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EFFECTIVENESS OF ONE ALTERNATIVE METHOD TO GRAM STAINING FOR BACTERIA DIFFERENTIATION

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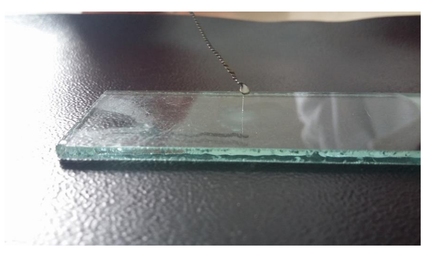
Abstract: Researchers presented in this study aimed to assess the applicability and the effectiveness of the KOH test, compared with the Gram method, in evaluation of the Gram-negative bacteria load in a cattle shelter, in order to reduce the working time required to evaluate the microorganisms from the air. The results obtained reflected that the number of Gram-negative bacteria in one cubic meter of air determined by the 3% KOH method is very close to value determined by the Gram method. A correlation coefficient (r^2) of 0.98 was obtained between the 3% KOH test and the Gram staining method, which means a 98% correlation between these two tests. The KOH test is a rapid test that can be used to differentiate between Gram-negative and Gram-positive bacteria in current laboratory practice *text*

• Introduction

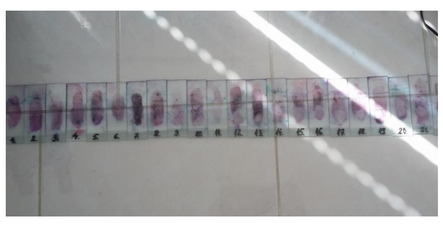
Gram staining method is still used as bacterial diagnostic techniques, but it is known that the main disadvantage of it is the premature discoloration of some bacteria, which are mistakenly identified as Gram negative bacteria and that often leads to diagnostic errors. The KOH test is based on the differences in the chemical structure of the cell wall in Gram-negative bacteria compared to Gram-positive ones. Due to this, the cell wall of Gram-negative bacteria is easily destroyed when exposed to dilute alkaline solutions. When the cell walls are broken, the suspension in KOH becomes viscous due to the release of DNA. Weak alkaline solutions have effectiveness of the KOH test, compared with the Gram no effect on the wall of Gram-positive bacteria. researchers presented in this study aimed to assess the applicability and the method, in evaluation of the Gram-negative bacteria load in a cattle shelter, in order to reduce the working time required to assess the microorganisms from the air. It is known the importance of taking measures prompt during the evolution of bacterial diseases in farm animals

• Material and method

This study aimed to assess the effectiveness of the fast method with the 3% KOH solution in classifying Gram negative and Gram positive bacteria, compared to the Gram method, in monitoring the total bacteria form the air in a cattle shelter. The free sedimentation method was used to assess the air microbial load (6). The examination of each colonies and identification of the bacterial type was performed simultaneously by the Gram method (13) and the 3% KOH method (8).



Positive reaction to 3% KOH test



Microscopic preparations stained by the Gram method

•Results and discussions

From the total of 110 bacterial isolates (colonies) examined in the 3% KOH solution test, a positive reaction was obtained in 50 colonies, which means that they were Gram-negative bacteria. As a result, it can be stated that in the cattle shelter the proportion of Gram-negative bacteria would correspond to a value of 45.45%.

The results obtained from the examination of the 110 bacterial isolates by the Gram method show that 48 isolates consisted of Gram-negative bacteria, which represents a proportion of 43.6% Gram-negative bacteria in a cubic meter of air in the air in the cattle shelter.

The difference between the proportion of Gram-negative bacteria tested by the 3% KOH method and that obtained by the Gram method was due of to two isolated colonies, which consisted of both Gram-positive cocci and Gram-negative bacilli, the latter in greater proportion .

Total number of isolated colonies	Number and percentage of colonies tested by the 3% KOH method		Number and percentage of colonies tested by the Gram method	
	with positive reaction	with negative reaction	positive	negative
14	5 / 35.7	9 / 64.3	9 / 64.3	5 / 35.7
14	7 / 50.0	7 / 50.0	7 / 50.0	7 / 50.0
22	10 / 45.5	12 / 54.5	14 / 63.7	8 / 36.3
18	7 / 38.8	11 / 61.2	11 / 61.2	7 / 38.8
42	21/ 50.0	21/ 50.0	21 / 50.0	21 / 50.0
110	50 /45.4	60 / 54.5	62 / 54.4	48 / 43.6

•Conclusions

•The KOH test is a rapid test that can be used to differentiate between Gram-negative and Gram-positive bacteria in current laboratory practice.

•There is a close correlation between the KOH test and the Gram staining method, as a result the two can be used and complete each other in situations where the diagnosis of bacteria is uncertain.

•The obtained results allow the recommendation of the use of the fast method with KOH to highlight Gram-negative bacteria in the assessment of microbial load in animal shelters or other environments. In this sense, research should be more extensive and to verify the correlation of this method with other methods of differentiating Gram-negative bacteria from Gram-positive ones.