Gram Stain Worksheet

The Gram stain procedure was originally developed by the Danish pharmacist Hans Christian Gram to differentiate pneumococci from Klebsiella pneumonia. The Gram stain is particularly useful in the presumptive diagnosis of bacterial meningitis, bacterial pneumonia, bacteriuria, gonorrhoea, and pyogenic infections of the brain, lung, abdomen, pelvis, and wounds.

Statistically about one third of all patients admitted to general hospitals have, or develop, infections, a large proportion of which are caused by bacteria and fungi. The investment of time spent in preparing and examining a Gram-stained smear when a specimen is first obtained often yields valuable results that cannot be obtained from culture of the specimen alone. The Gram-stained smear is also useful in judging the adequacy of the specimen obtained. In sputum and urine specimens, for example, a poorly collected or contaminated specimen can be recognised by the presence of many epithelial cells in the smear.

Information obtained from the Gram-stained preparation rarely permits definitive identification of organisms, but usually narrows the possibilities in diseases such as gas gangrene, pneumonia or meningitis, which have a variety of causative agents. Early diagnostic information obtained from Gram-stained smears often allows the physician to prescribe narrow-spectrum antimicrobial therapy, thereby reducing the risk of toxicity, superinfection, and the expense of broad-spectrum "poly-pharmacy".

Moreover, in certain infections, such as Vincent's angina (associated with fusobacteria and spirochetes), the organisms are not identifiable by the culture techniques employed in most diagnostic microbiology laboratories, and the Gram-stained smear together with the clinical findings form the basis for diagnosis.

Decide if these statements are true or false.
1 H. C. Gram developed the staining procedure because he had problems with pneumonia.
2 More than half of the patients in hospitals get an infection of some type.
3 Epithelial cells in the smear mean that the sample is of bad quality.
4 Thanks to the data from Gram-stain smear, we can identify a disease precisely.
5 In certain infections, the Gram-stain smear is the only basis for diagnosis.

Preparing a smear - video

Listening
A What are the steps to follow?
1 rinse with water
2 rinse with water
3 rinse with water
4 immediately rinse with water
5 dry the slide
6 decolourise with alcohol
7 put the Gram's iodine on (for 10 sec)
8 put safranin on (for 30 sec)
9 fix the smear
10 flood with crystal violet (for 30 sec)

B Answer these questions:
1 Why is Gram-stain called differential?
2 How long does it take to have a result?
3 What type of bacteria are resistant to penicillin?
4 Which of the stains is called mordant? What happens if you forget to use it?
5 For how long do you put alcohol on the stain?
6 What kind of the counter-stain is used here?
7 What do you do before you put the stain under the microscope?

Gram Stain : Use of English

The first consideration is the correct _____ (prepare) of the smear. Make a thin film of the material on a clean glass slide, using a sterile loop or swab. Air-dry, then heat-fix the slide by passing it several times through a flame. Failure to follow these _____ (direct) may cause staining artefacts and break the normal morphology of bacteria and cells.

The Gram staining _____ (proceed) itself involves the application of a solution of iodine (potassium iodide) to cells previously stained with crystal violet or gentian violet. The cells are next treated with a _____ (decolourise) agent such as 95 % ethanol or a mixture of acetone and alcohol. While Gram-positive bacteria retain iodine-dye complexes after the treatment with the decolourising agent, Gram-negative bacteria do not retain complexes when decolourised. To _____ (visual) decolourised Gram-negative bacteria, a red counterstain such as safranin is used after the decolourisation treatment.

Grammar Point

Verb patterns

53.2 Complete each sentence with one of the following verbs (in the correct form):

answer	apply	be	forget	listen	live
lose	make	pay	read	try	use

1 He tried to avoid ...answering... my question.
2 Could you please stop _____ so much noise?
3 I enjoy _____ to music.
4 I considered _____ for the job, but in the end I decided against it.
5 Have you finished _____ the newspaper yet?
6 We need to change our routine. We can't go on _____ like this.
7 I don't mind you _____ my phone, but please ask me first.
8 My memory is getting worse. I keep _____ things.
9 I've put off _____ this bill so many times. I really must do it today.
10 What a stupid thing to do! Can you imagine anybody _____ so stupid?
11 I've given up _____ to lose weight – it's impossible.
12 If you gamble, you risk _____ your money.

53.4 Use your own ideas to complete these sentences. Use -ing.

1 She's a very interesting person. I always enjoy ...talking to her_____ .
2 I'm not feeling very well. I don't fancy _____ .
3 I'm afraid there aren't any chairs. I hope you don't mind _____ .
4 It was a beautiful day, so I suggested _____ .
5 It was very funny. I couldn't stop _____ .
6 My car isn't very reliable. It keeps _____ .

54.2 Complete each sentence with a suitable verb.

1 Don't forget ...to lock... the door when you go out.
2 There was a lot of traffic, but we managed _____ to the airport in time.
3 We couldn't afford _____ in London. It's too expensive.
4 We've got new computer software in our office. I haven't learnt _____ it yet.
5 Mark doesn't know what happened. I decided not _____ him.
6 We were all afraid to speak. Nobody dared _____ anything.

54.3 Put the verb into the correct form, to ... or -ing. (See Unit 53 for verbs + -ing.)

1 When I'm tired, I enjoy ...watching... television. It's relaxing. (watch)
2 I've decided _____ for another job. I need a change. (look)
3 Let's get a taxi. I don't fancy _____ home. (walk)
4 I'm not in a hurry. I don't mind _____ . (wait)
5 Tina ran in a marathon last week, but she failed _____ . (finish)
6 I wish that dog would stop _____ . It's driving me crazy. (bark)
7 Our neighbour threatened _____ the police if we didn't stop the noise. (call)
8 We were hungry, so I suggested _____ dinner early. (have)
9 Hurry up! I don't want to risk _____ the train. (miss)
10 They didn't know I was listening to them. I pretended _____ asleep. (be)