NYCIII Culture Media for Gardnerella vaginalis and other fastidious anaerobes

Justin Perry, Cory Weimer, Warren G Lewis, Lewis Lab

Abstract

NYCIII (en-why-see-three) culture media
Suitable for the culture of Gardnerella vaginalis

Citation: Justin Perry, Cory Weimer, Warren G Lewis, Lewis Lab NYCIII Culture Media for Gardnerella vaginalis and other fastidious anaerobes. **protocols.io**
dx.doi.org/10.17504/protocols.io.ei4bcgw
Published: 12 Feb 2016

Before start

Heat inacticate your serum. Serum can kill many different types of bacteria.

Protocol

Step 1.
The quantities given are for 500 ml of liquid media.

Step 2.
Start thawing out a 50 ml conical of heat inactivated horse serum (Hemostat Labs shs500). Set aside for making solution B.

Step 3.
*To heat deactivate allow frozen serum to thaw at room temp. Once thawed, swirl to ensure serum is homogenous and transfer bottle to a 56C water bath (make sure water level does not extend above the cap). Incubate for 30 min, swirling every 5-10 minutes to ensure even heating. After incubation allow serum to return to room temp and sterile vacuum filter. To avoid multiple freeze-thaws aliquot the deactivated serum into 50ml conicals and store at -20C.

Step 4.
First, make your solution A.

Step 5.
HEPES solution (Cellgro 25-060-Cl): 4.2 mililiters
It is critical to use cell culture grade HEPES buffer

ANNOTATIONS
Warren G Lewis 20 Apr 2016
1M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). Non-cell-culture-grade HEPES from certain vendors has been identified a source of failed cultures of G. vaginalis in NYCIII liquid media. This is why we don't typically make this stock solution. So far, available brands of cell culture grade HEPES solutions have worked well in our hands

Step 6.

Proteose Peptone #3 (Fisher DF-122-17-4): 7.5 grams

Step 7.
NaCl (Sigma S3014-1kg): 2.5 grams

Step 8.
Glucose (Sigma G7528-250g): 2.5 grams

Step 9.
Yeast Extract (Fisher DF0127-17-9): 1.875 grams

Step 10.
Distilled water: 450 ml

Step 11.
• Adjust solution A to a final pH of 7.3. I use 50% solution (19M) of NaOH. It usually takes at least 200 ul to start approaching the proper pH.

Step 12.
• Use pH indicator strips for this, as the peptone will interfere with a pH probe

Step 13.
• Autoclave the solution. Normally I autoclave a 500 ml bottle for 45 minutes at 121C.

Step 14.
Let Solution A cool to near room temp (at least below 50C) prior to adding horse serum

Step 15.
• Pour the contents of the 50 ml conical into the cooled solution A.. Mix with a good swirl (or a stir bar).

Step 16.
• Pour the Soln A/horse serum mixture into a filter flask – sterile vacuum filter it.

Step 17.
• In order to achieve anaerobic conditions, the cap should be slightly loosened, and the flask left to equilibrate in the anaerobic chamber overnight.