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EU-compliant meat testing

Reference and alternative methods for testing meat products

The EU requires that slaughter and meat packing facilities regularly test their products. This is done through regulations that describe approved methods typically based on microbiological tests. Because the effort required for these methods cannot always be justified, the EU permits the use of comparable alternative methods, several of which have already been recognized by the authorities. The FreshDetect method will soon belong to this group of alternative approaches.

According to EU food hygiene regulations, companies are required to carry out microbiological tests on meat products to verify that the product is safe. Companies must therefore implement controls along every stage of the food processing chain in order to meet the constantly growing food safety and hygiene demands. The corresponding legal foundation is stipulated in (EC) 852/2004[i] and (EC) 2073/2005[ii], modified by (EC) 1441/2007[iii].

(EC) 2073/2005 defines the microbiological thresholds for specific types of food. It also describes the approved test methods, the method for taking samples, sample preparation and analysis, and also the measures to take when thresholds are not adhered to. The regulation differentiates between “safety criteria” for food available on the retail market, and “process hygiene criteria”, which is tied to the end of the manufacturing process. Food companies must fulfill both criteria.

Reference methods

The required reference methods are described in the appendix section of (EC) 2073/2005 and 1441/2007. They include:

- Total viable count: ISO 4833[iv]
- Enterobacteriaceae: ISO 21528-1/-2[v]
- Testing equipment surfaces and similar areas: ISO 18593[vi]
- Carcass testing: ISO 17604[vii]

The methods described here typically consist of a microbiological test comprising the following steps:

- Sample extraction
- Potential homogenization
- Dilution series
- Inoculation of the culture media
- Incubation
- Analysis
- Potential confirmation
- Final result

Until tested, the samples must be stored between 0 and 5°C – for deep-frozen products under -18°C. Smaller quantities of defined volumes and weight are then extracted under sterile conditions and hacked into smaller pieces if required.

The bacterial burden is then determined using the spatula, pour plate or drop plate method. At the end of the incubation period, all of the “typical” colony forming units (CFUs) are counted and the weighted average is calculated.

Approved alternative methods

In many cases however, the reference methods described here are of limited use, since the results of the analysis are normally available only several days later. Furthermore, these methods require considerable effort and the expertise of trained specialists. Article 5 of (EC) 2073/2005 therefore permits a degree of flexibility with respect to the analysis methods in order to streamline the availability of the results or simplify the actual process. These alternative methods for microbiological testing must offer the same degree of accuracy however – in other words they must be validated against the reference methods outlined in (EC) 2073/2005 – and approved by the appropriate authorities. Furthermore, testing for microorganisms other than those stipulated in (EC) 2073/2005 is allowed only when validating the process hygiene criteria.

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Following is a brief description of alternative methods that can be used[viii]:

1. Direct bacteria count

The bacteria are detected in a counting chamber using phase contrast microscopy. Highly active microorganisms are immobilized with a formalin solution.

2. Turbidity measurement

Bacteria enumeration occurs in a turbid sample suspension. The optical density of the solution is measured with a photometer or turbidity measurement instrument.

3. Membrane filtration

Membrane filters can be used to separate specific bacteria from a suspension solution. The bacteria are then enumerated in a culture medium by measuring the turbidity.

4. Spiral plate method

A defined volume of a sample solution is dispensed in spiral form over a rotating agar plate, from the edge to the middle, using a precision stylus. Bacteria count is carried out through image analysis, counting grids or a laser counter.

5. DEFT (direct epifluorescence filter technology)

With this method, the bacteria are captured on the surface of a membrane filter and stained with a fluorescent dye. Facilitated by image analysis, the bacteria are then counted in a fluorescence microscope.

6. Immersion plate method

With this method, a plastic plate is coated on one or both sides with culture medium and then immersed in the sample solution. After incubation in a tube, the bacteria concentration can be determined through a reference comparison.

7. Most probable number method (MPN)

With this method, test tubes filled with broth media from each weighted sample or dilution preparation are replicated and inoculated with the analyte solution. After the incubation period, a pattern of positive and negative tubes is observed and the results checked against an MPN table to determine the most probable number of microorganisms.

8. Petrifilm method

This ready-made system consists of a top and bottom film containing the dried medium and a soluble guar gum. The colonies are made visible with an indicator dye.

9. Bacteria limulus test

This test is based on the process of coagulation that occurs in the hemolymph of horseshoe crab (Limulus Polyphemus) in the presence of lipopolysaccharides of gram-negative bacteria, which results in a gel. The correlation between the gelation process and the endotoxin content can be used to draw conclusions about the number of gram-negative bacteria.

10. Impedance method

Bacteria growth results in an increase in metabolites, which triggers changes in electrical conductivity or resistance in a culture medium. The presence of more bacteria at the beginning permits faster detection of the change in impedance.

11. Bio and immune sensors

Bio or immune sensors are measurement probes furnished with sensitive biological elements such as enzymes, antibodies or DNA, which in turn are coupled to receivers that serve as evidence of bacteria.

12. Immunological method

Immunological analysis methods are based on an antigen-antibody reaction. The interaction between antigens and antibodies, which is highly specific, can be detected with various methods.

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13. Gene probes (hybridization technique)

Gene probes consist of single-strand DNA molecules that bind specifically to complementary DNA strands. The resulting double-strand is referred to as hybrid. Specific genes can be detected by tagging the probe with a molecular marker of fluorescent molecules, enzymes or other methods.

14. Automated DNA analysis

With this method, bacteria are inactivated through heat to extract the DNA. The DNA is then separated using gel electrophoresis and hybridization with a tagged probe.

15. Polymerase chain reaction

This method multiplies specific DNA fragments in vitro to permit the detection of even small amounts of original DNA material using various methods.

16. Bioluminescence (ATP measurement)

Since every living cell contains a relatively constant amount of adenosine triphosphate (ATP), the number of living cells can be indirectly determined using the ATP content. Microorganisms can be detected within less than a few minutes with the Luciferin-Luciferase reaction, which involves releasing light quanta whose energy content is proportional to the ATP concentration.

17. Flow cytometry

With flow cytometry, bacteria are stained and passed through a measurement cell with fluorescence stimulation. The resulting emission impulses are analyzed with a photodetector.

18. Colorimetric method

This method relies on a change in the pH value that occurs through the formation of carbon dioxide and organic acids. The system contains a sensor that changes color whenever microorganisms form acids or CO_2. As an alternative, the resulting color changes can be measured photometrically.

19. FT-IR (Fourier transform infrared spectroscopy)

After stimulating molecule atoms with infrared light, the corresponding spectra are compared to a database in order to map the microorganisms.

As discussed previously, the aforementioned methods, which require trained and experienced personnel to ensure reliable application, are generally based on invasive sample collection processes. Laboratories accredited in line with ISO/ IEC 17025[ix] and similar systems are required to assess and document the measurement uncertainty of the methods and analyses they use in accordance with ISO/ TS 19036[x]. This has to be done by running comparison analyses to verify and document these test methods against the reference methods, both before the method is implemented and on a regular basis. For this reason laboratories must carefully weigh the required effort and the time advantages that any test method brings. Apart from the delays in obtaining the test results, an additional issue for users in the meat industry is the time and effort required to carry out invasive sample collection.

New alternative method

Laser-induced fluorescence spectroscopy has recently become available as a rapid detection method. With this approach, metabolic end products from bacteria are stimulated with ultraviolet light and fluoresce. The fluorescent light is measured spectroscopically, after which the total viable count is calculated using an algorithm generated from the captured spectra. This non-invasive method of measuring bacterial burden has already undergone stringent scientific testing and documentation as part of various research projects and with independent laboratories. For this reason, fluorescence spectroscopy must be taken into consideration when developing rapid and non-invasive analytic test methods. Fluorescence spectroscopy is one of the most sensitive spectroscopic techniques for identifying, classifying, authenticating, quantifying and optimizing various parameters when handling, processing and storing foodstuffs[xii].

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In light of the low cost and high reliability of the freshdetect BFD-100, this method is an ideal tool for helping slaughter and meat packing operations satisfy the stringent hygiene regulations and quality assurance guidelines in every stage of the process. Furthermore, inspection authorities can employ the process on-site to increase the number of samples, while simultaneously keeping the number of laboratory tests within a reasonable framework.

[i] Regulation (EC) Nr. 852/2004 issued by the EUROPEAN PARLIAMENT AND COUNCIL, dated April 29, 2004, regarding food hygiene
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:139:0001:0054:de:PDF

[ii] EC Nr. 2073/2005 Regulation (EC) Nr. 2073/2005 issued by the Commission on November 15, 2005, regarding microbiological criteria for foodstuffs
2005http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0001:0026:DE:PDF

[iii] Regulation (EC) Nr. 1441/2007 issued by the Commission on December 5, 2007 - modification to Regulation (EG) Nr. 2073/2005 regarding microbiological criteria for foodstuffs
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:322:0012:0029:DE:PDF

[iv] DIN EN ISO 4833-2:2014-05
Microbiology in the food processing chain - Horizontal method for the enumeration of microorganisms - Part 2: Colony count at 30 °C by means of surface plating techniques (ISO 4833-2:2013 + Cor. 1:2014); German version EN ISO 4833-2:2013 + AC:2014

[v] DIN EN ISO 21528-1:2017-09
Microbiology in the food processing chain - Horizontal method for the detection and enumeration of enterobacteriaceae - Part 1: Detection of enterobacteriaceae (ISO 21528-1:2017); German version EN ISO 21528-1:2017

[vi] DIN ISO 18593:2009-12
Microbiology in food- and feed-stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs (ISO 18593:2004)

[vii] DIN EN ISO 17604:2015-12
Microbiology in the foodstuffs chain – carcass sampling for microbiological analysis (ISO 17604:2015); German version EN ISO 17604:2015

[viii] Mifek, Sabine (2009): Methods for EU-compliant microbiological analyses for meat and meat products. Graduate paper, University of Vienna

[ix] DIN EN ISO/IEC 17025:2017-02 - Draft
General requirements for the competence of testing and calibration laboratories (ISO/IEC DIS 17025:2016); German and English prEN ISO/IEC 17025:2016

[x] DIN ISO/TS 19036:2012-01; DIN SPEC 10125:2012-01
Microbiology in food- and feed-stuffs - Guidance for the estimation and expression of measurement uncertainty associated with quantitative results (ISO/TS 19036:2006 + Amd.1:2009)

[xi] Grimmler, Christina, Tamara Kuhfuß, Matthias Heiden, et al. (2017): Noninvasive assessment of the bioburden of minced pork using a handheld fluorescence device. tm Technical Measurements. 0(0): Retrieved 1 Feb. 2018, from doi:10.1515/teme20170092

[xii] Ahmad MH, Sahar, Hitzmann (2017): Fluorescence Spectroscopy for the Monitoring of Food Processes. Adv Biochem Eng Biotechnol. 2017;161:121-151, doi: 10.1007/10_2017_11.
https://link.springer.com/chapter/10.1007%2F10_2017_11

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