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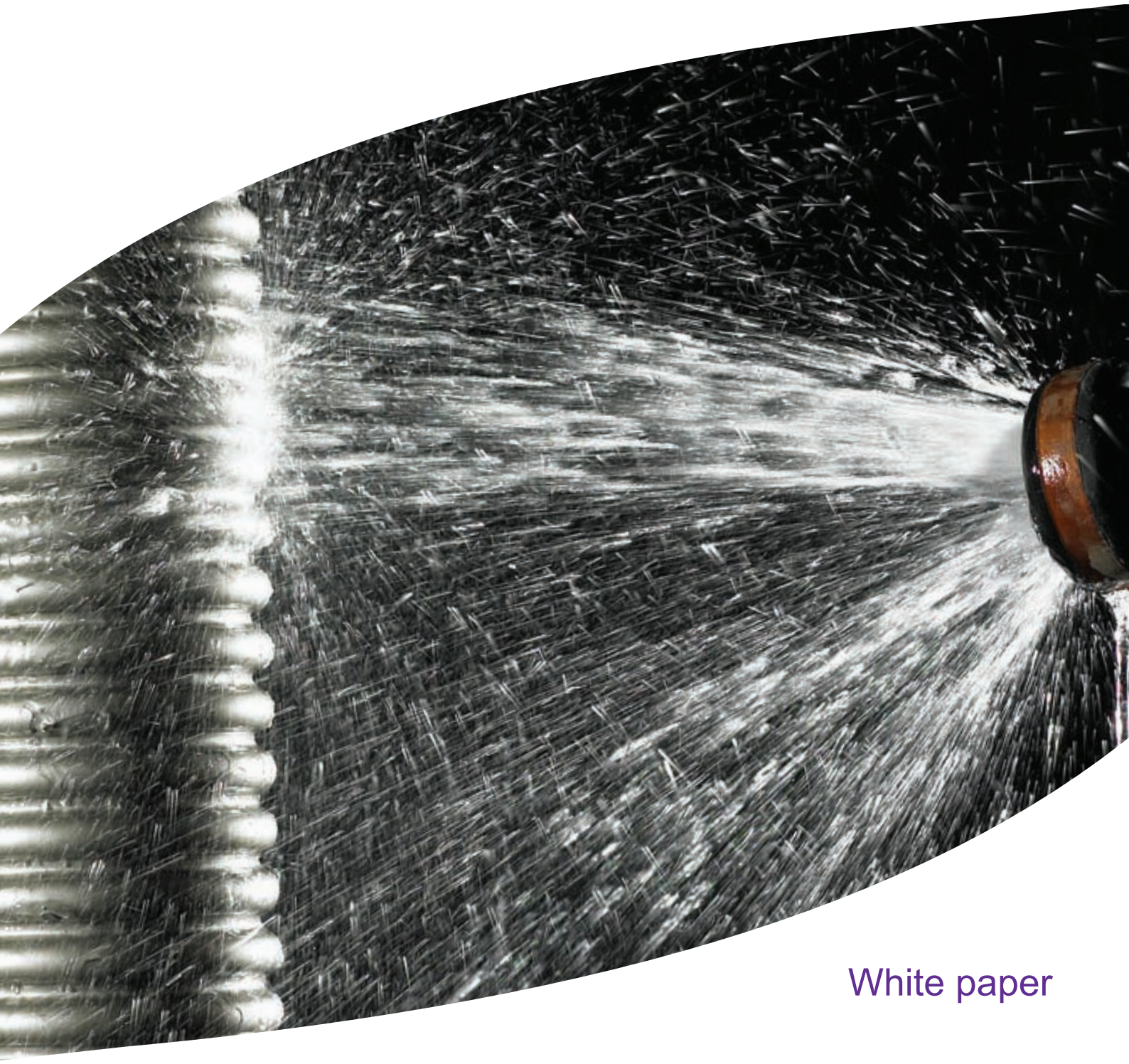
food and drink innovation

Rapid methods for hygiene determination



White paper

Campden BRI, Station Road, Chipping Campden, Gloucestershire, GL55 6LD. UK
Tel: +44(0)1386 842000
www.campdenbri.co.uk support@campdenbri.co.uk

Rapid methods for hygiene monitoring

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It is important in the food and drink industry that all production equipment is kept hygienically clean. Food and drink manufacturers have a legal obligation to demonstrate the efficacy of their hygiene procedures for equipment washers, manual cleaning practices and cleaning in place (CIP). There are many commercially available rapid methods to provide evidence of the effectiveness of hygiene actions. Manufacturers need to ensure the methods they use are cost effective, appropriately validated and meet verification requirements that demonstrate the effectiveness of the cleaning regime. This summary document has been written to help food and drink manufacturers understand the rapid methods for hygiene verification that are available to them.

If this is an area in which you need technical support, please get in touch.

Anna Falowska
anna.falowska@campdenbri.co.uk +44(0)1386 842271

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Contents

Rapid methods for hygiene monitoring

Hygiene procedures are designed to remove debris and contamination from a previous production run and sanitise equipment prior to starting the next run. Manufacturers must ensure that the established cleaning and sanitation procedures reduce the microbiological, allergenic and residual products to satisfactory level. Additional to this, the cleaning and sanitation solutions used should not leave a residue. The methods for hygiene verification should provide results that allow manufacturers to demonstrate that food safety risks control measures work. These methods must be simple, rapid and robust.

The most popular and established rapid hygiene monitoring techniques are ATP (based on the detection of adenosine triphosphate by bioluminescence) and protein detection. For each, there are kits designed to provide rapid results and to be used by unskilled personnel to assess the effectiveness of the cleaning procedure. However, these techniques can only be used as an indicator of general hygiene as they lack specificity. The ATP techniques detect ATP which is present in a wide range of cells from humans, microorganisms as well as food debris. Protein analysis uses a chemical colour change to detect total protein amount on a surface after cleaning to give an indication of allergen removal. This summary document reviews some of the latest hygiene methods for rapid, specific detection of contaminants to allow manufacturers to prevent and control food safety risks during production. The methods are divided for specific residues (microorganisms, allergens, meat species). The summary document describes the principle of working, strengths, limitations and provides the examples of commercially available kits/systems where applicable.

Microbiological contamination

Optical assay

Optical assays use a broth medium containing unique dyes which indicate metabolic activity as microorganisms grow. Microbial growth is detected by changes in either colour or fluorescence as metabolic processes take place. These changes (expressed as light intensity units) are detected by the optical sensor within the instrument and captured by the computer software for analysis. Various dyes, which are indicators of metabolic activity can be utilised in these systems. Swab samples are placed into pre-filled test vials containing ready to use growth media and indicators for analysis.

Most optical based detection technologies use test vials containing two compartments: an upper incubation zone, where the sample is added, and a lower reading zone. This allows the sensor to detect changes in colour or fluorescence without interference from components of the sample. The time to detection is recorded as the time taken for the organisms to reach the set threshold required to achieve a colour change within the medium. It is reported that approximately 100,000 cells/ml for bacteria, and 10,000 cells/ml for yeast and mould (include references for these limits). Typical times for detection are claimed to be within 8-18 hours for a single bacterium, 20-30 hours for a single yeast cell, and 35-48 hours to detect mould cells. Several factors influence the time for detection for a particular sample including the initial concentration of the microorganism, their generation time as well as exposure to stressful conditions such as cold storage or biocide treatments.

Commercially available optical systems on the market include BioLumix® and Soleris® supplied by Neogen. These systems can detect a range of organisms including total aerobic microbial count (TAMC),

total combined moulds and yeasts count (TCMY), enterobacterial count (bile tolerant gram-negative bacteria), *Escherichia coli*, coliforms, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella* and lactic acid bacteria.

Advantages of these types of systems are:

- Simple to use - minimal training required for changes in colour and swabbing. The vials are incubated in the instrument and monitored by instrument. The results are presented as soon as detection occurs without any operator involvement.
- Multiple tests can be operated simultaneously providing they have the same incubation temperature
- One person could operate multiple machines
- Broad detection range from single cells to highly contaminated samples
- Systems provide automated determination of detection time (DT) and data archiving
- Capability to set alerts for contaminated samples
- Flexibility of format for reporting results which can be communicated with any internet protocol (IP) network in real-time

Impedance microbiology

Impedance microbiology is based on the measurement of changes in electrical impedance of a culture medium or reaction solution resulting from bacterial growth. The level of bacteria is monitored by measuring changes in impedance at regular time intervals during growth at appropriate temperature (Moldenhauer, 2005). The measurement of impedance in culture media can be performed in either a direct or indirect way. Direct detection uses a pair of electrodes located in the bottom of the well which monitors the changes in impedance of the growth medium caused by bacterial metabolism. Changes in impedance are mainly produced by the release of ionic metabolites from live cells. One of the sources of ionic metabolites during microbial growth is the breakdown of larger molecules into smaller charged molecules (e.g. proteins into amino acids and polysaccharides or sugars into lactic acid).

Indirect detection of impedance uses electrodes immersed in a separate solution (usually a potassium hydroxide solution) instead of inoculated growth medium. Carbon dioxide produced as microorganisms grow is absorbed by the potassium hydroxide solution, which leads to a decrease in the conductance of the alkaline solution.

There are several commercial analytical instruments based on the impedance microbiology for food samples such as Bactomater (bioMérieux), rapid automated bacterial impedance technique (RABIT) (Whitley Scientific Ltd) and Malthus system (Malthus Instruments Ltd). The BacTrac® and µ-Trac® systems from Sy-Lab are designed for environmental monitoring and can detect Enterobacteriaceae, coliform, *E. coli*, *Salmonella*, *Listeria* spp., coagulase positive *Staphylococci*, yeasts and moulds as well as total viable count (TVC).

Advantages:

- Minimal training required for sample preparation and results reading (results are colour coded: pass = green, fail = red). Pre-prepared medium is supplied by the manufacturer to provide optimal impedance signals
- Sample incubation process is monitored online
- Automatic classification of results, measuring cycle and results recording.
- Reported time to result of 14-24 hours with changes in impedance detected faster than measuring turbidity

The individual measurement positions can be used whenever samples are available to be tested. Some of the systems allow more than one detection at the same time because of two different incubation zones within instrument

The ability to distinguish between viable and dead cells. Generally, the detection time requires bacterial concentrations to reach approximately 10^6 - 10^7 cfu/ml (Yang and Bashir, 2008). Higher contaminated sample needs shorter time to reach the detection threshold. As this method monitors impedance change in the medium it requires a selective medium to support growth of the target bacteria.

Colorimetric detection of carbon dioxide production

This techniques measures carbon dioxide (CO_2) produced as a metabolite as microorganisms grow in a liquid medium. CO_2 diffuses and interacts with liquid medium decreasing the pH and leading to changes in colour of the medium (e.g. yellow to green). The amount of CO_2 detected is dependent on the initial concentration of the microorganisms, with lower initial concentrations providing a slower detection response.

The kits/swabs are commercially available and supplied by companies such as Proteus (CLEANPIX™ Test) and ECOLAB® (Klerkit Sterikit). Environmental swabs are added to the culture bottle which is incubated, agitated and monitored for presence of microorganisms.

Advantages:

- Easy to use; the technology is designed to test surfaces directly without sample processing
- Minimal training and no specific laboratory equipment is required
- Easy to read; the positive result (colour changes) is easily identified by the naked eye
- Rapid results; depends on the level of contamination. Results are within 24-36 hours depending on the microorganism; however highly contaminated samples are detected within 6-8 hours
- Pre-moistened swab is capable of neutralising commonly employed detergents and sanitisers

Disadvantages:

- Variations in growth rates between strains makes this technology unable to use it for quantifying populations of mixed and/ or unidentified strains.

Chromogenic culture media

Chromogenic media are developed to detect enzyme activity known to be specific to the organism of interest. The media are produced in two formats - broths and agars and will typically contain a substrate which is bound to a soluble colourless molecules (chromogens), and a chromophore. As the target organisms grows, it produces the specific enzyme which breaks down the substrate allowing the chromophore to be released, producing coloured colonies or a change in broth colour. The distinctive coloured colonies on agar enable the target organisms to be detected more easily amongst other microbes which could be present in the sample.

Chromogenic media are available to detect a variety of microorganisms in food and environmental samples including *Listeria* spp., *Listeria monocytogenes*, *Salmonella*, coliforms, *E. coli*, Enterobacteriaceae, *Staphylococcus aureus*, yeasts and mould as well as the total viable count. There is a range of companies which offer chromogenic culture media for microbial detection; however most of them require self-preparation. Products such as SwabSURE (Technical Service Consultants Ltd), InSite™ Listeria/Salmonella (Hygiena), RIDA® STAMP and RIDA® COUNT (R-Biopharm Rhone Ltd), Compact Dry™ (TSC Biosciences), Liofilchem® Chromatic™ (Liofilchem®), CHROMID® Carba Smart (bioMérieux), RAPID' Chromogenic Media (Bio-Rad Laboratories Ltd) and Path - Chek® (Microgen Bioproducts) are examples of ready to use plates or self-contained tubes suitable for hygiene monitoring.

Advantages:

- Cost efficient working processes, time reduction
- Minimal training is required for sample preparation and running the test. The tubes/plates are simple and quick to use with no mixing of reagents or multiple steps
- Visual interpretation of results without the need of special skills or instruments. The colour changes are clearly distinguishable with the naked eye
- Sensitivity of 95.5 - 100% (Tavakoli *et al*., 2008)
- Selectivity (differential ability) of 98.5 -100% (Tavakoli *et al*., 2008)
- Availability of self-contained tubes, ready-to-use plates eliminate risk of contamination.
- Reduction in false positives due to the presence of inhibitors for non-targeted microorganism and growth enhancers for aimed microorganisms in the media. Hygiena® claims that 'InSite Environmental *Listeria* Species' is able to detect heat injured *L. monocytogenes* at low levels (10-50 cfu/ml of broth) even in the presence of high numbers of competing microorganisms.
- Reported to support the survival of damaged/stressed microorganisms (Tavakoli *et al*., 2008)

Disadvantages:

- Ready-to-use plates which do not use swabs can only be used on reasonably flat surfaces
- The method includes incubation step which requires block heater or incubator which are not supplied

Real time polymerase chain reaction (PCR)

One of the most commonly used nucleic acid-based methods for microbial detection is polymerase chain reaction (PCR). PCR operates by amplifying a specific target DNA sequence in a cyclic three step process. In the first step, the target double-stranded DNA is subjected to high temperature to split up the double stranded molecule into single strands. Secondly, two synthetic oligonucleotides (primers) specific to the selected target gene of interest bind (anneal) to the DNA strand. After the primers have annealed, DNA from the target region is synthesised by an enzyme known as DNA polymerase.

PCR amplification products (amplicons) can be detected by electrophoresis in gels containing ethidium bromide. Advances in technology have enabled DNA amplification to be measured as the reaction occurs using real timing fluorescence dyes that can report DNA synthesis. The amount of PCR amplicons produced is proportional to the fluorescence intensity (Omiccioli *et al*., 2009, Zhao *et al*., 2014).

There are many commercially available real time PCR systems for pathogen detection; however only selected assays are suitable for environmental monitoring. The examples of real time PCR kits include, BAX® System X5 (Hygiena™), BAX® System Q7 (DuPont Qualicon), TaqMan™ (Applied Biosystems™), MicroSeq® (Applied Biosystems) and foodproof® (Biotecon Diagnostics). The assays for hygiene monitoring detect microorganisms such as *Salmonella*, *L. monocytogenes* and *Enterobacter sakazakii*.

Advantages:

- Fast processing time which is typically 1-3.5 hours after enrichment step
- User friendly commercial PCR systems with software to analyse raw data and give automated results which eliminates the need an expert for interpretation
- The method is highly sensitive; with reported limits of detection 10^4 cfu/mL after enrichment (Law *et al*., 2014)

Disadvantages:

- Enrichment step required before PCR set up of 10-24 hours depending on assay and media
- Sample preparation requires trained, skilled personnel
- Additional equipment often required such a pipettes heat blocks and microcentrifuges
- A potential for DNA cross contamination to occur; which can be reduced using good lab practice and a closed tube system
- PCR can amplify DNA from both live and dead cells, however some commercial detection systems include steps that eliminate the impact of DNA from dead cells. These include:
  - Additional sample dilution and growth stage pre-PCR to reduce the signal from dead cells below the limit of detection
  - use of reagents that block DNA amplification from ‘dead’ DNA

Loop-mediated isothermal amplification (LAMP) of DNA

Loop-mediated isothermal amplification (LAMP) synthesises microbial DNA by auto-cycling strand displacement driven by a robust Bst DNA polymerase. The LAMP system uses four primers comprising two inner and two outer primers that target six specific DNA regions. LAMP occurs at a single temperature between 59°C and 65°C, typically taking 60 minutes to complete. DNA amplification by LAMP produces cauliflower-like DNA structures bearing multiple loops as well as team loop DNAs of different sizes. Large amount of DNA amplificons can be produced by LAMP within 60 minutes. LAMP amplification products can be detected in real time as the reaction progresses by an increase in turbidity, bioluminescence or fluorescence. The use of instrument-based detection systems eliminates the need for staining with ethidium bromide and gel electrophoresis (Abirami *et al*., 2016; Fortes *et al*., 2013).

Off the shelf LAMP systems on the market include Molecular Detection System (3M™), ANSR® (Neogen) and Loopamp detection kit (Eiken Chemical Company Ltd). These assays enable the detection of pathogens from environmental samples such as *Salmonella*, *Listeria* species, *E. coli* O157, *Cronobacter* or *Legionella*.

Advantages:

- High sensitivity and fast results. The system detects 10^2-10^4 cfu/mL within 75 minutes after enrichment time.
- Easy to operate. LAMP systems do not require thermal cycling system compared to conventional PCR. The sample preparation process includes only two transfer steps, instead of the more complex DNA extraction and purification (typically used in PCR).
- Reduction of training needed. Some of the systems offer solutions where a sample preparation protocol is this same for all target pathogens and multiple organisms can be tested in a single run. Results are automatically interpreted and easy to read.
- Ready to use and pre-dispended reagents and closed tube system reduces risk of amplicon contamination in the lab

Disadvantages:

- Enrichment of 8-30 hours required which is dependent on the media used

LAMP based on RNA detection

LAMP assays have been also developed for the detection of RNA from foodborne pathogens known as reverse-transcription LAMP. A commercially available rtLAMP assay supplied by Neogen (ANSR® Listeria Right Now™) has been specifically designed for *Listeria* detection from environmental samples. rRNA is present in *Listeria* cells at higher levels than DNA (with 1000 - 10,000 copies RNA per cell vs 1 copy DNA per cell). The substantially higher concentration of rRNA per cell enables a reduced time to result compared to many DNA detection methods.

Advantages:

- No enrichment required resulting in to a rapid turn around time
- Good sensitivity of 4 cfu per swab
- 95% confidence and the cleaning fluids do not impact the assays performance.

Phage based pathogen detection

Over recent years, methods have been developed to detect pathogens using the viruses that infect them (known as bacteriophage or phage). The phage is highly specific and will only attach to the species or sub species that they infect. Phage-based detection uses the specificity of the virus to bind the target pathogens in food and environmental samples. Sample analysis is typically carried out in an instrument-based system. The samples are prepared with an off-line incubation step to enable growth of the organisms above the limit of detection. After enrichment, a small portion of the sample is usually heat treated before addition into a strip containing pre-prepared reagents and placed in the instrument ready for analysis. The samples are loaded from the sample strip by the instrument into a receptacle containing the capture virus molecules which are tethered on the solid receptacle surface. On incubation in the system, target pathogens bind to the capture virus molecules tethered onto a solid receptacle surface. Once the cells of interest are stuck to the capture molecules, the receptacle is then washed to remove non-target cells and molecules prior to the detection stage. The detection is carried out by incubating the receptacle with a fluorescence labelled probe that attaches to the bound cells. The receptor emits light at a specific wavelength that is captured within the instrument. There are off the shelf phage-based detection systems assays available for *Salmonella* and *Listeria* detection including VIDAS UP® supplied by bioMérieux.

Advantages:

- Easy to use with limited hands on time required as sample analysis is semi-automated
- Automatic instrument analysis of results
- Sensitive detection due to specificity of the phage capture and detector molecules

Disadvantages:

- Enrichment step of 26-52 hours needed as part of sample analysis

Allergens and meat species residues

Enzyme-linked immunosorbent assays

The enzyme-linked immunosorbent assay (ELISA) is an immunochemistry format based on specific binding between an antigen (Ag) and an antibody (Ab). Commonly allergen-specific immunoglobin G (IgG) antibodies are raised in animals such as rabbits, goats and sheep. There are two techniques for antigen detection that depends on the size of the target molecules. The most common is a ‘sandwich’ technique used to measure intact allergenic proteins. The second method is competitive format and is used for the detection of proteins fragments or small peptides which contain only one binding site for the antibody.

The sandwich ELISA format consists of a pair of antibodies: ‘capture antibody’ and an enzyme labelled (detector) antibody. The capture antibody is bound to the inner surface of microtiter plate wells in a fixed amount. Once the solution (extracted sample) with target component (allergen) is added to the wells the antigen is bound to the antibody. After washing, to remove unbound compounds the enzyme linked antibody is added and reacts with the antibody - antigen group to form a ‘sandwich’. After binding the antibodies an enzyme substrate is added and colour is developed.

The colour intensity is proportional to the concertation of the allergen. The amount of an unknown sample can be determined in comparison to known amounts of suitable calibrant (calibration curve).

ELISA plates are recommended for conducting tests for cleaning validation and can be used as a tool to demonstrate if control measures for allergens/meat species work efficiently. There is a wide range of supplier of commercially available ELISA kits such as Allergen-Check™ allergen ELISA kits (Bio-Check, UK), ELISA kits (ELISA Systems), AgraQuant ® ELISA Tests (Romer Labs), RIDASCREEN®FAST ELISA (R-Biopharm AG), Veratox® Test Kits (Neogen Europe) and many more. These systems can detect various amounts of allergenic materials such as milk (including total milk, casein and β- lactoglobulin), almond, cashew, crustacea, egg, fish, hazelnut, lysozyme, lupin, mustard, peanut, pistachio, sesame, soya, walnut, crustacean or gluten.

Examples of ELISA sandwich-based kits for meat species detection are Species-Check™ (Bio Check, UK), F.A.S.T. Kit (Neogen Europe) and Raw Meat Species Kits (Elisa-Tek). These kits are suitable for monitoring of surface residue such as cow, horse, pig, poultry and sheep meat.

Advantages of ELISA methods for allergen and meat species detection are:

- Specific-test detects the actual allergenic protein/meat species from the source of concern
- Rapid, quantitative results: the processing time is 30 minutes - 4 hours
- The detection limits are generally in the low milligram per kilogram (ppm) range
- The result is determined by the development of visible colour which allows a quantitative analysis through colorimeter or a semi-quantitative measurement through a visual evaluation
- Multiple samples can be tested at the same time when testing for the same analyte

Disadvantages of ELISA method for allergen and meat species detection are:

- Cross-reactivity: antibodies may react with not only the target allergen but also with related or sometime unrelated substances
- Only one target allergen/meat species can be detected/quantified per test; food containing potentially three allergens require three different ELISA assays
- The processing steps required can change the allergen/meat species causing them to lose their immunological properties and lead to the false negative or underestimation
- The sample preparation requires training
- Affected by processing and matrix: protein detection may be affected by processing, e.g. thermal treatments or food matrix such as acidic, high sugar or salt containing foods
- It is very expensive when analysing several allergens in the same matrix
- Trained personnel are required who understand the products and select the most appropriate kit for detection to avoid false negative results

It is essential to do a robust and accurate assessment of the allergenic risk with a product/cleaning procedure. The nature of the allergen of interest may affect the results. If the sole source of milk residue is β- lactoglobulin, an ELISA kit that detects casein would not be appropriate for assessment of milk residue as very little or no casein would be present in the formulation. A negative result would be expected, however, potentially hazardous levels of β-lactoglobulin could be present and undetected.

Lateral flow immunoassay

The Lateral flow immunoassay (LFD) commonly known as rapid lateral flow devices, dipstick or pen-side test is a qualitative immunochromatographic form of an ELISA. The difference with this system is that the application of the sample and subsequent interaction with antibodies and conjugate are conducted simultaneously on a short one-step process. Immunological reaction is carried out on the chromatographic paper by capillary action.

Extracted sample with phosphate-buffered saline (PBS) or other buffer supplied by the kit manufacturer is applied to the sample/reagent zone. When the liquid sample is placed on the sample pad, the antigen in the sample forms an immunocomplex with the antibody labeled with latex beads or colloidal gold. If a specific allergenic protein is present, the protein will bind to the conjugated antibody in this zone. The coupled allergen-antibody migrates to the test zone which contains the second allergen-specific IgG antibody. If the allergen residue is present in the samples the coupled antibody-allergen will bind to the allergen-specific IgG and generate a colored line. A visible line indicates the positive presence of specific allergen. Any unbound antibody conjugates will migrate through the test line and continue to the control line where species-specific IgG is immobilised. The conjugated antibody will bind and form a visible line which allows the user to know that the LFD ran as expected. A negative sample will result in the development of a colored control line only, whereas the positive will be indicative of a visible test and control line. To correct for the hook effect, some of the devices contain an additional line (overload line) in the test zone which helps to indicate if high concentrations of the allergen are present in the sample.

Commercial LFD kits are available for detection of the following food allergens: gluten/gliadin, egg, milk (including detection of total milk, casein and β-lactoglobulin), sesame, peanuts, tree nuts (including almond, Brazil nut, cashew, hazelnut, macadamia nut, pistachio and walnut), crustacea, fish, mustard, soybean and lupin. Examples of suppliers of commercially available LFD kits are FlowThrough™ (Bio-Check, UK), AgraStrip® Allergen (Romer Labs Diagnostic), AllerFlow Gluten (Hygiena International Ltd), Reveal ®3-D Test Strips (Neogen), RIDA®QUICK (R-Biopharm®), AllergenControl™ LFD Detection Kits (Microbiologique) and Lateral Flow Tests for the Detection of Allergen-Residues (Bioacid Diagnostics).

The LFD kits can be used for detection meat species such as pork, cow, poultry, sheep, beef, or horse. The examples of available kits are FlowThrough™ Swab (Bio Check), Bio Kits F.A.S.T.® Screening Kit and Reveal® (Neogen) or Porcine Detection Kit (Perkin Elmer). Some of the kits are only suitable either with raw or cooked meat species.

Advantages:

- Minimum sample preparation; hand shaking of the sample in a vial with the buffer or 1-5 minutes to extract in the water bath for up to 15 minutes with shaking
- Ease of use: minimal operator training for running and results interpretation. Visual assessment of the line determines the positive/ negative presence doesn’t require high skilled personnel.
- Sensitivity limit of 1-10 ppm depending on the matrix being tested, however the test is qualitative; cannot provide information on the level of allergen present
- Rapid on- site analysis (< 5 minutes)
- Cost effective; no requirement for additional equipment

Disadvantages:

- The detergent or sanitizer residues may interfere with the LFD. For many kits neutral pH is needed for correct measurement of allergen residues. Too acidic or alkali pH gives possibilities of false/positive results.
- Very high concentration may reduce the intensity of the test line or suppress its formation completely
- Trained assessor required to ensure the test is fit and appropriate for the product being tested
- Some are designed for rinse water only and not for food matrices
- Subjective assessment of presence of faint lines

Real - time polymerase chain reaction (qPCR)

The principle of working is described in microbial detection section (page 5). It was mentioned that real time PCR detects and amplifies a target DNA sequence using specific oligonucleotide primers and fluorescence probes. The qPCR can be used for the detection and quantification of bacteria, viruses, GMOs as well as meat species and markers for allergens.

The qPCR can be used to detect food allergens for which suitable ELISAs are not available, such as celery. The commercially available qPCR kits for hygiene monitoring include foodproof ® Allergen MR 800 (Biotecon Diagnostics), SureFood® Allergen/Plant (R- Biopharm) and Genesig® allergen detection kit (Primerdesign). The above-mentioned kits detect DNA in allergenic food such as soya, hazelnut, peanut, walnut, gluten, almond, brazil nut, cashew, macadamia, pecan, pistachio, sesame, crustaceans, fish, molluscs, lupin and mustard.

Examples of commercially available real time PCR kits for meat and fish species detection/quantification are Bio Kits DNA Extraction Kit (Neogen), SureFood® Animal ID and SureFood® Fish ID (Congen), Real Time PCR detection kit from QIAGEN and Primer Design. These kits allow manufacturers to screen meat species such as horse, cow, mule, donkey, beef, swine, buffalo, deer, chicken, turkey, duck, ostrich goose and fish species.

Advantages:

- Systems highly sensitive; the limits of detection: 0.1 ppm-4 ppm and limit of quantification 0.8 ppm-25 ppm depends on the allergen
- Analysis results in less than 2-2.5 hours
- The DNA extraction and PCR set up can be automated; however, kits for manual DNA extraction are available
- PCR can be applied in processed food matrices
- Can test large volumes of same samples at same time

Disadvantages

- The technology is an indirect assay, it does not detect the target proteins but the marker DNA which may or may not correlate with the amount of the allergen in the food product. The absence of DNA does not indicate absence of protein.
- Sample preparation and analysis require skilled personnel

- Unstable in fermented, acidic environments (e.g. tomato sauce production)
- Not suitable for products such as milk or eggs (insufficient sensitivity as eggs contain very little, or in case of egg white, no DNA despite having a high allergenic potential due to the presence of specific proteins)
- High risk of cross contamination when small amount of target DNA from previous assays contaminate the PCR mix and generate false positive results
- Laboratories operating real time PCR require separate areas (samples preparation, qPCR mix preparation, PCR and post PCR handling rooms) for minimising the risk of cross contamination with amplified DNA

Emerging microbial detection

Academic groups are developing a system with the potential to detecting food resides or bacteria on cleaned food processing surfaces and equipment using fluorescence. Current innovations in this area use cameras to detect differences in fluorescence signals to monitoring microbial contamination. Due to the levels of fluorescence and the signal detection, these systems are currently limited to gross levels of contamination (>1x10^4 CFUs/g) so are not ideally suited to the food and drink industry.

Research at Oxford University is looking at developing a system which will use a combination of fluorescence concentrators to collect most of the fluoresce from the surface in combination with single photon detectors that are significantly more sensitive than pixels in cameras. These factors combined with excitation using a UV light source creates different systems with the potential to detect microbial, allergens and meat species residues. The invention is still in a developmental stage but appears to be a promising for the food industry as a potentially simple, inexpensive and easy to use system with range of targets for residue detection.

Large surface detection

Fresh Check UK is developing a chemical spray which visibly reacts in the presence of different types of contamination such as bacteria, cleaning chemical or food residues left on the surface after cleaning procedure. The product includes a dye compound which changes colour by factors such as by-products of microorganism, extreme pH changes as well as reactive agents that are used in industry (quaternary amines etc.)

Advantages:

- Provide general information about contamination on large surface areas compared to swabbing
- Easy to use: the product is designed to test surfaces directly without sample processing
- Requires no additional equipment
- Results from the spray are attained after 30 seconds
- The colour changes are clearly distinguishable with the naked eye (blue/purple-clean surface, any other colour changes indicates a contamination)
- Large surface detection, simplicity of use and rapid results makes the product a tool for training staff cleaning processes

Disadvantages:

- Only used for indicating contaminated surfaces, swabbing still required for contaminate analysis
- Cannot distinguish the type of contamination
- Wiping and rinsing with water after test is required to remove any product residues

Sampling devices

Hygiene monitoring typically involves swabbing the areas where microorganisms, allergens or meat species residues may accumulate, for example: rough welds, dead legs, joints. The advantage of swab/sponge techniques is that they can be used on both flat and surfaces with crevices as well as areas difficult for other techniques such as under surfaces, in narrow openings or behind equipment. The difference between swabs and sponges is that the latter are designed for sampling larger surface areas. Suppliers such as Hygiena™, Technical Service Consultants Ltd, 3M Food Safety, Bio-Check (UK), Hardy Diagnostics, Puritan Medical Products or Starplex™ Scientific offer a wide range of sterile and non- sterile swabs and sponges in a range of different formats such as:

- Pre-moistened devices which helps to improve the recovery of residues from both wet and dry surfaces
- Swab/sponge moisturiser which neutralises detergents and sanitisers. It is claimed that the wetting agent neutralises the effect of residual detergent and sanitisers remaining on the surfaces after cleaning. These types of swabs help avoid interference of the cleaning fluid that can produce false results.
- Swab/sponge moisturiser preserves the integrity of the sample - the wetting agents ensures that the samples introduced into the different microbiological broths are representative of the sample taken
- Swab sticks with a special break point - the swabs have special ‘break point’ to simplify the transfer of samples into the detection broth

Commercially available sampling devices are offered in different sizes and formats to suit specific application. It is essential that the swab and extraction/swabbing solution are appropriate for the methods used for the residue detection. The swabs used may need to be validated as not all swab types and swabbing solutions are right for use with specific residue detection tests. Some sampling devices are designed specifically for microbiological sampling and may contain allergenic components such as milk powder or preservatives that may influence results if are used for allergen detection.

Summary

This summary document highlights that there are many rapid methods for microbial, allergens and meat species detection from environmental samples. These alternative methods can provide information about the effectiveness of cleaning and sanitation procedures within 8-48 hours (some methods may be shorter than this) to support manufactures with critical decisions. Suppliers of some of the systems described in this summary document claim that the methods are comparable to the reference methods but with shorter detection times which allow manufacturers to make quicker

decisions. Many of the systems described do not require skilled workers to operate the equipment which can help to support manufacturers in a busy environment where significant skill shortages are a day-to-day reality. There are a reduced variety of systems compared to microbial determination available for allergenic proteins and meat species. With regards to the selection of an appropriate method for hygiene monitoring no method is 100% specific or capable of providing results without hands - on time and investment. All described methods have strengths and limitations. If manufacturers are unsure about the best methods to use it is important to consult a skilled analyst.

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For more information, please contact:

Anna Falowska
anna.falowska@campdenbri.co.uk
+44(0)1386 842271



About Campden BRI

Campden BRI helps food and drink businesses succeed

We do this through practical scientific, technical and knowledge support

We work closely with industry to ensure the absolute relevance of all our activities - from analysis and testing, process validation and safety assurance to product innovation, consumer studies and training, events, databases and publications

All our activities are underpinned by a strong programme of research - steered by industry for maximum relevance

Membership-based, we provide services to companies all along the supply chain

Vision

To be the partner of choice for the development and application of technical knowledge and commercially relevant solutions for the food and drink chain

Mission

Practical application of technical excellence for the food and drink chain

Campden BRI, Station Road, Chipping Campden, Gloucestershire, GL55 6LD, UK
+44(0)1386 842000

support@campdenbri.co.uk
www.campdenbri.co.uk