AMERICAN COLLEGE OF EMERGENCY PHYSICIANS
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Cutting-Edge Diagnostic Modalities for Infectious Diseases: Useful or Useless?

The diagnosis and management of infectious diseases are controversial due to continuing developments in this facet of emergency medicine. Emerging infections, antibiotic-resistant bacteria, and the new antibiotic therapies contribute to this quandary. The most current approach to the assessment and evaluation of infectious diseases will be described.

- Describe new techniques for identifying infectious agents and how they apply to clinical medicine.
- List the current applications of tests such as PCR, and what can be expected in the near future.

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FACULTY

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Cutting Edge Diagnostic Modalities for Infectious Disease

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I. Course Description

Recent technological advances potentially offer expanded opportunities to accurately diagnose infectious diseases in the emergency department. As tests become accessible and affordable they can increase both the sensitivity and the specificity of our diagnoses. This can help us offer correct or more specific therapy for various infections. It can also change the time course of our interventions by providing quicker results or reliable results earlier in the disease processes. All of this can lead to better individual patient care and better public health.

Specific modalities to be discussed are DNA-based technologies (including polymerase chain reaction [PCR]) and magnetic resonance imaging (MRI) in the emergency diagnosis of infectious diseases.

II. Objectives

At the conclusion of this course the participant will be able to list the indications for emergency MRI, describe the principles of PCR, list the current diagnostic uses of PCR and discuss potential future indications for DNA-based diagnostic modalities in emergency medicine.

III Course Outline

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A. Magnetic resonance imaging

1. Epidural abscess
2. Osteomyelitis
3. Pyomyositis
4. Septic arthritis
5. Encephalitis
6. Necrotizing fasciitis
7. Septic bursitis
8. Transverse myelitis

B. *Helicobacter pylori*

In addition to invasive (endoscopic) diagnostic modalities there are non-invasive tests available including serologic examination, CLO test, and carbon 13 labeled urea breath test (UBT).

Consideration can be given to empirical treatment based on clinical symptomatology.

C. Biological warfare

Microbial agents and toxins that could be used in biological warfare include anthrax, botulinum toxin, *yersinia pestis* (plague), staphylococcal enterotoxin B, and Venezuelan equine encephalitis virus. Despite different characteristics they can all be dispersed in aerosols of particle size enabling them to remain suspended for hours in the air when certain weather conditions prevail. If inhaled they penetrate to distal bronchial and terminal alveoli and cause severe, often fatal, illness.

While the specific diagnosis generally is confirmed either by culture or serology, and care is often ineffective after symptoms have developed, clinical diagnosis and appropriate reporting could result in public protective measures that could save many lives. For this reason, it is important for emergency physicians to be alert to the clustering of cases of severe and sudden respiratory illness.

Anthrax is, perhaps, the most likely agent for biological warfare and could, theoretically, kill or disable over 200,000 people if dispensed by airplane upwind from a population center of 500,000 unprotected persons.

Early diagnosis of inhalational anthrax is difficult and requires a high index of suspicion. In the first stage of the illness patients develop a spectrum of non-specific symptoms including fever, dyspnea, cough, headache, vomiting, chills, weakness, abdominal pain, and chest pain. Hours to days later, sometimes after a brief period of apparent recovery, patients progress to the second stage with sudden fever, dyspnea, diaphoreses, and shock. Massive mediastinal lymphadanopathy which can be hemorrhagic can lead to stridor and a widened mediastinum on chest x-ray. Up to half of the patients develop hemorrhagic meningitis with delirium and obtundation. Hypoxia and hypotension progress rapidly and death occurs sometimes within hours. [reference 25]. Rapid diagnostic tests such as enzyme linked immunosorbent assay (ELISA), which detects protective antigen, and PCR are available only at national reference laboratories. While these may not help an individual patient, rapid initiation of

diagnostic testing could confirm the diagnosis and determine invitro susceptibility to antibiotics. This testing can, also, be used for investigation and management of anthrax hoaxes. Standard blood culture can confirm the diagnosis in 6-24 hours.

The clinical combination of a **widened mediastinum on chest x-ray** in a previously healthy patient **with evidence of overwhelming flu-like illness** is essentially diagnostic of **advanced inhalational anthrax**. [reference 25]

D. Polymerase chain reaction (PCR) [References 5, 6]

1. Description

PCR is a technique for the selective exponential amplification of a single fragment of DNA. If tissue from an organism contains the specific, identified sequence of DNA nucleotides that is being tested for, PCR can theoretically take one double stranded DNA molecule and amplify it into millions of identical strands that can then be easily detected. This confirms the presence of the specific DNA in the sample. PCR is basically a three step process that is repeated many times.

Step One: Separating the two strands of the double helix. This is termed **"denaturing"** the double-stranded DNA and it is accomplished by heating the DNA to 95^{o} C.

Step Two: Attaching specific segments of nucleotides to the single strands. This is called **"annealing"** (attaching) the **"primers"** (strings of nucleotides, typically about 20 nucleotides long, the order of which corresponds to a known sequence of nucleotides in the DNA being tested for). This must be done at a temperature of 55^{o} C.

Step Three: **Replication** of the DNA single strands starting where the primers have attached. This synthesis is catalyzed by a naturally occurring enzyme called a **polymerase** which causes nucleotides to attach sequentially, resulting in double strands of replicated DNA This polymerase reaction occurs at 72^{o}C.

Thus, one cycle of the process will replicate a double stranded DNA molecule, producing two identical double-stranded molecules

if and only if portions of the DNA in the sample match the nucleotide sequence of the specific primer that was added to the solution. These primers are strings of nucleotides that are constructed to match a segment of the gene that is unique to the organism or trait being tested for ("specific homology" for the target DNA). For example, one 20 (or so) nucleotide primer has a sequence of nucleotides that is found only in the herpes virus. Another primer has a sequence found only in HIV.

This synthesis of DNA by the polymerase enzyme becomes a **"chain reaction"** when it is repeated many times. With each cycle the number of DNA strands doubles. If it is repeated 20-40 times then millions of identical DNA strands will be produced. This large volume of DNA can then be readily detected, usually by gel eletrophoresis, giving a positive result.

2. General Application

PCR can detect the presence of extremely small numbers of specific nucleotide sequences that are known to be found only in one specific genome. Thus the test can identify a specific type of virus, bacteria, cancer gene, gene related to a genetic disease, or even, in forensic analysis, a DNA nucleotide sequence unique to one single human being's genome.

3. Limitations

a. Sensitivity

The test is so sensitive that a single strand of DNA (for example from a previous test performed with the equipment) could lead to contamination and a false positive result.

b. Time

Since the temperatures must be changed between each step and the cycle must be repeated 20-40 times, the amount of time it takes to change the temperature of the solution limits how quickly the test can be done. Currently tests require about 6 hours to run.

c. Cost

Because of the technician time involved, the equipment.

necessary and various patents on components of the procedure, the cost of PCR can be significant

4. Specific Application in Emergency Medicine

a. Herpes simplex encephalitis (HSE)
Currently the best confirmatory test for HSE other than brain biopsy.

b. *Chlamydia trachomatis*
PCR appears to be more sensitive and specific than traditional methods of testing for chlamydia. The technique can be applied to non-invasive urine samples from both men and women. [References 7, 9,10, 11, 20].

c. *Borrelia burgdorferi* (Lyme disease)
PCR can be used to identify the presence of spirochetes in infected ticks as well as various body fluids including plasma, synovial fluid, CSF, serum and even urine. [Reference 8].

d. HIV
Serology is the current standard for diagnosing HIV (with a repeatedly positive ELISA followed by a positive Western blot). False negative results are only about 0.3% even in a high prevalence population, and false positive results are only 0.0007% in a low prevalence population. Home kits are available and rapid resting is possible with latex aggluntination assays (which have good sensitivity but poor specificity necessitating confirmation of positive results).

PCR offers the advantage of earlier potential detection (prior to seroconversion), and the ability to quantitatively measure viral load. This could have a significant impact on the emergency department evaluation of acute primary HIV infection and early treatment of infected persons. [References 12, 13, 14, 15, 16].

e. Mycobacterium tuberculosis
PCR could speed and improve the identification of this notoriously fastidious organism. [References 17, 18].

5. Other potential applications [Reference 5]

a. *Mycoplasma pneumonia*

b. *Chlamydia pneumoniae*

c. *Bordetella pertussis*
d. *Pneumocystis carinii*
e. *Trichomonas vaginalis*
f. *Treponema palladium*
g. *Neisseria gonorrhoeae*
h. *Giardia*
i. hepatitis C
j. forensics
k. unculturable infectious agents [Reference 19]

E Summary

. Polymerase chain reaction and other DNA-based modalities may significantly expand the capabilities of emergency physicians to impact the course of infectious diseases. Recognition of the signs of primary HIV infection, coupled with early confirmation of the diagnosis by PCR techniques, could enable earlier treatment that might significantly improve the course of the disease. PCR might also enable mass screening of frequently asymptomatic communicable diseases, such as chlamydia, and thus allow us to significantly impact public health. In all cases we must remain aware of the indications, costs, and limitations of these new diagnostic modalities.

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