Far-Forward Diagnostics in Toxic Industrial Chemical and Material Exposure Scenarios and Biomarker Identification

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This study describes key technical solutions for detecting environmental toxicants and diagnosing adverse health effects in military operational settings as outlined at a symposium cosponsored by the Department of Defense and the Johns Hopkins University-Applied Physics Laboratory (October 27 to 28, 2015). Such technologies are urgently needed in order to provide critical decision-aid tools and prognostic assessment of potential clinical sequelae. This review summarizes the state-of-the-science on (1) prioritization of adverse health effects, (2) existing technologies and diagnostic tools available for use in theater, (3) challenges to advancing diagnostic tools far-forward, and (4) the potential utility of anchoring diagnostic tools to adverse outcome pathways. Emerging technologies are increasingly available for physiological, environmental, and individual exposure monitoring. Challenges to overcome in austere environments include cold chain requirements and determination of adequate sampling intervals.

Global population estimates have identified 20 megacities in the world today. The number of these cities estimated to contain more than 10 million inhabitants is predicted to grow to 41 by 2030. Many of these cities are in less developed regions where rapid and unplanned growth could threaten economic, environmental, and political infrastructures. Political instability leading to military conflicts may involve US troop deployments.[1,2] The megacity operational environment represents unique challenges to the current and future operating force. Military medicine will have to address these challenges with improved far-forward diagnostic and treatment options. The US medical directorates must be prepared to rapidly screen and assess Service Members for adverse health effects in areas of increased risk of exposure to industrial toxicants, pollutants, chemicals, and materials. In order to address these challenges, the United States Army Center for Environmental Health Research (USACEHR) of the Department of Defense (DoD) cosponsored a 2-day symposium with the Johns Hopkins University Applied Physics Laboratory (JHU-APL) on October 27 to 28, 2015. The second session in the symposium brought together key military stakeholders and experts in technology development to identify the current state-of-the-science for far-forward diagnostics in nonagent toxic industrial chemical and toxic industrial material (TIC/TIM) operational environments.

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HISTORY OF BIOMONITORING EFFORTS IN THE US MILITARY

Beginning in 2000 to 2002, the Institute of Medicine and the Joint Chiefs of Staff issued a report recommending the development of a serum repository for biobanking biological specimens from deployed personnel. The rationale for the serum repository was to improve biomonitoring of both exposures and health outcome during the deployment life cycle.[3,4] Recent supplements to the *Journal of Occupational and Environmental Medicine* and *Military Medicine* summarize the latest proposed advances recommended for the serum repository, as well as data and performance gaps relating to specimen identification, tracking, and correlation to biomonitoring and environmental monitoring. Pre- and postexposure assessments are complicated by uncertainties in exposure parameters, including time from exposure to blood draw.[5,6] As logistical concerns are addressed, the serum repository will continue to be a valuable asset in patient monitoring.

Current exposure assessments are based on dosimetry quantifying exposure to known environmental chemical threats. The US Army Public Health Center assets monitor air pollution and other environmental exposures onsite (eg, soil and water specimens). Health risk assessments are based on literature review and integration of safety factors to determine organs at most risk and to set safe exposure limits for specific biological consequences.[7] However, some exposures are difficult to track and document, especially prolonged exposure to low levels of chemicals. These chemical hazards can significantly impair operational readiness. Further, standard monitoring is not feasible in some operational environments (eg, some hostile or in-theater operations).[8]

In response to credible threats (eg, measurements exceeding exposure standards and/or questionable effectiveness of preventive measures), a decision-tree approach could facilitate decisions to use biomarkers of exposure and/or effect to document exposures and associated health consequences. Historical examples of biomonitoring and environmental exposure monitoring conducted in deployed settings include whole blood chromium monitoring, elevated lead levels in air sampling, biological surveillance initiative (eg, metals, volatile organic compounds, sister chromatid exchanges plus physiological tests), serum dioxin, and testing for chemical warfare agents in the environment or blood.[8,9] For biomonitoring to be most strategically beneficial, biomarkers must be validated and there must be suitable documentation and archiving procedures and concepts of operations in place available for assessing exposures and making operational responses to them.[5]

There are currently limited capabilities for far-forward diagnostics that meet regulatory standards of development and validation. To fill this capability gap will require overcoming technical challenges related to testing in austere or hostile operational environments and expanding biomarkers of exposure to determine likely adverse health effects after exposure scenarios occur.

TECHNOLOGIES FOR FAR-FORWARD DIAGNOSTIC DEVELOPMENT

Unique technological challenges limit the utility of many prototype diagnostics for use in a far-forward operational setting. A review of the current and emerging diagnostic device literature

TABLE 1. Current and Emerging Technologies for Point-of-Care Diagnostics

Examples of Current Capabilities[10]	Format	Applications
POC glucose assays	Electrochemical (enzymatic oxidation of glucose)	Diabetes management
Lateral flow assays	Lateral flow immunochromatographic assay	Pregnancy and ovulation confirmation, screening (infectious disease, drugs of abuse), clinical diagnosis of heart attack, deep-vein thrombosis, stroke
iSTAT handheld[11]	Measures electrolytes, other markers, and blood gases	Diagnostics in whole blood
Examples of Emerging Capabilities	**Format**	**Applications**
Microfluidics devices (lab-on-a-chip)[12]	Glass, paper (nitrocellulose or cellulose), silicon, polycarbonate, plastics (cyclic olefin copolymers or polystyrene)	Microarray (microRNA, mRNA)
		Optofluidic (eg, microscopic optical imaging, fluorescence detection)
		Immunosensor (eg, pesticide, bacteria, food pathogen detection)
		enzymatic sensor (eg, glucose, urea)
		noninvasive sensor (eg, glucose)
Nanotechnology[13]	Gold nanoparticles	Diagnostics; detection of low-concentration biological molecules
	Magnetic nanoparticles	Fluorescence and magnetic resonance imaging techniques
	Quantum dots	Imaging and detection
	Carbon nanotubes	Detection, monitoring, therapy of diseases; enhanced biological action of drugs and reduced toxicity
	Liposomes	Imaging and drug delivery
	Flodots	Detection, diagnostics, bioanalysis
	Dendrimers	MRI/NIR contrasting agents and cancer therapies evaluated by flow cytometry and confocal microscopy
Next-generation iSTAT[11,14]	EncompassMDx system	Fully automated PCR reaction
	rHEALTH (nanostrip fluorescence technology)	Multiplexing tests on a single drop of blood (electrolyte, marker, blood gases)
	AgPlus (compatible with silver nanoparticle test system)	Different samples types (blood, urine, saliva)
	Mobile-linked systems (eg, Scanadu; Cue)	Physical sampler linked to user's smartphone; results delivered by mobile application
	BioFire	Detects biological warfare agents to guide treatment decisions in military situations
Rapid molecular detection of bacteria[15]	PCR-based amplification (eg, Epistem)	Detection of *Mycobacterium tuberculosis* complex from sputum in tuberculosis patients

EncompassMDx, trade name technology for an automated, modular, molecular analytical system for performing a range of molecular tests on multiple sample types; iSTAT, trade name of the iSTAT blood analyzer; MRI/NIR, magnetic resonance imaging/near-infrared spectroscopy; PCR, polymerase chain reaction; POC, point-of-care; rHEALTH, reusable Handheld Electrolyte and Lab Technology for Humans; RNA, ribonucleic acid.[10–15]

identified that current technologies are being replaced by microfluidics, nanotechnology, and other point-of-care diagnostics as miniaturization facilitates bench-to-bedside implementation (Table 1).[10–15] Frequently portable and disposable, these designs may be amenable to military field settings[16,17] (Fig. 1). Further research, clinical trials, and validation are needed to advance the devices beyond the prototype stage.[18] Ruggedization and miniaturization of laboratory prototypes are significant challenges in fielding these devices[19] (Fig. 1).

BIOMARKER DEVELOPMENT AND VALIDATION

Most biomarkers are in the development stage, and few pass the rigorous criteria for utility in a far-forward diagnostic setting. Recent publications by the Joint Chiefs of Staff emphasize operational and mission readiness in terms of human performance.[6] Biomarkers useful in a soldier's life cycle range from pre-mission susceptibilities to deployment-related health effects, and extend into post-deployment epidemiological surveillance. Biomarkers indicative of adverse effects on performance readiness include disease or injury after exposure to an unknown milieu of chemicals and toxicants, biological warfare agents, chemical warfare agents, and nonagent TIC/TIMs. Performance parameters include fatigue, vigilance, and stress, all of which could be adversely affected by xposure to environmental hazards and chemicals.[20] Pre-mission performance parameters such as chemical or material susceptibility could inform duty decisions and evaluations of deployment readiness.

Recent studies in DoD laboratories have indicated that panels of biomarkers can be more effective than single markers in allowing for early, pre-clinical detection of low-level exposure and real-time monitoring in hazardous environments. Many DoD laboratories have invested heavily in multiomics strategies to characterize risk and design biomarker panels and assays.[21–24] Biomarker discovery and development involves integrated systems biology approaches, bioinformatics data analysis pipelines, assay development, and transition to the field. For example, multiomics assessment has been used to identify panels of biomarkers indicating subclinical toxic kidney injury.[25,26]

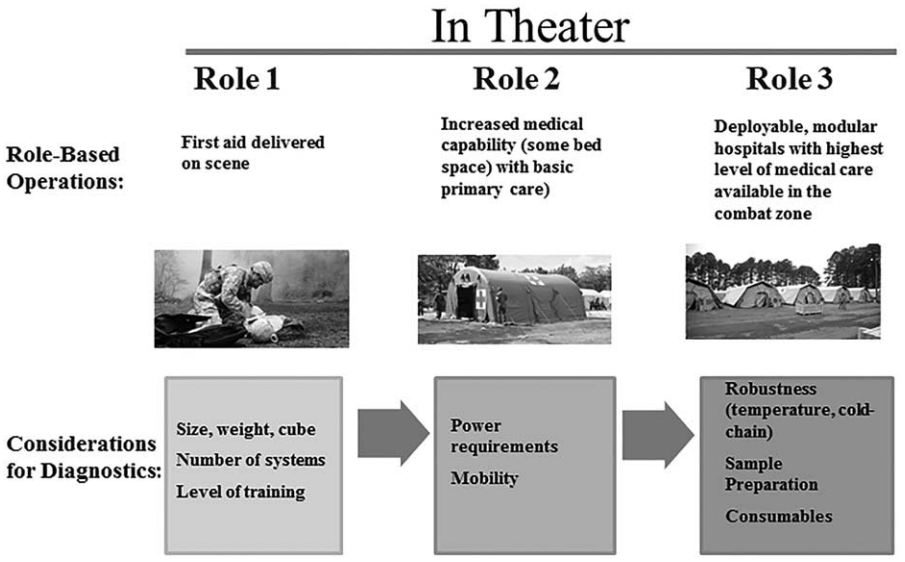

FIGURE 1. Considerations for current and emerging biomedical diagnostics in the Department of Defense (DoD) Medical Concept of Operations (CONOPS) describing role-based care (FM 4-02-283).[17]

The ability to detect the presence of chemical or biological hazards affecting Service Member health in far-forward field environments is currently limited by a number of factors, including our limited ability to 1) miniaturize bench top, laboratory-based systems; 2) automate manual laboratory methods so that they can be operated with limited training in austere environments; 3) eliminate or lessen logistic burdens, especially those needed in the case of disposable reagents, cartridges, tubes, etc.; and 4) develop systems with small utility footprints such as the need for water and power. Advances in electronics,[15] microfluidics,[13,27] nano-technologies,[10] and other fields of engineering have increased the feasibility of integrating advanced diagnostic technologies into portable platforms (Table 1). Over the past few years, the movement toward bedside diagnostic platforms[11,12,28] and at-home, do-it-yourself testing[29] fueled a belief that the smaller, lighter systems developed for these purposes could be deployed directly into far-forward field environments; however, that potential remains only partially realized because bedside diagnostic technologies are still targeted to medical settings where profit margins are high, large numbers of tests are run by experienced staff, and where size, power, and logistics issues are not as important. Therefore, while the push to bedside diagnostics has led to the development of systems that meet some of the requirements of a far-forward device, these systems still lack many critical capabilities necessary to operate in a far-forward field environment (Fig. 1). For example, small, portable thermal cyclers for patient bedside diagnosis of disease are now available.[14] These systems have addressed size and power requirements, but the systems still require a reagent cold chain, manual sample preparation, experts to interpret final results, take roughly an hour or more to yield a final result, and they have achieved their smaller footprint at the expense of sample throughput.

Although a number of systems are currently under development that might target one or two of these issues, no developmental system currently addresses all the issues required to field systems far-forward in resource-limited environments. Without significant financial incentives, it appears unlikely that large companies with proven technologies will be willing to take those final steps needed to convert their patient bedside diagnostics into truly fieldable devices.[30]

Medical laboratory methods have historically been based on the presence of a patient with symptoms to provide a primary diagnosis with laboratory-based diagnostics providing confirmation. Therefore, most, if not all, biological and chemical diagnostic methods have been developed for the medical laboratory setting wherein diagnostic systems target very specific markers of acute disease.[31] Due to the required specificity of these systems, detection of many different compounds in a single system requires that the system be capable of running tens, hundreds, or even thousands of high complexity assays at the same time, including appropriate standards and controls for each assay. Attempting to adapt present-day chemical and biological diagnostic approaches to far-forward applications might, on the surface, seem natural, but it is not clear whether or not our current approaches are truly the best suited for transitioning to field-based detection systems, especially for low-level, subclinical exposures. An alternative to simply shrinking current large, complex, difficult-to-operate laboratory-based detection systems could be developing entirely new approaches, or delivering current methods in completely new formats.[32]

In the case of field-forward all-hazard detection, there is no detectable disease at the time of exposure. Techniques for development need to be broad-based for initial detection and capable of detecting initial low-level exposures. Follow-up procedures would include more specific identification methods as needed to identify a particular compound of cause of future disease. Because they are so specific, approaches for detection of many different compounds require high complexity assays where hundreds or thousands of different toxicants might need to be detected simultaneously.[33] It may be preferable to develop new methods or approaches specifically tailored toward field-forward detection of exposure scenarios, as opposed to adapting or miniaturizing current laboratory-based approaches.

Physiological Monitoring

Developing a broad-based diagnostic detection platform has been proposed to monitor soldiers in the field for physiological responses triggered by initial exposure.[34] This approach involves building very small, portable, wearable sensors that could integrate directly onto the soldier's body or clothing to measure changes. Wearable devices can be broadly divided into two main categories: 1) monitors that can be worn on, near, or even inside the body and utilize a variety of different sensing strategies to detect physiological changes, such as body temperature, heart rate, blood pressure, oxygen level, gait, exhaled breath, and others, and 2) sensors for identifying and quantitating biomarkers of stress, exposure, or disease by actively measuring changes in the chemical makeup of human body fluids such as blood, sweat, saliva, urine, and interstitial fluid.[35] Driven by the sports and electronics industries, there have been huge technical leaps forward in the development of physiological sensors over the past few years with many sports

clothing manufacturers integrating sensors directly into their products.[36] In addition, a large health industry has developed to manufacture sensor systems that are worn on the wrist and, through a computer interface, report a variety of health-related data sets to the user.[37] This industry will continue to rapidly expand, but data sets from large populations are just now being analyzed to determine whether or not these physiological changes can be used to identify individuals or populations that have been subjected to low-level stresses. Wearable devices for the detection of biomarkers remain relatively underdeveloped largely due to the fact that few useful markers of disease or exposure have been identified, although a few prototypes have been developed.[30]

Paper-Based Networks Evolving From Chip-Based Approaches

A third approach involves adapting cell-based diagnostic systems to chip-based approaches in cell-free systems. Synthetic cell-based systems represent robust, accurate, and reproducible tools for medical diagnostics, but the translation capability has been limited by challenges of storing and maintaining cellular systems, especially in austere military operational environments. Reconstructing gene networks in vitro in cell-free systems could overcome these hurdles in theater. Recent developments have moved the clinical utility of synthetic systems closer to reality by demonstrating how paper can be used to manipulate gene circuits using freeze-drying strategies, thus obviating the need for freeze-thawing cycles, temperature controls, and technical hurdles surrounding sample preparation.[38,39] Freeze-drying strategies have been implemented that maintain enzyme activity necessary for transcription and translation in synthetic, paper-based gene-networks. Entire inducible expression systems could be reconstituted on the paper-based platform using freeze-dried discs. This strategy uses Adobe Illustrator to generate the patterns for printed arrays, and then prints the arrays directly onto chromatography paper with a standard Xerox printer. The dehydrated discs are stable for more than a year at room temperature, and can be rehydrated and evaluated by colorimetric changes observable by semi-quantitatively evaluating fluorescent or bright-field (colorimetric) images. Paper and quartz microfiber discs could be constructed with multiple toe-hold switches for independent activation and measurement of complex gene networks in cell-free systems.[38] These toehold switch mRNA sensors have distinct advantages over current antibody-based techniques in terms of development time and cost. Whereas Ebola sensors were constructed on paper in under 12 hours at $21/sensor, standard antibody-based diagnostics require between 2 and 6 months for development at a cost ranging from $4000 to $30,000.[38] As paper-based gene circuits continue to progress, these options will be valuable diagnostic tools in far-forward, rugged environments encountered by the US military.

Adverse Outcome Pathways

Once validated, biomarkers must show utility in medical decision trees and provide actionable information to care providers. Currently, there are very few treatments available in the field for TIC/TIM exposures. Exposures of unknown etiology are common, and treatment in these cases is usually supportive.[9] Comprehensive medical surveillance requires personal and environmental monitoring for biomarkers of exposure and effect leading to a matrix of risk assessment, mitigation, and medical follow-up.[40] One of the most essential needs is to identify patterns of injury that predict an actionable medical response based on objective reference ranges and thresholding criteria. Thus, it is essential to identify the top prioritized health effects requiring far-forward diagnostics in the megacity operational environment, preferably linked to a mechanistic development of the pathology in question [eg, adverse outcome pathways (AOPs)]. Effect-based diagnostics are an attractive complement to exposure-based diagnostics. Biomarkers of effect approximate end-organ pathology based on a select limited number of toxidromes (ie, a syndrome or series of symptoms suggestive of a specific toxic response) rather than relying on the logistically impractical number of exposure-based assays.

AOPs are gaining traction in the regulatory arena, as the US Environmental Protection Agency develops a framework to describe pathogenesis in terms of disruption of discrete biological pathways.[41,42] The most complete AOPs can make quantitative, predictive determinations about key events leading to pathologies. These models have the least uncertainty and therefore the most likelihood of regulatory usefulness. In Europe, the skin sensitization AOP was among the first to gain regulatory acceptance given the availability of quantitative predictive models.[43]

AOPs are chemically agnostic, modular devices for linking physiological effects with measurable biomarkers.[44] In the AOP framework, an exposure leads to a molecular initiating event, which triggers a cascade of molecular signaling pathways to lead to the pathology in question.[42,45–47] High-throughput in vitro assays can identify pure ligands as functional units of prediction for specific key events causally linked in the pathway. For example, in fish, inhibition of the enzyme aromatase leads to a reduction in estrogen synthesis, cascading to an inhibition in vitellogenin production in hepatocytes. Vitellogenin deficiencies are causally linked to a reduction in fertility by causing impairment in oocyte development and ovulation at the single animal level. Reduced fertility can be extrapolated to the population level to infer a population trajectory in a given region.[48] The linear description of AOPs is a good theoretical construct, but in practice AOPs interlink in networks. Key events can be shared by multiple AOPs, or key event relationships shared by multiple AOPs. Significant relationships in the network have predictive and prognostic value for key events, and can be good candidates for biomarkers mechanistically linked to disease pathogenesis. Examples include membrane disruption leading to respiratory failure in fish (ie, narcosis), hepatocellular proliferation leading to cancer, skin sensitization initiated by covalent protein binding, and reproductive dysfunction caused by aromatase inhibition.[42,44] Internet-based tools, including AOPXplorer (http://aopxplorer.org) and the AOP wiki (https://aopwiki.org), allow the research community to share data and collectively build and review AOPs, allowing for transparency in data collection, review, and analysis.

Some of the most well characterized AOPs (eg, steatosis) have experimental evidence of a linear relationship between key events and pathogenesis; inhibiting DHB4 (peroxisomal multifunctional enzyme type 2, an enzyme involved in β-oxidation and fatty acid biosynthesis), for example, leads to steatosis with virtually 100% accuracy.[44] The AOP framework would allow investigators to characterize the health impact of TICs and TIMs at the level of the pathology. Implications for soldier health include developing a causal network affected by environmental chemical exposure and matching the diagnostic tests with biomarkers associated with key events in the AOPs.[48] Although still largely experimental, AOP-based biomarker assays promise an effect-based diagnostic indicator that might inform pharmacological interventions and/or protective measures. Ultimately, the AOP framework could improve the interpretation of diagnostic tests by adding prognostic projections and characterizing the extent of disease progression, allowing for integration into a limited number of toxidromes to predict end-organ injury and project disease prognosis.

CONCLUSIONS

As megacities continue to grow in number and population size, there is an unmet need to understand the health threats of Service Members exposed to toxic chemicals and environmental hazards unique to the megacity operational environment. Properly validated markers of exposure and effect can integrate with current biomonitoring systems throughout the soldier deployment lifecycle to inform medical decisions during and after exposure events, especially in polluted megacity operational environments with an increased risk of nonagent

chemical exposure. Further research is needed to ensure the fieldability of ruggedized detection systems in the megacity environment.

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