LAB WORK 9.

Subject: Staining Microbial Structures: Endospore (Spore).

Session Purpose: Methods for detection and visualization of intracellular polymers stored.

Objectives:

1.Acquaintancewith the methods of Endospore staining.
2. Endospore staining by Ozheshko method.
3. Performexercises 1, 2.

An endospore is a special resistant, dormant structure formed within a cell that protects the microorganism from adverse environmental conditions. Although endospores are relatively uncommon in bacterial cells, they can be formed by seven genera of bacteria.

Because endospore are highly refractive, they can be detected under the light microscope but cannot be differentiated from inclusions of stored material.

Endospores cannot be stained by ordinary methods, such as simple staining and Gram staining, because the dyes do not penetrate the wall of the endospore.

The most commonly used endospore stain is the Schaeffer-Fulton endospore stain and Ozheshko method.

Lab Exercise 1.Schaeffer-Fulton endospore staining.

Methodical instructions: Malachite green, the primary stain, is applied to a heat-fixed smear and heated to steaming for about five minutes. The heat helps the stain to penetrate the endospore wall. SO, when malachite green is applied to a heat-fixed smear of bacterial cells, the stain penetrates the endospores and stains them green.

Then the penetration is washed for about 30 seconds with water to remove the malachite green stain from all of the sell`s parts except the endospore.

Next, safranin, a counterstain, is applied to the smear to stain portions of the cell other than endospore. When safranin (red) is then applied, it stains the remainder of the cells red or pink.

In a properly prepared smear, the endospore appear**green** within **red or pink cells**.

Procedure:

1.Prepare the smear-Initially make a thin smear of bacteria on a clean, non-greasy dirt free slide, dry it and heat fix the smear on slide.

2. Apply the dye-Flood the smear with malachite green and heat the slide by steaming, which will cause enhanced penetration of the impermeable spore coat of endospore by malachite green. Steam for 5 minutes, without letting the smear to dry by adding more stain to the smear from time to time.

3. Time to Counter-stain-Wash the slides under slowly running tap to remove excess dye and then apply counter stain safranin for 30 seconds. After then wash the slide and make it complete dry using absorbent/ blotting paper.

4. Colourfull cells are waiting-Place a drop of oil-immersion on slide at smear area and enjoy viewing green colored endospore in pink coloured cells. Do not forget to use 100x objective else you will not be able to see the beauty of these tiny creatures.

The endospore appear**green** within **red or pink cells**.

Lab Exercise 2.*Ozheshko* endospore staining.

Methodical instructions: The spores are stained by using special process that help dyes to penetrate the spore wall known as ***Ozheshko*** staining procedures and can be performed following these sequential steps.

Procedure:

1 step. Stainedcellswith spores.

1. On fat-free glass prepare thin bacterial smear.
2. The slide is air dried, without heat fixation.
3. 0.5%solution of hydrochloricacid (HCl) is applied to a smear and heatedfor 2 min, holding high above theburner flameuntilvapors.
4. Then the smear is washed for about 30 seconds with water to remove the acid.
5. Smearcoverfilter paper. The fuchsinZiehl's solution, the primary stain, is applied to a smear and heated about 7 minutes. Until thevapor(notto boiling).As evaporationof dyeadded toit periodically, not giving the drugto dry.It is important thatthe dyeevaporates, but the paper does notdry out.
6. Remove the paper. Wash the specimen with water to remove the dye. Carefullyblottedwith filter paper.

As a result ofthis treatment,cells werestainedwith the sporesevenly. Next,desaturatethe cytoplasm of cells, but not spores.

2 step. Discolorationof the cytoplasmof cells.

1. 1 % solution of hydrochloricacid (HCl) or sulfuric acid (H_2SO_4) is applied to a smear for decolorizing of cytoplasm of cells during 15-30 seconds.
2. Wash the decolorizedsmear by water to remove the acid.

3 **step.** Counterstain.

1. Stainedthe decolorizedsmear with methyleneblue for 2minutes.

In a properly prepared smear, the endospore appear**red** within **blue cells.**

Equipment:

- Microscope
- Slide
- Several cover glasses
- Dropper bottle of water
- Disinfectant tray
- Culture of sporulation Bacteria in slant tubes
- Inoculation loop
- Burnerflame
- Immersion oil
- Gloves
- Filterpaper.
- Staining material:

- Malachite green
- Safranin
- Loeffler's methylene blue
- Methylene blue
- Ziehl's solution (fuxin)

- Mordant ant decolorizingagents:

- 0.5%HCl

-1%HCl

-1 % H_2SO_4