

TO THE POINT CLASS LECTURES

**MILK AND MILK PRODUCTS INSPECTION
(MICRO 404)**

FINAL COMPLETE

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BEST OF LUCK !!!

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Addition of Formaldehyde in the Milk

It is frequently used in the milk as preservative. It is highly toxic to the kidneys (nephrotoxic). It forms aldehyde compounds and fix the proteins that leads to toxic syndrome. Adulteration of formaldehyde in the milk is not recommended and discouraged all over the world. Its use is illegal from any point of view.

Addition of formaldehyde

Under specific circumstances (for research purpose), it is allowed to use 1 part of formaldehyde in 200,000 parts of milk as milk preservative (1:200,000). It is recommended for those patients having gastric ulcers. As formaldehyde will precipitate the protein, so in such patients it will absorb from the intestine rather than the stomach.

Test for Formaldehyde Detection

Test to declare the milk as formaldehyde free or not, is very simple.

Procedure:

Take 10 ml milk sample and add 5 drops of ferric chloride (10%). Then, add 2 ml of H_2SO_4 . Shake it well and then stabilize it.

Interpretation:

There will be orange red colored ring at top of milk sample. This ring indicates presence of formaldehyde in the milk. Negative test presents no such ring formation.

Different products (even yogurt) can not be manufactured/prepared from formaldehyde treated milk. This test must be conducted before collecting the milk in bulk cans.

Boiled Milk

Boiling practice of milk is done in order to enhance the keeping quality of the milk. Boiling procedure is safe, and milk does not contain any toxin after boiling. But a variety of vitamins, essential enzymes and amino acids are lost through boiling of milk.

Commercially, it has got less price than the raw milk.

Test for Boiled Milk

To differentiate a raw and boiled milk

Peroxidase activity is completely lost if milk is boiled beyond 79 °C.

Procedure:

Take 15 ml milk & add 2-3 drops of starch solution (10%). Then add 5-6 drops of KI (10 %) and finally 1-2 drops of H_2O_2 .

Result:

If milk remains white colored, it indicates boiled milk.

If blue color appears, it indicates milk is not boiled, it is raw milk.

Interpretation:

Starch used as substrate and if milk contain peroxidase enzyme, then KI gives blue color by reacting with H_2O_2 . Peroxidase enzyme destroyed when milk boiled at 79 C for 5 minutes.

Milk Quality

Criteria for Milk Quality:

The milk must have / contain:

- (i) Low number of bacterial count
- (ii) No / Low number of potential pathogens
- (iii) No drug residues
- (iv) Minimum microbial/chemical toxins.

The ultimate goal for maintaining milk quality is basically consumers' health protection. Quality standards must laid on milk and milk products.

Quality:

It refers to "different things for different people". Their standards vary from time to time.

Quality Standards:

The quality standards change slowly but surely. Quality standards for milk come under Food Safety. Criteria are important for production of milk and safety in terms of consumer. Among quality standards, Somatic Cell Count (SCC) is one of such standards.

Somatic Cell Count

These are epithelial cells and leukocytes coming down in the milk.

Somatic Cell Count (SCC) for milk of healthy cow: 200,000 / ml

If there is an increase of SCC: 100,000 / ml in the milk, --> 300,000 /ml; it means milk is from mastitic cow. SCC in the milk is the big indicator for health of the animal.

Somatic Cell Count / ml of milk	% of quarter affected
300,000	6.2
400,000	12.8
750,000	24.3
1000,000	32.6

Standard Somatic Cell Count in Milk (SCC / ml) for Different Countries

United States: 750,000

Australia: 400,000

Canada: 500,000

Pakistan: 950,000-1200,000

Somatic Cell Count: Australia < Canada < USA < Pakistan

Health impact varies much on somatic cell count (SCC).

An increase of 100,000 SCC / ml in the milk, leads SCC value from 400,000 to 500,000 results into 25% less cheese production.

Somatic Cell Count (SCC) provides three important functions:

- a) Monitoring of prevalence of mastitis in dairy cows.
- b) Act as indicator of raw milk in all processes.
- c) Act as indicator of hygienic conditions of milk at a dairy farm.

Drug Residues in Milk

It is the normal phenomenon in animals treated in infectious diseases with various antibiotics. Physicians neglect the withdrawal period of every product. Withdrawal period depending upon the half life of the drug. It should be strictly follows. Irrational use of antibiotics is practiced for improving keeping quality of the milk.

Effects of Drug Residues:

- a) Consumers may acquire drug resistance.
- b) Allergic reactions
- c) Sensitized to various kinds of drugs.
(Consumer may have complaint for vomiting, diarrhea and enteritis)

Beside other effects, economic impact of industry is also hampered due to presence of drug residues in the milk.

Under WHO (World Health Organization), FDA (Food and Drug Authority) determines standards for drug residues in the milk.

Permissible level of drug:

It is level of drug residue in the milk which has no deleterious effect on the consumers' health. It is determined by FDA and withdrawal period of a drug is determined on it.

FDA recommendations for standard drug residues are accepted by health monitoring authorities of all countries irrespective to the quality of the milk.

Tests for detection of Drug Residues

PHAST (Pre-harvest antibiotic sensitivity test)

It is mostly used in European dairy industries.

Urine sample of the animal is taken and tested whether body is cleared residues or not from drug. It takes about 18-24 hours (about 1 day).

STOP (Swab test on premises)

It is mostly used in American dairy industries.

This test can be used on both urine and milk samples for drug residues.

It gives result within 10-12 hours.

STAF (Swab test on animal food)

It was devised in UAF (2004), mostly used in Asian countries.

It can be performed on premises. It takes only 10-12 hours and can be used for milk, meat and eggs to detect the drug residues. This single test is applicable for detection of 38 different antibiotics commercially used in animal production system.

These tests work on the common principle but the variation is in their procedural details.

STAF Test (Swab Test on Animal Foods)

STAF test is used to detect the antibiotic residues secreted in the milk, urine, meat and eggs.

Requirements:

- Spore suspension of *Bacillus subtilis* (Gram +ve, brick shaped, non-pathogenic bacteria) It produce sub-terminal spores, found in local soil (indigenous strain). Basically, spores are sensitive to 38 different antibiotics.
- Plastic polystyrene plate
- Nutrient agar (Melted)
- Petri dishes

- Gamma-Sterilized cotton buds (like ear-buds)
- Standardize antibiotic disc e.g. Neomycin sulphate (5 µg)
- Milk / urine / meat / egg

Procedure:

- Take 15 ml of melted nutrient agar and add spore suspension @ 1×10^6 spores / ml.
- Make a layer of 5-6 mm thickness of this mixture in the plate.
- Stand it for solidification
- After solidification, take kenamycin disc and place it with sterilized forceps on the surface of the nutrient agar
- Now, take sterilized cotton buds and:
 - Soaked in milk or urine
 - If eggs are to be tested for drug residues, then separate egg white and yolk. Mostly antibiotics are fat-soluble and remain in the egg yolk. So, soak cotton bud with yolk.
 - If meat is to be tested for drug residues, then cotton bud must be placed in the juicy part of the meat and it must has to absorb meat juice.
- Now place these cotton buds (one or more) on the surface of nutrient agar plate and press it gently with the help of forceps. Distance between disk and cotton bud should be >1.5 cm.
- Then, incubate the plate at 35-37 C for 12 hours.

Interpretation:

- Zone of inhibition around antibiotic disc must be of diameter: 14-16 mm. It indicates that the test is valid or complete and nutrient agar layer was not more than 5-6 m thickness, spore dilution was proper, temperature & time during incubation was correctly maintained. Results can be expressed more confidentially, if inhibition zone around antibiotic disc is 14-16 mm in diameter.
- If zone of inhibition is less than it then repeat the whole procedure.
- If Inhibition zone around the cotton bud is equal or more than 2 mm then test is positive for antibiotic residues and the antibiotic residues are greater than permissible limits (not fit for human consumption).
- If swab zone is less than 2 mm then the STAF test is negative but antibiotic residues are still present but in permissible limits.
- If no zone around swab, no antibiotic residue.

Preservation of Milk

Objectives:

To ensure the supply of wholesome milk

Maintaining keeping quality of milk for maximum time

Methods:

There are three different categories:

- i) Pasteurization
- ii) Use of antimicrobial agents
- iii) Use of GRAS (Generally regarded as safe) substances

These substances are used in two forms:

- a) Act as a food additive
- b) Act as a food stabilizer

Pasteurization

It was started in early 1980's. It is defined as "Heating of milk at 62 C for 30 minutes".

Objective: is to kill and target all the microorganisms present in the milk.

Mycobacterium tuberculosis (human and bovine type) is also sensitive to pasteurization. It destroys by heating at 61.3 C for 30 minutes.

Modified Technique:

In another modified forms of pasteurization: Holding time is decreased but on the other hand temperature is increased.

Modified pasteurization protocol:

Temperature 71 C / 5 minutes

High Temperature Short Time (HTST)

Temperature 92 C / 15 sec

Process is comparatively faster.

Ultra High Temperature (UHT)

Temperature 171 C / 2-3 sec

Much faster and efficient regarding killing of all type of microorganisms

Phosphatase Test

This test is used to check the efficiency of pasteurization process; whether milk is pasteurized or under pasteurized. Milk is immediately collected after pasteurization. It based on certain principle. Time required for this test is about 15 minutes.

Principle:

The principle of this test is that when milk is mixed with phosphoric ester (as substrate), the peroxidase and oxidase enzymes will convert it to phenolic compounds. This phenol will react with the indicator and give rise to colour change. Phosphoric ester is available in the form of buffer.

Requirements:

There are two basic compounds which are required for this test:

- (i) Disodium phenyl phosphate (DSP): It is available in tablet form (1 tablet + 5 ml water) and called as buffer substrate.
- ii) 2,6, di-bromo quinone chloramide (BQC): It is available in tablet form. One tablet is dissolved in 5 ml of n- butanol.

Procedure:

Take 5 ml of milk sample and add 5 ml of DSP.

Then after shaking, incubate it at 37-40 C for 5-10 minutes.

Add 6 drops of BQC compound and mix it.

Note any color change.

Interpretation:

If color of the milk changes to blue ----- Under pasteurized milk

If color of the milk turns to yellow ----- Good pasteurized milk

Concept of control sample:

A control milk sample can also run with the other experimental milk samples. Control sample (5 ml milk) must be pasteurized at 77 C for 15 sec before performing this test procedure. Then, this sample will surely gives you blue color; indicating perfectness of the procedure.

There is also use of various types of preservatives like phenolic detergents and their residues. Mercuric chloride is excellent detergent for washing of utensils, pipe line. Use of alcoholic detergents is not recommended.

Factors influencing results of phosphates test

- (i) Age of the sample: If milk is stored after proper pasteurization, then pasteurization remains effective for 50 days if it is stored at low temperature.
- (ii) Use of preservatives: also affect the results of phosphatase test.
- (iii) HTST method: In this pasteurization technique, peroxidase enzyme will again activate (enzyme reactivation occur). Due to which HTST treated milk is not suitable for phosphatase test. This test is efficiently used for UHT milk.

Milk Quality

10 years ago, milk quality was determined on TBC (Total bacterial count). Raw milk with 10,000 – 30,000 bacteria / ml was considered the good quality milk.

But now in the modern era, quality standards has been improved/increased due to:

- a) Educational backgrounds
- b) Economic / social system
- c) Scientific information
- d) Ordinance / law / regulations

Demands by the consumer:

- Milk should be in pure form (no adulteration)
- It must have good flavour
- Products must be attractive

Initially milk was packed in sachet but now a days it is packed in tetra-pack. Keeping quality / shelf life of the product has been increased. These products are claimed with 3 months of keeping quality. People also prefer that milk with longer half life.

Laws regulations of different countries are remaining continuously in process.

Quality Control

It based on two things:

- (i) Selection of analysis (compositional analysis)
It emphasis on method of analysis
- (ii) Sensory evaluation (organoleptic)
It is associated with the evaluation of color, consistency and aroma of the milk and milk products.

Compositional analysis:

Each and every quality control lab will examine these two things. The lab will conduct different tests for compositional analysis.

Bacterial analysis:

It includes a number of tests including TBC on milk, coliform test on milk and tests for count of yeast and mould are also important.

Antibiotic residue test:

It is also performed by quality control labs to know about the antibiotic residues in the milk.

Ingredients _____ Processing _____ >>Products

After analysis of the ingredients, a certification is provided before taking it into the market.

Quality of the product depends on the quality of the ingredients. Therefore, quality of the ingredients is very important.

Quality Assurance:

Check system of ingredients before the start of processing is known as quality assurance.

It is much important than the quality of the product. Thus, people now investing more for quality assurance of the ingredients.

When ingredients are passed or a certification is issued, then they come to processing for manufacturing/preparing of the products. Hence more stress is given on the quality assurance.

Quality assurance does not encompass all the quality control measures of the products also. It may be excluded in certain situations such as: Lacking of quality by products due to improper packaging (low keeping quality). Then, it will not indicate the fault of quality assurance certification.

HACCP Certification

National Agency of Health issue certification of HACCP (Hazard analysis for critical control points) – and provides certification of ISO 9000 – 2002. This ISO certification gives more stress on managemental points.

HACCP is an important certification based on the very critical steps including:

- (i) Instrument calibration
- (ii) Product line check
- (iii) Pre-operational checks
- (iv) Lab analysis record
- (v) Equipment maintenance record
- (vi) Sanitation pest control checks
- (vii) Training / manual / program
- (viii) Self / external audit

Each and every step requires a certification. Next step can not be started or considered complete until the preceding step got a certification.

(i) **Instrument calibration:**

It is a very basic and fundamental step. Measuring instrument in the QC lab must be standardized with the instrument standard weights.

(ii) **Product line check:**

Processing should be according to the specifications. If milk is packed in tetra-pack then tetra pack must be of 6 layers. Tetra pack with >6 or <6 layers will not held for HACCP certification.

(iii) **Pre-operational checks:**

Before delivering raw milk to the processing unit, got certification that there is not pre-operational fault in the processing unit.

(iv) **Lab analysis record:**

This is the responsibility of QC lab (quality control lab) attendants that they should keep record of each analysis properly. Further processing can not be started until the compositional analysis.

(v) **Equipment maintenance record:**

For a complete processing, people have to maintain stand by instrument or equipments. A certification is given fulfilling requirements of this step.

(vi) **Sanitation pest control checks:**

As in food industry, presence of rodents, flies and lizards is considered most probable but undesirable at all. So they should be removed and pest-free environment is required. According to standard HACCP recommendations, the environment must have < 10 particles / cm³.

But 2 weeks ago, an experiment conducted to know the particles / cm³ in our existing environment. Then it was really shocked to know that our environment contain 3,80,000 particles / cm³.

Involvement of instrumentation is now practiced in the modern era to create an environment of low particles numbers. Such innovation is known as “Heating ventilation and air control system (HVAC)”.

(vii) **Training / manual / program:**

A certification is issued on this aspect that either industry provided technical training to the workers or not. Technical sessions and programs must be conducted for this purpose according to the HACCP recommendations.

(viii) **Self audit system or External audit system:**

It depends on the industry either it induct self audit system or external audit system. In case of external audit system, external team of audit inspects the products and issue a certificate for one year. After this certification, product is stamped with HACCP logo.

Beside HACCP certification, HCP (Halal certification point) is also introduced in the market for quality control improvement. The HCP certifies that during packaging/processing, against used were not belonging to the Non-Halal food.

GMP (Good Manufacturing products) is introduced now days as a quality control measure.

----- End of Final Course

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Important Note for Final Paper

- Mid course will be included (30% ≈ 4 marks)
- Final paper comprise of two parts;
- Objective part (1/3): may consist of MCQs, T/F etc.
- Subjective (2/3): consists of short questions
- From the mid course, no explainable question will be given.

DATE SHEET FINAL THEORY EXAM FOR DVM 7th Semester

Date	Day	Course No.	Course Title
22-01-2011	Saturday	MICRO 404	Milk and Milk Products Inspection
25-01-2011	Tuesday	SURG 402	Regional Surgery
27-01-2011	Thursday	PARA 404	Meat Inspection
31-01-2011	Monday	MED 404	Systemic Medicine – I
02-02-2011	Wednesday	AR 402	Reproductive Biotechnology
07-02-2011	Monday	LM 404	Equine and Camel Production

Timing: 12:00 – 02:00 PM

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