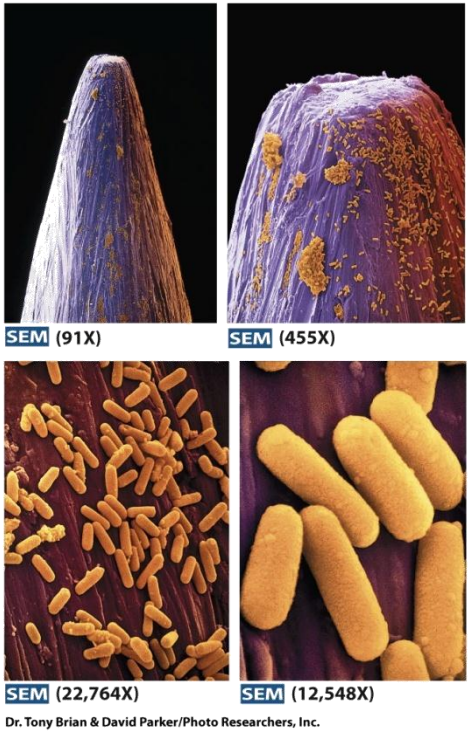
MICROSCOPY AND STAINING

CHAPTER 3



CHAPTER 3 MICROSCOPY AND STAINING- Most of the material in this chapter is much better presented in Bio 209 laboratory where you can actually do hands on work with the microscope and sample preparation. Hence, I will cover only those topics needed to understand material in subsequent chapters.

http://www.youtube.com/watch?v=77J4i3uyna0

Compound light microscope for those not taking the laboratory

http://www.youtube.com/watch?v=fToTFjwUc5M

Electron microscope for those not taking The laboratory

http://www.youtube.com/watch?v=O6JVAUgz0MU

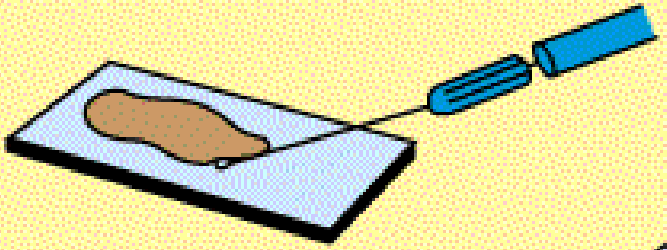
Fluorescent microscope for those not taking the laboratory

Techniques of Light Microscopy

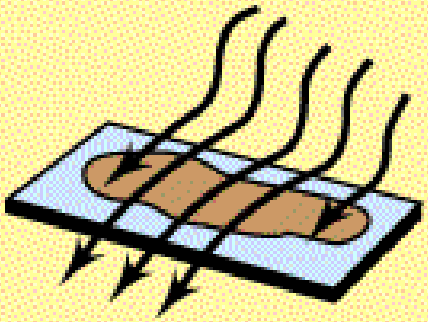
Smears: microorganisms from a loopful of medium are spread onto the surface of a glass slide, air dried, then **heat fixed.**

Heat fixation:

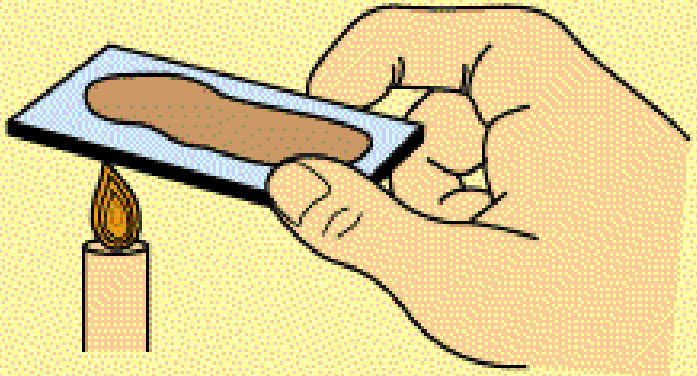
1. kills the organism
2. adheres the organism to the slide
3. allows the organism to better accept staining



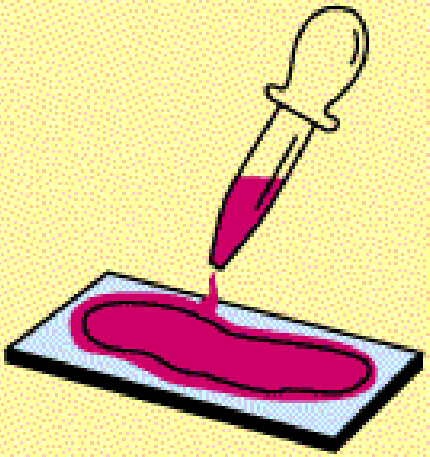
Spread culture in thin film over slide



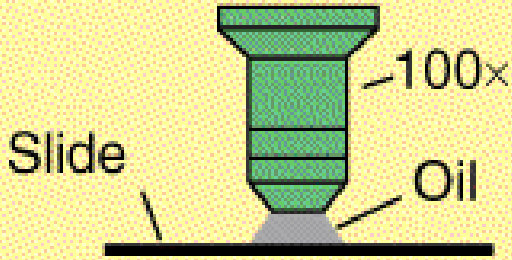
Dry in air



Pass slide through flame to fix



Flood slide with stain; rinse and dry



Place drop of oil on slide; examine with 100× objective

100x objective x 10x ocular (eyepiece) = 1000x magnification of sample (about the limit of resolution of a light microscope

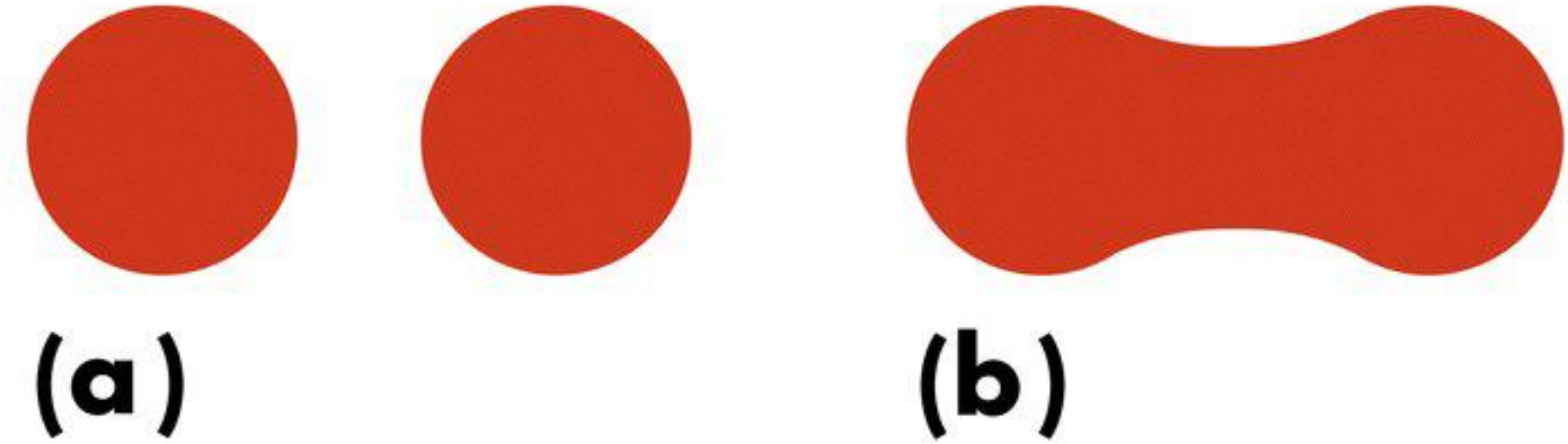


Figure 3-5 Microbiology, 6/e

Fig. 3.5 Resolution

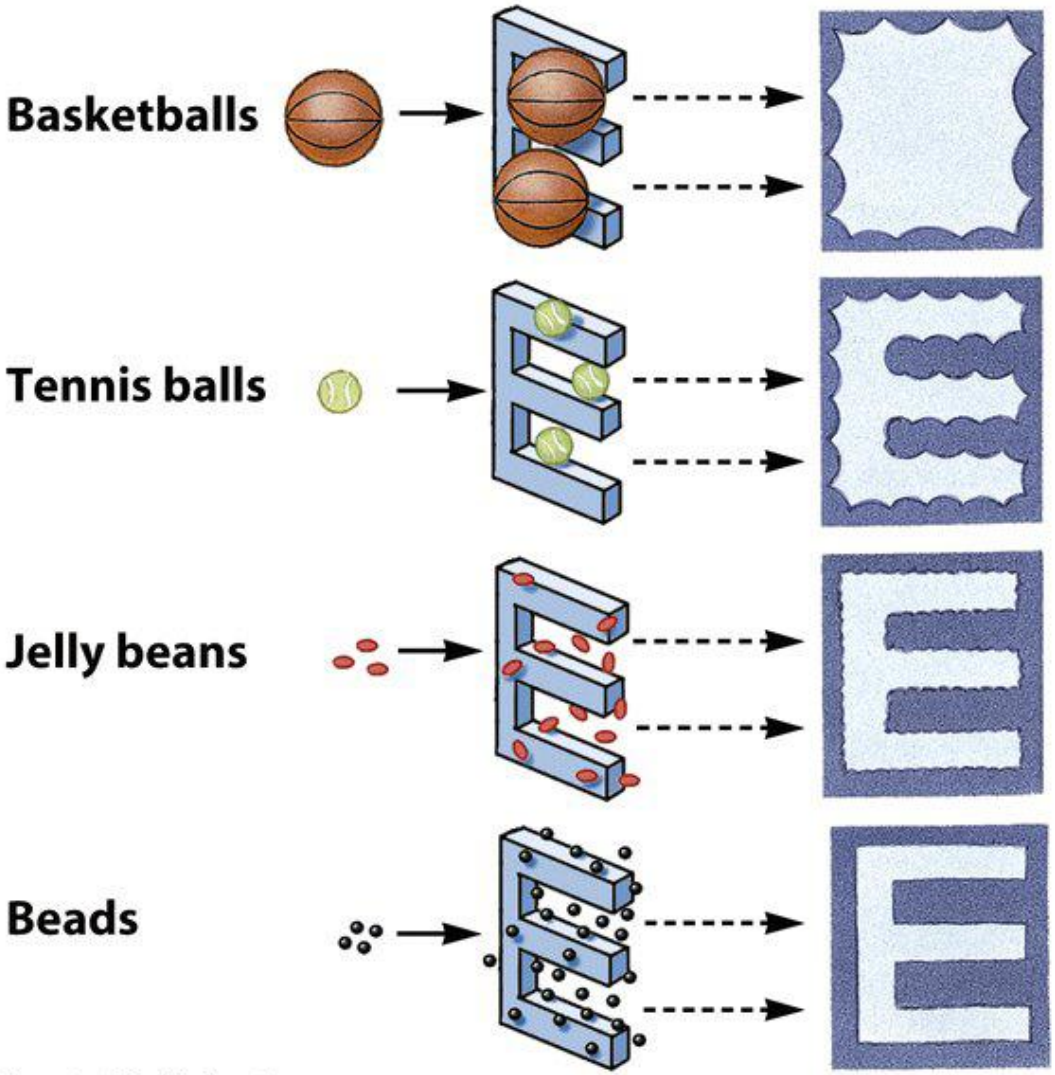
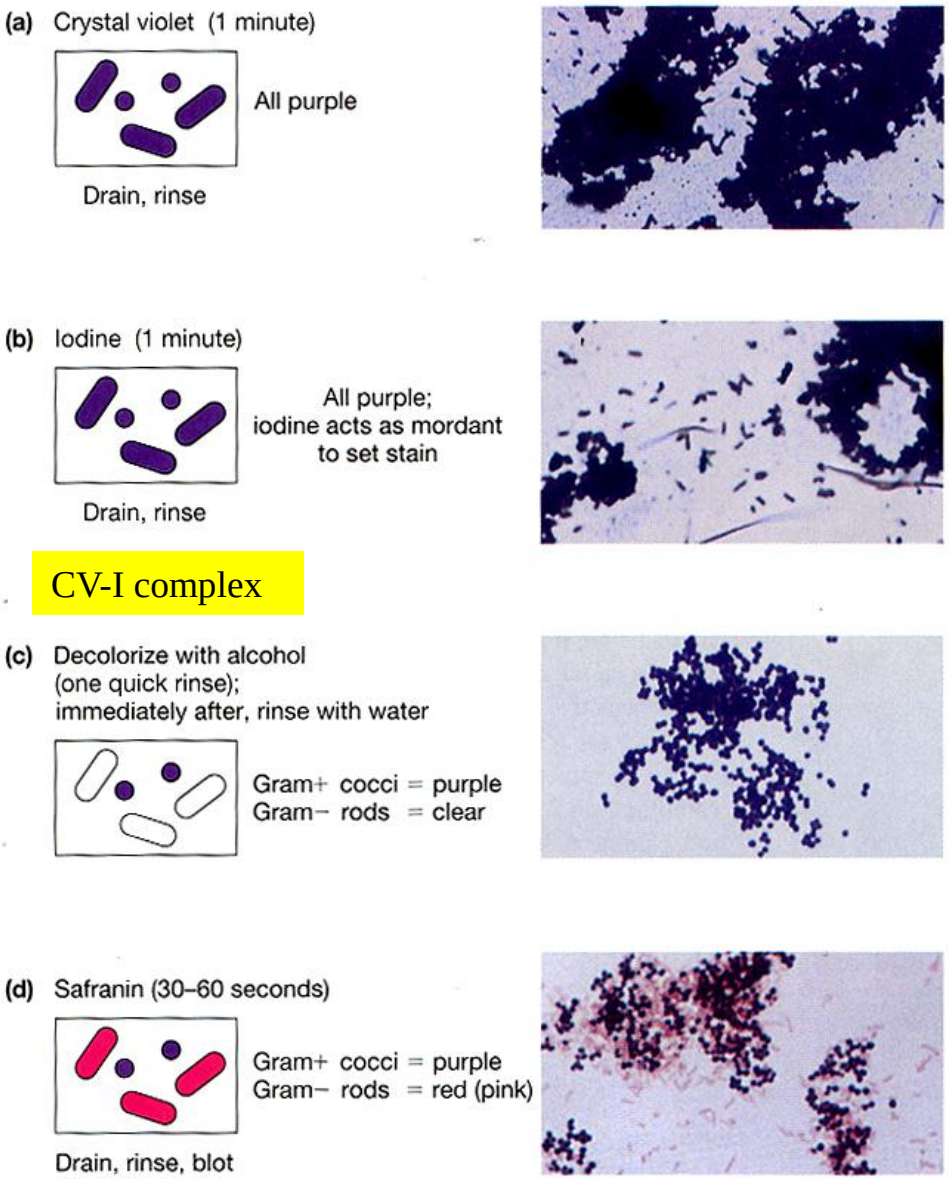


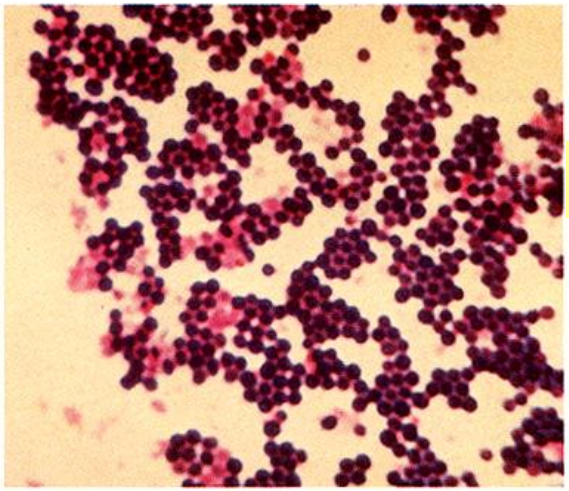
Figure 3-6 Microbiology, 6/e

Smaller objects (corresponding to shorter wavelengths) can pass more easily between the arms of the letter E, defining it more clearly and producing a sharper image

Fig. 3.6 An analogy for the effect of wavelength on resolution

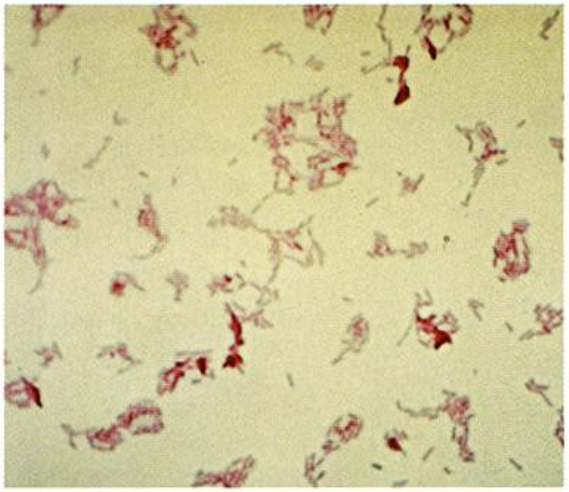


CV-I complex



(e)

Gram +

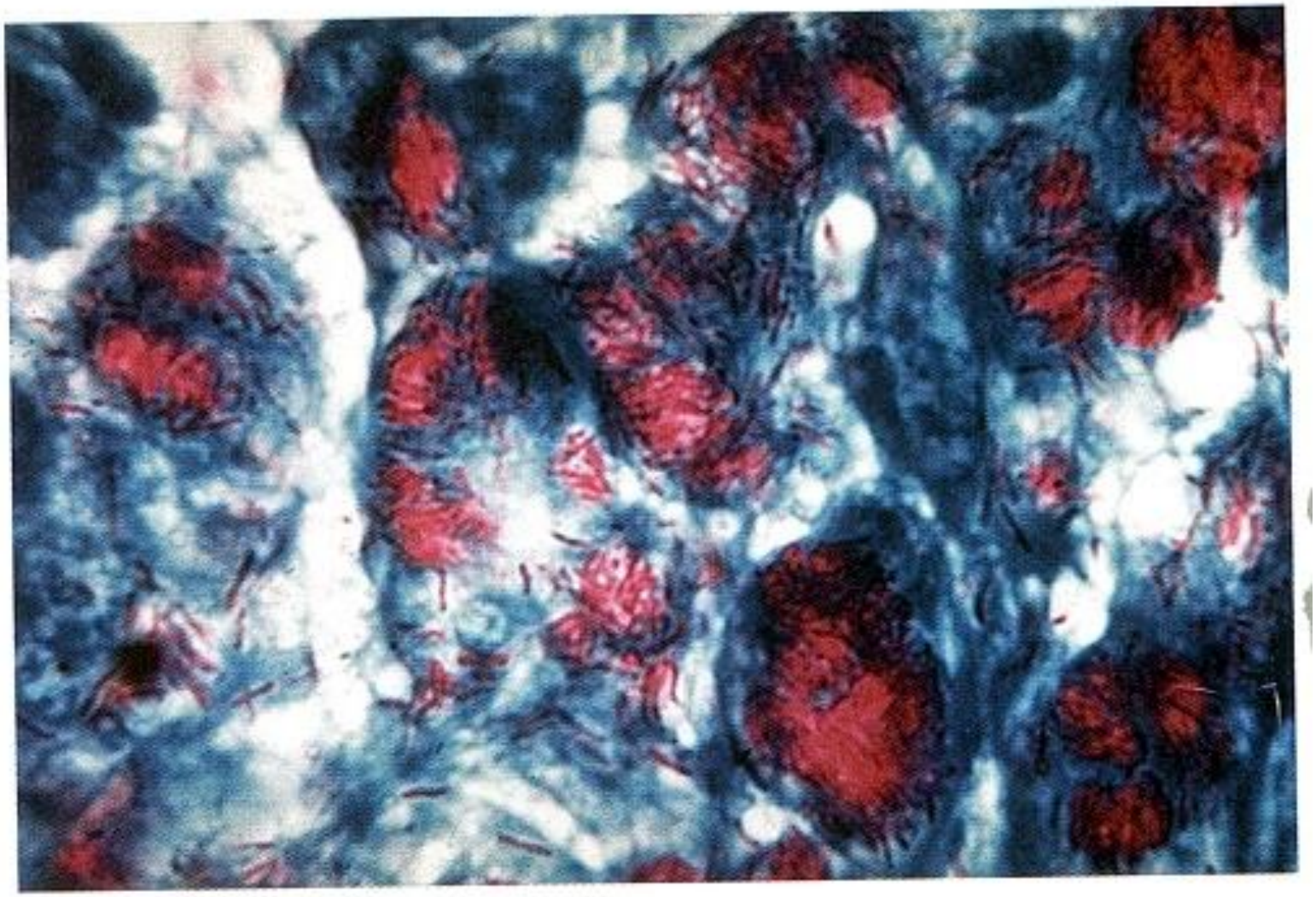


(f)

Gram -

CV-I complex is not extracted from Gram positive orgs.

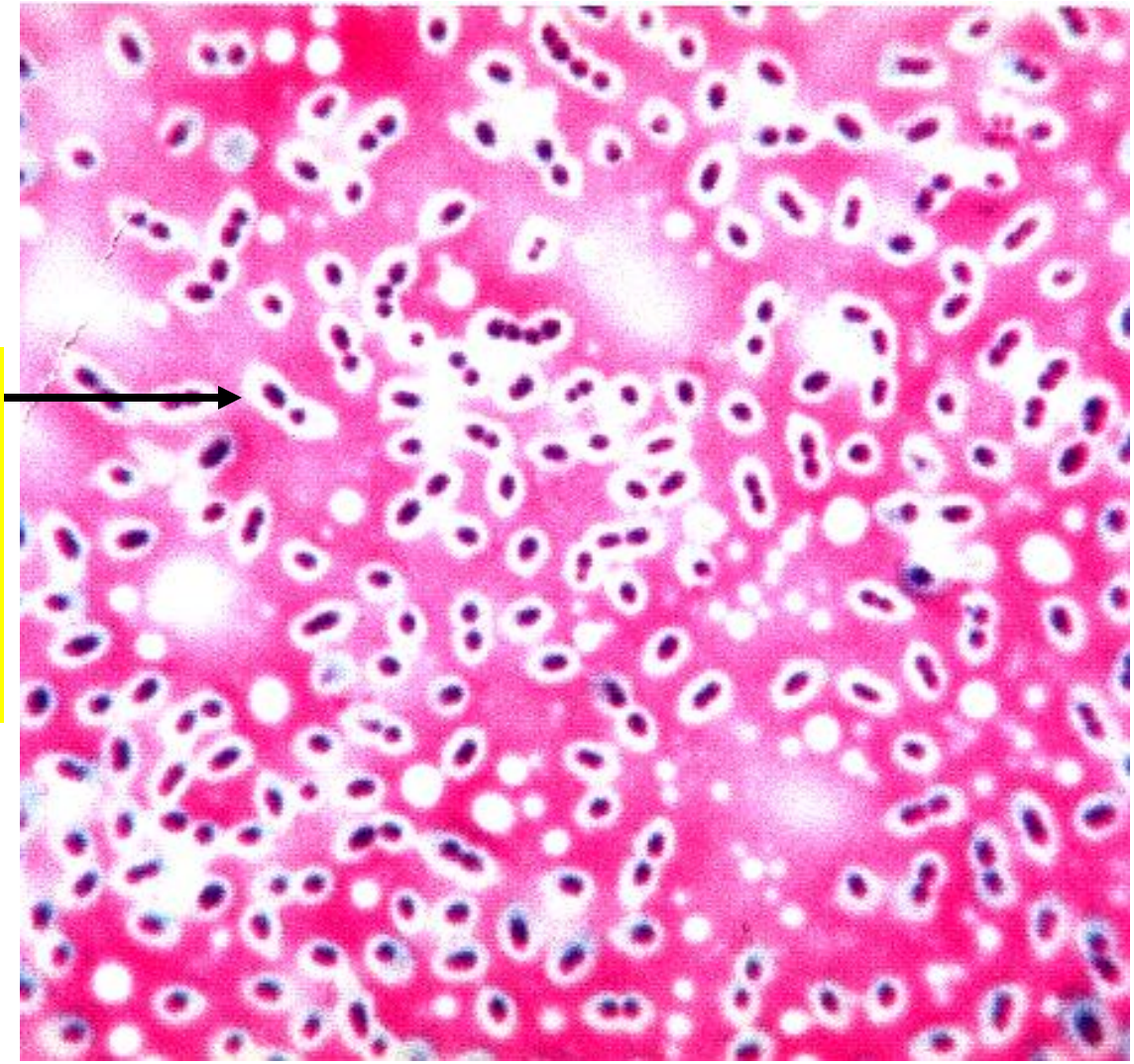
Fig. 3.3 Gram stain



This stain produces vivid red color in acid fast organisms such as *Mycobacterium leprae*, the cause of leprosy or *Mycobacterium tuberculosis*, the cause of tuberculosis**.**

Fig. 3. 31 The Ziehl-Neelsen acid-fast stain

The clear area around the crystal violet stained organism is the capsule- which does not accept the India ink .



Negative staining for capsules reveals a clear area (the capsule, which does not accept stain) in a dark pink background of India ink and crystal violet counter stain

Fig. 3.32 Negative staining

New TB Therapy Shows Promise A new three-drug therapy for tuberculosis appears to be highly effective and could dramatically shorten treatment times, according to a new study.

After two weeks of treatment, more than 99 percent of TB bacteria was killed in 85 patients, *BBC News* reported. Of the three drugs used in the therapy, one is new and another is not yet licensed.

The findings were published in *The Lancet*. Larger studies are now being conducted to further assess the therapy.

Currently, TB patients have to take drugs daily for six months. Drug-resistant TB is much more difficult and can require up to two years of treatment, *BBC News* reported.

TB kills about 1.4 million people a year worldwide, mainly in poor nations. **Remarkably, the 3-in-1 combo seems to work just as well against drug-resistant strains of TB, which are now spreading around the world. TB Alliance estimate the new regimen would eliminate the use of injectables and could slash the cost of MDR-TB therapy by as much as 90%. TB is currently the biggest killer of people with AIDS.**

Govt. gene sleuths stop superbug that killed 6 WASHINGTON (AP) — Over six frightening months, a deadly germ untreatable by most antibiotics spread in the nation's leading research hospital. Pretty soon, a patient a week was catching the bug. Scientists at the National Institutes of Health locked down patients, cleaned with bleach, even ripped out plumbing — and still the germ persisted. By the end, 18 people harbored the dangerous germ, and six died of bloodstream infections from it. Another five made it through the outbreak only to die from the diseases that brought them to NIH's world-famous campus in the first place. **It took gene detectives teasing apart the bacteria's DNA to solve the germ's wily spread**, a CSI-like saga with lessons for hospitals everywhere as they struggle to contain the growing threat of superbugs. It all stemmed from a single patient carrying a fairly new superbug known as KPC — **Klebsiella pneumoniae** that **resists treatment by one of the last lines of defense, antibiotics called carbapenems.** **Test** after test never found the bug on hospital workers' hands. Tainted objects like the ventilator couldn't be ruled out — but NIH adopted more complex and expensive decontamination, using robot-like machines to spray germ-killing hydrogen peroxide into the tiniest of crevices in all affected rooms and equipment. Still, November brought more bad news: The outbreak strain had escaped the ICU, as two patients who'd never been there now were carrying it. A new isolation room was built, and all 200-plus patients in the hospital started undergoing rectal testing. The outbreak now is over, the last carrier found in December. But NIH isn't dropping its guard. The isolation room remains, used every time one of the seven outbreak survivors returns to the hospital for their ongoing research studies — because they still carry the strain. Those rectal tests continue, hospital-wide once a month, to be sure no new KPC strain sneaks in. **Bacterial sequencing is becoming fast and cheap enough for most large hospitals to use during tough outbreaks,** said Dr. Lance Peterson, microbiology and infectious disease director at NorthShore University HealthSystem in Evanston, Ill.

From the antibiotic lecture in BIO 308

Carbapenems represent a relatively new group of bactericidal antibiotics with a two-part structure.

Example- Primaxin:

Imipenem/cilastatin is a broad spectrum beta-lactam antibiotic containing equal quantities of imipenem and cilastatin

TABLE 3.3

Comparison of Staining Techniques

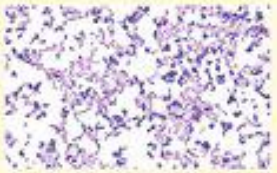
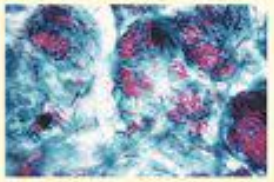
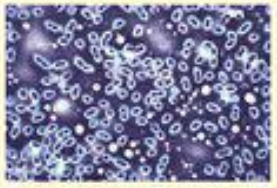
Type	Examples		Result	Uses
Simple Stains				
Use a single dye; do not distinguish organisms or structures by different staining reactions	Methylene blue Safranin Crystal violet →		Uniform blue stain Uniform red stain Uniform purple stain	Shows sizes, shapes, and arrangements of cells
Differential Stains				
Use two or more dyes that react differently with various kinds or parts of bacteria, allowing them to be distinguished	Gram stain		Gram +: purple with crystal violet Gram −; red with safranin counterstain Gram-variable: intermediate or mixed colors (some stain + and some − on same slide) Gram-nonreactive: stain poorly or not at all	Distinguishes Gram +, Gram −, Gram-variable, and Gram-nonreactive organisms
	Ziehl-Neelsen acid-fast stain		Acid-fast bacteria retain carbolfuchsin and appear red. Non–acid-fast bacteria accept the methylene blue counterstain and appear blue	Distinguishes members of the genera *Mycobacterium* and *Nocardia* from other bacteria
Special Stains	Negative stain		Capsules appear clear against a dark background	Allows visualization of organisms with structures that will not accept most stains, such as capsules

Table 3-3 part 1 Microbiology, 6/e

TABLE 3.3

Comparison of Staining Techniques

Special Stains

Identify various specialized structures	Flagellar stain		Flagella appear as dark lines with silver, or red with carbolfuchsin	Indicates presence of flagella by building up layers of stain on their surface
	Schaeffer-Fulton spore stain		Endospores retain malachite green stain. Vegetative cells accept safranin counterstain and appear red	Allows visualization of hard-to-stain bacterial endospores, such as members of genera *Clostridium* and *Bacillus*

Table 3-3 part 2 Microbiology, 6/e