CELL COUNTING USING THE SYSMEX

Taking samples

1. Fix sample by adding 0.4 ml culture to 1.6 ml formyl saline (0.9 % NaCl, 3.7% formaldehyde - fumes are toxic!). Store cells at 4°C (keep indefinitely).

For 500 ml: 50 ml of formaldehyde soln (approx 40%); 4.5 g NaCl. Keep in fume cupboard.

Using the sysmex counter

1. Check the waste is empty if not empty it.
2. Turn on the cell counter using the two switches (one at base and one at top)
3. **Dilution of your sample.** Rinse out the capillary tube of the cell counter by pressing the 'START' button at the top of machine when the light is on 'ASP', place a white cup under the capillary tube and then press again when the light is on 'DIL'. Repeat until no bubbles are left in the capillary tube.
4. Take 1ml of cell culture in an eppendorf.
5. Put the tip of capillary tube into your sample and press 'START', when the light is on 'ASP'; your sample will be taken up into the capillary tube. Place a white cup under the capillary tube, then press 'START' again when the light is on 'DIL'; your sample will be diluted 1:100.
6. *Optional: sonicate the sample in the white cup for 20-30 seconds - amplitude 5 um, 5 sec.*
7. Put the cup in the WBC/'S. pombe' marked holder and close the door.
8. Press 'count' on the keypad; cells are counted and a read-out is produced.
9. The number of interest is the 'WBC'. Multiply by x100 to take acccount of the dilution (or x500 if you take into account the dilution into formyl saline). Do at least 2 counts for each sample.
10. When you have finished clean the probe 3x with Isoton by pressing Count. Leave the probe in Isoton and press Clean for 10sec. Switch off still pressing Clean.
11. Empty the waste.

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Troubleshooting

1. Very long cells will not be counted. If your cells are very elongated then you should use a hemocytometer.
2. If the machine becomes clogged it will automatically recount. If it remains clogged then try cleaning it (press Clean for a few secs) and Flush and Fill. If it is still clogged then repeat everything, if you still cannot unclog it then go and have a cup of tea and try again later.
3. Nitrogen starved cells or spores may not count accurately as the lower discriminator which is set automatically, is in the wrong place (I don't know why). To change it go to the Menu page and press 4 (Manual Discrimination) and enter, press 1(WBC) and enter. You should now be on the Manual Discrimination page. Press enter to select the lower discriminator which will become a solid line when it is selected, move it with the arrow keys and then press enter and the new reading will be displayed. The upper discriminator does NOT affect the cell number, it is used to discriminate between different populations. To go back to the Front page press Select (X3). Unfortunately you have to reset the lower discriminator for each new sample.