agriculture, land reform
& rural development

Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

GUIDELINES FOR THE SAMPLING, TRANSPORTATION AND HANDLING OF SAMPLES FOR MICROBIOLOGICAL MONITORING OF MEAT.

MEAT SAFETY ACT, 2000
(Act No. 40 of 2000)

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Date: 30 September 2021

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1. DEFINITIONS

Act	The Meat Safety Act, 2000 (Act No. 40 of 2000)
Authorised person	A trained and competent person authorized by the National Executive Officer to perform meat sampling and related activities to ensure compliance of the meat to provisions of the Act
Cleaning	The process of removing unwanted substances, such as dirt, infectious agents, and other impurities, from an object or environment.
Composite sample	A composite sample is produced by mixing the primary samples (items) from a lot of pre-packaged products; or by mixing the primary samples (increments) from a bulk (not pre-packaged) lot
Consignment	means a quantity of some commodity delivered at one time. It may consist in either a portion of a lot, or a set of several lots. For inspection purposes, each consignment shall be considered as a new lot for the interpretation of the results. If the consignment is a set of several lots, before any inspection, the composition of each lot must be considered. A stratified sampling may be applied in case of non-homegenous lots within a consignment.
Control Measure	Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level as per voluntary or regulatory requirement.
Critical limit	A criterion, observable or measurable, relating to a control measure at a CCP which separates acceptability from unacceptability of the food
Direct supervision	means that the authorised person must be present at the establishment and directly supervising and monitoring the said processes. The authorised person takes responsibility of processes being supervised
Disinfection	Reduction by means of chemical agents and/or physical methods in the number of microorganisms on surfaces to a level that does not compromise food safety and suitability.
Lot	A lot is a definite quantity of some commodity imported, manufactured or produced under conditions, which are presumed uniform. For the purpose of these guidelines, a day's production or a single consignment in the case of exports and imports shall be considered as a new lot for inspection of products.

Microbiological criteria	means a risk management metric which indicates the acceptability of a food, or the performance of either a process or a food safety control system following the outcome of sampling and testing for microorganisms, their toxins/metabolites or markers associated with pathogenicity or other traits at a specified point of the food chain.
Monitoring	The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control.
Sample size	A representative set composed of item(s) selected by different means in a lot intended to provide information on a given characteristic of the lot from which it is drawn.
Sampling Officer	A trained person who is not an official, authorized by the controlling authority to perform meat sampling and related activities under the supervision of the NEO to ensure compliance of the meat to provisions of the Act
Sampling point	A point where samples for laboratory analysis are taken
Verification	The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.

2. INTRODUCTION

The National Executive Officer (NEO) designated under the Meat Safety Act, 2000 (Act No. 40 of 2000)("the Act") is responsible for the verification of compliance of product from registered slaughter, deboning and processing establishments to the Act..

3. LEGISLATION MANDATE

The Act provides for measures to promote meat safety and the safety of animal products. Under the Act, the NEO may examine, sample and test any meat or animal product. Paragraph 53 of the poultry meat regulations (R153 of 2006) and paragraph 55 of the red meat regulations (R1072 of 2004) provide for a hygiene management programme for regular checking for soiling on a representative sample of carcases throughout the day on a random basis and to determine the levels of contamination of carcases.

Paragraph 97(3) of the poultry meat regulations and paragraph 126(3) of the red meat regulations provide for veterinary procedures to be performed by the NEO whilst meat is stored at cold stores to confirm that no soiling, contamination or deterioration of the meat in any way

took place during transportation prior to storage and to conduct any other veterinary procedure necessary to ensure that the meat is safe and suitable for human consumption.

4. PURPOSE

The purpose is to provide guidance on good practices on sample collection, transportation and handling at the cold store and upon arrival at laboratories. Establishments must have procedures and sampling plans that define sampling sites.

5. SCOPE

These guidelines apply to abattoirs, import and export cold stores, cutting, deboning, and processing plants linked to an export abattoir and food safety laboratories (government, on-plant and independent laboratories).

6. SAMPLE HANDLING AND TRANSPORTATION TO THE ANALYSING LABORATORY

6.1. General

The establishment must document a standard operating procedure for the collection, preparing, handling and transportation of samples to the testing laboratories.

The transportation of samples must ensure that the integrity of the samples is maintained at all times. The authorised veterinarian responsible for the point of sampling may allow a third party (e.g. laboratory, cold store or courier service with compliant facilities to courier frozen or chilled products) to transport samples between point of sampling and laboratories.

Samples must be delivered at the testing laboratory within 24 working hours of being taken. If a delay in transport of the samples is expected, the product must be put aside and sampled at a time when the transport time and temperature requirements can be met. Any samples that do not reach the testing laboratory within 24 working hours after being taken, must be reported to the authorised veterinarian for a decision.

The testing of samples at the laboratory must be carried out within 48 hours after receipt.

Bags containing sample sponges must be firmly secured to prevent leakage.

6.2. Temperature requirements

The temperature of the frozen meat samples must be maintained below 7ºC in the case of red meat and below 4ºC in the case of poultry, offal and all other product samples at all times. It is recommended that the air temperatures within the transporting compartment be maintained

below 2ºC. Samples from warm carcases may be harvested and submitted to the on-site lab immediately for analysis.

The temperature of carcase swab samples must be maintained between 0°C and 4°C in the case of poultry and offal and below below in the case of red meat during transport to the laboratory. Carcase swabs must not be frozen for transportation to the laboratories.

The temperature of the meat must be taken during sampling by the authorised person and again at the laboratory upon arrival by the responsible laboratory person. The responsible laboratory person must also inspect the condition of the samples to confirm that they have been handled properly until receipt at the laboratory.

6.3. Maintenance of the microbiological status of the samples

The samples must be handled in such a manner so as to ensure that they are not contaminated in any way.

6.4. Maintenance of the identity / traceability of the samples

Each sample must be labelled, handled and packaged in such a manner so as to ensure that the traceability of the samples to the relevant specific source or consignment is maintained. This must include ensuring that the samples cannot be manipulated (altered / swapped / treated) at any stage of transportation.

6.5. Security of the samples

Samples must at all times be secured to ensure their integrity and also to ensure that they are not manipulated. When samples are kept at the sampling establishment for a time period until collection by a third party, the samples must be placed in a sealable container, sealed by or under direct supervision of an authorised person and the cold chain maintained until collection.

If the samples have to be transferred to a different container for transportation, the seal must be broken by or under direct supervision of an authorised person and the new container(s) sealed by or under direct supervision of an authorised person.

The transporter must verify the number of samples or sealed containers and seal numbers as recorded in the laboratory sample submission form.

6.6. Transportation

Samples must be transported in appropriate packaging and containers (clean, sanitised, dust-proof, sealable, etc.) which ensure that they remain hygienic and within temperature requirements during transport such that on receipt at laboratory, the temperature of the sample does not preclude their testing.

The transportation of the samples must comply with the regulations as set out in the National Road Traffic Act, 1996 (Act No. 93 of 1996) for the safe transportation of hazardous material

through the effective management of systems and processes. Standard operating procedures for collection and transportation of samples for diagnosis should be in accordance with Chapter 1.1.1 and Chapter 1.1.2 of the recent OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual). Where samples are send to network laboratories, additional compliance, packaging, transportation and sample submission forms may be required such as courier(s) certified by IATA for the transportation of biological samples.

6.7. Documents management

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. The required information must be included in the sample submission form.

The establishments must document their procedure of packaging of samples or adopt the International Air Transport Association (IATA) or a similar packaging and dispatch methodology.

Records must be kept for at least 5 years and must be made available to the authorised persons and NEO upon request. Minimum details that must be in the logbook are as per Veterinary Procedural Notice (VPN) 56 – *Requirements for registration of testing laboratories responsible for the analysis of samples for monitoring and verification of hygiene of meat and products of animal origin,* and the sample submission form.

7. HANDLING OF SAMPLES AT THE LABORATORY

7.1. Temperature

Where samples arrive at the laboratory at a temperature >7°C but <10°C in the case of red meat and >4°C but <8°C in the case of poultry and offal, analysis can proceed if the requested test is for detection and or serotyping of the organism(s). The samples outside the stipulated temperature limits must be disgarded if the requested test is for enumeration.

Analysis should not be carried out on samples that arrive at temperatures outside the limits stated in the paragraph above. Should the temperature requirements not be complied with, the laboratory must reject the samples and immediately notify the authorised person responsible for the establishment/cold store.

In all cases where high temperature precludes analysis the laboratory must notify the official responsible for the sampling and establishment. Another new sample from the same batch must be taken for replacement.

Based on the reasons for the non compliance of the samples to the temperature requirements, the authorised veterinarian may authorise another sampling of the product and may impose additional control measures to ensure the integrity of the samples

7.2. Laboratory processes

Prior arrangement with the laboratory must be made to ensure that the testing can be carried within 48 hrs of sample collection and where not applicable within the stipulated time frame as stated within the relevant international best practices.

On arrival at the laboratory, laboratory personnel must:

- where determined, ensure that they are not accepting samples beyond their effective and maximum capacity for official testing;
- verify the integrity and temperature of the samples; and
- in the case of enumeration tests, determine that analysis of the sample can commence immediately upon arrival or not later than 24 hrs following the time of receipt of the sample(s).

Laboratory analysing methods must operate according to a laboratory management system within the registration framework of the respective laboratory such as the VPN56 and other recognised government laboratory systems.

7.3. Reporting of results

The presentation of results must take cognisance of the sampling method.

In addition to all the information provided in the sample submission form, the laboratory report must contain the following details:

- Time and date of receipt of the sample(s) at the laboratory and temperature of sample(s).
- Proper identification of the sample(s) especially pertaining to the point/source of collection.
- Date and time of testing at the laboratory.
- Results of the analysis
- Name and professional registration number of the person approving the results.
- Range of criteria for evaluation of the results.

8. REPORTING OF NON COMPLIANCES BY THE ESTABLISHMENT MANAGEMENT

Establishments must report any non compliances to these guidelines to the authorised person and put measures in place to prevent further non-compliances from occurring.

9. SAMPLING

9.1. SAMPLING PERIOD

9.1.1. Carcases

Samples are to be collected prior to final chilling or freezing and where feasible prior to dispatch.

9.1.2. Primal cuts

Samples are to be collected prior to final chilling or freezing, before packaging, wrapping or bulk packing into cartons.

9.1.3. Packed meat including trimmings, mechanically deboned meat / mechanically recovered meat (MDM/MRM), offal and other meat products.

In the case of cutting plants, samples are to be collected immediately prior to closing and sealing of packages. In the case of abattoirs, samples are to be collected prior to chilling or freezing.

9.1.4. Chilled and frozen meat (carcases, cuts, MDM/MRM, trimmings offal and other meat products) at cold stores

Samples are to be collected prior to release.

9.2. SAMPLING SITES AND SIZE

Samples may be collected through the destructive or non destructive methods. The destructive method involves cutting a piece of the product for testing at a laboratory, whereas the non destructive method involves the usage of swabs to collect samples from the the product.

In general, the destructive method is the preferred method for sample collection. A sample size of <2 mm depth from the surface must be collected when collecting muscle and/or tissue samples. Depending on the required analysis, pooled samples in a sterile bag must weigh 100 - 650 grams each.

The non destructive method is commonly used for carcases and high value large intact cuts. When using the non-destructive method (swabbing) for this purpose, the sampling area from each of the carcass site must cover a minimum of 100 cm^2 and where not feasible, a minimum of 25 cm^2 of which a minimum of 4 sampling sites are required per carcase. Further guidelines on sampling sites can be obtained in ISO 17604.

When sampling for microbiological analyses, four or more risk based sites of each consignment must be sampled, however risk based deviation is permitted

In the case of sampling of lots or consignments at arrival or dispatch, at least five units (of which the unit may be a carcasse, carton, cut or package) must be sampled at random during each

sampling session. The sample size must take into consideration the number of units being in the lot and/or consignment.

large number of samples maybe be composited before examination if indicated in the sample submission form.

The authorised person may collect additional samples or a larger sample size to be tested as required.

9.3. SANITATION DURING HANDLING OF SAMPLES

Sampling personnel are to use aseptic procedures when collecting samples. The personnel must use effective cleaning, disinfection and sterilisation practices so as to prevent cross-contamination during samples collection and packaging.

Sanitize all non-disposable equipment before collecting samples. Immerse equipment e.g. chisel, template, bits, scalpels and forceps in 82°C water for 10 seconds or by using flaming method with denatured alcohol. Allow the equipment to cool before drilling so there is no heat damage to the bacteria in the collected samples.

9.4. SELECTING PRODUCTS TO BE SAMPLED

In case of imported meat, the authorised person must identify each shipping container to be sampled.

The authorised person must select random cartons, packages or carcases of meat and monitor the process of conveying the selected items to the sampling area.

The carton(s)/packages or carcases from which the laboratory sample was obtained must be identified by labelling with the sample label number.

9.5. SAMPLING PROCEDURE

9.5.1. DESTRUCTIVE METHOD

a. Equipment

The minimum equipment required are listed below:

i. sterile gloves;
ii. sterile sample bags;
iii. electric or hand drill with drill bit (22 mm or larger) and cork borer;
iv. electric saw;
v. sterile samples bags;
vi. template (50 x 50 mm, preferably of stainless steel wire);
vii. forceps, and scalpel, scissor or knife;
viii. hammer and chisel (19 mm or wider);
ix. denatured alcohol (methylated spirits) and lamp or lighter/ alcohol wipes;

x. depending on the arrangement and agreement, frozen chiller packs and foam polystyrene box provided by the establishment or cold store or laboratory;
xi. permanent marking pen

The following equipment must be used for the product specified:

i. Scalpel and forceps or cork borer: Applicable to chilled meat and offal
ii. Hammer and Chisel method: Applicable to frozen meat and offal
iii. Electric saw, electric/hand drill and other appropriate equipment: Applicable to frozen meat and offal

b Sampling of muscles and tissues

Samples must be collected from different sites in cartons/packages/carcases to be sampled.

The following procedure must be followed to sample frozen product (except frozen poultry carcases):

i. Loosen the enclosed frozen product by hitting the carton against a hard protected surface whilst ensuring that the package material is not torn during the process.
ii. Open the outer packaging;
iii. Disinfect the surface of the plastic wrapping the product with a disinfectant;
vi Wash and scrub hands thoroughly to the mid-forearm using antibacterial hand soap (or a hand sanitizer at 50 ppm chlorine equivalency).
v. Wear a pair of disposable gloves. A new pair of sterile gloves should be worn every time the sample is removed by means of hands to avoid cross contamination. The gloves must be worn as follows:
    - Peel and open the package of sterile gloves from the top without contaminating the exterior of the gloves;
    - Remove a glove by holding it from the wrist side opening inner surface. Avoid any contact with the outer surface of the glove;
    - Insert hand without puncturing the glove. Discard glove and use another sterile glove if there is a concern that it may have been contaminated;
vi. Carefully cut the plastic wrapping with a sterile scalpel. Care should be taken not to let the outer surface of the plastic wrapping touch the product.
vii. Cut the surface of the meat or offal at a depth of approximately 2mm thickness.
viii. For individually frozen portions not loosened, loosen the portions using a sterilized chisel and hammer and place the whole portion(s) inside a sterile sample collection bag.
ix. Open the sample collection bag without contaminating the interior, by grasping the side with fingers and pulling outwards.
x. Collect the sample with the gloved hand. Place the sample into the sample collection bag and close and label the bag and discard the glove.
xi. Properly label the sample and mark the carton/package.carcase from which the sample was collected with the same sample label identity.
xii. Place the sample in a cooler-box/container between layers of ice.

c. Sampling of frozen poultry carcases

The following procedure must be followed:

i. Open the outer packaging as explained above at point b.
ii. When carcases are individually packed, aseptically remove a wrapped carcase randomly from the box and place it in a sample bag. Alternatively separate the packed carcase from the rest and submit the carcase as an individual sample.
iii. When poultry carcasses are not individually packed, the sampling sites may include the neck skin, wings, back, thighs, drumstick and breast.

At the laboratory, sample preparation of poultry carcases must ensure that the neck skin, wings, back, thighs, drumstick and breast are included in the sample to be tested.

The fully completed sample submission form must accompany the sample.

9.5.2 NON DESTRUCTIVE SAMPLING (SWAB SAMPLES)

a. Equipment

The following equipment and materials must be used:

i. Container for carrying supplies
ii. Sterile gloves (optional with the alternate method)
iii. Sterile template
iv. Whirl-pack™ type Collection method: Sterile specimen sponge in sterile Whirl-pack™ - type bag or equivalent; or Microdiagnostics™ Collection Bag or equivalent (alternate method)

b. Diluents

The following diluents must be used:

i. For *E. coli* and APC sampling use:
   - 25 ml sterile Butterfield's Phosphate Diluent; or
   - 25 ml of 0.1% Peptone Salt Solution[1] or Buffered Peptone Water.
ii. For *Salmonella* sampling use 25 mL of Buffered Peptone Water.

c. Whirl-pack™ Method

A sampling sponge (which usually comes dehydrated and prepacked in a sterile bag) must be used to sample all the sampling sites on the carcase as follows:

i. Ensure that all bags have been pre-labelled and all supplies are on hand, including the sampling template.
ii. If a reusable template is used, it must be sterilised between each carcase

[1] Peptone Salt Solution - Dissolve 1g Peptone and 8.5 g sodium chloride in 1L of deionized water. Autoclave at 121 ±1°C for 15 min, pH after sterilization 6.9 ± 0.2, store in the dark at 0-5°C for one month

iii. Locate the sampling sites using relevant illustrations and directions as in the establishment procedures.
iv. While holding the sponge bag at the top corner by the wire closure, tear off the clear, perforated strip at the top of the bag.
v. Remove the cap from sterile diluent water bottle (diluents may differ depending on the target organism).
vi. Carefully pour about half the contents of the sterile diluent (approximately 10 ml) into the sponge bag to moisten the sponge. Recap the bottle.
vii. Close the top of the bag by pressing the wire closure together. Use hand pressure from outside of the bag and carefully massage the sponge until it is fully hydrated (moistened). Sponges may be pre-moistened prior to sampling the carcases.
viii. Prior to collecting the sample, carefully push the moistened sponge to the upper portion of the bag orienting one narrow end of the sponge up toward the opening. DO NOT open the bag or touch the sponge with your fingers.
ix. While holding the bag, gently squeeze any excess fluid from the sponge using hand pressure from the outside. The whole sponge should sit in the bag.
x. Open the bag containing the sponge, being careful not to touch the inner surface of the bag with your fingers. The wire closure at the top of the bag should keep the bag open. Set the bag aside.
xi. Put on a pair of sterile gloves.
xii. Carefully remove the moistened sponge from the bag with the thumb and fingers (index and middle) of your sampling hand.
xiii. With your free hand, retrieve the template by the outer edge, taking care not to contaminate the inner edges of the sampling area of the template.
xiv. Locate the sample site e.g. flank for beef and small stock; belly for pigs and place the template over this location.
xv. Hold the template in place with one gloved hand. Only the sponge should touch the sampling area. Take care not to contaminate this area with your hands.
xvi. With the other hand, wipe the sponge over the enclosed sampling area (10 cm × 10 cm or 5cm × 5 cm) for a total of approximately 10 times in the vertical and 10 times in the horizontal direction. The pressure of swabbing should be as if you were trying to remove a stubborn stain from the carcase. The pressure should not be so hard as to crumble or destroy the sponge. The template may need to be “rolled” from side to side during swabbing since the surface of the carcase may not be flat. This will ensure the 100 cm^2 or 25 cm^2 area is enclosed while swabbing.
xvii. Repeat these steps for the other sampling sites (brisket for beef and small stock; and leg part for pigs) using the same side of the sponge used to swab the previous site.
xviii. Reverse the sponge and swab the third site as detailed above (butt for beef; jowl for pigs; and mid-loin for small stock). For larger species, which would involve climbing the ladder or platform, ensure that you hold on to the rail with the hand used to hold the template, if necessary. Once at a convenient and safe height for sampling, transfer template back to climbing hand (hand used to hold onto the rail while climbing the ladder or platform), and take care not to contaminate the inner edges of the template.
xix. After swabbing all the sites, carefully place the sponge back in the sample bag. Avoid touching the sponge to the outside of the sample bag.
xx. Uncap the previously used diluent bottle. Add the additional diluent (about 15 ml) to the sample bag to bring the total volume to approximately 25 ml (this step can be carried

out back in the lab; taking care to use the corresponding sample bottle used to initially moisten the individual sponge).

xxi. Expel excess air from the bag containing the sponge and fold down the top edge of the bag 3 to 4 times to close. Secure the bag by folding the attached wire tieback against the bag. Place closed sponge bag into the second bag and close the second bag securely.

xxii. If samples are to be analysed at a laboratory, begin sample preparation without delay. Ensure that intervals between collection and testing of samples are minimal.

xxiii. If samples are to be analysed at an outside (offsite) laboratory, follow procedures detailed in the previous section on transport of samples to laboratory.

d. Microdiagnostics™ Method (Alternate Method)

The proposed alternate method consists of a Microdiagnostics™ Collection plastic bag with a press clip closure which contains a polyurethane sponge. The bag and sponge are irradiated for sterility. The procedure for sponge sampling is as follows:

i. The sample number must be written on the label of the bag.

ii. Part open the bag and pour in approximately 10 ml of diluent from a numbered bottle (25 ml).

iii. Moisten the sponge by squeezing the sponge a few times (from outside of the bag). Excess fluid should be squeezed out from the sponge.

iv. The bag is resealed and taken to the sampling site.

v. Open the plastic bag by holding the lugs above the seal.

vi. Hold the bottom of the bag and sponge in one hand then invert the bag over the hand with the other hand, making sure the inside of the bag does not contact any surface.

vii. Sponge the area to be sampled within the template (refer to methodology described above for Whirl-pack™).

viii. Invert the plastic bag, expel the air and seal the top.

ix. The bag may be folded then tied with a rubber band.

x. Transfer the sample to the laboratory. Reopen the bag and add the rest of the diluent (~ 15 ml) from the same bottle so as to make total volume of 25 ml.

xi. Test sample without delay if analysed on-site; or forward sample to external laboratory following procedures detailed in the previous section on transport of samples to the laboratory.