



# A Guide to Monitoring Surface Hygiene



A guide for

- kitchens
- supermarkets
- food industry
- food education
- health inspectors



## I History and Theory

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1.1	History of hygiene sampling .....	4
1.2	Old and new methods .....	4
1.3	Comparison of various methods .....	5
1.4	Significance of surface hygiene samples .....	5
1.5	Target surfaces and microbial groups .....	6
1.6	Visual inspection .....	7
1.7	Methods under development .....	8

## II Biofilm – Microbes' Defence against Cleaning

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2.1	Biofilms in industrial systems .....	9
2.1.1	Water systems .....	9
2.1.2	Process industry .....	10
2.1.3	Air-conditioning systems and humidifiers .....	11
2.2	Elimination of biofilms .....	11
2.2.1	Detergents and disinfectants .....	11
2.2.2	Summary of methods for preventing biofilm formation .....	11

## III Methods

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3.1	Methods .....	12
3.2	Sampling .....	12
3.2.1	Contact plates for total bacterial count .....	12
3.2.2	Petriefilm .....	13
3.2.3	Hygicult .....	13
3.2.4	Luminescence .....	14
3.2.5	Protein tests .....	16
3.3	Sampling sites .....	17
3.4	Incubation (microbial culture) .....	19
3.5	Sampling frequency .....	20
3.6	Hygienic premises .....	21
3.7	Disposal of cultures .....	21

## IV Practical Measures

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4.1	Personal hygiene .....	22
4.2	Analyses .....	22
4.2.1	Swabbing method .....	22
4.2.2	Contact method .....	23
4.3	Applications .....	23
4.3.1	Milk industry .....	23
4.3.2	Meat and fish industry .....	23
4.3.3	Kitchens .....	24
4.4	Reference values .....	24
4.4.1	L. ten Cate scale .....	24
4.4.2	Surface hygiene in kitchens .....	24
4.4.3	Bakeries .....	25
4.4.4	Meat processing premises .....	25
4.4.5	Slaughtering premises .....	25
4.4.6	Retail trade premises and institutional kitchens .....	25
4.5	Comparison of methods .....	26
4.6	Assessment of results .....	26
4.7	Preventive and corrective action and conclusions .....	27

## V Surface Hygiene as Part of In-House Control

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*Marjatta Rahkio*

5.1	Hygiene sampling in legislation .....	28
5.2	Risk control by regular sampling .....	28

## VI Other Reportable Premises and Activities

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*Keijo Houhala*

6.1	Potential harms or hazards to users .....	29
6.2	Elimination of harms and hazards .....	30

## VII Hand Hygiene

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*Sara Saari*

7.1	Why? .....	31
7.2	How? .....	32
7.3	Monitoring of hand hygiene .....	33

<b>Literature</b> .....	34
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# I History and Theory

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## 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 a so-called 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 then used by pressing them against the surface to be investigated. The method was called the agar-sausage method. Although the ten Cate's method of preparing agar slices never came into wide-spread use, his scale for quantifying microbial growth by the number of colonies on a 9 cm<sup>2</sup> agar surface was widely adopted and is still recommended also for official evaluations. The ten Cate assessment scale was and, still is, so popular that its colony counts were taken up as such for use with 25 cm<sup>2</sup> contact plates. This scale is still used by many food laboratories. Thus unnoticed the original ten Cate scale became stricter.

## 1.2 Old and new methods

Surface samples can be taken by various methods. At present the four major methodologies are:

- Swab methods using poured plates
- Contact plate methods using self-prepared or commercial plates
- Indirect methods such as  
ATP (adenosine triphosphate) bioluminescence  
or  
DEFT (direct epifluorescent filter technique)
- Methods for determining protein residues

Swab and contact methods such as Hygicult, Petrifilm and Redigel are commercial contact methods. ATP, DEFT and the protein tests are indirect methods in that they do not report actual bacterial counts but measure some characteristics related to the microbial mass.

### 1.3 Comparison of various methods

The number of bacteria detected by swab 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, 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 swab method. According to an extensive collaborative study, Hygicult gives the same result as the conventional contact plate or the swab method.

The contact method is best suited to even surfaces, whereas small tools, rounded forms or uneven surfaces are best examined by the swab method. The reliability of the swab method is crucially dependent on the skill of the sampling person. The swab should be applied at a pressure of 0.1 kg/cm<sup>2</sup>. The use of alginate or carbon swabs may decrease the sampling error. Although both swab 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 that come into contact with the surface concerned.

### 1.4 Significance of surface hygiene samples

The importance of surface hygiene samples has been recently emphasised 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 of entire production processes instead of checks on final products and sporadic quality control. The HACCP system is already commonly used in the food industry worldwide. In the retail trade, HACCP requires constructing flow charts (incoming, stored and outgoing goods), increasing visual inspections and applying more rigorous temperature controls.

Surface and other hygiene sampling is an integral part of monitoring the production process. Previously, the practical problem was that the

results were often available only after several days. Today, novel techniques allow surface cleanliness to be ascertained within minutes. This is important during food manufacturing where the slightest bacterial contamination may deteriorate the quality of the final product. The traditional hygiene control methods are suitable to monitoring less critical production or sales facilities.

## 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 paid primarily 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, afterwards 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 most easily cleanable material. 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.

Opinions on the importance of biofilms in the food industry are twofold: According to one opinion, understanding of the formation of biofilms is the solution to more or less all hygiene problems. According to another opinion, the importance of biofilms in the food industry is overemphasised. In any case it is certain that the longer surfaces are left uncleaned of organic matter, the more bacteria they will accumulate and the more difficult their 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 taken from “easy” sites, such as even surfaces, whose cleaning is easy and belongs to the cleaning routine. From the hygiene and contamination point of view, the critical point can be totally outside the cleaning programme. Similar overlooked sites may also exist in retail outlets.

The level of hygiene of cleaning equipment is a focus of increasing interest. Even pathogenic bacteria have been detected on cleaning equipment. The disinfectants used in cleaning have often been found to be too dilute.

### Bacterial groups

Usually total bacterial counts and sometimes coliform counts are determined from surface hygiene samples. Other possible sampling objects are

<i>Escherichia coli</i>	– indicator of faecal contamination
<i>Staphylococcus aureus</i>	– hand hygiene indicator
<i>Enterococci</i>	– grouping and origin unclear
<i>Listeria</i>	– potential indicator organism in future
<i>Yersinia</i>	– found in similar places as <i>Listeria</i> , floors
<i>Salmonella</i>	– air conditioning filters, sewage basins, floor drains
<i>Campylobacter</i>	– milk, meat, poultry, seafood, fruits, vegetables

Specific bacterial strains, moulds and yeasts are important in general, as well as being typical of 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.

Regarding different sampling methods, contact samples appear to correlate best with visual inspection. It is explained by that the contact

method measures more or less visible dirt, whereas the mechanical action of swabbing may yield more organisms, depending on the surface material.

## 1.7 Methods under development

The future prospects of hygiene sampling include for instance the following indirect methods:

- Turbidimetry
- Measurement of electrical conductivity
- Calorimetry
- Fluorometry
- Radiometry
- Microcalorimetry
- Catalase tests
- Endotoxin tests (limulus amoebocyte lysate, LAL)
- Biosensor applications



## II Biofilm – Microbes’ Defence against Cleaning

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***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 other bodies of water 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 withstanding antibacterial factors. In industrial equipment and circulation systems, biofilm protects microbes against cleaning and disinfecting agents.

#### 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. In cooling water systems, the most severe problem is loss in the heat exchange rate (even down to 10% of the optimum rate). Examples of other adverse effects in water systems are increased prevalence of pathogens, corrosion caused by microbes, increased resistance to water flow and local blockage of filters.

### 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 prevention of biofilms in the process industry is therefore fundamentally concerned with the properties of surface materials, such as smoothness, cracks, dead angles, etc. The formation of biofilm can be prevented by polishing or shining the surface electrically.

Gaskets easily collect dirt and nutrients enhancing the accumulation of microbes and formation of biofilms. The condition of gaskets should therefore be inspected regularly to avoid biofilms.

Valves have to 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.

Biofilm protects microbes against detergents, disinfectants and even heat sterilisation. Important microbes with regard to biofilm formation in the process industry include *Bacillus* sp., *Leuconostoc* sp., coliforms, enterobacteria, pseudomonads, listeriae, 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 Air-conditioning systems and humidifiers

Open circulation water systems may be connected to cooling or heating air-conditioning equipment. If a pathogenic microbe exists in the potential biofilm, the air-conditioning equipment may effectively spread the disease. For example *Legionella pneumophila* may exist in the circulating water systems of air-conditioning equipment. Legionella bacteria may be present without a biofilm, but the formation of a biofilm makes it difficult to remove the microbe from the circulation pipes.

Humidifiers often contain biofilms, which makes their regular cleaning (at least once a month) and maintenance very important.

## 2.2 Elimination of biofilms

### 2.2.1 Detergents and disinfectants

The development of a biofilm can be quantified in terms of microbial biomass and the amount of exopolysaccharides present. When eliminating biofilms, two important targets have 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, do they become effective.

### 2.2.2 Summary of methods for preventing biofilm formation

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

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

## III Methods

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### 3.1 Methods

Surface samples are taken either by directly inoculating a solid or liquid culture medium with the sample by the contact method or by transferring the sample with a swab 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	}	Monitoring of microbial contamination
■ Petrifilm		
■ Hygicult		
■ Luminescence	}	Monitoring of impurities
■ Protein tests		

### 3.2 Sampling

Hygiene samples are normally taken in the morning before start of work, when the premises are clean. In order to obtain 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<sup>2</sup>, the bottom of the plate is divided into 1 cm<sup>2</sup> squares. The surface area of the contact plate is generally 25 cm<sup>2</sup>.

### 3.2.2 Petrifilm

The medium is in dry form on a squared piece polyethylene-coated paper; the medium is protected on top by a polypropylene membrane. For surface sampling, the Petrifilm medium is moistened with 1 ml of sterile water which is spread onto the film with a special spreader and allowed to absorb for one minute. Because the film is flexible, the sampled surface does not need to be even. Total bacterial counts, coliforms, *E. coli*, yeast and mould counts can be obtained with Petrifilm. The medium is divided into 1 cm<sup>2</sup> squares, and the total surface area is about 20 cm<sup>2</sup>.

### 3.2.3 Hygicult

The validated Hygicult sampler 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 possible to close tightly the clear tube covering the paddle. The Hygicult paddle is ready for surface sampling as such. The paddle can be inoculated with sample by the contact method or with a swab. After sampling, the paddle is replaced in its tube where the sample can be safely transported. The surface area of one side of the paddle is about 9.6 cm<sup>2</sup>. 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.

The reproducibility and sensitivity of sampling can be improved by first spraying the surface with SprayCult reagent which disintegrates any biofilm without killing the microbes contained in it. Use of SprayCult helps to standardise the sampling procedure.

There are different Hygicult versions for determination of total bacteria, enterobacteriaceae,  $\beta$ -glucuronidase-producing bacteria (*E.coli* and *Shigella sp.* as well as certain strains of *Salmonella*, *Edwardsiella* and *Yersinia*), yeasts and moulds. The validated Hygicult TPC contains neutralising agents which remove any inhibitory effect of cleaning agent residues on bacterial growth. It is recommended that Hygicult TPC be incubated at room temperature for three days when monitoring general hygiene status. As many yeasts and moulds do not tolerate a temperature of 35°C, even Hygicult Y&F is incubated at room temperature. Hygicult E and Hygicult E/ $\beta$ -Gur are always incubated at 35°C.

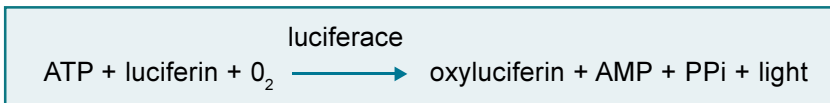
The Hygicult paddle is polypropylene, the tube polystyrene and the cap polyethylene.

### 3.2.4 Luminescence

The results of microbiological culture methods, that is visually detectable colonies on a culture medium, are usually not obtained until 24 h after sampling. Thus, there is a great interest in developing faster methods. The luminometric method is especially suited to analysing surface hygiene samples.

#### What is luminometry?

Luminometry is, as the name says, 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:



The two substances used in the reaction, luciferin and the enzyme luciferase, are both isolated from the light-emitting fire fly.

The amount of light formed in the reaction is directly proportional to the amount of ATP in the sample, and the amount of ATP, for its part, is related to the number of microbial cells in the sample. The amount of ATP contained in one eukaryotic animal, plant, yeast or mould cell is about  $10^{-12}$  g. One bacterial cell contains only 1/1000 of the ATP contained in an eukaryotic cell. The ATP content of a cell is not constant but depends on factors such as cell structure, stage of growth and growth temperature.

#### Measurement of ATP

In luminometry, samples are usually taken by swabbing a limited area with a swab moistened with sterile NaCl solution. The tip of the swab is then inserted in a reagent ampoule where the swab is rotated for about 20 s. The first step is to release the ATP of any bacteria in the sample, for instance by using a detergent to break the cells. Next the reagents for the luciferin-luciferase reaction are added.

The amount of light formed is measured using a special device known as a luminometer. The device contains a photomultiplier tube capable of measuring minute amounts of light energy. The result is usually expressed in relative light units (RLU).

Luminometer manufacturers report the sensitivity of the method to be 0.1–0.5 pg ATP, equivalent to the ATP content of about 100–500 bacterial cells. When measuring food samples with a luminometer, the biggest problem is how to differentiate the ATP originating from microbial cells from the ATP derived from other cells. In water analysis, this presents less of a problem since most of the ATP detected will be of microbial origin. As a rule, luminometry is not applicable to food samples because of their high animal or plant cell content.

In the case of surface hygiene samples, the aforementioned drawback can be turned into an advantage: the luminometry data also account for the presence of organic foreign matter which may constitute a nourishment source for microbes on a postcontaminated surface. Indeed, the expression "total hygiene monitoring" is often used in connection with luminometry.

### **Assessment of results**

When evaluating luminometric results, the same facts have to be kept in mind as with culture methods. Sampling is the critical phase as regards the representativeness of the data. Problems related to the shape of the surface under investigation or uneven contamination are some of the sources of error.

Avoidance of ATP contamination is an important consideration in luminometry. As the ATP content of human cells exceeds that of bacterial cells, a few exfoliated skin cells ending up in a surface sample may change the result significantly. It is therefore necessary to use disposable gloves both during sample taking and handling of reagents. Sterility of other sampling equipment is also an absolute requirement.

Luminometric methods have not been standardised. Hence, one has to follow the manufacturer's instructions which normally apply only if both the device and reagents come from the same manufacturer. The setting of action or alarm limits may be difficult although some manufacturers suggest values for clean and contaminated samples. In practice the

limits have to be determined locally on the basis of accumulated measurement data, which requires a lengthy run-in period when a luminometric system is introduced.

There are also on the market simple-looking luminometers that give a red, yellow or green light as an indication of cleanliness. These kinds of devices and the results obtained need to be thoroughly understood and critically evaluated. The introduction of luminometry always requires sufficient training of the luminometer user.

### **Luminometry and health inspection**

Luminometry is suited for internal hygiene monitoring in food production plants as part of a in-house control programme. Because the results are available almost immediately, action to correct wrong working methods, for instance, can be undertaken at once. On the other hand, it is not possible to compare the results for different production plants without sufficient standardisation of methods. Therefore, ATP measurement is unlikely to be suitable tool for official health inspection. Use of the method is also limited by price.

The authorities must, however, be aware of the specific features of luminometry and its use when considering industry's in-house control schemes and evaluating inspection findings.

#### **Summary**

- + Rapid results (within minutes)
- + Detects organic impurities in addition to microbes
- + Fairly simple to use (after training)
- + Results expressed numerical values
  
- Expensive investment
- Requires a rigorous routine to eliminate errors
- Not a standardised method

### **3.2.5 Protein tests**

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



The result of the test is given as “CLEAN” or “DIRTY” immediately, so a decision on re-cleaning can be taken before foodstuffs are handled on the surface or equipment concerned.

#### **Advantages**

- + Easy to use
- + Immediate response “CLEAN” or “DIRTY”
- + Inexpensive
- + Long shelf-life of test strips

If a clear colour change is obtained on the test strip, the inspected surface has to be re-cleaned.

### **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. Potential sites of sampling in the meat industry, for example, include

- Cutting boards, worktops, transportation hooks, bandsaws, conveyors, knives, basin carriages, scalding machinery, crust machinery, mesering 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, salteries, chopped meat departments, injection departments, cutting premises, storage premises for uncooked products and un-packed cooked products.

The contact method is best suited for even surfaces but for example wooden surfaces can be difficult sampling targets. On an uneven surface, the sampling agar may not fully contact the sampling point, resulting in an unrepresentative sample. The method is simple and requires little experience. 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 because one has to be able to apply the swab with a certain pressure against the surface while holding many items in one's hands at the same time. 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 experience of the sampling person. 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 swab method allows sampling in places inaccessible with Hygicult slides alone. Here, the site is swabbed in the normal way but the bacterial mass collected on the swab is spread on the Hygicult paddle surfaces as in plate culture in the laboratory.

Examples of the suitability of different methods							
Sampling target	Contact method				Swabbing		Lumi-nescence
	Contact plate	Hygicult	Petrifilm	Protein tests	Petridish	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)

Monitoring of the overall level of hygiene using plate count media involves incubation of the cultures at room temperature for three days. The presence of yeasts and moulds will be revealed, in addition to aerobic bacteria.

The cultures are inspected daily since heavily contaminated samples can be detected already after one day's incubation. Incubation at room temperature concerns to 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 cannot grow at 35°C. The incubation time at room temperature is up to four days for yeasts and moulds. The approximate level of hygiene is already apparent after two days' incubation.

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

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

If the results indicate a poor hygiene, corrective actions need to 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 risk 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 in order of hygienic importance as follows: surfaces of direct contact, surfaces affecting the product indirectly and other surfaces in the premises.

#### **Risk assessment and significance of risks, when evaluating the contamination danger 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 burn the cultures or immerse them in disinfectant solution overnight (Hygicult with caps open). The disinfectant-treated cultures can then be disposed of as ordinary waste.

## IV Practical Measures

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Easy and simple methods are needed in food production, processing, transportation and other handling 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 Analyses

#### 4.2.1 Swabbing method

A swab moistened with sterile 0.9% NaCl solution is used to swab an area of 10 cm<sup>2</sup>. The swabbing is performed twice with the second swabbing direction being perpendicular to the first one. During swabbing, the same pressure has to be applied throughout the sampled area. The area to be sampled is delineated using a sterile chablon. After swabbing, the swab is placed in a sterile test tube containing 10 ml of sterile 0.9% NaCl solution. Microbes caught on the swab are transferred to the solution by pressing the swab alternately against the wall and bottom of the tube. This is repeated 20 times, after which the swab is removed from the tube.

Alternatively a sterile solution containing 85 g of NaCl and 1g of peptone in 1000 ml of distilled water at pH 7.2 may be used. If chlorine or iodine compounds have been used for disinfection of the sampled surface, 0.1% of sodium thiosulphate is added to the solution. The THG culture medium consists of 5.0 g tryptone, 2.5 g yeast extract, 1.0 g glucose, 15.0 g agar and 1 000 g distilled water (NMKL 5/87).

### **4.2.2 Contact method**

The two contact method variants are plating and the ten Cate -method. In the plating method, a contact plate is pressed against the target surface. The plate is over-filled with medium, forming a convex contact surface. The ten Cate method has never become very popular, but the standard surface area of 9 cm<sup>2</sup> used today is derived from ten Cate's original technique. The purpose of the surface method is not the determination of exact colony counts but the approximate estimation of the hygienic status of a surface.

## **4.3 Applications**

### **4.3.1 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 receiving scales at dairies, tanks and other large, even surfaces.

### **4.3.2 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.

### 4.3.3 Kitchens

Surface hygiene in kitchens can be monitored by both contact and swab methods. The method is chosen on a case by case basis. Nevertheless, it is usually advisable to use the contact method to analyse even 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.

## 4.4. Reference values

4.4.1 L. ten Cate scale	
Description of growth	CFU/9 cm <sup>2</sup>
No growth	–
Minimal growth	< 10
Moderate growth	10 – 30
Abundant growth	30 – 100
Confluent growth	> 100

4.4.2 Surface hygiene in kitchens	
Description of hygiene level	Hygicult (CFU/10 cm <sup>2</sup> )
Good	< 20
Acceptable	20 – 100
Not acceptable	> 100



<b>4.4.3 Bakeries</b>	
Description of hygiene level	Hygicult (CFU/10 cm <sup>2</sup> )
Good	< 20
Acceptable	20 – 50
Not acceptable	> 50

<b>4.4.4 Meat processing premises</b>	
Description of hygiene level	Hygicult (CFU/10 cm <sup>2</sup> )
Good	< 18
Acceptable	18 – 50
Not acceptable	> 50

<b>4.4.5 Slaughtering</b>	
Description of hygiene level	Hygicult (CFU/10 cm <sup>2</sup> )
Good	< 18
Acceptable	18 – 40
Satisfactory	41 – 100
Not acceptable	> 100

<b>4.4.6 Retail premises and institutional kitchens</b>		
Description of hygiene level	Contact plate CFU/26 cm <sup>2</sup>	Hygicult CFU/10 cm <sup>2</sup>
Good	< 50	< 20
Acceptable	51 – 250	20 – 100
Not acceptable	> 250	> 100

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

Note:

No bacteria of enteric origin, such as *E. coli*, are acceptable on direct-contact surfaces.

## 4.5 Comparison of methods

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

Comparison of methods			
Description of hygiene level	Number of colonies by method		
	Contact plate 26 cm <sup>2</sup>	Petrifilm 20 cm <sup>2</sup>	Hygicult contact 10 cm <sup>2</sup>
Good	< 50	< 40	< 20
Acceptable	51 – 250	41 – 190	21 – 200
Not acceptable	> 250	> 190	> 100

Depending on the surface material, the contact method is able to pick up about 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.

## 4.6 Assessment of Results

Swab samples are normally cultured by the pour plate method, and the results are reported as CFU/cm<sup>2</sup> 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. The results can be reported either as the total number of CFUs on the plate or per unit (cm<sup>2</sup>) 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.

## 4.7 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. 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. The most critical sampling sites are issued action limits the exceeding of which initiates a predetermined chain of corrective and preventive measures. Such action, in other words, 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 re-cleaning to be performed before work can be started. Another reason for taking hygiene samples is, indeed, to train personnel in detecting poor hygiene.

The provisions of cleaning contracts often include a target level of hygiene as a condition for paying the fees or additional fees. 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.

## V Surface Hygiene as Part of In-House Control

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### 5.1 Hygiene sampling in legislation

The food industry, institutional kitchens and retail shops are required by law to carry out systematic hygiene sampling.

The operator is required to identify the hygiene-related dangers in food handling and set up an in-house control programme. Some of the biggest sources of risk are equipment and utensils. Regular hygiene monitoring helps to control these risks.

### 5.2 Risk control by regular sampling

Equipment and machinery are some of the major sources of risk in food handling. Regular sampling of such items helps to control these risks. The taking and inspection of hygiene samples contributes to risk control, the frequency of monitoring being determined by risk assessment. The recommended acceptable levels of microbes on various surfaces is also part of risk control.

Actions related to risk control should always be based on risk assessment. Risk assessment draws upon the existing information on risks in the form of hazards and adverse circumstances that cause health problems and illness. Comprehensive scientific risk assessment consists of identification and description of the hazard, evaluation of exposure and description of the risk.

This kind of risk assessment cannot be applied to surface samples since surface sampling concerns a factor which is only indirectly associated with the safety of a food product. Nevertheless, the scientific approach is applicable to decisions regarding the frequency of hygiene sampling.

Firstly, the level of hazard is determined on the basis of existing information about how dirty the surfaces are and what microbes prevail on them. When setting up a routine for hygiene sampling, it may be advisable to carry out a more extensive initial investigation of surfaces. The results can then be used to construct an assessment scale and to identify the most important sites for future routine sampling (see Chapter IV).

There is ample evidence of the importance of good hygiene in food production for food safety, for instance regarding food poisoning rates. As it is, most of the reported food poisonings are related to spoilt public meals, the spoilage often being due to wrong storage or cooling temperature. Cross contamination from surfaces and poor general hygiene are also mentioned in connection with food poisoning. It can be concluded that poor hygiene constitutes a risk factor or hazard. Therefore, restaurants and institutional kitchens should also include surface sampling in their in-house control programmes, in addition to visual inspection. The frequency of sampling depends on the extensiveness of the activity (see Chapter III).

## VI Other Reportable Premises and Activities

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The Health Protection Act (763/1994) 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, old people's 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.

<b>Action limits</b>		
Site	Hygicult CFU/10 cm <sup>2</sup>	Contact plate CFU/26 cm <sup>2</sup>
Moist premises	100	250
Barber's shops	50	125

## 6.2 Elimination of harms and hazards

<b>A few hints to prevent or eliminate harms and hazards</b>	
<b>Risk factor</b>	<b>Prevention or elimination</b>
<p><b>Moist premises:</b></p> <p>A lot of special groups among clients</p> <p>Corrosion-prone surface materials</p> <p>Warm surfaces</p>	<p>frequent check-up cleaning</p> <p>alkaline detergents, mild chlorines</p> <p>avoid chlorine-containing detergents</p>
<p><b>Barber's shops:</b></p> <p>Towels</p> <p>Neck trimmers</p> <p>Combs and scissors</p> <p>Neck supports on wash basins</p> <p>Spray bottles</p>	<p>wash at 70–90°C</p> <p>immersion disinfection, heating</p> <p>immersion disinfection, frequent replacement</p> <p>wipe disinfection</p> <p>frequent rinsing</p>
<p><b>Massage and dermatology premises:</b></p> <p>Equipment</p> <p>Towels</p> <p>Basins</p>	<p>immersion disinfection, heating</p> <p>wash at 70–90°C</p> <p>wipe disinfection</p>

## VII Hand Hygiene

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In-house control within the food sector often over-emphasises temperature measurements and food sample analyses.

In-house control should also include checks of cleaning functions. A good way to accomplish this is to monitor the results of surface hygiene testing. It is essential to systematically follow up the effectiveness of surface cleaning on the basis of changes in hygiene levels. Any impairment in hygiene levels calls for investigation of the cause and institution of corrective action. Subsequently the effectiveness of the corrective action will be controlled with new hygiene samples.

Hand hygiene is an important element in surface hygiene monitoring.

### 7.1 Why?

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 may exist on hands naturally. About 5–10% of 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 dried before continuing work (for example after handling dirty or spoilt foods or raw materials). As bacteria such as *Staph. aureus* cannot be fully removed from hands by washing or disinfection, heat-treated foods should not be touched with bare hands.

Protective gloves, too, may spread bacteria because the user may often perceive them as protecting the hands only. It should be borne in mind that protective gloves are also meant for the protection of food. Therefore, if a glove touches a potentially dirty object, e.g. a door handle, it should be changed.

*Staph. aureus* may multiply as the hands sweat in the gloves. Thus gloves need to be changed often and the hands washed at each change.

## 7.2 How?

Good hand care is based on cleaning and moisturising. A food worker will keep his/her nails short and attend to his/her cuticles. Rings or watches should not be worn at work because they tend to collect dirt, chemicals and cleaning agents. 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.

Hand hygiene samples can be taken, for instance, first at the time of employment and then after one or two months. It is today customary to monitor the hand hygiene of personnel at 12-month intervals. When food poisoning is suspected, health officials will take, in addition to food samples, hand hygiene samples. The purpose of these samples is to investigate the presence of food poisoning organisms (*Staph. aureus* and *Bacillus cereus*) on workers' hands. If hands and food products yield the same microbial strain, the reason for food poisoning is obvious. Hygicult or contact plates can be used for routine monitoring of hand hygiene.

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