Making Blood Agar Plates

Laura Sycuro[1], Ramon Cortez[1], sakonsch [1]

[1]University of Calgary

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Sycuro Lab

Ramon Cortez

Purpose

Blood agar is agar base enriched with 5% blood. It is an enriched medium often used to grow fastidious organisms and to differentiate bacteria based on their hemolytic properties.

Key concepts

The agar base can vary – Brucella is what is generally prefered for the growth of diverse vaginal organisms, but Casman and Columbia have also been used and shown to support a broad variety of organisms.

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Supplies

- Agar base (can be media base already containing agar, or liquid media base to which you add the agar separately)
- Distilled water
- Glass media bottles
- Blood (we usually use defibrinated sheep blood that is less than 1 month old)
- Sterile Petri dishes
- 25 mL serological pipet
- Serological pipettor

Equipments

- Autoclave
- Water bath
- Bunsen burner
- Stir plate

Wear a regular (fabric) lab coat when you pour plates. This helps ensure your sleeves/clothing won't become soiled with blood and won't come into contact with the Bunsen burner flame. Tie long hair back.

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Preparation

1 Mix appropriate amount of agar base into distilled water in a glass media bottle.

- **500 mL** agar in a 1 L bottle makes ~25 plates.
- Each bottle of powdered media lists the grams of powdered media needed for 1 L of media. Use half this amount for 500 mL.

1.1 Stir media with stir bar until completely dissolved.

Leave the stir bar in the bottle during autoclaving.

2 Autoclave

- For 1 or 2 bottles of Brucella agar media, autoclave for 15 minutes.
- For 3 or more bottles of Brucella agar media, autoclave for 30 minutes.
- For Columbia agar media, autoclave for 45 minutes.

Be sure to label the bottle **and the autoclavable pan with 'Agar' and requested autoclave time ('15 minutes'). Also can be helpful if you add ~1 inch of water to the bottom of the pan (they won't always do this for you).

3 After dropping off downstairs for autoclaving, turn water bath on and set to **55 °C** .
Remove blood from refrigerator and allow to warm on the bench.

- Check and make sure no one else needs water bath at lower temperature for the next 1-2 hours.
- Use the freshest blood available and generally do not use blood that is over 1 month old.

Pouring 30m

4 Pick up media from downstairs shortly after run completes (they should place it in a **55 °C** incubator downstairs after the run).

**Remember to bring thermal gloves with you when you go downstairs to retrieve your

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media from the incubator. It may still be hot!!

- Place in **55 °C** water bath for 20-30 minutes.
- Media is ready to pour when bottle is warm, but not hot to the touch.

5 Mix blood (at room temperature) by gently swirling (rubbing the bottle between your hands works well).

6 Prepare plates and pouring area.
- Clear and clean area of bench near Bunsen burner.
- Arrange sterile plates right side up in small stacks of 4-5.
- Stripe plates if desired.

7 Bring blood, a **25 mL** serological pipet, and the serological pipettor to the media making area (stir plate).

7.1 Remove 1 bottle of media from water bath and place on stir plate.

7.2 While media is being stirred, pipet **25 mL** of blood into **500 mL** of media (final concentration of **5 % (v/v)**)

7.3 Stir for 1 minute or until mixed.

8 Move to pouring area and pour plates immediately.
- Flame lip of bottle at the start of each stack of plates.
- When bottle is empty, carefully flame plates to remove bubbles.

9 Gently push plates to back of bench (away from Bunsen burner hose) to solidify and then clean up bench and rinse the media bottle.
- All papers and pipets with blood on them get disposed in the yellow biohazard waste bin

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(near fume hood).

10 Prepare to pour second batch of plates if needed.

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Completely finish with one batch of plates before removing a second bottle from water bath.

- Do not add blood to second bottle and then put back into water bath – carry out the pouring procedure from start to finish 1 bottle at a time.

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