

APPLICATION NOTE

Production of high quality blood agar – MEDIAJET in combination with the DOSE IT

The quality of blood agar plates is of prime importance for the reliable determination of hemolysis type – a common technique in medical diagnostics to classify Gram positive cocci. If combining the MEDIAJET Petri dish filler with the DOSE IT peristaltic pump, the blood can be added to the agar immediately before pouring the plate. This minimizes the time the blood is exposed to hot agar medium and thus efficiently reduces thermally induced lysis of erythrocytes.

Introduction

Determination of hemolysis type is an important classification system in microbiology and medical diagnostics. It is particularly useful in classifying streptococcal species. Streptococci are responsible for many cases of bacterial pneumonia, meningitis, endocarditis and other serious infection diseases. To detect hemolytic activities, blood agar plates made up of 5-10% defibrinated animal blood (e.g. sheep, bovine) are commonly used. Streptococcal colonies growing on blood agar plates show the ability to rupture red blood cells either completely, partially or not at all. These specific abilities can be differentiated by the hemolytic pattern on blood agar plates (Fig. 1).



Figure 1: α -hemolysis (*Streptococcus* spp., left) β -hemolysis (*S. mitis*, middle) and γ -hemolysis (*S. pyogenes*, right).

Alpha hemolysis is shown by a greenish halo around the colony. The halo is generated by partial hemolysis of the red blood cells. This is caused by hydrogen peroxide produced by the bacterium, which oxidizes erythrocyte hemoglobin. Oxidation of hemoglobin leads to green methemoglobin. Beta hemolysis is shown by a clear halo around the colony, caused by complete hemolysis of red blood cells. The complete lysis of erythrocytes is due to the endotoxin Streptolysin, an enzyme secreted by the bacterium. Streptolysin interacts with cholesterol in the membrane of eukaryotic cells, eventually leading to the break up of cells.

Gamma hemolysis is shown as no hemolysis or discoloration of the blood (Fig. 1).

Blood agar plates of high quality are essential for the reliable determination of hemolysis type. High quality agar plates have a typical light red color – a characteristic feature of intact erythrocytes. Agar plates with lysed blood cells appear brown colored, a feature that complicates the reading of hemolysis pattern. To avoid spontaneous lysis of erythrocytes, it is important to keep the temperature of the agar as low as possible. In addition to minimize cell lysis, the time the blood remains in the hot agar has to be as short as possible. Meeting these requirements is a challenge for most laboratories.



Figure 2: Brownish color of an agar plate if erythrocytes are lysed (left). Typical light red color of high quality blood agar plates (right).

Success factors for high quality blood agar

Using the INTEGRA media preparation system

This section describes an automated protocol that minimizes the time that the blood is exposed to hot agar medium and thus efficiently reduces thermally induced lysis of erythrocytes. By preparing the blood agar using an INTEGRA MEDIACLAVE media preparator, the dispensing temperature can be set and maintained at low temperature. Furthermore, by combining the MEDIAJET Petri Dish filler together with a DOSE-IT peristaltic pump and special silicone blood agar tubing, the blood can be added to the agar immediately before pouring the plate thereby reducing red blood cell lysis significantly.



Figure 3: MEDIAJET Petri dish filler in combination with the peristaltic pump DOSE IT and the blood agar tubing set.

The right dispensing temperature of the agar medium

The temperature of the agar during dispensing is essential to reduce thermal stress to erythrocytes. If too hot, the red blood cells will lyse. The lysis of erythrocytes appears as a changing of the agar color appearance from light red to brown. On the other hand, if too cold, the mixing performance with the blood is declined, resulting in inhomogeneous distribution of the blood on the plates. This becomes mainly apparent by the formation of streaks on the plates. Generally, these streaks do not impair growth of bacteria but the reading of hemolysis pattern is complicated. Therefore, the dispensing temperature has to be set as low as possible, ideally between 45°C and 47°C.

Blood at room temperature

Having the blood at room temperature is crucial for homogeneous mixing with the agar. Sterile blood is usually stored at low temperatures between 4-8°C. Before adding it to the agar, the refrigerated blood has to be tempered to room temperature. Cold blood below room temperature may lead to partial gelling of the agar within the silicone tubing and subsequently formation of streaks on the plates

Precise synchronization of MEDIAJET and DOSE IT

Setting the proper flow rate of the peristaltic pump DOSE IT is crucial for the precise synchronization with the MEDIAJET. Without accurate synchronization, the mixing performance is impaired and the blood volume varies between the dispensing steps. The flow rate of the DOSE IT has to be set such that the dispensing time is equal or slightly shorter than the dispensing time of the MEDIAJET.

Agitation of blood during dispensing

Because of their high specific density, erythrocytes sink below the plasma if the blood is allowed to stand for a prolonged time without agitation. For small blood containers (= 0.5 L), it is sufficient to shake them by hand just before starting the automated addition to the agar. If using larger blood containers, agitation might be necessary to avoid sinking of the red blood cells.

Tested instrument settings

The following instrument settings for producing blood agar plates were extensively tested by INTEGRA Biosciences and experienced blood agar producers.

MEDIACLAVE 10/30 settings:

Sterilization temperature:	121°C
Sterilization time:	15 min
Stirrer speed at sterilization:	150 rpm
Dispensing temperature:	47°C
Stirrer speed at sterilization:	100 rpm

MEDIAJET settings:

Dosing volume:	16 ml
Pump speed:	60 %
Pause time:	0.0 s
Rotor movement:	spread

DOSE IT settings:

Tubing-ID (mm):	2.0 mm
Volume:	0.8 ml
Flow rate (ml/min):	25.2

Conclusion

The INTEGRA Biosciences media preparation system facilitates the production of high quality blood agar. The media sterilizer MEDIACLAVE makes it possible to cool the agar medium down to the optimal dispensing temperature, minimizing blood cell lysis. Furthermore, combining the MEDIAJET with the DOSE IT, together with a special blood agar tubing set, minimizes the time the red blood cells are exposed to high temperature. Light red blood agar plates of high quality throughout the whole batch can be guaranteed if:

- The dispensing temperature of the agar medium is set as low as possible.
- The blood is tempered to room temperature.
- MEDIAJET filler and DOSE IT pump are precisely synchronized.
- Large blood containers are shaken during dispensing

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