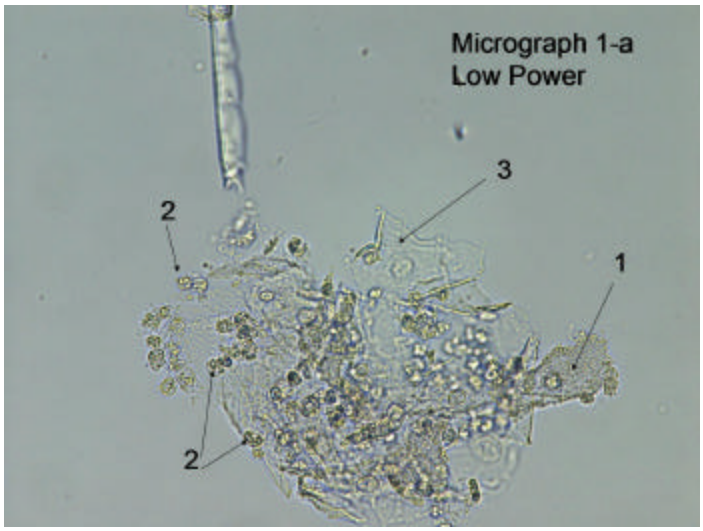
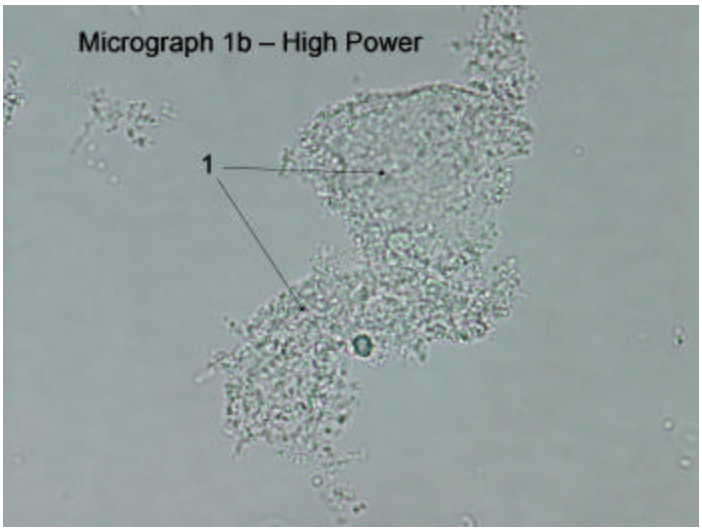
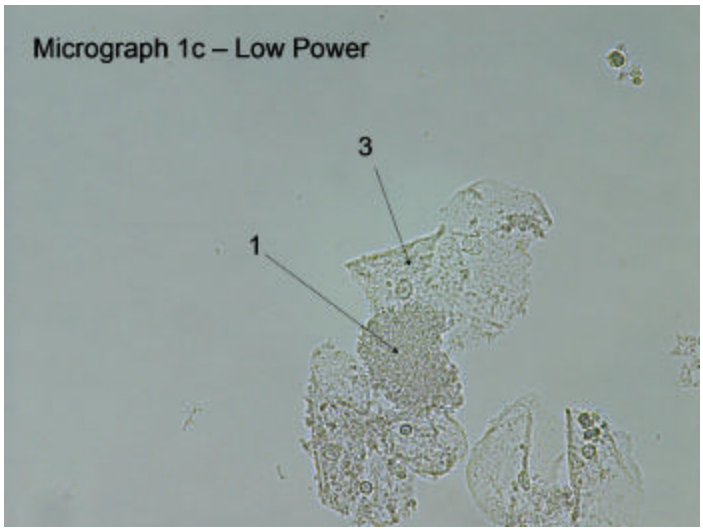
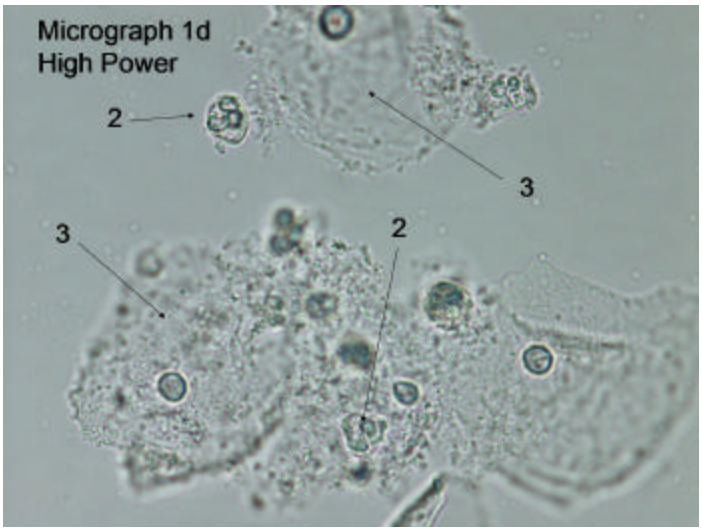
Wet Mount Proficiency Test 2007A
CRITIQUE



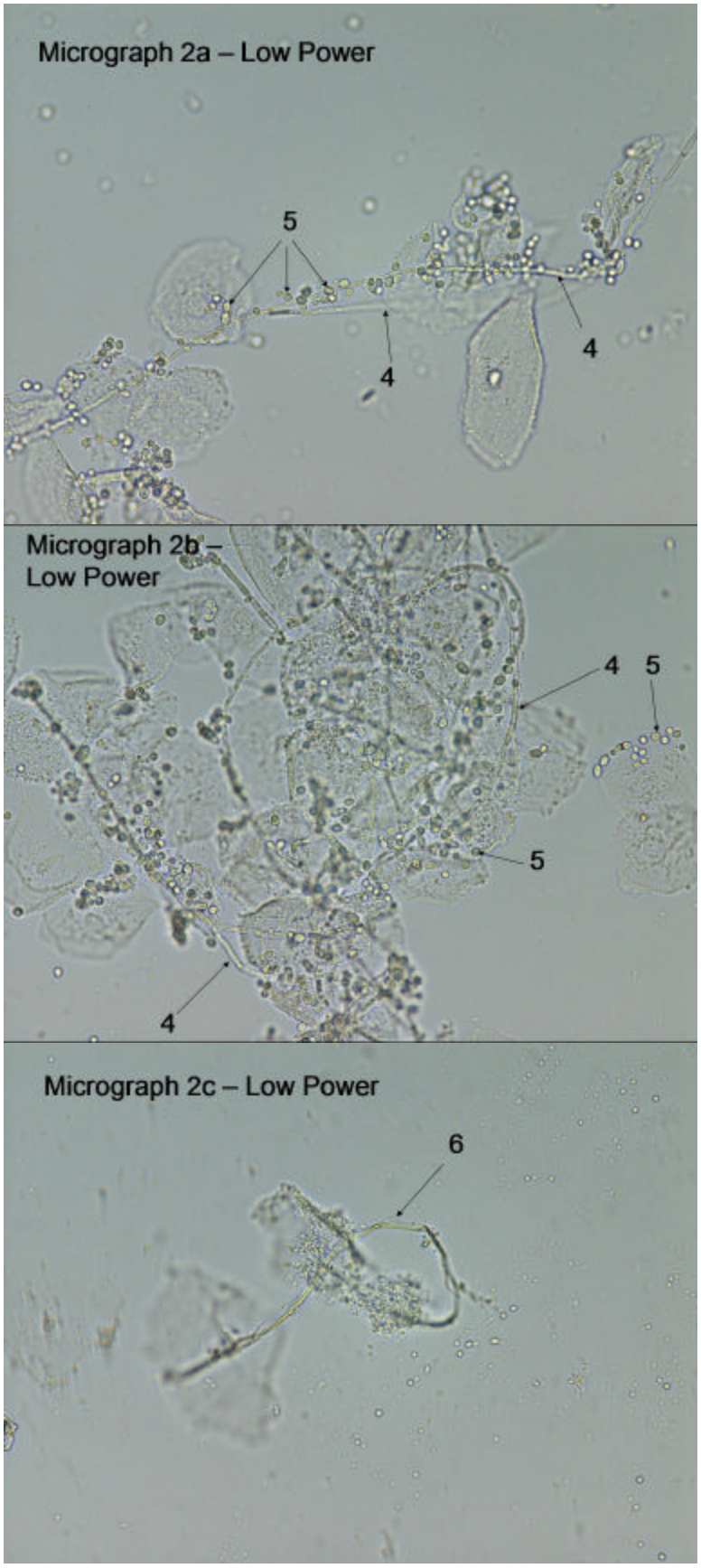






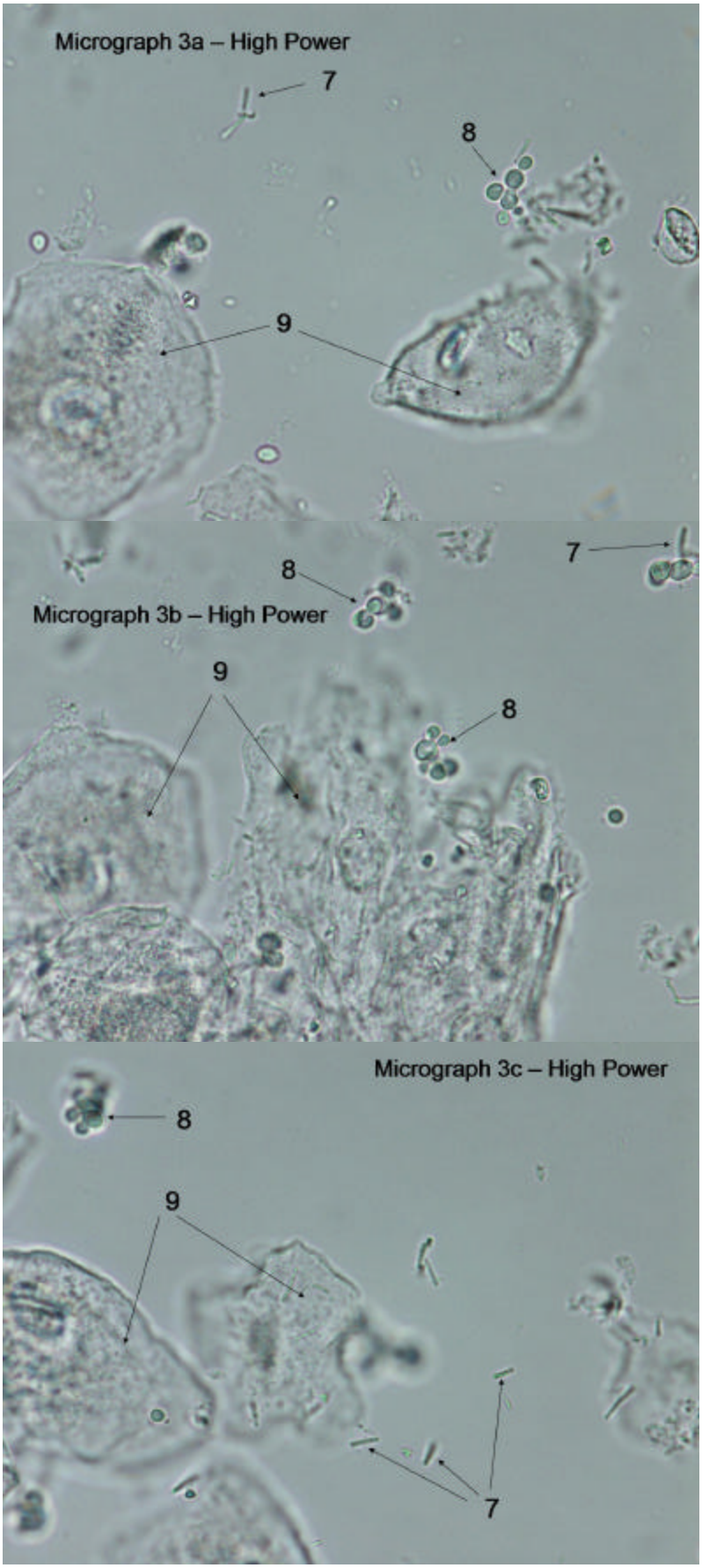
<u>Micrograph 1 – Expected Results</u>

Item 1: Squamous Epithelial cell – a clue cell
Item 2: White Blood Cell
Item 3: Squamous Epithelial cell – not a clue cell



Micrographs 2a, 2b, 2c

Item 4: Pseudohyphae **Item 5:** Yeast cell **Item 6:** Artifact



Patient 3, Micrographs 3a, 3b, 3c

Item 7: Bacteria **Item 8:** Yeast **Item 9:** Squamous epithelial cell – not a clue cell

Clue Cell (Item # 1)
Clue cells are squamous epithelial cells that are covered with a thick matte of bacterial cells and are associated with bacterial vaginosis. The traditional definition of a clue cell is that the bacterial overgrowth is so thick that all cellular detail (such as the cell nucleus and the cellular edge) is totally obscured. As described in previous critiques, it is sometimes possible to detect the nucleus in a clue cell by using the fine focus knob to focus throughout the cell.

Squamous Epithelial Cell, Not a Clue Cell (Items #3 and #9)
The examples shown here are typical of a normal squamous epithelial cell. The cell nucleus and the cell boundary are clearly observed.

White Blood Cell (Item #2)
These cells are larger than Red Blood Cells and are approximately the same size as the nucleus of a squamous epithelial cell. You can easily compare the relative size of the white blood cell with the size of the squamous epithelial cell nucleus by screening on low power. Under high power, the nuclear detail of the WBC becomes apparent. The WBC is characterized by a multilobed nucleus (usually three distinct lobes can be identified). The following micrograph shows four WBC in this field. The three lobes may not always be present (at least in the same focal field), but you should be able to see them by using the fine focus to focus up and down.

Yeast Cell (Items #5 and #8)
The cells vary in shape from circular to oval and are approximately 7.5 microns (µm) in diameter, making them slightly smaller than red blood cells. Yeast cells are more variable in shape than a red blood cell (see the description below). It is often possible to pick out the thick cell wall of the yeast cell. In budding yeast cells, a single bud is observed.

Red Blood Cell – no examples in this challenge, but may be confused with yeast
The cells are approximately 8 microns in diameter (smaller than white blood cells by about half, but larger than yeast cells). RBC possess a cell membrane, while yeast have a thick cell wall. Red blood cells are slightly larger and more uniform in shape than yeast cells. In fresh samples, RBC will be round. Because of the biconclave nature of RBC, a dimple may be observed in the middle of the cell. After 5-10 minutes, the RBC will crenate and get a jagged appearance. It is therefore best to analyze the wet mount sample as quickly as possible. The longer you wait, the more likely it is that RBC will crenate and lose their characteristic appearance.

Pseudohyphae (Items #4)
These are fragile tube-like structures that arise through elongation of the yeast form of Candida. Pseudohyphe may demonstrate a terminal swollen remnant of the original yeast cell. They are called pseudohyphae because they lack true branching as seen with mold like fungi. The side walls are parallel to each other which is an important characteristic that helps separate pseudohyphae from artifact whose side walls vary in width. Small oval structures called bastoconidia are often seen attached along the length of the pseudohyphae. The blastoconidia are smaller in size when compared to the yeast form of Candida. Pseudohyphae are most commonly seen to be associated with yeast cells or budding yeast. It is, however, possible to see pseudohyphae in the absence of yeast cells.

Artifact (Item #6)

One of the more troublesome artifacts is fibers that are sometimes confused with pseudohyphae. There are a few tips that may help in the differentiation.

1. Fibers are generally larger in size that pseudohyphae
2. Pseudohyphae have parallel sides with a consistent dimension between the sides while fibers show variable widths along the fiber.
3. Fibers tend to be birefringent. That is they change color when focusing up and down on the object. Colors are often gold or blue and result from the microscope light being refracted by the fiber.

Bacteria (Item #7)

The bacteria shown in micrograph 3 are characteristic of lactobacilli, which is normal flora in women following the onset on menses and will persist as normal flora until menopause. As described in the educational challenge, bacterial vaginosis results from the synergistic association of *Gardnerella vaginosis* (a small gram-positive or gram-variable rod) and *Mobiluncus* sp. (a small curved anaerobic gram-rod). These are the bacteria which coat squamous eqithelial cells as serve as a "clue" to the diagnosis of BV – hence the term "Clue Cell".

Educational Purposes Only – Not For Grading

Educational Picture #1: Gram stain of Squamous Epithelial Cell

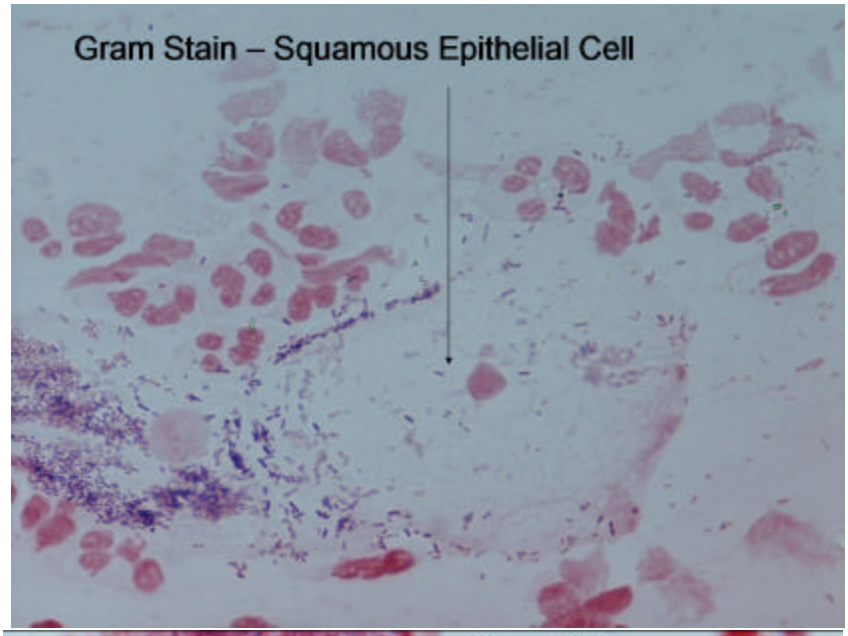
1. Normal appearance of an epithelial cell.
2. Nucleus is seen as a red circle
3. A few bacteria are seen on the surface

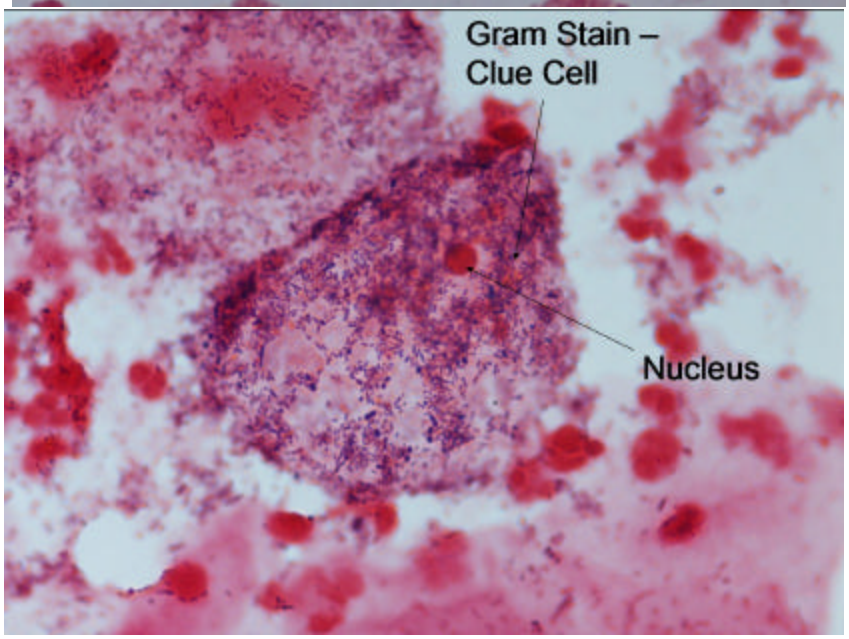
Educational Picture #2: Gram stain of Clue Cell

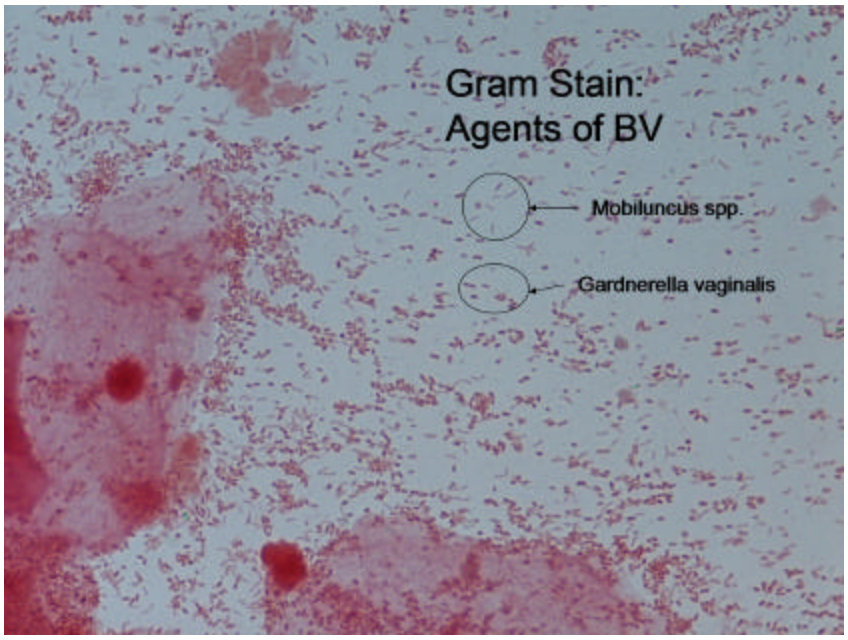
1. The surface is covered with bacteria – both coccobacilli and small, curved rods.
2. A nucleus is seen as a small red circle

Educational Picture #3: Gram stain from a patient with bacterial vaginosis

1. Gardnerella vaginosis – shown as red (gram negative) coccobacillus (small rounded rod)
2. Mobiluncus spp. – shown as red (gram negative), slender curved rods
3. Both are normally found in the vagina (i.e., normal flora), along with Lactobacillus – a large gram positive rod which is the predominant bacterial species. In BV the normal balance of bacteria in the vagina is disrupted. Lactobacilli decrease in number and the pH of the vagina increases (due to a decrease in the production of lactic acid as the population of Lactobacillus decreases). Other bacteria (such as Gardnerella and Mobiluncus) increase in number and degrade proteins in the vagina to produce volatile chemical compounds called amines. These amines give off the unpleasant "fishy" odor associated with BV. The odor of the amines are amplified by alkaline compounds (bases) such as potassium hydroxide (hence, the Whiff test) and semen (hence the common complaint of an unpleasant odor almost immediately after intercourse).


Gram Stain – Squamous Epithelial Cell


Gram Stain –
Clue Cell
Nucleus


Gram Stain:
Agents of BV
Mobiluncus spp.
Gardnerella vaginalis