

Surveillance for Respiratory Infections in U.S. Military Populations Using Classic and Novel Diagnostic Techniques

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ABSTRACT

Military populations are historically susceptible to outbreaks of acute respiratory disease. These epidemics disrupt training schedules, place a heavy burden on the military medical system, cause significant economic losses, and ultimately impact troop readiness and mission accomplishment. The U.S. Naval Health Research Center (NHRC) has provided population-based viral respiratory surveillance in select US military populations since 1996. Although classical methods of diagnosis (culture) are the gold standard, these techniques are laborious and time-consuming. Novel diagnostic techniques were recently explored, and show promise for providing rapid results for large numbers of specimens.

1.0 NAVAL HEALTH RESEARCH CENTER RESPIRATORY DISEASE LABORATORY

Established in 1996, the Naval Health Research Center Respiratory Disease Laboratory was created to address concerns of morbidity caused by emerging and re-emerging respiratory pathogens within the Department of Defense (DoD) [1]. Critical at this time was the pending loss of the adenoviral vaccine that was in use in our recruits since the early 1970s. The vaccine’s sole manufacturer was discontinuing production. Supplies of vaccine were rationed seasonally at recruit training centers until early 1999, when all supplies were exhausted. There was need to document the effect of this vaccine loss, thus initiation of surveillance efforts.

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The cornerstone of the developed program was population-based surveillance with laboratory diagnostic support at 8 recruit training sites within the Navy, Air Force, Army, Marines and Coast Guard (Figure 1). Dedicated civilian research associates (RA) were hired to perform the surveillance at each site, ensuring long-term success and minimizing the workload on local staff. The local RAs are responsible for monitoring sick call clinics and logs, and determining total number of individuals at each recruit training site that meet the case definition of: Oral temperature of 100.5°F, cough and or sore throat, and all non-bacterial pneumonias. From a selection of these individuals, a throat swab clinical specimen is taken, and stored at ultra-low temperatures. At least once a month, these samples are forwarded to our reference laboratory in San Diego for classic virology cell culture. RAs also collect the denominator data (the total number of recruits on site vulnerable to infection) at their respective recruit-training site. With this collected numerator and denominator information, rates are followed from season to season and year to year.

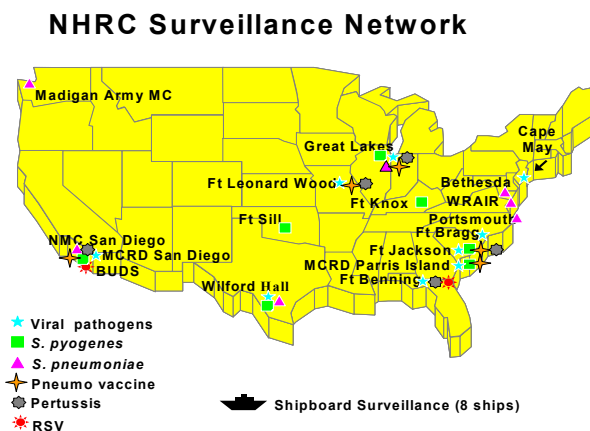


Figure 1

The data collected from this surveillance system since its inception in 1996 have been critical to the DoD. Numerous outbreaks caused by adenovirus have been documented with this laboratory-based surveillance system [2]. Critical data regarding the resurgence of adenoviral febrile respiratory illness in our recruits after the adenovirus vaccine was no longer available was also gathered [3, 4]. These data were invaluable for providing evidence that the adenovirus vaccine within our U.S. recruit populations was indeed needed; renewed efforts to find a new vaccine manufacturer were therefore initiated. Currently, Barr Pharmaceuticals is working to re-establish this vaccine. The currently projected availability year is 2009.

Figure 2 contains an example of information that is currently available on our internet site <http://www.nhrc.navy.mil/geis/sites/nhrc.htm>. This information is also shared via quarterly newsletters, and in weekly emails to concerned and engaged parties within the Department of Defense.

June 1998 - February 2004: Results of Diagnostic Testing of Febrile Respiratory Illness
Surveillance Participants
n = 14,327

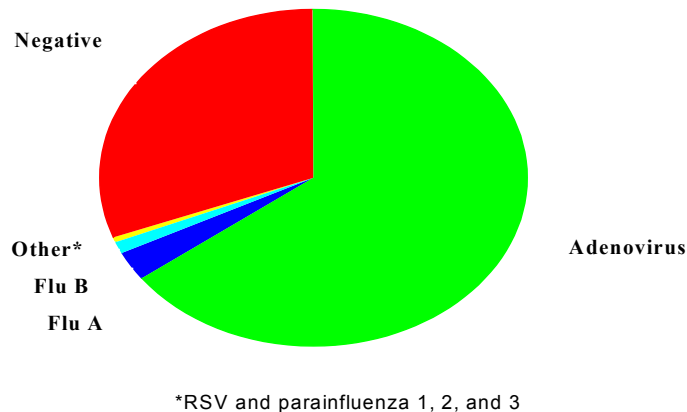


Figure 2: Results of diagnostic testing of samples received from recruits with a febrile respiratory illness. This diagnostic testing clearly shows adenovirus as the pathogen of greatest concern.

1.1 EXTENSION OF SURVEILLANCE TO REMOTE SETTINGS

As discussed, the developed febrile respiratory surveillance program proved very valuable within the U.S. recruit training settings. However, within the DoD, military members are exposed to a variety of other high-risk settings for acquiring respiratory infections. Morbidity from respiratory pathogens while on deployment can be crippling to mission accomplishment. To address these concerns, our surveillance capabilities were extended to a variety of other settings, often in remote sites.

Performance of surveillance onboard ships within the U.S. Naval Fleet is one such extension of our surveillance capabilities. As of March 2004, eight ships were taking part in our surveillance for febrile respiratory illnesses. Like surveillance in the recruit training setting, numerator and denominator data are collected, and a selection of individuals provide a diagnostic specimen for future testing. These samples are usually collected while the ship is at sea. They are stored at ultra low temperatures (liquid nitrogen or -70 degrees Celsius freezers), and upon return to their homeport at the end of their deployment, delivered to our respiratory disease laboratory for diagnostic workup. If a large respiratory outbreak were to occur at sea, appropriate supplies are on hand for collection of optimal diagnostic specimens at the time the outbreak is recognized. A special shipment could be arranged if needed for expedited laboratory processing. Figure 3 illustrates some of our results during the first 11 months of implementation in this setting.

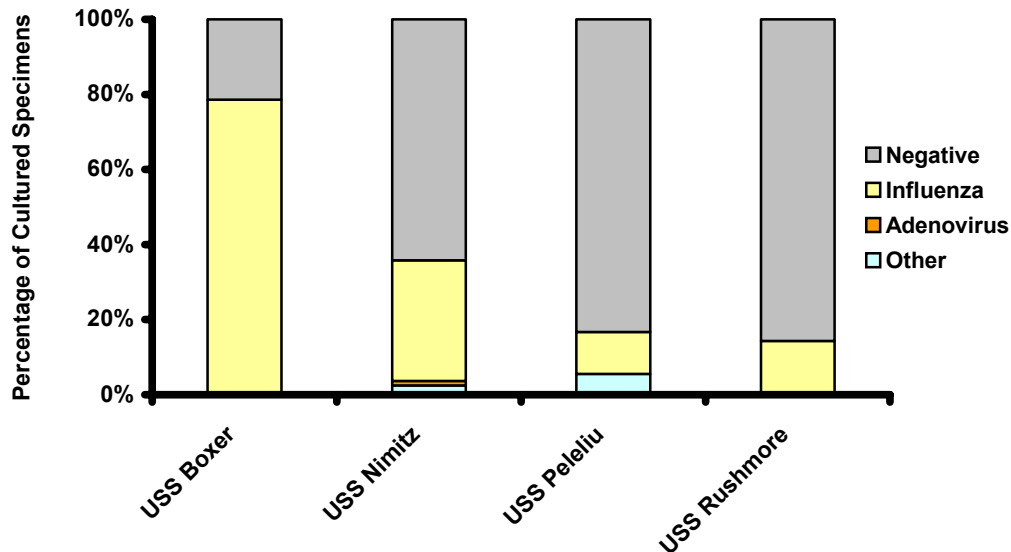


Figure 3: Results of diagnostic testing from samples acquired while on deployment from 4 U.S. Naval ships. Note influenza was diagnosed from samples on all 4 ships. Port stops prior to the outbreaks included Singapore and Hawaii.

Diagnostic testing capabilities were also provided to select ships that had Light Cyclers onboard. All consumables, controls, and an instruction manual were provided to each ship that requested diagnostic testing capabilities for influenza A, influenza B, and adenovirus. The testing takes approximately 4 hours for each pathogen. Although results were potentially available quickly, the testing still requires much time from the ship's laboratory technician. Given these time constraints, testing on-board ship was only rarely utilized.

Laboratory-based surveillance initiatives were also undertaken during the 2003 "Cobra Gold" exercise--a joint exercise located in Thailand. SARS transmission was not known to be present in Thailand in early 2003, and there were concerns that exercise participants could bring SARS into the country. Respiratory illness surveillance was initiated, including geographic exposure history for elucidating a SARS "suspect" case. Although no "suspect" SARS case was identified, 17 individuals met the case definition for febrile respiratory illness during this 4 week exercise; diagnostic specimens were obtained from 16. Laboratory testing identified influenza A in 7 samples (44%); sequence analysis on four of these demonstrated they were closely related to the Fujian-like influenza strain, which was the predominant strain found globally in 2003/2004. Two samples (13%) were positive for coronavirus OC43, 2 (13%) for respiratory syncytial virus, 1 (6%) for rhinovirus, and 4 (25%) were negative. Concern for SARS transmission was eased and knowledge of circulating respiratory pathogens was obtained as the logistics of implementing respiratory surveillance during a military training operation were overcome.

2.0 CLASSIC LABORATORY PROCESSING

Maintaining these classic methods of detection should always be high priority for any reference laboratory. They result in a critical product that none of the advanced molecular methods for pathogen detection are able to provide: a viable organism. The ability to *grow* the influenza virus is necessary for current influenza vaccine formulations. In addition, a viable organism allows testing of antigen/antibody interactions which can never be reproduced with only a portion of a non-viable genome. Complex tertiary and quaternary structures are absent in partial genome amplicons, so presence of the epitopes necessary to conduct such techniques is lacking. Our ability to perform in-vitro techniques that approximate the in-vivo antigen/antibody interaction is lost without viable, live organisms.

Clearly, classic methods of diagnosis for samples collected in our surveillance network have provided much-needed information. However, the potential disadvantages of laboratory-based surveillance as described are easy to discern. These classic methods are laborious, taking several weeks to process samples and requiring many man-hours for care of cell cultures. Consumables are numerous, from cells, to propagation media and reagents for performing detection methods such as immunofluorescence. There is also the need for specialized instrumentation such as the inverted microscope, the immunofluorescent microscope, and incubators. Results are not available in time for patient management decisions.

Another disadvantage of the classic methods is that any given cell line is only permissive to growth of a few viruses. Growing new, unknown pathogens would be unlikely in such a scenario. In addition, if a new virus was able to grow in the cells utilized, the detection methods require knowledge of what pathogen we are seeking. Antibodies can only be purchased against known organisms! Our ability to be sensitive to pathogen discovery is greatly reduced.

3.0 NOVEL DIAGNOSTIC TECHNIQUES

Molecular polymerase chain reaction (PCR) methods for detection are a frequently used alternative to classic cell culture methods. PCR offers the advantage of more timely results, often in 1 day or less. However, diagnosis is still essentially one specimen and one pathogen of interest at a time, and additional expensive instrumentation is required. Another disadvantage of these molecular techniques is that knowledge of the nucleotide sequence of the pathogen to be tested for is required. Pathogen discovery or identification of unknowns with these traditional PCR techniques is not possible. Multiplex platforms may allow detection of a few pathogens at a time, but often at the expense of sensitivity, with increasing risk of non-specific amplification or spurious band production ("primer dimers") as multiplexing efforts are increased. Newer "real-time" polymerase chain reaction such as the Light Cycler, Smart Cycler or TaqMan techniques make the need for gel electrophoresis for visualization of amplified product obsolete, thus reducing the potential turnaround time to hours. Again, additional costly instrumentation is required.

As we consider characteristics of the ideal diagnostic test, the following requirements should be considered:

1. Inexpensive for daily routine testing. Limited up front investments for instrumentation potentially could be supported.
2. Rapid, requiring hours rather than days or weeks.
3. Be useable in remote unsophisticated settings.
4. Not require extensive sample preparation prior to testing.
5. Be permissive to a variety of diagnostic specimens...from air samples to human samples to zoonotic samples.
6. Allow for processing large numbers of patient specimens for a large battery of pathogens simultaneously.
7. Be sensitive to unknown pathogens. The common pathogens, uncommon pathogens, and unknown pathogens should be detectable.

Clearly, these are challenging requirements to meet. In our new molecular age, however, the possibilities seem endless. A plethora of new sophisticated detection modalities are in various stages of development. One such technology is the micro-array format. Also requiring additional instrumentation, this technique does have the advantage of potentially testing a given specimen against a large battery of pathogens simultaneously. However, knowledge of the genetic sequence of the pathogen you are looking for is still needed with this technology.

One promising technology aggressively being pursued in our laboratory is called “Triangulation Identification for the Genetic Evaluation of Risks” (TIGER) through collaboration with Ibis Pharmaceuticals. This technology, although still in developmental stages, was successfully utilized in our surveillance efforts and fulfills many of the characteristics of the ideal testing platform.

The “triangulation” aspect of the TIGER acronym was coined from the concept of measuring MULTIPLE conserved and essential regions of pathogens present, as represented in Figure 4. There exist conserved regions of genomes that are essential for viability and replication of micro-organisms, and are therefore present in a genomically identical manner. Although the exact pathogens being sought may not be known, these conserved regions will be the same. Using PCR as the first amplification modality, these conserved regions are targeted and the region between is amplified. This nucleic acid sequence between the conserved portions differs in a pathogen specific manner.

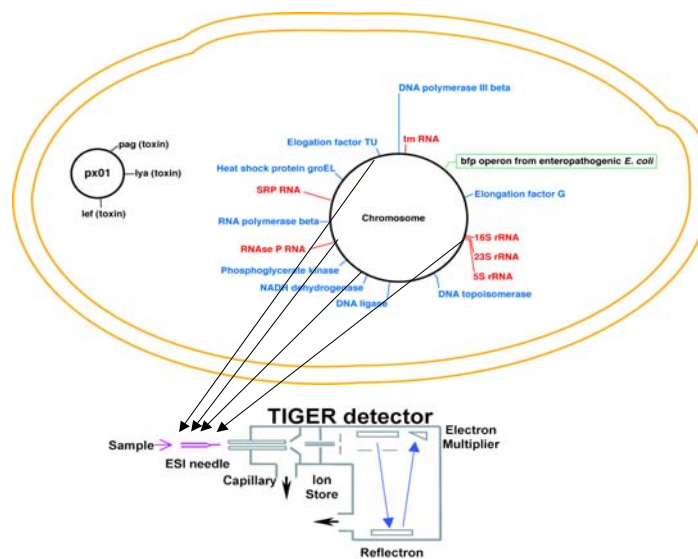


Figure 4: Triangulation identification of pathogens utilizing conserved genome portions for detection. In this manner, knowledge of the exact organism being targeted is not needed.

The TIGER technology, therefore, can take a complex mixture (specimen from any source: environmental, human, or animal), isolate the available DNA, and amplify from the conserved regions in this “triangulation” manner. A complex mixture of amplicons is produced with nucleic acid information between the conserved regions that is specific to their parent pathogen. These amplicons are then sprayed into a mass spectrometer that measures the specific molecular weight of each amplicon. Resolution of a complex mixture is achieved with high precision. The mass of each amplicon is determined to such high mass accuracy, that only one combination of nucleotides could result in that specific molecular weight. This specific nucleotide content is then often specific to only one pathogen, when results of all the triangulation amplicons are considered. See figure 5 and 6.

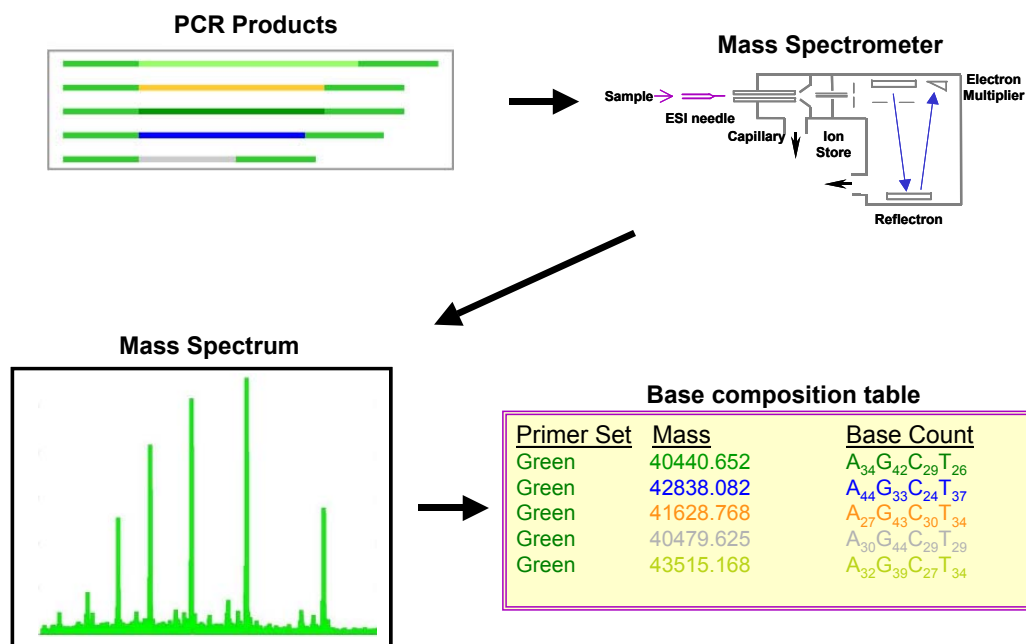


Figure 5: The process of determining the molecular weight of amplicons present in a complex mixture, and their specific base composition.

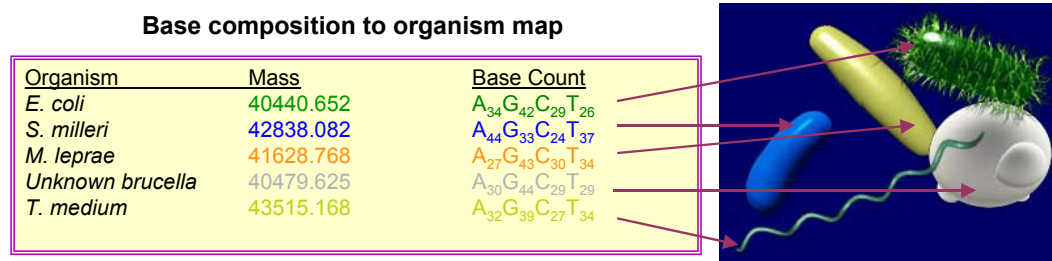


Figure 6: Taking the specific base composition and determining the pathogen of origin, using information from the triangulation and algorithms of known pathogen base count composition.

As a demonstration of the TIGER capabilities, an outbreak of Group A streptococcus (GAS, *Streptococcus pyogenes*) pneumonia among U.S. Marines was monitored and followed with this technique [5]. There was the need and desire to monitor the potential spread of the bacteria to other geographic sites. The questions that needed addressing: Are GAS outbreaks occurring in other geographic locations the same strain of GAS responsible for the Marine pneumonia outbreak? Is an unusually virulent clone causing these infections? Should we give prophylaxis to all groups that are exposed to troops from the original outbreak? Traditional classic and molecular methods of following the strains responsible for the illnesses were too laborious and time consuming to be effective epidemiologically. The TIGER technology was adapted for this purpose, and up to 600 samples could be processed overnight, giving sufficient resolution of genome variability or uniformity to follow the strains causing infection in other regions. We were able to quickly understand that the Marine GAS pneumonia strain was not causing wide spread illness in other military populations. Preventive medicine interventions were initiated with this information, information that was not attainable with traditional methods.

Adenovirus, as described above, is an important pathogen for military populations, particularly recruits. Knowledge of which serotypes are in circulation is important for renewed vaccination efforts. Traditional methods of determining serotype are again laborious and time-consuming. This TIGER technology was also successfully utilized for serotype determination of large numbers of adenovirus isolates.

Although additional instrumentation is needed with this TIGER technique, high throughput of original patient specimens against a countless number of potential pathogens is possible. In addition, using the broad priming techniques, amplicons that are not readily identifiable with given sequence information would alert one to the possibility of a unique pathogen being present. Many of the characteristics of the ideal diagnostic test could be fulfilled with this technology as it becomes refined.

4.0 DISCUSSION

Respiratory pathogens are of critical importance to the military. Only through laboratory-based surveillance efforts will we understand the pathogen specific risks in our different high-risk groups. Newer methods are being developed that will allow us to monitor and conduct this surveillance even more efficiently. We have a responsibility to remain knowledgeable about these developing techniques, as we strive to understand, diagnose and prevent illness among our constituents.

Classic techniques should not be abandoned, as their role is undeniably critical. However, for more timely capabilities at the point-of-care, alternative diagnostics must be pursued. Rapidly progressing technologies promise to provide greatly expanded capabilities in the near future, if we stay open to their potential. Validation of such techniques must be given priority, and must be thorough. As our ability to detect organisms becomes more robust, we must become more sophisticated in our ability to understand which organisms are actually responsible for disease. The commensal world must be better understood. However, if we remain dedicated to the cause, the rapid, accurate diagnosis of illness and heightened health and readiness status of those we are responsible for will be the reward.

REFERENCES:

- [1] Ryan M, Gray G, Hawksworth A, Malasig M, Hudspeth M, Poddar S. The Naval Health Research Center respiratory disease laboratory. *Mil Med* 2000;165(suppl 2):32-34.
- [2] Ryan MAK, Gray GC, Smith B, McKeehan JA, Hawksworth AW, Malasig MD. Large epidemic of respiratory illness from adenovirus types 7 and 3 in healthy young adults. *Clin Infect Dis* 2002;34(3):577-82.
- [3] Gray GC, Goswami PR, Malasig MD, Hawksworth AW, Trump DH, Ryan MA, Schnurr DP. Adult adenovirus infections: loss of orphaned vaccines precipitates military respiratory disease epidemics. *Clin Infect Dis* 2000;31(3):663-670.
- [4] Hyer RN, Howell MR, Ryan MAK, Gaydos JC. Cost-effectiveness analysis of reacquiring and using adenovirus vaccines in US Navy recruits. *Am J Trop Med Hyg* 2001;62(5):613-8.
- [5] Crum, NF, Hale, BR, Bradshaw, DA, Malone, JD, Chun, HM, Gill, WM, Norton, D, Lewis, CT, Truett, AA, Beadle, C, Town, JL, Wallace, MR, Morris, DJ, Yasumoto, Russell, KL, Kaplan, E, Beneden, Cvan, and R. Gorwitz. Outbreak of Group A Streptococcal pneumoniae among Marine Corp Recruits-California. 2003. *MMWR* article 52(6): 106-109.