

## DESCRIPTION

GRAM TEST STICK is a rapid test for differentiation of Gram-negative from Gram-positive micro-organisms. The sticks are impregnated with the reagent L-alanine-4-nitroanilide which, on contact with colonies of Gram-negative bacterial colonies, reacts by changing colour to blue-violet following addition of the detector reagent (Gram Test Reagent).

## CONTENT OF THE PACKAGES

Each package contains:

- 30 impregnated sticks contained in a heat-sealed container
- 1 Gram Test Reagent (detector agent)
- 1 instruction sheet

## PRINCIPLE OF THE METHOD

The reagent L-alanine-4-nitroanilide is hydrolysed by the enzyme amino-peptidase contained in the cell of Gram negative bacterial micro-organisms<sup>1,2</sup>. The stick impregnated with the reagent L-alanine-4-nitroanilide is placed in direct contact with the bacterial colony to be examined. Subsequently, through the action of the detector (Gram Test Reagent), an intense blue-violet colour will develop if the colony is of Gram-negative micro-organisms. Colonies of Gram-positive micro-organisms do not develop any colour.

## COMPOSITION

<b><u>GRAM TEST STICK</u></b>
L-alanine-4-nitroanilide
<b><u>Gram Test Reagent</u></b>
Dimethyl-amino-cinnamaldehyde 0.2% in hydrochloric acid 27%

## COLLECTION OF SAMPLES

The colonies to be subjected to the Gram test using the GRAM TEST STICK may be taken from either selective or non-selective media, which must, however, be colour-free. No reaction will be seen with bacterial colonies grown on media containing colouring matter such as Mac Conkey Agar, Cled Agar, XLD Agar etc.

## TEST PROCEDURE TEST

1. Take the container from the refrigerator and leave on the bench for some minutes until it reaches ambient temperature.
2. Select a well-isolated colony on the isolation medium.
3. Use tweezers to take a stick from the container.
4. With a loop of plastic, take the colony to be subjected to the test and smear it meticulously on the end of the stick.
5. Wait 5-10 minutes, leaving the stick at ambient temperature.
6. Add two drops of Gram Test Reagent.
7. Observe the immediate development, or non-development, of a blue-violet colouration, and interpret the results.

## INTERPRETATION OF THE RESULTS

With Gram-negative micro-organisms, a blue-violet colour develops. With Gram-positive micro-organisms, no colour develops. The test is used to identify bacteria which possess the enzyme amino-peptidase (Gram-negative) from those that do not have it (Gram-positives).

The test is particularly useful for differentiating:

- Gram-negative bacilli from Gram-positive ones;
- Gram-negative cocci from Gram-positive ones;
- Gram-negative coccobacilli from Gram-positive ones;
- Gram-negative diplococci from Gram-positives ones.

## QUALITY CONTROL

Each lot of GRAM TEST STICK is subjected to quality control using a culture of *Escherichia coli* ATCC 25922 for the positive control (development of the blue-violet colouration) and a culture of *Staphylococcus aureus* ATCC 25923 for the negative control (absence of colour).

## PRECAUTIONS

The pack of GRAM TEST STICK contains a 27% solution of hydrochloric acid in the Gram Test Reagent. At that concentration, the product can be classified as dangerous under current legislation; it is recommended that the safety data sheet be consulted for its use. GRAM TEST STICK is a disposable device. GRAM TEST STICK is only for diagnostic use *in vitro*. It is intended for use in a professional environment and must be used in a laboratory by adequately trained personnel using approved asepsis and safety methods for dealing with pathogenic agents.

## CONSERVATION

Store GRAM TEST STICK at 2-8°C in its original pack. Keep away from sources of heat and avoid excessive changes of temperature. In such conditions the product GRAM TEST STICK is valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration (change of colour of the stick or of the Gram Test Reagent).

## ELIMINATING USED MATERIAL

After use, GRAM TEST STICK and the material that comes into contact with the sample must be decontaminated and disposed of in accordance with current laboratory techniques for the decontamination and disposal of potentially infected material.

## BIBLIOGRAPHY

1. Carlone G.M., Valadez M.J. and Pickett M.J. 1983. *Methods for distinguishing Gram-positive from Gram-negative bacteria*. J.Clin.Microbiol. **16**:1157
2. Halebian S., Harris B., Finegold S.M. and Rolfe R.D. 1981. *Rapid method that aids in distinguishing*.
3. *Gram-positive from Gram-negative anaerobic bacteria*. J.Clin.Microbiol., **13**:44.

## PRESENTATION

Product	REF	Σ
<b>GRAM TEST STICK</b>	<b>88031</b>	<b>30</b>

## TABLE OF SYMBOLS

SYMBOL	MEANINGS	SYMBOL	MEANINGS
IVD	In Vitro Diagnostic Medical Device	⊗	Do not reuse
Manufacturer		Σ	Contains sufficient for <n> tests
REF	Catalogue number	⚠	Fragile, handle with care
Use by		⚠	Caution, consult accompanying documents
Temperature limitation		LOT	Batch code

