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PERFORMANCE TESTING OF MACCONKEY AGAR IN *E. COLI* STRAINS ISOLATION

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Abstract: *In any microbiology laboratory the accuracy of the obtained results largely depends on the used culture medium quality. As a dehydrated culture medium, the commercial MacConkey agar is rigorously tested by the manufacturer, in terms of quality, reproducibility and physico-chemical characteristics of the medium. But, once each batch is rehydrated and sterilized in a laboratory, it is necessary to test the performance of the culture medium. In this context, we set out through this study to test the performance of the two types of MacConkey agar: with and without cefotaxime supplement, used for the isolation of antibiotic-resistant Escherichia coli strains, tests otherwise absolutely necessary for all culture media used in any microbiology laboratory.*

• Introduction

MacConkey agar is a solid, selective, chromogenic medium used for the detection, isolation and counting of coliforms and intestinal pathogenic bacteria present in various biological samples, but also in water or dairy products.

The latest version of the Protocol for the *E. coli* commensal strains isolation and those producing ESBL/AmpC and carbapenems from fecal samples, is provided by point 4.1 of the Commission Implementing Decision (EU) 2020/1729 of November 17, 2020 Annex, regarding the monitoring and reporting of antimicrobial resistance of zoonotic and commensal bacteria. This version proposes the use of MacConkey agar in two different variants: with and without the addition of cefotaxime. The isolation of *E. coli* strains from cecal samples is described in detail in the 7th version of the Protocol (December 2019), published on the website of the European Reference Laboratory for Antibioresistance (EURL-AR) protocol that recommends the use of this agar.

• Material and method

Simple MacConkey medium was obtained by rehydrating 51.5 g of commercially available powder (Thermo Fisher Scientific, CM 115, lot: 3602179, validity: 20.01.2028) in 1000 ml of distilled water. The medium was then brought to boiling point to ensure complete dissolution. Afterwards, it was sterilized by autoclaving for 20 minutes at 121°C.

To obtain the cefotaxime supplemented MacConkey medium, 1 mg of cefotaxime was added to one liter of the medium, after cooling. The two culture medias were poured into Petri plates with a diameter of 90 mm, taking care not to exceed the amount of 15 ml/plate.

Physico-chemical characteristics and sterility control of culture media: in order to control the physical and chemical quality of both the simple MacConkey agar and the cefotaxime supplemented MacConkey agar, the following were observed: the filling volume/thickness of the medium in the Petri plates, the appearance of the medium, the color and homogeneity, the consistency of the agar, the water content, characteristics which were visually appreciated. In addition, the culture media pH was determined.

Microbiological quality control of culture media: in order to control the microbiological quality of MacConkey agar, the following reference strains were used: *Escherichia coli* ATCC 25922, as the target organism, *Staphylococcus aureus* ATCC 25923, as a non-target organism and *Pseudomonas aeruginosa* ATCC 27853, as a non-specific organism.

For the MacConkey agar microbiological performance testing purpose, the reference stock was obtained from the reference strain, following the manufacturer's instructions (fig. 1 and fig. 2).

Productivity represents the level of recovery of the target organism from a culture medium, under defined conditions, and for MacConkey agar, which is a selective solid agar, productivity is qualitative. **Selectivity** represents the degree of inhibition of the non-target organism on a selective culture medium, under defined conditions. **Specificity** (electivity) consists in demonstrating, under defined conditions, that non-specific microorganisms do not present the same visual characteristics as the target microorganisms.

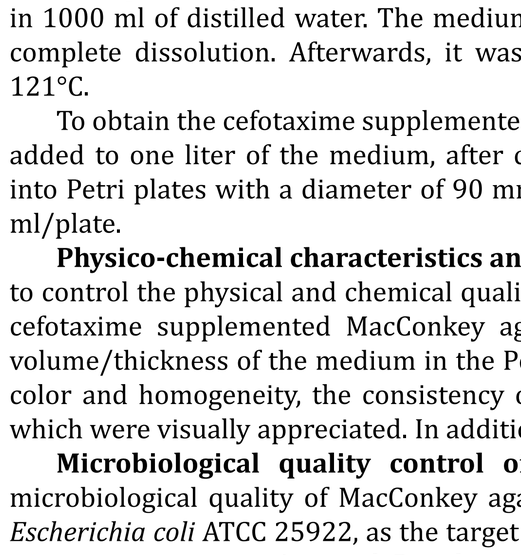

Fig. 1. Inoculum obtaining stages for microbiological testing of simple MacConkey agar

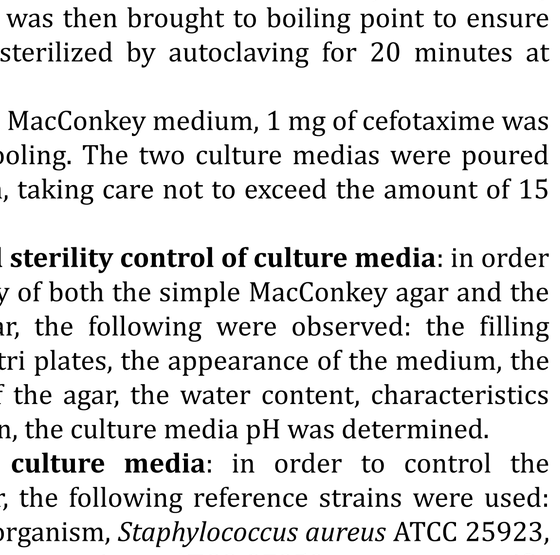

Fig. 2. Steps in obtaining inoculum for microbiological testing of cefotaxime supplemented MacConkey agar.

• Results and discussions

Microbiological quality of simple MacConkey agar: the determined microbiological parameters (productivity, selectivity and specificity) have been assessed visually, with a specific score to each parameter separately (fig. 3 and fig. 4).

Fig. 3. *E. coli*, respectively *S. aures* on simple MacConkey agar

Fig. 4. *P. aeruginosa* strain on simple MacConkey agar

For microbiological parameters, the following results were obtained: for productivity - score 2, specificity - score 0, and for selectivity a non-specific increase was observed, thus considering that all three parameters for testing the microbiological performance of simple MacConkey agar were met (Table 1).

Table 1

Results obtained in the microbiological testing of simple MacConkey agar and interpretation, based on the assigned scores

No.	Parameter	The bacterial strain used	Differential colonial characteristics	Result
1	Productivity	*E. coli*	Flat, dry, pink, non-mucoid colonies, with a darker pink surrounding area of precipitated bile salts	Score 2, parameter fulfilled
2	Specificity	*S. aureus*	No growth	Score 0, parameter fulfilled
3	Selectivity	*P. aeruginosa*	Colorless, flat, smooth colonies, 2-3 mm in diameter, with greenish to brownish pigmentation	Non-specific growth, parameter fulfilled

Microbiological quality of cefotaxime supplemented MacConkey agar

The performance of the cefotaxime supplemented MacConkey agar was as expected, namely a good growth for *E. coli* - positive control and the absence of growth for *E. coli* - negative control, thus the batch of cefotaxime supplemented MacConkey agar was considered valid and can be used to test the antibiotic resistance of *E. coli* strains isolated from fecal samples.

The frequency of additional validation with positive and negative control, for each medium batch must be established within each laboratory, but it is recommended that the testing be carried out weekly, in order to avoid canceling the results obtained in the case of mediums that have not been properly validated.

• Conclusions

- The accuracy of the results obtained in a microbiology laboratory largely depends on the quality of the used culture medium.
- By appreciating the physico-chemical, microbiological and sterility characteristics of a culture medium, it is ensured that correct results are obtained when reporting a valid result.
- The microbiological performance of a culture medium represents the response of that medium to the challenge represented by target organisms, non-target organisms and non-specific organisms for that medium.
- In the case of MacConkey agar, for the tested batch, appropriate scores were obtained, which were interpreted according to the SR EN ISO 1113:2014 standard, all microbiological parameters, namely productivity, selectivity and specificity, being fulfilled.
- The performed experiments demonstrated that cefotaxime supplemented MacConkey agar can be successfully used for the isolation of ESBL/AmpC-producing *E. coli* strains from feces, within the Program for testing antibiotic-resistant strains isolated from feces samples.