requirement of specific antibodies for each organism. Also,

background fluorescence from algae and other materials present in the sample interferes with these methods.

3.2.2 Culture Methods

Cultural methods have been used for the indicator and pathogenic bacteria and virus. An assay to culture pathogenic bacteria and viruses can take days to weeks to complete (Table 3-5). Larger volumes need to be assayed for the pathogens because they occur in lower numbers than indicator bacteria. Larger volume assays for the pathogens are also believed to be necessary because only a few viral or protozoan pathogens need be ingested to potentially cause an infection.

Animal cell culture assays (e.g., Buffalo Green Monkey Kidney Cells) are required to detect infectious human viruses in environmental samples. New cell culture methods have not been developed for many of the waterborne viruses, and cell lines may vary in sensitivity to virus types over time. Assay methods are not 100% efficient and are influenced by the analytical protocol and method used (e.g., the incubation time, volume assayed), recognition and numeration methods (plaque forming units versus cytopathogenic effects), and the number of cell passages used (number of times the cells have been subcultured into a new vessel) (Gerba et al., 2018). Studies of infectious viruses in water have largely been limited to enteroviruses, reoviruses, and adenoviruses.

3.2.3 Physiological Methods

These methods depend on the utilization of a substrate metabolism or by-product by bacteria, fungi, or protozoan. Physiological methods are not used for detection of viruses in environmental samples. With the use of selective media groups bacteria can be identified (i.e., coliforms) or species (*Escherichia coli*). Other approaches such as adenosine triphosphate (ATP) or measure of metabolites can be used to measure general microbial activity (excluding viruses), but not specific pathogens. Stable isotope probing is a molecular technique used for tracing fluxes of nutrients in the biogeochemical cycling of microorganisms, such as ^{13}C isotopes.

3.2.4 Antibody Methods

Antibody detection methods can be used for identification of organisms when linked to an enzyme which produces a color change in a solution. Another approach is to use a fluorescence detector e.g., a microfiber with antibodies attached. These methods require a specific antibody for each species of microorganism to be detected. They are not as sensitive as molecular methods and background fluorescence from other materials can interfere.

3.2.5 Nucleic Acid Methods

Cultural and polymerase chain reaction (PCR) assays are the most sensitive assays because they involve replication or amplification (the result is thousands or millions of signals that can be detected), which can result in the detection of 1 to 10 microorganisms in a sample. Unfortunately, this method cannot determine if microorganisms are viable or infectious, as it will detect pieces or fragments if present. In recent years, PCR methods have been developed

to determine viability of viruses and other microorganisms, but they have not been universally applied and extensively tested (Rodriguez et al., 2009).

Quantitative PCR (qPCR) methods have the advantages over regular PCR of being able to detect and quantify all types of microorganisms. The recent pandemic of coronavirus disease (COVID-19) has resulted in the first commercial test kit for detecting viral pathogens in water (IDEXX, 2020). The availability of test kits is expected to make it easier for more laboratories to conduct testing for waterborne pathogens. In addition, the development of digital droplet polymerase chain reaction (ddPCR) has allowed for more precise determination of the concentration of pathogens and increased the sensitivity of qPCR. This technique is based on the partitioning of the sample into thousands of wells of defined volume in aqueous droplets in oil. PCR then determines whether each droplet contains the nucleic acid of interest or not, thus allowing the absolute estimation of the number of molecules in the reaction. This method is similar to the most probable number method (MPN), allowing for a high degree of precision.

Table 3-5. Assay Parameters for Microorganism Detection in Water.

Microorganism	Typical sample volumes	Time for detection with cultural methods	Approximate cost (in U.S. dollars)
Bacteria	100 mL to 1 L	24 to 48 hours	$25 to 100
Protozoa	10 L to 100 L	Days	$350 to 500
Viruses	10 L to 1,000 L	Days to weeks	$500 to 1,200

The development of PCR to detect microorganisms has allowed us to detect any known organism in the environment by detecting the nucleic acid of a microorganism. The absence of detection by PCR indicates that viruses are not present, whether infectious or not.

Despite continued attempts to develop molecular methods that can indicate viability, significant limitations remain (Rodriguez et al., 2009; Knight et al., 2013, Wong & Molina, 2017). A major limitation of current methods is that the specific mechanism of virus inactivation must be known. This may vary with the type of disinfectant, environmental factors, and the type of virus. However, the application of molecular methods can be used to assess the removal of pathogens/indicators by physical removal and degradation of the microbial genome (Morrison et al., 2020b).

Metagenomics is a new approach that could be used to identify suitable indicators or surrogates of MAR performance. Because the costs of DNA sequencing continue to drop, metagenomics has allowed for identifying the strains in samples at a broad scale, looking at all genes from all members of the sampled communities, rather than limiting detection to specific target species. This approach also allows for identifying changes in the microbial population and for identifying pathogens resistant to removal (Zaouri et al., 2020).

3.2.6 Sensitivity of Analytical Methods

No available method targets all pathogens (i.e., no one method can detect and quantify all the different types of pathogens in a sample); therefore, their true number will always be underestimated to some extent. Our ability to detect pathogens in water depends upon several factors:

1. Sample size;
2. Presence of inhibitors which interfere with assay methods;
3. Pathogen pre-concentration efficiency;
4. Sample purification efficiency; and
5. Assay method and analytical sensitivity.

Due to the typically low concentrations of pathogens in treated effluent and MAR systems, and the human health risk associated with low concentrations, large sample volumes are usually processed (1 to 2,000 liters [L]). For viruses, up to 2,000 liters of groundwater have been processed in previous SAT studies (Morrison et al., 2020b). Theoretically, a single pathogen can be detected in these large volumes, making the methods for pathogen detection much more sensitive than the detection limits for chemicals.

Comparing the detection of a single virus in 100 to 1,000 liters on a weight basis illustrates this point. Viruses weight can be as low as 0.85 attogram (0.85×10^{-18} grams), resulting in a detection limit of approximately 10^{-23} to 10^{-24} on a weight per weight basis in 100 to 1,000 liters for viruses. In contrast, chemical tracer detection in water ranges from 10^{-6} to 10^{-12} on a weight-per-weight basis (as part per billion [ppb] or parts per trillion [ppt]).

The detection efficiency varies with the type and strain of the organism, the water quality (e.g., the type and concentration of organic matter present in the aqueous sample), pH, total suspended solids, and total dissolved solids. Furthermore, the volume processed, and method used for pre-concentration, such as ultrafiltration, glass beads, or filter adsorption elution, impact detection sensitivity (Ikner et al., 2012). Inhibitors from analytical controls are known to exist, although they have not yet been identified. Inhibitors may also be concentrated during sampling processing, which interferes with the assay methods. For these reason the true concentration of any pathogen in water is likely underestimated.

Positive controls are added to the sample to assess the efficiency of detection. Failure to detect any viruses can indicate an absence of pathogenic viruses and can be used to determine log removal credits (Morrison et al., 2020b). Likewise, indicators or surrogates, which are most resistant to removal, and the assessment of peak virus events and ranges of maximal pathogen concentration entering MAR systems are recommended to better determine the full range of log removal values (LRVs) that can be achieved and should be required under worst-case operating conditions (Morrison et al., 2020b; Betancourt et al., 2019; Betancourt et al., 2014).

3.3 Role of Pretreatment on Pathogen Concentrations in MAR Source Water

MAR systems in the United States have used a variety of surface water and treated domestic wastewater sources for recharge. Table 3-6 summarizes observed pathogen removal ranges via each treatment process (not from untreated wastewater) prior to recharge or injection, along with representative MAR examples in the United States and elsewhere (WHO, 2017; U.S. Environmental Protection Agency [USEPA], 2017).

Table 3-6. Log Removal of Pathogens by Treatment Processes.

Level of Wastewater Treatment	Typical Pathogen Log Removal Observed	MAR Site Examples
Secondary effluent, non-disinfected	• 1-2 log *Cryptosporidium* • 1-2 log *Giardia* • 0.5-1.7 log Enterovirus • 1-4 log Salmonella	Australia (Donn et al., 2020) United States (Pepper et al., 2015)
Secondary effluent, Bardenpho process, disinfected	• *1-2 log Cryptosporidium* • *1-2 log Giardia* • 3 -6 enteric viruses	United States (Morrison et al., 2020b) Schmitz et al., 2016 Schmitz et al., 2018
Tertiary treated effluent, disinfected	3->6 log virus, bacteria, protozoa	Montebello Forebay (CA) Upper Occoquan Service Authority (VA) Gwinnett County (GA), Langford (UK)
MF/UF + RO permeate	>6 log protozoa 2 to >6 log viruses >6 log bacteria	Big Spring, TX Orange County Water District, CA Perth (Australia) Singapore Wulpen (Belgium) Tucson, AZ. (Morrison et al., 2020a)

As can be seen from Table 3-6, different levels of wastewater pretreatment are in use by different facilities operating MAR systems in the U.S. Water suitable for use as MAR source water should at minimum have a low physical and biological clogging potential. The clogging potential depends primarily on the concentration of suspended solids, nutrients, and biodegradable dissolved organic carbon (DOC) as well as on the soil conditions and the hydraulic conductivity of the infiltration zone (including schmutzdecke) and underlying aquifer. Growth of algae and bacteria in the schmutzdecke can rapidly clog infiltration basins reducing infiltration rates, especially in the summer with warmer temperatures and longer sunlit days. This often requires plowing and cleaning of infiltration basins to restore infiltration rates.

The degree of wastewater treatment prior to recharge impacts the level of infectious pathogens and indicators that will be present in the MAR source water (Table 3-6). During secondary activated sludge treatment between 90 to 99% (1 to 2 logs) of the pathogens are commonly removed. Tertiary treatment involving filtration and chlorine disinfection can then further reduce infectious organisms by a total of 3 to 5 logs (Pepper et al., 2015).

Advanced treatment such as ultrafiltration and reverse osmosis can further reduce these numbers. Ultrafiltration is actual used as a pre-concentration step for detecting viruses in water. However, lab ultrafiltration devices differ from full-scale treatment systems since new filters are used every time and risk of potential damage or production flaws are low. With advanced treatment, the concentration of infectious organisms may be below the detection limits of culture-based methods but may still be detectable by molecular methods (qPCR) because, as discussed, these methods are more sensitive (Gerba et al., 2017; 2018) and can detect fragments of non-viable organisms. Very low to non-detect MAR source water concentrations make it very difficult to demonstrate and quantify pathogen, indicator, or surrogate removal either in the field site or in the lab. Consistent detectable concentrations in the source water are required to demonstrate LRVs to justify regulatory treatment credits.

The level of wastewater treatment before MAR influences more than just the extent of removal of pathogens prior to MAR. The resulting effluent quality also indirectly impacts the fate and transport of pathogens during aquifer recharge. As an example, removal of nutrients (nitrogen and phosphorus) during treatment results in more rapid infiltration of the water, reducing the time for pathogen inactivation and attachment to subsurface particles. The schmutzdecke may be less biologically active as a result and the removal of pathogens during infiltration may be reduced (Morrison et al., 2020b).

It has been speculated that nitrification of source water reduces the oxygen demand and increases the rate of MAR treatment by maintaining aerobic conditions longer resulting in shorter survival of viruses likely due to higher biological activity and predation. Whether this is indeed a driving factor for increased biological activity and predation is uncertain and the authors are not aware of field data supporting this hypothesis to date.

Water with low dissolved solids could impact the adsorption of viruses (Zhang et al., 2019) as well as the presence of metal oxides on the aquifer material (Chu et al., 2003). Salt concentrations impacts the adsorption of viruses to surfaces, with less adsorption occurring under conditions of low ionic strength. Also, adsorbed viruses may desorb and go back into the water phase when ionic strength is decreased, such as after rainfall events. Many metal surfaces have a greater positive surface charge density, which enhances the adsorption of the negatively charge viruses to these surfaces. The presence of certain types of particulate matter in the water may also affect the co-transport of pathogens (Walshe et al., 2010). These and other factors impacting the fate and transport of pathogens during MAR will be discussed in more detail in Chapter 4.

3.4 Selection of Appropriate Indicators and Surrogates for Pathogens

Indicators have traditionally been used to determine whether a water source is fecally contaminated and whether enteric pathogens might be present. Ideally, indicators for wastewater treatment and MAR system performance are organisms that should have all of the following characteristics, without being overly conservative:

- Present in numbers equal or greater than the target pathogens.
- No seasonal variation in the wastewater.
- Survival equal to or greater than the pathogens.
- Transport velocity equal to or greater than that of pathogens (e.g., similar size, shape, surface charge, etc.).
- Reasonable cost to assay/quantify.

For pathogens, surrogates are organisms, particles, or substances used in field and laboratory to study the fate, transport, and removal of pathogens in a specific environment. Ideally, surrogates for pathogens should have the following characteristics, without being overly conservative (Sinclair et al., 2012):

- Similar fate and transport behavior to pathogens (e.g., similar size, shape, surface charge, hydrophobicity, etc.).
- Appropriate resemblance of pathogen resistance, inactivation, and movement to estimate the number of pathogen concentrations in risk assessments.
- Available in high numbers or measurable concentrations.
- Common in the environment and easy to detect.

Table 3-7 below provides examples of microbial indicators for fecal contamination and surrogates for the fate and transport of pathogens. While there is no ideal organism that can relate exactly to the risk of infection to the ingestion of water, indicators and surrogates can be used to judge the performance of groups of pathogens in response to treatment processes. Some organisms can serve as both indicators and surrogates. For example, non-pathogens, such as male-specific coliphages (bacterial viruses that infect coliform bacteria) can also be used as surrogates for assessing pathogen removal. These viruses also typically occur in connection with fecal contamination.

To avoid confusion, these definitions for microbial indicators and surrogates differ from definitions commonly used for *chemical* indicators and surrogates. Chemical indicators (similar to pathogen indicators) are commonly defined as chemicals that can be correlated to a certain chemical target, are easy to measure, and are commonly found in the aqueous target environment. Chemical surrogates refer often to bulk or group parameters like total organic carbon (TOC) or ultraviolet absorbance (UVA) that contain specific target chemicals.

U.S. regulations of MAR systems have focused on monitoring fecal coliforms or *E. coli* as bacterial indicators for pathogen presence since they are inexpensive and easy to measure. (See Chapter 5 for further discussion.) However, today we recognize that there is rarely a direct correlation between the numbers of bacterial indicators and human pathogens (Pepper et al., 2015). Viruses and protozoan pathogens are more resistant to most treatment processes and survive longer in the environment, and infection occurs only after ingesting comparatively fewer organisms (Haas et. al., 2014).

Other surrogates can include dyed bacteria or artificial designed particles such as latex microspheres (Harvey at al., 1989). Clemens et al. (2020) have demonstrated that the attenuation and transport of DNA-labeled, glycoprotein-coated silica nanoparticles (DGSnp) very closely resembles that of rotavirus in subsurface saturated aquifer systems. Both particles showed greater reduction compared to the transport behavior of MS2 bacteriophages. The authors proposed that the biomolecule-modified silica nanoparticles DGSnp can serve as a new surrogate for rotaviruses. The design of human-made pathogen surrogates and their application in field tracer tests can indicate the treatment performance relative to pathogens and is useful as an “index or model organisms.” Such surrogates could potentially be used to develop models of survival and transport of pathogens in the environment. However, microsphere surrogates are typically of uniform composition while microorganisms are not. Most designed microspheres are not degradable, which make them appropriate for identifying removal by straining or adsorption but not from degradation and inactivation.

As previously discussed, viruses are expected to generally be more difficult to remove, especially in MAR systems with long travel times (weeks to months) compared to the bacteria and protozoa. Therefore, for SAT, ASR, ASTR, and injection sites, the absence of viruses or their significant reduction would ensure that bacteria and protozoa have been significantly reduced or limited below detect. Thus, selecting virus as a measure of site performance for pathogen removal credits or treated water quality may be useful. Reduction of coliphages, plant viruses (pepper mild mottle virus [PMMoV]), or groups of viruses (CRassphage) could measure the performance of such MAR sites. PMMoV are identified through RNA and not through infectivity, which is one limitation that makes this virus an overly conservative surrogate. However, PMMoV may be a good surrogate to indicate preferential flow paths in MAR systems, even though it is less suitable for quantitative microbial risk assessments (QMRAs).

With the development of PCR or qPCR to detect microorganisms, we have been able to assess their physical or biological degradation in treatment processes. With qPCR, almost any organism (viruses, bacteria, protozoa) can be detected, a significant advantage. qPCR is generally considered a conservative estimate of an MAR system's performance since the loss of infectivity is not considered. Metagenomic analysis of source or well water could help in monitoring the most abundant pathogens, indicators, or surrogates to assess MAR operations. Since viruses can be quantified with qPCR, quantification of MAR system input concentrations and measurement of extracted water in monitoring or production wells could be used to estimate conservative credits for infectious virus log removals (Morrison et al., 2020b). Predictive modeling could be used to support these estimates based on virus detection data to simulate the expected performance.

For MAR systems with short subsurface travel times and distances (such as IBF sites), protozoa are a key concern, along with viruses and indicators and surrogates should be selected to represent both pathogen categories (see the following Section 3.4.1 for further discussion).

3.4.1 Pathogen Surrogates for MAR Systems

Due to the cost of analysis, the low natural concentrations, and health risks associated with pathogens, surrogates have largely been used in field and laboratory studies. Laboratory studies seek to quantify and model the removal of pathogens, often using surrogates, in MAR systems at smaller scale and under more well-controlled conditions than full-scale field conditions. Coliphage MS2, for example, has been used extensively in laboratory and field studies as a surrogate. This virus shares many traits of human enteric viruses (i.e., enteroviruses, norovirus), can be grown in large numbers, and appears to be less retarded than many human enteric viruses during transport in MAR systems. *Escherichia coli* is not only used as an indicator but is also often used as a surrogate to model enteric bacterial removal in the laboratory and field.

Attenuation and decay rates differ by pathogen type. Bacteria are generally less able to survive in groundwater than viruses (Toze et al., 2010, Sidhu and Toze, 2012). For example, the die-off rate of *Salmonella* is 40 times higher than for rotavirus (see Section 4.2). Generally, the die-off rates of enteric bacteria have been ten to more than 100 times greater than of enteric viruses (Sidhu et al., 2010; Sidhu and Toze, 2012; Toze et al., 2010). To illustrate the relevance of this, in

an outbreak of hepatitis A, the virus was believed to have survived 22 months in groundwater in the Salento peninsula in southern Italy (Masciopinto et al., 2019). Another outbreak of hepatitis A in a rural river-island community on Orleans Island near Quebec City, Canada, was caused by contaminated well water and could be traced back to a cesspool at 60-meter distance and was still detected using immunocapture reverse-transcription polymerase chain reaction (IC-RT-PCR) six months after the initial contaminations when fecal coliform bacteria were no longer present (De Serres et al., 1999).

Different viruses may behave differently in transport through both the saturated and unsaturated zones in porous media (Jin et al., 2000; Shirasaki et al., 2018). Because they generally survive longer than bacteria and have the potential for greater long distant transport, the industry has generally focused on viruses as conservative pathogen indicators in MAR systems. Promising candidates are PMMoV and CrAssphage. These are not only the most abundant viruses in wastewater effluents year-round, but they can also be detected passing through reverse osmosis treatment and in the groundwater at MAR sites (Morrison et al., 2020a; Morrison et al. 2021) via PCR. All viruses are capable of penetrating RO full scale treatment operations, but detection of PMMoV and CrAssphage are more common because they occur in higher concentrations than human pathogenic viruses in wastewater, and thus they are more commonly detected in the permeate via PCR. Challenge tests assess the integrity and efficiency of membranes for pathogen removal and involve dosing a pathogen surrogate into the membrane feed water while monitoring the log removal across the membrane unit (Reeve et al., 2017). While challenge testing for virus removal by membranes may provide information at a particular time or with a laboratory scale test, they are not reflective of removal by full scale plants, where only few membrane fibers or elements out of the full process may exhibit integrity issues (Johnson and MacCormick, 2005).

Enteric bacteriophages, which are viruses infecting enteric bacteria, have been proposed as indicators for human viral pathogens. Coliphages, one taxonomic group of enteric bacteriophages, infect *E. coli* and have a likely fecal origin. They may serve as an indicator and surrogate for fecal pathogens. Bacteriophages that infect their hosts through receptors on the cell wall are called somatic. Male-specific coliphages infect hosts through receptors on F pili, hollow tubes used for connection to allow the transfer of genetic material. Schijven and Hassanized (2000) reviewed coliphages MS2, PRD-1, ΦX-174 and the FRNAPH coliphage (F-specific RNA bacteriophages), which are commonly used in column and field studies to model virus survival and transport. The authors concluded that FRNAPH as a group of naturally occurring viruses in wastewater are very useful viruses to model the behavior of viruses during subsurface transport. FRNAPH coliphages behave relatively conservatively—similar to MS2—and have been shown to be very persistent in the environment. In addition, FRNAPH that are naturally present adsorb poorly, more easily passing through soil and subsurface materials. Somatic coliphages usually occur in greater numbers than the FRNAPH but represent a larger distribution of particle sizes and shapes than the FRNAPH phages. The USEPA has published methods for both male-specific and somatic coliphages detection in water (USEPA, 2001).

Other surrogates and indicators become feasible options in MAR systems with relatively short retention times in the subsurface, such as IBF. Due to short residence times and travel

distances, oocysts are a special concern since they may go untreated if unrecognized in well water post-treated with chlorination only. Thus, under GWUDI regulations, federal and state regulations and guidance in the United States suggest or require monitoring of pathogen and surface water indicators such as total coliforms, *E. coli*, enterococci, coliphage, and the microscopic particulate analysis (MPA), a method that includes direct monitoring for the two pathogens *Cryptosporidium* and *Giardia*. Using USEPA Method 1623, Abbaszadegan et al. (2011) found Cryptosporidium generally absent in GWUDI well water samples from Sioux City and Cedar Rapids IA, with the exception of two out of a total of 24 samples.

The detection limit of *Cryptosporidium* in IBF wells depends on the sample size collected and the analytical method employed. In a study conducted by Weiss et al. (2005), oocysts were not detected in any well sample collected over the course of one year at three riverbank filtration facilities along the Ohio, Missouri, and Wabash Rivers. In this study, 100 L sample sizes were collected from well samples following the USEPA method 1623 (filtration / magnetic bead isolation/DAPI staining and fluorescence interference contrast microscopy). Log removals could not be determined for *Cryptosporidium* and *Giardia* due to low source water concentrations in the river. Total coliforms were also rarely detected in the well samples with 5-6 log removal reductions at one site. Other surrogates could be detected, and log removals quantified. Aerobic spore-forming bacteria were reduced between 0.8 to more than 3.1 logs, male-specific coliphages by more than 2.1 logs, and somatic bacteriophage by more than 3.2 logs, respectively. From these results, aerobic spores were revealed to have the most conservative log removal values compared to coliforms and bacteriophages.

Aerobic spores have been used in various IBF studies as a useful surrogate for *Cryptosporidium* in conjunction with total coliforms (Berger et al., 2018; Headd & Bradford, 2016.). Aerobic spores are about 1 micrometer in size, spherical, long-lived in the subsurface, dormant, and resistant, and they are commonly found in surface water, groundwater, and in groundwater closely connected to surface water. Analysis follows membrane filtration for concentration and growth to enumerate. The cost of analysis is typically less than $100 per sample, similar to coliforms (about $30 per sample). Because spores are long-lived, they have been detected in groundwater wells with no coliforms present. Limitations of spores as protozoa surrogates include that they vary in size, are smaller than *Cryptosporidium* and occurs ubiquitously even without contamination and therefore may be overly conservative.

A higher analytical sensitivity for *Cryptosporidium* detection can be achieved using qPCR. However, as discussed earlier, a positive result does not necessarily indicate infectious oocyst presence. Nevertheless, qPCR monitoring for oocyst genetic fragments can be a useful indicator and surrogate tracer. In a comprehensive study, Stokdyk et al. (2019) detected *Cryptosporidium* in 40% of 145 public water system wells in Minnesota by qPCR, and 62% of all positive samples were confirmed by immunofluorescence assay (IFA). The mean sample volume collected was 728 L (range: 140-1,783 L). Only two *Cryptosporidium*-positive wells had been previously categorized by the State of Minnesota as GWUDI for regulatory purposes; the remaining positive wells had not been regulated as GWUDI. The authors concluded that *Cryptosporidium* detection by qPCR in groundwater can occur, even when surface water influence is absent.

Note that many wells sampled in this study are located in karstic aquifer systems, which are not typically used for MAR systems because of their high transmissivity.

In past years, other surrogates have been suggested for MAR systems. Flow cytometry is a method that allows for quantifying cells and their size in groundwater. The method has recently received attention as an alternative surrogate for pathogen transport in groundwater systems (Safford and Bischel, 2019). Critics of this tool raise concerns that the method may not differentiate between cells of source water and aquifer origin.

3.4.2 Pathogen Surrogate Occurrence and Transport in MAR Systems

Pathogen removal during MAR operation can be measured to monitor the impact of operational changes. Coliphages have long been suggested as potential indicators, and significant effort has been expended to model their transport in groundwater (USEPA, 2015). The USEPA has a culture-based method to detect contamination of viruses in drinking water wells. For these methods, a ten-liter sample is collected to detect somatic and male-specific coliphages (USEPA, 2008a).

FRNAPH have been suggested as conservative indicators of virus removal by MAR (Schijven and Hassenizadeh, 2000). Sinton et al. (2000) compared the relative attenuation of rhodamine dye, *E. coli*, *Bacillus subtilis* endospores, and the F-Specific ribonucleic acid Bacteriophages (F-RNA) bacteriophage MS2 in an alluvial gravel aquifer at an MAR site at Burnham in Christchurch, New Zealand. The transport velocities of these indicators and surrogates were proportionate to the extent that they were excluded from small pore sizes and followed preferential flow paths. Larger particles exhibited the highest velocity (and smallest retardation). *E. coli* cells are 1,500 in diameter and 6,000 nm in length, B. *subtilis* endospores are 800 nm in diameter and 1,500-1,800 nm long, and MS2 phage is 27 nm in diameter. Rhodamine WT was assumed to be transported through all pore sizes. The travel velocity for *E. coli* J6-2 in the co-injection field test was only about 10% higher than MS2. How useful male-specific phages are as surrogates for enteric viruses still needs to be assessed.

Enteroviruses have been studied the most at MAR operations because a cell culture assay has been available for over a half a century. Infectivity assays are also available for several enteric viruses (see Table 3-4). However, special laboratories are needed, and the assays have a high cost and often take weeks, limiting their routine use. However, quantitative molecular methods have greatly increased our ability to study the concentration and range of viruses present in domestic wastewater, and all known pathogen/indicator now can be analytically detected if present. Several studies have applied this technology to assess the removal of microorganisms. For viruses, we now know that plant and bacterial viruses are the most abundant viruses in domestic wastewater. Several studies have shown that the plant virus PMMoV may be the most useful in quantifying virus removal during wastewater treatment processes (Kitajima et al., 2014; Symonds et al. 2018; Shirasaki et al., 2018; Farkas et al., 2020). This is mostly because, while very different in shape and size to human enteric viruses, PMMoV is always present in high levels and is significantly more resistant to removal than human enteric viruses. PMMoV is thus considered to be a good indicator of wastewater pollution. In case PMMoV is absent, typically human enteric viruses are as well.

PMMoV was the only viral indicator detected along with crAssphage at a MAR operation in Arizona (Morrison et al., 2020b). It has also been suggested as a conservative indicator of virus removal by MAR (Betancourt et al., 2014). Its significantly higher resilience compared to enteric viruses makes the PMMoV an inappropriate surrogate for risk assessments of fecal contamination.

Adenovirus have also been suggested as a potential indicator because they appear to occur in greater concentrations than the other human enteric pathogens and show little seasonal variation (Kitajima et al., 2014; Shirasaki et al., 2020).

Table 3-7. Advantages and Limitations of MAR Pathogen Surrogates and Indicators.

Surrogate / Indicator	Advantages	Limitations
Viruses		
Somatic coliphages (Indicator, Surrogate)	• Low-cost detection methods • Rapid analysis • Always present in wastewater • Limited seasonal variation. • Long survival • Present in larger numbers than FRNAPH	• Larger than some human viruses • Structure differs significantly from some human enteric viruses and FRNAPH
Male specific RNA coliphages (FRNAPH) (Indicator, Surrogate)	• Low-cost detection methods • Rapid analysis • Always present in wastewater • Limited seasonal variation • Long survival • Conservative removal rate through subsurface assumed	• May not represent the behavior of all human viruses (i.e., different survival, size, shape, persistence in the environment) • Present in smaller numbers than somatic coliphages
Enteroviruses (Indicator, Surrogate)	• Infectivity assays and detection methods well developed • Many studies on transport through subsurface conducted	• Concentrations in wastewater vary seasonally • Represent only a small fraction of all the human enteric viruses detectable in wastewater
Adenoviruses (Indicator, Surrogate)	• Infectivity assays and detection methods well developed. • Occurs in greatest concentration of all the enteric viruses in wastewater • Limited seasonal variation.	• Free DNA may persist longtime in the environment • Largest virus of all enteric viruses (~70 nm, see Table 3-1)
Aichi viruses (Indicator)	• Infectivity assay available • Limited seasonal variation	• Little known about transport through subsurface
Pepper mild mottle virus (PMMoV) (Indicator, Surrogate)	• Highest concentrations in wastewater year around • Long survival and persistence in the environment	• Can only be easily detected by qPCR • Too conservative for QMRAs
CrAssphage (Indicator)	• Smallest known size of all viruses	• Can only be easily detected by qPCR • Little known about concentrations from one location to another at present
Bacteroides phage (Indicator, Surrogate)	• Abundant in wastewater • Limited seasonal variation	• Laboratory methods require anaerobic chambers
Bacteria		
Total Coliforms (indicator)	• Abundant in wastewater • No seasonal variation • Low-cost detection methods	• Can occur from non-wastewater sources • Capable of growth in the environment • Not an indicator of virus removal
*E. coli (*indicator, surrogate for enteric bacteria)	• Abundant in wastewater • No seasonal variation • Low-cost detection methods	• Not an indicator of virus removal
Protozoa		
Aerobic spores (Surrogate for protozoa)	• Low-cost detection methods	• Not an indicator of virus removal • Abundant in soil
Microsporidium (Indicator, Surrogate for protozoa)	• Common in wastewater	• Not an indicator of virus removal • Detection methods require qPCR, immunofluorescence
Chemical Tracers		
Chemicals (i.e., nitrate, primidone, sucralose, PFAS, etc.) (Surrogates)	• Low-cost detection methods available	• Methods lack sensitivity • Solutes are transported differently than colloids • Not predictive of microbial removal. • Cannot assess microbial viability

CHAPTER 4

Current Understanding of Pathogen Occurrence and Fate and Transport

This chapter summarizes the current state of scientific knowledge on the mechanisms and factors influencing fate and transport of pathogens, indicators, and surrogates in MAR systems along with a summary of fate parameters for a variety of microbial agents in MAR systems as reported in literature. Various methods for monitoring and predicting pathogen removal in MAR systems are also discussed.

4.1 Factors Influencing Pathogen Fate and Transport

Pathogen removal in subsurface systems is a combination of die-off (decay or inactivation), predation, and physical attenuation (e.g., attachment). Microbial removal usually follows a biphasic or Weibull distribution, with initial greater removal/decay followed by a lower removal/decay (Figure 4-1). The retention time in a MAR system is generally assumed to be positively correlated with the level of pathogen removal. The infectivity of larger protozoan parasites usually decreases faster than that of viruses (Sidhu et al., 2015).

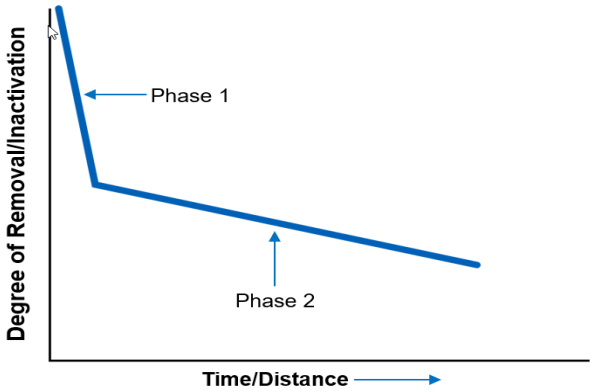

Figure 4-1. Schematic Representation of the Biphasic Nature of Pathogen Removal.
(In MAR groundwater recharge, where initial removal is high but later removal decreases (more information in Pang 2009)

However, other factors in the subsurface—among them redox conditions, mineralogy, attachment and detachment, and temperature—also have a major influence (see Table 4-1). Greater inactivation typically occurs near the soil surface and in the vadose zone where temperature and moisture content are more variable and biological activity and predation is greater. Soil drying during low flow conditions or SAT drying cycles can result in desiccation and higher UV-light exposure and, consequently, in lower pathogen survival. The greater microbial activity in the schmutzdecke diminishes pathogen numbers through predation. Thus, pathogens retained near the soil surface have a shorter survival rate than those reaching the saturated zone, as recently demonstrated for enteric pathogens in MAR systems in Australia (Sidhu and Toze, 2012).

Meschke (2001) investigated the long-term survival of viruses in saturated aquifers. He found that Norwalk virus and infectious Poliovirus type 1 were still detectable after up to 70 weeks, although virus concentrations had greatly declined. The mean ratio of reduction in infectivity to reduction in detectable nucleic acid for Poliovirus type 1 and MS2 were 1.7 and 3.2, respectively, indicating an over-proportionate decrease in infectivity compared to a reduction in detectable nucleic acid. It is also worth noting that pathogens that sorb to subsurface particles are not always permanently immobilized and can become remobilized, especially if changes in water quality or flow conditions occur (Masciopinto et al. 2008; Quanrud et al., 2003).

Table 4-1. Major Factors Driving Pathogen Transport and Removal during MAR.

Factor	Relevance / Examples
Organic matter in MAR source water and soil	Organic presence can extend pathogen survival, but cause-effect relationships are complex. Organic matter also supports a degree of higher and more complex lifeforms that increase predatory pressure on pathogens.
Infiltration rate	Higher infiltration rates can result in faster pathogen transport through the vadose zone and less chance for adsorption to the soil and geological material (Chapter 3).
Subsurface retention time	Virus removal is proportional to travel distance, but not necessarily in a linear/constant manner.
Regrowth	Regrowth of some bacteria (e.g., Salmonella) can occur under the right conditions (e.g., high moisture and organic load). Regrowth of viruses in the subsurface or in well casings is not expected for most viruses but could be relevant for bacteriophages such as coliphage and MS 2.
Die-off or decay rate	Enteric pathogens have a finite lifetime in the environment mainly driven by time and temperature.
Predation	By bacteria and protozoa.
Vadose zone depth	Generally greater retention of microbes occurs in the vadose zone compared to the saturated zone. Retention by adsorption depends on the hydrophobicity of the organism. For example, little movement of the coliphage MS2 occurs in the vadose zone because this virus associates with the air water interface, whereas the less hydrophobic ΦX-174 bacteriophage easily passes through the vadose zone.
Redox conditions and Microflora	Aerobic conditions result in shorter survival of viruses likely due to higher biological activity and predation.
Soil substrata and physico-chemical characteristics	Subsurface particle size, degree of sorting, layers of different porosity, grain chemistry, heterogeneities, and fractured bedrock material influence pathogen retention and transport.
Adsorption	The most important factor for the removal of viruses (Anders et al., 2004). Is influenced primarily by the isoelectric point (Dowd et al., 1998) and hydrophobicity of the organism and the surface characteristics of the soil and aquifer material. Generally, the lower the isoelectric point the less adsorption to the subsurface. Isoelectric points are both type and strain specific for microorganisms.
Hydrophobicity	Determines the adsorption behavior of pathogens. Important in the transport through the unsaturated zone because of the greater air-water interface.
Filtration	Considered only to be important for the transport of bacteria and protozoan parasites at MAR operations. Most virus removal is believed to be due to adsorption to the geological material (saturated) or air-water interfaces (unsaturated flow). Viruses attached to colloid material may be removed by filtration.
Retardation	The movement of microbes relative to the movement of the water in the subsurface under unsaturated and saturated conditions. This depends on the nature of the soil and aquifer substrata and the presence of preferential flow paths. Values in the literature range from 0.5 to 1.0 for all microbes. Under some conditions, viruses can move faster than the average groundwater flow because their movement is restricted to the larger pore spaces where the water moves faster.

(Continued)

Table 4-1. Continued.

Factor	Relevance / Examples
Velocity / hydraulic conductivity	Velocity depends upon the nature of the aquifer substrata and the hydraulic gradient.
Temperature / Climate	A critical factor for survival rates which are higher at lower temperatures.
Dynamic of water flow regimes and water quality changes in MAR systems	Dry-wet periods can influence the adsorption and desorption of bacteria and protozoa. Similarly, fluctuating water quality coming into the MAR system can alter the adsorption equilibrium in the subsurface. Pumping regime changes through shutoff/malfunction of pumps, for example, will affect the flow.
Level of wastewater pre-recharge treatment	Greater infiltration rates are generally achieved with water of lower turbidity and better nutrient removal. However, the schmutzdecke may be less developed in these systems due to faster transport of water (see Chapter 3). Additionally, whether disinfection is applied and what type (chlorination, ultraviolet irradiation, peracetic acid) is applied during wastewater treatment will influence infiltration the rate and concentrations of pathogens, surrogates, and indicators present in the source water.
Isoelectric points	Depends on viral proteins that may be exposed to the subsurface environment, influenced by water pH, and dissolved salts. The isoelectric point of viruses dictates charge, ionic strength, and adsorption potential (US EPA 2015).
Ionic strength and other source water quality parameters	Lower adsorption of microbes with lower ionic strength; previous adsorbed viruses can be de-adsorbed with reduced concentration of dissolved solids (Quanrud et al., 2003). Stormwater or rainfall may reduce salt concentrations.

4.1.1 Relevance of Site-Specific Factors on Pathogen Removal

Various physico-chemical factors affect the fate and transport of pathogens in the subsurface, and their interdependence dictates the observed removal. The relevance of individual factors for pathogen attenuation is discussed in the following sections. While we may be able to quantify correlations between specific parameters and pathogen removal, the interplay of multiple parameters must be considered.

4.1.1.1 Organic Carbon

Generally, the presence of dissolved and particulate organic carbon in the subsurface, especially as introduced from wastewater effluents or other MAR source waters, results in longer survival of viruses in the subsurface (Gerba et al. 1991).

While higher levels of carbon sources should theoretically increase the metabolic rate of indigenous microorganisms and thereby the decay (predation) rate of pathogens, the opposite effect is observed. Toze et al. (2010) found that the decay rate of *Cryptosporidium* was negatively correlated to biodegradable dissolved organic carbon (BDOC) concentrations present during an *in situ* decay study of a MAR site with infiltration of secondary treated wastewater. In another study, higher organic carbon decreased the decay rate for *E. coli*, MS2 phages, poliovirus, and Coxsackievirus (an enterovirus) in lab-scale experiments (Gordon and Toze 2003). This could either be because the introduced carbon surrounds the viral particles, preventing adsorption to the subsurface particles and preventing indigenous microorganisms from attacking the pathogens (Katzenelson, 1978), or because carbon acts as an alternative food source for the indigenous microorganisms (Gordon and Toze, 2003).

Microbial removal rates tend to be lower in carbon-rich aquifers than in pristine aquifers (Schijven et al., 2017). Dissolved organic matter in reclaimed effluent competes for adsorption sites with pathogens onto aquifer media and results in less removal than in pristine aquifers with low dissolved organic matter content (Schijven et al., 2017). Although BDOC and other

organic colloid matter in treated effluent competes with microbes for adsorption sites, pathogens can also adsorb onto the organic colloids and be co-transported with them. This protects pathogens from inactivation while reducing their concentration in the water phase (Pang 2009).

Organic compounds could also block available adsorption sites on aquifer media that may not be available any longer for pathogen attachment. Both organic carbon and viruses are predominantly negatively charged (Schijven et al., 2017). Virus surface charge is carried by viral proteins in the capsid or lipid membrane and is influenced by water pH and dissolved salts (Regnery et al., 2017).

4.1.1.2 Subsurface Retention Time

While greater retention time generally leads to greater removal, the relationship is not linear. The greatest removal occurs in the first few feet of infiltration, and removal rates decline subsequently with depth, contradicting conventional filtration theory (Regnery et al., 2017). Larger pathogens that are less negatively charged lose infectivity faster and will be removed at shorter distances than smaller, more negatively charged pathogens. In general, bacteria survive the shortest amount of time, followed by protozoa, and finally by viruses (Regnery et al., 2017; Page et al., 2018).

Most pathogens also show greater reduction in the vadose zone, since temperatures and moisture are more variable there, leading to greater microbial activity and predation contributing to pathogen inactivation (Regnery et al., 2017; Sidhu and Toze, 2012; Blaschke et al., 2016). Removal rates for viruses and bacteria in vadose zone media range typically from 0.1-1.0 log/meter for clay and silt, sand, gravels, and granite, and 1.5-4.8 log/meter for pumice sand, clay till, and occasionally uniform sand (Schijven et al., 2017). Vadose zone removal, which is primarily vertical via infiltration, is different than removal within an aquifer layer, which is primarily horizontal (Schijven et al., 2017). Page et al. 2010b suggests that an accurate understanding of subsurface residence time, coupled with accurate understanding of decay rates, allows MAR systems to be designed for a specific target log removal credit, as demonstrated for rotavirus, *Campylobacter spp*, and *Cryptosporidium*. While *in situ* tracer tests can be done, they are expensive and the obvious challenge of determining accurate pathogen decay rates remains. Likewise, transient conditions and changes in the subsurface make it difficult to accurately determine rates.

Retention time can decrease drastically during flooding events in IBF, also due to a drastic change in water chemistry, particularly ion strength (Bartak et al., 2015), and can mobilize previous sorbed pathogens. Produced water during and after flood events in IBF systems may contain elevated pathogen concentrations and require additional post treatment or a stop in production altogether (Derx et al. 2013). Not all MAR systems are prone to flood conditions, but flooding is common in RBF systems and in infiltration basins accepting stormwater runoff.

4.1.1.3 Predominant Redox Conditions

In lab-scale experiments, Gordon and Toze (2003) showed that aerobic conditions increased the decay of *E. coli* and MS2 in the subsurface, while anoxic conditions (oxygen concentrations not specifically stated) increased the persistence times (measured as the time it takes for viral titers

to be reduced by 90% [T_{90}]) for Coxsackievirus and poliovirus. Jansons et al. (1989) hypothesized that the higher viral decay rate under oxic conditions was caused by increased oxidation of the viral capsids (the protein shell of a virus, enclosing its genetic material), while Yates and Yates (1988) attributed this to increased indigenous organism predation. Likewise, decay rates of poliovirus were faster in the presence of higher dissolved oxygen (DO) concentrations (5.4 milligrams per liter [mg/L] DO, 0.09 $\log_{10}day^{-1}$ or 11 days T_{90}) than in lower DO concentrations (0.2 mg/L DO, 0.03 $\log_{10}day^{-1}$ or 18 days T_{90}). These results may have been influenced by microorganisms such as *Pseudomonas maltophila,* present at higher DO concentrations (John and Rose, 2005; Jansons et al., 1989). Hornstra et al. (2018) attributed more favorable removal under oxic conditions to improved attachment of pathogens to aquifer material (i.e., metal oxyhydroxides). Various other studies have confirmed inactivation to be slower under anoxic conditions (Frohnert et al., 2014; van der Wielen et al., 2008; Gordon and Toze, 2003).

However, recent work has demonstrated that low DO conditions of less than 1 mg/L (termed "suboxic" by some researchers) can also result in substantial indicator removal. Hornstra et al., (2018) demonstrated that 16 meters (50 ft) of suboxic and oxic transport (0.4-1.7 mg/L DO and 13-16 mg/L NO_3^-) resulted in 7 to 9 $\log_{10}$ (log) removals of MS2 and PRD-1 bacteriophages in the top layer of the subsurface. Few studies have directly compared pathogen removal under oxic and suboxic conditions. Additional work would be beneficial to verify whether differences exist and, if so, what the underlying mechanisms for removal and inactivation for these redox conditions are.

4.1.1.4 Geological Material and Aquifer Type

Subsurface material and aquifer type drive the setback distances needed between the point of recharge (e.g., injection wells) and extraction wells for drinking source water to achieve a certain amount of log removal. Using different subsurface types, Blaschke et al. (2016) compared the distance required to achieve the pathogen removal necessary to meet the public health goal of 10^{-4} enteric virus infections per person per year. The authors assumed an enteric virus concentration in the treated effluent of small biological wastewater treatment systems in decentralized locations of 2.3 $*10^9$/L. For sand aquifers, this distance varied quite a bit and was 39 to 144 meters (130 to 480 feet), 66 to 289 meters (220 to 950 feet) for gravel aquifers, and 1 to 2.5 km (0.6 - 1.5 miles) for coarse gravel aquifers (Blaschke et al. 2016).

To calculate the necessary setback distance, Blaschke et al. (2016) and Schijven et al. (2017) suggest that the most critical parameter information includes the vadose zone thickness, the vertical saturated aquifer thickness, groundwater hydraulic gradient, microbial removal rates, cation and anion exchange capacity, and subsurface type. Certain removal rates are assigned by subsurface type and hydrogeological conditions. Note that these relatively simple models for estimating pathogen removal were developed based on data collected from untypically well-sorted, homogenous aquifer sediments in the Netherlands. Much of the hydrogeology in the Netherlands is characterized by unusually uniform, well-graded sandy aquifers that can be quite accurately modeled. Schijven et al (2017) summarized typical microbial log removal efficiencies per meter transport distance in different soil types and aquifer materials from field studies, as well as soil cores >0.4 m long (Schijven et al 2017). The highest removal efficiencies were

observed for in limestone and sandy gravel aquifer (10^{-1} to 10^{-4} log removal per meter). The lowest removal efficiencies were observed in soils and vadose zone media (10^{1} to 10^{-1} log removal per meter). Heterogeneous aquifer conditions, which are prevalent in parts of the United States such as the Southwest, make groundwater modeling very challenging to the point where subsurface travel times cannot always be accurately predicted using deterministic models.

The hydraulic conductivity of subsurface materials determines the ease with which water can travel through the pores of subsurface media. Hydraulic conductivity is a material property and can be measured in the laboratory or with *in situ* tests. Values can vary over a large range and can naturally range over 3-5 orders of magnitude in the same lithology. For example, hydraulic conductivities for limestone can range from less than 1 to over 1,000 m/day (0.3 to 3,300 ft/d). Hydraulic conductivities for unconsolidated sand depend on the degree of sorting and the content of other materials such as gravel, silt, loess, or loam, and can range from 0.003 to 300 m/d (10^{-3} to 10^{3} ft/d). Clay materials have significantly lower hydraulic conductivities ranging from $3*10^{-7}$ to 0.03 m/d (10^{-7} to 10^{-1} ft/d).

The degree of sorting of materials in the vadose and saturated zone can be a significant factor in groundwater flow direction and velocity. Alluvial materials with similar K values but with different sorting (e.g., poorly graded sand versus well-graded sand) can significantly alter the tortuosity and pathways. Tortuosity is a property of the aquifer material and describes the ratio of the actual flow path length to the straight distance between the ends of the flow path. The soil material as well as the different vertical aquifer zones (e.g., hyporheic zone, underlying unconfined aquifer, confined aquifer, etc.) should also be considered when assessing groundwater flow and velocity.

The subsurface type dictates not only the ease and velocity with which water can pass through the media, but also whether removal can occur due to adsorptive processes between the pathogen and the media surface. In this case, the size range of pathogens (~50-10,000 nm, see Table 3-1) drives different transport and retardation processes (Hunt and Johnson, 2017). Pathogen transport is relatively fast compared to chemical transport, due to preferential flow paths (see earlier discussion in Section 3.4.2).

Pathogen (colloid) attraction to surfaces is governed by diffusion, gravitational settling, van der Waals forces, and electric double-layer repulsion (Hunt and Johnson, 2017). While adsorption of bacteria and viruses to subsurface particles decreases their transport velocity and distance, attachment to surfaces will increase pathogen survival in the subsurface (Regnery et al., 2017). Adsorption protects both viral and bacterial pathogens from enzymes that can degrade pathogens and are produced by native microorganisms. Adsorption of pathogens is estimated to increase their lifetime between 10 to 100 times (Hurst 1988; Pachepsky and Shelton, 2011). For this reason, inactivation of the bacteriophages MS2 and PRD-1 are different in loamy and sandy aquifer materials. Likewise, the decay of the pathogens poliovirus and hepatitis A varied. Therefore, the selection of appropriate surrogate organisms should consider the surface characteristics of the surrogate and target pathogens in addition to the aquifer material (John and Rose, 2005; Blanc and Nasser, 1996).

Aquifer material heterogeneities are widely known to make transport behavior challenging to monitor and predict. For example, hydraulic fracturing in clay can lead to rapid virus transport—much faster than in saturated, homogenous clay—and up to 100 times faster than the observed transport of chemical tracers (McKay et al., 1993). Allophanic soil (of volcanic origin), pumice sand (igneous porous rock formed during volcanic eruptions), and aquifers composed of schist (a metamorphic rock formed from mudstone or shale) have very high capacities for removing bacteria and phages due to their positive surface charge at pH <6. Their large specific surface areas facilitate attachment of negatively charged pathogens (Schijven et al., 2017). Loamy sand mixtures (fine sandy loam, sandy loam, and loamy sand) follow in sorptive capacity. These materials can effectively remove bacteria via straining (size exclusion), although virus removal is poor, specifically in clayey soils and clay loam caused by heterogeneities and cracks that create preferential flow paths (Schijven et al., 2017).

Certain surface-active minerals and metal oxides in aquifer material influence the sorption of pathogens and can shorten survival rates. Ryan et al. (2002) found that adsorption of PRD-1 to ferric oxyhydroxide (FeO(OH) patches in a sand aquifer significantly *increased* the inactivation of adsorbed phages compared to those in solution Ryan et al. (2002). This is an exception, since most surfaces that lack inactivation qualities, such as clay or organic matter, prolong pathogen survival as previously mentioned (Stagg et al., 1977; Liew and Gerba, 1980; Straub et al., 1992).

As mentioned in Section 3.1, the isoelectric point of viruses dictates charge, ionic strength, and adsorption potential of pathogens (US EPA 2015). For example, the bacteriophage ΦX-174 has generally a lower adsorption potential than MS2 at a neutral pH, since ΦX-174 has a higher isoelectric point than MS2, about 6.6 versus 3.9 (Jin et al., 2000) (see also Table 3-2 for typical isoelectric point value ranges for these viruses). This means that at a neutral pH, ΦX-174 carries an almost neutral charge, while the surface of MS2 carries a net positive surface charge. (The isoelectric point is the pH at which a particle carries no net electrical charge or is statistically electrically neutral.) Dowd et al. (1998) clearly showed this inverse relationship between isoelectric points and sorption onto sand for various viruses (meaning a lower isoelectric point resulted in higher retardation). Most aquifer materials (sand, organics, etc.) carry a net negative surface charge. Acidic soils have a higher anion exchange capacity than basic soils, which is important for pathogen transport (Pekdeger and Matthess 1983).

For this reason, surrogate organisms should have similar isoelectric points to pathogens. ΦX-174 has a similar isoelectric point as poliovirus (Jin et al., 2000) and can help predict polio adsorption to organic matter in the subsurface.

MAR systems could be designed to take advantage of intentionally selected materials to improve homogenous infiltration flow conditions and improve pathogen removal efficiency. Karakurt-Fischer et al. (2020a) established a novel sequential MAR concept using high-rate infiltration trench technology coupled with homogenous filter media that resulted in a sequence of controlled redox zones in the subsurface. At an effective velocity of 0.58 m/hr and an HRT of 12-13 hours, Karakurt-Fischer et al. (2020b) demonstrated a 1.7-3 log reduction of somatic and F+ phages, as well as murine norovirus and adenovirus. Spiking tests showed

removals of 3-5.6 log reduction values for MS2, ΦX-174, and murine norovirus (a natural pathogen affecting rodents).

4.1.1.5 Temperature

As with all chemical reactions and biological activity, temperature heavily influences the removal of pathogens. Viruses exhibit longer survival at lower temperatures (Gerba et al., 1991). In conjunction with higher oxygen levels, higher temperatures (28°C vs 15°C) have been shown to increase the activity of subsurface microorganisms responsible for the decay of *E. coli*, MS2 and poliovirus and Coxsackievirus (Gordon and Toze, 2003), and the presence of microorganisms was found to be more influential than temperature or DO on decay of pathogens.

The coliphages PRD-1 and ΦX-174 were deemed the most stable under elevated temperatures, followed by hepatitis A, adenovirus, and poliovirus (Bertrand et al., 2012; Regnery et al., 2017). Temperature-resistant viruses have been attributed to a more stable subset of the virus population than most. Adaptation, aggregation with other viral particles, or association with other substances found in the water are other possible factors (Regnery et al., 2017). As temperature increases, viral inactivation generally increases (Yates et al., 1990), and degradation by enzymes accelerates (John and Rose, 2005). For viruses and coliform bacteria, inactivation rates greater than 0.5 $\log_{10}day^{-1}$ (shorter than 2 days T_{90}) have been observed at subsurface temperatures greater than 20°C (John and Rose, 2005).

Direct temperature-based inactivation for bacteria has not been proven since bacteria replicate faster at higher temperatures in the presence of nutrients. Temperature is believed to act in conjunction with other factors to assist in bacterial inactivation (Hernandez-Delgado and Toranzos, 1995). In the United States and in other countries, temperature ranges of MAR systems can vary by region, and range from 0-30°C (John and Rose, 2005). While groundwater temperature can be influenced by the temperature of the infiltrated water, notably changing the subsurface temperature intentionally would require introducing a larger volume of reclaimed water via MAR, which may present other logistical and environmental and public health challenges.

4.1.1.6 Pore Size Exclusion

Larger microbes will move through preferential flow paths in the subsurface faster than chemical contaminants that can disperse, diffuse into, and/or sorb to aquifer materials. Therefore, pathogens can transport more quickly than chemical solutes because of size exclusion from small pore spaces with lower or stagnant flow conditions (Bradford & Harvey, 2017). For these reasons, viruses will be detected earlier and farther away from the source than chemicals or conservative tracers (e.g., nitrates, primidone, or sucralose) (McKay et al., 1993; Powelson et al., 1993). Fractures, heterogeneities, and preferential flow paths in the subsurface emphasize these transport differences and are site-specific (see Figure 4-2). The size and presence of soil pores play a notable role in subsurface transport: comparing the size of pathogens in wastewater (see Table 3-1 to the sizes of macro- (> 75 µm), meso- (30-75 µm) and micropores (~ 5-30 µm) reveals, for example, that most viruses and surrogates present in wastewater are up to 3 orders of magnitude smaller than micropores. For larger pathogens, straining (e.g., physical filtration) as well as movement along preferential macropathways facilitating the transport of liquids are important removal and transport mechanisms during subsurface travel.

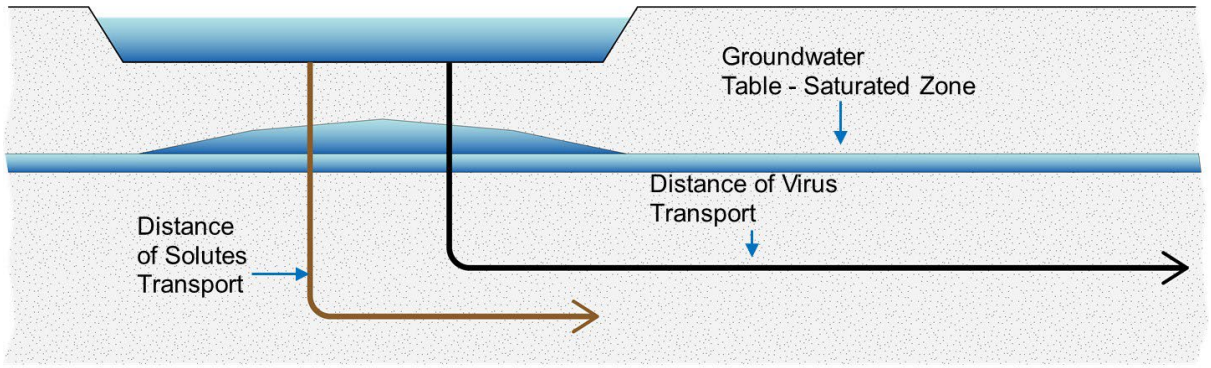

Figure 4-2. Comparison of Virus and Chemical Transport in Groundwater.

Viruses have been detected at greater distances than solutes in groundwater dependent on the heterogeneity of the subsurface while moving with the fastest moving fraction of the groundwater as particulates (McKay et al., 1993) and also because they can be detected at much higher sensitivities using PCR methods than chemical tracers (see discussion in section 3.2.6).

Water quality changes in MAR systems are often monitored through regular sampling of monitoring wells. These wells are typically located near the infiltration zone in either the vadose zone (dry wells) or in the saturated zone of an aquifer. When assessing whether monitoring wells will adequately reflect the occurrence of indicators or surrogates in production wells, operational field monitoring should consider the location of monitoring wells and their screening depth. Monitoring wells may collect water only from a limited subsurface area, as opposed to a production well, which may draw water from a much larger area at a greater pumping rate (Figure 4-3). Thus, sampling both monitoring, and production wells is needed to provide a more accurate assessment of pathogen transport and removal (Morrison et al., 2020b).

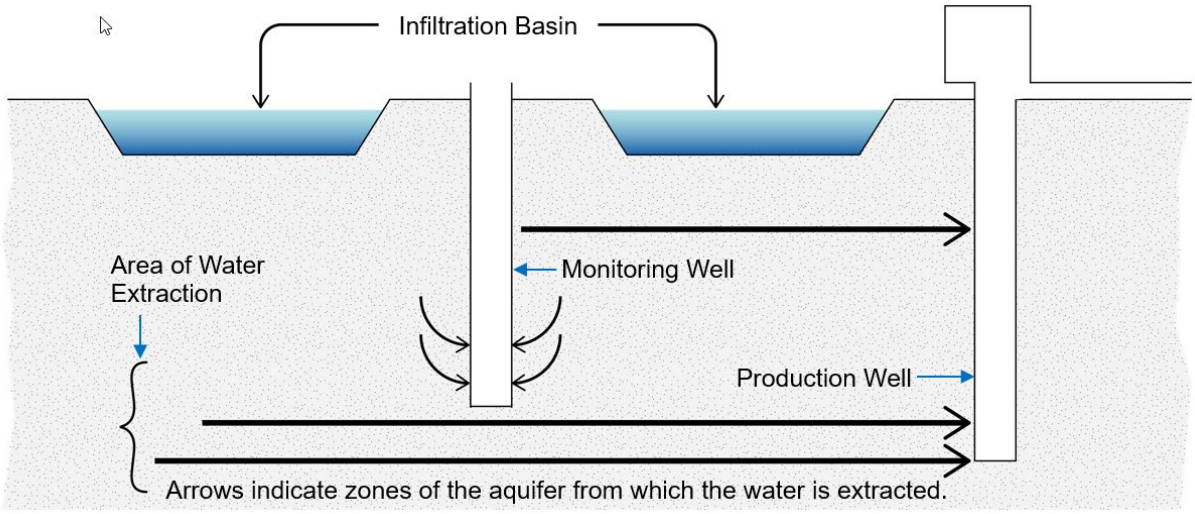

Figure 4-3. Schematic Representation of Groundwater Extracted by Monitoring and Extraction Wells.

This schematic shows the different areas from which the groundwater is extracted. Monitoring wells may represent a much smaller area from which the water is withdrawn.

Another consideration for surrogate monitoring in MAR systems is the impact of lenses of different permeability and porosity. These lenses may develop perched saturated zones that can result in more rapid subsurface transport of microbes compared to transport through vadose zones (Powelson et al., 1993) (Figure 4-4).

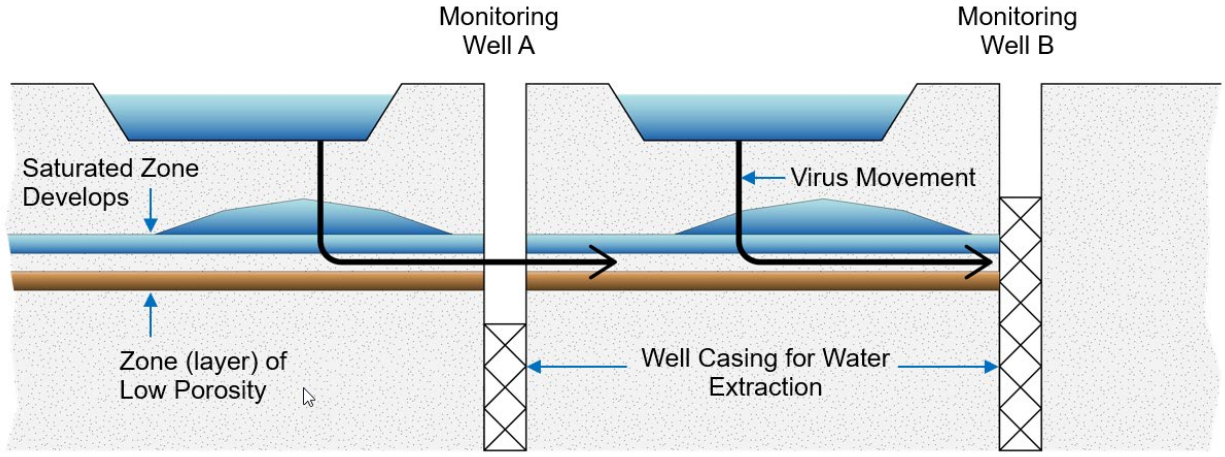

Figure 4-4. Impact of Subsurface Heterogeneities of Different Porosity on Virus Detection.

Virus will not be detected in Well A, but will be detected in Well B.

4.1.1.7 Dynamic of Water Flow Regimes

Changes in the pumping rates at MAR extraction wells can affect the flow through the aquifer, which can result in pressure waves that disturb the local adsorption equilibrium of pathogens. Because pumping is a physically controllable variable within MAR operation, it should remain relatively constant.

Wetting-drying cycles in recharge basins also influence pathogen fate and transport. Effective control of wetting-drying cycles, as site conditions allow, can increase pathogen retardation, since the removal rate in the vadose zone is inversely related to velocity, hydraulic conductivity, hydraulic loading, infiltration rate, and air-water interface inactivation (Schijven et al., 2017).

Several transport processes are highly sensitive to wetting / drying cycles. Blocking describes the process where available adsorption sites on the soil or aquifer material are filled, increasing the risk of colloid migration (Bradford et al., 2014). Straining is the trapping of colloid particles in down-gradient pore throats that are too small to allow particle passage. Straining depends on colloid and particle size and is inherently tied to pore size and pore space distribution (Bradford et al., 2003). Just as colloids can attach to soil and aquifer material, detachment can occur when the thermodynamics and hydrodynamics of the liquid/solid interface change (Bergendahl and Grasso, 2000).

The infiltration rate and desired hydraulic retention times in MAR systems can be controlled through adequate pre-recharge treatment. Feed water to MAR systems with low turbidity, total

dissolved solids, and organic matter content facilitates optimal infiltration conditions (Karakurt-Fischer et al., 2020a). Above-ground nitrification has been hypothesized to lower the oxygen demand of recharged water in the subsurface and thereby extend aerobic conditions in the aquifer thereby possibly resulting in shorter survival of pathogens. The authors are not aware of data or studies to support such a relationship. Aerobic conditions result in shorter survival of viruses and other pathogens likely due to higher biological activity and predation. Adequate pre-recharge treatment may be provided via nitrification, effluent reaeration, rapid sand, dual media, or membrane filtration, coupled with high-rate infiltration through gravel media prior to recharge (Karakurt-Fischer et al., 2020a).

4.1.1.8 Climate Change

Several other parameters, including humidity, seasonality, salinity, and precipitation patterns, have been linked to pathogen occurrence and removal (Semenza et al., 2012; Levy et al., 2016; European Environment Agency, 2017). As climate change alters rainfall, snowmelt, and the natural recharge cycle patterns of water, it is anticipated to affect pathogen occurrence and transport. Other factors that can change groundwater tables in MAR systems over years include changes to the level of pretreatment, groundwater pumping, or recharge rates. Masciopinto et al., 2008) observed increased pathogen contamination in household tap water in the Salento region of southern Italy because of more frequent spreading of wastewater on soil for irrigation due to the arid climate and droughts. Because of fractured aquifers, pathogens could be transported into the groundwater, resulting in unintended human exposure.

Major rainfall events can increase infiltration into recharge systems. In 1983, the Sweetwater SAT site in Tucson, Arizona, received unintended infiltration after a major rainfall event over several days from adjacent rivers. Conversely, periods of drought can cause groundwater tables to fall. Tucson has also seen a significant elevation increase in groundwater tables after reduced pumping of SAT water when more surface water from the Colorado River became available to the city. In recent years, recharge rates at the Sweetwater SAT site increased after treatment was upgraded to advanced treatment for nutrient removal. Such events could impact both the retention and transport of pathogens as a result of changes in salt concentrations, flow rates, and other environmental factors.

4.1.2 Operational Conditions

Infiltration rates, pumping rates, and wetting and drying cycles are key operational decisions to help control pathogen removal in MAR systems. Characterizing the subsurface prior to installing and operating a MAR system is critical to define the depth of the vadose zone. Infiltration rates are informed by the removal efficiency of pathogens in the vadose zone and the aquifer itself. Understanding the subsurface hydrogeology, dominant subsurface material, and type of prevalent heterogeneities can allow for predicting the most and least removed type of pathogens.

4.2 Inactivation Rates for Pathogens in MAR Subsurface Systems

Inactivation rates refer to a $\log_{10}$ reduction of pathogen per day and include decay and die-off rates observed at lab-, pilot-, and field-scale MAR studies. Most often, inactivation is represented as a first-order process as a function of retention time. As illustrated in Figure 4-1,

first order kinetics are often appropriate for short-term inactivation of pathogens but may overestimate removal in the long-term. This is one reason why inactivation rates reported in literature for pathogens can vary by several orders of magnitude. Appropriate selection of pathogen inactivation rates and experimental measurements should consider the study scale, temperature, and redox conditions.

Decay or inactivation rates can differ between measurements conducted under laboratory or field conditions. Laboratory studies may over or under-predict pathogen die-off compared to field conditions. Using membrane diffusion chambers implemented in aquifers of MAR systems, Sidhu & Toze (2012) demonstrated that *in situ* measured decay rates for various pathogens differed from those measured in laboratory microcosms. The chambers were inserted into groundwater wells below the groundwater level. Sidhu & Toze (2012) show the schematic design of the diffuser chamber used for *in situ* decay rate measurements. Using this device, Sidhu & Toze (2012) reported lower inactivation times (faster removal) for *Cryptosporidium* oocysts in situ compared to laboratory conditions, hypothesizing that this difference could have been caused by slightly lower temperatures in the laboratory experiment compared to the aquifer. Inactivation times for bacteria in the aquifer depended on the pore size of the membranes used in the chamber setup and were significantly higher (slower removal) when smaller pore-size membranes were used. The authors hypothesized that this may have been caused by a lower transfer of bio-available nutrients via diffusion into the cells protecting the seeded bacteria from autochthonous microorganisms. Adenovirus was also found to have a significantly higher inactivation time in the diffusion chambers compared to the laboratory studies. The authors proposed that lower dissolved oxygen concentrations in the aquifer could explain this difference.

Table 4-2, Table 4-3, and Table 4-4 compile inactivation rates for viruses, bacteria, and protozoa, respectively. Rates have been measured and reported for over 20 years. A meta-analysis of decay and die-off rates may be valuable to explore correlations with environmental site conditions.

Table 4-2. Inactivation Rates of Viruses.

Organism	Temperature (℃)	Mean Inactivation Rate (log_{10} day^{-1})	Inactivation Rate Range (log_{10} day^{-1})	Predominant redox condition	Experimental scale	Source
Polio virus	0-10	0.02	0.005-0.05	-	Groundwater	John and Rose, 2005; Yates et al., 1985; Yates et al., 1990
Polio virus	11-15	0.09	0.03-0.2	-	Groundwater	John and Rose, 2005; Yates et al., 1985; Yates et al., 1990
Polio virus	16-20	0.1	0.03-0.2	-	Groundwater	John and Rose, 2005; Yates et al., 1985; Yates et al., 1990
Polio virus	26-30	0.4	0.006-1.4	-	Groundwater	John and Rose, 2005; Yates et al., 1985; Yates et al., 1990
Polio virus	15-28	-	0.006-1.0	Aerobic	-	Gordon and Toze, 2003
Polio virus	28	-	0.013	Anoxic	-	Gordon and Toze, 2003
Polio virus	12	-	0.18	-	-	Blaschke et al., 2016; sourced from Yates et al., 1985

(Continued)

Table 4-2. Continued.

Organism	Temperature (˚C)	Mean Inactivation Rate (log_{10} day^{-1})	Inactivation Rate Range (log_{10} day^{-1})	Predominant redox condition	Experimental scale	Source
Polio virus	22	-	0.070	-	*In situ* groundwater study	Sidhu and Toze, 2012; sourced from Jansons et al., 1989
Polio virus	23	-	0.357-0.676	-	Laboratory	Sidhu and Toze, 2012; sourced from Yates and Gerba, 1985
Hepatitis A virus	0.-10	0.02	0-0.08	-	Groundwater	John and Rose, 2005
Hepatitis A virus	20-30	0.04	0.009-0.1	-	Groundwater	John and Rose, 2005
Echovirus	11-15	0.1	0.05-0.2	-	Groundwater	Yates et al., 1985
Echovirus	16-20	0.1	0.05-0.2	-	Groundwater	Yates et al., 1985
Echovirus	21-25	0.2	0.06-0.6	-	Groundwater	Yates et al., 1985
Coxsackievirus	8-20	0.06	0.002-0.2	-	Groundwater	John and Rose, 2005
Coxsackievirus	25-30	0.1	0.007-0.3	-	Groundwater	John and Rose, 2005
Coxsackievirus	15-28	-	0.002-0.098	Aerobic	-	Gordon and Toze, 2003
Coxsackievirus	28	-	0.007	Anoxic	-	Gordon and Toze, 2003
Rotavirus	3-15	0.4	One study		Groundwater	John and Rose, 2005
Rotavirus	22	0.03	One study	Aerobic (DO = 7 mg/L) DOC = 6.3 mg/L Total Nitrogen (TN) = 4.4 mg/L	*In situ* decay study in an aquifer	Toze et al., 2010
Adenovirus	4	0.0076	One study	-	-	Regnery et al., 2017; Toze et al., 2010
Adenovirus	12-22	0.028	0.01-0.047	-	-	Regnery et al. , 2017; Toze et al., 2010
Adenovirus	22	-	0.004-0.016	Aerobic (DO = 2 mg/L) DOC = 3 mg/L	*In situ* decay study in an aquifer	Sidhu and Toze, 2012
Adenovirus	20	-	0.047	-	Laboratory	Sidhu and Toze, 2012
MS2 phage	22	-	0.093-0.105	Aerobic (DO = 2 mg/L) DOC = 3 mg/L	*In situ* decay study in an aquifer	Sidhu and Toze, 2012
MS2 phage	20	-	0.174		Laboratory	Sidhu and Toze, 2012
MS2 phage	15-28	-	0.006-1	Aerobic	-	Gordon and Toze, 2003
MS2 phage	28	-	0.009-0.122	Anoxic	-	Gordon and Toze, 2003
MS2 phage	12	-	0.16	-	-	Blaschke et al., 2016; sourced from Yates et al. 1985

(Continued)

Table 4-2. Continued.

Organism	Temperature (˚C)	Mean Inactivation Rate (log_{10} day^{-1})	Inactivation Rate Range (log_{10} day^{-1})	Predominant redox condition	Experimental scale	Source
PRD-1	0-10	0.07	0.03-0.4	-	Groundwater	John and Rose, 2005
	21-25	0.2	0-0:8	-	Groundwater	John and Rose, 2005
ΦX-174 (Microviridae)	4	>365 days / log_{10}	-	-	Reagent grade water	Lee & Sobsey, 2011
ΦX-174 (Microviridae)	25	0.026	0.014-0.028	-	Reagent grade water	Lee & Sobsey, 2011

Table 4-3. Inactivation Rates of Bacteria.

Organism	Temperature (℃)	Mean Inactivation Rate (log_{10} day^{-1})	Inactivation Rate Range (log_{10} day^{-1})	Predominant redox condition	Experimental scale	Source
Coliforms	0-10	0.07	0.03-0.4	-	Groundwater	John and Rose, 2005
Coliforms	15-20	0.4	0.02-1.5	-	Groundwater	John and Rose, 2005
Coliforms	21-37	0.3	0.007-2.5	-	Groundwater	John and Rose, 2005
Enterococcus faecalis	22	-	0.109-0.597	Aerobic (DO = 2 mg/L) DOC = 3 mg/L	*In situ* decay study in an aquifer	Sidhu and Toze, 2012
Enterococcus faecalis	20	-	0.382	-	Laboratory	Sidhu and Toze, 2012
Salmonella	22	0.81		Aerobic (DO = 7 mg/L) DOC = 6.3 mg/L TN = 4.4 mg/L	*In situ* decay study in an aquifer	Toze et al., 2010
Salmonella	22	-	0.054-0.510	Aerobic (DO = 2 mg/L) DOC = 3 mg/L	*In situ* decay study in an aquifer	Sidhu and Toze, 2012
Salmonella	20	-	0.455	-	Laboratory	Sidhu and Toze, 2012
E. coli	20	0.539	-	-	Laboratory	Sidhu and Toze, 2012
E. coli	22	-	0.041-0.691	Aerobic (DO = 2 mg/L) DOC = 3 mg/L	*In situ* decay study in an aquifer	Sidhu and Toze, 2012
E. coli	15-28	-	0.009-0.909	Aerobic	-	Gordon and Toze, 2003
E. coli	28	-	0.010-0.161	Anoxic	-	Gordon and Toze, 2003

Table 4-4. Inactivation Rates of Protozoa.

Organism	Temperature (℃)	Mean Inactivation Rate (log_{10} day^{-1})	Inactivation Rate Range (log_{10} day^{-1})	Predominant redox condition	Experimental scale	Source
Cryptosporidium parvum	22	0.0254	0.0682-0.0824-0.072	-	*In situ* decay study in an aquifer	Toze et al., 2010
Cryptosporidium parvum	22	-	0.025-0.032	Aerobic (DO = 2 mg/L) DOC = 3 mg/L	*In situ* decay study in an aquifer	Sidhu and Toze, 2012
Cryptosporidium parvum	20	-	0.026	-	Laboratory	Sidhu and Toze, 2012

4.3 Field Data of Apparent Pathogen Removal in MAR Systems

Measuring log removal reduction of pathogens in MAR systems can be challenging. There are several reasons for this. Often times, pathogens are not detectable in groundwater samples since analytical methods are not sensitive enough or sample volumes are smaller than required. Field data may not be published and accessible for scientists or regulators. The large majority of (planned or de-facto) MAR systems may not conduct detailed sampling campaigns to quantify pathogens, indicators, or surrogates that are not required by their permit.

As discussed, MAR systems differ significantly and so does their pathogen removal performance. Rather than compiling a table summary of log removal rates from various studies and various MAR systems and organisms, we emphasize the importance of a science-based, mechanistic approach when assessing pathogen removal. In this project we have therefore compiled and reviewed studies that report log removal values for bacteria, viruses, and oocysts in MAR systems and have integrated this information into the respective text sections in Chapters 3 and 4 to discuss field results within their relevant context. This information spans from the upper end of anticipated removal efficiencies (observed in highly homogenous fine sand aquifers such as in the Netherlands) to the lower end of anticipated MAR performance (such as in heterogeneous karst aquifers).

4.4 Methods for Predicting Pathogen Removal in MAR Systems

4.4.1 Laboratory-Scale Studies

Most of our apparent understanding of pathogen removal in MAR systems comes from controlled laboratory-scale column or three-dimensional tank studies (Pang 2009; Regnery et al., 2017; Karakurt-Fischer et al., 2020a). Lab-scale experiments are easy to control and operate and can provide higher resolution monitoring at a lower cost than field studies. Additionally, lab-scale studies allow for quantifying cause-effect relationships prior to full-scale MAR implementation and study of how aquifer materials, redox conditions, organic carbon, and other factors influence pathogen removal. The state of Florida requires column studies lasting year to demonstrate RIB performance before full-scale operation can be permitted (see Chapter 5). Many larger utilities operating MAR systems operate lab-scale experiments in parallel for years to conduct performance tests under different conditions.

Nevertheless, laboratory studies have limitations. Pang (2009) compared microbial removal in laboratory and field studies and concluded that column studies can significantly overestimate

results by as much as two to three orders of magnitude. Lab-scale column experiments are usually fairly homogeneous, even if column material is sampled from the field (Torkzaban et al., 2019b; Hornstra et al., 2018). Preferential transport pathways in fractured rock, macropores, or cracks in clay are a major contributor to faster transport in the field. Varying velocities between column studies and field applications make it difficult to accurately simulate field conditions at column scale (Liu et al., 2016).

The following are additional considerations when extrapolating results from laboratory to field conditions:

1. The greatest removal rate for microbes has been reported to occur approximately within the first meter of the soil at infiltration systems. Thus, removal rates derived from short columns should not be extrapolated to field conditions beyond the initial infiltration zone. Removal rates are functions of velocity, grain size, solution and solid phase chemistry, and microbial activity that can change with distance.
2. Unlike microbes found in wastewater, microbial surrogates that are at times used in laboratory-scale studies are more uniform in size, shape, net charge, and survival, and thus may not reflect the behavior of more diverse microbial species in full-scale MAR systems.
3. Fate and transport parameters determined in laboratory microcosms may not be accurately accounting for adsorption and detachment, straining, predation, field conditions regarding temperature, nutrient and dissolved oxygen concentrations, and redox conditions. Small differences in fate and transport parameters determined under lab conditions may extrapolate to larger differences when applied to field conditions.
4. Predicting the long-term persistence of pathogens is difficult because of the biphasic nature of pathogen die-off (rapid initial decay of labile pathogens near the soil surface, followed by a much slower die-off rate of more resistant organisms, as shown in Figure 4-1). For example, Charles et al. (2009) observed inactivation of adenovirus type 2 in groundwater at a rate of 0.489 log/day for the first few days, decreasing to 0.002 log/ day over the next 380 days.
5. Heterogeneity of the subsurface is site specific and not well simulated in column tests. For example, flow through fractured porous media or clay lenses can significantly affect the transport and removal of viruses. Laboratory results can be extended to the field through the use of models that account for the influence of field heterogeneity (see for example Torkzaban et al., 2019a and Sasidharan et al., 2021).
6. Certain types of viruses are removed differently under saturated and unsaturated conditions (Zhunag and Jin, 2003). Column studies are typically conducted under saturated conditions. Research gaps remain in pathogen removal, specifically under unsaturated conditions. For these conditions, PRD-1 has been proposed to be an appropriate surrogate for virus transport (Schijven et al., 2017).
7. Most virus studies to date have focused on human enteroviruses and coliphages (Regnery et al., 2017). These may not be the most appropriate surrogates for viruses in all MAR systems. Enteroviruses are only a small proportion of all the viruses found in wastewater and, depending on treatment, can occur in far lower numbers than other enteric viruses (e.g., adenoviruses) (Gerba et al., 2017).

4.4.2 *In Situ* Experiments

In situ experiments are conducted in the field. Dialysis bags or chambers can be placed into wells to measure pathogen die-off or decay rates under ambient temperature and water quality conditions. Sidhu and Toze (2012) used Teflon chambers placed in the aquifers of MAR sites to measure decay rates of pathogens as a function of subsurface residence time. Pathogens or surrogates can be placed into the chambers in known concentrations, and groundwater can be allowed to flow through the chambers through exclusion membranes on each end (0.01 to 0.025 micrometer in pore size). Studies in Australian aquifers quantified a decay rate of 5.5 log per day for *Campylobacter*, 4.9 log over 419 days for *Cryptosporidium*, and 5.5 log over 1,020 days for rotavirus. Decay rates using this method differed from those determined in laboratory studies and depended greatly on groundwater temperature.

In situ experiments have been proposed to be more accurate than laboratory tests since they are performed within the actual recharged water matrix and aquifer. Per recent lab-scale experiments, *E. coli* cells can adapt their physico-chemical properties to the ambient water matrix. In these experiments, rainwater, impacted surface water, or secondary wastewater effluent altered the zeta potential and the hydrophobicity of the bacteria, changing their transport behavior and removal (Fan et al., 2020).

But even these experimental set ups have limitations as they neglect potential native predation by microorganisms (Sidhu et al. 2015) or possible inhibition by anti-microbial agents in the groundwater that cannot pass through the membranes. The smaller the membrane pore sizes the larger the experimental bias may be (Regnery et al., 2017). In-situ decay experiments, such as diffusion chambers, do not consider removal by attachment and solid phase inactivation because the setup in a well does not allow for direct interaction between the pathogens and the aquifer matrix. This can lead to overly conservative pre- or post-treatment requirements. In addition, in-situ decay experiments do not account for expected variability in decay due to subsurface heterogeneity (differences in water content, soil/sediment types, redox conditions, and microbial populations).

On the other hand, LRV obtained from such die-off experiments are conservative, since pathogen removal via filtration, adsorption, or predation is not considered. This may be helpful if experiments reveal higher decay rates than default regulatory LRV credits allow.

4.4.3 Full-Scale Field Studies and Fate and Transport Modeling

Full-scale field studies use tracer tests to determine groundwater flow direction, preferential flow paths, dilution ratios with native groundwater, residence times, and log removal values for pathogens (Bekele et al., 2014). When calculating removal rates using non-reactive tracers, groundwater dilution should be considered. The structure of the aquifer material (i.e., macropores and heterogeneities) generally has a greater influence on pathogen transport and the matrix material or texture influences pathogen removal (i.e., grain size and material type) (Schijven et al., 2017). Thus, a cracked clayey soil with greater pathogen transport should be considered over a homogeneously packed sandy material.

Groundwater monitoring networks in the field are needed to gather sufficient data for model set up and calibration and the detail of data that needs to be collected depends on MAR site characteristics. Monitoring networks can be costly and insufficient to accurately quantify pathogen arrival time and location, and concentrations. The design of the network is important and can otherwise bias the results. Models are useful to guide and interpret sampling results, especially when mass balance is not achieved even with conservative tracers.

Combining tracer tests with hydraulic modeling can yield reasonably accurate results for pathogen fate and transport. Tracer tests need to be designed to obtain mass balance otherwise they can provide misleading interpretation of results. Models can be used to design and quantify tracer results. However, field-scale tracer tests can be expensive. Often, chemical tracers such as bromide or isotopes (tritium, helium, oxygen-18, deuterium, etc.) are used. Wastewater-derived tracers such as chloride, boron, or persistent pharmaceuticals, and personal care products (e.g., sucralose, carbamazepine, or acesulfame) can be suitable choices given specific MAR site and source water conditions. Temperature is a less expensive tracer but can yield less reliable results in MAR systems with longer residence times (weeks to months) unless the tracer is introduced over a significant duration and significant volume. Several researchers have used a combination of different tracers to provide quality estimates of quantified residence times in the subsurface (Massmann et al., 2008; Engelhardt et al., 2013; Bekele et al., 2014).

Tracer test data can be analyzed via one-dimensional (1-D) or three-dimensional (3-D) curve fitting procedures. One-dimensional modeling approaches can be sufficient and appropriate for homogenous aquifers, such as large, sandy aquifers (e.g., MAR systems in the sand dunes in the Netherlands). Three-dimensional fate and transport modeling should be used for anisotropic and heterogeneous aquifers and may still not in result in satisfying estimates of aquifer residence times under highly heterogeneous aquifer conditions. The tracer breakthrough curves from monitoring and production wells can help identify velocity variations and preferential flow paths and the short-circuiting of pathogens. Bekele et al. (2014) recommend repeating tracer tests and using data from multiple observation wells to identify possible discrepancies that can improve conceptual models. Monitoring of additional site specific and water quality parameters can help in the interpretation of tracer tests and prediction of groundwater flow and transport direction.

Geophysical techniques including the time-domain (TEM) electromagnetic method, electrical conductivity (EC), and the self-potential method (SP) can assist in evaluating infiltration of recharged water, subsurface flow direction and magnitude, desaturation, and decoupling of surface and groundwater interface in multi-phase flow regimes (Shaaban et al., 2016; Cockett and Pidlisecky, 2014; Jasper, 2014).

4.4.4 Predictive Modeling Approaches

Numerous studies have dealt with modeling the fate and transport of pathogens using various desktop simulations models. Several models have been developed that incorporate first-order inactivation of free and attached viral particles in subsurface removal, as well as models that incorporate reversible adsorption and different time-dependent inactivation rate coefficients

(Sim and Chrysikopoulous, 1996). More recent work modeled virus transport in up to three distinct aquifer layers with different hydraulic conductivities in ASR using the infinite element domain feature of multiphysics software package (COMSOL) (Torkzaban et al., 2019a).

Extrapolating results from predictive models to column studies, and ultimately field scale, can be difficult and imprecise if aquifer conditions are not properly characterized and understood. Where applicable, the influence of preferential flow paths is critical for accurate modeling of pathogen transport to drinking water wells and accurate risk assessments (Bradford et al., 2017). Model predictions need to consider the distribution in travel times of groundwater in the subsurface. A small percentage of the total groundwater flow volume will take the fastest path. California MAR regulations chose for this reason to define the travel time conservatively as the time of breakthrough of 2% of the conservative tracer concentration.

Various model types are used to simulate pathogen fate and transport and risk assessments. These models might consist of an online decision support tool, which is applicable only to well-defined boundary conditions of a MAR system (e.g., IBF). Deterministic models build on mechanistic fate and transport relationships and equations and have been used to help describe experimental data from small- or large-scale transport studies. These models help identify factors that control the fate and transport of pathogens in the subsurface and improve our mechanistic system understanding. Stochastic models predict the probability of pathogen breakthrough for various subsurface heterogeneity conditions with selected mean, variance, and correlation lengths of saturated hydraulic conductivity. These models can help to assess uncertainty in predicting the flow, transport, and fate of pathogens at a larger scale. QMRA is a screening-level approach using probabilistic models to estimate a range of likely removals, which can determine the pathogens for which the removal during MAR should be optimized. Models can be incorporated into QMRA to inform the predicted concentration at a particular location, and to estimate the risk of infection if this water was ingested.

Uncertainty in modeling and scale-up of results for inactivation rates has been primarily attributed to aquifer heterogeneities that are not adequately captured (Rehmann et al., 1999; Masciopinto et al., 2008; Toze et al., 2010) and incorrect process descriptions. As discussed earlier, full-scale tracer tests are one possibility to better characterize heterogeneities and preferential flow paths to gain a higher resolution in models along the entire flow path. Pumping tests are less helpful in defining these aquifer characteristics due to scale of such measurements (Toze et al 2010). Tracer test monitoring is informative when using monitoring wells placed at strategic locations along a transect of the flow path. Tracer test results may in turn reveal that initial placement of monitoring wells is incorrect, in which case additional monitoring wells should be installed to better capture the fastest flow paths. Such monitoring wells are often not in place during the permitting stage of a MAR project. Monitoring wells along the transect alone may not capture the full mass balance of tracers or the arrival time and location. Moreover, the costs and logistics of monitoring well construction to construct tracer tests may be prohibitive if such costs were not considered early on in the project planning and implementation phase.

Simpler models have also been used to determine pathogen transport at MAR sites. The German Environment Agency's decision support tool for virus transport in bank filtrate water provides a predicted log reduction value depending on water quality characteristics present at a bank filtrate location (German EPA, accessed 2021). In the Netherlands, online calculator model (QMRACatch) has been used for source-targeted simulation of pathogen concentration in rivers and floodplain systems (RIVM, accessed 2021), which can be used to determine appropriate setback distances for wells. Scientific debate still focuses on how to appropriately model pathogen fate and transport parameters, such as size-exclusion, reversibility of retention, and pathogen release (Bradford et al., 2017). Limiting field data is a challenge to properly account for the spatial changes in groundwater flow and processes controlling pathogen retention and release (Bradford et al., 2017). Table 4-5 compares the pros and cons of different approaches to characterize pathogen removal in MAR systems.

Table 4-5. Comparisons of Scientific Approaches to Characterize Pathogen Removal in MAR.

Approach	Pros	Cons
One-Dimensional Column Studies	• Controlled conditions in the laboratory • Mathematical description of results • Identification of monocausal cause-effect relationships • Least-costly method • Regulatory / permit requirement by some states' agencies in the United States • Vadose zone and infiltration zone simulation.	• Can underestimate survival compared to field studies • Careful attention to conduct experiments under representative conditions, e.g., *in-situ* temperature. • Redox, dissolved oxygen and nutrient supply in aquifer demonstrate to impact inactivation times for bacteria and viruses are challenging to reproduce in the lab. • Reliance on few, homogeneous organisms (strains) grown in the laboratory could lead to misleading results. • Limited length of scale • Results often simulate only removal near the recharge surface (although longer scale tests can be designed)
Three-Dimensional Tank Studies	• More costly • can consider three-dimensional dispersion, diffusion, and advective flow • Improved simulation of heterogeneities	• Similar to cons for column studies
In situ field studies in controlled environments	• Identified as a verifiable method for determining decay rates by Australian reuse guidelines • Easy to construct • Useful for determining long-term removal	• Overly conservative LRVs, as other removal processes (filtration, adsorption, inhibition, predation) are not considered • Limited range of studies • Cost of membranes
Field Studies	• Consideration of the hyper-exponential rate of virus removal. • Site-specific heterogeneity of the subsurface and changing environmental conditions. • Considers heterogeneity in pathogen • Populations among naturally occurring viruses. • Captures changes in infiltration rates on pathogen removal.	• Requires collection of large volumes of water. • Dependent on the number and location of wells. • Pumping operations (duration and volume) • Costs

Approach	Pros	Cons
Predictive Fate and Transport Models	• Die-off and adsorption can be included in models • Using geophysical methods in combination with models can help with well placement • Can determine a representative flow path • Multilinear regression models can be used to more accurately incorporate influence of multiple parameters	• Models are only as good as their input data, especially difficult to get data for certain regions (e.g., Eastern/Southern Europe) • Machine learning predictive models limited to field-scale applications • Costs and complexity for calibration
Online decision support tool	• Helps determine the occurrence of viruses in bank filtrate and guides how to select pathogens for further monitoring • Decision tree shows predicted LRVs for bank filtrate depending on flow/DOC/substrate/DO/ionic strength of system • Middle point between no information and extensive modeling	• Only applicable to bank filtrate • Used for shorter residence times and short travel times

CHAPTER 5

Current U.S. Regulatory Practices and their Challenges

This chapter summaries relevant US federal and state and international regulations and regulatory guidance relative to groundwater and protection against pathogens in drinking water. The discussion highlights the pathogens, indicators, and surrogates that are targeted by the different rules, any quantitative pathogen removal requirements, and MAR system design, operation, and monitoring requirements set by different regulatory agencies. The chapter also summarizes justifications for certain regulatory requirements where relevant and accessible. This summary forms the basis for further recommendations on regulatory improvements summarized in Chapter 8, considering the current regulatory status and scientific understanding of pathogen fate and transport in MAR systems.

5.1 U.S. Regulatory Practices

5.1.1 Federal Regulations

5.1.1.1 U.S. EPA Underground Injection Control Program

ASR wells have been increasing throughout the United States, especially in areas of high population density, near intensive agriculture, and in high demand of water sources. USEPA's Underground Injection Control (UIC) program regulates aquifer and ASR injection wells, authorizing wells by rule or by permit. The well is typically authorized by rule if the owner or operator submit the well information and the well injection does not endanger underground sources of drinking water. The regulating agency may require an individual permit if additional operating requirements are needed to ensure Underground Source of Drinking Water (USDW) protection.

The UIC program does not regulate the recovery of the stored water. The regulation requires that "no owner or operator shall construct, operate, maintain, convert, plug, abandon, or conduct any other injection activity in a manner that allows the movement of fluid containing any contaminant into underground sources of drinking water, if the presence of that contaminant may cause a violation of any primary drinking water regulation under 40 CFR part 142 or may otherwise adversely affect the health of persons." (Code of Federal Regulations 2022). Pathogens are a specific concern that may enter aquifers if water is not disinfected prior to injection. Some states allow injection of raw water and treated effluent. Only some states have set regulations for injection wells using wastewater effluent that set requirements that go beyond the federal UIC program. For all other states the UIC program sets the permitting requirements for protecting aquifers from pathogen contamination. (USEPA, 2020).

5.1.1.2 U.S. EPA Groundwater Rule

USEPA's Ground Water Rule (GWR) protects drinking water quality from disease-causing microorganisms and fecal contamination. The GWR applies to all public water systems (PWSs) that use groundwater. The rule establishes a risk-targeted approach to identify groundwater systems (GWSs) that are potentially susceptible to fecal contamination and requires corrective action in cases of significant deficiencies to prevent source water fecal contamination in all

public GWSs. For potentially susceptible systems, the GWR requires 4-log of virus removal through chemical disinfection, membrane treatment, or alternative treatment methods.

The GWR monitoring program relies on the protections provided by the Revised Total Coliform Rule (RTCR) to protect undisinfected public water system wells. Source ground water monitoring is required only if RTCR monitoring identifies a total coliform detection in the distribution system. In addition, undisinfected public water system wells are protected by sanitary surveys and by state, tribal or local sanitary setback distances.

GWSs that provide at least 4-log treatment of viruses as a corrective action must conduct compliance monitoring. GWSs using chemical disinfection must monitor for the residual disinfectant concentration and meet the state-specified minimum concentration at or before the first customer. GWSs using membrane filtration or state-approved alternative treatment must also monitor the effectiveness of the treatment process.

Systems not achieving, or not performing compliance monitoring for, 4-log treatment of viruses (using inactivation, removal, or a state-approved combination of these technologies) must conduct triggered source water monitoring for the presence of at least one of the following fecal indicators: *E. coli*, enterococci, or somatic coliphage. The triggered monitoring requirements apply to systems that receive notification that a RTCR routine sample is total coliform positive (USEPA, 2008b).

The GWR requirements and associated permits apply to the operator of the drinking water recovery wells in MAR systems. Virus removal requirements set by USEPA in the GWR are superseded by state regulations that may require higher LRV for viruses from MAR system operators as part of their state potable reuse regulations (see discussion further below). Most states and well operators continue to rely on *E. coli* compliance monitoring under the GWR, although US EPA has emphasized the need and clear benefit in including or switching to coliphage monitoring to better assess the risk of fecal contamination and to verify the 4-log removal virus requirement using virus rather than bacteria surrogates. For this purpose, US EPA has developed standardized methods to make coliphage monitoring more sensitive and less costly. However, virus monitoring remains optional in the regulatory language and has not been broadly included as a requirement in permits by state agencies.

In Orange County, CA, one or more undisinfected public water system wells are located with 1 to 2 kilometers of wells injecting highly treated wastewater to prevent seawater intrusion. It appears that, given the long distance, the apparent large ground water residence time, and the advanced wastewater treatment, these wells are at low risk of infectious virus breakthrough.

5.1.1.3 U.S. EPA National Pollutant Discharge Elimination System

For the discharge of effluents to surface waters, EPA administers the National Pollutant Discharge Elimination System (NPDES) that regulates point sources that discharge pollutants to waters of the United States. NPDES permits can regulate the quality of effluents that may be source water for MAR systems. Fecal contamination is regulated through limits and disinfection and/or monitoring requirements applied to dischargers. Typically, microbial pathogen limits and monitoring requirements are defined for fecal coliforms as the target indicator group.

5.1.1.4 Groundwater Under Direct Influence Provisions of the Surface Water Treatment Rule

Groundwater Under Direct Influence (GWUDI) assessments apply to IBF or RBF systems that are mechanistically similar to the fate and transport of pathogens in the subsurface of other MAR systems. In the United States, GWUDI assessments were developed based on the Surface Water Treatment Rule (SWTR) (USEPA, 1989) to address groundwater systems that are potentially susceptible to risks by pathogens from nearby surface water bodies or from recent infiltrating recharge from the ground surface. According to the SWTR, GWUDI is defined as any water beneath the surface of the ground with:

1. Significant occurrence of insects or other microorganisms, algae, or large-diameter pathogens such as *Giardia*.
2. Significant and relatively rapid shifts in water characteristics such as temperature, conductivity, turbidity, or pH that correlate closely with climatological change or surface water conditions.

USEPA's guidance document (USEPA, 1990) establishes general recommendations for a three-step decision-making evaluation based on 1) hydrogeologic criteria of a given site, including proximity to surface water, 2) a sanitary well survey, and 3) analytical testing. Analytical testing may involve surface water tracers, such as temperature fluctuations or conductivity, to assess the hydraulic connection between surface and groundwater. If a possible risk of surface water contamination is identified based on this analysis, the MPA is generally performed to estimate the risk of pathogen intrusion into a well from a nearby surface water body.

In practice, protocols for GWUDI assessments of groundwater wells vary between states. Some distinctions between GWUDI assessment and state regulations are related to the selection and relative importance of certain microbial indicators. For example, some states focus exclusively on MPA, while others include additional microbial indicators that are not part of the MPA indicator suite. Other differences include the minimum number of MPA samples required, rule-of-thumb setback distances for wells from surface water bodies, and the exact definition of a "hydraulic connection." A good starting point for the comparative analysis of the GWUDI approaches in different states is provided by Chaudhary et al. (2009). Their report highlights relevant GWUDI assessment approaches related to pathogen indicators and surrogates and their removal assessments in aquifers.

If an IBF well is categorized as GWUDI, utilities must either follow traditional surface water treatment requirements after extraction or show the site-specific removal performance of the system for *Cryptosporidium* in demonstration studies to receive log removal credits exceeding the basic credit assigned by the LT2ESWTR. These IBF demonstration studies generally rely on the same methods, indicators, and tools used in GWUDI assessments to draw conclusions on the removal of *Cryptosporidium* in the aquifer. Site-specific demonstration studies have been conducted in states such as Colorado, Wyoming, California, and Washington to permit reduced post-extraction treatment requirements (e.g., Berger et al., 2018).

Debate still exists over which surrogates are most suitable and reliable for predicting transport and removal of pathogens, specifically oocysts, in GWUDI systems. Furthermore, the MPA method was not intended to assess the fate and transport of pathogens other than oocysts that

might be a public health concern. In past years, research has focused on identifying surrogates for pathogen transport that are diverse in structure, morphology, and culture to offer analytical methods for representing this diverse group of microorganisms.

5.1.2 U.S. State Regulations

Several states have developed regulatory requirements for MAR systems and pathogen log removal for the protection of public health. MAR regulations greatly accelerated the implementation of MAR systems over the past 50 years in the United States. Since the 1980s, several states have developed laws or rules specifically addressing some aspect of ASR systems. In states such as Oregon and Washington, ASR facilities' development was delayed while new regulatory programs were being established, after which ASR development accelerated rapidly. Today, Oregon and Washington have more than a half-dozen ASR sites in operational or pilot stages.

Arizona has multiple ASR facilities that developed after its regulatory program was established. Several MAR systems for underground water storage developed in the eastern Coastal Plain states during the 1960s, 1970s, and 1980s in response to designating regions where the over-extraction of groundwater was restricted to prevent saltwater intrusion, land subsidence, well interference, or other negative impacts (NRC, 2008).

Since these early days, MAR regulations have expanded into other states and expanded to different MAR system applications. Some states today have detailed requirements for different MAR systems, while others still do not have any regulations. Table 5-1 provides an overview of the regulations of states with most prevalent MAR and GWUDI systems. A few selected states are discussed in more detail in the following sections that illustrate interesting, alternative approaches for the protection of public health from microbial pathogens in MAR systems.

Table 5-1. Overview of MAR and GWUDI regulations in the U.S. by state.

State	Regulatory Agency	MAR Application	Source Water	MAR System Type	Applicable Regulation
Arizona	Department of Water Resources, Arizona Department of Environmental Quality (ADEQ)	recharge permit (Department of Water Resources [DWR]), Aquifer Protection Permit (ADEQ)	Surface water, recycled water	GW recharge	R18-11-4XX Aquifer Water Quality Standards, R19-11-3XX Reclaimed Water Standards
Arizona	ADEQ	Reclaimed water (WW effluent discharge	WW effluent	De facto infiltration	NPDES
California	California State Water Resources Control Board, Division of Drinking Water (DDW)	Surface spreading (Indirect potable reuse [IPR] GW recharge)	Disinfected Tertiary Effluent	SAT, GW spreading (surface application)	California Code of Regulations (CCR) Title 22 - §60320.108 (surface application)

(Continued)

Table 5-1. Continued.

State	Regulatory Agency	MAR Application	Source Water	MAR System Type	Applicable Regulation
California	California State Water Resources Control Board, Division of Drinking Water	Groundwater Injection (IPR, GW recharge)	WW Effluent + full advanced treatment (RO/AOP) (§60320.201)	GW injection (subsurface application)	CCR Title 22 - §60320.208 (subsurface application)
California	California State Water Resources Control Board, Division of Drinking Water	ASR	Any water treated to drinking water standards and treated pursuant to a California Department of Public Health (CDPH) domestic water supply permit	California State Water Resources Control Board, Division of Drinking Water	General Waste Discharge Requirements for Aquifer Storage and Recovery Projects that Inject Drinking Water into Groundwater. SWRCB 2021
California	California State Water Resources Control Board, Division of Drinking Water"	GWUDI	Surface water	IBF	CCR Title 17 - drinking water
Colorado	Colorado Department of Public Health and the Environment	GWUDI	Surface water or reclaimed water infiltration	ASR, IBF, or similar	Alternative GWUDI Determination Regulation
Florida	Florida Department of Environmental Protection (FDEP), Water management districts	IPR, GW recharge	Reclaimed water - disinfected	Recharge, ASR	Florida Administrative Code, Chapter 62.610
Florida	FDEP, Water management districts	RIB	WW, surface water - disinfected	Infiltration	No regulations to date.
Nevada	State of Nevada	Reuse category A+: Approved uses: Indirect Potable Reuse through spreading basins or injection wells;	Reclaimed water	GW recharge, ASR	Nevada Administrative Code (NAC): Chapter 445A- water controls. Reuse category A+: Water quality requirements (NAC 445A.2761)
Oregon	OR Department of Environmental Quality	Surface infiltration, subsurface/vadose injection	Surface water, disposal	MAR, IBF	OAR 340-040 (GW rules), 340-044 (UIC), 690-350 (ASR, AR)

(Continued)

Table 5-1. Continued.

State	Regulatory Agency	MAR Application	Source Water	MAR System Type	Applicable Regulation
Oregon	Oregon Water Resources Department, Health Authority Drinking Water Program, Department of Environmental Quality (DEQ)	IPR, GW recharge, ASR	Reclaimed water - oxidized WW - meet NPDES requirements	GW recharge, ASR	340-055 (recycled water), 340-044-0018 (setbacks and travel time for injection)
Texas	Texas Water Development Board, Texas Commission on Environmental Quality	GW recharge, ASR	Case-by-case permit	ASR	Title 2, Chapter 26-27
Texas	Texas Water Development Board, Texas Commission on Environmental Quality	Land application	Treated Effluent	De-facto recharge. Objective is to effluent disposal.	NPDES permits, Title 30, 210.33
Virginia	DEQ	IPR	Recycled water - secondary treatment plus disinfection	De facto infiltration	9VAC25-740; Virginia Pollutant Discharge Elimination System (VPDES), Virginia pollution abatement (VPA) - fecal coliform <14 CFU/100mL, *E. coli* <11 CFU/100mL, Enterococci <11 CFU/100mL if infiltration site is public; <200, <126, and <35, respectively, if infiltration area is public
Washington	Washington State Department of Health, Department of Ecology	IPR w/ GW Recharge, ASR (surface infiltration)	reclaimed water - Class A (tertiary treatment plus disinfection and 4-log virus removal) or B (secondary treatment plus disinfection)	GW recharge, ASR	Reclaimed Water Act - Chapter 90.46.040, 90.48.080, Washington Administrative Code (WAC) 173-219-320
Washington	Washington State Department of Health, Department of Ecology	IPR w/ GW Recharge, ASR (subsurface injection)	reclaimed water - Class A (tertiary treatment plus disinfection and 4-log virus removal)	GW recharge, ASR	Reclaimed Water Act - Chapter 90.46.042, WAC 173-219-320

5.1.2.1 California

California has set detailed requirements for log removal, multi-barrier protection, and monitoring requirements for pathogens in groundwater recharge systems using recycled water for surface and subsurface applications in the Title 22 California Code of Regulations (Title 22 CCR) that covers drinking water and recycled water (Title 22 CCR § 60320.108 - IPR Surface

Application - Pathogenic Microorganisms Control and § 60320.208 - IPR Subsurface Application - Pathogenic Microorganisms Control). These regulations pre-date the federal Groundwater Rule. Thus, operators of recharge operations are subject to California regulations, while drinking water well operators of MAR systems must comply with the USEPA Groundwater Rule. Drinking water extracted from groundwater basins that are not under the influence of wastewater are typically chlorinated or chloraminated only to meet distribution system residuals. Free chlorination to any log reduction is not required in California for potable reuse projects, either via spreading infiltration or injection.

Specifically, California regulations for IPR surface and subsurface applications include:

Reclaimed Water Pretreatment. Water used in groundwater recharge projects must be tertiary treated and disinfected for spreading or have received advanced treatment for subsurface injection. Based on Regulation Title 22 CCR §60301.230 tertiary, disinfected water has been demonstrated to inactivate and/or remove 99.999 percent (5 log removal) of the plaque-forming units of F-specific bacteriophage MS2, or polio virus in the wastewater. For IPR projects, the SWRCB no longer automatically credits 450 mg/L•minute contact times with chloramines as achieving 5-log virus credit, but now require site specific testing or offer a lower automated credit.

A virus that is at least as resistant to disinfection as polio virus may be used for site-specific performance demonstrations.

System Log Removal. The recycled municipal wastewater used as recharge water must receive treatment that achieves at least 12-log enteric virus reduction, 10-log *Giardia* cyst reduction, and 10-log *Cryptosporidium oocyst* reduction. These log removal requirements were initially proposed by the CDPH in 2011 as an expansion of a concept previously developed by the National Research Council (NRC,1998). The three pathogens that CDPH selected for this regulation had precedence in USEPA regulations for drinking water, in the 1998 SWTR, 1998 Interim Enhanced SWTR (IESWTR), Long-term1 SWTR (LT1ESWTR), and Long-term2 SWTR (LT2ESWTR). The state's regulations for groundwater IPR were finalized in 2014 and include pathogen removal requirements (Title 22 CCR § 60320.108 and 60320.208).

The proposed log removal requirements for each pathogen were selected to allow for sufficient pathogen removal between raw wastewater and final drinking water quality while not exceeding an annual health risk of 1 infection in a population of 10,000. Concentrations of pathogens in raw wastewater were based on maximum concentrations reported in peer-reviewed literature at the time. See Trussell et al. (2013) for a detailed explanation of the justification the log removal values and associated risk assessment assumptions.

The 12/10/10 log removal must occur between the raw wastewater and point of groundwater extraction (post-extraction disinfection not included) and must consist of at least three separate processes, each being credited with no less than 1.0-log reduction. Each separate treatment process may be credited with no more than 6-log reduction, except for pathogen log removals credits in the subsurface based on subsurface retention time. In the case of surface spreading, the entirety of the 12/10/10 requirement can be met underground.

Subsurface Virus Log Removal Credits. For each month retained underground, the recycled municipal wastewater or recharge water is credited with a default 1-log virus reduction for surface and subsurface applications. Surface application projects can receive more than 6 log removal credits for viruses with more than 6 months subsurface travel time. However, *subsurface* application projects cannot get more than 6 log virus removal, even if the subsurface retention time exceeds 6 months. Regulators decided to cap the removal credits for viruses in subsurface application projects to 6 logs because of public health concerns rather than scientific evidence (per Bob Hultquist, retired California SWRCB regulator, US Project Workshop communication, September 9-10, 2020).

The regulatory removal rate for viruses (1 log per month of residence time) dates back to a study published by Yates & Gerba (1985). This study determined the survival of a virus as a function of time for various viruses (poliovirus 1, echovirus 1, and MS2 coliphage) at various groundwater temperatures, since temperature was the only variable found to correlate significantly with decay rates of viruses among a broader group of physical and chemical characteristics initially tested. Note that this study reported decay rates of viruses, not a total removal rate in MAR systems, which should include other processes such as adsorption, desorption, and predation.

Protozoa Log Removal. After six months of subsurface travel time, a 10-log removal credit for protozoa is given for surface application projects, but not for subsurface application. This regulation was based on a number of MAR sites that demonstrated the absence of parasites in any downgradient wells after six months of retention time, one of these sites being the Montebello Forebay recharge site. The 6-month retention time requirement was supported by a 1987 California science advisory panel report that did not find problems (health risk) with reuse recharge that included those conditions (State of California Department of Water Resources, 1987). Based on this evidence, and under the concurrence of scientific advisors, regulators determined that a six-month filtration path after surface spreading was sufficient to reduce the protozoa risk by 10-log. The 10-log credit was included by regulators as they were trying to switch from criteria specifying a treatment train to a log reduction target for reference pathogens while accommodating previously permitted projects that were deemed to be safe. Because the groundwater replenishment regulation was adopted at the time as an emergency regulation during a drought the normal regulation adoption process, including a Statement of Reasons, was never prepared.

At the time of the rule, regulators had little data from injection projects on oocyst removal, and therefore had no basis to give the same credit to subsurface applications, since surface spreading treatment might have been a factor in the observed protozoa removal (Bob Hultquist, retired California SWRCB regulator, US Project Workshop, September 9-10, 2020).

Utilities can receive the same credit for less than six months of travel time if removal performance can be demonstrated.

Minimum Subsurface Retention Time. T22 CCR § 60320.124 defines a minimum response retention time for which water of a groundwater recharge replenishment project is retained underground necessary to allow a sufficient response time to identify treatment failures and implement actions

necessary for the protection of public health. This minimum subsurface retention time, for either a surface or subsurface application, is no less than two months.

The required minimum subsurface retention time for a 10-log removal credit for *Giardia* cyst or *Cryptosporidium oocyst* reduction in surface applications is six months for disinfected tertiary effluent or advanced treated effluent.

Log Removal Validation. For above ground treatment, each treatment process used to meet the log requirements for surface or subsurface applications must be validated for their log reduction by submitting a report for the Department's review and approval, or by using a challenge test approved by the Department that provides evidence of the treatment process's ability to reliably and consistently achieve the log reduction.

Site-specific Log Removal Demonstration Studies. Title 22 CCR allow for alternative demonstrations of LRV by conducting a site-specific demonstration for surface applications (subsection §60320.130) and subsurface applications (Title 22 CCR §60320.230). The required procedure is elaborate and requires the review and approval of a state test plan; public involvement; health impact assessments, including exposure and human epidemiologic studies; and an independent review of an independent scientific advisory panel.

According to Bob Hultquist (US Project Workshop communication, September 9-10, 2020), alternatives to the default 1-log per month virus removal in the subsurface can also be pursued under the "Log Removal Validation" section of the regulation— Title 22 CCR § 60320.108(d) and § 60320.208(c)—analogous to how credits are pursued for above-ground treatment processes using approved challenge tests. This does not require following the procedures under the "Alternatives" subsection of the Title 22 CCR (§60320.130 and §60320.230). However, as discussed above, only surface application projects can receive >6 log credit virus with >6-month travel time. This does not apply to subsurface applications.

MAR System Monitoring. Disinfected secondary and tertiary recycled water needs to be sampled at least once daily for total coliform bacteria. In addition, disinfected tertiary recycled water shall be continuously sampled for turbidity (Title 22 CCR § 60321).

Quarterly monitoring is required of the recycled municipal wastewater and the groundwater from the downgradient monitoring wells for priority toxic pollutants and chemicals that the State Water Board has specified for the specific groundwater recharge project. Regulators may approve reducing these monitoring requirements from quarterly to annual (Title 22 CCR § 60320.120). Typically, microbial monitoring simply requires monitoring of total coliform in the finished recovered water and at the monitoring wells.

Tracer Studies. To demonstrate the retention time underground, a tracer study using an intrinsic or added tracer must be implemented under hydraulic conditions representative of normal recharge operations. The retention time shall be the time representing the difference from when the water with the tracer is applied at the point of recharge to when either 2% of the initially introduced tracer concentration has reached the downgradient monitoring point or 10% of the peak tracer unit value observed at the downgradient monitoring point reaches the

monitoring point. The tracer study shall be initiated prior to the end of the third month of operation.

MAR Project Siting. To site a groundwater replenishment reuse project location during project planning, the recycled municipal wastewater or recharge water shall be credited with no more than the corresponding virus log reduction in column 2 of Table 5-2 for each month of retention time estimated using different methods.

Table 5-2. Virus Log Reduction Credits for Groundwater Recharge Systems in CA using Reclaimed Water.

Method used to estimate the retention time to the nearest downgradient drinking water well	Virus Log Reduction Credit per Month
Tracer study utilizing an added tracer.	1.0 log
Tracer study utilizing an intrinsic tracer.	0.67 log
Numerical modeling consisting of calibrated finite element or finite difference models using validated and verified computer codes used for simulating groundwater flow.	0.5 log
Analytical modeling using existing academically accepted equations such as Darcy's Law to estimate groundwater flow conditions based on simplifying aquifer assumptions.	0.25 log

Monitoring Well Placement. At least two downgradient monitoring wells must be constructed. One monitoring well must represent a retention time of more than 2 weeks but less than six months of travel through the saturated zone affected by the groundwater replenishment reuse project and be located at least 30 days upgradient of the nearest drinking water well (Title 22 CCR § 60320.1260).

5.1.2.2 Washington

Washington instituted reclaimed water regulations for surface and subsurface soil applications (Washington Administrate Code (WAC) Title 173 Chapter 173-320, Revised Code of Washington (RCW) Title 90, Chapter 90.49, Sections 90.46.040 and 90.46.040). For groundwater recharge projects, an engineering report is required that includes the following information:

1. Specific treatment and use of reclaimed water for application to recharge groundwater.
2. Project operation plan.
3. Conceptual model of the hydrogeologic system.
4. Environmental assessment and analysis of any potential adverse conditions or potential impacts to the surrounding ecosystem.
5. Project monitoring plan.
6. Pilot demonstration of project performance.

Criteria are established on a case-by-case basis. Compliance must be further demonstrated with the following:

- Groundwater standards.
- Drinking water maximum contaminant levels in finished reclaimed water or at alternative point of compliance (all states require compliance with drinking water standards).
- Minimum physical setback of 200 feet, and sanitary control area requirements.

Aquifer recharge requires Class A or B water quality, while ASR projects require Class A. Class B reclaimed water is disinfected secondary treated. Class A reclaimed water is tertiary treated and disinfected demonstrating at least 4-log virus removal or inactivation.

Aquifer recharge and recovery projects are in addition evaluated based on:

1. Aquifer vulnerability and hydraulic continuity.
2. Aquifer boundaries and characteristics.
3. Geotechnical impacts of project operation.
4. Chemical compatibility of surface waters and groundwater.
5. Recharge and recovery treatment procedures.
6. System operation.
7. Pilot demonstration project performance.

5.1.2.3 Oregon

Oregon sets general requirements for groundwater quality protection and two specific requirements for aquifer injection systems (State of Oregon Administrative Rules (OAR) Chapter 340 Division 40; OAR Chapter 340 Division 44; and AOR Chapter 690, Division 350-0110):

Subsurface Retention Time. No domestic drinking water wells can be present within 500 feet of the injection system. The injection system cannot be located within the two-year time-of-travel zone or closer than 500 feet to a public water supply well, whichever is more protective.

Reclaimed Water Pretreatment. Recycled Class A water must be oxidized, filtered, and disinfected wastewater. Before disinfection, wastewater must be treated with a filtration process, and the turbidity must not exceed an average of 2 nephelometric turbidity units (NTU) within a 24-hour period, 5 NTU for more than 5% of the time within a 24-hour period, and 10 NTU at any time. Furthermore, after disinfection, Class A recycled water must not exceed a median of 2.2 total coliform organisms per 100 milliliters, based on the results of the last 7 days that analyses were completed, and 23 total coliform organisms per 100 milliliters in any single sample.

Monitoring. Monitoring for total coliform organisms must occur once per day at a minimum.

Oregon allows for site-specific testing to demonstrate log removal requirements under the SWTR for IBF systems.

5.1.2.4 Florida

Florida set requirements for recycled water land application (Florida Administrative Code, Chapter 62.610).

Pretreatment. Treatment shall result in reclaimed water that meets, at a minimum, secondary treatment, and high-level disinfection. The reclaimed water shall contain no more than 5.0 milligrams per liter of suspended solids before the disinfectant is applied. By removing TSS before disinfection, filtration serves to increase the ability of the disinfection process to inactivate virus and other pathogens. Filtration also serves as the primary barrier for removing protozoan pathogens (*Cryptosporidium, Giardia*, and others).

Monitoring. Monitoring wells and groundwater sampling procedures need to be included in the monitoring program. Groundwater test wells resulting from hydrogeologic exploratory programs, background water quality determinations, or other requirements shall be collected.

Monitoring for fecal coliforms should occur monthly in the water recovered from the ASR system. Groundwater shall be monitored quarterly for all parameters that exist under groundwater standards. After the first year of operation, the Department may adjust the frequency of this monitoring and the list of parameters based on previous monitoring. Reductions in monitoring shall be considered only after the injected bubble of reclaimed water reaches a monitoring well. The complete list of all parameters for which groundwater standards exist shall be sampled at least once every five years.

Setback distances. A 500-foot setback distance must be provided from the edge of the wetted area to potable water supply wells that exist or have been approved.

Pathogen removal. ASR systems require that fecal coliforms are removed prior to application through high-level disinfection.

Florida requires one year of pilot testing as column studies to demonstrate water quality improvements prior to MAR operation for RIB systems. For example, Florida requires a 12-month pilot test for any MAR system involving wastewater or reclaimed water use for aquifer recharge. Testing must demonstrate Enterovirus, *Cryptosporidium*, *Giardia*, and helminths concentration removal below detection limits (Florida Administrative Code, PART V, Section 62-610.564).

5.1.2.5 Colorado

Colorado developed an alternative GWUDI assessment protocol using the following groundwater evaluation screening criteria (Colorado Department of Public Health and Environment Safe Drinking Water Program Policy Number DW-003, 2012); State of Colorado Design Criteria for Potable Water Systems, Sate Drinking Water Program Implementation Policy #5, 2017):

1. The source has passed visual well inspection.
2. The source depth is greater than 50 ft.
3. The groundwater flow path length is greater than 500 ft.
4. Aquifer recharge activities are occurring at greater than 300 ft from the source.
5. For Type III aquifers, time of travel must be greater than 50 days, and approved groundwater models can be used to support travel time assessments.

Aquifers not fulfilling all of these requirements will be assessed on a case-by-case basis. Groundwater quality performance testing shall be conducted based on the requirements included in Table 5-3. Besides the typical indicators included in the MPA method, the Colorado Department of Public Health, and the Environment (CDPHE) requires the analysis of aerobic bacterial spores.

Table 5-3. Groundwater Quality Performance Testing Requirements per CDPHE for GWUDI Assessments.

Parameter	Location	Frequency	Sampling Dates
Temperature, turbidity, conductivity	Well and surface water	2 times per 7-day period	March 1st – October 31st
Total coliform (w/ *E. coli*)	Well	1x per month	March 1st – October 31st
Total aerobic bacterial spores	Well and surface water	3 times as specified (concurrently with MPAs)	March 1st – April 30th July 1st – August 31st Sept. 1st - Oct. 31st
MPA	Well (surface water may also be required on a case-by-case basis)	3 times as specified	March 1st – April 30th July 1st – August 31st Sept. 1st - Oct. 31st
EPA Method 1622 / 1623 (*Giardia and Cryptosporidium*)	Case by case		

5.1.2.6 Other States

A number of other states have developed regulatory requirements for groundwater recharge operations. To our knowledge, none have specified log removal allocations for MAR system specifications (see Table 5-1). Arizona requires permits for aquifer recharge operations using surface or recycled water but has little descriptive regulatory requirements for microbial risk protection beyond total coliform monitoring. De facto infiltration occurring at some locations is controlled through quality requirements for effluents through the NPDES, which is typical for many other locations in the country.

Most states that have regulations require a minimum of tertiary treatment for surface discharge of wastewaters, such as California and Texas (California SWRCB, 2018; Texas Commission on Environmental Quality [Texas CEQ]). However, some also mandate disinfection, such as Washington, Virginia, Idaho, and Florida (Washington State Department of Health [WSDH]; Virginia Department of Environmental Quality [Virginia DEQ]; Idaho Department of Environmental Quality [Idaho DEQ]; Florida DEP). For subsurface applications using wastewater/direct infiltration, the treatment requirements are usually greater, and some states require advanced treatment followed by disinfection prior to recharge (California SWRCB; Oregon DEQ; Virginia Hampton Road Sanitation District [Virginia HSRD]).

Nevada requires the same log removal requirement as California for viruses, *Cryptosporidium*, and *Giardia* for indirect potable reuse systems, in addition to meeting primary and secondary drinking water standards. Specifically, 12-log enteric virus reduction must be demonstrated from where raw sewage enters a treatment works to the point of extraction from an aquifer for potable use. Ten-log *Giardia* lamblia cyst reduction and 10-log *Cryptosporidium* oocyst reduction must be demonstrated from where raw sewage enters a treatment works to the zone of saturation. Where reclaimed water in reuse category A+ is used for indirect potable reuse, the point of compliance is the zone of saturation. Generally, as is the case for other states, national primary drinking water regulations and secondary maximum contaminant levels must be met.

5.2 Regulatory Approaches in Other Countries

MAR, mostly in the form of IBF, has been the backbone of the public drinking water supply in Central Europe for more than 100 years. MAR has also been widely practiced in Australia, Israel, the Middle East, and India. The following highlights regulatory examples from these regions that follow alternative approaches to the United States for protecting human health from microbial risks in MAR systems using wastewater source water.

5.2.1 World Health Organization

The World Health Organization (WHO) 2017 Potable Reuse guidance puts forth management systems for drinking water providers and regulators on how to plan, design, and operate potable reuse schemes (WHO, 2017).

WHO sets default microbial removal performance targets of 8.5 log reduction of enteric bacteria, 9.5 log reduction of enteric viruses and 8.5 log reduction of enteric protozoa for the selection of treatment process combinations and operational monitoring requirements, to ensure that the LRVs are being achieved.

WHO reports a validated log reduction for SAT as 6 log removal for bacteria, viruses, and protozoa, respectively, based on reported pathogen removals in literature (WHO, 2017), acknowledging that removal performance is system specific and dependent on the nature of soil and retention time in the aquifer.

Microbial water quality monitoring acknowledges that *E. coli* alone is not a good indicator for enteric viruses and protozoa that are more resistant to environmental pressures. The guidance suggests considering other indicators for verification monitoring, including coliphages for viruses and *Clostridium* spp. for protozoa, though both indicators have limitations. Clostridium spores are far more resistant than protozoa and thus a conservative indicator that can be present long after contamination events. Coliphages can be present in high numbers in wastewater but a direct correlation to enteric viruses in drinking water has not been established.

The guidance identifies a number of potential reference pathogens, including *Vibrio cholerae*, *Campylobacter*, E. *coli* O157, *Salmonella*, *Shigella*, rotavirus, norovirus, enterovirus, *Cryptosporidium* and *Giardia*. WHO suggests that the selection of reference pathogens by different countries should be based on consideration of prevalence and severity of disease and source water characteristics.

5.2.2 Australia

In 2008 and 2009, the federal regulatory agency in Australia published Phase 2 of the Australian Guidelines for Water Recycling targeting the augmentation of drinking water supplies with recycled water and the practice of MAR, respectively (Natural Resource Management Ministerial Council et al. 2008; Natural Resource Management Ministerial Council et al., 2009).

In these guidelines, retention time is a key attenuation parameter for microbial and chemical contaminants, but no minimum retention time for MAR operation is requested. Instead, the guidelines require a risk assessment for each individual project taking into account different

end uses and site-specific conditions (including the appropriate retention time). For instance, a minimum retention time is required to meet 2 x 10^{-6} disability adjusted life years (DALYs)/person/year (pppy) for drinking water production (Environment Protection and Heritage Council 2008). Following this risk-based approach, very different treatment levels and retention times would result for recovering (e.g., urban stormwater for non-potable applications such as urban irrigation, compared to water reclamation for potable reuse, such as Ayuso-Gabella et al., 2011; Page et al., 2010a; Page et al., 2015). The Australian guidelines have also been adapted for use in India and China (Bartak et al., 2015).

Figure 5-1 shows the simplified risk assessment stages that Australian agencies follow to assess and manage the risk of microbial pathogens for groundwater recharge applications. For additional information see Natural Resource Management Ministerial Council et al. (2009). Compared to some statewide approaches in the United States, Australian guidelines are less prescriptive and instead emphasize the risk assessment process to develop performance-based outcomes for public-health protection.

Figure 5-1. Simplified Risk Assessment Approach in MAR Project Development from the Australian Guidelines Water Recycling MAR.

The entry-level assessment in Stage 1 shown in Figure 5-1 is based on existing information and regulations to assure the aquifer is suitable and the project conforms to the aquifer

management plans and local government requirements. This stage addresses issues such as intended uses of recovered water.

Stage 2 involves a maximal risk assessment to assess whether the project has low maximal (inherent) human health and environmental risks. This assessment is supported by data collection during field investigations, drilling, and basic modeling. This stage addresses issues such as

- Source-water quality.
- Groundwater quality.
- Soil, aquifer, and aquitard characteristics, and the fate of recharge water.
- Aquifer storage competence.
- Groundwater pressures and gradients.
- Reaction between recharge water, groundwater, and aquifer minerals.
- Water treatment options and their effectiveness.
- Management of clogging.
- Biodegradation and inactivation of contaminants.

In case this assessment identifies the cause for a high degree of difficulty, feasible preventive measures must be put in place or projects will not be feasible. Risk is assessed at two levels: 1) inherent risk (before controls are applied) and 2) residual risk. Residual risk prior to MAR system commissioning assesses whether the proposed preventive measures and operational procedures ensure an acceptable low residual risks to human health and the environment in Stage 3 of the assessment. Preventive measures might include source control and avoidance to prevent poor water quality in the first place, additional treatment, and management of end uses.

The residual risk is again revisited post-commissioning in Stage 4 of the assessment during MAR operation, and the risk management plan is refined as needed. Stages 3 and 4 are conducted on the basis of pilot trials and detailed system modeling. Emphasis is placed on assessing the effectiveness of preventive measures and operational controls, quantifying recovery efficiency, and conducting targeted studies to identify hazards.

This assessment approach was applied to the Groundwater Replenishment Scheme (GRS), Australia's first full-scale groundwater recharge project, which provided treated recycled water from the Beenyup Wastewater Treatment Plant after advanced treatment for injection into the Leederville and Yarragadee aquifers for later abstraction for drinking water use (Government of Western Australia, 2020). The first phase of this project was taken into operation in 2017. The expansion in 2020 increased its capacity to 28 gigaliters per year (20 million gallons per day). The treatment scheme consists of secondary treatment, followed by ultra-filtration, reverse osmosis, and UV disinfection prior to groundwater injection for subsurface water storage.

Australian's water recycling guidelines require a minimum of 9.5, 8, and 8.1 LRVs for enteric viruses, *Cryptosporidium*, and *Campylobacter* for the production of drinking water from untreated wastewater (Natural Resource Management Ministerial Council et al., 2008). For the Beenyup Wastewater Treatment Plant these log removal targets were applied to the above

ground treatment process (secondary treatment, ultrafiltration, reverse osmosis, and UV) prior to groundwater injection (Lozier, 2015). For this project, public health protection from pathogens is managed through several regulatory elements (Government of Western Australia, 2015):

- Regular monitoring of 17 chemical and 1 microbial indicator in the treated water prior to recharge to verify MAR source water quality. MS2 coliphage was selected as the key target microbial indicator to measure the effectiveness of the advanced water treatment process to remove microorganisms as one of the smallest viruses, is considered to have properties representative of fecally-derived viruses, and is more resistant to UV irradiation than other viruses. MS2 was used for validating the virus removal of individual unit processes (ultrafiltration, reverse osmosis, and UV disinfection) through challenge testing and to validate that the total log removal target for viruses of 9.5 was achieved. In addition, MS2was used for commissioning validation and ongoing verification monitoring of the final treated effluent post-UV of the advanced treatment process. The MS2 guideline value is set to < 1 plaque forming units (pfu/L) with a reporting limit of 0.6 pfu/L. This surrogate was selected to specifically indicate potential membrane degradation and damage or loss of membrane integrity that would trigger an alarm for a possible loss of LRVs for pathogens during source water pre-recharge treatment.
- Hazard Analysis and Critical Control Point (HACCP), including the following:
  - Monitoring of surrogates and operational parameters and
  - Event notifications for limit exceedance and alarms.
- Drinking water quality standards at the point of recharge.
- Definition of the Environmental Values (EVs), water quality objectives, and guidelines that the recycled water must meet at the point of recharge and at the boundary of the Recharge Management Zone (RMZ), defined as a radial distance of 250 meters (820 feet) from the recharge location. After that point, the recycled water is considered part of the environment (groundwater).

To prepare for the Phase 2 expansion in 2020, the Water Corporation conducted another assessment of the risks and mitigations of the GRS (Water Corporation, 2017). In this assessment, an extreme inherent risk rating was assigned to four specific pathogen groups: virus, bacteria, protozoa, and helminths. Each pathogen group was consequently represented by specific pathogen indicators and surrogates:

- MS2 coliphage.
- Somatic coliphage thermotolerant coliforms (TTC).
- Escherichia coli.
- Clostridium perfringens spores (a group of anaerobic spores).

Pathogen removal during conventional wastewater treatment was insufficient at reducing pathogens to below the water quality goal. Routine sampling of the advanced treatment process after ultrafiltration and challenge testing of the reverse osmosis process demonstrated sufficient performance in removing pathogens to below the water quality guidelines. The treatment scheme consistently met the treatment performance requirements for log reduction

of pathogens, which concluded that the residual risk was low. Monitoring of MS2 prior to recharge is used to verify consistent membrane treatment.

The Australian guidelines for water recycling using managed aquifer recharge allow to use pathogen removal credits from MAR operation in risk assessments to demonstrate that the DALY pppy risk target can be met (Natural Resource Management Ministerial Council et al., 2009). The guideline gives general direction on how to select target pathogens for validation and verification monitoring without prescribing specific organisms. It is strongly recommended to include enteric viruses in survival studies to validate the treatment capacity of an aquifer, suggesting adenoviruses as a suitable indicator. It is further recommended to include *E. coli*, enterococci, and coliphage in verification monitoring. The guidance document summarizes the days required for 1-log removal achieved in the subsurface for various pathogens for informational use from literature, acknowledging that log removals are primarily related to the residence time of the recharge water, the activity of the indigenous groundwater microorganisms, the redox state of the aquifer, and temperature. Retardation mechanisms, such as adsorption, dilution, or straining are not considered "treatment" per Australian guidelines. Therefore, unless there is sound site-specific evidence to the contrary, inactivation is the only factor that should be used to measure effectiveness of aquifer treatment.

5.2.3 European Union

The European Union (EU) has not set regulations for the design and operation of indirect potable reuse schemes. Instead, several European countries (i.e., Spain, France, Italy, Cyprus, Greece, and Portugal) have developed their own water reuse standards or regulations for groundwater recharge operations (Fawell et al., 2016; Yuan et al., 2016). All member states of the EU are subject to compliance with the European Drinking Water Directive (DWD) (European Commission, 1998) in their national drinking water legislation.

The following highlights regulatory approaches of several European countries with broad established MAR operations.

5.2.4 The Netherlands

The Dutch Drinking Water Decree applies strict water quality standards and prescribes a risk-based approach for managing pathogen risk in groundwater recharge systems. The maximum acceptable annual infection risk by pathogens is the same as used by USEPA (1 per 10,000 inhabitants annually).

The Dutch Drinking Water Act of 2011 requires Dutch drinking water suppliers to conduct a QMRA for possible infections using the following index pathogens:

- Enterovirus.
- Campylobacter.
- *Cryptosporidium*.
- *Giardia*.

Risk assessment must be periodically conducted at least once every four years to assess the microbial safety of finished drinking water. For every groundwater production site, utilities have

to demonstrate that the four index pathogens and any other pathogens are sufficiently removed by monitoring index pathogen concentrations in the source water and finished drinking water. In the QMRA, source concentrations of pathogens are estimated, along with recovery efficiencies, treatment removals, final drinking water concentrations and consumption, exposure, dose-response, and infection risk (Schijven 2015). Groundwater recharge systems must also have a retention time of at least 60 days to assure proper inactivation. Well monitoring requirements targets fecal indicator organisms (Smeets et al., 2009).

The Dutch drinking water guideline prescribes how to conduct the QMRA process for drinking water production from groundwater. The approach is currently being published by the Ministry of Infrastructure and Water Management (as of 2020) (Ministry of Infrastructure and Water Management, 2020) and summarized in Figure 5-2.

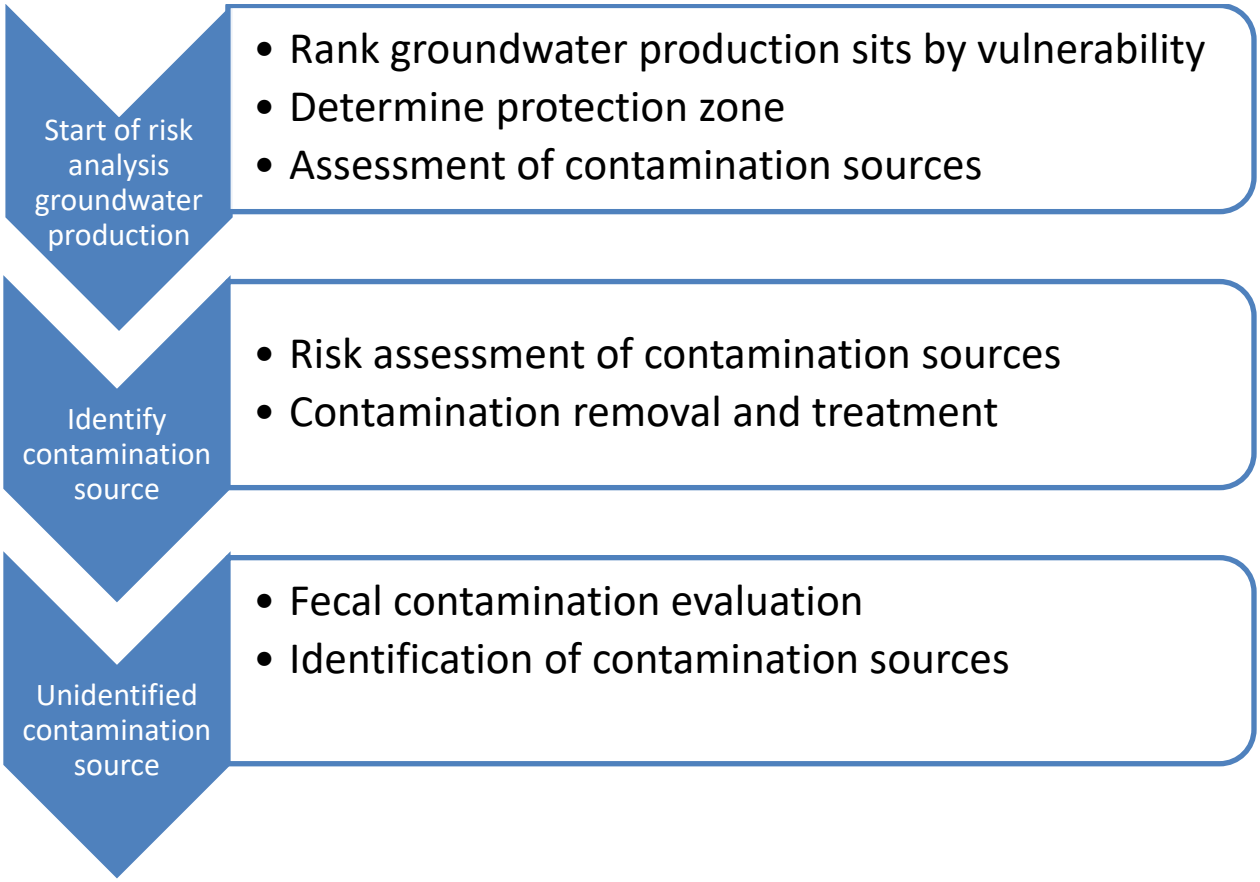

Figure 5-2. Simplified QMRA Decision Tree for Dutch Groundwater Production Sites.

In this QMRA procedure, the vulnerability of the geohydrologic system for pathogens is determined by a list of intrinsic properties that affect the survival of an index pathogen between the contamination source to the production well. These properties are similar to those considered in US GWUDI assessments. Based on the following factors, a risk analysis is conducted to assess the system vulnerability for pathogen breakthrough:

- Physical aquifer properties.

    - Presence/absence of less permeable, protective clay layers (clay layers make an aquifer less vulnerable).
    - Permeability of layers of the geohydrologic system (less permeable is less vulnerable).
    - Thickness and depth of the geohydrologic system (thicker and deeper is less vulnerable).
    - Position of well screen deeper is less vulnerable).
    - Grain size of the sand (coarser sand is more vulnerable).
    - Heterogeneity of the soil and subsurface material (more heterogeneous can be more vulnerable, like the presence of preferential, fast flow paths).
    - Pumping rate (a higher rate implies more dilution, but also faster transport, which results altogether in more vulnerable conditions).
    - Anisotropy (layers in the geohydrologic system may imply slower transport in vertical direction, which implies lower vulnerability if the well screen is situated deep enough).
    - Thickness off the unsaturated zone (the thicker, the less vulnerable).
- Physico-chemical conditions of the groundwater.
    - pH, ionic strength (lower ionic strength and higher pH are more vulnerable).
    - Temperature (temperature is almost constant in the Netherlands).
    - Ion composition.
    - Organic matter content (organic matter may prevent attachment due to blocking of attachment sites).
    - Redox conditions (microorganisms are not removed from anoxic groundwater as effectively because they do not attach very strongly to soil particles).

The required protection zone (setback distance) around the production well is defined based on the fate and transport modeling focusing on viruses as the key group of concern. Viruses, specifically enteroviruses, are considered to be the most relevant “index” pathogen in MAR systems due to their small size, usually poor attachment to sand (the prevalent geological material in the Netherlands), slow inactivation, and high infectivity.

The model-predicted minimum travel time to reduce the virus infection rate to less than 1 in 10,000 starts with the source water virus concentration and assumes homogeneous geohydrologic conditions in the groundwater aquifer, certain log removal rates for enteroviruses depending on the prevalent redox conditions in the subsurface, specific sticking factors for virus adsorption, and dilution with native groundwater in the aquifer. This model quantifies setback distances and was developed by Schijven et al. (2010). It was made available as the online calculator model QMRAcatch (RIVM, accessed 2021). Note that groundwater used for drinking water is typically not disinfected in the Netherlands. The pathogen removal goals must be met at the point of extraction, and post-chlorination is typically not applied prior to distribution.

In case a pathogen contamination source is identified, a risk assessment is conducted that involves transport modeling simulating advection, dispersion, dilution, attachment/detachment, inactivation, and straining of viruses in the subsurface. Proposed models for estimating pathogen concentrations in the production well include QMRAcatch and U.S. Geological Survey modular finite-difference flow model (MODFLOW). Depending on the

modeling results, additional dilution at the point of extraction or treatment may be required to adequately protect public health.

If the protected zone is smaller than the model-predicted required protection zone around the wellhead, the utility must conduct a four-year monitoring program, where monitoring must then be repeated every four years. This program entails target sampling for somatic coliphages to report any detection in least 3 samples of 100 liters, and 1000 liters of well water sample volume if possible. (The sample volume might be practically restricted during sampling by filter clogging.) This target sampling focuses on the indicator and surrogate somatic coliphage instead of the index pathogen enteroviruses, since somatic coliphage may appear in concentrations 1000 to 10,000 times higher than enterovirus concentrations. Detection of somatic coliphage indicates fecal and viral contamination of the geohydrologic system. If the monitoring program indicates sufficient virus removal, then bacteria removal is deemed sufficient as well. Oocyst monitoring is not routinely conducted in the Netherlands, since *Giardia* and *Cryptosporidium* are generally removed sufficiently in the Netherland-specific homogenous, sandy aquifers.

5.2.5 Germany

Germany regulates groundwater protection zones, which also apply to IBF and MAR systems, on the federal level by the Water Management Act (§ 51052WHG 2009). Specific regulatory requirements for required subsurface residence times may differ among individual federal states within Germany. Typically, federal states require a minimum retention time in the subsurface of at least 50 days in the Protection Zone II to protect drinking water from bacterial contamination. For comparison, Switzerland sets a minimum retention time of 10 days and a minimum distance of 100 meters in the subsurface for bacteria and viruses to achieve proper inactivation (Water Protection Act 2018, 814.201, Appendix 4, Section 123). The distribution of groundwater retention time is demonstrated through site-specific hydrogeological tracer studies.

In 2015, the German EPA suggested a new procedure for drinking water utilities to assess microbial risk in de facto MAR and IBF systems. This procedure recommends a quantitative microbiological risk assessment for MAR systems following a decision analysis shown in Figure 5-3. This procedure has been recently implemented by some utilities in Germany (German EPA / Umweltbundesamt 2015), and the German EPA intends to assess its usefulness in proving pathogen risk mitigation in the coming years.

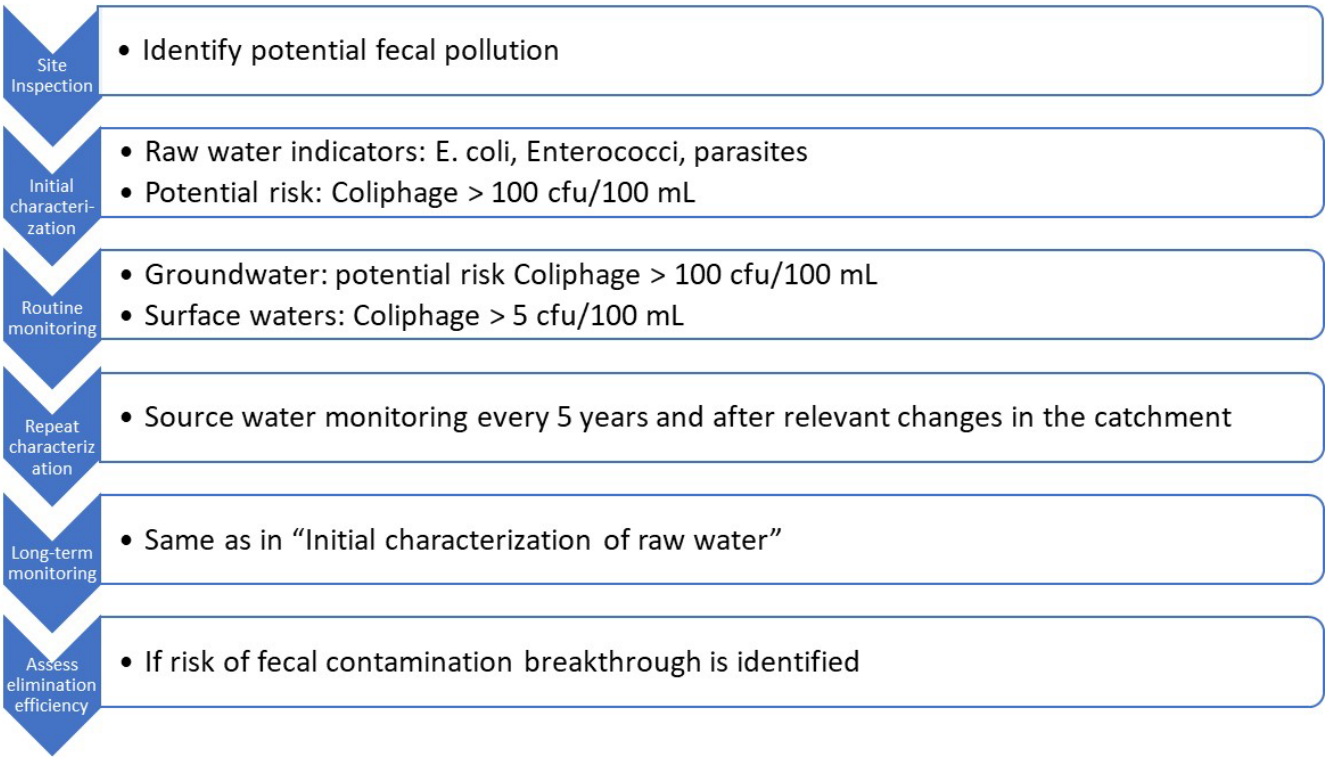

Figure 5-3. Summary of Proposed Approach for Assessing Microbial Hazards in MAR Systems in Germany (Pfu = Plaque Forming Units; Cfu = Colony Forming Units).

The assessment attributes a relevant risk for microbial contamination for groundwaters if the coliphage concentrations in surface water recharged exceeds a level of 100 pfu/100 mL, and if *E.coli* and Enterococci are present at concentrations exceeding 100 cfu/100 mL. This approach has similarities to the bin classification used by USEPA per LT2ESWTR for categorizing the microbial risk of surface waters based on the concentrations of *Cryptosporidium* and *Giardia*.

If these microbial thresholds are exceeded in the source water of planned or unplanned MAR systems, the German EPA recommends five steps as part of the QMRA procedure:

1. Identify personnel responsible for conducting the risk analysis and assessment.
2. Characterize the hydrogeology and microbial risks in the catchment area of the MAR well.
3. Employ a monitoring program for quantitative microbial characterization of the source water(s) to identify possible pathogen sources. This involves regular sampling for the following:
   - *E. coli*.
   - Enterococci.
   - *Cryptosporidium* and *Giardia*.
   - Coliphages.
4. Evaluate risk mitigation factors in the catchment area.
5. Evaluate risk mitigation factors through additional drinking water treatment and disinfection.

The German EPA recommended somatic coliphages as indicators and surrogates for fecal contamination because they are similar to viral human pathogens and live longer in the environment than other bacterial surrogates. In addition, the concentrations of coliphages exceed other viruses, such as adenovirus or rotavirus, by more than 10- to 100-fold. Just like *E. coli*, somatic coliphages are excreted by humans at all times, not just at times of infection.

The German EPA further recommended conducting the initial characterization of the catchment area routinely each quarter in a calendar year and in addition two times after extreme hydrological events, such as heavy precipitation, flooding, snow melt, or droughts. This characterization should be repeated every five years or if relevant changes to the catchment area are made.

5.2.6 Canada

Canada does not have federal regulations for MAR systems and instead allows individual provinces to develop their own regulations. MAR systems in Canada apply primarily to GWUDI systems of bank filtration. Some provinces have no official rules for GWUDI assessments, while others (e.g., Ontario and Quebec) have developed very detailed regulations and guidelines.

Ontario has developed a regulatory paradigm for GWUDI systems that uses a different approach than US GWUDI and MAR regulations. Specifically, Ontario does not prescribe requirements for treatment credits for pathogen removal and log removal targets based on subsurface travel time. Prescribing requirements did not seem defensible given that subsurface travel times are a distribution of various travel times that may apply to chemical tracers but not to particle transport. The actual maximum travel time in the subsurface for pathogens was considered uncertain and variable given the dynamic hydrological conditions in GWUDI systems and the heterogeneous aquifer conditions. Therefore, Ontario regulators did not consider travel time an adequate “surrogate” to assess and regulate pathogen removal in the subsurface for public health protection.

Instead, the regulatory focus is on direct monitoring of key water quality parameters for public health protection in the well water. Monitoring programs focus on possible changes of baseline water quality in wells, setting stringent alert levels to draw attention to changes from baseline water quality conditions. Data reporting and interpretation received extra attention during the regulatory development because analyses of well water frequently resulted in “non-detects.” Ontario focuses more on well monitoring and post-treatment requirements of the recovered water, and less on what may happen in the ground.

5.2.7 Summary of Log Removal Targets

Table 5-4 summarizes the log removal targets different regulatory agencies and organizations have defined for MAR systems fed by treated wastewater source water and the rationale or method on which this requirement was based.

Table 5-4. Summary of Log Removal Targets for MAR Systems Set Forth by Selected Countries and Regions.

Agency/Organization	Log Removal Values Requirements	Rationale
Australian Guidelines	Enteric bacteria (*Campylobacter*): 8.1 log Enteric viruses (noroviruses): 9.5 log Enteric protozoa (*Cryptosporidium*): 8 log Applied between the raw wastewater and finished drinking water.	Used 95th percentile of reference pathogen concentrations in untreated wastewater from large metropolitan WWTPs to take into account observed variability and increases observed during outbreaks of disease. These concentrations were used to calculate minimum performance targets to meet the health outcome of 10-6 DALYs pppy (WHO, 2017).
California, Nevada	Enteric viruses: 12 log *Cryptosporidium*: 10 log *Giardia*: 10 log Applied between the raw wastewater and point of groundwater extraction.	Used 95th percentile of maximum reported concentrations of enteric pathogens in wastewater to reduce the level of risk to below an acceptable level of 10^{-4} infections per year.
Germany	Site specific.	Germany is early in the process of setting regulatory procedures and requirements. Developed an assessment method that is currently under pilot testing to evaluate source water risk and risk mitigation for pathogens from wastewater influenced MAR systems.
Ontario	Not applicable.	Ontario does not use LRV for regulating MAR systems. Instead, key water quality parameters in the well water are monitored for public health protection.
The Netherlands	Site specific.	Log removal values are determined for each site using QMRA.
World Health Organization	Enteric bacteria: 8.5 log Enteric viruses: 9.5 log Enteric protozoa: 8.5 log Applied between the raw wastewater and point of groundwater extraction.	Minimum performance targets identified to meet the health outcome of 10^{-6} DALYs pppy.

CHAPTER 6

Managing Microbial Risk

Different regulatory approaches for pathogen removal in MAR systems cannot be evaluated without considering the human health protection goals, exposure risks assessments, and risk management approaches underlying these programs. The USEPA, Australia, Europe, and the World Health Organization (WHO) have established pathogen reductions needed for drinking water treatment based on a QMRA approach (Regli et al., 1991; Sano et al., 2016). For the United States and the Netherlands, a goal was set to treat water so that the risk of infection was no more than 1:10,000 per year. The WHO employs a disability adjusted life years (DALY) approach with a tolerable goal of 10^{-6} DALYs per person per year. DALYs are used to assess the severity and duration of disease and the number of people affected. The 10^{-6} DALYs per person per year is approximately equivalent to 1 case of diarrhea per 1,000 people per year (WHO, 2017). This cannot be directly compared to the goals of less than 1:10,0000 infections per year used in the US, as not every infection results in illness. The probability of illness per infection varies by pathogen, for example 0.2 for *Cryptosporidium* and 0.7 for Norovirus (WHO, 2017). The WHO determined pathogen concentrations equivalent to 10^{-6} DALYs per person per year as 2.0×10^{-5} enteric bacteria (*Campylobacter*)/L, 1.1×10^{-5} PCR detectable units (PDU)/L of enteric viruses (norovirus), and 1.2×10^{-5} oocysts/L (WHO, 2017).

This health outcome target is also used by Australia and numerous other countries. Treatment requirements were determined to achieve this level for the various pathogens, depending on their initial concentration in the source water. Although the WHO specifies that as much site-specific data as possible should be collected for a QMRA, default concentrations in raw sewage and treated wastewater are typically being used when data cannot be obtained (WHO, 2017).

As covered in the Chapter 5, different regulatory approaches have been developed to assure public health risk goals are met in MAR systems. These regulatory approaches differ among countries and among state agencies within those countries. The WHO 2017 Potable Reuse guidance assigns a categorical maximum of 6 logs reduction for pathogens to soil-aquifer treatment based on challenge testing (WHO 2017). California, Arizona, and Texas require a certain level of enteric virus removal from wastewater prior to use for agricultural purposes (Gerba et al., 2017). A 6-7 log reduction of enteric viruses has been suggested for reusing wastewater for agricultural crops that may be consumed raw (Drechsel et al., 2010). Currently, California requires 10 log removal for *Cryptosporidium* and *Giardia* and 12-log for viruses as performance targets for indirect potable reuse, with a maximum 6-log reduction assigned to individual unit processes regardless of actual performance. California further requires a six-month underground retention of recycled water at MAR operations to obtain a 6-log retention time credit for virus removal. A minimum of two months is allowed in California if the project demonstrates it can identify and respond to a treatment failure in that interval granted that the remaining log removal required is demonstrated with above ground treatment. In that case, only 2-log virus reduction credit is required in the subsurface. Australia requires a minimum of 9.5/8/8.1 LRVs for enteric viruses, *Cryptosporidium*, and Campylobacter be achieved between

untreated wastewater and finished drinking water. (CDPH, 2014; USEPA, 2017; Natural Resource Management Ministerial Council et al., 2008).

Most of these regulatory requirements were defined before the development and broader application of molecular detection methods to MAR systems for pathogen screening. These methods became available only in the last decade. While these methods do not quantify *infectious* pathogens and are thus conservative in their quantification, the higher detections, and occurrences of known and previously unknown pathogens and indicators cannot be ignored. Today, virus levels may be 100 times greater than previously thought, before molecular methods were used (Gerba et al., 2017; 2018). Theoretically, in using updated raw sewage norovirus densities from Eftim et al. (2017), Soller et al. (2018a) proposed that viruses would need to be reduced by 14 logs or more to consistently achieve the 10^{-4} annual risk of infections per year health target for direct potable reuse. This new data poses new questions and challenges on how to relate higher de-facto occurrence densities of pathogens obtained via molecular methods to infectivity rates for public health protection that seem unchanged since before this knowledge was available.

The variability in treatment processes may affect the concentrations of pathogens in the wastewater supplied to MAR operations, and variability in the ability of MAR should be considered (Soller et al., 2018b). Instead of using prescriptive or deterministic LRVs requirements and pathogen concentrations, which is standard regulatory practice in assigning minimum and maximum LRVs for unit treatment processes, the uncertainty and variability inherent to pathogen removal and presence in treatment trains can be better described using probability distribution functions (PDFs). By using PDFs to characterize both pathogen presence and LRVs attributed to a unit treatment process, evaluating different percentiles of the attributed risk can help operators, legislators, and public health officials determine which LRVs are truly required on a case-by-case basis. Such approaches have already been implemented in QMRAs of direct potable reuse trains (Pecson et al., 2017; Soller et al., 2017; Soller et al., 2018 a, b) as well as indirect potable reuse trains including MAR (Ayuso-Gabella et al., 2011; Zhiteneva et al., 2021).

Not only would such a method help determine critical control points in a treatment train, but a switch to probabilistic assessments and characterizations of residence time and decay rate could also help identify proper placement of monitoring wells, especially for MAR operations. These wells could be placed at strategic locations intersecting the transport path of the water to enable high-resolution monitoring and early detection of potential contamination or inadequate removal. They would also demonstrate which LRVs can be achieved on a site-specific basis, which could be higher than the maximum 6 LRVs allowed in Australian and Californian regulations.

CHAPTER 7

Critical Knowledge Gaps

This study identified the following knowledge gaps through the literature review summarized in this report and recommendations solicited from participants of the national and international expert workshops. Chapters and sections where the context for these knowledge gaps is further discussed are referenced where applicable:

1. **Suitability of Surrogates and Indicators.**
   - Metadata-analysis of indicators and surrogates from studies conducted globally would be beneficial to identify trends and correlations relative to pathogen, indicator, and surrogate characteristics, removal, occurrence, and fate and transport in various aquifer materials. Such trends and correlations would provide a more refined scientific basis for the selection of site appropriate indicators and surrogates and definition of LRVs for certain types of MAR sites than used by practitioners and regulators today (see Section 4.2)
   - Application of metagenomics to characterize entire population of pathogen removal in laboratory column studies and compare to field studies. Metagenomics could identify the strains in samples at a broad scale, looking at all genes from all members of the sampled communities, rather than limiting detection to specific target species. This approach also allows for identifying changes in the microbial population and for identifying pathogens resistant to removal (see Section 3.2). It is noted that broad sequencing does not have the same sensitivity than qPCR to detect pathogens and is therefore a good complementary technique.
   - Aerobic spores have been proven reliable as surrogates for *Giardia* and *Cryptosporidium* in GWUDI assessments. USEPA has collected a dataset of aerobic spore concentration, spore log removal, and *Giardia* and *Cryptosporidium* detections at various IBF sites in the United States. It would be useful to assess whether spores are suitable surrogates to quantify the removal performance of protozoa in SAT or ASR sites (Section 3.4.1).
2. **New indicators and surrogates opportunities.** Approaches for assessing fate and transport characteristics of target pathogens that extend beyond indicators organisms (Section 3.4), including the feasibility of silica beads with virus-specific proteins, free DNA or RNA encapsulated in polymers, online flow cytometry, or DNA binding dyes to detect viability in PCR.
   - Metagenomics to determine the role of indigenous organisms and inactivation of pathogens. This approach can be used to define optimal operation of MAR for pathogen removal by monitoring organisms that are agonistic to their survival/removal.
   - ATP as an indicator of biological activity. This approach can be used to define optimal operation of MAR for pathogen removal by monitoring organisms that are agonistic to their survival/removal.
   - New surrogates that be used to determine/monitor MAR operations, i.e., plant-based surrogates (e.g., PMMoV) or CrAssphage. These would be conservative indicators of

removal relative to the human pathogenic viruses, which occur in lower numbers in wastewater.

3. **Monitoring of pathogen and indicator removal by MAR in near or real time.** In response to the SARS-Cov-2 pandemic automated systems have been developed for the concentration and detection of viral pathogens in wastewater/water. These automated concentration and detection systems by digital droplet PCR (ddPCR) are capable of processing 100 samples a day. The Centers for Disease Control is setting up 100 sites to monitor the wastewater in the United States for emerging pathogens. This should make this technology easily available to the wastewater industry since pandemic wastewater monitoring for viruses has become common around the world (Section 3.1).
4. **Aquifer recharge systems not covered in this study.** Pathogen occurrence, fate, and transport understanding, as well as suitable indicators and surrogate definitions, are needed for aquifer recharge systems not explicitly covered in this study. These include:
   - Dry wells used for injection of stormwater for vadose zone treatment. USEPA has no design requirements for dry wells, and responsibility is left to local authorities. For example, in California, cities or counties set design specifications for the minimum distance between dry well release points and groundwater. Common standards require only 3 ft (Office of Environmental Health Hazard Assessment, 2014).
   - Stormwater infiltration.
   - Liquid effluent disposal sites through aquifer recharge. These systems are not permitted as MAR sites and groundwater monitoring requirements are limited.
5. **Fate and transport modeling.**
   - Fate and transport models do not yet adequately reflect the dynamics of IBF systems, such as release pulses, attachment, and detachment process. Some of these models are currently being developed, but they are not yet published or available for utilities. Certain regulatory agencies, such as Australia, are not considering retardation processes in risk assessments for required log removal calculations (Section 4.4 and 5.2).
6. **Testing of procedures for demonstration studies for alternative LRV at MAR sites.**
   - This study developed recommendations for site-specific demonstration studies for alternative LRV to permit agencies. These recommendations should be tested in pilot studies to flush out guidelines and best management practices for regulatory consideration by state agencies (see Chapter 8).

CHAPTER 8

Conclusions and Recommendations

This report summarizes various perspectives and scientific implications for different stakeholders in the field of public health protection from pathogens in MAR systems using treated wastewater impacted source water. We have compiled the state-of-the-science understanding of pathogens, indicators, and surrogate occurrence, monitoring and detection, fate and transport, emerging trends, and remaining knowledge gaps relevant to MAR systems.

The report also analyzes the regulatory approaches currently in place in the United States for public health protection in MAR and GWUDI systems relative to microbial agents. It provides reference for justifications for these regulations as well, and contrasts regulatory requirements to alternative approaches used in other countries.

This chapter summarizes conclusions and final recommendations that can help develop robust and sound procedures for the selection of suitable indicators and surrogates and monitoring programs. Based on the scientific findings of this study, we suggest a stepwise procedure that can help regulators and utilities determine site-specific log removal values for pathogens to define minimum design and operational criteria for MAR operation that are neither too conservative nor risk groundwater or drinking water contamination.

8.1 General Microbial Risk Assessments Challenges

Regulators and utilities operating MAR or GWUDI system commonly expressed two specific challenges.

Regulatory indicators and surrogates currently used to monitor MAR and GWUDI systems are challenging to interpret, specifically over whether public health is adequately protected. This is because pathogens are typically not detected and fecal coliforms, commonly used indicators, and surrogates, are not considered adequately protective.

Regulatory accepted guidance for how to conduct site-specific testing needs to be developed. GWUDI assessments follow a two-step process: 1) screen the site to determine if adequate pathogen removal is apparent, and 2) demonstrate that the site is actually providing the required pathogen removal, and add treatment as needed. MAR systems are being screened for pathogen removal risks. However, site-specific demonstration studies for regulators are not conducted. Instead, regulations follow empirical LRVs.

8.1.1 Recommendations

Adopting the concept of demonstration studies from GWUDI assessments would not only help verify that public health goals are indeed achieved and maintained, but they can also reveal whether design assumptions are overly conservative in meeting public health goals.

A standard regulatory practice in assigning minimum and maximum LRVs for unit treatment processes, is using prescriptive or deterministic LRVs requirements and pathogen

concentrations. PDFs might be a better approach to describe the uncertainty and variability inherent to pathogen removal and presence in treatment trains. By using PDFs to characterize both pathogen presence and LRVs attributed to a unit treatment process, evaluating different percentiles of the attributed risk can help operators, legislators, and public health officials determine which LRVs are truly required on a case-by-case basis.

8.2 Recommendations for Indicators and Surrogate Selection

The following recommendations were developed among experts supporting this study and are listed in order of priority:

1. **Right Level of Conservatism.** Indicators and surrogates should be selected to be conservative but not overly conservative, since they may otherwise eliminate MAR operation from consideration or make it more costly than necessary to meet regulatory accepted human health risk goals. Generally, regulations and site-specific studies should clearly state why specific surrogates and indicators were selected.
2. **Reference Indicator and Surrogate Selection.** Commonly used indicators and surrogates don't provide relevant information on fate and transport of pathogens. Most state regulations for MAR sites require monitoring of E. coli and total coliforms. However, the general scientific consensus is that these are not useful indicators or surrogates. Instead, multiple diverse indicators and surrogates should be selected depending on the pathogens of concern and site conditions. Viruses are a relevant group due to comparatively fast transport, high source water concentrations, and low infectivity. Current regulatory monitoring programs do not reflect this.
3. **Viral Indicators / Surrogates.** An increasing number of viruses and bacteria have been found to be resistant to disinfection, making the fate and transport of these pathogen groups a higher priority in MAR systems. Traditionally, the regulatory emphasis for pathogen monitoring has been on oocysts and bacteria. Public health protection from viruses has been considered adequate, since recovered well water from GWUDI and MAR wells is subjected to chlorine disinfection. However, testing male-specific and somatic bacteriophages as surrogates in IBF demonstration studies and requiring routine groundwater monitoring permits should be considered.
4. **Toolbox Approach.** A single indicator type does not provide a comprehensive microbial risk assessment. For example, focusing only on bacteria or only on viruses is too narrow. A toolbox approach would be helpful to consider all pathogen types (virus, bacteria, and protozoa) and to characterize fate and transport at all scales (laboratory and field site investigations along with modeling). Multiple indicators and surrogates need to be considered, depending on the situation:
    - Process-specific (above-ground vs. subsurface treatment).
    - Type of system – SAT, IBF, ASR or ASRT with RO treatment, ASR or ASRT with advanced non-RO treatment.
    - Type of aquifer – aquifer material, heterogeneities, retention time, saturated vs. unsaturated transport, etc.
    - Certain indicators and surrogates may be scale dependent. Some are useful for field demonstration studies, while others are useful in laboratory column experiments.

- Selecting specific surrogates suitable for retardation and then differentiating them from other surrogates suitable for pathogen survival, for example, could be useful.

5. **Toolbox Bins.** Surrogates and indicators should reflect the properties of the pathogens present at the site. An analysis of common and important pathogens and relevant surrogates/indicators would provide significant benefit to operators of MAR systems. Categorizing viruses into bins based on relevant properties and fate parameters could be helpful to guide informed selection of indicators and surrogates. This is similar to defining indicator chemicals for monitoring that represent a larger family of chemicals of similar functional groups, structures, and associated behavior. Many characteristics matter and should be considered, including stickiness, size, shape, survival rate, etc.
6. **Detection Sensitivity.** Indicator and surrogate organisms that occur at highest concentrations in MAR source water at a specific site should be considered as reference organisms. Metagenomic screening could help in this identification.
   While molecular methods are most sensitive, results are conservative since they do not represent viability. Cultural assays are less sensitive. At a minimum, precision must be sufficient to demonstrate the overall 12/10/10 log removal target in groundwater recharge systems. Therefore, the range of the occurrence concentration and sensitivity of analytical method (detection limit) must be appropriate to be able to demonstrate the highest required removal rates.
7. **Regulatory Consistency within a State or Region.** From a utility perspective, site-specific regulatory approaches (e.g., monitoring parameters) on a case-by-case basis for MAR systems can be challenging. Site-specific approaches need to be balanced against the need for regulatory consistency at minimum within a state / region to avoid misinterpretations and / or public confusion.

Table 8-1 summarizes opportunities and limitations of various pathogen surrogates and indicators compiled from the shared experience of various national and international experts. This list can help with selection (and data interpretation) of common options.

Table 8-1. List of Advantages and Limitations of Indicator and Surrogate Candidates.

	Opportunities	Limitations
Viruses		
PMMoV	• Fecal marker / indicator • Most abundant RNA virus in human feces • Conservative indicator for presence of viruses in subsurface • Conservative useful surrogate for virus Fate and transport (F&T) in aboveground treatment in in subsurface as it can be quantified • High concentrations, detectable in MAR sites • Indicator for preferential flow paths in MAR systems • Low seasonal variability	• Overly conservative as indicator and surrogate for pathogens (RNA virus and very persistent) • Too conservative for QMRA • Analysis via RNA (not infectivity) so detection of PMMoV does not mean that infectious pathogens are present
F-specific RNA Coliphages & Somatic bacteriophages (Enteroviruses)	• Validated EPA method available for groundwater (1642/1643) • Direct relevance to human health / gastrointestinal illness • Can be measured by infectivity, culturable assays • Vary in size and stickiness • Required to be used for challenge phage spiking test in The Netherlands • Coliphage monitoring as a whole group is worthwhile to help identify those found for detailed analysis • Surrogate for viruses in above ground treatment and in subsurface • Already in the California water recycle regulations	• 10-12 taxonomic groups of coliphages with varying size and adsorption affinity • Need to consider shape, size, and whether enveloped or non-enveloped if used as surrogate • Lower concentrations than plant or certain bacterial viruses
Bacteriophage MS2	• Suitable surrogate for spike tests in field. • Even for short travel distances (about 10 ft) effective removal can be demonstrated towards the California 12-log removal requirement	
CrAssphage	• Fecal marker / indicator • Smallest known virus • Present in high concentrations • Indicator for viruses in above ground treatment • Low seasonal variability	• Analysis via DNA (not infectivity)
Adenoviruses	• Resistant to most above ground treatment / UV disinfection • Conservative indicator for viruses for above ground treatment and in subsurface. • Prevalent in source water • Useful indicator for short HRT systems (e.g., IBF) • Non-seasonal indicator	• Free DNA may persist longtime in the environment • Largest of all enteric viruses
Rotavirus	• Suggested by WHO • Useful indicator for short HRT systems (e.g., IBF)	• Not present as often
Silica beads covered with virus specific proteins	• Designed to simulate fate close to real viruses • Employed in New Zealand	
Chemical Tracers	• Well known, often monitored, • Can often be detected in groundwater • Conductivity useful indicator for membrane integrity	• Chemical tracers are not suitable surrogates, since analytical sensitivity is lower than for viruses and viruses follow preferential flow path

(Continued)

Table 8-1. Continued.

	Opportunities	Limitations
Bacteria		
	• Drinking water regulations need to be met, but bacterial indicators and surrogates are not adding much value since bacteria are typically not present in MAR systems	
Protozoa		
Cryoptospridium and *Giardia*	• Relevant pathogens with high infectivity	• Rarely detectable in source water and not detectable in MAR systems with longer residence times • Too large for surrogates
Algae	• Direct injection in Melbourne study (small enough as proxy for viruses) • Successfully used for spike test in ozone (O3)/ Biological aerated filters (BAF) treatment.	• Ubiquitous occurrence without contamination
Aerobic Spores or Anaerobic Spores (Clostridium spores)	• Aerobic spores proven reliable surrogate in IBF systems • Persistent • Anaerobic spores index organism in MAR application in Australia	• Might be too conservative for Cryptosporidium, since it is much smaller and occurs ubiquitously without contamination • Need to understand presence / range of spores in source water and detection limit by source water type • Vary in size
Fecal DNA markers (HF 183, HF 182)	• Sensitive detection method.	• Not a useful surrogate related to survivability of Cryptosporidium. • Nucleic acid methods generally overly conservative as pathogen indicators; detection may still not pose a threat to public health
Microsporidium	• Smallest known protozoa	
Engineered particles		
Plastic Microspheres Free DNA DNA encapsulated in polymers	• Desired surrogate properties (size, stickiness, etc.)	• Application is scale dependent (column vs. field study)
Chemicals Persistent CECs PFAS Isotopes, etc.	• Useful for field tracer tests to determine average travel times; however, consider that pathogens may travel faster than chemicals • Chemical tracer test useful in first step to verify recovery of spiked material in the system and for placing monitoring wells to represent general flow path	• Limit of detection higher than for biological organisms • Transport velocity in subsurface possibly slower than viruses due to pore size exclusion of viruses

8.3 Fate and Transport Understanding of Pathogens in Subsurface

Our knowledge of viral pathogens is rapidly developing. Over 50 new viruses have been discovered that could be transmitted by wastewater over the last 50 years. An estimated 100,000 new viruses that infect humans are believed to be undiscovered. Moreover, viral biodiversity and evolution results show that some will survive longer and are becoming resistant to chlorine disinfection (Rodriguez et al., 2018).

Wastewater characteristics, treatment of the recharged water, and subsurface characteristics impact pathogen removal. Water characteristics such as pH, TOC, colloidal matter, and total dissolved solids (TDS) are important for virus and bacterial removal in the subsurface. Viruses may attach to colloidal matter, which affects their transport, removal, and survival. Removal of nutrients, specifically nitrogen in recharged water, generally increases infiltration rates because of the lack of algal mat formation, resulting in a higher risk of virus transport into deeper subsurface depths. Subsurface characteristics, including redox conditions, organic carbon, and iron oxide content, impact virus attenuation during MAR.

MAR sites can be modified to enhance pathogen removal. The University of Arizona found that adding aluminum shavings to soil enhances virus removal in soil columns. The Technical University of Munich, Germany, developed the SMARTplus concept, which has maintained strictly controlled redox conditions and demonstrated enhanced virus attenuation under extended aerobic conditions during subsurface travel (Karakurt-Fischer et al., 2021). A recent study showed that adding compost to the surface of a MAR site enhanced trace organic compound removal but had no effect on bacterial removal.

Models simulating fate and transport have been successfully used to predict pathogen removal in homogenous aquifers, but they also have value in heterogeneous aquifers to inform and help interpret tracer test studies and surrogate transport monitoring results. Modeling can supplement sampling and monitoring programs that are limited to a number of target agents that may not represent the diversity of known and unknown pathogens present in MAR systems.

Model outputs can be valuable for assessing the conditions of potential risk, minimum recommended setback back distances, or residence times needed to achieve a certain log reduction, particularly when parameter inputs can be determined and calibrated to laboratory or field measurements followed by validations in the field. One-dimensional models are appropriate for homogenous, simple, alluvial aquifers and simple lab scale studies. These models are feasible for most utilities without academic support. Three-dimensional models are needed for heterogeneous aquifer systems with various wells and complex hydrogeological site conditions.

However, some sites are too heterogeneous or not sufficiently characterized to be adequately modeled. Some systems are too complex for various reasons: multiple sources of water or influenced by other subsurface flows, variability in operation, substrata with lenses of lower permeability, preferential flow paths or changing water table levels, affecting the depth of the unsaturated zone. In complex hydrogeological conditions model set up, calibration and

validations can be too costly for utilities, and they may not have the means to do so without long-term academic support.

8.4 Recommendations for Site-Specific MAR Performance Demonstrations

Field monitoring strategies, laboratory simulations, and hydrogeological modeling have made substantial scientific advances in recent years that are not adequately reflected in today's MAR regulations. In combination these methods can help utilities develop site specific log removal value estimates that can be used in support of permitting decisions.

A stepwise procedure recommended for log removal determination in MAR sites is graphically summarized in Figure 8-1. Requesting to change the LRVs assigned to a MAR site from the regulatory default removal credits could be done at multiple points in the procedure, which has been denoted in Figure 8-1 as "variance request".

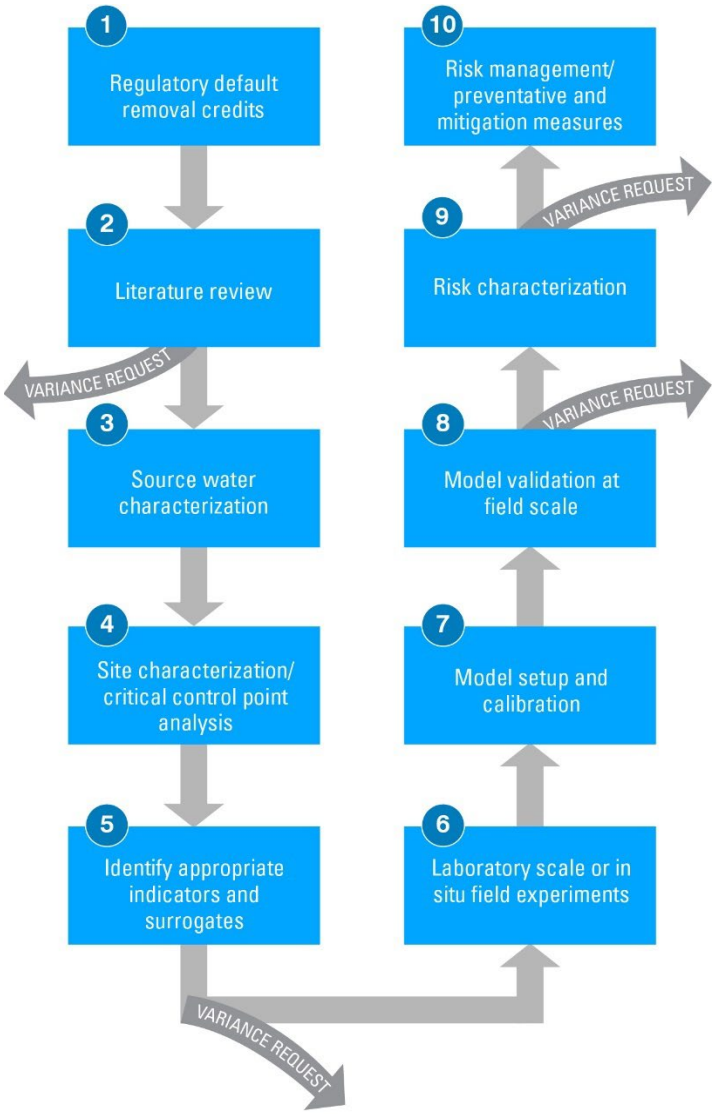

Figure 8-1. Proposed Tiered Approach for Site-Specific Pathogen Removal Credit Demonstrations in Wastewater or Wastewater-Influenced Source Water MAR Systems.

The tier approach consists of the following steps:

1. **Regulatory default credits.**
   a. Adopt conservative default log removal credits set by regulators as a starting point.
2. **Literature Review.**
   a. In case a set of default regulatory credits are significantly inconsistent with existing literature (i.e., more conservative), a utility may choose to update the default LRVs with site specific consideration and updated scientific literature. Literature review is an appropriate first step in when attempting to better understand pathogen removal at specific MAR site. A review of observed inactivation rates for the pathogen(s) of interest (e.g., as provided in Tables 4.2 – 4.4) can help establish the potential range of removal. Documented performance from laboratory or field studies published in peer-reviewed literature carried out under similar conditions (e.g., temperature) to the MAR site may

further provide insight to help determine if default credits can be adjusted to reflect site specific conditions without additional study.
   b. In some cases, this step may be sufficient as a justification basis for a variance request. In these cases, it may not be necessary to proceed with Step 3.
3. **Source water characterization.**
   a. For some MAR systems, the reference pathogens, indicators, or surrogates selected by regulators may not be the most appropriate to demonstrate LRVs or conduct human health risk assessments. For example, given the source water quality, site specific above ground treatment process configuration, or hydrogeological conditions target organisms may not be present in high numbers. In these cases, a MAR operator may choose to select alternative pathogens, surrogates and indicators to demonstrate appropriately conservative LRVs.
   b. Screen source water for pathogens, surrogates and indicators for virus and oocyst pathogens.
   c. Consider use of metagenomics to identify highest concentration occurrences.
4. **Site characterization.**
   a. Characterize relevant conditions of MAR system for pathogen fate and transport (e.g., hydrogeological conditions, vadose vs. saturated flow, redox conditions, aquifer material, etc.).
   b. In case, the site-specific characterization is sufficient to select relevant and appropriately conservative LRVs from literature using the regulatory default reference pathogens, indicators or surrogates, this step may be sufficient as a justification basis for a variance request. In these cases, it may not be necessary to proceed with Step 5.
5. **Identify appropriate indicators and surrogates.**
   a. Select suite of indicators and surrogates from toolbox that are appropriate given the pre-recharge treatment process, source water quality, and MAR system characteristics.
   b. Note that surrogates may differ between lab tests and field monitoring.
   c. Document justification for indicator and surrogate selection for different types of pathogens.
   d. Determine log removals during pretreatment process through either a literature review (Step 2) or laboratory scale or field sampling (Step 6).
6. **Laboratory scale or *in situ* field experiments.**
   a. Assess published characteristics of index organisms. Assess the need for additional laboratory or pilot tests for verification or development of relevant fate parameters.
   b. Consider laboratory tests to simulate relevant model inputs under controlled conditions depending on MAR system characteristics, such as the following:
      i. Removal rates during initial infiltration zone.
      ii. Fate and transport under vadose or saturated conditions.
      iii. Temperature controlled decay / inactivation rates.
      iv. Adsorption and desorption coefficients.
   c. Consider *in situ* tests to quantify model input parameters under field conditions.
   d. Develop simplified models to simulate fate and transport at lab scale.

e. Laboratory scale or *in situ* field experiment results may be sufficient justification to determine site-specific LRVs for a variance request. In these cases, it may not be necessary to proceed with Step 7.

7. **Model set up and calibration.**
   a. Use modeling tools to assess the fate and transport of pathogens in the subsurface and appropriate residence times. Scale up the model and calibrate it to field-scale conditions based on information collected in Steps 2 through 5. Demonstrate through modeling and tracer tests the level of calibration accuracy and the level of accuracy of the model to simulate groundwater flow and pathogen fate and transport.
   b. Estimate parameters for tracer and surrogate field tests (estimated concentrations, flow paths, monitoring locations).
   c. Consider spike tests if background concentrations are too low.
8. **Model validation at field scale.**
   a. Evaluate / validate tracer tests and surrogates transport study with models to estimate preferred flow path, residence time, dilution, and possible heterogeneities.
   b. Assess degree to which full scale can be reliably modeled.
   c. Groundwater modeling results may be sufficient justification to determine site-specific LRVs for a variance request. In these cases, it may not be necessary to proceed with Step 9.
9. **Risk analysis.**
   a. Based on the results of Steps 1 through 7, identify vulnerabilities and critical control points.
   b. Consider system dynamics and all operational conditions.
   c. Conduct quantitative microbial risk assessment. Consider using probabilistic models.
10. **Risk management / Preventative and mitigation measures.**
    a. Define preventative measures to reduce pathogen load in MAR system, such as pretreatment reliability, monitoring, MAR system design improvements, etc.
    b. Define mitigation measures, including long-term routine monitoring and monitoring well location, and acceptable thresholds, post treatment, maximum system capacity limit, dilution, etc., to justify a site-specific variance request.

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