AIDIAN

A Guide to Monitoring Surface Hygiene

A guide for kitchens, supermarkets, food industry, food education, and health inspectors.

1 History and theory

1.1 | History of hygiene sampling

Rapid and reliable methods of hygiene sampling have been actively sought for decades. Probably the first microbiological hygiene samples were taken by pouring molten agar on the surface of interest, after which the solidified agar was detached from the surface in a sterile way and transferred for incubation. In the 1960s, ten Cate developed an "agar-sausage" method for making contact agars. He poured molten agar into a sausage-like tube, which he cut into slices after solidification. The agar slices were pressed against the surface to be investigated. Although the ten Cate´s method never came into wide-spread use, his scale for quantifying microbial growth by the number of colonies on a 9 cm^2 agar surface was widely adopted. The ten Cate assessment scale was so popular that its colony counts were taken up as such for use with 25 cm^2 contact plates.

Dipslide tests were developed for easy on-site testing for microbial growth. A dipslide is a plastic paddle covered on both sides with agar. Aidian's first dipslide, Uricult test for Urinary tract infection detection, has been on the market since 1968. Over the years, more products have been developed for numerous different applications. Easicult tests are designed for liquid contamination monitoring and Hygicult tests for surface hygiene monitoring. Using dipslides does not require any microbiological expertise, advance preparation or equipment, and the slides can easily be used and cultivated on-site. The test paddle within the tube can also be used as convenient transport media if further analysis of the samples is needed.

1.2 | Direct and indirect methods

Surface samples can be taken by various methods. The two main methodological categories are:

Direct culture methods such as

- Swab, sponge, or cloth methods using poured plates
- Contact plate methods using self-prepared or commercial plates
- Contact methods using Dipslides (e.g. Hygicult)
  - Sampling by dipping dipslides directly into liquid (Easicult and Hygicult)
  - Sampling using swab and a dipslide (Hygicult)

Indirect methods such as

- ATP (adenosine triphosphate) bioluminescence
- Methods for determining protein residues (e.g. Clean Card PRO)

Swab and contact methods such as Hygicult are commercial contact methods. ATP and Clean Card PRO protein tests are indirect methods which do not disclose actual bacterial counts but measure some indirect characteristics related to the cleaning status, which acts as an early warning for organic matter that is a food source for microorganisms.

1.3 | Comparison of various methods

The number of bacteria detected by swabbing or contact methods correlates with the true contamination level of the surface. However, the proportion of total bacteria released into the sampling medium varies widely (1-50%) and by contacts method it varies 1-20%, mainly depending on the surface material. The biofilm formed by bacteria may be broken by swabbing, thus revealing the bacteria in it. Conversely, the contact method may under certain circumstances reveal more bacteria than the swabbing method. According to an extensive collaborative study[1], Hygicult gives the same result as the conventional contact plate or the swabbing method.

The contact method is best suited to flat surfaces, whereas small tools, rounded forms or uneven surfaces are best examined by the swabbing method, or easy combined use of Hygicult and swab. The reliability of the traditional swabbing method is crucially

dependent on the skill of the sampling person. The swabbing should be applied at a pressure of 0.1kg/cm^2. The use of alginate or carbon swabs may decrease the sampling error. Although both swabbing and contact methods only reveal a proportion of the microbes present on a surface, the microbes detected are likely to be the ones that would contaminate products which come into contact with the surface.

1.4 | Significance of surface hygiene samples

The importance of surface hygiene samples has been emphasized both in the food industry and retail shops. Apart from the food chain, surface hygiene sampling has been increasingly recognised as an indicator of health hazards in premises such as health care establishments, saunas and gyms.

Much of the increase in hygiene sampling has been brought about by the introduction of the Hazard Analysis – Critical Control Points (HACCP) system which involves monitoring the poten-tial risk areas (CCP's), instead of examining the final products and sporadic quality control. The HACCP system is commonly used in the food industry worldwide and it has led to improvements in the product shelf lives.

Validation of the cleaning with surface and other hygiene sampling is an integral part of monitoring the production process. This is often described as mandatory in the Food regulation and is crucial during food manufacturing where the slightest bacterial contamination may deteriorate the quality of the final product with loss of customer satisfaction, potential recalls and media coverage as a result.

1.5 | Target surfaces and microbial groups

Hygiene samples are usually taken from cleaned surfaces at the start of the working day. Sometimes the sampling has to take place on the preceding day, immediately after cleaning. In process control, however, attention is primarily paid to the contamination level of the equipment and surfaces just before work and production begins. The adhesion of microbes onto different surface materials varies significantly. First the adhesion is reversible, after attachment it can become irreversible. The smoother the surface is, the less adhesion there is. There is less adhesion onto a glass surface than onto rubber or steel. Controversial results have been published on bacterial adhesion onto plastic (polyamide, polyvinyl chloride, polypropylene), aluminium and steel. Stainless steel appears to be the material, which is most easily cleaned. Irreversible adhesion is generally due to the formation of biofilm. The formation of the polysaccharide-based biofilm requires the existence of a physical or chemical gradient, such as temperature, pH or hydrophobicity versus hydrophilicity between the surface and the bacteria. The gradient may be based on the temperature difference between the surface and the bacteria, on acidity or hydrophobicity or hydrophilicity. The bacteria multiply within the biofilm, thus increasing the mass of the biofilm and making cleaning of the surface difficult.

The longer surfaces contain organic matter, the more bacteria will accumulate, and the more difficult cleaning will be later on. Washing and cleaning must always be started with removal of visible dirt and residues before the use of cleaning agents or disinfectants.

In industry, hygiene samples are often taken from "easy" sites, such as flat surfaces, where cleaning is easy and is part of the cleaning routine. From the hygiene and contamination point of view, the critical point can be totally outside the cleaning program. Similar overlooked sites may also exist in retail outlets.

The level of hygiene of cleaning equipment is also very important. Pathogenic bacteria may be detected on cleaning equipment. The cleaning detergents should be appropriate, and properly diluted, for the surface in question.

Microoganisms

A common protocol is to determine the total bacterial count, and in some cases, also the coliform count from surface hygiene samples.

Other possible target organisms are

- Total count: an indicator of the general hygienic status and often direct comparable with the shelf life on the product; a low Total count gives long/full shelf life, high Total count gives short shelf life.
- Listeria: in the production of RTE (ready-to-eat) products and in fish and dairy industry, Listeria is typically considered an indicator of poor hygiene, found in drains and places where there is water reservoirs
- *Escherichia coli*: indicator of relatively fresh faecal contamination
- *Staphylococcus aureus*: indicator of hand hygiene
- Enterococci: grouping and origin often unclear
- Yersinia: found in similar places as Listeria, floors
- Salmonella: air conditioning filters, sewage basins, floor drains
- Campylobacter: milk, meat, poultry, seafood, fruits, vegetables
- Yeast and molds: typically a problem that arises from ventilation systems

Specific bacterial strains, moulds and yeasts may be typical for certain industries and products.

1.6 | Visual inspection

Visual hygiene control refers to monitoring general cleanliness. It can also involve observations on staff hygiene, contamination risks, cleaning techniques, temperatures and even the educational level of personnel. The conclusions from visual inspections do not always concur with microbial findings. This may be explained by the fact that visual inspection sometimes fails to target the most relevant items. On the other hand, it is not relevant to take a microbial sample on a visible dirty (food residues) surface, since it will contain microorganisms.

Regarding different sampling methods, contact samples appear to correlate best with visual inspection. It is explained by that the contact method detects more visible dirt, whereas the mechanical action of swabbing may yield more organisms, depending on the surface material.

2 Microbial burden, biofilms, and process design

Biofilm – Microbes' defence against cleaning

Biofilm is one of the ways in which microbes protect themselves against antibacterial agents. Most microbes are capable of adhering onto various surface materials, both organic and inorganic. Indeed, microbes exist attached to surfaces in numerous ecosystems. They require minimal amounts of liquid and nutrients to form microbial layers known as biofilms. The tendency to form biofilms can be considered microbes' general survival strategy by which they optimise the utilisation of available nutrients. The best and oldest examples of biofilm are found on stones on the bottom of seas and the hulls of ships. With the development of technology, biofilm has created problems in process equipment and pipe systems in the form of contamination risks or energy losses.

2.1 | Biofilms in industrial systems

The formation of biofilm begins when a microbial cell adheres onto a surface. Although adhesion does not necessarily lead to biofilm formation, it is a prerequisite for the process. Adhesion is often preceded by accumulation of organic dirt on a surface, which in turn favours adhesion. Biofilm is a stress phenomenon and one of microbes' means for tolerating antibacterial factors. In industrial equipment and circulation systems, biofilm protects microbes against cleaning and disinfecting agents. Besides causing problems in cleaning and hygiene, biofilm can cause energy losses and blockages in condenser tubes, cooling fill materials, water and wastewater circuits and heat exchangers. Microbial corrosion is also observed in processing equipment and it leads to huge losses in different industrial areas, e.g. piping and cooling water systems.

2.1.1 | Water systems

The large surface areas and availability of nutrients activate the formation of biofilms in industrial water systems. In drinking water systems, the presence of biofilm can cause lowering of water quality. Problems with *Legionella pneumophila*, a dangerous organism that is able to form biofilm, can occur in hot and temperate water systems. Mechanical rinsing and shock treatment with e.g. chlorine-based agents or ozone should be performed when biofilms have been observed. In cooling water systems, the most severe problem is loss in the heat exchange rate (even down to 10% of the optimum rate). The primary colonisation of bacteria and moulds in cooling water systems is often followed by accumulation of algae and clams. Examples of adverse effects in water systems are increased prevalence of pathogens, corrosion caused by microbes, increased resistance to water flow and local blockage of filters. Regular mechanical and chemical cleaning and treatment with biocides can control the level of microbes in cooling systems.

2.1.2 | Process industry

Biofilm-derived problems are most evident in the food and animal feed industries, where organic material is handled. Because any biofilm mostly consists of water, the volumes of biofilms on dry surfaces are only a fraction of those in liquid. The nature of surface material is an essential determinant of biofilm formation. The formation of biofilm can be prevented by polishing or shining the surface electrically. Gaskets easily collect dirt and nutrients which can lead to formation of biofilms. The condition of gaskets should therefore be inspected regularly. Valves should be designed according to hygiene requirements. Poorly designed sampling valves can create problems by spoiling the process and distorting the sampling data. Cleanliness of food industry equipment, handling surfaces and process machinery play a major role in the quality of the products. If cleaning is ineffective, the equipment forms a major contamination source. Significant microbes to biofilm formation in the process industry include *Bacillus* spp., *Leuconostoc* spp., coliforms, enterobacteria, pseudomonas, listeria, yeasts and moulds. Contamination of line lubricants is a frequent problem in the food industry, dairies, and breweries. Water-containing lubricants are particularly susceptible to microbial contamination, and the biofilm surrounding such microbes makes them both resistant to cleaning agents and a potential source of contamination.

2.1.3 | Hygienic design

A hygienic design of process equipment has a tremendous impact on diminishing the risks of contamination of foods during production and hence on the products' shelf life. If the process equipment is of poor hygienic design, it is difficult to clean it from

microbes. Poor hygienic design of process equipment and components used in the food processing industry is a risk for food contamination. With a good hygienic design, the lifetime of the equipment will increase, and the maintenance and manufacturing costs will be reduced. The European Hygienic Engineering and Design Group (EHEDG) develops practical guidance for hygienic design benchmarking and risk assessment. The choice of materials and their surface treatments, e.g. grinding and polishing, are important factors in inhibiting the formation of biofilm and in promoting the cleanability of surface. The process equipment is easy to clean if the surface materials are smooth and in good condition. Dead ends, corners, cracks, crevices, gaskets, valves and joints are vulnerable points for biofilm accumulation. Biofilm is easily formed on measuring probes and the inner parts of equipment because these are usually difficult to clean. The porous matrix on the surface, which diminishes the effect of sanitation and sterilisation procedures, hampers the penetration of disinfectants and heat.

2.1.4 | Air conditioning systems

The quality of air in food production facilities is very important for the final product quality. The microbial population in the air channels depends on the environment, filtration membranes and the sites of air holes. Formation of biofilm in air conditioning systems does not occur without a water reservoir of some kind. Normally, there is no water in the air conditioning systems, but it can accumulate unintentionally through condensation. The harmful Legionella pneumophila has been isolated from water systems that were connected to an air conditioner. When the air conditioning system is cleaned and disinfected, it is very important that the disinfection medium penetrates the biofilm and does not simply flow through the system with the air. The membranes in the air conditioning system and the walls in the air conditioning channels are locations where biofilms start to grow. Biofilm growth can be prevented in ducts by using effective filters and appropriate maintenance procedures.

2.1.5 | Biofilm problems in food industry

The hygiene status of surfaces, instruments and equipment in the food industry essentially affect the quality of the processed products. Biofilms have been found in the food processing lines which produce canned products, meat and poultry products, pastries, biscuits, pizza, fish cakes, cheese, milk products, beer, spices, vegetable, and salad products. Problems are found in various sites: air handling systems, blancher extractors, conveyors, cooling systems, floors, drains, food contact surfaces of stainless steel; gaskets, heat exchangers, manufacture line for paper-based packaging material, milk transfer lines, mixers, poultry processing equipment, rubber fingered pluckers, slicers, brine injectors, packaging machines, pasteurizers, ultrafiltration and reverse osmosis membranes and vegetable lines. It can be seen from the list that problems originating from biofilm can occur anywhere in the food process if the design or maintenance is improper. The occurrence of slime-forming microbes is a major problem in the sanitation and disinfection of process equipment. Common contaminants on surfaces contacting food are enterobacteria, lactic acid bacteria, micrococci, streptococci and *Pseudomonas fragi*. Once a biofilm is formed it can be a source of contamination for foods passing through the same processing line. For example, *L. monocytogenes* is difficult to remove from the factory environment once it has become a part of the house microbiota. In food processing environments microbes can attach to stainless steel with various surface finishes and increase in number relatively rapidly. This development can be limited by regular application of cleaning and disinfection procedures.

Production of paper and cardboard packaging material for food products must be controlled similarly as the production of the food. Paper machines offer microbes a favourable environment, and thus their numbers may be quite high. Microbial contamination of paper machine environments may result in significant problems in the end-product safety because of spoilage of raw materials, slime build-up or contamination of the end-product by microbes or microbial metabolites.

2.2 | Elimination of biofilms

2.2.1 | Detergents and disinfectants

The biofilm development can be quantified in terms of microbial biomass and the amount of exopolysaccharides present. When eliminating biofilms, two important targets need to be met: a microbicidal effect and loosening of the biofilm or breaking up of its polysaccharide layers. The cleaning result depends on the chemical composition of the washing and disinfecting agent, the mechanical effects of the cleaning, temperature, and time. Typically, microbicides alone are ineffective against microbes protected by layers of biofilm because they often fail to penetrate the biofilm. Only after the biofilm has been broken and the cells exposed, they become effective.

2.2.2 | Summary of methods for preventing biofilm formation

Preventive measures available to the food and process industries comprise four approach sectors:

- Choice of construction materials and surface treatment
- Process management
- Good design practice
- Staff training

3 Methodological options for detection of contamination

3.1 | Methods

Surface samples are taken either by directly inoculating a solid culture medium or liquid reagent. Inoculation can be done by the contact method or by transferring the sample using a swab, sponge, or cloth onto the medium or into a reagent tube. For reproducible and comparable results, various performance-tested media or reagent-equipment combinations can be used for surface monitoring. With solid media, microbes are visualised as colonies after incubation at room temperature or in an incubator. When reagent-equipment combinations are used, the presence of microbes is detected chemically. The following methods apply to surface monitoring:

- Contact plates
- Hygicult and Easicult

→ Monitoring of microbial contamination

- ATP-bioluminescence
- Protein tests (such as Clean Card PRO)
- NAD/NADH tests

→ Monitoring of impurities (such as food residues)

In addition, Molecular biology: MALDI-TOF MS, microarray, PCR, qPCR and multiplex PCR enable bacterial identification, but each requires different sample preparation, has different cost of use, run time, reagents, preparation conditions, and results analysis. These methods tend to be more labour intensive and costly but provide accurate identification.

3.2 | Sampling

Hygiene samples are normally taken in the morning before start of work, when the premises are clean. For comparable results, it is important to regularly monitor the hygiene status of same sites. If poor hygiene is suspected, random samples may also be taken outside the monitoring program. This can make it easier to pinpoint the contamination source.

3.2.1 | Contact plates for total bacterial count

Contact plates are prepared by pouring the medium onto a 5.5 cm diameter polystyrene plates so that the surface of the medium rises clearly above the edge of the plate. For sampling, the lid of the plate is removed and the plate is pressed firmly against the surface under inspection for about three seconds after which the lid is replaced. The plate is incubated at 35–37°C for 24 to 48 h or at room temperature for three to five days. After incubation the colonies are counted. To facilitate expression of the colony count per cm^2, the bottom of the plate is divided into 1 cm^2 squares. The surface area of the contact plate is generally 25 cm^2.

3.2.2 | Hygicult

Hygicult tests are reliable, economic and timesaving on-site surface hygiene monitoring tests. The validated Hygicult contact slide is a hinged plastic paddle covered with culture medium on both sides. The hinge facilitates surface sampling. The plastic paddle is fastened to a cap, which makes it is possible to tightly close the clear tube covering the paddle.

The Hygicult test is ready for sampling as such. The paddle can be inoculated with sample by the contact method, with a swab, or it can be dipped into a liquid sample. After sampling, the paddle is placed back in its tube where the sample can be cultured or safely transported if needed. The surface area of one side of the paddle is about 9.4 cm^2. To sample a larger area, the two sides of the paddle are pressed against adjacent sites or against random points on the surface. During sampling, inadvertent touching of the culture medium should be avoided. Results are easily interpreted by comparing the density of the growth to a model chart. No counting of colonies is needed.

Hygicult is available in five versions. Hygicult TPC is intended for general hygiene monitoring as most common bacteria, yeasts and moulds can grow on it. Hygicult E and Hygicult E/ß-GUR are intended for the detection of Enterobacteriaceae, a large group of primarily intestinal bacteria. Additionally, Hygicult E/ß-GUR differentiates ß-glucuronidase-positive organisms, especially *Escherichia coli*. Hygicult CF supports the growth of coliform bacteria while the growth of gram-positive organisms is inhibited. Hygicult Y&F (Easicult M) is intended for the detection of yeasts and moulds; no bacteria grow on this slide.

Hygicult TPC is intended for monitoring general hygiene status. The agar contains neutralising agents which remove any inhibitory effect of cleaning agent residues on microbial growth. It is recommended that Hygicult TPC is incubated at room temperature, or at temperature below 35-37°C since many yeasts and fungi do not tolerate high temperatures. Hygicult Y&F (Easicult M) test is incubated at room temperature, or at 27-30°C. Hygicult CF, Hygicult E and Hygicult E/ß-Gur are incubated at 35-37°C.

The Hygicult production is certified according to ISO 13485:2016. Hygicult products are widely used in different industries. The Hygicult TPC and Hygicult E dipslides have been validated in a collaborative study in twelve laboratories. Hygicult TPC validation report has been approved by NMKL/Nordval. The NordVal International Certificate states that the results obtained in the study published in Journal of AOAC International have been evaluated and there was no statistical difference in the performances between the Hygicult® TPC and the Trypticase soy agar (TSA) culture plates and contact plates.

3.2.3 | Easicult

Easicult tests are reliable and easy-to-use culture tests intended for detecting microbial contaminations in the industrial fluids, such as in cutting fluids, cooling waters, fuel and oil tanks or fluids in paper and pulp industry. The tests are also useful in the cosme-tics industry, as well as in the paint and varnish industries. Easicult can be used almost anywhere where microbial contaminations can cause problems. The Easicult test is a plastic paddle covered with culture medium on both sides, and it is ready for sampling as such. Sampling is performed by dipping the slide into the liquid. Spraying or holding the paddle under fluid stream is also possible. Easicult tests are available in three versions.

Easicult Combi is a two-medium dipslide. TTC agar allows almost all aerobic bacteria to grow, while Rose Bengal agar supports the growth of yeasts and moulds. Easicult Combi is intended for simultaneous estimation of total bacterial counts, yeasts and moulds. The test is incubated at room temperature, or at 27–30°C.

Hygicult Y&F (Easicult M) contains malt agar where the growth of bacteria is inhibited. The medium supports the growth of yeasts and moulds. Hygicult Y&F (Easicult M) is intended for monitoring fungal contaminations. The test is incubated at room temperature, or at 27–30°C.

Easicult TTC contains TTC agar that allows almost all aerobic bacteria to grow. It is intended for estimation of total bacterial counts. The test is incubated at room temperature, or at 27–37°C.

Results are easily interpreted by comparing the density of the growth to a model chart. No counting of colonies is needed. The Easicult production is certified according to ISO 13485:2016.

3.2.4 | Luminescence

The luminometric method is especially suitable for analysing surface hygiene samples. Luminometry is measurement of light. In the case of surface sampling, measurement of light is used to quantify the amount of biological energy in a sample. The most important energy store in all living cells is adenosine triphosphate, or ATP. The energy contained in the phosphate bonds of ATP can be converted to light in the following chemical reaction:

$$\text{ATP + luciferin + } O_2 \xrightarrow{\text{Luciferace + } Mg^{2+}} \text{oxyluciferin + AMP + PPi + light}$$

The two substances used in the reaction, luciferin and the enzyme luciferase, are both isolated from the light-emitting firefly.The amount of light emitted in the reaction is directly proportional to the amount of ATP in the sample, and the amount of ATP, is related to the number of ATP containing cells in the sample.

Measurement of ATP

In luminometry, samples are usually taken by swabbing a limited surface area with a moistened sterile swab. The area should be clean of physical dirt since excess physical matter affects the reaction and causes inaccurate results. The swab is then inserted to a reagent tube. ATP sampling test kits including all the reagents within a sampling tube are available. The amount of light from the reaction is measured using a luminometer. The device contains a photomultiplier unit capable of measuring small amounts of light energy. The result is usually expressed in relative light units (RLU).

The luminometry data also account for the presence of organic foreign matter which may constitute a nourishment source for microbes on a post-contaminated surface. Indeed, the expression “total hygiene monitoring” is often used in connection with luminometry.

Avoidance of ATP contamination is an important consideration in luminometry. As the ATP content in human cells is high, exfoliated skin cells alter the results. It is therefore necessary to use disposable gloves both during sample taking and handling of reagents. Sterility of other sampling equipment is also a requirement.

The introduction of luminometry requires sufficient training. The user has to follow the manufacturer’s instructions for the calibration the luminometer. Some manufacturers suggest values for clean and contaminated samples.

Luminometry and health inspection

Luminometry is suitable for internal hygiene monitoring in food production plants as part of an in-house control program. Because the results are available almost immediately, action to correct wrong working methods can be undertaken at once. Use of the method may be limited by price.

Summary

Advantages

+ Rapid results
+ Detects organic impurities in addition to microbes
+ Results most often expressed as numerical values

Disadvantages

– Requires investment in the luminometer instrument
– Requires some training to perform
– Instrument needs calibration
– Users may not understand the meaning of the results
– The result indicates the level of cleaning, not sterility (dirt increases the result)

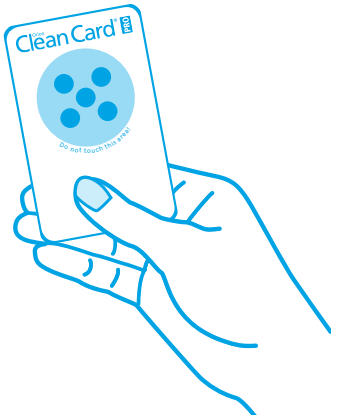
3.2.5 | Protein tests and Clean Card PRO

Protein tests are used to assess proteinaceous impurities on various types of cleaned surfaces. The test can be used to complement the in-house control of cleaning of food production premises, where proteinaceous dirt is an excellent growth medium for microbes.

Clean Card PRO is a good way to monitor cleaning and surface hygiene e.g. in the food industry and in hospital environments. Each Clean Card PRO device contains a reagent pad which is impregnated with reagents. The Clean Card PRO device is wiped on a moistened surface, resulting in a visually readable colour change if proteins are present. The result of the test is given immediately, so a decision on re-cleaning can be taken before foodstuffs are handled on the surface or equipment.

Advantages

+ Easy to use
+ Response (e.g. color change) in a few seconds/minutes
+ Easy to interpret (clean or dirty)
+ Long shelf-life of test strips
+ No instrument or special training required



Disadvantages

– Unspecific, measures protein impurities not the bioburden
– Result is not numeric
– Water must be clean (drinking quality)

3.3. | Sampling sites

Surfaces that come into direct contact with foodstuffs must be hygienically adequate. Samples are taken using the contact method or, in the case of difficult-to-reach sites, using the swab method. The sampling sites may comprise direct contact surfaces, machinery, and equipment. For example, potential sampling sites in the meat industry include:

Cutting boards, worktops, transportation hooks, bandsaws, conveyors, knives, basin carriages, scalding machinery, crust machinery, measuring drums, meat mincers mills, cubing equipment mills, chop cutters, knife-sharpening stones, plastic boxes, packaging equipment and packaging materials.

Surfaces touched manually, e.g. doors, door handles, packaging supplies and equipment, scale keyboards, packaging materials and aprons.

Surfaces in storage rooms and warehouses, e.g. carcass and cold storage rooms, saltеries, chopped meat departments, injection departments, cuttering premises, storage premises for uncooked products and unpacked cooked products.

The contact method is best suited for flat surfaces. On uneven surfaces, the sampling agar may not fully contact the sampling point, resulting in an unrepresentative sample. The method is simple and does not require any special training. Although the contact method does not meet the swabbing method in accuracy on an uneven surface, the results still adequately reflect the hygiene level of the sampled surface.

Swabbing together with a chablon is applicable to almost any surface although it may sometimes be difficult to obtain representative and reproducible samples. The reproducibility of results is very much dependent on the skill of the sampling person. Successful use of the method requires practice and skill. After sampling, the swab has to be placed in the test tube without unwanted contamination.

The method requires laboratory facilities for culturing the samples on Petri dishes. Compared with the contact method, the swabbing method is more time consuming, more expensive and requires more experienced sampling personnel. On the other hand, with favourable surface materials the results may be more precise than those of the contact method.

Combined use of Hygicult slides with the swabbing method allows sampling in various places. Here, the site is swabbed, and the bacterial mass collected on the swab is spread on the Hygicult paddle surfaces. If the tested area is dry, the swab can be moistened with sterile water before taking the sample. It is important to use the same sampling method in order to monitor the possible change in the results.

Examples of the suitability of different methods

Sampling target	Contact method				Swabbing		Luminescence
	Contact plate	Hygicult	Petri film	Protein tests	Petri dish	Hygicult	
Worktop	+	+	+	+/–	+	+	+
Cutter	–	+/–	–	+/-	+	+	+/-
Meat mill	-	+/-	-	+/-	+	+	+/-
Dishes	+	+	+	+	+	+	+
Display cabinet	+	+	+	+	+	+	+
Door	+	+	+	+/-	+	+	+/-
Handle	+	+	+	–	+	+	+/–
Water tap	+	+	+	-	+	+	+/-
Hands	+/-	+	+/-	-	+	+	+/-

Scale: + suitable – unsuitable; +/– suitable for certain applications

3.4. | Incubation (microbial culture)

It should be acknowledged, that incubation temperature (and lenght) is dependent on the type of microbes present in the specific production facility or in the product. For example, microbes in fish production may be derived from the sea, therefore they may grow in lower temperatures than microbes originating from animals in meat factories.

Monitoring the overall level of hygiene using plate count media involves incubation of the cultures at room temperature for approximately three days. The presence of aerobic bacteria, yeasts, and moulds can be detected, depending on the culture media.

The cultures are inspected daily since heavily contaminated samples can be detected already after one day's incubation. Incubation at room temperature concerns samples taken from room temperature or chilled premises. Samples taken from room temperature may also be incubated at 35°C, in which case the results can be read after 24–48 h. Coliforms and Enterobacteriaceae should be incubated at 35°C for 24 h. The detection of yeasts and moulds requires incubation at room temperature since many are unable to grow at 35°C.

3.5 | Sampling frequency

Surface hygiene monitoring should:

- be integrated in the self-monitoring of operations
- support the actual operations
- serve to ensure the sufficiently high quality of operations

The frequency of hygiene monitoring is determined by the nature and extensiveness of operations and by the requirements set by regulatory authorities. The number of samples has to be sufficient to prevent sporadic factors from distorting the results. Surface hygiene samples are also needed to verify the findings of visual assessment. Hygiene sampling is thus used as a control of visual inspection. Visually clean surfaces need to be checked if they really are as clean as they appear. Visually dirty surfaces should not be tested, they should be recleaned and then tested.

The following table presents examples of sampling frequencies in various activities:

Sampling site	Number of samples	Sampling frequency per year
Small retail shop	4–6	2
Large retail shop		
meat counter	4–6	4
worktop	4–6	4
utensils 3–6 pcs	6–10	4
meat mincers	4–6	6
Small grill restaurant	6–10	2
Large grill restaurant	6–10	4
Institutional kitchen	10–15	4
Catering service	6–10	3
Meat industry	8–16	52
Fish industry	8–16	52
Dairy industry	8–16	52

Examples of sampling frequencies in various activities

If the results indicate a poor hygiene, corrective actions should be taken. After this, sampling and corrective actions are repeated until the cause of the problem has been found and an acceptable level of hygiene has been reached.

An experienced sampling person will also make notes about structural matters such as the visual cleanliness and condition of surface materials.

3.6 | Hygienic premises

In food handling it is important to monitor the hygiene of the premises since worktops and utensils may become dirty and hands can be contaminated from dirty taps or handles. There should be a continual effort to reduce hygiene hazards although a zero contamination level may be impossible to reach.

Premises and surfaces should be classified according to their importance for the hygienic quality of final products. A risk analysis can help to determine the likelihood of food contamination from an unhygienic surface. Surfaces may be classified based on the hygienic importance.

Risk assesment and significance of risks, when evaluating contamination in a food shop due to unhygienic surfaces

1. Surfaces of direct contact
2. Surfaces affecting indirectly to the foodstuff
3. Other surfaces in the working site

3.7 | Disposal of cultures

Because the cultures contain microbes, they have to be disposed of without endangering the environment. The safest way is to incinerate the cultures or immerse them in disinfectant solution overnight (Hygicult and Easicult products with caps open). The disinfectant-treated cultures can then be disposed of as ordinary waste. Dispose the contents according to national and local law.

4 Practical measures

Easy and simple methods are needed in food production, processing, transportation, and other actions for the determination of hygiene levels of surfaces and products. The methods for the determination of hygiene levels vary according to the surfaces and other sites monitored. The tests should also be rapid and relatively inexpensive.

4.1 | Personal hygiene

The numbers of microbes on one's hands can be reduced significantly by washing with a mild detergent, depending on the bacterial strain and the condition of the skin. In places where the personnel is engaged in both customer service and serving food, the use of disinfecting hand rinses can prevent potential pathogens from being transmitted to customers.

4.2 | Applications

4.2.1 | Milk industry

Milk production, transportation and processing operations should undergo hygiene sampling whenever it appears justified. In addition to cases of suspected contamination of milk, sampling is routinely performed as part of hygiene monitoring, and random samples are taken in conjunction with regulatory inspections. The sampling frequency will depend on the local circumstances.

Swab samples can be taken from milk churns, dairy farm equipment, milk pipes, tanks, milk meters, bottling and packaging machinery, milk buckets, milk jugs, and drinking glasses.

Contact sampling is suitable for scales at dairies, tanks and other large containers, and flat surfaces.

4.2.2 | Meat and fish industry

Surface samples in the meat and fish industry should be taken at sites where products come into direct contact with surfaces that, if unhygienic, will probably or inevitably cause product contamination during the working day and significantly impair the microbiological quality of products. Such sites include knives, cutting boards, conveyors, trolleys and other vessels, worktops, saw blades and parts of machinery that are in direct contact to foodstuffs. In industry, the choice of sampling method is largely dictated by requirements of speed and the level of hygiene imposed by regulatory surveillance. On the other hand, the range of applicable methods is set forth in legislation.

4.2.3 | Kitchens

Surface hygiene in kitchens can be monitored by both contact and swab methods. The method is chosen based on the tested area. Nevertheless, it is usually advisable to use the contact method to analyse flat surfaces. Potential sampling targets include:

- Worktops
- Cutting boards
- Various cutters and mills
- Knives and other utensils
- Kitchen appliances
- Towels

In other words, all sites or items that are in direct contact with foods are potential targets for sampling. The principles mentioned above are also applicable to other food processing facilities.

More applications for testing can be found on Aidian's webpage: www.aidian.eu.

4.2.4 | Liquid foods

There are several guidelines for testing consumable liquids. The recommendations for testing depend on the manufacturing processes, for example whether the product is pasteurized or not. Hygicult and Easicult tests can be used to test liquid foods and beverages in the food manufacturing process. Testing is easy since the test slide can be dipped into the sample. Dilution of the liquid could be recommended depending on the sample quality, for example if the liquid is viscose. Hygicult and Easicult tests enable monitoring of the microbial burden level, which is recommended in the Hazard Analysis Critical Control Points (HACCP) process.

National guidelines may also require the testing of specific bacteria such as species of *Salmonella, Listeria*, or *Yersinia*. The testing specifications depend on whether the manufacturing processes eliminate the growth of the bacteria (heating, freezing), and also on the consumer group to which the product is targeted. For example, there are special requirements for infant foods.

4.3. | Reference values

4.3.1 | L. ten Cate scale

Description of growth	CFU/9 cm²
No growth	–
Minimal growth	< 10
Moderate growth	10–30
Abundant growth	30–100
Confluent growth	> 100

4.3.2 | Surface hygiene in kitchens

Description of hygiene level	Hygicult (CFU/10 cm²)
Good	< 20
Acceptable	20–100
Not acceptable	> 100

4.3.3 | Bakeries

Description of hygiene level	Hygicult (CFU/10 cm²)
Good	< 20
Acceptable	20–50
Not acceptable	> 50

4.3.4 | Meat processing premises

Description of hygiene level	Hygicult (CFU/10 cm²)
Good	< 18
Acceptable	18–50
Not acceptable	> 50

4.3.5 | Slaughtering

Description of hygiene level	Hygicult (CFU/10 cm²)
Good	< 18
Acceptable	18–40
Satisfactory	41–100
Not acceptable	> 100

4.3.6 | Retail premises and institutional kitchens

Description of hygiene level	Contact plate (CFU/26 cm²)	Hygicult (CFU/10 cm²)
Good	< 50	< 20
Acceptable	51–250	20–100
Not acceptable	> 250	> 50

This table is based on the 1995 Consensus Statement by Finnish Laboratory Veterinarians on the Assessment of Hygiene Samples.

It should be noted that no bacteria of enteric origin, such as *E. coli*, are acceptable on direct-contact surfaces.

4.4 | Comparison of methods

Comparison of various sampling methods for total bacteria after three days' incubation at room temperature:

Description of hygiene level	Number of colonies by method		
	Contact plate 26 cm^2	Petri film 20 cm^2	Hygicult contact 10 cm^2
Good	< 50	< 40	< 20
Acceptable	51–250	41–190	21–100
Not acceptable	> 250	> 190	> 100

Depending on the surface material, the contact method is able to detect approximatelyt 10–20% of the microbes present on the surface investigated. Therefore, it is important to always use the same method when monitoring a particular surface.

4.5 | Assessment of results

Swab samples are normally cultured by the pour plate method, and the results are reported as CFU/cm^2 according to the usual colony counting rules. In the case of contact plates, interpretation becomes difficult when there are more than 200 colony forming units per plate, this can be reported as TNTC (Too Numerous To Count). The results can be reported either as the total number of CFUs on the plate or per unit (cm^2) of plate surface area.

With Hygicult, the counting error margin remains around 10% up to colony counts of 200. Higher numbers are more difficult to count, but with the help of model charts it is possible to estimate microbe concentrations up to 600 CFU/Hygicult side with a margin of error around 30%. A model chart for the interpretation of Hygicult results is supplied with each kit, allowing approximate assessment of hygiene level on the basis on colony density on the agar.

4.6 | Preventive and corrective action and conclusions

Targeting critical sites

The basic idea of systematic surface hygiene sampling is to avoid and prevent dangers inherent to food handling and processing, but clean production environment also gives longer shelf life on products. Accordingly, systematic and continual hygiene monitoring is performed in food processing, transportation, handling, retail trade and preparation. All phases of food production and handling are evaluated systematically for risks, and additional assurance is obtained by taking hygiene samples. The whole process is evaluated phase by phase, identifying phases that pose a safety risk or a potential source of other defects in the food product. Such phases are crucial targets for hygiene monitoring. Action limits are issued to the most critical sampling sites. If the limits are exceeded, a predetermined chain of corrective and preventive measures are initiated. Such action is aimed at minimising or totally removing the detected hazard at the critical site.

When to take samples

Surface hygiene samples are also taken from surfaces and cleaning equipment after cleaning, before the start of work. At this phase, a skilled eye or nose can also detect poor hygiene, causing need for re-cleaning before work can be started. Another reason for hygiene sampling is to train personnel in detecting poor hygiene.

It should be borne in mind, however, that the purpose of cleaning is not to reach sterility everywhere but to maintain a good general tidiness and keep bacterial counts and types under control. The microbiological level of hygiene pursued is determined by the character of the activity and by official regulations.

5 Surface hygiene as part of in-house control

5.1 | HACCP and risk assessment

The hygiene level of food processing equipment affects on the hygiene level and safety of the processed products. The machinery and surfaces can be the major sources of risk in food production and handling. Regular sampling of the equipment and production surfaces helps to prevent, control, and manage the microbiological risks. Systematic and continuous hygiene monitoring is performed in food processing, transportation, handling, retail trade, and preparation.

Surface sampling is one of the Risk Management Operations. In addition to monitoring the product or process, the product safety and safe shelf life can be based on the monitoring of surfaces.

The need and frequency of the surface sampling are based on the risk assessment and HACCP system. In addition to the raw material related hazards, also the working environment related hazards should be included to the complete Hazard Analysis and all the production steps should be evaluated as a source of hazards. Some production steps, in which the hazards can be eliminated or reduced to acceptable levels, are identified as critical control points (CCP) and monitoring-verification operations are established to separate approved lots from non-approved. However, there are several production steps, in which this kind of operating is not possible. Cutting, slicing, chopping, and packing are production steps, where prevention of contamination is needed, although there is no individual step or operation, which could be chosen as a CCP. These kinds of production steps are usually called as critical points or GMP (Good manufacturing practise) points and the chosen risk management operation can be the hygiene level monitoring of the surfaces and equipment.

The type of bacteria and the critical limits of surface bacteria are also part of the risk management. Actions related to risk management should always be based on risk assessment which draws upon the existing information about risks in the form of hazards and adverse circumstances that cause health problems and illness. Comprehensive scientific risk assessment consists of identification and characterisation of the hazard, exposure assessment and characterisation of the risk. This kind of risk assessment can seldom be applied to surface samples since surface sampling concerns a factor which is only indirectly associated with the food product's safety. Nevertheless, the scientific approach is applicable to decisions regarding the frequency of hygiene sampling.

Since there is a lot of knowledge associated with *Listeria* contamination, sampling *Listeria* from the surfaces is emphasized in the EU regulation on microbiological criteria (2073/2005). Manufacturers of dried infant formulae or dried foods for special medical purpose intended for infants below six months which pose a *Cronobacter* spp. risk must monitor for Enterobacteriaceae. Specific regulations and local requirements should always be followed.

The need for sampling other pathogens is evaluated by HACCP. First, the pathogens related to manufactured food product are identified and the cleanliness of the production environment is evaluated. Then, the possibility of the pathogens to be derived from equipment is estimated. A hygiene survey may be needed for the estimation. In addition to the results and knowledge of the production plant, the hazard identification can be based on general knowledge.

The high total bacterial count is an indicator for the risk of pathogens. The recommendations on total counts given at chapter 4 are based on general experience. In principle, the establishments and plants should carry out initial investigation of surfaces and take several samples from different surfaces and then create their own critical limits, construct an assessment scale, and identify the most important sites for future routine sampling. When this is not possible, general guidelines such as the critical limits and sampling sites given in this guide can be used.

5.2 | Risk control by regular sampling

The monitoring program for surface sampling is part of the prerequisite programme (in-house control plan). The most important part of the monitoring is the visual control, which is usually done daily. The microbiological samples are needed to verify the visual inspection. The frequency of the sampling is based on the HACCP, on the character of the products, and on the possible legislation.

When the results are above the critical limits, corrective actions are needed and further samples must be taken to control the effect of these actions.

The visual control and the maintenance control of the equipment can be performed at the same time as the sampling.

The frequency of the sampling is related to the risks of the products and to the size and type of the production. See also the table in Chapter 3.5. The common practise is to take samples at least four times per year in small-scale production.

When ready-to eat food are produced at factory level, the importance of the cleanliness of the surfaces is easy to demonstrate and understand. However, most of the reported food poisoning outbreaks occur in catering business. The most important factors related to these outbreaks are mistakes with cooling, chilling and storage temperature. In addition, unhygienic working methods, unwashed hands and cross contamination from surfaces are reasons for outbreaks. It can be concluded that poor hygiene constitutes a risk factor or hazard. Therefore, in addition to visual inspection, restaurants and institutional kitchens should also include surface sampling to their in-house control programs. The frequency of sampling depends on the extensiveness of the activity. If the outbreak or food poisoning occurs, one of the needed actions is to increase the frequency and number of samples taken.

6 Other reportable premises and activities

Health protection in Finland is regulated through the Health Protection Act (763/1994). The objective of the Health Protection Act is to maintain and promote the health of people. In addition, it is aimed at preventing, reducing, and removing factors in the environment that might present health hazards. The Health Protection Act requires a written notification to be made when taking into use premises for activities that may be harmful or hazardous to the health of the users of such premises. From the surface hygiene point of view, such premises comprise lodging and food premises, public saunas, indoor swimming pools, outdoor swimming baths, spas and other similar facilities.

6.1 | Potential harms or hazards to users

Potential risk sites in lodging premises, public saunas, indoor swimming pools, outdoor swimming baths, spas, kindergartens, elderly homes and gyms include sanitary facilities and various exercise equipment involving skin contact. In moist premises, various surface pathogens such as bacteria, viruses, yeasts, moulds and protozoans may present a risk. The number and character of these harm or hazard factors must be controlled.

The risk sites in barber's shops, hairdresser's, beauty salons, massage facilities and dermatology salons comprise wash basins, treatment appliances and other work equipment. In these places, bacteria, viruses, moulds, yeasts and other noxious agents such as chemical residues can be found. Moreover, poor general hygiene and untidiness often make gyms and barber's shops less pleasurable.

Action limits

Site	Contact plate (CFU/26 cm^2)	Hygicult (CFU/10 cm^2)
Moist premises	100	250
Barber's shops	50	125

6.2 | Elimination of harms and hazards

A few hints to prevent or eliminate harms and hazards

Risk factor	Prevention or elimination
Moist premises	
Many special groups among clients	Frequent check-up cleaning
Corrosion-prone surface materials	Alkaline detergents, mild chlorines
Warm surfaces	Avoid chlorine-containing detergents
Barber's shops	
Towels	Wash at 70–90°C
Neck trimmers	Immersion disinfection, heating
Combs and scissors	Immersion disinfection, frequent replacement
Neck supports on wash basins	Wipe disinfection
Spray bottles	Frequent rinsing
Massage and dermatology premises	
Equipment	Immersion disinfection, heating
Towels	Wash at 70–90°C
Basins	Wipe disinfection

7 Hand Hygiene

7.1 | Hand hygiene is an important element in surface hygiene monitoring

Hands are an efficient route for microbes to spread from uncooked foods to cooked foods. Typically, the bacterial flora is scarce in cooked food. Microbes transferred via hands to a suitable growth surface at suitable temperature (10–60°C) are very likely undergo heavy multiplication. Microbes exist on hands naturally, also many people carry *Staphylococcus aureus* on their hands. Such people are allowed to work in food production as long as they recognise the importance of careful washing and disinfection and act accordingly. Microbes of faecal origin reach food product through poor personal hygiene. It is important to wash hands with sufficient care using the right technique after visiting the toilet. If production hygiene is not under control, microbes can be transferred via hands from dirty surfaces onto clean surfaces or directly into food. Personnel handling food should refrain from simultaneously handling other things, such as money or uncooked foodstuffs, washing dishes and cleaning surfaces or customer premises.

When working, unhygienic practices such as touching the face or nose, combing hair, etc. should be avoided. Should one's hands for some reason become contaminated, they should be washed, rinsed, and properly dried before continuing work (for example after handling dirty or spoiled foods or raw materials). Because some bacteria cannot be completely removed from hands by washing or with hand disinfectant, heat-treated foods should not be touched with bare hands. Protective gloves may also spread bacteria because the user may often perceive them as protecting the hands only. It should be kept in mind that protective gloves are mainly meant for the protection of food. Therefore, if a glove touches a potentially dirty object, e.g. a door handle, it should be changed. Bacteria such as *S. aureus* may multiply as the hands sweat in the gloves. Thus, gloves need to be changed often and hands should be washed at each change.

7.2 | Good practice in hand care

Good hand care is based on cleaning and moisturising. Food workers should keep their nails short and care for their cuticles. Rings and watches should not be worn at work because they tend to collect dirt, chemicals and cleaning agents. They also offer microbes a warm and moist place to grow. Since frequent washing wears out the skin, hands should be washed with lukewarm, not hot, water and the detergent should be mild. In food production, towels should be disposable.

Hands are washed as follows:

1. Wet your hands
2. Apply detergent
3. Wash both hands carefully, including thumbs, backs of hands, between fingers, fingertips, underneath the nails
4. Rinse well using lukewarm, not hot, water
5. Dry your hands on a disposable towel
6. Turn off the tap with the towel
7. Apply moisturising lotion only after work

7.3 | Monitoring of hand hygiene

Hand hygiene samples should be taken on a regular basis, especially in catering and food production. The first sampling should be done when the person starts in the position, then after one or two months, and then the sampling should be continued on regular intervals. When food poisoning is suspected, health officials will take both food and hand hygiene samples. The purpose of these samples is to investigate the presence of food poisoning organisms (*S. aureus* and *B. cereus*) on workers' hands. If hands and food products yield the same microbial strain, the reason for food poisoning is obvious. Hygicult contact slides monitor the level of contamination, not specific microbes, and they can easily be used for routine monitoring of general hand hygiene.

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