PURPOSE

The present Procedure has the purpose of describing the operating procedures that must be applied by the personnel in charge for the execution and management of sampling and the related transports in order to ensure the correct management of the activities in the agro-food and enviromental fields. This procedure is also intended to allow a rapid identification of the equipment to use as well as of the reference standards for the described activities.

REFERENCES

- **Legislative Decree 02/02/2001, no. 31**
- **Legislative Decree 11/05/1999, no.152 and following amendments/updatings**
- **REGULATION (EC) no. 401/2006 OF COMMISSION of the 23rd February 2006 and following amendments/updatings**
- **TRACEABILITY PROCEDURE OF MAIZE SEMOLINA IN THE ITALIAN BEER SUPPLY CHAIN ASSOBIRRA , last edition**

DEFINITIONS AND ABBREVIATIONS

4.1 Definitions

Sampling: definite procedure whereby a part of a substance, material or product is taken to provide a representative sample of the whole for the purpose of the analysis to be performed on the sample itself; sometimes the sampling procedure is provided by the Customer.

Collection / Pick-up: collection of samples from customers and subsequent transport to the laboratory.

SAMPLING OF WATERS INTENDED FOR HUMAN CONSUMPTION AND SURFACE WATERS

Materials required for the samples collection

- Single-use sterile containers (two one liter sterile jars for drinking water sampling for microbiological determinations for each sampling point);
- Two clean one liter plastic bottles with cap for each sampling point, or a 2 liter bottle for the chemical analyses;
- Portable flambator/Bunsen with a spare gas bottle and a lighter;
- Sample collection forms;
- Thermometer for temperature recording;
- Permanent marker and pens;
- Spray with denatured ethyl alcohol;
- Latex gloves;
- Masks;
- Lab coat (in tow);
- Headgear (hairnet and helmet);
- Sodium thiosulphate 0,1 M to be added if the water has recently been subjected to chlorination in quantities of 0,2 ml per sterile jar.

Operating procedures

General warnings: The maximum asepsis precautions should be observed during sampling in order to prevent the accidental introduction into the bottle of microorganisms foreign to the water to be tested. For this purpose, care shall be taken during the sampling to ensure that the inside of the cap and the neck of the sterile container/bottle do not come into contact with any source of contamination, particularly with the operator's hands. For the sampling of tap drinking water, check with the customer, before sampling, whether the water is chlorinated or not. If in doubt use the bottle with sodium thiosulphate. The bottles used for taking samples for microbiological analyses shall never be rinsed at the time of collection. Bottles should never be fully filled in order to allow an efficient mixing, by stirring, at the time of examination. It is recommended to avoid overflowing of the water to be sampled during the sampling.

Sampling procedure for waters intended for human consumption to be tested for microbiological analyses

Sampling from a tap

1) Remove plastic and/or rubber tubes or gaskets, if present;

2) Sterilize with flame the tap spout, only on metal taps;

3) Open the tap and let the water run for 1-3 minutes;

4) At the time of sampling, open the sterile bottle, being careful not to touch the inside of the cap which will come into contact with the sample taken, nor the inside of the bottle neck ;

5) Take the sample without changing the flow of the tap during the operation and without rinsing. Fill 2 sterile bottles of 1 liter each;

6) Avoid filling the bottle completely to allow an effective homogenisation of the sample during the analysis;

7) Immediately close the bottle cap;

8) Record the sampling references on the container with permanent markers and place the sample in a portable fridge equipped with ice packs.

9) Place the sample as soon as possible in a refrigerator connected to the battery of the controlled temperature machine (maximum temperature 8 ºC) and transport it to the laboratory.

Sampling procedure for waters intended for human consumption to be tested for chemical analyses

1) Remove all rubber parts from the tap where the sampling will take place;

2) Open the tap and let the water run for about 1 minute;

3) Fill the plastic bottle;

4) Record the sampling references on the container with permanent markers; the same data shall be entered on the sampling report with the sample description;

5) Place the samples in the portable fridge previously equipped with ice packs.

SAMPLING OF WASTE WATERS (CHEMICAL PARAMETERS) (*)

Materials required for the samples collection

- ✓ Two clean 2 liter plastic bottles with caps for each sampling point,
- ✓ Gloves,
- ✓ Sample collection forms,
- ✓ Permanent markers and pens;
- ✓ Spray bottle with denatured ethyl alcohol;
- ✓ Latex gloves;
- ✓ Masks;
- ✓ Lab coat (in tow)
- ✓ Stick with final container

Operating procedures

General warnings: maximum precautions must be observed during the sampling in order to avoid damages to the sample and to the operator.

1) Take the sample from the last well before the discharge in the public sewer, unless otherwise indicated;

2) Take about two liters of water with the stick at the end of which the container is placed;

3) Fill the plastic bottle;

4) Record the sampling references on the container with permanent markers; the same data shall be entered on the sampling report with the sample description;

5) Record the same references on the data collection form with the addition of all the necessary information for a more adequate identification of the sample by the company, and the time of collection;

6) Place the samples in the portable fridge.

(*) In the event that it is necessary to carry out a sampling of waste water for the research of microbiological parameters, it is required to operate as described for the sampling of drinking water by filling 2 sterile bottles with a capacity of 1 liter.

STORAGE AND TRANSPORT OF WATER SAMPLES FOR MICROBIOLOGICAL PARAMETER

Samples must be kept refrigerated and protected from the light from the time of sample collection to the time of analysis. The Customer, or the third subject that carries out the sampling on behalf of the Customer, is directly responsible for the correctness of its execution and relieves the Laboratory from any liability deriving from improper ways of execution. The Laboratory reserves the right not to accept samples presented in anomalous ways and which, in its opinion, do not allow the execution of the requested tests in compliance with the standards established by the Laboratory Quality System.

Air sampling for microbiological analysis – SAS method (Surface Air System)

Materials required for the sampling

- ✓ Latex gloves
- ✓ Permanent markers and pens
- ✓ masks
- ✓ lab coat (in tow)
- ✓ plastic bags to contain the sampled plates
- ✓ paper masking tape
- ✓ Petri dishes with agarized culture media
- ✓ SAS portable instrument with two suction heads
- ✓ Sterile cloth and disinfectant

Operating procedures

1. Turn on the instrument;
2. N.B. The instrument is set with both suction heads at 500 litres. Through the instrument control panel it is possible to modify both the aspirated volumes and the number of suction heads;
3. Remove the covers from the two suction heads, taking care not to contaminate the surfaces;
4. Insert the plates, removing the protective cover from them;
5. Replace the covers on both heads;
6. Start the sampler;
7. At the end of the sampling, remove the covers from the heads and extract the plates, taking care not to contaminate the surfaces.

8. Place the protective cover over the plates and secure the cover to the plate with paper masking tape. Place the closed plates in properly labelled plastic bags;
9. Soak the sterile cloth in disinfectant and disinfect the head covers;
10. Proceed with the next sampling from point 3) or switch off the instrument.

Air sampling for microbiological analysis – Static sampling

Materials required for the sampling

- ✓ Latex gloves
- ✓ Permanent markers and pens
- ✓ Masks
- ✓ Lab coat (in tow)
- ✓ Plastic bags to contain the sampled plates
- ✓ Paper masking tape
- ✓ Petri dishes with agarized culture media

Operating procedures

1. Place the plate still covered on a surface, at least 1 meter from the ground and 1 meter from any relevant fixed obstacle;
2. Remove the cover from the plate, exposing the agarized medium;
3. After 30 minutes, replace the cover on the plate, taking care not to contaminate the surfaces;
4. Replace the cover on the plate and secure it with paper masking tape;
5. Place the plate in a properly labelled plastic bag;

Air sampling for microbiological analysis – Compressed air sampling

Materials required for the sampling

- ✓ Latex gloves
- ✓ Permanent markers and pens
- ✓ Masks and lab coat (in tow)
- ✓ 250 mL glass bottles with inclined agarized medium
- ✓ flambator

Operating procedures

1. Flamb the compressed air outlet nozzle, only on metal nozzles;
2. Place the open bottle near the compressed air dispenser, taking care that the nozzle does not touch neither the neck of the bottle nor the medium it contains;
3. Let the compressed air out for thirty seconds;
4. Close the bottle properly labelling the sample.

5.2 INSTRUCTIONS FOR SAMPLING FOOD OR SWABS

Materials required for the sampling

- ✓ Sterile knife and blades, tweezers, spoon;
- ✓ Sterile stomacher bags;
- ✓ Portable Bunsen with a spare gas cilinder and lighter (to be used if, for example following an accidental contamination of the sampling materials, it would be necessary to sterilize it again);
- ✓ Sample collection forms;
- ✓ Thermometer for checking the transport temperature of the samples;
- ✓ Permanent marker and pens;
- ✓ Spray bottle with denatured ethyl alcohol;
- ✓ Latex gloves;
- ✓ Masks;
- ✓ Lab coat;
- ✓ Headgear;
- ✓ Physiological solution
- ✓ REFRIGERATOR with device to maintain the temperature to be attached to the vehicle's cigarette lighter and in any case equipped with refrigerated blocks or REFRIGERATED BAG equipped with refrigerated blocks. The refrigerated container is in any case the preferential device to be used for the transport of samples. If not present, use the cooler.
- ✓ Suitable sterile or sterilized containers (sterile disposable plastic containers, sterile plastic bags)
- ✓ Disposable sterile plastic bottles or containers (if water sampling is foreseen).
- ✓ For the sampling of bacteriological swabs, use ready-to-use sterile swabs (SRK typology) which can be kept in the sampling transport bag. Take out from the same area the sterile masks to be used for sampling.

Operating procedures for swabs sampling

The procedures for sampling, transport, and conservation for environmental surfaces are reported in the latest edition of the ISO 18593 Standard.

1. Carry out the sampling avoiding direct contact between the operator and the material and/or the sample;
2. Open the sterile package and take out the swab for the sample collection;
3. Identify and number the surface to be sampled, marking the relative test tubes as previously described;
4. Rub by rolling the swab tip on the surface determined by the sterile mask. If the surface is dry, it is advisable to pre-wet the swab in the solution contained in the tube or with a sterile physiological solution for dry swabs, to facilitate the sampling.
5. Depending on the model of swab used, break or not-break the swab inside the tube.

For the qualitative analyses of pathogens, a swab must be prepared for each required test and the response will be given indicating the presence or absence of microorganisms.

For the quantitative analyses, only one swab per surface to be checked, is sufficient.

Operating procedures for food sampling

During the sampling, the sample must not be damaged, modified, or contaminated.

1) Open the sterile bag or container and, after removing any protective wrapping of the product, take with a sterile spoon or a sterile knife the quantity necessary for the analytical investigation to perform. Reseal the bag and place in the shipping container.

2) For foods packed in vacuum containers, take the package and place it in the transport container.

3) The sample must always be accompanied by information such as type of food, producer name, production batch, production date, deadline to be delivered to the laboratory .

4) introduce the sample in sterile bags or other sterile containers, operating quickly, preventing the sample from coming into contact with the air.

5) Record the data as required by the relevant sampling and transport form.

Food sampling for representative analysis: instructions for sampling meat, fish, vegetables, bakery products and egg products (milk and milk by-products excluded). The indicated quantities are approximate.

Parameters	Minimum quantity of sample	Containers	Notes
Microbiological parameters qualitative analysis	100 g	Sterile bags or other sterile containers	Use sterile disposable or flame sterizable sampling tools
Microbiological parameters 5 aliquots exam (qualitative analysis)	50 g X 5	Sterile bags or other sterile containers	Use sterile disposable or flame sterizable sampling tools
Microbiological parameters 5 aliquots exam (quantative analysis)	20 g X 5	Sterile bags or other sterile containers	Use sterile disposable or flame sterizable sampling tools
Chemical parameters	200 g	Bags or original containers	/

5.4 TEMPERATURE-CONTROLLED TRANSPORT

The samples that need temperature-controlled transportation are:

- perishable foods
- swabs
- potable water

All containers used for the refrigerated transport of samples are equipped with data loggers for continuous recording of the temperature; the Data Loggers are equipped with an alarm system which signals the case in which the temperature goes outside the acceptability range.

Hot samples

For short transport times (less than 4 hours) the samples must be transferred to the laboratory with no need of temperature reduction, otherwise it is necessary to adequately bring down the temperature (possibly at the customer premises) and proceed as for frozen samples.

Frozen and deep-frozen samples

The frozen samples must be transported using the electric cooler, for the transport from the sampling point to the means of transport and for short distance transfers (less than 4 hours) the refrigerated container with pre-frozen ice blocks can be used. If the temperature on arrival is higher than -15°C but lower than +4°C (and if the transfer takes less than 12 hours), it is necessary to proceed with the analyses at the soonest.

GENERIC TRANSPORT

Samples that do not require controlled temperature are still treated in such a way to preserve the sample own characteristics, integrity, and hygiene. The samples are placed in containers to avoid damages during the transport, to protect them from direct light sources, and to ensure suitable hygienic conditions.

Transportation of swabs and waters intended for human consumption

Considering that waters intended for human consumption must be analysed within 24 hours from the collection and that swabs must be analysed within a maximum of 48 hours from collection, if kept adequately refrigerated, these two types of samples must be delivered to the laboratory as soon as possible. If the preservation of these samples was carried out, in exceptional circumstances, at temperatures or times different than those foreseen, this should be reported with all its details on the document accompanying the samples.

5.5 – PART 1 SAMPLING CARRIED OUT BY NEOTRON PERSONNEL

The sampling procedures carried out by NEOTRON are performed against prescriptive documentation which can be of internal or external origin. Documentation of internal origin refers to the present guideline and to an internal list named “Mod.NEOT-DIR/007/45” which specifies the quantity of sample necessary for carrying out the analyses. On the other hand, the external origin documentation includes the Standards and Laws that give specific details on the sampling procedures. When an external origin documentation already exists, NEOTRON uses it.

5.5 – PART 2 SAMPLING CARRIED OUT BY CUSTOMERS

In case of samplings made by customers, the heads of the NEOTRON laboratory sections are available to provide information regarding sampling, collection, and transfer of the sample to the laboratory.

5.6 RECORDING ACTIVITY

Neotron operators fill out the form named ***"NEOT-DIR/008/73 "Sampling/ Samples collection"*** in every part, also recording the time of collection and deliver the sample to the laboratory within the end of the day of collection unless logistical impossibilities; if a sampling activity is carried out, the following data are reported in the form: date and place of sampling, reference to any sampling plan or offer, details on the environmental conditions during the sampling and which may have affected the sampling results, a reference to the sampling procedure and any variation from it.

The operators maintain the dark conditions in case nutritional analyses are requested (this warning is in any case always suggested);

The Sample Reception Department evaluates the conformity of the sample during the acceptance phase on the basis of macroscopic aspects: transport times, package integrity, presence of liquid, thawing, etc…; it measures the temperature on the sample surface with an infrared temperature probe for perishable samples or on customer demand. The Sample Reception Department reports any non-compliance to the Section Manager and in case it is not possible to perform the requested analyses, the need to repeat the sampling is communicated to the customer by the Sales & Marketing Department.

For the determination of pesticides on water, the water is taken with glass bottles and the conditions of refrigeration are maintained; for the determination of chlorinated solvents on water, the sampling is carried out with glass bottles with emery caps until the water overflows.