UNIVERSITY OF GONDAR
COLLEGE OF VETERINARY MEDICINE AND ANIMAL SCIENCES
DEPARTMENT OF PUBLIC HEALTH AND EPIDEMIOLOGY

1. Course Information

Course Title	Veterinary Public health -II	
Course Number (Code)	VETM -4271	
Credit Hours	3 (5ECTS)	(1cr laboratory practice)

Instructor (s) for Laboratory practice course

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Course Description

Practical: Determination of milk composition and quality control of milk; detection of adulteration and preservatives in milk and milk products. Collection of milk samples for chemical and bacteriological examination, grading of milk; test for pasteurization and plant sanitation; microbial examination of raw and pasteurized milk, milk products and water for processing plant; isolation and identification of organisms of public health importance..

Student Learning Objectives/Outcomes

Course objectives

At the end of the course the students should:

- To introduce students with the basic principles of veterinary public health and the activities of public health veterinarian practical oriented course ;
- To acquaint students with composition, microbiology, milk processing and quality control of milk and milk products
- To familiarize students with the major activities in laboratory processing of milk and milk products
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Required Textbooks and Materials

Required Texts

LAB SESSION ONE

1. Collection of Milk Sample for Biochemical and Bacteriological Examination

General Precautions

Milk samples may become contaminated with bacteria from the hands of the sample collector, the environment, and the teat skin or teat canal. It is important that proper sample collection techniques are used in order to avoid contamination of the milk sample.

The laboratory examination of milk is one of the most valuable and necessary aids to the overall milk quality control program. Without this service the safety and sanitary quality of milk is difficult to determine

For certain analyses, milk samples can be preserved and stored. Samples of milk or cream for butterfat analysis can be preserved using formalin or potassium dichromate. Milk processors usually pay for milk or cream on the basis of butterfat analysis, and a single butterfat test may be used to determine the butterfat content of thousands of litters of milk or cream. Therefore, an accurate and representative sample must be obtained.

Milk must be mixed thoroughly before sampling and analysis to ensure a representative sample

Label the sample tube with a permanent marker before sample collection as milk fat will cause the ink to smear

Make sure samples are cold or frozen until they are delivered to the lab to avoid excessive growth of bacteria, which can lead to misleading results

If the milk is in bulk containers it must be mixed with a sterile plunger before a sample is collected from well below the surface with a sterile dipper and poured into a sterile stopper or screw cupped bottle with a capacity of about 125ml

Here are steps to aseptically collecting milk samples:

1. Wash your hands and put on new disposable gloves.
2. Using a permanent marker, label a new sample tube with the date, cow ID, and the quarter that the milk will be collected from (RF for right front, LF for left front, RR for right rear, LR for left rear). Keep the sample tube closed until the sample will be collected.
3. Make sure that the udder and teats are clean and dry. Pre-dip the teats with an effective germicidal teat dip and leave the dip on for 30 seconds.
4. Wipe each teat dry with a single-use paper or cloth towel, making sure there is no teat dip left behind on the teat, as it will kill the bacteria in your milk sample.
5. Discard 3 to 4 streams of milk to minimize risk of contamination of the sample with bacteria in the teat canal.
6. Scrub teat ends with a cotton ball or gauze pad soaked in alcohol. Scrub until the cotton ball or gauze pad comes away clean. If sampling more than one quarter of the same cow, scrub far teats before scrubbing near teats. Use a new cotton ball or gauze pad for each teat. Teats should not be dripping with alcohol, as the alcohol will kill the bacteria in your milk sample.
7. Open the sample tube immediately before the sample is taken. Do not let your hands or the teat end come into contact with the inside of the tube, including the lid. Collect milk until the sample tube is ⅓ to ½ full, holding the tube at an angle to prevent loose dirt or hair from falling into it. Immediately close the tube once filled.
8. Immediately put the sample tube in the refrigerator or on ice. Samples that will not be plated within 24 hours should be frozen. It is best to freeze samples before shipping to the lab.

LAB SESSION TWO

2. Milk quality tests

A) Rapid platform tests

i. Organoleptic Test

During organoleptic examination Milk is examined for its appearance or colour, consistency and flavour (taste and smell)

- **Appearance or colour**
  Observe and evaluate the colour of milk. The normal colour of milk is whitish yellow.

- **Consistency: it** determining the viscosity and sticking ability of milk
  Determining the viscosity of milk
  Place drop of milk on one side of a glass slide; hold this perpendicularly and determine the rate of flow of the drop of milk;. Fresh milk flows fast, while spoiled or sour milk flows very slowly.
  Determine the sticking ability of milk
  Moisten a piece of paper with milk. Stick this on a flat surface. Good quality milk sticks.

- **Smell:** Bring the milk sample close to your nose and notice the smell.

- **Taste**: Take a teaspoonful of milk and determine its taste. Normal taste is sweet due to the presence of lactose. Abnormal taste: Sour, salty or bitter.

ii. PH determination

Milk **pH** is measured by using indicator paper and with **pH** meter.

- **indicator paper**

Method
immerse indictor paper into milk and read the **pH** by compare the color. the normal PH of milk

lies between 6.3—6.75. Value below 6. 3 suggests Microbial contamination, Presence disinfectants in milk, Excessive lactic acid production and Value above *6.75* indicates mastitis

- **pH meter**

Determination of pH depends on the potential difference set up between two electrodes when they are in contact with a test sample. A reference electrode whose potential is independent of the pH of the solution and an electrode whose potential is proportional to the hydrogen ion concentration of the test sample are used. Saturated calomel electrodes are usually used as reference electrodes, and glass electrodes are used to measure pH. Combined glass and calomel electrodes are also available.

Instruments which measure the current produced by the difference in potential between the glass and calomel electrodes are called pH meters.

Preparation of the pH meter

1. Read the pH meter instruction manual carefully.
2. The pH meter should be kept in a clean, dry atmosphere.
3. Before using a new glass electrode, or a glass electrode which has been stored for some time, soak it in N/10 HCl for about five hours.
4. Care should be taken not to scratch glass electrodes against the sides of beakers or other hard surfaces during storage or testing.
5. Check the level of saturated potassium chloride in the calomel electrode before making pH measurements. Crystals of potassium chloride should be present in the solution within the electrode.
6. Remove the rubber stopper or cap on the filling arm of the calomel electrode before making a test.

iii. **Clot on boiling test (COB)**

allows you to check whether the milk has high acidity (pH <5.8).

Principle:
Fresh milk can be boiled (pasteurised) without being precipitated. However, when the casein is dissociated, the milk precipitates on heating. Acidity decreases the heat stability of milk. The clot-on-boiling test is used to determine whether milk is suitable for processing, as it indicates whether milk is likely to coagulate during processing (usually pasteurization). It is performed when milk is brought to the processing plant—if the milk fails the test it is rejected.

The test measures the same characteristics as the alcohol test but is somewhat more lenient (0.22 to 0.24% acidity, as opposed to 0.21% for the alcohol test). It has the advantage that no chemicals are needed. However, its disadvantage is that at high altitude milk (like all liquids) boils at a lower temperature and therefore the test is even more lenient.

Materials : One boiling water bath,Test tubes and Timer (a watch or clock is adequate) Milk sample Test tube holder

Procedure

1. Place about 5 ml of milk in a test tube (the exact amount is not critical) and place the test tube in boiling water for 5 minutes.

2. Carefully remove the test tube and examine for precipitate. The milk is rejected if any curd forms.

iv. Alcohol test

The alcohol test, together with the acidity test, is used on fresh milk to indicate whether it will coagulate on processing. Milk that contains more than 0.21% acid, or calcium and magnesium compounds in greater than normal amounts, will coagulate when alcohol is added.

Principle
Casein (milk protein) is found in milk as calcium hydrogen cascinate. When lactic acid develops, thc calcium is removed from hydrogen cascinatc (colloid form) is changed to acidic cascin (precipitate

Material: Milk sample, test tubes, pipettes test-tube racks or blocks of wood with holes bored to hold the test tubes.

Reagents: 68-75% alcohol solution. This is usually prepared from 95% alcohol by mixing with distilled water in the proportion of 79 parts of 95% alcohol to 21 parts of distilled water.

Procedure

1. Put equal volumes of milk and 75% alcohol in a test tube.
2. Invert the test tube several times with the thumb held tightly over the open end of the tube.
3. Examine the tube to determine whether the milk has coagulated. If it has, fine particles of curd will be visible.

LAB SESSION THRE and FOUR

B) Additional milk quality tests

i. Determination of milk specific gravity

Specific gravity is the relation between the mass of a given volume of any substance and that of an equal volume of water at the same temperature. Since 1 ml of water at 4°C weighs 1 g, the mass of any material expressed in g/ml and its specific gravity (both at 4°C) will have the same numerical value. The specific gravity of milk averages 1.032, i.e. 1 ml of milk weighs 1.032 g at 4°C.

Since the mass of a given volume of water at a given temperature is known, the volume of a given mass, or the mass of a given volume of milk, cream, skim milk etc can be calculated from its specific gravity.

Example one litre of water at 4°C has a mass of 1 kg, and since the average specific gravity of milk is 1.032, one litre of average milk will have a mass of 1.032 kg.

Apparatus

• Lactometer this is a hydrometer (a device for measuring specific gravity) adapted to the normal range of the specific gravity of milk. It is usually calibrated to read in lactometer degrees (L) rather than specific gravity per se.

The relationship between the two is:

L 1000 + 1 = specific gravity (sp. gr.) Thus, if L = 31, specific gravity = 1.031

- A tall, wide glass or plastic cylinder
- A thermometer (the lactometer may have a thermometer incorporated).

Procedure

1. Heat the sample of milk to 40°C and hold for 5 minutes. This is to get all the fat into a liquid state since crystalline fat has a very different density to liquid fat, and fat crystallises or melts slowly. After 5 minutes, cool the milk to 20°C.
2. Mix the milk sample thoroughly but gently. Do not shake vigorously or air bubbles will be incorporated and will affect the result.
3. Place the milk in the cylinder. Fill sufficiently so that the milk will overflow when the lactometer is inserted.
4. Holding the lactometer by the tip, lower it gently into the milk. Do not let go until it is almost at rest.
5. Allow the lactometer to float freely until it is at rest. Read the lactometer at the top of the meniscus. Immediately, read the temperature of the milk; this should be 20°C. If the temperature of the milk is between 17 and 24°C, the following correction factors are used to determine L:

Temp°C	17	18	19	20	21	22	23	24
Correction	-0.7	-0.5	-0.3	0	+0.3	+0.5	+8	+1.1

For example if the lactometer reading is 30.5 and the temperature is 23°C:
Corrected lactometer = Lc = 30.5 + 0.8 = 31.3

Calculations

Calculations always use Lc, the corrected lactometer reading. To calculate the specific gravity, divide the corrected lactometer reading by 1000 and add 1.
In our example: Sp. gr. = 31.3/1000+1=1.0313

Lactometer method

The total solids content of milk is the total amount of material dispersed in the aqueous phase, i.e. SNF = TS– % fat.

The only accurate way to determine TS is by evaporating the water from an accurately weighed sample. However, TS can be estimated from the corrected lactometer reading. The results are not likely to be very accurate because specific gravity is due to water, material less dense than water (fat) and material more denser than water (SNF). Therefore, milk with high fat and SNF contents could have the same specific gravity as milk with low fat and low SNF contents.

TS =Lc/4 + (1.22 x fat %) + 0.72

SNF = TS – fat %

or =Lc/4 + (0.22 x fat %) + 0.72

It should be noted that the relationship between Lc and TS varies from country to country depending on milk composition. The above formulae are called the Richmond formulae and were calculated for Great Brita

ii. Fat determination

The main tests used to determine the fat content of milk and milk products are the Gerber (Europe) and Babcock (USA) tests. Automated methods for testing milk are now used in central laboratories and at large processing centers. Fat in milk exists in the form of an emulsion which is stabilised by phospholipids and proteins. The theory of the Gerber method is based on the fact that the fat globules are de-emulsified by the addition of concentrated sulphuric acid (H2SO4). The free fat, with a lower density than the surrounding medium, may be separated rapidly by centrifugal force. Amyl alcohol addition gives a clearer dividing line on the butyrometer scale between the fat layer and the other material. While the Rose Gottlieb ether extraction method is the recognised standard procedure for the determination of fat in milk and dairy products, the Gerber method gives results which are in agreement with those of the Rose Gottlieb.

The procedures outlined below are used to determine the butterfat content of milk, skim milk, buttermilk, cream and whey.

Materials:

- ✓ Gerber butyrometer calibrated to read 0–8% or 0–5% and graduated at 0.1% intervals
- ✓ Butyrometer stoppers
- ✓ Milk pipette volume to match the butyrometer in use
- ✓ 10 ml double-bulb pipette* for pipetting sulphuric acid
- ✓ 1 ml bulb pipette* for pipetting amyl alcohol
- ✓ Thermometer to read 1–100°C
- ✓ Water bath
- ✓ Gerber centrifuge
- ✓ Alternatively, automatic dispenser scan be used for delivering 10 ml of sulphuric acid and1 ml of amyl alcohol.
- ✓ Milk samples

Reagents

- ✓ Sulphuric acid (density of 1.815 g/ml ± 0.002)
- ✓ Amyl alcohol (density between 0.810 and 0.812 g/ml).

Procedure

1. Mix the milk sample (temperature about 20°C) thoroughly, taking care to minimize incorporation of air. Allow the sample to stand for a few minutes to discharge any air bubbles. Mix gently again before pipetting.
2. Pipette or dispense 10 ml of sulphuric acid into the butyrometer.
3. Pipette the required volume of milk into the butyrometer. Care must be taken to avoid charring the milk, by ensuring that the milk flows gently down the inside of the butyrometer.
4. Pipette or dispense 1 ml of amyl alcohol.
5. Clean the neck of the butyrometer with tissue or dry cloth.
6. Stopper the butyrometer tightly using a clean, dry stopper. Shake and invert the butyrometer several times until all the milk has been absorbed by the acid.
7. Place the butyrometer in a water bath at 65°C for 5 minutes.
8. Centrifuge for 4 minutes at 1100 rpm.
9. Return the butyrometer to the water bath for 5 minutes. Ensure that the water level is high enough to heat the fat column.
10. Read the fat percentage by bringing the graduation mark to eye level If necessary, the fat column can be adjusted by regulating the position of the stopper.

Hazards

Sulphuric acid is toxic, highly corrosive and will also cause severe burning if it comes in contact with the skin or eyes. • When mixing the butyrometer contents, considerable heat is generated. • If the stopper is slightly loose, leakage may occur during mixing, centrifuging or holding in the water bath.

Precautions

- ✓ Wear protective eye goggles. • Avoid all spillage and drops of sulphuric acid from acid dispensers.
- ✓ Transferring milk to the butyrometer; (b) reading the fat result from the butyrometer.

- ✓ When mixing, hold the butyrometer stopper firmly to ensure that it cannot slip. Use a cloth or glove to protect the hands when mixing. • Do not point the butyrometer at anyone when mixing..
- ✓ Causes of unsatisfactory tests with the Gerber method
- ✓ Incomplete separation of the fat and aqueous phases may be caused by:
- ✓ Impure or incorrect amounts of amyl alcohol
- ✓ Below strength sulphuric acid
- ✓ Using acid or milk at low temperatures.
- ✓ Charred material in the fat column may be caused by:
- ✓ Too strong or too much sulphuric acid
- ✓ Using acid or milk at high temperatures
- ✓ Using dirty or burnt rubber stoppers.

Other factors affecting the accuracy of the Gerber test include incorrect centrifuge speed and incorrect temperature of reading the test.

LAB SESSION FIVE

4. Determination of Bacterial Lodes of the milk

a. Methods for quantification by Direct test

Standard plate count (SPC): The plate count or pour plate method determine the degree of bacterial contamination on surfaces of equipment, tools, and premises as well as in milk, meat and meat products It is often used for estimating the number of viable number of micro-organisms in liquid, reconstituted or suspended the sample. Bacteria can often only be counted after diluting the liquid. A number of 10-fold serial dilutions of the sample are prepared.

Materials

Sterile scalpel, balance, aluminium foil, forceps, scissors, flasks, Pipette, test tubes, stomacher, incubator, sterile plastic bags, physiological salt solution, incubator, buffered peptone water, meat sample or Milk sample

Procedure

1. Meat sample (10 grams meat + 90 ml sterile saline water or 0.1% peptone water). Homogenize in stomacher. First dilution. Or milk sample(1ml + 9ml sterile saline water)
2. Transfer 1 ml from first dilution (10^1) to second test tube (Test tube contains 9 ml. of sterile saline water) (2^{nd} dilution or 10^2) then from second test tube transfer 1ml to the third tube (3^{rd} dilution or 10^3) and so on up to the 4^{th} or 6^{th} or 9^{th}dilution.
3. Place 1 ml of the dilution into each of two sterile Petri dishes.
4. Add about 15 mL of molten clear agar, tempered to 44–47°C, to each plate (e.g. plate count agar for a total colony count).
5. Mix each plate well by moving it five times in a vertical, clockwise, horizontal and anticlockwise direction as shown, then allow the plates to set. let the agar solidify with out further disturbance(about 30minute) and invert the plate.
6. Incubate all plates as appropriate for the organisms for a total aerobic colony count; incubate for 12 to 24 hours at 35 to 37°C, alternatively 72 hours at 30°C.
7. Results Count the bacterial cells grow to recognizable colonies that can be counted. Plates with 30-300 colonies are selected for counting all colony forming units (CFU), including those of pinpoint size. Colonies could be counted using Colony Counter. Plates with more

than 300 colonies cannot be counted and are designated as too numerous to count (T'NTC). Plates with fewer than 30 colonies are designated as too few to count (TFTC).

8. Calculate and interpret the result Plates with 30-300 CFU.

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2) \times (d)}$$

where:
N = Number of colonies per ml or g of product
$\sum$ C = Sum of all colonies on all plates counted
n_1 = Number of plates in first dilution counted
n_2 = Number of plates in second dilution counted
d = Dilution from which the first counts were obtained

Example plate n1=200, 276, d1=10^{-2}, n2= 30, 31, d2=10^{-3}

$$N = \frac{(200+276+30+31)}{[(1x2) + (0.1x2)] \times 10^{-2}}$$

= 537/0.022
= 24,409
≈ $2.4x10^4$

Membrane Filtration Method This method is suitable for water, beverages and liquid food products. Any measured volume of sample that is compatible with the equipment available may be used, so this method is particularly useful for examining larger sample sizes such as 100 mL or 1L. If the sample is likely to contain high numbers of organisms, the use of a small volume or preparation of serial decimal dilutions is recommended.

Materials: sterile membrane filtration membrane with pore size 0.45 µm, filtration funnel, Vacuum filtration Samples.

Procedure

1. Filter a measured volume of the sample or dilution using sterile membrane filtration equipment and a membrane with pore size 0.45 µm. For sample volumes of less than 10 mL, aseptically pour 20 mL of sterile diluent into the filtration funnel before addition of the measured volume of sample. Vacuum filtration is recommended.
2. After filtration, remove the filter membrane with sterile forceps and place it on a culture pad previously soaked in appropriate culture medium or on the surface of a suitable agar medium (see Section 6 for guidance).
3. Incubate the culture pad or agar plus filter membrane as appropriate for the organisms sought (see Section 6 for guidance).
4. **After incubation** Count the number of colonies on the membrane and relate the number of colonies to the volume (and dilution) of sample filtered to obtain a count per mL.

b) *Indirect tests*

1.1 Titratable acidity test

The production of acid in milk is normally termed “souring” and the sour taste of such milk is due to lactic acid. The percentage of acid present in milk is a rough indication of its age and the manner in which it has been handled.fresh milk has an initial acidity due to its buffering capacity.

Apparatus Using N/10sodium hydroxide

- ✓ White enameled or porcelain basin
- ✓ Stirring rod
- ✓ A 10 ml or 17.6 ml pipette
- ✓ Burette
- ✓ Burette-stand.

Reagents

- 1.6% alcoholic solution of phenolphthalein
- N/9 sodium hydroxide.

Procedure

1. Put 10 ml of milk in a porcelain dish using a pipette..
2. Titrate with N/9 sodium hydroxide Adjust the level of NaOH in the burette to the top mark, the lowest reading being at the upper end.
3. If milk, skim milk or buttermilk is to be tested, deliver 10 or 17.6 ml of milk into the porcelain dish. If cream is to be tested, use a 9 ml pipette (for cream weighing about 1 g/ml).
4. Add 3 to 5 drops or 0.5 ml of 1.6% solution phenolphthalein to the sample in the cup.
5. Note the reading of the NaOH in the burette at the lowest point of the meniscus.
6. Allow the NaOH to flow slowly into the cup containing the sample and stir continuously. When a faint but definite pink color persists, the end-point has been reached.
7. Take the reading of the burette at the lowest point of the meniscus. Subtract the first reading from the second to determine the number of milliliters of alkali (NaOH) required neutralizing the acid in the sample.

Calculation

Lactic acid (%) = W/ V
Where: W =volume of N/9 NaOH required (ml)
V =volume of milk taken for analysis (10 ml

Lactic acid (%)= ml N⁄10 alkali × 0.009 × 100/ ml of sample

5. Additional tests on milk quality, proper pasteurization and sterility