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Diagnosis and Differentiation of Microorganisms through Citrate Utilization Test

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SUMMARY

Citrate utilization test is one of the important tests to differentiate a wide range of microorganisms on the basis of their ability to utilize citrate. Citrate is a ubiquitous natural compound which can be utilized as a carbon and energy source by many bacterial species. This test is also helpful to identify the members of species of Enterobacteriaceae family. The growth of the organism is indicative of the utilization of citrate as it is an intermediate metabolite in the Krebs cycle. The enzyme citrase breaks down citrate into oxaloacetate and acetate, where oxaloacetate is further broken down to form pyruvate and carbon dioxide. A positive test is demonstrated by growth with a colour change from green to intense blue while A negative test results no growth and no colour change.

INTRODUCTION

The citrate utilization test is a part of the IMViC test (Indole, Methyl Red, Vogues-Proskauer, and Citrate Test) that differentiates organisms on the basis of their ability to use citrate as a sole source of energy (Ausubel, 1987). The citrate test is performed along with the other IMViC tests to differentiate Gram-negative bacilli of the Enterobacteriaceae family. It is an important test that allows the species-level identification of the members of the Enterobacteriaceae family. With the exception of a few species, *Salmonella*, *Edwardsiella*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Serratia*, and *Providencia* usually give a positive reaction, and *Escherichia*, *Shigella*, *Morganella*, and *Yersinia* give a negative reaction. *Proteus* is a citrate variable. The test is also called Simmon's citrate test as it utilizes Simmon's citrate agar that contains citrate as the major source of energy. The medium further contains ammonium hydrogen phosphate (NH4H2PO4) as the sole source of nitrogen. Organisms that give a positive result in this test should be capable of fermenting citrate in the presence of the enzyme citrase (Hofwegen, 2016).

Principle: Citrate agar is used to test the ability of an organism to utilize citrate as a source of energy. The used agar medium contains citrate as the sole carbon source and inorganic ammonium salts as the sole source of nitrogen. The release of carbon dioxide induces the metabolism of ammonium salts, causing the formation of ammonia or sodium carbonate, both of which increase the alkalinity of the medium. Shift in pH turns the bromothymol blue indicator in the medium from green to blue. The reaction above pH 7.6 are given below

Citrate → Oxaloacetic acid → Pyruvic acid + CO2

Excess of sodium from sodium citrate + CO2 +H2O → Na2CO3

Growth of the organism on the medium followed by the change in the colour; as a result, citrate metabolism gives a positive citrate test.

Media Used: Simmon's Citrate agar is used as the medium to test the ability of an organism to utilize citrate as a sole source of energy. Simmon's Citrate agar is sold commercially by different vendors in the form of dehydrated powder (Winn *et al.*, 2016). However, it can also be prepared in the laboratory if the necessary ingredients are available. The composition of Simmon's Citrate agar are prepared by mixing ammonium dihydrogen phosphate (1.0g), Magnesium sulfate (0.2g), Dipotassium phosphate (1.0g), Sodium citrate (2.0g), Sodium chloride (5.0g), Bromothymol blue (0.08g), Bacteriological Agar (15g) at pH 6.8 ±0.2 and temperature (25°C)

Procedure

Preparation of the media: Media is prepared by adding 24.28 grams of the dehydrated powder in 1000ml distilled or deionized water. The solution is then heated to bring it to a boil in order to dissolve the medium completely. The dissolved medium is then dispensed into tubes and autoclaved at 15 lbs pressure (121°C) for 15 minutes. Once the autoclaving process is complete, the tubes are taken out and cooled at a slanted position to a temperature of about 40-45°C. The position should be maintained in order to obtain butts of 1.5 – 2.0 cm depth.

Utilization test: A well-isolated colony is taken from an 18-24 hour culture with a sterile inoculating needle. The citrate agar tubes are inoculated by streaking the surface of the slant. The slant should be streaked back and forth with the loop or the inoculating stick. The cap of the test tubes should be left loosened to ensure adequate aeration. The tubes are then incubated aerobically at 35-37°C for up to 4 days. Test tubes should be examined daily for 4 days before discarding the result as a negative.

Quality Control: Aseptic Simmon's Citrate Agar should appear as forest green colored slightly opalescent gel slants.

Result: The agar should be inspected for evidence of freezing, contamination, cracks, dehydration, and bubbles prior to use. Any tubes that might appear blue before use should be discarded. A positive test is demonstrated by growth with a colour change from green to intense blue along the slant. A negative test is demonstrated by no growth and no colour change, and the color of the slant remains green (Fig 1). The positive and negative control for the test are:

Klebsiella pneumoniae: Citrate Positive- Positive Control

Escherichia coli: Citrate Negative; Negative Control

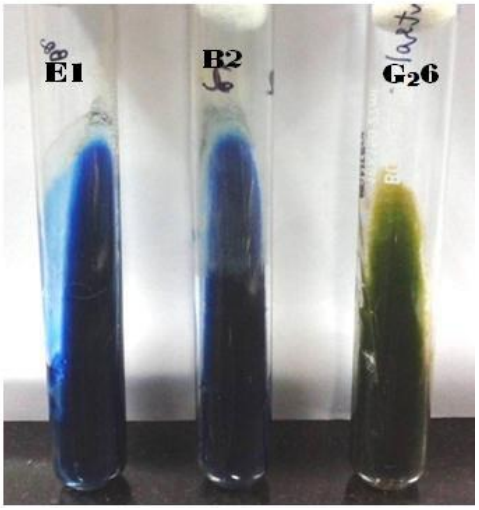

Fig 1: Result of citrate utilization test by color changing from green to blue (Makki *et al.*, 2014)

Table 1: Demonstrates the result of Citrate utilization of important medical bacteria:

S.N	***Organism***	***Growth***	***Citrate Test***
1.	*Escherichia coli*	Inhibited	Negative; the colour of the medium remains green.
2.	*Enterobacteraerogenes*	Good,Luxuriant	Positive reaction; change in the colour of the medium from green to blue.
3.	*Salmonella Enteritidis*	Good-Luxuriant	Positive reaction;
4.	*Salmonella Typhimurium*	Good-Luxuriant	Positive reaction;
5.	*Shigella dysentriae*	Inhibited	Negative;

CONCLUSION

This citrate utilization test is one of the excellent methods to diagnose the microorganisms on the basis of their species level. Citrate utilization tests the ability of an organism to grow aerobically with sodium citrate as the sole carbon source and with ammonium phosphate as the sole nitrogen source. Now-a-days, many kits from several companies are available in the market which lessen the time. This method is very easy to conduct. The change of colour from green to intense blue testify the species as a positive one while no colour change is negative. But, only the colour change can't be the determining factor of the microorganism to the species level (Hall, 1982). Therefore, to identify up to species level, some other tests need to be conducted parallel with this citrate utilization test.

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