

## Reference testing procedure

To receive results with reference accuracy from somatic cell counting using LACTOSCAN SCC the following steps must be done:

### **Samples taking:**

Samples must be taken with accordance to the recommended sampling method given in ISO 707|IDF 50.

### **Samples storage:**

Samples must be stored with accordance to the recommendations in ISO 13366-1 | IDF 148-1:2008, page 4:

#### "7.1 Storage

Prior to testing or preservation, store the test samples at a temperature of  $4\text{ °C} \pm 2\text{ °C}$ .

Analyse the test samples within 6 h after sampling. In the case of longer storage, add chemical preservatives such as boric acid, bronopol or potassium dichromate. The final concentration of boric acid shall not exceed 0,6 g per 100 ml of test sample. The final concentration of bronopol shall not exceed 0,05 g per 100 ml of test sample. The final concentration of potassium dichromate shall not exceed 0,1 g per 100 ml of test sample. Store the thus preserved test samples at a temperature of  $4\text{ °C} \pm 2\text{ °C}$  for no longer than 6 days.

For environmental reasons, it is recommended to restrict the use of potassium dichromate to samples that require a long shelf life only."

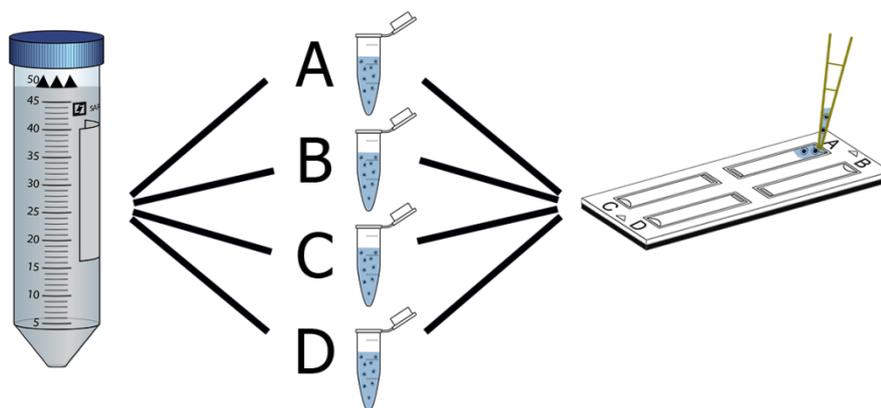
**Note!** Bronopol is the preferred preservative for preparing samples for analysis with LACTOSCAN SCC.

### **Sample Preparation procedure recommended by IDF Reference laboratory:**

Heat the sample in a water bath with the temperature between 38 and 40°C (100 – 104°F) for a period of 10 minutes. Shake vigorously for 2 minutes and then emerge the vial in the water bath for an additional 10 minutes. Cool the sample to the temperature, for example to 20 °C.

### **Testing:**

To do this the following consumables will be required: 1 x X4 cartridge, 4 x eppendorf tubes with Sofia Green dye and 8 x pipette tips.



- After sample preparation take 100µL of sample and put it into an eppendorf tube. Do this a total of 4 times from the vial.
- Incubate for 5 minutes while mixing frequently.
- After incubation do a final mixing and take 7.5 µl of the sample and put into the X4 cartridge – do this a total of 4 times, each time with sample from a new eppendorf tube.

**Note!** A new tip must be used for preparing each of the 4 samples and a new tip must be used for putting the sample in each chamber A, B, C, D.

- Let the cartridge sit for 30 seconds before testing in order the cells to stop moving inside the chambers.
- Press button  to select "Reference testing mode".
- Fill in the data for the tested milk sample.



- Press button  to start the testing.

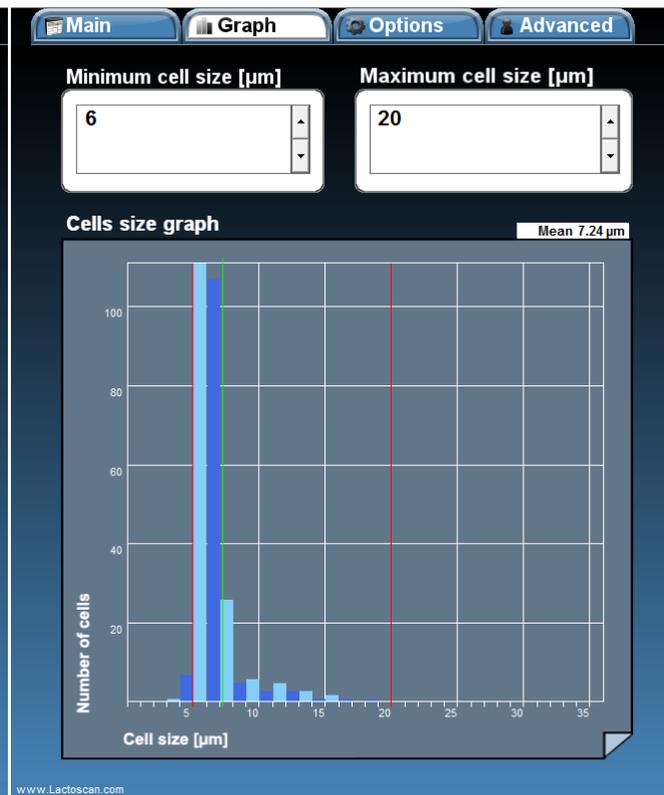
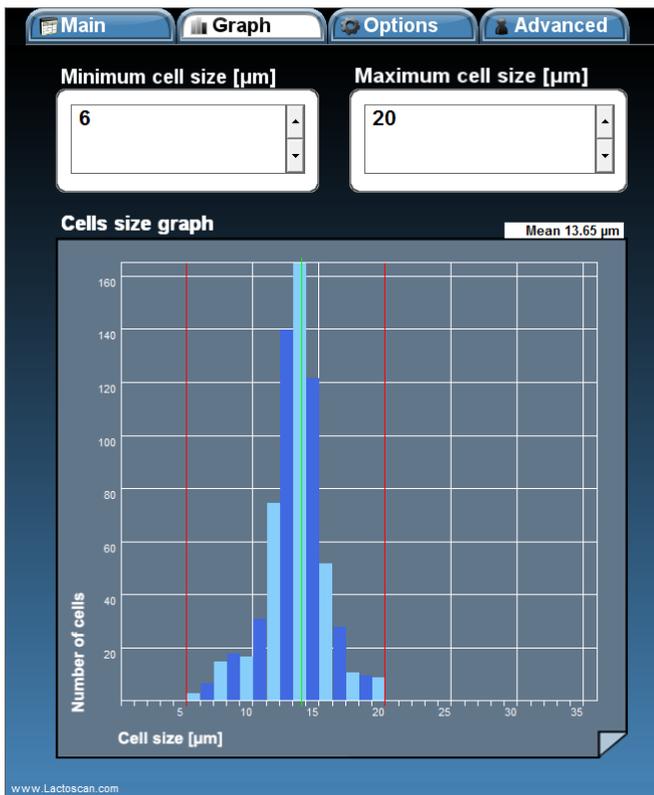
LACTOSCAN SCC performs the test and gives final result. The final result is an automatically calculated average result from the results of the tests of chambers A, B, C, D.

**Coefficient of variation (CV) %:**

Coefficient of variation (CV) % is measured in Reference mode following the instruction for Reference testing procedure. It is determined by analysis of standard reference milk samples from **certified dairy laboratories**. To determine the reference coefficient of variation (CV) % only such standard reference milk sample must be used. If nonstandard milk samples are used, a deviation of the CV% may be received. The standard reference milk samples do not include a large amount of cell fragments and do not include yeast cells. The presence of yeast cells in milk can easily be determined by examining the graph of the analyzed milk sample. Below you will find examples for graph of normal milk without yeast cells and graph of milk with yeast cells:

**Normal milk**

**Milk with yeast cells**



**Calculating Coefficient of variation (CV) %:**

To calculate the Coefficient of variation (CV) %, following the above procedure, you have to test 10 times milk sample with somatic cell concentration 50000-200000 cell/ mL, to test 10 times milk sample with somatic cell concentration 400000-600000 cell/mL and to test 10 times milk sample with somatic cell concentration 800000-1400000 cell/mL. Then to fill in the received results in the table below.

## Sample tables:

### Empty

### Filled in

Raw data	Sample 50000- 200000 cell/mL 1	Sample 400000- 600000 cell/mL 2	Sample 800000- 1400000 cell/mL 3	Raw data	Sample 50000- 200000 cell/mL 1	Sample 400000- 600000 cell/mL 2	Sample 800000- 1400000 cell/mL 3
Measurement 1				Measurement 1	142 000	571 000	1 153 000
Measurement 2				Measurement 2	161 000	563 000	1 164 000
Measurement 3				Measurement 3	147 000	548 000	1 146 000
Measurement 4				Measurement 4	157 000	530 000	1 142 000
Measurement 5				Measurement 5	142 000	543 000	1 146 000
Measurement 6				Measurement 6	145 000	584 000	1 122 000
Measurement 7				Measurement 7	151 000	558 000	1 100 000
Measurement 8				Measurement 8	150 000	584 000	1 154 000
Measurement 9				Measurement 9	140 000	539 000	1 120 000
Measurement 10				Measurement 10	145 000	570 000	1 155 000
Mean value				Mean value	<b>148000</b>	<b>559000</b>	<b>1140200</b>
Standard deviation				Standard deviation	6815	18708	19904
Coefficient of variation %				Coefficient of variation %	4,60	3,35	1,75

Excel formulas for calculating:

- Mean Value: =AVERAGE(Measurement 1: Measurement 10)
- Standard deviation: =STDEV(Measurement 1: Measurement 10)
- Coefficient of variation %: =100\* Standard deviation / Mean Value

The above table can be downloaded in Excel from LACTOSCAN.COM from Downloads menu in LACTOSCAN SCC product page [http://lactoscan.com/somatic-cell-counter-\(29,1,1,product=22\)](http://lactoscan.com/somatic-cell-counter-(29,1,1,product=22))

### LACTOSCAN SCC setting:

For measuring standard reference milk samples from certified dairy laboratory and calculating the Coefficient of variation (CV) %, LACTOSCAN SCC must be set as follow:

- Minimum cell size in micrometer : 6
- Maximum cell size in micrometer : 20