NYC III medium – version 16 april 2019

This medium is used for growth of fastidious anaerobes like *Gardnerella vaginalis* and *Lactobacillus iners*. In my work I use it also for growth of other lactobacilli such as *Lactobacillus crispatus* although they will also grow on regular MRS medium. The reason for this is that in most cases I want to compare growth of these different bacteria, and want to keep as many other parameters in the experiment constant.

I adjusted this recipe from the ATCC protocol
https://www.atcc.org/~/media/FA8074C3B4B9450899EE2542D6AD7116.ashx:
For 500 mL NYCIII medium:

HEPES (CellGro)	1.2 gram
Proteose Peptone No. 3	7.5 gram
Yeast Extract	1.9 gram
NaCl	2.5 gram
Glucose	2.5 gram (I use monohydrated glucose which means that I need 10% more = 2.75 gram)
water	450 mL

I use less HEPES compared to the ATCC protocol to better allow acidifcation, and do not adjust the pH. The pH is generally around 6.7 before autoclaving and addition of the Horse Serum. After autoclaving and cooling down I add 50 mL of heat inactivated horse serum.

In case of liquid medium I include a filtration step through a 0.22 uM filter.

In case I want to poor plates, I add 5 grams of agar (1%) before autoclaving. I autoclave this mixture at 121°C for 20 minutes and let cool down to 60°C, warmer medium will solidify the protein in the serum. I warm the horse serum to 37°C and mix right before pooring the plates (the medium should be slightly too hot to hold).

NYC III medium without glucose, 1.1x

I use this medium in case I want to test various carbon sources other than glucose. I use the same recipe as above except that I leave out glucose and only add 400 mL of water. In order to supplement with alternative carbon sources I add 10% of the final volume of a carbohydrate solution (such as glycogen or glucose dissolved in water), with water as the control. The concentration of the carbon source is 10x higher concentration than the final concentration.
So for a 1 mL culture in NYCIII medium with 0,5% of a carbon source I add:
900 uL NYCIII medium without glucose 1.1x
100 uL of a 5% glucose of glycogen solution in water or water as a control.