



## KOH string and Vancomycin susceptibility test as an alternative method to Gram staining

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### Abstract:

Gram staining is a common technique to differentiate Gram positive and Gram negative bacteria. They may be gram variable (incorrectly identified) due to rapid decolorization of Gram positive bacteria and poor decolorization of Gram negative bacteria. There are few alternative techniques. Potassium hydroxide (KOH) string test and Vancomycin susceptibility test were done in addition to Gram stain to differentiate bacteria and were compared. The study was done in Academy of Medical Sciences, Pariyaram, Kannur. Total 300 various isolates were collected and analysed for its bacterial identification; various samples were subjected to Gram stain, KOH string test and Vancomycin susceptibility test. Of all Gram positive cocci, 91(100%) showed KOH string test negative and 89(97.8%) Vancomycin sensitive. Out of all Gram negative bacilli, 209(100%) showed KOH string test negative and Vancomycin resistant. KOH string test and Vancomycin susceptibility tests can be used as alternative or in addition to Gram stain for rapid identification of bacteria and better accuracy.

**Key words:** KOH string test, Vancomycin sensitivity test

### Introduction:

In most instances, the initial identification and classification of unknown bacteria is done by Gram staining. Over-decolorization of Gram positive bacteria and poor decolorization of Gram negative bacteria are the major drawbacks with this technique. Factors like composition of culture medium, age of culture and antibiotic treatment can affect the Gram positive bacteria by fast decolorization.<sup>1</sup> Several modifications in this procedure and also introduction of several tests have been made to overcome the shortcomings.<sup>2, 3</sup> Among these tests, demonstration of aminopeptidase enzyme<sup>4</sup>, disruption of Gram negative bacterial cell wall by exposing to alkali<sup>5</sup> and Vancomycin<sup>6</sup> (5µg) susceptibility test are few.

In the current study, bacterial isolates collected from different cultures were differentiated into Gram positive and Gram negative bacteria using Gram staining. KOH string test and Vancomycin

susceptibility test were done for all the isolates and were compared to Gram reaction.

### Materials and Methods:

A total 300 various bacterial isolates were collected from the different positive cultures analysed in the Department of Microbiology, Academy of Medical Sciences, Pariyaram. They were analysed for its bacterial identification using Gram stain. KOH string test and Vancomycin susceptibility test were done for all the isolates.

**Gram staining:** Crystal violet stain was added to the smear for one minute. Then it was washed under tap water and Gram's iodine was added for one minute. Again after washing under tap water, it was exposed to acetone for decolorization and then washed immediately with tap water. Then, dilute carbol fuchsin was added as counter stain and washed after 30seconds. After drying, microscopic examination was done under oil immersion objective to

see Gram reaction, morphology and arrangement of bacteria.

**KOH string test:** A loopful of bacterial colony from the culture plate was emulsified over glass slide in suspension of 3% KOH. The suspension was stirred continuously for one minute and then loop was gently pulled up from it. The test was considered positive if string was seen within first 30 seconds after mixing in KOH solution.<sup>7</sup>

**Vancomycin susceptibility test (Modified Kirby-Bauer's method):** A lawn culture was made on Mueller Hinton agar using inoculums corresponding to 0.5 McFarland turbidity standards ( $1.5 \times 10^8$  CFU/ml). Vancomycin disc (5µg) was placed on the lawn culture and incubated at 37° C overnight. Any zone of inhibition was taken as positive<sup>7</sup>.

The standard strains *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were used as control. *Staphylococcus aureus* (ATCC 25923) was Vancomycin sensitive but negative for KOH string test, while *Pseudomonas aeruginosa* (ATCC 27853) was Vancomycin resistant and positive for KOH string test.

## Results:

Among 300 isolates, 91 (30.33%) were Gram positive cocci and 209 (69.67%) were Gram negative bacilli. Gram positive isolates included *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp. Gram negative rods included *Pseudomonas* spp., *Klebsiella* spp., *Proteus* spp., *Escherichia coli*, *Enterobacter* spp., *Salmonella typhi*, *Salmonella paratyphi* and *Acinetobacter* spp. In this descriptive study, results of the

KOH string test and Vancomycin susceptibility are mentioned in the **Table I**.

## Discussion:

In diagnostic clinical microbiology and clinical medicine, Gram staining helps in the initial identification of the organism, which in turn helps in presumptive treatment. But equivocal results arise with some Gram positive and Gram negative bacteria<sup>8</sup>, eg. *Bacillus macerans*, *Listeria* spp., *Achromobacter* spp, *G. vaginalis*, etc. In our study, negative KOH string test was shown by all the isolates of Gram positive cocci (100%) but 97.8% strains were susceptible to Vancomycin (>6mm zone diameter). The resistant strains 2.2% were *Enterococcus* spp. identified by culture and biochemical reactions. In the studies of Arthi et al<sup>9</sup> and TJ Chandra et al<sup>7</sup>, similar findings were seen.

Of the Gram negative bacilli, Vancomycin resistance and KOH string test was positive in 100% isolates. Similar findings were seen in Arthi et al<sup>9</sup> study. In Shushan H et al<sup>6</sup> study, 30% of the gram-negative anaerobic bacteria gave a false-negative KOH reaction. False-negative tests (i.e., no string formation) could still occur in *Achromobacter* spp, *G. vaginalis*, *K.kingae*, *Moraxella* spp. and *Acinetobacter* spp. The former two are practically always Gram negative and give negative Vancomycin tests. The false result may be observed with *Moraxella* spp. and *Acinetobacter* spp. in both the tests but were differentiated easily by presence of coccoid forms on solid media and non-motile diplobacillary forms in liquid media.<sup>10</sup>

**Table I: Result of KOH string test and vancomycin susceptibility test of GPC and GNB**

	KOH String Test		Vancomycin Sensitivity	
	Positive	Negative	Sensitive	Negative
<b>Gram positive cocci (91)</b>	0 (0%)	91(100%)	89 (97.8%)	2 (2.2%)
<b>Gram negative bacilli (209)</b>	209(100%)	0 (0%)	0 (0%)	209 (100%)

GPC- Gram positive cocci, GNB- Gram negative bacilli

**Conclusion:**

Gram staining has been very useful method for bacterial differentiation but gives inconclusive and incorrect results due to operator technique and inherent properties of certain organisms. KOH string test and Vancomycin susceptibility tests can be used to classify bacteria into Gram positive and Gram negative. With these tests, there will be less of false positive or false negative result. So these tests can be used as a definitive, stain free test that decreases variability or in addition to Gram staining for better diagnosis.

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