Recent developments in antibiotics screening tests

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Screening tests, such as inhibitor tests, ELISA, receptor assays and simple diagnostic kits are frequently used for the purpose of detecting antibiotics residues in food of animal origin. In the last decade, some tests have been improved and new rapid tests have been developed, especially with regard to the inspection of raw milk on arrival at the dairy factory. Various trends can be distinguished within these developments.

Shortening test times

Microbiological inhibitor tests are based upon the general principle that anti-infection products inhibit the growth of a test organism. That inhibition may be determined, e.g. by measuring an inhibition zone in plate tests or being attentive to a change of colour of a pH indicator such as bromo cresol purple in agar diffusion tests. Formerly, efforts were made with a view to shorten the incubation step that is inherent to microbiological inhibition tests by using another measuring principle, e.g. an ATP measurement (Lumac Rapid Antibiotic test, Lumac bv - 90 minutes) or a measurement of the light produced by a genetically modified luminescent test organism by means of a luminometer (Valio T-102 test, Valio Ltd.). The gain in time was however relatively small. DSM-Food Specialties developed the Delvotest Accelerator, which is a Delvotest version (incubation time of 3 hours) with a shorter incubation time. Increasing the number of spores in the culture medium accelerates acid production but makes the moment of colour reading more critical. This problem is solved by introducing continuous colour reading during incubation and by using a flexible incubation time (between 105 and 140 minutes) that depends on the moment when in a predefined number of cups of the microtiter plate the colour of the agar turns yellow.

A more significant time gain was achieved by using rapid tests. The first rapid test that was developed for screening raw milk on the presence of B-lactam antibiotics was the so-called Penzym test (UCB-Bioproducts s.a.), an enzymatic (carboxypeptidase) colometric test which produced a result after 20 minutes. Subsequently, several B-lactam screening tests were placed on the market in the 1980s and 1990s, including the SNAP receptor test (Idexx Laboratories, Inc. - 9 min), ßeta-s.t.a.r. (Neogen Corporation - 5 min), Charm MRL Beta-lactam Test (ROSA) (Charm Sciences Inc. - 8 min), and the immunoassays Lactek (Idexx laboratories, Inc. - 8 min) and Parallux (Idexx Laboratories, Inc.- 5 min).

Still to the aim of gaining time, a certain number of tests have been recently modified or new tests have been developed for which the test time is 3 minutes or less. These rapid tests make it possible to perform the inspection when the milk is collected, before pumping up the farm tank milk and not on the tank truck milk on arrival at the dairy factory. This method makes it possible to avoid destruction of large amounts of milk. Currently the following 3 minutes tests are available: Charm MRLBL3 (Charm Sciences Inc.); BetaXpress & TwinExpress Milk (Unisensor s.a.) en ßeta-s.t.a.r. Combo 3.0 (Neogen Corporation); as well as the following 2 minutes tests: ßeta-s.t.a.r. 1+1 (Neogen Corporation) and Charm MRLBTET2 & MRLBLRTET2 (Charm Sciences Inc.) and a 1 minute test: Charm MRLBL1 (Charm Sciences Inc.).
Widening the detection spectrum

Most of the rapid tests can only detect one substance or a few substances belonging to the same family of antibiotics. Whereas before the spectrum was usually limited mainly to screening tests on β-lactam (milk), tetracyclines, sulfamethazine or chloramphenicol, we nowadays have generic rapid tests at our disposal for detecting aminoglycosides, (fluoro)quinolones, sulfonamides,… Moreover, many combined tests that allow simultaneous detection of β-lactam- and tetracycline antibiotics in milk have been recently developed, e.g. TwinSensor Milk & TwinExpress Milk (Unisensor s.a.), Charm MRL Beta-Lactam/Tetracycline Combo Test & Charm MRLBLTET2 (Charm Sciences Inc.), βeta-s.t.a.r. Combo 3.0 (Neogen Corporation) and SNAPduo (ST) (Idexx Laboratories, Inc.). The generic Trisensor (Unisensor s.a.) dipstick screening test allows simultaneous detection of β-lactam, tetracycline and sulfonamide residues in milk. Using the 4Sensor Milk (Unisensor s.a.) or the βeta-s.t.a.r. 4D (Neogen Corporation) makes it possible to screen milk in less than 10 minutes for the presence of β-lactam, tetracycline, streptomycin and chloramphenicol residues. Finally, with the Parallux Milk Residue Testing System (MEDEXX CO., Ltd.) residues of 6 major β-lactam compounds, tetracyclines, spectinomycin, neomycin, streptomycin, spiramycin, sulfonamides and quinolones can be detected in milk in 4 minutes by means of one single test.

For testing honey on residues may be used the Bee4Sensor multiple test strip for detecting tylosine, (fluoro)quinolones, sulfonamides and chloramphenicol by means of one single test.

Improving detecting capacity

Continuous efforts are made to improve the composition of the culture medium of microbiological inhibitor tests in order to make it possible to detect a larger number of components at EU-MRL level. Eclipse 3G (ZEU INMUNOTEC), Charm Blue-Yellow II (Charm Sciences Inc.) and the Delvotest T (DSM Food Specialties) are each enhanced versions of an already existing test. Another approach consists in using several test plates that contain a medium of a different composition or another testing organism.

On the other hand, the detecting capacity of some rapid tests has also been improved recently in order to meet the expectations of customers. Some examples: the SNAP ST (Idexx Laboratories, Inc.) that can determine a larger number of β-lactam compounds at EU-MRL level while at the same time detecting cephalosporins in a less sensitive way and within a smaller range of the MRL standard. Some screening tests were modified for the inspection of foodstuffs intended for the Russian market, which requires compliance with specific residue standards, such as the Charm MRLBLRFTET2 (Charm Sciences Inc.) and the Tetrasensor Tissue 10 ppb (kit 036, Unisensor s.a.). At the request of the dairy industry in Australia and New Zealand a test has been developed that has a corrected detection capability for cephalonium, i.e. the Charm SL Kiwi test.
Simplifying the carrying out of tests (reading, incubation, …)

Most of the microbiological inhibitor tests and rapid tests can be visually read. Now, the subjective colour reading of agar diffusion tests can be replaced by reflectometric colour reading using a flatbed scanner driven by specific software [Delvoscan & Cscan (DSM Food Specialties), Premiscan (R-Biopharm AG) and GVSCAN (Charm Sciences Inc.)]. For some tests [Delvotest Accelerator (DSM Food Specialties), BRT Incubator & BRT Scan (AiM GmbH) and Eclipse & Explorer 2.0 with an i-Reader (ZEU INMUNOTEC)] colour is monitored during incubation and the software determines the end of the incubation. The same development also took place recently in the field of rapid tests, with Charm Sciences Inc. developing the EZ-Reader, a combined incubator/reader that does no longer require putting the test strip into a separate reader. This finally is a real ‘ROSA’ or ‘Rapid One Step Assay’. In this test, the reader is not only a “reading instrument” as such, but the milk flow over the test strip is monitored and deviations are recorded by specific software. Moreover, the software can decide to extend the time of incubation slightly in order to improve the distinctness of the result.

Some other kit manufacturers rather focused on a test that might be used at ambient temperature and would no longer require the use of a heating device. Some examples: the SNAP ST & SNAPduo ST (Idexx Laboratories, Inc.) and the BetaSensor Milk (Unisensor s.a.). The design of these tests is such that they can be performed at the holding of the producer without requiring any laboratory equipment.

Several rapid tests are also commercially available for testing honey: TetraSensor & SulfaSensor Honey (with field test protocol) (Unisensor s.a.); Chloramphenicol, Tetracyclines & Quinolones Residue Rapid Inspection Test Devices (Hangzhou Nankai Biotech Co., Ltd.) and the Chloramphenicol, Tetracycline, Quinolones, Sulfadiazine & Penicillins Drug Residue Rapid Test Devices (SmarKIT, Zhejiang Huazheng Import & export Co., Ltd.).

A completely different and for food laboratories less relevant phenomenon is the development of new technologies such as biosensors, cellular bioassays, transcriptomics and proteomics as well as optical biosensors based upon surface plasmon resonance (SPR). The first applications of molecularly imprinted polymers (MIP) are now also available for residue analyses; in most cases, MIPs are used as a selective absorbent in solid-phase extraction methods during the clean-up of samples. Some new electrochemical and optimal immunosensors, flow cytometric immunoassays and biochip array technology applications (Evidence, Randox Laboratories) are already or will soon become available for use in residue analysis. Moreover, applications of aptamers (artificial oligonucleotids (RNA of DNA)) are to be expected.

Finally, over the last few years mass spectrometric methods have been used more and more often for the purpose of highly selective and specific multi residue detection, in spite of the high cost.

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