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Defibrinated vs. Citrated Blood Agar: Assessing the Impact of Blood Form on Bacterial Growth and Morphology

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Abstract: ***Blood agar plates are a cornerstone in microbiology for cultivating and characterizing bacteria. This study investigated the impact of blood processing methods (defibrinated vs. citrated) on bacterial growth and morphology when cultured on sheep blood agar plates. Both defibrinated and citrated blood agar supported bacterial viability for all tested strains (Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, and Streptococcus pyogenes). Hemolysis patterns, a key factor in bacterial identification, remained consistent between the two blood agar types for most strains, exhibiting alpha hemolysis (Streptococcus pneumoniae), beta hemolysis (Staphylococcus aureus, Streptococcus pyogenes), and gamma hemolysis (Enterococcus faecalis and Escherichia coli). While all strains grew on both media, subtle variations in colony size, opacity, and surface texture were observed, particularly for Streptococcus pneumoniae, Staphylococcus aureus and Streptococcus pyogenes colonies on defibrinated blood agar (shinier and more opaque, respectively). These findings suggest that both blood agar types can be suitable for routine bacterial culture, although defibrinated blood agar might offer advantages for detailed colony morphology analysis. Future studies could explore a broader range of bacterial species and quantify colony morphology variations to further elucidate the influence of blood processing methods. This study can contribute valuable insights into optimizing blood agar preparation for reliable and consistent bacterial analysis in clinical laboratories.***

Keywords: Sheep blood agar, Defibrinated sheep blood, Citrated blood, Hemolysis

1. Introduction

The choice of growth media in microbiology labs can have a significant impact on the growth and appearance of bacterial cultures. These plates offer vital nutrients—vitamins, minerals, and amino acids—that are required for the growth of bacteria. (Bonnet et al., 2019) . Because of their flexible substrate, sheep blood agar plates are a widely utilized medium that may facilitate the cultivation, isolation, and characterization of diverse microorganisms for research, diagnostic, and industrial applications. This medium provides an excellent environment for the growth and cultivation of a wide range of microorganisms, particularly bacteria. Sheep blood is added to enhanced microbiological medium in the form of nutrient - rich agar base and usually in amounts between 5% and 10% (Yeh et al., 2009) . Furthermore, the presence of blood makes hemolytic activity detectable, which helps identify and categorize bacteria. (HRV et al., n. d.) . The physical characteristics and patterns of bacterial development, however, can be impacted by variations in blood composition, especially when using citrated or defibrinated blood. (Anand et al., 2000)

The fibrinogen protein, which is required for blood coagulation, has been extracted from the blood used to make defibrinated blood agar plates (GLUCONE, n. d.) . This method ensures that the blood stays liquid and makes it easier to incorporate the blood into the agar media. In microbiology labs, defibrinated blood agar plates are widely used for routine bacterial culture and isolation procedures. (Russell et al., 2006)

Blood in citrated blood agar plates, is treated with sodium citrate, an anticoagulant that chelates calcium ions to inhibit blood clotting (Mann et al., 2007) . Citrated blood agar plates are a substitute for defibrinated plates that maintain the blood components' integrity while inhibiting coagulation (Yeh et al., 2009) . However, citrate has the power to alter the metabolic conditions of the medium, which in turn affects the bacteria's growth characteristics.

The choice between defibrinated and citrated blood agar plates can influence bacterial growth kinetics, colony morphology, and hemolytic activity (Russell et al., 2006) . Defibrinated blood agar plates may support more robust bacterial growth due to the presence of intact blood components, promoting hemolysis and facilitating the visualization of distinct colony morphologies. (Yeh et al., 2009) .

The aim of the study is to observe the differences between blood agar plates that are citrated and defibrinated, as well as how those differences impact the cultures of bacteria.

2. Materials & Methods

Collection of blood &quality check

The defibrinated and citrated blood were purchased from local distributors (CNK imports and export). Defibrination is done by manually rotating the sterile glass beads (6 mm) in 500 mL sterile Screw cap glass bottle.

Citrated blood received in blood bag which contains Anticoagulant Citrate Phosphate Dextrose Adenine Solution I. P - 49 mL.

The quality of the blood is cross - checked once it has been received in the laboratory. The physical checking was conducted by examining the blood for any signs of contamination, such as discoloration, clots, or unusual odors. The sterility test is carried out by aseptically transferring a small quantity (2 ml) of blood to a sterile brain - heart infusion broth. Incubate the broth for bacterial growth at 37°C. (24–48 hr). The broth is examined daily for any signs of turbidity or microbial growth in the broth, indicating contamination of the defibrinated blood. If turbidity is observed, streak the broth in the soybean casein digest medium to find the source of the contamination.

Preparation of blood agar

Sheep blood agar media were prepared and supplemented with 5% citrated sheep blood, defibrinated sheep blood based on the below composition.

Ingredients	Quantity (gm/L)
Pancreatic Digest of casein	14
Beef extract	3
Sodium chloride	5
Yeast extract	3
Agar	14

Bacterial strains

The bacterial strains used in this study are ATCC strains (5 nos.), NCTC strains (2 nos), and clinical strains (5nos each) as indicated in Table 1. The clinical strains were procured from Little flower hospital laboratory, Angamaly, Kerala.

Bacterial Standard Strains	Clinical Isolates
Streptococcus pneumoniae ATCC 6303	*Streptococcus pneumoniae*
Streptococcus pneumoniae NCTC 1936	*Streptococcus pyogenes*
Staphylococcus aureus NCTC 7443	*Staphylococcus aureus*
Staphylococcus aureus ATCC 25923	*Staphylococcus saprophyticus*
Enterococcus faecalis ATCC 29212	*Enterococcus faecalis*
Escherichia coli ATCC 2592	*Escherichia coli*
Streptococcus pyogenes ATCC 19615	*Klebsiella pneumoniae*

Inoculation &incubation

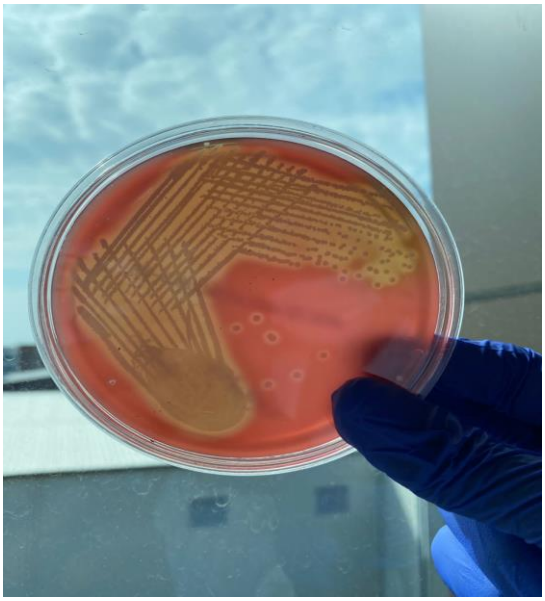
A suspension equal to the 0.5 McFarland standard (1.5 x 108 CFU/ml) was prepared using reference and all the fastidious organisms of ATCC & NCTC and clinical strains (…Streptococcus pneumonia, Streptococcus pyogenes) inoculated in Brain Heart Infusion broth, Nonfastidious were (other all bacteria in the above list) inoculated in peptone water and 100 microliter of suspension were plated in triplicate on citrated and defibrinated blood agar plates. The inoculated plates were incubated at 37 °C for 18–24 hours, and the results were recorded in accordance with the colony morphology, hemolysis pattern, and viable count. (Satzke et al., 2010) .

3. Results and Discussion

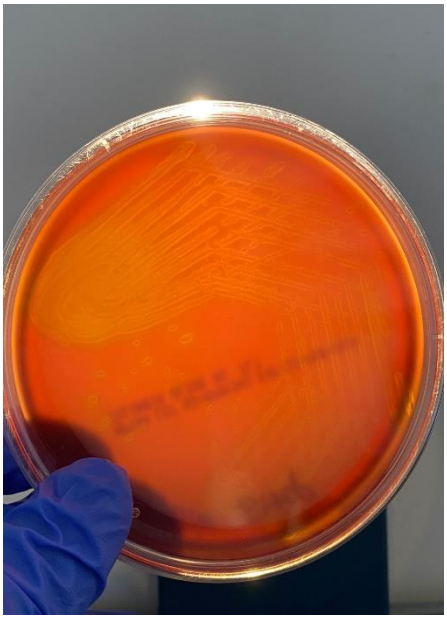
This study investigated the potential influence of blood preparation methods (defibrinated vs. citrated) on bacterial growth and morphology when cultured on sheep blood agar plates. Here, we will analyze the obtained results and their significance for microbiology practices. The outcomes from both citrated and defibrianted blood agar are presented here. While alpha and beta hemolysis indicate partial and total lysis of red blood cells, respectively, the gamma hemolytic strain exhibits no lysis pattern at all.

Strains		Citrated Sheep Blood agar	Defibrinated Sheep Blood agar
Streptococcus pneumoniae (ATCC 6303)	Colony Characteristics	Dull, grey, mucoid,	Shiny, grey, mucoid,
	Colony size (mm)	Pinpoint	Pinpoint
	Haemolysis	Alpha	Alpha
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Staphylococcus aureus (ATCC 25923)	Colony Characteristics	Opaque white, smooth, translucent, raised colonies	Opaque white glossy, smooth, glistening, translucent, raised colonies
	Colony size (mm)	1 mm	1 mm
	Haemolysis	Beta	Beta
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Enterococcus faecalis (ATCC 29212)	Colony Characteristics	Grayish - white, small, smooth,	Grayish - white, small, smooth,
	Colony size (mm)	Pinpoint	Pinpoint
	Haemolysis	Gamma	Gamma
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Escherichia coli (ATCC 2592)	Colony Characteristics	Small, Moist, circular, and low - convex with entire edges	Small to medium, circular, Moist and low - convex with entire edges
	Colony size (mm)	3 mm	3 mm
	Haemolysis	Gamma	Gamma
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
	Colony Characteristics	Grey, Smooth, clear margins,	White, Smooth, clear margins,
	Colony size (mm)	0.4 mm	0.6 mm

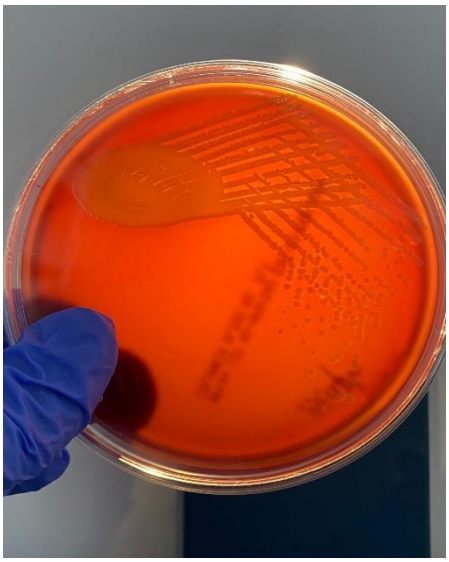
Streptococcus pyogenes ATCC19615	Haemolysis	Beta	Beta
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Streptococcus pneumoniae NTCC 1936	Colony Characteristics	Dull, grey, mucoid,	Shiny, grey, mucoid,
	Colony size (mm)	Pinpoint	Pinpoint
	Haemolysis	Alpha	Alpha
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Staphylococcus aureus NTCC 7443	Colony Characteristics	Opaque white, smooth, glistening, translucent, raised colonies	Opaque white glossy, smooth, glistening, translucent, raised colonies
	Colony size (mm)	1 mm	1 mm
	Haemolysis	Beta	Beta
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Streptococcus pneumoniae	Colony Characteristics	Dull, grey, mucoid,	Shiny, grey, mucoid,
	Colony size (mm)	Pinpoint	Pinpoint
	Haemolysis	Alpha	Alpha
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Escherichia coli	Colony Characteristics	Small, Moist, circular, and low - convex with entire edges	Small to medium, circular, Moist and low - convex with entire edges
	Colony size (mm)	2.5 mm	2.5 mm
	Haemolysis	Gamma	Gamma
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Staphylococcus saprophyticus	Colony Characteristics	Creamy white,	Creamy Bright white,
	Colony size (mm)	6 mm	8mm
	Haemolysis	Gamma	Gamma
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Staphylococcus aureus	Colony Characteristics	Opaque white, smooth, glistening, translucent, raised colonies	Opaque white glossy, smooth, glistening, translucent, raised colonies
	Colony size (mm)	1 mm	1 mm
	Haemolysis	Beta	Beta
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Enterococcus faecalis	Colony Characteristics	Grayish - white, small, smooth,	Grayish - white, small, smooth,
	Colony size (mm)	Pinpoint	Pinpoint
	Haemolysis	Gamma	Gamma
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Klebsiella Pnemoniae	Colony Characteristics	Small, grey, moist, mucoid	Small, grey, moist, mucoid
	Colony size (mm)	4 mm	5 mm
	Haemolysis	Gamma	Gamma
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)
Streptococcus pyogenes	Colony Characteristics	Grey, Smooth, clear margins,	White, Smooth, clear margins,
	Colony size (mm)	0.3 mm	0.5 mm
	Haemolysis	Beta	Beta
	Growth	luxuriant (Viable after 24hours of inoculation)	luxuriant (Viable after 24hours of inoculation)



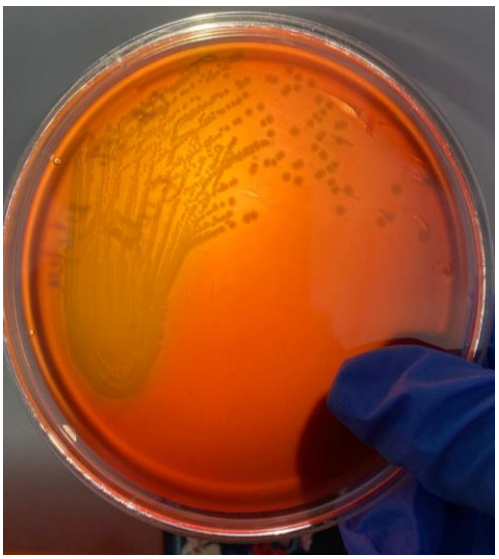
Defibrinated – *Staphylococcus aureus*



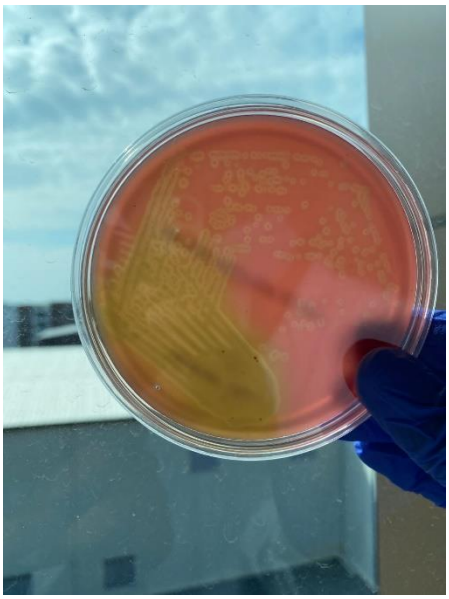
Citrated- *Streptococcus pneumoniae*



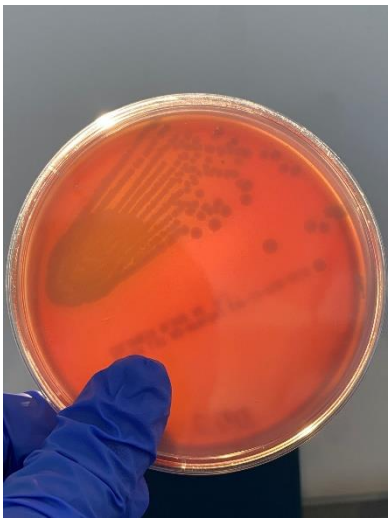
Citrated- *Staphylococcus aureus*



Defibrinated – *E.coli*



Defibrinated – *Streptococcus pneumoniae*



Citrated- *E.coli*

Bacterial Growth and Hemolysis:

The observed bacterial growth patterns across all strains on both defibrinated and citrated blood agar plates suggest that

both media can support bacterial viability. All strains exhibited comparable growth (luxuriant) after 24 hours of incubation, indicating that neither blood processing method significantly hindered bacterial survival.

Hemolysis patterns, a crucial factor in bacterial identification, were largely consistent between defibrinated and citrated blood agar for most strains. Alpha hemolysis (partial red blood cell lysis) was observed for *Streptococcus pneumoniae* strains (ATCC 6303 and NCTC 1936), while *Staphylococcus aureus* strains and *Streptococcus pyogenes* strains (ATCC 25923 and NCTC 7443) displayed beta hemolysis (complete lysis). *Enterococcus faecalis* (ATCC 29212) and *Escherichia coli* (ATCC 2592) exhibited gamma hemolysis (no lysis) on both blood agar types. These observances were significantly comparable with the clinical isolates. These findings are in line with established characteristics of these bacterial species.

Colony Morphology:
While all strains grew on both blood agar types, some variations in colony characteristics were observed. These included subtle differences in colony size, opacity, and surface texture. For instance, *Streptococcus pneumoniae* colonies appeared pinprick in size on both media, but with a slightly shinier appearance on defibrinated blood agar. Similarly, *Staphylococcus aureus* colonies displayed comparable sizes, but those on defibrinated blood agar appeared slightly larger and more opaque. The physical characteristics (shape, size, color) of *Streptococcus pyogenes* colonies from both control and clinical samples were easier to see on defibrinated blood agar compared to citrated blood agar.

4. Interpretation and Future Considerations

The current study provides initial insights into the potential effects of blood processing methods on bacterial growth and morphology. The observed consistency in bacterial viability and hemolysis patterns for most strains suggests that both defibrinated and citrated blood agar can be suitable for routine bacterial culture in clinical settings. However, the noted variations in colony morphology warrant further investigation. Future studies could explore a broader range of bacterial species and quantify colony size measurements to determine the extent of these morphological differences. Additionally, analyzing the composition of defibrinated and citrated blood might reveal specific components influencing the observed variations.

5. Limitations

This discussion acknowledges the limitations of the proposed study design.

Here are some aspects to consider:

- The origin of the defibrinated and citrated blood (species source) might influence their growth - promoting properties.
- The chosen bacterial strains may not represent the full spectrum of organisms encountered in a clinical setting. Additional factors beyond blood preparation, such as media composition or incubation conditions, could also affect bacterial growth.

6. Conclusion

In conclusion, the choice between defibrinated and citrated blood agar can have significant implications for studying bacterial growth and morphology in the laboratory. While defibrinated blood agar provides optimal conditions for robust bacterial growth and accurate assessment of colony characteristics, citrated blood agar offers specific advantages, such as preventing clot formation and enabling specialized testing procedures. By understanding the differences between these two types of blood agar, microbiologists can make informed decisions when selecting growth media for their experiments, ensuring reliable results and accurate interpretation of bacterial behavior.

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