



## Original article

# A study of 2 rapid tests to differentiate Gram positive and Gram negative aerobic bacteria

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### Abstract

293 aerobic bacterial pathogens were isolated from various clinical samples. All the isolates were subjected to Gram stain (GS), potassium hydroxide (KOH) string test, and Vancomycin susceptibility test. All Gram negative bacilli i.e. 100%, showed resistance to Vancomycin and KOH string test positivity ( $p=0.000$ ). 97.8% of gram positive bacteria were sensitive to Vancomycin and 100% were KOH string test negative ( $p=0.000$ ). As KOH string test and Vancomycin tests are simple and inexpensive, these can be used in addition to Gram staining, for rapid identification of bacterial isolates.

**Key words:** KOH string test, Vancomycin, Gram stain, Gram positive cocci, Gram negative bacilli

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Gram staining (GS) is the principal staining technique used for microscopic examination of bacteria. Unknown bacteria can be classified into gram-positive or gram-negative by GS, where de-colorization is the major pitfall, as some gram positive bacteria decolorize more rapidly, and incorrectly identified as gram-negative. Adding to this, factors like composition of growth medium, age of culture<sup>1</sup> and antibiotic treatment<sup>2</sup> may allow crystal violet to wash out, and the sample may appear gram-variable, with some cells staining pink and others staining purple. To overcome these difficulties several modifications have been developed in gram staining procedures<sup>3</sup> and also several tests have been introduced to differentiate bacteria. Of these tests, a few are: demonstration of aminopeptidase enzyme<sup>4</sup>, disruption of gram negative bacteria cell wall by exposing to alkali<sup>5</sup> and Vancomycin (5µg) susceptibility<sup>6</sup>.

In the current study, potassium hydroxide (KOH) string test and Vancomycin susceptibility test were

used to differentiate bacterial isolates and these results were compared with standard GS.

### Materials and methods

This study was conducted in the department of Microbiology, GSL Medical College, Rajahmundry, Andhra Pradesh from 01<sup>st</sup> April 2011 to 15<sup>th</sup> June 2011. During the above period a total of 293 pathogenic aerobic bacteria were isolated from various clinical samples. All the strains were subjected to gram staining, KOH string test and Vancomycin susceptibility test. Standard strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used as controls.

### Gram staining

Smears were flooded with crystal violet for one minute and then washed gently in tap water. In the second step, smears were exposed to Gram's iodine for one minute, and then washed with tap water. In the third step, slides were exposed to acetone for de-colorization and washed immediately with tap

water. Finally, dilute Carbol Fuchsin was added as the counter stain and washed after 60 seconds. After drying, stained slides were examined under oil immersion (100X) to note Gram reaction, morphology and arrangement.

### KOH string test

A loopful of growth from a bacterial colony was emulsified on the surface of a glass slide in a suspension of 3% KOH. The suspension was stirred continuously for 60 seconds after which the loop was gently pulled from the suspension. The test was considered positive if string occurred within the first 30 seconds after mixing the bacteria in KOH solution.

### Vancomycin susceptibility test

Using an inoculum corresponding to 0.5 McFarland turbidity standard (i.e.  $1.5 \times 10^8$  CFU / ml) a lawn culture was made on Mueller Hinton agar. Vancomycin discs (5µg) were placed on the lawn culture and plates were incubated at 37°C overnight. Any zone of inhibition was considered as sensitive.

### Results

Out of 293 isolates, 90 (31%) were Gram positive cocci, and the remaining 203 (69%) were Gram negative rods. Gram positive cocci included *Staphylococcus* species (spp), *Streptococci* spp and *Enterococci* spp, and Gram negative rods included *Pseudomonas* spp, *Proteus* spp, *Esch. coli*, *Klebsiella* spp. Results of the KOH string test and Vancomycin susceptibility are given in the table 1.

**Table 1.** Results of the KOH string test and Vancomycin susceptibility.

	KOH string test		Vancomycin sensitivity	
	Positive	Negative	Sensitive	Resistant
GPC	0 (0%)	90 (100%)	88 (97.80%)	02 (2.20%)
GNB	203 (100%)	0 (0%)	0 (0%)	203 (100%)

GPC: Gram positive cocci,

GNB: Gram negative bacilli

### Discussion

GS is one of the preliminary and essential procedures in diagnostic microbiology to classify bacteria into Gram positive and Gram negative. In our study, 88 (97.8%) Gram-positive cocci were susceptible to

Vancomycin and 02 (2.2%) were resistant, and all the strains were KOH string test negative ( $p=0.000$ ). Both the resistant strains were *Enterococcus* species. In the study by Arthi et al<sup>7</sup> Gram-positive spp showed 100% sensitivity to Vancomycin.

All Gram-negative bacteria in our study showed resistance to Vancomycin, and tested positive with the KOH string test ( $p=0.000$ ). Our results concurred with those of Arthi et al<sup>7</sup> where Vancomycin resistance and positive KOH string test results among Gram negative bacteria were 99.6% and 98.8% respectively. The difference in results was due to *Acinetobacter* species. A total of 18 *Acinetobacter* isolates were present in the study by Arthi et al<sup>7</sup>, which were absent in our study. There is a highly significant association between the results of GS with KOH string test and Vancomycin susceptibility ( $p=0.000$ ).

### Conclusion

KOH string test and Vancomycin susceptibility tests are rapid and can differentiate bacteria well into Gram positive and Gram negative. Hence these can be used as alternative tests to GS, in the laboratories with heavy work-loads.

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**Conflict of interest:** None

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