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T Level Technical Qualification in Healthcare Science

Occupational specialism assessment (OSA)

Assisting with Healthcare Science

Assignment 3 - Standard Operating Procedures

Assignment brief insert

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Standard Operating Procedures

Contents

Gram stain standard operating procedure

1. Introduction

The Gram stain is a complex and differential staining procedure that remains a useful test performed in microbiology laboratories. The staining procedure differentiates organisms of the domain bacteria according to cell wall structure. Organisms are classified according to their Gram staining reaction, Gram positive and Gram negative. The name ‘Gram’ comes from its inventor, Hans Christian Gram. Gram positive bacteria have thicker and denser peptidoglycan layers in their cell walls.

Iodine penetrates the cell wall in these bacteria and alters the blue dye to inhibit its diffusion through the cell wall during decolourisation. Gram positive bacteria must have an intact cell wall to produce a positive reaction. Gram negative cells which do not retain the methyl/crystal violet are stained by a counterstain. Neutral red, safranin or carbol-fuchsin may be used as the counterstain.

2. Safety considerations

Iodine is toxic and so inhalation, ingestion, or skin contact should be avoided.

Ethanol and acetone are flammable. 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.

3. Equipment Required

- appropriate waste containers for potentially contaminated materials
- frosted microscope slide
- forceps to hold slide
- sink with tap
- slide staining rack
- microscope with 100x and oil immersion lens, slides and cover slip
- immersion oil
- microscope wipe
- slide labels and/or pencil
- sterile inoculating loop
- sterile distilled water
- hot plate to fix slide
- agar plates
- Gram staining reagents kit to include:
    - distilled water in bottle
    - crystal violet

- Gram iodine solution
- Gram's differentiator (require safe store as this is flammable)
- safranin

4. Method

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

- prepare a smear (using the sample) and heat gently to fix
- flood the slide with 0.5% crystal violet and leave for 30 seconds
- tilt the slide, and rinse slide gently with water
- flood on sufficient (1%) Lugol's/Gram's iodine to rinse off excess water, cover with fresh Lugol's/Gram's 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 reaction

5. 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.

6. Quality control organisms

A culture containing Gram positive and Gram negative organisms may be used for quality control.

7. Technical Information

Common errors in Gram staining procedure

These are the errors that arise depending on the method and techniques used and which could result in a Gram positive organism staining Gram negatively. They include:

- smear preparations being too thick
- excessive heat during fixation
- low concentration of crystal violet

- excessive rinsing between steps during the staining procedure. This could cause the step of the crystal violet or the dye-iodine complex to be washed off from the Gram positive cells
- insufficient iodine exposure
- prolonged decolourisation
    - over-decolourising will lead to an erroneous result where Gram positive cells may stain pink to red indicating a Gram negative result, and under-decolourising will lead to an erroneous result where Gram negative cells may appear blue to purple indicating a Gram positive result - the degree of decolourising required is determined by the thickness of the smear
- excessive counterstaining
- uneven staining or decolourisation due to insufficient reagent being used for staining
- decolourising step missed or need to increase time of decolourising step

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

ELISA standard operating procedure

1. Introduction

This Interleukin-6 detection kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of the levels of Interleukin-6 protein in human serum. It is intended for use in clinical laboratories for diagnosis and management of patients with suspected inflammatory disorders that exhibit raised levels of Interleukin-6.

Interleukin-6 is a protein that in humans is normally produced as a normal part of the immune response, however persistent presence of the protein can be linked to a range of autoimmune disorders that involve persistent inflammation even when no infection is present.

2. Principle of the method

This kit is a solid phase antibody ELISA assay in which microtitre plate wells are precoated with antibodies that will bind to Interleukin 6. The patient's serum sample is added, during the first incubation step, any Interleukin-6 will be captured in the wells.

After washing out all the other components of the sample the Interleukin-6 protein, captured on the solid phase, is detected by the addition of conjugated anti-Interleukin-6 antibody.

In order to quantify the levels of Interleukin-6 present in the patient samples a series of reference samples with known concentrations of Interleukin-6 are run on the plate at the same time. The value returned by the spectrophotometer can then be compared with those obtained from the known concentrations to quantify the concentrations of Interleukin-6 present in the patient sample.

3. Specimen collection and storage

Blood collected by venipuncture should be allowed to clot naturally and completely, the serum must be separated from the clot as early as possible as to avoid haemolysis of the red blood cells.

Samples need to be stored at 2°C to 8°C for maximum of 3 days or in line with national guidelines.

4. Equipment required

- appropriate waste containers for potentially contaminated materials
- 1000 µl, and 200 µl pipette (single), disposable pipette tips
- 96 well plate
- microcentrifuge tubes
- marker pen

5. Reagents required

- positive control ready for use (800 pg/µl Interleukin-6 reference standard)
- specimen diluent ready for use
- specimen sample (patient serum)

6. Method for ELISA sample preparation and plate loading

1. Allow the reagents and serum samples to reach room temperature (18°C to 30°C) for at least 15 minutes before use.

2. Set out 10 microcentrifuge tubes and label 1 to 10.

3. In tubes labelled 2 to 10, add 250 µl of sample diluent to each and dispose of the pipette tip.

3. Prepare the reference curve samples by taking the 800 pg/µl Interleukin-6 reference standard and dilute it into the labelled microcentrifuge tubes as follows:

- tube 1: add 500 µl of the 800 pg/µl reference sample – this is the **800 pg/µl** reference sample
- tube 2: add 250 µl of the 800 pg/µl reference sample to the 250 µl of sample diluent and mix using the pipette – this is the **400 pg/µl** reference sample
- tube 3: add 250 µl of the 400 pg/µl reference sample to the 250 µl of sample diluent and mix using the pipette – this is the **200 pg/µl** reference sample
- tube 4: add 250 µl of the 200 pg/µl reference sample to the 250 µl of sample diluent and mix using the pipette – this is the **100 pg/µl** reference sample
- tube 5: add 250 µl of the 100 pg/µl reference sample to the 250 µl of sample diluent and mix using the pipette – this is the **50 pg/µl** reference sample
- tube 6: add 250 µl of the 50 pg/µl reference sample to the 250 µl of sample diluent and mix using the pipette – this is the **25 pg/µl** reference sample
- tube 7: add 250 µl of the 25 pg/µl reference sample to the 250 µl of sample diluent and mix using the pipette – this is the **12.5 pg/µl** reference sample
- tube 8: add 250 µl of the 12.5 pg/µl reference sample to the 250 µl of sample diluent and mix using the pipette – this is the **6.25 pg/µl** reference sample
- tube 9: add 250 µl of the 6.25 pg/µl reference sample to the 250 µl of sample diluent and mix using the pipette – this is the **3.125 pg/µl** reference sample. Dispose of the pipette tip.
- tube 10: 0 pg/µl reference sample – leave as 250 µl of sample diluent - this is the **0 pg/µl** reference sample

4. Prepare the patient sample in a microcentrifuge tube labelled P by diluting the separated serum 1:10 as follows:

- add 450 µl of sample diluent and dispose of the pipette tip
- add 50 µl of serum and mix using the pipette

5. Load 100 µl each of the reference samples into the 96 well plate in duplicate, recording where in the plate each sample is loaded on the LIMS system, and do the same for the diluted patient samples

6. Inform the biomedical scientist that the preparation of the plate is complete so they can complete the ELISA and analyse results.

Document information

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