Preparation of liquid cultures

This protocol describes how to prepare liquid medium for culturing mycelium in liquid phase

Materials: pressure cooker, scale, distilled water, malt extract powder*, plate with well growing mycelium, scalpel, gloves, ethanol, jar, wadding, rubber injection ports (optional)
(*light malt extract is better because you can monitor what's going on in the jar)

Part 1: Weigh Materials

Component	Quantity	Custom Quantity	
Malt extract	2 g	multiplier	g
Distilled water	100 ml	x	ml

Weigh and dissolve the malt extract in the water and distribute the solution in the vessels

Part 2: Prepare the jars

- Make 2 holes in the lid;
  - Push wadding cotton through the hole (enables pressure cooking, and air exchange for the mushroom)
  - (optional) push an injection port through the second one
- Close the jars and cover the lid with aluminum foil

Part 3: Sterilize In Pressure Cooker

- Sterilize in the pressure cooker for 50 minutes (No air tight containers, they burst!!!)
- Take off the lid from the cooker, and let the jars cool down (the bigger the volume, the longer the time needed)

  Note: Note on the jar the volume and mark the liquid level. This will help while adding new liquid substrate or when inoculating new substrate

Part 4: Inoculate

- In sterile conditions, cut a piece of mycelium from the PD and put it in the jar (take a piece from the outer part of the mycelium, where the young and fast spreading hyphae are growing) (Don't worry about transferring some agar too)

- close the lid, and monitor the growth:

HINT: On the bottom, the mycelium will look cloudy, slimy and difficult to break later on. If grown on an agitator or mixed on a magnetic stirrer (add magnetic bar before sterilising), the hyphae will be fragmented and optimal for solid substrate inoculation. Mycelium at the surface will build a layer and cover the liquid.