The Brewing-Science Institute

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In-House Testing

This guides you through the basic steps of microbiological testing. For a complete set of microbiological protocols, visit our website for a complimentary copy of the *Brewers' Laboratory Handbook*. Please practice good lab safety techniques while working, and feel free to call us if you have any questions.

Where & When To Sample

Sample	***Frequency***	***Sample Size***	***Common Contaminants***	***Tolerance***
water supply	1/week	100ml, filtered	enteric, molds	≤10 cfu*
wort	every brew	1ml	enteric, acetic & lactic, wild yeast	≤10 cfu; 0 cfu wild yeast
pitching yeast	every crop	1ml	enteric, acetic & lactic, wild yeast	≤10 cfu; 0 cfu wild yeast
fermenting beer, days 1-2	every tank	1ml	enteric, acetic & lactic, wild yeast	≤10 cfu; 0 cfu wild yeast
fermenting beer, days 3-5	every tank	1ml	acetic & lactic, wild yeast	≤10 cfu; 0 cfu wild yeast
storage tank	3/week	100ml, filtered	acetic & lactic	≤10 cfu
finishing tank	3/week	100ml, filtered	lactic	≤10 cfu
bottling tank	1/month	100ml, filtered	lactic	≤10 cfu
bottled beer	every batch	100ml, filtered	acetic & lactic	≤10 cfu
CIP rinse water	every CIP	100ml, filtered	-	0 cfu
CIP'd surfaces	every CIP	swab	-	0 cfu

*cfu = colony-forming units, or the number of colonies growing on the test plate

Sampling & Swabbing

Sample tubes are used to sample *liquids*, such as water, yeast, wort and beer.

- Wash and dry hands thoroughly.
- Sterilize intervening "outside" surfaces, such as valves, with 70% alcohol and flame, not with sanitizer.
- Do not uncap sample tube until immediately before sampling.
- Do not touch inside of tube or cap to any surface.
- If possible, allow sample to flow or crumble directly into tube without using utensils.
- Do not allow sample to overflow onto outside of tube.
- Recap tube as soon as possible and label.
- Keep cold until ready to test; test as soon as possible.

Sample swabs are used to sample *surfaces*, such as vessels, hoses, valves and bottles.

- Wash and dry hands thoroughly.
- Unwrap swab without touching the cotton tip or inside of sterile packaging.
- Firmly swab area to be tested, twisting swab to expose entire tip to area in question.
- Immediately place exposed swab back into sterile packaging and label.
- Keep cold until ready to test; do not store for more than 72 hours after exposure.

Wort Stability Test

This test determines whether or not brewing organisms -- including yeast -- are present without allowing for their identification. Using aseptic technique as described in "How To Draw Samples" section, draw about 25-ml of cooled, aerated wort into the sterile tube and cap it loosely. Allow to grow in a warm area (~86ºF/30ºC) for 72 hours. Clear, bubble-free wort indicates the sample contains no viable brewing organisms. Cloudiness and/or bubbles indicate that live organisms are present, which means either your sanitizing regime is inadequate, or sterile materials and surfaces are being exposed and contaminated. If you are unsure of your interpretation, tighten the cap, shake the tube vigorously, then loosen the cap; if any gas escapes, the sample contains organisms. Tubes may be sterilized and reused.

Don't pull that heat exchanger apart for cleaning until you've run a wort stability test on the cooled wort. If the test results are negative, you've saved yourself considerable effort and know you must look elsewhere for the contaminant's point of entry.

Pouring Plates

Each bottle contains 250ml of sterile, prepared media. It is important that your media does not become contaminated during handling. Please follow these directions carefully.

- Submerge bottle in boiling water and boil media is completely liquefied, shaking bottle as needed to break up clumps.
- NOTE: Plan to dispense all media from the bottle; saving partially-used bottles for reheating is not a good idea.
- Wipe down countertop or table with a 5% Lysol, bleach or other sanitizing solution.
- Label bottom of sterile plates with media name, the current date and your initials. Turn plates lids-up before pouring!
- Invert bottles before opening to suspend any insoluble components of media.
- Pass lip of bottle through flame to sterilize.
- Divide each bottle among 30 plates, lifting covers just enough to allow for pouring.
- Do not disturb plates until media has solidified.
- Check fresh plates for contamination by examining them after 48 hours of incubation at ~86ºF/30ºC.
- Invert and store plates refrigerated in a clean plastic box.
- NOTE: Bottles and caps are made of very durable polypropylene which can be sterilized* and used again.
  *15 minutes @ 15psi in a pressure cooker, autoclave or sterilizer.

Plating Samples

Plated media are generally used to test the purity of liquid samples such as wort, yeast slurry and bottled beer. It is important that the plates do not become contaminated with anything other than the sample you want tested, so store your plates refrigerated and upside-down in a clean plastic box until you are ready to use them.

- Choose an area that is enclosed or away from drafts and messy or dusty operations.
- Wipe down countertop or table with Starsan or a 5% Lysol or bleach solution.
- Wash and dry hands thoroughly.
- Inspect each new plate for contamination; label underside with date, sample name and sample site.
- Beer: Draw 100ml of beer through a 0.45μ filter and place the filter *face-up* on the agar, making sure the membrane's entire underside is in contact with the media.
- Equipment: streak used swab tip over entire media, twisting swab to expose all of tip to media surface and immediately replace plate lid.
- Wort, pitched or unpitched: Place 0.1ml (2 drops) of wort on media using a sterile pipette, gently spread over entire surface without gouging media and immediately replace plate lid.
- Yeast slurry: If using LMDA, place 0.1ml (2 drops) of yeast on media using a sterile pipette, gently spread over entire surface without gouging media and immediately replace plate lid. If using LCSM, LWYM, RDMA or wort medium, the above method will render the test unreliable. Instead, dilute sample with sterile water until sample is ever-so-slightly-cloudy (about 10^6 cells/ml) and use 0.1ml to inoculate the plate.
- Wipe down a plastic box with Starsan or a 5% Lysol or bleach solution.
- Place plates in box upside-down, loosely fit box lid and allow to grow in a warm area (~86ºF/30ºC) for 72 hours. Do not wrap or seal plates in any way.

About the Media

LMDA (SDA)

This agar test may be used aerobically or anaerobically to determine whether or not brewing bacteria are present, and allows for quick and easy genus identification. It supports the growth of all the most common types of brewing bacteria, but suppresses the growth of most brewing yeasts. Whatever growth is visible may be considered bacteria. If colonies arise, note the color, texture and size of each type (see pictures of various bacteria on LMDA on our website under the "Products" section). Also, note whether colonies have changed the color or cloudiness of the media immediately surrounding them. Put a drop of drugstore-strength peroxide on each colony and see if bubbles are produced. Check your observations against the list below. *Keep refrigerated; shelf life is indefinite.*

acetic acid bacteria (*Acetobacter, Acidomonas)*

Gram-negative, strictly-aerobic rods common in plant material such as fruit and grain. Normally encountered in stored or fermenting wort and bottled beer. Produce acetic acid, which lowers pH and lends a vinegary flavor/odor. Recommended limit: 5 per 1-ml sample or per 100-ml yeast-free sample.
colony: greenish-blue
colony size: 1-2mm
colony texture: smooth
Changes media color or cloudiness?: YES
Bubble formation when exposed to peroxide? YES
Turns royal purple when exposed to oxidase reagent? NO *(Acetobacter)*, YES *(Acidomonas)*

lactic acid bacteria *(Lactobacillus, Pediococcus)*

Lactobacillus (rod) and *Pediococcus* (coccus) are gram-positive, facultative anaerobes common in plant material such as fruit and grain. Encountered in all stages of brewing. Produce lactic acid, which lowers pH and lends a tart, sour flavor/odor. Recommended limit: 3 per 1-ml sample or per 100-ml yeast-free sample.
colony: yellow-green *(Pedio)*, white with bluish center *(Lacto)*
colony size: 1-3mm
colony texture: smooth *(Pedio)*, rough or smooth *(Lacto)*
Changes media color or cloudiness?: YES
Bubble formation when exposed to peroxide? NO
Turns royal purple when exposed to oxidase reagent? NO

enteric bacteria (*Citrobacter, Enterobacter, Hafnia, Klebsiella, Obesumbacterium)*

Gram-negative, facultatively-anaerobic rods common in water, soil, and plant material. Normally encountered in stored and fermenting wort. Produce sulfur compounds, fusel alcohols, phenolics and acetaldehyde, which cause a variety of off-flavors/odors. Recommended limit: 8 per 1-ml sample or per 100-ml yeast-free sample.
colony: greenish-blue, yellowish-green
colony size: 2-5mm, may spread to cover entire plate
colony texture: smooth, slimy
Changes media color or cloudiness?: NO
Bubble formation when exposed to peroxide? YES
Turns royal purple when exposed to oxidase reagent? NO *(all except Obesum)*, YES *(Obesum)*

other bacteria (Zymomonas)

Gram-negative, facultatively-anaerobic rods, common in water, soil and plant material. Normally found in areas where there is little or no oxygen, such as in bottled or cask beer and in CO2 recovery systems. Tolerates up to 10% alcohol. Rare in microbreweries. Produces sulfur compounds and acetaldehyde. Recommended limit: 5 per 1-ml sample or per 100-ml yeast-free sample.
colony: bluish-green
colony size: 1-2mm
colony texture: round, shiny
Changes media color or cloudiness?: NO
Bubble formation when exposed to peroxide? YES
Turns royal purple when exposed to oxidase reagent? NO