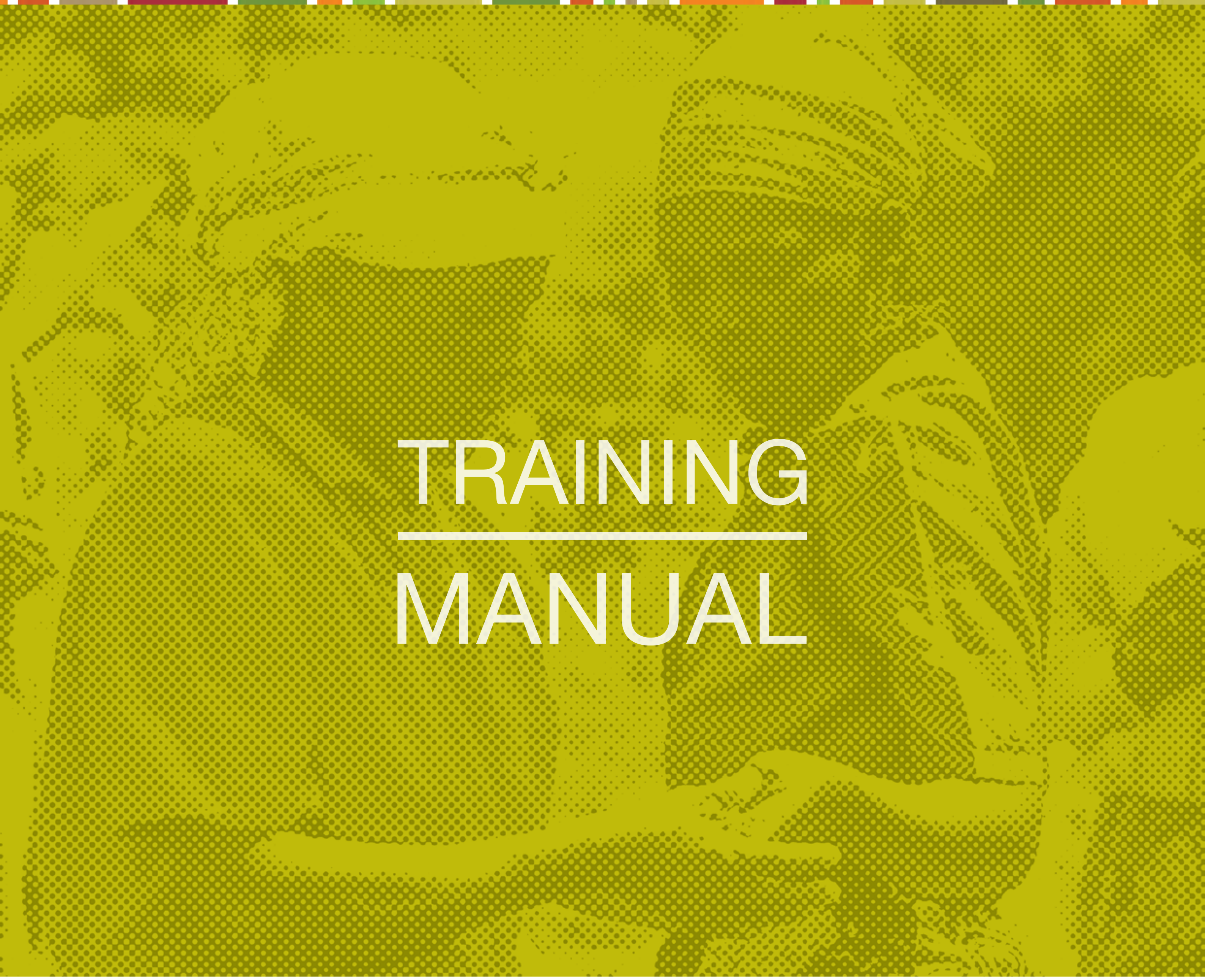

TRAINING MANUAL

- PLANTS HEALTH -

SAMPLING AND PEST DETECTION METHODS

COLEACP

This training manual was produced and designed by the Training, Information and Communication services of COLEACP. This publication was written by Steve Homer, Morag Webb and Nursel Gumusboga.

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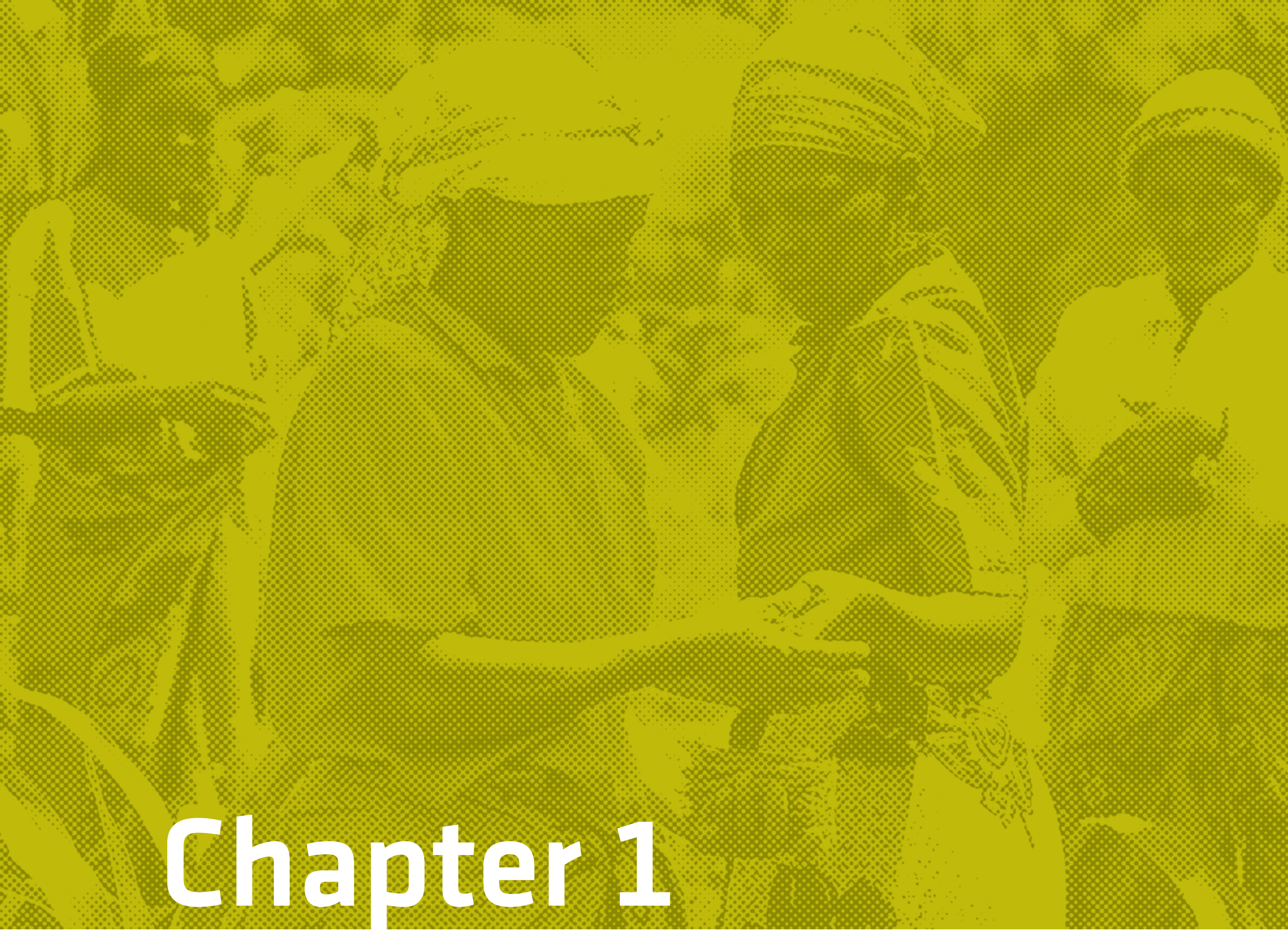
SAMPLING AND PEST DETECTION METHODS



Chapter 1

Methods for detecting quarantine pests (insects, roundworms)

1.1. INTRODUCTION

1.1.1. Background

The approach to **detecting quarantine pests** comes directly from the World Trade Organization's agreements on sanitary and phytosanitary measures (SPS),[1] which state, amongst other things: "*With the purpose of securing common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control, the contracting parties undertake to adopt the legislative, technical and administrative measures specified in this Convention and in supplementary agreements…*".

One of the main aims of the methods for detecting quarantine pests is to **verify compliance with regulations**. The proper detection and identification of pests is crucial for applying phytosanitary measures correctly. Indeed, underestimating or failing to detect a pest may lead to serious consequences for animal and plant health. Conversely, confusion about the identity of a pest may lead to inappropriate and disproportionate decisions regarding real risks, with economic consequences that can prove to be disastrous.

National Plant Protection Organizations (NPPOs) establish regulated diagnostic protocols for pests in order to adequately fulfil their responsibilities to them, particularly regarding surveillance, the inspection of imports and the certification of exports.

The notion of 'pest' covers several types of organisms, such as prokaryotes, fungi, viruses, insects and roundworms, as well as certain parasitic plants. **The detection methods are specific for each of these types of organisms.** Detection analyses are then carried out by taking into account, the types of agents to search for and the regulatory values set out.

The legislation applies to all products, meaning those on the national market and those exported or re-exported before being placed on the market of a third country, unless the importing country possesses specific legislation. The analyses must therefore consider international regulations and legislation in force in the destination country.

1 The Agreement on the Application of Sanitary and Phytosanitary Measures (the 'SPS Agreement') entered into force with the establishment of the World Trade Organization on 1 January 1995. It concerns the application of food safety and animal and plant health regulations.

1.1.2. Normative basis

All **international standards for the phytosanitary measures (ISPM)** may be found in a downloadable booklet found on the FAO Web site: www.fao.org/home/en.

They help readers to understand the whole system, but Standard 27 deals especially with detection (ISMP No. 27 (2006) – Diagnostic protocols for regulated pests).

An international standard deals specifically with criteria for laboratory operations. Laboratory accreditation by an independent national or international body is the guarantee that the results are recognized in all countries. Accreditation is bestowed according to the criteria of Standard ISO 17025, which describes the general requirements regarding the competence of calibration and testing laboratories.

The above standards are general ones that describe the diagnostic protocols to implement or organizational protocols for the laboratory and quality system.

They contain no technical information for analysing a particular pest.

Even if the protocols as defined in Standard ISPM 27 are approved by the authorities,[2] **for their research, laboratories must use the specific methods cited in the protocol.**

For each pest, several specific analysis methods exist according to the research context. In France, the specific methods used are published in the Official Bulletin of the Ministry of Agriculture, which is the competent authority on the subject (agriculture.gouv.fr/bulletin-officiel). Le lien ne fonctionne pas

For example, when searching for whole insects, whether alive or dead, **entomological methods** must be used to help to identify and count them. Their number and status help to estimate the size of the population and whether the invasion is just beginning, well under way or reaching its end. With this information, it is possible to evaluate the risk posed by products coming from the region.

Inspecting products calls more for techniques, such as observing defects (holes in wood, attacked cereal grains, etc.) and searching for fragments.

Molecular biology methods may be used to formally identify the origin of fragments or the identity of insects that could be very similar species from a morphological point of view, or to identify them at early stages of development (larvae, eggs, etc.).

2 An example may be viewed at the following link: www.ippc.int/sites/default/files/documents//1336039694_DP_01_2010_En_2012-05-02.pdf.

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1.2. GENERAL ASPECTS OF METHODS FOR DETECTING QUARANTINE PESTS

1.2.1. Sampling

Sampling is the first step of the analysis. It determines the entire validity of an interpretation made from the result regarding the compliance of a batch. In order for the sample to be representative, the rules of sampling must allow verification of the validity of the entire product batch.

Sampling conducted as part of official controls must comply with the regulatory texts in force in the country and internationally. Sampling to validate a batch must be conducted according to the specific rules that ensure the representativeness of the batch via the sample.

Furthermore, to ensure reliability, sampling must be performed in conditions that prevent the samples from becoming contaminated by the sampler or the environment.

However, it is important to systematically consider the sample as potentially containing a pest, so the sample may not be allowed to contaminate the environment and create a centre of infestation itself.

1.2.2. Transporting samples

Sampling sites are generally located outside the laboratory. It is therefore a good idea to ensure that the conditions of transport are not likely to alter the results, for example by destroying pests in extreme temperatures or fostering their development during transport that takes an excessively long time in optimal temperatures. This would lead to overestimating the amount present in the batch.

1.2.3. Analyses quality

In all spheres, the reliability of the result must be demonstrable. Therefore, the quality of the analyses is paramount. It will only be recognized if the laboratory in question is committed to a quality approach.

1.2.4. General rules for detecting quarantine pests

The methods for detecting quarantine pests are primarily analytical methods to which all the general rules applicable to the analysis methods described below also apply.

The main techniques implemented for detection include:

- Trapping for control over areas of production, whether through pheromone-based traps or sticky yellow panels. These techniques are linked to entomological analyses and taxonomy. These trapping techniques may also be conducted during transport by placing traps with the cargo.
- Phytosanitary diagnostic kits based mostly on the enzyme-linked immunosorbent assay technique (ELISA), for example to determine quickly the presence or absence of disease on plant cuttings.
- Molecular biology techniques for precise identification.

The design of the laboratory must comply with the safety requirements dependent on the type of organism sought.

1.3. RULES TO FOLLOW FOR DETECTING QUARANTINE PESTS

We should remember that analyzing plants and animals to search for quarantine pests may lead to the production of populations that could become sources of contamination if they are not controlled.

These populations, which are not dangerous or only slightly dangerous at first, become sources of contamination unless they are dealt with properly before being eliminated.

1.3.1. Protecting the operator

Aside from the fact that samples must be protected from any contamination, it is important to remember that the organisms we search for can be dangerous to humans. Furthermore, the operator may be a vector to the outside for the organism sought, either through contact (skin and mucous membranes) or through inhalation. Therefore, protection must be provided for individuals (masks, gloves and combinations) and groups (laminar flow cabinets, cleanroom etc.) with possibility for operators to fully wash themselves if necessary.

1.3.2. Protecting the environment

As mentioned above, analysis of quarantine pests leads to their proliferation. If they are released into the environment, either via air or waste disposal, they can multiply and cause an infestation (like the spread of the Asian hornet (*Vespa velutina*), for example, which destroys beehives throughout South-Western France after the introduction of only a few specimens.

Therefore, depending on the regulations of the country concerned, it is necessary to respect the external release of air from the laboratory to control air contamination. All waste must then be decontaminated with a proper system to avoid the accidental spread of contamination during waste processing. Dry heat systems are not recommended and autoclaving remains the surest way to destroy the organisms.

1.3.3. Practical aspects of methods for detecting quarantine pests (insects, roundworms)

For each of the paragraphs below, a distinction is made between:

- trapping tests in production areas;
- tests by culture ;
- laboratory tests with phytodiagnostic kits;
- molecular biology tests (PCR).

1.3.3.1. Controls through trapping in production areas

Although trapping mainly takes place outdoors, steps still need to be made to prepare the traps for counting and identification.

Thus, there must be:

- traps or panels storage area that includes a room for preparing the traps or panels (e.g. identification). This room must be cleaned and must not allow contamination or the introduction of organisms before the test area. The traps are also prepared and protected during transport to the control area. Equipment may also be used to repackage the traps and panels to ensure that the trapped organisms are not lost and do not spread during the return trip;
- a reception room for the traps where the samples are brought by those requesting analysis (controllers). This is the only room accessible to outsiders. It leads directly to the room for temporary sample storage. Depending on the analysis required, the samples are transferred from this room to the entomological examination room or to the phytodiagnostic testing room;
- an entomological examination room that must contain all the material necessary for testing and all the safety measures must be in place so that no living insect taken out of its trap can escape;
- plants must be grown in the appropriate material in a room dedicated to that purpose or in a greenhouse that meets all safety criteria;
- a room nearby can make recording of the results easier without having to leave the secure perimeter;
- a washing room for cleaning and sterilizing the material and for waste disposal.

1.3.3.2. Plant tests

Plant tests are performed by cultivating cuttings or plants to observe the development of diseases during their growth. This is done in greenhouses that are secure enough to prevent contamination both outside and inside the laboratory.

1.3.3.3. Laboratory tests with phytodiagnostic kits

- A reception room for the traps where the samples are brought by those requesting analysis (controllers or actors in the agricultural food chain). This is the only room accessible to outsiders. It leads directly to the temporary sample storage area. According to the analysis required, the samples are transferred from this room to the entomological examination room or to the phytodiagnostic testing room.
- If plants are deemed necessary, they will be grown in the appropriate material in a room dedicated to that purpose.
- The room for conducting ELISA tests must contain material for carrying them out (plate reader, extraction, deposit of extracts on plates, etc.).
- A room nearby can make recording of the results easier without having to leave the secure perimeter.
- A washing room for cleaning and sterilizing the material and for waste disposal.

1.3.3.4. PCR analyses

- A DNA extraction room.
- A room for preparing the polymerization reagents mix (completely free of DNA).
- A PCR room that contains thermocyclers.

1.3.3.5. Laboratory tests with phytodiagnostic kits and PCR analyses

To the greatest extent possible, the connection between these rooms must follow the order cited, with communication between the rooms using a service hatch to limit staff movements.

1.3.4. Analysis procedure

1.3.4.1. Choosing the method

As indicated above, to make it easier to read results and facilitate implementation, it is preferable to choose standardized or official methods. Most pathogens have already been studied and protocols have already been published for them, such as those described in the Official Bulletin of the French Ministry of Agriculture, an example of which can be found at the following link: info.agriculture.gouv.fr/gedei/site/bo-agri/recherche/resultat_recherche_rapide?titre=agents+pathog%C3%A8nes (in French).

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1.3.4.2. Implementation

The first step to implementing the methods selected is verifying that the laboratory has material available, as well as the reagents and consumables cited in the reference text.

It is then recommended to test the whole protocol on a matrix to ensure that it is entirely functional.

1.3.4.3. Validation

When the procedure is operational in the laboratory, the protocol must be validated to ensure that the method meets all the requirements meaning that it is selective (it only shows the organisms searched for), sensitive (it can detect organisms at the regulatory value level) and repeatable (with several analyses of the same sample giving the same result). For this purpose, analysis must be performed on samples whose count is known and the characteristic values of the method must be determined. These values may then be compared with those given in the reference text. The laboratory must also participate in inter-comparison tests with other laboratories.

After this validation is complete, the laboratory must possess a written document that includes:

The origin of the method

1. Identification of the target organism
2. Biology
3. Host plans
4. Symptomatology
5. Field of application.
6. Schematic representation of the detection

If ELISA methods are used

Description of the ELISA method:

1. Products and consumables:
    - buffers, serological reagents
    - other consumables (if any)
2. Equipment and material
3. Controls and indicators
4. Steps of the analysis :
    - grinding the sample
    - conducting the test
5. Results
    - validating the results
    - interpreting and formulating the results

If PCR methods are used

Description of the RT-PCR method in real time

1. Products and consumables:
    - buffers
    - other consumables (if any)
2. Equipment and material
3. Controls and indicators
4. Steps of the analysis:
    - grinding the sample
    - extraction
    - PCR in real time – Amplification of DNA
5. Results:
    - validation of the results
    - interpreting and formulating the results

In all cases

Elimination and conservation of material

1. Eliminating materials likely to be contaminated
2. Retaining remnants of the material used

1.3.4.4. Sampling

It is important to avoid mixing the methods to control pests with the methods to identify them, even if both objectives may apply. For example, insect traps are a way to fight them, but counting or identifying insects helps determine their presence while also, through recounts, determines whether the population is growing (beginning of an invasion) or decreasing (disappearance). In both cases, both the number and position of the traps are specific to the goal.

As we have seen, sampling is very important. In most cases, a third party, the controller of the competent service (plant or animal) performs the sampling. In this case, the sampler has the competence to avoid contaminating the sample and will have carried out a visual inspection of the area or product before beginning sampling.

The laboratory must define the number of samples that it is to receive for interpretation. The sampler may increase this number upon inspection.

Essential information and precautions to take during sampling

The usual precautions to keep the sample in its state must be respected during the sampling procedure. Certain information is essential for the laboratory's analysis or test report:

- an accurate description of the product, which is necessary for identifying the organisms to search for if the controller has not already specified the same in his/her request;
- the product's place of origin for imported products.

The European and Mediterranean Plant Protection Organization (EPPO – **www.eppo.int/**) provides a free downloadable software programme at the site **newpqr.eppo.org/download.php** in order to learn about the pests to search for according to the geographical area.

Transport of the sample to the laboratory

Transporting samples is an important step that determines the laboratory's performance of the analyses. This operation must be organized, prepared and carried out with care.

Reception at the laboratory

When the samples arrive at the laboratory, the staff must verify the integrity of the sample containers to detect any possible contamination that may have occurred after sampling or any potential risks of contaminating the environment.

The samples are then kept in conditions to guarantee that they will not deteriorate and that the organisms will not proliferate.

1.3.4.5. Analyses

Tests with trapping in production areas

Traps are used to capture pests. An entomologist or a pest specialist conducts the analyses to clearly demonstrate whether they are present or absent. Note that it is sometimes easier to observe traces left by these pests (holes, cysts etc.) than the pests themselves.

For some insects, like termites, for example, radiation methods may also be used to detect their presence in wood.

Termite colony

Plant tests

Tests on plants consist firstly of culturing the sample when possible, based on the status of the sample for the purposes of:

- observing the symptoms of the disease or the pest;
- obtaining enough substrate to be able to carry out an ELISA test or a molecular biology test.

Laboratory tests with phytodiagnostic kits

Suppliers' recommendations regarding storage conditions before use and conservation during use must be followed. In lieu of these, the laboratory sets the conditions that it deems best.

The list of buffers needed to carry out the method must be recommended by the serological reagents' supplier.

The preparation of buffers and their duration and conditions of conservation must conform to the supplier's recommendations in all respects.

The laboratory uses specific serological reagents (polyclonal antibodies or universal monoclonal antibodies). These reagents must be able to detect all strains sought.

The reference laboratory may be able to suggest names of suppliers of serological reagents. It is therefore advisable to contact the laboratory before each analysis campaign.

In all cases, the laboratory cannot use a reagent that is tested then declared unsatisfactory by the reference laboratory.

Other consumables (if applicable)

- Analytical quality water (*i.e.* demineralized, distilled, treated by reverse osmosis) to ensure that the performance criteria expected for the tests are met.
- Microtiter plates (and lids): use certified flat-bottomed microtiter plates that ensure a reaction quality at least equivalent to that given in the reference method.
- A virucidal product to disinfect work surfaces and material (e.g. bleach with 0.96% active chlorine).

Equipment and material

To implement this method, the laboratory must have the equipment described in the official method for ELISA analysis techniques.

Different grinding systems may be used based on the equipment available in the laboratory.

All methods are considered valid, provided that measures are implemented to prevent the risk of cross-contamination. In particular, the grinding material used must be disinfected easily in case the packaging containing the material breaks.

It is recommended to use a ball mill in a plastic grinding bag fitted with a nylon mesh gauze filter. The homogenate may then be recovered directly from the bag.

Grinding harder materials is easier when a press is used beforehand.

Controls and indicators

Reading references must be added to each microtiter plate. As required by the official method, these references include:

- Healthy Controls (HCs). HCs of the same nature and species as the matrices to analyse are advisable. It is preferable that the form of use (refrigerated, frozen), physiological status (young bodies, senescent bodies etc.) and HCs culture conditions are identical to those of the samples. The HCs are dealt with in parallel to and under the same conditions as the samples. There are at least two, at a rate of two wells per control. The use of three controls (or six wells) is recommended. These HCs are used to determine the thresholds of positivity and negativity.
- Sick Controls (SCs). SCs ensure that handling is being performed correctly. Each series of analyses may use contaminated samples, samples prepared in the laboratory (naturally contaminated samples of reference) or positive commercial controls (lyophilised, glycerolised) to prepare according to the supplier's recommendations.
- Buffer Indicators (BIs). This is a blank test that solely consists of the grinding buffer to control the background noise on the grinding buffer and to verify that it is free from any contamination likely to change the results of the analysis.
- Substrate Indicators (also called substrate wells). A column of microtiter plates is filled with analytical quality water at each step of the deposit, except in the last step when it is filled with substrate solution. This allows for a 'blank' or zero optic on the microtiter plate spectrophotometer reading.

If the reading device does not automatically produce corrected absorbance values, the average absorbance of the substrate wells is subtracted from the raw absorbance of the tests.

These references are used to validate the proper conduct of the various steps of the analysis and the results obtained from the different microplates. Observing and/or reading them and their degree of compliance with the values reached are a prerequisite to the results obtained from the samples subject to analysis.

Steps of the analysis

The distribution plan (plate plan) and the plan for identifying the extracts are established carefully.

Each sample is repeated at least once (or two wells per sample).

It is not recommended to use wells on the edge, unless the laboratory can prove that there is no border effect.

Grinding the sample

If not already prepared, the sample is divided evenly then mixed. It is a good idea to keep a fraction of each sample prepared this way at a temperature lower than -18 °C to be able to start the analysis again in the event of an indeterminate result.

Since the sample in the divided form has very limited conservation at room temperature, efforts must be made to keep the samples in positive cold (in the refrigerator or on ice) and quickly proceed to grinding.

The material should be ground in the grinding buffer recommended by the supplier of serological reagents and according to the mass/volume ratio recommended by the supplier (generally 1/10 or 1/20, or respectively, 1 g of fresh material for 10 ml or 20 ml of grinding buffer). For a test sample of dehydrated (lyophilized) material, the mass to be implemented is that resulting from dehydration of the same fresh material.

If the deposit is not made immediately, the homogenates must be kept refrigerated at +5 °C or +4 °C for four hours maximum.

After the deposit is made, a fraction of each homogenate must be kept at a temperature lower than -10 °C in an attempt to obtain a definitive result. These fractions may be used for possible confirmation.

Conducting the test

Priority should be given to following the protocol of analysis of the reagent supplier (steps, times of incubation, volumes and dilutions). Following the recommendations of the reference method is mandatory.

For reference purposes, the steps are carried out as follows:

- coating (Immunoglobulin G – IgG): when used, the IgG antibodies are diluted then homogenized in a coating buffer;
- fill the microplate wells with the volumes indicated;
- incubate at +37 °C or at room temperature for a duration of two to four hours; or at +5 °C overnight;
- washing: at least three washes, each involving incubation time of two to four minutes for the wash buffer in the wells before emptying;
- deposit the extracts: filling the wells with the volumes indicated in the extracts;
- incubate at room temperature for two hours or at +5 °C overnight;
- take all possible precautions to avoid any contamination from one well to another (change the pipette cone, deposit extracts at the bottom of the wells without creating aerosols);
- washing: at least five washes, each involving incubation time of two to four minutes for the wash buffer in the wells before emptying;
- conjugate (IgG-E: Immunoglobulins coupled with the enzyme): when used, the IgG-E antibodies are diluted, then homogenized in a conjugate buffer. Fill the wells with the volumes indicated;
- incubate at +37 °C or at room temperature for a duration of two to four hours; or at +5 °C overnight;
- washing: at least three washes, each involving incubation time of two to four minutes for the wash buffer in the wells before emptying;
- substrate: for labelling with alkaline phosphate (the most common), the substrate p-nitrophenyl phosphate is dissolved in the substrate buffer at a concentration of 1 mg/ml. Fill the microplate wells with the volumes indicated. Incubate at room temperature;
- reading: for the phosphatase alkaline marker and p-nitrophenyl phosphate as a substrate, the absorbance reading is conducted at 405 nm. Several readings may be performed at different times.

For example, if the reagent supplier does not provide reagents for blocking the enzymatic reaction, the readings may be made after around 30 minutes, one hour or two hours, or even later if necessary (slow reaction) after adding the substrate solution.

The reference reading used to calculate the thresholds may be performed after around two hours.

Notes: to avoid uncontrolled variations in environmental conditions during the period, since environmental conditions could affect the development of different reactions, it is advisable to carry out these operations in a defined and constant environment (regarding temperature, humidity and light). The use of lids on the microplates and keeping them in the dark at controlled temperatures throughout all stages of incubation may be preventive measures that help to achieve this goal. All these simple measures must be taken with care, consistency and rigor, including washing operations.

Results

- *Validation of the results*

  The results may only be interpreted once all validation criteria for the microplates in the official method of analysis are verified.

- *Interpretation and formulation of the results*

  In lieu of explicit recommendations from the reagent supplier, the results may be interpreted based on the calculation of two thresholds, denoted S1 and S2:

  S1 = Average corrected absorbance values of Healthy Controls x 2

  S2 = Average corrected absorbance values of Healthy Controls x 3 or 4 according to the laboratory's customary practice

  Corrected absorbance = raw absorbance – substrate absorbance (zero optic carried out in the substrate wells).

Other methods may be used to calculate the thresholds, or even to establish a single threshold. Each laboratory should describe and validate these methods.

As the analysis is qualitative, three categories of results are defined: positive, indeterminate and negative.

For interpretations using a single threshold, the categories of the results may be positive or negative.

Positive: the test's absorbance value is higher than or equal to S2.
Result: the organism detected in the sample analysed according to the ELISA technique.

Indeterminate: the test's absorbance value falls between S1-S2.
Result: the method cannot determine the status of the sample and additional analyses are necessary. These may be molecular biology analyses via PCR.

Negative: the test's absorbance value is less than S1.
Result: the organism not detected in the sample analysed according to the ELISA technique.

1.3.4.6. Molecular biology tests (PCR)

This molecular biology technique is commonly used today specifically to detect traces of DNA of known organisms. It is also increasingly used as an identification technique because it is very specific and quick.

DNA extraction

The first step is extracting DNA, which must be done in a clean room because contamination by DNA traces from another sample or from an operator could disturb the analysis. This extraction is based on commercially available ready-to-use extraction kits. It consists of destroying cells to release the DNA, which is then purified on small columns that are often silica-based. After this step, the purified DNA is recovered in solution in a microtube.

Preparing the mix

The mix is the combination of reagents necessary for the polymerase reaction, which re-synthesizes DNA based on the DNA of viruses or bacteria. To be selective for the virus and organism being sought, a small sequence of known DNA is required, which serves as the primer for the polymerase. The preparation room must be completely free of DNA because the slightest trace will then be amplified and could disturb the normal development of the analysis.

Amplification

This operation is automated and carried out in thermocyclers that reproduce various temperature cycles to multiply the number of strands of the desired DNA. The detection may either be performed by migration in polyacrylamide gels or directly in real time with a thermocycler with a computer control and results collection system.

1.3.5. Reporting the results

1.3.5.1. The test report

The test report must provide the party requesting the analysis with all the information necessary to interpret the results. For this purpose, it must at least contain the following information (listed in regulation ISO 17025):

a. a title (e.g. 'Test Report' or 'Calibration Certificate');
b. the name and address of the laboratory, and the location where the analyses were carried out, if different from the address of the laboratory;
c. unique identification of the test report (such as the serial number), and identification on each page so the page can be recognized as part of the test report or calibration certificate, and clear identification of the end of the test report or calibration certificate;

d. the name and address of the client;
e. identification of the method used;
f. a description, the condition, and the unambiguous identification of the item(s) tested or calibrated;
g. the date of receipt of each item subject to analysis because this makes it possible to know the delay between sampling and the analysis and is thus critical to the validity and application of the results; the same applies for the date(s) of each analysis;
h. reference to the sampling plan and procedures used by the laboratory or other bodies, if known, as they are relevant to the validity or application of the results;
i. the analysis results with units of measurement;
j. the name(s), positions(s) and signature(s) or equivalent identification of person(s) authorizing the test report or calibration certificate;
k. where relevant, a statement to the effect that the results relate only to the items subjected to the test.

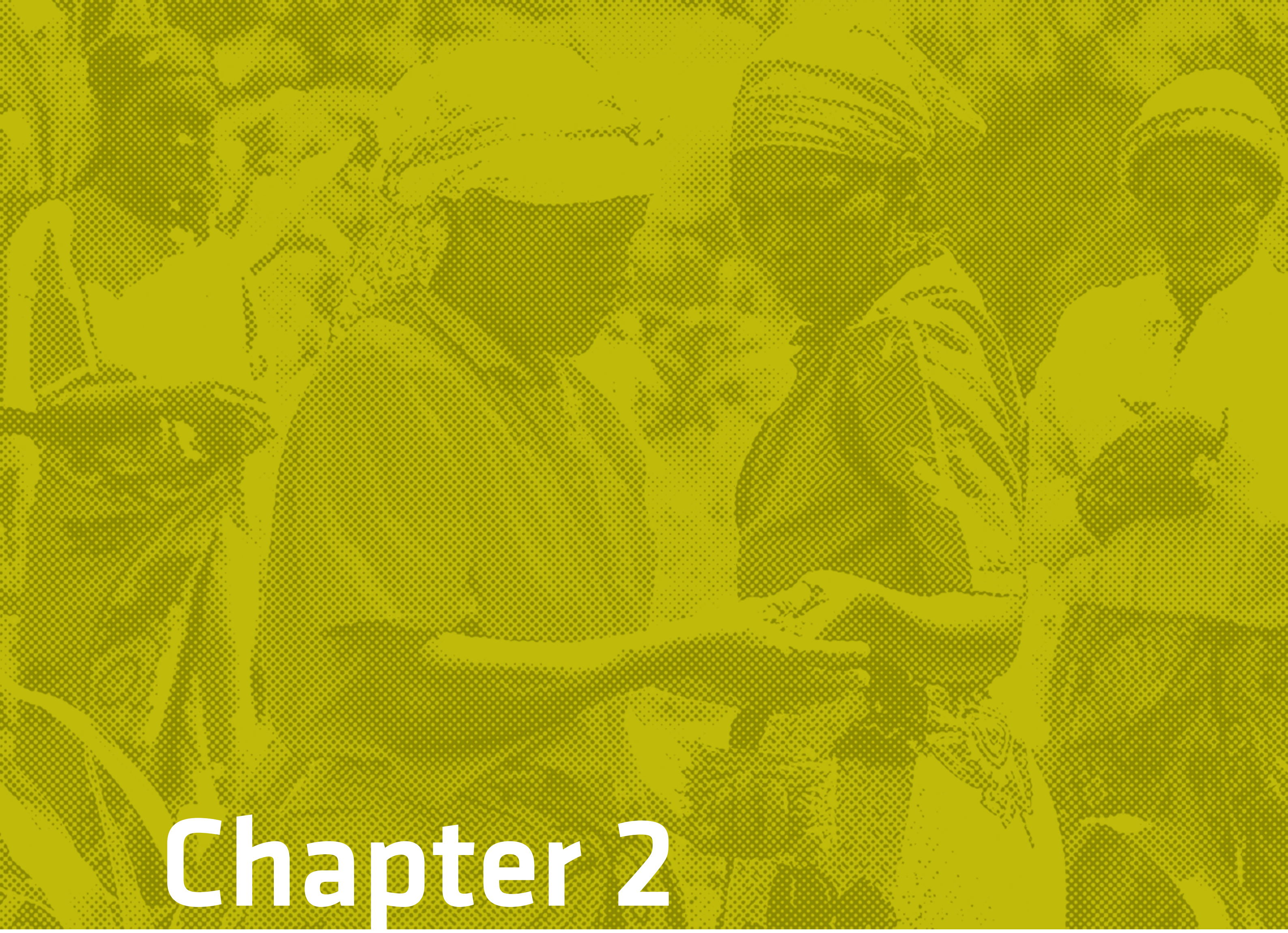
Paper copies of the test reports and calibration certificates should also contain page numbers and state the total number of pages.

It is recommended that the laboratories insert a warning specifying that the test report or calibration certificate must not be reproduced, except in full, without written authorization from the laboratory.

1.3.5.2. Conclusions of the analysis report

When the laboratory draws a conclusion that is included in the test report, it must ensure that:

- the criteria chosen for the interpretation are relevant;
- the sample is representative of the batch and the sampling conditions have not changed the characteristics of the sample. Otherwise, it is crucial to mention that the report only deals with the sample submitted for analysis.



Chapter 2

Sample planning within the framework of official controls

2.1. PURPOSE

The main aim of the controls based on sampling plans for statistical quality control is to suggest decision rules for controlled goods.

This chapter is intended to set the modalities of sampling plans for the statistical control of the proportion of non-compliant items in a lot of goods, in order to seek out and note infringements.

The results obtained in the samples extracted from one or more lots portray an image of the quality and actual safety of these goods and can be used to assess the extent of the fraud, if necessary.

The rules are based on statistical methods to assess the proportion of items in a lot of controlled goods that do not comply with the regulations.

The inspecting officer, supported by direct observations or the laboratory report, must decide whether the lot(s) of controlled goods:

- comply with the regulations in force;
- do not comply with the regulations in force.

2.2. SCOPE

This chapter sets out the situations most frequently encountered in statistical quality control.

These plans do not involve the statistical quality control when it is defined by mean content.

Similarly, this chapter does not cover situations relating to the controls of consumer safety provisions.

It only covers goods likely to be individualised by basic samples representative of them, for example:

- pre-packaging;
- a receptacle or spoon containing a quantity determined by the sampling plan of homogeneous bulk goods extracted from a lot, for example:
  - quantity of milk or wine stored in a tank;
  - a quantity of homogeneous goods sampled from a conveyor belt etc.

Are excluded:

- heterogeneous bulk goods; the statistical control of such goods requires special procedures, given the difficulty involved in taking basic representative samples of the goods due to their heterogeneity;
- the endurance tests of a product (reliability) that also call on special statistical techniques.

2.3. SPECIFIC SAMPLING VOCABULARY FOR STATISTICAL QUALITY CONTROL

2.3.1. Quality control

In a broad sense, this covers all operations (prevision, coordination, execution) intended to maintain or improve quality and establish the most economical production that takes into account the user satisfaction.

It is verifying compliance of the product with its definition or its specifications.

2.3.2. Statistical quality control

Quality control using statistical methods, mainly control charts and sampling plans.

2.3.3. Sampling plan

This is a plan whereby one or more samples are taken, each one made up of several increments, to provide information to be compiled and a decision to be made, where necessary. It sets the rules stipulating the sampling modalities and can, if appropriate, be supplemented by fixing acceptance or rejection conditions of the controlled lot.

Every 'Quality-Safety' sampling plan must state the following instructions and include a decision rule for the controlled lot:

- the nature or objective of the control;
- the type of sampling used;
- the type of sample taken and prepared;
- the number of increments of each sample taken;
- the main stages in the sampling procedure, particularly:
  - the increment modalities;
  - the modalities for preparing samples based on these increments to create the sample intended for the physical and chemical analyses;
  - the modalities for identifying each lot and each sample taken;
  - the acceptance or rejection conditions for the controlled lot.

2.3.4. Statistical control of the percentage of non-compliant items in a lot

The statistical control of the percentage of non-compliant items in a lot is intended to ensure by statistical methods, based on results obtained from samples, that the quality of the controlled lot complies with the Acceptable Quality Level (AQL) set by the sampling plan.

The acceptable quality level (AQL) of a lot in a sampling plan is a quality level that corresponds to a high probability of acceptance of lots with this quality level.

The AQL is in fact a 'quality' objective and the control plans to target this level will be such that the lots showing this level of quality will be accepted with a high probability of acceptance.

2.3.5. Attribute statistical control of the percentage of non-compliant items in a lot

This is a method for estimating the quality of a lot. It involves noting, for each increment making up a sample extracted from a lot, the presence or absence of a certain qualitative characteristic called an attribute, then counting how many of them possess this attribute.

The acceptance rule for the controlled lot fixed by the sampling plan is based on a maximum number of increments with the defined attribute; this maximum number is also set by the sampling plan. The attribute can be determined by counting or by measuring.

Example:

Attribute control, using a sample of eight fruit, on the appearance of a lot of fruit with an AQL set at 1.5%:

- the compliant characteristic of one fruit (attribute of one fruit) is the following: no spots on the skin of the fruit;
- given the size of the sample and the AQL value, the acceptance rule for the controlled lot is as follows: there must be no non-compliant increment in this sample of eight fruit;
- it is therefore a question of counting the non-compliant increments in the sample of eight fruit: determining the non-compliance of the increment – **its attribute** – is achieved by counting the spots on the skin of each fruit;

Example:

Attribute control, using a sample of eight increments of the fat content in a lot of whole UHT milk, with an AQL set at 1.5%:

- the control uses a sample of eight increments;
- the attribute for an increment is as follows: the increment is compliant if its fat content is higher than 3.5%;
- the acceptance rule for the controlled lot is the following, given the size of the sample and the AQL value: no non-compliant increment in this sample of eight increments;
- **the attribute** – and the non-compliance – of the increment is determined by measuring the fat content of each increment, but the decision concerning the lot is made after the number of non-compliant items in the sample of eight increments has been counted.

2.3.6. Variable statistical control of the percentage of non-compliant items in a lot

This method assumes that the controlled characteristic can be measured and that the numeric values taken by this characteristic are distributed according to the Gaussian probability distribution, called the "normal distribution".

Differences from the attribute statistical control of the proportion of non-compliant items in a lot:

- the increments are no longer qualified by an attribute;
- the decision rule is no longer taken following the result of counting non-compliant increments in the sample;
- the decision is taken from the results from comparing the numeric value of the arithmetic mean of the measures taken in each increment in a sample and the numeric value of an algebraic formula set by the sampling plan; this formula is based on estimating the standard deviation in the results of the measures taken.

2.3.7. Lot

Defined quantity of goods determined, manufactured or produced in conditions that are assumed to be uniform.

The term 'lot' means a lot destined to be controlled by sampling, *i.e.* a quantity of material or a set of items from which a sample has been taken and examined.

2.3.8. Homogenous lot

This is a lot for which no identifiable cause for variation in components and raw materials occurred during production, preparation, manufacture, transport or storage.

In terms of statistics, a lot is considered to be homogeneous in relation to a given property, if the distribution of values noted for characteristic parameters of this property follows an approximately normal probability distribution. Where the distribution is not normal or the value of its standard deviation is high, the lot must be considered as heterogeneous.

Items in a lot can be considered as homogeneous for a given property and heterogeneous for another simultaneously.

2.3.9. Item

It relates to:

- either an object or a defined quantity of material where a set of observations can be made;
- or the result of previous observations, be they qualitative (the increment is qualified as compliant or noncompliant) or quantitative (measurement result).

2.3.10. Increment

If the lot comprises:

- individualised units, this is an item in the lot taken to form a sample;
- bulk goods, it is a small quantity of material taken once, at a point in the lot, to form a sample; this quantity is then individualised permanently in a receptacle (box, spoon etc.) for observations or measurements.

2.3.11. Characteristic

Property used to distinguish the individuals of a population. A characteristic can be qualitative (compliant or non-compliant) or quantitative.

A quantitative characteristic is said to be continuous if, in its field of variation, it can be assimilated with a continuous variable in the sense of mathematical analysis: for example, the protein content of a food.

A quantitative characteristic which can only take integer values is called discrete: for example, the number of pre-packs in a box.

2.3.12. Sample

Set of one or more increments taken in a lot of goods intended to provide information on these goods or the process that produced them; this information can potentially be used as a basis for a decision.

2.3.13. Sample size

Number of items or increments making up the sample.

2.3.14. Non-compliant increment

Increment with one or more defects defined in the sampling plan based on the regulations or standards or professional use.

2.3.15. Sampling

Procedure for sampling goods to form a sample.

2.4. RULES TO BE FOLLOWED TO PERFORM THE STATISTICAL QUALITY CONTROL

2.4.1. General rule

When the results of the survey increment control are found to be non-compliant with the plans mentioned above, the inspecting officer can consider that:

- the facts noted only require a regulatory reminder: he must write to the professional concerned, setting out the modalities and results of the control and inviting him to make the necessary corrections as soon as possible;
- the facts noted suggest a legal follow-up: he must implement the set procedure, mainly by taking three identical, homogeneous samples constituted according to the sampling plan chosen, for the purposes of a joint expertise.

2.4.2. Preparing samples

Samples, and in particular their characteristic, representative of the lot controlled and the amount of material contained in the increments, are prepared according to the instructions and guidelines.

2.4.3. Principle of the statistical control by sampling the percentage of non-compliant items in a lot

The statistical control of the percentage of non-compliant items in a lot is intended to ensure, by statistical methods based on results obtained from samples, that the quality of the controlled lot complies with the Acceptable Quality Level (AQL) set by the sampling plan.

The acceptable quality level (AQL) of a lot in a sampling plan is a quality level that corresponds to a high probability of acceptance of the lots with this quality level.

The AQL is in fact a quality objective, and the control plans to target this level will be such that the lots showing this level of quality will be accepted with a high probability of acceptance.

2.4.4. Producing increments for all sampling plans

The increments are chosen randomly.

2.4.5. Precautions to be taken: the ratio between the size of the sample and the size of the lot must be no more than 0.15

When the ratio between the sample size and the lot size is not low enough, the formulas used to decide on the controlled lot are no longer valid. To avoid invalidating the results obtained, make sure that the ratio between the sample size and the lot size is no more than 0.15.

Statistical quality controls should be avoided when this ratio is higher than 0.15.

2.4.6. Choice of the sampling plan: comparison of advantages and drawbacks of attribute and variable sampling plans

A 'Quality-Safety' statistical control of the proportion of non-compliant items in a lot, where the AQL is set by the sampling plan, can use either a variable sampling plan or an attribute control. But there are also situations where both types of plan can be used, for example, the fat content of milk.

How can we choose?

2.4.6.1. Attribute sampling plan

No condition imposed on the factor studied.

Advantage: no limit on their use

Simple implementation, as the decision is taken following the counting of non-compliant items in the sample.

The attribute (non-compliant characteristic) can be determined qualitatively (fruit with or without spots) or quantitatively (the result of a measurement qualifying the increment as compliant or non-compliant).

When the attribute is set as a result of a measurement, there is no condition on the mathematical distribution of values of the variable measured.

As these plans – unlike variable plans – set no condition on the factor studied (qualitative or quantitative), there are therefore no limits to their use.

Drawback: the size of the samples is higher than for variable plans

For a same AQL and a same effectiveness, the size of a sample in increments is greater in an attribute sampling plan than in a variable plan.

Circumstances where attribute sampling plans are recommended

- checking a non-measurable quantitative content stated on a document (advertising, labelling, invoice etc.);
- the number of parts containing in pre-packaging (screws, sweets, sheets of paper etc.);
- the number of pages in an exercise book;
- checking the measurable quantitative content stated on a document (advertising, labelling, invoice, etc.) and checking compliance with one or more regulatory values (maximum, minimum, range of values) applicable to a product when they can be measured;
- the percentage of an ingredient stated in the 'ingredients' list on a food label (this percentage is a minimum value, but there is no guarantee that this value is distributed according to a 'normal' probability distribution);
- the maximum fat content of cheese; this content is assessed in relation to the dry matter content of the cheese and there is no guarantee that the value of the ratio between the fat content and the dry matter content follows a 'normal' probability distribution;
- the maximum depth of a tyre wear indicator.

2.4.6.2. Sampling plan by measuring

The values of the measurable variable must follow the normal distribution.

Advantage: smaller sample sizes

For a same AQL and a same effectiveness, the sample size is smaller than in the attribute plans.

Drawbacks: the values of the measurable variable must follow a normal distribution

They cannot be used in all circumstances; before they are implemented, it is essential to ensure simultaneously that:

- the characteristic controlled can be measured, i.e. can be the subject of physical and chemical measurement (determining the value of a temperature, an electrical resistance, a content of useful principles etc.);
- the variation in values of the controlled characteristic follow the Gaussian probability distribution known as 'normal distribution'.

Circumstances where variable sampling plans are recommended

These plans should be implemented every time the compliance of the controlled characteristic with the specification can be measured and is assessed by a value (maximum, minimum, included in a range) where the law of distribution is a normal law:

- the nutrient content of a food intended for a special diet;
- the painted surface stated on the label of a tin of paint.

2.4.6.3. Summary table for choosing a control plan

The plan is chosen based on the response to the questionnaire below:

QUESTIONNAIRE	CHOICE OF AN ATTRIBUTE PLAN	CHOICE OF A VARIABLE PLAN
1. Can the factor studied be measured?	Choose the attribute plan if the answer to question 1 is no. Example: counting parts inside prepackaging	If the answer to question 1 is yes, wait for the answer to the second question before choosing. Example: the fat content of a cheese, the qualitative criterion for premium hams.
2. Are the values taken by the measurable variable distributed according to a normal distribution?	Choose the attribute plan if the answer to question 2 is no. Example: the fat content of a cheese, the qualitative criterion for premium hams.	Choose the variable plan if the answer to question 2 is yes. Example: the painted surface stated on a tin of paint.

2.4.7. Characteristics of sampling plans for the control of the proportion of non-compliant items in a lot

The AQL is set at 1.5 %, regardless of whether the plan is an attribute or a variable plan. This means that the lots with a non-compliant rate equal to 1.5 % pass the control in nearly 90 % of cases and are rejected in nearly 10 %.

2.4.8. Attribute sampling plan for statistical controls

2.4.8.1. Principle of the method

Having been analysed, tested and measured, each increment in the sample, according to the regulatory or normative requirements, is considered compliant or non-compliant; this is the attribute of the increment of the sample.

The number of increments or items that are non-compliant with the sample are then counted:

- where there are no non-compliant increments in the sample taken of eight increments, the controlled lot is
- accepted and the goods are assumed to be compliant;
- where there is at least one non-compliant increment in the sample taken, the goods are assumed to be non-compliant.

2.4.8.2. Specifications of a sampling plan

1. ***Defining the non-compliant increment***
   - see § 2.3;
   - where there are no guidelines, liaise, if necessary, with the competent technical office for the product.
2. ***Sample size***

Unless the regulations or the product data sheet states otherwise, the sample size is eight increments.

3. ***Decision after implementation of the plan***
   - Lot acceptance. This conclusion is drawn when no defective increment is found in the sample of eight increments.
   - Lot rejection. This conclusion is drawn when at least one defective increment is found in the sample of eight increments.

2.4.9. Variable sampling plan for statistical controls

2.4.9.1. Essential precautions before implementing a variable sampling plan

1. ***It must be possible to measure the controlled characteristic***

   It must be possible to measure the characteristic controlled physically and chemically, for example determining the value of a temperature, an electrical resistance, a content of necessary ingredients etc.

2. ***The values of this characteristic are distributed according to a so-called 'normal' probability distribution***

   The values of the controlled characteristic must be distributed following the Gaussian probability distribution known as 'normal distribution'.

   To avoid making an erroneous conclusion on the compliance of controlled goods, it is absolutely essential to make sure of this before the plan is implemented.

2.4.9.2. Principle of the method

- Measuring the controlled characteristic in each increment of the sample;
- Based on the results of these measurements, determining the arithmetic mean and estimating the standard deviation for the sample;
- Deciding about the controlled lot, after having compared this arithmetic mean with the numeric value of the set algebraic formula.

2.4.9.3. Specifications of a sampling plan

1. ***Types of sample to be taken and prepared***

- see the product's data sheet;
- where no sheet exists, liaise, if necessary, with a laboratory and/or the competent technical office for the product.

2. ***Sample size***

Unless the regulations or the product data sheet states otherwise, the sample size is five increments.

3. ***Acceptance of a lot if compliant with the regulations in force***

The items in the lot are compliant if the value of the measured variable is lower than or equal to the upper limit that must not be exceeded, for example the maximum sodium content of a product for a low salt diet.

2.5. APPENDIX: THE NORMAL DISTRIBUTION

In probability theory, it is said that a real-valued random variable X follows a normal distribution (or Gaussian distribution) of expectation μ and a strictly positive standard deviation σ (therefore with variance σ2) if this realvalued random variable X accepts for probability density the function p(x) defined, for any real number x, by:

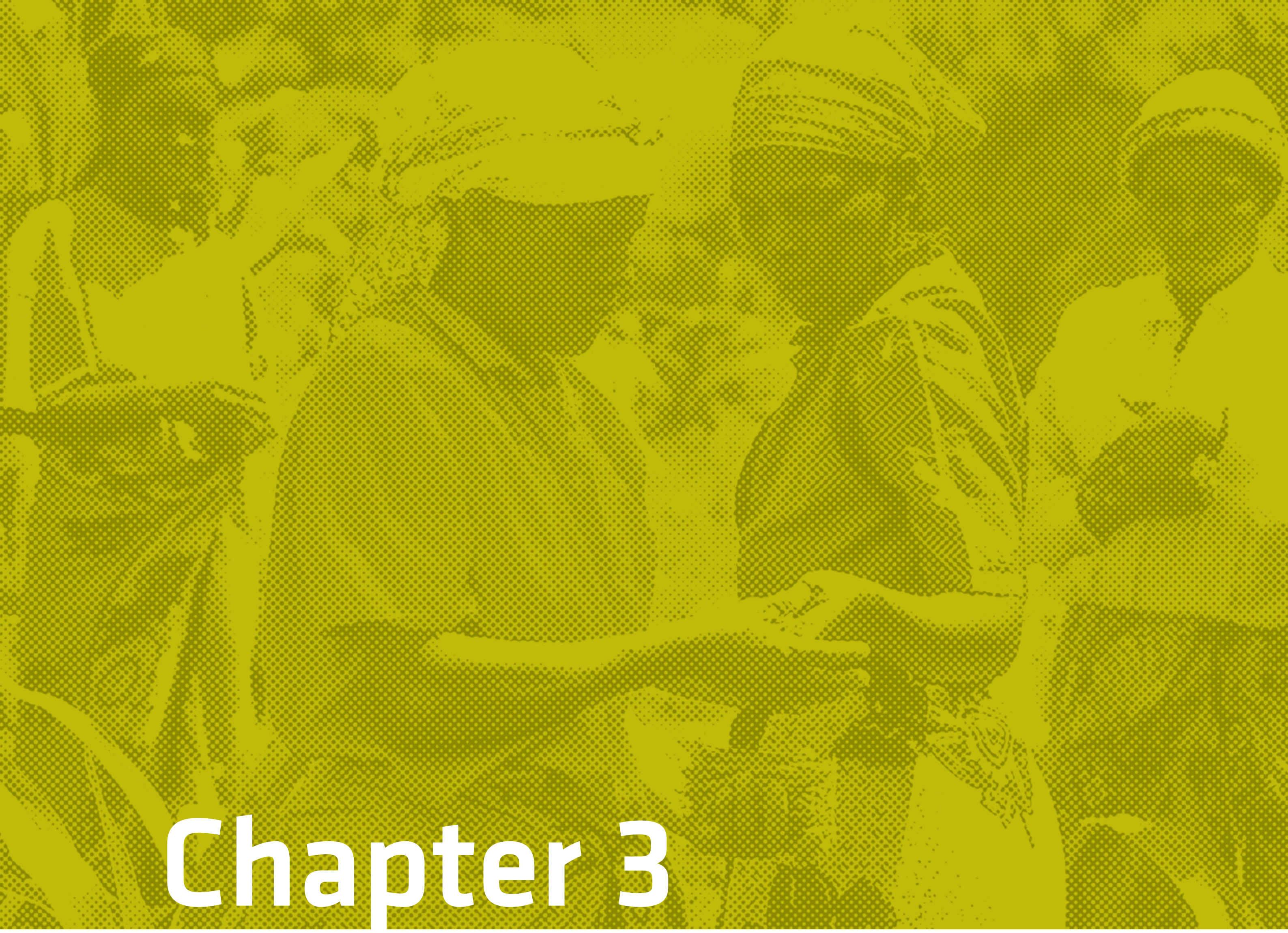
$$p(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2}$$

Such a random variable is then called a Gaussian variable.

It is usually written as follows:

$$X \sim N(\mu, \sigma^2)$$

The normal distribution is one of the main probability distributions. It was introduced by the mathematician Abraham de Moivre in 1733 and used by him to approach probabilities associated with binomial random variables with a very large parameter n. This distribution was highlighted by Gauss in the 19th century and is used to model many biometric studies. Its probability density draws a curve called a bell curve or Gauss curve.



Chapter 3

Sampling methodology during official controls

3.1. INTRODUCTION

3.1.1. Purpose

This chapter is intended to set general rules about sampling techniques (constitution, execution) on the control site, to check the compliance of all types of goods with a specification, whether or not regulatory. It does not include decision rules on the lot of controlled goods.

3.1.2. Scope

Sampling covers all sampling operations on all types of goods. It ceases at the first operation of a physical and chemical analysis on a homogeneous portion of the sample.

Appendix summarises all operations for sampling goods.

3.2. SPECIFIC VOCABULARY RELATED TO SAMPLING

3.2.1. Lot

It is a defined quantity of goods determined, manufactured or produced in conditions that are assumed to be uniform.

The term 'lot' means a lot destined to be controlled by sampling, i.e. a quantity of material or a set of items taken as a sample.

In the meaning of this chapter for sampling controls, the lot defined above must also be sold or intended for sale as it is.

3.2.2. Homogeneous lot

It is a lot for which no identifiable cause for variation in components and raw materials occurred during production, preparation, manufacture, transport or storage.

In terms of statistics, a lot is considered to be homogeneous in relation to a given property if the distribution of values noted for characteristic parameters of this property is approximately normal.

Where the distribution is not normal or the value of its standard deviation is high, the lot must be considered as heterogeneous.

Items in a lot can be both considered as homogeneous for a given property and heterogeneous for another.

3.2.3. Item

It relates to:

- either an object, a unit or a defined quantity of material where a set of observations can be made;
- or the result of previous observations, be they qualitative (the increment is qualified as compliant or noncompliant) or quantitative (measurement result).

3.2.4. Increment

If a lot comprises individualised units, it is an item in the lot taken to form a sample.

If the lot is bulk goods, it is a quantity of material taken once, at a point in the lot, to form a sample; this quantity is then individualised permanently in a receptacle (box, spoon etc.) for observations or measurements.

3.2.5. Characteristic

Property used to distinguish the individuals of a population. A characteristic can be qualitative (attribute) or quantitative.

A quantitative characteristic is said to be continuous if, in its field of variation, it can be assimilated with a continuous variable in the sense of mathematical analysis: for example, the protein content of a food.

A quantitative characteristic which can only take integer values is called discrete: for example, the number of pre-packs in a box.

3.2.6. Sampling

Sampling relates to a procedure for sampling goods to form a sample.

3.2.7. Sample

It is a set of one or more increments taken in a lot of goods intended to provide information on these goods or the process that produced them. This information can potentially be used as a basis for a decision.

3.2.8. Sample size

This is the number of items or increments making up the sample.

3.2.9. Sample plan

It is a plan whereby one or more samples are taken to provide information to be compiled and a decision to be made where necessary.

It sets the rules stipulating the sampling modalities and, if appropriate, these rules can be supplemented by fixing acceptance or rejection conditions of the controlled lot.

Every 'Quality-Safety' sampling plan must state the following instructions and include a decision rule for the controlled lot:

- the nature or objective of the control;
- the type of sampling used (see nomenclature of samplings in 3.3.12);
- the type of each sample taken and prepared (see nomenclature of samples in 3.3.13);
- the number of increments of each sample taken.

The principal stages in the sampling procedure are mainly:

- the increment modalities;
- the modalities for preparing samples based on these increments to create the sample intended for the physical and chemical analyses;
- the modalities for identifying each lot and each sample taken;
- the acceptance or rejection conditions for the controlled lot.

3.2.10. Non-compliant or defective increment and non-compliant or defective item

It is a defective increment or item with one or more defects defined in the sampling plan, based on the regulations or standards or professional usage.

3.2.11. Defect

It is the non-compliance of an item or increment to the stipulations imposed for a characteristic by the sampling plan.

3.2.12. Sampling types

If appropriate, the samplings listed below can be used to further characterise the goods to be controlled.

3.2.12.1. Random sampling

It is a sample made up of items in a lot that all have the same chance of being part of the sample.

Where the lot is not made up of individualised parts, it is divided mentally into virtual items.

All the items in a lot (real or virtual) have two distinct numbers:

- the number of items in a sample is determined or designated randomly, using random numbers generated by a table or a calculator or statistical software;
- all possible combinations of n items in a lot of N items have therefore the same probability of being taken.

3.2.12.2. Stratified sampling

The strata are the different parts into which the population to be sampled is divided.

Stratified sampling involves taking a sample of items or increments from each stratum; all these samples make p the sample intended for the physical and chemical analysis.

3.2.12.3. Multi-stage sampling

For the sake of convenience, the items making up the goods to be controlled can be grouped into primary units (sometimes called clusters) so that every item belongs to a primary unit and only one.

The items are then secondary units. Two-stage sampling involves taking a sample of primary units at the first stage, then at the second stage, a sample of secondary units from each primary unit taken at the first stage.

The primary sample is the sample taken in a lot at the first stage of multi-stage sampling.

Consequently, the sample taken in the primary sample is called the secondary sample.

Samples with more than two stages can therefore be considered under a similar mechanism, with the final sample corresponding to the final sampling stage.

Example of three-stage sampling in a lot of pre-packs of cigarettes controlled on the manufacturing line:

- primary unit: a box;
- secondary unit: a pack;
- third unit: one cigarette.

3.2.13. Samples types

The samples defined below will be executed according to the instructions in the sampling plan.

3.2.13.1. Representative sample of the controlled lot

Sample that reflects the best the controlled goods; the sample taken at random is a representative sample.

3.2.13.2. Global sample

This is the quantity of goods formed by uniting all the increments.

3.2.13.3. Reduced sample

This is the quantity of goods coming from the reduction of the global sample or a partial sample in conditions guaranteeing the representativeness of samples; this sample reflects as closely as possible the sample intended for the physical and chemical analysis and the amount of matter it contains cannot be less than the amount required for the physical and chemical analysis.

3.2.13.4. Laboratory sample

This is a small quantity of goods representative of the reduced sample and intended for the physical and chemical analysis in a laboratory.

3.2.13.5. Reference sample

This is a sample prepared at the same time as the laboratory sample which is stored to serve as a laboratory sample, mainly in the event of a counter-expertise.

3.2.14. Preparing a sample

For bulk goods, this involves all physical operations such as mixing, dividing etc., required to convert the global sample into the state of laboratory sample.

Preparing a sample for the laboratory must ensure the representativeness of the sampled lot.

3.3. SAMPLING MEANS AND METHODS

3.3.1. General rules

These rules aim to obtain and prepare a sample to determine one or more characteristics of the controlled product.

The sampling must be the most representative of the controlled goods.

3.3.1.1. Equipment used

See Investigator guide in Appendix 1.

3.3.1.2. Preliminary thoughts on the sampling operation

See Investigator guide in terms of quality approach sampling.

Preliminary thought should be given to the following questions before starting the sampling operation.

- Aim of the operation?
- Gathering information on the quality and/or safety of the goods?
- Noting an infringement?
- Type of constraints imposed by the operation?
- Suitability of the equipment?
- State of the goods (solid, liquid, gaseous), degree of homogeneity of the goods, dimension of the goods?
- Storage conditions?
- Nature of the characteristic(s) to be controlled?

3.3.1.3. Instructions of a sampling plan

Sample taking must follow the instructions in a sampling plan chosen in advance.

1. ***Which sampling plan should be used?***

The plan is fixed:

- either by the data sheet;
- or by a standard relating to the controlled product or a sampling plan;
- or by special instructions from the laboratory which will then be included in a product sheet.

2. ***Standard structure of a sampling plan***

Every plan to be used must include all the headings listed and expanded below. Failing that, the professionals controlled may dispute its validity.

The control nature or objective

- Based on statistical principles;
- Or every plan to be used must include all the headings listed and expanded below. Failing that, the professionals controlled may dispute its validity.

The type of sample taken and prepared

See nomenclature of samples in 3.12.

Size of each sample taken as increment

See the product's data sheet.

Modalities for identifying each sample taken

See Investigator guide.

Type of sampling used

See nomenclature of samplings in § 3.

Main stages in the sampling procedure

- Quantity of goods to be sampled. See the relevant product data sheet or liaise, if necessary, with an official laboratory.
- Increment modalities. See below.
- Modalities *for preparing samples from these increments.* See specific sampling provisions in the product sheet; liaise, if necessary, with an official laboratory.

Acceptance or rejection conditions for the controlled lot

Liaise with an official laboratory.

3.3.1.4. Sampling procedure

The following sampling procedure must be followed when taking samples.

1. ***Identifying the lot before any samples are taken***

Note:

- the size of the lot (volume, weight, number of items included in it etc.)
- the inscriptions on the labels or commercial documents.

2. ***Examining the lot before any samples are taken***

Note:

- the characteristics of its environment, mainly the temperature;
- the state of the goods (damaged parts, heterogeneous parts etc.).

3. ***Heterogeneous lots***

It is essential to have information on all the different heterogeneous parts in a heterogeneous lot; these are processed as separate lots and subjects for increments:

- on the damaged parts;
- on the fractions of the lot obtained by dividing them virtually (mentally) into fractions with more similar properties.

Nevertheless, these operations are not mandatory if information is available on all the heterogeneous parts of the lot; this information is sent to the analysis laboratory.

4. ***Taking increments***

Precautions to be taken

Protecting sampling operations (controlled products and control equipment) from any contamination such as rain, heat, dust or modification to receptacles in which the increments are placed.

Choosing increments at random

Whenever possible, to avoid any challenge to the representativeness of the sample increment, increments should be chosen at random without fail. Proceed as follows:

- if the lot is not formed of individualised parts, for example milk in a tank, wheat stored in a hopper or wine stored in a barrel, the lot is divided mentally into virtual items;
- all the items in a lot (real or virtual) have two distinct numbers;
- all the parts of the controlled lot, whether or not individualised virtually, must have the same chance of being taken; this requirement is ensured by using random sampling. This involves making up a sample with items in a lot (real or virtual) that all have the same chance of being part of the sample;
- the number of items (real or virtual) taken and included in a sample is determined by chance, using random numbers generated by a table or a calculator or statistical software.

When it is impossible to take items at random (for example, in a huge warehouse where the products are badly stored), the following precautions must be taken:

- avoid choosing the most accessible items systematically;
- do not systematically choose items that stand out with a clear characteristic;
- where there is a periodic phenomenon that can give highly biased results and which can potentially distort the validity and representativeness of the results, for example an out-of-adjustment dosing machine so that every x seconds the product conditioned by this machine shows defects, or a food contaminant that is distributed selectively at the bottom of a hopper, avoid taking every k second or every kth packet or every k^{th} centimetre or taking a unit every 'n' pallet, carton or pre-pack etc.

A periodic phenomenon is assessed, where appropriate, by:

- examining manufacturing control charts for the goods;
- information to be compiled on the product storage from a laboratory, control networks or professional organisations.

Avoid choosing items resembling each other, for example those corresponding to a short manufacturing time or which are contained in the same pallet or carton.

Goods in the form of individualized objects

For example, pre-packs or manufactured objects.

Case of instruments or manufactured objects and pre-packs where the content is less than the minimum quantity to be sampled for physical and chemical analyses

It is preferable to keep the goods in their original packaging when controlling the average value of a characteristic, in order to maintain the initial state:

- take sufficient numbers of pre-packs, instruments or manufactured objects to reach the quantity set by the instructions from the official laboratory to constitute an increment;
- the sample intended for the physical and chemical analysis is then made up of all increments set by the sampling plan.

Case of instruments or manufactured goods and pre-packs where the content is more than the minimum quantity of goods to be sampled for physical and chemical analyses

Where necessary, it is preferable to keep the goods in their original packaging in order to maintain their initial state. Unless specified otherwise in the sampling plan, the sample size is made up of a pre-pack of instruments or manufactured goods.

Given the inaccuracy of investigations based on a single sample, the sample size and analytical determinations must be increased to obtain a significant result.

Bulk goods

1. Quantity of goods to be sampled

- where the goods are covered by a data sheet, take the amount fixed by the sheet;
- where the goods are not covered by a data sheet, liaise with an official laboratory.

2. Operating procedure

It is recommended to carry out the sampling when loading or unloading the goods from the means of transport, or during the storing process, when the goods are being moved

Goods are taken randomly. They are no longer goods that participate in a draw, but virtual units (quantity of goods unloaded in x seconds, content of a receptacle etc.).

For example, if unloading takes four hours, samples are taken at the 10th, 21st and 37th minute; the range of minutes chosen was determined by random prior to the sampling operation.

A firm operating technique is required in a lot of moving goods; this means cutting through the entire thickness of the stream so that the global sample corresponds to a full section of the stream, which can be heterogeneous given the different densities and particle dimensions, or possess heterogeneity factors generated by the goods' manufacturing, storage or circulation techniques.

This type of operation can only be envisaged if automatic sampling tools are available.

General case

Wherever possible, to offset any effects of a selective distribution of components through the density, the samples should be taken in the entire depth of the bulk goods.

Where no information on the distribution of components or contaminants in the bulk goods is available, the increments should be multiplied in the bulk space.

The sampling probe must be plunged in several directions using a turning movement, except for liquids and moving goods.

Powdered goods

The sampling equipment must have a large enough opening to be able to take the largest particles in the goods.

Failing suitable automatic sampling equipment, probes with a large enough opening should be used. Wherever possible, they penetrate the goods in several directions at a regular speed until they reach the bottom.

Liquid or semi-liquid products stored in drums or barrels

The product must be mixed carefully, for example with a stirring rod long enough to reach the bottom and stir all the product, and light enough to be handled quickly.

Avoid creating a foam. The walls and bottom should be scraped if necessary.

A dipping receptacle of a size relating to the minimum quantity is then plunged into the liquid.

The constant plunging speed must be estimated so that the receptacle fills up as it descends and is completely full when it reaches the bottom.

Liquid products stored in large receptacles and tanks

Where it is impossible to stir the contents mechanically to mix them up, samples are taken at several points.

Pastes and semi-solid products, depending on the consistency, form and weight of the goods to be sampled

One of the following techniques is to be used, depending on the consistency of the goods:

- hard consistency: sampling using a knife or saw; making several cuts which direction depends on the nature of the product;
- other cases: sampling using a probe as indicated above.

Gases

Consult an official laboratory before sampling.

5. ***Constituting samples from increments***

Follow the instructions in the sampling plan (*cf.* 3.2.9).

6. ***Sampling report***

All samplings must have a written sampling report according to defined modalities.

3.3.2. Rules specific to the products

3.3.2.1. Existence of data sheets

See the sheets established by each of the authorising offices.

3.3.2.2. Lack of data sheets

If appropriate, follow the general rules of this chapter and liaise with an official laboratory for any specific additional sampling modalities.

3.4. SAMPLING AND PROCESSING

3.4.1. Purpose

The aim of the data sheet, included in this chapter, is to define the minimum quantities to be taken for the samples intended for the laboratories.

It does not deal with the sampling modalities (number of units per sample, sampling modalities) intended to ensure the representativeness of the sample in the lot (see regulations or product data sheets when they exist).

3.4.2. Scope

This chapter covers the samplings taken under the 'Quality and Safety' controls, whether they be intended for the laboratory or set aside for an expertise, if necessary. It states the weight (or volume) of the sample to be sent to the laboratory so that it can make all routine determinations in a given product.

3.4.3. Specific vocabulary

3.4.3.1. Sampling, sample, unit

A sampling can be made up of one or more samples (e.g.: three samples for the PO_3, one sample for the PE1); each sample can be made up of one or more units (example: one PO_3 of ham, made up of three samples each comprising eleven units).

3.4.3.2. Sales unit

Sales units are normally lots of two to six (or more) product units (e.g. a six-pack of yoghourt pots).

3.4.3.3. Minimum quantity

Amount of product used by the laboratory to carry out all routine determinations.

N.B.: the minimum quantity must be respected for each unit in the sample.

3.4.4. Quantities to be taken

The quantities to be taken, shown in the headings of this chpater, are per sample (and per unit). They have been assessed as the minimum required by the laboratory to carry out the routine determinations on the products in question.

For the products not listed in the headings, the quantity will be assessed by comparison, or even better, after having obtained the information from the concerned laboratory. This is particularly true, wherever possible, for cosmetics, maintenance products and industrial chemical substances, where counting has deliberately been limited and which require multiple determinations, given their complex composition. The same applies to industrial equipment and products.

In case of doubt, especially when assessing the contamination rate of pollutants, contact must be made with the competent laboratory to determine the exact quantity to be taken, or a lesser quantity than planned when the analysis is focused on determining a reduced number of criteria.

For goods conditioned in small units (butter, cheese and other dairy products, biscuits, sugar, detergents etc.), the sampling must gather enough unit packs to obtain the quantity indicated.

3.4.5. Special conditions for transporting and storing samples

The investigators may usefully refer to the procedures of the investigator guide (see annex 1) or product sheets (when they exist).

3.4.5.1. Special transport conditions

The product must be left in the original packaging whenever possible. This is especially true for olive oils, coffees, chicory coffees, teas, household or agricultural pesticides, cosmetic and hygiene products and distilled or demineralised waters.

Additional protection must nevertheless be guaranteed to maintain the characteristics of the goods, for example:

- to prevent increasing the proportion of fragments in coffee;
- to prevent some compounds like formaldehyde from evaporating (floors, materials in contact with foodstuffs etc.); contact should be made with the competent laboratory to determine how to proceed (type of overpack to be used etc.);
- to prevent cross-contamination during the transport of samples intended to find materials that are in contact with food or pesticide residues and samples for microbiological analysis;
- to contamination through samples of fruit and vegetables intended to seek out pesticide residues coming into contact with other processed products, ventilated bags must be used (mesh nets are not suitable);
- to prevent the degradation of mycotoxins, the samples must be transported, wherever possible, in opaque packaging;
- to prevent the degradation of specific components contained in foods intended for particular nutritional use (e.g. most vitamins), it is important to protect them from potential degradation as much as possible.

If fractioning takes place, the label or original packaging must be sent, whenever possible, to the laboratory with its intended sample.

It is mandatory to store samples of perishable products using the cold chain, in accordance with the instructions of the investigator guide (see appendix).

3.4.5.2. Sample storage conditions

All samples must be stored in the best conditions, if necessary away from light, heat or humidity, depending on the nature and the indications marked on their packaging.

Only two chemical preservatives – potassium dichromate and salicylic acid – can be used in the following conditions with the added preservatives stated on the label:

- potassium dichromate used for bulk raw milk at a rate of 0.25 g per 250 ml bottle,
- salicylic acid used for vinegary wines at a rate of 1 g per bottle. A sample without the addition of this preservative is sent to the laboratory at the same time.

3.4.6. Food products

3.4.6.1. Food additives and processing aids

Flavouring agents	200 g
Other additives (emulsifiers, thickeners, texturising agents, nutritive value improvers etc.):	
• pure (purity criteria)	50 g
• dosage in food products	200 g
• in formulation (composition)	200 g
Oenological products:	
• all products except enzymes	500 g
• enzymes	50 g

3.4.6.2. Animal feed

Mineral compounds, simple or compound feeds	> = 500 g to be adapted to the number of determinations requested
Additives	250 g minimum
Dioxin, PCB	See § "Pollutants and contaminants"

3.4.6.3. Drinks

Table, spring and mineral waters		1 l (excluding microbiological analysis) + 1 l if micro-pollutants
Fruit and vegetable juices, nectars, non-alcoholic drinks		1 l.
Concentrated fruit juices		250 ml
Cordials		500 ml
Lemonades and fizzy drinks	1 sample for CO_2 dosing and 1 sample for the other determinations + 1 sample for volume measurement	2 x 500 ml (3 if greater volume)
Preparation for drinks		100 g
Wines	General case	750 ml
	NMR analysis: 1 additional sample	750 ml
	Fresh grapes for micro-vinification for NMR	10 kg min
	Search for ochratoxin A	See Mycotoxins
Ciders	1 sample for NMR analysis and 1 sample for the other determinations	2 x 750 ml
Aperitifs		700 ml
Spirits		700 ml
Natural flavours of cordials		750 ml

3.4.6.4. Fruit, vegetables, mushrooms

1. ***Pesticides- residues***

Refer to international standards for the quantities to be taken (e.g. *Codex* Standard: **Recommended Methods for Sampling for Pesticide Residue Determination for Compliance with MRLs– *CAC/GL 33-1999 or Laboratory Guidelines***

2. ***Nitrates***

Lettuce and spinach	Nitrate dosing	10 lettuce or 1 kg of spinach

3. ***Heavy metals, mycotoxins, radioactivity***

See relevant chapters.

4. *Other determinations not specified elsewhere*

Dried fruit and vegetables (nuts, prunes, apricots, peanuts etc.)		Humidity, varietal identification, rancidification, calibre, preservatives	1 kg (at least 5 units for coconuts)
250 g (or 1 sales unit)			250 g (or 1 sales unit)
Potatoes		varietal identification	at least 30 tubers
Champignons	fresh	varietal identification	500 g
	dried	varietal identification	30 g
Truffles	whole or in bits	varietal identification	a number of pieces or bits representative of the lot representing 25 g or 1 sales unit
	fragments	varietal identification	50 g or 1 sales unit
	peel	varietal identification	25 g or 1 sales unit
	juice	varietal identification	100 ml

3.4.7. Milk, milk products, eggs and egg products

Natural milks:		
• milk from all animal species, in raw state, pasteurised, sterilised, flavoured		250 ml (or 1 sales unit)
• milk from all animal species, for a limited control seeking antibiotics or identification of the producing species		150 ml
Processed milks:		
• concentrated milks		150 g (or 1 sales unit)
• powdered milks		100 g (or 1 sales unit)
• fermented, gelled, renneted		120 ml (or 1 sales unit)
• creams and cream desserts		100 g (or 1 sales unit)
• milk desserts		100 g (or 1 sales unit)
• cheese	General case	150 g
	cheese: dosing of the natamycin	200 cm^2 surface crust
Eggs		6 units
All types of egg products		250 g

3.4.8. Fats

Butter	more than 100 g or 1 sales unit
Other fats (all types: fats, oils) (*)	250 g (100 ml minimum for frying oils)

(*)Packaged olive oil is always kept in its original packaging.

3.4.9. Microbiology

Refer to the product data sheets or laboratory guidelines for the quantities to be taken.

3.4.10. Genetically modified organisms (GMO)

Seeds	oilseed rape	200 g minimum
	corn and soy bean	1 kg minimum
Raw products (grain etc.)		10 000 or their equivalent weight with a maximum of 3 kg
Products of first-stage processing (semolinas, flour, grits, meals etc.		1 kg
Liquid products		500 ml
Paste and viscous products		500 g
Finished products (conditioned)		2 individual packages or 100 g

3.4.11. Pollutants and contaminants

3.4.11.1. Asbestos

Asbestos detection	pre-packed products	as is
Asbestos detection	materials (sheets of fibre cement etc.)	5 x 5 cm surface area

3.4.11.2. Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs	Human and animal food	500 g

3.4.11.3. Heavy metals

Pb, Cd, Hg	pre-packed products		liquids: 1 unit
			solids: 1 to 10 packs or units depending on the lot volume t
	bulk products	Minimum weight	1 kg made up of three to ten increments according to the weight of the lot
		However, the rules below can be applied to the following products. For the others, contact the laboratory (additives, herbs and spices etc.).	
		cereals as grain or flour	300 g
		fruit, vegetables, mushrooms, potatoes	300 g if unit weight < 25 g 1 kg if unit weight > 25 g
		fish	3 units (500 g minimum)
		shellfish, crustaceans, molluscs	1 kg
		Animal feed	500 g minimum

3.4.11.4. Mycotoxins

Mycotoxins in food intended for human consumption:	
Ochratoxin: cereals and grape	1 to 10 kg depending on lot weight
Ochratoxin: wine and grape juice	1 litre
Patulin: juice, stewed fruit	1 to 10 packs or units
Alfatoxins B and G: dried fruit, oil crops, cereals	3 to 30 kg depending on lot weight
Aflatoxins B and G: spices	1 to 10 kg depending on lot weight
Aflatoxin M1: dairy products	500 g minimum or 1 litre minimum
Other mycotoxins, other processed products	500 g minimum
Mycotoxins in products intended for animal feed	
Aflatoxin B1	500 g minimum per global sample (the number of global samples depends on the size of the lot)

3.4.11.5. Nitrates in plants

Nitrates in plants	See products

3.4.11.6. Polychlorinated biphenyls (PCBs) and dioxins

PCB and dioxins	human food	1 kg (for the PE1, a 500 g sample is deemed sufficient, even 200 g for high-fat products)
PCB and dioxins	Animal feed	500 g
Detecting MCPD (monochloropropane diol)		1 sales unit of 100 ml minimum (liquids) or 200 g (solids)

3.4.11.7. Radioactivity

Fruit and vegetables	detecting radioactivity	1 kg
Cereals and legumes	detecting radioactivity	1 kg
Animal foodstuffs	detecting radioactivity	1 kg
Milk	detecting radioactivity	1 l
By-products	detecting radioactivity	1 kg (or 1 l)
Aromatic plants and dried mushrooms	detecting radioactivity	200 g

3.4.11.8. Pesticide residues

Pesticide residues	See products

3.4.12. Special food products and nutritional labelling

3.4.12.1. Minimum required for a full analysis

Basic analysis and other determinations	200 g or 400 ml
Vitamins and polyphenols	300 g or 400 ml
Minerals and trace elements	50 g

3.4.12.2. Quantities by type of determination

Caloric value and dietary fibres	100 g or 200 ml
Amino-acids, sugars, polyols, sweetening agents, preservatives	100 g or 200 ml
Miscellaneous nutritional additives (creatine, carnitine etc.)	100 g or 200 ml
Common vitamins: C, B1, B2, B5, PP, B6, H, B9, A, E, b.carotene	150 g or 250 ml
Vitamin D, K, nucleotides, choline, carotenoids	200 g or 300 ml
Polyphenols (flavonoids, isoflavones, OPCs)	50 g or 100 ml
Minerals – trace element: amount to meet most requests	50 g
Sodium, potassium, calcium, magnesium	10 to 20 g
Phosphorous	10 to 20 g
Zinc, copper, manganese, iron, chromium	10 to 20 g
Iodine, selenium	20 g minimum
Food supplements based on plants and released medicinal plants: macro- and microscopic examination, dosing of main active ingredients	50 g
Protein allergens	20 g per type of allergen

The quantities taken must be accumulated for a full analysis

3.4.13. Aromatic products, soups and seasonings

3.4.13.1. Flavouring, spices and essential oils

Flavourings and spices:	
orange blossom water	300 ml
vanilla pods or powder	100 g
vanilla extract	100 ml
peppers (all types)	25 g
saffrons	5 g for basic analysis
other flavourings or spices	50 ml or 50 g
Flavouring agents:	
base product (… flavouring, … extract), concentrated or otherwise	25 ml
compound flavourings, aromatic compositions, floral waters	250 g (or 250 ml)
natural flavourings	See products

3.4.13.2. Infusion plants

Infusion plants, fresh medicinal plants	dosage of pesticide residues	200 g
Infusion plants, fresh or dried medicinal plants	identification	25 g

3.4.13.3. Coffees, teas, chicory

Green coffees	identification	350 g
Roasted coffees, whether or not decaffeinated, as beans or ground	identification	250 g
Coffee substitutes	identification	100 g preferably
Chicory coffees	identification	100 g packed
Teas	identification	100 g
Soluble extracts or liquids of coffee, chicory, tea or coffee/chicory	identification	100 g (or 100 ml)

3.4.13.4. Stocks and soups

Liquid or paste products	200 g (or 200 ml)
Powdered products	100 g

3.4.13.5. Vinegars

All types of vinegar	500 ml

3.4.13.6. Other seasonings (condiments and salt)

Mustards, condiments, sauces	100 g
Salts (all types)	100 g
Brines (fresh preparations before any use)	250 ml

3.4.14. Meat-based products

3.4.14.1. Cooked meats, meat- and poultry-based products

Meats and offal (including minced meat)	200 g
Meat-based products (to determine the species)	lean meat (no bard, rind, crust etc.)
Meat-based products (for the histological examination)	50 g
Cooked meat preparations in bulk, canned or semi-preserved (cassoulet, choucroute etc.)	one sales unit (if physical and chemical analysis on meat content, 200 g minimum of meat content)
Cooked meats (all preparations, including hams)	300 g (so that 200 g remain after removing the fat, rind, bard, jelly, crust etc.)
Coated cooked meats (barded, with crust, in jelly etc.)	600 g (so that 200 g remain after removing the fat, rind, bard, jelly, crust etc.)
Truffled preparations (to determine the proportion of truffles)	600 g
Foie gras and (chemical-)based preparations	200 g
Foie gras and foie gras-based preparations (% of bits)	1 sales unit
Foie gras and foie gras-based preparations (for histological examination only)	50 g (or 1 sales unit)

3.4.14.2. Fishery and aquaculture products

Whole fish (including species identification)	400 g or several specimens
Bits of fish, fillets etc. (including species identification)	250 g
Fresh, frozen and deep-frozen pre-shelled crustaceans	250 g
Fresh, frozen and deep-frozen unshelled crustaceans	400 g
Scallops	300 g
Frozen shellfish	250 g of the edible part
Canned fish, crustaceans and miscellaneous pre-packs	1 sales unit
Canned food for histamine determination	9 units from the same lot
Canned and deep-frozen food for metrological control (net weight, glazing)	20 units from the same lot
Miscellaneous	200 g
Polyphosphates	200 g

3.4.14.3. Gastropods, amphibians

Gastropods		12 à 24
Amphibians	Ionisation detection	200 g minimum

3.4.15. Cereal products

Cereals (for human consumption)	500 g
Flours and dusting agents	200 g
Breadmaking flours (to determine the W value)	1 kg
Breads (all types, including toasts) – Crispbreads (all types, must be in original packaging)	200 g
Dietary pasta (all types)	200 g
Fresh pastries and industrial pastries, biscuits, gingerbreads	200 g
Semolinas, tapiocas	200 g

3.4.16. Sugar-based products

Sugars	150 g
Vanilla sugars	100 g
Honeys and hive products	150 g
Royal jelly	20 g
Confectionery products (including candied fruit)	250 g
Natural flavourings	400 g
Jams, marmalades, jellies	250 g
Cocoas, chocolate products and breakfasts	150 g
Chocolates and confectionery products containing inclusions (e.g. hazelnut chocolates, nougats with dried fruit etc.)	300 g
Ices, ice creams, sorbets	200 g
Preparations for making ices, ice creams and sorbets	200 g

3.4.17. Industrial materials and industrial products

3.4.17.1. Fibres, textiles and leather

Materials, in lengths or samples	l/3 of a 20 cm strip across the whole width
Patterned materials in lengths or samples	as above and with at least two full patterns
Items for the feet (stockings, socks etc.)	1 pair
Clothing	1 complete item if it has different parts and colour
Detecting azo dyes in the textiles and leathers	1 complete item or a representative part of all colours present, minimum 5 g per colour
Flammability test	1 unit per maintenance mode stipulated on the labelling

3.4.17.2. Personal care, cosmetics

Aerosol products	general case	2 generators
	control of the regulation under pressure	6 generators
Perfumery and grooming products containing alcohol		25 ml or 25 g
Products not necessarily containing alcohol:		
Products intended for contact with the mucous membranes:		
• dental and mouth care products		50 ml or 50 g
Products intended for other uses:		
• beauty products (mask, cream, emulsion, gel, oils, foundations, powders); products presented as affecting the skin (sun, sun tanning, anti-wrinkle products); deodorants, anti-perspirants; products containing surface agents (shampoo, bath product, toilet soaps etc.)		100 ml or100 g
• products for hair (all types)		2 packs
• products for the nails (varnish, removers)		25 ml
• tattooing inks		2 x 50 ml
• PPE • Sunglasses	for optical control	1
	for control against standard	5

3.4.17.3. Hardware products

Aerosol products (general case)	2 generators
Control of the regulation under pressure	6 generators
Other products:	
• cleaning products for materials entering into contact with foodstuffs (disinfectants not subject to authorisation, dishwashing rinsing products etc.)	250 ml or 250 g
• disinfectant or anti-pest products not subject to authorisation (household products, products for industries, descaling agents, bleach etc.)	250 ml or 250 g
• soap (all types), detergents, softeners, scouring products, cleaning products (waxes, greases, polishes), strippers, products for windows, wood varnishes, glosses, waterproofing agents, primers, stain removers, solvents (trichloroethylene, turpentine, benzene etc.), paints, varnishes, driers, adhesives, linseed oil, inks and any hazardous or poisonous substances or preparations (phosphoric, hydrochloric or sulphuric acid, soda, potassium, ammonium etc.)	100 ml or 100 g
Distilled or demineralised waters	1 pack
Methylated spirits, denatured alcohols	1 pack

3.4.17.4. Fuels, petroleum products

Petroleum fuels (unleaded and leaded petrol, engine mixes)	2 l

3.4.17.5. Toys

Soft toys with removable clothes	European safety standard	2 units
Toys	request for tests relating to toy standards	1 unit
	for electric toys, when tests under standard EN 50088 are also requested	1 additional unit
Percussion primers for priming pistol		minimum 100 primers

3.4.17.6. Conditional material for food contact

Wood for PCP analysis	see product data sheets or the laboratory	200 cm²
Rubber		10 g
Ceramics, glass		4 units
Metal materials		3 units and at least 60 cm²/unit
Plastics		12 units
Plastics in the form of film		12 units of A4 format size

3.4.17.7. Metals and alloys

Metals and alloys	all types excluding precious metals	100 g
Jewels	to detect allergenic metals, false precious stones	1 jewel 2 pearls

3.4.17.8. Furniture, home decoration

Furniture	safety, identification of the wood	1 unit
Garland	constructive analysis and IP index	1 unit

3.4.17.9. Tools

Electric tools	1 (2 if EMC)
Thermal motor tools	1

3.4.17.10. Small household goods and domestic equipment

Electrical goods	e.g. robots, hairdryers, diffusers, light fittings etc.	1 (2 if EMC)
Domestic equipment	e.g. kitchen utensils	to be specified according to the type of equipment

3.4.18. Agricultural and assimilated products

3.4.18.1. Plant health products

Agriculture pest control and assimilated products	
• pre-packed products	1 sales unit
• bulk products	500 g

3.4.18.2. Seeds (excluding GMO)

Cereals	
• corn – basic seed of inbred lines	250 g
• rice	500 g
• buckwheat	600 g
• oats, wheats (durum and soft), corn (basic seed apart from inbred lines and certified seed), barley, rye, sorghum, triticales	1 kg
Vegetables	
N.B. : • For F1 hybrids, the minimum weight can be reduced to 1/4 of the fixed weight, without dropping below 5 g and include at least 400 grains; • For lots packed in small packages, the sample can be reduced to 2 bags without the number of grains dropping below 400 per sample.	
• celery, watercress, marjoram, purslane, savory, thyme	5 g
• dill, basil, carrot, lettuce, sorrel, parsley, dandelion	10 g
• chicories (curly endive, scarole, witlof and wild, large leaved)	15 g
• orach, aubergine, chervil, Chinese leaves, lamb's tongue, turnip (root), parsnip, leek, roquette, tomatoes	20 g
• cabbage (including broccoli and cauliflower), Welsh onions, chives, cucumber-gherkin, garden cress, fennel, onion	25 g
• salsify, black salsify	30 g
• hot pepper-pepper	40 g
• cardoon, radishes	50 g
• spinach, rhubarb	75 g
• asparagus, garden beetroot, melon, Swiss chard	100 g
• artichoke, courgette	150 g
• water melon, New Zealand spinach, pumpkin-gourd	250 g
• squashes, marrow	350 g
• lentils, peas	500 g
• beans	700 g
• broad beans, Spanish beans, sweetcorn	1 kg

Industrial beet and chicories	
• industrial chicory	50 g
• beet	500 g
Oil and fibre plants	
• poppy seed	50 g
• mustards (brown and black)	100 g
• oilseed rape, common turnip	200 g
• flax (oil and textile)	300 g
• white mustard	400 g
• hemp	600 g
• soy bean, sunflower	1 kg
Forage plants	
• yarrow, crested dog's tail	25 g
• bentgrass (all species), yellow oat grass, Bermuda grass, timothy (bulbous, field), oxeye daisy, bluegrass (all species)	50 g
• brome (other than ceratochlea), foxtail millet	90 g
• orchard grass, fescue (tall, sheep's, meadow or red), Harding grass, plantain, soapwort, meadow foxtail	100 g
• kidney vetch, ceratochlea brome, swede, kale, festulolium, tall oat grass, bird's-foot trefoil, rye grass (all species), white clover, hybrid clover, Persian clover, rutabaga	200 g
• crown vetch	250 g
• alfalfa, black medick, forage radish, phaceliata nacetifolia, purple clover	300 g
• sainfoin grain, Spanish sainfoin grain, Alexandria clover	400 g
• brachypodium	450 g
• fenugreek, crimson clover	500 g
• sainfoin fruit	600 g
• burnet, subterranean clover	650 g
• forage sorghum	900 g
• field beans, lupin (all species), forage and protein peas, Spanish sainfoin fruit, vetch (all species)	1 kg
• potatoes (seed potatoes only, do not confuse with table potatoes)	0,5 kg

3.4.18.3. Other industrial materials

Baby soothers	15
Imitation weapons (ball pistols, not toys)	1 unit
Articles imitating foodstuffs	1 unit
Natural and artificial Christmas trees	1 unit
Decorative candle arrangements	1 unit
Other industrial material	see with the laboratory

3.4.18.4. Other industrial product

Cements	measurement of the hexavalent chromium rate	5 kg
Other		see with the laboratory

3.5. APPENDICES

A.1. Investigator guide in terms of quality approach sampling

Sheet 1: Necessary preparations prior to field surveying

1. ***Provide***
   - Sampling labels;
   - Sampling report;
   - Reimbursement form;
   - Sampling equipment: thermometer, sterile bags, sampling pouches, seals, wax;
   - sealing clamp, single-use gloves, disinfectant wipes, gas jet etc;
   - Review any laboratory instructions about the products.

2. ***Prepare the insulated boxes***
   - Clean and disinfect the box if necessary.
   - Insert the eutectic plates according to size (capacity) of the box and the desired temperature.

Capacity of the insulated box for example	Used to transport fresh products above 0 °C		Used to transport deep-frozen and frozen product	
	Number of small plates (-3 °C)	Number of large plate (-3 °C)	Number of small plates (-21 °C)	Number of large plate (-21 °C)
12 litres	2 placed on the side	1 placed in the slide of the box lid	2 placed on the side	1 placed in the slide of the box lid
30 litres	4 placed on the side	1 placed in the slide of the box lid	4 placed on the side	1 placed in the slide of the box lid

These minimum rules must be applied strictly, except for samples without temperature restrictions.

Advice

- It is advisable to add extra plates in extreme heat.
- The use of an additional icebox can limit opening times when repeated samples are taken in several companies.

When using a thermo button

A numbered thermo button is placed in the insulated boxes (do not put the thermo button in a plastic food bag to avoid disrupting it).

Indicate the exact position of thermo buttons.

3.5.18.1. Sheet 2: Sampling in the company

Preamble

The sampling is a extremely important administrative act.

A contentious procedure, even the administrative closure of a company can depend on it.

The sampling technique and conditions, the type, quantity and quality of samples and their storage and transport conditions must therefore comply with precise, defined and encoded procedures that are listed below.

The sampling will be rejected by the laboratory if there is a failure to comply with these guidelines.

Therefore, at any time in the 'inspection-laboratory' sequence, do not hesitate to seek advice from the laboratory technician so that you take the samples under conditions that comply with regulation.

The inspectors must have in their possession the contact details of the Control and Test Body that governs their control structure.

1. ***Samplings***
    - Carry out the samplings according to the laboratory or administrative instructions (quantities to be sampled).
    - It is essential to note the time of each sampling on the label(s) and repeat it in the relevant sampling report.
    - Seal them (to make the products inaccessible) with the labels, checking that they have been signed by the inspector and the holder (see § 2).
    - **Caution:** for microbiological sampling, the sample is placed firstly in a sterile bag or container and then in a second bag. Only the second bag is sealed with the sampling label.
    - Refrigerated and frozen products must not be placed in the same container.
    - Hot-sampled products must cool without fail on the sampling site before being placed in a box.
    - Load the samples (if appropriate, change the thermo button between the samples) and close the insulated box.
    - In the summer and/or extreme heat, it is preferable to group the samplings in the same locality if possible or to use several iceboxes.

2. *Labels*

Caution: fill in all the headings!

- **origin of samples:** sampling location and origin of products making up the sample; it is advisable to be as precise as possible on the manufacturing conditions of the sampled product;
- **name and brand:** where appropriate;
- **sampling date**;
- **date of manufacture**;
- **sampling number**;
- **analysis to be made:** to be specified or otherwise in physico-chemistry (in microbiology, the non-specification of the determination leads to the application of a pre-established search table).

Do not forget to have the labels signed by the professional and the investigator(s).

3. *Sampling report*

Write the report filling in all the headings, deleting as appropriate, indicating the number of deleted words and lines and paginating the document.

- The sampling time written on the label is repeated by the inspector in the body of the sampling report.
- For samplings with no value (frying oil, calf urine etc.), the statement will be "Amount of the value declared by him: nil" (delete the statement "estimated by the inspector").

Where the regulations make provision for this:

4. *Reimbursement slip*

All units sampled are reimbursed, including those left in the professional's care.

Fill in the reimbursement slip requesting supporting documentation for the value of the sample. The value of samples to be reimbursed must be indicated before signing the report (no deferred value statement). If the professional does not declare a value, the inspector estimates it.

The purchase price or cost price including VAT should be indicated.

3.5.18.2. Sheet 3: Operations to be carried out when returning to the department

For samplings under controlled temperature

1. ***Tranship the samplings into the refrigerator or freezer***

For temporary storage in the department.

2. ***When using a thermo button: read it***
   - For the iceboxes, the data recorded by the thermo button must be read upon your return to the department, on the specific PC.
   - The information recorded every five minutes on the thermo button is saved for seven days, then deleted. It is therefore essential to read the temperature and print the corresponding curve when returning from each survey.
   - **Caution:** temperatures taken into account are those included in the time interval between the sampling and the transfer of samples into the cold cabinet in the department (adjust the time based on the programming and the time of year – summer or winter).
   - If the reading is non-compliant, contact the laboratory to find out what should be done with the samplings.

For all the samplings

3. ***Record the sampling in the department's administrative log***
   - The number allocated to the sampling must be included in the report or the sample taking report which is then filed.
   - This number must also be shown on the label(s) of sampling(s) sent to the laboratory and those stored in the department.
   - Make sure that data agree between the sampling labels, the report and the entries in the administrative log in the department.

4. ***Classifying samples (where there are no guidelines)***
   - Each department states here how to identify samples when this information is given in their instructions (example: coloured stickers).
   - Each department states how to store samples as described in their instructions.

3.5.18.3. Sheet 4: Sending the sample to the laboratory

Important: Who prepares and who sends? See instructions.

1. ***Meet the deadlines for the shipment to the laboratory***

- The samples must be sent as quickly as possible to the laboratory.
- For the samples with a limited lifetime, a maximum time must be fixed between the sampling date and the shipment date; by the regulations:

As a guide (shipment time normally applied in Europe):

- **For the microbiological analysis:**

  < 48 hours for fresh produce

  < 10 days for frozen produce

- **For the physico-chemical analysis:**

  < 36 hours to 72 hours for prepared products (ready-to-use products, trimmed beans etc.) and for fruit and vegetables

  < 15 days for other fresh produce and frozen produce.

- Shipment must be made respecting the fixed temperature and in sufficient time for the laboratory to commence its analysis before the best before date.
- The shipment day in the week must take account of the shipment time, working days and the possibility of being taken in charge until the laboratory deposit phase.
- For products with no limited lifetime, the maximum time between the sampling date and the shipment date to the laboratory is thirty days.

2. ***Checking the sampling***

Check the indications on the back of the label:

- either '**routine analysis**' or '**standard analysis**';
- or precise '**analytical research**' requested by the inspector.

Attach any documents that may be useful to the analysis (manufacturing sheet etc.)

3. ***Prepare and ship the package***

- Make sure the contents will not move before you ship.
- Special case of samplings subjected to temperature constraints: do everything possible to ensure transport that complies strictly with the temperature constraints (additional eutectic plates etc.).

4. ***Checking the acknowledgement of receipt of the package***

- Acknowledgement of receipt at the return from the laboratory: to be filed in the department.
- If the laboratory states a non-conformity on the acknowledgement of receipt, draw up a non-conformity sheet.

3.5.18.4. Sheet 5: Monitoring the sample on its return from the laboratory analysis

1. Confirm that the test report agrees with the record in the department (contact the laboratory if they do not match).
2. Inform the professional holder and/or person in charge of the first marketing immediately of the test report conclusions.
3. Where appropriate, recover and file the third sample.
4. File the documents (test report, letter to the professional etc.).

A.2. Control flow chart per sampling

Stages	
Preliminary reflection on the sampling operation to be carried out	See instructions in 3.4.1
Follow the instructions in the sampling plan	See instructions in 3.4.1
Identifying the lot before any sampling	See instructions in 4.1 n° 1
Examining the lot before any sampling	See instructions in 3.4.1 n° 2
Essential homogeneity of increments taken in heterogeneous lots	See instructions in 4.1
Taking increments	See instructions in 4.1
Constituting samples from increments	See instructions in 4.1
Sampling report	See investigator guide (see A.1)

A.3. Example of random sampling of large quantities of pre-packed products: 50 bottles of fizzy drinks in a warehouse

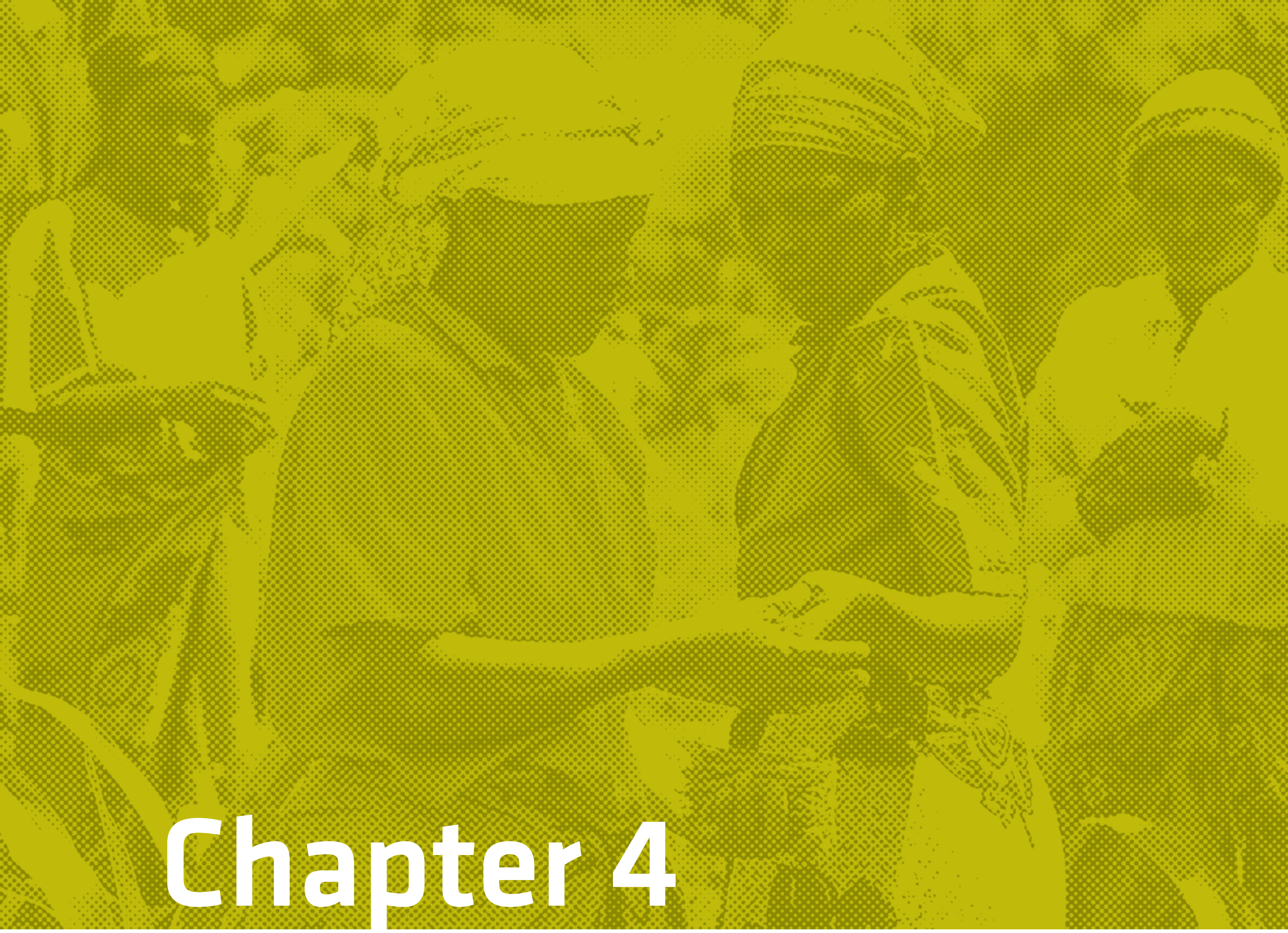
Lot composition:

- 25 pallets, each holding 100 boxes
- 6 bottles in each box

1. Application of the sampling plan if one exists.
2. Otherwise:

Constitution of a first level sample	Note for each pallet: • the lot number, • the date and time of manufacture, • give each pallet a virtual number if there is no lot number, • put them in an urn and pick five numbers at random, • identify and mark the five pallets.
Constitution of a second level sample	Choose ten boxes from each pallet thus designated, *i.e.* a total of fifty boxes. Choice of boxes: front, rear, top, bottom, left and right. Note all the indications applied to each box.
Constitution of a third level sample (final sample)	Take one bottle of fizzy drink from each box thus defined. Choice of bottles: front, rear, middle, left and right. Note the lot numbers, date and time of manufacture and all markings that identify the sample to be analysed.

The sample of 50 bottles is constituted this way.



Chapter 4

Organisation of a plant health laboratory

4.1. THE TASKS OF A PLANT HEALTH LABORATORY

4.1.1. The regulatory context

Certain vegetables, vegetable products and other objects present an unacceptable risk since they may have pests. Vegetable health is, indeed, very important for crop production, forests, natural spaces and planted areas, natural ecosystems, ecosystem services and biodiversity with the European Union. Plant health is threatened by the introduction or extension of pests that damage crops and vegetable products. The risk of introduction (e.g. within the European Union) is increased by globalisation of trade and climate change.

To combat this threat, measures must be taken to **determine the risk to plant health presented by pests and reduce this risk to an acceptable level**. Such measures (referred to as Sanitary and Phytosanitary – SPS – measures) have long proved their worth. They have been the subject of **international agreements and conventions**, including the *International Plant Protection Convention* (IPPC) of 6 December 1951, concluded within the Food and Agriculture Organization (FAO) of the United Nations and its revised text approved at the 29th session of the FAO Conference in November 1997.

The Agreement on the Application of **Sanitary and Phytosanitary Measures (the "SPS Agreement")** sets out the basic rules on food safety and animal and plant health standards. Countries are permitted to establish their own standards but it is also stated that such legislation and regulations must be based on **scientific principles** and may be applied only to the extent necessary to protect the life and health of humans (e.g. the application of Maximum Residue Limits (MRLs) for pesticides for food products or even maximum limits on heavy metals), animals (e.g. limiting animal transport to prevent zoonosis or plants (e.g. with regard to plant health it may involve regulations imposing restrictions on the import of crops likely to harbour pests such as fruit flies or harmful pathogens such as viruses, funguses or bacteria). Nor may SPS measures arbitrarily or unjustifiably discriminate between countries where identical or similar conditions prevail.

SPS measures may take many forms; countries may, for example, impose a requirement for products to come from an area free from disease, be inspected, undergo treatment or to specific processing, or establish maximum residual levels for pesticide residues. Public health measures (human and animal health) phytosanitary measures (crop protection) must apply to foodstuffs of domestic origin and local animal or crop diseases as well as to products from other countries.

To be able to take suitable measures, it is essential to be able to achieve a valid diagnosis, that is to say to be able to pinpoint and identify, using effective sampling and detection methods, the pests present in a batch of vegetable products. This calls for **a well-equipped laboratory with competent staff**.

4.1.2. The tasks entrusted to a plant health laboratory

The tasks to be entrusted to a plant health laboratory (also referred to as a 'plant clinic') are the following:

- The **detection and identification** of so-called 'quarantine' pests (note that a quarantine pests is a regulated pest for which urgent measures laid down by statute apply if it is detected).[3] This is the **basic mission** of the laboratory. It calls for particular skills and equipment.
- **Notification:** States, professional operators and the public are required to undertake notification. This makes it possible for effective action to be taken rapidly when the presence of a quarantine pest is noted or suspected. A professional operator or any other person (including the laboratory performing the analysis) who suspects or notes the presence of a quarantine pest must notify the competent authority (although not all states require laboratories to notify the authorities; this is sometimes the sole responsibility of the producer).
- **Inspection:** it is extremely important to prevent and detect the presence of pests as soon as possible in order to ensure their rapid and effective eradication. States must therefore undertake inspections (visual examinations, sampling campaigns etc.) in production locations to investigate the presence of pests in regions where this presence has not as yet been noted. Given the number of quarantine pests and the time and resources required for such monitoring, States need to establish risk-based multi-annual inspection programmes (a Pest Risk Analysis – PRA – is therefore recommended).
- **Recommendations:** the laboratory must help the competent authorities to take the SPS measures necessary to protect human and animal health and life and to protect plants based on a scientific risk assessment. SPS measures are applied:
  - to protect the human and animal life from the risks arising from additives, contaminants, toxins or pathogens present in foodstuffs;
  - to protect human life from illnesses conveyed by plants or animals;
  - to protect animal life or preserve plants from parasites, illnesses or pathogens; or
  - to prevent or limit, in a country, other damage arising from the entry, establishment or dissemination of parasites.

3 The following link provides the Alert List for the principal pests targeted by European and Mediterranean Plant Protection Organization (EPPO): www.eppo.int/QUARANTINE/Alert_List/alert_list.htm.

A competent laboratory is therefore a powerful tool in managing phytosanitary emergencies.

- **Technical support for scientists (researchers and trainers)**: familiarising them with the detection tools for plant diseases. The laboratory serves to provide a scientific reference point for learning (e.g. for practical work or work placements and in entomology, phytopathology and other related disciplines).
- **Phytosanitary risk management plan:** based on tests conducted by the laboratory and its recommendations, operators (notably those intervening in the value chain) can establish a **'risk management plan'** ensuring and demonstrating a high level of competence and awareness of phytosanitary risks as regards critical points in their professional activities and justifying special control arrangements with the competent authorities. This type of plan is aimed in particular at:
    - helping farmers, companies and NGOs providing support to farmers to resolve plant health problems encountered in their crop areas;
    - helping farmers to put in place integrated crop protection systems for smallholdings;
    - helping individuals and managers in the public and private sectors to manage problems, preferably sustainably (e.g. by putting in place integrated protection or biological control methods).

4.2. ORGANISATION OF THE LABORATORY

4.2.1. The ISO/CEI 17025:2005 standard

It is no longer possible now to speak of the organisation of a test or analysis laboratory without putting it into the context of the reference standard in the appropriate area. This is all the more true of a body which serves official monitoring bodies since it means providing incontrovertible results. Consequently, to meet the performance criteria for a laboratory, the **international standard ISO/CEI 17025:2005 serves as the reference point**. It makes it possible for laboratories to gain accreditation for a specific purpose (a scope, domain) under this standard.

The accreditation of laboratories by an independent national or international body **guarantees recognition of the findings** in all the countries concerned. Accreditation is granted on the basis of the criteria laid down in the ISO/CEI 17025:2005 standard for use by laboratories in developing their management system for quality, administrative and technical operations. This international standard specifies the general requirements for the competence to carry out tests (generally referred to as 'analyses') and/or calibrations, including sampling. It covers testing and calibration performed using standard methods, non-standard methods, and laboratory-developed methods. It is **applicable to all organizations performing tests and/or calibrations**. ISO/CEI 17025:2005 is **applicable to all laboratories** regardless of the number of personnel or the extent of the scope of testing and/or calibration activities.

ISO/CEI 17025:2005 is an excellent tool for the organisation of a laboratory. Section 4 of this standard deals with the management system for quality, while section 5 deals with technical aspects, such as:

- accommodations and environmental conditions;
- skills of personnel;
- equipment;
- test and calibration methods and method validation;
- estimation of measurement uncertainty;
- data management;
- measurement traceability;
- reference standards and reference materials;
- sampling;
- handling of test and calibration items;
- assuring the quality of test and calibration results;
- reporting the results, opinions and interpretations.

We will look at certain of these points and return later to the question of quality management *per se*.

4.2.2. The building and organisation of the premises within the building

4.2.2.1. The laboratory building

This must be a building devoted primarily, or preferably exclusively, to the operations of the laboratory. It may include research and development laboratories or specialised control laboratories and related areas based on a plan that allows them to function smoothly.

It must also include common areas, such as:

- circulation areas (accesses, corridors, stairways, lifts, goods lifts);
- hallways and reception desks;
- reception areas;
- meeting and conference rooms;
- documentation areas;
- spaces for relaxation;
- sanitary facilities and cloakrooms etc.

All these areas, which are not specific to laboratory buildings, must, however, be taken into account in determining the size and plan for the building. The various areas in the building can be organised most rationally by taking due account of the foreseeable relationships between those areas in the knowledge that the solution will be a compromise between the various objectives.

The siting of this building on the land concerned must take into account its relationship with its environment. It will need to be placed within a retaining tank to avoid accidental spills or fire extinction water being dispersed into the natural environment. Some rooms, where products presenting specific risks (e.g. acids, solvents or toxic reagents etc.) will also need their own retention facilities.

4.2.2.2. Organisation of the premises within the building

Inside the laboratory building, the layout of the rooms will need to respond to the following objectives:

- to facilitate the prevention of risks at the level of the building as a whole;
- to reduce the distances to be covered in order to reduce the risk of accidents and foster work;
- to promote common use of certain heavy equipment;
- to facilitate exchanges between research and development laboratories and control laboratories.

Consequently, the following arrangements are recommended:

- to group together in a same sector laboratories and rooms that need to work together (a preliminary study is essential);
- to favour a location on the ground floor due not only to the risk of falling down staircases but also to the virtually systematic and by no means negligible risk of fire. If this solution is not practical, the number of floors should be limited. The siting of laboratories underground should be prohibited;
- there should be no floor below the premises where there are significant risks of explosion.

4.2.2.3. Circulation within the building

Circulation within the laboratory building should meet the following objectives:

- provide direct access to the laboratories and related areas, while complying with any rules on containment;
- facilitate flows, handling and supplying of the rooms;
- avoid risks of falling or collision.

Consequently, the following arrangements are recommended:

- a free width of a minimum of 2 m (allowing for any fixed equipment, such as cupboards, that may be planned). For the work of the operators, a free space of at least 2 m in front of laboratory bench tops and other work stations, 3 m if there are two laboratory bench tops or two extractor fans facing each other;
- no obstacles, such as steps or curbs (favour ramps);
- the fire doors dividing the passageways should preferable be equipped with an oculus at eye level and be kept open by positive safety devices linked to the fire alarm system (e.g. sliding doors that close themselves if there is a fire thanks to a counterweight system);
- the doors to room will open towards the corridor without protruding into it; they will be equipped with an oculus at eye level making it possible to prevent collisions when they are opened.

For circulation with the laboratory, there should be a passage way that takes into account the staff and the likely space occupied by equipment and handling equipment.

Passage ways will be place more **than 1 m away from fume chambers (or laminar flow hoods)** to ensure that passages do not disrupt the pulsed air.

4.2.2.4. Containment and the requirements therefor: classification of laboratories based on the products handled

Containment Level 1

In a Containment Level 1 (L1) laboratory, the biological material handled **is not pathogenic**. It is therefore a **standard laboratory**, separated from other areas by at least one door and having the following characteristics:

- a sufficient work space for each technician (e.g.: 5 m^2 on the ground, with 2 m linear of laboratory work top);
- smooth (wall, ground, work tops) and impermeable surfaces that are easy to clean and are resistant to cleaning and disinfecting agents;
- no place that is difficult to access for cleaning;
- a washbasin or handbasin for washing hands;
- a changing room (ideally, separation of men and women).

No special containment equipment is required.

Containment Level 2

In a Containment Level 2 (L2) laboratory, the **biological material handled is deemed to be pathogenic**.

An L2 laboratory has the following characteristics:

- marking of the containment level and 'biological hazard' sign with pictogram at the entrance to the laboratory;
- regulated and lockable access. The names of the head of the L2 and authorised persons will be displayed on the door; separate changing room for personal effects;
- access to the laboratory through at least one door, with separation from other rooms;
- the presence of a window making it possible to see the occupants;
- adequate space for each technician;
- means of communicating with those outside the room (telephone, intercom): not to be used with gloves being use for an ongoing experiment;
- ventilation of the room through a mechanical suction system;
- smooth surfaces (walls, floors, work tops) that are easy to clean and decontaminate;
- no place that is difficult to access for cleaning;
- door and windows that must be closed during performance of work;
- airtight room for the purposes of disinfection.

Containment Level 3

In a Containment Level 3 (L3) laboratory, the **biological material handled is a quarantine agent**.

An L3 laboratory has **the same characteristics as an L2 with the following** properties in addition:

- one access to the laboratory through a sluice access system comprising:
  - a double set of doors that cannot be opened simultaneously;
  - changing rooms to change shirt and put on personal protection equipment necessary for operations;
  - a shower, if possible, so that staff can be decontaminated in the event of an accident;
- keeping the L3 under lower pressure than neighbouring areas. There must be an **alarm** to warn of any change in pressure;
- the window must be unbreakable and hermetically closed;
- filtration of the air extracted (and if possible, entering) using a HEPA (High Efficiency Particulate Air)-type absolute filter capable of stopping all micro-organisms;
- the room must be hermetically sealed when work is performed;
- an emergency ventilation system;
- emergency electrical energy is strongly recommended.

Table of requirements based on the confinement levels

Containment measures	Containment levels		
	L1	L2	L3
a) Design of the laboratory			
1. Laboratory signing (biological hazard pictogram)	No	Yes	Yes
2. Laboratory separated from other room by at least one door	Yes	Yes	Yes
3. Access to the laboratory *through* a SAS	No	No	Yes
4. Regulated and lockable access. Access solely for authorised staff	No	Yes	Yes, through a SAS
5. Possibility of hermetically sealing the work place for the purpose of disinfection (fumigation)	No	Optional	Yes
6. Filtration of air extracted from the work place	No	No	Yes, through a HEPA filter

7. Filtration of air entering the work place	No	No	Optional
8. Presence of an observation window or equivalent system making it possible to see the occupants	No	Yes	Yes
9. Means of communicating with the outside	No	Yes	Yes
10. Negative pressure kept in the laboratory as compared with neighbouring areas	No	No	Yes
11. Alarm for detecting any unacceptable change in air pressure	No	No	Yes
12. Supply of emergency electrical energy	No	No	Optional
13. An emergency ventilation system	No	No	No
b) Requirements for internal fixtures			
1. Microbiological safety cabinet	No	Yes	Yes
2. Protective clothing	Yes	Yes	Suitable clothing and overshoes
3. Fittings for storing protective clothing in the laboratory	No	Yes	Yes
4. Shower for decontaminating staff	No	No	Optional
5. Hand washing: washbasins with taps that can be turned on and off without the use of hands	No	Yes	Yes
6. Water-resistant surfaces that are easy to clean and without areas that are difficult to clean	Yes (floors)	Yes (floors)	Yes (floors, walls and ceilings)
7. Waterproof laboratory work tops that are resistant to acids, alcalines, solvents and disinfectants	Yes	Yes	Yes
8. Effective control of vectors, for example rodents and insects	Yes	Yes	Yes
9. Presence of an autoclave	Yes, on the site	Yes, in the building	Yes, in the laboratory, with a double entry
10. Specific basic equipment in the laboratory (marked equipment)	No	No	Yes

c) Requirements for operations			
1. Storage of biological agents in a safe place	Yes	Yes	Yes
2. Handling of infected materials	Prohibited	Optional	Yes
3. Use of specific containers for collecting soiled sharps and blades	Yes	Yes	Yes
4. Control of the dissemination of aerosols that are formed	Minimised	Minimised	Prevented
5. Gloves	Optional	Optional	Yes
6. Inactivation: contaminated material and waste	Yes	Yes	Yes
7. Decontamination of equipment before it leaves the laboratory (extractors, etc.)	Yes	Yes	Yes
8. Inactivation of effluents: washbasins and showers	No	No	Yes

4.2.3. Design of laboratory installations

4.2.3.1. Surfaces to be provided

The first step in design is to **determine the overall surface necessary** for working safely in the laboratory. The laboratory surface must be determined to that it can contain the following items:

- an area where products and materials can be received and removed;
- spaces for work and the circulation of technicians and handling devices;
- supply areas for products, materials and glassware required;
- laboratory work tops for other work;
- rooms in which operations that are or may be dangerous or emissive can be conducted;
- equipment such furnaces, ovens and pumps, some of which may be pollutant and require specific capture as close to the source as possible;
- storage furniture, for equipment, products to be stored temporarily or for scrap or waste;
- locations for gas bottles.

The ratios between these surfaces must be determined by the designed based on the number of staff, the activity and the likely needs of the users.

The design of the laboratory must also comply with **health and safety requirements.** Allowance must also be made for the space needed for equipment essential for the proper operation of a laboratory, such as dustbins, showers and eye baths, extinguishers, fire blankets, storage for personal protection equipment (respirators, goggles, lab coats) and in particular those for use by visitors.

4.2.3.2. Basic rules

A certain number of rules need to be followed:

- **Ceiling height:** this must be chosen on the basis of the maximum height of the equipment to be installed in the laboratory, allowing for collective protection equipment, ventilation systems etc. A height to the ceiling of 3 metres is generally adequate. If a false ceiling is planned, it must be ensured that gases and vapours cannot build up in the gap and the installation within the gap of equipment that needs maintenance must be avoided.
- **Work stations:** given that the laboratory's activities involve analysis, work stations should preferably be containment areas. These work stations will be supplemented by bench tops for the materials and products being used (and any computer equipment).
- **Tables:** serving primarily for writing, consulting documents, undertaking minor activities without major products or materials. They should not replace the office area but are justified by the need for somewhere close to the work station.
- **So-called 'dry' bench tops:** for materials that do not use water. This is the case, for example, of certain materials for physical analysis, computers, etc.
- **So-called 'wet' bench tops:** equipped with a water supply and drainage. These are characterised by a high-resistance waterproof coating and have equipment for the use of water, air, specific gases, electricity, etc.
- **Working height:** between 500 and 900 mm, but generally between 720 and 900 mm. The height of the bench top should ensure the best possible posture under the conditions of the work station. This means that it will be close to the upper limit for work performed upright and requiring close observation, closer to the middle for work performed sitting down and close to the lower limit for work on large equipment. It is even recommended that there be "low" bench tops (around 300 mm) for assemblies that are relatively high.
- **Work top:** when justified by the hazards presented by the products that may be handled, the work top should be edged with a trim with a height of 5 to 10 mm for containment. This will prevent any liquid spread accidentally on the bench top from running along the front surface and entering into contact with the operator, often leaning against the edge of the bench top. It also has the advantage of being able to stop an object from rolling and falling onto the ground. The depth of the work top should be a compromise between the need for space, notably to store materials, and the need for the entire surface to be accessible from the front of the bench top. This is generally between 600 and 900 mm.
- **The controls and connections to fluids:** electrical sockets and fluid controls (taps) should ideally be placed on the side of the table rather than on the backsplash (pilaster) since this position makes them easier to handle, particularly in an emergency. Such sockets and controls must be protected from any spills. Electricity sockets must be in significant number, around 5 per metre of bench top, to make connection easier and reduce the number of wires.

- **Drainage system:** small sinks, also referred to as 'fonts', that are only to be used for water for cooling and often placed at the back of bench tops may be advantageously replaced by easily accessible drainage ducts (close to the front). With regard to the pipes for water for cooling, they should be equipped, whenever possible, with a quick release nozzle in order to avoid the risks of threading pipes to 'nipple' tips. Sinks placed at the extremity are to be avoided unless there are specific reasons for so doing. If they are intended for hand washing, they must be located at the entry or exit from the laboratory and be equipped with a foot control. If they are intended for washing dishes, they must be incorporated into the work station designed for this.
- **The use of a closed-circuit cooling system:** to which a rapid connection can be made not only saves water but also protects against the risks of water spillage on the bench top (danger for electrical equipment).

4.2.3.3. Washing and decontamination stations

Decontamination operations and the cleaning of specific equipment are an integral part of laboratory work and must be performed in the laboratory. The standard manual washing station consists essentially of a double sink with fittings that should preferably be controlled by a foot pedal and storage islands upstream and downstream.

Work tops for washing must have rims for confining and draining water from washing and preventing objects from falling. The size and weight of objects to be washed should be considered in determining the size of these elements in order to limit the risks inherent in handling them. The upstream island generally has a draining rack, generally affixed to the wall; the space required for installation and fixing must be allowed for.

If solvents are to be used for washing, the work station must be equipped with:

- a ventilated enclosure;
- a device for recovering used solvents with a suction device (suction funnel, suction sink etc.).

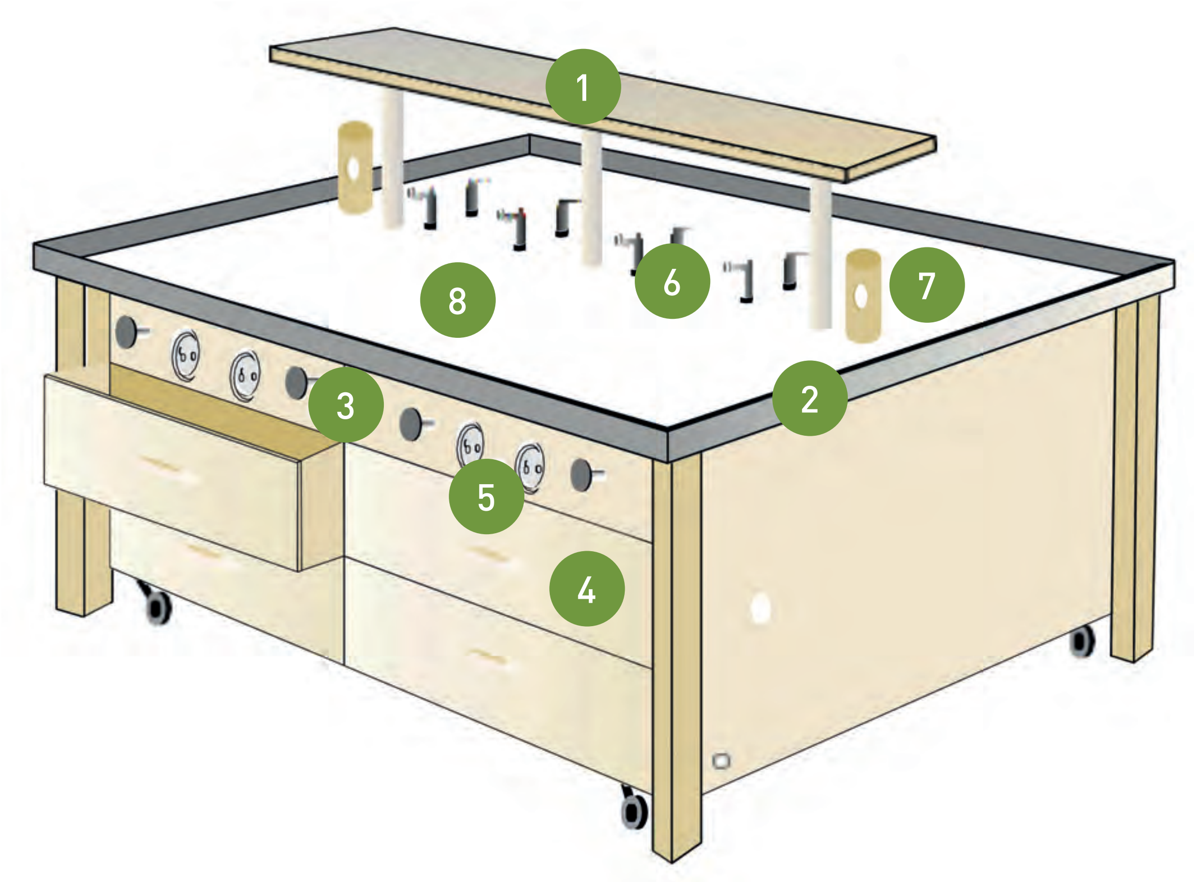


Figure 1 - Example of a work top
Caption: 1. *Central tablet – 2. No-drip edging – 3. Fluid controls*
4. Wheeled drawers – 5. Electrical sockets – 6. Fluid influxes
7. Water drainage ducts – 8. Wheeled storage unit

- **Laboratory ventilation**: the concentration of pollutant emissions in the room must be kept as low as possible and below the professional exposure limits, if any. To achieve this objective, ventilation systems, containment devices and emissions capturing devices must be used as close to the emission point as possible. Emissions must be discharged outside the building containing the laboratory, away from compensating air intakes, after any cleansing. The devices used may be mobile or non-mobile devices for capturing at source (suction nozzles, suction funnels, suction rings, ventilated tables, suction backsplashes etc.) and above all ventilated enclosures. When ventilation devices are installed in a laboratory, it is essential to allow for one or more compensatory air intakes located so that they do not interfere with the operation of these devices.
- **Siting of equipment**: equipment such as ovens, furnaces and pumps must be sited in dedicated areas so that they can be accessed easily for use and maintenance. They do not need to be place in a hood or under a hood, provided they are equipped with a connection to the gaseous pollutants extraction network.

- **Storage furniture**: storage furniture is generally located under the hood or under the bench top, saving space and limiting movements within the laboratory. For chemical products and waste, they need to be specially designed. With regard to the nature of the furniture itself, to facilitate the work of the operator, furniture with drawers and the use of trolleys (equipped with brakes) should be favoured for heavy or voluminous materials. A storage area should be set aside for these trolleys. Wheeled furniture has the following advantages:
  - easy to move to install a seated work station;
  - easier access to equipment located under the work top for maintenance operations;
  - easy to clean the floor up to the skirting boards.

  Since access to heights is easy at between 1 metre and 1.20 metres and acceptable up to 1.50 metres, the shelving must be fixed between these limits.
- **Product reception and/or collection area**: the size must be determined on the basis of the like activities of the laboratory, provided with signage and marked out on the floor. A suitable trolley or handling device must be provided for this purpose.

4.2.3.4. Design of the premises

- **The changing rooms:** the changing rooms must have a sufficient surface area to accommodate two lockers per operator (casual clothes and lab coats). If possible, there should be a partition for men and women. The rooms should preferably be separate and close to the laboratory. If justified by the risk, they will act as a SAS for entering the laboratory and will be equipped with showers and washbasins (for hand washing).
- **The offices:** to limit the operators' exposure to products and the noise of certain equipment, facilitate office layout and protect sensitive equipment (IT in particular), the offices area should be independent but in the immediate proximity to the laboratory. This layout also ensures the concentration necessary for office work (calculations, drafting, telephone calls etc.). In order to limit any contamination of this area, access to the laboratory will be through a **SAS**, enabling operators to put on or take off their work clothes. To facilitate monitoring of ongoing operations and communication, there must be transparent partition walls between the offices and the laboratories. For the purposes of evacuation and to limit the number of exposed persons (avoid outsiders crossing the laboratory), **there must be direct access to the offices** without going through the laboratory.

- **Weighing area:** while ordinary weighing can be performed in the laboratory, some weighing requires a specific environment due to:
  - sensitivity to movements or air quality (high precision weighing);
  - fragility of the equipment (precision scales);
  - hazards presented by the products to be weighed (active ingredients, for example);
  - The physicochemical properties of the product to be weighed (powders that are fine, light or that have a static charge, volatile liquids etc.).

  The weighing area should preferably be:
  - sheltered from draughts;
  - blind (without windows) in order to avoid any disruption caused by variations in external lighting.
- **Distillation area:** certain activities, the processing of flammable solvents (purification, regeneration, dehydration, etc.) may require the installation of a specialised laboratory with specific preventative measures:
  - adequate ventilation;
  - vapour detection;
  - confinment;
  - means of extinction etc.
- **Washing area**: decontamination and cleaning operations may be undertaken in a wash room that may be common to a number of laboratories. In general, manual and machine washing stations may coexist in this area. The standard manual washing station consists essentially of a double sink with the taps ideally being controlled by a foot pedal and with trolleys on either side. Work tops must have rims for confining and draining water from washing and preventing objects from falling. The size and weight of objects to be washed should be considered in determining the size of these elements in order to limit the risks inherent in handling them. The trolley on the right generally has a draining rack, generally affixed to the wall; the space required for installation and fixing must be allowed for.

  The machine washing station must have one or more machines with trolleys on either side. To limit the risk of breakages, dishes should preferably be transported on trolleys equipped with containers or trays with edges. The organisation of the wash room, work stations and accesses must take allow for the width of these trolleys.

More generally, there should be:

- lockers for cleaning products and materials;
- a place for emergency equipment;
- a place for waste paper bins and bins for glass;
- a place for containers and transport trolleys that may serve as islands;
- if necessary, a location for the drying area;
- a non-slip floor equipped with floor drains that are plugged during normal operation to prevent accidental pollution;
- one or more intakes for demineralised water or space for installing a demineralisation apparatus;
- general ventilation calculated to ensure air renewal at around 5 to 6 volumes/hour.

- **Storage areas:** to limit operator exposure or the risks of impairment of equipment by products and vice versa, equipment and products and samples to be kept must be stored in separate rooms intended for their respective purposes. The size of rooms for storing equipment must be determined on the basis of the quantity, size and storage method for the equipment concerned. There must also be sufficient space for shelving or lockers and for access to the equipment. Particular attention must be paid to correctly determining the strength of such shelving and its stabilisation to prevent its fall.
- **Service areas:** to limit the nuisance caused by certain equipment, to facilitate their operation and maintenance and prevent the exposure of staff responsible for their operation and maintenance, service areas or rooms must be created. They may contain, for example: refrigeration units, ice machines, water heaters, central ventilation units, pumps, demineralised water stations, special gas distribution stations, shut-off valves, etc., and even fluid networks. The size, design and equipping of the areas must be such as to accommodate the equipment to be placed there and make it easy to operate and maintain. The size and layout of these service areas and rooms will depend primarily on the organisation and size of the laboratory building. These areas should therefore be laid out in a manner that avoids the risks of cross contamination through the technical ducts that cross through the walls (potential ingress from one area to another).

4.2.3.5. Access to the premises

- No outsider may be authorised to enter the areas where laboratory work takes place.
- The laboratory doors must remain closed.
- Children may not be authorised to enter the work areas.

4.2.3.6. Working conditions

The environmental conditions must make it possible:

- not to impair samples or the quality of culture media (e.g. dry them out);
- not to contaminate samples or environment, notably by controlling air intake or by using climate control devices. Particular attention should be focused on this in hot climates. The temperature of the room must be controlled to prevent any disruption of plant cultivation, change the growth conditions for micro-organisms being researched or even interfere with incubation or reaction temperatures during the use of diagnostics kits (e.g. ambient temperature exceeding the recommended temperature for the reaction).

- **Temperature:** the normal ambient temperature is 20 °C, with certain tolerances depending on the type of experiment or measurement to be performed. Variations in temperature within the tolerance interval must be progressive. Temperature regulators are a key element of regulation and control systems. Temperature regulators detect the actual values in the environment (laboratory), and change the temperature until it reaches the recommended value. The simplest and most well- known temperature regulator is the thermostat.
- **Humidity:** the humidity must be low since it accelerates the oxidisation of metal instruments. Nevertheless, it is recommended that laboratory humidity should not be less than 50%.
- **Pressure:** generally, in laboratories, the pressure should be slightly over atmospheric pressure (25 Pa or more) to prevent the intake of air when the access doors are opened. In the case of laboratories presenting biological hazards (handling of infectious agents), the situation must be the opposite, that is to say, air that may be contaminated must not be able to leave the laboratory; in this case, the pressure in the environment must be slightly lower than the atmospheric pressure.

4.2.3.7. Hygiene recommendations for laboratory work areas

The laboratory must be kept clean and tidy and free from all products or objects that are not required for the work. Work tops must be decontaminated if they are contaminated by potentially hazardous products and at the end of the working day. All contaminated materials, samples and cultures must be decontaminated before being thrown out or cleaned for reuse. If windows can be opened, they must be fitted with screen panels to prevent the ingress of arthropods.

4.2.3.8. Waste processing

There are three key questions to be posed before disposing of an object or biological material from a laboratory working on plant tissue or potentially hazardous micro-organisms:

1. Have these objects or biological materials been properly sterilised or disinfected using one of the approved tried and tested procedures (e.g. procedure for disposing of biological waste)?
2. If not, have they been packaged using an approved method for their immediate incineration on site or their transport to another establishment capable of doing so?
3. Does the disposal of sterilised or disinfected objects or biological material involve additional biological or other risks for the staff responsible for their immediate disposal on site or for persons outside the laboratory who likely to be in contact with that waste?

4.2.4. Hygiene and protection of staff

4.2.4.1. Requirements with regard to hygiene

In the field of staff hygiene, the following precautions must be taken to **avoid the contamination of samples and culture media** to avoid risks of staff infection or the spread of pests.

- The wearing of laboratory clothing properly fastened, clean and in a good state, made of material that limits flammability hazards. This clothing must not be worn outside the testing premises and possibly in the changing rooms. Changing clothing in the SAS and applying a rigorous cleaning procedure.
- Wearing protection for hair and beards, if necessary.
- Keeping nails clean and, preferably, short, and wearing gloves if necessary.
- Cleaning hands carefully with warm water, preferably from a tap not controlled manually before and after microbiological examinations and immediately after going to the toilet. Use a liquid or solid soap, possibly a disinfectant, issued preferably by a distributor with a good state of cleanliness. Dry hands using disposable paper towels or tissues. These precautions are valid for both laboratory staff and visitors.
- During work with samples, cultures or contaminated environments and during sowing, avoid speaking, coughing etc.
- Persons with skin infections or illnesses must take specific precautions if micro- organisms from this infection or illness are likely to contaminate the samples and risk falsifying the findings.
- Not eating or drinking in the laboratory and not putting food for personal consumption in laboratory refrigerators or freezers.

4.2.4.2. Personal protection

- Wearing lab coats and gloves is mandatory for work in the laboratory.
- Wearing safety goggles, a face screen (visor) or other protection equipment is mandatory when it is necessary to ensure that the eyes or face are protected against splashes, the impact of objects or artificial ultraviolet radiation.
- Protective clothing may not be worn outside the laboratory or, for example, in the canteen, cafeteria, offices, library, staff room or toilets.
- Protective clothing worn in the laboratory must not be stored in the same changing rooms or cupboards as casual clothing.
- The washing of these clothes must be organised by the laboratory (under no circumstances may lab coats be taken home for domestic cleaning.

4.2.4.3. Compliance with Good Safety Practices

All plant health laboratories must have a "Manual" or "Code of Good Practice" (a practical manual of good practices, laboratory health and safety guide, security manual etc.) which sets out the safety instructions to ensure chemical, electrical, fire safety, protection from radiation and equipment safety (actual and potential hazards and how to eliminate or at least reduced them to the minimum).

The safety instructions to be followed:

1. **Pipetting by mouth must be strictly forbidden.**
2. **Materials must not be placed in the mouth.** Labels must not be licked.
3. All technical procedures should be performed in a way that minimizes the formation of aerosols and droplets.
4. All spills, accidents and overt or potential exposures to infectious materials must be reported to the laboratory supervisor. A written record of such accidents and incidents should be maintained and the report filed.
5. A written **procedure for the clean-up of all spills** of products of any nature must be developed and followed.
6. Contaminated liquids must be decontaminated (chemically or physically) before discharge to the sanitary sewer.
7. An effluent treatment system may be required, depending on the risk assessment for the agent(s) being handled.

A breakdown in the containment of pathogenic organisms may be the indirect result of chemical, fire, electrical or radiation accidents. It is therefore essential to comply fully with safety rules in any laboratory to prevent such accidents (WHO, 2005).

4.2.5. Equipment necessary in a plant heath laboratory

A plant health laboratory requires minimum equipment to operate.

4.2.5.1. What basic equipment must be acquired?

- **Scales:** a balance for weighing ingredients (e.g. preparing the mediums) or assessing the masses of samples received or to be taken. An analytical balance ensures a very high degree of precision (to the mg); balance-beam scales give a precision of a tenth or even a hundredth of a gram.
- **Bunsen burner:** a Bunsen burner to produce a single open gas flame, which is used for heating, sterilization, and combustion.
- **Autoclave:** to sterilize using high-pressure (several bars) steam. For equipment to be deemed to be sterile, the theoretical probability of isolating a germ must be less than 1 to 1 million.
- **Platinum loop:** for separating bacteria in a sample. Isolation makes it possible to obtain different colonies spaced apart from each other.
- **Laminar flow hood(s) (or fume cupboards):** to work without risk of outside contamination (e.g. Isolation, transplanting onto a medium etc.).
- **Ovens:** to conduct various thermal treatments (e.g. to dry samples or maintain Petri dishes at a constant temperature for several days in the case of isolation) at a set temperature.
- **Double boiler:** to heat materials not too strongly and to control the temperature while avoiding almost all risks of calcification, even partial.
- **Heat bath:** it provides precise temperature control, for example to heat to 37 °C to perform an enzymatic digestion.
- **Environmental chambers or climatic chambers:** an enclosure, often insulated and with forced ventilation to optimise homogeneity used to monitor the characteristics of a sample, or more generally to test the effects of specified conditions on a chemical substance.
- **Optical microscope:** to detect and sometimes identify a pathogen taken directly from the plant to be tested or after a period of isolation.
- **Binocular microscopes:** to observe samples at low magnification lit from above.
- **Hot plate:** to heat various objects.
- **Refrigerators and freezers:** to preserve and protect samples.
- **Thermostat:** to keep a system (equipment, machine, etc.) at a relatively stable temperature.
- **pH-meter:** to measure the pH of a solution or adjust the pH of a medium.
- **Tube holder:** to hold a large number of test tubes and for samples.
- **Washing-up basin:** for washing and cleaning laboratory equipment. Glass or plastic laboratory equipment can be cleaned by hand in a basin using the soaking method.

- **Distiller:** to obtain water that is theoretically free from certain mineral salts and organisms that can be found in "natural" water. It makes it possible therefore to have distilled water.
- **Fire-proof cabinet:** to store flammable products **to protect against fire or an abnormally high heat** source.
- **Computer:** to record data, etc.
- **Drill:** for crushing samples (leaves) and extracting the juice for potential analysis.

4.2.5.2. Special equipment and its use

- **Centrifuge:** a laboratory centrifuge is an instrument essential for separation, purification and increasing the concentration of enzymes, cells, stem cells, DNA and other molecules, and for developing production methods, e.g. for new chemical compounds, diagnostic methods and new plant varieties.
- **Equipment for triggering a polymerase chain reaction (PCR):**
  - **Thermal cycler:** a device automating a PCR. The device has a thermal block with holes where tubes holding the reaction mixtures can be inserted. This is used to establish the best annealing temperature for a given pair of primers.
  - **Micropipettes:** make it possible to remove and transfer small volumes of liquid with great accuracy. They enhance the findings of pipetting samples by excluding the deposit of sample residues in the pipette tip.
  - **Cuvette:** with a plug and transformer, used for electrophoresis of agarose gel. It is a method used to separate DNA and RNA. The smallest molecules move more rapidly and go further than larger molecules.
  - **Generator:** for electrophoresis of DNA: makes it possible to detect products amplified by electrophoresis in agarose gel.
  - **Reader:** for detecting products amplified by fluorometry.
- **Equipment for enzyme-linked immunosorbent assay (ELISA) tests**
  - **Incubator:** a thermostatically controlled enclosure in laboratories (room for the culture of bacteria, fungus, etc.), generally set to 37 °C.
  - **Micropipettes:** *cf. supra*.
  - **96-well plates:** specially designed for storing samples, the culture of micro-organisms, the extraction of nucleic acid etc.
  - **Reader:** enables automated reading of the findings of the samples in the various wells in the plate.
  - **Tips:** makes it possible to move a sample solution to wells on the plate.

- **Spectrometer:** a device that makes it possible to measure the optical density of a sample.
- **Electron microscope:** observation using an electron microscope (sometimes combined with a technique for specific marking using antibodies targeting the antigenic patterns of the pathogen) may be used to detect and identify viruses.
- **Fluorescence microscope:** makes it possible to detect and identify a pathogen. It is linked to a computer to display the observations.

4.2.5.3. Glassware

- **Petri dish:** a Petri dish is a cylindrical dish that is used for the culture of micro-organisms (bacteria, fungus).
- **Weighing bottles:** weighing bottles are laboratory equipment used for precise measurement of solid samples.
- **Pestle and mortar:** the mortar is a recipient for crushing material (samples) to be changed into a paste or powder by using the pestle.
- **Pipette:** it is a tool used to remove a liquid or solution then transfer it from one container to another.
- **Beakers:** are probably the glassware item that is the most used: shaking, preparation of solutions and mixtures, heating etc.
- **Funnel:** an instrument in the form of a cone, ending in a tube and used for pouring a liquid, powder from samples etc., into a recipient with a small opening.
- **Watch glass:** is laboratory equipment, with a concave form, in glass. It is used primarily to cover a beaker, to evaporate a liquid, to perform certain reactions for identification or to weigh a quantity of a sample (also in solid form).
- **Condenser:** Condensers are special glassware used to recover a liquid, or distillate, during distillation through condensation or to avoid the loss of materials as a result of evaporation, particularly in the case of heating relating to reflux.
- **Test tubes:** mostly of glass, the test tube makes it possible to use small quantities of reagents thanks to its small diameter.
- **Dropping funnel** (also called a **pouring funnel**): this is a laboratory instrument in glass used to pour a reagent drop by drop.

4.2.5.4. Safety equipment

- **Glove box:** it is a sealed container designed to allow objects to be manipulated where a separate atmosphere is desired.
- **Fire blanket:** device designed to combat fire. It is used to smother a conflagration.
- **Fire alarm:** a manual fire alarm is a device is a device activated manually used to warn of the presence of a fire.
- **Smoke detector:** a safety device that reacts to the presence of smoke.
- **Safety shower:** or fixed emergency shower, is used to help those who are being burnt chemically or thermally.
- **Eyewash:** first-aid device serving to help those whose eyes have been contaminated by chemicals.
- **Portable safety shower:** a device that often looks like an extinguisher but that serves to help those who are the victims of thermal or chemical burns.
- **PPE:** personal protective equipment protects an individual against a given hazard depending on the activity he will be required to perform. In general, the whole of the body can and should be protected. This generally relates to work ware (coat), mask etc.
- **Fire extinguisher:** a fire fighting device capable of projecting or spreading an appropriate substance, called an extinguishing agent, to put out an incipient fire.
- **Fume hood:** a device for extracting toxic fumes from products used during operations to protect the operator. The fumes are extracted from the volume of the working area, then either processed by filtration or vented to the outside.
- **Vertical laminar flow hood:** makes it possible to work under sterile conditions, but never be used for work with pathogens since they risk being vented to the outside world.
- **Horizontal laminar flow hood:** Makes is possible to work under sterile conditions (sterile air)
- **Chemical fume hood:** a device in which chemical waste is placed (solids and liquids) before their destruction by specialised companies.
- **Bottle of ethanol**: makes it possible to disinfect the workplace in a laboratory. The efficacy of ethanol depends on its concentration. Thanks to a laboratory experiment conducted, it has been shown that the ideal concentration of ethanol to kill all micro- organisms around us was 70% (http://www.my-microsite.com/ethanol/La- desinfection/).

Le lien ne fonctionne pas

4.2.6. Design of a greenhouse adjoining the laboratory

In many cases, identification requires a greenhouse since it makes it possible to create a mini climate favourable to the development of diseases in plants. The greenhouse should consist of common areas, such as circulation spaces (accesses, corridors, etc.), potting rooms, rest areas, sanitary facilities and cloak rooms etc. Each cloakroom should have:

- a metal cupboard for each user;
- a rack for boots and shoes;
- a system for suspending wet clothing;
- one or two benches;
- washbasins and mirrors should be planned for the cloakrooms or in the vicinity.

4.2.6.1. The different types of greenhouse

There are several types of greenhouses that vary primarily in their form or in the material from which their walls are made. The **roofing material must be carefully chosen** (plastic, polycarbonate, glass).

- **The polytunnel:** its principal advantage is its low cost. It is a structure in steel to which a hard-wearing plastic film is attached. The plastic does however make it relatively fragile. The plastic films would have to be replaced every few years and be washed from time to time for maximum light absorption. Tough double-wall plastic film must be used. Stabilised polyethylene resistant to ultraviolet rays should preferably be used. Polycarbonate can mould to the shape of the roof and generate energy savings of up to 30% due to its double walled structure. It allows 80% of light to pass through. This material transmits light in the same way as glass but is lighter.

Figure 2 - Example of a polytunnel

- **Glass greenhouse:** this type of greenhouse has a clear advantage: it guarantees optimum light; in addition, since the condensation runs down the windows, there is no risk of planting being damaged. Glass has an undeniable advantage over plastic: its life. This type of greenhouse also has an opening, making it possible to eliminate the surplus heat generated; and the fact that there is immediate entry into the greenhouse may be of advantage to certain people, making it easier to tend to plants. However, it cost is much higher than for a plastic greenhouse.

Figure 3 - Example of glass greenhouse

- **Oblique walled greenhouse:** this offers the same advantages as its cousin, the glass greenhouse, but is even more accentuated. This type of greenhouse, even more expensive, is for those who wish to obtain the best results.

4.2.6.2. Choosing a location for installing a greenhouse

Preference should be given to locations exposed to the morning sun rather than exposed in the afternoon. The best solution is to choose a location exposed to the sun throughout the day since sunlight promotes plant growth. If there are trees or bushes close to the greenhouse, it must be ensured that their shadows do not shade the greenhouse before the end of the afternoon.

A **location close to an energy source** must be chosen: Most greenhouses need lighting and ventilation to maintain the optimum temperature (sometimes a heating system if temperatures, in particular at night, are too low). The quality of light depends on the true distribution of the light spectrum, a factor which has a major impact on plan growth, fruit yield, fruit quality etc. Greenhouses should be lit using LED lamps since:

- they speed up the harvest cycle: the harvest cycle for market garden crops accelerates by 15 to 20% with LED lights;
- they have a long life: LED plant grow lights have a life of 50,000 hours and do not require maintenance for at least 8 years, on the basis of 16 hours of operation per day. There is no longer any need to replace expensive and ineffective light bulbs;
- they have a low operating temperature: no plants are burnt, while money and resources are economised;
- they have environmental durability: LED horticultural lights are low energy, 100% recyclable and do not contain any toxic agents or substances such as mercury.

A **location that does not retain water must be chosen**. It must be possible to evacuate excess rainwater. It is possible to install a tank to collect rainwater from the greenhouse roof. Savings in water and electricity will help to keep the operating costs of your greenhouse at a low level.

The **size of the location for the greenhouse must be chosen:** the size of the greenhouse must be chosen carefully, whether it is a brand new construction or a prefabricated assembly. The size of the greenhouse and cubicles will vary depending on the purpose for which they are constructed, the budget, etc. The most popular size for cubicles is 2.4 m by 1.8 m. The bigger the greenhouse, the greater the construction costs (and any heating costs).

4.2.6.3. Controlling the temperature in the greenhouse

Fans must be placed at the corners of the greenhouse, diagonally. They must operate continuously when the weather is cold to even out the temperature throughout the greenhouse. Skylights must be installed in the greenhouse roof for ventilation and to vent the carbon dioxide. They must be adjustable and opened wide when it is hot.

Ventilation is therefore necessary for proper control of temperature and humidity. Heating also sometimes plays a very important role in dehumidification.

A number of **thermometers and thermostats** must be installed to be able to record temperature. They must be placed at different levels in order to be able to control the greenhouse temperature at all times.

The **ability to provide water continuously** must be ensured: the best way is to have water lines and tanks.

4.2.6.4. Greenhouse equipment

Crop support

- install semi-mobile tables (in aluminium, galvanised steel, concrete etc.);
- the space used by the tables and shelves should be as much as possible;
- the space used for alleys should be kept to the minimum and they should be in non-slip concrete.

Container nursery

- flat cultivation area, provide a slope for collecting excess water for its removal or recycling;
- sheltered from high winds;
- staking system for supporting tall plants.

Storage bins

- put on one side;
- accessible to delivery lorries through alleyways capable of bearing heavy loads;
- cleaning station;
- provide an area outside for cleaning tools and boots (water point, basin, boot washer);
- the water point must be located close to the greenhouses, nursery and cloakrooms.

Motorised vehicles

- plan a large area that is easy to access and ventilated;
- establish a 'maintenance workshop' 'corner' with an equipped workbench, and a cupboard for storing tools.

The gallery

The repotting gallery should be equipped with fixed repotting tables, wall panels (for putting up posters, illustrations, small tools and instruments etc.). There should be a table affixed to the wall (resistant to dust and humidity).

Building for storing small tools and instruments

- plan for tool racks (tools with handles), shelves, cabinets (cupboards), pinboards etc., for storing different types of tool.

4.3. LABORATORY STAFF QUALIFICATION

Laboratory staff must have undergone training specific to their position; they must have sufficient knowledge and experience.

4.3.1. Entomological skills required

Insects pose many major challenges for entomology: identification of difficult specimens. Flies that resemble wasps or bees, spiders that mimic the appearance of ants, butterflies that pass for beetles... Many arthropods are masters of disguise. Such strategies serve to confuse prey or predators.

A well-trained entomologist is capable of distinguishing between two orders of insects. But the **more identification needs to be precise, the more complicated it is**, particularly at species level. Mimicry is, unfortunately, just one of the many reasons that make the identification of insects a complicated task, even for professionals. For example, there are a number of cases where the visible morphological difference between two species comes down to the number of hairs on the insects head, to the designs formed by the grooves on the wings, or even the anatomy of the genitalia!

In a laboratory, identification requires considerable time and expertise. Given the multitude of arthropods that exist throughout the world, an entomologist often specialises in just one particular group.

As a specialist in insects, their way of life, their role in ecosystems and the impact on the environment, an entomologist conducts research with a view to making a concrete contribution to the monitoring and protection of invertebrates. In particular, he is responsible for establishing measures for combating crop pest or even methods for making controlled use of appropriate insects for agricultural needs, forestry, the environment or other areas. He conducts field work (observations, species identification, inventories, assessments, diagnostics, instructions on treatment, etc.) and provides services and advice on projects relating to the environment, ecotourism and the dissemination of scientific knowledge in entomology.

His role in the laboratory: analysis of insects that are vectors for diseases, tests, breeding, notably to establish measures for biological or chemical control of harmful species.

Specialised staff (responsible for diagnostics) should therefore have followed studies in biological, agronomic and environmental engineering and specialised in entomology.

4.3.2. Phytopathological skills required

The detection and identification of pathogens underlying symptoms observed is a key element in combating plant diseases. Plant diseases can be caused by various organisms: fungus, oomycetes, bacteria, viruses, viroids, phytoplasma, protozoa, nematodes and parasitic plants.

The **first approach to a diseased plant is primarily visual**. In certain cases, a pre-diagnostic test may be conducted based on the symptoms shown by the plants (malformations, damage, discolouration etc.) or directly based on the presence of organisms or their traces (insects, eggs, exudates, tunnels etc.). But in general, **laboratory confirmation is required**. This all the more true given that certain pathologies present are **latent or asymptomatic**.

In the laboratory, analysis methods are based on **various principles for identification**. The majority are conducted in the following categories: observation of the phytopathogen (with the naked eye or with an optical instrument).

The objective of these analyses is to seek the **origin of the symptoms observed on a crop or plant** (www.fredon-centre.com/Rub_28/Une-CLINIQUE-du-vegetal-au-service- des-professionnels-et-desparticuliers.html). Le lien ne fonctionne pas

The phytopathologist must:

- have vast scientific knowledge (in botany, biology, but also in maths, physics and chemistry);
- learn continuously and read specialised literature;
- know how to diagnose diseases;
- know how to collect field samples;
- know how to conduct analyses and experiments in a laboratory;
- know how to grow plants in a greenhouse or in a laboratory;
- conceive effective treatments;
- know how to use state-of-the-art computer equipment and techniques.

4.3.2.1. Virological skills required

Diseases caused by plant viruses have specific characteristics which distinguish them from those caused by other pathogenic micro-organisms. Virologists should be familiar with the characteristics of viral diseases, which may vary depending on the virus, the variety or the species affected, the environment and the physiological state of the plants.

A virologist conducts research relating to the structures, development, effects and processes enabling viruses to infect plant cells and exploit cellular mechanisms in reproducing. His ultimate objective is to discover how they can be eliminated. To this end, he conducts analyses and experiments in a laboratory using precision instruments, such as electron microscopes. He uses techniques such as isolation: he is capable isolating, characterising and identifying a particular virus. Not being able to grow it directly, since he cannot replicate it outside a fume cabinet, he grow vegetable cells before inoculating them with the virus.

The symptoms obtained may be confused with those caused by other viruses. To prevent confusion, the virologist must combine this technique with another serological or molecular technique.

The virologist must:

- have vast scientific knowledge (in biology, but also in maths, physics and chemistry);
- know how to conduct chemical and biological analyses and experiments;
- learn continuously and read specialised literature;
- know how to use state-of-the-art technical and computer equipment;
- comply with rules on safety.

4.3.2.2. Nematological skills required

Phytopathogenic nematodes, microscopic worms present in the soil, are major parasites for many crops grown. A nematologist must understand better how such nematodes manage to infest plants by studying the saliva secretions of the nematode and the processes involved in the formation of nursery sites, referred to as 'giant cells', essential for the development of the nematode within a plant.

To be able to recognise nematodes, scientists must be capable of:

- conducting research and observing the symptoms of a nematode attack;
- collecting samples of soil and plant tissues;
- extracting nematodes from samples;
- identifying nematodes;
- estimating the density of nematodes;
- analysing damage caused by nematodes;
- taking decisions on control methods.

Nematologists must have a good knowledge of animal biology, zoology, genetics, molecular biology, chemistry, agronomics, etc.

4.3.2.3. Botanical skills required

It is important in such a laboratory to be able to identify plants (in particular invasive plants).

Botanists have in-depth knowledge in floristry (the science of flowers) and systematics (determination and classification of plant species). Like all researchers, botanists divide their time between activities in the field and laboratory analyses.

In the field, in contact with nature, botanists draw up inventories of plants, flowers and trees. They observe their diversity, development and life cycle. They draw up botanical maps of the locations they are responsible for studying and take samples of flora for research purposes.

In the **laboratory**, botanists analyse such samples, grow them and organise herbariums. They compare notes taken in the field and the findings of laboratory analyses, then draw up a report in which they present their conclusions. They conduct taxonomic, morphological, anatomical, physiological and phytochemical analyses of organisms. In other words, they study their classification, form, internal structure, the way in which they work and the chemical reactions they catalyse. Their task is also to analyse the development, heredity, reproduction and biodegradability of plants, that is to say their decomposition by micro-organisms.

In addition, botanists must be able to:

- grow plants in a greenhouse or in a laboratory;
- use state-of-the-art computer equipment and techniques;
- collect field samples;
- learn continuously and read specialised literature.

Botanists come through the universities or higher-educational engineering institutions: botanists are high-level scientists specialising in plant biology, ecology, genetics, statistics, chemistry etc.

4.4. MANAGEMENT AND QUALITY CONTROL IN THE LABORATORY

4.4.1. Definition

Laboratory quality can be defined as the accuracy of the analysis findings. Laboratory findings must be as accurate as possible, all aspects of the laboratory operations must be reliable, and reporting of the findings must be correct so that they can be used for plant health purposes.

4.4.2. Organisation

In order to achieve a quality management system that operates effectively, the structure and management of the laboratory must be organised so that quality procedures can be established and implemented.

The complexity of laboratory processes means that a number of factors must be taken into account to ensure the quality of a laboratory. These factors include, *inter alia*:

- the laboratory environment;
- the quality control procedures;
- communications;
- archiving;
- competent and well informed staff;
- good quality reagents and equipment.

The environmental conditions in the laboratory, such as lighting, temperature and space available for conducting tests properly.

In a quality management system, all aspects of the laboratory operation, including the organizational structure, methods and procedures, need to be addressed to assure quality.

Laboratories produce the results of analyses that are widely used for clinical or public health purposes, and the benefits for health depend on the accuracy of those analyses and the reporting of the findings. If inaccurate reports are issued, the consequences can be very serious, for example inappropriate treatment, further and pointless analyses etc. The consequences result in an increase in cost and time and contribute nothing of value to plants.

To achieve the highest level of accuracy and reliability, it is essential to conduct all the laboratory processes and procedures to the highest standard possible. Consequently, a quality management system model that encompasses the system as a whole is essential in order to ensure that a laboratory operates smoothly.

4.4.3. Equipment

The different types of equipment already cited must function properly. Choosing the right equipment, installing it correctly, assuring that new equipment works properly, and having a system for maintenance are all part of the equipment management programme in a quality management system.

4.4.4. Purchasing and inventory

The management of reagents and supplies in the laboratory is often a challenging task. However, rational management of purchasing and inventory can produce cost savings in addition to assuring supplies and reagents are available when needed. The procedures that are a part of management of purchasing and inventory are designed to assure that all reagents and supplies are of good quality, and that they are used and stored in a manner that preserves their integrity and reliability.

4.4.5. Process control

Process control is comprised of several factors that are important in assuring the quality of the laboratory testing processes. These factors include **quality control** for testing, **appropriate management of the sample**, including collection and handling, and **method verification and validation**.

4.4.6. Information management

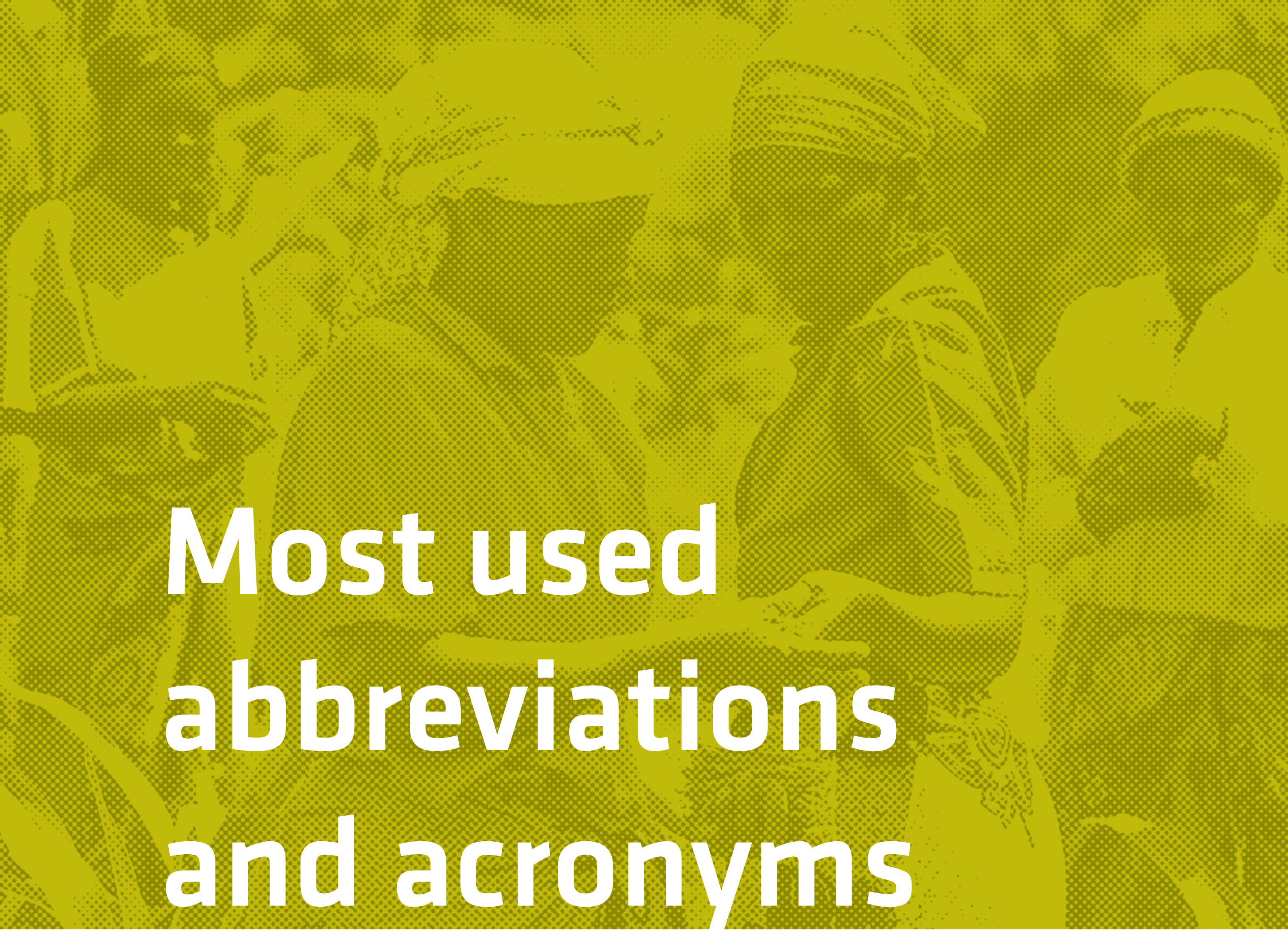
The finished product of the laboratory is information, primarily in the form of test reporting. Information (data) needs to be carefully managed to assure confidentiality of the findings, as well as accessibility to the laboratory staff. Information may be managed and conveyed with either paper systems or with computers.

4.4.7. Occurrence management

A laboratory 'occurrence' is an error or an event that should not have happened. A system is needed to detect these problems, to handle them properly, to learn from mistakes and take action so that they do not happen again.

4.4.8. Assessment

The process of assessment is a tool for examining laboratory performance and comparing it to existing standards or benchmarks, or even the performance of other laboratories. Assessment may be internal, or performed within the laboratory using its own staff, or it may be external, conducted by a group or agency outside the laboratory. Laboratory quality standards are an important part of the assessment process, serving as benchmarks for the laboratory.

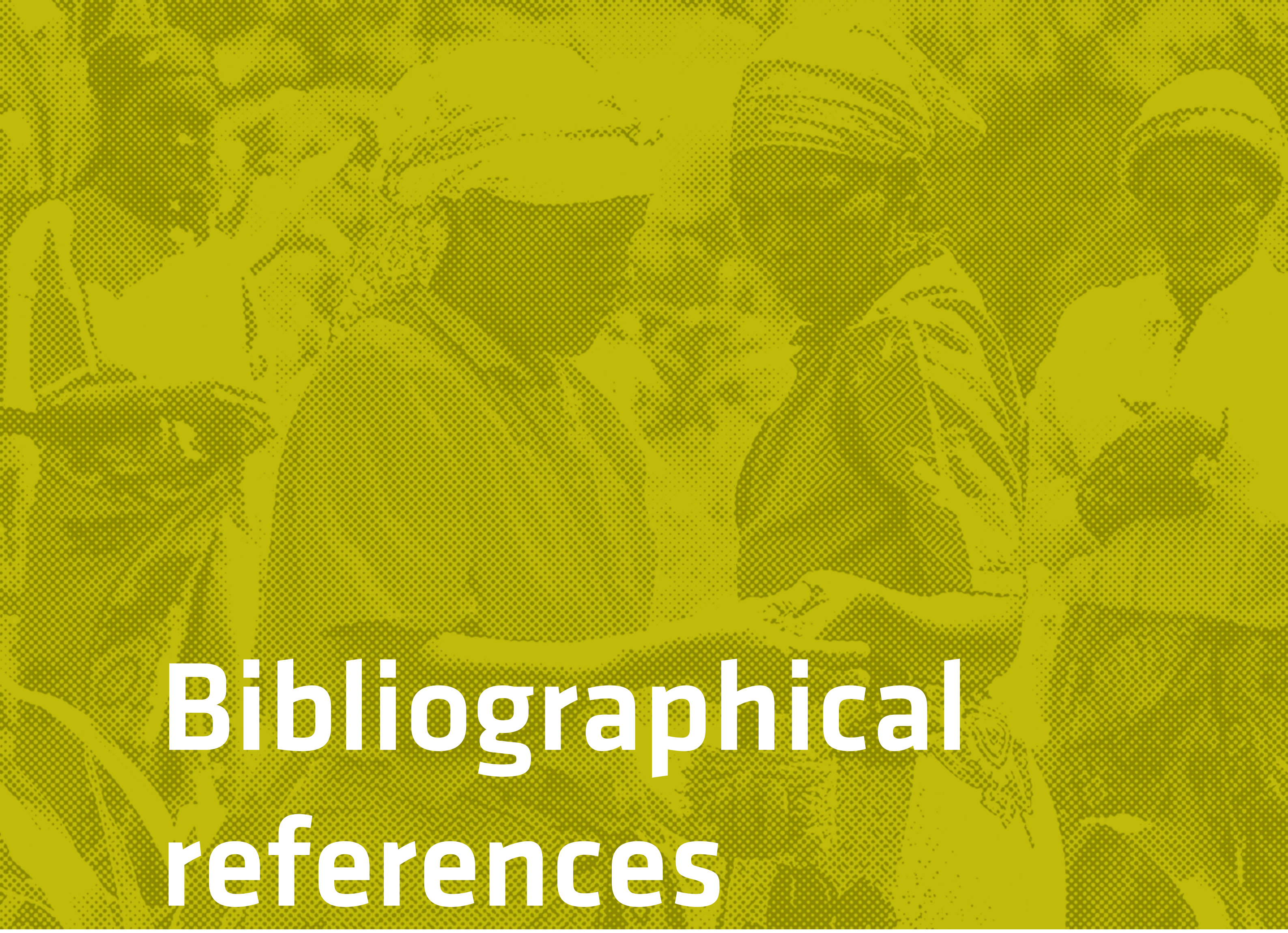


Most used abbreviations and acronyms

MOST USEFUL ABBREVIATIONS AND ACRONYMS

ACP	Africa – Caribbean – Pacific (countries of the ACP Group, having signatories of a series of specific agreements with the EU called the "Cotonou agreements")
AQL	Acceptable Quality Level
BI	Buffer Indicators
CA	Competent authority
Cd	Chemical symbol of cadmium
CO_2	Chemical symbol of carbon dioxide
DNA	Deoxyribonucleic acid
EC	European Community
ELISA	Enzyme-linked immunosorbent assay
EMC	Electromagnetic compatibility
EPPO	European and Mediterranean Plant Protection Organization
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
GMO	Genetically modified organism
HACCP	Hazard analysis and critical control point
HC	Healthy Controls
HEPA	High Efficiency Particulate Air
Hg	Chemical symbol of mercury
IgG	Immunoglobulins
IgG E	Immunoglobulin G coupled to enzyme
IP	Index of protection
IPPC	International Plant Protection Convention
ISO	International Organisation for Standardisation
ISPM	International standards for the phytosanitary measures
MLR	Maximum residue level

NGO	Non-governmental organisation
NMR	Nuclear magnetic resonance
NPPO	National Plant Protection Organisations
OPC	Procyanidolic oligomers
PAH	Polycyclic aromatic hydrocarbons
Pb	Chemical symbol of lead
PC	Personal computer
PCB	Polychlorobiphenyls
PCP	Pentachlorophenol
PCR	Polymerase Chain Reaction
pH	Power of hydrogen
PPE	Personal protective equipment
PRA	Pest Risk Analysis
RNA	Ribonucleic acid
RT	Reverse Transcriptase
Scs	Sick Controls
SPS	Agreement on the Application of Sanitary and Phytosanitary Measures
UHT	Ultra-high temperature
VAT	Value added tax
WTO	World Trade Organisation



Bibliographical references

BIBLIOGRAPHICAL REFERENCES

Engvall, E. and Perlman, P.,
"Enzyme-linked immunosorbent assay (ELISA).
Quantitative assay of immunoglobulin G", *Immunochemistry*,
vol. 8, 1971, pp. 871-874.

FAO, *International Convention for Plant Protection*,
Rome, 1992.

FAO, "Glossary of phytosanitary terms",
FAO phytosanitary Bulletin, 38 (1) 1990, pp. 5-23.

ISPM, *Guidelines for pest risk analysis*,
Pub. 2, FAO, Rome, 1996.

ISPM, *Requirements for the establishment of pest free areas*,
Pub. No. 4, FAO, Rome, 1996.

Kindundu, N., "Rapport de stage à la clinique des plantes
de l'Université Catholique de Louvain: Diagnostic d'agents phytopathogènes
et conseils en matière de lutte intégrée",
Graduation work, UCL, 2013.

Legrève, A. and M. Clausse, M.,
"Phytopathologie. Maladies causées par des champignons",
Notes of practical works, 2013.

Triolet, J., Capois, J., Gautret de la Moricière, G.,
Lê Quang, X., Petit, J.M., Protois, J.C. et Rocher, M.,
"La conception de laboratoire de chimie",
Cahiers de notes documentaires Hygiène et sécurité du travail,
No. 188, 3rd quarter 2002, pp. 7-26.

WTO,
*Agreement on the Application of Sanitary
and Phytosanitary Measures*, Geneva, 1994.

Useful Websites

USEFUL WEB SITES

Au jardin info
www.aujardin.info

CNRS
www.cnrs.fr/index.php

Codex Alimentarius
www.fao.org/fao-who-codexalimentarius/en Le lien ne fonctionne pas

COLEACP
www.coleacp.org

EPPO
www.eppo.int

Eur-Lex
eur-lex.europa.eu/homepage.html?locale=en

FAO
www.fao.org/home/en

IPPC
www.ippc.int/en/

ISO
www.iso.org/home.html

Ministry of Agriculture FR
agriculture.gouv.fr

World Organisation for Animal Health (OIE)
www.oie.int/en/

Wallonia Portal
wallex.wallonie.be

WHO
www.who.int/en

WTO
www.wto.org/index.htm

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