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Evaluation of Rapid Water Microbial Test Technology

Morgan Russell, M.S., CTR
Doug Lewis, PhD
Stephaney Shanks, PhD, CTR
Mike Brothers, PhD, CTR
Claire Butkus, M.S., CTR

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Air Force Research Laboratory
711 HPW/RH
2510 Fifth St., Bldg. 840
Wright-Patterson AFB, OH 45433-7913

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TAMERA G. BORCHARDT, Lt Col, NC	GUY R. MAJKOWSKI, Col, BSC
Branch Chief, Biomedical Impact of Flight	Division Chief, Warfighter Medical Optimization

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Mike Brothers, PhD, Claire Butkus, M.S.

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14. ABSTRACT
The United States (U.S.) Air Force Medical Service (AFMS), and largely the U.S. Army, lack the ability to rapidly detect bacteria in drinking water on-site, without incubators, and in austere environments. Recent Air Force guidance has identified the need for improved microbial detection capabilities in water. In garrison, U.S. Air Force Bioenvironmental Engineering (BE) and the tri-services conduct routine testing of installation drinking water in accordance with the Safe Drinking Water Act (SDWA) and the Revised Total Coliforms Rule (RTCR). These standards are based on the detection of fecal indicator bacteria (*Escherichia coli* and total coliforms) to determine the microbial quality of potable water and is represented as Colony Forming Units per 100 milliliter (CFU/100 mL). The U.S. Environmental Protection Agency has a Maximum Contaminant Level Goal (MCLG) for total coliforms of 0 CFU/100ml [5] as this concentration has been associated with protecting public health. Typical laboratory equipment required for these tests is not designed to operate under the environmental extremes of a military-forward operating environment. Quick and easy-to-interpret methods are needed to meet these standards and to better protect service members by providing results more rapidly. Military personnel are at risk of exposure to microbial contaminants until bacteriological test results are concluded [1] and standard methods require at least 18 hours to complete. Furthermore, future military campaigns will be a delicate balancing act of protecting military health, while operating in low resource environments with often limited available technology to identify microbial pathogens.

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1.0 SUMMARY

The United States (U.S.) Air Force Medical Service (AFMS), and largely the U.S. Army, lack the ability to rapidly detect bacteria in drinking water on-site, without incubators, and in austere environments. Recent Air Force guidance has identified the need for improved microbial detection capabilities in water. In garrison, U.S. Air Force Bioenvironmental Engineering (BE) and the tri-services conduct routine testing of installation drinking water in accordance with the Safe Drinking Water Act (SDWA) and the Revised Total Coliforms Rule (RTCR). These standards are based on the detection of fecal indicator bacteria (*Escherichia coli* and total coliforms) to determine the microbial quality of potable water and is represented as Colony Forming Units per 100 milliliter (CFU/100 mL). The U.S. Environmental Protection Agency has a Maximum Contaminant Level Goal (MCLG) for total coliforms of 0 CFU/100ml [5] as this concentration has been associated with protecting public health. Typical laboratory equipment required for these tests is not designed to operate under the environmental extremes of a military-forward operating environment. Quick and easy-to-interpret methods are needed to meet these standards and to better protect service members by providing results more rapidly. Military personnel are at risk of exposure to microbial contaminants until bacteriological test results are concluded [1] and standard methods require at least 18 hours to complete. Furthermore, future military campaigns will be a delicate balancing act of protecting military health, while operating in low resource environments with often limited available technology to identify microbial pathogens.

The goal of this report was to identify emerging microbial water testing technologies that may be able to meet these Air Force needs and to develop a roadmap for future Air Force guidance to meet this challenge. The approach included three methods of research: conducting a literature review, interviewing key stakeholders, and discussing collaborative opportunities with the U.S. Army. The literature review was separated into three core research areas: enzyme substrate reactions, engineered bacteriophages, and biosensor development. Key stakeholders included Air Force BE Personnel and Independent Duty Medical Technicians (IDMT) who conduct Force Health Protection activities and perform water quality monitoring both in garrison and in deployed locations.

Many technical challenges remain in developing final end-state water detection solutions capable of eliminating an incubator, meeting size requirements, detecting and quantifying indicator bacteria (i.e., total coliforms and *E. coli*) separately and determining the presence and viability of microbial pathogens. Cross-reactivity with non-target bacteria (leading to false positives) and sample matrix interference (leading to false negatives) are common deficiencies encountered with biosensor systems. Potential avenues for research and development aimed at addressing these challenges for Air Force requirements for advanced water quality monitoring capabilities are presented.

2.0 INTRODUCTION

Water sampling and testing is critical to the tri-services. Each branch of service conducts drinking water testing based on different needs and mission requirements. While in garrison, Air Force Instruction 48-144 *Drinking Water Surveillance Program* serves as the regulation for developing a drinking water Sampling, Analysis and Monitoring plan (SAM) for the protection of public health. For field water in deployed locations, AFMAN 48-138_IP, *Sanitary Control and Surveillance of Field Water Supplies* also serves as the tri-service field water standard. The Army identifies this document as TB MED 577, while the Navy and Marines use NAVMED P-5010-10. These regulations serve as the Military Field Water Standards (MFWS) and apply to land-based deployment operations. All branches of service have the same goal: “Ensuring the water produced, treated, and provided by U.S. Military Services and contactors in the operational environment is of the highest quality possible and that it will support and sustain the health and performance of deployed personnel”[3].

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The Air Force Science and Technology Strategy Vision for 2030 has recently identified the need for a change in strategic environment that will require a change in how the tri-services approach water quality assessments. This is a paradigm shift from the previous 20 years of facing non-state adversaries with limited technology to emerging nations equipped with state-of-the-art capabilities [2]. Large centralized bases will become smaller dispersed units for dynamic airbase operations that can be rapidly deployed. The results will present new challenges for future operations in a contested, degraded and operationally limited environment. Airmen will be forced to operate in an expeditionary environment in austere conditions while being subjected to a lack of supplies under limited communications, including lack of internet connectivity. There is an urgency for producing and optimizing technologies to reduce weight, size, power and personnel needs, while improving functionality in austere conditions. A top priority among these technological challenges is the ability to determine the safety of local drinking water supplies in order to eliminate the need for transporting large quantities of bottled water into a forward operating environment.

Drinking water sampling has been identified in the Research Development Document (RDD) for Expeditionary Medicine (EM-RDD) by the Air Combat Command Surgeon General (ACC/SG) [1]. The EM-RDD states the need for autonomous capabilities, including microbial water quality testing, with near real-time analysis. In the document, this is addressed as the ninth priority of 31 capability gaps with the title *Drinking Water Sampling* with the description stating: "The AFMS lacks the capability to test bacteria in drinking water on-site without incubators and at reduced time in an austere environment." The associated research objectives are to: 1) Reduce/eliminate the need for an incubation period; 2) Kit Properly sized to fit ADVON team equipment configuration; 3) Develop Technology capability similar to HAPSITE DCR; and 4) Determine viability of pathogens. These requirements present challenges that require a multidisciplinary approach with a foundational knowledge of engineering to include: systems, mechanical, biomedical, electrical, and chemical. In addition, microbiology, synthetic biology, and data science are all critical disciplines required for the success of this effort.

3.0 BACKGROUND

TB MED 577 states "Total coliform and *E. coli* testing may be performed using any EPA-approved testing method, including the membrane filter technique, Colilert® and Colisure®" (IDEXX Laboratories, Inc., Westbrook, Maine.)[3]. Currently, Air Force relies on the Colilert® Test Kit to determine the presence or absence of *E. coli* and coliforms in drinking water during routine water sampling. This requires the use of limited shelf-life reagents, an incubator (and associated electrical power source) and an 18-24 hours incubation period. Furthermore, special equipment is needed to distinguish between *E. coli* and coliforms. Contamination and cross-sensitivity may result in false positives or false negatives.

There are approximately 500 known waterborne pathogens capable of causing health concerns, including viruses, bacteria, protozoa, and fungi [4]. Given this broad cellular diversity, numerous challenges exist to identify, speciate and enumerate microbial indicators and pathogens in the environment. Examples include environmental interference, viable but non-cultural (VNBC) bacteria, including the persistence of chlorine-shocked cells in potable water samples, and sensitivity and specificity, and relevant limits of detection that may require pre-concentration or sensor signal amplification. Furthermore, water samples usually require tedious preparation and filtration steps to obtain sensitive and accurate results. While new and emerging technologies are rapidly evolving to meet these needs, no single technology to date is capable of achieving the Air Force requirements for water quality analysis in an expeditionary environment.

4.0 MATERIALS AND METHODS

4.1 Key Stakeholders

A meeting with three BE and one IDMT was held on 14-Sep-2018 to discuss the use-cases for a replacement drinking water technology. Both career fields conduct water quality testing of tap and/or bottled water using Colilert® 18. The DREL test kit, manufactured by Hach®, is used for additional water testing, such as pH, and free chlorine, that can be associated with drinking water treatment efficacy. One of the BE personnel had recently returned from Al-Udeid Air Base in Qatar, while the other from Al Dhafra Air Base in United Arab Emirates where they conducted water quality testing. Bottled water is procured locally and must be tested on a lot-by-lot basis with approximately 10% of each lot being tested for microbial contamination.

IDMTs test water quality in forward-operating locations where water buffalos are often used as the only potable water supply. An IDMT, who was also an Iraq War veteran, was also present at the stakeholder meeting. He was responsible for testing the water stored in water buffalos in Iraq, including levels of free chlorine. He discussed how he was instructed to add chlorine to the water without proper instruction on how to determine the appropriate amount to add to remove the microbial threat, while preventing an excess of chlorine in the drinking water supply. The training he received was "the more, the better." Excess chlorine can cause serious health issues beyond those posed by the presence of microbial contaminants; therefore, a strong need exists for a microbial water quality testing technologies to accurately assess drinking water quality in austere environments.

U.S. Army Presentative Medicine Specialists also conduct bacterial analysis of field drinking water. Given the similarities for the end-state of the deliverable, efforts were made to partner with U.S. Army Center for Environmental Health Research (USACEHR). USACEHR has extensive knowledge of available technologies for contaminated water detection and is familiar with the associated challenges and limitations. Similar to the USAF, the intent of USACEHR is on rapid detection (<8 hours) of viable fecal indicator bacteria with reduced or no cross-sensitivity to non-targeted bacteria. However, USACEHR prefers that the technology is capable of meeting U.S. EPA approval under the Alternative Test Procedure. Furthermore, there is greater emphasis on meeting EPA drinking water standards (i.e., RTCR MCLG). There is mutual interest in a joint Army/Navy/Air Force collaborative research effort for both short-term and long-term objectives; however, it is worth noting that there are differences between Air Force objectives and USACEHR.

4.2 Literature Review

Scholarly articles were searched and reviewed periodically from July 2018 to August 2019. Search databases included Web of Science, SpringerLink, and Scopus. Keyword searches included "Microbial detection", "drinking water", "bacteria", "rapid *E. coli* detection", "sensor", and "phage". Results were typically refined to the publications in the past 5 years with preference given to those with more citations. The literature review identified recent and improved technologies that may be worth additional consideration for use by the Air Force.

Primary research areas were divided into novel enzyme substrate reactions, biosensor development, and engineered bacteriophages. These technologies may be used in a variety of platforms and require a computing device, and data visualization. To meet portability needs, the use of smartphones with unique interfaces have become increasingly popular [4]. The technologies identified by the literature review and their attributes are provided in the following table (Table 1).

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Table 1. Literature Review and their Attributes

Detection	Portability	Conc.	LOD	Selectivity	Time to Detect	Power Req.	Consumables	Misc.	Ref.
portable NMR (pNMR) sensor	32 cm x 24 cm x 14 cm.	0.45 µm syringe filter and 30 min. incubation	76 CFU/mL	*E. coli* O157:H7	Prep time: 30 min Detect time: 1 min (0.5 hrs)	Yes	Antibodies with magnetic nanoparticles.	Estimated to require a fair amount of power but not stated.	[5]
electrochemical sensor -Nanotube based electrochemical sensor and nucleotide analysis		immunomagnetic bead separation to isolate active bacteria	3 cfu/100mL	*E. coli* +VBNC	2 hrs			Espira has developed a Biohazard Water Analyzer (BWA) for field-based rapid pathogen detection. https://www.sbir.gov/sbirsearch/detail/408193	[6]–[9]
Paper based ELISA			10,000 CFU/mL	*E. coli* O157:H7	3 hrs				[10]
T7 Bacteriophage-Conjugated Magnetic Probe (colorimetric change in presence of β-galactosidase)		T7 bacteriophage-conjugated magnetic beads were used to capture and separate *E. coli* BL21 from drinking water	10,000 CFU/mL	*E. coli* +VBNC	2.5 hrs		Phage magnetic beads		[11]
enzyme-based electrochemical method using T7 bacteriophage		Not used, however, could use filtration or immunomagnetic beads) and pre-enrichment steps	100,000 CFU/mL (1 CFU/100mL if preconcentration and enrichment steps used)	*E. coli* +VBNC	3 hrs		Phage	Differential pulse voltammetry (DPV) with Platinum electrode.	[12]
Smart Phone with colorimetric reactions	Yes but no details	Syringe Filter		*E. coli* Total Coliforms	.5 hrs			Mobile Water Kit (MWK)	[13]
impedance spectroscopy and resistance change (conjugated molecules designed with suitable moieties) not antibodies			10 CFU/mL Impedance 10^7 resistance change (max threshold)	*E. coli*	1-2 min		Sensing film, reagents		[14]
T4 bacteriophage to release β-galactosidase reaction on paper-based portable culture device. Colorimetric luminometer		0.45µm pore filter	10 CFU/mL	*E. coli*	5.5 hrs		Paper assay Bioluminescence assay	bioluminescent β-galactosidase	[15]

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Detection	Portability	Conc.	LOD	Selectivity	Time to Detect	Power Req.	Consumables	Misc.	Ref.
MUG Substrate hydrolyzed by *E. coli* to yield fluorogenic (4-MU) product.			10,000 CFU/mL	*E. coli*	3 hrs		Fluorescence Assay		[16]
Antibody coated microbeads for agglutination assay- Scattered light captured for quantification using iPhone	Yes	40 µm cell strainer and Amicon Centrifugal filter pore size 30nm.	10 *E. coli* O157:H7 cells/10 mL	*E. coli*	10 min		Microfluidic Chip, membrane filtration	iPhone CMOS image camera. Requires centrifuge	[17]
T7 Phage genetically engineered to express luciferase (NanoLuc)		Cellulose membrane	10 CFU/mL	*E. coli*	3 hrs		Filter Plate	Cellulose captures NanoLuc and then is centrifuged or separated by gravity prior to detection.	[18]
X-Gluc and REG chromogenic enzymatic substrates using MetGlu as an inducer		0.45-µm pore size filter with	Presence/Absence	*E. coli*	2 hrs			Requires Incubation	[19]
Graphene based field effect transistor device		*E. coli* antibodies on gold nanoparticles	Presence/Absence	*E. coli*					
Tryptophan-like Fluorescence spectrophotometry	Yes	None		Thermotolerant (faecal) coliforms (TTCs).	Instant		LEDs from Fluorimters	Potentially VBNC Capable	[20]
LED fluorimeter & Raman & Flow cytometry	425 (l) × 300 (w) × 225 (d)mm 3.5 kg			Detects surrogates for organic and microbial matter.					[21]
Lateral flow immunoassays (LFIA)		Gold nanoparticles	1 cfu.ml *E. coli* with enrichment 10^7 cfu/ml–10^4 cfu/ml Salmonella	Salmonella, *E. coli*					[22]
UV LED Fluorescence sensors for online monitoring of dissolved organic matter- fluorescence excitationeemission matrix (EEM)	Yes	None	Protein-like fluorescence and humic-like fluorescence.	protein-like biopolymers	Instant		LEDs		[23]

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Detection	Portability	Conc.	LOD	Selectivity	Time to Detect	Power Req.	Consumables	Misc.	Ref.
Portable fluorimeter		None	>100 CFU/100 mL					portable fluorimeter as an initial screening tool for indicative microbial water quality	[24]
Quantiphage Assay	Yes	No	1PFU/10mL 1PFU/mL	enabled cultured based detection of somatic and F+ coliphages after 2 and 3 h	2.5-4 hours			Still requires a portable incubator to activate bacterial cells (40 min).	[25]
Bluephage: A rapid method for the detection of somatic coliphages used as indicators of fecal pollution in water	Yes	No	1 somatic phage	Somatic coliphage	3.5-5.5 hours			MPN and Presence Absence Testing	[26]
Modified Bluephage Method that uses *E. coli* strain CB12 to detect both somatic and F-specific coliphages	Yes	No	10PFU/well	Somatic coliphage and F-specific RNA coliphage	3.5 hours			MPN and Presence Absence Testing	[27]
Automated bacterial concentration and recovery system (ABCRS)	No			*E. coli*				Uses two peristaltic pumps for enrichment.	[28]
Innova Prep Concentrating Pipette									[29]
In-line digital holography microscopy (DHM)				Various				TRL-5 Requires microbial library.	Rapid Innovation Fund-TDA Research Inc.
fluorescence reader and en masse pathogen capture technology	Yes	Coated Filters		Various	1 minute	Battery- 17 hours	Coated filters for Pathogen detection	TRL-6	Rapid Innovation Fund- Sonoran Analytical

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Novel Enzyme Substrate Reactions

Coliforms and *E. coli* contain enzymes including beta-galactosidase and beta-D-glucuronidase that catalyze reactions during metabolic activity (in actively growing cells) that can be harnessed for detection of these cells in water samples. The products of these enzymes can be detected through colorimetric change, electrochemical response or fluoresce under ultraviolet (UV)-light [11], [30]–[32]. These substrates could provide a low-cost, portable, and rapid method for identifying coliforms and *E. coli* in water, but the speed of enzymatic reactions must be increased and incubation periods reduced. New substrates are being examined and optimized to address this gap [19]. Recently, Wu, et al. examined the substrates 5-Bromo-4-chloro-3-indolyl β-D-glucuronide (X-Gluc) and Resorufin β-D-glucuronide (REG) for rapid detection of *E. coli*. Additionally, alterations to incubation temperature and pH were included to further enhance the speed of the enzymatic reactions for *E. coli* detection in 1-7 h; however, these experiments were performed under ideal laboratory conditions and did not include coliform detection. Future tests may focus on accelerating enzyme productions and faster growth of *E. coli*. Additional research is needed to confirm results in real-world environmental samples. Despite the potential benefits of these techniques, the requirement of an incubator and the time required for substrate reactions remain two primary concerns.

Engineered Bacteriophages

Alternative techniques for detection of *E. coli* and coliform in water rely on modified bacteriophages, specifically coliphage (also known as phage) [11], [15], [31], [33]. Bacteriophage, like all viruses, have a narrow range of host specificity, which can be exploited for detection systems by reducing the potential for false positive detection. For ambient waters, coliphages are being considered by the EPA as possible indicators of fecal contamination [34]. Phage-based assays show promise in rapid detection of *E. coli* for presence/absence tests and quantitative analysis. Phages are natural predators to bacteria and modify the host genome for production of daughter virions upon completion of the lytic cycle, which can occur in as little as a few minutes. Unfortunately, current detection methods for these indicator phages can take hours.

Recent efforts to develop detection schemes for microbial contamination of water utilizing the phage lytic cycle include a study by Hinckley et al. They used T7 coliphage [18] to detect < 10 CFU/mL of *E. coli* in 3 hours. T7 coliphage were genetically modified to produce the enzyme luciferase (NLuc). Once infected by the T7 phage, *E. coli* expressed NLuc for subsequent detection of the luminescent signals. Other approaches have relied on colorimetric reactions following cell lysis. Muniesa et al. used a genetically modified phage to induce host overexpression of the β-glucuronidase [26]. Once the host cell has been lysed, a color change can be observed following enzyme reaction with the substrate in the growth medium.

Other researchers have begun exploring ways to expand the host range of certain phages, either in a cocktail or genetically modified together. Exploiting the presence of multiple *E. coli* serotypes present in seawater and sewage influent in Osaka, Japan, Namura et al. exposed these bacteria to a cocktail of two modified phage candidates, IP008 and IP052 [35]. The phage *gene e* was tagged with green fluorescent protein (GFP) to suppress lytic activity. Additional GFP was fused to the phage small outer capsid (SOC) proteins to increase visibility of the infected bacteria

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under the microscope through increased fluorescence. The phage cocktail detected a broader range of *E. coli* serotypes than either phage alone. Mahichi et al. modifiedT2 and IP008 to enhance detection of *E. coli* [36]. They combined the site-specific recombination of the T2 phage tail fiber genes (genes 37 and 38) with the long tail fiber genes of IP008. Despite the opportunities for rapid detection of microbial contaminants provided by phage, their narrow host specificity requires can present challenges for multiplexed detection (i.e., simultaneous detection of indicator species and pathogens). Additionally, incubators are still required to catalyze bacterial host cell growth and the virus/host cell interaction.

Biosensors and Bioassays

Bioassays and biosensors typically rely on the manipulation of a key biomolecule in order to generate signals that can be read by standard optical and/or electrochemical readers. The problem is more difficult when the desired limit of detection is lower, as the assay must process more sample. In order to come within orders of magnitude of the EPA limit of 1 CFU/100 mL of drinking water, pre-concentration of microbes is essential to enable bioassays within the limits of detection.

Real-time detection systems, while ideal, are not necessary for these platforms, as there is an inherent lag-time built-in to the sample preparation. Additionally, current real-time electrochemical and optical detectors are underdeveloped for microbial/viral contamination, have limits of detection substantially higher than other assay techniques, and/or are currently in form-factors that are not amenable to deployment in austere conditions. Therefore, point-of-care (POC) assays are preferred and can be packaged into transportable platforms for deployment. The two most common POC assays used commercially are immunosorbent assays and molecular diagnostic assays due to their ability to amplify signals using molecular techniques.

Molecular diagnostics rely on lysis of the pathogen to provide access to the genome/transcriptome. DNA primers then hybridize to specific sites on the bacteria or viral genome/transcriptome and replicate the target gene area using polymerase chain reaction that can then be detected, typically via fluorimetry or other optical means. Both platforms benefit greatly from sample pre-concentration to overcome inherent background noise and improve LOD. Commercially available molecular diagnostic techniques, such as the BioFire, use a microfluidic pouch and comprehensive electronics to perform the following: 1) Isolation of the virus/bacteria from the media, 2) Transfer into optimal media, 3) PCR of relevant genes, and 4) Detection. While this technique can detect down to $1x10^3$ pathogens**,** the technique requires 1 hour total to complete and costs ~$125 per sample for the consumable diagnostic, as well as a cost for the device, as well as the power-supply to run the device [37]. Isothermal amplification holds substantial promise to reduce the need for power due to reducing thermal cycling [38]**.** However, widespread adaptation has yet to occur, as well as the same time lag compared to traditional PCR [39]**.** Additionally, identifying a sufficient number of target DNA sequences to provide broad spectrum analysis of bacteria as well as multiplexing increases both the complexity and the demands on the assay.

The time to generate a result as well as the complexity of the machinery to perform molecular diagnostics has led to immunosorbent assays being more commonplace in point-of-care settings, most commonly the pregnancy assay that detects the protein human chorionic gonadotropin in as short as three minutes. Immunosorbent assays rely on capture and measurement of a specific target already present without further amplification of the analyte, but

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typically rely on amplification of reporter molecules. Standard targets for pathogen detection can range from nucleoproteins that are found in high abundance, to membrane proteins or lipopolysaccharides that can immobilize whole bacteria. Immunosorbent assays can be performed both on whole-cells/viruses [40] or on lysed cells/viruses, measuring proteins that are found in much quantity/concentration such as nuceloproteins [41]; the tradeoff being that extra preparation steps are needed to measure the lysate. Immunosorbent assays used for proteins, bacteria, viruses, and other larger particles useful for water pathogen detection rely on 1) immobilization of a biorecognition element (BRE) on a surface, 2) capture of an antigen by the BRE, 3) immobilization of a BRE containing a reporter tag on a bioorthoganal site, and 4) amplification and reading of the reporter. This is most often contained with the confines of a piece of paper/nitrocellulose known as a lateral flow assay.

Lateral flow assays, most identifiable by the ubiquitous pregnancy test, use paper microfluidics to run immunosorbent assays with reagents deposited on strips perpendicular to flow to create a mini bioreactor within the paper-fluidic device. Lateral flow assays have been demonstrated to detect as low as $1x10^3$ CFU/mL [42] depending on the virus and the concentration of nucleoproteins. However, depending on the matrix, substantial sample work-up is required initially. Additionally, since all reagents are immobilized and solubilized within the assay, no mixing or other steps are possible to further enhance sensitivity and/or improve analyte capture.

Therefore, in order to perform more complex assays, to multiplex against multiple antigens, and/or to automate sample preparation, lab-on-a-cd has been demonstrated, where the assay can be controlled by moving fluid through the device and causing mixing through controlling the speed of a compact disc [43]**.** In short, the speed of the disc produces centrifugal force sufficient to move fluid through hydrophobic burst valves in a staged fashion, enabling precise control. Rapid mixing is also enabled through reversing the centrifugal force applied, creating substantial turbulence to increase molecular collisions. This has caused the reduction of enzyme-linked immunosorbent assays from 2 hours down to 41 minutes, where 75% of the process time was for antigen capture [44]**.** Further biochemical optimization has promise to bring the time down further.

Finally, novel technologies are constantly arising using biological systems. Most notably, luciferase reporter genes in b-cells specific for antigens have been demonstrated to be sensitive down to 50 pathogen particles [45]. However, the batch to batch reproducibility as well as storage conditions necessary to field the b-cells limits their utility in austere environments. Further development should be monitored and the viability of deployment in austere conditions should be scrutinized heavily.

In summary, lab on a chip/lab on a CD type assays provide the greatest promise for rapid, highly selective and sensitive detection, either using isothermal PCR or adapted immunosorbent assays. R and D efforts into the miniaturization and optimization of both speed and sensitivity of these techniques provides the most likely path forward in the foreseeable future.

5.0 RESULTS/DISCUSSION

Currently there are no single technology that can address all the requirements desired by the USAF. Literature review results are summarized into the five most promising technologies below. Technologies listed were intentionally diversified based on scientific principles of operations.

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1. “Reporter bacteriophage T7 NLC utilizes a novel NanoLuc::CBM fusion for the ultrasensitive detection of *Escherichia coli* in water”
    - Uses T7 engineered phages as lab on a filter technology to quantify *E. coli*. using colorimetric or bioluminescent signals. Future work could investigate applying a mix of phages other to additional pathogenic bacteria.[46]
2. “New approach for the simultaneous detection of somatic coliphages and F-specific RNA coliphages as indicators of fecal pollution”
    - Uses the simultaneous rapid detection of both somatic and F-Specific RNA coliphages in under 3.5 hours. Results are interpreted by a colorimetric change for presence absence or by using MPN.[27]
3. “Portable LED fluorescence instrumentation for the rapid assessment of potable water quality”
    - Previous research has indicated there is a relationship between the fluorescence of dissolved organic matter and microbial water quality. Organic carbon (Peak C) and Microbial activity (Peak T) for tryptophan-like fluorescence can be measured using a portable and rugged fluorometer. Combining this technology with advancements in analytics, portable computational power, and other technologies may provide additional research opportunities. [21]
4. “Rapid Waterborne Pathogen Detection with Mobile Electronics”
    - Uses membrane filtration for pre-enrichment followed by an immunoagglutination reaction for quantitative detection for specific pathogen detection. This is combined with a microfluidic device for detection using a smartphone. [17]
5. “Detection of Escherichia coli in Drinking Water Using T7 Bacteriophage-Conjugated Magnetic Probe”
    - T7 bacteriophage conjugated to magnetic beads are used to target and concentrate bacteria (*E. coli*). Phage lyses and releases B-gal to be detected using colorimetric assay.[47]
6. “Detection of Escherichia coli with Fluorescent Labeled Phages That Have a Broad Host Range to *E. coli* in Sewage Water”
    - IP008 and IP052 sewage influent isolates when tagged with GFP and utilized in a cocktail together have the ability to detect a wider host of *E. coli* species.
7. “Site-specific recombination of T2 phage using IP008 long tail fiber gene provides a targeted method for expanding host range while retaining lytic activity”
    - The genetic recombination of two phages, T2 and IP008, increased the host range exponentially.

Other Noteworthy Technologies:

Innova Prep Concentrating Pipette.

- A portable and user-friendly device for concentrating bacteria, viruses, spores and parasites from environmental samples. Could be applied to other technologies for

rapid pathogen detection. (InnovaPrep Concentrating Pipette Select, www.Innovaprep.com)

"Development of a low-cost paper-based ELISA method for rapid Escherichia coli O157:H7 detection"

- Uses filter paper with pretreated chitosan and glutaraldehyde incubated with *E. coli* O157:H7 antibodies. Results were determined with a smart phone but detection limit is $1x10^4$ CFU/mL. [10]

6.0 CONCLUSIONS

This report concludes that there is no single technology capable of explicitly meeting the Air Force requirements. From Section 5, seven technologies were chosen to be monitored over the next two to five years to determine if there are additional innovations. Big data sources are increasingly available and can be processed through portable methods. Processing power is continuing to increase significantly with smaller and more robust chip sets. By combining this with data analytics, machine learning, and the rapidly evolving artificial intelligence this could develop a water testing capability meeting USAF needs.

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ABBREVIATIONS

ACC/SG	Air Combat Command Surgeon General
AFMS	Air Force Medical Service
BE	Bioenvironmental Engineering
BRE	Biorecognition element
CFU	Colony Forming Units
EM	Expeditionary Medicine
GFP	green fluorescent protein
IDMT	Independent Duty Medical Technicians
MCLG	Maximum Contaminant Level Goal
MFWS	Military Field Water Standards
POC	Point-of-care
RDD	Research Development Document
REG	Resorufin β-D-glucuronide
RTCR	Revised Total Coliforms RulR
SDWA	Safe Drinking Water Act
SAM	Sampling, Analysis and Monitoring
SOC	Small outer capsid
U.S.	United States
USACEHR	U.S. Army Center for Environmental Health Research
UV	Ultraviolet
VNBC	Viable but non-cultural
C-Gluc	5-Bromo-4-chloro-3-indolyl β-D-glucuronide