|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| MCVI 3.60 Bioterrorism Protocol | | | | | | | | |
| **Purpose** | This procedure provides instruction for the recognition and safe handling of isolates that may be used as agents of bioterrorism. | | | | | | | |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. | | | | | | | |
| **Special Safety Precautions** | 1. Remember to always follow standard precautions. If you are suspicious of an organism, perform all preliminary identification under a BSC until it can be ruled out. 2. Do not use automated systems (Vitek 2, MicroScan, MALDI) if you are suspicious of an organism. 3. Primary and subculture plates should be taped or shrink sealed 4. Avoid sniffing organisms on plated media. Always work with CAP Lab Preparedness Surveys and MDH Challenges in a Bio Safety Cabinet. 5. Be suspicious of hazy growth or no growth day one cultures. Use red/orange NG1 stickers to alert others of potential bioterrorism agent. 6. Refer to MDH/ CDC/ LRN manual flowcharts to guide workups. Other references available include APHL, ASM, and CDC websites. 7. Microbiologists are subject to occupational risks associated with specimen handling. 8. [*Biohazard Containment*](file:///\\kidsnet.childrenshc.org\chcdfs\dept\Lab%20Procedures\Micro%20Procedure%20Manuals\MC%20200%20%20%20%20Safety\MC%20201%20%20%20Biohazard%20Containment.doc) 9. [*Biohazardous Spills*](file:///\\kidsnet.childrenshc.org\chcdfs\dept\Lab%20Procedures\Micro%20Procedure%20Manuals\MC%20200%20%20%20%20Safety\MC%20204%20%20%20Biohazardous%20spills.doc) 10. [*Safety in the Microbiology/Virology Laboratory*](file:///\\kidsnet.childrenshc.org\chcdfs\dept\Lab%20Procedures\Micro%20Procedure%20Manuals\MC%20200%20%20%20%20Safety\MC%20202%20%20%20Safety%20in%20the%20Microbiology%20Lab%20Policy.doc) | | | | | | | |
| **Abbreviations** | MDH-Minnesota Department of Health  CDC-Center for Disease Control  LRN-Laboratory Response Network  APHL-Association of Public Health Laboratories  ASM-American Society for Microbiology  MALDI-Matrix-assisted laser desorption/ionization  BT-Bioterrorism agents  BSC-biosafety cabinet | | | | | | | |
| **Procedure** | 1. On all cultures with hazy growth or no growth on day 1, work up the culture under the BSC. Place red/orange NG1 stickers on plates and tape plates closed. Do not open plates on bench top until it can be determined if BT organisms are a concern. 2. Follow MDH flow charts and the following descriptions of each bioterrorism (BT) agents. 3. When unable to rule out a BT organism, call **MDH-PHL at 612-282-3723.** 4. Send organism to MDH with category B packaging. 5. Indicate on submission form if we require further identification if BT agents are ruled out. 6. Indicate on submission form if we suspect a specific agent. 7. MDH will call and fax all positive results. 8. MDH will not call negative results, but we will receive a fax. 9. MDH may perform additional testing to rule out all of the small Gram negative BT agents. 10. All materials (plates, slides, tubes, bottles) will be placed in a bucket to reduce exposure. After testing had been confirmed and completed, bleach will added to the items in the bucket, held for 24 hours and disposed of in a biohazard bucket. | | | | | | | |
| ***Bacillus anthracis, Bacillus cereus* biovar *anthracis*** | These are the steps to recognize, presumptively identify and rule out these organisms from clinical specimens in Sentinel Clinical Laboratories.   1. *Bacillus anthracis*, the agent of anthrax, is a zoonotic disease that is transmissible to humans through consumption or handling of contaminated products. It is an aerobic, spore-forming, non-motile, large gram positive bacteria.      1. *Bacillus cereus* biovar *anthracis* are non-hemolytic, motile, and resistant or sensitive to penicillin. 2. *B anthracis* and *B cereus* biovar *anthracis* are classified as Tier 1, Category A because of the suitability for use as biothreat agents. 3. To rule out *B anthracis* and *B cereus* biovar *anthracis* requires a combination of morphological assessment including gram stain and three simple tests. Automated systems have no place in the identification of this organisms due to the danger of misidentification and close relationship to other species within the *Bacillus* genera.  |  |  |  |  | | --- | --- | --- | --- | | Characteristic | B. anthracis | B. cereus | B cereus biovar anthracis | | Hemolysis | - | + | - | | Motility | - | + | + | | Penicillin | S | R | S/R |  1. All patient samples can be handled using BSL-2 practices. Wear gloves and gown and work in the certified Class II biosafety cabinet when performing activities having a high potential for aerosol production. 2. *B anthracis* and *B cereus* biovar *anthracis* are large gram positive rods, often occurring inlong chains. Both organisms grow well on BAP and CHOC, but not MAC.     http://www.ndhealth.gov/microlab/docs/bt/Bacillus%20anthracis%20Identification%20Flowchart.pdf   1. Colonies are round with irregular edges, flat of slightly convex with a ground glass appearance. There are often comma-shaped projections from the edge of the colony. They have a tenacious consistency that when teased with a loop, will stand up like beaten egg whites.     https://www.health.ny.gov/guidance/oph/wadsworth/bacillus\_anthracis.pdf   1. Perform all testing in a BSC. Perform catalase and motility. 2. *B anthracis* and *B cereus* biovar *anthracis* cannot be ruled out if the isolate fulfills the following characteristics:    * Large Gram positive rods    * Growth on BAP is non-pigmented, white colonies having a ground glass appearance with edges that are slightly undulated (Comma-shaped)    * No growth on MAC    * Non-hemolytic on BAP    * Catalase positive    * Non-motile 3. Contact the LRN Reference Laboratory for guidance regarding the need to refer the isolate for confirmatory testing.     Sentinel Level Clinical Laboratories Guidelines 2018 | | | | | | | |
|  |  | |  | |  | | |  |
| ***Brucella spp.*** | These are the steps to recognize, presumptively identify and rule out this organisms from clinical specimens in Sentinel Clinical Laboratories.   1. Brucellosis is a zoonotic disease, with four species causing infections in humans. *B. melitensis, B. suis, B. abortus,* and *B. canis. B. melitensis* is thought to be the most virulent and causes the most severe cases of brucellosis. 2. Brucellosis is seen in people that work with animals that have not been vaccinated, people that ingest unpasteurized dairy products and laboratory workers. 3. All patient samples can be handled using BSL-2 practices, wearing gloves and gown and work in the certified Class II biosafety cabinet. Subcultures should be performed in the BSC. Plates should be taped shut and all further testing should be performed only in the BSC.      1. *Brucella spp.* is an aerobic, small, gram negative coccobacillus. Be suspicious of slow growing organisms especially from cultures of lymph nodes. The Gram stain will be a very tiny, faintly staining gram negative coccobacillus.     Courtesy of Dr. Bryan Schmitt DO   1. In blood culture media, Brucella can appear as Gram positive cocci or rods, including some cocci in chains, suggesting Streptococci.     Courtesy of Dr. Bryan Schmitt DO     1. *Brucella spp*. will be pinpoint or not visible at 24 hours and will be small, creamy, and non-hemolytic on Chocolate or Sheep Blood agar at 48 hours. This organism will not grow on MacConkey agar, but can grow on CNA agar. As soon as *Brucella* *spp.* is suspected, the culture should be worked up under the BSC.     http://www.ndhealth.gov/microlab/docs/bt/Brucella%20Identification%20Flowchart.pdf   1. *Brucella spp.* grows without the addition of a staph streak, is non-hemolytic. 2. *Brucella spp*. is oxidase positive, catalase positive, nitrate positive and urease positive. 3. Since this organism does grow slowly, blood culture may require an extended incubation. 4. Further identification should not be attempted with commercial automated systems due the danger of aerosol production. 5. *Brucella spp.* cannot be ruled out if the isolate fulfills the following characteristics:  * Faintly staining tiny gram negative coccobacillus. * Growth on BAP without a staph streak * No growth on MAC. * Oxidase positive. * Catalase positive. * Urease positive. * Nitrate positive  1. Contact the LRN Reference Laboratory for guidance regarding the need to refer the isolate for confirmatory testing.     Sentinel Level Clinical Laboratories Guidelines 2018 | | | | | | | |
| ***Burkholderia mallei and Burkholderia pseudomallei*** | These are the steps to recognize, presumptively identify and rule out these organisms from clinical specimens in Sentinel Clinical Laboratories.   1. *Burkholderia mallei* is the agent that causes glanders. It is non-motile, aerobic, Gram negative coccobacillus. 2. *Burkholderia pseudomallei* causes melioidosis. It is oxidase positive, aerobic, Gram negative bacillus that is straight air slightly curved. 3. Glanders is seen in people that work with infected horses. 4. Melioidosis is detected in the water in the rice paddies in Vietnam and Thailand and can be reactivated in returning soldiers from the Vietnam War. 5. All patient samples can be handled using BSL-2 practices, wearing gloves and gown and work in the certified Class II biosafety cabinet. Subcultures should be performed in the BSC. Plates should be taped shut and all further testing should be performed only in the BSC. 6. *Burkholderia mallei* is a small, straight or slightly curved Gram negative coccobacillus. Cells are arranged in pairs, parallel bundles or Chinese-letter form.     http://www.ndhealth.gov/microlab/docs/bt/Burkholderia%20mallei%20Identification%20Flowchart.pdf   1. *Burkholderia pseudomallei* is a straight or slightly curved Gram negative bacillus.   Gram-stain of B pseudomallei isolate VB976100, phase contrast microscopy with a 100 x oil immersion objective (Microscope Leica DM4000B).  Healthandwelfare.idaho.gov   1. *Burkholderia mallei* will be pinpoint or not visible at 24 hours. They will be smooth, gray, and translucent without pigment or distinctive odor on Chocolate or Sheep Blood agar at 48 hours. This organism will grow on MacConkey agar, but do not look like typical Gram negative rods. As soon as *Burkholderia mallei* is suspected*,* theculture should be worked up under the BSC.     http://www.ndhealth.gov/microlab/docs/bt/Burkholderia%20mallei%20Identification%20Flowchart.pdf   1. *Burkholderia pseudomallei* will form small, smooth creamy colonies which will gradually change to dry, wrinkled colonies after a few days.   burkholderia pseudomallei, bacteria, grown, blood agarburkholderia pseudomallei, illness, melioidosis, categorized, acute, localized, infection  https://pixnio.com/science/microscopy-images/glanders-burkholderia-mallei/burkholderia-pseudomallei-the-illness-melioidosis-can-be-categorized-as-an-acute-or-localized-infection   1. *Burkholderia mallei* is catalase positive, oxidase negative but may be oxidase positive, indole negative and does not grow at 42ºC. 2. *Burkholderia pseudomallei* is catalase positive, oxidase positive, indole negative and will grow at 42ºC. 3. Further identification should not be attempted with commercial automated systems due the danger of aerosol production. 4. *Burkholderia mallei* cannot be ruled out if the isolate fulfills the following characteristics:  * Small gram negative coccobacillus. * Growth on BAP is smooth, gray, translucent in 2 days without hemolysis or odor * Weak or no growth on MAC. * Oxidase variable, mostly negative. * Catalase positive. * Indole negative. * Does not grow at 42ºC.  1. *Burkholderia pseudomallei* cannot be ruled out if the isolate fulfills the following characteristics:  * Typical Gram negative bacillus, can demonstrate bipolar staining * Growth on BAP is creamy that change to dry and wrinkled in a few days. * Non-hemolytic * Musty or earthy odor. * Grows on MAC. * Oxidase positive. * Catalase positive. * Indole negative. * Grows at 42ºC.  1. Contact the LRN Reference Laboratory for guidance regarding the need to refer the isolate for confirmatory testing.     Sentinel Level Clinical Laboratories Guidelines 2018 | | | | | | | |
| ***Yersinia pestis*** | These are the steps to recognize, presumptively identify and rule out this organism from clinical specimens in Sentinel Clinical Laboratories.   1. *Yersinia pestis,* the causative agent of the plague, is a zoonotic pathogen transmitted from animals and their infected fleas. Most cases occur in the late winter to summer months and are associated with flea contact. 2. *Yersinia pestis* is acquired by humans through the bite of infected fleas, direct contact with contaminated tissue or inhalation. 3. All patient samples can be handled using BSL-2 practices, wearing gloves and gown and work in the certified Class II biosafety cabinet. Subcultures should be performed in the BSC. Plates should be taped shut and all further testing should be performed only in the BSC. 4. *Yersinia pestis* is a plump Gram negative coccobacilli that are seen mostly as single cells or pairs, which may exhibit bipolar staining, referred to as safety-pin like.   [Image result for yersinia pestis gram stain](https://www.google.com/url?sa=i&rct=j&q=&esrc=s&source=images&cd=&ved=2ahUKEwig6IKo7LfkAhUPIjQIHSn9AiMQjRx6BAgBEAQ&url=https://doh.sd.gov/lab/resources/bt/yersinia/gram.aspx&psig=AOvVaw0UwDbM4oEzSlL7z8A4zRLx&ust=1567709812375697)  South Dakota Department of Health   1. *Yersinia pestis* will be grey-white, translucent and usually too small to be seen as individual colonies at 24 hours. At 48 hours, colonies are gray white to slightly yellow and opaque. Older culture may have a ‘fried egg’ appearance. This organism will grow on MacConkey agar as small, lactose fermenting colonies. As soon as *Yersinia pestis* is suspected, the culture should be worked up under the BSC.   [Related image](http://www.google.com/url?sa=i&rct=j&q=&esrc=s&source=images&cd=&cad=rja&uact=8&ved=2ahUKEwiQlPKK7bfkAhVBIDQIHR2lBvQQjRx6BAgBEAQ&url=/url?sa%3Di%26rct%3Dj%26q%3D%26esrc%3Ds%26source%3Dimages%26cd%3D%26ved%3D%26url%3Dhttps://www.health.ny.gov/guidance/oph/wadsworth/yersinia_pestis.pdf%26psig%3DAOvVaw3CriOSi57O29Di98euouv9%26ust%3D1567709928335011&psig=AOvVaw3CriOSi57O29Di98euouv9&ust=1567709928335011)  https://www.health.ny.gov/guidance/oph/wadsworth/yersinia\_pestis.pdf   1. *Yersinia pestis* grows without the addition of a staph streak, is non-hemolytic. 2. *Yersinia* *pestis* is oxidase negative, indole negative, urease negative and catalase positive. 3. Further identification should not be attempted with commercial automated systems due the danger of aerosol production. 4. *Yersinia pestis* cannot be ruled out if the isolate fulfills the following characteristics:  * Plump gram negative bacillus. * Slow growing on BAP after 24 hours * Non lactose fermenter on MAC. * Oxidase negative. * Urease negative * Indole negative. * Catalase positive.  1. Contact the LRN Reference Laboratory for guidance regarding the need to refer the isolate for confirmatory testing.     Sentinel Level Clinical Laboratories Guidelines 2018 | | | | | | | |
| ***Francisella tularensis*** | These are the steps to recognize, presumptively identify and rule out this organism from clinical specimens in Sentinel Clinical Laboratories.   1. *Francisella* *tularensis* is a zoonotic pathogen with an extremely wide host range that includes mammals, birds and amphibians. The primary hosts are believed to be small rodents. Ticks, mosquitos and biting flies have been implicated as capable vectors. 2. *Francisella tularensis* is the agent that caused tularemia. Most patients have a recent history of animal contact (bite, scratch) of arthropod bite. It is also a known risk factor for laboratory workers due to its low infectious dose (<10 organisms) and ability to be acquired by inhalation. 3. All patient samples can be handled using BSL-2 practices, wearing gloves and gown and work in the certified Class II biosafety cabinet. Subcultures should be performed in the BSC. Plates should be taped shut and all further testing should be performed only in the BSC. 4. *Francisella tularensis* is a tiny, faintly staining Gram negative coccobacillus. The Gram stain may be difficult because the cells are minute and faintly staining. It is smaller than *Haemophilus influenzae* and bipolar staining is not a distinctive feature of *Francisella*.     https://www.health.ny.gov/guidance/oph/wadsworth/francisella\_tularensis.pdf   1. *Francisella tularensis* requires cysteine supplementation. It may grow on BAP at first, but not subsequent sub cultures. 2. *Francisella tularensis* will be gray-white, opaque on Chocolate agar and usually too small to be seen at 24 hours on Sheep Blood agar. At 48 hours, the colonies are white to grey, opaque, flat with an entire edge, smooth and shiny. This organism will not grow on MacConkey agar. As soon as Francisella is suspected, the culture should be worked up under the BSC. Be suspicious of slow growing organisms especially from cultures of lymph nodes.   [Image result for francisella growth on sb](http://www.google.com/url?sa=i&rct=j&q=&esrc=s&source=images&cd=&ved=2ahUKEwjh5Zi847fkAhVgFjQIHTLvCZwQjRx6BAgBEAQ&url=http://www.asmscience.org/content/book/10.1128/9781555817381.mcm11.ch46?crawler%3Dtrue%26mimetype%3Dapplication/pdf&psig=AOvVaw2m5jAFMHwaWlPAJIE2NZOe&ust=1567707429962380)  https://www.health.ny.gov/guidance/oph/wadsworth/francisella\_tularensis.pdf   1. *Francisella tularensis* grows without the addition of a staph streak, is non-hemolytic. 2. *Francisella tularensis* is oxidase negative and catalase negative or weakly positive. 3. Since this organism does grow slowly, blood cultures may require an extended incubation. Incubate for 10 days and perform terminal sub cultures. 4. Further identification should not be attempted with commercial automated systems due the danger of aerosol production. 5. *Francisella tularensis* cannot be ruled out if the isolate fulfills the following characteristics:  * Faintly staining tiny gram negative coccobacillus. * Scant growth on BAP without staph streak. * Not growing on MAC. * Oxidase negative. * Catalase negative or weakly positive. * Β-lactamase positive.  1. Contact the LRN Reference Laboratory for guidance regarding the need to refer the isolate for confirmatory testing.     Sentinel Level Clinical Laboratories Guidelines 2018 | | | | | | | |
| **Rule-Out Algorithm** | Sentinel Level Clinical Laboratories Guidelines 2018 | | | | | | | |
| **References** | MDH Sentinel Laboratories Bioterrorism Wet Workshop 2018 reference manual  MCVI 3.2 Safety in the Microbiology Lab | | | | | | | |
|  |  | | | | | | | |
| **Training Plan/ Competency Assessment** | **Training Plan** | | | | | **Initial Competency Assessment** | | |
| 1. Employee must read the procedure. 2. Employee will observe trainer performing the procedure. 3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer. | | | | | 1. Direct observation. | | |
|  |  | | | | | | | |
| **Historical Record** |  |  | |  | | |  | |
|  | **Version** | **Written/Revised by:** | | **Effective Date:** | | | **Summary of Revisions** | |
| 1 | Susan DeMeyere | | 9/27/2019 | | | Initial Version | |
| 2 | Susan DeMeyere | | 10/4/2021 | | | Added procedure section for further instructions | |
|  |  | |  | | |  | |
|  |  |  | |  | | |  | |  |  |
|  |  | |  | | |  | |
|  |  | |  | | |  | |
|  |  |  | |  | | |  | |