



## **BEE PRODUCT PROCESSING- LEVEL- II**

Based on October 2019, Version 2 Occupational standards (OS)

**Module Title: -Preparing and Monitoring Mead  
Yeast Propagation Processes**

**LG Code: IND BPP2 M12 LO(1-5) LG(37-41)**

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**Instruction sheet**

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Identifying and confirming propagation requirements
- Preparation of yeast cultures
- Confirming products and materials availability
- Preparing product for propagation
- Confirming services availability

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Identify and confirm propagation requirements.
- Prepare yeast cultures for use.
- Confirm products and materials and available to meet propagation requirements.
- Prepare product to meet specified propagation requirements.
- Confirm services as available and ready for operation.

**Learning Instructions:**

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-checks” which are placed following all information sheets.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).
6. If you earned a satisfactory evaluation proceed to “Operation sheets
7. Perform “the Learning activity performance test” which is placed following “Operation sheets” ,
8. If your performance is satisfactory proceed to the next learning guide,
9. If your performance is unsatisfactory, see your trainer for further instructions or go back to “Operation sheets”.

## Information Sheet 1- Identifying and confirming propagation requirements

### 1.1 Introduction

Yeast is the next ingredient to consider and once again, there are several choices. Yeast is living organism that metabolizes sugars in honey to carbon dioxide and ethyl alcohol. Cultured wine yeast is commonly used to make mead. In general, those that are used for white wines, especially *sauterne yeast* work well. The yeast used for wine and mead fermentation is *Saccharomyces cerevisiae*. The purpose of a yeast starter is not to produce an enjoyable fermented beverage but rather to produce a sufficient quantity of yeast for subsequent fermentation. Propagation conditions should be such that a maximal amount of yeast is produced which provides optimal fermentation performance once pitched.

### 1.2 Types of Yeast

- a) **Baker's Yeast** (Quick-Rise Yeast:-Active Dry Yeast, Baker's Compressed Yeast).  
Yeast dissolved directly in liquids and mixed with flour Compressed yeast
- b) **Nutritional Yeast**- this type of yeast is available in both powder and pill form.  
Nutritional Yeast is powdered yeast without leavening power, marketed for its protein and vitamin content.
- c) **Brewer's Yeast**- is dried and inactive yeast that has no fermenting power. It is sold for its nutritional qualities as it is very high in at least 10 separate B-vitamin factors, including: Riboflavin, Thiamin Pyridoxine, Niacin, Folic acid, Inositol, Choline, Biotin, Paraminobenzoic Acid and Pantothenic Acid

Different yeast strains produce different levels of these compounds and therefore impart their own subtle characteristics to the wort in which they are pitched. Although yeasts may all be the same species, *Saccharomyces cerevisiae*, they are not created equal. Indeed bread yeast does not make good beer and the same may be said for some wine yeast. Beer yeasts also differ and anyone who has split a batch of beer and pitched different yeasts will attest to the difference they can have in brewing. So

just as hops, malts, and water must be chosen for a specific beer, so must the yeast. The propagation and maintenance of yeast at home adds yet another level of control to the brewing process, allows for experimentation, and aids in the consistent production of unique high quality beers.

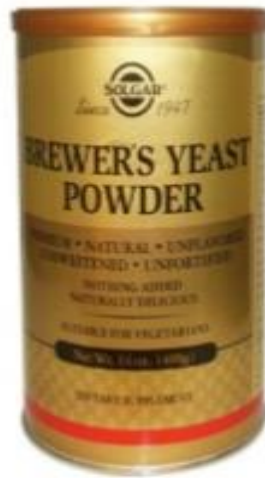


Figure 1: Brewer's Yeast

Brewer's yeast products are usually found ***in the form of powders, flakes or tablets, or in liquid form***. Liquid yeast contains enzymatically digested yeast for better digestion, absorption and utilization. Brewer's yeast should not be confused with "brewer's type yeasts", which are pure yeasts usually grown on enriched cane or beet molasses under controlled production conditions, and are not by-products of the brewing process.

Self-check 1	Written test
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

**Test: Short Answer Questions (10 points)**

1. What is yeast? (1 points)
2. Mention the three types of yeast and discuss them (6 points)
3. Mention the form of brewer's yeast products (3 points)

**Note:** Satisfactory rating - 15 points

Unsatisfactory - below 15 points

Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

**Short Answer Questions**

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_
3. \_\_\_\_\_  
\_\_\_\_\_
4. \_\_\_\_\_  
\_\_\_\_\_

## Information Sheet 2- Preparation of yeast cultures

Cultured wine yeast is commonly used to make mead. The yeast used for wine and mead fermentation is *Saccharomyces cerevisiae*. Matching the appropriate yeast culture to the honey variety is key operation to developing the desired taste and mouth feel of mead. In addition to sugar, yeast needs nitrogen, phosphorus and potassium for growth. Ingredients such as urea, peptone and potassium phosphate are used to supply these nutrients. It is also possible to buy packaged nutrients specially designed for mead.

### 2.1 Principles of Yeast Growth and Fermentation

Yeast is a facultative anaerobe which is just a fancy way of saying that it can survive and grow in the presence (aerobic) or absence (anaerobic) of oxygen. The presence of oxygen determines the metabolic fate of the cell. In terms of the yeast cell, its survival, growth and metabolism is optimal in the presence of oxygen. In this case, yeast will rapidly grow to high densities and will convert sugar (glucose) to carbon dioxide and water. Under anaerobic conditions, yeast grows much more slowly and to lower densities and glucose is incompletely metabolized to ethanol and carbon dioxide. It is important to realize that optimal yeast growth is distinct from fermentation. Therefore, the conditions and methodologies used for propagating and maintaining yeast need not be identical to those used for fermenting wort.

### 2.2 Preparation of yeast cultures

A yeast starter should be composed of a 1.040 gravity wort that is supplemented with amino-acid based nutrients (*BrewTek nutrient or Superfood*). It should be aerated well before adding yeast. Once the yeast is added it should be kept at room temperature (~75 °F) and shaken as often as possible (or better yet, constantly stirred). All media used to store brewing yeast (slants, plates, etc.) should contain yeast nutrients, some malt, and should have an acidic pH.



## 2.3 Homemade Starter

Equipments and Ingredients used for starter culture are listed below

Equipments:-

- 1) Vessel: - used to grow yeast. It must be sanitized.
- 2) Pot- used to boiling of the DME and water mixture and it transfer in to flask for fermentation. It must be stainless steel.
- 3) Emerlyser Flask-used to fermentation. Can sanitise with heat and used for boiling as well as fermentation. At this time the flask must be resist heat and fermentation shock.
- 4) Sanitizable glass vessel (1 liter and larger capacity)
- 5) Airlock and stopper or foam stopper fit to propagation flask. Can greatly accelerate yeast growth. Drives off CO<sub>2</sub>. Keeps yeast in suspension. Allows more air/yeast contact, potentially allowing more oxygen access.
- 6) Stove –used to heating purpose
- 7) Measuring cylinder and cup- used to measure water ingredients
- 8) Weighing balance- used to measure ingredients
- 9) Aluminum foil- for covering during cooling the boiled mixture /wort/. Foil over the top is fine while CO<sub>2</sub> is being produced.
- 10) Cooling bath /sink/- for cooling purpose



*Figure 2: Emerlyser Flask and boiling starter wort*

## Ingredients:-

### 1) Yeast to be propagate

You need one if you use start from small amounts of yeast. Slants or plates, culturing from bottle dregs, etc. Quality of starting yeast is important. Bad parents will result in more likelihood of demented children. There are ways to reculture suspect yeast, but that is a whole new topic. Try to avoid “stressed” and over-used yeast. Each propagation risks more mutations. Yeast used in high gravity brewing is not recommended (its well and truly passed its best). Try to get yeast closest to the “source” of known good yeast in following preference:

- Liquid yeast packet from yeast supplier
- Slant or stored yeast from above
- Yeast collected from Krausen
- Yeast from secondary
- Yeast from primary
- Yeast from bottle
- Yeast just found lying around the shed floor :p ( this is commonly known as an infection)



### 2) Starter Wort

- Dry malt extractor (DME)- sugar nutrient from many fermentation. 1gm per 10 ml for ~ 1.030 SG. Should be boiled up to ensure it is sanitised.
- Liquid malt extract- Can get at HBS and sometimes low quality types at supermarket
- Saved wort- Must be sterile to store at room temp. Can be frozen, but needs to be sanitised after defrosting

### 3) Others

- Yeast nutrient:- FAN (Nitrogen)-Generally available from the malt in the starter wort. Specialised nutrient:-Contains minerals such as Zinc and Magnesium, yeast hulls, diammonium phosphate etc. It will give the best overall results
- Oxygen- Oxygen is critical to yeast growth.

### 4) Water- is used to make a mixture with DME

## Procedures required following starter culture preparation

In order to prepare Wort we will perform the following operations:-

- Activate the yeast for a few minute
- Measure water (the proportion of 10ml of water will be prepare for 1gm of dry malt extractor (DME)
- Addition of nutrient and boil
- Pour wort into flask (if the boiling was on pot)
- Do it in the emerlyser flask if you have one with some foil mostly over the top will sanitise it at the same time.
- Cool down to pitching temperature using it with flashing water on flask or inserting the flask in water bath.
- Make sure propagating container is sanitised (Sanitize stopper and yeast after cool).
- Bring it up to room temperature
- Incubation-

Time-to reproduction and fermentation, incubation is complete between 12-24 hrs after pitching yeast in to wort

Temp. -Fairly warm to promote high cell metabolism and activity- incubate the starter at 70-75<sup>0</sup>F even larger yeast

Oxygen-swirl the flask during inoculation put it in incubator much aeration

- Ready to pitch- pouring the incubated yeast in to primary fermenter.

If you culture yeast frequently, you will probably want to prepare your own starter tubes. Starter tubes are fairly easy to prepare, but slants and plates are more challenging. Capped glass tubes for starters and slants are available from scientific supply houses. You may be able to reuse empties from prepared commercial starter tubes.

## 2.4 Stages of Yeast Growth

When yeast is added to wort or media, it goes through 5 basic stages of growth. The diagram shown below is a typical change in yeast cell number when introduced into liquid media at very low concentration. The various phases of growth are shown.

- Note that the actual time it takes to reach stationary phase varies depending on growth conditions, yeast strain, and amount of yeast inoculated.

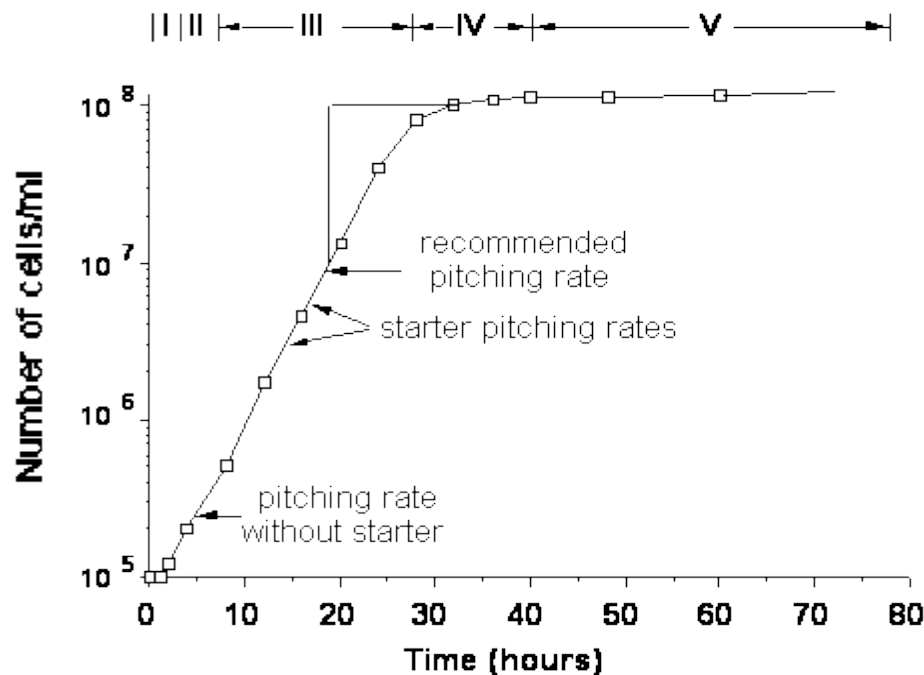


Figure 3: Growth curve for brewer's yeast

### Keys

I- Large phase-Period of adaptation

II- Accelerating growth phase-initiation of yeast growth

II-Exponential growth phase- rapid yeast growth, doubling every 2-3 hours

IV-Decelerating growth phase- nutrient supply depleted

V-Stationary phase- no yeast growth, cell viability begins to decline

- When a small amount of yeast from a slant is inoculated to a tube containing 10 ml of wort or media, it will undergo the growth curve shown in figure 3 over a 1-3 day

period. The precise length of time will vary depending on the yeast strain, how old the slant is, the media used, the level of aeration, etc.

a) ***Initial phase.***

Initially there is a lag phase occurs during the first few hours after addition of the yeast. During this time there are no apparent signs of fermentation or growth. The yeast is becoming acclimated to their new environment. If the previous media (or starter) is similar to this new one, acclimation will occur rapidly and the lag phase will be short. If there are major differences in the gravity, temperature, or wort composition, the yeast may be surprised or shocked and it may take some time to adjust to this new environment. Major changes occur within the yeast at this time, they are absorbing all of the oxygen in the wort, using it to synthesize all the enzymes and other metabolic machinery necessary for growth and fermentation, and storing oxygen up in the form of sterols for later use. This stage is critical to fermentation and should occur as rapidly as possible, preferably within a few hours.

b) ***The second phase***

The second phase is the accelerating growth phase during which yeast cells start to grow and divide. Signs of fermentation will also become apparent. The yeast begin storing sugar in the form of glycogen for later use.

c) ***The third phase***

The third phase is the exponential phase where yeast reproduction and metabolism is in high gear. Cells are dividing every 90 - 180 minutes and fermentation begins. During this time the number of yeast cells may increase as much as 1000-fold (or 3.0 logs) within 24 hours. The extent to which the cells divide is dictated primarily by the pitching rate. If appropriate pitching rates are used, the yeast are pitched at high concentrations (5-15 million yeast cells per ml) and undergo approximately 3 generations (23- or an 8-fold increase in cell number) to yield 80-100 million cells per ml. 100 million cells per ml is about the maximal concentration of yeast attainable in

fermenting wort. Fermentation is also very active and a krausen may be beginning to form.

**d) *The fourth phase***

The fourth phase is the decelerating growth which should occur 12-24 hours after pitching. At this time the oxygen is fully depleted and fermentation and CO<sub>2</sub> production is taking over. Fermenting wort should be in high krausen. Maximal fermentation occurs during 12-48 hours; heat is being generated and there should be rapid CO<sub>2</sub> evolution (bubbling).

**e) *The final phase***

Finally several days later, the yeast enters a stationary phase. During this time the fermentable and nutrients are completely consumed. All yeast growth has stopped and they are beginning to fall out of suspension or flocculate. The sterol and glycogen stored up during early growth are beginning to be broken down and used to continue growth. Prolonged exposure in this phase (weeks) can lead to autolysis or total breakdown of the cell.

<b>Self-Check – 2</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

**Test I: Short Answer Questions (21 points)**

1. What type of yeast used for wine and mead fermentation? (1 points)
2. Discuss the principle of yeast growth and fermentation (2 points)
3. Mention equipments used for starter culture preparation (10 points)
4. Mention ingredients used for starter culture preparation (3 points)
5. What are the stages of yeast growth? (5 points)

**Note:** Satisfactory rating - 21 points

Unsatisfactory - below 21 points

**Answer Sheet**

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

**Short Answer Questions**

1. \_\_\_\_\_
2. \_\_\_\_\_
3. \_\_\_\_\_
4. \_\_\_\_\_
5. \_\_\_\_\_

### Information Sheet 3-Confirming products and materials availability

Before the propagation of culture, the processor needs to be confirmed on the availability of the required materials and products next to the equipments. Products and materials are confirmed and available to meet propagation requirements for mead preparation. There are different types of products and materials described below. Such as culture yeast slopes/slants, oxygenated wort, yeast nutrients, oxygen supply and Sterilization materials are the main inputs for the propagation process

## Products and Materials used for yeast culture propagation

### *Oxygen supply and Oxygenated Worth*

Oxygen or aeration is essential for good yeast growth and is the driving force behind many aspects of yeast metabolism including fermentation. In terms of the yeast cell, its survival, growth and metabolism is optimal in the presence of oxygen. Oxygenated wort is used as ingredient for pitching of yeast which means addition of a specific amount of yeast to freshly, at the correct fermentation temperature. Yeast can be new, first generation, or reused from previous fermentation. Yeast can be reused 5-10 times. Pitch more yeast for high gravity beers.

### *Yeast Nutrients*

The addition of yeast nutrients and certain salts can also improve yeast growth and are a worthwhile addition to starters. Yeast nutrients usually are of two types, one which is ammonium phosphate-based, and the other which is amino acid/peptide and vitamin-based (similar to the peptone and yeast extract in the laboratory media described below). Both serve the same basic function which is to increase the nitrogen content of the wort and yeast.



A mixture of different nitrogen sources have been shown to enhance both growth and fermentation and suggest that the amino acid/peptide-based nutrients may be more appropriate than diammonium phosphate. Also rapidly growing yeast such as those in starters have a higher than normal nitrogen requirement. Thus starter wort should be supplemented with yeast nutrients so that nitrogen is not limiting

### ***Sterilization materials***

Clear away all materials cluttering your work area on the laboratory bench. Remove a pre-moistened disinfectant wipe from the canister and clean down the entire area. Allow the disinfectant to evaporate - do not clean dry!

- Use disinfectants such as alcohol (isopropanol or 70% ethanol) or phenolic compounds (o-phenylphenol) .
- To prevent **aerosolization**, or the production of a fine mist containing bacterial cells, and spread of microbial contaminants, avoid dispensing disinfectant from a squeeze bottle.
- Desiccation of microorganisms is one of the most effective ways to decontaminate surfaces.
- Even if someone has recently used the laboratory bench and the bench top was wiped down with disinfectant, ALWAYS begin your laboratory time by wiping down the bench.

Self-check 3	Written test
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions (10 points)

1. Mention products and materials used for yeast culture propagation (4 points).
2. Describe the importance of each products and materials in yeast culture propagation (6 points).

**Note:** Satisfactory rating - 10 points

Unsatisfactory - below 10 points

#### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

#### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

## Information Sheet 4- Preparing product for propagation

Propagated yeast is among the basic ingredient for propagation. It has its own purpose. The first purpose is to control beer quality propagation and there by fresh yeast supply is necessary. The second purpose is in the fermentation process yeast mutates and change performance over time as well as infection sooner or later becomes a problem.

The objective of propagation is to produce large quantities of yeast with known characteristics for the primary role of fermentation, in as short a time as possible. Most brewers use a simple batch system of propagation, starting with a few milliliters of stock culture and scaling up until there is enough yeast to pitch a commercial brew. Scale-up introduces actively growing cells to a fresh supply of nutrients in order to produce a crop of yeast in the optimum physiological state.

### Optimum Propagation Temperatures

There is a wide variety of recommendations in this instance as well. Some brewers prefer to propagate their yeast at temperatures identical to those employed during fermentation in order to prevent temperature shock to the yeast.

### Propagation Plants

The propagation plant usually consists of anywhere from one, two or more closed stainless steel vessels of increasing volume, which are usually situated in a separate room to minimize contamination of risk.

## Yeast Maintenance

After a determined number of repitchings, new yeast should be used, either obtained in a dry form or propagated by a third party or in-house. See yeast bank. Propagations are typically started from a stock culture. Yeast stocks should be kept at cold temperatures to maintain the integrity of the DNA through time; spontaneous mutations do occur and can affect the characteristics and performance of yeast. To protect yeast strains against mutation for a long period of time, cryopreservation is recommended, with the safest method being storage in the gas phase of liquid nitrogen in a specific container. Working stocks can be maintained frozen at  $-80^{\circ}\text{C}$  for long-term storage.

Agar slants may be kept at  $4^{\circ}\text{C}$  ( $39^{\circ}\text{F}$ ); however, this is only for short-term storage because there is a higher risk of mutation and contamination. The number of times a yeast culture can be reused depends on numerous factors; however, it is well documented that cultures should be replaced regularly to ensure fermentation performance and consistency. Although this is the norm, there are exceptions, and some breweries have been reported to have used a single yeast culture for years or even decades without notable mutation or loss of vitality. The genetic stability of the strain used, hygiene process, brewing frequency and schedule, the yeast maintenance program, and type of beer produced will eventually determine how many times a particular yeast culture can be re pitched.

<b>Self-Check – 4</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

**Test I: Short Answer Questions (8 points)**

1. What type of starter Wort used in yeast culture propagation? (2 points)
2. What type of yeast used in yeast culture propagation? (2 points)
3. What type of yeast nutrient used in yeast culture propagation? (2 points)
4. What type of oxygen used in yeast culture propagation? (2 points)

**Note:** Satisfactory rating - 8 points

Unsatisfactory - below 8 points

**Answer Sheet**

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

**Short Answer Questions**

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_
3. \_\_\_\_\_  
\_\_\_\_\_

## Information Sheet 5- Confirming services availability

Confirming services availability is the task to be ready for any operation. In beverage processing industry services like electricity, water, steam, compressed air and Oxygen are basic to be confirmed.

### Services used for yeast propagation

#### 1) Energy

This is basically the conservation of energy on all levels. To improve the conservation of energy one must focus on the site, the orientation of the building, the site layout and how different functions of the site work together. The need is there to make use of modern-day technologies that maximize energy efficiency. The production of harvesting honey involves the process of pasteurization which involves the use of mechanical energy such as the exposure to extensive heating and cooling in a circuit.

#### 2) Water

“Preserve the existing natural water cycle and design site and building improvements such that they closely emulate the site’s natural “predevelopment” hydrological systems. Emphasis should be placed on the retention of storm water and on-site infiltration and ground water recharge using methods that closely emulate natural systems. Minimize the unnecessary and inefficient use of potable water on the site while maximizing the recycling and reuse of water, including harvested rainwater, storm water, and grey water.”

#### 3) Lighting

Lighting is usually brought in through high roof windows to avoid visual distractions for workers. Lux (symbol: lx) is the unit of illumination which is used to measure the intensity of light that hits or passes through a surface, as seen from the human eye. It measures luminous flux per unit area and is equal to one lumen per square meter. In an industrial environment, the spaces are deep thus artificial lights are the dominant

source of light. The use of sky lights and jig saw roofs are adopted in the design to allow natural lights to reach deep spaces.

#### **4) Energy and Services (sustainability)**

Efficient ventilation is made possible by utilizing the prevailing winds in conjunction with an intelligent roof design which invites fresh air in and allows the hot air from the production area to be sucked out and dissipated through perforations in the glass facade. Through geothermic methods the air is maintained at a steady 20°C. It is naturally treated in underground pipes (3 m deep) using just 7 mechanical fans (37 HP total consumption). This air is incorporated into working areas. During the day, the building only uses natural zenith lighting.

#### **5) Steam**

Steam is an efficient and effective energy medium which is widely used in industry worldwide to produce everything from food to chemicals to paper and building materials. Steam heat plays an integral role in food production in numerous ways. Let's explore the most common and essential applications.



*Figure 4: Process steam*

Various industry sectors, such as food processing, brewing & beverage, dairy, pharmaceuticals, chemicals & building materials, require steam in their processes.

The consumers don't often realize it, but the products we use daily, often go through processes utilizing high temperatures.



Figure 5: *Steam Honey Extractor*



Figure 6: *Sterilization and Disinfecting*

Here are some common applications of steam in the food and beverage industry:-

### ***Sterilization and Disinfecting***

Food safety is always among the top priorities for food processing companies. To ensure the foods and beverages produced are safe for public consumption, it's essential for food processing plants to clean, sterilize, and disinfect all tools, utensils, and surfaces used in food production.

### ***Reducing Microbiological Risks***

Surface contamination isn't the only concern with food safety; there are also inherent risks of microbiological contamination on or in the foods themselves bacteria and microbes that cause food-borne illnesses. Steam pasteurization is an effective method of controlling the microbial risks of food processing. Having quality steam from an industrial boiler makes this process a lot easier to carry out.

### ***Cooking, Curing, and Drying***

Some food processing companies do a lot of cooking before their food is ready for the general public. While there are many ways to cook or pre-cook food, doing so with both steam and hot water produced by boilers is one of the best ways to ensure the food is smooth, soft, and easy to digest once it gets to consumers. More complex processes like curing and drying certain food products require steam, too. Since food



processing is typically a large-scale operation, large volumes of steam and hot water are needed at least some of the time.

### ***Heating the Facility***

Food processing companies rely not only on efficient machinery but also on their employees' hard work. Climate control is essential in these plants to maximize productivity and keep workers safe. Thus, in many cases, the same steam boiler system that helps with the various stages of food production also provides heat for the plant itself.

## **6) Compressed Air**

Compressed air has a wide variety of applications across industries. In the food and beverage industry, air compressors are used in production chains, packaging and cleaning. Generally, there are three different types of compressed air systems in the food and beverage industry:

- **Contact:** Compressed air that comes in direct contact with food products is categorized as a contact system. In their Compressed Air Best Practice Guideline, the British Compressed Air Society recommends a pressure dew point of -40 degrees Fahrenheit for contact compressed air.
- **Non-Contact High-Risk:** When compressed air is used in the production environment but does not come in direct contact with food products, it is considered a non-contact high-risk system. For example, when compressed air is used to create packaging that will later come in contact with the food product; this is a non-contact high-risk system. The same safety standards applied to contact compressed air should be upheld for non-contact high-risk compressed air systems.
- **Non-Contact Low-Risk:** In some instances, compressed air does not come in direct contact with any food products or food contact surfaces. When used in pneumatic systems, compressed air powers machines, control valves, air motors or other pneumatic devices. When compressed air does not come in contact with food or food contact surfaces, it does not need to keep such

stringent purity standards and should be maintained at a pressure dewpoint of 37 degrees Fahrenheit.



*Figure 7: Food & Beverage Safe Air Compressors*

A food and beverage air compressor is a tool used by the food industry to provide clean, safe air for packaging, processing, and storing products. From cooking, storing, maintaining, and dispensing food, our food and beverage grade air compressors are made to the highest industry standards for safe handling. Here are some common applications of air compressors in the beverage industry:

- **Fermentation Processes:** Compressed air may be used during fermentation when producing wine or beer. The compressed air increases oxygen levels that help bacteria complete the fermentation process.
- **Aeration:** Air compressors can be used to add more oxygen into a liquid product in a clean manner.
- **Water Sterilization:** Compressed air can create ozone gas that is used to sterilize water. Ozone works as an oxidizing agent to remove undesirable compounds from water, such as iron or magnesium.
- **Moving Product:** Compressed air is used in beverage production to move liquid products through pipes. Beverages must go through many processing stages, such as blending, pasteurization and bottling. Compressed air allows the product to move more quickly through the production line.

- **Creating Packaging:** Bottles for sodas, juices, wines and water are often created using blow-molding and then cooled with compressed air. Labels are applied or indented onto packaging through mechanical arms that are often pneumatically-powered.
- **Cleaning and Filling Packaging:** Before bottles are filled, they are cleaned with compressed air. Compressed air dries bottles to remove any excess water vapor or moisture. The liquid contents are then poured into the bottle with the help of an air compressor for consistent and efficient filling. Air compressors used for filling reduce waste and ensure products are filled precisely.
- **Sealing Bottles:** After bottles are filled, pneumatically-powered machines are often used to seal bottle caps.
- **Bottling Beer:** Air compressors are used in bottling beer to reduce the residual oxygen content. Bottles are flushed with CO<sup>2</sup> using air compressors and then filled with beer using pneumatically-powered machinery.
- **Dispensing Soft Drinks and Draft Beer:** Soda machines often use air compressors to push sodas through the machine's lines and dispense it in an even stream. Draft beer is also dispensed using air compressors that push the beer from the keg to the tap using air pressure.

<b>Self-check 5</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions (8 points)

1. What type of services required in food and beverage industry? (2 points)
2. What is the importance of energy in food and beverage industry?(2 points)
3. What is the importance of steam in food and beverage industry? (2 points)
4. What is the importance of compressed air in food and beverage industry? (2 points)

**Note:** Satisfactory rating - 8 points

Unsatisfactory - below 8 points

#### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

#### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_
3. \_\_\_\_\_  
\_\_\_\_\_
4. \_\_\_\_\_  
\_\_\_\_\_

Operation Sheet 1- Preparing yeast culture for propagation	
Operation Title: Preparing yeast culture for propagation	
Purpose	To acquire knowledge, skill and attitude by performing Prepare yeast culture for propagation
Equipment, tools and materials	<p>Supplies and equipment needed or useful for sampling and testing of honey include:</p> <ul style="list-style-type: none"> <li>• Vessel, Pot, Emerlyser flask, glass vessel, airlock and stopper, stove, measuring cylinder/cup, weighing balance, aluminum foil and cooling bath.</li> <li>• Yeast Starter Wort, Yeast nutrient, oxygen &amp; distilled water</li> </ul>
Conditions or situations for the operations	<ul style="list-style-type: none"> <li>• Services, equipment's and materials should be available</li> <li>• Appropriate working area for receiving, sampling and testing and storing.</li> </ul>
Procedures	<ol style="list-style-type: none"> <li>1. Wear the appropriate personal protective cloth</li> <li>2. Prepare the required equipments, ingredients and services for yeast culture preparation</li> <li>3. Activate the yeast for a few minute</li> <li>4. Prepare water</li> <li>5. Addition of nutrient in to water and boil</li> <li>6. Pour wort into flask (if the boiling was used with pot)</li> <li>7. Cool down to pitching temperature</li> <li>8. Sanitise the propagating container</li> <li>9. Bring it up to room temperature</li> <li>10. Incubation by considering the requirement of time, temperature and oxygen.</li> <li>11. Take and record measurements</li> <li>12. Report the appropriate personnel</li> </ol>
Precautions	<ul style="list-style-type: none"> <li>• Care should be taken while handling and using equipments and yeast preparing</li> </ul>
Quality criteria	<ul style="list-style-type: none"> <li>• Incubation- Time - complete between 12-24 hrs after pitching yeast in to wort, Temp. - at 70-75<sup>0</sup>F even larger yeast Oxygen-swirl the flask during inoculation put it in incubator much aeration</li> </ul>

LAP TEST	Performance Test
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Name..... ID.....

Date.....

Time started: \_\_\_\_\_ Time finished: \_\_\_\_\_

**Instructions:** Given necessary templates, tools and materials you are required to perform the following tasks within **12-24** hour. The project is expected from each student to do it.

Project: Yeast Culture preparation for propagation

**Task:** Prepare yeast culture for propagation

**Task:** Take records and report the appropriate personnel

<b>LG #38</b>	<b>LO #2- Prepare equipment for culture preparation</b>
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### Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics:

- Checking equipment whether cleaned and sanitized
- Selecting, cleaning and sanitizing propagation equipment
- Selecting, cleaning and sanitizing transfer equipment

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Check cleaning and sanitizing equipment to confirm readiness for use.
- Select, clean and sanitize propagation equipment according to workplace procedures.
- Select, clean and sanitize transfer equipment as required.

### Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-checks” which are placed following all information sheets.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).
6. If you earned a satisfactory evaluation proceed to “Operation sheets
7. Perform “the Learning activity performance test” which is placed following “Operation sheets” ,
8. If your performance is satisfactory proceed to the next learning guide,
9. If your performance is unsatisfactory, see your trainer for further instructions or go back to “Operation sheets”.

## Information Sheet 1- Checking equipment whether cleaned and sanitized

Before you begin the mead making process, there will start out making sure all equipment is clean and sanitized. Anything that touches the must (unfermented honey and water mixture) should be sanitized; this will of course include the brew pot. If we are a home brewer, we may depend on the boil to "sanitize" our brew pot. But with mead, we will not be boiling. So, it is important to clean and sanitize everything. To start the main operation of the processes there should check the hygiene and sanitation of all equipment used to that processing. Inspection by the processor or supervisor is one of the instruments to check the equipments whether cleaned or not.

### 2.1 Inspection

The purpose of an inspection is to identify whether work equipment can be operated, adjusted and maintained safely with any deterioration detected and remedied before it results in a quality and safety risk. Not all work equipment needs formal inspection to ensure safety and, in many cases, a quick visual check before use will be sufficient. However, inspection is necessary for any equipment where significant risks to health, quality and safety may arise from incorrect installation, reinstallation, deterioration or any other circumstances. The need for inspection and inspection frequencies should be determined through risk assessment.



*Figure 8: Clean Pot for boiling*



## 2.2 What should the inspection cover?

This will depend on type of work equipment, its use and the conditions to which it is exposed. This should be determined through risk assessment and take full account of any manufacturer's recommendations. The advice of others, such as trade associations and consultants, as well as other sources like published advice on health and safety, may also be helpful.

An inspection should concentrate on those safety-related parts which are necessary for the safe operation of work equipment and, in some cases, this may require testing or dismantling. However, not all safety-critical features on a particular item of work equipment may require inspection at the same intervals.

An inspection can vary in its extent, as the following demonstrate:

- quick checks before use (eg electric cable condition on hand-held power tools, functional testing of brakes, lights on mobile machinery)
- weekly checks (eg presence of guarding, function of safety devices, tyre pressures, and the condition of windows, mirrors and etc.)
- more extensive examinations, undertaken every few months or longer (eg general condition of a ladder, close examination of a safety harness, portable appliance testing)

Records are not normally required to be made for the simplest pre-use checks.

## 2.3 Who should carry out the inspection of work equipment?

Equipment can be inspected by anyone who has sufficient knowledge and experience of it to enable them to know:

- what to look at
- what to look for
- what to do if they find a problem

The necessary level of competence will vary for inspections, according to the type of equipment and how / where it is used. The nature of these inspections does not have to be determined by the same person who undertakes them, provided the person

determining them is competent. This can often be done in-house by experienced staff, taking account of:

- the manufacturer's recommendations
- industry advice
- their own experience of the equipment, its use, the particular factors of the workplace and the people using the work equipment

<b>Self-Check – 1</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

**Test I: Short Answer Questions (10 points)**

1. Describe the importance of inspection to confirm checking equipment whether cleaned and sanitized (5 points)
2. What should the inspection cover? (3 points)
3. Who should carry out the inspection of work equipment? (2 points)

**Note:** Satisfactory rating - 5 points

Unsatisfactory - below 5 points

**Answer Sheet**

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

**Short Answer Questions**

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_
3. \_\_\_\_\_  
\_\_\_\_\_

## Information Sheet 2- Selecting, cleaning and sanitizing propagation equipment

Cleaning and sanitizing is probably in one of most important aspects in food and beverage processing plants, an effective sanitation program is the key factor to guarantee product quality. Cleaning is the complete removal of food soil using appropriate detergent chemicals under recommended conditions. It is important that personnel involved have a working understanding of the nature of the different types of food soil and the chemistry of its removal. Sanitation is treatment of a cleaned surface with a chemical or physical agent to destroy disease/spoilage causing organisms.



*Figure 9:-Equipments and Indigents for propagation*

### 2.1 Types of Sanitizers:

- Chlorine / Bleach Sanitizers:
- Quat Based Sanitizers:
- Iodophor Sanitizers:
- Peracetic Acid – Peroxyacetic Acid (PAA):
- Acid Anionic Based Sanitizers:

## 2.2 Propagation Environment

Lab propagation environment is important that propagation room is kept clean. Ideally it should be completely tiled. Walls, floor, ceiling sprayed with suitable sanitizer at regular intervals. Extractor fan and ideally should be a de-humidifier. Further precautions: UV lights, foot baths, double doors, positive pressure

## 2.4 Brewery Propagation Environment

- Floor should be easy cleanable
- Periodical Sanitation for the appropriate equipments and materials should be consider
- Have regular protocols. Walls, floor, ceiling sprayed with suitable sanitizer at regular intervals.
- Environmental testing is necessary. Further precautions: UV lights, foot baths, double doors, positive pressure.

The key considerations in brewery propagation are:-

- Wort- sterility, type, gravity- ~ 1.020 to 1.040
- Conditions - hygiene, aeration/agitation, pure O<sub>2</sub>
- Contamination-vessel, wort, air, CIP system, valves
- Temperature - gradual decreases – 26<sup>0</sup>C
- Time - transfer yeast in log phase (stat phase later in prop)
- Batch or Fed-Batch for Brewery Propagation

Self-Check – 2	Written test
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions (15 points)

1. Mention the five types of sanitizers (5 points)
2. What should be brewery propagation environment looks like) (5 points)
3. What are key considerations in brewery propagation? (5 points)

**Note:** Satisfactory rating - 15 points

Unsatisfactory – below 15 points

### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_
3. \_\_\_\_\_  
\_\_\_\_\_

### Information Sheet 3- Selecting, cleaning and sanitizing transfer equipment

Equipments used for transferring the yeast culture for propagation is available in the kit of yeast culture kit (brewer's kit) which may or may not come with starter yeast and instructions. To maintain the propagated yeast quality, using aseptic technique is preferable. The success of aseptic processing and sterile fill-finish operations relies on mitigating contamination from each of these sources. Aseptic processing and sterile fill-finish operations can be applied to a range of container systems made of either glass or plastic, including vials, syringes, bottles, cartridges, and ampoules. For ophthalmic preparations, single dose blow-fill-seal (BFS) containers molded from plastic and newer multidose, sterile-delivery packaging may also be options. Glass containers typically undergo a pre-sterilization process to remove foreign matter.

## 3.1 Equipment and Process

Sterile fill-finish operations can take several forms, including hand-filling of clinical trial material (CTM), fully automated high-speed filling lines, and sterile lyophilization to name a few. In this section, we will focus on solution filling of glass vials and syringes, which is performed with semi-automated or fully-automated fillers.

## 3.2 Sterile technique

Sterile technique is ESSENTIAL when working with microorganisms! It is important to protect strains from contamination with other strains and from the many undefined microbes in the environment. Large numbers of diverse microorganisms are all around us - in the air, on laboratory surfaces, on your skin and on your clothing. True to their name, microorganisms are too small to be detected by the eye, but they grow rapidly in laboratory culture media. Correct transfer techniques and the use of sterile reagents are usually enough to prevent contamination. Some simple precautions will reduce the possibility of contamination:

- Wipe down a small working area on the lab bench with 70% ethanol.
- Light a Bunsen burner in your work area while working with strains. The burner produces an updraft that prevents airborne microorganisms from falling into cultures.

- Use sterile reagents, micropipette tips, and test tubes. Tips and micro-centrifuge tubes should be kept in covered containers when not in use.
- Minimize contamination from clothing and body surfaces. Pull back and secure long hair. Avoid touching or breathing on sterile surfaces that will contact microorganisms.
- Avoid talking when you are transferring strains.
- Work quickly! Minimize the time that tops are removed from vessels containing microorganisms or media.
- Keep caps right-side up to prevent contamination from airborne microbes.

Aseptic technique refers to a set of routine procedures done to prevent sterile solutions and cultures from becoming contaminated by unwanted microorganisms in the laboratory. Such techniques are essential for experiments that require growing cells.



Figure 10:-1) Aseptic technique (lighter)

2) Aseptic environment

Aseptic technique make in small scale or at home or in the laboratory. The figure above showed that making aseptic environment using lighter (fig. 12-1) or in controlled mechanism (fig. 12-2) according to your industry level and access during.



Liquids from a bottle, flask, or tube with caps, never place the cap on the bench when aspirating. Instead, hold the cap in the same hand as pipette aid while manipulating the vessel containing the liquid with the opposite hand as shown.

**Repeat the sterilization before adding yeast to each container.**

- Use the same process to add yeast to each container, but remember to heat the inoculation loop to sterilize it between each transfer, and then cool it in alcohol.
- Yeast cultures grown at home have a relatively high chance of contamination, so using multiple, separately-grown cultures increases the odds that some of your cultures end up usable.

<b>Self-Check – 3</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. What are forms of sterile fill-finish operations can take for equipment and process? (4 points)
2. Describe sterile technique? (2 points)
3. Mention four of simple precautions will reduce the possibility of contamination (4 points)

**Note:** Satisfactory rating - 10 points

Unsatisfactory - below 10 points

#### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

#### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_
3. \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Instruction sheet**

This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics:

- Conducting transfer operations
- Conducting culture propagation process, products and materials
- Making scale up addition
- Monitoring control points
- Monitoring equipment
- Identifying, rectifying and reporting defected product and equipment

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Conduct transfer operations to meet propagation requirements.
- Conduct the culture propagation process and products and materials according to workplace procedures.
- Make scale-up additions according to workplace procedures.
- Monitor control points to confirm performance maintained within specification.
- Monitor equipment to confirm operating condition.
- Identify, rectify and report out-of-specification product, process and equipment performance

**Learning Instructions:**

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-checks” which are placed following all information sheets.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).
6. If you earned a satisfactory evaluation proceed to “Operation sheets
7. Perform “the Learning activity performance test” which is placed following “Operation sheets” ,
8. If your performance is satisfactory proceed to the next learning guide,
9. If your performance is unsatisfactory, see your trainer for further instructions or go back to “Operation sheets”.

## Information Sheet 1- Conducting transfer operations

The propagated yeast should multiply for use in bulk in beverage industry. It is the same as pitching process. Once the wort has been topped off and aerated, it is time to transfer the yeast at the correct fermentation temperature. The transfer operation will be free of contamination using the aseptic transfer. The technique should be consider in propagation is discussed below.

### **1.1 Principle and Purpose of Basic Culture Technique: (Aseptic Transfer)**

The use of pure cultures of microorganisms is essential to properly performing any microbiological experiment. The ability to maintain such cultures is dependent upon aseptic technique, that is, a set of practices and procedures to prevent contamination of one organism with another. Also, aseptic technique is used to prevent the introduction of a microbe to an object, person, medium, etc. In the health-care realm, aseptic technique involves applying the strictest rules to minimize the risk of infection. In the microbiology laboratory, aseptic technique requires common sense and dexterity. The latter comes from practice, practice, practice. The exercise describe below will provide trainees the opportunity to not just practice the method of aseptic technique, but to master this critically important skill.

### **1.2 Objectives of Aseptic Transfer**

1. To acquire the skill of aseptic technique in the field of Microbiology.
2. To prevent contamination of cultures and media from microbes in the environment.
3. To transfer cultures from one medium by inoculating another medium. This is called subculturing.
4. To isolate a microorganism from a mixed culture to obtain a pure culture.
5. To prevent lab microorganisms from being spread in the environment and/or infecting the investigator.

### 1.3 Procedures of transfer operations

**Add the yeast to the surface of the wort, following this step carefully.**

- Leaving the lid off for as short a time as possible, move the inoculation loop lightly over the surface of the starter wort in one of your containers.
- This transfers yeast onto the hopefully germ-free and nutrient-rich wort.
- To minimize the chance of contamination, immediately attach the lid again.
- Turn petridishes upside down, or cap starter tubes to about 3/4 tightness.
- The process of adding a micro-organism to the plate is called "streaking" by microbiologists.

<b>Self-Check – 1</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. Describe the aseptic culture transfer? (2 points)
2. Mention the objective of Aseptic Transfer? (5 points)
3. What are the steps to be follow during the transfer of yeast to the surface of the wort? (5 points)

**Note:** Satisfactory rating - 12 points

Unsatisfactory - below 12 points

#### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

#### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_
3. \_\_\_\_\_  
\_\_\_\_\_

<b>Information Sheet 2- Conducting culture propagation process, products and materials</b>
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Yeast propagation is the act of propagating; continuance or multiplication of the kind by generation or successive production; as the propagation of animals or plants. It is the act of producing offspring or multiplying by such production. Yeast propagation is producing from a single cell sufficient amount of yeast for fermenting a batch (brew or fermenter size) of wort. The yeast should be 100% uninfected. The yeast should be propagated with effective aeration in order to achieve a sufficient cell count. The preparation of a starter culture of yeast is used particularly for larger batches. The following proportions are for such a starter batch. The final must therefore consist of:

- a) a sugar and water mix, at a ratio according to previously mentioned criteria;
- b) nutrients added in the same quantities per liter as given for the starter batch below and
- c) the yeast starter batch at 2% by volume of the total must.

## **2.1 Propagation of Yeast**

Yeast is that wonderful microbe which converts sweet wort into an enjoyable alcoholic beverage. In addition to converting sugar to alcohol, yeast can also influence the taste, flavor, bouquet, and even the color of mead/beer. They do this by secreting a variety of compounds at very low levels. The propagation and maintenance of yeast at home adds yet another level of control to the brewing process, allows for experimentation, and aids in the consistent production of unique high quality meads/beers.

The development of pure yeast strains and their importance in the brewing process has been going on for over a century and is still an active area of research. In 1883, Emil Christian Hansen described the first techniques for successfully isolating single yeast cells and propagating them to a larger scale. This was a landmark finding since up until then all yeasts were a mixture containing various forms of brewing yeast, wild yeast, bacteria, and molds. Brewing with these mixtures of micro-organisms was difficult. Beer spoiling was common and there was wide variability in beer quality. Hansen's techniques changed all that and were quickly applied to improving

large scale beer production; first in the Carlsberg brewery and a few years later in American breweries. Current propagation techniques remain similar to those first described by Hansen. Further characterizations of yeast physiology and fermentation technology, however, have also influenced the current methods used to propagate and maintain yeast.

## 2.2 Propagation parameters:-

- ✓ Temperature- High temperature will give fast propagation. But also cause stronger flavour impact from the propagated yeast
- ✓ Aeration:- The more effective the aeration - the higher the cell count. Continuous is conducted by interval, Flow rate and Foam problems
- ✓ Propagation Step:-From sterile to sterile conditions 1:100 (1:200) and from sterile to woldwort conditions 1:10 (1:20).

## 2.3 Basic process of Propagation starters in the Laboratory

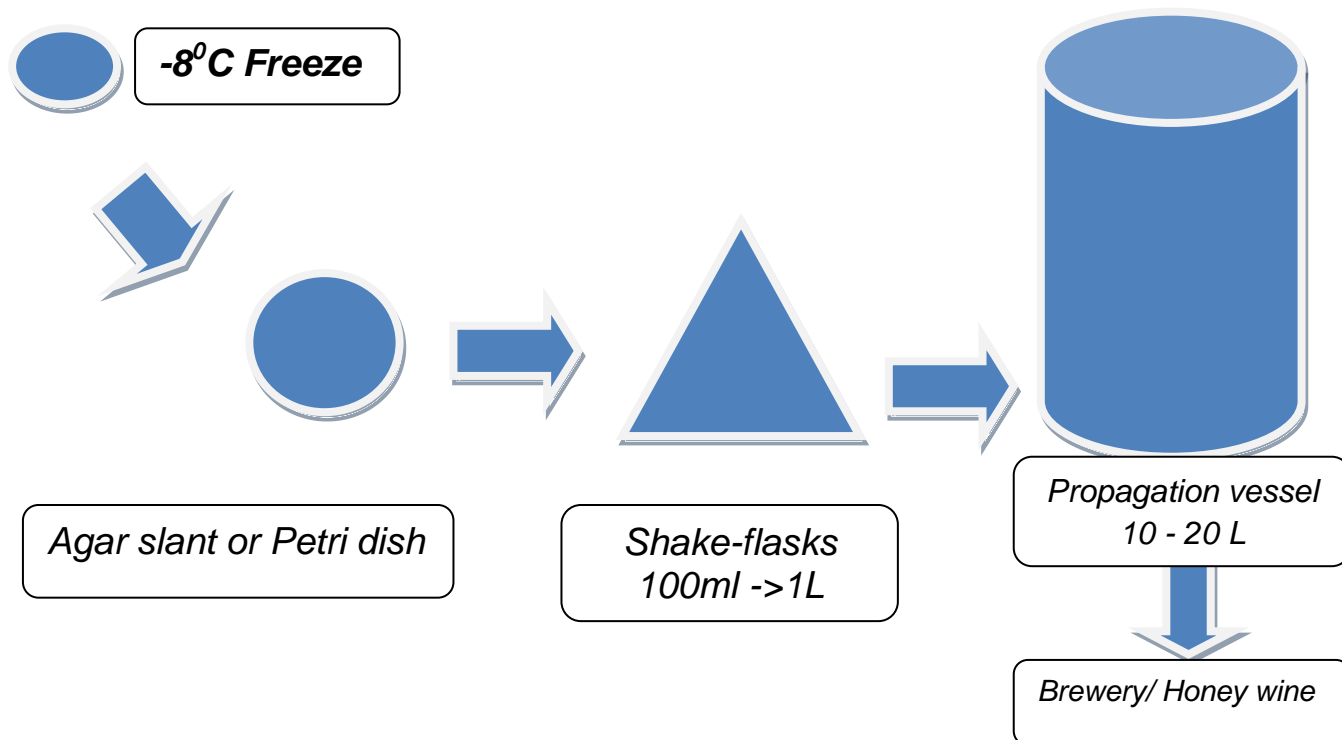


Figure 11: The flow diagram of Propagation starts in the Laboratory



## Key considerations of laboratory propagation

- Aseptic technique!
- Sterile growth media
- Do not exceed 1->10 volume increments
- Aeration (but remember Crabtree effect - may be best to use weak wort



*Figure 12:-Yeast propagation system in large industry*

<b>Self-Check – 2</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. Define the propagation of yeast (2 points)
2. Mention the three parameters of propagation (3 points)
3. Draw the flow diagram of propagation of starter in Laboratory (5 points)

**Note:** Satisfactory rating - 10 points

Unsatisfactory - below 10 points

#### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

#### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_
3. \_\_\_\_\_  
\_\_\_\_\_

### Information Sheet 3- Making scale up addition

With the ever-growing demand for foods, nutraceuticals, pharmaceuticals, fuels, and materials as well as for sustainable development of economy and environment, microbial fermentations using low-cost and renewable feed stocks have become increasingly important. Great effort has been endeavored for improving yields, titers, and productivities of aimed products through bioprocess engineering strategies since the very early beginning of the fermentation industry. Among them, optimization and scale-up method toward industrial process shows great importance.

#### 3.1 Making Scale-Up

Cambridge Dictionary defines scale-up as increasing something in size, amount, or production. Microbial processes involve cultivation of microbes in bioreactors (also referred to as fermentors) to produce a product, as well as the subsequent recovery and purification of the product and disposal of associated wastes. Scale-up of microbial processes is undertaken typically for a commercial purpose, specifically to provide product benefits to customers and to generate a financial return for investors.

Scale-up of large industrial processes is preferably done in two stages if there is a high degree of novelty in the process and/or the commercial product. The first stage is a pilot plant (pilot scale) with 100–10,000 L fermentors and matched downstream equipment.

#### 3.2 Why does Scale-up Matter?

Its purpose is to translate the lab-scale process into a realistic scaled-down version of the manufacturing process. In most cases, the process is not fully integrated; i.e. each individual unit operation is operated batch-wise. The selected pilot scale is a judgment based on the size, availability, and cost of representative scaled-down equipment and required product sample sizes.

The second stage of scale-up is a demonstration plant (demo scale) with 10 000–100 000 L fermentors and matched downstream. It serves to minimize the risk of a large capital investment in the full-scale manufacturing plant by further validating the process, the supply chain (from raw materials to commercial product application), and market demand. Laboratory-scale bioreactors typically have a working volume that varies from about 0.2 L to 20 L. Such bioreactors can be used with a high power input, resulting in rapid mixing of the fermentation broth and high mass-transfer rates.

### **3.3 Fermentation Scale-up**

Scale-up for the growth of microorganisms is usually based on maintaining a constant dissolved oxygen concentration in the liquid (broth), independent of reactor size. One key to a scale-up is to have the speed of the end (tip) of the impeller equal the velocity in both the laboratory pilot reactor and the full-scale plant reactor. If the impeller speed is too rapid, it can lyse the bacteria; if the speed is too slow, the reactor contents will not be well mixed. Typical tip speeds range from 5 to 7 m/s.

### **3.4 Yeast Propagation and Scale-up**

The objective of propagation is to produce large quantities of yeast with known characteristics for the primary role of fermentation, in as short a time as possible. Most brewers use a simple batch system of propagation, starting with a few milliliters of stock culture and scaling up until there is enough yeast to pitch a commercial brew. Scale-up introduces actively growing cells to a fresh supply of nutrients in order to produce a crop of yeast in the optimum physiological state.

#### ***Laboratory Phase***

The process initially begins in the laboratory when cultures are taken from the "working" master culture and grown in a progression of fermentations of increasing size until enough yeast is produced to transfer to the propagation plant. The number of transfer steps in the laboratory varies according to the final weight of yeast required for the propagation plant. Of course, the more transfers, the greater the risk of infection. Most yeast culturing is done in a laminar flow hood.

### ***Plant Phase***

After rigorously cleaning the yeast-propagation vessel (in the case of smaller breweries, a production fermenter) it is then filled with hot or cold wort and aerated with sterile air. Preferably the wort should be of the same quality as that used in fermentation. During propagation, temperature is maintained at a set level and the propagating yeast is intermittently aerated.

### ***Aeration***

As mentioned, air or oxygen is passed continuously into the vessel through an efficient gas sterilizer to encourage yeast growth. Oxygen is preferable since it is sterile, whereas an air supply may contain impurities that must be removed before the air enters the vessel. The optimum rate of oxygenation for a system must be found by experiment, as the rate will affect the total crop produced.

### ***Optimum Propagation Temperatures***

There is a wide variety of recommendations in this instance as well. Some brewers prefer to propagate their yeast at temperatures identical to those employed during fermentation in order to prevent temperature shock to the yeast.

### ***Propagation Plants***

The propagation plant usually consists of anywhere from one, two or more closed stainless steel vessels of increasing volume, which are usually situated in a separate room to minimize contamination of risk.

<b>Self-Check – 3</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. Define the scale-up process (2 points)
2. What are the two stages done in large industry? (2 points)
3. What is the objective of propagation? (2 points)
4. Mention and discuss the states of scale-up to produce a crop of yeast. (10 points)

**Note:** Satisfactory rating - 16 points

Unsatisfactory - below 16 points

#### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

#### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_
3. \_\_\_\_\_  
\_\_\_\_\_
4. \_\_\_\_\_  
\_\_\_\_\_

## Information Sheet 4- Monitoring control points

The main control points related to food safety (critical), quality and regulatory control points and inspection points should be monitored in the propagation process. After rigorously cleaning the yeast-propagation vessel (in the case of smaller breweries, a production fermenter) it is then filled with hot or cold wort and aerated with sterile air. Preferably the wort should be of the same quality as that used in fermentation.

### 4.1 Monitoring quality

One of the most difficult raw materials to manage in the production of beer, in terms of monitoring quality, is the yeast culture, because it consists of living organisms in which health and the ability to ferment can be affected by a multitude of factors. One must carefully store cropped yeast and then rejuvenate it in the correct physiological condition to ferment the wort. Doing so to produce the desired beer alcohol and flavor profile in the mandated time period requires a thorough knowledge of yeast physiology and an understanding of the environment to which the yeast will be exposed at various times in the process. Numerous quality tests can assist in terms of examination of viability, vitality, and culture purity. Because yeast performance is tied to such a multitude of factors in the brewing process, careful attention to detail especially in terms of documentation throughout the process is a key factor in a successful quality program.

### 4.2 Control points of Propagation process

The propagation yeast, the spent yeast, the washed spent yeast before disruption and the macerated yeast suspension were only used after passing quality control. Several factors influence both yeast growth (and fermentation) and therefore should be considered when propagating and maintaining, and yeast. The most important are oxygen, pH, temperature, and wort composition.

## ***Oxygen***

Oxygen or aeration is essential for good yeast growth and is the driving force behind many aspects of yeast metabolism including fermentation. Oxygen is quickly absorbed by yeast and is used to synthesize unsaturated fatty acids and sterols which form the cell membrane. These molecules are important for both growth and fermentation and serve as a means of storing oxygen within the cell. They are also necessary for increasing cell mass (growth), improving the overall uptake of nutrients, and determining alcohol tolerance. Oxygen also stimulates synthesis of molecules necessary for yeast to metabolize and take up maltose, the primary sugar in wort.

## ***Temperature***

Another important factor which influences yeast growth and metabolism is temperature. Temperature is somewhat neglected in terms of its role in influencing growth rate and fermentation performance. Most brewing yeasts will actually grow and ferment at temperatures up to 98 °F (37 °C). These high temperatures are not optimal for yeast propagation or fermentation, since they produce numerous esters and affect the overall viability and stability of the yeast. **86 °F (30 °C) is the usual temperature for the growth and propagation of laboratory yeast** but this is still too high for brewing yeast. Room temperature or 77°F (25 °c) is the recommended temperature for *propagating* brewing yeasts.

## ***Wort or media composition***

Wort (or media) composition also determines yeast growth and fermentation performance and is important in maintaining and storing viable, stable yeast. In terms of fermentation, standard brewing wort contains most of the ingredients necessary for fermentation. Problems arise only if the nitrogen composition is low. This occurs only if a cheap or poor quality malt extract is used or if there are a large amount of adjuncts added. In terms of propagation, the closer the starter media is to the fermentation wort the better. Wort with an original gravity of 1.040 works well for most fermentations and is recommended for use in most brewing situations. If pitching into



high gravity wort, a standard starter may get shocked from the change in osmotic pressure. In this case a higher gravity starter (O.G. =1.065) may be necessary. Lower gravity starters (O.G. = 1.020) are commonly used by homebrewers and routinely produce higher concentrations of yeast but do not perform well when pitched into normal brewing worts.

## **pH**

The last factor to affect yeast growth is pH (a measure of acidity). Yeast grow well at acidic pHs. They grow best between pH 4 to pH 6. Normal wort is acidic with a pH near 5.2. During growth and fermentation the pH drops to about 4.1-4.2 and in some cases even lower. The further acidification of the wort helps to prevent bacterial infection. (Most bacteria cannot tolerate acid pH). Yeast can survive at very low pH, as low as 2.0. This is the basis of acid washing where the bacterial load of yeast slurry is reduced prior to repitching by lowering the pH to 2.2. Most bacteria will be destroyed at this pH while a good percentage of the yeast will survive. Interestingly, diluted unfermented honey is more acidic than wort and the production of more acid during fermentation actually slows down its fermentation. To make matters even worse most mead makers add acid blend to the honey. Although the acid does help to balance out the flavor, it will inhibit fermentation and therefore should only be added after fermentation is complete. In fact some mead makers will add a small amount of calcium carbonate to buffer the acidity and raise the pH. This can significantly accelerate fermentation.

### **4.3 Yeast Culture Contamination**

It frequently happens that brewing yeasts carry a persistent low level of contaminants such as *Obesumbacteriumproteus*, acetic acid bacteria, and slow-growing *Torula*-type yeasts. These organisms are generally regarded as harmless because their numbers never reach a point where they are likely to have adverse effects on the beer. On the other hand, *L. pastorianus*, *Z. anaerobia*, and *S. carlsbergensis* are strains considered harmful at low levels.

## ***Microscopic Examination***

Microscopic examination of the yeast culture can be useful in assessing the overall health of the population. Abnormal-looking or irregularly shaped yeast cells are signs of cell stress, possibly indicating potential problems with wort composition, aeration, poor yeast handling, or fermentation conditions. Microscopic examination is also useful in detecting extraneous particles such as diatomaceous earth, trub, grain particles, etc. that may interfere with proper yeast performance.

In general monitoring may involves:

- checking that hygiene and sanitation standards,
- safety standards and pre-start requirements are met and that equipment is operational
- Checking the calibration status of measuring instrumentation

<b>Self-Check – 4</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. Why the yeast culture is one of the most difficult raw materials to manage in the production of beer in terms of monitoring quality (2 points)
2. What are factors influence both yeast growth and fermentation? (4 points)
3. Discuss the control points of each factors to be consider as a recommendation? (4 points)
4. What are the general monitoring control points in the propagation process? (3 points)

**Note:** Satisfactory rating - 5 points

Unsatisfactory - below 5 points

#### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
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3. \_\_\_\_\_  
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4. \_\_\_\_\_  
\_\_\_\_\_

## Information Sheet 5- Monitoring operating condition equipment

This is the part of completing the process of yeast propagation process. When monitoring equipments used the yeast propagation process include

- the use of production data,
- sampling,
- checking temperature,
- gravity,
- cell counts,
- viability,
- oxygen levels, and
- Visual inspection.

Among them some are monitoring not only at the end of processing but also on processing. The monitoring operation used to observe if there is defect or not on equipment operation condition. Depending on the level of defect the corrective action will be done. The correction may be taken by operator/who done inspection/ or reported to the appropriate personnel to take an action to bring the defect to the normal operation condition.

Different formats should be available by the industry manager as the requirement. The format should contain all the necessary parameters of maintenance requirements (you should be learn under learning guide of Conducting Routine Maintenance of machineries).

<b>Self-Check – 5</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. What are parameters to be considered during equipment of yeast culture propagation monitoring? (5 points)

**Note:** Satisfactory rating - 5 points

Unsatisfactory - below 5 points

### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_

<b>Information Sheet 6- Identifying, rectifying and reporting defected product and equipment</b>
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The maintaining and monitoring process of the propagation is required throughout the process to identify, rectify and/or report when there is out-of-specification on product, process and equipment performance. The way you handle your culture media from storing it before preparation, to lifting the agar plate lid, to incubation affects your results. Selective media cannot select for pathogens if the starting materials are poor quality, compromised or contaminated. There are problems caused by faulty culture media preparation which includes:

- Reduced growth/recovery rates
- Atypical colonial morphology
- Inhibition of target organism
- Failure to inhibit competing flora
- Reduced shelf life of prepared medium
- Bacteria and other microbes use a culture medium

Reporting the above faults or defects from the propagation process will protect the final product from the intended quality. The measures to be taken beyond you may take a correct action by your supervisor or other professional.

Different formats should be available by the industry manager as the requirement. The format should contain all the necessary parameters of maintenance requirements.

<b>Self-Check – 6</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. What are problems caused by faulty culture media preparation needs to be report? (6 points)
2. Why need report to your supervisor when any defects may occur? (4 points)

**Note:** Satisfactory rating - 10 points

Unsatisfactory - below 10 points

#### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

#### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_

<b>Operation Sheet 1- Propagating yeast</b>	
<b>Operation Title: Propagating yeast</b>	
Purpose	To acquire knowledge, skill and attitude by performing Propagating yeast for fermentation scale up.
Equipment, tools and materials	<p>Supplies and equipment needed or useful for sampling and testing of honeyinclude:</p> <ul style="list-style-type: none"> <li>• Propagation vessel, Emerlyser flask, starter tube, airlock and stopper, measuring cylinder/cup, aluminum foil, starter plate and cooling bath.</li> <li>• Starter culture, Nutrient rich worth, oxygen &amp; distilled water</li> </ul>
Conditions or situations for the operations	<ul style="list-style-type: none"> <li>• Services, equipment's and materials should be available</li> <li>• Germ free environment /working area for propagation in aseptic condition.</li> </ul>
Procedures	<ol style="list-style-type: none"> <li>1. Wear the appropriate personal protective equipments</li> <li>2. Prepare all equipments, ingredients and services</li> <li>3. Prepare aseptic transfer environment</li> <li>4. Take cultures from the "working" master culture</li> <li>5. Grow in a progression of fermentations</li> <li>6. Transfer cultured yeast in to propagation vessel on the surface of the wort.</li> <li>7. Pass air or oxygen continuously</li> <li>8. Store for fermentation scale up</li> <li>9. Record all the necessary workplace information</li> <li>10. Report the appropriate personnel</li> </ol>
Precautions	<ul style="list-style-type: none"> <li>• Care should be taken while making germ free condition (if used fire)</li> </ul>
Quality criteria	The propagation process should be with temp. at 77 <sup>0</sup> F (25 <sup>0</sup> C), aeration:- until you see the higher the cell count, sterile conditions 1:100 (1:200) and the original specific gravity of wort (O.G) is 1.040



<b>LAP TEST</b>	<b>Performance Test</b>
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Name..... ID.....

Date.....

Time started: \_\_\_\_\_ Time finished: \_\_\_\_\_

**Instructions:** Given necessary templates, tools and materials you are required to perform the following tasks within 2 hour. The project is expected from each student to do it.

**Project title:** Propagating Yeast

**Task-1:** Performing propagating yeast for fermentation scale-up process

**Task-2:** Record and report for appropriate personnel

<b>LG #40</b>	<b>LO #4- Complete propagation activities</b>
<b>Instruction sheet</b>	
<p>This learning guide is developed to provide you the necessary information regarding the following <b>content coverage</b> and topics:</p> <ul style="list-style-type: none"> <li>• Completing the propagation process</li> <li>• Dismantling and preparing equipment</li> <li>• Collecting, treating and disposing wastes</li> <li>• Guidelines to avoid work hazards</li> <li>• Conducting work as workplace environmental guidelines</li> </ul> <p>This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:</p> <ul style="list-style-type: none"> <li>• Complete the propagation process according to workplace procedures.</li> <li>• Dismantle and prepare equipment for cleaning.</li> <li>• Collect, treat and dispose of, or recycle waste generated by both the process and cleaning procedures according to workplace procedures.</li> <li>• Conduct work in accordance with workplace environmental guidelines to avoid work hazards</li> </ul>	
<b>Learning Instructions:</b>	
<ol style="list-style-type: none"> <li>1. Read the specific objectives of this Learning Guide.</li> <li>2. Follow the instructions described below.</li> <li>3. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.</li> <li>4. Accomplish the “Self-checks” which are placed following all information sheets.</li> <li>5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).</li> <li>6. If you earned a satisfactory evaluation proceed to “Operation sheets</li> <li>7. Perform “the Learning activity performance test” which is placed following “Operation sheets” ,</li> <li>8. If your performance is satisfactory proceed to the next learning guide,</li> <li>9. If your performance is unsatisfactory, see your trainer for further instructions or go back to “Operation sheets”.</li> </ol>	

## Information Sheet 1- Completing the propagation process

After the propagation process the final product should be store in appropriate condition. Because cultured and propagated yeast is prepared not for one cycle but for more products. Good storage is key. Don't put your dehydrated medium packs on any old shelf in the lab; pay attention to the surrounding environment. Investing in time and staff for culture media preparation pays off only if you pay attention to storing the finished plates and broths. Storing in the right conditions maintains product quality to ensure optimal growth and culture conditions for microbial isolation. Correct storage can also extend shelf life and reduce waste.

### 4.1 Yeast Storage

**Move successful cultures to the fridge.**

- Now that the successful cultures have been activated, wrap the containers completely in electrical tape or another light-blocking material, since light can destroy or damage yeast colonies.
- Store these in the fridge, ideally at 34–36°F (1–2°F) or slightly warmer, to slow their growth and prevent them running out of nutrients.
- When you wish to use one in a brew, remove it from the fridge in advance to bring it up to room temperature before adding (pitching) into the wort.

In most breweries, yeast is stored during the period between cropping and re-pitching. Pitching yeast may be stored within the brewery as slurry in a yeast collection vessel, or as slurry stored under a layer of water or beer, or as pressed cake.

#### ***Yeast Collection Vessels***

Most modern breweries store their yeast in sophisticated collection vessels under filtered sterile air or inert sterile gas pressure with external cooling and equipped with low shear stirring devices.

## ***Slurried Storage Systems***

Slurried storage systems are usually self-contained, thereby reducing the risks of contamination. Slurry yeast has the advantage over pressed yeast in that it gives more vigorous fermentations, requires lower pitching rates, and can be stored for longer periods (usually fewer than 4 weeks) without affecting viability.

## ***Pressed Cake***

Alternatively, yeast can be stored as pressed cake. The yeast recovered is stored at 0°C before re-suspension in wort for pitching.

## **4.2 Methods of Yeast Maintenance**

Maintaining and storing your own yeast stocks is both convenient and cost-effective. Three major things must be considered when choosing a method of yeast storage. These are yeast strain purity, viability and genetic stability. Each of these differs depending on the method of preservation. The one most suitable for homebrewers is somewhat controversial. Each method has its own advantages and disadvantages and depends on personal preference as well as access to specialized equipment.

**Table 1:** Methods for yeast storage

<b>Method</b>	<b>Shelf-life (years)</b>	<b>Advantages /Disadvantages</b>
Liquid Media	0.5	Convenient but low viability and stability, questionable purity
Agar Plate/slopes	0.2-1	Pure cultures but unreliable shelf-lives.
Agar Slant	1-2	Easy, reliable, but moderate shelf-life
Agar Stab	2-4	Easy, reliable, good shelf-life, but messy.
Dried	3-6	Inconvenient, requires purification
Frozen	>5	Need special freezer or liquid nitrogen

Homebrewers are faced with a variety of options on maintaining their yeast (summarized in Table above). The method of choice depends solely on the needs of the individual and their equipment. We are fortunate that there is an ever increasing number of inexpensive commercial sources of yeast so long-term storage by the homebrewer is not the necessity it once was. No matter what source of yeast or how it is stored, further propagation along with adequate aeration and fermentation at the correct temperature is sure to improve the quality of the beers you make at home.

<b>Self-Check – 1</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. What is the reason of using yeast storage? (2 points)
2. What is the purpose of the use of the correct storage? (2 points)
3. Mention the three options used to store the yeast within the brewery as slurry (3 points)
4. What are parameters to be consider when selecting the appropriate method of yeast storage? (3 points)
5. What are methods used for yeast storage? (5 points)

**Note:** Satisfactory rating -15 points

Unsatisfactory - below 15 points

### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

### Short Answer Questions

1. \_\_\_\_\_
2. \_\_\_\_\_
3. \_\_\_\_\_
4. \_\_\_\_\_
5. \_\_\_\_\_

## Information Sheet 2- Dismantling and preparing equipment

Make sure it is in working condition and your employees know how to run it. All equipment should be thoroughly cleaned and sanitized.

### 2.1 Check list of common equipment in wine/mead industry:

- **Equipment such as fork lift, shovels, pitchforks, paddles, plastic 5 gallon buckets, and other items** used for unloading should be washed and sanitized with chlorine compounds and hot water.
- **Crusher/stemmer:** Check to see if electrical and mechanical parts are in working condition. If it needs repair, fix it before the crush begins. If metal parts are exposed, apply a coat of paint for food processing equipment.
- **Press:** Regardless of what kind or type of press you have, your first job is to clean and sanitize it. Be sure it is in working order. Have some spare parts in stock that are not too expensive which you think you may need. It is best to have a factory representative check it for you. This may cost you some, but will save you money in the long run because you cannot afford a press breakdown during crush. Anticipating problems and planning ahead is the key here.
- **Must pump, must chiller, transfer pumps, etc: All pumps** should be cleaned and repaired if necessary- especially checks seals. Any part of the pump that comes in contact with Must should be made of stainless steel. If it is made of any metal, be sure to paint it. Must lines and hose should be thoroughly cleaned and sanitized. Check for leaks or damage and have some extra hose and clamps on hand. If you are using a must chiller, make sure it is working properly. It is best to have it checked by someone who knows refrigeration.
- **Cooperage and containers:** Receiving and crushing bins, hold tanks, steel tanks, wooden barrels, and other storage containers must be cleaned and sanitized. Check valves and tank fittings and make all necessary repairs.
- **Cleaning equipment:** Power washers should be in working order. Have some high pressure hose and a spare nozzle on hand. Replace worn-out brushes and brooms. Plenty of hot and cold water is a must.

- **Miscellaneous:** There are other items that you may need and should have on hand. Such items are tools, food grade grease and grease gun, electrical tape, machine oil or lubricant, hydraulic oil, fuses, flashlight and bulbs, first aid kits (particularly to help with bee stings), safety glasses, masks, gloves, boots, a couple of 5 gallon sprayers, drain cleaners, etc.

You may add other items to this list based on your work. Have a technical person check out your refrigeration system and equipment if you have jacketed tanks for cold fermentations and/or a room for cold stabilization.



<b>Self-Check – 2</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. Mention equipment needs to prepare in wine/mead processing industry (5 points)

**Note:** Satisfactory rating - 5 points

Unsatisfactory - below 5 points

#### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

#### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_

### Information Sheet 3- Collecting, treating and disposing wastes

Wastes from the propagation process is collecting, disposing and recycling /treating/ according to alcoholic beverage guideline and the industry capacity. Recycling after treating is the main management in yeast propagation processes in beverage industry. In mead or brewing yeasts carry a persistent low level of contaminants such as *Obesumbacterium proteus*, acetic acid bacteria, and slow-growing *Torula*-type yeasts. These organisms are generally regarded as harmless because their numbers never reach a point where they are likely to have adverse effects on the beer. On the other hand, *L. pastorianus*, *Z. anaerobia*, and *S. carlsbergensis* strains considered harmful at low levels.

## 3.1 Waste Disposal

Waste from the food and beverage industry may be disposed or treat or recycle after collection. Disposal waste from food and beverage industry measured by the following parameters:-

- Are the non-edible by-products and other refuse removed as quickly as possible from rooms where food is present so as to avoid their accumulation?
- Are the non-edible by-products and other refuse deposited in closable containers or any other appropriate foot operable container to prevent contamination?
- Are the containers made of an appropriate construction, kept in sound condition, easy to clean and, where necessary, to disinfect?
- Is there adequate provision made for the storage and disposal of waste, non-edible by-products and other refuse?
- Are the refuse stores are designed and managed in such a way as to enable them to keep clean and, where necessary, free of animals and pests?
- Is all waste eliminated in a hygienic and environmentally friendly way in accordance with state pollution control board's consent and does not constitute a direct or indirect source of contamination?

### 3.2 Waste management:

Removal of waste from the facility is required to maintain an environment that does not offer the potential of adulteration.

- Payment of accounts must be kept current to maintain this utility.
- Personnel throughout the facility will, per department supervision, be responsible for placing non-recyclable waste in appropriate receptacles. Those receptacles will be moved in timely manner to collection points.
- A timely pick up schedule will be determined with the third party supplier of this utility.
- Audits of the plant for waste and sources of waste will be performed regularly
- These actions must be documented.

<b>Self-Check – 3</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. What type of contaminants which carry a persistent low level in mead or brewing yeasts (3 points)
2. Mention strains considered harmful at low levels (3 points)
3. Discuss at least two of parameters measured among disposal waste from food and beverage industry (4 points)

**Note:** Satisfactory rating - 10 points

Unsatisfactory - below 10 points

### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_
3. \_\_\_\_\_  
\_\_\_\_\_

## Information Sheet 4- Guidelines to avoid work hazards

The propagation work exposed with the workplace environment like physical, biological, chemical, dangerous or hazardous substances. Protect the quality and safety of the final product depending on the control measure of such problems occurred from the process, the product or from the equipments.

### Workplace Hazards

#### 1) Safety hazards:

Safety hazards are unsafe working conditions that that can cause injury, illness, and death. Safety hazards are the most common workplace risks. They include:

- Anything that can cause spills or trips such as cords running across the floor or ice
- Anything that can cause falls such as working from heights, including ladders, scaffolds, roofs, or any elevated work area.
- Unguarded and moving machinery parts that a worker can accidentally touch.
- Electrical hazards like frayed cords, missing ground pins, and improper wiring
- Confined spaces.

#### 2) Biological hazards

Biological hazards include exposure to harm or disease from working with animals, people, or infectious plant materials. Workplaces with these kinds of safety hazards include, but are not limited to, work in schools, day care facilities, colleges and universities, hospitals, laboratories, emergency response, nursing homes, or various outdoor occupations. Types of things you may be exposed to include:

- Blood and other body fluids
- Fungi/mold
- Bacteria and viruses

- Plants
- Insect bites
- Animal and bird droppings

### **3) Physical hazards**

Physical hazards can be any factors within the environment that can harm the body without necessarily touching it. This include:-

- Radiation: including ionizing and non-ionizing (EMF's, microwaves, radio waves, etc.) materials
- High exposure to sunlight/ultraviolet rays
- Temperature extremes – hot and cold
- Constant loud noise

### **4) Chemical hazards:**

Chemical hazards are present when a worker is exposed to any chemical preparation in the workplace in any form (solid, liquid or gas). Some are safer than others, but to some workers who are more sensitive to chemicals, even common solutions can cause illness, skin irritation, or breathing problems.

Beware of:

- Liquids like cleaning products, paints, acids, solvents – particularly if chemicals are in an unlabeled container
- Vapors and fumes that come from welding or exposure to solvents
- Gases like acetylene, propane, carbon monoxide and helium
- Flammable materials like gasoline, solvents, and explosive chemicals
- Pesticides

### **5) Ergonomic hazards**

Ergonomic safety hazards occur when the type of work, body positions, and working conditions put a strain on your body. They are the hardest to spot since you don't always immediately notice the strain on your body or the harm that these hazards

pose. Short-term exposure may result in “sore muscles” the next day or in the days following the strain, but extended exposure can result in serious long-term issues. This includes:

- Improperly adjusted workstations and chairs
- Frequent lifting
- Poor posture
- Awkward movements, especially if they are repetitive
- Having to use too much force, especially if you have to do it frequently
- Excessive vibration

<b>Self-Check – 4</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. What does workplace hazard mean? (1 points)
2. Define biological, physical, chemical and Ergonomical hazards? (4 points)
3. Mention type of hazards under the four workplace hazards? (10 points)

**Note:** Satisfactory rating - 15 points

Unsatisfactory - below 15 points

### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

### Short Answer Questions

1. \_\_\_\_\_  
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2. \_\_\_\_\_  
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3. \_\_\_\_\_  
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<b>Information Sheet 5- Conducting work as workplace environmental guidelines</b>
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The work of yeast culture preparation and propagation is under the beverage /wine/mead production processing. The impact of environment of yeast preparation is similar with wine processing. The SOPs had to incorporate the modernized GMPs is similar. Environment consideration is the main and mandatory in food and beverage processing. As we discussed under waste management and hazard control, work should be conduct based on the environment guidelines to avoid workplace hazards it may be biological, physical or chemical.

Wastes from product, processes and equipments have a contribution for environmental pollution. Knowing the guide line of environment of each process can minimize the problem related to the environment. Environmental pollution is a series problem in the world.

<b>Self-Check – 5</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. What does mean environmental guideline? (2 points)
2. Discuss the consideration of environmental guideline in food and beverage industry? (3 points)

**Note:** Satisfactory rating - 5 points

Unsatisfactory - below 5 points

### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
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\_\_\_\_\_  
\_\_\_\_\_

**Instruction sheet**

This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics:

- Recording workplace information

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Record workplace information in the appropriate format
- Record information a well manner

**Learning Instructions:**

10. Read the specific objectives of this Learning Guide.
11. Follow the instructions described below.
12. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
13. Accomplish the “Self-checks” which are placed following all information sheets.
14. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).
15. If you earned a satisfactory evaluation proceed to “Operation sheets
16. Perform “the Learning activity performance test” which is placed following “Operation sheets” ,
17. If your performance is satisfactory proceed to the next learning guide,
18. If your performance is unsatisfactory, see your trainer for further instructions or go back to “Operation sheets”.

## Information Sheet 1- Recording workplace information

### 1.1 Introduction

Workplace information is recording throughout the process in food and beverage industry. This work may protect from any mistake of product selection and its amount, the procedure of the process, the monitor of the required parameter of the product, etc. If any defect is happen from the process, we can cross check with the record and can take the appropriate correction action.

#### Recording workplace information like:-

- Standard Operating Procedures (SOPs)
- specifications
- production schedules and instructions
- routine maintenance schedules
- work notes
- Material Safety Data Sheets (MSDS)
- manufacturer instructions
- Verbal direction from manager, supervisor or senior operator



Record keeping is essential, especially to comply with food safety and quality requirements. The formats should develop for keeping records. The food safety plan of the enterprises will specify what records need to keep.

The following are some records in prepare and monitor yeast cultures with respect to yeast propagation production processes might keep:

- cleaning and sanitizing schedule for the extracting room
- cleaning and sanitizing schedule for each piece of equipment
- register of date/time of checks of containers for cleanliness and condition
- approved Suppliers list
- batch numbers
- number of supers and their identification numbers
- number of containers filled for each type of yeast culture and propagated yeast
- samples collected, including identification numbers to link them to batches of products and suppliers or the required test of yeast culture and propagated yeast at storage
- temperature, pH, sugar content and other parameters' test during the process
- Encountered problems in quality and safety
- Take measurements

<b>Self-Check – 1</b>	<b>Written test</b>
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Name..... ID..... Date.....

**Directions:** Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

### Test I: Short Answer Questions

1. Mention workplace information need to be record? (8 points)
2. What are records to be keep during monitor with respect to yeast propagation production processes might keep? (at least 5 of them) (5 points)

**Note:** Satisfactory rating - 13 points

Unsatisfactory - below 13 points

#### Answer Sheet

Score = \_\_\_\_\_

Rating: \_\_\_\_\_

Name: \_\_\_\_\_

Date: \_\_\_\_\_

#### Short Answer Questions

1. \_\_\_\_\_  
\_\_\_\_\_
2. \_\_\_\_\_  
\_\_\_\_\_

## List of Reference Materials

1. Chester R. Cooper, Jr. 2020, Microbiology Laboratory (BIOL 3702L), Page 1 of 11
2. Erin R. Sanders, 2012, Aseptic Laboratory Techniques: Volume Transfers with Serological Pipettes and Micropipettors, J Vis Exp. 2012; (63); 2754
3. Gibson, B. R., S. J. Lawrence, J. P. Leclaire, C. D. Powell, and K. A. Smart. Yeast responses to stresses associated with industrial brewery handling. FEMS Microbiology Reviews 31 (2007): 535–69.
4. Inteaz Ali, 2004, Food Quality Assurance, Principles and Practices, Boca Raton London New York Washington, D.C.
5. Jianye Xia, Guan Wang, Jihan Lin, Yonghong Wang, Ju Chu, Yingping Zhuang and Siliang Zhang, 2015, Advances and Practices of Bioprocess Scale-up, AdvBiochemEngBiotechnol DOI 10.1007/10\_2014\_293.
6. MaltogeSalcong, 2014, Yeast Propagation and Maintenance: Principles and Practices, California Homebrew Club of the 2014.
7. Neal r. Baughman, 1999, craft brewery HACCP: prerequisite programs based on good manufacturing practices developed for boulevard brewing company, Kansas City, mo., a report submitted in partial fulfillment of the requirements for the degree, B.S., Kansas State University.
8. Russell, 2016, Brewing materials and processes a practical approach to beer Excellence, pages 77-96, Elsevier Inc.
9. Sherman F (2002) Getting started with yeast. Meth Enzymol 350: 3-41. Werner-Washburne M, Braun E, Johnston GC & Singer RA (1993) Stationary phase in the yeast *Saccharomyces cerevisiae*. Microbiol Rev 57: 383-401.

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**The trainers who developed the learning guide**

<b>No</b>	<b>Name</b>	<b>Level</b>	<b>Educational background</b>	<b>Region</b>	<b>E-mail</b>
1.	Alemayehu Tolera Gemed	A	Animal production	Oromia	toleraalex@gmail.com
2.	Ayele Mengesha Mosisa	A	Animal nutrition	Oromia	ayelemengesha@ymail.com
3.	Kebebush Tessema Beyene	A	Food science and post-harvest technology	Sidama	Kebebush04@gmail.com