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Detection of inhibitors and antimicrobial residues in milk and dairy products by screening methods – Guidance on preparation of the test portion

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Detection of inhibitors and antimicrobial residues in milk and dairy products by screening methods – Guidance on preparation of the test portion

FOREWORD

The use of antimicrobials in lactating cows coud present a risk of contamination of milk by antimicrobial residues. Various national and international regulations are in place to define the levels of residues that are considered as acceptable or as non-compliant.

These levels, described as maximum residues limits (MRL), are set for raw bovine milk and there are only a few countries with regulations for semi-transformed or transformed dairy products. All tests available for the screening of antimicrobial residues have been developed, and often validated, on raw milk and are not immediately applicable to dairy products.

Under the supervision of IDF, laboratories routinely working with milk products have provided information on the use of screening methods. This has led to recommendations on the preparation of the test portion for the intended field of application. IDF involvement guarantees the scientific objectivity of the recommendations.

This article presents various ways of preparing dairy products to obtain test samples that are applicable for most available screening methods for the detection of antimicrobial residues in a consistent manner.

IDF would like to thank the members of the Project group A02 “Detection of inhibitors/antimicrobial residues in milk and milk products by screening methods” including:

Adriany Ansgar (DE), Aktipis Anastasios (GR), Baumgartner Christian (DE), Brenne Egil (NO), Broutin Pierre (FR), Chocilowska-Choluj Joanna (PL), Costa Fabiano Freire (BR), Diserens Jean-Marc (CH) – leader, Dominguez Elena (ES), Ferrini Anna Maria (IT), Ganesh Panta (US), Gaudin Valerie (FR), Granier Benoît (BE), Heynen Olivier (BE), Jamieson Paul (NZ), Kerkhof Jan (NL), Kraehenbuehl Karin (CH), Kreis Birgit (DE), Mitchell Mark (CA), Ordoñez Alfredo (MX), Pecou Anne (FR), Pikkemaat M.G. (NL), Portmann Reto (CH), Reybroeck Wim (BE), Salter Robert (US), Scott Gavin (NZ), Urbsiene Laima (LT), Van Den Bijgaart Harrie (NL).

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Nico van Belzen, PhD
Director General
International Dairy Federation
Brussels, May 2014

Detection of inhibitors and antimicrobial residues in milk and dairy products by screening methods – Guidance on preparation of the test portion

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1. INTRODUCTION

The use of antimicrobials in lactating cows could present a risk of contamination of milk by antimicrobial residues. Various national and international regulations are in place to define the levels of residues that are considered as acceptable or as non-compliant.

Acceptable levels, described as Maximum Residues Limits (MRL), are set for raw bovine milk and there are only a few countries with regulations for semi-transformed or transformed dairy products (e.g. The Russian Federation, Technical Regulation for Milk and Milk Products, Federal Law dated 22.07.2010 No. 163-FZ). Hence, in the EU a (semi-)transformed dairy product containing residues of a veterinary drug at any concentration needs to be considered as non-compliant. The worldwide trade of dairy products is increasing and it is not always possible to trace back the origin of the products. Results of the analyses performed to detect antimicrobial residues in the raw milk used to manufacture such products are not always available. Information on how the analysis was performed is also often missing.

The dairy industry needs tools to analyse dairy products in order to check their quality and safety and ascertain the absence, or presence within permissible limits, of violative antimicrobial residues before processing.

All tests available for the screening of antimicrobial residues have been developed, and often validated, on raw milk and are not immediately applicable to dairy products such as whole milk powder, skimmed milk powder, whey, condensed milk, buttermilk, etc.

This article presents various ways of preparing dairy products to obtain test portions that are applicable for most available screening methods for the detection of antimicrobial residues.

2. MAIN DAIRY PRODUCTS EXPORTED WORLDWIDE

Table 1 has been extracted from the FAOSTAT website and gives an idea of the size of the world trade in cow dairy products.

Table 1. World exports of cow dairy products 2010

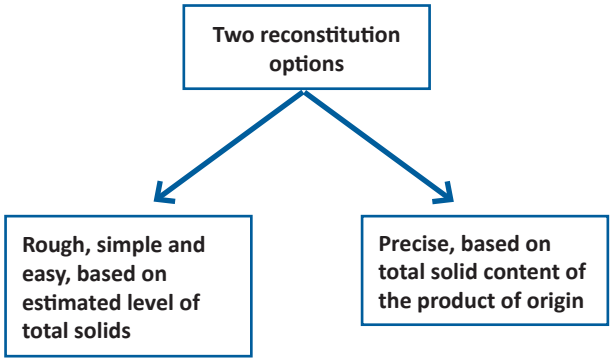
Item	Tonnes[a]
Cow milk, whole raw	8 113 498
Cheese of whole cow milk	5 133 247
Milk, whole dried	2 355 944
Milk, skimmed dry	2 039 371
Whey, dry	2 032 907
Butter, cow milk	1 573 735
Milk skimmed of cows	1 285 070
Buttermilk, curdled, acidified milk	1 247 130
Cream, raw	1 189 350
Milk, whole evaporated	989 459
Whey, condensed	606 888
Milk whole condensed	322 920
Processed cheese	263 239
Whey, raw	80 658
Milk, skimmed condensed	74 119
Milk, dry buttermilk	43 012
Ghee, butter oil of cow milk	30 818
Whey cheese	5 467
Cheese of skimmed cow milk	469

[a]Data taken from FAOSTAT, http://faostat.fao.org/site/569/DesktopDefault.aspx?PageID=569#ancor

3. RECONSTITUTION OF DAIRY PRODUCTS

To be able to use screening tests to detect possible antimicrobial residues, dairy products must be reconstituted to reach a composition, pH and viscosity that are as close as possible to the original raw dairy product.

Two approaches can be applied for the reconstitution of dairy products: (i) simple reconstitution to obtain milk containing an approximate concentration of dry matter or (ii) a more scientific approach whereby the exact amount of water that was eliminated during processing of the dairy product is added to the concentrated or powdered product.



In the end, there is not much difference between the two procedures!

One should not forget that screening tests do not provide precise quantitative results.

4. TOTAL SOLIDS

Processed dairy products lose water during processing. To reconstitute a product and obtain the same product, containing the same water content as before processing, the lost water must be re-introduced.

The amount of water to be added is calculated on the dry matter of the product of origin.

The average dry matter or total solids of various dairy products are listed in Table 2. The levels of total solids are used to calculate the dilution factor for reconstitution of the various dairy products.

Table 2. Content of dry matter/total solids in various dairy products

Dairy product	Average dry matter/ total solids (g/100 g)
Milk whole raw	12.3
Skimmed milk pasteurized	8.5
Sweet whey liquid	6.3
Buttermilk	9
Yoghurt	18
Sweetened condensed skimmed milk	24
Milk sweetened condensed	28
Milk evaporated	25
Evaporated skimmed milk	20

4.1. Test suitability

Most screening tests are suitable for the detection of antimicrobials in dairy products. The manufacturer of the test must be contacted in case of doubt on the applicability of the test to the dairy product to be analysed.

4.2. pH

Most screening tests require the pH of the reconstituted dairy products to be between 6.5 and 7. If this is not the case, the pH should be adjusted with 1 N or 2 N NaOH or HCl.

In most cases, pH adjustment is not necessary because the pH of dairy products is normally between 6.7 and 7. However, with acidified milks and whey products the control and adjustment of pH is very important.

5. RECONSTITUTION PROCEDURES

5.1. Liquid dairy products

Liquid dairy products like whole milk, skimmed milk, pasteurized or UHT milk, liquid whey, buttermilk, etc. are analysed as such. The screening test used must have been tested before analysing samples for release. The pH of the samples must be checked and adjusted if necessary.

- Transfer a volume of liquid sample to a sample bottle

- If necessary, adjust the pH to 6.5–7.0 with 1 or 2 N NaOH or 1 or 2 N HCl
- After cooling, the obtained solution is used for the screening test

5.2. Viscous liquid products

For condensed and evaporated milks a two time dilution (1:2 (w/v)) is applied.

- Weigh 10 g of condensed or evaporated milk
- Add 10 ml distilled water (at 45°C if necessary)
- If necessary, adjust the pH to 6.5–7.0 with 1 or 2 N NaOH or 1or 2 N HCl
- After cooling, the obtained solution is used for the screening test

5.3. Powder products

Powder products include milk powder, powdered infant formulae, whey powder, casein powder, etc. As mentioned above, there are two reconstitution procedures that can be followed.

a. Simple procedure

Prepare a 10% dilution of powdered dairy product in demineralized or distilled water (1:10, w/v).

- Weigh 10 g powder in a sample bottle
- Add 10 ml demineralized water
- Wait between 30 s and 2 min to allow the powder to rehydrate
- Mix with a spoon until all lumps have disappeared and a thick creamy solution is obtained
- It might be necessary to use warm water (45°C) to completely dissolve the sample
- Check for the absence of lumps
- Add another 80 ml of demineralized water (at 45°C if necessary)
- If necessary, adjust the pH to 6.5–7.0 with 1 or 2 N NaOH or 1 or 2 N HCl
- After cooling, the obtained solution is used for the screening test

b. Precise procedure

As seen before, each dairy product has a different level of total solids. The level of total solids is taken into consideration so that the dry dairy product is reconstituted with the precise amount of water to obtain the same viscosity and total solids as that of the product of origin.

If a more precise reconstitution procedure is required, the exact level of dry matter of the dairy product of origin must be obtained in order to calculate the reconstitution factor.

c. Lumps

The presence of lumps (undissolved powder particles) can interfere with the test. This is why it is very important to eliminate them. If lumps are still present after shaking, use a glass rod to squeeze the lumps against the bottom or the walls of the flask. This is the best way to eliminate them.

Table 3. Reconstitution of dairy products

Dairy product	Average dry matter/ total solids (g/100g)	Dilution	Reconstituted dairy product
Sweetened condensed milk	28	2×	Sweetened milk
Evaporated milk	25	2×	Whole milk
Evaporated skimmed milk	20	2×	Skimmed milk
Full cream milk powder	–	8×	Whole milk
Skimmed milk powder	–	12×	Skimmed milk
Sweet whey powder	–	16×	Sweet liquid whey
Dry buttermilk	–	11×	Buttermilk

5.4. Full cream milk powder

- Weigh 1.3 g of powder in a sample bottle
- Add 9 ml distilled water
- Wait between 30 s and 2 min to allow the powder to rehydrate
- Mix vigorously with a spoon or with a mechanical shaker until all lumps have disappeared
- It might be necessary to use warm water (45°C) to completely dissolve the sample
- Check for the absence of lumps
- If necessary, adjust the pH to 6.5–7.0 with 1 or 2 N NaOH or 1 or 2 N HCl
- After cooling, the obtained solution is used for the screening test

5.5. Skimmed milk powder

- Weigh 1 g of powder in a sample bottle
- Add 10 ml distilled water
- Wait between 30 s and 2 min to allow powder to rehydrate
- Mix vigorously with a spoon or with a mechanical shaker until all lumps have disappeared
- It might be necessary to use warm water (45°C) to completely dissolve the sample
- Check for the absence of lumps
- If necessary, adjust the pH to 6.5–7.0 with 1 or 2 N NaOH or 1 or 2 N HCl
- After cooling, the obtained solution is used for the screening test

5.6. Whey powders

- Weigh 0.6 g of whey powder in a sample bottle
- Add 9 ml distilled water
- Wait between 30 s and 2 min to allow powder to rehydrate
- Mix vigorously with a spoon or with a mechanical shaker until all lumps have disappeared
- Some whey powders develop a lot of bubbles and/or foam while shaking. If this is the case, use a magnetic stirrer to dissolve the whey powder
- It might be necessary to use warm water (45°C) to completely dissolve the sample
- Check for the absence of lumps
- If necessary, adjust the pH to 6.5–7.0 with 1 or 2 N NaOH or 1 or 2 N HCl
- After cooling, the obtained solution is used for the screening test

5.7. Dry buttermilk

- Weigh 1 g of powder in a sample bottle
- Add 10 ml distilled water
- Wait between 30 s and 2 min to allow powder to rehydrate
- Mix vigorously with a spoon or with a mechanical shaker until all lumps have disappeared
- It might be necessary to use warm water (45°C) to completely dissolve the sample
- Check for the absence of lumps
- If necessary, adjust the pH to 6.5–7.0 with 1 or 2 N NaOH or 1 or 2 N HCl
- After cooling, the obtained solution is used for the screening test

6. SPECIAL CASES

Some dairy products require special preparation before being used for the screening test.
As already mentioned, screening tests for antimicrobials have been developed for raw milk. The analysis of other dairy products requires sample preparation and, even after such procedures, some tests are not suitable for such extracts.

6.1. Cream

- Centrifuge 40 ml cream for 10 min at 2000 × g
- Remove the cream layer by suction or collect the watery phase with a pipette through the fat layer
- Pour the watery phase into a sample bottle
- If necessary, adjust the pH to 6.5–7.0 with 1 or 2 N NaOH or 1 or 2 N HCl
- After cooling, the obtained solution is used for the screening test

6.2. Butter and butter oil (ghee)

- Put some butter into a sample bottle
- Melt the butter in a water bath (45°C) for a maximum of 15 min
- Centrifuge for 10 min at 2000 × g
- Remove the fat layer by suction or collect the watery layer with a pipette through the fat layer
- If necessary, adjust the pH to 6.5–7.0 with 1 or 2 N NaOH or 1 or 2 N HCl
- After cooling, the obtained solution is used for the screening test

6.3. Yoghurt

- Adjust the pH to 6.5–7.0 with 1 or 2 N NaOH or 1 or 2 N HCl
- The obtained solution is used for the screening test
- If the yoghurt is very thick, it must be diluted twice before testing

6.4. Hard and semi-hard cheese

- Take a sample from the centre part of the cheese
- Do not take any part of the rind
- Cut into small pieces
- Weigh 30 g in a Stomacher bag
- Add 70 ml antibiotic-free milk (at approximately 45°C)
- Mix in a Stomacher for 4 min
- Filter or centrifuge to obtain a watery extract
- If necessary, adjust the pH to 6.5–7.0 with 1 or 2 N NaOH or 1 or 2 N HCl
- The obtained solution is used for the screening test

Individual varieties of cheeses are all different and a procedure suitable for one kind of cheese may not be suitable for another cheese and must be adapted.

7. TEST PROCEDURE

Some tests require sample preparation such as. defatting, cooling, centrifugation, buffering, etc. Even raw milk may require some pretreatment and milk from animal species other than the cow can require a special sample pretreatment before testing.

The detection of antimicrobial residues in reconstituted dairy products must follow the procedure described in the kit protocol.

Every combination of dairy product and antimicrobial screening test must be validated before being used for routine analysis of samples.

A negative and a positive sample must be analysed with every series of samples, or at least once a day, when analysing the same type of samples.

8. EXPRESSION OF RESULTS

Because MRLs are set for raw bovine milk and not for transformed or semi-transformed dairy products, the results obtained must be interpreted as positive or negative on the reconstituted dairy product. A (semi-)transformed dairy product with presence of a veterinary drug residue needs to be considered as “possibly non-compliant”.

Such results must be confirmed by confirmatory methods such as, for example, described in the Commission Decision 2002/657/EC (CEC 2002). These are methods that provide full or complementary information enabling the substance to be unequivocally identified and, if necessary, quantified at the level of interest.

An agreement with the dairy product supplier or manufacturer could be made to recognize the value of the results obtained by screening tests on well-defined dairy products, applying a well-described procedure.

9. DETECTION LIMITS OF TEST KITS ON DAIRY PRODUCTS

The detection limits provided by the manufacturers are for raw (bovine) milk and not for other dairy products.

The applicability and detection limits must be determined by the user of the test on the dairy product of interest.

In some cases, manufacturers have already established procedures for dairy products and can be contacted to seek confirmation on the applicability of their test.

Recovery trials, repeatability and reproducibility should also be tested for validation of the sample preparation and the screening method.

10. AVAILABLE KITS

Most test kits are suitable for the analysis of antimicrobial residues in dairy products.

The procedure must be adapted to the product analysed and the procedure must be tested using blank samples and samples containing known added levels of antimicrobials.

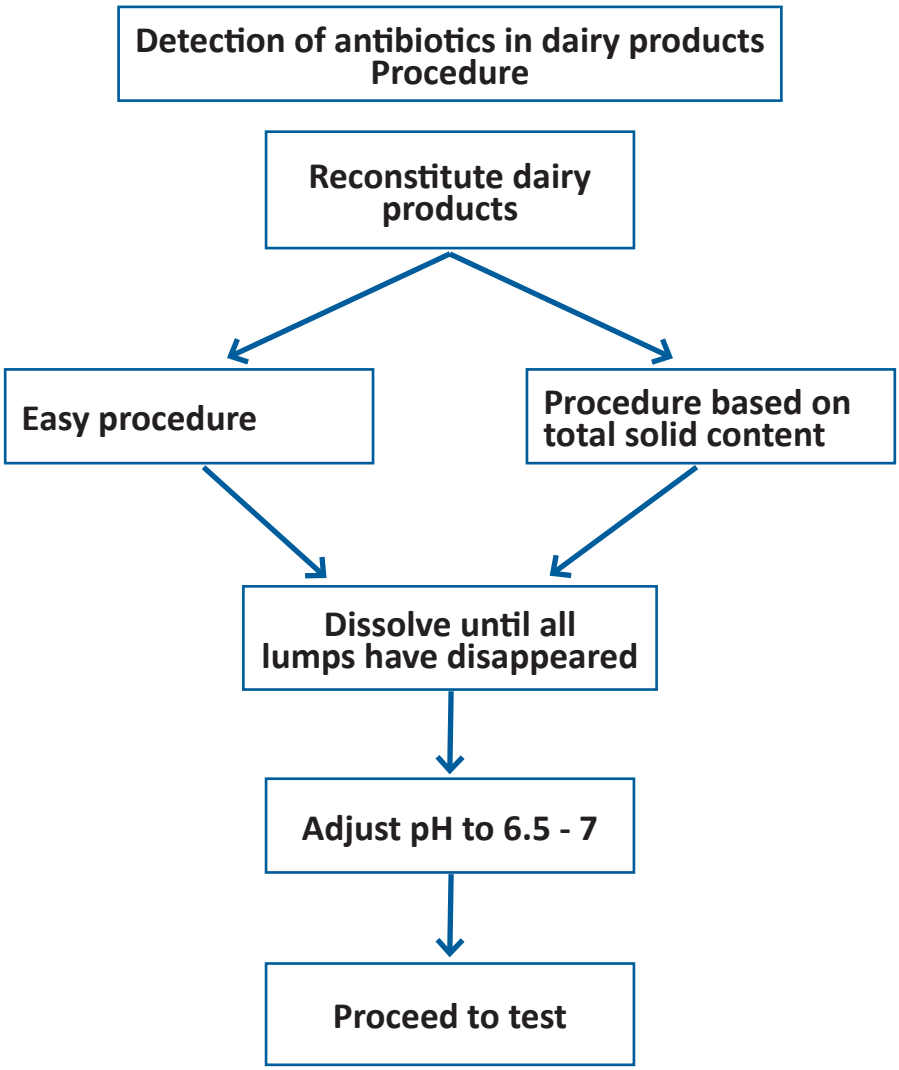
Manufacturers must be contacted in case of doubt.

11. CONCLUSION

The detection of antimicrobial residues in most dairy products is feasible with a few preparative steps. The viscosity and pH are very important for good performance of the test.

The parallel analysis of blank and positive control samples in similar matrix material is essential for checking the performance of the test with the dairy product being analysed. If similar material is missing, spiking a portion of the test sample with a known amount of analyte could be an alternative.

An agreement with the supplier or manufacturer of the dairy product on the method and procedure to be applied for the detection of antimicrobial residues could be the best way to eliminate discrepancies in results.



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DETECTION OF INHIBITORS AND ANTIMICROBIAL RESIDUES IN MILK AND DAIRY PRODUCTS BY SCREENING METHODS – GUIDANCE ON PREPARATION OF THE TEST PORTION

Jean-Marc Diserens and members of the Project Group

ABSTRACT

Treatment of lactating cows with antimicrobial agents can result in the presence of drug residues in milk. Tests available for the screening of antimicrobial residues have been developed, and often validated, on raw milk and are not immediately applicable to dairy products such as whole milk powder, skimmed milk powder, whey, condensed milk, buttermilk, etc. Many dairy products require special preparation before they can be used in screening procedures routinely used for raw milk. This article presents ways of preparing various dairy products to obtain appropriate test samples. Detailed procedures are given for the reconstitution of dried samples such as milk powder and for the preparation of samples of cream, butter, yoghurt and cheese. Particular attention is paid to the dilution necessary for test samples of various types.

Keywords: Antimicrobial, Contaminant, Dairy, Food safety, Milk, Drug residues, Sample preparation, Screening, Veterinary medicines

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"	Usually double quotes and not single quotes
? !	Half-space before and after question marks, and exclamation marks
±	Half-space before and after
microorganisms	Without a hyphen
Infra-red	With a hyphen
et al.	Not underlined nor italic
e.g., i.e.,...	Spelled out in English - for example, that is
litre	Not liter unless the author is American
ml, mg,...	Space between number and ml, mg,...
skimmilk	One word if adjective, two words if substantive
sulfuric, sulfite, sulfate	Not sulphuric, sulphite, sulphate (as agreed by IUPAC)
AOAC INTERNATIONAL	Not AOACI
programme	Not program unless a) author is American or b) computer program
milk and milk product	rather than "milk and dairy product" - Normally some latitude can be allowed in non scientific texts
-ize, -ization	Not -ise, -isation with a few exceptions
Decimal comma	in Standards (only) in both languages (as agreed by ISO)

No space between figure and % - i.e. 6%, etc.

Milkfat	One word
USA, UK, GB	No stops
Figure	To be written out in full
1000-9000	No comma
10 000, etc.	No comma, but space
hours	Ø h
second	Ø s
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