University of Tikrit

College of Veterinary Medicine

Dept of Vet Public Health

Meat Hygiene

FOOD HYGIENE

Microbiological Examination of Meat

Microbiological Examination of Meat:

microbiological examination aims to evaluate the abattoir and the meat in cases of:

1- All emergency slaughter animals (except injury cases

2- Imperfect bleeding and poor setting

3- Trimmed carcasses, i.e., carcass with organs missing at veterinary examinations

4- Septic conditions and carcasses heavily contaminated during dressing

5- PH value higher than 6.5 or more 24 hrs after slaughter

Meat must be of a high microbiological quality in order to ensure that the consumer receives a product that is not spoilt or does not carry food borne disease

An important function of meat related legislation and enforcement is to ensure that meat is indeed prepared under conditions of acceptable hygiene However, the modern trend is that the abattoir assumes more and more responsibility for the microbiological condition of its product Apart from visual inspection, it is necessary do microbiological tests to ensure that the hygiene measures that cost money and time are indeed effective By regular microbiological evaluation of the abattoir Before embarking on microbiological examination of a foodstuff it is logical that the

specific purpose of the examination be clear and made clear to the laboratory technician.It is also logical that before choosing a microbiological test the suitability of the test for the particular purpose be evaluated.

Microbiological test

1-Evaluation of the abattoir and the meats Counts on solid samples (meat products, etc) are expressed as colony-forming units (CFU) per gram

2-Counts on liquid samples (water) are expressed as CFU per milliliter

3-Surface counts are expressed as CFU per 10 square centimeters

As well as The presence of faecal coli forms or E.coli constitute a much greater potential for faecal contamination and hence for the presence of enteric pathogens.

Factors that may influence the choice of microbiological method:

Performance which includes Sensitivity, specificity, accuracy, precision, reproducibility, repeatability.

Time include Total test time (presumptive/confirmed results), 'hands-on' time, time constraints

Ease of use Complexity, automation, robustness, training requirement,

sample throughput, result interpretation

Standardization Validation, accreditation, international acceptance

Cost per test, capital outlay, equipment running cost, labour cost.

Microbiological examination of foods and food ingredients can be placed into three categories

1- Tests for pathogens and their toxins (for example, *Salmonella, Staphylococcus aureus* and its toxin, *Listeria monocytogenes, Clostridium botulinum* toxin).

2- Tests for organisms/groups of organisms (for example, *Pseudomonas* spp. as spoilage organisms.)

3- Tests for sc. indicator organisms (for example indicators of hygiene, indicators for the potential presence of pathogens).

Tests for indicator organisms and agents can be grouped into 4 categories:

1-To assess numbers of micro-organisms and/or microbial activity

aerobic plate count

*psychotro*pic count

mesophilic count

Pseudomonas spp count

yeast and mould count

direct microscopic count

pH determination

(Change in the expected pH)

2- **Presence and potential presence of pathogens:**

• *Staphylococcus aureus*

• *Escherichia coli* (certain strains like *E.coli* O157:H7)

• *Salmonella* spp.,

• *Clostridium perfringens,*

• *Campylobacter jejuni/coli*,

• *Bacillus cereus,*

• *Listeria monocytogenes*

3- **Indication of potential faecal contamination*:***

• *Enterobacteriaceae*

• *coliform bacteria*

• *faecal coli forms*

• *enterococci*

4- Metabolic products of pathogens that indicate a potential health hazard:

• *Staphylococcus aureus* toxins/thermo nuclease test

• phosphatase test.

Microbiological examination usually consists of

sampling, transportation and storage of the samples and their evaluation

sampling and sampling methods

The choice of sampling site,

sample size and the

number of samples to be taken for microbiological

Sampling methods

Non-destructive methods break up the colonies of bacteria (e.g. swabbing, rinsing) and counts are usually higher than with those methods that replicate intact surface colonies (e.g. agar sausage, PetrifilmTM and Rodac plates).

Destructive techniques a sample is removed and macerated or blended before counting. This usually gives higher and less variable results than contact plate and swab methods.

Surface Count Methods

Agar sausages

Petrifilm TM

Rodac plates

Swab

Storage of Samples

1- The storing of samples to be used in total counts must be avoided as far as possible.

2- Bacteria present in the sample may start multiplying under favourable conditions and this could lead to false high counts.

3- If storage is unavoidable, the sample may be kept in a refrigerator or on ice at 0 to 2°C.

4- Frozen samples must be allowed to thaw in a refrigerator between 1°C and 5°C for 18 to 24 hours

The Agar Sausage Method

This is a direct contact method used to determine total counts on flat, dry surfaces. It is most commonly used in the testing of plant equipment and utensils for efficacy of cleaning and disinfection and also to obtain total aerobic counts on slaughtered carcasses.

Principle of the method

An agar medium is solidified in a container such as a big syringe (50 - 60 ml) with a known diameter and with the front end cut off.

Using the plunger of the syringe, about 5 mm of the agar is pushed out and the front surface of the agar is pressed firmly against the surface to be tested .

A slice (2-3 mm thick) is cut off, placed in a Petri dish, and incubated

After incubation the number of colonies that have grown on the slice is counted and corrected to an area of 10 cm2

The result is then expressed as the number of CFU/10 cm2. It must be stressed, however, that this method is only used as a screening test

The result obtained are not a reliable indication of the actual number of bacteria present on the tested surface, especially irregular surfaces such as carcasses, because the agar is not able to pick up all the bacteria

Sampling procedures

• Remove the aluminium cover and using the plunger, press out about 5 mm of the agar.

• Sterilise the knife by immersing it in the sterilising fluid and then flaming it. Repeat this process betweeneach item.

It is not normally necessary to sterilise the knife between successive slices taken from thesame item or area. Sterility controls may be made and incubating them.

• Cut off a 2 - 3 mm thick slice and discard it.

• Press out another 5 mm of agar with the plunger and press the surface of the agar firmly against the surface to be sampled, taking care not to wipe the agar over the surface. Remove immediately.

• With the sterilised knife, cut off a 2-3 mm thick slice and transfer it, inoculated side uppermost, to an empty Petri-dish with the blade of the knife.

• Up to 5 slices may be sampled from one item and placed into one Petri-dish. However, three slices per item usually give a good idea of the efficacy of cleaning and disinfection, depending on the size of item

The Petri-dishes containing the slices are not inverted during incubation which is for 24 - 48 hours at 30°C. Samples from carcasses should be incubated for 48 hours, Counting and interpretation of the result, After incubation the colonies on all the slices per Petri dish are

counted by using a colony counter and then totalled. Regard this figure as the number of viable cfu on the test area. Correct this figure to an area of 10cm2.

Example:

The square surface of one slice is 8 cm. If three slices per item were used, the total area sampled is therefore 8 x 3 = 24 cm2. The number of colonies counted on all three slices totalled for instance 252 colonies. Correct this figure to an area of 10 cm2 by dividing 252 by 2,4 = 105 cfu / 10 cm

Rapid Methods To Asses Numbers Of Organisms

ATP Photometry

Direct Epifleurescent Filter Technique

Electrical Impedence Method

ATP Bioluminescence

The ATP bioluminescence procedure is referred to as a “real time” procedure because an incubation period is not required and cleanliness can be determined in 1 or 2 minutes. This technology thus allows processors to determine the effectiveness of their sanitation procedure immediately and to make corrections before production begins. The technique measures the adenosine triphosphate (ATP) present in a swab from an equipment surface. It uses an enzyme and its substrate (sc. luciferin – luciferase system) from fireflies in the determination. In the presence of ATP a reaction takes place and light is produced

The method measures all ATP whether from micro-organisms or from meat residues or exudate. Since all these sources of ATP are undesirable on thoroughly clean and sanitised surfaces, it is a most useful procedure. (There are special techniques to determine whether the source of ATP is microbiological or animal with the instruments)

Culture Media:

Culture media serves a number of purposes in practical microbiology. They used to:

1- Grow a wide range of organisms in a particular culture group e.g. bacteria

2- maintain organisms in culture collections

3- distinguish between different types of microorganisms

4- select specific grups of microorganisms from the environment e.g. a food material

5- help identify microorganisms

6- assay nutrients or antimicrobial compounds

Types of culture media:

1-General purpose : which allow the growth of wide range of either bacteria, yeasts or moulds e.g. nutrient agar ,plate count agar.

2- selective media :media which contain ingredients that inhibit the growth of certain organisms but allow the other to grow e.g. modiefied charcoal campylobacter agar for isolation of *campylobacter jejuni/coli*

Eosin methylene blue agar (EMB) for isolation of *E.coli* and mannitol salt agar for isolation of *staphylococcus aureus*

3- enrichment media which is a broth contain selective ingredients shift the growth of mixed population of bacteria in the direction of specific organisms which will be the dominants e.g. selenite broth for salmonella due to its content of sodium biselenite which is toxic to all organisms but less toxic to salmonella.

4- differential media which is purpose to distinguish between organisms groups e.g. blood agar according to hemolysis due to hemolysin producing a clear zone around colonies.

5- selective/differential media which is designed to isolate a group of closely related organisms and differentiate between them. E.g. macConckey agar which contain e bi;le salts as a selective agents tend to make the medium selective for Enterobacteriaceae.

6- chemically defined media which is of chemical nature e.g. mineral modified glutamate medium.

7- elective media designed to promote the growth of specific organisms that have special nutritional requirements by adding particular ingredients to the medium improved the growth e.g. tomato juice agar for culturing of lactobacilli .

8- living media include culture of host cells for the growth of viruses e.g. chick embryo or tissue cultures.

Basic Laboratory Equipment

Microscope with 10x and 40x objectives and 90x or 100x oil immersion objective.

Immersion oil - Glass slides and cover slips – Balance - Bunsen burner – Camera – Autoclave – Incubator – Refrigerator – Homogeniser –

Centrifuge - Stomacher lab blender - PH meter – Thermometer - Candle jar - colorimeter/- photometer - luminometer - Commercial identification – kits - Ringer solution - Ready poured cultured media - Anaerobic culture system – 3% rogen peroxide - Specimen transport media - Antibiotic disc dispenser and discs - Oxoid sensitivity test agar – Stains(Gram ,Giemsa, methylene blue, zielh-neelsen, leishman) - Test tubes, holding and staining racks - Durham tubes - Centrifuge tubes - Petri dishes - Beaker and flasks, measuring cylinders,universal containers - Absorbent cotton wool swabs,gauze swabs - Platinum loopself adhesive cellophane tape - Specimen collection equipment(scalpel, forceps, spatula, spoons).