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MINISTRY OF AGRICULTURE

Abattoir Laboratory Guideline

For meat hygiene and safety analysis.

Export Abattoir Inspection and Certification Directorate
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LIST OF ABBREVIATIONS

AA	Approved Agreement
APC	Aerobic Plate count
BHI	Brain Heart Infusion
ES	Ethiopian Standards
CSA	Central Statistical Agency
CFU	Colony forming Unit
EAICD	Export abattoir inspection and Certification Directorate
MoA	Ministry of Agriculture
NSAID	Non-steriodal Anti-inflamatory drugs
SOP	Standard Operating procedure
SPS	Sanitary and Physio-sanitory Standards
TPC	Total Plate Count
TVC	Total Viable count
UTI	Urinary Tract Infection
HUS	Hemolytic Uremic Syndrome

FOREWORD

This technical document entitled **"Abattoir Laboratory Guideline, for Meat safety and quality analysis"** is one of the guidelines and Standard Operating Procedures (SOPs) developed by the Ministry of Agriculture (MoA) with the support of the EU-funded Health of Ethiopian Animals for Rural Development (HEARD) project.

The guideline is intended to provide inspectors and management of licensed export abattoirs with information required to establish standardized laboratory facility and activity safe and quality meat and meat products according to the international standards.

This laboratory guideline includes criteria for the basic facilities and materials requirements of abattoir laboratory with different standardized operating procedures for identification of pathogens and laboratory safety rules.

At this point, the Export Abattoir Inspection and Certification Directorate (EAICD) would like to thank the HEARD Project for the support given while developing this guideline.

Finally, Special thanks go to Dr.Gedion Yilma for facilitating and supporting in the preparation of this guideline. I would like also to thank Dr. Daba Gudeta for his determination for the realization of this guideline and thank Dr. Melaku Assefa, Mr. Yohannes Woinue, Dr Fikirte Lemma and those participated from VPH Directorate, NAHDIC and VDFACA for their active participation in preparation of this guideline.

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1. INTRODUCTION

Ethiopia ranks first in Africa and 10th in the world in total livestock population. As per Central Statistics Agency (CSA) 2020), the number of cattle for the rural sedentary areas at country level is about 61.6 million, Sheep 32.86 million and goats36.8million. There are two types of livestock export trends in Ethiopia. These are: chilled meat and frozen offal and live animal export. However, the country's animal and animal product export potential remains low despite increasing number of export abattoirs and existing livestock population.

The main reasons for low livestock and livestock product export are poor animal health management, poor animal product processing quality meeting SPS standard requirement and illegal animal trade.

Export legislation requires all exporters to have registered establishments producing meat and meat products to comply with the relevant Ethiopian Standard and to satisfy importing country requirements. The Ministry of Agriculture, Export Abattoir Inspection and Certification Directorate (EAICD) verifies compliance and certifies that products from export registered slaughter and further processing establishments have been produced in accordance with these requirements. To compete in the global meat market export slaughtering plants must comply with national and international standards of meat hygiene through establishment of laboratory analysis as part of safety and quality control mechanism in addition to routine ante-mortem and post-mortem inspection. However, there is no harmonized system that can support implementation of laboratory activity in all export abattoirs.

Therefore, there was a need to prepare standard guideline to evaluate, monitor and harmonize laboratory activities in each export abattoir to meet with requirements and as a response this standard guideline is prepared. This will eventually increase meat export quality and volume by improving meat quality management system of the country.

2. OBJECTIVE

The main objectives of this guideline are:

- ✓ To improve hygienic status of personnel, establishment, working environment, equipment and utensils, and finally the product/meat and by-products of the abattoir.
- ✓ To ensure meat safety and quality that complies with importing countries and international food safety requirements.
- ✓ To establish standardized laboratory activity in all abattoirs
- ✓ To monitor, validate and verify that the export abattoir food safety management system is effective

3. SCOPE OF THE GUIDELINE

This guideline applies to all Ethiopian export abattoirs for conducting and monitoring meat safety and quality through microbiological and physicochemical analysis.

4. BASIC FACILITIES AND REQUIREMENT OF ABATTOIRLABORATORY

a. Approval

All laboratories undertaking testing under the scope of this manual must apply in writing and be approved by EAICD. Laboratories must apply in writing to the directorate for approval.

b. Abattoir Laboratory room requirement

- ✓ There should be a system either group related activities in a single room, or clearly delineate bench space for specific activities. Measures must be taken to prevent cross-contamination of samples.
- ✓ Rooms should have a high ceiling to ensure proper ventilation, and walls and ceilings should be painted with washable, glossy paint or coated with a material suitable for cleaning and disinfection.
- ✓ The floor must also be easy to clean and disinfect and have no edges between the walls and floor.
- ✓ Laboratory work benches should be constructed of materials that are durable and easy to disinfect.

- ✓ All areas of the laboratory rooms are cleaned and maintained on a regular basis which includes bench tops, walls, floor etc.
- ✓ Abattoir laboratory facility for meat safety and quality test must include energy source, water supply, lighting and environmental condition for correct performance of tests.
- ✓ The abattoir management shall ensure that the environmental conditions in the laboratory do not compromise the validity of the test results.
- ✓ Abattoir laboratory should have effective separation from production area to avoid cross contamination.
- ✓ Abattoir laboratory shall have sufficient rooms for office, laboratory testing area, reagent preparation area, reagent storage and washing and sterilization room.
- ✓ The floor and wall shall be white in color, non-absorbent, washable, without cracks, easy to clean and disinfect.
- ✓ Access to the laboratory must be restricted to only authorize personnel.
- ✓ Must be kept clean and free from dust.
- ✓ A dedicated hand wash station must be provided.

c. Abattoir laboratory personnel requirement

Abattoir laboratory staff shall ensure competence of workers to operate specific tests and equipment, manage results and interpretation, and take responsibility for test reports.

The Ministry of Agriculture (MoA) and the abattoir shall employ and ensure the competence of all staff based on appropriate education, training, experience and/or demonstrated skills as appropriate. The laboratory shall maintain current job descriptions for all staff involved in testing and shall maintain records of relevant staff competencies including training. The MoA shall authorize the laboratory personnel to perform and monitor defined duties with impartiality and without work overload.

d. Basic materials requirements in abattoir laboratory

All materials that are used to conduct tests indicated in Standard Operating Procedure (SOP), should be available in the abattoir laboratory. Materials should be recorded, arranged and stored with clear labeling.

Records shall be retained for equipment which can influence test results.

- ✓ Equipment should have a procedure for handling, transport, storage, use and planned maintenance of equipment to ensure proper functioning and to prevent contamination or deterioration.
- ✓ Defective or malfunctioning Equipment should not be used until calibrated or tested to that it performs correctly.
- ✓ Required laboratory equipment &related software should be available. The equipment must be able to achieve accuracy required and comply with relevant specifications. A correctly used calibration program for key quantities to ensure that results are valid and accurate.
- ✓ Anything related to equipment, hardware and software should be safeguarded from arbitrary adjustments as these can result in invalidate results.
- ✓ All equipment and its subsidiary measurements having a significant effect on validity of results must be well calibrated and properly maintained before being put into service.

The materials should be arranged based on type and use with traceable label and records. Measurement equipment shall be calibrated with correct degree of accuracy.

e. Abattoir laboratory safety rules

Many pathogenic organisms encountered in veterinary medicine are potentially pathogenic to human. Staff in laboratory should be instructed on the careful handling and disposal of specimen submitted for laboratory investigation. The practice of good laboratory management needs to be followed. Laboratory management should ensure that defined procedures are followed when handling dangerous pathogen.

All employees working with potentially pathogenic microorganisms should be instructed in proper safety procedures. All staff entering the laboratory should be aware of the hazards present and should meet the defined entry requirements.

Moreover, an abattoir laboratory should follow special safety rules to avoid cross-contamination between production and biological wastes of abattoir laboratory.

The following safety measures should be taken in abattoir laboratory.

- ✓ Never eat, drink, or smoke while working in the laboratory.
- ✓ Read labels carefully.
- ✓ Do not use any equipment unless you are trained and approved as a user by your supervisor.
- ✓ Wear safety glasses or face shields when working with hazardous materials and/or equipment.
- ✓ Wear gloves when using any hazardous or toxic agent.
- ✓ If you have long hair or loose clothes, make sure it is tied back or confined.
- ✓ Properly dispose of all used material in appropriate containers.
- ✓ If leaving a lab unattended, turn off all ignition sources and lock the doors.
- ✓ Never pipette anything by mouth.
- ✓ Clean up your work area before leaving.
- ✓ Wash hands before leaving the lab and before eating.
- ✓ Treat every chemical as if it were hazardous.
- ✓ Never allow a solvent to come in contact with your skin. Always use gloves.
- ✓ Never "smell" a solvent!! Read the label on the solvent bottle to identify its contents.
- ✓ Dispose of waste and broken glassware in proper containers.
- ✓ Clean up spills immediately.
- ✓ Do not store food in laboratories.
- ✓ Never do unauthorized experiments.
- ✓ Never work alone in laboratory.
- ✓ Keep your lab space clean and organized.
- ✓ Never taste anything.

- ✓ Microbial cultures should be under strict quarantine to avoid escaping of biological agents into slaughterhouse.
- ✓ Biological wastes, especially bacterial culture should be decontaminated before disposal.
- ✓ All equipment used for preparing, dispensing and storing media must be sterilized before use to prevent contamination from the atmosphere.
- ✓ All sample processes should be carried out under bio-safety cabinet.
- ✓ Avoid direct exposure of abattoir laboratory to production room.

5. GENERAL DUTIES OF ABATTOIR LABORATORY PERSONELS

5.1. Duty and responsibility of governmental laboratory personnel

- ✓ Monitor laboratory activity.
- ✓ Ensure and monitor fulfillment of needs for laboratory requirement and materials.
- ✓ Forward recommended corrective action on production quality
- ✓ Analyze and interpret laboratory results including statement of conformity or opinion.
- ✓ The leader or responsible person should have capacity or overall responsibility for ensuring that laboratory operations are conducted in compliance with requirement set in the SOP given by Ministry.
- ✓ The laboratory leader has major responsibilities, including but not limited to assigningan appropriate number of personnel to cover workload, verify that items on the job application are correct, develop complete and thorough job descriptions for each employee, train each employee in their specific duties and provide training for new employees. New employees need to be trained on laboratory practices and SOPS to ensure competence to perform laboratory activity independently.
- ✓ Laboratory personnel performing specific tasks shall be qualified on the basis of appropriate education, training, experience and/or demonstrated skills.
- ✓ Laboratory personnel shall be trained on the Good Laboratory practices and Standard Operating Procedures.
- ✓ Abattoir laboratory leader should check the competence of all who operate specific equipment, perform tests/calibrations, and evaluate results, sign reports / certificates.

- ✓ Laboratory needs to authorize specific personnel to perform particular types of sampling, test, issue reports and certificates and give opinions, interpretations; and operate particular types of equipment.
- ✓ Ensure personnel are free from undue pressures/influences that may adversely affect quality of work.
- ✓ There should be means of motivation for laboratory workers working in the lab.
- ✓ Report review and authorize results
- ✓ Give judgment based on laboratory results.
- ✓ Conduct laboratory tests as required.
- ✓ Development and modification, verification and validation of methods
- ✓ Checking balance of reagent, presence and functionality of necessary equipment and preparation of reagents and equipment specifications
- ✓ Monitoring laboratory activity
- ✓ Ensure and monitor fulfillment of laboratory requirement and materials.
- ✓ Forward recommended corrective action
- ✓ Analyze results including statement of conformity or opinions and interpret laboratory results.
- ✓ Overall responsibility for the laboratory tests and to review, authorize test result reports.
- ✓ Give judgment based on laboratory result.
- ✓ Conduct laboratory tests as required.
- ✓ Ensures the implementation of agreed preventive actions in the laboratory

5.2. Duty and responsibility of private abattoir laboratory personnel

- ✓ She/he is responsible to collect samples.
- ✓ She/he is responsible to ensure fulfillment of all laboratory equipment and requirements in collaboration with abattoir management.
- ✓ She/he shall conduct routine laboratory work at abattoir level in collaboration with government laboratory personnel.
- ✓ He/she should report laboratory result and record and retain data properly.

- ✓ He/she shall follow and implement corrective actions recommended as a result of laboratory report in collaboration with abattoir management and government laboratory personnel.
- ✓ With respect to department microbiology monitoring programs, establishment management have responsibility to:
- ✓ Develop and implement a program compliant with the requirements of this manual and relevant to the establishment's export registration and incorporate this into their Approved Arrangement (AA)
- ✓ Ensure that personnel collecting, and handling samples are competent to perform these functions and have been properly trained and monitored and that these activities are properly recorded and that these records are available to the department.
- ✓ Ensure all testing is performed at a department approved laboratory using department approved methods.
- ✓ Where appropriate, notify laboratories of testing and reporting requirements associated with particular samples.
- ✓ Authorize and instruct testing laboratories to provide all relevant test results to department on-plant officers before or at the same time they are provided to establishment management.
- ✓ Make and retain records of all results of screening tests, confirmatory tests and comments.
- ✓ Support department verification activities undertaken to ensure programs are functioning correctly and efficiently.
- ✓ Ensure that all training and verification records are maintained and available for review.
- ✓ Ensure that a Request for Permit is not validated until all relevant final microbiological results relating specifically to product certification are known.
- ✓ In the event of a result that indicates failure of a standard or market requirement, establishment management must ensure that:
- ✓ Implicated product is retained or can be traced and quarantined.
- ✓ The department on-plant officer is notified without delay. Where the department officer is not present at the establishment.

6. LABORATORY BASED QUALITY REQUIREMENTS OF ABATTOIR

To meet international food safety and SPS standard, an export abattoir has to fulfill quality and safety requirements of product and production processes which should be confirmed by laboratory analysis. Especially microbial and physicochemical quality requirements should be confirmed by laboratory analysis described below.

6.1. Microbiological quality requirements

Food safety is a significant barrier to social and economic development throughout the world, particularly in developing countries. Food consumers in developing countries, including Ethiopia suffer from food-borne diseases such as bacterial illnesses, especially from those of *Salmonella spp.*, *Shigellaspp.*, s*taphylococcus aureus* and *Bacillus cereus.* Food-borne diseases result from ingestion of a wide variety of foods contaminated with pathogenic microorganisms,microbial toxins,or chemicals.

Meat is one of the most perishable foods, and its composition is ideal for the growth of a wide range of spoilage and pathogenic bacteria. It is prone to contamination at various stages from primary production to when it is ready for consumption (farm-to-fork). Contaminated meat is one of the main sources of food-borne illnesses and death caused by agents that enter the body through ingestion. Food-borne diseases are diseases resulting from ingestion of bacteria, toxins, and cells produced by microorganisms present in food. It is generally recognized that the most significant food-borne hazards from fresh meat are bacteria that can cause disease in humans (pathogenic bacteria), such as *Salmonellae* species, *Staphylococcus aureus, Listeria monocytogenes, Campylobacter* species, and *Escherichia coli* O157:H7. Some of these, particularly *E. coli* O157:H7, require only a few bacteria to cause food poisoning in humans. The main sources of contamination are the slaughtered animals themselves, the workers and working environment, and to a lesser degree, contamination from air via aerosols and from carcass dressing water.

i. **Salmonella*:*** Foods of animal origin are considered to be the major sources of food-borne salmonellosis. Salmonella species are Gram-negative, usually motile, facultative anaerob, non-spore-forming, and rod-shaped bacteria. Salmonella have been recognized as major foodborne pathogens for humans and animals and remain a tremendous medical and economic

burden. Most human pathogenic Salmonellae belong to *Salmonella enterica subsp. enterica* (*S. enterica*), which are divided into more than 2,600 serovars (serotypes). The most common Salmonella serovars that cause human diseases include *Salmonella typhi, Salmonella enteritidis, Salmonella paratyphi, Salmonella typhimurium,* and *Salmonella choleraesuis.*

ii. **Campylobacter*:*** Campylobacter spp. are both zoonotic pathogens and the leading cause of foodborne gastroenteritis in humans worldwide. *Campylobacter spp* is a frequent cause of foodborne illness worldwide. They are small gram-negative, non-spore forming helical bacteria with a distinctive darting' motility and are catalase and oxidasepositive. *Campylobacter spp* can be found in the reproductive organs, intestinal tracts and oral cavity of animals and humans. They are the leading cause of bacterial diarrheal disease worldwide, resulting mainly from the contamination of poultry or other meats, raw milk, milk products and surface water. Campylobacteriosis symptoms can range from mild diarrhoea to bloody diarrhoea, and the infections with these bacteria can lead to long term detrimental neurological consequences in a form of post-infection Guillain-Barrésyndrome. Among the genus Campylobacter,*C. jejuni*and *C.coli* are the most prevalent species

iii. ***Escherichia coli:*** *E. coli* is a natural inhabitant of the gastrointestinal tract of mammals. While most strains of *E. coli* do not cause disease in humans, some are known to cause severe illness due to the production of toxins and/or other virulence factors. One of the most concerning pathotypes of *E. coli*is Shiga-toxin producing *E.coli*(STECorVTEC).A subset of STECstrains can cause a frequently fatal entero-haemorrhagic colitis (HC) and the haemolytic uremic syndrome (HUS). STEC strains that can cause HC or HUS are classified as enterohaemorrhagic *E. coli* (EHEC). Among STEC, serotype O157:H7 is well known to cause food borne illness, STEC serotypes associated with food borne illness. The infectious dose of *E. coli*O157:H7canbeaslittle as 10–100 cells in a susceptible individuals. Cattle are a known reservoir of this pathogen, and several outbreaks of *E. coli*O157:H7havebeenassociated with consumption of dairy products.

*Escherichia coli O157:H7*is one of the most important food-borne pathogens, causing diarrhoea, hemorrhagic colitis and haemolytic-uremic syndrome in humans worldwide. Cattle are the major reservoirs of *E. coli O157:H7* followed by sheep and goats. The pathogen is carried in the intestinal tract and excreted in faeces. Consumption of raw or under cooked foods, especially under cooked minced beef has been found to be the most common means of transmission of the

pathogen.

iv. **Listeria*:*** *Listeria monocytogenes* is an intracellular pathogen transmitted to humans and animals through the consumption of contaminated food. It causes listeriosis and affects mostly at-risk people causing high hospitalization rates and death. To develop improved control measures for listeriosis, it is important to understand pathogenic mechanisms of *L. monocytogenes*.

v. **Staphylococci** are normal inhabitants of the skin and mucous membranes of animal and human. Pathogenic strains are usually coagulase positive and have been found to cause disease in their hosts throughout the world. *Staphylococcus* species cause mastitis and wound infection in livestock and food poisoning in humans through ingestion of contaminated foods, including meat and dairy products.

Staphylococcus aureus: *Staphylococcus aureus* can cause a variety of diseases including skin and soft tissue infections, and systematic and life-threatening infections. Meanwhile, *S. aureus* is one of the major food-borne pathogens causing food poisoning both in humans and animals. The pathogenesis of *S. aureus* causing food poisoning is attributable to the production of many staphylococcal bacteria.

Staphylococcus aureus can cause disease ranging from mild skin infection to life-threatening sepsis. It is the most common cause of community-associated cellulitis, endocarditis, and is a common cause of bacteraemia. Moreover, *S. aureus* evolves various drug resistance mechanisms, subsequently results in difficulty in the management of infections. *S. aureus* strains were once nearly uniformly susceptible to semi-synthetic penicillinase-resistant β-lactams (e.g. methicillin, oxacillin), the most commonly used class of antibiotics for skin infection. However resistant strains have evolved and these strains were termed ‘methicillin resistant *Staphylococcus aureus*, or MRSA, a term that implied cross-resistance to all β-lactams including all penicillin and cephalosporin.

To ensure the microbial quality of meat samples should be taken from meat, processing water, contact surfaces, personnel and other critical points based on the following microbial tests.

A. **The Aerobic Plate Count (APC)**

Description: The Aerobic plate Count (APC) estimates the number of viable aerobic bacteria or fungus per g or ml of animal product or feed. A portion of the sample is mixed with a specified agar medium and incubated under specific conditions of time and temperature. It is assumed that each viable aerobic bacterium will multiply under these conditions and give rise to a visible colony which can be counted.

The load of microorganisms on fresh meat, including all spoilage and pathogenic bacteria determines safety, hygiene and shelf life of meat. The presence of microorganisms on meat in excess quantities indicates poor hygiene in slaughtering process or cross contamination. Estimating the population of microorganisms on meat is important to determine the level of meat hygiene. Based on the result obtained, corrective actions are indicated when counts exceed the acceptable limit for meat safety.

This technique enumerates the total bacteria count on fresh and chilled meat and hence determines the level of contamination. A *viable count*, which is a cultural method, assumes that a visible colony (colony forming unit-CFU) develops from one viable organism (bacteria). And therefore, by counting the number of CFU, it is possible to estimate the bacterial load in the meat food and its hygienic status by comparing against a set standard. Excessive numbers of bacteria above standard indicate poor hygiene.

For Performance criteria: Refer table 1

Sampling method: Refer annex 1

Test method/procedure: Refer annex 9

B. **Yeast and mould count**

Description: The yeast and mould count estimates the number of viable aerobic mould and yeast per gram or ml of product. A portion of the feed/food homogenate is mixed with a specified agar medium and incubated under specific conditions of time and temperature. It is assumed that each viable aerobic mould and yeast will multiply under these conditions and give rise to a colony.

C. **Detection salmonella**

Description: Samples can be plated directly onto highly selective and differential agars such as *Salmonella–Shigella* (*SS*) agar, xylose-lysine-deoxycholate (XLD) agar, xylose lactose tergitol 4 (XLT-4) agar, Hektoen enteric (HE), and MacConkey's agar plates. However, many types of specimens, such as food, blood, vomitus, and tissues may contain only a very low concentration of *salmonellae,* sometimes with large numbers of other bacteria, may need to pre-enrich and enrich in non-selective media, then culture in selective or differential media to form single colonies for identifying *Salmonella*.

For Performance criteria: Refer table 1

Sampling method: Refer annex 1

Test method/procedure: Refer annex 10

D. **Detection and enumeration of coliforms**

Description: Coliforms are a group of bacteria which are rod-shaped gram negative non spore forming and lactose fermentors with the production of acid and gas when incubated at 35-37^{0}c. The presence of these organisms on the surface of carcasses is an indicator of faecal and environmental contamination. The majority of coliforms are not disease causing by themselves, but their presence in the food can indicate presence of other pathogenic bacteria. This procedure describes the method for microbial examination of carcass to determine the coliform count.
Graduated amount of diluted sample are transferred to a series of fermentation tubes containing lactose or lauryl sulphite tryptose broth of proper strength and inoculate to three fermentative tubes. The tubes are incubated at 35$\pm$ 0.5 ^{0}C for 24 and 48hrs. The formation of gas in any of the tubes with in 48hr, regardless of the amount, constitutes as positive for coliform and the absence of gas formation within this period considered as negative for coliform. Confirm the coliform by Brilliant Green Bile medium (BGB).

For Performance criteria: Refer table 1

Sampling method: Refer annex 1

Test method/procedure: Refer annex 12

E. **Detection and enumeration of *S. aureus***

Description: The enumeration of *S. aureus* involves inoculation of the surface of the selective agar medium with a specified volume of a 10^{-1} and other appropriate decimal dilutions of the test sample and incubation at 37°C for 48 hours. Calculation of the number of *S.* aureus cfu per gram or ml of sample is made from the number of typical and/or atypical colonies obtained on the selective medium and subsequently confirmed by DNase and coagulase tests.

For Performance criteria/specification: Refer Table 1

Sampling method: Refer annex 1

Test method/procedure: Refer annex 3

Result interpretation and judgment on microbiological quality

The standard is described in two measurements based on the method of sample collection employed. These are:

1. CFU/g(in destructive sample collection method, to indicate the number of bacteria per one gram of meat) and
2. CFU/cm2 (in swab sample collection, to indicate the number of bacteria per one centimeter square of meat surface)

Table 1: Specification for microbial quality of meat according to Ethiopian Standards

No.	Sample	Characteristics	Test method	Specification	Specification. No.
1.	**Meat and meat products of Beef**	Aerobic plate count	ES ISO 4833	10^6 CFU/g	ES 1111: 2019
		E-coli	ES ISO 16649-2	10^2 CFU/g	
		Coagulase positive *Staphylococcus Aureus*	ES ISO 6888-1	10^2 CFU/g	
		Clostridium perfringenes (for vacuum packed products)	ES ISO 7937	Absent in 25 grams	
		Salmonella	ES ISO 6579	Absent in 25 grams	
		Listeria monocytogens	ES ISO 11290-1	Absent in 25 grams	
2.	**Mutton and goat carcass and cuts**	Aerobic plate count	ES ISO 4833-1	10^6 CFU/g	ES 1202:2015
		E-coli	ES ISO 16649-2	10^2 CFU/g	
		Coagulase positive *Staphylococcus Aureus*	ES ISO 6888-1	10^2 CFU/g	
		Clostridium perfringenes (for vacuum packed products)	ES ISO 7937	Absent in 25 grams	
		Salmonella	ES ISO 6579-2	Absent in 25 grams	
		Listeria monocytogens	ES ISO 11290-1	Absent in 25 grams	

Table 2: Microbial criteria of slaughterhouse and contact surfaces swab, meat handler and water.

Sample category	Microorganism	Interpretation		Method Reference
		Satisfactory	unsatisfactory	
All contact surface, personnel and room swab	Total viable counts	0 – 10/cm2	>10/cm	
	Enterobacteriaceae	0 – 1/cm2	>1/cm2	
	Salmonella	not detected	If detected	
	E-Coli	not detected	If detected	
Air plate count (environment)	TVC	1-15/plate	>15/plate	
Water	Coliform	0-1cfu/ml	>1cfu/ml	
	APC	0-1cfu/ml	>1cfu/ml	
	E. coli	0-1cfu/ml	>1cfu/ml	

6.2. Physiochemical analysis of meat

The use of veterinary drugs such as antimicrobials, growth promoters, Non-steriodal Anti-inflamatory drugs (NSAIDs), tranquilizers, etc. in food animals, can lead to human health problems.

6.2.1. Antimicrobial residual test in meat

Residues of such drugs may be present in foods of animal origin, including meat and poultry, milk and dairy products, eggs, fish and seafood, and honey. These drug residues may lead to immediate toxicities such as allergic reactions or longer-term health problems such as cancer or disturbances of the human microbiota. Perhaps most importantly, antimicrobial drug use in food animals can cause antimicrobial resistance to develop in bacteria which may lead to severe, untreatable, human infections.

6.2.2. Anthelminthic residual test in meat

The presence of veterinary drug residues found in animal-derived foods can occur through both legal and prohibited uses and can occur *via* direct administration of the drug to the food animal, through the environment, or in other ways, with varying levels of residues depending upon on animal characteristics and administration methods.

6.2.3 .Residual test for Banned substances in meat.

Drug and chemical residual test should be carried out at highly equipped laboratory center like **animal origin, feed and veterinary drug quality assessment center** to ensure meat safety in export abattoirs.

- ✓ Chloramphenicol residual test in meat
- ✓ Nitrofurans residual test in meat

PH analysis of meat, Chlorine concentration analysis of potable water and pH analysis of potable water should be analyzed on daily basis

7. NECESSARY TESTS AT ABATTOIR LABORATORY LEVEL

Routine hygiene monitoring tests like total plate count and coliform count should be implemented in an abattoir laboratory. These tests are used to monitor meat hygiene, personal hygiene of meat handlers, contact surfaces, laboratory room and environmental hygiene. In addition, simple tests like pH analysis of meat and water as well as chlorine concentration analysis of process water should be routinely carried out in abattoir laboratory.

Other required tests like detailed microbial characterization, diagnostic tests as well as physico-chemical analysis are recommended to be carried out at well-equipped external laboratories like **animal origin, feed and veterinary drug quality assessment center.**

8. DOCUMENTATION

Laboratory documentation should be readily available to the Quality Control Department:

- ✓ specifications;
- ✓ procedures describing sampling, testing, records (including test worksheets and/or laboratory notebooks), recording and verifying;
- ✓ a procedure for the investigation of out of specification and anomalous results and out of trend results;
- ✓ procedures for and records of the calibration/qualification of instruments and maintenance of equipment;
- ✓ testing reports and/or certificates of analysis;
- ✓ data from environmental (air, water and other utilities) monitoring, where required;
- ✓ validation records of test methods, where applicable

Some kinds of data (test results, yields, environmental controls) should be recorded in a manner permitting trend evaluation. Any out of trend or out of specification data should be addressed and subject to investigation.

In addition to the information, which is part of the batch documentation, other raw data such as laboratory notebooks and/or records should be retained and be readily available.

9. ASSURING THE QUALITY OF TEST RESULTS

Laboratories must have appropriate documented procedures for monitoring the validity of test results including media quality control and verification of test results as per relevant standards and guidelines. Laboratories must be furnished with all equipment necessary to ensure the validity of tests. Equipment must be properly maintained and calibrated as per relevant standards and guidelines where applicable.

Control cultures must be used in daily test and media quality control. Laboratories must have documented procedures for handling, maintaining, preparing and use of control cultures.

10. INTERPRETATION OF TEST RESULT

Interpretation of results for carcass *E. coli* and APC1 is based on a 'three-class sampling plan'. The performance of a three-class sampling plan is defined by a value at or below which the results are acceptable (m), a value above which the sample is unacceptable (M) and the number (c) of marginal samples (>m but ≤M) in a defined sample window of (n) samples e.g.:

- ✓ Acceptable – less than or equal to the acceptable value ≤ m)
- ✓ Marginal – greater than m but not higher than the upper limit of acceptability M(>m, but ≤ M])
- ✓ Unacceptable – greater than M (>M).

Four categories of microbiological quality shall be used based on standard plate counts, levels of indicator organisms and the number or presence of pathogens. These are satisfactory, marginal, unsatisfactory and potentially hazardous.

- ✓ **Satisfactory:** results indicate good microbiological quality. No action required.
- ✓ **Marginal: R**esults are borderline in that they are within limits of acceptable microbiological quality but may indicate possible hygiene problems in the preparation of the food.
- ✓ **Action**: Re-sampling may be appropriate. Premises that regularly yield borderline results should have their food handling controls investigated.
- ✓ **Unsatisfactory:** results are outside of acceptable microbiological limits and are indicative of poor hygiene or food handling practices.

Performance Standard - *E. coli* and APC on carcasses

The department has established target limits for generic *E. coli* and APC that are assessed on a moving window of 15 consecutive samples to allow for continuous evaluation of performance. The latest result is compared to the previous 14 results (n-1) to determine if the performance standard has been met.

In order to allow for corrections in the process to be evaluated the window must be 'reset' after each failure and implementation of corrective and/or preventive action.

A window of n=15 samples will fail if the number of marginal results (>m but ≤M) exceeds 'c'; or a single result exceeds M. Such results will trigger an 'ALERT '.

10.1. CORRECTIVE ACTIONS

If the result is exceeding the acceptable limit of bacterial count, the following corrective actions are indicated.

- ✓ Consideration should be given to the withdrawal of any of the food still available for sale or distribution and, if applicable, recall action may be indicated.
- ✓ An investigation of food production or handling practices should be investigated to determine the source/cause of the problem so that remedial actions can commence.
- ✓ Further samples are taken for testing.
- ✓ If these return good or acceptable results no action is taken.
- ✓ If these return unacceptable results, the business is inspected to determine if food handling controls and hygiene practices are adequate.
- ✓ A product withdrawal may be considered while further testing occurs Inspect supplier to determine if food handling controls and hygiene practices are adequate; consider a product recall.

11. REPORTING

All test results with one or more typical colonies must be reported irrespective of the minimum acceptable level of contamination for that particular sample. A zero result (i.e. no colonies on plates) is reported as less than (<) the limit of detection of that method (this must include consideration of the dilutions used by the laboratory).

Laboratories must report the actual result i.e. less than the lower limit of detection, greater than the upper limit of detection or an actual number between these limits, as appropriate.

Laboratories must ensure that enough dilutions are tested to enable quantitative results to be reported down to the lower limit of detection for the method used to analyse the sample.

Results of swab testing for generic microbial test on carcass must be reported as CFU/cm2 of carcass surface.

All samples tested for *Salmonella* are reported as 'Negative' or 'Positive'based on the test result being not detected or detected.

The report should fully and accurately reflect the raw data. This means that everything which happened during the study should be reported, but does not necessarily mean that every, single item of raw data must be included in the report. The report should, however, allow the reader to follow the course of the experiment and the interpretation of the data without the need to refer to other material not included. The report should not be a selection of the "highlights" of the test leaving out the parts that did not "work" or where restarts were needed for one reason or another. The report should certainly include any aspects where the test conduct deviated from that laid down in the protocol or SOPs, whether this is considered to have impacted on the test integrity or not. All reports usually contain the following information:

- ✓ Title of the test, date tested, date of report, name of abattoir, reporter name and approved name.
- ✓ Purpose of the test
- ✓ Description of the test
- ✓ Identification of the test
- ✓ Description of the test proceduretest procedure
- ✓ Test results
- ✓ Data summaries (tables, graphs, etc.) if available
- ✓ Computations and analysis
- ✓ Conclusions and comparisons to standards and expectations
- ✓ Appendices with supporting material

It is particularly important to annotate the "test results" section so that the reader can understand the significance of each table, graph, etc. Conclusions and comparisons to theory and expectations should be quantitative whenever possible. Variation expressed as a percentage usually carries greater significance than absolute variation. Reasons for the variations must also be given whenever possible.

The report need not be lengthy or elaborate. However, it must be neat and well organized. Use good grammar and correct spelling.

The laboratory shall have appropriate documentation and procedures for the reporting of results. This must include procedures for reporting results directly to department on-plant staff or central office while they are reported to the establishment management. In the case of third-party laboratories sub-contracted to perform certain tests the laboratory must instruct such laboratories to report results directly to the department at the same time they are reported to the establishment.

All reports issued by the laboratory should have as a minimum the following information:

- ✓ Unique report number;
- ✓ Laboratory/establishment name;
- ✓ Date of report;
- ✓ Identification of the method used;
- ✓ Identification of any ambiguous conditions associated with the test;
- ✓ Date of receipt of the sample including unique sample identification;
- ✓ Date of testing;
- ✓ Test results including units e.g., CFU/cm2;
- ✓ Name and signature of the person authorizing the report.

If requested the laboratory can include in the report as needed opinions and interpretations. Justification for these opinions and interpretations shall be documented by the laboratory.)

Table 3: Verification of environmental quality report

Date of sampling: _____ Date of analysis: _____Date of reporting: ____

Q.C. Code	Area of Exposure/point of collection	Type of microbial test and result				Remarks
		TPC	Coliforms	Yeasts & Moulds	Other /specifiy	

Performed by: _____ Verified by: _____ Date: _____

Table 4: Verification of effectiveness of hand wash report

Date of sampling: _____ Date of analysis: _____Date of reporting: _____

Q.C. code	Name of the Food Handler	Type of microbial test and result (CFU/cm2)				Remarks
		Coliforms / per hand	Total Plate count	*Salmonella*	Other/specify	

Performed by: _____ Verified by: _____ Date: _____

Table 5: Verification of sanitation of product contact surface report

Date of sampling: _____ Date of analysis: _____Date of reporting: _____

Q.C. code	Sampling area/Type of material	Type of microbial test and result in CFU/cm2				Remarks
		Total plate count	Coliforms	*Salmonella*	Other/specify	

Performed by: _____ Verified by: _____ Date: _____

Table 6: Meat Microbiological Examination report format

Date of sampling: _____ Date of analysis: _____Date of reporting: _____

Q.C. Code	Sampling Location	APC / cm^2	*E. coli* /cm^2	Coliforms/ cm^2	Y&M /cm^2	*S. aureus* /cm^2	*Salmonellae* / cm^2

Performed by: _____ Verified by: _____ Date: _____

Table 7: Chlorine concentration and pH analysis of process water report format

Date of sampling: _____ Date of analysis: _____Date of reporting: _____

Q.C. Code	Date	Point of collection	pH	Chlorine concentration	Remark

Performed by: _____ Verified by: _____ Date: _____

Table 8: Microbiological water analysis report format

Date of sampling: _____ Date of analysis: _____Date of reporting: _____

Q.C. Code	Source/point of collection	Type of microbial test and result per 100ml			
		TPC/ml	Coliforms /100ml	*E. coli* /100ml	*Salmonellae* /100ml

Performed by: _____ Verified by: _____ Date: _____

Table 9: pH analysis of meat

Date of sampling: _____ Date of analysis: _____Date of reporting: _____

Q.C. Code	Date	Point of sample tested	pH	Remark

Performed by: _____ Verified by: _____ Date: _____

Table 10: General result reporting format

Q.C code	Test Type	Sample	Result obtained	Overall judgment against standard			Remark
				satisfactory	Marginal	unsatisfactory	

Prepared by: Name_____sign_____Date_____

Approved by: Name_____sign_____Date_____

Table 11: Register for residual chlorine in process water and sanitizing solutions.

Date of sampling: _____ Date of analysis: _____Date of reporting: _____

Date	Time	Q.C. Code	Location/point of collection	PPM of free Chlorine	Remarks

Performed by: _____ Verified by: _____ Date: _____

Annex I: SOP for sampling and sample processing for monitoring microbial quality test in abattoir.

The main aim of abattoir laboratory is to monitor hygiene of the meat production process. Only limited types of laboratory test are performed at abattoir level. The main tests include meat microbial test, slaughterhouse hygiene monitoring, personal and equipment hygiene monitoring and some simple diagnostic tests like gram staining. Detailed and confirmatory laboratory tests should be done at higher laboratory centers outside abattoir in case any suspicious microbial agent is found. Overall procedure of sample collection, processing as well as result interpretation of each test is discussed in this topic.

i. Surface swab sampling method

Swab method is applicable to the collection of bacterial samples from flat surfaces like external part of carcass, equipment and personnel. In the swab method sterile cotton swab is rubbed vertically and horizontally against the limited surface area of material to be sampled. This method is used to collect swab sample from flat surfaces to estimate sanitation level. Approximately 25cm^2 will be covered by moistened swab (swab should be pre-moistened using sterile rinse medium). Cotton tipped sterile swabs can be moistened using sterile distilled water or brain-heart infusion broth (BHI) while taking samples from different surfaces. The swab should be stroked in close parallel sweeps over the defined sampling area while being slowly rotated. Sampling of the same area should be repeated, stroking the same swab perpendicular to the initial sweep. After sampling the sample site surface should be cleaned to remove any residue of the rinse medium. Surface swab samples will be collected from different areas of carcass, slaughterhouse, meat handlers, different contact surfaces and equipment.

ii. Destructive sampling method

This sampling technique is used to determine the bacterial count in a particular weight of meat and other food materials and the result is expressed in terms of colony forming unit per gram of sample (cfu/g). The destructive method of sample collection applies to determination of bacterial count in gram of sample like totalviable count (TVC), coliform count, *Salmonella* test, *Listeria monocytogens* and other spoilage and pathogenic microorganisms for samples collected from meat and other solid food materials.

At the relevant places of the carcass, about 2mm thick samples are cut delineated by sterile templates using sterile scalpels and forceps. Clean and sterilize scalpels and forceps after each sampling as follows:

a) Clean with cotton wool dipped in 70% ethanol.
b) Dip in 70% ethanol in a bottle

Burn the ethanol off; if the use of a naked flame is hazardous, and then allow the ethanol to evaporate.

Sample labeling and sample code

Sample code is very important in microbiological test result. During reporting every abattoir should have been similar sampling code for each date of sampling under every months of the year of each test results.

Entering the details of the test sample:

- ✓ Ensure that all the test sample details are entered in the incoming sample registers before samples are taken for testing.
- ✓ For the sake of convenience each of the testing laboratory can open different incoming sample registers for different types of the products as mentioned below
    a) Meat Sample
    b) Meat Byproduct Sample
    c) Water Sample
    d) Others (contact surface sample, hand wash sample)
- ✓ Allot QC codes for all the test samples to uniquely identify the sample during testing process.
- ✓ QC code are to be given as per below system.
    - QC code: **TX-Y better** (ATX-Y), A stands for Abattoir, others are the same

  Where:

“T” stands for type of product/Sample.

1. Meat sample
2. Meat Byproduct
3. Water Sample
4. Others Sample

“X” stands for Month Code.

A. September
B. October
C. November
D. December
E. January
F. February
G. March
H. April
I. May
J. June
K. July
L. August

M. “Y” stands for the sample number received in month.

In case the number sample, exceeds more than 99, these numbers can be further continued.

Examples for above illustration,

QC Code: 1A-01

Where:

1-is meat sample.

A-is for month September.

01-is the first sample of meat received in the laboratory in the month September. Like this please allot numbers throughout the month. Next months start against from with next month code and 01.

- ✓ After allotting QC Code for the test sample. Ensure that the test sample is labeled with QC code with marker pen or sticker.

Annex II: SOP for selection of carcass for sampling

In most instances, the number of carcasses tested is proportional to the production volume. Carcasses from different shifts, slaughter chains, species and/or chillers must be sampled and tested independently based on the production volume for each shift, chain, species or chiller. Carcasses should also be selected randomly from those available for sampling.

One of the key elements of the microbiological testing is to assess and validate chiller performance. Therefore, sampled carcasses should not be selected from one chiller only, but all chillers must be included in the sampling frame for the selection of sample carcasses.

Where these sampling frequencies do not achieve a minimum of one test per day for indicators of process control i.e. *E. coli* and APC, establishments should seek to achieve this minimum level of testing.

Sampling location/time

Carcasses are sampled according to the requirements identified in Table 1. The department recognizes that the sampling time may vary from operation to operation, however the time of sampling should be consistent within an operation to the extent possible

Table 12: Required sampling time for different species.

Species	Sampling time
Cattle, pigs,	After a minimum of 12 hours active chilling
Sheep and goats	After a minimum of 4 hours active chilling
Hot boned/warm cutting carcasses	After completion of dressing at a point immediately prior to exiting the slaughter floor and following any final carcass wash or hot water decontamination treatment that may be employed
Hot bagged carcasses	Before bagging (i.e. similar to hot boned carcasses).

Where it is not possible to begin testing of samples within 24h of collection, selected carcasses should be appropriately retained for sampling at a more suitable time e.g. carcasses randomly selected on Friday afternoons, Saturdays, Sundays or public holidays.

Sampling of Carcasses

For species other than wild game, separate carcasses or carcass sides must be selected for sampling for *Salmonella* and indicator organisms (*E. coli* and APC).

Note: In the case of wild game, the same swab can be used for all tests.

The number of samples required to be collected for a shift is based on the establishment's throughput for that shift.

A sterile template defining the specific area to be swabbed should be used to aid in the collection of the sample. Commercially available disposable templates can be used, alternately clean templates can be sterilized by one of the following methods;

- ✓ Immersion in 82°C water for 10 seconds. Remove the template and air dry.
- ✓ Dipping into ethyl alcohol followed by flaming.
- ✓ Wiping with 70% isopropyl alcohol and air dry, or
- ✓ Autoclaving at 121°C for 15 minutes.

Sterile templates should be placed on each of the identified carcass sampling sites and the site swabbed with a sponge. Templates must be sterilized between carcasses or a new sterile template used for each carcass.

Swabbing Materials used for sampling are:

- ✓ Whirl-packTM or
- ✓ MicrodiagnosticsTM.

For further details about swabbing techniques, see Appendix 1.

Note: Other suitable sponges may be available and can be used as long as they are approved by the department.

The intent of this program is to sample carcass at sites that represent the greatest level of contamination. Sites listed in this section for the different slaughter species have been identified in the scientific literature to be the sites most likely to be contaminated during slaughter and dressing.

If there is evidence that these sites do not represent areas of likely contamination at individual establishments then alternate sites may be nominated by the establishment and agreed to by the department. Reasons for selecting alternate sites may include:

Unavailability of sites due to dressing procedures at the establishment

Treatment of the sites in such a way as to render them non-representative of the carcass as a whole i.e.

- ✓ Intensified targeted trimming of these sites.
- ✓ Treatment of the site with an antimicrobial intervention (i.e., steam vacuum) that is not uniformly applied to the carcass surface.

Large stock

Bovine (Cow/Bull & Steer/Heifer)

Sampling sites (S1, S2 and S3) for large animal carcasses are shown in Figure 1. Three sites each of 100 cm2 must be sampled. Use one side of the sponge to sample the flank and brisket and use the other side to sample the butt.

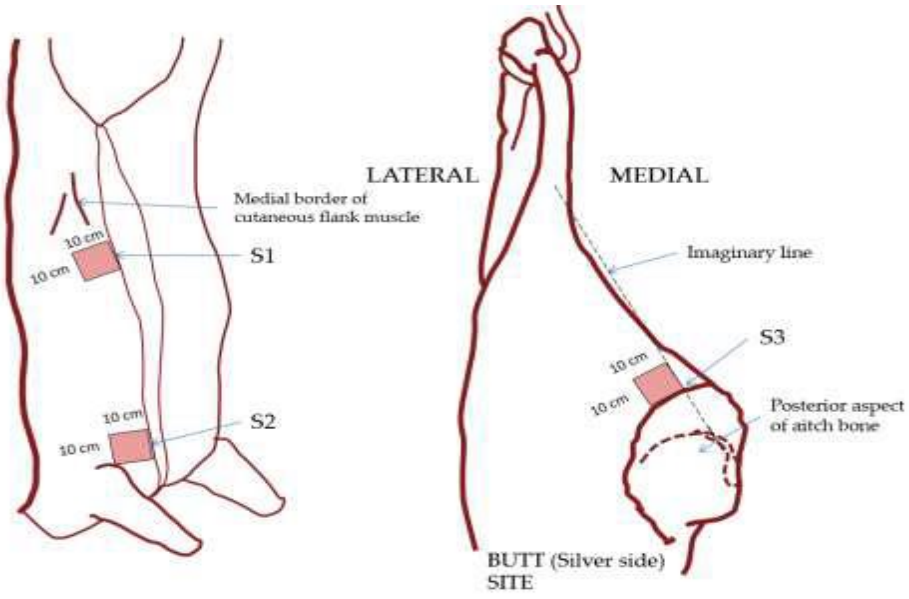

Figure 1: Sampling sites for large stock carcasses

S1, locate the cutaneous flank muscle (external abdominal oblique) and follow the medial border of the muscle anterior until it comes approximately within 7.5 cm from the mid-line. Place your template and swab the area with a sponge starting from the midline.

For S2, locate the elbow of the carcass. Draw an imaginary line straight across (medially) to the midline cut.

For S3, locate the posterior aspect of the aitch bone. Draw an imaginary line towards the Achilles tendon. At the point where the line intersects the cut surface of the round is the starting point for the butt sample. Measure 10 cm up the line leading to theAchilles tendon, then 10 cm over (laterally), then 10 cm back to the cut surface on the round, then 10 cm along the cut surface to form the 10 cm square area.

Samples are collected in the sequence of S1 first, then S2 and S3.

Small stock

Ovine (sheep), Caprine (Goats), Calves and lambs

Sampling sites (S1, S2 and S3) for small stock are shown in Figure 2. Three sites each of 25 cm2 are sampled. Use one side of a sponge for flank and brisket and use the other side of the sponge for the mid-loin.

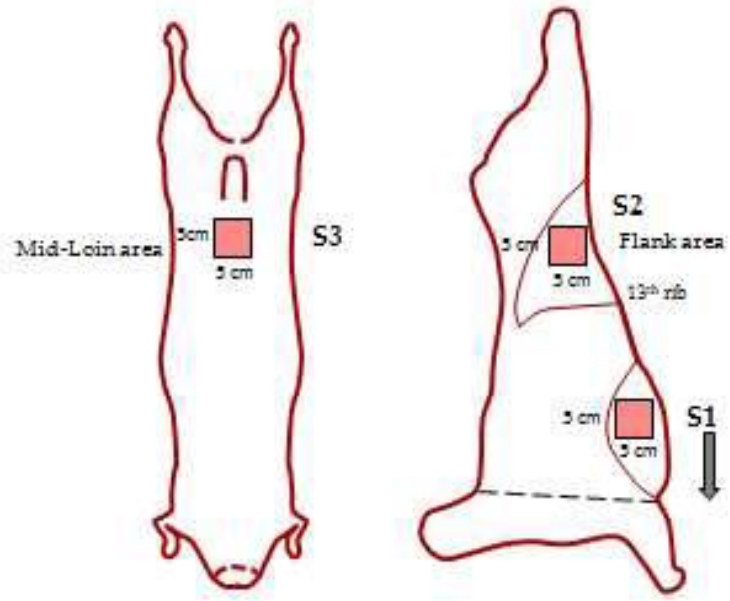

Figure 2: Sampling sites for small stock

For S1, Locate the elbow of the carcass. Draw an imaginary straight line from the angle of the elbow dorsal to the midline. Please note that the site of testing will be as pointed by the arrow.
For S2, locate the caudal edge of the 13th rib. Place your template 7.5 cm above the caudal edge of the 13th rib going outside (latero-dorsally).
For S3, locate the base of the tail. For sheep/lambs/ goats this is approximately 8 cm below the base of the tail, and for calves and deer approximately 12 cm below the base of the tail.
Samples are collected in the sequence of S2 first, then S1 and S3.

Annex III: SOP for carcass sampling frequency

Carcass Sampling Frequency *E. coli* and APC

Samples are taken at a frequency based on the volume of production. Minimum sampling rates for each slaughter class are provided in Table 2. The intention of these minimum rates is to ensure that at least one sample is collected daily at the establishment.

The sampling frequency must be determined separately for each slaughter class, chain and shift. Analysis for generic *E. coli* and APC can be performed from the same swab.

Table 13: *E. coli* and APC sampling frequency and area for different species

Class of Stock*	**Sampling Frequency**	**Sampling Area**
Steer/Heifer, Horse, Donkey, Mule, camel, cow/Bull	1 test per 300 carcasses	300 cm2
Pig	1 test per 1000 carcasses	300 cm2
Sheep/goat/lamp/calf/	1 test per 1000 carcasses	75 cm2

Establishments (abattoir) should collect a minimum of one sample per day for indicators of process control i.e. *coli* and APC.

Salmonella

Samples shall be taken at a frequency based on a slaughter establishment's volume of production at the minimum rates detailed in Table 3.

Class of Stock*	**Sampling Frequency**	**Sampling Area**
Steer/Heifer, camel, cow/Bull	1 test per 1500 carcasses	300 cm2
Pig	1 test per 5000 carcasses	300 cm2
Sheep/goat/lamp/calf/	1 test per 5000 carcasses	75 cm2

Table 14: *Salmonella* sampling frequency for different species

Annex IV: SOP for sample handling and transport

The following requirements for the handling and transport of specimens apply across all testing programs in this manual. Where samples are tested for APC, a temperature range of 0-5°C must be used. Where samples are not being tested for APC a wider acceptable temperature range can be used, i.e., 0-7°C.

All relevant documentation pertaining to the samples must be sent with the samples to ensure adequate identification of samples and notification of testing requirements to the laboratory. This information must include the date of collection, and the time that the first sample was collected.

Samples must be transported in appropriate packaging and should maintain sample temperature during transport such that on receipt at laboratory, the temperature of the sample does not preclude its testing.

Swab Samples

- ✓ Carcass swab samples must be packed in such a manner as to maintain the sample temperatures described above during transport to the laboratory. The procedure for preparing samples for transport to achieve this outcome must be documented in the establishment.
- ✓ Carcass or surface swabs must not be frozen for transport. If a delay in transport of the sample is expected, the carcasses should be put aside and sampled at a time when the transport time/temperature objectives can be met.
- ✓ Samples should be dispatched on the day of collection and analysis commenced on the day following collection and no later than on the second day following collection.
- ✓ Bags containing sample sponges should be firmly secured to prevent leakage.

Samples of fresh meat

- ✓ Samples of fresh meat must be packed in such a manner as to maintain the sample temperatures described above during transport to the laboratory. The procedure for preparing samples for transport to achieve this outcome must be documented in the establishment.
- ✓ Samples should be dispatched on the day of collection and analysis commenced on the day following collection and no later than on the second day following collection.
- ✓ Unless otherwise specified, unfrozen fresh tissue samples must not be frozen prior to or during transport.

Samples of frozen meat

- ✓ Frozen meat samples can be maintained frozen for up to 7-days after collection. Frozen meat samples can be held frozen during transport or transported at 0-5°C/0-7°C to allow thawing during transportation. Frozen samples must not be re-frozen once thawed or transported at 0-5°C/0-7°C. Samples arrive above specified temperatures.
- ✓ Where samples arrive at the laboratory at a temperature >7°C but <10°C, analysis for enteric bacteria (*E. coli*, *Salmonella*, etc.) can proceed but the laboratory must contact the department and the establishment to determine if the results of analysis are meaningful. (Samples tested for APC must be between 0°C and 5°C)
- ✓ Analysis should not be carried out on samples that arrive at temperatures >10°C without written approval from the department.
- ✓ In all cases where high temperature precludes analysis the laboratory must notify the department and establishment and a new sample provided.

Laboratory

On arrival at the laboratory, laboratory personnel must:

- ✓ Verify the integrity and temperature of the sample.
- ✓ Determine that analysis of the sample can commence on the day of arrival or no later than the day following receipt of the sample.
- ✓ Notify the department and the establishment should the time and/or temperature requirements not be achieved.

Establishment management

- ✓ If advised of a failure to control temperature during transportation, either due to loss of integrity, traceability or unacceptable temperature or time of arrival, establishments must notify the department immediately and put procedures in place to prevent further failures from occurring.
- ✓ The department Central Office (Residues and Microbiological Policy, Export Standards Branch) will determine if analysis of the sample can proceed or if a new sample is required to be collected by the establishment.
- ✓ If agreed that a new sample is required, the procedures for repeating sample collection should be initiated (by the establishment).

Annex V: Sample processing for microbial test

i. Serial dilution

In microbial quality analysis, sample collected from the designated object is dissolved in sterile saline water or peptone water and serially diluted to avoid overlapping of bacterial colony during culturing. The steps in preparation of serial dilution are discussed below.

a) Label 3 or more test tubes containing 9ml sterile peptone water from 10^{-1} - 10^{-3}
b) Shake the sample well and transfer 1ml from 100ml sample bottle to test tube labeled $10^{-1}$by using 1ml sterile pipette and homogenize for 5 to 10 seconds using vortex mixer.
c) From 10^{-1} test tube transfer 1ml to 10^{-2} labeled test tube, then homogenize using vortex mixer.
d) From 10^{-2} test tube transfer 1ml to 10^{-3} labeled test tube, then homogenize using vortex mixer and continue this step for all tubes according to the figure below.

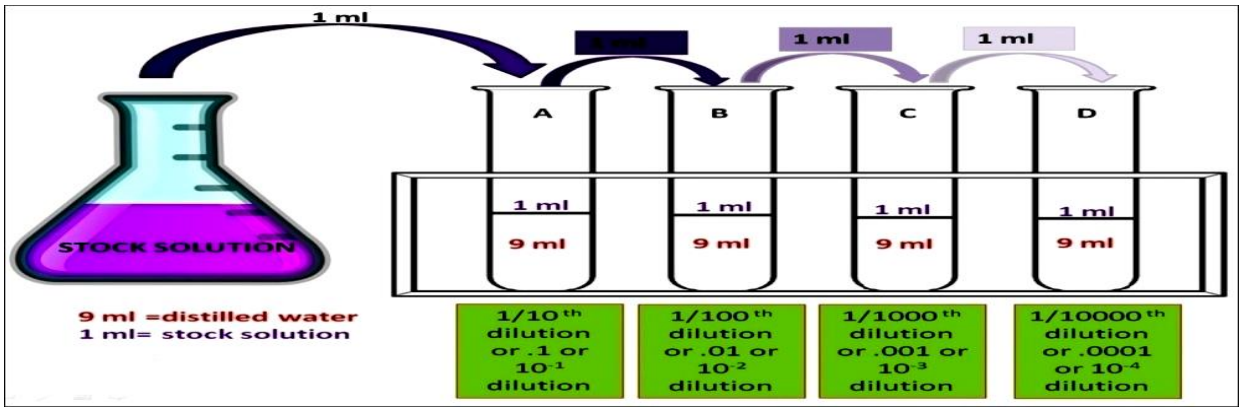

Figure 3: Serial dilution

Inoculation /plating

Inoculation or plating can be done by using spread plating or by pour plating technique.

a. Spread plate method.

If plate count agar is already transferred to petri-dish, warm the agar and transfer 0.1ml to two petri-dishes (double plating) from each dilution starting from high dilution until10^{-1} dilution. Then spread the transferred inoculums on the agar surface by using sterile bent Pasteur pipette and leave to dry.

Finally incubate at 37^0C for 24 up to 48 hours.

b. Pour plate method.

- ✓ Boil previously prepared and sterility checked plate count agar in water bath or on Bunsen burner with tripod stand.
- ✓ Once boiled transfer the test tube containing the molten agar to a water bath having temperature of 46 ± 1^0C
- ✓ While the agar is maintained at the required temperature in water bath, label sterile empty petri-dish in duplicate per dilution made in safety cabinet and invert so that the lid is on upper side.
- ✓ Transfer 1ml to two petri-dishes from each dilution (1ml per petri-dish) starting from higher dilution (10^{-3}) to lowest dilution (10^{-1}).
- ✓ Add 20ml of molten agar aseptically per petri-dish and carefully mix by swirling.
- ✓ Allow the agar to solidify.
- ✓ Invert the petri-dish and incubate at 37^0C for 24 up to 48 hours.

Colony counting and calculation.

Following growth of bacterial on the media, the colony is counted using colony counter or manual method and calculated to determine the number of bacteria per unit area or unit weight of sample which determines the bacterial load on the object. The formula is described below.

The formula for spread plate technique.

$$\text{CFU/100ml} = \frac{\text{average cfu/plate X 10 X total volume of water sample}}{\text{Dilution Factor}}$$

Example: If growth is observed on 2^{nd} serial dilution (10^{-2} dilution) from serial dilution made until 10-3 and if total bacterial count of plate A=4CFU and count of plate B=6CFU which gives average cfu/plate of (4+6)/2=5, because 0.1ml sample is taken from 100ml water sample.

$$\text{CFU/100ml} = \frac{5X10X100}{} = \mathbf{5x10^5 cfu/100m \text{ of water sample}}$$

10^{-2}

The formula for pour on plate technique.

$$\text{CFU/100ml} = \frac{\text{average cfu/plate X 10 X total volume of water sample}}{\text{Dilution Factor}}$$

Example: If growth is observed on 2nd serial dilution (10^{-2} dilution) from serial dilution made until 10-3 and if total bacteria count of plate A=4CFU and count of plate B=6CFU which gives average cfu/plate of (4+6)/2=5, because 1ml sample is taken from 100ml water sample.

$$\text{CFU/100ml} = \frac{5X100}{10^{-2}} = \mathbf{5x10^4 cfu/100m \text{ of water sample}}$$

Annex VI: List of general-purpose abattoir laboratory equipment

- ✓ Sensitive balances
- ✓ Thermometers
- ✓ Incubator
- ✓ Autoclave
- ✓ Hot air oven
- ✓ Automatic pipettes
- ✓ Microscope
- ✓ Bunsen burner
- ✓ Inoculating needle
- ✓ Test Tubes
- ✓ Plugs
- ✓ Petri dishes
- ✓ Flasks
- ✓ Refrigerator
- ✓ Water bath
- ✓ Centrifuge
- ✓ Safety cabinet
- ✓ Laminar flow bench
- ✓ Homogenizer and blender
- ✓ Colony counter
- ✓ Pasteur pipette
- ✓ Postmortem kit
- ✓ Icebox
- ✓ Templates
- ✓ Graduated cylinder.
- ✓ Sampling bottle
- ✓ Cotton swab
- ✓ Vortex Mixer
- ✓ Universal bottle
- ✓ Graduating cylinders
- ✓ Beaker
- ✓ Anaerobic jar and gas pack
- ✓ Stainless still tiffin for sampling
- ✓ Ice box with ice pack
- ✓ Burette
- ✓ Burette stand.
- ✓ Screw cupped test tube.
- ✓ Petri plate rack
- ✓ Test tube rack
- ✓ Inoculating loop

Annex VII: SOP for temperature measurement of air, carcass and cartons

Electronic digital thermometers should be used for all manual meat-related temperature measurements. The thermometer's probe should be of stainless steel, adequate length and not more than 3 mm in diameter.

About using Thermometers

- ✓ Thermometers must be calibrated at regular intervals
- ✓ Ensure the sensing tip of the thermometer is located at the point in the meat or air where the temperature is to be measured
- ✓ Avoid exposing the shaft of the thermometer probe to large temperature differences between the material being measured and its environment. This precaution will minimize thermal conduction from hot to cold areas, thereby eliminating the risks of conduction errors. To do this, insert the thermometer along the 'longest thermal path', leaving as little as possible of the shaft of the probe exposed to external temperatures whilst ensuring the sensing tip is located precisely at the area to be measured. Alternatively, wrap the exposed probe section with insulating material.
- ✓ Leave the thermometer in place until its reading stabilizes. In some cases this may require a minute or more
- ✓ Allow sufficient time for the thermometer to settle when it is moved from one environment to another, such as ambient conditions of 25°C to a freezer store at sub-zero temperature
- ✓ Clean the probe before use and regularly during use.

Calibration

The accuracy of thermometers should be checked regularly as part of a quality assurance program. Check the accuracy by measuring the temperature of a stirred mixture of ice and water in a vacuum flask. The ice/water mixture should be mostly ice; drain off excess water if the ice is floating. The temperature of this mixture is 0°C, assuming that reasonably pure water is used to make the ice. If the thermometer does not read 0°C, adjust the calibration of the instrument if possible, or make an adjustment to the temperature readings. For example, if the thermometer reads +0.8°C in ice, deduct 0.8°C from the reading of meat temperature. As with hand-held thermometers, it is important to check the calibration of temperature control systems regularly.

Air temperature measurement

Air circulating in cold rooms will vary in temperature; it will be coldest at the outlet of the refrigeration unit and warmest (on average) at the inlet. However, local variations will occur. For

example, the air will be warm near hot carcasses. It is important therefore to locate the sensor with care so that it measures the temperature at an appropriate position.

Measure air temperatures at the inlet to a refrigeration unit. Place the sensor in the air stream at least several centimeters away from the coils to avoid false temperature readings. Alternatively ensure that the sensor is shielded by means of a cover. This will eliminate errors due to radioactive heat exchange.

For manual routine measurements and spot checks, care should be taken to ensure that open doors and other openings do not result in misleading temperature readings being taken. The probe must be dry since a wet probe will result in a falsely low reading due to evaporation of moisture.

Measurement of deep butt temperature

Probes should be at least 150 mm, and preferably 180 mm, long and 3 mm in diameter. Correct deep butt measurements are dependent on correct insertion of the thermometer probe. The true deep butt temperature is measured by locating the tip of the thermometer probe against the Trochanter Major, which is the knob of bone on the opposite side of the femur to the hip joint.

Chilled beef

With beef, this point is approached by inserting the probe upwards through the Pope's eye at an angle of 15-20°. The tissue surrounding the bone can be felt through the thermometer probe. Aim the probe at an imaginary vertical line approximately one third of the distance from the Achilles tendon to the last tailbone.

Frozen beef hindquarters, small stock carcasses

Drill a hole matching the diameter of the thermometer probe from the outer surface of the frozen beef hindquarter until the surface of the Trochanter Major is reached.

Remove the drill and insert the probe firmly. Minimize the risk of conduction errors by angling the hole so that most of the length of the probe is inserted or wrap the exposed probe section with insulating material. Note the reading when it has stabilized.

In the case of lambs and whole pig carcasses, the best way to measure deep butt temperature is through the topside. Push the probe downwards through the topside of the hanging carcass, aiming for the junction of the femur bone and H-bone. It may be necessary to first drill a hole.

Surface temperature measurement

There are two methods of surface temperature measurement - contact and non-contact.

Contact measurement.

Measurement may be with a probe with a temperature sensor in the tip using any of the following methods:

- ✓ Insert the tip of the probe up to 5 mm under the carcass surface, the probe should be inserted parallel to and just under the surface.
- ✓ Press the side and tip of the probe hard against the fat or meat surface. The probe can be shielded from room air by holding the probe against the carcass surface with a dry sponge in a sterile plastic bag.
- ✓ Place the probe between the pressed together surfaces of two adjacent carcasses. Alternatively use loggers if they are available.

Non-contact measurement

Non-contact infrared thermometers can be used for carcass surface temperature measurement. Infrared thermometers are accurate, easy to use and have a response time of less than one second.

These instruments measure the radiant heat emitted by the surface and from this determine the temperature. The radiant heat emitted depends on the temperature and emissivity (a measure of the efficiency of emitting infrared radiation) of the surface.

Measurement in frozen cartons

In frozen cartons, drill a hole that matches the diameter of the thermometer probe. Drill from the centre of one side or end of the carton and parallel to the bottom of the carton. Use a clean drill of adequate length. Then insert the thermometer probe and wrap the exposed section of the probe with insulating material. Take care to locate its sensing tip at the thermal centre of the carton. Record the reading when it has stabilized.

Note that the thermal centre of a carton may be slightly above its geometric centre if, for instance, there is a substantial insulating air gap between the lid and the top surface of the meat. Check by drilling another hole approximately 20 mm above the first one and measuring a second temperature.

For cartons where thawing or softening of the surface of the meat block is evident, open the carton and insert the probe parallel with a top edge and within 5mm of the edge so that the sensing tip is at the location to be measured. Alternatively press the probe firmly along a top edge and insulate it with a dry sponge in a plastic bag.

Annex VIII: SOP for Total Plate Count (TPC) in meat

The load of microorganisms on fresh meat, including all spoilage and pathogenic bacteria determines safety, hygiene and shelf life of meat. Presence of microorganisms on meat in excess amount indicates poor hygiene in slaughtering process or cross contamination. Estimating population of microorganisms on meat is important to determine the level of meat hygiene. Based on the result obtained, corrective actions are indicated when exceeding the acceptable limit of meat safety.

This technique enumerates the total bacteria count on fresh and chilled meat and hence determines the level of contamination. A *viable count*, which is a cultural method, assumes that a visible colony (colony forming unit-CFU) develops from one viable organism (bacteria). And therefore, by counting the number of CFU, it is possible to estimate the bacterial load in the meat food and its hygienic status by comparing against a set standard. Excessive numbers of bacteria indicate poor hygiene.

This procedure applies to all bacterial count determination for samples taken by swab method from the Carcass at all stage of production.

Procedure

i. Collect surface swab sample from different parts of chilled or fresh carcass.
ii. Prepare sample in the laboratory based on the sampling technique within 1 hour of sample collection or store at refrigerator if the analysis time is extended.
iii. Perform serial dilution from 10^{-1} up to 10^{-6}
iv. Inoculation /plating: Inoculation or plating can be done by using spread plating or by pour plating technique.
v. Incubate at 32^{O}C for 24-72 hours.
vi. Count all bacterial colony on the plate containing countable colony using colony counter (Countable bacterial colony is between 25-300 colonies)
vii. Calculate number of bacteria per cm2 (cfu/cm2).

N.B: Further identification and isolation techniques should be employed at external laboratory for characterization of bacterial species (if required).

1. Coliform count in meat

Coliforms are a group of bacteria which are rod-shaped gram negative non spore forming and lactose fomenters with the production of acid and gas when incubated at 35-37^{0}c. The presence of these organisms on the surface of carcasses is an indicator of faecal and environmental contamination. Majority of coliforms are not disease causing by themselves, but their presence in the food can indicate presence of other pathogenic bacteria. This procedure describes the method for microbial examination of carcass to determine the coliform count.

Procedure

- ✓ Collect surface swab sample from chilled or fresh meat.
- ✓ Dissolve swab in sterile saline water and make serial dilution.
- ✓ Inoculate the sample on MaCconky agar using pour plate or spread method.
- ✓ Incubate at 32^{O}C for 24-72 hours.
- ✓ Count Pink colored colonies (coliforms) using colony counter (Countable bacterial colony is between 25-300 colonies)
- ✓ Calculate number of bacteria per cm2 (cfu/cm2).
- ✓ Compare the result against the set standards and report the result obtained**.**

Result Interpretation

All colonies with pink color which ferment lactose on MaConkeyagar are counted as coliform bacteria. Their presence at high levels provides a warning that unhygienic food handling may have occurred, or processing was not effective.

N.B: Further identification and isolation techniques should be employed for characterization of bacterial species.

Annex IX: Detection of *E.coli* in meat

Escherichia coli (E. coli) is a Gram-negative, short rods (1–2 μm in length), aerobic, and generally motile organism. Most*E. col*i strains are non-pathogenic and exist harmlessly in the intestinal tract of humans and animals. Pathogenic *E. coli* strains cause a variety of diseases including gastroenteritis, dysentery, hemolytic uremic syndrome (HUS), urinary tract infection (UTI), septicemia, pneumonia, and meningitis. However, the major concern in recent years has

been the increasing numbers of outbreaks of enterohemorrhagic *E.coli*, due to consumption of contaminated meat, fruits, and vegetables primarily in developing countries.

The objective of the test is **t**o check the *E.col*i bacteria load on the carcass (meat sample) for the purpose of ensuring safety of meat, before approving for human consumption. This procedure applies to *E.col*i bacteria for swab sample collected from carcass at all steps of production.

Procedure

Media preparation:

- ✓ Suspend 41.53gm of violet Red Bile Agar in 1000ml sterile distilled water and boil with stirring to dissolve the contents thoroughly. Do not autoclave the media, overheating may result in decrease productivity.
- ✓ Store sterile medium in the dark no longer than 2 weeks before use and remelt agar in following sterile steam, boiling water, or in a microwave oven. Cool to 48^{o}C before use; pH, 7.0-7.2
- ✓ Add one vial of MUG supplement to 500ml of VRBA medium prior to overlay for identification of *E.col*i within coliforms.
- ✓ Suspend Brilliant Green Bile Broth 2% in 1000ml of distilled water. Boil to dissolve contents completely, distribute 10ml in to test tubes having inverted Durham's tubes and sterilized by autoclaving at 15Ib pressure for 15 minutes.
- ✓ Suspend 37gm of *EC* (*E.coli*) Broth Hi media in 1000ml distilled water. Heat to dissolve the media. Dispense in to test tube containing inverted Durham's tubes and sterilized by autoclaving at 15Ib pressure for 15 minutes.

Sample collection, preparation and examination

- ✓ Collect sample using destructive or swab method as sampling protocol.
- ✓ Prepare sample in the laboratory based on the sampling technique within 1 hour of sample collection or store at refrigerator if the analysis time is extended.
- ✓ Perform serial dilution.
- ✓ Inoculate the sample on violet Red Bile Agar using pour plate or spread method.
- ✓ Incubate for 48 h at 35°C.

- ✓ Count Green metallic shine colonies that are 0.5mm or larger in diameter and surrounded by zone of precipitated bile acids. Plates should have 25-250 colonies.
- ✓ Calculate number of bacteria per cm2 (cfu/cm2) for swab method, and per gram of meat cfu/g for destructive method.

Interpretation

- ✓ **Colony characterization:** purple red in color, 0.5mm in diameter or larger and are surrounded by a zone of precipitated bile acid is screened out to be *E.Coli* and should be further characterized at external laboratory.

N. B: This procedure is only screening of suspected E. coli species in meat, not necessarily pathogenic strain. Further identification and isolation techniques should be employed at external laboratory for characterization of bacterial species (if there is suspected organism).

Annex X: Detection of Salmonella in meat

Salmonella species are enteric bacteria and can be found in the intestinal tract of animals, including birds. As such, *Salmonella* can frequently be isolated from raw foods of animal origin. Environmental contamination can also result in *Salmonella* being present in a wide variety of foods, although generally at lower numbers. Their presence in ready-to-eat foods may be a result of under cooking, poor handling practices and cross contamination.

Salmonella is a group of bacteria that includes several pathogens of significance in human food poisoning disease. They mainly arise from faecal contamination but can also arise from the processing environment. Ready-to-eat foods should be free of *Salmonella* as consumption of food containing this pathogen may result in food borne illness called salmonellosis. Salmonellosis is an important zoonotic infection, and human salmonellosis causes widespread morbidity and economic loss, the presence of this organism indicates poor food preparation and handling practices such as inadequate cooking or cross contamination. Consideration may also be given to investigating the health status of food handlers on the premises who may have been suffering from salmonellosis or asymptomatic carriers of the organism.

The objective of this test is to ensure production of *salmonella* free meat by regular quality monitoring test that can detect presence of *salmonella* species in meat before approving for

human conception. This technique applies to detection and enumeration of the *salmonella* species for sample collected from carcass by using selective media only. Detailed identification method is not indicated in this technique.

Principle of the test

The ten-fold dilution of food homogenates was pre-enriched with peptone water for 24 hours at 35 ± 1^{O}C. To enhance the recovery of Salmonella, pre-enrichments were followed by enrichment on Rappaport vassiladise medium (RV broth) 42 ± 0.5^{O}C for 22-24h. Detection of Salmonella was aided using the enriched cultures streaked onto Xylose Lysine Desoxycholate agar (XLD). The inoculated selective agar was incubated at 35 ± 1^{O}C for 18-24 hours and examined for typical *Salmonella* colonies. Large sized and completely black colonies were suspected of Salmonella.

Procedure

Media Preparation

- ✓ **Pre-enrichment:** Suspended 20gm of Buffered Peptone Water, in 1000ml-distilled water. Sterilize by autoclaving at 15Ibs pressure for 15 minutes.
- ✓ **Selective enrichment:** Suspend 49.2gm of Rappaport Vassiliadis Medium, in 1000ml distilled water, dispense into 10ml into test tube and sterilize by autoclave at 10Ibs(115^{O}C) pressure for 15 minutes.
- ✓ **Agar Media for Streaking:** The following two media to be used simultaneously for streaking.
    - Suspend 59gm B.G. Sulpha Agar in 1000ml of distilled water. Heat to boiling to dissolve the completely. Sterile by autoclaving at 15Ibs pressure for 15minutes. Pour into petri-dishes to prepare settle plates for streaking.

Prepare settle plates using XLT4Agar Base (xylose LusineTergitol 4 Agar), as per the manufacturer's instructions.

Sampling procedure

The sample collection method is destructive (template excision method).

At the relevant places of the carcass, about 25gm of 2mm thick meat samples are cut delineated by sterile templates using sterile scalpels and forceps.

Weigh 25 gm of meat sample into a sterile blender jar, other sterile jar, or a whirl-Pak or stomacher bag, if the sample size is 50g run pre-enrichment in duplicate.

- ✓ Add 225ml buffered peptone water. Blend two minutes or shake thoroughly.
- ✓ Incubate at $35 \pm 1^{O}C$.
- ✓ Transfer 0.1ml into 10ml RV broth test tubes.
- ✓ Incubate at $42 \pm 0.5^{O}C$ for 22-24h
- ✓ Samples are homogenized using a peristaltic stomacher at a speed of 250 cycles/min for 2 minutes using 100ml of MRD. The suspension contained in the stomacher bag is not a dilution and is represented in the calculation as the 10^{0} dilution.
- ✓ Streak on both BGS and XLD agar plates. Do not subdivide plates for streaking multiple samples; streak the entire agar plate with a single sample enrichment.
- ✓ Incubate at $35 \pm 1^{O}C$
- ✓ Examine in 18-24h. Re-incubate all plates for an additional 18-24h. Re-examine initially negative plates and those yielding non confirming salmonella colonies from the initial selection.
- ✓ Examine of and picking colonies from plating media. Pick typical well-isolated colonies.
    - **BGS.** Select colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium. On very crowded plates, look for colonies that given a tan appearance against a green background.
    - **XLD.** Select black colonies or red colonies with black centers. The rim of the colony may still be yellow in 24h; later it should turn red.

Counting and calculation

- ✓ Count (On XLD Agar) black colonies or red colonies with black centers.
- ✓ Count (on BGL agar) colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium.

Colony characterization

- ✓ On BGS. Colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium.
- ✓ On XLD. Black colonies or red colonies with black centers. The rim of the colony may still be yellow in 24h; later it should turn red.
- ✓ Bacteria of the genus *Salmonella* are Gram-negative, facultatively anaerobic, non-spore forming, usually motile rods belonging to the family Enterobacteriaceae.

Compare the result against the set standards, interpret and report the result.

N. B: This test does not necessarily indicate the presence of pathogenic salmonella species.Further identification and isolation techniques should be employed at external laboratory for characterization of bacterial species (if suspected agent is found).

Annex XI: Detection of yeast and moulds

Yeast and mold are eukaryotic with defined chitin and/or cellulose cell walls, nuclei, and other cellular organelles. All yeasts and moulds are heterotrophic. Heterotrophs lackchlorophyll and, thus, are dependent upon an outside source to meet their energy needs. This is the reason why they are commonly found as contaminants of foods and feeds - in the field, in storage during and after processing, and in the home. The basic units of structure of all molds and some yeasts are multicellular, filamentous, threadlike strands called hyphae.

Because of their slow growth and relatively poor ability to compete with bacteria successfully, the yeasts and molds are most likely to be found in the foods in which the environment is less favorable for bacteria growth, e.g., low pH, low moisture, high salt or sugar content, low storage temperature, the presence of antibiotics, or exposure to irradiation. Yeasts and molds present a problem in foods in that they discolor food surfaces, cause off-odors and off-flavors, cause various degrees of spoilage, alter substrates allowing for the outgrowth of pathogenic bacteria, and can produce mycotoxins in certain instances.

Objective: To check the yeast and mould load on the meat sample for the purpose of ensuring the quality of meat, before approving to human conception. This procedure applies to yeast and mold for swab sample collected from carcass at all s

Principle

In the past, acidified media were used to enumerate yeasts and moulds in foods. Such media are now recognized as inferior to antibiotic supplemented media that are formulated to suppress bacterial colony development, enhance resuscitation of injured fungi, and minimize precipitation of food particles. A medium, containing (a) adequate nutrients for growth of most yeasts and moulds and (b) antibiotics for inhibition of most bacteria, is inoculated with a given quantity of the product or with scrapings from equipment or the manufacturing environment. It is incubated at 22-25^{o}C for 3-5 days. Colonies appearing on the medium are then counted and/or examined.

Procedure

Sampling frequency

- ✓ The time and sampling frequency is governed by a lot of factors including the production volume and other risk factors.
- ✓ For practical purpose five carcasses on one day per week, after dressing and before chilling (Enterobacteriaceae at 37°C) could be enough.
- ✓ The day of sampling should be changed each week to ensure that every day of the week is covered.

Sample collection

- ✓ Apply sterile template of different size (20cm^2, 25cm^2 or 50cm^2 for carcass and personnel & 25cm^2, 50cm^2 or 100cm^2 for floor and wall sampling) on the area of sample collection.
- ✓ Moisten a swab in 10ml MRD.
- ✓ At each selected carcass site, press the template 25cm^2 (to provide a total of 100 cm^2 from the 4 sites) hard on to the surface.
- ✓ Rub the swab over the whole area first horizontally and then vertically so that all sides are covered.
- ✓ Place the swab into the diluent used to wet the swab, breaking of the wooden shaft against the inside of the bottle.

- ✓ Then with a dry swab, sample the area again as above and place this swab into the same container of the diluents. This is when conducting separate counts on those specific sites.
- ✓ For pooled sample counts(100cm^2), all the four swabs shall be diluted in 100ml of the diluents (equal to the surface area) and 1ml of this initial suspension represents 1cm^2 and the result is reported as per cm^2
- ✓ Every carcass should have an equal chance of being selected for sampling.
- ✓ Regarding sampling points according to Ethiopian standard (ES) ES ISO 17604: 2006 for beef four sites (brisket, flank, flank groin, round lateral) sites and lamb four sites (abdomen (flank), thorax(lateral), crutch, breast (lateral) representing a total area of 20 cm^2. These sites are selected on the bases of contamination prevalence (See Annex).
- ✓ Carcass identification: Code, date and time of sampling should be recorded for each sample.

Sampling: The four individual samples from each sample site are pooled and the 4 swabs from each carcass are then placed in a sterile container containing 100 ml of sterile diluent.

Media preparation: Suspend 32.15 gm of Rose Bengal Chloramphenicol Agar in 1000ml distilled water. Boil to dissolve the medium completely. Sterile by autoclave at 15Ibs pressure for 15 minutes.

Interpretation

Yeast: contain only a single cell. In appearance white and thread usually oval in shape

Mould: contains multiple identical nuclei. It grows in the form of hyphae of filaments. It has a fuzzy appearance and can be an orange, green, black, brown, pink or purple in color. It can be found in several shapes.

Annex XII: Total coliforms count in meat handlers.

The presence of coliforms on the body is an indication of faecal and environmental contamination. Majority of coliforms are not disease causing by themselves, but their presence in the food can indicate presence of other pathogenic bacteria. This procedure describes the method for microbial examination of meat handlers' hand to determine the coliform count.

Procedure

- ✓ Collect hand swab sample from selected meat handler.
- ✓ Prepare sample in the laboratory based on the sampling technique within 1 hour of sample collection or store at refrigerator if the analysis time is extended.
- ✓ Perform 2-5 serial dilution.
- ✓ Inoculate the sample on MaConkeyagar using pour plate or spread method described in guideline.
- ✓ Incubate at 32^{O}C for 24-72 hours.
- ✓ Count Pink colored colonies (coliforms) using colony counter (Countable bacterial colony is between 25-300 colonies)
- ✓ Calculate number of bacteria per cm2 (cfu/cm^2) on the hand.
- ✓ Compare the result against the set standards, interpret and report the result.

Result Interpretation

All colonies with pink color which ferment lactose on MaConkey agar counted as coliform bacteria.

Their presence at high levels provides a warning for ineffective hand washing.

Corrective action must be taken in exceeding the set standard.

N.B: Further identification and isolation techniques should be employed for characterization of bacterial species.

Annex XIII: SOP for detection of *E. coli* in meat handlers

A majority of *E. coli*strains are non-pathogenic and exist harmlessly in the intestinal tract of humans and animals. Pathogenic *E.coli*strains cause a variety of diseases including gastroenteritis, dysentery, hemolytic uremic syndrome (HUS), urinary tract infection (UTI), septicemia, pneumonia, and meningitis. The objective of the test is to check the *E. coli* bacteria load on the hand of meat handlers.

Procedure

- ✓ Collect swab sample from hand of selected meat handlers.
- ✓ Collect sample on the hand of meat handler based on the swab method with swabbing area of 25cm^2.
- ✓ Suspend 41.53gm of violet, Red Bile Agar in 1000ml sterile distilled water and boil with stirring to dissolve the contents thoroughly.

- ✓ Store sterile medium in the dark no longer than 2 weeks before use and remelt agar in following sterile steam, boiling water, or in a microwave oven. Cool to 48^{o}C before use; PH, 7.0-7.2
- ✓ Add one vial of MUG supplement to 500ml of VRBA medium prior to overlay for identification of *E. coli* within coliforms.
- ✓ Suspend Brilliant Green Bile Broth 2% in 1000ml of distilled water. Boil to dissolve contents completely, distribute 10ml in to test tubes having inverted Durham's tubes and sterilized by autoclaving at 15Ib pressure for 15 minutes.
- ✓ Suspend 37gm of *EC* (*E. coli*) Broth Hi media in 1000ml distilled water. Heat to dissolve the media. Dispense in to test tube containing inverted Durham's tubes and sterilized by autoclaving at 15Ib pressure for 15 minutes.
- ✓ Compare the result against the set standards, interpret and report the result.
- ✓ Forward corrective action if the result does not meet the standard.

N.B: Further identification and isolation techniques should be employed at external laboratory for characterization of bacterial species (if there is suspected organism).

Interpretation

- ✓ **Colony characterization:** Purple, red in colour, 0.5mm in diameter or larger and are surrounded by a zone of precipitated bile acid.

Annex XIV: SOP for detection of *Salmonella* on meat handlers

Objective of this test is **t**o check the *Salmonella* bacteria load on meat handlers.

Procedure

- ✓ Swab sample is taken from the hand of meat handlers with swab area of 25cm^{2}.
- ✓ Add 225ml buffered peptone water to swab sample. Blend two minutes or shake thoroughly.
- ✓ Incubate at 35 ± 1^{O}C
- ✓ Transfer 0.1ml into 10ml RV broth test tubes.
- ✓ Incubate at 42 ± 0.5^{O}C for 22-24h
- ✓ Streak on both BGS and XLD agar plates. Do not subdivide plates for streaking multiple samples; streak the entire agar plate with a single sample enrichment.

- ✓ Incubate at 35 ± 1^{O}C
- ✓ Examine in 18-24h. Re-incubate all plates for an additional 18-24h. Re-examine initially negative plates and those yielding non confirming *salmonella* colonies from the initial selection.
- ✓ Examine of and picking colonies from plating media. Pick typical well-isolated colonies.
    - **BGS.** Select colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium. On very crowded plates, look for colonies that given a tan appearance against a green background.
    - **XLD.** Select black colonies or red colonies with black centers. The rim of the colony may still be yellow in 24h; later it should turn red.

Counting and calculation

- ✓ Count (On XLD Agar) black colonies or red colonies with black centers.
- ✓ Count (on BGL agar) colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium.
- ✓ Calculate the number of N of microorganisms per cm^2.

Colony characterization

- ✓ On BGS. Colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium.
- ✓ On XLD. Black colonies or red colonies with black centers. The rim of the colony may still be yellow in 24h; later it should turn red.
- ✓ Bacteria of the genus *Salmonella* are Gram-negative, facultatively anaerobic, non-spore forming, usually motile rods belonging to the family Enterobacteriaceae.

Compare the result against the set standards, interpret and report the result.

Presence of pathogenic salmonella indicates poor hygiene and unsafe meat handling.

N.B: This test does not necessarily indicate the presence of pathogenic salmonella species. Further identification and isolation techniques should be employed at external laboratory for characterization of bacterial species (if suspected agent is found).

Annex XV: SOP for Total Plate Count on contact surface and equipment

The load of microorganisms on hand of food handler indicates the hygienic status. Presence of microorganisms indicates poor hygiene and inadequate hand washing. Regular detection of personal microbial load is important to ensure hygienic the meat handling practice. Based on the result obtained, corrective actions are indicated when exceeding the acceptable limit of meat safety.

Procedure

Sample Collection

Swab method is applicable for collection of samples from different equipment used in meat production like knife, weighing balance, chains, hook and wall of chilling room. In swab method sterile cotton swab is rubbed vertically and horizontally against the limited surface area of material to be sampled. Approximately 25cm^2 will be covered by moistened swab (swab should be pre-moistened using sterile rinse medium).

i. Prepare sample in the laboratory based on the sampling technique within 1 hour of sample collection or store at refrigerator if the analysis time is extended.
ii. Perform serial dilution from 10^{-1} up to 10^{-6}
iii. Inoculation /plating: Inoculation or plating can be done by using spread plating or by pour plating technique.
iv. Incubate at 32^{O}C for 24-72 hours.
v. Count all bacterial colony on the plate containing countable colony using colony counter (Countable bacterial colony is between 25-300 colonies)
vi. Calculate number of bacteria per cm2 (cfu/cm^2).

N.B: Further identification and isolation techniques should be employed at external laboratory for characterization of bacterial species (if required).

Corrective action is taken and monitored in case the result exceeds the set standard.

Corrective actions:

- ✓ Re assessment of cleaning facility and cleaning effectiveness
- ✓ Training and awareness creation on hygienic meat handling practice
- ✓ Interruption of meat production until good hygiene is ensured.

N.B: Further identification and isolation techniques should be employed at external laboratory for characterization of bacterial species (if required).

Annex XVI: SOP for coliform count on count surface and equipment

This procedure describes the method for microbial examination of product contact surfaces and slaughter room including wall of chilling room to determine the coliform count.

Procedure

- ✓ The procedure of Sample collection is like the point described.
- ✓ Prepare sample in the laboratory based on the sampling technique within 1 hour of sample collection or store at refrigerator if the analysis time is extended.
- ✓ Perform 2-5 serial dilution.
- ✓ Inoculate the sample on MacConkey agar using pour plate or spread method described in guideline.
- ✓ Incubate at $32^{O}C$ for 24-72 hours.
- ✓ Count Pink colored colonies (coliforms) using colony counter (Countable bacterial colony is between 25-300 colonies)
- ✓ Calculate number of bacteria per cm^2 (cfu/cm^2).
- ✓ Compare the result against the set standards, interpret and report the result.

Result Interpretation

All colonies with pink color which ferment lactose on MacConkey's agar counted as coliform bacteria.

Their presence at high levels provides a warning that unhygienic food handling.

Corrective action must be taken in exceeding the set standard.

N.B: Further identification and isolation techniques should be employed for characterization of bacterial species.

Annex XVII: SOP for detection and isolation of *E. coli* species on equipment and slaughter house

A majority of *E. coli*strains are non-pathogenic and exist harmlessly in the intestinal tract of humans and animals. Pathogenic *E. coli*s trains cause a variety of diseases including gastroenteritis, dysentery, hemolytic uremic syndrome (HUS), urinary tract infection (UTI), septicemia, pneumonia, and meningitis. Objective ofthe test is **t**o check the *E. coli* bacteria load on equipment, product contact surfaces and wall of slaughter house.

Procedure

Media preparation:

- ✓ Suspend 41.53gm of violet Red Bile Agar in 1000ml sterile distilled water and boil with stirring to dissolve the contents thoroughly. Do not autoclave the media, overheating may result in decrease productivity.
- ✓ Store sterile medium in the dark no longer than 2 weeks before use and remelt agar in following sterile steam, boiling water, or in a microwave oven. Cool to 48^{o}C before use; pH, 7.0-7.2
- ✓ Add one vial of MUG supplement to 500ml of VRBA medium prior to overlay for identification of *E. coli* within coliforms.
- ✓ Suspend Brilliant Green Bile Broth 2% in 1000ml of distilled water. Boil to dissolve contents completely, distribute 10ml in to test tubes having inverted Durham's tubes and sterilized by autoclaving at 15Ib pressure for 15 minutes.
- ✓ Suspend 37gm of *EC* (*E. coli*) Broth Hi media in 1000ml distilled water. Heat to dissolve the media. Dispense in to test tube containing inverted Durham's tubes and sterilized by autoclaving at 15Ib pressure for 15 minutes.

Sample collection, preparation and examination

- ✓ Collect sample on the hand of meat handler based on the swab method with swabbing area of 25cm^2.
- ✓ Sample preparation, processing, examination and calculation is similar to that of the technique described in 8.1.3.
- ✓ Compare the result against the set standards, interpret and report the result.
- ✓ Forward corrective action if the result does not meet the standard.

Interpretation

- ✓ **Colony characterization:** purple, red in color, 0.5mm in diameter or larger and are surrounded by a zone of precipitated bile acid.

N.B: Further identification and isolation techniques should be employed at external laboratory for characterization of bacterial species (if there is suspected organism).

Annex XVIII: SOP for microbial analysis of process water

In export abattoir water is extensively used for washing of carcass and other materials of slaughtering process. The water that comes in contact with edible meat should be free of any harmful agents, must contain microorganisms only at acceptable level and the chemical component should meet the requirements of potable water. The microbial and chemical quality of water should be regularly monitored by laboratory tests.

There are different microbial, chemical and biochemical test of water among which Bacteriological water analysis is a common method of analyzing water to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality. It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations. This process is used to routinely confirm that water is safe for carcass washing and human consumption.

According to common water quality standard, drinking water and food processing water must be free from any colony of bacteria. Sterility of water that used for washing of carcass in abattoir should regularly checked using different microbiological techniques.

Annex XIX: Plate Count (Total Bacterial count) in water

Total bacterial count of water is a technique used to evaluate the sterility of water. It is important for enumeration of all groups of bacteria present in water. The plate count method relies on bacteria growing a colony on a nutrient medium so that the colony becomes visible to the naked eye and the number of colonies on a plate can be counted. To be effective, the dilution of the original sample must be arranged so that on average between 30 and 300 colonies of the target

bacteria are grown. Fewer than 30 colonies makes the interpretation statistically unsound whilst greater than 300 colonies often result in overlapping colonies and imprecision in the count. To ensure that an appropriate number of colonies will be generated several dilutions are normally cultured.

The laboratory procedure involves making serial dilutions of the sample (1:10, 1:100, 1:1000, etc.) in sterile water and cultivating these on nutrient agar in a dish that is sealed and incubated. Typical media include plate count agar for a general count. The total number of colonies is referred to as the Total Viable Count (TVC). The unit of measurement is cfu/ml (or colony forming units per millilitre) and relates to the original sample. Calculation of this is a multiple of the counted number of colonies multiplied by the dilution used.

Scope

The procedure applies to total bacterial count for water sample collected from abattoir processing water and potable water.

Purpose

The purpose of this test is to ensure the water used for drinking to workers and for processing in abattoir is hygienic and safe.

Principle of the test:

The water sample is inoculated in plate count agar and incubated at 37^0C, the growth of bacteria is observed after 24 to 48 hours. The growth of colony indicates that the water is not sterile. The colony is then counted, and the result described in cfu/ml.

Procedure

Sampling procedure

Sample from taps that are representative of the water distribution system (water collection tank, distribution lines, etc) using the following procedures.

A. Remove attachment from faucets including aerators, hose, water filters or tubes. If disinfectant is applied before sampling, flush it to remove.

B. Turn on the water to run steadily for minimum of five minute.
C. Before taking sample, turn the water down to a thin stream (about width of pencil) and allow to run for one minute.
D. Open the sample bottle to take water sample. To avoid contamination by your hand, wear sterile glove and hold its bottom with one hand and hold its cap by other hand. The side of cap in contact with bottle should not come in contact with any surface.
E. Hold the bottle under the stream under the stream of water and take the sample until neck of the bottle (100ml).
F. Replace the cap, label properly and keep the sample in the icebox with icepack.

Sample maintenance, storage and transport

I. Make sure that the sample is labeled with necessary information (type of sample, date and hour of the sampling)
II. The sample is stored between 0-4^{0}C (using icebox and ice pack) and transported to the laboratory under chilled condition (0-4^{0}C)

N.B: The time between sampling and laboratory examination should not exceed 24 hours.

Performing serial dilution

a) Label 3 test tubes containing 4.5ml sterile peptone water from 10^{-1} - 10^{-3}
b) Shake the sample well and transfer 0.5ml from 100ml sample bottle to test tube labeled $10^{-1}$by using 1ml sterile pipette and homogenize for 5 to 10 seconds using vortex mixer.
c) From 10^{-1} test tube transfer 0.5ml to 10^{-2} labeled test tube, then homogenize usinga vortex mixer
d) From 10^{-2} test tube transfer 0.5ml to 10^{-3} labeled test tube, then homogenize using a vortex mixer.

Inoculation /plating and incubation

Inoculation or plating can be done by using spread plating or by pour plating technique as describe on general guideline.

Annex XX: Enumeration of coliform in process water

Coliform count in water is test of water contamination in which the number of colonies of coliforms bacteria per 100ml is counted. The result is expressed in CFU/100ml of water. This procedure applies to coliform bacterial count for water sample collected from abattoir processing

water and potable water.This procedure describes the method for microbial examination of water to determine level of contamination.

Principle of the test:

Coliform bacteria are a commonly used bacterial indicator of sanitary quality of foods and water. This is because coliform bacteria are originally coming from feaces of animal or human and their presence indicates faecal contamination and poor hygiene.

Procedure

Media and reagent preparation

Before collecting water sample, the broth media used for titration, and the MacConkey agar for plating, 1m pipettes used for titration shall be prepared, sterilized and sterility check by pee-incubation (only for media). For media preparation, the manufacturer's recommendation should be followed.

Sampling and sample preparation serial dilution

Sampling technique and serial dilution method is similar to that of total plate count method described above.

Inoculation /plating and incubation

Inoculation or plating can be done by using spread plating or by pour plating technique.

Spread plate method.

If MacConkey agar is already transferred to petri-dish, warm the agar and transfer 0.1ml to two petri-dishes (double plating) from each dilution starting from high dilution until10^{-1} dilution. Then spread the transferred inoculums on the agar surface by using sterile bent Pasteur pipette and leave to dry. Finally incubate at 37^0C for 24 up to 48 hours.

Pour on plating method.

- ✓ Boil previously prepared and sterility checked MacConkey agar in water bath or on Bunsen burner with tripod stand.
- ✓ Once boiled transfer the test tube containing the molten agar to a water bath having temperature of 46 ± 1^0C
- ✓ While the agar is maintained at required temperature in water bath, label sterile empty petri-dish in duplicate per dilution made in safety cabinet and invert so that the lid is on upper side.
- ✓ Transfer 1ml to two petri-dishes from each dilution (1ml per petri-dish) starting from higher dilution (10^{-3}) to lowest dilution(10^{-1}).

- ✓ Add 20ml of molten agar aseptically per petri-dish and carefully mix by swirling.
- ✓ Allow the agar to solidify.
- ✓ Invert the petri-dish and incubate at 37^0C for 24 up to 48 hours.

Counting colony and calculation

Pink colored colony which ferments lactose on MacConkey agar are considered to be coliforms and counted directly or by using colony counter.

Calculation is done to evaluate the number of coliforms per a given volume of water.

Annex XXI: Sample dispatching protocol

Submit complete information with all samples including:-

- ✓ Veterinarian's name, address and phone number.
- ✓ Tests requested.
- ✓ Identify sample: tissue or fluid, Infection site, Date and time of collection.
- ✓ Protect the submission from from moisture by enclosing it in a waterproof bag.
- ✓ Each specimen should be placed in a separate, clearly labeled container.
- ✓ Label tubes numerically and reference the numbers to animals' IDs on submittal form)

Table 15: Sampling size and frequencies

Test required	Sample	Sampling frequency
Antibiotic residual test	Meat	Every three months
Antihelminthic residual test	meat	Every three months
Hormonal test	Meat or blood	Every three months
Microbial identification	Culture or suspected sample	When required

Table 16: Sample collection and dispatching format

Name of abattoir:abattoir: _____ Date_____								
No	Date of collection	Sample code	Type of sample	Animal Species	Sample size/Qty/	Test required	Collection area / production Stage/	Preservative
Total sample size: _____								

Sample collected by: _____Sign_____ Date_____ Phone_____

Sample to be submitted to _____Address _____Tel_____

Received by: _____Sign: _____ Date_____ Phone; _____

Table 17: Water sample collection format

S/n	Sample type	Volume of sample	Number of samples	Frequency of sampling	Test to be performed	Remark
	Process water collected from point of uses.		1	Weekly	TVC Total coliform Faecal coliform Free coliform	
	Process water collected from point of uses.		3	Weekly once	Color Odor Taste Turbidity PH Total Hardness	
	Process water collected from point of uses.		1	Quarterly once	As per Ethiopian standard	To be tested externally
	Process water collected from point of uses.		1	Daily	Free chlorine	
	Soft water from softener		1 per softener	Daily	PH Hardness	

Table 18: Microbial performance criteria for carcasses of various species

Species/ catagory	Windo w (n)	Marginal result (c)					m# (CFU/cm2)				M (CFU/cm2)				Method Ref.
		APC	Coliform	*E. Coli*	*Salmonella*		APC	Coliform	*E. Coli*	*Salmonella*	APC	Coliform	*E. Coli*	*Salmonella*	
Steer/Heifer/cow/Bull															
Sheep and goat															
Mule/donkey/camel/horse															

Table 19: Microbial performance criteria for product contact materials and environment

Species/ catagory	Window (n)	Marginal result (c)					m# (CFU/cm2)				M (CFU/cm2)				Method Ref.
		APC	Coliform	*E. Coli*	*Salmonella*		APC	Coliform	*E. Coli*	*Salmonella*	APC	Coliform	*E. Coli*	*Salmonella*	
Meat handler															
Equipment															
Slaughterhouse/air															
Chilling room/wall															
Chilling room/air															

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