The Brilliant Black Reduction Test (BRT)

Background and Basic Information

What are inhibitors?

Inhibitors are substances which may be present in milk which have the effect, even in small quantities, of inhibiting micro-organisms (bacteriostatic effect), or of killing them (bactericide effect). Inhibitors are frequently the cause of reject produce, especially in the manufacturing of fermented milk products, and are thus the cause of considerable financial losses in the dairy industry. Such inhibitors include, in particular, veterinary medicines, certain ingredients in animal feed, cleaning fluids and disinfectants, and natural inhibitors.

What are anti-infectives?

All substances used to treat bacterial infections come under the heading of **anti-infectives.** Antibiotics (penicillins, tetracyclins, aminoglycosides, etc.) belong to this group, as do other substances (sulfonamides etc.) which have an antibiotic effect.

The **use of anti-infectives** is indispensable for the treatment of illness in animal farming. However, it must be taken into account that animals treated in this way excrete the anti-infectives administered in both altered and unaltered form through their urine, faeces, and also in their milk. The **treatment of mastitis,** in particular, requires that antibiotics and other anti-infectives be administered directly into the udder; these are then discharged in the milk. Contamination of milk is therefore unavoidable during treatment of mastitis with anti-infectives, and also for a certain period after conclusion of treatment. **Statutory withdrawal times** must therefore be observed after the use of veterinary medicines before the next milk delivery.

According to the German Food and Feeding Stuff Law, LFGB (before Food and Consumer Goods Law, LMBG) and EEC Regulation No. 2377/90, in order to **safeguard the consumer** from inhibitory substances or residual anti-infectives milk may only be brought into circulation if the **statutory maximum** (MRL = Maximum Residue Limit) in the milk be not exceeded.

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Inhibitors in milk and anti-infectives in particular are a significant problem, both from the point of view of consumer health and from that of dairy technology. It is therefore in the interest of both, milk producers and milk processing companies, as well as their legal responsibility, only to circulate milk and milk products which are free of inhibitory substances or residues.

In order to guarantee this, control inspections of the milk are necessary. Such inspections are carried out by the milk producers and the milk processing companies, as well as by independent or governmental inspection laboratories, using reliable, standardised test methods.

Detection of inhibitors and residual anti-infectives in milk

Microbiological inhibitor test systems are the most generally accepted world-wide for the detection of inhibitors or residual anti-infectives. Microbiological inhibitor tests are especially suitable for routine inspections on account of their low cost, easy execution, relatively broad detection range and, in some cases, short test time (e.g. 2-3 hours for the most widely used test bacterium, *Geobac. stearothermophilus* var. *calidolactis* C953 (before *B. stearothermophilus*).

Test principle of the microbiological inhibitor test

Microbiological inhibitor tests operate by making use of the sensitivity of specific micro-organisms - so-called test bacteria - to antibiotics, sulfonamides and other inhibitors. The inhibitors lessen or prevent metabolic activity and thereby also the growth of the test bacteria. The thickness of the bacteria or the opacity of the test medium can be used as a gauge to measure the amount of test bacterium growth. Acidification and reduction processes are used to measure metabolic activity with the help of coloured indicators. The colour of the indicator changes in the acidification process in response to the acidic metabolic products of the growing test culture. In the reduction process, such as the Brilliant Black Reduction Test, the growing test culture reduces specific indicators (e.g. brilliant black), which also leads to a colour change (e.g. from blue to yellow). Activity can however also be measured using other indicators, such as pH value, degree of acidity, amount of lactic acid present, curdling time, or conductivity.

One of the best-known and most widely-used procedures is the **Brilliant Black Reduction Test (BRT).**

The Brilliant Black Reduction Test (BRT)

The Brilliant Black Reduction Test (BRT) was first described in 1967 by KRAACK and TOLLE. Because of its reliability and easy execution it very quickly established a wide distribution base in dairy circles. The BRT stood out as a result of its stability in the presence of different influencing factors. Various different milk products and also samples of preserved milk can thus be tested using the BRT. Furthermore, automation of the test procedure and also of the evaluation (photometric evaluation) is possible with large quantities. Since details of the BRT were first published, it has been and continues to be modified in different ways, with the aims of improving sensitivity of detection with regard to selected anti-infective residues, simplifying the execution procedure, or shortening the test time.

Experiences with the BRT show that this test method is easily standardised. The BRT has established itself as an internationally recognised standard method, and is used not only in milk quality payment but also in quality control in dairy industry and in monitoring inspections as a screening method for the detection of anti-infectives. Alongside its function as the **BRT Inhibitor Test**, the BRT thus also has a role in routine inspection, in its modified form, as the **BRT MRL Screening Test**.

Test principle of the Brilliant Black Reduction Test

With the BRT, the test medium is contained in cavities, or wells, in microtiter test plates or ampoules. This test medium is a mixture of nutrients, test bacteria *Geobac. stearothermophilus* var. *calidolactis* C953 (before *B. stearothermophilus*), brilliant black, and other supplements which help improve detection sensitivity towards chosen inhibitors. The milk samples are pipetted into the wells. Any inhibitors present can then diffuse throughout the test medium. During incubation the growing test bacteria shift the redox indicator (brilliant black) to its yellow or colourless reduction stage through the division of double azocompounds, and the test medium changes from blue to yellow. If inhibitors are present in the sample, growth will be minimal or non-existent. There will then be no reduction of the colouring agent, or to only a very small degree, and the test medium will remain blue.

Official description of the method

Within the European Union, uniform European **maximums** (**MRL** = Maximum Residue Limit) for veterinary medicinal residues in foodstuffs of animal origin, and thereby also in milk, were set down by law in EEC Regulation No. 2377/90. A microbiological inhibitor test for the qualitative detection of antibiotics and sulfonamides in both raw and heat-treated milk which is required to contain the test bacterium *Bacillus stearothermophilus* var. *calidolactis* is described in the decision of the Commission (91/180/EEC). The Brilliant Black Reduction Test is one such procedure.

In 1982 the BRT was recognised in Germany as an official inspection method for the detection of inhibitors in tanker milk, and was accorded a place in the official register of inspection procedures according to § 35 LMBG (now § 64 LFGB). Since then the method has been amended many times, most recently in February 1996.

The BRT systems of Analytik in Milch GmbH

AiM offers the following BRT systems:

BRT Inhibitor Test

This test was developed especially for the detection of inhibitors in milk, and distinguishes itself by its particularly high sensitivity to β-lactam antibiotic residues. The maximum residue limits (MRLs) laid down by the EU are reliably encompassed by the BRT Inhibitor Test (see following table); other antibiotics and sulfonamides present will also be detected.The BRT Inhibitor Test is particularly designed to fulfil the demands of milk quality regulation in Germany, but can also be used successfully in other countries in quality evaluation and payment for tanker milk. It is produced according to Commission Decision 91/180/EEC and § 64 LFGB (before § 35 LMBG) Method 01.01-5.

BRT MRL Screening Test

The MRL Screening Test is a modified BRT which is distinguished by an increased sensitivity towards certain antibiotics and sulfonamides. Besides the statutory maximums (MRLs) for β-lactam antibiotics, the test also encompasses substances from the sulfonamide group, macrolide group and aminoglycoside group to MRL level. Other substance groups are also detected with greater sensitivity than in the BRT Inhibitor Test (see following table). The MRL Screening Test is used as a screening method for the detection of anti-infectives in quality control in dairy industry as well as in monitoring tests. It is produced according to Commission Decision 91/180/EEC and § 64 LFGB (before § 35 LMBG) Method 01.00-11.

Further information

BRT – Background and Basic Information
BRT – Product Information
BRT – Instructions for Use of Plates
BRT – Instructions for Use of Tubes
BRT – The Correct Incubation
BRT – Use of Preserved Samples
BRT – The Visual Evaluation
BRT – The Photometric Evaluation
BRT – Use of Milk Products
Standards, lyophilisated – Product Information
Penicillinase Product Information

Should you have any further questions or require any special information, please do not hesitate to contact us directly.