

A risk assessment for working with HIV

Action	Risk	Solution
Blood test	The right tubes?	Use preferably PBMC tubes(peripheral blood mononuclear cells) where the gel barrier separates erythrocytes and granulocytes underneath and where the Ficoll –Hypaque medium keeps the lymphocytes, monocytes and platelets in plasma.
Transport	Avoid any mess under transportation	Transport each glass in separate plastic tubes collected in a plastic box. Keep them in an isopore box.
Centrifugation	Avoid aerosol when centrifuging.	Always keep the cap on when centrifuging.
	Defect separation of blood cells and plasma	Do not keep the samples on ice before centrifuging a CPT-sample. Do the centrifuging on Ullevål at room temp. A cold CPT-gel gives bad separation.
Preparing for work	Products for decontamination and preparation	Prepare every time: Virkon powder. 1- 3% made in the bench (irritating) Active:Pink colour. Inactivat:no colour. Bleach 10%, (Sodium hypochlorite) keep dark and cold: Dilute to 1% ..(1:9)
	Everything in contact with blood or plasma need to be decontaminated	For large pipettes: Prepare 1,6 L 1,5% Virkon in the graduated cylinder and keep it in the plastic beaker not to tilt. For larger tips: Prepare 1,5% Virkon one or two plastic beaker , <u>not too full</u> . For blood samples: Fill a plastic box ¼ with 3% Virkon. For decontamination Prepare 1% Bleach.
	Is the bench clean?	Always wipe of with 70% disinfection ethanol. Use preferable the same bench every time.
	Infection work	Use only yellow racks.
Starting the work	Avoid aerosol removing the cap after centrifuging.	Always remove the cap in the bench. Keep the hatch down
	Decanting the plasma	Avoid the drop when decanting, and if contaminating the bench – immediately decontaminate with 1% bleach for 30 min. Afterwards 70% ethanol.
	Gel fouling in the sample	Filter the plasma. Wash the filter three times with PBS to save the cells. Centrifuge and remove the supernatant from the Falcon tube with a long pipette. After washing there is not much free virus left, mostly in the cells.
Waste	Pipettes and tips loaded with Virkon.	Leave over night and dispose of in the sink. Rinse the equipment thoroughly.
	Box with blood samples.	Close it thoroughly. Shake it and dispose of standing in the yellow container. Always click the lid firmly on the yellow boxes for biologic waste (11 clicks)
Analyzing	Counting for estimate	Learn to use a Bürcker – easier than disinfecting the cell counter.
	Separation of cells	Only use fixated cells on the flow. Wait 30 min before opening the chamber.