T-LEVELS | Institute for Apprenticeships & Technical Education

T Level Technical Qualification in Science

Occupational specialism assessment (OSA)

Laboratory Sciences

Assignment 2 - Part A

Assignment brief

v1.1: Additional sample material
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Assignment brief

Assignment 2

Part A

SAMPLE

Contents

Experimental practical

Scenario

You work in a microbiology laboratory for a research company. A doctor has asked you to run some tests to determine the causative bacteria responsible for a patient's skin infection that is proving difficult to treat. The result will enable the doctor to find a suitable antibiotic to treat the patient.

You have been provided with an agar plate containing bacterial colonies grown from a sample collected by a clinician.

You must perform initial Gram staining experiments. This will identify whether the bacterial infection is likely to be a result of a Gram-positive or Gram-negative bacterial species.

You will need to complete the following tasks:

- use laboratory equipment to carry out an experiment to assist in identifying the likely cause of bacterial infection
- evaluate data from the test results using appropriate statistical techniques
- report your findings

Task 1

You have three hours to complete tasks 1(a) and 1(b).

1(a) Carry out Gram staining in order to identify the causative bacteria following the provided standard operating procedure (SOP). Observe the stained bacteria using a light microscope with a x100 oil immersion lens and record your observations and results using a suitable format. During the practical you will be observed by an assessor who will make judgements on your ability to carry out the experiment with due care.

1(b) From your recorded observations and using the information below (from Tripathi N, Sapra A. Gram Staining. StatsPearls, 2021), identify which species of bacteria you suspect is present in the sample. Explain and justify why you have identified the species you have as the likely source of infection.

(12 marks)

Findings on Gram stain that suggest underlying bacterial infections:

- Gram-positive cocci in clusters: usually characteristic of *Staphylococcus* species such as *S. aureus*
- Gram-positive cocci in chains: usually characteristic of *Streptococcus* species such as *S. pneumoniae, B group streptococci*
- Gram-positive cocci in tetrads: usually characteristic of *Micrococcus* spp
- Gram-positive bacilli, thick: usually characteristic of *Clostridium* spp., such as *C. perfringes*, *C. septicum*
- Gram-positive bacilli, thin: usually characteristic of *Listeria* spp
- Gram-positive bacilli branched: usually characteristic of *Actinomyces* and *Nocardia*

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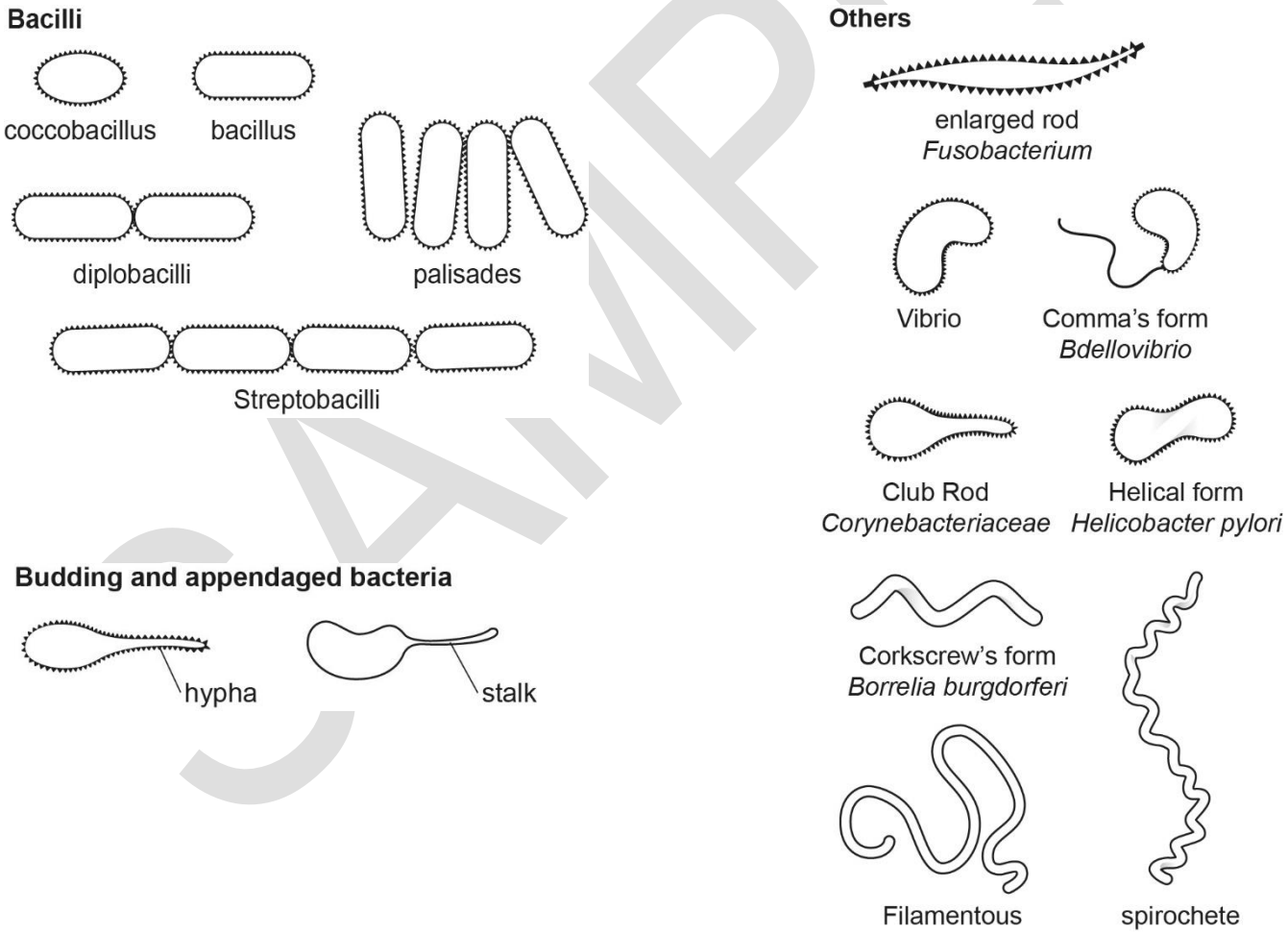
- Gram-negative diplococci: usually characteristic of *Neisseria* spp., such as *N. meningitidis*

(**Note:** *Moraxella* spp., and *Acinetobacter* spp., are often diplococcal in morphology. Acinetobacter can sometimes appear as Gram-positive cocci, and they can be pleomorphic)

- *Coccobacilli*: usually characteristic of *Acinetobacter* spp., and they can be Gram-positive, Gram-negative, or Gram variable
- Gram-negative bacilli, thin: usually characteristic of *Enterobacteriaceae*, such as *E. coli*
- *Coccobacilli*: usually characteristic of *Hemophilus* spp., such as *H. influenzae*
- curved: usually characteristic of *Vibrio* spp., *Campylobacter* spp., such as *V. cholerae*, and *C. jejuni*
- thin needle shape: usually characteristic of *Fusobacterium* spp

Figure 1 – Basic morphological differences between bacteria



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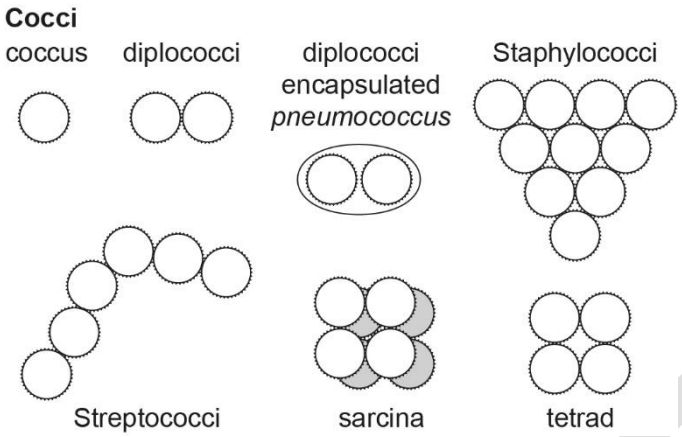
Cocci
coccus
diplococci
diplococci
encapsulated
pneumococcus
Staphylococci
Streptococci
sarcina
tetrad

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Standard operating procedure (SOP)

Process title: Gram staining to determine the bacterial species in a sample

Introduction

Apparatus

- frosted microscope slides
- forceps to hold slides whilst fixing/rinsing
- access to sink with tap (create a rack using secured long glass rods that run across the sink)
- a slide staining rack per work area
- microscope with x4, x10, x40 and x100 achromatic objectives, which when used with an x10 lens has a magnification range of x40 to x1000 and a spring-loaded front lens that retracts the lens into the objective on contact with the side
- immersion oil
- microscope wipes
- slide labels and/or pencil
- sterile inoculating loops
- sterile distilled water
- Bunsen burner/hot plate to fix slides
- prepared culture of bacteria on an agar plate
- agar plates and growth media
- Gram staining reagents kit to include:
  - distilled water in a bottle
  - crystal violet
  - Gram iodine solution
  - Gram's differentiator (need a safe store as this is flammable)
  - safranin
- Pasteur pipettes (larger volume for Gram staining if not using a bottle with a pourer lid or squeezy bottle)
- alcohol wipes or equivalent to wipe down
- digital timer
- paper towels/blue roll

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Safety considerations

Iodine solutions are toxic. Above 1 mol dm^{-3} the solutions are harmful so inhalation, ingestion, or skin contact should be avoided.

Ethanol and acetone are flammable so extra care is required if using a Bunsen burner rather than a heat plate.

They both cause irritation to skin, eyes and intoxication when ingested or inhaled for a long period of time.

Follow local control of substances hazardous to health (COSHH) and risk assessments when performing all staining procedures.

Method

Hucker's modification of Gram stain for examination of smears:

- label the slides
- begin preparing a smear by adding a drop of distilled water to the slide
- sterilise the inoculating loop in the Bunsen burner flame and allow it to cool
- use the cooled, sterilised inoculating loop to transfer the sample bacteria to the slide
- mix with the distilled water gently
- use the Bunsen burner or heat plate to heat gently to fix the sample
- flood the slide with 0.5% crystal violet and leave for 30 seconds
- tilt the slide, and rinse the slide gently with distilled water
- flood on sufficient (1%) Lugol's iodine (also known as Gram's iodine) to rinse off excess water, cover with fresh iodine and allow to remain for 30 seconds
- tilt the slide and wash off the iodine with water
- decolourise with 95% to 100% ethanol or acetone until colour ceases to run out of the smear
- rinse with water
- flood the slide with 0.1% counterstain safranin and leave to act for about 30 seconds to 1 minute (note: it can be counterstained for longer if using other dyes, for example, neutral red for about 2 minutes)
- wash briefly with water and blot dry
- examine the slide using an oil immersion objective to observe cell morphology and Gram status

Interpretation

Positive result Gram-positive organisms stain deep blue/purple. Negative result Gram-negative organisms stain pink/red.

Note: other counterstains (such as carbol fuchsin) used may give more intense colours.

Taken from
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/802769/TP_39i3.pdf

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Risk assessment

Substance, equipment, or procedure	Hazard	Risk	Controls
Bunsen burner/heat plate	Heat	Risk of burns.	Exercise caution around open flames. Leave on the yellow safety flame when not in immediate use. Turn off either Bunsen burner or heat plate when not required for protocol.
Bacterial plates	Microorganism	Infection	Use sterile loops to handle the colonies, either disposing of or flaming after use to sterilise. Use correct PPE. Wash hands after contact with plates.
Iodine	Toxic	Toxic if inhaled, ingested or in contact with skin.	Avoid leaving bottles open. Clean up any spillages immediately. Use correct PPE. Follow local COSHH forms.
Ethanol/Acetone	Flammable irritant	Risk of fire. Both cause irritation to skin and eyes. Cause intoxication when ingested or inhaled for a long period of time.	Do not use around the heat source required. Use correct PPE. Clean up and spillages. Avoid leaving bottles open. Follow local COSHH forms.

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Owner: Head of Assessment Design

Change History Record

Version	Description of change	Approval	Date of issue
v1.0	Additional sample material		01 September 2023
v1.1	Sample added as watermark	November 2023	20 November 2023

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