## Conclusions from 20190122 Experiment about biological replicates

* Pretty consistent data also on biological replicates
* However, second subsample from an aliquot had higher counts
  + Liwei pointed out that this is probably due to the fact that later subsamples come from a deeper area
  + We have to transfer all supernatant first and then take subsamples/aliquots from that supernatant (or filter)
* We still have 10x lower counts than Vandeputte et al
  + Maybe our homogenization step is not efficient yet: try with higher dilutions from the start (Friday)

## Goals Friday experiment:

* Test whether we can make the homogenization step more efficient and thus get higher cell counts:
  + Start with 15 µl buffer per mg aliquot, e.g. 1500 µl buffer on a 100 mg aliquot.
  + Have 2 aliquots in a 15 ml tube, where we even start with 40 µl buffer per mg aliquot, i.e. 4 ml on a 100 mg aliquot and then homogenize in the 15 ml tube
* Compare filter vs low centrifugation:
  + See what is more convenient and whether the cell counts differ
  + Remember to transfer all supernatant first and then take the “100 µl” aliquots
* Test maybe more convenient ways to get the pellet back in solution after the washing instead of squeeze
* Get Liwei’s unstained samples clean ☺ (and keep Sandra’s clean☺)

## Counting how many plates we will need overall:

The suggestion here assumes that the unstained samples are clean, and we can always only have unstained from the big control sample (and maybe one or two extra checks)

Pilot Study:

* We agreed on:
  + For one person we have 6 aliquots per time point, with 4 timepoints we have 24 aliquots in total
  + For each of the 24 aliquots do 3 technical replicates >> 3 \* 24 = 72 wells
  + On top 1 aliquot of the Control, 3 technical replicates plus 2 unstained + 1 buffer only >> 72 + 6 = 78 wells = 1 plate
  + So 1 plate per person >> 5 plates for pilot study (2.5 weeks)

DIRECT study:

* 1 aliquot per person with 3 technical replicates
* Then 25 persons go on 1 plate: 25 \* 3 = 75 wells + Control 3 technical replicates + 2 unstained + 1 buffer only = 81 wells
* 1 plate per 25 persons
* If both Sandra and Liwei can manage 1 plate a day, then we have 50 persons a day
* 1500/25 = 60 plates in total, and 30 analysis days (a 2 plates) >> 15 analysis weeks >> 4 months

>> If it’s possible that each of you could handle one plate per day, you could tell Lau that you would need

* 5 times the machine for 1 plate for the pilot study
* 30 - 35 times the machine for 2 plates for the direct study (assuming 1500 - 1750 samples)