Dept. of Vet .Public Health //MEAT HYGIENE Course:

Bacteriological examination of carcasses:

- the actual operation of slaughter and dressing may introduce bacteria to the blood, tissues and organs. These organisms are usually a mixed flora of a non-specific type but can include food poisoning organisms. On the other hand, the bacteria present may be of a specific and pathogenic type and the presence of these in organs or tissues such as the spleen, muscular tissue or lymph nodes can only be attributed to the fact that a generalized septic or bacteremic infection existed in the animal at the time of slaughter.

☒ **Bacteriological exam is required in the case of animals which:**

1. Have been slaughtered in emergency.
2. where slaughter has taken place without the prescribed ante mortem inspection.
3. have been slaughtered on account of a disease associated with systemic disturbances.
4. have been slaughtered on account of acute inflammation of intestine , udder, uterus, lungs , pleura, joints, tendons, umbilicus and peritoneum, or because of systemic illness associated with suppurative or gangrenous wounds.
5. show pathological changes on P.M inspection that lead to doubt as to suitability of the meat for human consumption , even though the animal was found healthy on ante-mortem inspection.

➢ **The following samples shall be taken for submission to the lab for bacteriological exam**:

1. Two complete muscles, with their fascia, one from forequarter, and one from hindquarter, or cubes of muscle not less than 7.5 cm in diameter.

2. the prescapular lymph node (or axillary) and internal iliac node.

3. the spleen.

4. a kidney 5. liver with gall bladder.

6. parts showing pathological change .

7. a portion of small intestine together with mesenteric L.N.

Types of bacteria found: The bacteria found may belong to

1-non specific group :which comprises species that are non pathogenic or only potentially pathogenic, e.g *streptococci, enterococci. E. coli* in adult animals except serotype 0157:H7, clostridia , non hymolyic staphylococci. These species are present naturally in the intestinal flora. 2-specific group :include all species regarded as specific pathogens and include the hemolytic *streptococci,,* hemolytic staphylococci, *pasteurella, salmonella, E coli* in newborn animals , *Bacillus anthracis*

Method detection of meat spoilage:

1. Mesuring of normal change:such increase pH and change of degree of electrical contact and surface tension

2. By chemical change:as aresult of produce some substance like ammonia ,indol,hydrogen sulfate

3. bacteriological examination:by count of number of total bacterial count(direct microscopic count) or by indirect microscopic count which divided in to SPC and Dye reductation test

Significant bacteria in meat:

1. Action of bacteria in split meat in to number of chemical substance such as gaseous and foul smelling.
2. decomposend protein and fat also carbohydrate
3. Un pleasant odour or fluavor in fat due to :

A. Absorbtion foreign odour,as in tainting meet or butter stored in champer previously used for fruit
B. Atmospheric oxidation
C. Action of miroorganisim

Coliform bacteria :

are defined as rod-shaped Gram-negative non-spore forming able to ferment lactose with the production of acid and gas when incubated at 35–37°C.One source of these organism is intestinal tract of warm blooded animal,these organisim are classified to

- *Klebsiella*
- *Escherichia*
- *Citrobacter*
- *Enterobacter*
- *Hafnia*
- Serratia
-

The 2 major coliform type:

1. Fecal type (E.Coli): indicate contamination by fecal
2. Non fecal type : Enterobacter indicate contaminate by soil, dust,water

Procedure of coliform count :

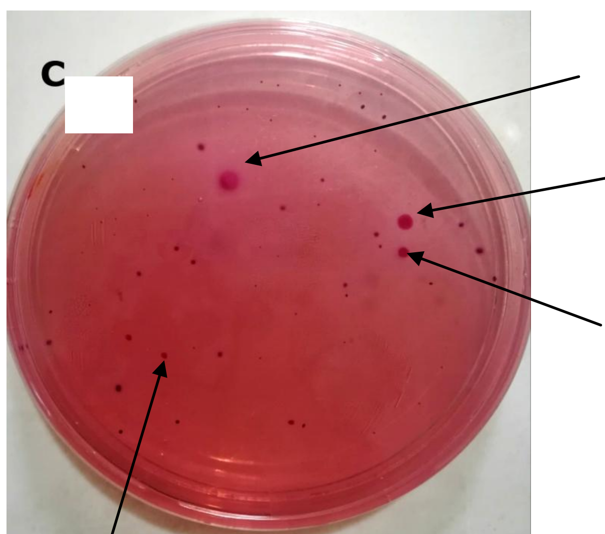
1. sample is decimally diluted in diluents
2. Transfer 1ml of each sample on to sterile plate
3. Add to each plate 10 to 15ml of VRB tempered to about 45 -50° C ,mixed content of plates

4. Allow mixture to solidify(5-10 minutes) then distribute on additional 3 to 4 ml of plating medium as an over lay to inhibit surface colony formation and ,invert and incubate plates at 37 ˚C for 24 houres
5. Count the dark red lenticular colonies on un crowded plates which contain about **(((15 to 150)))** colonies and then multiply by dilution factor to give (CPC) per gm.or ml.

- **Afinding of coliform in food is indicative of possible presence enteropathogenic or toxigenic microorganisim which could constitute a public health hazard**

Disadvantages in looking for pathogens in foods:

1. Many pathogens are fastidious and difficult to grow in routine test
2. Pathogenic agent are few in number per ml or gram ,allowing them to go un detected
3. Many pathogenic agent are difficult to identify accurately
4. Procedure are relatively time-consuming
5. When grown in food testing laboratory ,pathogenic cultures constitute additional hazard to laboratory personnel



(Coliform on VRB Agar)

In order to differentiate between Ecoli and Enterobacter take selective media to allow growing colony called (Eosin methyline blue) EMB also another biochemical test such as IMVIC(indol ,methyl red, voges proskour,citrate)

E.Coli :

Indol(+), methyl red(+) ,citrate(-)

Enterobacter:

Indol(-), methyl red(-) ,citrate(+)

E .coli appear in EMB green metallic sheen while Enterobacter appear pink to colourless(fish eye colony) convex center and smooth circular

Standerd plate count// (spc):

This method consists of growing the bacteria in a nutrient culture petridish or (petrifilm) and counting colonies which develop.

- It can be used for meat and all types of dairy products and is generally used in the examination of Grade A raw and pasteurized milk. This method used to determine the general quality of the milk supply.

Procedure

1. Wash the hands and disinfect the stage.
2. Mixing of sample.
3. Make a series dilutions of sample.
4. Transfer a suitable dilution to a petridish by sterile pipette.
5. Add a suitable amount (12 ml) from SPC agar on the dilution and mixing gently by moving the dish on different direction.
6. Incubate 48 hours at 32°C .
7. Count colonies and report results.

Note: Choosing the plates with 30-300 colony.

SPC(Cfu/ml)= Average number of colonies x Reciprocal of the dilution used

<u>Counting</u>: mean estimate of bacterial populations and it is not exact because:-

1. The agar used in method (SPC agar) is not suitable for growing all species of bacteria.
2. Incubation Degree which used in method (32°C) is not suitable for growing all species of bacteria.
3. Sharing more than one bacteria to form one colony.

Note: SPC(Cfu/ml)= Average number of colonies x Reciprocal of the dilution used

Duplicate plate dilution

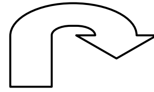
Note: SPC(Cfu/ml)= Average number of colonies x Reciprocal of the dilution used

Q//////What Estimated <u>SPC</u> IF Lower dilution (175 ,208 and higher dilution (16 ,17)

<table>
<tr><th>1/100</th><th>1/1000</th><td rowspan="4">Only one dilution yields plates with 30-300 colonies compute the mean for that dilution as the basis for the SPC .</td></tr>
<tr><td>175</td><td>16</td></tr>
<tr><td>208</td><td>~~17~~</td></tr>
<tr><td colspan="2">175+208=383 383÷2=191.5 191.5x100=19150….SPC=19000</td></tr>
</table>

1/100	1/1000	Both dilutions yield plates with 30 to 300 colonies: Average the mean count for each dilution as the basis for SPC.
230	32	
246	36	
230+246=476 476÷2=238 238x100=23800…SPC=23800	32+36=68 68÷2=34 34x1000=34000 SPC=34000	
23800+34000=57800 ÷2= 28900=29000		

ملاحظة مهمة ؟اذا كان معدل عدد البكتريا بالتخفيف العالي ضعف معدل التخفيف الواطىء او اكثر فيهمل التخفيف العالي



1/100	1/1000	***unless*** **the count computed for the higher dilution is more than twice the computed for the lower dilution .In the latter instance , use the lower computed count as the SPC.**
138	**42**	
162	**30**	

138+162=300 300÷2=150 150x100=15000… SPC=15000	42+30=72 72÷2=36 36x1000=36000…SPC =36000	

1/100	1/1000	Both dilutions yield plates with no colonies: Estimate the SPC as less than (<) 1 times the lowest dilution .
0	0	
0	0	
Lower than 1x100 ESPC=lower than 100		

1/1oo	1/1000	Both plates ;one of them(lower dilution) TNTC ,the other (high dilution)with 10 - 100 colonies per cm^2 : use the estimated count of the ESPC. In this case take the average of (4 square cm^2).
TNTC	14,20,34,12 TNTC	
SPC(Cfu)=Average of(4 square cm)x 65 cm^2xreciprocal of dilution = 20 x 65 x 1000		

1/100	1/1000	Both plates ;one of them(lower dilution) TNTC ,the other (high dilution)with less than 10 colonies per cm²: use the estimated count of the ESPC. In this case take the average of(12 square cm²).
TNTC	3,6,7,8,2,5,3,2,7,9,6,3	
SPC(Cfu)=Average of(4 square cm)x 65 cm² x reciprocal of dilution = 5 x 65 x 1000		