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| Table of  Contents  [[Introduction]](http://docs.google.com/title.html)  [[Prediction/](http://docs.google.com/pred-hypo.html)  [Hypothesis]](http://docs.google.com/pred-hypo.html)  [[Data]](http://docs.google.com/data.html)  [[Conclusion]](http://docs.google.com/conclusion.html)  [[Daily Log]](http://docs.google.com/dailylog.html)  [[Bibliography]](http://docs.google.com/biblio.html) | Procedure  [[Vibrio harveyi]](#30j0zll) [[Vibrio fischeri]](#2et92p0) [[System Diagram]](http://docs.google.com/sysdiag.html)   |  |  | | --- | --- | | Procedure for Vibrio harveyi  [[Top]](#gjdgxs)  Part One: Obtaining the water samples  Water samples to experiment on must be obtained from a variety of sources with differing levels of pollution.  We obtained five:  1) Distilled water  2) Pleasanton tap water  3) Rapidly moving water from the Arroyo Del Valle creek  4) Stagnant water from the Arroyo Del Valle creek  5) Bleached water  Part Two: Growing the Bacteria  We first attempted to do the experiment using Vibrio harveyi, a bioluminescent strain of bacteria.  This is grown as follows.  1) A broth is prepared by dissolving the following in 100 mL distilled water:   2 g NaCl   1 g bactotryptone   .5 g bacto-yeast extract | Culture of Vibrio harveyi | | **Autoclave** | 2) The broth is autoclaved using a pressure cooker. Autoclaving makes sure that the broth is sterile. This is done for 20 minutes at a temperature of 270 degrees Celsius and a pressure of 15 psi. (Autoclave shown at right.)  3) 40 mL of this broth is then inoculated from a culture of Vibrio harveyi (ours was provided to us by Dr. Bonnie Bassler of Princeton University).  An inoculating loop sterilized with flame should be used (a Bunsen burner works perfectly).  The loop is used to scrape off some of the live culture and then dipped in the prepared broth.  4) The resulting mixture is lightly capped in a flask and shaken, using a gyratory shaker (more commonly known as a water bath shaker), overnight (24 hours) at 30 degrees Celsius and 200 rpm.  Once the bacteria is grown, the broth, at first deep yellow, should take on a chalky white color and should glow in the dark. | | **Water Bath Shaker** | **Inside Water Bath Shaker** | | Part Three: Preparing the Fresh Water Samples  1) Take fresh water samples out to make a 10% saline solution of them. We do this in order to keep an iso-osmotic situation for the marine dwelling bacteria.  2) Pour 40 mL of sample into 250 mL flask using a pipette.  3) Add 1.2 g NaCl to the fresh water sample. Cap flask and swish in a circular motion.  Part Four: Doing the Experiment  1) The broth containing bacteria is divided into test tubes, 5 mL per sample.  Readings of the light output should be taken for each sample, using a light sensor.  After these initial readings have been recorded, 1 mL of media is added, using a different media for each sample.  The bacteria should then be allowed to sit in the media for a minimum of two hours.  2) After the bacteria has been given time to respond the various media, measurements from each are taken with a light sensor.  The light output is recorded and compared to the initial readings. | **Note:**  Ideally, we would have liked to have actually grown the bacteria in varying media.  However, we had access to only one gyratory shaker, and as a result had no way of incubating five flasks of inoculated broth at once.  In addition, our access to the shaker was limited, so returning several times to grow the various flasks of broth separately would not have been an option either.  In the end, the only feasible way to conduct the experiment was to grow the bacteria all together and add the media afterward.  Unfortunately, we could not even do that because we could not get the bacteria to glow.  It would grow -- the broth would turn from a deep yellow to a chalky white, indicating growth -- but there was no light output that could be detected by our eyes or by the light sensor.  Consequently, we had to abandon Procedure One and design a new protocol. | |  |  | |  |  | |  |  | |  | Procedure for Vibrio fischeri  [[Top]](#gjdgxs)  Part One: Obtaining the water samples  [Same as in Procedure 1](#1fob9te)  Part Two: Growing the bacteria  For the redesigned procedure, we ordered a new kind of bioluminescent bacteria, called Vibrio fischeri, from Carolina, a biological supply company.  Along with the bacteria culture, we ordered 20 prepared agar slants (in test tubes) on which to grow our own samples.  This is done as follows.  1) As with the broth, an inoculating loop sterilized with flame must be used (a Bunsen burner works perfectly).  The loop is dipped in the live culture and brushed in a criss-cross pattern on an agar slant (refer to picture at left [illustration provided by Carolina Biological Supply Company]).  2) The newly inoculated agar slant is lightly capped and placed in complete darkness at room temperature for at least 18 hours, at which point it should glow brightly.  The bacteria will continue to glow until around 48 hours after it is inoculated.  Part Three: Preparing the Fresh Water Samples  [Same as in Procedure 1](#3znysh7) | | Part Four: Performing the experiment  The experiment should be performed once several agar slants are properly inoculated and glowing.  1) As in Procedure One, a different media should be added to each bacteria sample.  We added 5 mL rather than just one this time around.  Also, instead of taking the light readings before the media was added, as in Procedure One, we elected to take the readings immediately after adding the media.  This was done because we wanted all the readings to be of the bacteria in a liquid environment.  (This would not have had to be a consideration had we been able to grow the bacteria in a broth, as that already is liquid.)  2) Once again, the bacteria should be allowed to sit in the media for a minimum of 2 hours.  After the bacteria has been given time to respond to the different media, new measurements of light output are recorded and compared to the initial readings. |  | |  |  |   [[Top]](#gjdgxs) |