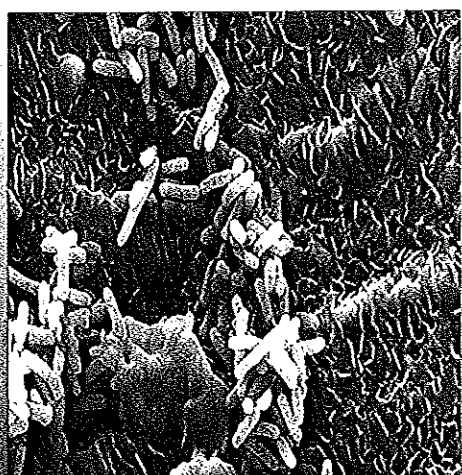


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Biofilms in the food and beverage industries

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Sampling and quantification of biofilms in food processing and other environments

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Abstract: In the food industry, assessment of food contact surfaces is necessary to determine whether equipment is properly cleaned and/or sanitized and whether living problematic microorganisms are present. Existing quantitative detection technologies are limited by the inability to directly detect living cells in sporadically dispersed biofilms on large surface areas. Thus, precise and accurate sampling strategies must be coupled with detection technology. This chapter discusses sampling methods and standard (e.g., plating and ATP-bioluminescence) and emerging (e.g., spectrometry, immunosensor, and nucleic acid-based) quantitative techniques to detect biofilms on food contact surfaces with a survey of function, analytical performance, and limitations.

Key words: biofilm, sampling methods, spectrometry, immunosensors, nucleic acid-based.

21.1 Introduction

Definitions of biofilms vary but most investigators agree that biofilms usually consist of four main components: (i) water, (ii) the living microbial population, (iii) the surface to which the cells are adhered, and (iv) the associated extracellular matrix, which can consist of secreted polymers (2, 17, 20, 107). These polymers, usually polysaccharides and/or protein, allow the cells to form three-dimensional structures but are not required for biofilms to proliferate on a surface (79). Biofilms can be contiguous, but are more likely to form networks of micro-colonies and yield patchy distributions (Fig. 21.1). Microorganisms are thought to contaminate 'clean' surfaces by the following sequential steps: transfer to the surface, attachment

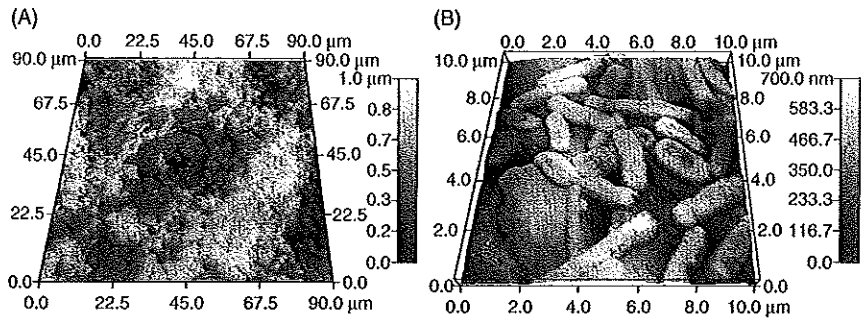


Fig. 21.1 3D z-sensor AFM image of air dried *Escherichia coli* O157:H7 biofilm (1-hour inoculation in 10^7 cells/ml and 8-hour biofilm development in minimal salts media) on stainless steel surface. Imaged in air with AC240 cantilever in AC mode. A) $90\ \mu\text{m} \times 90\ \mu\text{m}$ and B) $10\ \mu\text{m} \times 10\ \mu\text{m}$.

or entrapment on the surface, and biofilm development. Sources of the initial colonizing microorganisms are air, the food product, human contact, rinse and cleaning solutions, or an up-stream surface in sequential step food processes.

Attachment occurs when the forces associated with surfaces (electrostatic, van der Waals, hydrogen bonding, etc.) overcome forces associated with transport (e.g., convection). This process can also occur in protective microenvironments such as scratches, grooves, or interfaces between different materials. Development of biofilms occurs when the adhered cell(s) have proper environmental conditions (e.g., temperature and humidity) and suitable source of nutrients to grow. Once colonized on surfaces, biofilms can live in single or multi species microenvironments and through detachment become a source of contamination for other sites.

On food processing equipment, detection assays are necessary to provide evidence mainly for (i) determining that living problematic microorganisms (e.g., pathogens) are not present and/or (ii) determining whether equipment is properly cleaned and/or sanitized. Idealized monitoring technologies should provide automated, continuous, rapid, inexpensive, and rugged operation. Other desirable attributes for the idealized detection system include the ability to scan large surface areas to accurately quantify and to selectively identify all potential problematic microorganisms without sample preparation. Obviously, this idealized technology does not exist; however, progress has been made towards the development of technology that can achieve some of these goals. One of the major limitations of existing methodology is the lack of techniques that can be used to directly detect living cells in sporadically dispersed biofilms on large surface areas associated with food processing equipment. To circumvent this problem, many food industries have incorporated sampling techniques, such as rinsing and/or swabbing, as a step prior to detection and analysis.

Precise and accurate sampling of biofilms requires cells to be efficiently removed, collected, and prepared for the analytical detection method of choice. To quantitatively remove biofilms from microenvironments, the sampling technique should provide enough force to overcome the intermolecular forces that secure the biofilm matrix or cells within the biofilm to the surface. In sampling, biofilm removal is usually accomplished using shear forces. Removal can be achieved by providing convectional forces from rinsing and/or contact shear forces from methods such as swabs or sponges. For many assays, the goal of sampling is to remove living cells under conditions that avoid cell death in order to obtain an accurate assessment of the number and type of living microorganism present. Chemicals such as detergents can be added to sampling solutions to facilitate removal; however, in many cases, these chemical treatments can kill cells, thereby systematically biasing data. After removal from the surface, the cells or fragments from the biofilm must be removed from any sampling materials and quantitatively transferred to a sterile/clean and sometimes enzyme free container for analysis. Some assays require enrichment in a growth medium to increase the number of cells to a concentration above the minimum detectable quantity and reduce the interferences from sample matrices. Although necessary, enrichments can bias quantitative results since all growth media has a certain degree of selectivity, especially in multiplexed analysis schemes; and accurate models of growth are rarely determined or applied.

Recently, advances in bio-analytical chemistry and molecular microbiology are providing a new understanding of quantitative microbiology; and new techniques are being developed for the food industry. These efforts have led to the development of novel immunosensor, immunoassay, and nucleic acid detection technologies that allow more rapid and selective data to be obtained. This chapter will discuss sampling methods as well as standard (widely utilized in the food industry) and emerging/non-routine bio-analytical techniques (novel and not widely utilized) that can be coupled with these sampling technologies and detect the presence of problematic species within biofilms on food contact materials (Fig. 21.2). The chapter is not intended to be an exhaustive review of quantitative techniques applied to sampled biofilms, but instead a survey of techniques with highlights of key recent work and reviews in the hopes of providing an overall sense of the function, analytical performance, limitations, and scope of standard and state-of-the-art detection/identification platforms.

21.2 Sampling surfaces

On food contact surfaces, the US Public Health Service recommends no more than 100 colony forming units (CFUs) per 50 cm² sampled (28). However, in most cases the type of microorganism is more important than

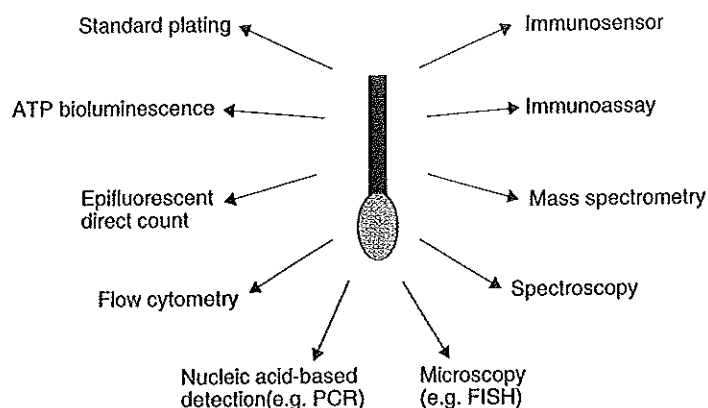


Fig. 21.2 Standard (black) and emerging detection technologies (gray).

the number. Sampling and testing are the best means to monitor microbial activity on process surfaces; and for meaningful results, the appropriate sampling strategy for examining surfaces needs to be selected. Which sampling strategy used is dependent on the sampling surface material, surface structure, location, and expected microbial contamination level (109). Some sampling strategies include random, representative, selective, and convenience sampling (56). Food processing companies should develop a sampling program to selectively test identified hazards and risks at critical control points (CCPs) within their Hazard Analysis Critical Control Point (HACCP) system (28). Assessment should be performed at all sites with the potential to harbor microorganisms that may directly or indirectly contaminate the product. For instance, sampling should occur at points where there is higher risk of residual moisture and also at points that are representative of the production equipment. Thus, focus should not only be on direct food contact surfaces because microorganisms can be transferred from indirect contact surfaces to direct contact surfaces. Direct contact surfaces include the inside of pipes, conveyors, storage vessels, fillers, and mixers; and indirect contact surfaces include the outside of equipment, walls, floors, and tools. Sampling should always be performed sterily to avoid contamination of the sample. Moreover, the sample should be properly labeled, stored, and transported and quickly analyzed after being collected (13).

Sampling methods can be qualitative or quantitative. Qualitative sampling detects the presence or absence of microorganisms at numbers above a given threshold; whereas, quantitative sampling determines the number of microorganisms on the sampled surface. Sampling methods inherently increase random error which decreases precision and systematic errors which cause deviations from true values (56). Errors in sampling are associated with the removal, recovery, and dispersal of living microorganisms

within a sampled biofilm isolated from a processing surface. In quantitative sampling, approximately 95% of the replicate results should lie within plus or minus two standard deviations of the mean (59).

There are two main sampling methods that have been found acceptable: indirect and direct sampling (13, 28). In indirect surface sampling, the most common method is the rinse test. The rinse method can be used to sample containers and processing equipment systems such as tanks, pipelines, and fillers or areas inaccessible to direct sampling. The test involves rinsing a large area of the surface and collecting the rinse water. Water used for rinsing should be heat sterilized, filter sterilized, or chlorinated followed by neutralization (28). The rinse water is usually added upstream of the process and collected downstream at various points in the process for sampling. The number of CFUs is then obtained from the rinse samples. Indirect sampling has the advantage of sampling a larger surface area and areas inaccessible without disassembling equipment. The disadvantages of indirect sampling are that it may not possibly provide enough shear force to remove biofilms physically attached to the surface and that large rinsing volumes yield high material and disposal costs (13).

Microbial assessment of contact surfaces is generally tested by direct contact methods. In direct surface sampling, the most common method is the swab test. The swab contact method involves using a sterile nonabsorbent swab (e.g., cotton, calcium alginate, Dacron, or rayon) to sample the surface (13, 27, 28). The swab is first moistened with rinse solution and then rubbed slowly and thoroughly over the surface to be sampled with rinsing after each swab. Generally, swabs are used to wipe a defined area (e.g., 25 cm²) of the surface and can be used for surfaces with cracks, corners, or crevices. Flexible stencils made of inert materials like Teflon® or silicon can be used to mark the area to be sampled. Since sampling is mechanical, attached biofilms can be sampled. After sampling, the swab is broken or cut and collected in small vials containing buffered rinse solution with appropriate neutralizers. The sample can be used for both qualitative and quantitative analyses. For quantitative analysis, accurate enumeration depends greatly on the ability of the swab to remove microorganisms from the surface, the release of the microorganisms from the swab into an enrichment media. The type of swab and media used for sampling greatly affects the number of microorganisms detected (74). Underestimation may be due to microorganisms becoming injured during swabbing (73). Other disadvantages of swabbing include a small sample area and low reproducibility and repeatability due to variation among sampling technique such as amount of pressure applied when rubbing (86). For instance, applying too little pressure may cause microorganisms to remain adhered to the surface and applying too much pressure can damage the swab (98). Self-contained media-based swabs include a semi-solid selective agar culture tube for microbial detection (73). These swabs have been found to be more sensitive than traditional swabbing techniques but only give qualitative data. Sampled

areas should be carefully cleaned after use with 70% alcohol to prevent agar residue from promoting growth (13).

An alternative to swabs are sponges, which can sample large surface areas and identify areas that harbor pathogens for better control. The sponge contact method involves using sterile cellulose or polyurethane sponges free of antimicrobial preservatives (28). When sampling, sterile tongs or gloves should be used to hold the sponge. The sponge is moistened with rinse solution and rubbed over the surface (up to several meters). After sampling, the sponge is placed in a sterile plastic bag. The sponge can be introduced directly into enrichment broth or diluent can be added to the bag. Before analysis the sponge should be massaged in the diluent to release the microorganisms.

Another commonly used method involves using solid culture media in the form of a RODAC plate (Replicate Organism Detection and Counting), which consists of a plastic dish containing convex culture medium (6). The RODAC plate (agar contact) method is a simple method for sampling flat surfaces that have been previously cleaned or sanitized. It should not be used for crevices, curved and irregular surfaces, difficult to access areas, or heavily contaminated areas. During sampling, a representative and random number of sites should be obtained (101). The RODAC plate should be free of condensation and pressed against the surface so that the entire agar meniscus contacts the surface for 5–10 seconds (28). Applied pressure and contact time need to be optimized. This method is more sensitive than swabbing when the surface density of attached cells on surfaces is low with a recovery rate of 80% versus 1%, respectively (86). When surface density is high, the RODAC plate method will underestimate the number.

Commercially available alternatives to RODAC plates are PetriFilm™ (2M medical-Surgical Division, St. Paul, Minn.), Con-Tact-It (Birko Chemical Corp., Dever, Colo.), and dip slides (BIOSAN Laboratories, Inc., Warren, Mich.) (28, 73). The Petrifilm™ aerobic count or direct-contact method is a simple way to sample flat or curved surfaces but should not be used for surfaces with crevices or cracks. Plates come sealed in foiled pouches and need to be prehydrated before use with sterile water. When sampling, the top film with the gel should be lifted and pressed against the sample surface. Then the top film is placed back onto the bottom film and incubated. The Con-Tact-it® method is also a simple method to sample surfaces. When sampling, a fresh piece of tape is removed from the dispenser and pressed onto the surface to be tested. Then it is pressed onto the Con-Tact-it Petri plate of selective media, leaving one zone as a control, and incubated. Dipslides, which consist of nutrient agar on a sampling paddle, are another method to sample surfaces. When sampling, the contact slide is removed from its tube and pressed against the surface to be tested. Then the slide is turned over and pressed again against the surface. The slide is returned to the tube and incubated for 24–48 hours. Contamination levels are assessed by using a comparison chart provided in the commercially available kits.

Given all sampling techniques have limitations that bias results, it is recommended to compare results from more than one method to obtain representative data (98). Good hygienic design of equipment should minimize problematic sites (e.g., joints, pipe corners, gaskets, and o-rings, and fasteners) where contamination may occur. Furthermore, materials used in food processing should withstand a wide range of temperatures and be durable so that the surface is free of cracks, scratches, and pits, and most importantly microorganisms (66).

21.3 Quantitative detection technologies for sampling

21.3.1 Standard detection technologies

Plating assay

Culture-based methods are traditional and still useful approaches for identification of bacteria isolated from food-processing surfaces due to the ease of use and low cost. These methods typically involve three steps: (i) growth of microorganisms in nonselective and selective enrichment to greater than detectable limits (detection), (ii) obtaining a pure culture of the targeted organism (isolation), and (iii) identifying the targeted microorganism and applying a confirmatory assay (identification and confirmation) (1). Given their reliability, they generally are considered the 'gold standard' for microbiological analysis (8). For nonselective enrichment, methods such as the aerobic plate count (APC) or standard plate count (SPC) are used to detect viable microorganisms (42). Selective enrichment and differentiating media are then used to test for desired species of bacteria and to prevent overestimating by accounting for harmless bacteria. Isolated colonies are often further characterized and/or identified using bacterial identification based-systems such as test kits (e.g., API 20E and API Listeria test strips) or nucleic acid-based assays to confirm the microorganism detected is correctly identified. Recently, emerging light-scattering technology using a laser (BARDOT) has been shown to nondestructively detect and distinguish bacterial species growing on a Petri dish with high specificity in seconds (8). The technique, however, is limited by the inability to detect microorganisms on membrane filters and the lack of defined scatter signatures for transparent colonies. Other promising emerging confirmatory assays are: mass spectrometry (18, 106), vibrational spectrometry (49, 50, 61, 77), and hyperspectral imaging (39, 71), which are discussed later.

A major drawback of culture-based methods is that only a small percentage of viable bacteria obtained from any environmental sample are culturable on a given medium. Conventional techniques recover less than 1% of the total species in a sample (70). Compounding the problem, injured or stressed bacteria may need time to recover and increase to detectable limits; and the lack of cultivability may be due to a variety of factors such as inappropriate growth medium or growth conditions, including concentrations of

oxygen or carbon dioxide that may not be optimal for the bacteria. Many bacteria from natural environments are in what has been termed a 'viable but nonculturable' or more recently referred to as an 'active but not culturable' state (68). These bacteria, although still active in the environment and potentially problematic in terms of food spoilage or sources of food poisoning, will not grow immediately on a selected culture medium. Another issue affecting accuracy is cell aggregation or clumping, which results in an underestimation of the number of bacteria present. Other disadvantages of conventional cultivation are that it is time-consuming, labor intensive, and may take days before results are obtained, particularly when the sample is a complex of microbial species.

ATP-bioluminescence assay

Adenosine triphosphate (ATP) bioluminescence measurement is a commonly used method for monitoring the presence of surface contamination (43). The technique determines the amount of total organic soil on surfaces based on ATP content (86). Luciferase and luciferin are added to the sample in a magnesium-containing buffer. The enzyme luciferase uses the chemical energy in ATP to drive the oxidation of luciferin to oxyluciferin. For each ATP, one photon of light is emitted and detected with a luminometer within 15 minutes (42, 47). However, since ATP is within all actively metabolizing cells, the method does not differentiate between microorganisms on the surface and ATP present in food residues. Additional steps need to be performed to segregate the microbial ATP, which are laborious and time consuming. Drawbacks of the ATP-bioluminescence assay are the lack of selectivity and dependence of the results on cell physiology. The limit of detection is 10^3 – 10^4 CFU/ml (86).

Epifluorescent direct count

Direct count (DC) methods using standard optical microscopy offer an excellent method to visualize and enumerate extracted remnants of biofilms. The methods detect all types of cells including: living, 'viable but nonculturable', and intact dead cells. Furthermore, visualization of the sample can identify systematic errors associated with cell aggregates or particles. To improve accuracy, the sample can be treated with a detergent or other dispersing agents to separate cells prior to visualization.

Although microorganisms can be directly visualized, stains such as acridine orange (12, 90) or 4',6-diamidino-2-phenylindole (DAPI) (68, 90) are commonly used to enhance visualization. For example, acridine orange binds to nucleic acids and when exposed to ultraviolet (UV) light, causes the cells to fluoresce in the green and orange wavelengths. To improve the limit of detection, the sample can be filtered onto commercially available stained black 0.2 micrometer pore size filter membranes. Another epifluorescence method involves binding an antibody with a fluorescent label to targeted microorganisms for visualization. Direct counting methods have

excellent limit of detection of approximately 10^3 microorganism/ml and provide great insight into sample consistency and make-up of biofilms. However, the approach is labor intensive, has difficulties differentiating live and dead cells, and cannot accurately determine the number of counts from clumped or particle associated biofilms.

Flow and chemical cytometry

Cytometry is a cell counting method that measures physicochemical properties of single cells. In flow cytometry, cells from a liquid suspension are hydrodynamically focused onto a small column with internal dimensions similar to the size of a single cell and are passed one by one through a detection zone that measures changes in optical or electrical properties. Cytometers can also be used to nondestructively sort cells to be used in further studies (19). Optical parameters that are measured include absorbance, light scattering, and fluorescence techniques. For instance, light scattering techniques can be used to determine the size of the particle passing through the detection zone; however, bacteria can be difficult to discern from some culture debris since they are similar in size. Thus, better analytical performance is obtained with larger cells such as mammalian cells. To increase selectivity, microorganisms can be stained or tagged with fluorescent antibodies for counting (110). Multiple tags can be used to detect and quantify multiple targeted microorganisms in a single sample suspension. The technique for homogeneous microbial populations has a limit of detection of $<10^3$ CFU/ml (24). The technique is useful for on-line monitoring to obtain a population distribution of a heterogeneous sample; but it also may require enrichment and use of sample preparation steps to ensure that only single cells are present in the analytical sample. In addition, cytometry use has been limited due to its cost and complexity with the need for trained operators to properly handle samples and waste (24, 68, 100).

Chemical cytometry detects chemical biomarkers obtained from a lysed cell. Biomarkers that can be analyzed are nucleic acids, proteins, and metabolites (19). The technique is currently slow and destroys the cells, but offers the capability of quantitative analysis and confirmatory assays in a single detection system. The technique has many configurations but can be coupled with other automated analytical separation techniques including one and two-dimensional capillary electrophoresis and amplification detection techniques (e.g., quantitative polymerase chain reaction technology). Using microfluidics and other advances in nanotechnology, investigators are working to miniaturize the technology for use in microchip format (7, 112).

Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay (ELISA) based on immunological techniques couples the specificity of antibody and antigen interactions with the sensitivity of enzyme assays to detect analytes (in this case

problematic microorganisms or their biomarkers) in a 96-well microtiter plate format (64). Common enzymes linked to specific antibodies in these assays are alkaline phosphatase, horseradish peroxidase, and beta-galactosidase. Enzyme-labeled antibodies bound to antigens then catalyze the conversion of a substrate into a measurable product. For example, alkaline phosphatase will dephosphorylate *p*-nitrophenyl phosphate (substrate) to produce *p*-nitrophenol, which is a detectable yellow-colored product with absorbance at 405 nm.

Different types of ELISA include direct, indirect, sandwich, and competitive. In the direct method, the antigen is bound to the surface of the plate and an enzyme-labeled antibody binds the antigen forming a surface bound complex. After washing to remove excess antibodies, the appropriate substrate for the enzyme is added and reacts to give a signal. If the signaling molecule accumulates in the well, the signal will be amplified. The amount of analyte containing the antigen (problematic cells or biomarkers) is quantified using a calibration curve. The indirect method is similar except it uses an unlabeled primary antibody and an enzyme labeled second antibody. Direct ELISA eliminates the risk of cross-reactivity of the second antibody but lacks signal amplification; and indirect ELISA has increased sensitivity but may result in additional nonspecific binding.

Sandwich ELISA involves sandwiching the antigen between two layers of antibodies. In this assay, a capture antibody is used to bind the antigen to the surface of the plate. After washing, a second enzyme-labeled antibody is added and also washed away. An appropriate substrate is used to quantify the amount of bound antigen; thus, the measured signal is proportional to the concentration. Competitive ELISA on the other hand uses labeled and unlabeled antigens to compete for surface-bound antibodies. Since the labeled antigens are used to quantify the concentration, the measured signal is inversely proportional to the concentration. The limit of detection for commercial ELISA kits is 10^5 – 10^6 CFUs/ml (95). Thus, a pre-enrichment step is needed for detection. ELISA assays can be used to quantify problematic microorganisms; however, the assay is prone to problems associated with non-specific adsorption (leading to false positives) and is time consuming with multiple reagent addition and sample washing steps.

21.3.2 Emerging or non-routine quantitative detection technologies

In biofilm research, various analytical technologies have been used to characterize biofilms and biofilm components (26, 105), study interspecies and intraspecies interactions, identify various types of 3-dimensional microbial architecture (52, 93), and study real-time dynamic responses and processes occurring within biofilms (80). Capital cost and the complexity of instrumentation currently may preclude their routine use in the food industry; however, several of these bio-analytical fields of study may potentially provide quantitative information and/or confirmatory results. Furthermore,

companies are in the process of developing application specific miniaturized equipment that may prove to be useful in the future.

21.3.3 Vibrational and spectrometry detection technologies

Vibrational spectrometry

Spectroscopic techniques, which measure quantized energy states of matter, have been used to detect absorption peaks or bands that indicate the presence of biofilms or components of biofilms, such as proteins and polysaccharides in wavelengths ranging from UV-visible to mid-IR. Mid-IR (10 to 2.5 μm) absorption spectrometry (typically Fourier transform infrared (FT-IR)) measures quantized vibration modes that indicate the presence of specific functional groups within molecules of a sample. With IR spectrometry, the absorption bands with the greatest intensity are associated with vibrational modes which produce changes in dipole moments. Fourier transform infrared spectrometry analysis allows the rapid determination of relative amounts of biomolecules (e.g., proteins and polysaccharides) from isolated dried or hydrated biofilms. For example, biofilms have been removed from surfaces, suspended in water, spotted directly onto IR-cards, dried, and analyzed by transmission methods (99). In real-time analyses, specialized substrata that totally reflect (attenuated total reflection) IR radiation are used to analyze biofilm formation (79). In addition, Fourier transform infrared spectrometry, which uses an interferometer, has been used to tentatively identify bacteria from isolated colonies by comparing unknown spectra with library data (49, 50, 61, 77). Limitations in FT-IR analysis of microorganisms include: selectivity, high limit of detection (approximately 10^8 cells), and interferences from water. Selectivity is a potential problem because infrared bands are rarely unique to biofilms and relative intensities of the bands within a given spectrum are dependent on: (i) the physiology of the cells within the biofilm, (ii) the expression of phenotypic makers, and (iii) the species and serotype of microorganism present. Thus, with spectral fingerprinting analysis, cells within biofilms should be isolated and/or enriched under the same growth conditions as those used for library development. In addition, water strongly absorbs mid-IR radiation and must either be removed from the sample (e.g., analysis using dried biofilms on IR-cards) or subtracted from the sample spectra (analysis of aqueous samples using attenuated total reflectance). In response to the need for inexpensive technology, many investigators and some analytical instrument companies are developing small handheld and less expensive spectrometers that could potentially be used for routine monitoring and/or confirming the presence of problematic microorganisms (21–23).

Near-IR absorption spectrometry (770 to 2500 nm) uses similar instrumentation to visible spectrometry, but provides more spectral information. The technology measures spectral bands that are associated with overtones

and vibrational modes, which yield excellent fingerprint data for identification of unknown materials with less interferences due to water. The technology provides similar spectral information to mid-IR spectrometry, with the advantage of being very inexpensive. The technology has been used in conjunction with multivariate data analysis to successfully identify and classify *Lactococcus lactis*, *Listeria innocua*, *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas mendocina* suspended in saline solutions (4).

Raman, like IR spectrometry, can be used to detect vibrational modes in molecules and to characterize and help identify chemical structure. With Raman spectrometry, light interacts with the sample, and some of the radiation is scattered at wavelengths shifted from the incident radiation (referred to as inelastic scattering). Chemical shifts are caused by vibrational modes of bonds in the sample molecule. The vibrational energy can be added to or subtracted from the incident radiation. Raman spectral data is considered to be complementary to IR data. In general, Raman active vibrational modes associated with changes in polarizability can detect totally symmetrical vibrations, which are not detected with IR spectrometry. Raman is considered to have a poor limit of detection because the inelastic scattering of light has low efficiency. The efficiency of inelastic scattering can be greatly improved when the energy of incident light approaches the energy required for an electronic transition. This phenomenon is referred to as resonance Raman spectrometry and can lead to signal enhancement up to 10^6 . Resonance Raman has been used to fingerprint bacteria. For example, the technique has been used to identify eight different strains of lactic acid bacteria from yogurt (34).

Surface enhanced Raman spectrometry (SERS) is another technique that dramatically increases the intensity of the Raman scattered light. However in this case, molecules are absorbed onto specialized surfaces. The surface enhancement occurs when light of specific wavelengths interacts with free electrons in the conducting band (surface plasmon) producing an intensified electromagnetic field on thin roughened metal surfaces or metal colloids (51). Metals are usually silver or gold with silver having a potential toxic effect on microorganisms. The stimulation of the plasmon creates an intense Raman signal with enhancements ranging from 10^4 to 10^{15} . SERS has been used to detect a number of bacteria including *Bacillus anthracis*, *Escherichia coli* K12, and *Salmonella typhimurium* (92). Micro-Raman technique has also been used to detect single bacteria and offers the potential to tentatively identify problematic microorganisms without enrichment (45, 46, 96, 102).

Mass spectrometry

Mass spectrometry is a vacuum technique that creates, separates, and detects mass ions (mass to charge ratio) from molecules of interest and has many types of configurations and platforms for various types of applications (37).

The technique is conceptually simple: the samples are ionized in a source and the ions are separated by a mass analyzer that in turn focuses the ions onto a mass detector. However, selection of the proper mass spectrometry platform requires knowledge of the molecule(s) of interest, potential interferences that may also be contained within the sample matrix, and the type of data required. Platforms vary by the sample preparation technique (e.g., chromatography), the type of ionization source, and the types and number of mass analyzers. For example, mass spectrometry is routinely interfaced with gas chromatography (electron ionization/chemical ionization source) and liquid chromatography (electro spray or atmospheric pressure chemical ionization) to analyze cellular components (biomarkers) that are specific to a particular microorganism. Matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry is used to identify proteins in proteomic research and to create spectral fingerprint patterns of bacteria isolated from agar plates for tentative identification (18, 106). As with mid-IR and near-IR, researchers and companies are working toward miniaturization of the technology while maintaining enough application specific analytical performance. Recently, a 10 kg tandem mass spectrometer (33) with a mass to charge limit of 2000 has been developed. The mass spectrometer uses an electrospray ionization source and a resonant ion ejection technique to analyze ions from proteins that were as large as 17000 Daltons (57). With further development, miniature mass spectrometers may also play a role in detection of biofilm contamination on surfaces or also be used as a detection system for a confirmatory assay.

Camera/imaging spectroscopy

Owing to the sporadic nature of biofilms on food processing equipment, no analytical technologies offer the ideal attributes that will allow inexpensive, noninvasive, rapid, and direct detection of biofilms on the surfaces of food processing equipment. Camera technology offers the ability to provide images that directly detect problematic regions on food processing equipment without the need for sampling. However, microcolonies can be translucent and small in size and thus are not readily detectable with standard visible light cameras. Hyperspectral or imaging spectroscopy is an emerging technology that is being used for detecting deleterious effects in food and agriculture systems (41). The technology, originally designed for use in remote sensing (38), collects simultaneous spatial and spectral information to help locate regions of interest on surfaces with proper illumination. For example, the spatial data is collected using a visible NIR camera system that is sensitive between 400 and 1000 nm, while the spectral data is generated with either acousto-optic or liquid crystal tunable filters. The results are typically in the form of a data cube, where the x and y axes represent the 2-D images, and the z-axis plots the spectral information collected from each pixel. The system can be operated in reflectance, transmission, or fluorescence imaging modes. Once a given system is well characterized with

hyperspectral data, multispectral imaging is useful in reducing the amount of data for storage. Multispectral imaging collects relevant 2-D images showing significant differences at selected wavelengths. The technology has been used to detect defects in foods such as apples (71) to allow more efficient sorting and fungal infections on citrus fruits before they are detected visually (39).

21.3.4 Antibody antigen-based detection technologies

A biosensor uses a biological element (e.g., proteins) to selectively recognize a targeted analyte (e.g., chemicals, biomolecules, viruses, pathogens, etc.) and create a signal, which in turn can be detected with an analytical transducer. The transducer converts the signal to an electrical form for further processing and potential quantification. Biosensors are usually inexpensive, potentially easy to use, and at least in part reusable or disposable. Typically, biosensors are classified by the biological recognition element and type of transducer used for detection.

Immunosensors

Biosensors using antibody (monoclonal or polyclonal) and antigen interactions to mediate detection are referred to as immunosensors; and use of the technology in the food industry has recently been reviewed (108). Common analytical transducers for immunosensors use: electrochemistry (3, 113), surface plasmon resonance (SPR) (9, 54), and optical (11), or piezoelectricity (44, 63) for detection. Measurements are made directly by capturing analytes (the antigen directly or the cell with the antigen) with attached, unlabeled antibodies using piezoelectric, SPR, or impedance immunosensors. Indirect measurements detect the label (e.g., enzymes, fluorescent, chemiluminescent, and visible) linked to the antibodies which usually lowers the limit of detection but adds cost to the system. As with most immuno-technologies, the antibody antigen interaction and non-specific adsorption play a key role in determining the selectivity of the device.

Piezoelectricity yields an electrical potential response on anisotropic materials (e.g., quartz or LiTaO_3) when a mechanical stress is applied to its surface. Piezoelectric immunosensors attach analyte-specific antibodies to the active surface of either quartz crystal microbalances (104) or surface acoustic wave (SAW) devices (44, 63). These immunosensors typically use the inverse piezoelectric effect, where an alternating potential is applied to or across the material to establish a surface bound wave. Quartz crystal microbalances typically create oscillations by coating an electrode on both sides of thin AT-cut quartz crystals to measure mass changes at its surface and have been used to monitor biofilm formation directly in liquids (78). The frequency of the oscillation is dependent on a number of factors including material and medium properties, temperature, and most importantly for sensors, adsorbed mass. Surface acoustic wave devices use two sets of

interdigitated electrode patterns, one to transmit the wave and the other to receive it, to create surface waves across surfaces (44). Depending on the cut and type of anisotropic material, different types of surface waves can propagate across the surface. These devices are smaller, more rugged, and most importantly operate at higher frequencies and thus are more sensitive to mass changes. Applications usually involve attaching the antibodies onto the surface of the device and exposing the surface to a liquid medium containing the pathogen or biomarker of interest to generate a change in frequency and/or phase. For example, *Escherichia coli* (*E. coli*) O157:H7 cells were detected using a pure shear horizontal SAW immunosensor (10); however, best results were obtained when the sensor was removed from solution and dried. In another study, approximately 2000 *Bacillus thuringiensis* spores were detected in aqueous solutions using antibodies attached to polystyrene or polyimide coated shear horizontal SAW immunosensor on LiTaO_3 (15). The SAW devices offer promise, but the technology has not been fully developed for wide spread utilization in the food industry.

Surface plasmon resonance (SPR) is another phenomenon that has been used to quantify antibody and antigen interactions in solution. SPR occurs in the free electron (conducting band) at interfaces of thin metal films and is excited with light (9, 54). At particular angles of incidence and under conditions for total reflection (light traveling through a transparent material with a higher refractive index), light impinging off the metal interface excites free electrons in a metal film (plasmon), which in turn causes measurable changes in the properties of the light at photodetectors. Although the underlying physics is conceptually complex, the instrumentation and resultant signals are relatively straightforward to understand. For example, *Listeria monocytogenes* cells were detected using the single-chain variable-fragment (scFv) antibody physically adsorbed onto a SPR sensor with a limit of detection of 2×10^6 cells (76). Laboratory-based systems have been commercialized and used to study the kinetics and thermodynamics of antibody and antigen interactions. A number of advancements in optoelectronics, integrated optics, microfluidic and nano-fabrication techniques have made the technology more attractive for pathogen detection in the food industry (53).

Impedance measurements involve applying an oscillating voltage perturbation onto a system of electrodes and monitoring the alternating current response. In impedance microbiology, commercially available instrumentation has been used for a number of years to quantify microbial growth or activity in fermentation by directly measuring changes in conductance of a liquid medium. This conventional technology usually has a limit of detection ranging from 10^6 to 10^7 cells/ml and by measuring increases in biomass, can be used to distinguish living from dead cells (103). Initial application of the technology for pathogen detection involved the development of growth medium that (i) optimizes the sensitivity of

the response and (ii) increases the medium specificity for a particular pathogen or group of pathogens (e.g., *Salmonella* in foods) (35). More recently, impedance spectroscopy, which stimulates the electrode system at a wide range of frequencies, has been used to develop immunosensor technology. The presence of pathogens can be determined using double interdigitated electrode arrays in which a pair of addressable array of finger-like parallel electrodes is fabricated onto surfaces in an interlacing pattern (67, 113, 114). These interdigitated electrode systems have been used to follow the growth of *Salmonella* and *E. coli* (62). The technology was further improved with the miniaturization of the electrode systems onto a microchip device and the integration of a concentration technique using dielectrophoresis (DEP) (40). DEP is the translational motion of particles as a nonuniform electric field acts on dipoles or induced dipoles (88, 89). The concentration technique used microfluidics, a cell deviation electrode, and a set of interdigitated microelectrodes to concentrate two species of *Listeria* by a factor calculated to be between 10^4 or 10^5 . Once concentrated, the cells are transferred to a smaller chamber using impedance and a second interdigitated microelectrode system for detection. This concentration step decreased the response time by several hours to approximately 1 hour for a culture with a final concentration of 8×10^5 *L. monocytogenes* cells. In a different microchip set-up, 9×10^5 *E. coli* cells/ml were detected using a microfluidic chip, antibodies, and impedance base detection system (14). In that study, the antibodies were coated onto a glass coverslip on a surface opposite of the electrode system. As the cells become trapped by the antibodies on the glass coverslip, the number of cells in solution decreases, thereby attenuating the conductance signal, which in turn is used to quantify the number of cells.

Magnetoelastic immunosensors have been developed to improve the sensitivity of direct immunosensors (97). The transient response to a magnetic impulse applied to the sensor is captured and converted using a fast Fourier transform to obtain the resonant frequency, which provides chemical and environmental information. In the food industry, magnetoelastic immunosensors have been used to detect pathogens (e.g., *E. coli* O157:H7) by monitoring antibody-antigen reactions (97). An enzyme catalyzes a reaction to form an insoluble product that deposits on the sensor surface, which changes the mass and in turn the resonance frequency. Changes in resonance frequency can then be correlated to cell concentration (CFU/ml) by a calibration curve. Recently the technology has also been used to detect in real-time pathogens in liquid medium (55). The microorganisms' consumption of nutrients and growth change the viscosity, which changes the resonance frequency. The technology is highly sensitive with a limit of detection of 10^2 to 10^6 CFU/ml (55, 97). Other advantages of magnetoelastic sensors include being cost effective and small, while yielding comparable results to optical, electrochemical, and mass-sensing methods (97).

Although many antibody antigen-based configurations show promise, no platform is able to detect pathogens directly from a food contact surface, thus enrichment steps such as growth in a liquid medium are required prior to detection. Enrichment is advantageous in some ways since it: (i) provides a mechanism to increase the concentration of cells, (ii) minimizes the effect of interferences, and (iii) may decrease the amount cell aggregations or biofilm fragments in swabbed or rinsed biofilm samples. Drawbacks associated with enrichment are the dramatic increase in analysis time, the capability to enrich only a subset of the population, and the introduction of systematic errors associated with variables with lag or inhibitors when modeling the growth data. In addition, some of these detection systems may also require washing steps to remove materials that non-specifically adsorb to the antibody. Improvements in the analytical performance of these techniques are being made with new microchip technologies that integrate sample preparation steps.

Lateral flow immunoassays

Similar to an immunosensor, an immunoassay involves antibody and antigen interactions in an assay to detect analytes but without an on-device transducer. Therefore, in the strictest sense immunoassays should not be classified as a biosensor. Immunoassays have many configurations, with some of the simplest to use being lateral flow immunoassays (LFIAs) (Fig. 21.3), which are also sometimes referred to as immune-chromatographic assays (91). LFIAs involve applying a test liquid onto a sample pad and allowing capillary forces to draw the sample through the device. Liquid applied to the sample pad is wicked over a conjugate pad (containing reagents) and onto a chromatographic strip membrane (e.g., nitrocellulose) and onto a chromatographic strip membrane (e.g., nitrocellulose)

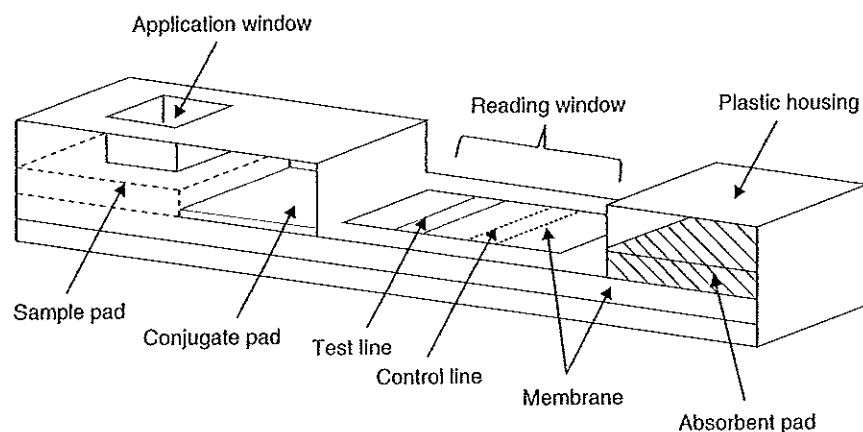


Fig. 21.3 Lateral flow immunoassay (LFIA) adapted from Posthuma-Trumpie, Korf *et al.* 2009 (91).

with zones (e.g., lines) of dried and/or immobilized reagents. The zones are used to provide selectivity and/or a means to visually detect the analyte and control. Excess sample is trapped by an absorbent pad.

As in ELISA, sandwich and competitive assay formats exist for LFIAs. The sandwich assay typically has labeled antibodies in the conjugate pad and a second antibody at the test line. When analyte is present in the sample and passes across the conjugate pad, it binds to a labeled antibody. This antibody-labeled analyte complex will then form a sandwich complex with the antibody at the test line and yield a positive signal. In contrast, a competitive assay has labeled analytes in the conjugate pad and an antibody at the test line. Thus, when an analyte is present in the sample, it will compete with the labeled analytes in the conjugate pad for the antibody at the test line. Generally, labels are colored or fluorescent nanoparticles. Color signals can lead to ambiguous results in cases where the analyte concentration is near the limit of detection resulting in false positive and false negatives, whereas fluorescent signals are quantitative and have a better limit of detection but require devices to readout the signals. In order to eliminate ambiguity and obtain more quantitative results, manufacturers are looking to develop and launch handheld and portable LFIA readers (29).

Presently, immunoassays are most often used in medical or home diagnostic kits (e.g., pregnancy test) but are also being used to detect pathogens in food samples due to rapid detection (10–20 minutes) (91). Typically, pathogens from enrichments are detected directly with sandwich LFIAs. For the food industry, commercially available kits are available for detection of many pathogens, including *Escherichia coli* O157, *Listeria*, and *Salmonella* to allow screening of food samples after enrichment. This pathogen LFIA technology is similar to ELISA commonly used in a 96-well format, where in sequential steps a targeted pathogen is captured with a surface-bound antibody, tagged with second enzyme labeled antibody, and quantitatively detected when the enzyme interacts with a substrate to produce a detectable signal. LFIAs can also be used to detect RNA, DNA and protein from specific pathogens (16).

21.3.5 Nucleic acid-based detection technologies

Polymerase chain reaction-based approaches for bacterial detection

The polymerase chain reaction (PCR) is a powerful tool for the identification of bacterial pathogens on food preparation surfaces. Real-time PCR can be used to quantify target sequences, and it has a broad dynamic range. PCR primers may be developed to be specific for a certain organism/gene or can be designed to detect certain genera or classes of organisms. For example, studies have been designed to characterize the presence of *E. coli* O157:H7 by targeting the PCR primers to be specific for Shiga toxin genes

or to the genes encoding the O157 antigen. In contrast, more general primers may target the *E. coli* ribosomal RNA genes (particularly the 16S rRNA gene). Ribosomal RNAs contain regions that are highly conserved, for identifying general classes of organisms, including the different domains, Bacteria and Archaea, as well as regions that are more divergent. The diverged regions may be used to identify organisms to the genus and often the species level.

Polymerase chain reaction analysis of 16S rDNA may be used to study community structure. Denaturing gradient gel electrophoresis (DGEE) is used to separate the PCR products based on the annealing properties of the double-stranded DNA molecule, rather than the size of the PCR product (32, 58, 75). For these studies, a 'clamp' is added to the end of the PCR product by using PCR primers with a 5' end that is rich in guanine/cytosine bases. This gives the PCR product a region that does not denature. Using a denaturing gradient gel, the PCR products migrate on the gel based upon their denaturing properties (i.e., when the remainder of the PCR product melts to single-stranded form), allowing separation of the products based on their sequence, rather than their size. For community structural analysis, each community will give a different banding pattern on a DGEE gel. For each community, the bands on the gel may be extracted and sequenced for species identification.

Two potential drawbacks for using DGEE for biofilm community profiling are PCR amplification bias – some sequences may amplify more efficiently than others, and the resolving power of the DGEE gels – the community may have many more species than may be resolved on the gel. The development of high-throughput sequencing approaches has allowed greater resolution of microbial community structures. For these experiments rather than using electrophoresis to resolve PCR products, the PCR products (for example, targeting 16S rDNA of a microbial community) are cloned *en masse* into a plasmid vector. Each PCR insert may then be sequenced, providing a qualitative assessment of community structure, generally displayed using a phylogenetic tree, as well as a semi-quantitative analysis, providing information on the number of different types of organisms obtained. The PCR amplification and sequencing strategy also suffers from potential amplification bias.

Another potential drawback of PCR amplification strategies (either DGEE or cloning) is that the PCR reaction cannot distinguish between viable cells, dead cells, or simply DNA released into the environment by cell lysis. Recently, Nocker *et al.* used a combination of PCR with propidium monoazide to enrich for PCR products from only viable cells (81). DNA-intercalating dyes are used to differentiate live versus dead cells, since they often only penetrate into cells with compromised membranes (dead cells). Once intercalated into the DNA of dead cells or extracellular DNA, propidium monoazide inhibits the PCR reaction. Using this dye, only DNA from cells with intact membranes is amplified, providing an indication of

not only the community structure, but also the viable cells in that community. This approach has been tested on pathogens that contaminate food, including *Listeria monocytogenes*, *Salmonella typhimurium*, and *Escherichia coli* O157:H7 (82).

Quantitative polymerase chain reaction and quantitative reverse transcriptase polymerase chain reaction

Gel-based approaches for PCR analysis are generally not quantitative since a PCR amplification step is required. However, quantitative PCR (qPCR) and quantitative reverse transcriptase PCR (qRT-PCR) now provide excellent approaches for determining the number of specific DNA or RNA molecules in a sample, respectively (36, 48, 60, 83). qPCR and qRT-PCR are very sensitive and may be used on samples with small amounts of biological material, such as swab samples from food processing surfaces. The techniques also have a large and generally linear dynamic range. Commonly used qPCR methods are the SYBR green labeling approach and the dual-labeled probe approach. In the SYBR green approach, a fluorescent dye is incorporated into the PCR product. As the amount of PCR product increases during the PCR cycles, the amount of fluorescence that is detected also increases exponentially. A threshold cycle (Ct value) is obtained during the exponential increase of the PCR product/fluorescence and compared to a linear curve of values for standards with known amounts of starting DNA. The amount of starting DNA for a particular gene may then be determined for each unknown sample. In the dual-labeled probe approach, a DNA probe that is specific for the PCR product of interest is labeled with both a fluorescent molecule and a fluorescence quenching molecule. While both molecules are contained on the DNA probe, fluorescence is quenched. As the probe is degraded during the exponential increase of the PCR product by *Taq* polymerase, the quenching molecule is released from the fluorescence molecule, resulting in increased fluorescence. Fluorescence is detected, and a Ct value is obtained, and compared to standard curves. qPCR may be applied directly to the DNA isolated from samples. For analysis of gene expression, mRNA is isolated from cells and converted to cDNA by reverse transcriptase prior to performing the qPCR reaction.

The advantage of the SYBR-green approach is that it is the most cost-effective. In addition, primer specificity may be determined by a melt-curve analysis. Following the PCR reaction a melt curve analysis may be performed. If only one peak is observed that generally indicates that the PCR primers were specific for a unique PCR product. While the cost of the dual-labeled probes is higher than that of the SYBR-green dye, an advantage of dual-labeled probe analysis is that it allows multiplexing. Different probes may be labeled with different color fluorescent dyes. Therefore, multiple PCR products (for example, designed for different genes or for different organisms) may be produced in one

reaction, allowing more rapid analysis of community structure or multiple gene expressions.

For detailed protocols on isolation of DNA and RNA from samples with small amounts of biological material, please refer to protocols by Perez-Osorio and Franklin (84, 85).

21.3.6 Microscopy detection technologies

Fluorescence in situ hybridization (FISH)

While PCR techniques allow amplification of DNA or cDNA from low biomass samples, approaches are available that allow direct probing of the samples without an amplification step. Fluorescence *in situ* hybridization (FISH) allows direct probing of samples using nucleic acid probes that are specific for a target sequence. As with PCR-based techniques, the probes may be specific for a particular genus or more general, to detect different domains of organisms. Generally, rRNA is targeted for hybridization, since the rRNA is the most abundant RNA in the cells, and therefore provides the greatest signal (5, 25, 72). Targeting mRNA is also possible, but generally requires an amplification step, since individual mRNAs are in low abundance. *In situ* reverse transcription polymerase chain reaction (IS-RT-PCR) was used to amplify mRNA present in low copy number from intact cells (69). However, this technique is not quantitative for the gene expression since the amplification step is required.

Using FISH, cells are treated to allow penetration of the probes into the cells. The fluorescently labeled probes then hybridize to the target molecule, usually the rRNA target, and cells from a given group are quantified using epifluorescence microscopy. Recently, difficulties with FISH, such as lack of penetration of the probes through extracellular matrix material and low fluorescence intensities, have been addressed by modifying the approach. For example, PNA-FISH uses peptide nucleic acid probes (65). These probes while still specific for a certain target sequence use a peptide backbone that has increased penetration into cells and through biofilm matrix material. CARD-FISH (catalyzed reporter deposition) is another advantage, in that the probes provide increased fluorescence intensity and therefore enhanced detection of target sequences (30, 31, 87).

Most of the PCR and probing techniques described above are specific for target nucleic acids. However, the techniques generally do not provide an indication of cell viability or activity. FISH is often combined with microautoradiography (MAR) to determine cell activity. For MAR, cells are exposed to a radiolabeled substrate, allowing the cells to incorporate the label into cellular material. The active cells may be detected by microautoradiography, using a photographic emulsion. This approach, when combined with FISH allows cell identification as well as an indication of cell activity. The FISH-MAR technique can be tedious and is not easily amenable to the rapid study of many samples (111).

Live-dead staining

Alternative approaches for examining cell viability involve differential staining with fluorescent stains followed by epifluorescence or confocal scanning laser microscopy. Among the stains commonly used are the 'live-dead' stains. The 'dead' stain, such as propidium iodide primarily enters cells with compromised membranes, and therefore does not generally stain viable cells. Counterstains that stain all cells are then used to distinguish the viable cells from the dead cells. This approach must be tested on each system to ensure that the individual stains are specific for the live and/or the dead cells. Tetrazolium dyes such as 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) can be useful for studying actively respiring cells within a population. CTC is a soluble, non-fluorescent substrate that forms a red fluorescent precipitate when it is reduced by respiring bacteria. Therefore, actively respiring cells may be detected using CTC. As with the live-dead stain, the specificity of CTC dye must be tested for each system. Recently, Rani *et al.* used pulse labeling with the thymidine analog 5-bromo-2-deoxyuridine to label cells that are actively synthesizing new DNA (replicating cells) (94). Following DNA labeling, the brominated DNA was probed with fluorescently labeled antibodies. This approach represents an alternative to examining cell viability by characterizing cells that are actively incorporating nucleotides into their DNA.

21.4 Conclusions

In general, successful monitoring of food contact surfaces with respect to microbial contamination is ultimately dependent on the attributes (e.g., limit of detection, specificity, precision, accuracy, etc.) of the analysis methodology and the statistical sampling plan. However, the implementation of a particular analysis strategy is not solely dependent on the merits of the method but also on economic considerations. Thus, evaluation of analysis strategies are based on the ability of the assay to: (i) provide precise and accurate data in a timely and cost-effective manner, (ii) provide the proper degree of sensitivity and specificity, and (iii) provide a statistically significant result. Furthermore, although presence/absence assays may suffice, accurate quantification of the number of viable microorganisms is desirable since it provides a measure of the level of risk associated with the contaminated product. Once quantified, a confirmatory assay that has a high degree of selectivity should be applied to verify results.

Ideally, quantification of microorganisms on food contact surfaces could be performed directly to eliminate the sampling step, which introduces sampling error. However, currently no such technique exists, so sampling strategies must be considered when developing a sampling plan in order to minimize error and obtain precise and accurate results. Accurate assessment of food contact surfaces integrates both representative and critical

control points. These critical control points should include areas identified as problematic such as corners, cracks, and interfaces, which can harbor microorganisms and are difficult to clean. Since all sampling methods are limited, using more than one sampling method is recommended to obtain representative data.

Plate count assays quantify the number of bacteria growing at a given set of nutrients, thereby providing an estimate of the number of viable bacteria in the sample. The methodology is sensitive but provides only limited specificity and does not provide rapid results. Delays in obtaining results are a source of lost revenue and are a major reason why companies are seeking new methods. Plate count assays can underestimate populations since no plating medium supports growth of all types of microorganisms. The lack of specificity can be a crucial flaw if the assay is compromised by the presence of viable but nonculturable problematic microorganisms (underestimates risk) or by culturing harmless bacteria (overestimates risk). Other sources of systematic errors are biofilm fragments causing cell aggregates or contaminants detected on growth plates. Plating on selective medium should be considered presumptive evidence, and should be verified by a confirmatory test. Bioanalytical techniques including infrared, Raman, and mass spectrometry can be used for confirmatory testing if more inexpensive instrumentation that allows fingerprinting and library searches from bacterial isolates becomes available.

Immunosensors and immunoassays like impedance-based immunosensors which can detect living cells may prove to offer more rapid analysis and be more selective than plating assays. However, these detection systems currently have problems associated with selectivity because of nonselective binding of samples. Therefore, a second confirmatory assay should also be used with these sensors and assays to verify results. In addition, investigators should recognize that samples may contain food ingredients and biofilm fragments that will complicate detection and/or quantification. Additional sample preparation steps may be necessary for more accurate and precise results.

Molecular microbiological detection strategies have variable specificity allowing the detection of all bacteria, as well as particular groups of bacteria (e.g., *Salmonella* species), or even a distinct strain or serotype (e.g., *E. coli* O157:H7). Thus, nucleic acid based analysis strategies can be used as indicator and confirmatory assays. For example, determination of the total bacterial concentration of the product can indicate a problem, while analyzing for specific harmful bacteria confirms the existence of the problem. Instruments capable of automated and real-time detection of PCR products are presently available and provide results in hours rather than days.

Each of the quantitative detection technologies for sampling of food contact surfaces surveyed in this chapter have limitations and fall short of attributes for an ideal detection method: cost effective, rapid, selective,

sensitive, and rugged. Advancements and continued developments in the various technologies are making progress toward these goals. The majority of these technologies have the potential to be used for sampling of food when fully developed.

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