

MacConkey Agar Plates Protocols

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Information

History

MacConkey agar was the first solid differential media to be formulated. It was developed at the turn of the 20th century by Alfred Theodore MacConkey, M.D, then Assistant Bacteriologist to the Royal Commission on Sewage Disposal, in the Thompson-Yates Laboratories of Liverpool University, England. The goal was to formulate a medium that would select for the growth of gram-negative microorganisms and inhibit the growth of gram-positive microorganisms. Dr. MacConkey first developed a bile salt medium containing glycocholate, lactose and litmus, to be incubated at 22°C (MacConkey, 1900). This formula was soon altered by the replacement of glycocholate with taurocholate and the incubation temperature was raised to 42°C (MacConkey, 1901). MacConkey later changed the recipe again by substituting neutral red for litmus (MacConkey, 1905), following the suggestion that neutral red be used as an indicator in bile salt lactose medium (Grunbaum and Hume, 1902). The final media formulation was designed to support growth of *Shigella* and is the one that is most commonly used today.

Purpose

MacConkey agar is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. It has also become common to use the media to differentiate bacteria by their abilities to ferment sugars other than lactose. In these cases lactose is replaced in the medium by another sugar. These modified media are used to differentiate gram-negative bacteria or to distinguish between phenotypes with mutations that confer varying abilities to ferment particular sugars.

Theory

MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae and the genus *Pseudomonas*. The inclusion of crystal violet and bile salts in the media prevent the growth of gram-positive bacteria and fastidious gram-negative bacteria, such as *Neisseria* and *Pasteurella*. The tolerance of gram-negative enteric bacteria to bile is partly a result of the relatively bile-resistant outer membrane, which hides the bile-sensitive cytoplasmic membrane (Nikaido, 1996). Other species specific bile-resistance

mechanisms have also been identified (Provenzano, et al. 2000; Thanassi et al. 1997).

Gram-negative bacteria growing on the media are differentiated by their ability to ferment the sugar lactose. Bacteria that ferment lactose cause the pH of the media to drop and the resultant change in pH is detected by neutral red, which is red in color at pH's below 6.8. As the pH drops, neutral red is absorbed by the bacteria, which appear as bright pink to red colonies on the agar.

The color of the medium surrounding Gram negative bacteria may also change. Strongly lactose fermenting bacteria produce sufficient acid to cause precipitation of the bile salts, resulting in a pink halo in the medium surrounding individual colonies or areas of confluent growth. Bacteria with weaker lactose fermentation growing on MacConkey agar will still appear pink to red but will not be surrounded by a pink halo in the surrounding medium.

Gram-negative bacteria that grow on MacConkey agar but do not ferment lactose appear colorless on the medium and the agar surrounding the bacteria remains relatively transparent.

Lactose can be replaced in the medium by other sugars and the abilities of gram-negative bacteria to ferment these replacement sugars is detectable in the same way as is lactose fermentation (for example Farmer and Davis, 1985).

RECIPE

Peptone (Difco) or Gelysate (BBL)	17.0 g
Proteose peptone (Difco) or Polypeptone (BBL)	3.0 g
Lactose	10.0 g
NaCl	5.0 g
Crystal Violet	1.0 mg
Neutral Red	30.0 mg
Bile Salts	1.5 g
Agar	13.5 g
Distilled Water	Add to make 1 Liter

Adjust pH to 7.1 +/-0.2. Boil to dissolve agar. Sterilize at 121° C for 15 minutes. (Holt and Krieg, 1994, Remel 2005)

PROTOCOL

Streak a plate of MacConkey's agar with the desired pure culture or mixed culture. If using a mixed culture use a streak plate or spread plate to achieve colony isolation. Good colony separation will ensure the best differentiation of lactose fermenting and non-fermenting colonies of bacteria.



Streak plate of *Escherichia coli* and *Serratia marcescens* on MacConkey agar. Both microorganisms grow on this selective media because they are gram-negative non-fastidious rods. Growth of *E. coli*, which ferments lactose, appears red/pink on the agar. Growth of *S. marcescens*, which does not ferment lactose, appears colorless and translucent.

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the [ASM Curriculum Recommendations: Introductory Course in Microbiology](#) and the [Guidelines for Biosafety in Teaching Laboratories](#).

COMMENTS AND TIPS

This section is to evolve as feedback on the protocol is discussed at ASMCUE. Please contact the project manager for further information.

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