

Fungal Diseases Laboratory b-roll description

- :07 The laboratorian is setting up polymerase chain reaction (PCR) tests to detect fungal DNA in cerebrospinal fluids (CSF) from patients with suspected fungal meningitis. She is working in a biosafety cabinet to prevent contamination. The laboratorian is using a pipet to collect the fungal DNA extracted from a patient CSF.
- :09 The DNA is then dispensed with the pipet into a plate containing the test reagents that will be used to copy the DNA.
- :15 The pipet tip is discarded and a new tip is used to collect DNA from a second patient sample. The process is repeated.
- :33 The laboratorian is changing pipets after discarding the tip. She is dividing a DNA sample into several different tubes for storage.
- 1:02 The laboratorian is demonstrating proper use of a pipet to transfer reagents from one vessel/tube to another.
- 1:54 The laboratorian is sub-culturing *Exserohilum rostratum*, the fungus responsible for most of the outbreak infections, by transferring a portion of growing fungal cultures from one culture plate to a fresh plate. She is working in a biosafety cabinet with culture plates.
- 2:04 She is using a sterile loop to capture fungal hyphae from the growing culture and transfer the fungus to a fresh plate.
- 2:33 The laboratorian places the cover on the culture plate to prevent contamination with other organisms.
- 2:39 A special container is kept in the biosafety cabinet for disposal of contaminated loops.
- 2:57 The laboratorian is transferring another culture. She keeps the culture plates face-down while working in the hood.
- 3:37 Black mold is growing in the culture plate. The laboratorian scrapes fungi from the outer edge of this growth for transfer to a fresh plate.
- 4:45 The electric burner is used to sterilize the transfer loop between each fungal transfer.
- 5:03 The laboratorian is performing antifungal susceptibility testing. She holds the drug plates to the light to check for fungal growth.
- 5:36 The culture plate contains a plastic test strip impregnated with antifungal drugs that diffuse into the culture medium. This strip is called the Etest, and the fungus is not growing near the top of the strip where the drug concentration is higher.
- 5:44 The numbers on the Etest indicate the concentration of the antifungal drug in micrograms/mL on that portion of the strip. The highest amount of drug is at the top of the strip.

- 6:08 Broth microdilution is another antifungal susceptibility test. Antifungal drugs are dispensed in decreasing dilutions across the plate. A mirror is used to detect the presence or absence of fungal growth in the plate.
- 6:29 The laboratorian checks the plate for fungal growth. The lowest drug dilution at which the fungus does not grow is known as the minimum inhibitory concentration (MIC). The MICs for each drug are recorded by the laboratorian.
- 7:10 The laboratorian is working in a biosafety cabinet sub-culturing fungi growing on media slants. She is sterilizing the loop in the electric burner between cultures to prevent contamination.
- 8:27 The laboratorians are checking fungal cultures for growth.
- 8:45 The laboratorian is streaking a culture plate with fungus in the biosafety cabinet. She is using disposable plastic loops to transfer fungus from growing slants to clean plates. She disposes of the plastic loops in the biohazard container between each transfer to avoid contamination of the cultures.
- 9:32 The laboratorian is counting and recording the number of fungal cultures.
- 9:49 The laboratorian is adding DNA to an agarose gel for sizing and visualization. The DNA was amplified from patient cerebrospinal fluid using the polymerase chain reaction (PCR) and may contain DNA from fungal organisms such as *Exserohilum rostratum*.
- 10:00 Each well in the gel receives a different PCR product DNA, and each represents a different patient sample. An electric field will be applied to the gel and the products will move through the agarose. Smaller pieces of DNA move faster than large pieces. A blue dye is added to the PCR products to visualize the PCR products as they move through the gel.
- 10:23 A fresh pipet tip is used before pipetting the next PCR product in order to avoid contamination of the samples.

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