T Level Technical Qualification in Healthcare Science

Occupational specialism assessment (OSA)

Assisting with Healthcare Science

Assignment 3 - Standard Operating Procedures

Assignment brief insert

v1.2: Specimen assessment materials
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Standard Operating Procedures

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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 structure24. 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 counterstain25. Neutral red, safranin or carbol-fuchsin may be used as the counterstain25.

However, while Gram staining is a valuable tool for the identification of a bacterial organism, not all bacteria can be definitively classified by this technique. This has given rise to gram-variable (organisms that may stain either negative or positive) and gram-indeterminate groups (which do not respond to Gram staining and, therefore, cannot be determined as either Gram positive or Gram negative, for example, acid fast bacteria).

This technique has also been used for staining of certain fungi such as candida and cryptococcus which are observed as Gram positive yeasts.

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. Method

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

- prepare a smear 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 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 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)

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- wash briefly with water and blot dry
- examine the slide using an oil immersion objective to observe cell morphology and Gram reaction

4. 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.

5. Quality control organisms

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

6. Technical Information

Modification for anaerobic bacteria

There are many suggested modifications and recommendations for the original Gram's stain targeted at improving the visualisation of anaerobic bacteria. These include the Kopeloff modification for Gram positives, the Willis and Phillips recommendation and the Wadsworth Manual's suggestion to enhance the staining of Gram negatives.

For further details on the use of the different Gram stain modifications, refer to the Anaerobe Reference Unit, Cardiff.

Gram's stain observations

The Gram staining procedure does not always give clear-cut results. Examples of these are:

Some Gram positive bacteria regularly appear Gram negative, in whole or in part, for example, rapidly growing streptococcus species, involution forms of streptococcus pneumoniae and some strains of bacillus species. For this reason, it is recommended that very young cultures from non-inhibitory media are used for this procedure after growth has become visible on culture plates.

Some gracile Gram negative bacteria such as haemophilus species might easily be missed if stained by the Gram method (see Sandiford's modification).

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

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- 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

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ELISA standard operating procedure

1. Introduction

This Hepatitis B antibody detection kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative determination of antibodies to Hepatitis B virus in human serum. It is intended for use in clinical laboratories for diagnosis and management of patients with suspected Hepatitis B virus infection.

Hepatitis B is an enveloped, icosahedral virus double stranded DNA virus. Infection with Hepatitis B can cause both acute and chronic infection.

2. Principle of the method

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

After washing out all the other components of the sample the specific Hepatitis B antibody captured on the solid phase is detected by the addition of conjugated antihuman antibody.

During the second incubation, the conjugated antibody will specifically react only with Hepatitis B antibodies and after washing to remove the unbound conjugate, chromogen solution is added into the wells. The colourless chromogen is hydrolyzed by the bound conjugate to a blue colour. Wells containing samples negative for Hepatitis B antibodies remain colourless.

The blue colour turns yellow after stopping the reaction with sulphuric acid and the colour intensity can be measured using a spectrophotometer.

This process is completed within an enclosed environment on an analyser.

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
- pipette (single or multichannel), disposable pipette tips

5. Reagents required

- negative control ready for use
- positive control ready for use
- specimen diluent ready for use
- HRP conjugate reagent ready for use
- chromogen solution ready for use

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- stop solution ready for use
- stock wash buffer dilute before use, the concentration must be diluted 1 to 20 with distilled water

6. Method

1. Allow the reagents and samples to reach room temperature (18°C to 30°C) for at least 15 to 30 minutes before use.
2. Centrifuge samples and separate serum using appropriate pipette.
3. Prepare positive and negative QC samples
4. Rack sample and QC samples ready to load onto analyser.
5. Load reagents onto the analyser.

7. Results and analysis

Inform biomedical scientist that the preparation of ELISA is complete, and analysis can commence.

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