

Teri Fahrendorf's
SMALL BREWERY LAB PROCEDURES MANUAL

TABLE OF CONTENTS

Introduction	Below
HLP Offer from Siebel Institute	1
Minimum Requirements	1
Objective and Purpose of Small Brewery Labwork	2
What is HLP Media?	2
Supplies for Setting Up the Lab	3
Photos of Finished Incubator	4
Wiring Diagram for Incubator	6
Instructions to Build Incubator	7
Culturing Supplies Information	9
The Triple Rock Technique	11
Special Techniques: Pipetting	13
What is "Dry Labbing?"	13
Analyzing Lab Results	14
Recording Your Results	16
Example Form: Weekly HLP Test Log	18
Example Form: Accumulated Lab Results, by Tank	19

INTRODUCTION

From 1989-1990 I was employed as Head Brewer at Triple Rock Brewing Company in Berkeley, California, one of California's first brewpubs. I had learned about and used HLP (Hsu's Lactobacillus and Pediococcus) media while attending the 1988 Siebel Diploma course, but it was at Triple Rock where I learned the value of disciplined weekly "labwork."

In my opinion every brewery, no matter how small, would benefit from doing HLP labwork weekly. And in my opinion there is no brewer who is too busy to schedule 1 to 1.5 hours per week to discover what is going on inside of her/his tanks, by doing this labwork.

This manual was designed to provide a broad overview of HLP labwork that is possible in a small brewery. It will also guide you in building a lab incubator and setting up what you need to test your in-process and finished beer with HLP. It will teach you basic lab procedures, and will outline the minimum lab practices that are recommended for any brewpub or small brewery.

See more brewing articles at www.terifahrendorf.com

HLP OFFER FROM SIEBEL INSTITUTE:

I googled “Siebel HLP” on 9/7/2010, since Dr. Hsu worked for Siebel when he developed HLP Media, and I found a great offer for commercial breweries to get started using HLP. The offer is on ProBrewer.com and is dated 8/24/2010, so I consider it current. The ProBrewer link is <http://www.probrewer.com/vbulletin/showthread.php?p=56958>. Keith Lemcke, Vice President of Siebel writes:

Try Siebel HLP Media for Free

To help brewers get more comfortable with doing simple but effective lab work, we have put together a free (yes, free) kit that we would like to send to any U.S. or Canadian commercial brewery to try their skills at sampling, incubating & evaluating their beer. In the kit we have Siebel HLP lactic bacteria detection media as well as some test tubes, and sanitary pipettes to get you started. We have even posted an instructional video on YouTube at <http://www.youtube.com/watch?v=UThdqdUfKpw> to make it as simple as possible to try.

Using lab media can be really easy to do, and once you try it you will be on your way to building your own QC/QA program. If you would like us to send you a kit Again, USA and Canada only), please send an email to klemcke@siebelinstitute.com requesting an order form for the lab media trial kit or download the order form from www.beercountry.net/mediakit.pdf.

MINIMUM LABWORK REQUIREMENTS

Teri's Fabulous Four

- 1. Build an Incubator before opening day & HLP test all beers every 2nd or 3rd day pre-opening:** On all tanks containing beer, including test batches.
- 2. Weekly HLP testing on all fermenters:** No less!
- 3. Monthly HLP testing on all servers:** Monthly is the minimum. Weekly is preferred.
- 4. Monthly water pH, temperature and flavor testing:** Keep a record of each of these monthly. (Note: Instructions on how to test and record these items is not included in this Small Brewery Lab Procedures Manual, but I do consider them important.)

OBJECTIVE and PURPOSE of SMALL BREWERY LABWORK

OBJECTIVE:

1. To test your beers for:
 - A. Lactic Acid- and Diacetyl-creating bacteria.
(Specifically Lactobacillus and Pediococcus.)
 - B. Generic aerobic bacteria.

PURPOSE:

1. To determine if yeast should be repitched into another brew.

Most small breweries use expensive liquid yeast cultures. You need to keep these yeast cultures going for 6 to 15 generations before you buy another fresh culture. You need to know if there is anything besides yeast growing in your beers, and thus in your yeast, before you repitch the yeast. Test beer from the fermenter at least two days before you plan to repitch the yeast.

2. To troubleshoot an off-flavored beer.

Not all off-flavors stem from bacteria, but if the beer has a sour taste to it, chances are it was caused by a bacterial infection. Lab culturing could show whether bacteria is the cause or not. HLP results are not always conclusive, although they do help. If results for bacteria are negative, the off-flavor could be caused by wild yeast or unwanted esters due to unfavorable fermentation conditions, especially temperature. HLP labwork is one tool in your toolbox.

You may find you have bacteria growing in a decent tasting beer. A very small amount will probably not affect flavor, however **DO NOT REPITCH YEAST** from that beer, otherwise you are simply propagating that bacteria, and it could grow to tasting threshold next brew. If you have a bit of bacteria growing in your sample, I hope you are a brewpub and it is in a serving tank of fast-moving beer! If you find substantial bacteria in any sample, I recommend you both caustic & acid wash that tank before you fill it with a new beer.

HOW TO ACHEIVE THE OBJECTIVE:

1. By building the incubator.
2. By culturing your beer for bacteria in HLP media.

WHAT IS HLP?

Hsu's Lactobacillus-Pediococcus Media, invented by Dr. Hsu of the Siebel Institute of Technology and J.E. Siebel Sons' Co. in Chicago. It is an agar or media that supports the growth of the specific bacterium, Lactobacillus and Pediococcus. HLP contains Actidione, which suppresses the growth of yeast. (It kills the yeast.)

WHY USE HLP?

Because no microscope is needed! The individual cultures of each bacteria organism grow large enough to see and identify with the naked eye. Also, HLP testing does not require an autoclave (pressure cooker). Only a single burner hot plate is needed to heat the media.

SUPPLIES FOR SETTING UP THE LAB

Note: These costs do not include shipping charges.

Catalog No.	Item	Cost
From Crosby & Baker (www.crosby-baker.com):		
#9533	500 gram bottle of HLP	\$132.50
From Fisher Scientific (www.fishersci.com):		
14-959-40B	Case of 500 test tubes	\$259.86
13-676-10G	Case of 1000 pipettes	\$252.63
14-792-11	Test tube half rack (Order 2: \$29.41 each)	\$58.82
10-040H	500 ml Erlenmeyer flask (6-Pack: \$44.69 find a friend)	\$7.45 (See below*)
S41877	Alcohol Burner	\$16.90 (See below*)
From Home Science Tools (www.hometrainingtools.com):		
CE-FLA500	500 ml Erlenmeyer Flask	\$6.90 (*alternative)
CE-LAMP	Alcohol Burner	\$7.95 (*alternative)
CE-PIPFILL	Pipette Filler (optional)	\$4.50
CE-EVAPDSH	Weighing Dish	\$2.20

From the Hardware Store:

15-watt bulb.

Light bulb socket with 1/8 FPT and screws to connect wires.

1.5-inch nipple (1/8 MPT - 1/8 MPT).

Nut for 1/8 nipple.

One foot of lamp cord.

Several brass washers that fit on 1/8 nipple.

3 feet of 3-wire plug cord (white, black, and green wires).

120 Volt grounded plug (3-prong plug).

Small 1/4-inch screw (for grounding wire).

Two long screws and nuts for mounting the thermostat.

Propane torch.

Denatured Alcohol.

Gallon of Distilled Water.

Roll of paper towels.

(You can certainly find some of the Amazon.com items listed on the next page at your local hardware store as well, and definitely without the shipping charges.)

From Amazon.com:

American Weigh AMW-600 Silver Precision Digital Pocket Scale w/wt.	\$18.99 (SSS)
Hobart 770080 Single Flint Striker (or matches)	\$2.99
Taylor Digital Instant-Read Pocket Thermometer	\$10.07 (SSS)
Honeywell T6031A1136 Remote Bulb Refrigeration Thermostat	\$48.70
Proctor-Silex 34101 Fifth Burner	\$14.99 (SSS)
Rubbermaid 2A1104MODRD 10 Quart Ice Chest	\$14.67
8" x 8" Brown Flameguard Terry Pot Holders	\$2.29
Reynolds Aluminum 08010 Reynolds Wrap Aluminum Foil	\$1.99
Tolco Empty Spray Bottle 8 oz.	\$1.99
Strait Line China Marker, White	\$0.42
Saunders Recycled Hardboard Clipboard, Letter Size	\$2.49
(Some of the above may be found locally.)	(SSS = Super Saver Shipping)

From Home:

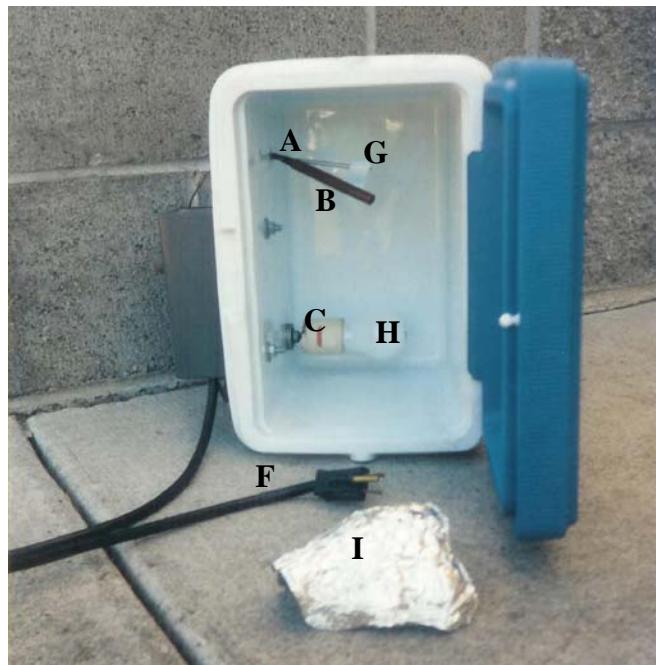
Regular spoon
Styrofoam peanut
Sharpie/permanent marker
Drill & Bits
Screwdrivers
Wire clipper

PHOTOS OF FINISHED INCUBATOR

Below are photos of the incubator I built for Steelhead Brewing Company in Eugene, Oregon.

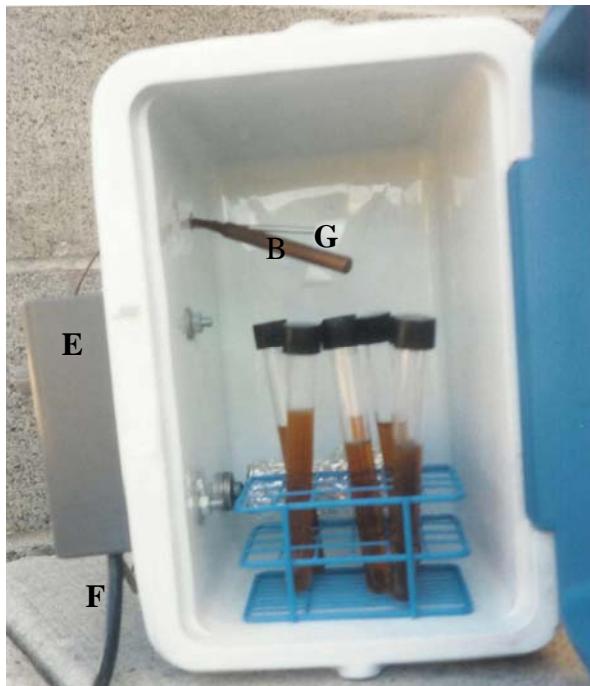


OUTSIDE: THERMOSTAT VIEW



INSIDE: PROBE AND BULB VIEW

INSIDE VIEW WITH TEST TUBES



INCUBATOR PARTS SHOWN IN PHOTOS:

- A. Thermometer (0-160°F, accurate).
- B. Bulb capillary (Thermo-probe for testing air temperature).
- C. Light bulb fixture (For heat, not light).
- D. Lamp cord (Connects light bulb to thermostat).
- E. Thermostat (To control light bulb & switch on automatically).
- F. 120 Volt grounded plug (3-prong) and 3-wire cord.
- G. Styrofoam peanut.
- H. 15-watt bulb.
- I. Aluminum foil (To keep light off of samples).

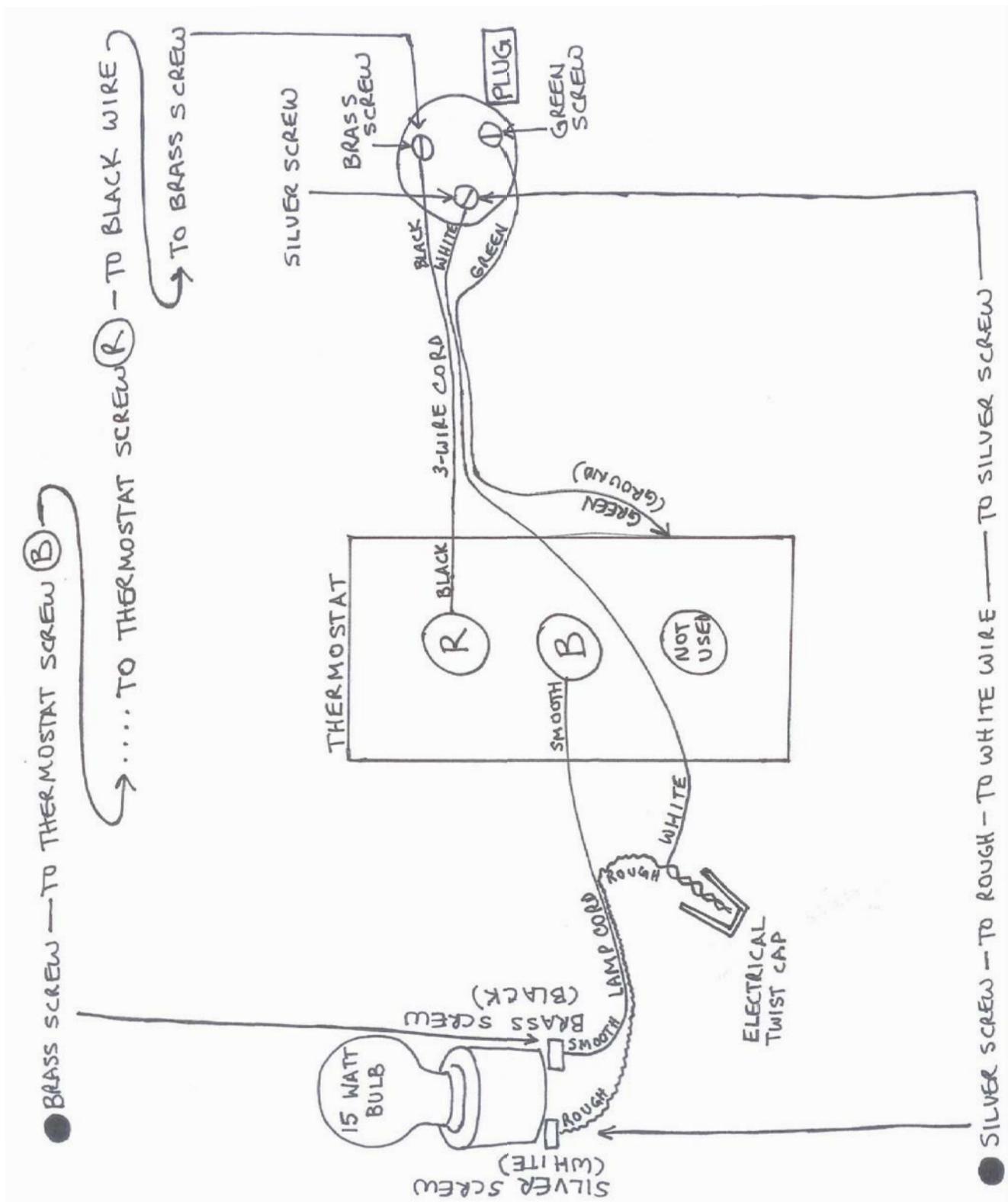
Alternative incubators which have been suggested but not tested:

1. Aquarium with heater as a water bath.
2. Yogurt making kit.

There are other designs of incubators you could create, but the above is the one that is referred to throughout this HLP Lab Manual.

Please see next page for wiring diagram.

INCUBATOR WIRING DIAGRAM



INSTRUCTIONS TO BUILD INCUBATOR

1. Make sure your picnic cooler has a hinged lid, otherwise you will have to buy hinges to attach to it. Make sure the lid closes snugly if you install your own hinges.
2. Take off the handle, you won't need it and it's in the way.
3. Tip the incubator up on one side, the lid should look like a door with the hinge on your right. This is how the incubator will look sitting on your shelf.
4. Put the bulb socket together, attaching the lamp cord according to the electrical diagram on the previous page.
5. Usually the bulb socket will have a silver and a copper colored screw. The lamp cord will probably have a rough and a smooth coated wire, connected to each other along their length. Sometimes the lamp cord will have two different colored wires connected along their length.
6. After you have attached the lamp cord, feed it through the 1/8 nipple, and screw the nipple onto the bulb socket.
7. Put a bulb in the socket, so you can estimate where you need to drill the hole for the 1/8 nipple. You do not want the bulb to touch either the back or the bottom of the incubator. Leave room for a test tube rack. Mark with permanent marker inside the box where your hole will go. Then transfer your mark to the outside of the box.
8. Determine where you want the capillary bulb hole to be. It should be fairly close to the top of the side of the incubator, so that the bulb won't touch the test tubes as it hangs down.
9. Next look at your thermostat. Determine where you want the mounting holes to be, and mark on your box.
10. If you don't have drill bits the right size, ask the construction contractors working on your brewpub site to drill the above holes for you.
11. Also, if you have trouble with the wiring diagram, ask the electrician on site to take a look at it for you. Otherwise see if someone at the hardware store can help you.
12. Once the holes are drilled, insert your bulb socket-nipple apparatus through the hole, with the lamp cord trailing out. Put a brass washer on the inside of the box, if necessary, to keep the socket steady.
13. Put as many washers as are necessary on the outside of the box onto the nipple. Secure with the nut.
14. Attach the other end of the lamp cord, and the 3-wire cord to the thermostat, as shown in

the electrical diagram. You will have to punch out the disk on the bottom of the thermostat.

15. Make sure the tiny internal dial on the thermostat is set to its smallest setting (3). This is the amount of variance the thermostat will allow as it cycles (ie: 3 degrees above or below the set point). If the thermostat cycles to a wider degree, this dial could have been set as high as 16. Reset it to 3.

16. The green grounding wire will be attached with the small screw to an unused small hole in the back of the thermostat.

17. Attach the thermostat to the box.

18. Stretch the bulb capillary gently, and insert it into the upper hole until fully inserted.

19. Push the thermometer into the same hole.

20. Put the styrofoam peanut between them so they don't touch each other.

21. If there is more space in that hole, fill it with bits of styrofoam.

22. Put the 3-prong plug and 3-wire cord together, using the wiring diagram.

23. Cover the light bulb loosely with the foil.

24. Your incubator should now be complete. Plug it in.

25. Test the incubator by turning the thermostat dial to a higher temperature than the thermometer reading. The light bulb should go on. With any luck, the thermostat should kick the light bulb on within 3 degrees of the thermometer reading. If not, check the small cycle variance dial inside the thermometer. Also, your thermostat could run "hot" or "cold" in comparison with the actual temperature. Always use the thermometer to set the thermostat dial, as the thermostat temperature indication may not be accurate.

26. Set the incubator in a safe place, and set the thermostat for 86°F or 30°C. Watch it for a few days, and adjust the dial according to the actual temperature on the thermometer. The incubator will never maintain 86°F, but should average 86°F.

27. Do labwork as soon as you have beer to test! Also, test your brewery water source. Other things to test with HLP include beer in kegs. Kegs returned from the distributor. Beer coming out of your filter, and darn near any liquid you want to test.

CULTURING SUPPLIES INFORMATION

1. Test tubes.

Polystyrene disposable 16 x 150 mm test tubes with screw caps. Sterile and individually wrapped. 500 to a case.

Test tubes and pipettes shall be ordered individually wrapped, since you will only be using about 10-20 at a time. Do not order them 50-per box because the other 30-40 will no longer be sterile once the box is opened.

2. Pipettes.

Polystyrene disposable 1 ml pipette with cotton plug and .01 graduations. Sterile and individually wrapped. 1000 to a case.

Although we only use .5 ml of beer to inoculate our cultures, we don't use .5 ml pipettes. To accurately deliver .5 ml of liquid, the last few drops must be blown out. That is not sanitary, so we use the 1 ml capacity pipette. We can measure .5 ml without blowing into them.

3. Test tube rack.

Test tube rack for 16 mm diameter test tubes. A half rack is the perfect size for the incubator and will fit 24 test tubes. Two racks will work just fine. Three racks are even better.

4. Erlenmeyer flask.

500 ml Erlenmeyer flask should be sufficient. For every approximately 8 test tubes of HLP, you will need 100 ml of distilled water in the flask. (This will test approximately 8 tanks of beer.)

5. Alcohol burner.

This is used to create a "flame hood" in your lab work area. A flame hood causes the air to rise, and sterilizes the air it heats. Hopefully any bacteria in the air will be toasted and moved up and out of the way, and won't fall on your labwork.

6. HLP.

Hsu's Lactobacillus-Pediococcus Medium, 500 gram jar. You may take a year to use up this quantity of HLP. HLP is very hygroscopic, which means it absorbs water from the atmosphere. If you don't use it up quickly, it will literally turn into a rock. Don't store the HLP bottle in a humid place, like the brewery itself. Put the bottle in a one-gallon Ziploc bag. Store it in a Rubbermaid box in the mill room if you must. HLP is sold with an expiration date. Check your date when you get your bottle, it should be a year away. Otherwise send it back.

7. Propane torch.

This will be used in conjunction with your denatured alcohol spray bottle to sterilize the sample port before you pull a sample of beer.

8. Aluminum Foil.

Used to cover the opening of the Erlenmeyer flask when it is heating.

9. Pot holders.

Used to handle the hot Erlenmeyer flask.

10. Single burner Hot Plate.

Used to heat the solution in the Erlenmeyer flask.

11. Denatured Alcohol.

Burned in the alcohol burner, sprayed on your work surface before starting, and used to sterilize your tank's sample port (zwickle), your gram scale dish and your HLP spoon.

12. Distilled Water.

Usually found in plastic one-gallon jugs. If you can find it in a glass jug, get that.

13. Paper Towels.

For wiping dry the denatured alcohol-sanitized spoon and gram scale dish. Also for wiping off your work surface after spraying with denatured alcohol.

14. Small spray bottle.

Fill with denatured alcohol, and use it to spray and wipe down your "lab" counter/work space. Also used to spray your beer tank's sample port (zwickle), and spray off your gram scale dish and your HLP spoon.

15. White grease pencil.

Grease or wax pencil, also called a "china marker." For marking the caps of the test tubes with the name of the fermenter or server, ie: F1, S2.

16. Clipboard.

To post the Lab sheets on, to record your results everyday for four days after doing the labwork. Hang next to your incubator.

17. Gram scale.

Digital gram scale, as accurate as you can afford. For approximately every 8 test tubes of HLP solution, you will weigh out 7 grams of HLP powder. Look for 0.1 gram variance at the most.

18. Regular spoon.

For spooning HLP powder from the bottle to the weighing dish.

19. Incubator.

To incubate .5 ml beer samples in tubes of HLP solution for 2 or more days at 86°F. For complete results, let test tubes sit in incubator until the next week's labwork. Then record results under comments on the lab sheet if over 4 days.

THE TRIPLE ROCK TECHNIQUE

Arrange all your lab supplies before you begin. If you do not have a lab, arrange to use the Manager's Office undisturbed for the next hour.

Prepare the lab area by closing doors and windows. Turn off fans that are blowing. Wipe off workspace counter with the denatured alcohol spray. Light up the alcohol burner anytime the Erlenmeyer flask or an open test tube is near. Continue to work near the flame.

Take a clean 500 ml Erlenmeyer flask which has been hanging upside-down on a dowel or peg-board hook. That way it is not dusty or dirty and does not need to be sanitized, nor rinsed with city water. Measure 100 ml of distilled water into it and put it aside.

Take the weighing dish off the gram scale and spray it top and bottom with the denatured alcohol. Wipe off with a clean paper towel and replace on the gram scale. Spray the spoon as well and then wipe it off.

Tare the gram scale with the weighing dish on it. Measure 7 grams of HLP into the dish. (Use 100 ml distilled water and 7 grams of HLP for every approximately 8 tanks to test.)

Pour the HLP powder into the flask, put a clean piece of aluminum foil over the opening of the flask, and swirl gently. Put it on the single burner hot plate.

Plug the hot plate in and turn it to high. Bring to a boil and boil the HLP solution for (+) or (-) 2.5 minutes. Turn off the hot plate, and with a hot pad, remove the Erlenmeyer flask and place it on another hot pad to cool.

While it is cooling, take the clipboard with a fresh *Weekly HLP Test Log* form and write down the required information about each beer that will be tested. Tank number, Beer type, Brew number, Date brewed, Yeast number, Prior yeast tank, Yeast generation number, etc.

It is imperative that labwork be done on the fermenters weekly, in order to propagate only healthy yeast. If something has to slide in labwork due to time constraints, let it be the serving tanks. However, it is an excellent idea to test the servers in order to get an overall view of how your sanitation and transfer procedures are holding up.

Extremely cloudy one-day old beer will often leave dead yeast in your test tube. Sometimes it is difficult to see through the haze. If you are concerned, you may want to do labwork twice a week.

While the HLP is still cooling, take as many test tubes out of their plastic wraps as tanks you will be testing. By now the HLP solution is probably cool enough to handle. Use the hot pads if necessary. Pour solution into a test tube until it is about 1/2 to 2/3 full and cap it off. After all test tubes are full, allow them to cool in the rack some more. Discard excess HLP solution, or use it to test your beer engine, water, filter, etc, with the excess. Put hot test tubes aside to cool.

Take another series of test tubes out of their plastic wraps and place them in another rack. Mark the caps with the white grease pencil, ie: F1, S2, etc. You will collect beer samples in these fresh test tubes.

You will collect beer samples from the sample ports (zwickles). Sanitize the stainless sample port with denatured alcohol spray, and then flash it for a few seconds with the propane torch. Be careful not to "nuke" the nut as it contains plastic parts.

When you open the sample port, let the first sample hit the ground, as your sample port is so hot it is actually sterilizing the beer coming out and will give false "clean labwork" results. After a portion has hit the ground, put a test tube under the stream, being careful not to touch the opening to anything. Also be careful nothing touches the edge of the test tube cap. If you have not already pre-marked the caps with the grease pencil, do so now as you collect each sample.

After collecting all the samples in the test tube rack, the HLP tubes have probably cooled down to about 104°F (40°C). Feel the HLP tubes. If they feel pretty hot, let them cool further. If you inoculate them too hot, they will sterilize the beer sample, once again giving a false "clean lab-work" reading.

To inoculate the first sample: Mark an HLP tube cap with the beer tank number; take the wrapper off a pipette, open the beer sample tube, put the pipette into the beer sample tube, being careful not to touch the end of the pipette to the edge of the test tube. *More detail on Pipetting Technique below.*

Then open the HLP tube (hold cap with pinky finger); carefully fill pipette with 1 ml of beer. Inoculate HLP solution with .5 ml of beer (half of pipette); discard the pipette and the beer sample tube; reseal the HLP tube; tip sealed tube upside-down twice to mix; mark cap with grease pencil if you haven't yet; place it back in the HLP test tube rack; and go on the next sample. It takes a great deal of coordination to not contaminate what you should not be contaminating!

After all the HLP test tubes have been marked and inoculated, place the test tube rack into the incubator at 86°F. (If you forgot to tip each test tube twice to mix, tip twice now, but no more than twice.)

Monitor temperature with the thermometer frequently the first time you use the incubator. Adjust the thermostat manually until it keeps an average temperature of 86°F (30°C). Check the test tubes for the next four days. Record your results daily. Results are usually seen after 48 hours. See Analyzing Results, below.

NOTE: Some breweries like to know if they even have a minute quantity of bacteria, so they run a second test and inoculate the HLP test tubes with 3x as much beer as the first test. If you choose to do this, you may want to get a variety pack of colored grease pencils (china markers).

Then, if test #1 (0.5 ml of beer) is negative, but test #2 (1.5 ml of beer) is positive for bacteria, you probably have a very teeny infection. This is a good test if you bottle your beer, as bottled beer may spend a long time before consumption, allowing any resident bacteria to grow.

SPECIAL TECHNIQUES

TO MOUTH PIPETTE: Open the beer sample test tube first.

Turn a pipette so the cotton plug end is up. Take the wrapper off the pipette. Discard the wrapper. Hold pipette with right thumb and index finger. (If you're a lefty, you'll have to translate this yourself.) Be aware of the other end of the pipette so that you don't touch it to anything. Put the pipette into the beer, being careful to avoid any sediment at the bottom of the test tube.

Pick up an HLP tube with your left hand. Wrap your right pinky around the cap, and twist tube with your left hand to get the cap off.

Put both the plug-end of the pipette, and your right index finger into your mouth. Suck the beer up slowly to the 1 ml level, avoiding the sediment, and then put your finger over the end of the pipette so the beer doesn't slip out. The beer should stay put until you barely remove your finger to release the beer into the HLP tube. As long as you suck and don't blow, the cotton plug should protect the beer sample from your germs.

Before releasing the beer, check the level of the beer on the pipette graduations, beneath the foam level. If this 1 ml pipette was exactly full, it would read 0. If it was nearly 3/4 full, it would read .3. (The test tube graduations seem upside-down.) If you add .5 to the beer level reading, the answer is the level the beer should be at after you release exactly .5 ml. The best way to not let the beer get away from you, is to watch the ending beer level reading for the approach of the beer. It is much harder to watch the beer itself - a moving target.

Discard the pipette, and reseal the test tube. Tip the test tube upside-down twice to mix. If you haven't already, mark the test tube cap with the grease pencil to identify the beer sample, and put the test tube back in the HLP rack (A third test tube rack is very handy here, but I am trying to save you money!)

PIPETTING HELP...

If the above mouth pipetting procedure seems too difficult to master, you may want to purchase the optional Pipette Filler, listed on the supply page. I have never used a Pipette Filler, so you will have to read the directions on how to use it.

WHAT IS “DRY LABBING”?

I learned the name of this unfortunate technique while on my 2007 road trip. I once employed a brewer who faked his labwork. He put HLP test tubes straight into the incubator without any beer in them. Of course nothing grew, so it looked like his tanks were always bacteria-free. When he left my employ, I discovered he had a low-level contamination in all the fermenters and servers, so I spent six months bringing in new yeast and acid washing every tank after every batch. Faking labwork and filling out perfect paperwork is called “dry labbing.” If your employees will be doing this labwork and not you, you must require that they mark the date, with a Sharpie permanent pen on the test tube, not on the cap, each time. Only then can you be sure they at least have to brew up fresh HLP each time. However, I hope they are diligent, and dedicated employees, and that they appreciate the reason for labwork as much as you do!

ANALYZING LAB RESULTS

Clear

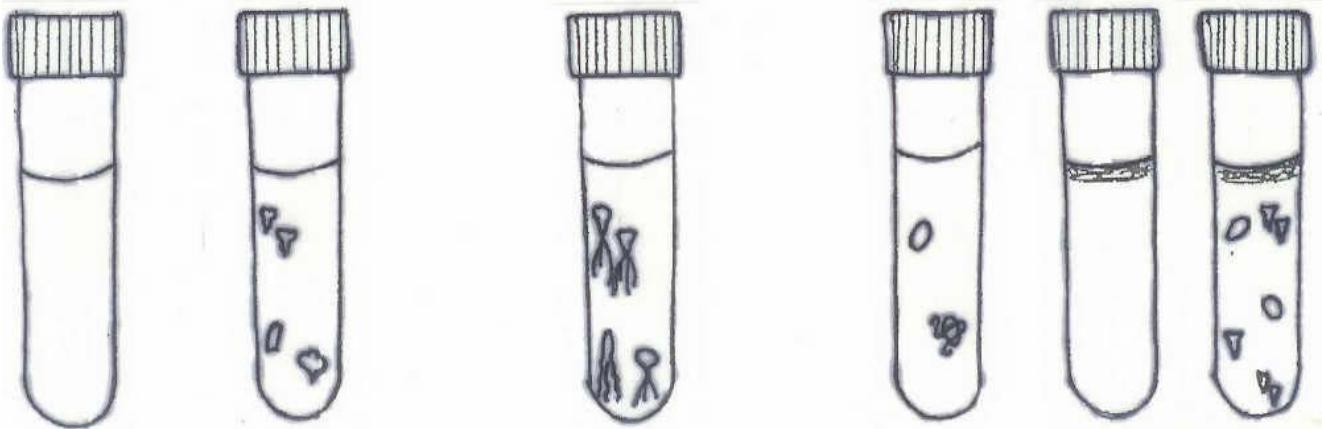
Lactobacillus

Old Lacto Colonies

Pediococcus

Aerobic

All



1. Totally Clear: Looks just like when you inoculated it.

(Nebulous floating cloudiness is usually dead yeast.) Probably means your beer contains no Lactic acid or aerobic bacteria. Might also mean that you inoculated your test tubes while the HLP solution was too hot, or you took a hot beer sample out of the sample port, destroying any bacteria present. If effect, you just sterilized your bacteria with heat. The HLP solution should be allowed to cool to 104°F (Hot tub temperature) before inoculation. When taking a beer sample, let the sample port cool down before you dip your test tube in the stream.

2. Lactobacillus: White, tornado-shaped colonies.

Colonies are suspended in the medium. Each tornado is one colony, each of which grew from a single bacteria cell. Lactobacillus colonies can also look like small white ghosts, or like bumpy roundish shapes, each with a point on the bottom. (Like a bumpy radish.) It is the total number of colonies that is important, not the size of each. The total number of colonies should be recorded. If you have only one or two colonies, they will appear quite large. If you have many colonies, they will appear quite small. The size is limited by the amount of nutrient in the medium, consequently if you only have one or two colonies, there is more nutrient per colony, and they can grow to a larger size. Just count and record total number of colonies.

The more colonies you see, the more bacteria present, the more likely you will taste it! (Lactic acid tastes like lemon juice or sour milk, and can cause cloudiness.) Also, Lactobacillus is a Diacetyl producer. (Diacetyl smells and tastes like butterscotch.) Just a few colonies will not affect the taste of the beer very much, but do not repitch the yeast from that beer and propagate the bacteria! The taste threshold will be different for each person's taste buds. One can definitely taste it at 30 colonies, and perhaps not at all at 1-10 colonies.

Lactobacillus is anaerobic (does not like oxygen), so it will affect your wort and beer before it is bottled or kegged. It grows best in a non-oxygen environment, such as your wort after it cools to 140°F (60°C) or less, and your beer before kegging. That is why it is so important to cool your wort fast and get your yeast in it as soon as possible. A slow start, not enough oxygen (or too much), or poor sanitation procedures will nearly guarantee the presence of this bacterium. *Let's be careful out there!*

If your culture tubes sit in the incubator for a few extra days, the colonies will tend to spread out and will not be as recognizable. (See figure on previous page.) Sometimes, if your tubes sit in the incubator for an extra week or two, a formerly clean lab result will be seen to grow some small colonies. My guess is that you have a very weak Lactobacillus infection. With a strong start in the next brew, the yeast from that beer probably did not propagate these weak bacteria very much, however, caution should be used. If you are finding a lot of old tubes that are sprouting colonies, it would be prudent of you to order a fresh lot of liquid yeast.

3. Pediococcus: White, swollen football-shaped colonies.

Colonies will be suspended in the medium. Each football, or more accurately, rugby-ball, represents a colony that has grown from a single cell. The total number of colonies is important and should be recorded. The size of each individual colony is not important. Sometimes the colony can be surrounded by a white swirl that looks like cigarette smoke, and sometimes the colony can appear flat instead of three-dimensional.

Pediococcus is a Lactic acid producer, as well as an even more prolific producer of Diacetyl. It is also anaerobic. It is considered more dangerous than Lactobacillus as it is harder to eradicate from the brewery. If your beer has this bacterium, please do not repitch the yeast.

4. Generic aerobic bacteria: Cloudiness at top of the medium.

Any cloudiness appearing in the top half-inch of the medium in the test tube indicates some sort of aerobic, or oxygen-loving bacteria. It is probably an acetic acid bacterium, which tastes like vinegar. It grows best in an oxygen-rich environment, such as your beer after aerating it, by accident, during bottling or kegging. That is why it is so important to counter-pressure fill kegs and bottles, and to keep the splashing down in all ways. This bacteria is aerobic, it loves oxygen, and needs it to grow.

5. Any combination: of 2, 3, and 4 above is entirely possible.

Any combination of lactobacillus, pediococcus, and aerobic bacterial infection is entirely possible. Test your spit to see what this looks like.

RECORDING YOUR RESULTS

You will record your lab results on a *Weekly HLP Test Log*. See the end of this manual for a sample *Weekly HLP Test Log* form and a sample *Accumulated Lab Results, by Tank* form.

Below is an example of how to fill out the *Weekly HLP Test Log*. The Name, Date & Time line you fill out when you start the lab procedures. You will also fill out the first 6 positions on each line when you do your lab procedures. The information should correspond exactly with the information on your *Brew Log*. (See www.terifahrendorf.com/Brew-Log.pdf for an example.)

Starting the day following lab day, you will begin collecting the results for the next four days. Mark your results as shown in the example below. The first letter represents the presence of any generic aerobic bacteria found in the sample. (Cloudiness in the top half-inch of the media.) The result is marked as either Y or N, standing for Yes or No, in the first position.

Then a slash-mark is made, and the second position is marked by a number. This represents the number of anaerobic bacteria colonies that were counted. No differentiation is made between lactobacillus or pediococcus colonies. (If you want to, you can make a notation in the comment column to differentiate.) This number can be anything from 0 on up. Please record your results everyday for the first four days. Also, do labwork weekly!

NOTE: If you have bacteria colonies of any kind, please refer to the *Brew Log* for that beer and mark "NO" next to "**Repitch?**" in the Yeast section, otherwise leave blank. Whenever you plan to repitch a yeast, always check the *Brew Log* for "Repitch? NO".

SMALL BREWERY LABWORK **Weekly HLP Test Log**

Name Teri F. Date 10/3/2010 Time Start 8:16am Time End 9:22am

Tank No.	Brew Name	Batch No.	Date Brewed	Yeast No.	From Fermenter	Yeast Generation	Results	1 day	2 days	3 days	4 days
F1	Amber	548	10/1/10	546	F4	3	N/0	N/1	N/1	N/2	
F2	Stout	547	9/29/10	542	F3	2	N/0	Y/0	Y/0	Y/0	
...											

Every month or so you will transfer your weekly lab results to the *Accumulated Lab Results, by Tank* form. Each *Weekly HLP Test Log* sheet is condensed to one line on the accumulated lab sheet. Put down the 4th day result. If there is any bacteria present, please put a square around your result on the accumulated sheet. (Note: On the example I couldn't draw squares, so results are in **red**.)

At the bottom of the accumulated lab sheet, you will add up the totals for each fermenter. In the example below, please note that the first letter, formerly Y or N, has become a number. This is the number of times listed on the accumulated sheet that the fermenter in question has been tested as having aerobic bacteria present. (ie: the number of Y answers in the column.)

The second number represents the number of times listed on the accumulated sheet that the fermenter in question has been tested as having anaerobic bacteria present. (ie: the number of non-zero answers in the column.) By putting a square around any results with bacteria present, you should be able to add up your totals quickly without missing any. (Since I can't draw squares with this software, I have made the squared results **red**.)

You are looking for patterns here. If you find that a particular tank tests positive again and again, over time, with either aerobic or anaerobic bacteria showing up, then you need to look at that tank closely. Examine the structure of the tank for faults (ie: check the stainless), and examine your procedures closely. Has the same person brewed into that tank for the last 8 brews? Coincidences happen. Track down the causes of your results. Look for the big picture.

Dates 8/1 to 10/31/2010

SMALL BREWERY LABWORK
Accumulated Lab Results, by Tank

Date	Fermenters								Servers				
	F1	F2	F3	F4	F5	F6	F7	F8	S1	S2	S3	S4	S5
10/3	N/2	Y/0	N/0	N/0	N/0	Y/3	N/1	N/0	Y/1	N/0	N/0	N/2	Y/0
10/10	N/0	Y/0	N/0	N/0	N/0	N/1	N/0	N/0	Y/1	N/0	Y/0	N/0	N/1
.	...												
.	...												
.	...												
.	...												
10/17	N/0	N/1	N/0	N/0	N/0	N/0	Y/2	N/0	N/1	N/0	N/0	N/4	Y/3
	Fermenters								Servers				
	F1	F2	F3	F4	F5	F6	F7	F8	S1	S2	S3	S4	S5
Totals:	0/1	2/1	0/0	0/0	0/0	1/2	1/2	0/0	2/3	0/0	1/0	0/2	2/2

Fermenters shown in **red** are normally boxed or circled. Somehow you want them to stand out!

Collect your *Accumulated Lab Results* into a 3-ring binder. Look at them quarterly. Are certain tanks showing up repeatedly on the Totals line? Keep your Accumulated Lab Results forever, so that you can track long-term trends. Once a year you may toss out (recycle) your *Weekly HLP Test Logs*.

SMALL BREWERY LABWORK**HLP Test Log**

(Example: 8 Fermenters/5 Servers)

Name _____ Date _____ Time Start _____ Time End _____

Tank No.	Brew Name	Batch No.	Date Brewed	Yeast No.	From Fermenter	Yeast Generation	Results				Comments
	1 day	2 days	3 days	4 days							
F1											
F2											
F3											
F4											
F5											
F6											
F7											
F8											
S1											
F1											
F1											
S1											
F1											
Equip Name	Brew Name		Other Info	Results				Comments			
Beer Engine				1 day	2 days	3 days	4 days				
Control Test											
Other											

Dates _____ to _____

SMALL BREWERY LABWORK

Accumulated Lab Results, by Tank (Example for 8 Fermenters/5 Servers)