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Validation report Quantum BT-CC

(Cat. n°: W1530/W1560)

(ProGnosis Biotech S.A., Larissa, Greece)

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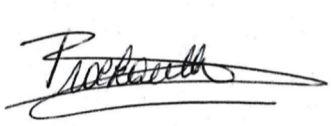

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1. Introduction

Quantum BT-CC (ProGnosis Biotech S.A., Larissa, Greece) is a qualitative one-step 2 to 5 minutes rapid lateral flow test kit for the simultaneous detection of β-lactams, tetracyclines, ceftiofur and cefalexin in cows', sheep and goats' milk.

This test is validated at ILVO-T&V (Technology & Food Science Unit of the Flanders research institute for agriculture, fisheries and food) according to ISO Technical Specification 23758 | IDF RM 251 (ISO/IDF, 2021), Commission Implementing Regulation 2021/808 and to the EURL Guidance document on screening method validation (*Anon.*, 2023). The following analytical parameters were checked: detection capability, test specificity, rate of false positives, repeatability of test and reader and test robustness. Furthermore, the suitability of Quantum BT-CC to screen different milk types (UHT milk and reconstituted milk powder) and milk from species other than the cow (goat and ewe) was evaluated. The test was also included in a interlaboratory study organised by ILVO in April 2025.

2. Test procedure

Test preparation
All reagents should be at room temperature (21-25°C) before use. Open as many foils with cassettes as needed. Shake the milk vigorously by hand or vortex to ensure milk sample homogeneity (no precipitation or clotting). The ideal temperature of the milk sample is between 8 and 25°C. In this validation study raw milk temperature was standard 8°C.

Test procedure
Step 1: Use a disposable plastic fixed-volume pipette included in the kit and add the milk sample in the circular window of the cassette(s). Maximum 4 samples can be run simultaneously.
Step 2: Place the cassette(s) inside the plastic holder of the reader and press SCAN using the S-flow software. All cassette(s) must be facing up. A 5 minute countdown starts immediately.
Step 3a: After 2 minutes, a First Quantum read is performed. The device automatically scans the cassettes and if all simultaneous tested samples are free of antibiotics (or contain a concentration below the detection limit), the analysis stops and the results are interpretated negative with no ratio values given.
Step 3b: If one of the simultaneous tested samples is suspected positive, the analysis continues until it completes 5 minutes.
Step 4: When completing these 5 minutes a final Quantum read is performed where the S-flow software will use the ratio of the test line and the control line to calculate the result. Both an interpretation of the result and a ratio is given.

With a 5 minute incubation, the cassette could also be visually read and interpreted.

The control line should always be visible, if not the test is invalid. For the test lines (T1 – T4) following counts.
Negative: If the test line is stronger/darker than the control line, the milk sample contains no antibiotics or contains antibiotics at lower level than the detection limits.

Positive: If the test line is equal to the control line, the milk sample contains antibiotics close to the detection limits. If the test line is weaker/lighter (less intense) than the control line, the milk sample contains antibiotics above the detection limits.

2.1 Configuration of Quantum BT-CC test strip

The configuration of Quantum BT-CC is shown in Figure 1.

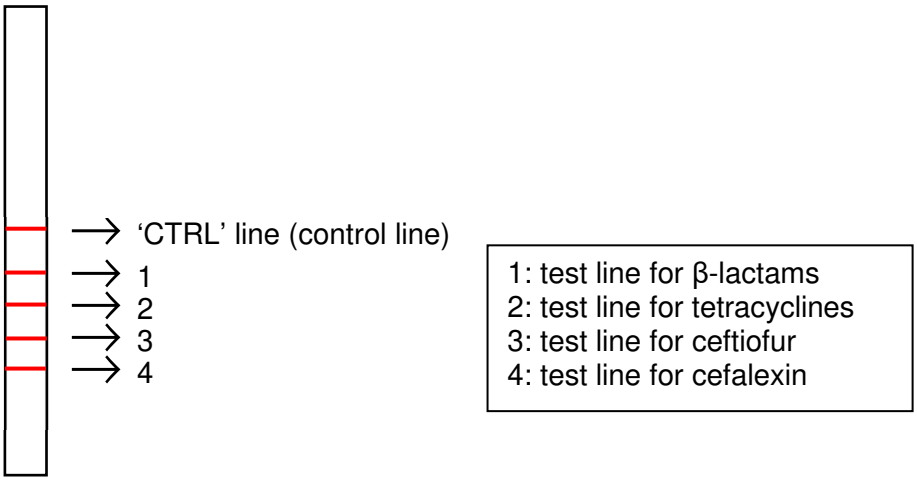

Fig. 1. Configuration of a Quantum BT-CC test strip.

2.2. Instrumental interpretation of the test

The S-Flow reader (ProGnosis Biotech S.A.) is comparing the intensity of each test line with the intensity of the control (reference) line and calculates for each channel a ratio = intensity test line / intensity control line. This ratio for each test line is compared to a fixed cut-off value (ratio = 1.10). The ratio cut-off levels are given in Table 1.

Table 1. Instrumental reading: interpretation of the test results.

Ratio	Interpretation	Ratio	Interpretation	Ratio	Interpretation
R>1.1	negative	0.9≤R≤1.1	weak positive	R<0.9	positive

Note: R: ratio.

In this validation no discrimination was made between 'weak positive' and 'positive' but both categories were just considered as positive.

Fig. 2. S-Flow Reader for instrumental reading.

2.3 Visual interpretation of the test

Visual reading of Quantum BT-CC test is also possible. The intensity of the test line is compared to the intensity of the reference (i.e. control) line. Negative: If the test line is stronger/darker than the control line, the milk sample contains no antibiotics or contains antibiotics at lower level than the detection limits. Positive: If the test line is equal to the control line, the milk sample contains antibiotics close to the detection limits. If the test line is weaker/lighter (less intense) than the control line, the milk sample contains antibiotics above the detection limits. The interpretation is shown in Figure 3.
Visual reading was not checked in this validation study.

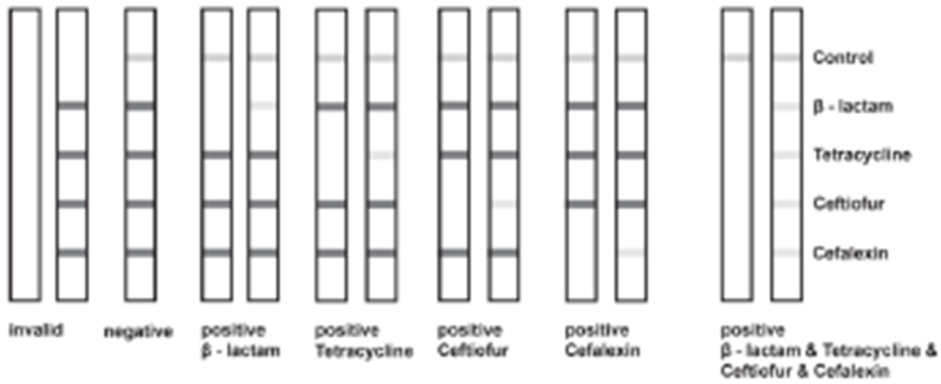

Fig. 3 . Visual interpretation of the color formation on Quantum BT-CC (*Anon.*, 2025).

3. Detection capability

Methods and Materials:

Spiking of antibiotic-free (blank) raw milk with β-lactams (penicillins and cefalosporins, including ceftiofur and cefalexin) and tetracyclines.
Blank milk was collected from 4 individual cows in mid-lactation which had not been treated with any veterinary drug for the last 2 months and which had a low to moderate number of somatic cells in the milk. The milk was collected in sterile containers and kept below 4°C to limit the bacterial count. The maximum period for the cold storage of the fresh raw milk was 56 hours which is shorter than the local milk collection interval (3 days in Belgium). Milk of at least 4 animals is commingled and is considered as a sample of standard blank matrix, free from antimicrobial residues and β-lactamase.
At least four such samples are used for the determination of the detection capability when testing 20 replicates. If 40 or 60 replicates need to be tested to determine the detection capability, minimum eight or twelve different blank milk samples are used, respectively.

The detection capability of Quantum BT-CC was determined for all different compounds belonging to the β-lactam, including ceftiofur and cefalexin, and tetracycline family mentioned as marker residue in Table 1 of the annex of Commission Regulation (EU) No 37/2010. The spiking was performed as described in the ISO TS 23758 | IDF RM 251 (ISO/IDF, 2021). Each compound was individually spiked in blank raw milk at fixed concentrations. For each compound a minimum of 2 concentrations around the test sensitivity (test detection capability) were tested. The increment between the concentrations tested for each compound was dependent on the level of spiking and the closeness to the respective MRL (Table 2).

Each concentration was tested 20, 40 or 60 times in a time period of at least three days.

- Tested concentration ≤0.5 MRL: 20 times
- Tested concentration >0.5 – <0.9 MRL: 40 times
- Tested concentration ≥0.9 – ≤1.0 MRL: 60 times
- Tested concentration >MRL: 20 times

Table 2. Increment between the concentrations tested for each compound was dependent on the level of spiking.

Concentration (in µg/kg)	Increment (in µg/kg)
<1	0.2
1-10	1
11-20	2
21-50	5
51-100	10
101-250	25
251-500	50
501-1,000	100
1,001-5,000	500

The detection capability is defined as the lowest concentration tested where at least 19 out of 20 tests, 38 out of 40 tests or 57 out of 60 tests were positive, respectively.

Every day the following standards were also tested:

- blank raw milk free from antimicrobials (fresh and frozen) - twice
- blank raw milk spiked with benzylpenicillin at 2 µg/kg and oxytetracycline at 45 µg/kg - twice
- blank raw milk spiked with ceftiofur at 70 µg/kg- twice
- blank raw milk spiked with cefazolin at 35 µg/kg and cefalexin at 6 µg/kg - twice

These standards were used for the robustness analyses.

A positive control (lot number BTDP1224 (exp. date 12/2025)) and negative control (lot number BTDN1224 (exp. date 12/2025)) included in the kit were also tested daily. The positive control contains benzylpenicillin at 4 µg/kg, tetracycline at 100 µg/kg, ceftiofur at 100 µg/kg and cefalexin at 100 µg/kg.

Detection capability tests were performed with three different lot numbers of reagents: W1560004 (exp. date 01/011/2025), W1560005 (exp. date 06/02/2025) and W1560006 (exp. date 01/12/2025) following the manufacturer's instructions. The intensity of color formation of each test line was compared to the intensity of the control line and was interpreted by means of a S-Flow Reader and S-Flow Software version 2.0.5.5 and 2.0.3.12 (latter from 13/2/2025 onwards). The cut-off value is 1.10 (>1.10: negative; ≤1.10: positive). All results (reader values) were collected in a database.

Certified reference material from following different reagent suppliers was used: Dr. Ehrenstorfer GmbH (Augsburg. DE), HPC Standards GmbH (Borsdorf. DE), LGC Mikromol (Luckenwalde. DE), Sigma-Aldrich N.V. (Overijse. BE) and Toronto Research Chemicals (TRC) (Ontario. CA). Detailed information of all standard material is given in Table 3.

Table 3. Standard material used in this validations study.

Active compound	Origin	Product number	Lot number	Solvent
4-epimer chlortetracycline	Dr. Ehrenstorfer	DRE-C13175500	1401757	MeOH*
4-epimer oxytetracycline	Dr. Ehrenstorfer	DRE-C13179000	1288752	MeOH*
4-epimer tetracycline	Dr. Ehrenstorfer	DRE-C13179500	1398177	MeOH*
Amoxicillin	Dr. Ehrenstorfer	DRE-C10242500	G1193514	ACN/H_2O 50/50*
Ampicillin	Dr. Ehrenstorfer	DRE-C10243080	G1276647	ACN/H_2O 50/50*
Benzylpenicillin	Dr. Ehrenstorfer	DRE-C15935000	G1367278	H_2O
Cefacetrile	HPC Standards	679543	812729 825011	ACN/H_2O 50/50*
Cefalexin	Dr. Ehrenstorfer	DRE-C11064000	G1408939	ACN/H_2O 50/50*
Cefalonium	LGC Mikromol	MM3169.00	1244778	ACN/H_2O 50/50
Cefazolin	Dr. Ehrenstorfer	DRE-C11064100	G1339724	H_2O
Cefoperazone	Dr. Ehrenstorfer	DRE-C11064300	G1408543	ACN/H_2O 50/50
Cefquinome	Dr. Ehrenstorfer	DRE-C11064700	G1382333	ACN/H_2O 50/50*
Ceftiofur	Dr. Ehrenstorfer	DRE-C11065000	G1338950	10% FA + H_2O ACN/MeOH
Cephapirin	Dr. Ehrenstorfer	DRE-C11064071	G14619900	H_2O
Chloramphenicol	Dr. Ehrenstorfer	DRE-C11120000	G1399096	MeOH*
Chlortetracycline	Sigma-Aldrich / Supelco	PHR1520	LRAD3654	H_2O
Clavulanic acid	Dr. Ehrenstorfer	DRE-C11668545	G1342649	H_2O
Cloxacillin	Dr. Ehrenstorfer	DRE-C11692100	G1067932	H_2O
Colistin	Sigma-Aldrich / Supelco	PHR1605	LRAD7352	H_2O
Dapsone	Dr. Ehrenstorfer	DRE-C11963000	G1143440	MeOH*
Desacetylcephapirin	HPC	682120	827796 825422	ACN/H_2O 50/50*
Desfuroylceftiofur	TRC	TRC-D289980	4-SUB-46-2	ACN/H_2O 50/50
Dicloxacillin	Dr. Ehrenstorfer	DRE-C12560500	G1226254	H_2O
Doxycycline	Dr. Ehrenstorfer	DRE-C13084280	1402730	MeOH*
Enrofloxacin	Dr. Ehrenstorfer	DRE-C13170000	G1364961	MeOH*
Erythromycin	Sigma-Aldrich / Supelco	PHR1039	LRAD3079	MeOH*
Lincomycin	Sigma-Aldrich / Supelco	PHR1657	LRAC9565	H_2O
Nafcillin	Dr. Ehrenstorfer	DRE-C15402400	1409448	H_2O
Neomycin	Sigma-Aldrich / Supelco	PHR1491	LRAD3724	H_2O
Oxacillin	Dr. Ehrenstorfer	DRE-C15755100	G1262742	H_2O
Oxytetracycline	Dr. Ehrenstorfer	DRE-C15820000	G1409502	H_2O
Phenoxymethylpenicillin	Dr. Ehrenstorfer	DRE-C15935010	G1069505	H_2O
Sulfadiazine	Dr. Ehrenstorfer	DRE-C16990500	G1390658	1M NaOH*
Tetracycline	Dr. Ehrenstorfer	DRE-C17396150	G1357741	H_2O
Trimethoprim	Dr. Ehrenstorfer	DRE-C17875000	G1341882	MeOH*

Note: *dissolved in a small amount of solvent and further diluted with H_2O; FA: formic acid.

Results:

A summary of Quantum BT-CC detection capabilities is given in Table 4.

Discussion:

Quantum BT-CC is capable to detect residues of all β-lactams including cefalexin and ceftiofur, and tetracyclines with a MRL in milk (EU-Regulation 37/2010 and amendments).

All β-lactams, including ceftiofur and cefalexin, and tetracyclines (parent drugs), can be detected at least in 95% of the samples at their respective MRL. The exact 95% detection capability of the 4-epimers of tetracyclines were not determined, but were tested twice at MRL. It is worth noting that those 4-epimers are generally not found in milk (ILVO, internal communication).

Doxycycline, not for use in animals from which milk is produced for human

consumption, was detected at least in 95% of the replicates at 70 µg/kg.
The test is fulfilling the acceptance criteria and could be approved by the Belgian Federal Agency for the Safety of the Food Chain (FASFC) as test used by the Belgian dairy companies to check incoming milk on the presence of β-lactam residues (*Anon.*, 2024).

Table 4. Detection capability (in µg/kg) of Quantum BT-CC (ProGnosis Biotech S.A., Larissa, Greece) in raw bovine milk with instrumental reading (S-Flow Reader and S-Flow software) with cut-off ratio = 1.10. Detection capability defined as the lowest concentration tested giving minimum 19, 38 or 57 positive results out of 20, 40 or 60 samples, respectively.

Antibiotic Group/ antibiotic	EU MRL (µg/kg)	Detection capability (µg/kg)			
		BL channel	TET channel	CEFTIO channel	CEFALEX channel
Penicillins					
benzylpenicillin	4	2			
ampicillin	4	2			
amoxicillin	4	2			
oxacillin	30	3			
cloxacillin	30	3			
dicloxacillin	30	3			
nafcillin	30	5			
phenoxymethylpenicillin	(25[a])	<25*			
Cefalosporins					
ceftiofur	100[b]	125		60	
desfuroylceftiofur	100[b]			60	
cefquinome	20	5			
cefazolin	50	30			
cephapirin	60[c]	3			
desacetylcephapirin	60[c]	7			
cefacetrile	125	7			
cefoperazone	50	3			
cefalexin	100				6
cefalonium	20	2			
Tetracyclines					
tetracycline	100[d]		50		
4-epimer of tetracycline	100[d]		**>100***		
oxytetracycline	100[d]		40		
4-epimer of oxytetracycline	100[d]		**>100***		
chlortetracycline	100[d]		50		
4-epimer of chlortetracycline	100[d]		≤100*		
doxycycline	--[e]		70		

Notes: Bold and red font detection capabilities are above the drug MRL. Purple font: detection capability above MRL, but compound is detected below MRL on a compound specific test channel. *: No exact detection capability searched, tested twice at MRL. MRL: Maximum Residue Limit. Regulation (EC) No 470/2009 of the European Parliament and of the Council and Commission Regulation (EU) No 37/2010 and amendments (current situation). BL: β-

lactam; TET: tetracycline, CEFTIO: ceftiofur; CEFALEX: cefalexin.
[a]: No MRL in milk, MRL based on Commission Implementing Regulation (EU) 2018/470;
[b]: The MRL of 100 µg/kg is applied on the sum of all residues retaining the β-lactam structure expressed as desfuroylceftiofur;
[c]: The MRL of 60 µg/kg in milk is applied on the sum of cephapirin and desacetylcephapirin;
[d]: The MRL of 100 µg/kg in milk is applied on the sum of parent drug and its 4-epimer;
[e]: Not for use in animals from which milk is produced for human consumption.

4. Test selectivity and rate of false positive results

4.1. Test selectivity

Methods and Materials:

The selectivity of the different test lines of Quantum BT-CC was tested by analysing milk spiked with β-lactam, tetracycline, ceftiofur and cefalexin compounds and by analysing milk spiked with compounds belonging to different antibiotic or chemotherapeutic families (1 per family) to check the selectivity of the β-lactam, tetracycline, ceftiofur and cefalexin test line. Raw milk was spiked at a high concentration (100×MRL or 100×RPA (reference point for action; Commission Regulation 2023/411) or 100×MMPR (Minimum Method Performance Requirement; *Anonymous*, 2022)) in raw milk. All testing was completed in duplicate. In case of a positive result also lower concentrations were tested.

Following compounds were used: all penicillins and cefalosporins used for the testing of detection capability, oxytetracycline (tetracyclines), sulfadiazine (sulfonamides), neomycin B (aminoglycosides), enrofloxacin (quinolones), colistin (polymyxins), chloramphenicol (amphenicols), erythromycin (macrolides), lincomycin (lincosamides), clavulanic acid (β-lactamase inhibitors), trimethoprim (diamino pyrimidine derivatives) and dapsone (others chemotherapeutics).
More details about the standard material can be found in Table 5.

Results:

A summary of the test selectivity is given in Table 5.

Table 5. Ratios obtained for β-lactams, tetracyclines, ceftiofur and cefalexin compounds of other families spiked in raw milk and tested with Quantum BT-CC.

Family	Compound	MRL/ RPA/ MMPR (µg/kg)	Conc. spiked in milk (µg/kg)	BL		TET		CEFTIO		CEFALEX	
				Ratio	Res.	Ratio	Res.	Ratio	Res.	Ratio	Res.
Penicillins	benzylpenicillin	4	400	**0.01**	**+**	2.71	-	3.01	-	3.70	-
	ampicillin	4	400	**0.01**	**+**	2.65	-	3.01	-	3.60	-
	amoxicillin	4	400	**0.01**	**+**	2.63	-	2.90	-	3.40	-
	oxacillin	30	3000	**0.01**	**+**	2.65	-	3.16	-	3.78	-
	cloxacillin	30	3000	**0.04**	**+**	2.47	-	2.76	-	2.97	-
	dicloxacillin	30	3000	**0.01**	**+**	2.61	-	3.03	-	3.53	-
	nafcillin	30	3000	**0.01**	**+**	2.53	-	2.84	-	3.39	-
Cefalosporins	ceftiofur	100[a]	10,000	**0.14**	**+**	4.62	-	0.01	**+**	4.56	-
			100	**1.34**	**+**	3.11	-	**0.71**	**+**	3.83	-
	desfuroylceftiofur	100[a]	10,000	**0.07**	**+**	2.74	-	**0.01**	**+**	1.18	-
			7,500	**0.01**	**+**	2.79	-	**0.01**	**+**	2.02	-
			5,000	**0.03**	**+**	2.62	-	**0.01**	**+**	2.11	-
			2,500	**0.07**	**+**	2.74	-	**0.01**	**+**	2.45	-
			100	1.76	**-**	3.16	-	**0.42**	**+**	4.10	-
	cefquinome	20	2,000	**0.01**	**+**	2.52	-	**0.23**	**+**	2.74	-
			500	**0.01**	**+**	2.78	-	**0.73**	**+**	3.51	-
			20	**0.01**	**+**	2.75	-	2.78	-	3.78	-
	cefazolin	50	5,000	**0.31**	**+**	2.82	-	3.20	-	3.74	-
	cephapirin	60[b]	6,000	**0.01**	**+**	2.50	-	2.69	-	1.99	-
	desacetylcephapirin	60[b]	6,000	**0.01**	**+**	2.75	-	3.07	-	1.95	-
	cefacetrile	125	12,500	**0.01**	**+**	2.72	-	3.02	-	3.56	-
	cefoperazone	50	5,000	**0.01**	**+**	2.76	-	3.01	-	1.45	-
	cefalexin	100	10,000	**0.01**	**+**	2.69	-	3.10	-	**0.18**	**+**
			2,500	**0.01**	**+**	2.62	-	2.84	-	**0.13**	**+**
			100	**1.86**	**-**	3.41	-	3.89	-	**0.40**	**+**
	cefalonium	20	2,000	**0.01**	**+**	2.57	-	2.94	-	3.33	-
Tetracyclines	oxytetracycline	100[c]	10,000	2.56	-	**0.01**	**+**	3.83	-	4.60	-
Sulfonamides	sulfadiazine	100[d]	10,000	2.45	-	3.39	-	**3.73**	-	4.56	-
Aminoglycosides	neomycin B	1,500	150,000	2.15	-	3.08	-	3.37	-	4.07	-
Quinolones	enrofloxacin	100[e]	10,000	2.67	-	3.39	-	3.78	-	4.48	-
Polymyxins	colistin	50	5,000	2.40	-	3.35	-	3.60	-	4.59	-
Amphenicols	chloramphenicol	0.15[f]	15	2.78	-	3.49	-	4.00	-	4.82	-
Macrolides	erythromycin A	40	4,000	2.59	-	3.21	-	3.42	-	4.31	-
Lincosamides	lincomycin	150	15,000	*	-	*	-	*	-	*	-
β-lactamase inhibitors	clavulanic acid	200	20,000	**0.02**	**+**	2.83	-	3.10	-	3.83	-
			5,000	**0.01**	**+**	2.77	-	3.06	-	3.57	-
			200	*	**-**	*	-	*	-	*	-
Diamino pyrimidine derivatives	trimethoprim	50	5,000	2.58	-	3.49	-	3.76	-	4.74	-
Others	dapsone	5[g]	500	*	-	*	-	*	-	*	-

Notes: MRL: Maximum Residue Limit. Regulation (EC) No 470/2009 of the European Parliament and of the Council and Commission Regulation (EU) No 37/2010 and amendments (current situation). Conc.: concentration; Res. Result; BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel and CEFALEX: cefalexin channel.

*: read with QUANTUM: negative results after first QUANTUM read, no values obtained.
[a]: The MRL of 100 µg/kg is applied on the sum of all residues retaining the β-lactam structure

expressed as desfuroylceftiofur;
[b]: The MRL of 60 µg/kg in milk is applied on the sum of cephapirin and desacetylcephapirin;
[c]: The MRL of 100 µg/kg in milk is applied on the sum of parent drug and its 4-epimer;
[d]: The combined total residues of all substances within the sulfonamide group should not exceed 100 µg/kg;
[e]: The MRL of 100 µg/kg in milk is applied on the sum of enrofloxacin and ciprofloxacin;
[f]: Prohibited substance, RPA or reference point for action for chloramphenicol, Commission Regulation (EU) 2019/1871;
[g]: Prohibited substance, Minimum Method Performance Requirement (MMPR) (*Anon.*, 2022).

Discussion:
Clavulanic acid, a β-lactamase inhibitor, gave an interference at the β-lactam channel at high concentrations (25×MRL). This interference is expected since this molecule contains a β-lactam structure resembling that of the penicillin, except that the fused thiazolidine ring of the penicillins is replaced by an oxazolidine ring (Anon., 2005). The exact 95% detection capability of clavulanic acid was not determined, but negative results were obtained at MRL (= 200 µg/kg).
The beta-lactam channel detected also high concentrations of cefalexin and ceftiofur (both beta-lactams), but not at MRL, hence the presence of a specific channel for the compounds.
On the ceftiofur channel, also high concentrations of cefquinome (25×MRL) were detected, but at MRL (=20 µg/kg) negative results were obtained.
On the tetracycline and cefalexin channels no interference was noted.

Quantum BT-CC is a highly specific test for detection of β-lactams and tetracyclines in milk and does not detect compounds from the sulfonamides, aminoglycosides, quinolones, polymyxins, amphenicols, macrolides, lincosamides and diamino pyrimidine derivatives, nor dapsone.

4.2. Test for false-positive results

Methods and materials:
300 blank farm and 300 blank tanker load milk samples were tested with Quantum BT-CC. In case of positive results, the samples were tested with other microbiological and receptor screening tests to determine whether it is a false-positive result. When it remained inconclusive, the sample was analysed with LC-MS/MS.

Results and discussion:
All 300 farm and 300 tanker load milk samples tested negative for β-lactams, including ceftiofur and cefalexin and tetracyclines, on Quantum BT-CC. So in total no false positive results (0%) are obtained upon 600 samples on all channels.
Most results were obtained at 1st Quantum read, for which no values are given only the interpretation “negative”.

It is worth noting that one farm sample was found positive on the beta-lactam channel (ratio 0.92). By use of beta-lactamase ES, the presence of a beta-lactam antibiotic was confirmed, proving that the test is capable of detecting antibiotic residues due to veterinary use.

5. Reader and test repeatability

5.1 Repeatability of the reader

Methods and Materials:
Samples of 10 blank,10 low positive samples and 10 high positive samples for each channel were measured twice after drying for half an hour. For the spiked samples. any compound found positive could be used for the testing of the reader repeatability.

Results:
The results of the repeatability of the reader on Quantum BT-CC results are summarized in Table 6. For the spiked milk only the relevant data for the different channels are presented.

Table 6. Repeatability of the reader

Reader repeatability	β-lactam channel			Tetracycline channel			Ceftiofur channel			Cefalexin channel		
	Mean ratio	s_r	CV%	Mean ratio	s_r	CV%	Mean ratio	s_r	CV%	Mean ratio	s_r	CV%
Blank milk	2.13	0.05	2.27	2.84	0.05	1.70	3.14	0.05	1.65	3.52	0.04	1.28
Low pos	0.87	0.01	1.56	0.83	0.01	1.27	0.74	0.01	1.69	0.78	0.01	1.77
High pos	0.45	0.01	1.88	0.40	0.01	2.00	0.40	0.01	2.00	0.52	0.01	2.30

Notes: mean: mean ratio on the respective channel; s_r: Standard deviation of repeatability; CV(%): Relative standard deviation.

Discussion:
The repeatability of the reader was very good; low standard deviations of repeatability were obtained (expectation for lateral flow reader: <5%); the highest variance value was 2.30%.

5.2 Repeatability of the test

Methods and Materials:
Ten blank. 10 low positive samples and 10 high positive samples for each channel were analysed in duplicate. For the spiked samples. any compound found positive could be used.

Results:
The results of the repeatability of Quantum BT-CC are summarized in Table 7. For the spiked milk only the relevant data for the different channels are presented.

Table 7: Repeatability of the test

Reader repeatability	β-lactam channel			Tetracycline channel			Ceftiofur channel			Cefalexin channel		
	Mean ratio	s_r	CV%	Mean ratio	s_r	CV%	Mean ratio	s_r	CV%	Mean ratio	s_r	CV%
Blank milk	2.28	0.19	8.13	3.24	0.13	4.11	3.60	0.17	4.64	4.30	0.23	5.26
Low pos	0.89	0.10	11.54	0.98	0.09	9.19	0.95	0.05	5.59	1.00	0.09	9.19
High pos	0.53	0.11	20.57	0.53	0.04	7.88	0.51	0.05	10.56	0.44	0.06	13.61

Notes: mean: mean ratio on the respective channel; s_r: Standard deviation of repeatability; CV(%): Relative standard deviation.

Discussion:
The repeatability of the test was good. The highest variance value were obtained for high positive samples, with as highest value 20.57% on the beta-lactam channel. However the expectation for lateral flow tests is <20%, this is still very acceptable as it concerns high positive samples far away for the cut-off (mean ratio on the beta-lactam channel: 0.53). For blanks and low positive samples, the variance is much lower (<11.55%).

6. Test robustness

6.1. Influence of changes in the test protocol on the test results

Quantum BT-CC in combination with S-Flow reader and software has automated incubation/reading.

6.2. External influences

6.2.1. Impact of the milk temperature

Methods and Materials:
Tests were performed (4 samples) with milk of 8°C (= reference) and of 20°C in order to check if the milk temperature is influencing Quantum BT-CC results. Besides blank milk also spiked milk samples containing benzylpenicillin at 2 µg/kg and oxytetracycline at 45 µg/kg or containing ceftiofur at 70 µg/kg or containing cefazolin at 35 µg/kg and cefalexin at 6 µg/kg were used.

Results:
The results of the impact of the milk temperature are summarized in Table 8. For the spiked milk only the relevant data for the different channels are presented.

Discussion:
The milk temperature (20°C) did not significantly impact the Quantum BT-CC results. Blank milk always resulted in negative results and for spiked milk samples positive results on the respective channels were obtained.
At 20°C, it was noticed that milk containing ceftiofur at 70 µg/kg also gave two border positive results (ratio 1.06 and 1.07) on the beta-lactam channel. This is however normal as ceftiofur belongs to the beta-lactam family.

Table 8. Impact of the milk temperature on the Quantum BT-CC result.

	Milk temperature							
	8°C (REF)				20°C			
	BL	TET	CEFTIO	CEFALEX	BL	TET	CEFTIO	CEFALEX
Blank milk								
mean	2.39	3.20	3.56	4.32	2.42	3.21	3.65	4.22
min	2.23	3.09	3.49	4.12	2.40	3.07	3.45	3.94
max	2.63	3.37	3.62	4.49	2.45	3.42	4.00	4.71
Milk spiked with benzylpenicillin at 2 µg/kg and oxytetracycline at 45 µg/kg								
mean	0.28	0.46	3.07	3.61	0.24	0.43	3.05	3.61
min	0.20	0.39	2.90	3.40	0.20	0.40	2.86	3.28
max	0.35	0.50	3.25	3.84	0.26	0.45	3.42	4.21
Milk spiked with ceftiofur at 70 µg/kg								
mean	1.23	3.01	0.81	3.99	1.15	2.90	0.81	3.90
min	1.19	2.89	0.77	3.63	1.06	2.73	0.74	3.62
max	1.26	3.19	0.83	4.45	1.26	3.02	0.85	4.20
Milk spiked with cefazolin at 35 µg/kg and cefalexin at 6 µg/kg								
mean	0.60	2.74	3.21	0.67	0.63	2.70	3.13	0.67
min	0.54	2.65	3.07	0.62	0.57	2.55	2.96	0.57
max	0.67	2.77	3.34	0.71	0.69	2.94	3.43	0.76

Notes: REF: reference; mean: mean ratio; min: minimum ratio; max: maximum ratio; BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

6.3. Milk quality and milk composition influences

Methods and Materials:

Somatic cell count

Normal milk samples and milk samples with a high somatic cell count (>10^6 per ml) were analysed and the ratios obtained were compared in order to study the impact of the somatic cell count on the Quantum BT-CC result. The milk samples with a high number of somatic cells were selected at the milk control station based on Fossomatic 7 (FOSS. Hillerød. DK) measurements.

Total bacterial count

Normal milk samples and milk samples with a high total bacterial count (TBC >1.1×10^6 CFU per ml) were analysed and the ratios obtained were compared in order to study the impact of the total bacterial count on the Quantum BT-CC result. The milk samples with a high total bacterial count were obtained by keeping normal milk samples during 4-6 hours at room temperature. The final bacterial count was determined by performing a spiral plate count (Eddy Jet. IUL sa. Barcelona. ES) on Plate count agar plates after 3 days incubation at 30°C.

Fat content

Normal milk samples and milk samples with a low (≤1.76 g per 100 ml) or a high (≥6.31 g per 100 ml) fat content were analysed and the ratios obtained were compared in order to study the impact of the fat content on the Quantum BT-CC result. The milk samples with a low and high fat content were natural milk samples with a low and a high fat content selected at the milk control station based on infrared spectroscopic results with a MilcoScan 7 (FOSS. Hillerød. DK).

Protein content

Normal milk samples and milk samples with a low (≤3.02 g per 100 ml) or a high (≥4.04 g per 100 ml) protein content were analysed and the ratios obtained were compared in order to study the impact of the protein content on the Quantum BT-CC result. The milk samples tested were natural milk samples with a low and a high protein content. These samples were selected at the milk control station based on infrared spectroscopic results with a MilcoScan 7 (FOSS. Hillerød. DK).

pH:

Milk samples with a normal pH (6.7 - 6.9) and milk samples with a low pH (6.0) or a high pH (7.5) were analysed and the ratios obtained were compared in order to study the impact of the milk pH on the Quantum BT-CC result. The low and high pH milk samples were prepared by adding 1 M HCl or 1 M NaOH. Respectively. to milk with normal pH. Afterwards the pH of the milk was brought exactly to 6.0 and 7.5 by fine-tuning with 0.1 M HCl and/or 0.1 M NaOH.

Lactation stage

Milk samples from an early lactation (<30 days after calving but no colostrum milk) and from a late lactation stage (>270 days after calving) were analysed and the ratios obtained were compared in order to study the impact of the lactation stage on the Quantum BT-CC result.

Results:

With respect to the impact of the milk quality (high somatic cell count. high total bacterial count) and composition (fat and protein content. pH and lactation stage). the mean. the highest and lowest reader value for each milk type are given in Figures 4 to 7 and Table 9. For blank samples, quantum read was not used in order to obtain values, except for low and high pH samples (no values given for blanks).

The legend for the different situations in figures 4 to 7.

1 = Reference: normal raw milk;
2 = SCC >10^6/ml
3 = TBC ≥1.1×10^6 CFU/ml
4 = Low fat content (≤1.76 g/100 ml);
5 = High fat content (≥6.31 g/100 ml);
6 = Low protein (≤3.02 g/100 ml);
7 = High protein (≥4.04 g/100 ml);
8 = Low pH (pH = 6.0)
9 = High pH (pH = 7.5)
10= Early lactation (<30 days post calving; no colostrum)
11= Late lactation (>270 days post calving)

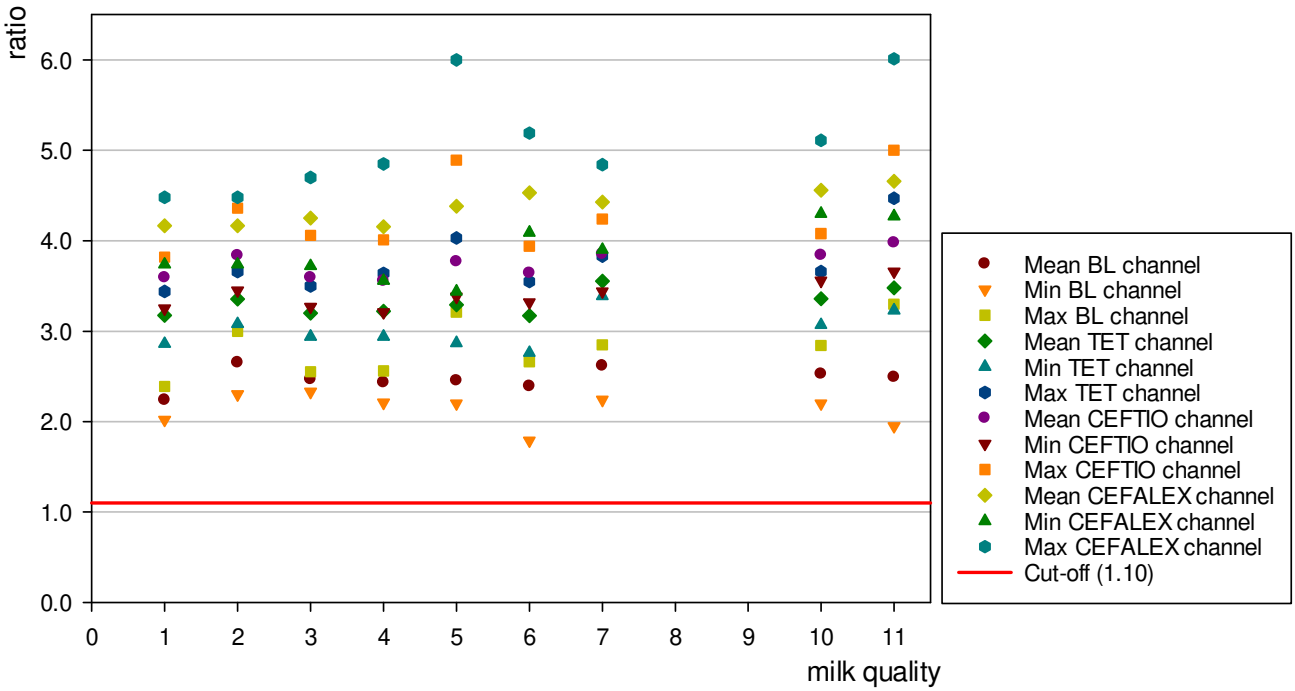

Fig. 4. Results for blank milk. 10 samples. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

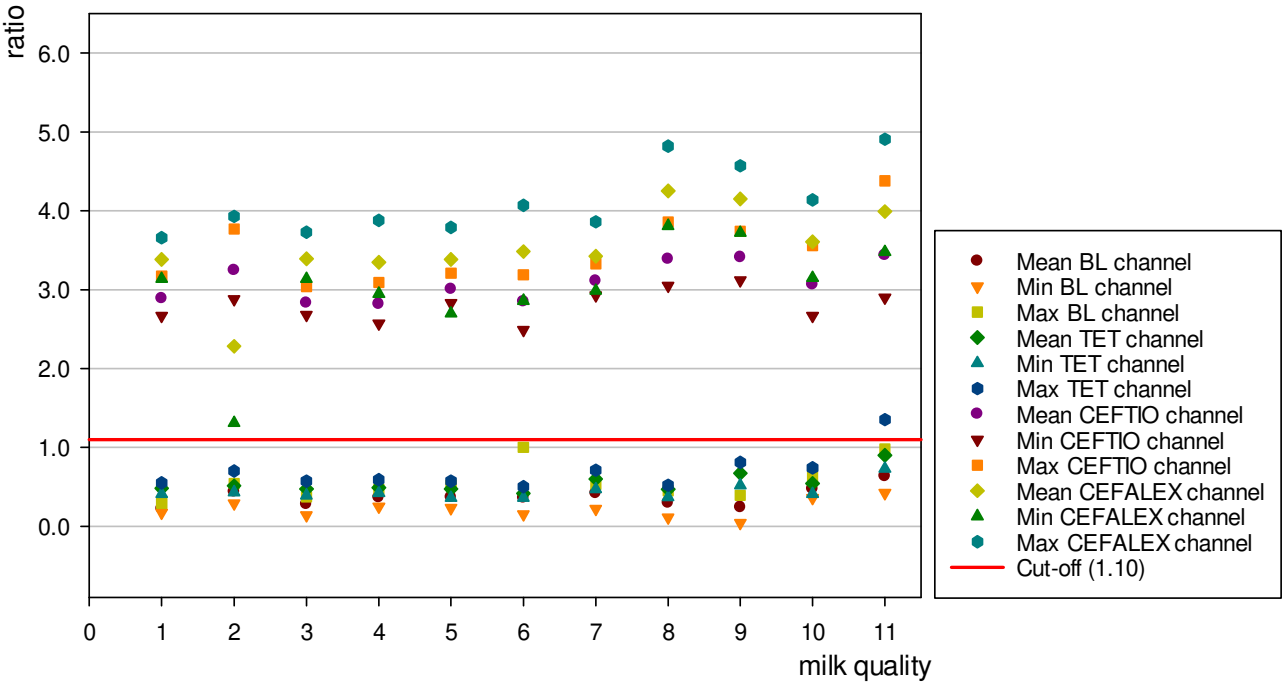

Fig. 5. Results for milk spiked with benzylpenicillin at 2 µg/kg and oxytetracycline at 45 µg/kg. 10 samples. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

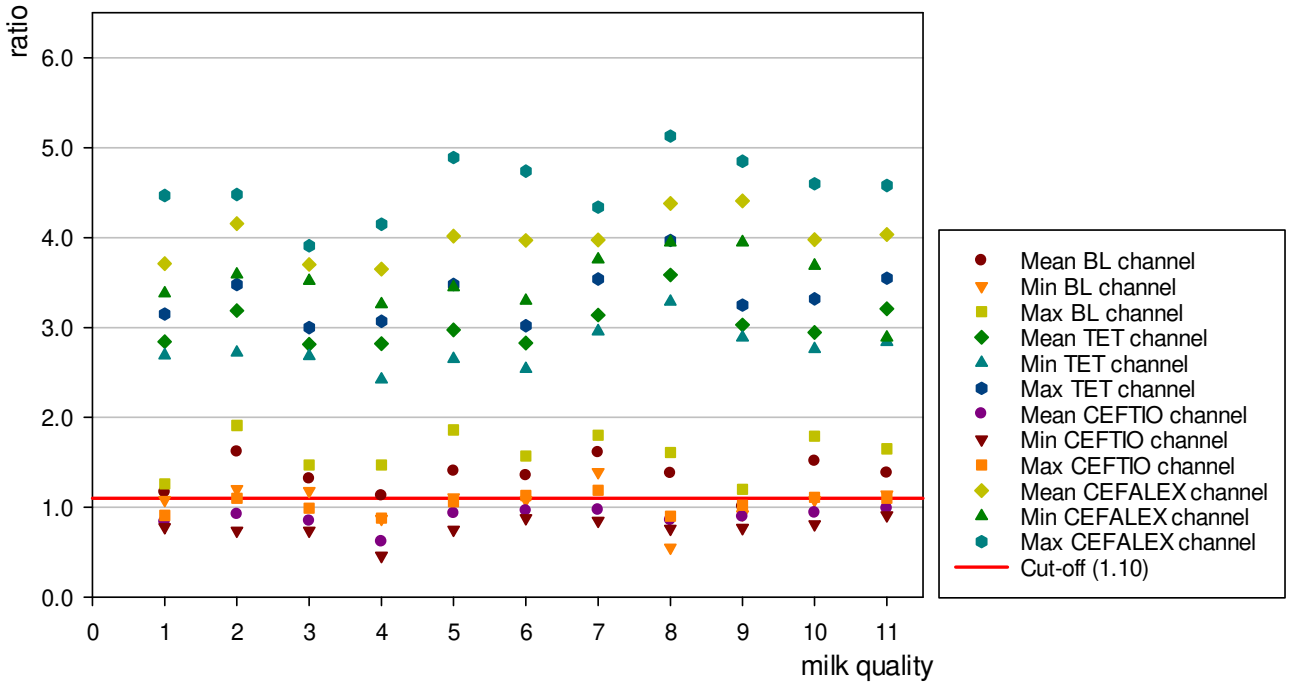

Fig. 6. Results for milk spiked with ceftiofur at 70 µg/kg. 10 samples. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

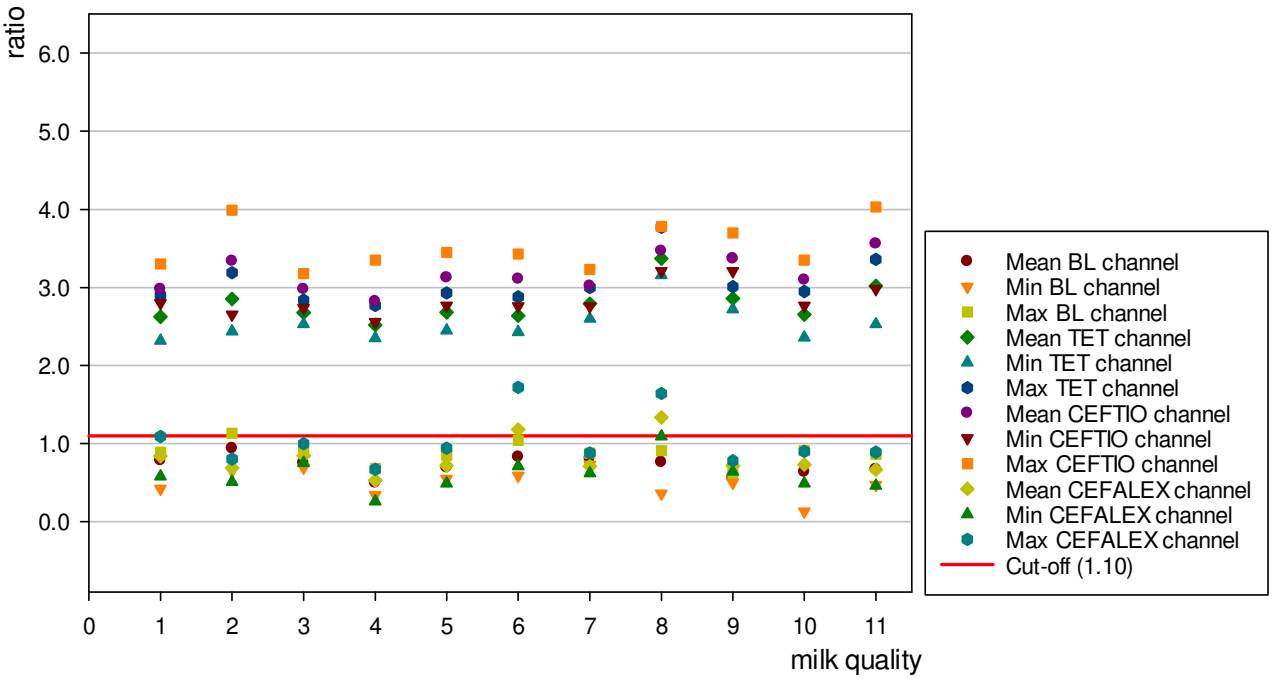

Fig. 7. Results for milk spiked with cefazolin at 35 µg/kg and cefalexin at 6 µg/kg. 10 samples. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

Table 9. Quantum BT-CC results for blank and spiked normal raw cows' milk and for blank and spiked milk of special quality or composition (10 samples).

	Ratio											
	BL channel			TET channel			CEFTIO channel			CEFALEX channel		
	mean	min	max	mean	min	max	mean	min	max	mean	min	max
Blank raw cows' milk												
normal milk = reference	2.24	2.02	2.39	3.18	2.86	3.44	3.59	3.25	3.82	4.16	3.74	4.48
SCC >10^6/ml	2.65	2.30	3.00	3.36	3.08	3.66	3.84	3.45	4.36	4.16	3.74	4.48
TBC ≥1.1×10^6 cfu/ml	2.47	2.33	2.55	3.20	2.94	3.50	3.59	3.27	4.06	4.25	3.72	4.70
low fat ≤1.76 g/100 ml	2.43	2.21	2.56	3.22	2.94	3.64	3.56	3.21	4.01	4.16	3.56	4.85
high fat ≥6.31 g/100 ml	2.45	2.20	3.21	3.29	2.87	4.03	3.77	3.38	4.89	4.38	3.44	6.00
low protein ≤3.02 g/100 ml	2.39	1.79	2.66	3.17	2.76	3.55	3.64	3.32	3.94	4.53	4.09	5.19
high protein ≥4.04 g/100 ml	2.62	2.24	2.85	3.56	3.39	3.83	3.85	3.44	4.24	4.43	3.90	4.84
low pH (pH = 6.0)	Quantum read: no values; all negative											
high pH (pH = 7.5)	Quantum read: no values; all negative											
early lactation*	2.52	2.20	2.84	3.36	3.07	3.66	3.84	3.56	4.08	4.56	4.30	5.11
late lactation**	2.49	1.95	3.30	3.48	3.23	4.47	3.98	3.66	5.00	4.66	4.27	6.01
Milk spiked with benzylpenicillin at 2 µg/kg and oxytetracycline at 45 µg/kg												
normal milk = reference	0.22	0.17	0.29	0.48	0.41	0.55	2.89	2.67	3.17	3.38	3.14	3.66
SCC >10^6/ml	0.44	0.29	0.54	0.51	0.43	0.70	3.25	2.88	3.77	2.28	1.31	3.93
TBC ≥1.1×10^6 cfu/ml	0.28	0.14	0.37	0.47	0.39	0.57	2.83	2.68	3.04	3.39	3.14	3.73
low fat ≤1.76 g/100 ml	0.36	0.25	0.47	0.49	0.42	0.59	2.82	2.57	3.09	3.35	2.95	3.88
high fat ≥6.31 g/100 ml	0.37	0.23	0.52	0.47	0.36	0.57	3.01	2.83	3.21	3.39	2.70	3.79
low protein ≤3.02 g/100 ml	0.36	0.15	1.00	0.42	0.36	0.50	2.85	2.49	3.19	3.48	2.86	4.07
high protein ≥4.04 g/100 ml	0.42	0.22	0.53	0.60	0.47	0.71	3.11	2.93	3.33	3.42	2.99	3.86
low pH (pH = 6.0)	0.30	0.11	0.45	0.47	0.37	0.52	3.39	3.05	3.86	4.25	3.81	4.82
high pH (pH = 7.5)	0.24	0.04	0.39	0.67	0.52	0.81	3.41	3.12	3.74	4.15	3.72	4.57
early lactation*	0.47	0.36	0.63	0.54	0.41	0.74	3.06	2.67	3.56	3.61	3.15	4.14
late lactation**	0.63	0.42	0.98	0.90	0.73	**1.35**	3.44	2.90	4.38	3.99	3.48	4.91
Milk spiked with ceftiofur at 70 µg/kg												
normal milk = reference	1.17	1.08	1.26	2.84	2.69	3.15	0.84	0.78	0.91	3.71	3.38	4.47
SCC >10^6/ml	1.62	1.20	1.91	3.19	2.72	3.48	0.92	0.74	1.10	4.16	3.59	4.48
TBC ≥1.1×10^6 cfu/ml	1.32	1.18	1.47	2.81	2.68	3.00	0.85	0.74	0.99	3.70	3.52	3.91
low fat ≤1.76 g/100 ml	1.13	0.87	1.47	2.82	2.42	3.07	0.62	0.46	0.88	3.65	3.26	4.15
high fat ≥6.31 g/100 ml	1.40	1.11	1.86	2.97	2.65	3.48	0.93	0.75	1.06	4.02	3.45	4.89
low protein ≤3.02 g/100 ml	1.35	1.08	1.57	2.83	2.54	3.02	0.96	0.88	**1.13**	3.97	3.30	4.74
high protein ≥4.04 g/100 ml	1.61	1.39	1.80	3.14	2.96	3.54	0.97	0.85	**1.19**	3.98	3.76	4.34
low pH (pH = 6.0)	1.38	0.55	1.61	3.59	3.29	3.97	0.86	0.76	0.90	4.38	3.95	5.13
high pH (pH = 7.5)	1.01	0.92	1.20	3.03	2.89	3.25	0.89	0.77	1.02	4.41	3.95	4.85
early lactation*	1.51	1.08	1.79	2.95	2.76	3.32	0.94	0.81	**1.11**	3.98	3.69	4.60
late lactation**	1.38	1.14	1.65	3.21	2.84	3.55	0.99	0.91	1.10	4.04	2.89	4.58
Milk spiked with cefazolin at 35 µg/kg and cefalexin at 6 µg/kg												
normal milk = reference	0.79	0.42	0.89	2.63	2.32	2.90	2.98	2.80	3.30	0.84	0.58	1.09
SCC >10^6/ml	0.94	0.65	**1.13**	2.85	2.44	3.19	3.34	2.65	3.99	0.69	0.51	0.80
TBC ≥1.1×10^6 cfu/ml	0.75	0.69	0.89	2.68	2.53	2.84	2.98	2.74	3.18	0.84	0.75	1.00
low fat ≤1.76 g/100 ml	0.49	0.34	0.68	2.52	2.35	2.77	2.82	2.56	3.35	0.53	0.26	0.67
high fat ≥6.31 g/100 ml	0.69	0.55	0.85	2.68	2.45	2.93	3.13	2.77	3.45	0.72	0.49	0.94
low protein ≤3.02 g/100 ml	0.83	0.59	1.04	2.64	2.43	2.88	3.11	2.76	3.43	1.18	0.71	**1.72**
high protein ≥4.04 g/100 ml	0.81	0.73	0.88	2.79	2.60	3.00	3.02	2.76	3.23	0.71	0.62	0.88
low pH (pH = 6.0)	0.76	0.36	0.91	3.37	3.16	3.77	3.47	3.21	3.78	**1.34**	1.09	**1.64**

high pH (pH = 7.5)	0.56	0.50	0.62	2.86	2.72	3.01	3.37	3.21	3.70	0.72	0.64	0.78
early lactation*	0.64	0.13	0.91	2.65	2.36	2.95	3.10	2.77	3.35	0.73	0.49	0.90
late lactation**	0.67	0.47	0.86	3.02	2.53	3.36	3.56	2.98	4.03	0.67	0.46	0.89

Notes: *: early lactation: <30 days post calving, no colostrum; **: late lactation: >270 days post calving. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel. min: minimum; max: maximum ; SCC : somatic cell count; TBC: total bacteria count; cfu: colony forming unit.

Discussion:

The milk quality and composition had some influence on the performance of the Quantum BT-CC. No false positives were obtained with the blank milk. Quantum read was not used to obtain values, except for testing blank low and high pH milk samples (no values, only interpretation).

For all spiked samples positive results on the respective channels were obtained, except for two milk samples out of 10 samples with a high somatic cell count containing 35 µg/kg of cefazolin obtaining borderline negative results (ratio 1.10 and 1.13) on the beta-lactam channel. On the tetracycline channel, one borderline negative result (ratio 1.35) was obtained for a milk sample from late lactation containing oxytetracycline at 45 µg/kg on a total of 10 spiked samples. On the ceftiofur channel, one borderline negative result (ratio 1.13) was obtained for low protein milk, one (ratio 1.19) for high positive milk and two (ratio 1.10 and 1.11) for early lactation milk, each time on a total of 10 milk samples spiked with ceftiofur at 70 µg/kg. On the cefalexin channel, some hampering of detection occurred for low protein samples (5 negative results on a total of 10 results with rations between 1.13 and 1.72) and for samples with a low pH (9 negative results on a total of 10 with ratios between 1.21 and 1.64) for milk containing cefalexin at 6 µg/kg.

So, a slightly higher 95% detection capability is expected especially for cefalexin in low protein and low pH milk. But since these samples were spiked far below MRL and also several positive results were obtained, it is expected that all compounds will be detected at MRL. Only for low pH milk samples, results should be interpreted with care, in fact, it is recommended to adjust the pH to normal milk and retest.

6.4. Type of milk and animal origin influences

Methods and Materials:

Raw milk, UHT milk and reconstituted milk powder were analysed in order to determine if the Quantum BT-CC is a suitable test for these types of milk. For UHT milk, both skimmed, semi-skimmed and full cream milk was used. Also raw goats' and raw ewes' milk samples were analysed to determine if Quantum BT-CC is a suitable test for milk from these animal species. For goats' and ewes' milk, both individual milk samples as well as tank milk samples were used. For blank samples, Quantum read was not used in order to obtain values.

Results:

With respect to the impact of the milk type (UHT and reconstituted milk powder). goats' and ewes' milk. the mean. the highest and lowest reader value for each milk type are given in Figures 8 to 11 and Table 10.

The legend for the different situations in figures 8 to 11.
1 = Normal raw milk = reference
2 = UHT milk
3 = Reconstituted milk powder
4 = Goats' milk
5 = Ewes' milk

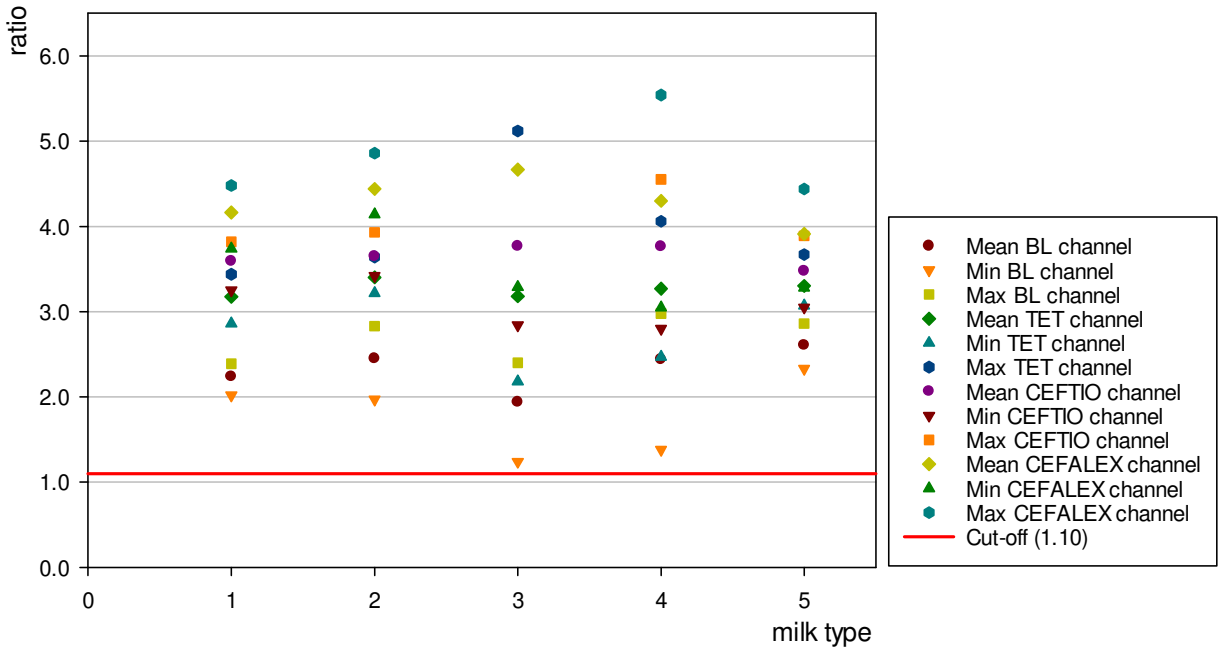

Fig. 8. Results for blank milk. 10 samples. except for goats' and ewes' milk. 20 samples. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

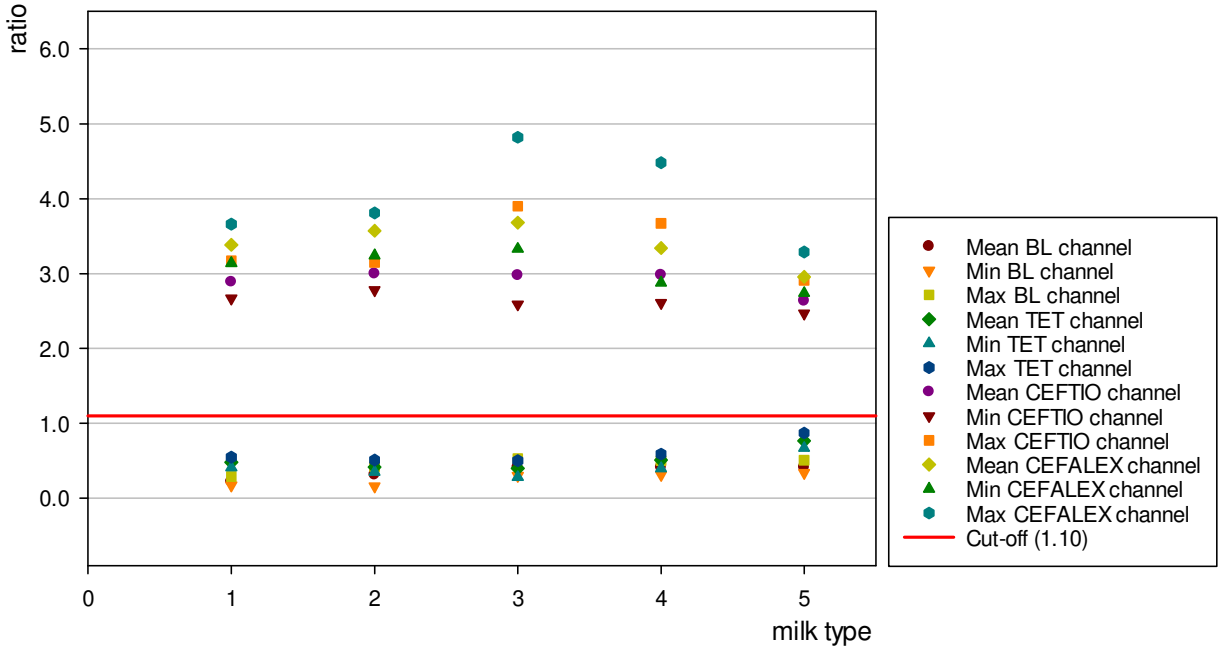

Fig. 9. Results for milk spiked with benzylpenicillin at 2 µg/kg and oxytetracycline at 45 µg/kg. 10 samples. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

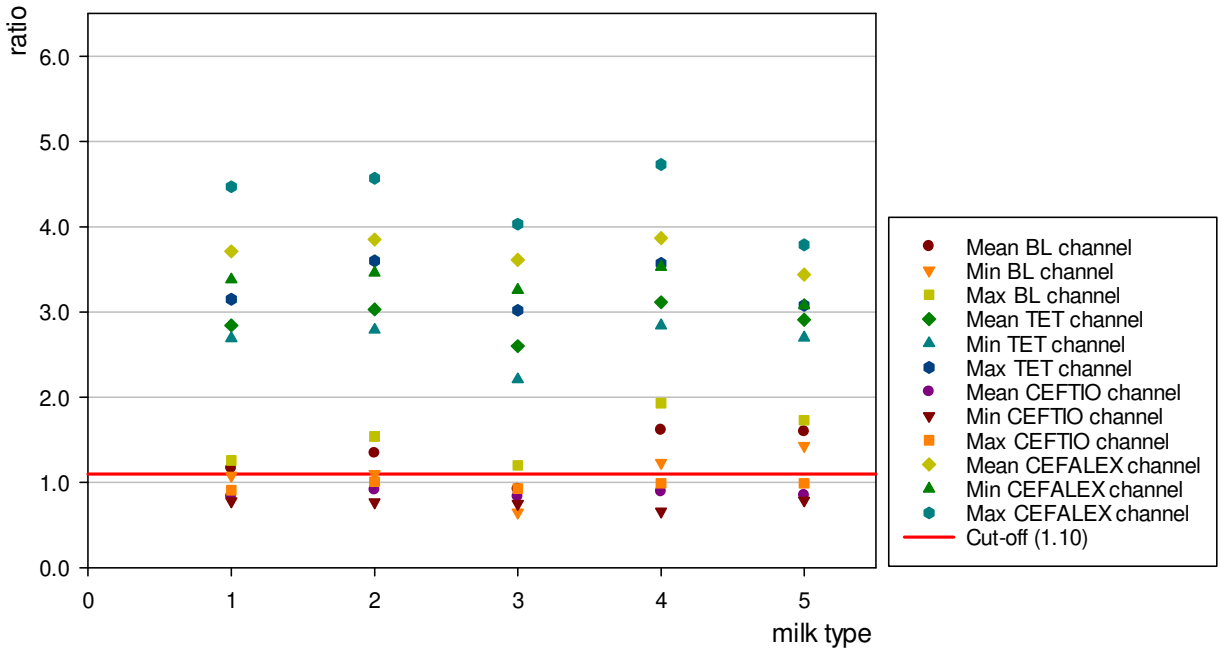

Fig. 10. Results for milk spiked with ceftiofur at 70 µg/kg. 10 samples. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

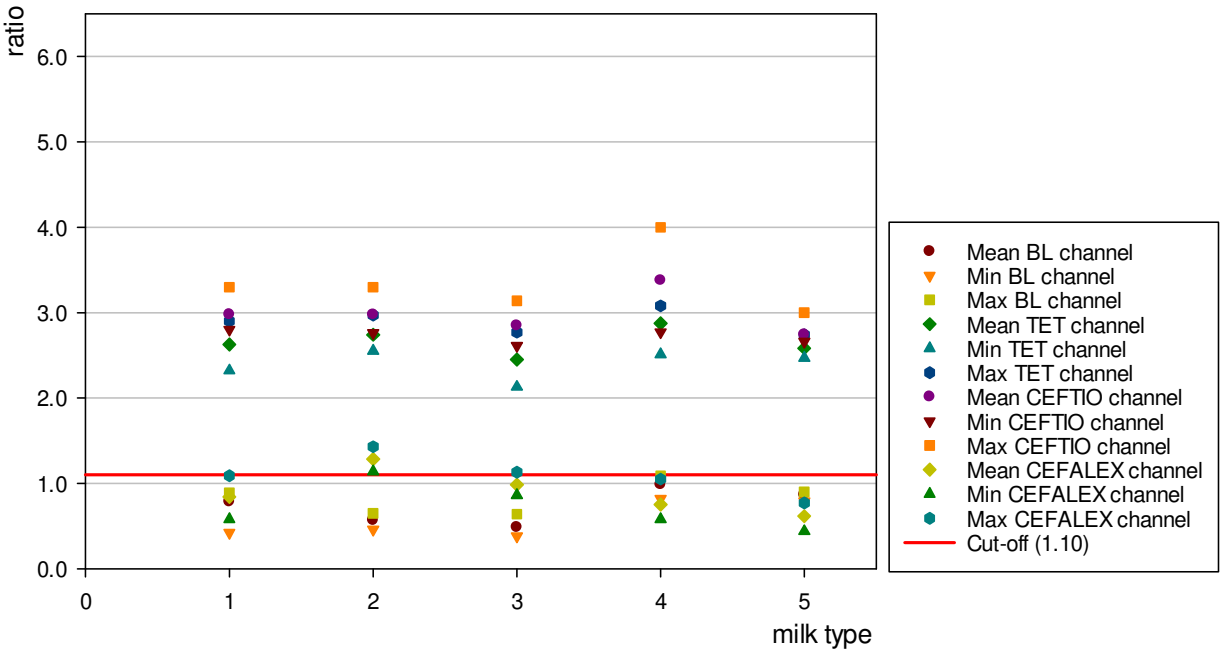

Fig. 11. Results for milk spiked with cefazolin at 35 µg/kg and cefalexin at 6 µg/kg. 10 samples. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

Table 10. Quantum BT-CC results for blank and spiked normal raw cows' milk and for blank and spiked milk of special milk type or other animal origin (10 samples, except for 20 samples of blank goats' and ewes' milk).

	Ratio											
	β-lactam channel			Tetracycline channel			Ceftiofur channel			Cefalexin channel		
	mean	min	max	mean	min	max	mean	min	max	mean	min	max
Blank raw cows' milk												
normal milk = reference	2.24	2.02	2.39	3.18	2.86	3.44	3.59	3.25	3.82	4.16	3.74	4.48
UHT milk	2.45	1.97	2.83	3.40	3.22	3.64	3.65	3.42	3.93	4.44	4.14	4.86
Reconstituted milk powder	1.94	1.24	2.40	3.18	2.18	5.12	3.77	2.84	7.54	4.67	3.29	9.61
Goats' milk	2.44	1.38	2.98	3.27	2.47	4.06	3.76	2.80	4.55	4.30	3.05	5.54
Ewes' milk	2.60	2.33	2.86	3.30	3.07	3.67	3.48	3.05	3.89	3.91	3.28	4.44
Milk spiked with benzylpenicillin at 2 µg/kg and oxytetracycline at 45 µg/kg												
normal milk = reference	0.22	0.17	0.29	0.48	0.41	0.55	2.89	2.67	3.17	3.38	3.14	3.66
UHT milk	0.31	0.16	0.42	0.41	0.35	0.51	3.00	2.78	3.15	3.57	3.24	3.81
Reconstituted milk powder	0.41	0.30	0.53	0.40	0.28	0.50	2.98	2.59	3.90	3.68	3.33	4.82
Goats' milk	0.41	0.31	0.50	0.51	0.40	0.59	2.98	2.61	3.67	3.34	2.88	4.48
Ewes' milk	0.41	0.34	0.51	0.77	0.67	0.87	2.64	2.47	2.91	2.96	2.74	3.29
Milk spiked with ceftiofur at 70 µg/kg												
normal milk = reference	1.17	1.08	1.26	2.84	2.69	3.15	0.84	0.78	0.91	3.71	3.38	4.47
UHT milk	1.34	1.10	1.54	3.03	2.79	3.60	0.91	0.77	1.01	3.85	3.46	4.57
Reconstituted milk powder	0.92	0.65	1.20	2.60	2.21	3.02	0.83	0.75	0.93	3.61	3.26	4.03
Goats' milk	1.61	1.23	1.93	3.12	2.84	3.57	0.89	0.66	0.99	3.87	3.53	4.73
Ewes' milk	1.60	1.43	1.73	2.91	2.70	3.08	0.85	0.79	0.99	3.44	3.08	3.79
Milk spiked with cefazolin at 35 µg/kg and cefalexin at 6 µg/kg												
normal milk = reference	0.79	0.42	0.89	2.63	2.32	2.90	2.98	2.80	3.30	0.84	0.58	1.09
UHT milk	0.57	0.46	0.65	2.74	2.55	2.97	2.98	2.76	3.30	**1.28**	**1.14**	**1.43**
Reconstituted milk powder	0.49	0.38	0.64	2.45	2.13	2.77	2.85	2.61	3.14	0.98	0.86	**1.13**
Goats' milk	0.99	0.82	1.09	2.88	2.51	3.08	3.38	2.77	4.00	0.75	0.58	1.05
Ewes' milk	0.87	0.79	0.90	2.58	2.47	2.74	2.74	2.66	3.00	0.62	0.44	0.77

Notes: min: minimum; max: maximum.

Discussion:

There could be interest to use the Quantum BT-CC, although developed for the testing of raw cows' milk, to test UHT milk or reconstituted milk powder or to test milk from other animal species, such as goats' or ewes' milk.

For all tested milk types different from raw cows' milk, all blank milk tested negative. For all spiked samples, all positive results were obtained on the beta-lactam, tetracycline and ceftiofur channels. On the cefalexin channel a hampering of detection was observed for UHT milk (all 10 negative results) and reconstituted milk powder (one borderline negative result (ratio 1.13) on a total of 10 samples). The results indicate a higher 95% detection capability for cefalexin in UHT milk and possibly also in reconstituted milk powder compared to Tabel 4. For reconstituted milk powder at MRL no problems are expected. For the UHT samples all analyses were negative, therefore, three UHT milk samples with cefalexin at MRL were analysed in duplo, obtaining all positive results (with ratios between 0.76 and 1.02). These results indicate a

hampering of detection on the cefalexin channel for UHT milk at low concentrations, but at MRL cefalexin will be detected. Based on the results, it is expected that a 95% detection at MRL in UHT milk will be achieved, but it is advised to verify to be sure.
For spiked goats' and ewes' milk, all positive results were obtained on all channels.

6.5. Stability of reagents - Daily control samples

Methods and material:
The following control samples were analyzed daily with Quantum BT-CC to check the stability of the reagents and consistency of results:

- blank raw milk free from antimicrobials (fresh and frozen) - twice
- blank raw milk spiked with benzylpenicillin at 2 µg/kg and oxytetracycline at 45 µg/kg - twice
- blank raw milk spiked with ceftiofur at 70 µg/kg - twice
- blank raw milk spiked with cefazolin at 35 µg/kg and cefalexin at 6 µg/kg - twice

Each day. also a negative and positive control as provided in the kit were analysed. The positive standard contains benzylpenicillin at 4 µg/kg. tetracycline at 100 µg/kg. ceftiofur at 100 µg/kg and cefalexin at 100 µg/kg. The negative control was dissolved with 200 µl of HPLC water. while for the positive control 200 µl of blank milk was added.

Results:
The results of the daily control samples and negative and positive control samples are presented in Figure 12 to 15. A summary is provided in Table 11.

Discussion:
Stable ratio values were obtained for daily control samples with Quantum BT-CC reagents over the test period on all four tests lines. Always correct values were obtained for the different daily standards. All blank milk standards gave a negative result on all channels and all spiked milk samples gave positive results.
Also the negative and positive controls inserted in the kit always gave correct results.

Table 11. Quantum BT-CC results (ratio values) for the daily standards.

	β-lactam channel				Tetracycline channel				Ceftiofur channel				Cefalexin channel			
	mean	min	max	SD	mean	min	max	SD	mean	min	max	SD	mean	min	max	SD
Blank milk																
Fresh	2.41	1.54	2.94	0.34	3.27	2.62	3.95	0.24	3.68	2.94	4.39	0.27	4.39	3.54	5.48	0.33
Frozen	2.72	1.97	3.38	0.25	3.48	3.04	4.13	0.23	3.88	3.38	4.49	0.25	4.50	3.90	5.28	0.33
Milk spiked with benzylpenicillin at 2 µg/kg and oxytetracycline at 45 µg/kg																
	0.45	0.23	0.70	0.11	0.80	0.53	0.97	0.10	3.12	2.80	3.49	0.18	3.63	3.25	4.25	0.23
Milk spiked with ceftiofur at 70 µg/kg																
	1.69	1.20	1.98	0.17	2.87	2.61	3.18	0.14	0.94	0.80	1.08	0.07	4.04	3.61	4.79	0.25
Milk spiked with cefazolin at 35 µg/kg and cefalexin at 6 µg/kg																
	0.86	0.57	1.02	0.12	2.90	2.57	3.20	0.15	3.21	2.80	3.71	0.22	0.78	0.61	0.93	0.08
Controls included in the kit																
Neg	2.27	1.45	2.73	0.28	3.18	2.38	3.69	0.32	3.76	2.59	4.49	0.40	4.39	3.02	5.01	0.46
Pos	0.09	0.01	0.35	0.07	0.28	0.01	0.40	0.06	0.85	0.56	1.05	0.13	0.16	0.08	0.27	0.05

Notes: SD: standard deviation; min: lowest ratio; max: highest ratio

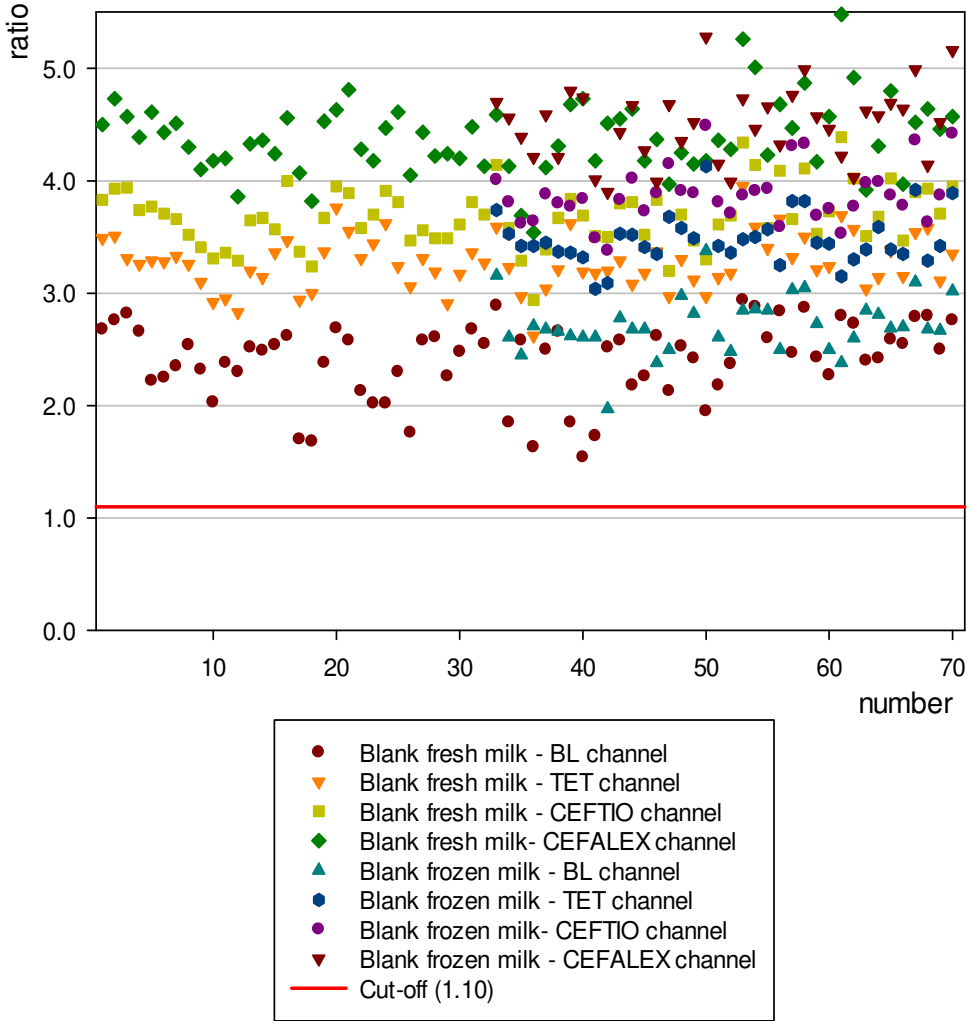

Fig. 12. Quantum BT-CC results (ratio) for the blank control samples (fresh and frozen) of the daily standard. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

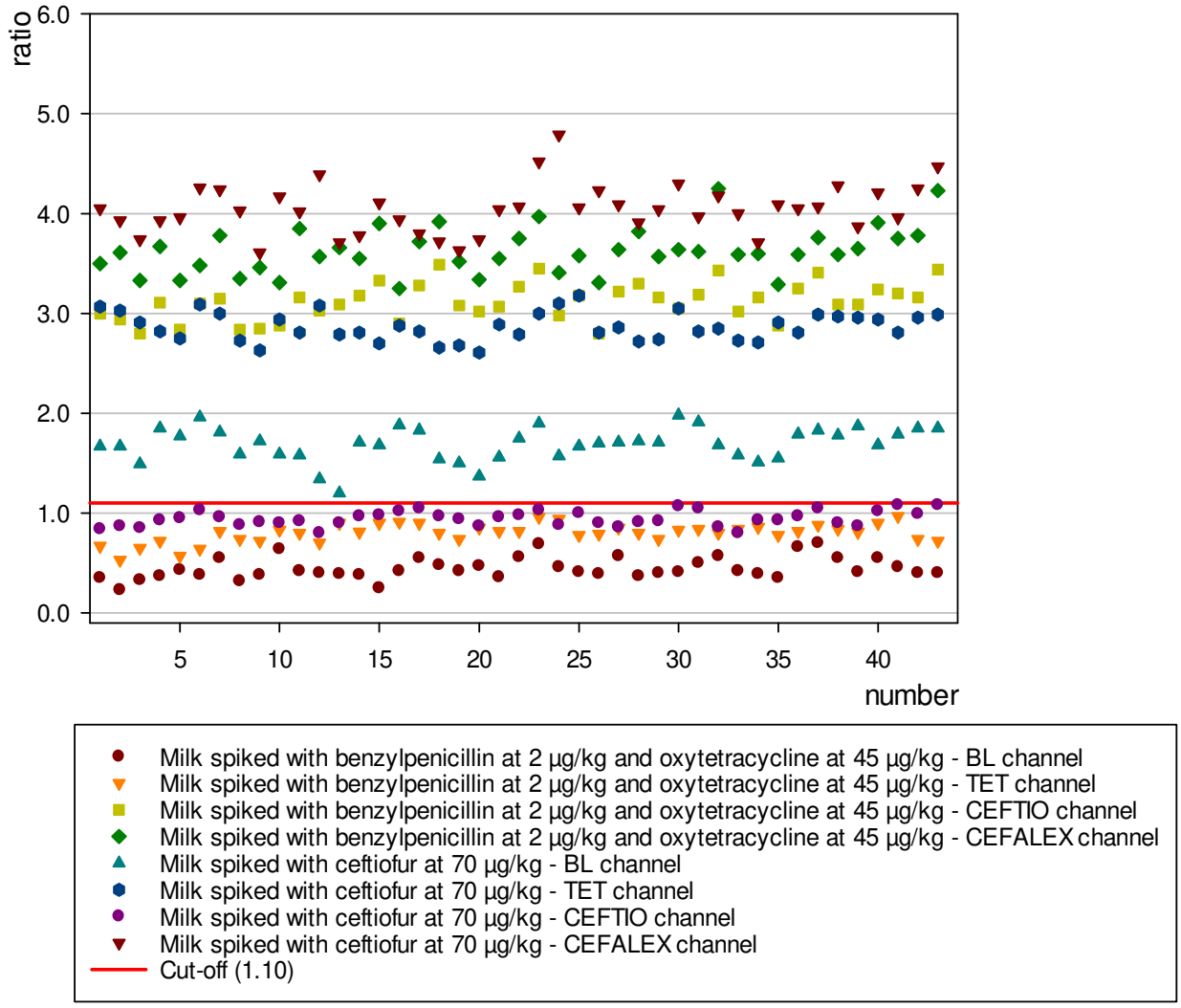

Fig. 13. Quantum BT-CC results (ratio) for the spiked samples with benzylpenicillin at 2 µg/kg and oxytetracycline at 45 µg/kg or containing ceftiofur at 70 µg/kg of the daily standards. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

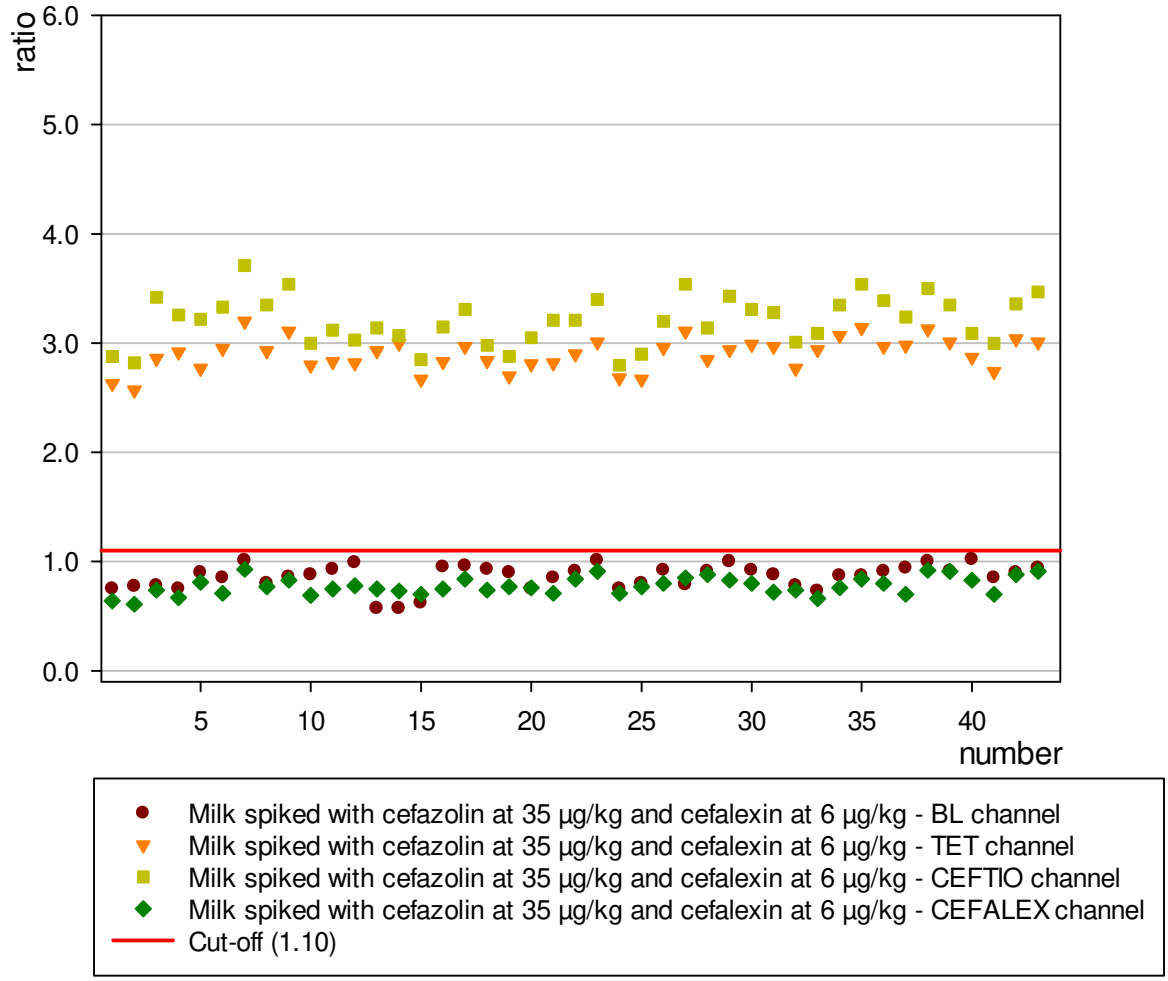

Fig. 14. Quantum BT-CC results (ratio) for the spiked samples with cefazolin at 35 µg/kg and cefalexin at 6 µg/kg of the daily standards. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

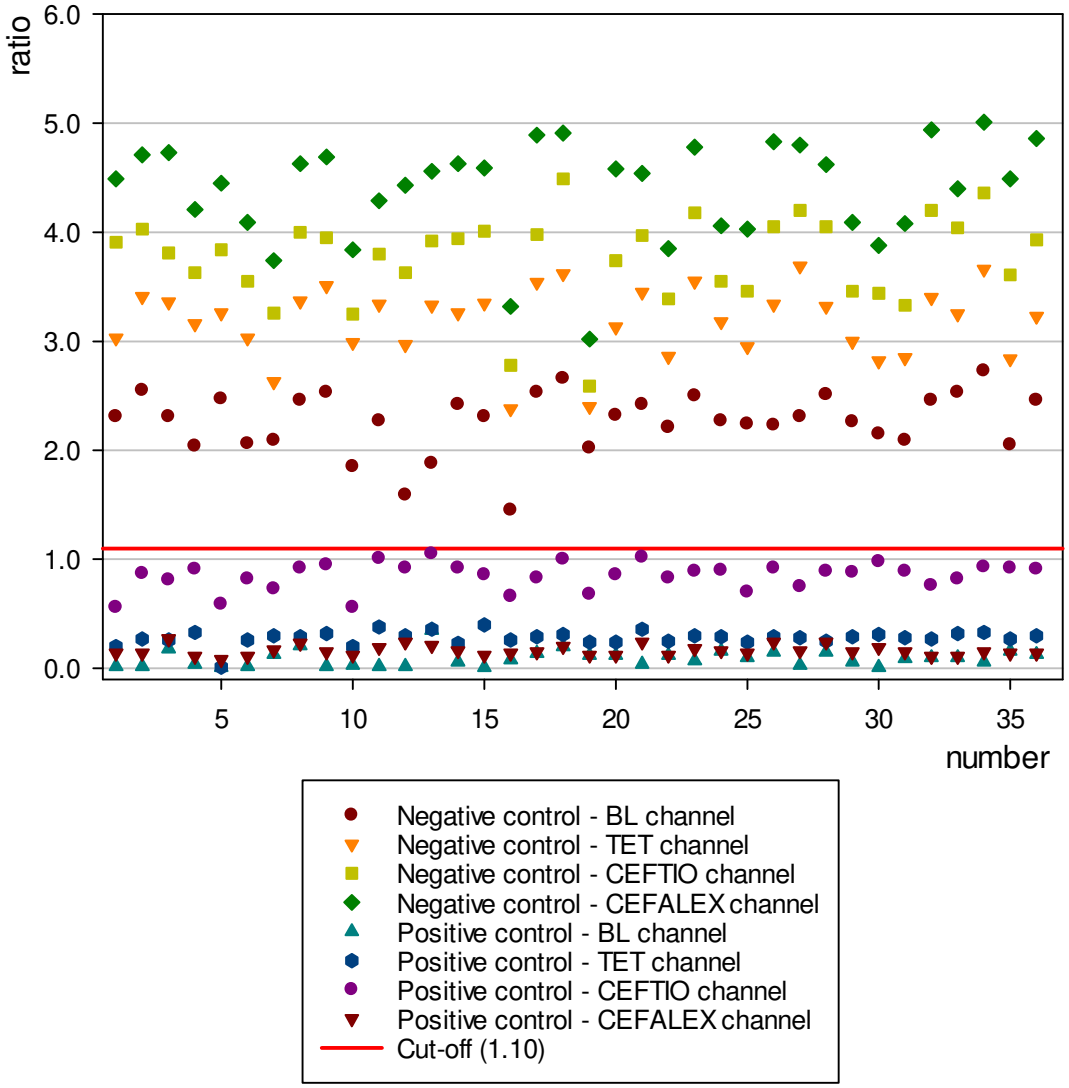

Fig. 15. Quantum BT-CC results (ratio) for the negative and positive controls inserted in the kit. BL: β-lactam channel; TET: tetracycline channel; CEFTIO: ceftiofur channel; CEFALEX: cefalexin channel.

7. Reliability of the instrumentation

Few points of attention that occurred during validation:

- It is important to check that the screen of the reader remains clean to assure a correct read. When dirty, the screen can easily be removed and cleaned.

8. Interlaboratory testing - National ring trial

Methods and material:
T&V-ILVO is organizing twice a year a national ring trial for the (Belgian) dairy industry regarding the detection of residues of antibiotics in milk by microbiological and rapid tests. In the ring trial of April 2025, Quantum BT-CC was integrated as rapid test.

Results:
Results are given in Table 12.

Identification of the samples:

Sample	Drug	Conc. (µg/kg)	MRL (µg/kg)
I	Cephapirin	20	60[a]
J	Cloxacillin	10	30
K	Benzylpenicillin + Oxytetracycline	3+50	4/100[b]
L	Cefoperazone	25	50
M	Blank	-	-
N	Amoxicillin	3	4
O	Benzylpenicillin	2	4
P	Cefalexin	50	100

Notes: [a]: sum of cephapirin and desacetylcephapirin, [b]: sum of oxytetracycline and its 4-epimer.

Table 12. Results of Quantum BT-CC in the national ring trial of April 23rd, 2025 (Ooghe & Broekaert, 2025).

Visual verification									Instrumental reading (ratio + result)								Lot number Expiry date Type of reader
I	J	K	L	M	N	O	P		I	J	K	L	M	N	O	P	
Beta-lactam (BL)																	
+	+	+	+	-	+	+	-		0.01 POS	0.01 POS	0.17 POS	0.01 POS	1.97 NEG	0.41 POS	0.41 POS	1.93 NEG	
Tetracycline (TET)																	
-	-	+	-	-	-	-	-		2.46 NEG	2.4 NEG	0.61 POS	2.31 NEG	2.9 NEG	2.78 NEG	2.59 NEG	3.08 NEG	W156004 11/2025 S-Flow Reader
Ceftiofur (CEFT)																	
-	-	-	-	-	-	-	-		2.76 NEG	2.73 NEG	3.09 NEG	2.71 NEG	3.53 NEG	3.17 NEG	3.13 NEG	3.63 NEG	
Cefalexin (CEFA)																	
-	-	-	-	-	-	-	+		3.44 NEG	3.28 NEG	3.83 NEG	3.36 NEG	4.33 NEG	3.91 NEG	3.67 NEG	0.44 POS	

Note: The cut-off value of the S-Flow Reader is 1.10. Milk samples generating a ratio above this cut-off value are considered negative.

Discussion:
Good results were obtained with Quantum BT-CC.

All milk samples fortified with β-lactam antibiotics (samples I, J, K, L, N, O & P) were screened positive with Quantum BT-CC, either on the specific cefalexin channel or on the β-lactam channel. This is in line with the respective detection capabilities obtained at ILVO (cephapirin ccβ = 3 µg/kg; cloxacillin ccβ = 3 µg/kg; benzylpenicillin ccβ = 2 µg/kg; cefoperazone ccβ = 3 µg/kg; amoxicillin ccβ = 2 µg/kg and cefalexin ccβ = 6 µg/kg).
The milk sample fortified with 50 µg/kg of oxytetracycline (sample K) was screened positive on the tetracycline channel of Quantum BT-CC, which is in line with the detection capability obtained at ILVO for oxytetracycline (40 µg/kg).
Negative results were obtained on all channels for the blank milk (sample M) and for the milk samples spiked with antibiotics that are supposed to give a negative result on the respective channels. So, there were no false positive results with Quantum BT-CC.

9. Final conclusion

The validation of Quantum BT-CC (ProGnosis Biotech S.A.. Larissa. Greece) was executed following ISO Technical specification TS 23758 / IDF RM 251.

Quantum BT-CC is capable to detect residues of all β-lactams including cefalexin and ceftiofur, and tetracyclines with a MRL in milk (EU-Regulation 37/2010 and amendments).
All β-lactams, including ceftiofur and cefalexin, and tetracyclines (parent drugs), can be detected at least in 95% of the samples at their respective MRL. The exact 95% detection capability of the 4-epimers of tetracyclines were not determined, but were tested twice at MRL. It is worth noting that those 4-epimers are generally not found in milk (ILVO, internal communication).
Doxycycline, not for use in animals from which milk is produced for human consumption, was detected at least in 95% of the replicates at 70 µg/kg.

The test is fulfilling the acceptance criteria and could be approved by the Belgian Federal Agency for the Safety of the Food Chain (FASFC) as test used by the Belgian dairy companies to check incoming milk on the presence of β-lactam residues (*Anon.*, 2024).

Quantum BT-CC is a highly specific test for detection of β-lactams and tetracyclines in milk and does not detect compounds from the sulfonamides, aminoglycosides, quinolones, polymyxins, amphenicols, macrolides, lincosamides and diamino pyrimidine derivatives, nor dapsone.

No false positive results (0%) were obtained on in total 600 milk samples (300 blank farm and 300 blank tanker load) on all 4 channels. And repeatability of both reader and test are good.

It is worth noting that one farm sample was found positive on the beta-lactam channel (ratio 0.92). By use of beta-lactamase ES, the presence of a beta-lactam antibiotic was confirmed, proving that the test is capable of detecting antibiotic residues due to veterinary use.

The test shows to be robust to changes towards the test protocol: changes in milk temperature has no impact. Incubation and reading is automated and a bulb pipette included in the kit is used for sample volume.
The milk quality and composition had some influence on the performance of the Quantum BT-CC. No false positive results were obtained for blank milk samples. But results indicate a higher 95% detection capability than given in Table 4 for cefalexin for low protein milk and for low pH milk. For low pH milk it is strongly recommended to adjust pH to normal milk before (re)testing.
On the other channels, a borderline negative result was obtained for e.g. high somatic cell count (BL channel), late lactation (TETRA channel), low and high protein and early lactation (CEFTIO channel). Nevertheless, all the samples were spiked so far below the compounds' MRL, that including cefalexin, all compounds will be detected at MRL.

The test could also be used to screen UHT milk and reconstituted milk powder on the presence of residues of β-lactams, including ceftiofur and cefalexin and tetracyclines. On the cefalexin channel hampering of detection occurred for UHT milk, however, extra testing indicates that the test is capable to detect cefalexin in UHT milk at MRL concentrations. Based on the results, it is expected that a 95% detection at MRL in UHT milk will be achieved.
The test can also be used to screen goats' and ewes' milk.

Good results were obtained with the test in a ring trial.

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