Explanation

Microbiology and sampling during the preparation/processing of fruit, vegetables, potatoes

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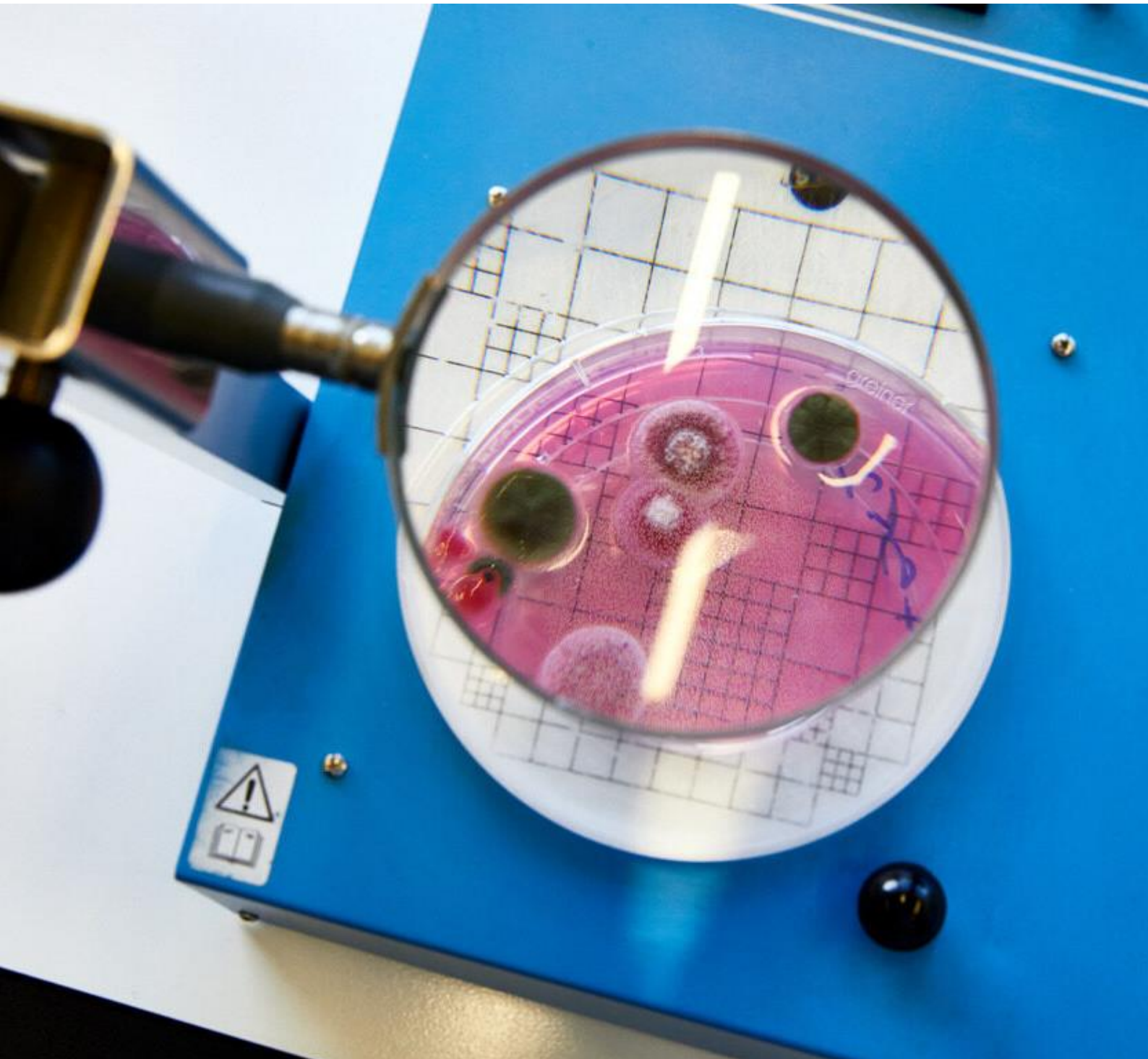

Contents

1 Basic Principles

This supporting document provides information on microbiology and sampling. It serves as an orientation aid for the implementation of the requirements outlined in the guideline "Preparation/Processing Fruit, Vegetables, Potatoes" as well as in annex 11.2 to the QS-GAP guideline "Requirements for preparation processes". Furthermore, this supporting document serves as an orientation aid for the implementation of the microbiological monitoring in the branches of the food retail. The information contained here can be used to help to decide on company-specific measures. The information contained in this supporting document does not claim to be complete. The requirements described in the guidelines are authoritative for independent inspections.

2 Background

In addition to natural processes such as breathing, water release and the breakdown of reserve sub-stances, microorganisms can have a considerable influence on the quality of fruit and vegetables and can cause them to decay. Fruit and vegetables contain fruit acids and other substances with an anti-microbial effect which protect them for a while against microbial decay. Appropriate storage is nevertheless important for maintaining the quality of fruit and vegetables. During storage, a compromise has to be found between the requirements for maintaining microbial and general quality, i.e. the air humidity and temperature must be selected in such a way that they comply with the physiological requirements of fruit and vegetables while inhibiting the growth of microorganisms at the same time. They can be stored in a normal storage area, fresh air store, regular refrigerated storage area or so-called CA store (CA = controlled atmosphere). The storage behaviour of fruit and vegetables is crop-specific on the one hand while it depends on agricultural measures such as fertilization, irrigation, harvesting time etc. on the other.

The decay of fruit often starts from the surface and is usually caused by fungi, because they are well able to tolerate the acidic milieu in the interior of the fruits. Fungi exist in the natural environment of fruit and penetrate into the fruit tissue via damage in the surface and natural openings or directly via intact dermal tissue.

Vegetables have a higher pH-value, which is why bacteria in addition to fungi can lead to decay. In addition to that, vegetables often grow in the direct vicinity or in the soil, therefore the harvest products always have natural contact with fungi and bacteria. If mycotoxin-forming fungi occur in in-creased numbers, consumption constitutes a health hazard. Furthermore, pathogenic (morbid) microorganisms (bacteria, viruses) or parasites (helminth eggs) can be taken with the consumption of fruit or vegetables. These pathogens can get on the fruit and vegetable e.g. through organic fertilizers or washing or irrigation with water contaminated with faeces. Salmonella, Shigella, Escherichia coli and other bacteria, as well as viruses, protozoans and helminth eggs can be transmitted in this way. Chopped salads (Fresh Cut) are particularly susceptible from a microbiological point of view. The natural protection of the plant is largely destroyed by the chopping, the associated increase in surface area and the release of cell juice. The breathing activity inside the packaging produces a particular gas composition which promotes the propagation of pathogenic bacteria such as Salmonella and Listeria monocytogenes, thus constituting a potential health hazard.[(1)]

Note: *To avoid the risk of infection, inter alia low-germ raw materials should be used and the cold chain should be maintained without interruption.*

Microorganisms can significantly affect the quality of fruits and vegetables and cause their decay. Ac-cordingly, microbiological examinations are required. The specific QS requirements for the microbio-logical monitoring of the products and the microbiological examinations within the company facilities can be found in the respective QS guidelines.

3 Microorganisms

Microorganisms are microscopically small living creatures (bacteria, mould fungi, yeasts) or organic structures (viruses). Their size varies from approx. 0,02 to 0,04 µm for viruses via approx. 0,5 to 5 µm for bacteria, approx. 5 to 10 µm for yeasts to approx. 1-2 mm for mould fungi.

In the production of foods microorganisms - but for a few exceptions - are undesired as they can restrict their durability and suitability for human consumption.[(2)]

Classification of microorganisms and their effect:

- Spoilage germs

- Lead to decay of food (smell, taste, consistency, appearance) by release of metabolic prod-ucts and/or by release of certain enzymes
- e.g. Yeasts, mould fungi, clostridia

- Pathogens
  - Pathogenic microorganisms have the ability to harm an organism (pathogen = causing a dis-ease)
  - e.g. Salmonella, *Listeria monocytogenes*, Campylobacter

- Food intoxication
  - Formation of toxins in the food by the microorganism. It is not the germ itself that is "toxic", but its metabolic products formed in the food products
  - e.g. *Clostridium botulinum, Staphylococcus aureus*

- Foodborne pathogens
  - The microorganism itself is pathogenic. Harmful effect takes place in the host organism itself
  - e.g. Salmonella, *Listeria monocytogenes*

- Toxic infection
  - Microorganism forms toxin after ingestion in the host organism, which then damages the host organism
  - e.g. EHEC (enterohaemorrhagic *Escherichia coli*)

3.1 Transmission paths and temperature influence

Microorganisms can find their way into foods in various ways. The following points represent a list of possible transmission paths:

- Food residues: Changes through chemical, physical and microbiological processes
- Deposits: Formation of insoluble salts
- Biofilms: Creation of extracellularly formed polymer substances from microorganisms
- Contamination and dirt: dust, abrasion residues, sealing and lubricating grease
- Transfer from dirty rinsing water after cleaning and disinfection
- Air
- Staff

In addition to chemical and physical influences, temperature is a significant external factor which in-fluences the survival and propagation of microorganisms:

- Cold: Inhibits propagation
- Warmth: Moderate heat promotes propagation. Most microorganisms propagate quickest be-tween 20 °C and 40 °C.
- Heat: Most microorganisms are killed off at temperatures above approx. 62 °C. Several spores survive to approx. 134 °C.[3]

Table 1 shows the influence of different temperatures on microorganisms and how warmth and cold treatments are applied to foods.

Tabelle 1: Temperature influence on microorganisms

Temperature	Treatment of Foods	Microorganisms
140°C (for secs.)	UHT (Ultra high temperature) process for milk	Milk becomes practically germ-free
120°C (for mins.)	Sterilization of preserves	Almost all resistant bacteria spores are killed off
100°C (for mins.)	Boiling point of water	
90°C (for mins.)	Temperature range of pasteurization	Salmonella and other heat-labile bacteria are killed off in a few minutes Over 60 °C – bacteria are no longer capable of growing
62°C (for min. 30 mins.)		
65°C	Danger zone	
40°C	Critical danger zone	Optimal growth temperatures for microorganisms
20°C		
10°C	Danger zone	
-18°C	Storage of deep-frozen products	Bacteria growth has stopped, but bacteria are not killed off. Growth and propagation start up again under favourable conditions (defrosting, heating)

Source: modified according to Almedica AG 2012, P. 18[4]

3.2 Aerobic mesophilic colony count

Aerobic (oxygen-loving) mesophilic (heat-loving) germs are bacteria which grow best under oxygen and at moderate temperatures. The colony count is a measure of the general microbial condition of a foodstuff. Depending on the type of the food high colony counts can indicate shortcomings in process-/personal hygiene. In addition to the food test, the aerobic mesophilic colony count is also used as a hygiene indicator and for cleaning controls.[5]

Note: *For the evaluation of the aerobic mesophilic colony count e.g. the nature of the food, the growth and harvest conditions as well as the processing processes need to be considered.*

3.3 Enterobacteriaceae

Various bacteria types, which occur in the intestines of humans and animals but which can also be found in soil, water and plants, are grouped together under the term Enterobacteriaceae. Because they are sensitive to salt and heat and have low nutrition requirements, they can propagate well on insufficiently cleaned surfaces. The enterobacteria include a large number of disease pathogens *(e.g. Salmonella, Shigella, EHEC/STEC/VTEC).*

Note: *For the evaluation of the colony count of Enterobacteriaceae the nature of the food, the growth and harvest conditions as well as processing processes need to be considered. The detection of Enterobacteriaceae in cooked foodstuff implies deficiencies during the heating process and/or subsequent contamination of the food.*[6]

3.3.1 *Escherichia coli* (*E. coli*)

E. coli is a germ that occurs naturally in the intestines of birds and warm-blooded mammals. It is a component of the intestinal flora of humans. Certain strains of *E. coli* can produce serious illness in animals and humans (*EHEC/STEC/VTEC*)[7].However, a variety of *E. coli* strains are apathogenic and thus not hazardous to human health.

Preventive measures:

- Strict monitoring of staff and production hygiene and the water supply[8]
- Optimization of raw material management

Note: *E. coli is a typical faeces indicator. If an increased colony count of E. coli occurs in food, this indicates hygiene deficiencies or errors in the production process*[6]

3.3.2 EHEC – Enterohaemorrhagic *Escherichia coli*

Enterohaemorrhagic *Escherichia coli* (EHEC) respectively shiga toxin-producing *Escherichia coli* (STEC)/ vero-toxin-producing *Escherichia coli* (VTEC) naturally occur in the intestines of ruminants and are excreted by the animals' faeces. These germs can be transferred directly or indirectly from animals to humans or from humans to humans and cause diseases (diarrhea, nausea, vomiting, possible com-plications (haemolyticuraemic syn-drome). The most important source of infection for humans are raw or not sufficiently heated beef products and raw milk.[1] EHEC germs can be transferred to fruit and vegetables by EHEC contaminated water or by organic fertilizers. In addition, cross-contaminations can occur during the preparing foods when germs from meat are transferred to a ready-to-eat food (such as salad). The contamination of plant-based foods can also occur via hands and/or kitchen utensils.[8] EHEC strains have a strong acidity tolerance and survive storage in foods at 4 °C and freezing temperatures of -20 °C to -80 °C for 9 months.[9]

Preventive measures:

- Strict monitoring of personal and production hygiene and the water supply[10]
- Optimization of hygiene
- If applicable, consideration at the HACCP (Hazard Analysis Critical Control Point) concept[1]

3.3.3 *Salmonella* spp.

The rod-shaped bacteria of the species *Salmonella* spp. are significant diarrhoea-causing pathogens in humans. The main reservoir for Salmonella is the intestinal tract of numerous animals, such as pigs, cattle, calves, poul-try, game, rodents, pigeons and seagulls. The transmission usually occurs from animals to humans by the con-sumption of animal-based foods.[11] Salmonella are determined particularly common in poultry stocks. Foods containing raw eggs and meat products are the most important infection sources of Salmonella. The transmis-sion to other foods occurs via cross-contaminations during or rather after the preparation of charged foods (de-frosting water of deep-frozen poultry).[1] In addition, vegetables can be contaminated via fertilization or wash-ing with faecal contaminated water. Mixed salads are extremely vulnerable to microbiological contamination be-cause the natural protection of the plant is largely destroyed through shredding. Salmonella can multiply partic-ularly well in the low-oxygen atmosphere of the film packaging, thus constituting a health risk.[1]

However, Salmonella can also be determined in other plant-based foods. For example, herbal teas can cause Salmonella infections especially in infants if they are prepared with not sufficiently heated water. Furthermore, spices, seasoning and sesame seed are often contaminated with Salmonella.[12] The spread of Salmonella is en-couraged by insufficient cooling of foods. Their ideal temperature is 37 °C. They are killed at high temperatures above 70 °C with appropriate holding time (at least five minutes).[11]

Preventive measures:

- Strict compliance with the cold chain
- High hygiene requirements on worktops, equipment and staff[10]

3.4 *Listeria monocytogenes* (*L. monocytogenes*)

L. monocytogenes is a bacterium which is very common in agriculture (in soils, on plants, in silage, faeces, wa-ter and wastewater), in aquacultures and in the food processing environment. It is resistant to many different environmental conditions, such as high salt or acid concentrations.

L. monocytogenes grows in a low-oxygen atmosphere and low refrigeration temperatures and can survive for a long time in the environment, on foods, in the preparation/processing plant and in consumer's refrigerators. *L. monocytogenes* causes the invasive listeriosis by overcoming the mucous membrane barrier of the gastrointes-tinal tract which then leads to infections in the body. Infections with *L. monocytogenes* can cause light flu-like

illnesses but also gastrointestinal diseases, meningitis and/or encephalitis and can lead to miscarriage or premature birth.

L. monocytogenes has been isolated from various foods such as raw vegetables, raw poultry, raw and processed meat, salmon and raw milk cheese. The germ can also multiply even at low concentrations in an affected food during storage at refrigeration temperature. Important factors that influence the propagation of Listeria in food products are among others the temperature during the storage, the duration of storage, the pH as well as the water activity (aw-value) of food products.

Listeria can persist in those areas of working premises that are difficult to achieve with cleaning and disinfection measures (e.g. in sewage systems, the insides of pipes and places with condensed water). They are also able to form biofilms in whose matrix other microorganisms can grow, too.(1)

Preventive measures:

- The cold chain must be continuously maintained
- Avoidance of recontamination of the products
- Hygienic equipment and maintenance

3.5 Coagulase-positive Staphylococci

Staphylococci are bacteria which occur naturally in humans and animals. The significance of coagulase-positive Staphylococci is in terms of food hygiene their ability to form Staphylococci enterotoxins (SE) and enterotoxin-like SAgs (SE-like) termed super antigens (SAgs). Food intoxication by coagulase-positive Staphylococci presupposes that the pathogens have been able to sufficiently multiply in the food and have formed heat-stable enterotoxins.(13)

3.5.1 *Staphylococcus aureus* (*S. aureus*)

The most important representative of the coagulase-positive Staphylococci is *Staphylococcus aureus*, which is regarded as one of the most common pyogenic organisms. *S. aureus* can be transferred to foods via open wounds, especially on the hands. In about 30 to 40% of all healthy persons, the bacterium can be detected in the stool, the mucous membranes of the nasopharyngeal area and on the scalp and in the hair. Several *S. aureus* strains have the capability to produce enterotoxins which can be the cause of food poisoning. All carbohydrate- and protein-containing foods with a high water con-tent, on which the germs were transferred, provide ideal conditions for the propagation of the bacteria and the formation of enterotoxins.(1) The predominant symptoms of a Staphylococcus intoxication are vomiting, nausea, diarrhoea and circulatory symptoms. Already very low amounts of toxin can be sufficient.(13)

Preventive measures:

- Good staff hygiene (e.g. special measures for employees with inflammations on their hands or other skin areas)
- Cleaning and disinfection of hands, clean working clothes
- Hair and beard cover
- Sufficient heating of foods
- Refrigeration of non-sterile products
- Avoidance of lengthy idle times at temperatures below 65° C(1)

3.6 Presumptive *Bacillus cereus*

Bacillus cereus (B. cereus) occurs naturally in normal soil. Together with other species (e.g. *Bacillus anthracis*, *Bacillus thuringiensis* and *Bacillus cytotoxicus*), it forms the *B. cereus* group. The spore forming bacterium can be transferred to animal and plant-based foods via spore-containing soil particles and dust. Although heat treatment kills the germ, the spores survive. The vegetative form of *B. cereus* grows in a range of 10 to 50 °C, with a temperature optimum between 30 and 40 °C and above a pH of 4,8. Individual cold-tolerant strains also reproduce at 4 to 6 °C. Complete prevention of contamination is hardly possible. However, a low germ count does not present a hazard to the health of the consumer. Poor storage conditions result in the germination of the spores and/or the propagation of the germs on the food.(14) During its growth, *B. cereus* produces toxins which can trigger either the diarrhoea syndrome or the vomiting syndrome.(1)

Bacillus thuringiensis is widespread in the environment, too. In addition, some strains are used as organic insecticide for plant production. On the basis of investigations that have performed to date, the ability of *Bacillus thuringiensis* to form cereulide toxin is not been proved. But there are evidences that some of the *Bacillus thuringiensis* strains used as insecticides are able to form enterotoxins. On the basis of the close genetic relation of *Bacillus cereus* and *Bacillus thuringiensis* a differentiation of both germs within the routine diagnostics is not possible with certainty.(15)

In order to reduce the risk based on the *Bacillus cereus* and *Bacillus thuringiensis*, the European Food Safety Authority (EFSA) recommends to use *Bacillus thuringiensis* preparations for plant protection strictly in accordance to the manufacturers' specifications. To reduce a germ multiplication, the EFSA also recommends to comply a storage temperature along the food chain of ≤ 7°C but better of ≤ 4°C.[16]

Preventive measures:

- As temperatures below 100 °C enable the survival of individual spores. Therefore, an immediate cooling after heat treatment is necessary in order to prevent germination
- Compliance with the cold chain[14]

3.7 *Campylobacter* spp.

Currently, *Campylobacter* spp. are the most common bacterial food associated diarrhoeal pathogens for humans in Germany. The infectious dose amounts to 500 CFU (colony forming units). *Campylobacter* spp. occur in the intestinal tract of animals and in the dung of mammals and birds. They also can be found in the environment (soil, insects) as well as contaminations on various foods (mainly on poultry meat, also pork, fruit and vegetable). As reservoir for the germ, especially poultry but also pork, cattle and pets are described.

Human campylobacter infections express themselves through fever, dizziness, diarrhoea, vomiting, stomach-, head- and muscle pain. Joint inflammations are described as complications, too (Guillain-Barré-syndrome).

3.8 Yeasts

The single-cell subgroup of fungi propagates by sprouting. With oxygen (aerobic conditions), intensive propagation results with the formation of lots of carbon dioxide and little alcohol. Without oxygen (anaerobic conditions) there is no or only poor growth but alcoholic fermentation. This causes undesirable sensory changes of sweet or sour foods. When the fermentation process has occurred, the food is inedible.[17]

Preventive measures:

- Good staff and process hygiene
- Raw materials management
- Destruction of the yeasts by heating

3.9 Mould fungi

Mould fungi are microorganisms which spread via spores and propagate on the surface of foods. Mould fungi can grow on relatively dry materials. They are sensitive to heat and grow well in acidic environments.[17] The thread braid (mycelium) is in the interior of the food. Several mould fungi form mycotoxins that are harmful to humans, such as the very heat-stable aflatoxin, which has a severe carcinogenic effect.[17]

Mould fungi are the most important spoilage agent in fruits because they well tolerate the acidic milieu, caused by the high concentration of fruit acids. Fruits may become contaminated with fungi be-fore and after harvesting via the soil, the fruit trees, dead plant parts etc. Depending on the conditions, some fungi continue to grow during storage and thereby cause considerable storage damage. The most significant storage damage for fruits are botrytis rot (grey mould), gloeosporium rot (rusty blight), sclerotinia rot (monilia) and penicillium rot (green or blue mould). However, vegetables and potatoes can also be infected with mould fungi causing them to rot. An overview about important mycotoxins, their occurrence and their effects on humans and/or animals is shown in the table 2.[1]

Table 2: Overview of important mycotoxins

Toxin	Common diseases and effects on humans and/or animals	frequently infected foods
Aflatoxins	Liver cancer, liver cirrhosis, teratogen	Peanuts, pistachios, cereals
Ergot alkaloids	Ergotism	Rye flour bread
Ochratoxin A	Nephropathy, enteritides, genotoxic, mutagenic, possibly cancerogenic	Cereals, coffee, beer

Toxin	Common diseases and effects on humans and/or animals	frequently infected foods
Patulin	Nausea, organ toxicity (liver, kidneys, lungs etc.)	Fruit
Fusarium toxins		
Zearalenone	Oestrogen effects, abortions, sterility	Cereals
Trichothecenes	Skin, mucous tissue damage	Cereals
Deoxynivalenol (DON)	Immune suppressive, vomiting	Cereals
Fumonisins	Possibly cancerogenic	Maize
Citrinin	Teratogen	Cereals, rotting tomatoes
Citreoviridin	Nephrotoxic, cardiac beriberi	Rice
Sterigmatocystin	Liver cancer	Nuts, cereals

Source: Krämer (2011)[(1)]

Preventive measures:

- Agricultural measures (healthy seed, proper fertilization, plant protection, appropriate harvesting)
- Optimal storage and transport of harvested products
- Inhibiting of fungal growth on non-sterile products through conservational measures such as refrigeration, freezing, the addition of preservatives, aw-value reduction or CA storage[(1)]
- Removal of mouldy products and/or rejection of contaminated lots
- Deactivation of existing mycotoxins through technological processes
- Killing of fungi in the intermediate or final product through sterilization or pasteurization
- Avoidance of secondary contamination by suitable packaging

3.10 Viruses

In contrast to the bacteria, yeasts and mould fungi, viruses have no own metabolism. To propagate they rely on a suitable host organism. The stability of the viruses depends on various factors. Since viruses do not live in the proper sense they cannot be killed but rather become "inactivated". De-pending on the virus, an inactivation can occur through physical impacts like temperature, humidity/drying, through radiation or through chemical impacts (inter alia alcohols, proteins, salts, deter-gents).

3.10.1 Hepatitis A viruses

Hepatitis A viruses are pathogen for humans. Humans are the main hosts and reservoir for the hepatitis A viruses. The virus also can be proofed in waste and use water, as well as a contamination on various foods (inter alia meat, fruit, vegetable). Clinically, a hepatitis A infection appears in humans through an inflammation of the liver which come along with gastrointestinal diseases, fever, yellow discoloration of the skin and the mucous membranes as well as with pale stool and dark urine. Even itching and skin rashes are described.

3.10.2 Noroviruses

Noroviruses are pathogen for humans. The infectious dose is up to 10-100 virus particles. Currently, noroviruses are the most common pathogens for human gastro-intestinal infections in Germany. Humans are the reservoir for noroviruses. Noroviruses can also be found in waste and use water and as a contamination on various foods (inter alia meat, fruit, vegetable). Clinically, the infection of hu-mans with noroviruses appears

through an acute gastroenteritis with projectile vomiting and severe diarrhoea which is accompanied by nausea, headache and faintness. The clinical symptoms usually only last 12-48 hours.

4 Microbiological Examinations

4.1 General information on food hygiene including cleaning and disinfection

In accordance with the **Regulation (EC) No. 853/2004** under "food hygiene" measures and precautions are understood, which are necessary to control hazards and to guarantee that foodstuff in con-sideration to its purpose is suitable for the human consumption. The term "food hygiene" can be broadened and include such measures and precautions, which are taken for the assurance of the product quality and the durability of a food.

The penetration of microorganisms into the production environment is prevented by:

- Cleaning and disinfection plans
- Working equipment (chopping boards etc.) cleaned and disinfected in accordance with the cleaning and disinfection plan
- Flush toilets and hand wash basins (hot and cold water, soap, disinfectants)
- Clean transport vehicles and packaging
- Staff and production hygiene measures
- Hygiene Trainings

In this regard inter alia cleaning and disinfection measures can play an important role. In the following some important terminologies on the aspect "Cleaning and disinfection measures" from the **DIN 10516 Food hygiene - Cleaning and disinfection** are listed:

- Contamination: Every undesirable substance, including product residues, microorganisms, residues of cleaning agents and disinfectants
- Cleaning: Removal of contamination
- Disinfection: Chemical and/or physical process to kill microorganisms to a level which is neither harmful to health nor influences the quality of the food
- Clean: Visually free of contamination
- Disinfectants: Biocidal product intended to achieve a disinfection
- Exposure time: Time that is needed to remove contaminations and/or to achieve a required disinfection

In food producing, preparing and processing companies, a combined hygiene measure according to the current state of the art, a combined hygiene measure consisting of dry cleaning, wet cleaning and disinfection according to **DIN 10516** is usually carried out. This hygiene measure proceeds in the following six steps:

- 1. Step: Coarse pre-cleaning
- 2. Step: Pre-cleaning
- 3. Step: Main cleaning
- 4. Step: Post cleaning including drying phase
- 5. Step: Disinfection
- 6. Step: Clear rinsing

The specific QS requirements for microbiological testing within the facility can be found in the respective QS guidelines.

4.2 Sampling, sample transport and documentation

4.2.1 General notes on the planning of sampling

In order to make microbiological examinations meaningful, considerations regarding suitable sampling points must be made in advance. The following aspects should be considered:

- Identification of sources (Identification of niches)
- Identification of the flow of goods
- Identification of staff routes
- Identification of working and cleaning areas
- Identification of potential sources of cross-contamination
- Sampling of end product required for verification, but secondary for root cause analysis

Microbiological examinations should be carried out on a scientific basis and objectively. There are different reasons for microbiological examinations, e.g.:

- Verification that the specification is met
- Verification that legal requirements are met (e.g. confirmation that a lot is marketable)
- Specific examinations required by law (e.g. **Regulation (EC) No. 2073/2005**)
- Validation of processes
- Verification of processes and of corrective actions
- Process management
- "Baseline-Study" → What is statistically "normal" when the process is under control
- Monitoring/ trend analysis → Indication that process is no longer under control
- Identification of risk factors, also based on current events or rapid alerts (for example, by the RASFF (Rapid Alert System for Food and Feed)

4.2.2 Sampling

The sampling is an important step in microbiological testing which has a decisive influence on the validity of the result. Sampling errors can have a much more severe effect on the overall result than testing errors. The following must be observed when taking samples[18]:

- The immediate environment of the sampling point should be as germ-free as possible. Hands must be washed and disinfected prior to taking the sample. The sampler should not cough or sneeze.
- During and after sampling, it is important not to touch the sample excessively.
- For fruits and vegetables: deliberately sample ''worst-case'', i.e., not only spotless produce.
- When samples are taken from open foods, this should be done as quickly as possible.[19]
- Sample containers and equipment must be sterile when the sample is taken and sampling must be performed under aseptic conditions. Sterile disposable material is recommended.
- When sampling, it is particularly important that a representative sample is taken (from various points on the product in question). Depending on the batch size, a single sample does not pre-sent the quality of the batch sufficiently. In such cases the representativeness can be increased by the drawing of a bulk sample assembled from various single samples.
- To avoid cross-contamination,
  - disposable food-safe gloves should be worn when taking samples from unpacked products. These should be changed after the sampling of each lot.
  - each sample should be packed separately in a new, sufficiently large container which is suitable for the purpose (e.g. plastic bag, sterile sampling bag) and closed tightly. Contamination through contact with other samples, sample tags etc. has to be avoided. Samples must be clearly marked (waterproof/indelible ink).
  - It should be avoided to place and transport several samples next to each other in the same container (e.g. plastic bag, transport box).
- Refrigerated products must be sent to the laboratory in compliance with the cold chain.
- If (several) product samples have been taken and transport is staggered, the samples should ideally be stored in a refrigerated area (e.g. cold storage/chamber).
- The storage time of samples should not exceed 24 hours.
- Microorganisms can be killed off by freezing. The reduction in the germ count does not usually alter the susceptibility of the defrosted product to decay, but it would produce false results.[1]

Before sampling, the sequence of procedures should be defined ("step-by-step"). Sampling requires practice and should be carried out by trained specialists. This includes persons who are familiar with the subject and who have received an appropriate training. Evidence to this effect can be provided by certificates for the participation in training courses, advanced training, information events, testimonies.

4.2.3 Sample transport

Samples should arrive at the commissioned laboratory the same day and no later than the following day. For microbiological samples, it is important to ensure that an express shipment option is commissioned. Ideally, the individual sampling vessels/containers (bags) are placed in a thermobox and cold packs are enclosed. A cooling temperature of 3-5 °C during transport has proven to be suitable.

When placing fruit and vegetable samples in the transport container, it is important to ensure that it is well protected and sealed for transport. It must be avoided that the products (samples) are bruised or injured. In addition, samples must be protected from external contamination and should not become a source of contamination themselves.

4.2.4 Sample related data and sampling documentation

For each sample, the packaging (of the sampling bag or container) must include at least the following information:

- Name of the sampler/company
- Date of sampling
- Unique identification for traceability (sample number, lot, pitch, etc.)

In addition, an appropriate sampling protocol must be filled out for each sample in order to assign it to the sample. The more detailed the sampling protocol is filled out, the easier and faster the sample can be processed in the laboratory. Laboratories usually provide appropriate sampling protocols upon request.

4.2.5 Sampling plans

Sampling plans must be drawn up for the microbiological tests. In-house self-assessment processes must ensure compliance with the sampling plans and documentation of microbiological status. Proof of the microbiological quality of the products must be provided. The microbiological analyses of the products must be performed based on the risk analysis and then considered reasonable if there is reason to believe that a microbiological problem or risk exists and the testing will help control the problem or reduce the risk.

At least, the legal requirements regarding the microbiological criteria for foods must be met according to **Regulation (EC) No. 2073/2005**. It must be ensured that the products are safe during the shelf life and have their specific sensory characteristics.

The specific QS requirements for microbiological monitoring of the products can be found in the respective QS guidelines.

If risk-oriented product groups are formed for microbiological examinations, the following aspects should be considered:

- Growth conditions during primary production comparable? E.g.
  - Tubers/berries/fruits/…
  - Outdoor/greenhouse
  - In the soil/near ground level/distant from soil/shrub/tree/nutrient solutions
- Harvest conditions comparable? E.g.
  - Domestic/foreign goods
  - Harvest season/harvest temperature
  - Hygiene conditions
- Conditions during transport/storage comparable?
- Characteristics of the products comparable? E.g.
  - pH
  - aw
  - Sugar content
- Preparation/processing processes comparable? E.g.
  - Cooling
  - Frosting
  - Blanching
  - Peeled/unpeeled
  - Shredding
- Packaging conditions comparable? E.g.
  - Inert gas
  - Vacuum
  - Plastic film
  - Cartons
  - Wooden box

At comparable framework conditions e.g. apples and pears or raspberries and blackberries can be respectively summarized in one product group, meanwhile e.g. strawberries and apple cannot be summarized in one product group because of their different growth, harvesting and storage conditions as well as their different product characteristics (pH value, sugar content, shelf life).

Unspecific plans, e.g. quantity-oriented, may help to reclaim a batch, but not to identify sources of entry and risks from cultivation and processing. Therefore, the situation on site (field, product, harvesting process, packing operation, procedures) should always be evaluated.

Due to systematically risk-oriented sampling plans, targeted derivations for the company can be made by means of analyses. In this way, possible stresses can be identified preventively and important information can be obtained about safety aspects in relation to the environment, processes and products.

The creation of a risk-based sampling plan for fruit and vegetable products should include the following aspects:

- Target of the planned microbiological tests
- Determination of the relevant test parameters ("target organism")
- Determination of the respective sampling procedure
  - Product
  - Surface
  - Human
- Place/location and time of sampling in the value chain
  - Crop management
  - Harvesting processes
  - Preparation (workplaces, surfaces)
  - Packing processes
  - Infrastructure
- Growing area and environment
  - Manure application
  - Urban areas
  - Topography
  - Surrounding (usable) areas

4.3 External factors influencing product and environment samples

Microbiological examinations should show to how much the production environment and the products themselves are contaminated with microorganisms. It should be ensured that the products comply with the hygiene requirements for foods. When consumed, fruit and vegetable products can be direct vehicles for the transmission of germs. For example, listeria, salmonella, or E. coli can get on products during the growing, harvesting, processing process and the following process steps. Fruits and vegetables are often consumed unwashed and unprocessed and therefore represent an increased risk. The contamination of the products can be attributed to a variety of external factors. Product and environmental sampling in fields, greenhouses, or packing companies provides an opportunity to monitor contamination risks. It is important to include not only the product but also used resources (including irrigation water, fertilizers) in the sampling plan.

In the following, it will be explained which possible influencing factors exist at the respective process stages and how they can be monitored.

4.3.1 Influencing factor field and cultivation: agricultural process water

Enterobacteria (Salmonella, *Campylobacter*, VTEC, and noroviruses) take a predominant position in this context. Water sources and piping systems (e.g., wells, sprinklers, drippers) are relevant, especially in relation to the positional behaviour of the particular crop (near-ground growth such as lettuces, soft fruits, outdoor vegetables). Irrigation of crops with water that is potentially faecally contaminated and that comes into direct contact with the edible parts of the products is a risk of contamination. Possible entry routes can be, on the one hand, domestic and wild animals (also bird excrements) in freely accessible areas, on the other hand, non-maintained well installations and pipe systems or missing covers of reservoirs.

Sampling of process water directly at the water supply sources (wells, drippers) makes sense. If the results of the microbiological analyses of the process water are not adequate, specific corrective measures should be established.

4.3.2 Influencing factor field and cultivation: fertilizers

Applied manure, compost or sewage sludge can be a source of contamination for products. Salmonella, VTEC and also noroviruses can get on the products on this way. Especially heavy rainfall on the cultivated areas increases the risk of contamination by sprayed soil and fertilizers. The risk is also in-creased if waiting periods between manure application and harvest are not observed (e.g. 60 days for leaf vegetables). In order to assess the operational risk in this regard, sampling of source materials can be carried out. If, for example, *E. coli* can be detected in the corresponding samples, this indicates a contamination with faecal germs.

4.3.3 Influencing factor field: climate and location

Climatic contamination events (irrigation, flooding, rainfalls), especially just before harvest, can pose another contamination risk. Therefore, the transmission of human pathogenic germs can occur while the crop is still on the field, often during the harvest. Products with growth close to the ground are problematic. Harvest and transport containers can also come into direct contact with the soil during the harvest, as well as harvesting tools (e.g., when cleaning outer lettuce leaves).

4.3.4 Influence factor field: animal hosts

If animal production occurs next to the cropland or is located near urban areas, there is a potential source of contamination. Domestic, farm, or wild animals have appropriate access to the fields as well as to water supply sources. Naturally occurring germs, e.g. in the intestines of birds or warm-blooded mammals, can get on edible parts of the products. *E. coli* is a typical faecal indicator. Regular pre-harvest inspections are important to detect possible traces, faeces or feeding damage and to act accordingly (sampling of products).

4.3.5 Influence factor field: staff hygiene/ harvest teams

The risk of contamination, especially with faecal germs, and the transmission of human pathogenic germs to the products can be caused by poor hygiene of the harvesting staff and direct contact with the product. A particularly high level of cleanliness (including working clothes) and hand hygiene facilities must be provided. In this context, the availability of (mobile) WCs is also an important component. In order to check the success of the implemented measures, impression samples of the hands of individual staff members (palms of the hands) should be taken for microbiological examinations. Details on the different sampling methods can be found in chapter 4.4.

4.3.6 Influence factor field: hygienic conditions transport containers and harvesting equipment

During the harvest, it is important to ensure that the products are stored and transported in clean harvest and transport containers. The used harvesting tools (knives, cutting tools) should also be clean. During the work, direct contact with the ground should be avoided. For harvest and transport containers, the use of a protective pad is possible.

Unclean and damaged containers increase a possible (cross) contamination risk. Products can be damaged mechanically, which can result in possible entry points for germs. The transmission of hu-man pathogenic germs is often due to harvesting operations.

In order to monitor the efficiency of implemented cleaning and disinfection measures, impression and contact methods, e.g. in harvest containers or cutting tools, are useful. Swab or sponge samples can also be used. Details on the different sampling methods can be found in chapter 4.4.

4.3.7 Influential factor packing operation: washing and preparation processes

In the course of the packaging steps, raw produce is subjected to further processing steps as well as washing, transport and cooling processes after arrival from the field. During this process, the natural protective barrier formed by the epidermis against the development of microbes on the surface of the fruit is damaged or removed. As a result, further processing steps and the following storage can lead to increased decay and finally to an increased risk of contamination inter alia by *E. coli* or listeria. Washing processes are one of the most important causes of (cross-)contamination.

In order to check the quality of the used water regularly, frequent water analyses are recommended. Details on this topic can be found in the QS supporting document "Water Quality".

4.3.8 Influential factor packing operation: contact surfaces (machines, conveyors, devices, containers)

When raw materials are emptied after arrival at the packing company, the goods are transferred to further transport containers for storage or brought directly to the packing lines (conveyor belts, sorting tables, workstations). Residues and contaminations from possibly preceding and contaminated batches can cause cross-contamination of the batch in process. The formation of so-called biofilms also represents a special risk. In these biofilms, spoilage agents and food-associated pathogens such as Listeria, EHEC or Salmonella can multiply particularly well. The shelf life of products can be limited and consumers can get sick if they consume contaminated food.

To check the success of cleaning and disinfection processes on work surfaces, machine parts or screw connections and transport containers as well as conveyor belts, smear or swab methods with dry or moistened swabs are suitable. Similarly, sponges can be used to swab various surfaces to check the samples for microbiological contaminations. Details on the different sampling methods can be found in chapter 4.4.

4.3.9 Influence factor packing operation: staff hygiene

As is the case for harvesting staff, staff hygiene also plays a central role for employees of packing companies regarding possible contamination risks. Depending on its characteristics, many products require complex processing steps to prepare the raw material (cleaning, weighing, sorting). This re-quires intensive manual handling of the products. In particular, hand hygiene of employees on packing lines is essential to limit transmission routes through infected employees.

To check the status of staff hygiene, as well as the success of cleaning and disinfection measures of working equipment, contact and smear methods can be used. For example, the insides of employees' hands can be sampled using the impression method, and knives and other work equipment can be swabbed with a sponge. Details on the different sampling methods can be found in chapter 4.4.

4.4 Sampling for microbiological examinations of surfaces and furnishings

In order to prevent the establishment of germs in the production environment and the associated (re)contamination of the products, environmental examinations are essential. Surfaces can be sampled with different objectives. These include, for example, control of cleaning and disinfection success, step control, as well as listeria monitoring.

If a disinfection of the operational facility is carried out in the company, microbiological examinations of surfaces in the preparation and processing rooms should be carried out regularly for control purposes. The sampling is carried out on the relevant food contact surfaces (e.g. equipment, installations, conveyor belts, knives, palms) and on other relevant surfaces (e.g. tables, door handles, counters, containers, boxes). These sampling points must be determined based on a risk analysis and documented in a sampling plan.

The sampling plan should ensure that all planned points in the company are sampled in a defined period of time. To check the disinfection success, samples should be taken at least monthly during the production months. In addition to these minimum requirements, the frequency of sampling is to be selected and adjusted (increased if necessary) on a risk-oriented basis:

- Size of company
- Existing facilities (places where washed products are handled)
- Microbiological sensitivity of the produced products
- Results of previous investigations

The sampling methods outlined below can be carried out independently by staff members. Conventional sampling methods include the smear or swab method on the one hand and the contact or im-pression method on the other hand. Furthermore, it is possible to use bioluminescence methods with which adenosine triphosphate (ATP) can be detected (e.g. ATP test).

4.4.1 Smear or swab method

In the **ISO 18953:2018** "Microbiology of the food chain - Horizontal methods for surface sampling" different techniques for surface sampling are described. Using the smear or swab method, surfaces are wiped either with a dry swab (single swab) or moistened and dry swabs (wet-dry swab technique) or as well with sponges or other suitable materials. Any microorganisms present in the swabs or sponges after sampling are applied to suitable nutrient media in further steps (in the laboratory) and counted after incubation (cf. e.g. **DIN 10113-1** "Determination of the surface microorganism con-tent on furnishings and commodities in the food sector"). Unlike the impression method, the smear or swab method is particularly well suited for uneven surfaces, machine parts, valves, screw fittings and so on.

Note: *Personnel must have basic microbiological knowledge in order to perform this method.*

Procedure:

- Using the wet-dry swab technique, a defined surface area is smeared with a moistened swab. This is then repeated with a dry swab. The heads of the swabs are then shaken out into a sterile thinning solution.
- Only one swab (dry or moist) is used with the single swab technique.
- With extremely germ-free surfaces or when looking for specific germs, the specimen collecting tube can be filled with a specific nutrient broth instead of thinning solution prior to incubation.
- Used with aerobic mesophilic colony counts for example.[21]

Fig. 1: Swab

Source: LfL "Options and Evaluation of Hygiene Checks in the Household"

4.4.2 Impression or contact method

When using the contact method, a suitable culture medium is usually pressed directly on the surface so that any germs on the surface adhere to the culture medium and can subsequently be counted after incubation of the culture medium. However, the surface can also be sampled, for example, with the aid of sterile films or adhesive strips, which are then placed or pressed on to a culture medium (indirect contact method), which can then be incubated and evaluated.

Note: *As with the swab or smear method, personnel must also have basic microbiological knowledge to use the impression or contact method.*

For the direct impression methods, two different systems are widely used:

- Replicate Organism Detection and Counting (RODAC–plates) (Figure 2):
  - The RODAC plate consists of a transparent culture medium carrier of plastics material onto which a culture medium with a convex arch is attached.
  - The culture medium can be closed with a lid for transport and storage.
- culture medium-coated contact carriers, called "contact slides" or "dip-slides" (Figure 3):
  - They consist of a flexible plastic carrier coated with a culture medium. The culture medium is wrapped in sterile packaging, usually in the form of a tube, which acts as a reclosable transport container and incubation chamber after contact has been made with the surface.
  - The germ carrier is flexibly connected to the lid of the sample vessel and the carriers them-selves have a certain degree of flexibility. Impression and contact methods are well suited for level, smooth surfaces.[20]


Fig. 2: RODAC plate
Source: LfL "Options and Evaluation of Hygiene Checks in the Household"


Fig. 3: Contact Slides
© International PBI S.p.A.

Procedure – Obtaining a Contact Sample

Note: *The instructions for use must always be observed with ready-to-use products!*

The contact sample is prepared by first removing the protective packaging and marking the adhesive labels. The contact plate should then be opened without touching the surface and pressed against the surface to be tested for 10 seconds with a pressure of approx. 500g. The hand should be kept as steady as possible here (no wiping or twisting movements). When taking a contact sample of a hand, three fingertips (inside of the hand) are pressed onto a contact plate as described above. The sample container should be reclosed immediately after the sample has been taken and put back into the original protective packaging. RODAC plates should be sealed securely with adhesive tape prior to transport. Any culture medium residues should be thoroughly removed from the sample point after sampling.[22]

Advantages of contact samples :

- Motivation of staff towards more self-responsibility
- Promotion of interest in hygiene within the company
- Increase of the hygiene standard

Microorganisms that can be detected with contact tests:

- Bacteria (e.g. Enterobacteria, Coagulase-positive Staphylococci)
- Yeasts and mould fungi

Incubation of impression samples

As bacteria usually require higher temperatures for growth and propagation, samples are incubated in incubators.[3] The reclosed plastic containers are placed in the small pre-heated incubator (25 °C for yeasts/mould, 30 °C for fungi, 37 °C for enterobacteria/Staphylococci) and kept there for 24 to 48 hours. Plates used for the proof of mould fungi have to be incubated for four days. A propagation process takes place during this time, resulting in a reflection of the germ contamination of the tested surface.[23]

The culture medium must be disposed properly after incubation as otherwise the germs can be spread, grow or propagate on the premises or infect personnel.

The following precautionary measures have to be observed:

- Do not place the incubator in rooms in which foods are processed or stored.
- Use each test once only.
- Disposal of the incubated culture medium according to the manufacturers' specifications or rather according to legal regulations.
- Store the tests protected against daylight and draughts and keep them at room temperature.
- Do not open used tests.
- If contact is made with bacteria colonies, disinfect the affected areas of the skin and/or other are-as (floors, objects)[4]

4.4.3 Bioluminescence methods

ATP is detected with the help of bioluminescence methods. ATP is the abbreviation for adenosine triphosphate, a metabolic molecule that is present in all cells of lower and higher organisms and therefore also in cells of plants and animals as well as in microorganisms. Depending on the intensity of cleaning and disinfection, varying ATP concentrations of microorganisms and product residues that have not been completely removed remain on surfaces. In this way, the detected overall ATP serves as a contamination indicator which can be used for the control and monitoring of the hygiene level and cleaning efficiency.

Unlike traditional methods of determining the germ count, the ATP method does not detect the microorganisms themselves but the ATP, which is contained in all animals, vegetable and microbial cells as an energy store. As ATP from bacteria, yeasts and somatic (animal and/or vegetable) cells is detected simultaneously without differentiation, ATP bioluminescence is not exclusively a microbial quick test but rather a quick test for the detection of organic and metabolically active contaminants. Therefore, the results from microbiological surface examinations are not directly comparable with the results from ATP measurement.

To determine the ATP concentration, a swab is used to take a sample from the control point to be examined in accordance with **DIN 10124** "ATP measurement - Principles for determining the hygiene status by means of bioluminescence". The extraction of the ATP and subsequent reactions between the added enzyme-substrate mixture of luciferin-luciferase and ATP result in the release of light energy. The released quantity of light is recorded per luminometer as "Relative Light Units" (RLU) and is proportionate to the existing ATP quantity. The result of the test can be seen after a few minutes.

4.4.4 Special notes on listeria monitoring

Producers of ready-to-eat foods that may pose a risk to consumer health due to *Listeria monocytogenes* are required by **Regulation (EC) No. 2073/2005** to test samples from processing areas and equipment for *Listeria monocytogenes* as part of the sampling plan. Sampling and analysis must be performed by qualified persons and appropriate procedures must be used. If residual effects of disinfectants are expected, de-inhibitors must be used to neutralize disinfectant residues. The sampling frequency for routine environmental monitoring depends on the factors described previously. The sampling time depends on the question. Possible sampling times can be, for example, after/during production, after/during cleaning and disinfection, or immediately before the start of production. To achieve a representative overview, the sampling times should vary.

When sampling surfaces, the largest possible areas should be sampled when testing for Listeria. The use of sponges with which areas of several 1,000 cm² can be wiped off has proven successful. A wipe sample should

be taken in such a way that any existing biofilms are also removed. Gullies and drains should also be sampled. The use of swabs should be limited to hard-to-reach places and niches.

It is recommended that the environmental samples be divided into zones depending on the risk of contamination to the food posed by the particular spot. At least a subdivision into "product-contacting" and "non-product-contacting surfaces" should be made. The subdivision into further zones may also be useful. Enclosed is an example in which 4 zones are differentiated:

- Zone 1
  - Food contact surfaces such as table surfaces, belts, knives, inside of tubes and pipes, conveyor belts, boxes, paloxes, gloves, packaging material
- Zone 2
  - Non-food contact surfaces in direct proximity to food and food contact surfaces such as equipment/machine surfaces, operator terminals, transport carts if applicable walls, floors, drains
- Zone 3
  - More distant non-food contact surfaces located within or near production areas where food is handled and may result the contamination of Zone 1 or Zone 2, e.g., forklifts, lift trucks, transport carts, walls, floors, drains, hygiene sluices, boot/shoe soles
- Zone 4
  - Non-food contact areas that are outside of production areas where food is handled and where listeria could be imported from the environment, such as locker room areas, cafeterias, box storage areas

4.4.5 Evaluation scheme for microbiological examinations of surfaces in the production environment and of equipment

There are currently no legally prescribed limit values available for evaluating the microbiological status of surfaces in the production environment and of equipment. It is therefore the responsibility of the food business operator to define company-specific limit values. Orientation for the determination of limit values is provided by **DIN 10516** as well as the various QS guidelines, annexes and supporting documents. From this, for example, the evaluation scheme shown in Table 3 can be derived.

Tabelle 3: Evaluation schemes microbiological examination of surfaces in the production environment and of equipment (based on DIN 10516, QS guidelines for the butchery[17] and the supporting document Listeria prevention for slaughtering, deboning and processing[20])

Area and time of sampling	Germ type	Limit value
Surfaces with food contact immediately after cleaning and disinfection	Aerobic mesophilic germ count	< 100 CFU/100 cm^2
	Enterobacteriaceae	0 CFU/100 cm^2
	Listeria monocytogenes	Not detectable (area if possible > 1000 cm^2)
Surfaces with food contact immediately before production	Aerobic mesophilic germ count	≤ 10 CFU/cm^2
	Enterobacteriaceae	≤ 1 CFU/cm^2
	Listeria monocytogenes	Not detectable (area if possible > 1000 cm^2)

In case of not acceptable results, the following measures are primarily relevant:

- Review of the cleaning and disinfection measures
- If necessary, change the type of cleaning or the cleaning agent/disinfectant
- Training of the staff
- Repetition of cleaning and disinfection
- Renewed Test after repeated cleaning[21]
- For listeria: Exclusion of a possible influence on food safety, market-related measures if necessary

5 Abbreviations and terms

- ATP = adenosine triphosphate
- aw value = water activity
- B. cereus = Bacillus cereus
- CA = Controlled atmosphere
- CFU = Colony-forming unit
- Coagulase (lat. coagulare = curdle) is an enzyme which causes the blood to congeal. Some bacteria produce this enzyme. These are then described as coagulase-positive bacteria.
- EFSA = European Food Safety Authority
- EHEC = enterohaemorrhagic Escherichia coli
- HACCP = Hazard Analysis Critical Control Point
- L. monocytogenes = Listeria moncytogenes
- RASFF = Rapid Alert System for Food and Feed
- RODAC = Replicate Organism Detection and Counting
- S. aureus = Staphylococcus aureus
- SAgs = superantigene
- SE = staphylococcal enterotoxins
- STEC = shigatoxigenic Escherichia coli
- UHT = ultra high temperature
- VTEC = verotoxigenic Escherichia coli

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Explanation

Microbiology and sampling during the preparation/processing of fruit, vegetables, potatoes

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