



Instituut voor Landbouw- en Visserijonderzoek

FASFC ACCEPTANCE CRITERIA FOR MICROBIOLOGICAL INHIBITOR TESTS: FULFILLMENT BY NEW TESTS

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EuroResidue VII, May, 16th 2012

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Beleidsdomein Landbouw en Visserij



INTRODUCTION

Regulation (EC) No 853/2004: milk collected from milk production holdings must be checked on the presence of antibiotic residues so that the raw milk placed on the market is not containing residues in a quantity $>$ MRL

In most countries, each delivery of milk:

- sampled
- checked on antimicrobials: each sample (BE, NL,...) or less
- screening with a microbiological test
- post-screening with a microbiological test and/or a receptor assay

Result: - penalty for the farmer

- withdrawal of collection of the next production (in some cases)

⇒ Number 1 in residue testing in quantity of tests

e.g. BE (2011): testing of inhibitors in ex-farm milk: 1 287 262 tests

652 (0.051%) positive samples

TESTING OF EX-FARM MILK

Screening: mostly based on a microbiological growth inhibition test with *Geobacillus stearothermophilus* as test organism

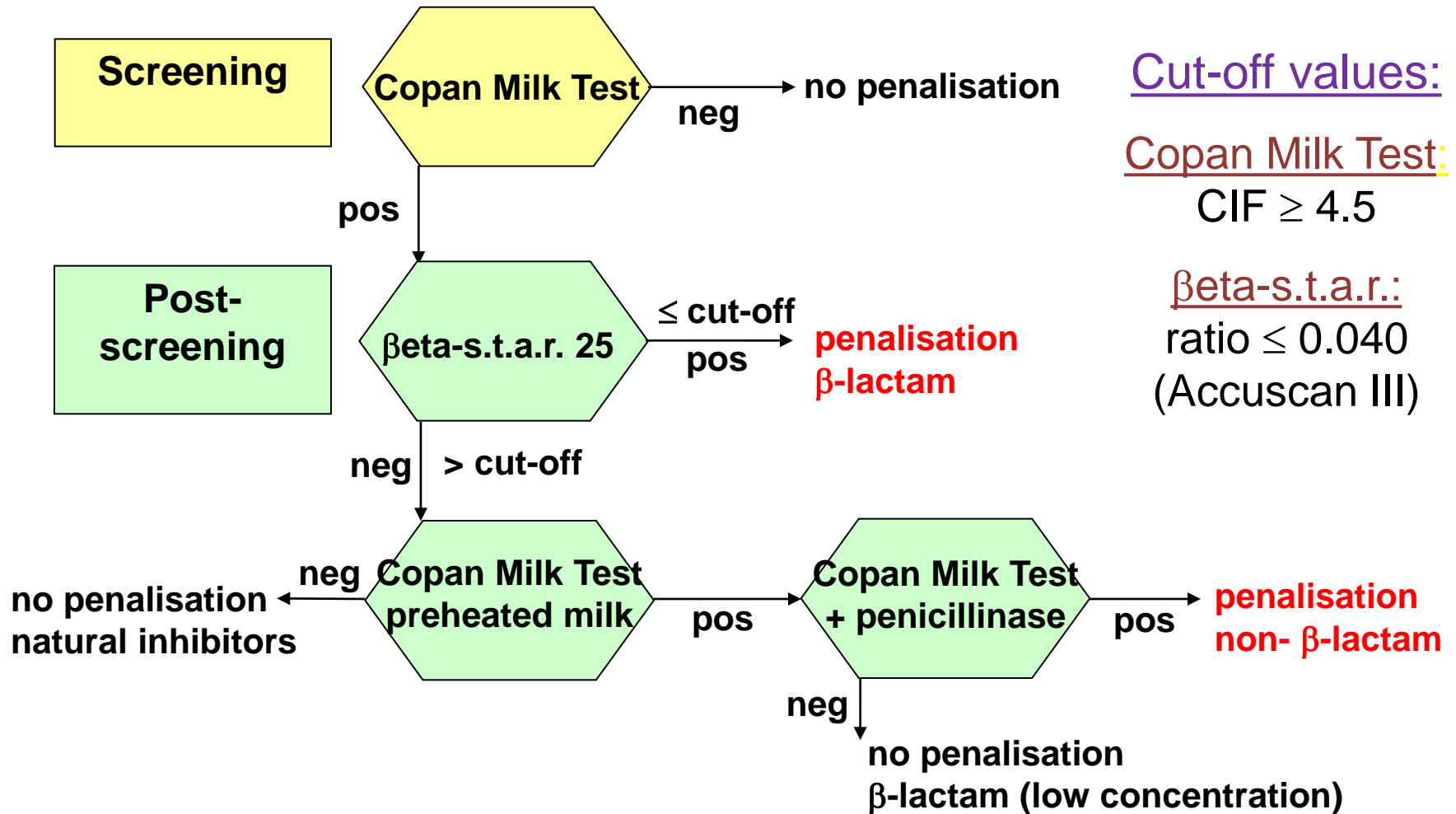
⇒ not all marker residues detected at MRL

⇒ no clear requirements set in the European legislation

Choice of test mainly based on:

- price
- possibility of automation (pipetting, reading,...)
- ...

ACTUAL TEST PROCEDURE FOR INHIBITORY SUBSTANCES IN MILK BY THE IO IN BE



ACCEPTANCE CRITERIA MICROBIAL INHIBITOR TEST

http://www.afsca.be/labor/zugelassenelabore/algemeines/_documents/2010-10-15CriteresTestSubstancesInhibitrices-en-de.doc

GDP/LB/LABO/556040 of 15/10/2010

- result can be interpreted by means of an instrument
- good correspondance with visual reading
- total test time ≤ 4 h
- robustness: no disturbance by a high somatic cell count, certain microflora, lipolysis,... unless the use of an adapted test procedure preventing that false-positive results could lead to penalties
- shelf life of reagents minimal 3 months, with a stable detection capability
- no large batch differences ($<25\%$ of the $cc\beta$), tested for a penicillin, a sulfonamide and a tetracycline

ACCEPTANCE CRITERIA MICROBIAL INHIBITOR TEST

- manufacturer of reagents:
 - sufficient production & storing capacity + guarantee of 'in-time' delivery
 - to provide information about the quality control (if defective batches are found in practice: each batch shall be tested by the Belgian NRL)
 - criteria for blank milk + a penicillin, a sulfonamide and a tetracycline
- incubation period shall be mentioned for each batch
- test shall be validated by the NRL in accordance with Directive (EC) No 2002/657/EC
- test approved by FASFC upon assessment of a validation file compiled by the NRL; whenever necessary the Scientific Committee of the Agency will be requested to give its opinion
- notification of each change in composition

FASFC ACCEPTANCE CRITERIA

- Detection capacity

- 85% or minimal 12 upon 14 β -lactam compounds* detectable at MRL

amoxicillin, ampicillin, benzylpenicillin, cloxacillin, nafcillin, cefalexin, cefalonium, cefazolin, cefoperazone, cefquinome, ceftiofur, desfuroylceftiofur, cephapirin, desacetylcephapirin

- 75% of minimal 3 upon 4 sulfonamides* & sulfones detectable at MRL/recommended concentration for detection

sulfadiazine, sulfadimethoxine, sulfadoxine, dapson

**: marker residues of all compounds with a brand registered in Belgium for use in lactating cows;*

MRL: Regulation (EU) No 37/2010 and amendments

FASFC ACCEPTANCE CRITERIA

- Detection capacity
 - 100% or 2 upon 2 tetracyclines* detectable at 2×MRL
chlortetracycline, oxytetracycline
 - 35% or minimal 6/16 of other compounds* detectable at 3×MRL

spiramycin, tylosin, lincomycin, pirlimycin,
dihydrostreptomycin, gentamicin, kanamycin, neomycin,
spectinomycin, danofloxacin, enrofloxacin, marbofloxacin,
rifaximin, colistin, clavulanic acid, trimethoprim

**: marker residues of compounds with a brand registered in Belgium for use in lactating cows in Belgium;*

MRL: Regulation (EU) No 37/2010 and amendments

VALIDATION OF NEW MICROBIOLOGICAL SCREENING TESTS

Validation at ILVO-T&V respecting Commission Decision 2002/657/EC

Eclipse 50 (ZEU-INMUNOTEC S.L., Spain)

Delvotest Accelerator (DSM-Food Specialties, The Netherlands)

Charm Blue-Yellow II (Charm Sciences Inc., US)

Eclipse 3G (ZEU-INMUNOTEC S.L., Spain)

Delvotest T (DSM-Food Specialties, The Netherlands)

- Test repeatability
- Detection capability at target concentration (FASFC approval criteria)
- Robustness
 - milk parameters
 - somatic cell count, fat content, protein content
 - rate of false-positive results & false-negative results
 - batch differences
 - participation in (a) ring trial(s)

TEST REPEATABILITY

Test	s_r			
	Blank milk	Spiked milk		
		Low pos	Medium pos	High pos
Eclipse 50	0.044	0.036	0.040	0.041
Delvotest Accelerator	0.44	0.57	---	0.48
Charm Blue Yellow II	0.77	0.75	---	0.51
Eclipse 3G	0.050	0.018	0.037	0.040
Delvotest T	0.46	0.39	---	0.51

DETECTION CAPABILITIES versus FASFC CRITERIA

Group	Minimum n detected	Number of compounds detected at target concentration				
		Eclipse 50	Delvotest Accelerator	Charm Blue Yellow II	Eclipse 3G	Delvotest T
β-lactams	12/14	9	12	13	12	12
sulfonamides & sulfones	3/4	---	4	3	4	4
tetracyclines	2/2	2	0	2	2	2
other	6/16	---	7	10	6	7
total	23/36	---	23	28	24	25

SUMMARY OF ROBUSTNESS

Milk parameter	% False positive results					
	Charm BY II		Eclipse 3G		Delvotest T	
	n*	%	n*	%	n*	%
milk with normal composition	1717	0.2	435	0.9	403	2.7
bacterial count >10 ⁸ per ml	20	0.0	nt	nt	nt	nt
scc 5×10 ⁵ - 10 ⁶ per ml	232	6.5	198	15.7	68	19.1
somatic cell count >10 ⁶ per ml	145	33.8	117	32.5	69	47.8
fat content <2 g per 100 ml	10	0.0	nt	nt	nt	nt
fat content >6 g per 100 ml	82	12.2	72	29.2	46	8.7
protein content <2.5 g per 100 ml	10	30.0	nt	nt	9	0.0
protein content >4 g per 100 ml	10	40.0	nt	nt	nt	nt
bacterial bacteriocins		yes		yes		yes
pH >7.0		yes		yes		yes

n*: number of samples tested

nt: not tested

CONCLUSIONS

By setting acceptance criteria, kit manufacturers are forced to improve their screening tests

New generation of microbiological screening tests

- improved detection capability, especially for tetracyclines
- increased rate of false-positive results

⇒ important to find a good balance

⇒ positive results should be confirmed by a test based on a different test principle

Results used by the FASFC for approval

BE: LIST OF APPROVED TESTS

In Belgium: list of FASFC (Federal Agency for the Safety of the Food Chain) approved tests for the official determination of the quality and composition of ex-farm milk delivered to buyers

Screening for inhibitory substances:

Routine method: microbiological inhibitor test

Delvotest MCS (DSM, The Netherlands) (till 31/08/2012)

Copan Milk Test (Copan Italia, Italy) (till 31/08/2012)

Charm Blue-Yellow II (Charm Sciences, US)

Eclipse 3G (ZEU-INMUNOTEC, Spain)

Delvotest T (DSM-Food Specialties, The Netherlands)

Rapid test: β -s.t.a.r. 25 with a special cut-off ratio of 0.040:

benzylpenicillin 3 μ g per kg: negative

benzylpenicillin 4 μ g per kg: positive



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This was the last presentation of EuroResidue VII \Rightarrow time to rest

Thank you for your attention

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