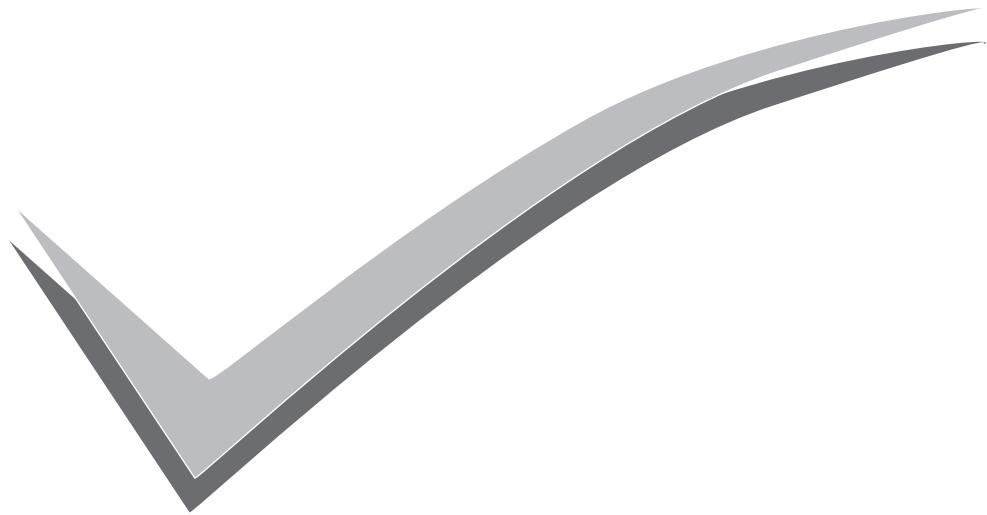




ISO 9001: 2008  
EN ISO 13485: 2012

# Performance Evaluations



## **GRAM STAIN KIT**

To differentiate between Gram positive and Gram negative bacteria



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ISO 9001: 2008  
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## SCIENTIFIC REPORT

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1.	Excellent correlation of an indigenous Gram Stain from Microxpress with certified Gram Stain from Sigma Aldrich, U.S.A.

## **GRAM STAIN KIT**

To differentiate between Gram positive and Gram negative bacteria



## Excellent correlation of an indigenous Gram Stain from Microxpress with certified Gram Stain from Sigma Aldrich, U.S.A.

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### ABSTRACT

**Objective** - To evaluate performance and assess the quality of Gram Stain kit from MICROXPRESS, A Division of Tulip Group of Companies against a certified Gram Stain kit, from Sigma Aldrich U.S.A.

### Study Design and Methods

Specimens from different hospitals were used in the study. The specimens were analyzed using Gram Stain kit, from MICROXPRESS and a certified Gram Stain kit, from Sigma Aldrich, U.S.A in all the hospitals. The results of both the stains were compared for their accuracy in identification of organism morphology, differentiation between Gram positive & Gram negative organism, clarity of the smear & visibility of the organism against the background.

### Results

Data from selected hospitals were analyzed in the present study on the basis of the stain quality. Since Crystal Violet, Iodine and Safranin stain from Sigma Aldrich is a certified stain, the study revealed that Crystal Violet, Iodine and Safranin stain provided in Mycostain kit from MICROXPRESS was correlating well with the used reference stain. Both the stains

showed excellent performance when compared for the characteristics like – smear clarity, visibility of organism, differentiation between Gram positive & Gram negative organism & background distinction against microorganism.

### Conclusions

The study shows that the stain quality and purity play a significant part to make microscopy an excellent tool in diagnosis. For exact analysis of the organism morphology and their distinction from one another based on cell structure, the stain used for microscopy should be certified. The result comparison done by different hospitals using Gram Stain, MICROXPRESS and Gram Stain from Sigma Aldrich, U.S.A. for the same samples following similar techniques revealed that the quality of Gram stain kit is very well comparable with certified reference stain.

It was also observed that the performance of Gram Stain, MICROXPRESS in differentiating Gram positive and Gram negative organism, clear background distinction, visibility of organism morphology as well as smear clarity was equivalent to standardized Gram Stain kit from Sigma, Aldrich, U.S.A.

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### Background

Gram staining is an empirical method of differentiating bacterial species into two large groups - Gram positive and Gram negative based on the chemical and physical properties of their cell wall. The method was named after his inventor, Hans Christian Gram, who developed the technique in 1884 (Gram 1884) while for the most infections bacterial culture is the ultimate test for the diagnostic, the Gram stain is much quicker (minutes v/s days) and provides the immediate feedback on the severity of the infection and the quality of samples. The technique is used as a tool for the differentiation of Gram positive and Gram negative bacteria as a first step to determine the identity of a particular bacterial sample.

Gram stain remains one of the most valuable methods we have for identifying isolates accurately and rapidly. Despite our long standing familiarities with the method it still warrants careful attention at every step of a way

from preparation and QC of reagents to staining and interpretation. Mainly the preparation of the reagents and their concentration plays a critical roles in staining and thus influencing the interpretation directly. Most commercially available reagents can undoubtedly help in mere staining the cells, but the performance of the standardized reagents when used are outstanding when it comes to the interpretation of the result based on the cell morphology, their distinction and differentiation. It is essential for the stains used in the Gram staining to fulfill the following criteria in order to produced accurate results.

- 1) Clear visibility of the organism
  - 2) Clarity of the smear
  - 3) Differentiation between Gram positive and Gram negative organism
  - 4) Distinction between background and bacilli/ cocci
  - 5) Any particle if found in the smear
- and these criterias are only fulfilled when a known certified Stain are made used of.

## Material And Methods

Stains used in the Gram Staining procedure are Crystal Violet reagent, Grams iodine, Decolorizer (acid alcohol) and Safranin. In this study three reagents provided in the Gram stain kit, from MICROXPRESS (Lot No.: GM-901T, Mfg – Dec2009, Exp – Nov2012) is compared with certified Crystal Violet reagent, Grams Iodine and Safranin Reagent of Gram stain kit, from Sigma Aldrich, USA. (Lot No.: 1434447, Mfg – Oct 2009, Exp – Sep2012) on its performance based on above mentioned criteria.

**Preparation of Smear –** The smear was prepared by standard protocol as mentioned in Clinical Diagnosis & Management by Laboratory Methods, Todd Stanford. 17<sup>th</sup> Edition 1988 and Practical Medical Microbiology, Mackie & Mc Cartney, 13<sup>th</sup> Edition.

Gram Stain kit from MICROXPRESS and Sigma Aldrich were provided to three reputed hospitals. All were asked to use their routinely applied staining methods and microscopic examination protocols. They were asked to evaluate both the kits using same samples collected by them from volunteers.

### Pitfalls In The Interpretation Of Grams Stain

Gram Stain is a first step to determine the identity of a particular bacterial sample. Gram positive cocci in clusters suggest *Staphylococci* and in chains suggest *Streptococci*. Gram positive lancet shaped diplococci are characteristic of *S. pneumococcus*. The entire differentiation is based on cell morphology and it is the critical part of Gram staining which can be enhanced only by making use of known Standardized stains.

Following is the interpretation chart which shows that if the stain used are not certified or standardized can make a huge difference in depicting the cell morphology and differentiation, thus resulting to mis- interpretation of the results.

ORGANISM	RESULTS		COMMENTS
	Using certified stain	Using commercially available stains	
	<b>Classic Presentation</b>	<b>Variant Presentation</b>	
<i>Streptococcus pneumoniae</i>	Gram positive lancet shaped	Elongated cocci resembling short cocci	May be mis interpreted as mixed organism
Acinetobacter species	Gram negative cocci	Gram negative cocci, Gram positive cocci	- May be mistaken as <i>Neisseria sp.</i> ( <i>Acinetobacter</i> demonstrate elongated forms which are not seen in <i>Neisseria</i> ) - May be mistaken for <i>S. pneumoniae</i> and reported as Gram positive cocci. <i>Pneumococci</i> , in addition to having coccal forms, retain CV tentiously during decolorization.
<i>Clostridium perfringens</i>	Box -car shaped Gram positive bacilli	Gram negative or Gram variable bacilli	May be mistaken for Gram negative
Yeast especially <i>Cryptococcus</i>	Gram positive round or oval cells with budding	Gram variable cells	May be mistake for artifact, size and shape distinguish them from bacteria.

Table 1: Pitfalls in the interpretation of Gram's Staining\*

### Analysis Of Results

Microscopy result with 10X low power magnification were interpreted and reported by the hospitals according to the standard recommendation.

Data from all the hospitals were analyzed in the present study on the basis of quality of stains (Crystal Violet, Grams Iodine and Safranin) present in Gram Stain kits from MICROXPRESS and Sigma Aldrich, U.S.A. Since Crystal Violet, Gram's Iodine and Safranin Reagents from Sigma Aldrich are certified stains, the study revealed that Crystal Violet, Gram's Iodine and Safranin provided in Gram Stain kit MICROXPRESS was well comparable with the certified stains by Sigma.

Hospitals	Number of Samples analyzed	
	Brand A Gram Stain, Sigma Aldrich, U.S.A.	Brand B Gram Stain, MICROXPRESS
HOSP 1	24	24
HOSP 2	25	25
HOSP 3	27	2

Table 2: Number of Samples analyzed with Gram Stain Kits from MICROXPRESS and Sigma, Aldrich, U.S.A.\*

\* Data on MICROXPRESS file

The Kits were also compared on the following criteria in order to evaluate the performance of the stain (Table: 3 & 4)

Sr. No.	CHARACTERISTICS OBSERVED	REMARKS for Gram Stain Kit from	
		Sigma Aldrich U.S.A. (poor/good/excellent)	MICROXPRESS (poor/good/excellent)
1.	Clarity of the smear	Good	Good
2.	Visibility of the organism	Good	Good
3.	Differentiation between Gram positive and Gram negative organism	Good	Good
4.	Distinction of background against organism	Good	Good
5.	Any particles if found in the smear.	Good	Good

**Table 3: Performance comparison of Gram Stain Kits from MICROXPRESS and Sigma, Aldrich, U.S.A.\***

Hospitals	RESULTS OBSERVED				
	Clarity of smear (poor/good/excellent)	Visibility of the organism (poor/good/excellent)	Differentiation b/w Gram negative & Gram positive organisms (poor/good/excellent)	Distinction b/w background (poor/good/excellent)	Any particles if found any (poor/good/excellent)
HOSP 1	Good	Good	Good	Good	Good
HOSP 2	Good	Good	Good	Good	Good
HOSP 3	Good	Good	Good	Good	Good

**Table: 4 – It depicts the important criterion's that play a critical role in microscopy.\***

From the analysis it is clear that - the quality and other parameters of a particular stain plays a significant part to make microscopy an excellent tool in diagnosis. For exact analysis of the morphology of organism and distinction of one organism from another, the stains have to be of certain standard.

Comparison of the results obtained by using Gram Stain kits from MICROXPRESS and Sigma Aldrich, U.S.A. following similar Gram Staining technique revealed that the quality of Gram Stain reagents (Crystal Violet, Gram's Iodine & Safranin) in Gram Stain kit from MICROXPRESS correlates well with Gram Reagents (Crystal Violet, Gram's Iodine & Safranin) provided in

certified Gram Stain kit from SigmaAldrich, U.S.A.

It was also observed that the performance of Gram Stain, MICROXPRESS in differentiating pink colored Gram negative organisms from purple colored Gram positive, clear distinction of background against organisms, visibility of (cell morphology) organisms, no observation of particles in the smear as well as clarity of the smear was comparable to standardized Gram Stain kit from SigmaAldrich, U.S.A.

## DISCUSSION

For exact microscopic identification and result interpretation it is very critical to analyze the cell morphology of the organism. There are various criteria on which the interpretation depends like - Visibility of the organisms, Clarity of the smear, Differentiation between Gram positive and Gram negative organisms, Distinction between background and organism, Any particles if found in the smear. And these mentioned criterion's are only fulfilled when a standardized stain is made use of.

A standardized stain is a stain in which the concentration of the dye is optimal in order to fulfill all the above characteristics. And when these standardized dyes are certified by a globally known body, it is then called as a certified stain. The reference stain used in this study (Gram Stain, from Sigma Aldrich, U.S.A.) is certified by Biological Stain Commission and is ISO 9001 certified. Since the study depicts that the performance of Gram Stain, from MICROXPRESS showed excellent correlation with Gram Stain, from Sigma Aldrich, U.S.A., we can therefore conclude that Gram stain, from MICROXPRESS is equivalent to a certified stain.

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