

## LYSIS OF BLOOD

### **Procedure for lysis of horse blood by 3 x freeze-thaw (work in lab bench with gloves)**

1. Use 50 mL defibrinated horse/cattle/ blood.  
**NB: The blood only keeps 24 hours at room temperature and 3 weeks at 5°C.**
2. Shake the bottle and pour 20 mL in each tube (50 mL conical plastic tubes).
3. Write the batch no. of the blood on the plastic tubes.
4. Freeze the tubes at -20°C until the next day.
5. Next morning, take the blood from the freezer and thaw at room temperature.
6. Repeat steps 4 + 5 until the blood has been frozen and thawed three times.
7. You may choose to check the lysis of the blood by microscopy: Use 100x enlargement if all/almost all erythrocytes are lysed.
8. Add 20 mL sterile MilliQ water to all plastic tubes containing blood.  
Invert the tubes a few times.
9. Seal the tubes and centrifuge at 5000 rpm for 30 minutes.
10. Carefully, decant the supernatant into a new tube, leaving the pellet in tube. Alternatively, use a 20mL disposable syringe/pipette to remove the supernatant. Throw away the pellet.
11. Write on the tube the batch no. of the blood along with date and initials
12. Store the lysed blood in a freezer (-20°C)

**50% lysed horse blood keeps for four months at -20°C**

**50% lysed horse blood keeps for 24 hours at 5°C**

## **PREPARATION OF A NEW BATCH MH + BLOOD**

### **Procedure for preparing a new batch of Mueller Hinton broth supplemented with 5% lysed blood (work in lab bench with gloves)**

1. Thaw the lysed blood (40 mL makes 40 tubes of 10mL MH+blood)
2. Use a batch of MH-bouillon in 10 mL test tubes – preferably a batch that does not expire until four months later
3. Register the batch no. of the test tubes with MH along with the batch no. of the blood. Determine the expiry date of the MH+blood as the shortest of
  - Four months from preparation date (MH+BLOOD keeps for four months at 5°C)
  - Expiry date of the MH broth
  - Expiry date of the blood
4. Invert the tube with the blood a few times and transfer 1000µl to each test tube. Invert the test tube a few times.
5. Incubate the batch at 35-37°C until next day
6. Next day: Examine the batch for contamination (towards light, look for pellet in the bottom)
7. Store the new batch at 5°C.
8. Measure the pH in two tubes. Temperature when measuring: 25° C. Record the pH in a log book along with the no. of contaminated tubes

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1mL lysed horse blood (50%) is added to 10-11 mL MH broth =>

The final concentration of the lysed blood in the broth is 4,1-4,5%