

**Circulating Tumor Cells TheRapeutic APheresis: a
novel biotechnology enabling personalized therapy for
all cancer patients**

**SOP.9. Manually counting and spiking of
cells in blood**

UNIVERSITY OF TWENTE.



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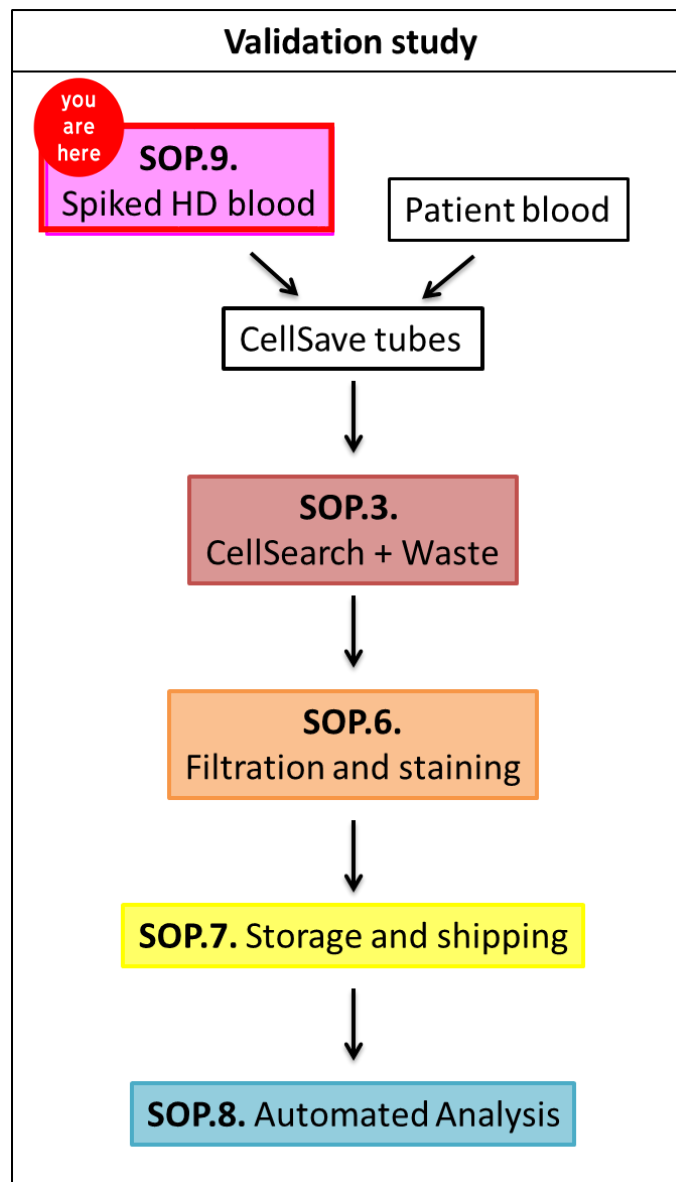


Introduction

This Standard Operating Procedure (SOP) describes the spiking of cell manually in blood samples. These samples will be used to determine the recovery of cell line CTC after CellSearch, CellSearch Waste Filtration (**SOP.3.**) and staining (**SOP.6.**).

This is SOP.9. Manually counting and spiking; version 1.0-092015/VS

Workflow of procedures in the CTCTrap program Validation Study



SOP.9. Manually counting and spiking of cells in blood

For the validation study, cells will be manually spiked in healthy donor (HD) blood samples. The cell lines used for this are PC3 prostate cancer cells and MDA-MB-231 breast cancer cells. Both of these cells are (partially) EpCAM negative and will be found after CellSearch and after filtration.

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1. Cells

Time: 30 min

Note: Cells have to be added to the CellSave blood sample on the day of draw.

1. Dissociate the cells for spiking with trypsin/EDTA. After dissociation, transfer the cells to a tube and add CellSave. Add 100 μ L CellSave for every 5 mL medium with cells. Mix thoroughly (see Figure 1).
2. Determine the concentration of cells with a counting chamber.

2. Preparation

Time: 30 min

2.1. Cells

3. Dilute the cells you will spike to a concentration of 15,000 cells per mL in 1 or 0.5 mL PBS/BSA1%. This will be 15 cells per μ L.
4. Add Hoechst-33342 at 2 μ g/mL final concentration and incubate for 10 minutes at 37°C (see Figure 2).

2.2. Microscope slide

5. Put the glass microscope slides in PBS/BSA1% for 10 minutes (see Figure 3).
6. Rinse the slide with MQ and blow dry with air.

2.3. CellSave blood sample

7. Mix the CellSave blood sample thoroughly. Divide the blood samples over the conical tubes with each 7.5 mL blood.
8. Label each tube with a code, separating for each site: unspiked healthy donor blood (HD) (A), spiked PC3 cells (B), spiked MDA-MB-231 cells (C).

3. Counting

Time: 20 min per slide for counting and spiking

9. Add 10 drops of 2 μ L cell suspension on the edge of the slide (see Figure 4).
This will yield approximately 10x 30 cells and can be counted before all the drops dry out.
10. Count the cells with aid of a cell counter under the microscope with 4x magnification using a UV filter cube.
11. Note the amount of cells in each drop (in case you go wrong during counting).

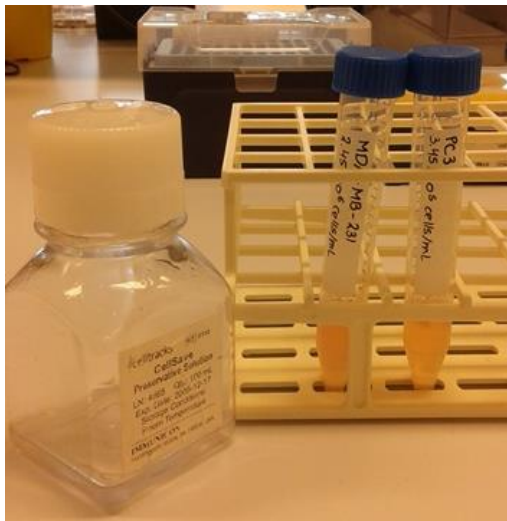


Figure 1 Medium with cells after dissociation, with manually added CellSave (from stock or from CellSave tubes).

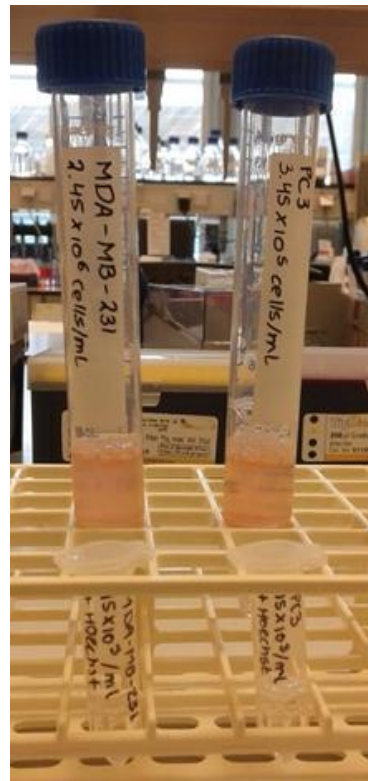


Figure 2 Dilution of cells to $15 \cdot 10^3$ cells/mL for counting with Hoechst for nuclei staining.



Figure 3 Incubating microscope slides in PBS/BSA 1%.



Figure 4 Ten droplets of 2 μ L with cells on a microscope slide for easy manual counting.

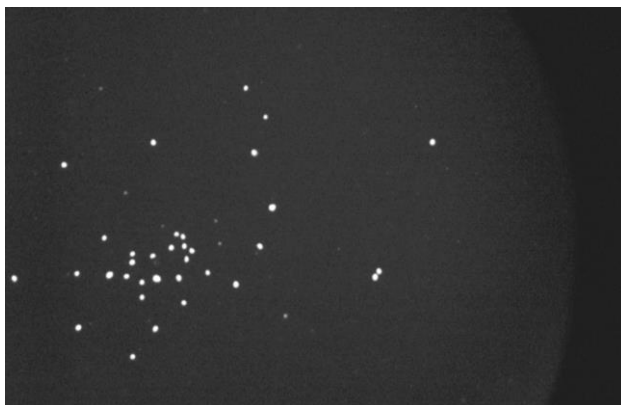


Figure 5a In the droplet you can count 34 Hoechst positive cells. Small specks can be easily distinguished from the cells. This image was taken with a 4x objective.

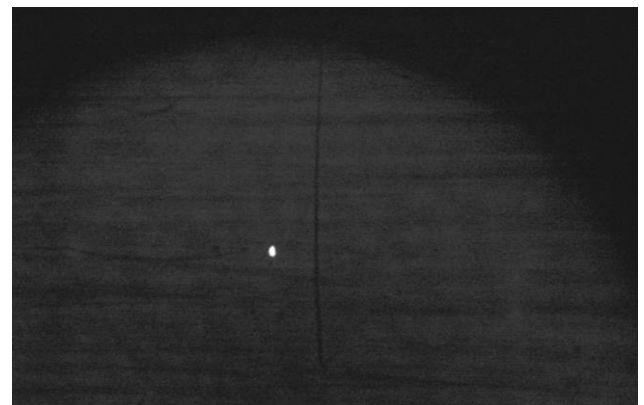


Figure 5b After spiking the cells from the glass slide, the imprint of the drops can be easily spotted with the microscope and any remaining cells in it, as well. Here 1 cell remained behind, so this is deducted from the total count.

4. Spiking

12. Use 1 mL CellSearch Dilution buffer (stored at RT) to rinse the slide and flow the cells in the blood sample (see Figure 5).

Be careful not to spill, because then you will lose cells.

13. Check the clean slide under the microscope and count the remaining cells.
14. Fill in the tables below, by adding all the counts from every drop, and then deducting the amount of cells that remained on the slide.
15. Add 1 mL of CellSearch Dilution buffer to the HD blood tube as well.

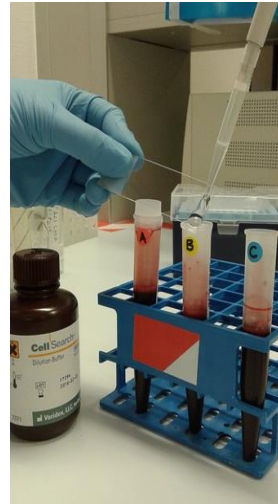
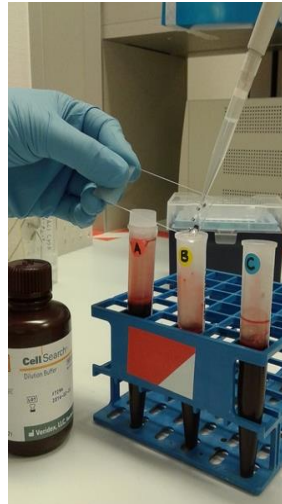
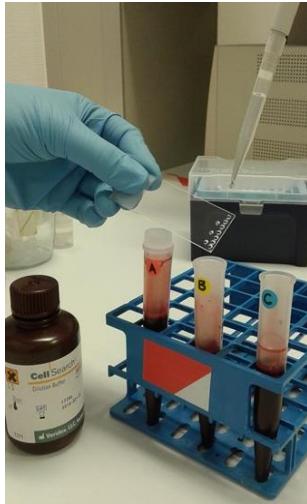


Figure 6 Spiking the cells in the blood with 1 mL CellSearch Dilution Buffer.

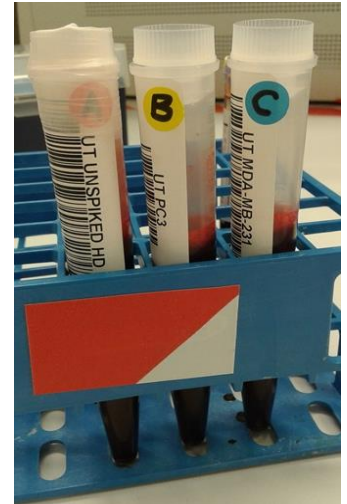


Figure 7 Spiked blood samples designated with cell type and site. Tube A is wrapped for shipment.

5. Counts

Tubes collection 1

Operator		Date: ____ - ____ - ____	
Site	Cell count per cell type		
	HD (A)	PC3 (B)	MDA-MB-231 (C)
ICR			
IGR			
IOV			
LMU			
UFT			
UT			

Tubes collection 2

Operator		Date: ____ - ____ - ____	
Site	Cell count per cell type		
	HD (A)	PC3 (B)	MDA-MB-231 (C)
ICR			
IGR			
IOV			
LMU			
UFT			
UT			

Tubes collection 3

Operator		Date: ____ - ____ - ____	
Site	Cell count per cell type		
	HD (A)	PC3 (B)	MDA-MB-231 (C)
ICR			
IGR			
IOV			
LMU			
UFT			
UT			

6. Shipping

16. Collect the tubes for shipment. Make sure the tubes are securely closed with a conical tube cap. Wrap parafilm around the the caps (see Figure 6).

17. Isolate the tubes sufficiently to protect them from temperature changes during shipment.

Note: All tubes have to be shipped on the day of blood draw, to ensure processing the sample 24 hours after draw.