Standard operating procedure for milk sampling and milk cell isolation

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<u>Purpose</u>

Collecting milk samples directly from the cow's teats for milk cell isolation. Samples are taken from cows with a somatic cell count > 50,000 cells/ml per udder quarter

<u>Materials</u>

- 50ml tubes, 15ml tubes, low-bind DNA tubes (2ml)
- permanent marker and/or tape
- disposable gloves
- ice
- paper towels or wipes
- centrifuge (for 50ml tubes, 15ml tubes and 2ml tubes)
- wide-bore tips and standard pipette tips
- 1X PBS
- FlowMi cell strainers
- Biocoll
- Cellometer Auto2000

Milk sampling

- 4x 50ml tubes are labeled with cow number and quarter with tape or permanent marker
- milk sample is collected immediately subsequent to a regular machine milking in the falcon tube without allowing the container to touch the teat end or any other object
- the cap from the tube is removed and held facing down (without the inside of the cap touching your gloves) in the same hand you will be holding the sample tube
- the tube is held at a 45° angle, angled away from the teat to prevent debris from falling into the tube and filled
- tube is capped immediately after sample is collected and stored on ice
- repeat 3x times for the same quarter
- store sample in refrigerator and process within 4 hours

Cell isolation from milk

- tubes are centrifuged at 3000 g for 10 min
- fat layer is removed leaving about 2 ml of the milk over the pellet and the inside of the tubes is wiped clean using cellulose
- 20 ml 1X PBS is added and the pellet is carefully resuspended using wide-bore tips
- tubes are centrifuged at 3000 g for 10 min
- supernatant is removed
- 10 ml 1X PBS is added and the pellet is carefully resuspended using wide-bore tips

- tubes are centrifuged at 3000 g for 10 min
- supernatant is removed
- 1 ml 1X PBS is added and the pellet is carefully resuspended using wide-bore tips
- cell suspension is transferred into a 2 ml low-bind DNA tube with a standard pipette tip and a FlowMi cell strainer
- 8 ml Biocoll are placed in a 15 ml falcon tube and all cell suspensions are carefully pipetted onto the side wall of the falcon tube
- falcon tube is centrifuged at 800 g for 30 min (brake set to off)
- the middle layer is carefully pipetted into a 2 ml low-bind DNA tube
- cell concentration and viability are measured using a Cellometer Auto2000 (Nexcelom)