

Emerging Illness Update

Newsletter for Emerging Illness Studies at the Naval Health Research Center

Emerging Illness Surveillance Expands at Naval Health Research Center

Introduction

The Adenovirus Examiner and "Invasive Investigator" have merged into one, more expansive newsletter, the "Emerging Illness Update." This newsletter will keep you up to date on the progress of the emerging illness studies being conducted at the Naval Health Research Center including, the surveillance of adenovirus, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Ureaplasma urealyticum*.

Adenovirus

Before the implementation of a vaccine, adenovirus infected 10% of military recruits and caused 90% of recruit pneumonia. These costly outbreaks have been effectively controlled with AV type 4 and 7 vaccines for nearly 30 years, but now pose a serious health threat to military populations due to unavailability of the vaccine. Recent studies suggest that less common adenovirus types not protected by present vaccines may be more endemic in high-risk populations than they were 30 years ago.

Invasive *Streptococcus pyogenes* Surveillance Continues

There have been several additional cases of invasive *S. pyogenes* infection reported since June 1997. We would like to remind everyone that a **positive blood culture is not necessary** to meet the case definitions for this study. A positive culture from a sterile site (blood, skin, etc.) in conjunction with toxic shock syndrome, also meets the case definition. The complete case definition was described in the last issue of the "Invasive Investigator," January 1997. Invasive *S. pyogenes* infection is a rare event, so we need to make sure that every case is identified. We appreciate the help and cooperation we have received from our colleagues. These data are very important and positive cultures are found in the following for a better evaluation of the incidence of invasive disease. Thank you for your good work.

New *Streptococcus pneumoniae* Study begins at NHRC

Naval Training Center, Pines, IL
Aerobics Corp., San Diego, CA
Port Jackson, Columbia, MO
Port of Naval Warfare, Houston, TX
Rockwell Aircraft Co., Houston, TX.

New *Ureaplasma*

A viral culture was obtained on every respiratory specimen following

1. Oral temperature of $\geq 100.5^{\circ}\text{F}$, and
2. One or more respiratory symptoms (cough, sore throat, runny nose, wheezing, dyspnea, chest tenderness, or physical exam consistent with respiratory tract infection).

Specimen Collection

Quality specimens can only be obtained from quality collection. Several important factors are critical to successfully isolate and identify an infected person; two of the most important are:

1. The specimen must be collected during the acute phase of the illness. This is in the early stages of the disease (3 to 5 days after onset of infection) and is usually associated with a high fever.

Storage and transport temperatures are critical since viruses are heat labile. For this reason, the specimen should be frozen at -70°C as soon as possible after collection, and then transported on dry ice for the best possible chances of isolation.

Laboratory Procedures

Viral Isolation: To avoid the risk of viral contamination of stock cultures, the laboratory work area at NHRC is divided into two separate spaces, a dedicated "clean room" for the propagation of continuous cell lines, and a separate working laboratory for inoculations and isolations. Stock cultures of A-549 cells are maintained and split 1:6 weekly in the clean room.

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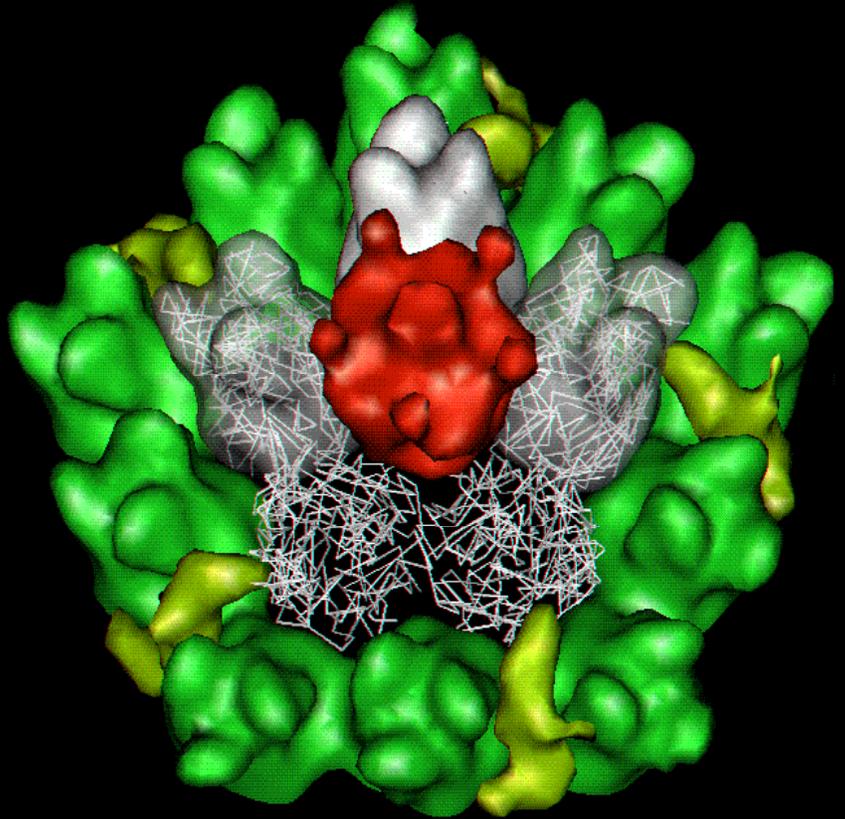
Once the nasopharyngeal swabs reach the laboratory, they are removed from the dry ice packaging, briefly examined for acceptability, cataloged, then immediately frozen at -70°C until all materials are ready for inoculation. Batched samples are inoculated in duplicate along with controls held for up **effect (CPE)**. If CPE is noted, spot slides are prepared, air-dried, fixed in chilled acetone, then examined by a direct immunofluorescence assay for the presence of adenovirus using the technique shown below. The slide is stained by a fluorochrome conjugate, then exami to 7 days. After 2 to 4 days, if virus is present, it will cause **cytopathic** ned under a fluorescent microscope. The presence of specific cytoplasmic fluorescence in the infected cells with a lack of fluorescence in the control cells is diagnostic of adenovirus and constitutes an **adenovirus isolation**, although further tests would be necessary to determine the serotype.

Serotyping: The adenoviruses include 47 known serotypes that have been recovered from virtually every human organ system and have been associated with a number of clinical syndromes. Certain serotypes are more commonly associated with specific syndromes, for example: upper respiratory (AV-1-3, 5, & 7) and lower respiratory (AV-3, 4, 7, & 21) illness and acute respiratory disease (AV-4 & 7). The focus of this surveillance is to identify those adenovirus serotypes most commonly associated with respiratory disease, which are thought to include subtypes 1-5, 7 and 21.

Subtyping involves the neutralization of adenovirus with specific monoclonal antibodies. We use a microneutralization assay that combines the sensitivity, conservation of reagents, and ease of handling of microtiter assay with the speed, simplicity, and objectivity of spectrophotometric analysis. This technique was developed and perfected by our collaborator, Dr. David Schnurr at the Viral and Rickettsial Disease Laboratory, California Department of Health Services, Berkeley, CA.

Rapid Diagnostic Methods: At a Triservice Acute Respiratory Disease Surveillance meeting in November in San Antonio, the need for a rapid diagnostic method was agreed upon by all. Rapid laboratory confirmation of the etiologic agent would allow quick decisions to be made regarding

Adenovirus Penton region

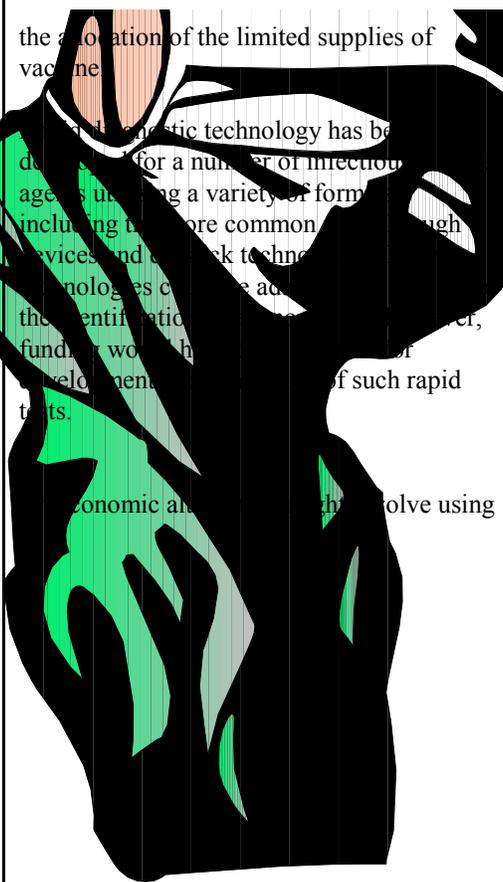
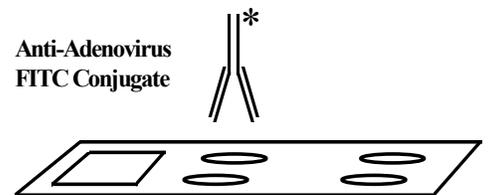


Fitting of hexon polypeptide into cryo-em map.
P.L. Stewart, R. Burnett, & S.D. Fuller

the allocation of the limited supplies of vaccine. Diagnostic technology has been developed for a number of infectious agents using a variety of forms including the more common techniques and diagnostic technologies currently used for the identification of such rapid tests.

immunofluorescent stain of slide made from nasopharyngeal swab smears. This is a potentially inexpensive method, however, its sensitivity and specificity is unknown. We plan to evaluate this procedure by preparing swab smears from all samples received. The results will be compared with those of standard viral isolation method.

Current DoD Laboratory Capability



As pointed out at the recent Triservice Acute Respiratory Disease Surveillance meeting, the DoD currently lacks good laboratory surveillance capabilities. The DoD has:

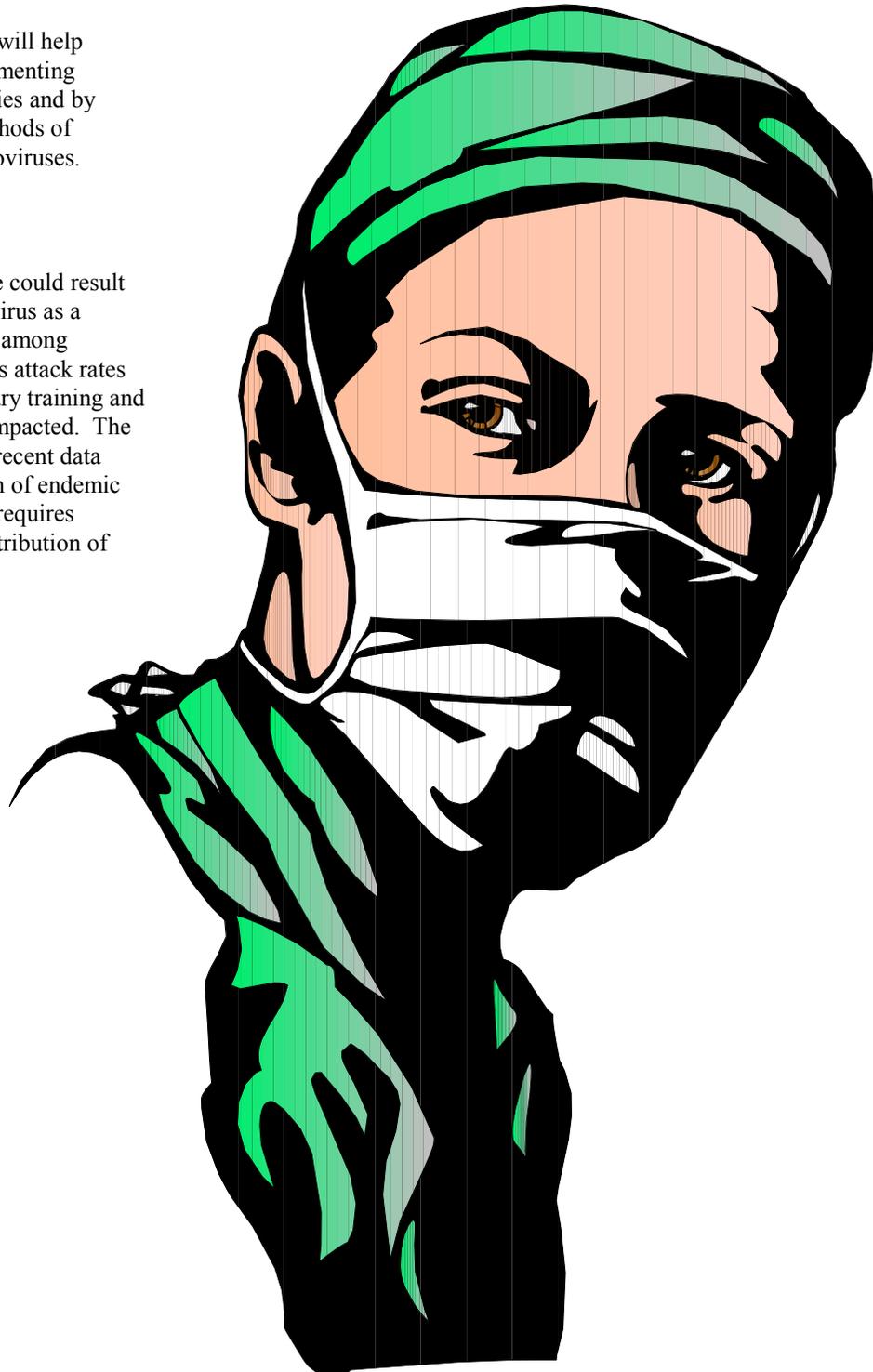
- * minimal capability for virus isolation
- * no recognized/resourced public health laboratory capable of supporting DoD needs
- * a need for advanced technology and rapid diagnosis for adenovirus
- * uncertainty regarding how much routine

laboratory support is needed other than in "outbreak" situations

The surveillance in this study will help address these needs by supplementing existing viral culture capabilities and by developing more efficient methods of screening and subtyping adenoviruses.

Summary

The impending loss of vaccine could result in the re-emergence of adenovirus as a significant cause of morbidity among military recruits. If adenovirus attack rates reach prevaccine levels, military training and readiness would be severely impacted. The loss of vaccine, coupled with recent data suggesting that the distribution of endemic serotypes may have changed, requires examination of the current distribution of adenovirus serotypes.



**CASE REPORT FORM
Adenovirus Study**

The Department of Defense is conducting surveillance for adenovirus infection among military recruits (basic trainees). A viral culture (throat) should be obtained on every recruit who meets the case definition for acute respiratory disease (ARD) (fever \geq 100.5 and at least one respiratory symptom). This form must be completed for each viral culture taken.

Instructions for Viral Culture

1. Obtain throat culture using a sterile Dacron swab.
2. Place swab directly into viral culture media and break off swab tip so that it remains in the vial.
3. Cap vial, place barcode sticker on vial, and hand-label vial with the barcode number.
4. Also place identical barcode stickers on this Case Report Form and on monthly log.
5. Store vial in -70°C freezer until batch is ready for shipping.
6. As soon as possible, complete this form for each specimen obtained.

Barcode: _____
Place sticker here

Sex: M F (Circle one)

Age: _____

Date throat culture taken: _____
mm dd yy

Highest oral temperature _____ F

Current oral temperature _____ F (at time culture taken)

Number of days since symptoms began: _____

Currently hospitalized with pneumonia? Y N

Previous adenovirus vaccine? Y N
mm dd yy

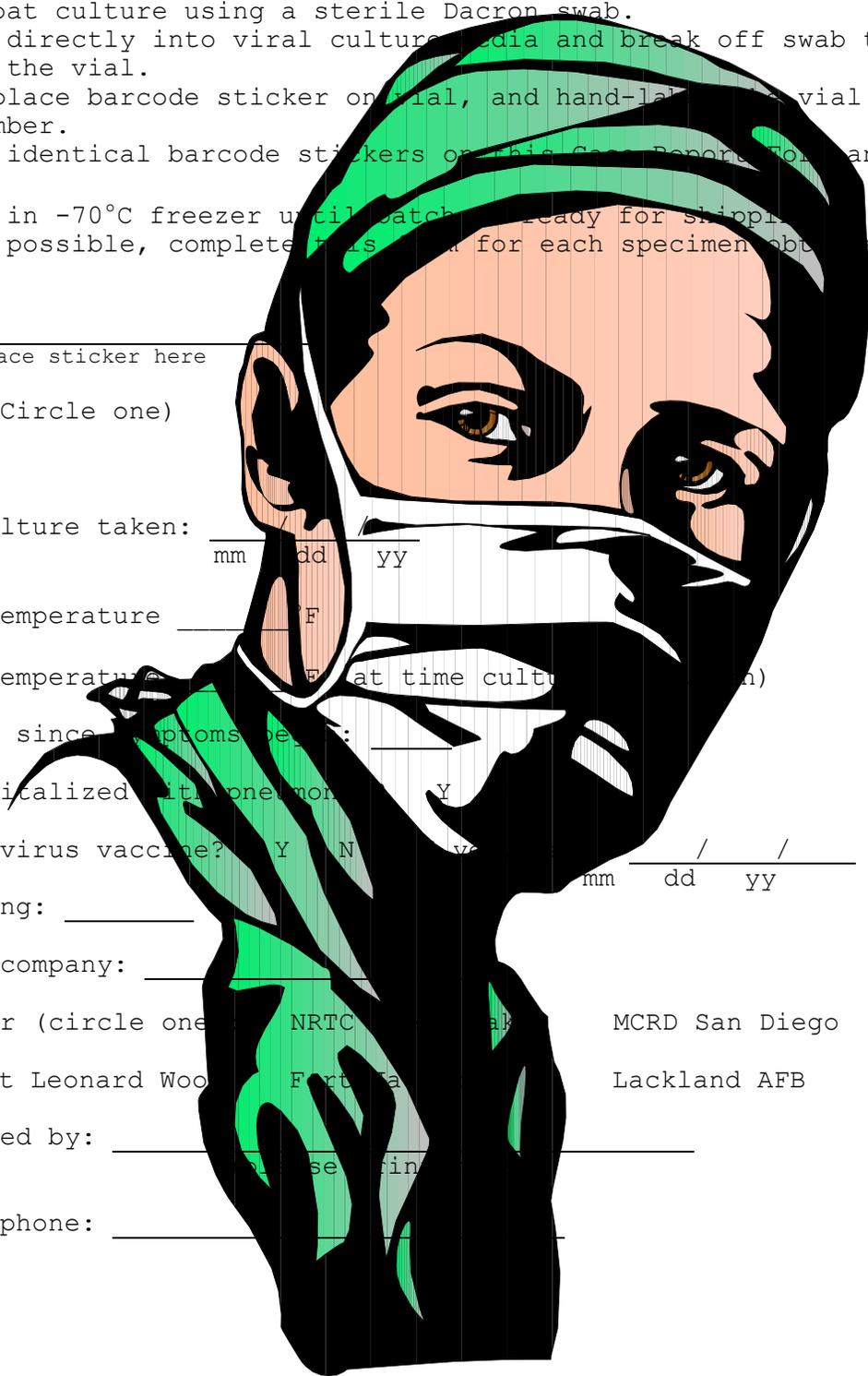
Week of training: _____

Training unit/company: _____

Training center (circle one) NRTC Fort Jackson MCRD San Diego
Fort Leonard Wood Fort Rucker Lackland AFB

Report completed by: _____
Please print name

Telephone: _____



4-WEEK SUMMARY FORM
Adenovirus Study

Please send this form to the principal investigator every 4 weeks to coincide with shipping of the viral cultures. Each reporting period should begin on a Monday and end on a Sunday 4 weeks later. The first reporting period will be from 30 September 1996 to 27 October 1996.

1. 4-week reporting period: / / to / /
mm dd yy mm dd yy
(Monday) (Sunday)

2. Number of recruits (basic trainees) cultured: _____

3. Number of recruits who met case definition for ARD¹: _____

Training center (circle one): MCRF Great Lakes MCRF San Diego
 Fort Leonard Wood Fort Jackson IAWA

Report completed by: _____
(please print name)

Telephone: _____

¹ The case definition for acute respiratory disease (ARD) (which must apply):
- Fever \geq 100.5
- At least one respiratory symptom

