# Preparations at the lab

## Leukocyte depleted blood

## Materials

* Silicone tubing to connect catheter bag to leukofilter
* Clamps
* Leukocyte filter (BioR O2 plus BS PF, Fresenius Kabi, Bad Homburg, Germany)
* Tie wraps
* 2L beaker
* Parafilm tape/cling foil

## Protocol

1. Attach the leukocyte filter to the outlet of the catheter bag. And attach a funnel to the top of the tubing.
2. Put the tie wraps to the top sides of the catheter bag.
3. Close all the tubing underneath the bag and fill the bag with **1L** of blood (2 person job).

Repeat this step with another liter of blood.

1. Fill the leukocyte filter and de-air by holding it upside down.
2. Attach the system to a suspension hook a place the beaker underneath.   
   Attach the tube to the beaker with the red clip.
3. Open the outlet of the catheter bag and de-air the leukocyte filter, by keeping it upside down until blood leaves the filter.
4. When the blood has passed the filter, close the clamp.
5. Devide the leucocyte-depleted blood in 4 special centrifuge bottles.
6. Centrifuge the blood 20 min. 3000 g. 20°C.
7. Put a silicon tubing with a 50 ml syringe on the bottom of the bottle and remove the RBCs.   
   Put the RBC in a separate bottle.
8. Wash the RBC (pellet) with an equal volume of 1x PBS (1:1)
9. Centrifuge the RBC 20 min. 3000 g. 20°C
10. Put a silicon tubing with a 50 ml syringe on the bottom of the bottle and remove the RBCs.

## Cleaning

1. Put all the used instruments in a 5L beaker with water and some biotex.
2. After a while clean everything with water and rinse with demi water.
3. Use the air tap in the fume hood to get rid of the excess water in cannulas.