

BioMI 290Case Study in Microscopy (The Fatal Flu)The Disease

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Types of pathogens could be viral respiratory pathogens such as influenza types A & B. Human Respiratory Syncytial Virus, paramyxovirus, measles, mumps, human parainfluenza viruses, Human Adenovirus, Rhinovirus, Human Coronavirus, henipaviruses, metapneumovirus, mycoplasma, chlamydia. Also, they can be bacteria, such as Legionella, Pseudomonas, and Haemophilus.

The Gram Stain Results

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2) Gram stain is one of the important differential stains. Gram stain differentiates bacterial cells with an outer lipid layer and thinner cell wall (Gram- type) from those with thicker cell walls and no outer membrane (Gram+ type).

The crystal violet-iodine complex gets trapped inside bacteria with a thick cell wall, and those cell (Gram +) remains purple. In bacteria with a thin cell wall, the crystal violet-iodine complex leaks out, <sup>-when?</sup> and the cells become colorless. Because of counterstain with safranin, the Gram- cells will be stained pink. The end result is purple-colored Gram(+) cells and pink-colored Gram(-) cells. The viral pathogens, not bacterial pathogens, will be ruled out.

In this case, the results indicate that patient is possibly affected by Gram(+) bacteria, and patient 2,3, 4 are hard to tell whether they are affected by bacteria or not, the pink stained cells are hard to be distinguished whether they are human cells or bacteria cells.

Patient 3 may possibly be affected by Gram(+) bacteria.

*So what pathogen can you rule out?*  
(-1) the cells in the sputum samples are too large to be bacteria when compared to the bacteria in the positive controls; the background on patient 1 sputum

	Patient 1	Patient 2	Patient 3	Patient 4
<b>Sputum sample</b>	2 Spherical purple stains that are about 5um in diameter each, it looks like some smaller spherical purple stains around 1um in diameter each are grown in grape-like clusters.	Spherical pink stains that are about 10um in diameter each.	Spherical purple pink stains that are around 10um in diameter each.	Spherical pink stains that are around 10um in diameter each. It seems there are some smaller spherical stains grown in chains.

### The DFA Results

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3) The direct fluorescent antibody (DFA) technique detects the presence of a particular antigen, typically a specific protein on the surface of a virus, bacterium or other microbe.

Fluorescent chemicals are attached to the constant region of an antibody. The antibody binds to generate a very specific, very sensitive protein tag if the antigen is present.

In this case, the DFA results showed that patient 1 and patient 4 was not affected by the four viruses, Human adenovirus, Influenza, Human Respiratory Syncytial Virus (RSV), Rhinovirus, as the DFA results showed similar pattern as the healthy person, these samples do not emit light, indicating there are no presence of the viral antigen. Patient 2 and Patient 3 are possibly affected by Human RSV, but not the other three viruses. The two samples only emit light because of the presence of Human RSV antigen.

*was detected using a fluorescent microscope*