The Brilliant Black Reduction Test (BRT)

The BRT Inhibitor Test
The BRT MRL-Screening Test
The BRT hi-sense

Agar Diffusion Procedure for the Detection of Inhibitors and Medicinal Residues in Milk and Milk Products

Instructions for use

The BRT is carried out as follows:

- Remove the sealing foil from the test plate
- Pipette 0.1 ml of the milk sample into one well (a second insertion is also recommended)
- Pipette 0.1 ml of the negative control (Inhibitor Free Milk) into one well
- Pipette 0.1 ml of the positive control (Penicillin G Standard) into one well
- Seal the test plate with the perforated sealing foil
- Incubate the test plate in a water bath or thermo-block at 65 °C until the negative control has turned yellow (BRT Inhibitor Test, 2 hrs 15 +/- 15 min; BRT MRL-Screening Test, 2 hrs 30 +/- 15 min, BRT hi-sense 3 hrs 30 +/- 30 min)
- The milk can then be poured away before evaluation
- The test results are read from the bottom of the test plate

The procedure is the same for test strips, single 48 or tubes. The test is standardised, and each batch is analysed according to the procedural conditions described above. If the procedure is not followed correctly there can be no guarantee for the accuracy of the results.

The test systems BRT Inhibitor Test, BRT MRL-Screening Test and BRT hi-sense are produced according to Commission Decision 91/180/EEC, § 64 LFGB Methods L 01.00-11 and L 01.01-5.

Analytik in Milch
Produktions- und Vertriebs- GmbH
Kaiser-Ludwig-Platz 2
D-80336 München

Phone
+49 89 5307512-0
Fax
+49 89 5307512-2

Email
info@aim-bayern.de
Internet
www.aim-bayern.de

Important notes for execution of the test

Storage and use-by date

The Brilliant Black Reduction Test must be kept cool, and should be stored on delivery at 6-10 °C. Under these circumstances the BRT is usable for at least 4 months (BRT ESL 9 months; BRT single 48 / BRT tubes 12 months) from production. After a period of storage the incubation time may be longer (by about 15-30 minutes, according to length of storage). The detection sensitivity of the product will not, however, be affected by long storage.

Sample material

The milk samples should be of acceptable quality and should be tested as soon as possible. Polluted samples, sour samples (pH <5.8) and samples with high bacteria count or high somatic cell count are unsuitable to be tested with BRT. For short-term preservation a storage temperature of 6-10 °C is sufficient. For a longer storage period the material should be frozen at -15 to -30 °C to prevent inactivation of any residue. Samples stored in this way should be thawed in a water bath at 45 °C, and must be thoroughly mixed before inspection.

Controls/Standards

In line with Good Laboratory Practice it is essential that microbiological inhibitor tests are controlled continuously. This is not only to fulfil the requirements of the officially described method, it also has many advantages for users. The end point of incubation is clearly indicated by the complete colour change of the negative control to yellow. The correct reactions of the positive and negative control samples give clear evidence to the user that the system is functioning properly and therefore validates all the results of samples investigated in the same batch. Comparing the colours of the test samples to the control samples makes it easy to interpret the test results. The negative and the positive controls should always be composed and tested in exactly the same way as the sample material. The influence of the test material on the test system is thereby taken into account.

The **Inhibitor Free Milk** is used in the BRT as a negative control. The Inhibitor Free Milk is delivered freeze dried and can be pipetted into the test wells after re-suspension. The well containing Inhibitor Free Milk must turn from blue to yellow during incubation.

The **Penicillin G Standard** serves as a positive control. The Penicillin G Standard is delivered freeze dried and has to be re-suspended according to the instructions for use to obtain the desired dilution, before it can be pipetted directly into one of the test wells. The well containing the Penicillin G Standard must remain blue after incubation.

The **Penicillinase solution** can be used for the identification of penicillinase-unstable penicillins. The Penicillinase solution is delivered ready for use and can be used as follows:

Pipette 10 µl of the Penicillinase solution into one of the wells. Add 0.1 ml of the positive test sample and incubate by the described manner. If the milk sample has been

contaminated by penicillinase-unstable penicillins/ß-lactams, the well will show a negative reaction (yellow colour) after incubation. In the presence of other inhibitors, it will show a positive reaction (blue colour). This negative reaction, as described above, clearly indicates the presence of penicillins/ß-lactams in the milk sample.

In the case of contamination the solutions or the Inhibitor Free Milk may be rendered unusable or may lead to incorrect results. The controls must therefore always be kept clean and carried out with care.

Sealing of test containers

The test plates (strips, tubes) should be sealed before being placed in the water bath, BRT single 48 tubes will be incubated unsealed. If water penetrates the reaction system, the test result may be inaccurate.

Incubation

Incubation can take place in a **water bath, a thermo-block** or in an **incubation chamber**. The water bath or the thermo-block is fundamentally preferable, in that temperature transfer is more direct and heat distribution more even than in an incubation chamber. For all of these methods it is important that the temperature remains constant and that there are no great discrepancies in the set temperature, either at different periods of times or in different spaces of the incubator.

Incubation temperature

The incubation temperature for the water bath and for the thermo-block is 65 °C. In an incubation chamber the temperature must be set individually according to the type of apparatus, and can be between 65-69 °C. Using a water bath or thermo-block for incubation is recommended because of the easier and more reliable regulation of temperature compared with the use of an incubation chamber. If the incubation temperature deviates from the ideal 65 °C (as measured within the test system), the incubation time will increase considerably (by 30 minutes or more).

Incubation time

The incubation time is dependent on the temperature set as well as on a constantly even distribution of heat. Under ideal circumstances the incubation time of the BRT Inhibitor Test is 2 hrs 15 +/- 15 min, of the BRT MRL-Screening Test, 2 hrs 30 +/- 15 min time and of the BRT hi-sense is 3 hrs 30 +/- 30 min. The incubation time may, however, increase as a result of factors such as differing storage times. The test must be incubated until the negative control is completely discoloured.

Evaluation

A correct evaluation can only be made if at least one positive and one negative control have been included on the test plate or strip or in one of the test tubes, and both have reacted correctly.

The interpretation of the test results should be in accordance to the official regulations; these regulations may differ in various countries.

In Germany there are, according to regulations, two possible interpretations of the results:

Inspection tests for **milk quality payment** (detection of inhibitors in bulk milk in accordance with the official test procedures according to § 64 LFGB Method L 01.01-5) are to be evaluated as follows:

- All reaction systems which display at least the colour intensity of the positive control (Penicillin G 4 µg/kg standard) are to be evaluated as positive.

Inspection tests carried out in accordance with the German Food, Feed and Consumer Goods Code (screening procedure for the presence of anti-infectives in milk in accordance with the official test procedures according to § 64 LFGB Method L 01.00-11) are to be evaluated as follows:

- All reaction systems which display a colour which is clearly to be differentiated from the colour of the negative control are to be evaluated as positive or suspect.

BRT tests can be evaluated by visual examination as well as by instrumental (e. g. photometric) evaluation methods – if their suitability has been proven.

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