Milchprüfring Bayern e.V.

Monitoring of Antibiotic Residues in Milk – Development of the New Biosensor System MCR 3 for Routine Practical Analyses

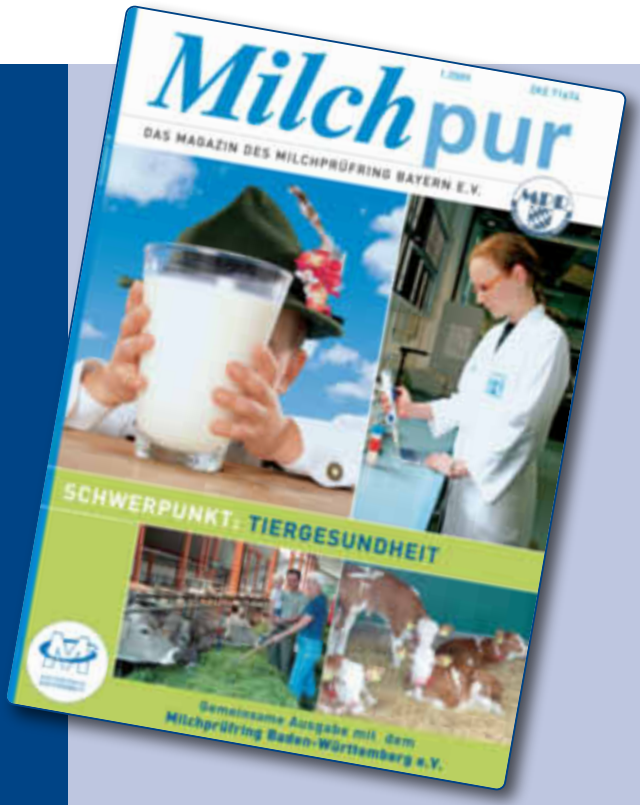
Innovation – Networking – Transparency – Information

Cluster Ernährung

The Biosensor MCR 3 – Improving Milk Safety

"Residues in milk?! Antibiotic residues have been a serious problem for dairy farmers for generations. Farmers in the past were primarily concerned with economic losses at both the farm and the dairy but today there are also serious legal issues to address.

Inhibitor-positive tested milk has been a problem for the Foodstuffs Control ever since the EU Food Hygiene Regulation came into effect in 2006." (Milchpur 01/2008)



When milk contains inhibitors (= antibiotics) it cannot be used as a food source. However a major problem for the dairy industry is that milk from different origins is routinely mixed at various points along the dairy food chain making it difficult to prevent contamination or to keep it to a minimum level. It is therefore important to exclude contaminated milk at various points in the food chain. But how?

Using current testing methods it is not possible to ensure compliance with the statutory residue limits for different antibiotics on-site for analytical reasons and also due to the high costs involved. In addition, if contamination is detected later on in the dairy food chain, for example in the final collection tanks, the economic loss is high. However a solution is in sight!

The answer is MCR 3!

The MCR 3 (Munich Chip Reader of the third generation) prototype is being evaluated at the laboratory of Milchprüfring Bayern e.V. in Wolnzach in Bavaria/Germany. The new Biosensor is part of an integrated system for the prevention of antibiotic residues and can be used alongside routine classical methods. For the first time, there is a system that can be used to monitor and control the dairy food chain reliably and cost effectively at an early stage.

The Use of Antibiotics

Residues in food are undesirable but unavoidable and this fact has caused some confusion and concern for consumers.

Milk and milk products are generally considered to be a healthy and natural food. The presence of antibiotic residues in dairy products neither fits with this idea nor with the many advertising messages, which show such products to be good for the consumer. There is apparently a contradiction since on the one hand, the application of veterinary drugs such as antibiotics when treating sick milk cows is necessary for animal protection, on the other hand even with minimized use and the observance of all preventive measures this treatment is inextricably linked with the risk of residues in milk. The consumer has a legitimate interest in residue-free foods since residues, such as those coming from the treatment with antibiotics, have an allergy potential and can lead to the formation of resistant germs and may even have a direct toxic effect.

Current Situation in Food Inspection

To protect the consumer, legislation has imposed strict regulations for the treatment of animals with antibiotics. At the same time, legislation has determined maximum residue limits (MRL) for possible residues in foods of animal origin, which are not to be exceeded.

Since the existence of the respective EU Regulation 2377/90[1] over twenty years ago, it has been clear that the concept of regulating MRL values in foods can only be implemented successfully if methods for qualification and quantification of the single substances are available on-site for use during routine monitoring.

The current system for safeguarding the raw milk chain is based on the analysis of the delivered milk from the dairy farms in line with the milk quality ordinance (MilchGüV) - in Bavaria by the Milchprüfring Bayern e.V. - as well as the self-monitoring of the dairies at the milk reception in the processing plant. Due to technical and economical constraints, only detection methods are employed which do not ensure complete monitoring in compliance with all MRL values. Methods which are capable of this are applied sporadically with only some hundred samples per year being tested by the Food Inspection Service according to the national residue inspection plan.

[1] now replaced by the EU Regulations 470/2009 and 37/2010

How are Tests done today?

At the dairy farm level normally a cost-efficient microbiological residue test (BRT= Brilliant Black Reduction Test) is performed. This allows the detection of a broad range of residues, however in the case of a positive reaction it fails to identify the specific residue present. Furthermore, this detection method is not suitable for time-critical areas of application due to its relatively long testing duration of at least two hours.

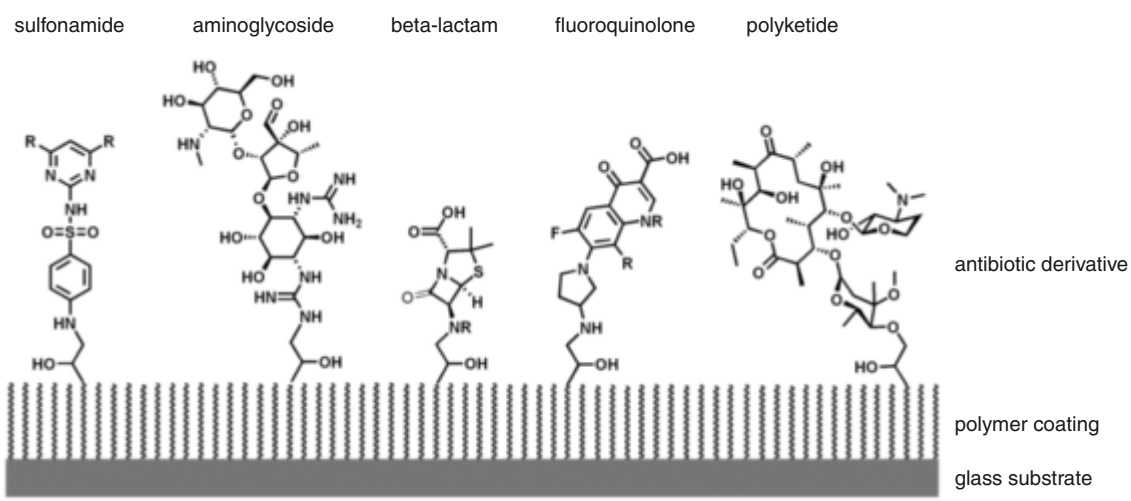
Dairies normally use receptor tests - so-called "screening tests" - for the incoming inspection, which means testing before unloading the milk from the milk collecting truck. The result is normally available within minutes. Such tests are using an antibody to detect a single or a group of antibiotics with definable detection limits. However, all other antibiotics are not detected. Since the most common screening tests are directed at the most commonly used antibiotics (i.e. beta-lactam group), non-beta-lactam antibiotics are undetected. About 10 % of the antibiotic agents used are not covered by the analytical tests applied in the incoming inspection of the dairy. This gap could be filled by applying standard analytical methods with liquid chromatography and mass spectrometry. Only recently it has been possible to use these methods to identify and to quantify antibiotics reliably and such methods require the use of expensive equipment and a high level of technical expertise. They are also very time-consuming and cost-intensive due to the complex sample preparation required and therefore it is only possible to use these in special laboratory application areas.

The MCR 3 creates New Opportunities

To enable practical, on-site implementation of the food hygiene requirements, test systems ideally should record as many potential antimicrobial residues as possible in parallel and within a short period of time using a single measurement. With this goal in mind, two FEI-research scientists including the Chair of Hygiene and Technology of Milk of the Department for Veterinary Sciences of the LMU München (Prof. Dr. E. Märtlbauer) and the Chair of Analytical Chemistry of the TU München (Prof. Dr. R. Nießner) developed a new Biosensor System to prototype maturity. This Biosensor System is able to detect the most important antibiotics used in dairy production, directly from raw milk without sample preparation within a few minutes. The measuring principle of the Biosensor MCR 3 is based on an interaction between a specific antibody and a respective antibiotic. 14 pharmaceutical residues are currently detectable which are the most commonly applied antibiotics[2] used in veterinary medicine.

- sulfonamide – sulfamethazine (100) and sulfadiazine (100)
- beta-lactam – penicillin G (4), ampicillin (4), cloxacillin (30) and nafcillin (30)
- cephalosporin – cephapirin (60) and ceftiofur (100)
- aminoglycoside – streptomycin (200), neomycin B (1,500) and gentamicin (100)
- polyketide – erythromycin A (40) and tylosin (50)
- fluoroquinolone – enrofloxacin (100)

[2] respective MRL values (in µg/L) in accordance with EU Regulation 37/2010 in parentheses

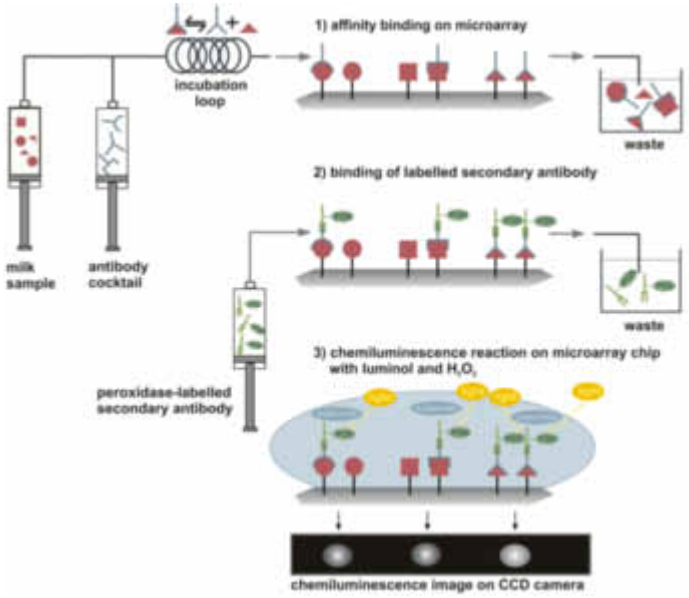


Analytical Results with MCR 3 can be achieved in Three Steps

The MCR 3 testing procedure is performed in three simple steps. In the first step, a cocktail of antibodies is mixed into the milk sample to be tested; these antibodies specifically react with the 14 antibiotics most commonly used. If there is an antibiotic present in the milk, the corresponding antibody will bind to the respective antibiotic in the milk sample.

In the second step, the reaction takes place on the glass surface of the microarray chip where 14 antibiotics have been arranged in small spots, printed in a grid (array) on each chip. The antibody milk mixture is passed over the chip and any unbound antibodies in the milk react with the antibiotic spots on the glass surface of the chip. If an antibiotic is present in the milk sample it will have already bound to the specific antibody in the cocktail and depending on the concentration of the antibiotic in the sample, this antibody would be prevented or reduced from binding to the corresponding antibiotic spots on the glass surface.

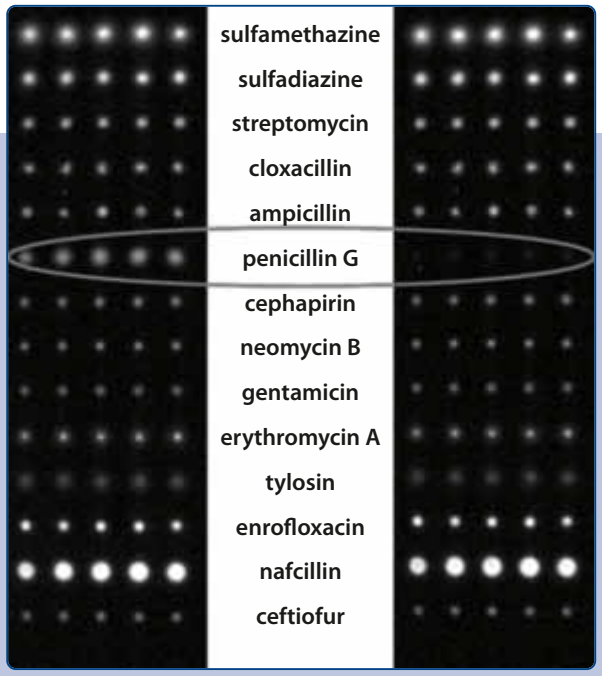
In the third step, the linked antibodies on the glass surface are made visible by adding a second detection antibody which produces light due to a chemical reaction and can in turn be measured using evaluation software. This chemical light reaction is also used in forensic science for example to detect blood traces. In this case the spots to which the most antibodies are bound then light up the brightest. If an antibiotic is present in the milk, less antibodies are available to bind to the antibiotic on the chip, in turn less light will be produced and the corresponding spot will appear darker. At a very high concentration of antibiotics no light will be visible at all. The luminous intensity of the spots on the microarray chip is recorded by a camera and identified using evaluation software. This will not only prove that an antibiotic is present in the milk sample but will also show how much of the antibiotic is present.

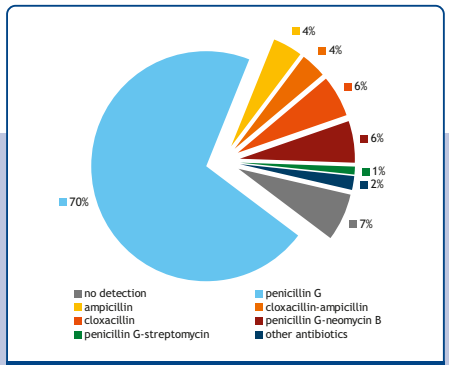


What makes the MCR 3 so special?

Each antibiotic chip is specially calibrated, which means standard samples are measured and analysed one by one. All samples contain measurable antibiotics in different concentrations (0-1,000 µg/L). The data analysis is done by evaluation software, which measures light intensity and calculates the concentration of the antibiotic in the milk sample. To ensure the accuracy of each analysis, every antibiotic is tested five times on each chip. This complex, yet highly reliable testing procedure only takes about six minutes. After the analysis the chip is not thrown away but can be regenerated up to 100 times. MCR 3 not only confirms the presence of inhibitors in the milk but also specifically detects the antibiotic present and measures the concentration enabling the source of contamination to be determined more effectively. The employees of the dairies and of the Milchprüfring Bayern e.V. as well as the official veterinarians find this approach useful for their investigative and advisory activities. When certain combinations of antibiotics are detected conclusions can possibly be drawn as to the source of the contamination and the drug used.

At the Milchprüfring Bayern e.V. more than 3,000 inhibitor-positive milk samples have been analysed and evaluated on the MCR 3 so far. The results confirm the practical use of the MCR 3 and have shown that considerable progress has already been made in safeguarding the dairy food chain against antibiotic residues.



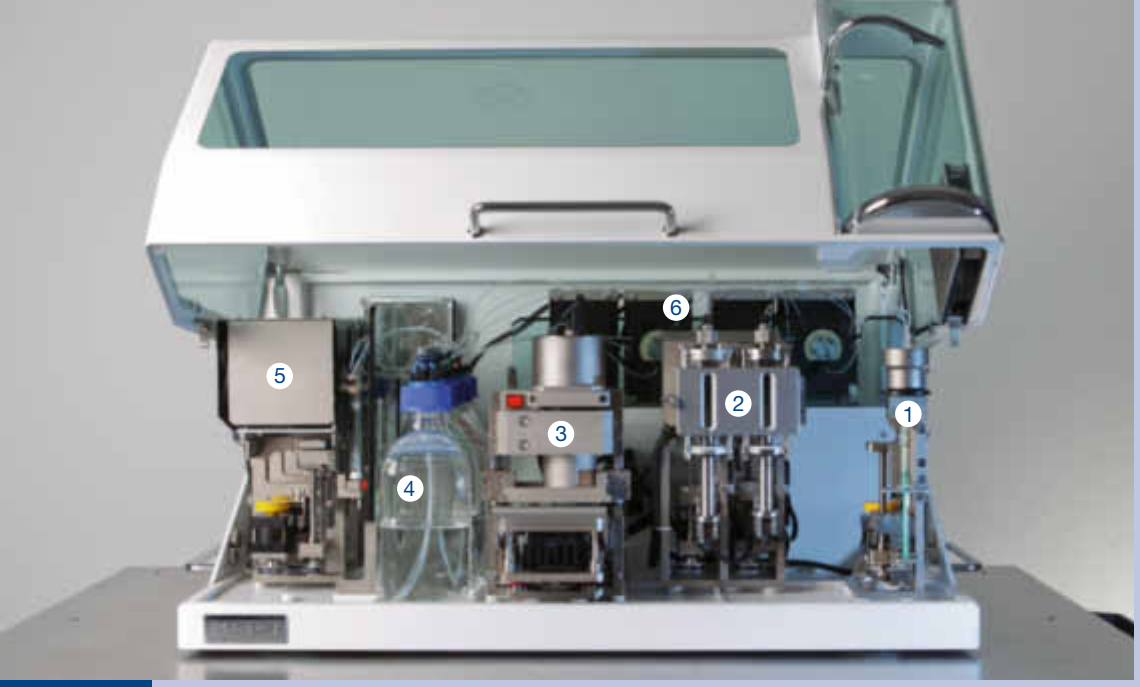


Using the new groundbreaking MCR 3, multiple antibiotics can be identified and quantified simultaneously, quickly and cost effectively without sample preparation.

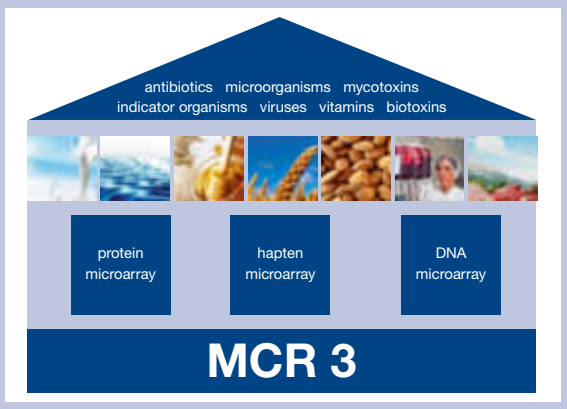
New Dimension in Food Safety

The MCR 3 has successfully completed the basic validation phase at the Milchprüfring Bayern e.V. and is now approaching its first practical application in the Bavarian dairy industry.

The development and validation phase of the project was made possible using public funding through the Federal Government (FEI/BMWi) and the State of Bavaria (StMELF). Current efforts are being made to develop the MCR 3 into a finished system, which can be marketed for routine, practical use to the dairy industry, worldwide. R-Biopharm AG (Darmstadt) and the equipment manufacturer GWK Präzisionstechnik GmbH (München) are working together with the consortium of developers, the Milchprüfring Bayern e.V. and its affiliated company AiM GmbH (München) to optimize the MCR 3 to make it fit for purpose at a reasonable cost.



1 sample handling
2 antibody syringes
3 chip camera unit
4 reagent reservoir
5 syringe pumps
6 valve unit



With the MCR 3, all elements are now available in Bavaria/Germany to establish a true centre of excellence for "antibiotic residues in milk" together with the project participants. The cluster project "monitoring of antimicrobial residues in milk" which established the new Biosensor System MCR 3 for routine practical analysis has helped to make this possible. This system will ensure that the Bavarian dairy industry is the market leader in ensuring the absence of residues and controlling the safety of its products!

Following the project to control antibiotics in milk, the test range for the MCR 3 can be extended to include other groups of substances and other matrices. It is possible to develop further biosensors for the detection of other pharmaceuticals in addition to toxins, hormones, viruses, pesticides, pathogenic bacteria and other analytes on the MCR 3 platform. As the monitoring of residues in milk, the MCR 3 could be refined for drinking, process or waste water and other foods such as meat, honey or eggs. Some promising projects in cooperation of TU München and LMU München are already underway.

Photos/Pictures: AiM GmbH · Fotolia · Landesvereinigung der Bayerischen Milchwirtschaft e.V. Milchprüfring Bayern e.V · Technische Universität München

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