WHEN IS A BIOTERRORISM AGENT NOT A BIOTERRORISM AGENT?

SHARON MASTER, PH.D., BIOLOGICAL SCIENCES BUREAU CHIEF

Ever since the 2001 anthrax attacks, bioterrorism has been flung into global notoriety, developing into a household term. Bioterrorism agents are microbiological organisms, such as viruses, bacteria, fungi, or toxins of biological origin, which could cause severe, potentially lethal infections in humans, or affect crops and livestock. Biological terrorism dates back to ancient times when the Romans hurled feces into the faces of enemies, while in the 14th century bubonic plague-ridden corpses were catapulted across enemy lines in an attempt to decimate the population and to force surrender. Although a large number of pathogenic microorganisms exists that could be utilized for bioterrorism, the Centers of Disease Control and Prevention have compiled a "select" list of these agents that have high infectivity and mortality if left untreated.

When not used for bioterrorism these microbiological organisms thrive in soil, insects, or animals, in pockets around the world, where they occasionally cause disease in both humans and animals. New Mexico, probably because of its vast unpopulated areas and balmy climactic conditions, provides a haven to a few of these microscopic yet devastating pests. These include the bacteria *Yersinia pestis* and *Bacillus anthracis* that cause bubonic plague and anthrax respectively (see Table 1 for more information).

Name of Agent	Type of Agent	Natural Reservoir	Disease Caused
Yersinia pestis	Bacterium	Rodents including squirrels, rabbits, hares, and fleas	Plague (bubonic or pneumonic)
Bacillus anthracis	Bacterium	Spores in soil, herbivores	Anthrax (cutaneous or inhalation)
Coccidiodes immitis, posadasii	Fungus	Soil	Coccidioidomycosis (valley fever)
Brucella abortus, mellitensis	Bacterium	Cattle, swine, goats, sheep	Brucellosis
Francisella tularensis	Bacterium	Rodents including squirrels, rabbits, hares, ticks, fleas, and mosquitos	Tularemia
Coxiella burnetti	Bacterium	Ticks, birds, ruminants, domestic animals	Q-fever

TABLE 1. BIOTERRORISM AGENTS THAT ARE ENDEMIC TO NEW MEXICO

Bioterrorism agents, to most lay people, might conjure up visions of the Black Death or boils on the ancient Egyptians. However, because they are endemic to New Mexico, for the laboratorians of the Biological Sciences Bureau (BSB) at the SLD, these agents represent the relatively routine testing and surveillance of clinical and animal specimens, and occasional food, water, and environmental testing, in keeping with the core functions of a Public Health Laboratory. The specimens that the Bureau receives, for such testing, are diverse in their nature ranging from lymph node aspirates, skin scrapings, blood culture bottles, tissue, serum samples, isolates, bits of liver or spleen, milk samples, raw and

processed food samples, environmental swabs, or water samples. The origins of these specimens are also varied and include humans, cats, dogs, rabbits, cattle or other animals. These agents are tested in the BSB, using a wide spectrum of tests, within one or more of four sections depending on the type of specimen or the urgency of the result.

The General Microbiology Section tests raw specimens and isolates using archetypal, highly detail-oriented microbiological techniques. Culturing, or growth, of these organisms, from raw specimens, represents the most important aspect of identification and includes growth in liquid broth or onto selective agar plates, which can sometimes take several days. Once grown, colony and microscopic morphology of the agents are determined by studying shape, size, structure and cell wall properties. This is accomplished either by direct visualization, through microscopy via the use of stains that are colorimetric or fluorescent, or via biochemical analysis. *Coccidioides immitis/posadasii* is specifically identified via a DNA probe assay where a sequence-specific probe is hybridized to a target in the DNA of the microorganism.

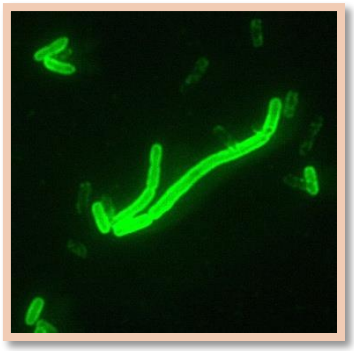

FIGURE1. FLUORESCENT STAINING OF THE PLAGUE BACTERIA OBSERVED THROUGH A MICROSCOPE

The Molecular Biology Section exploits state-of-the-art molecular technologies and equipment to identify these select agents from both raw specimens and isolates. The primary assay of choice for the agents listed in Table 1 is a Real-Time Polymerase Chain Reaction (Real-Time PCR). Microbial DNA is first extracted in automated medium-throughput extractors. Specific sequences of this extracted DNA are then amplified, in real time, up to ten billion molecules, using cycles of heating and cooling. Real-Time PCR is a convenient, albeit expensive, tool that permits rapid identification of agents from raw specimens. Occasionally, however such identification from raw specimens becomes tricky and necessitates growing of the organism before accurate molecular identification can be accomplished.

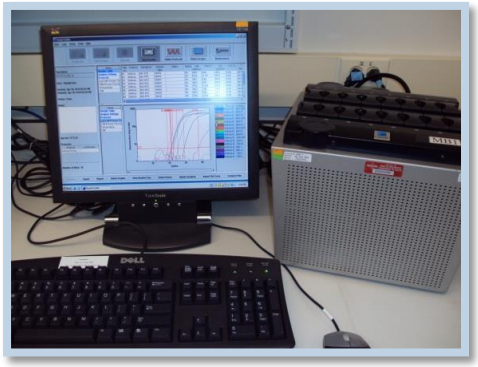

FIGURE 2. REAL-TIME PCR MACHINE WITH GRAPH OUTPUT OF RESULTS

The Virology/Serology Section has an entirely different perspective to the testing they conduct. They do not test for the specific agent directly but instead examine whether or not a person or animal has been exposed to a particular agent. An immunocompetent mammal (including humans) when exposed to a microbiological agent mounts an immune response producing various types of antibodies. These antibodies are often produced before clinical symptoms appear and hence early detection of antibody levels becomes critical in initiating prompt treatment. Serum from potentially exposed humans and animals are tested in the section through agglutination assays which detect antibodies that are produced specifically against these select agents.

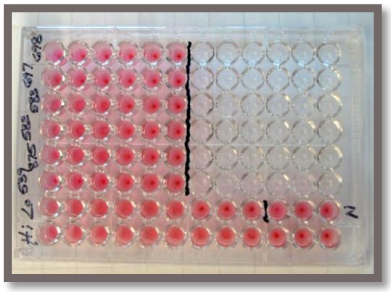

FIGURE 3. IMMUNOASSAY OF A SERUM SAMPLE TO TEST FOR TULAREMIA

The Environmental Microbiology Section also plays an important role in the testing of these agents. They have the capacity to test food (as well as milk), water and environmental swabs from dirt or almost any surface including vacuum bags. The Section employs an assortment of traditional microbiological techniques such as broth enrichments and culturing, using some of the same techniques as the General Microbiology Section does along with more modern molecular methods such as Real-Time PCR. Some of the processes, however, require additional steps. For example, food samples must first be broken down in a stomacher before the agent can be isolated for further testing.

FIGURE 4. A TYPICAL WORKBENCH IN THE ENVIRONMENTAL MICROBIOLOGY SECTION SHOWING EXAMPLES OF SELECTIVE MEDIA USED IN GROWING MICROBIAL ORGANISMS

In the Biological Sciences Bureau at the SLD, identification of such microbiological agents occurs mostly as part of routine testing and occasionally as part of an emergency response. The analysts that execute these investigations must undergo a series of rigorous proficiency tests as mandated by the agencies that regulate the lab. The Bureau is a member of the Food Emergency Response Network (FERN) that is under the jurisdiction of the USDA and the FDA, the Laboratory Response Network (LRN) that falls under the auspices of the CDC, and is also audited by the College of American Pathologists (CAP) that regulates all clinical testing. Hence, the laboratorians here in the Bureau, through their expertise allow for an early rapid response in the removal or cleansing of contaminated food, water or the environment as well as in the diagnosis and treatment of affected individuals or animals.