Milk and Milk Products

Checks on residues of antibiotics in milk
Integrated system

In order to guarantee high technological quality and safety for consumers, an integrated system has been developed for milk that covers two aspects: the use of various methods and the sharing of responsibility between dairy farmers, processing industry and food inspection. The system applies some elements of the HACCP concept (Hazard Analysis & Critical Control Points) to minimize risks and to guarantee the safety of food.

In the integrated system (Fig. 1) each level has its own responsibilities, e.g. the farm’s vet responsibilities pertain to diagnosing, administering or supplying veterinary drugs and giving relevant advice to dairy farmers. The dairy farmer should observe the time lag set for drugs. Raw milk supplies must regularly be tested on the presence of residues of bacterial growth inhibitors by means of a microbiological inhibitor test (penalty system). Dairy factories must apply self-checking (in pursuance of article 14 of EEC Directive 92/46) to be sure that the MRLs (maximum residue limits, see EU Regulation No 47/2010) are not exceeded and that the milk is free of forbidden substances, such as chloramphenicol, dapsone, nitrofurans and nitroimidazoles. For that purpose, a suggestion is made to test milk in the collection tanks regularly on β-lactam antibiotics (and tetracyclins) by means of a rapid test or to test silo tank milk before starting production by means of a microbiological inhibitor test and, in addition, by means of tests for the detection of compounds for which the sensitivity of microbiological tests is not sufficient (e.g. quinolones, aminoglycosidases ...) or forbidden compounds (chloramphenicol, dapsone, ...).

Fig. 1. Integrated milk control system (according to HEESCHEN & SUHREN, 1996).
Food inspection or other competent authorities shall identify any residues that are detected and determine whether the MRL has been exceeded. Physico-chemical tests are used to that aim, e.g. HPLC or LC-MS/MS.

Such a system is process oriented and may be defined as a barrier system.

**TESTS PERFORMED IN BELGIUM**

**Tests within the framework of official quality testing**

The detection of antibiotics residues in milk has been part of the official raw milk quality testing since 1966. For this official testing all milk supplies are sampled by means of a well defined sampling system. The milk samples are tested by branch organisations on their composition (to calculate basic prices) and on quality. Since November 2000 all supplies are tested on the presence of bacterial growth inhibitors. According to the testing scheme in force (see Fig. 2) the Coplan Milk Test (DSM-Food Specialties, Delft, NL) is used as a screening test. The Copan Milk Test is a microbiological large spectrum test using (traces of) Geobacillus stearothermophilus var. calidolactis as testing organism. The absence of inhibitors in the milk during incubation leads to acidogenesis resulting in a change of colour of the pH indicator (bromocresol purple) from purple to yellow. Bacterial growth inhibitors in milk may inhibit this acidogenesis so that the colour of the test medium remains the same (i.e. purple). For reflectometric reading is used a flat bed scanner with specific software (C scan).

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**Fig. 2. Diagram for the detection of non-milk bacterial growth inhibitors in farm milk by branch organisations**

- **Screening**
  - Copan Milk Test
    - **pos** → no penalisation
    - **neg** → Confirmation

- **Confirmation**
  - **beta-s.t.a.r. 25**
    - **neg** > cut-off → no penalisation
    - **pos** ≤ cut-off → **penalisation**
      - **beta-lactam**

- **natural inhibitors**

- **preheated milk**
  - Copan Milk Test
    - **pos** → **penalisation**
      - non- **beta-lactam**
    - **neg** → no penalisation

- **penicillinase**
  - Copan Milk Test
    - **pos** → **penalisation**
      - non- **beta-lactam**
    - **neg** → no penalisation

**Cut-off values:**

- **Copan Milk Test:**
  - CIF ≥ 4.5
- **beta-s.t.a.r.:**
  - ratio ≤ 0.25
The excessive sensitivity of the screening test for most of the β-lactam compounds is compensated at the stage of confirmation. To that end the βeta-s.t.a.r. 25 reflector test with a special cut-off is used as first confirmation test. A testing line/reference line ratio that is lower than the target cut-off leads to a penalty for the supplier involved. If the ratio measured is higher than the target cut-off, the second stage of confirmation is performed in which the origin of the growth inhibition is examined: natural inhibitors that are present in the milk, such as an increased lactoferrin or lysozyme content. If such is not the case, a test is performed to find out whether the growth inhibition still occurs after incubation of the milk in the presence of penicillinase. If it does, that phenomenon reveals the presence of a non-β-lactam compound and the supplier will as yet incur a penalty.

**Tests at the dairy factory (self-checking)**

In order to guarantee the safety of the production of fermented dairy products (yoghurt, cheese,…) as well as compliance with EC regulation No 853/2004 a large number of tests on antibiotics are also performed by dairy factories. In pursuance of these provisions, food business operators of the dairy sector are not allowed to place on the market raw milk with an antibiotics residues content that is higher than the MRL. More than 90% of all milk supplies are tested on receipt by means of a rapid test that is focused almost exclusively on the group of penicillin and cephalosporin, the group of which residues occur most frequently. However, a rapid test does not allow a quantitative determination of the residue content. That can be done only by means of a physico-chemical confirmatory test. If a confirmatory test is not performed, milk with a positive screening test result is considered as being unsafe.

Milk with a residue content above the MRL is not fit for human consumption and must not be processed. It is not allowed to dilute residues to a content below the limit.

In addition to the test on receipt, silo tank (industrial) milk is sometimes tested on antibiotics residues before the start of production by means of a large spectrum microbiological test.

**Tests related to MONIMILK or Food Agency monitoring**

Tests for detecting residues of veterinary drugs are also performed on a wide range of dairy products (individual farm milk, collection tank milk, consumption milk, milk powder, sheep’s and goat’s milk). These tests are performed, partly for the BCZ-CBL (Belgian Dairy Confederation) within the MONIMILK programme and partly for the Food Agency, within the national monitoring scheme.

This monitoring aims at maximum detection of substances at MRL level. These substances are then identified and quantified. This gives a general view of the problems related to veterinary drugs residues in Belgian dairy products. In addition to tests on anti-infectants (antibiotics and chemotherapeutic substances), products also undergo tests for the detection of antiparasitic agents (benzimidazoles and macrocyclic lactones) as well as non steroidal anti-inflammatory agents.

Three different microbiological inhibitor tests and a receptor test are performed in order to detect antibiotics residues in dairy products. Part of the samples are immunologically screened on chloramphenical, (dihydro)streptomycin and 3-amino-2-oxazolidone (AOZ), a furazolidon metabolite.
Tests performed at the farm
Dairy farmers also perform increasingly more tests on antibiotics in order to decide whether the time lag set will be sufficient. To do so, the milk of animals that were treated is tested on expiry of the time lag by means of a microbiological test (e.g. Delvotest SP Mini or Single Copan Milk Test in bulb) or a rapid test. Especially after having used products to dry off dairy cows, it is recommended to test the milk of the animals systematically or to have it tested. In the case of early calving, the time during which milk must be kept apart according to requirements, is often insufficient. The dairy farmer may avoid penalties related to inhibitors by taking precautionary measures in order to prevent milk with antibiotics residues from being added to the tank.

Conclusion
Milk and milk products are among the foodstuffs that are tested in a most optimal way on antibiotics residues. In Belgium, all milk supplies are tested on inhibitors by means of a large spectrum microbiological inhibitor test. In addition, most dairy factories also perform a rapid test on antibiotics residues on entries and at random tests are conducted in which milk is tested on a very wide range of veterinary drugs (antibiotics, chemotherapeutics, antiparasitic agents,...) by means of a large number of testing methods. On each level of the chain, each of the actors has his own responsibilities. In an integrated residue control system microbiological, immunological and receptor tests all have their interest and value in addition to the physico-chemical tests.

References


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Developments in the field of standards and legislation


**Standards:**

ISO 3890-1|IDF 075-1:2009 - Milk and milk products - Determination of residues of organochlorine compounds (pesticides) - Part 1: General considerations and extraction methods
ISO 3890-2|IDF 075-2:2009 - Milk and milk products - Determination of residues of organochlorine compounds (pesticides) - Part 2: Test methods for crude extract purification and confirmation
ISO 8196-1|IDF 128-1:2009 - Milk - Definition and evaluation of the overall accuracy of alternative methods of milk analysis - Part 1: Analytical attributes of alternative methods
ISO 8196-2|IDF 128-2:2009 - Milk - Definition and evaluation of the overall accuracy of alternative methods of milk analysis - Part 2: Calibration and quality control in the dairy laboratory
ISO 12080-1|IDF 142-1:2009 - Dried skimmed milk - Determination of vitamin A content - Part 1: Colorimetric method
ISO 11870|IDF 152:2009 - Milk and milk products - Determination of fat content - General guidance on the use of butyrometric methods
ISO 11865|IDF 174:2009 - Instant whole milk powder - Determination of white flecks number
ISO 26323|IDF 213:2009 - Milk products - Determination of the acidification activity of dairy cultures by continuous pH measurement (CpH)
ISO/TS 27265|IDF/RM 228:2009 - Dried milk - Enumeration of the specially thermoresistant spores of thermophilic bacteria

**Other interesting IDF publications in 2009:**

Bulletin of the IDF No. 436/2009 - Environmental/Ecological Impact of the Dairy Sector - E-Form

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