Survey of Antibiotic Test Methods for Raw Milk

Commissioned by
The Dairy Industry Federation

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HANNAH
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Survey of Antibiotic Test Methods - Dairy Industry Federation 1999

Introduction

Immediately prior to the end of the Milk Marketing schemes the Joint Committee of England & Wales re-issued the "Blue Book" under the title of "Methods for the assessment of Raw Milk Quality". Subsequently the Milk Quality Forum (whose members are the Dairy Industry Federation, National Farmers Union and the UK Federation of Milk Producer Organisations) was set up and agreed to keep this manual up to date.

The Dairy Industry Federation successfully obtained funding from MAFF from its Sector Challenge programme for the development of a raw milk quality programme. Part of this programme was a revision of the "Blue Book". The Milk Quality Forum was aware that the section on antibiotic testing was in particular need of revision, with a large number of antibiotic test kits being offered to milk purchasers. Taking into account the specialised nature of this subject, and the desirability of carrying out practical evaluations of the various tests, the Dairy Industry Federation commissioned the Hannah Research Institute to undertake an examination of the tests available.

Although at the outset of the survey it was anticipated that a single test might be found to serve all the needs of industry it quickly became clear that this objective was unlikely to be met because of the diverse needs of individual dairy companies for antibiotic testing and because of the diversity of the test methods available. These complications precluded the use of an exactly similar protocol for evaluation of the test methods. A compromise was reached where all methods were evaluated in a similar way. The differences being confined to the number of replications of the test at a particular concentration of antibiotic and to the concentration levels of antibiotic tested. The information about each Antibiotic Test Kit is reported in a common format.

This report comprises: (a) Definition of the testing method and rating system, (b) Individual reports on all test methods submitted to us and, (c) A brief summary of our findings.

Definition Of Testing Methods And Rating System

Test Samples

Morning milk was collected under near aseptic conditions from healthy cows in the Institute herd that had no history of antibiotic treatment for mastitis or any other condition. The cows were all in mid-lactation and the somatic cell count of the herd was consistently less than 150,000 and the bacterial count index was less than 50 (Bactoscan). The milk was cooled in ice immediately after collection and stored under refrigeration until required. The milk from 5 animals was mixed in equal proportions to give a commingled sample. Spiked samples were prepared by (1) Dilution of antibiotic (0.250g; Penicillin G, sodium salt monohydrate; or Cloxacillin; Sigma Chemical Corp.) in distilled water (100ml), (2) Dilution of the master

solution (1.0 ml) in distilled water (1000 ml) and (3) Addition of the diluted master solution to milk to obtain concentrations of Penicillin G in the range 0-8 mg kg^{-1} PPB) and concentrations of Cloxacillin in the range 0-100 mg kg^{-1} (PPB). (No correction was applied for the purity of the antibiotic. However, the equivalent concentration of free base is reported based on purity of penicillin G of 93.7% and Cloxacillin of 90.8% derived from data on product label.)

Spiked milk samples were used on the day of preparation and for up to 2 days after collection (stored under refrigeration). As a result, over the testing period for a single antibiotic test kit it was usual for several batches of spiked sample to be used. Care was taken to ensure that the complete range of test concentrations of antibiotic was evaluated for each batch of milk to avoid confounding between batches and the antibiotic concentration. There was no evidence of batch-to-batch variation. During testing, either milk from individual cows was used or the co-mingled milk of 5 animals, depending on the recommendation of the supplier of the test method.

Two antibiotics were selected as the test stimuli - penicillin G and Cloxacillin. The choice of antibiotic was based on the universal acceptance of penicillin G as a benchmark. Cloxacillin was selected as the second test material because of its widespread therapeutic use in the UK. (Note: Procaine penicillin and Cloxacillin rank first and second as active ingredients of the most numerous of licensed proprietary formulations in the UK). A series of concentrations of the two antibiotics in milk were prepared. The choice of levels to be tested was discussed at length with technical experts from the companies supplying the antibiotic tests. Levels were chosen to encompass the expected threshold for detection.

It is important to note that the threshold levels for detection of different antibiotics vary between tests. The test protocol sought to confirm the thresholds for Penicillin G and Cloxacillin. It should also be noted that the reported spectrum of antimicrobials and inhibitory substances detected by the various test methods varies significantly. It was not possible to confirm these claims within the scope of the current study. However, a table reporting the manufacturer specification for test sensitivity of other antimicrobial compounds is included in the report sheets for information.

Reporting of test results

The results of the detection tests are reported in tabular form. The recording of a result was according to the instructions of the kit supplier. The exact interpretation of the results is detailed in the individual report forms.

Ease of Use Statistic

An important aspect of the method is its ease of use. To avoid subjective assessment, we have devised an 'Ease of Use Statistic' which is objectively based. The potential for deviation from the test protocol is associated with the number (and type) of unit operations (individual

manipulations) which comprise the overall analysis. Thus, by determining the number of independent manipulations in a test, an overall rating for complexity (the inverse of 'ease of use') can be deduced. In the scheme adopted here, low indices indicate a user-friendly format. Each test was rated according to the following criteria detailed in Table 1.

Testing Schedule

The 17 test kits listed in Table 2 were evaluated. In each and every case a Technical Expert from the supplier visited HRI. On this visit the objectives of the survey were described and, in discussion with the Technical Expert, the number of replications of the test and the concentration levels of the spiked milks were agreed. Hannah personnel were then trained by the Technical Expert. Training was deemed complete when both parties were satisfied that the testing method was fully understood by HRI and a number of tests had been successfully completed. No representatives of the suppliers were present during subsequent evaluations. The results of the individual evaluations were sent to each Technical Expert for comment and the description of the Test, but not the results, were amended in the light of these comments.

Threshold for Detection of Antibiotic

The ideal performance of an antibiotic test is shown in Figure 1. The response would take the form of a step change where the test would report negative results up to a threshold then, in a step change, switch to reporting positive results. In reality, the response usually takes the form of the actual curve shown in Figure 1. In this case, the probability of reporting a positive result increases from 0 to 1 over a transitional range of antibiotic concentrations. This range and the response curve can be defined by very extensive testing but such an approach was impossible within the resource available for the project. In this study, the threshold value for a test was considered to be the point at which 95% or more of the replicated tests at a particular antibiotic concentration were positive (i.e., 19/20 or 19/30 positive). This approach is conservative but ensures that a sample is not condemned as contaminated unless there is no reasonable doubt that an antimicrobial compound is present.

Summary of Findings

The test methods examined in this study fell within three categories:

- First, inhibition tests which detect the presence of a wide range of antimicrobial compounds;
- Second, tests specific for a class or limited range of antimicrobial compounds based on an analytical routine with instrumental detection. Such tests require skilled operators.
- Third, special tests designed for use by operators with rudimentary training.

The assignment of test kits into these categories is shown in Table 3.

Performance - sensitivity to penicillin G and cloxacillin

The sensitivity of all the tests for the detection of penicillin G is reported in Table 4. The Maximum Residue Limit (MRL) for this compound are currently set at 4 ppb (1 ppb = 1 g kg ^{1}) within the European Union. Although there was a wide range in sensitivity, with the exception of the Penzyme method, all tests detected penicillin G at or below the MRL. It should be noted that the BetaScreen technique was particularly sensitive to this antimicrobial compound.

The corresponding results for cloxacillin (MRL 30 ppb) are also shown in Table 4. There was greater divergency in the detection limit for cloxacillin - the detection threshold ranged from 5 to 73 ppb. Although not unusually sensitive to penicillin G, the BetaStar and LacTek methods had a detection thresholds for cloxacillin below 7 ppb.

Performance - ease of use statistic

The ease of use statistics are shown in Table 5. Within each of the categories shown in Table 3, there were significant differences in ease of use. The BetaScreen (EOU -23) and Charm II (EOU - 23/24) tests were more complicated than the Delvo-X-Press (EOU - 10) and LacTek (EOU -11) tests. However, the instrument used for the BestaScreen test also supports the Fluorophos alkaline phosphatase test for checking the efficiency of pasteurisation and the Charm II also may be used in a wider range of applications. The other test methods were either easy to use (BetaStar100, Penzyme 100, Charm AIM; EOU - 9-11) or very easy to use (BetaStar 25, Penzyme 20, Charm MRL, Charm VN, Charm MV, Delvtest P, Delvotest SP and Delvo MCS; EOU - 5-7)

Performance - length of test

The relative time taken for sample evaluation and the number of samples that may be tested at the same time are shown in Table 6. There are two distinct types of test - those developed for mass screening or where time is not of the essence (Charm AIM, VN & MV, Delvotest P, SP & MCS) and the rapid tests designed for tanker acceptance/rejection at the dairy reception. Of the rapid tests most were complete in 6-12 minutes. However, the BetaScreen and Penzyme tests were completed in 20 minutes.

Conclusion

The 'consumer' has a choice of test method for detection of antibiotic in milk. Demand can be divided into various choices:

- First, mass screening tests where cost is particularly important or rapid screening tests where time is of the essence.

- Second, tests which have the greatest sensitivity for the widest range of antibiotics or tests that are tuned to detect at the EU maximum residue limits.
- Finally, tests that are highly selective which have a broader spectrum of response.

This survey has clearly revealed that the 'consumer' has a choice within each category. For individual company use this choice must be welcomed because it allows individual companies set their own quality criteria. For example, where dairy companies have a dedicated milk pool, from individual producers under contract or from a producer co-operative, suitable criteria can be mutually agreed and the most appropriate test chosen.

However, when milk is subject to trade outside these close confines the Dairy Industry is faced with a dilemma because it is preferable that equivalent tests are used for application to freely traded milk. The decision on this equivalence and on the tests which meet the needs of the Industry are matters for the Milk Quality Forum to decide. Nevertheless, provided the milk meets the EU Maximum Residue Limits - it conforms to the statutory requirements.

Supplementary Information

At the request of the DIF additional information is provided in the Appendices on:

(a) The Manufacturers' claimed sensitivity to antibiotics accorded fixed and provisional Maximum Residue Limits by the European Union.
(b) Prices at June 1999.
(c) Contact addresses.

DDM/NW
26 July 1999

Table 1. Calculation of the Ease of Use Statistic

Process		Code	Minimum	Maximum
Preparation	Set up instrument [a]	1	0	2
	Set up incubation conditions [b]	2	0	1
	Fluid transfers [c]	3	0	n
Analysis	Fluid transfers [c]	4	0	n
	Timings [d]	5	1	n
	Other manipulations [e]	6	1	n
Interpretation of results [f]		7	0	1
	Total rating [g]		n	n

Notes on rating criteria:

[a] *0 = not required; 1 = switch on and allow to warm up, 2 = switch on, allow to warm up, calibrate.*

[b] *0 = not required, 1 = set up dry incubator or water bath*

[c] *0 = none, n = number of fluid transfers*

[d] *0 = none, n = number of separate timed operations*

[e] *0 = none, n = number of other manipulations e.g., transfer of test strip to incubator, wash tubes.*

[f] *0 = decision printed by instrument, 1 = comparison of band density or interpretation of colour change*

[g] *Total rating = arithmetic sum of ratings for operations coded 1-7*

Table 2. Summary of Tests Completed at Friday, 23 April 1999

Kit	Report	Outcome
BetaScreen, Advanced Instruments		
BetaStar 25, UCB		
BetaStar 100, UCB		
Penzyme 20, UCB		
Penzyme 100 UCB		
Charm II		
Charm AIM		
Charm MRL Beta-lactam test		
Charm Farm test VN		
Charm Farm test MV		
Lactek, Guildhay		
Paralux, Guildhay		Withdrawn[a]
SNAP, IDEXX		
DelvoExpress		Re-tested[b]
Delvo P		
Delvo SP		
Delvo MCS		

[a] The instrument supplied was a prototype and did not perform according to expectations of the manufacturer. The evaluation was therefore abandoned at the request of the supplier. It is anticipated that the test be re-submitted for evaluation later in the year.

[b] The system did not perform according to expectations in our laboratory, despite re-testing. Previous independent tests have revealed no problem. In collaboration with the manufacturer we are currently investigating the problem.

Table 3 Assignment of Test Methods into Categories

Category 1	Category 2	Category 3
Charm AIM	BetaScreen	BetaStar 25/100
Charm Farm VN	Charm II	Charm MRL
Charm Farm MV	DelvoExpress	Penzyme 20/100
Delvotest MCS	Lactek	SNAP
Delvotest P		
Delvotest SP		

Table 4. Sensitivity of test methods to penicillin G and cloxacillin (g kg^{-1})

Test	Pen. G	Cloxacillin	Test	Pen. G	Cloxacillin.	Test	Pen. G	Cloxacillin
BetaScreen	0.9	20	Charm AIM	2.8	32	Delvo-X-Press	4.0	60
BetaStar 25	2.8	5.4	Charm MRL	2.8	27	Delvotest P	2.8	36
BetaStar 100	2.8	5.4	Charm VN	2.8	36	Delvo SP	2.8	18
Penzyme 20	4.7	73	Charm MV	2.8	27	Delvo MCS	1.9	14
Penzyme 100	5.6	73	LacTek	3.3	6.8			
Charm II	2.8	18	SNAP	3.7	54			

Maximum residue limits set by EU are: penicillin G = 4.0 g kg^{-1}, cloxacillin = 30.0 g kg^{-1}

Table 5. Ease of use statistics (EOU; lower values indicate easiest to use).

Test	EOU	Test	EOU	Test	EOU
BetaScreen	23	Charm AIM	10	Delvo-X-Press	10
BetaStar 25	6	Charm MRL	5	Delvotest P	5
BetaStar 100	9/10	Charm VN	6	Delvo SP	5
Penzyme 20	6	Charm MV	7	Delvo MCS	5
Penzyme 100	10	LacTek	11		
Charm II	23/24	SNAP	7		

Table 6. Relative time taken for sample evaluation and number of samples which may be tested simultaneously.

Test	No. samples	Time, min.	Test	No. samples	Time, min.	Test	No. samples	Time, min.
BetaScreen	5	20	Charm AIM	96	240	Delvo-X-Press	5	8
BetaStar 25	4	6	Charm MRL	10	10	Delvotest P	40	150
BetaStar 100	8	6	Charm VN	150	210	Delvo SP	40	210
Penzyme 20	8	20	Charm MV	72	180	Delvo MCS	150	150
Penzyme 100	8	20	LacTek	5	10			
Charm II	5	12	SNAP	6	10			

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	Beta-Screen EU (Fluorophos®)
Supplier	Advanced Instruments Inc., Norwood, MA, USA
UK Agent	QuadraChem, Riverside, Forest Row Business Park, Forest Row, East Sussex RH18 5DW
Expert Technical Contact	Jeffrey P. Guilbert (jeffg@aitests.com)
Recommended application	Qualitative test for beta-lactam antibiotic residues in commingled bovine milk including: penicillin G, amoxicillin, ampicillin, cloxacillin, dicloxacillin and oxacillin. Applicable to pasteurised milk.
Principle of test	BetaScreen is a competitive enzyme-linked immunoassay. Milk and enzyme-conjugate are added to a tube coated with an antibody to beta-lactam antibiotic. If no antibiotic is present in the milk, the binding sites are complexed only with the enzyme-conjugate. After washing, when a substrate is added, the enzyme releases a fluorescent material that is detected by a fluorimeter. When antibiotic is present in milk, it competes with enzyme conjugate for the binding sites on the coated tube. As a result, a mixture of enzyme-conjugate and antibiotic are bound to the tube. Therefore, after washing and addition of substrate less fluorescent material is released. The decrease in fluorescence is related to the concentration of antibiotic.
Cross-reactivity	BetaScreen does not cross react at a concentration of 100 parts per billion with ceftiofur, cephalosporin-C, cephapirin, chlorothiazide, chlortetracycline, dexamethasone, doxycycline, dipyrone, erythromycin, furosemide, gentamicin, ivermectin, neomycin, novobiocin, oxytetracycline, oxytocin, para-aminobenzoic acid, phenylbutasone, pirlimycin, streptomycin, sulphadiazine, sulphadimethoxine, sulphamethazine, sulphanilamide, sulphapyridine, sulphathiazole, tetracycline, thiabendazole, tilmycosin and trichlormethiazide.
Interference	Sensitivity and selectivity are not adversely affected by somatic cell numbers up to 800,000 cells per ml.
Apparatus supplied	In start up kit:-Tray organiser, timer, graduated cylinder (50 ml), wash bottle (500ml), pipette (500 µl) and disposable tips, foam assay tube holder.
Extra apparatus required	Fluorimeter (Fluorophos Test System), vortex mixer and cuvettes.
Reagents supplied	Beta Standard and diluent, Beta-conjugate, Fluorophos substrate, substrate buffer, stop solution, wash concentrate, assay tubes.
Extra reagents required	Distilled or deionised water
Time (set-up)	30-40 minutes
Time per test	20 minutes (5 samples are tested together; time per test 3 minutes)
Operator skill level	Training required by an agent of Advanced Instruments Inc.; previous experience in analytical procedures is helpful.
Instructions	Detailed and unambiguous.
Safety advice	Hazard warnings are detailed on reagent bottles.
Reporting method	Instrument prints out 'positive' or 'negative' result
Ease of use	Requires careful adherence to instructions (Rating = 23).

Test results (antibiotic concentration in μg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	20	0	0	0	20	0
0.25	0.23	20	3	10	9.1	20	19
0.5	0.46	20	8	15	13.7	20	17
1.0	0.94	20	20	20	18.2	20	15
2.0	1.9	20	20	22.5	20.4	20	20
3.0	2.8	20	20	30	27.2	20	20
				37.5	34.1	20	20

Positive attribution when ratio of Test/Standard readings 1.0

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity (μg kg^{-1})
Penicillin G	1
Amoxicillin	10
Ampicillin	3
Cloxacillin	20
Dicloxacillin	30
Oxacillin	20
nafcillin	10

Additional Comments

The fluorimeter used to measure the extent of reaction is common to Fluorophos Test systems for alkaline phosphatase (used to detect efficient pasteurisation of milk) and a test under development (acid phosphatase) for confirmation of the efficient pasteurisation of meat.

DDM/NW March 12, 1999 (Final revision)

Test Report

Test method: Beta s.t.a.r 25
Beta s.t.a.r 100

UK supplier: Axient Laboratories

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	**Beta s.t.a.r. 100**
Supplier	UCB Bioproducts, Braine-L'Alleud, Belgium
UK Agent	Axient, Axient Laboratories, Unit 20, Goldthorpe, Rotherham S63 9BL
Expert Technical Contact	Jacques Degelaen (jacques.degelaen@ucb-group.com)
Recommended application	Qualitative test for beta-lactam antibiotics, including penicillin G, ampicillin, amoxicillin, nafcillin, cloxacillin, oxacillin, dicloxacillin, cephapirin, cephalonium, cefoperazone and ceftiofur. Beta s.t.a.r 100 is designed for screening milk in any laboratory where an answer is needed with respect to contamination of milk by beta lactam antibiotics.
Principle of test	The test involves a specific beta-lactam receptor linked to gold particles An initial incubation of a specific amount of receptor with milk containing antibiotics results in a complex between the receptor and the antibiotic. Second, the solution is transferred onto an immunochromatographic medium. The first band captures receptors that have not complexed with antibiotic. The second band serves as a reference band.
Cross-reactivity	Not available at time of test
Interference	Operator should not be under medical treatment by beta-lactam antibiotics.
Apparatus supplied	None.
Extra apparatus required	Dry incubator or water bath at 47°±0.5C, micropipettes (25 & 100µl), pipette to deliver 1350µl.
Reagents supplied	2 vials of receptor, 4 x 25 dipsticks (receptor can be stored in deep freeze for more than a month)
Extra reagents required	Deionised water
Time (set-up)	Warm-up time of dry incubator, preparation of solutions
Time per test	6 minutes (8 samples may be tested in one batch)
Operator skill level	Minimal
Instructions	Detailed and unambiguous.
Safety advice	No specific hazard
Reporting method	After development, the test strips normally show two distinct bands, an upper (reference) and a lower. If the lower band is denser than the reference, the sample is negative; if the bands are similar in density then a positive with caution (+/-) result should be applied; if the lower band is less dense than the reference (+) *or* absent (++) then the sample is positive.
Ease of use	Relatively easy to use (rating = 9/10).

Test results (antibiotic concentration in µg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	20	0	0	0	20	0
2	1.8	20	0	2	1.8	20	0
2.5	2.3	20	1	3	2.7	20	0
3	2.8	20	20 (-/+)	6	5.4	20	20 (+)
4	3.8	20	20 (+)	9	8.2	20	20 (+)
5	4.7	20	20 (++)	12	10.9	20	20 (++)
6	5.6	20	20 (++)				
8	7.50	20	20 (++)				

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity (µg kg^{-1})
Penicillin G	2-4
Amoxicillin	2-4
Ampicillin	2-5
Nafcillin	8-20
Oxacillin	5-10
Cloxacillin	5-10
Dicloxacillin	5-10
Cephapirin	8-16
Cephalonium	7.5-15
Cefoperazone	5-8
Ceftiofur	75-150

Additional Comments

Reacted strips can be stored as a permanent record.

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	Beta s.t.a.r. 25
Supplier	UCB Bioproducts, Braine-L'Alleud, Belgium
UK Agent	Axient, Axient Laboratories, Unit 20, Goldthorpe, Rotherham S63 9BL
Expert Technical Contact	Jacques Degelaen (jacques.degelaen@ucb-group.com)
Recommended application	Qualitative test for beta-lactam antibiotics, including penicillin G, ampicillin, amoxicillin, nafcillin, cloxacillin, oxacillin, dicloxacillin, cephapirin, cephalonium, cefoperazone and ceftiofur. Beta s.t.a.r is designed for screening milk in any situation where an answer is needed with respect to contamination of milk by beta lactam antibiotics.
Principle of test	The test involves a specific beta-lactam receptor linked to gold particles An initial incubation of a specific amount of receptor with milk containing antibiotics results in a complex between the receptor and the antibiotic. Second, the solution is transferred onto an immunochromatographic medium. The first band captures receptors that have not complexed with antibiotic. The second band serves as a reference band.
Cross-reactivity	Not available at time of testing
Interference	The operator should not be under medical treatment using beta-lactam antibiotics.
Apparatus supplied	Spring syringe and 25 disposable tips.
Extra apparatus required	Dry incubator or water bath at 47°±0.5C
Reagents supplied	25 individual vials of receptor, 25 dipsticks
Extra reagents required	None
Time (set-up)	Warm-up time of dry incubator
Time per test	6 minutes (4 samples may be tested at one time)
Operator skill level	Minimal
Instructions	Detailed and unambiguous.
Safety advice	No specific hazards
Reporting method	After development, the test strips normally show two distinct bands, an upper (reference) and a lower. If the lower band is denser than the reference, the sample is negative; if the bands are similar in density then a positive with caution (+/-)result should be applied; if the lower band is less dense than the reference (+) *or* absent (++) then the sample is positive.
Ease of use	Very easy to use (Rating = 6)

Test results (antibiotic concentration in µg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	20	0	0	0	20	0
2	1.8	20	0	2	1.8	20	0
2.5	2.3	20	0	3	2.7	20	0
3	2.8	20	20 (+)	6	5.4	20	20 (+)
4	3.8	20	20 (++)	9	8.2	20	20 (+)
5	4.7	20	20 (++)	12	10.9	20	20 (++)
6	5.6	20	20 (++)				
8	7.50	20	20 (++)				

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity (µg kg^{-1})
Penicillin G	2-4
Amoxicillin	2-4
Ampicillin	2-5
Nafcillin	8-20
Oxacillin	5-10
Cloxacillin	5-10
Dicloxacillin	5-10
Cephapirin	8-16
Cephalonium	7.5-15
Cefoperazone	5-8
Ceftiofur	75-150

Additional Comments

Reacted strips can be stored as a permanent record.

DDM/NW March 12, 1999 (Final revision)

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	CharmII® Beta-lactam Test for Maximum Residue Limits
Supplier	CHARM Sciences Inc., 36 Franklin Street, Malden MA USA
UK Agent	FOSS UK Ltd, Parkway House, Station Road, Didcot Oxon OX11 7NN
Expert Technical Contact	Bob Salter, VP.Regulatory Affairs, E.mail to charm1@world.std.com
Recommended application	The Charm II beta-lactam Test detects beta-lactams at or below the maximum residue limits in raw, comingled bovine milk. It is designed for use by milk, intake, laboratory, field and regulatory personnel.
Principle of test	The test uses bacteria with specific receptor sites that bind all beta-lactam drugs. The bacteria are added to a milk sample together with a minute amount of ^{14}C labelled penicillin G. Any beta-lactam already in the milk competes with this labelled penecillin G. The amount of ^{14}C labelled penicillin G that binds to the receptor sites is measured compared to a previously determined control point. The greater the amount of ^{14}C labelled penicillin G measured, the lower the beta-lactam concentration in the sample.
Cross-reactivity	No interference (at 100ppb) from sulfadiazine, sulfanilamide, sulfathiazole, sulfamethazine, sulfapyridine, sulfadimethoxine, tetracycline, oxytetracycline, chlortetracycline, doxycycline, gentamicin, neomycin, streptomycin, ivermectin, erythromycin, novobiocin, furosemide, trichlormethiazide, chlorothiazide, oxytocin, phenylbutazone, dexamethasone, PABA and dipyrone.
Interference	Intra-family cross reactivity with cefadroxil (15ppb), cefotaxime (4ppb), cephalexin (15ppb), cephradine (15ppb), hetacillin (5ppb), piperacillin (8ppb) and ticarcillin (35ppb)
Apparatus supplied	None
Extra apparatus required	Charm scintillation counter, test tubes, caps for test tubes, cotton swabs, pipette tips (5 ml & 1 ml)
Reagents supplied	Beta-lactam tablet reagents, operators manual, MRL standard , zero control standard, standards manual, typical counts sheet
Extra reagents required	Deionised water, scintillation fluid
Time (set-up)	1 hour
Time per test	12 minutes (a control and 5 tests may be run at the same time)
Operator skill level	Moderate; training required; videotape available
Instructions	Concise and clear
Safety advice	Explicit
Reporting method	Positive or negative in relation to a pre-determined control point
Ease of use	Requires training and attention to detail (Ease of use = 23/24)

Test results (antibiotic concentration in µg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	30	0	0	0	30	1
1.0	0.9	30	6	15	13.7	30	25
2.0	1.9	30	23	20	18.2	30	29
3.0	2.8	30	30	25	22.8	30	30
4	3.8	30	30	30	27.3	30	30
6	5.6	30	30	35	31.9	30	30
8	7.5	30	30	40	36.4	30	30

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity (µg kg^{-1})
Penicillin G	2
Cefazolin	15
Ceftiofur	40
Cefquinome	20
Cephapirin	3
Amoxicillin	5
Ampicillin	4
Cloxacillin	30
Dicloxacillin	20
Oxacillin	30
Penethamate	2
Nafcillin	30

Additional Comments

The contents of the ^{14}C labelled penicillin G supplied with the Charm Test are sufficiently low that they are exempt from Nuclear Regulatory Commission regulations. Each tablet contains less than 0.15 kilobecquerels of ^{14}C labelled penicillin G. Solid waste may be disposed of without additional precautions. Liquid waste contaminated with ^{14}C labelled penicillin G can be flushed with water down the drain. However, the liquid waste contains scintillant which may be collected and disposed of with waste solvent.

DDM/NIV March 12, 1999 (Third revision)

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	Charm AIM-96™ Antimicrobial Inhibition Monitor for 96 Multiwell Plates
Supplier	CHARM Sciences Inc., 36 Franklin Street, Malden MA USA
UK Agent	FOSS UK Ltd, Parkway House, Station Road, Didcot Oxon OX11 7NN
Expert Technical Contact	Bob Salter, VP.Regulatory Affairs, E.mail to charm1@world.std.com
Recommended application	The Charm AIM-96 is a microbial inhibition assay designed for high volume, broad spectrum screening of raw, pasteurised, homogenised or skim milk. Cream may be run if pre-treated.
Principle of test	The test detects inhibition of growth of bacteria. A dye is present in the growth medium which changes colour when bacterial growth occurs. If growth is inhibited, no colour change occurs.
Cross-reactivity	Test detects a very wide range of microbial inhibitors
Interference	
Apparatus supplied	Multiwell plates, sealing strip, plate lid
Extra apparatus required	AIM-96 Incubator, automatic pipette (50 l),Repitter or octapette with reservoir; reference colour strip
Reagents supplied	Lyophilised medium, B.stearothermophilus spore tablets
Extra reagents required	Deionised water, antimicrobial drug free milk
Time (set-up)	30 minutes
Time per test	3-4 hours, 90 test samples plus 3 negative and three positive controls may be conveniently run at the same time
Operator skill level	Moderate; some skill in pipetting required
Instructions	Concise and clear
Safety advice	No specific hazard
Reporting method	Positive or negative based on reference to colour chart
Ease of use	Requires minimal training (Ease of use = 10)

Test results (antibiotic concentration in μg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	30	0	0	0	30	0
1.0	0.9	30	0	20	18	30	9
2.0	1.9	30	8	25	23	30	14
3.0	2.8	30	29	30	27	30	19
4	3.8	30	30	35	32	30	30
6	5.6	30	30	40	36	30	30
8	7.5	30	30	50	45	30	30

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

(Antimicrobial drugs listed are typical of their respective families)

Antibiotic	Sensitivity* (μg kg^{-1})
Penicillin G	3-5
Sulfamethazine	10-50
Gentamicin	30-100
Oxytetracycline	150-300
Tylosin	40-60

- First level = colour 4, Second level = colour 5

Additional Comments

None

DDM/NW March 12, 1999 (Third revision)

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	Charm MRL™ Beta LactamTest
Supplier	CHARM Sciences Inc., 36 Franklin Street, Malden MA USA
UK Agent	FOSS UK Ltd, Parkway House, Station Road, Didcot Oxon OX11 7NN
Expert Technical Contact	Bob Salter, VP.Regulatory Affairs, E.mail to charm1@world.std.com
Recommended application	The Charm MRL™ Test is a rapid receptor assay designed for detection of beta lactam drugs in raw, commingled bovine milk at or near the European Union maximum residue limit. The test is designed for use by milk intake, laboratory, field and regulatory personnel.
Principle of test	The test uses receptors that bind to beta lactam drugs. As the milk flows through the test strip a line forms the test position when no beta lactam is present in the milk. When beta lactams are present in the sample this line is less dense or absent. The test line is compared to a control line designed to discriminate beta lactams close to the maximum residue limit. The control and test lines were compared using a CHARM LUM-T with imager.
Cross-reactivity	The following drugs at 100ppb show no interference: sulfadiazine, sulfanilamide, sulfathiazole, sulfamethazine, sulfapyridine, sulfadimethoxine, tetracycline, oxytetracycline, chlortetracycline, doxycycline, gentamicin, neomycin, streptomycin, ivermectin, erythromycin, novobiocin, furosemide, trichlomethiazide, chlorothiazide, oxytocin, phenylbutazone, dexamethasone, PABA and dipyrone.
Interference	Other beta-lactam drugs are detected.
Apparatus supplied	None
Extra apparatus required	Strip incubator, 300 Imocropipette and disposable tips; optional Imager
Reagents supplied	MRL Beta lactam Test Strips, operators manual; 4ppb Penicillin G standard
Extra reagents required	Antimicrobial drug free milk
Time (set-up)	20 minutes to allow heater block to equilibrate
Time per test	<10 minutes, 10 test samples plus negative and positive controls may be conveniently run at the same time if 3 x 4 place incubators are used.
Operator skill level	Basic; some skill in pipetting required
Instructions	Concise and clear
Safety advice	No unusual hazard.
Reporting method	Positive or negative reported by strip reader
Ease of use	Requires minimal training (Ease of use = 5)

Test results (antibiotic concentration in µg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	30	0	0	0	30	0
1.0	0.9	30	12	10	9.1	30	6
2.0	1.9	30	23	15	13.7	30	10
3.0	2.8	30	29	20	18.2	30	20
4	3.8	30	30	25	22.8	30	23
6	5.6	30	30	30	27.3	30	30
8	7.5	30	30	35	31.9	30	30
				40	36.4	30	30
				50	45.5	30	30

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity (µg kg^{-1})*
Penicillin G	3
Amoxicillin	4
Ampicillin	4
Ceftoifur	100
Cephapirin	10
Cloxacillin	30
Oxacillin	50

*100% positive using Imager

Additional Comments

None

DDM/NW March 24, 1999 (Final revision)

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	Charm Farm Test-Vial for Beta lactams, sulfonamides and other antimicrobial drugs
Supplier	CHARM Sciences Inc., 36 Franklin Street, Malden MA USA
UK Agent	FOSS UK Ltd, Parkway House, Station Road, Didcot Oxon OX11 7NN
Expert Technical Contact	Bob Salter, VP.Regulatory Affairs, E.mail to charm1@world.std.com
Recommended application	The Charm Farm Test-Vial is a microbial inhibition assay designed for detection of beta lactam drugs in raw, commingled milk at or near the European Union maximum residue limit.
Principle of test	The test detects inhibition of growth of bacteria. A dye is present in the growth medium which changes colour when bacterial growth occurs. If growth is inhibited, no colour change occurs.
Cross-reactivity	Test detects a very wide range of microbial inhibitors. The following drugs at 100ppb show no interference: ivermectin, novobiocin, furosemide, trichlormethiazide, chlorothiazide, oxytocin, phenylbutazone, dexamethasone and dipyrone
Interference	Lacteal secretions from mastitic animals have been reported to contain natural inhibitors that cause positives. No interference from somatic cell (10^6 SCC) or bacteria ($3x10^5$ cfu/ml)
Apparatus supplied	None
Extra apparatus required	Incitronic incubator or mini incubator or water bath or Delvo incubator, water bath rack, reference colour strip and timer
Reagents supplied	B.stearothermophilus spore tablets, Charm-VN test vials, 200 l micropipette and disposable tips; 4ppb penicillin G standard
Extra reagents required	Antimicrobial drug free milk
Time (set-up)	20 minutes to allow heater block to equilibrate
Time per test	<3½ hours, 150 test samples plus negative and positive controls may be conveniently run at the same time
Operator skill level	Basic; some skill in pipetting required
Instructions	Concise and clear
Safety advice	No unusual hazard.
Reporting method	Positive or negative based on reference to colour chart
Ease of use	Requires minimal training (Ease of use = 6)

Test results (antibiotic concentration in μg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	30	0	0	0	30	0
1.0	0.9	30	0	10	9.1	30	1
2.0	1.9	30	2	15	13.7	30	2
3.0	2.8	30	30	20	18.2	30	2
4	3.8	30	30	25	22.8	30	3
6	5.6	30	30	30	27.3	30	16
8	7.5	30	30	35	31.9	30	28
				40	36.4	30	30
				50	45.5	30	30

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity (μg kg^{-1})
Penicillin G	3-4
Amoxicillin	4
Ampicillin	4
Ceftoifur	50
Cephapirin	10
Sulfamethazine	50-100
Gentamicin	100-300
Oxytetracycline	100-150
Tylosin	40-50

Additional Comments

None

DDM/NW March 24, 1999 (Final revision)

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	**Charm Farm Test Mini-Vial for Beta lactams, sulfonamides and other antimicrobial drugs**
Supplier	CHARM Sciences Inc., 36 Franklin Street, Malden MA USA
UK Agent	FOSS UK Ltd, Parkway House, Station Road, Didcot Oxon OX11 7NN
Expert Technical Contact	Bob Salter, VP.Regulatory Affairs, E.mail to charm1@world.std.com
Recommended application	The Charm Farm Test Mini-Vial is a microbial inhibition assay designed for detection of beta lactam drugs in raw, commingled milk at or near the European Union maximum residue limit.
Principle of test	The test detects inhibition of growth of bacteria. A dye is present in the growth medium which changes colour when bacterial growth occurs. If growth is inhibited, no colour change occurs.
Cross-reactivity	Test detects a very wide range of microbial inhibitors. The following drugs at 100ppb show no interference: ivermectin, novobiocin, furosemide, trichlormethiazide, chlorothiazide, oxytocin, phenylbutazone, dexamethasone and dipyrone
Interference	Lacteal secretions from mastitic animals have been reported to contain natural inhibitors that cause positives. No interference from somatic cell (10^6 SCC) or bacteria ($3x10^5$ cfu/ml)
Apparatus supplied	None
Extra apparatus required	Air incubator or water bath or Delvo incubator with mini-vial adaptors, water bath rack, tweezers, 0.1 ml micropipette and disposable tips, reference colour strip and timer
Reagents supplied	Media tablets, Charm-MV test vials (96 per pack), zero control milk powder, 4ppb penicillin G standard, 12x8 well strip sealing tape
Extra reagents required	None
Time (set-up)	20 minutes to allow heater block or incubator to equilibrate
Time per test	< 3 hours, 72 test samples plus negative and positive controls may be conveniently run at the same time
Operator skill level	Basic; some skill in pipetting required
Instructions	Concise and clear
Safety advice	No unusual hazard.
Reporting method	Positive or negative based on reference to colour chart
Ease of use	Requires minimal training (Ease of use = 7)

Test results (antibiotic concentration in µg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	30	0	0	0	30	0
1.0	0.9	30	0	10	9.1	30	2
2.0	1.9	30	6	15	13.7	30	4
3.0	2.8	30	30	20	18.2	30	4
4	3.8	30	30	25	22.8	30	27
6	5.6	30	30	30	27.3	30	30
8	7.5	30	30	35	31.9	30	30
				40	36.4	30	30
				50	45.5	30	30

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity (µg kg^{-1})
Penicillin G	3-4
Amoxicillin	4
Ampicillin	4
Ceftiofur	50
Cephapirin	10
Sulfamethazine	50-100
Gentamicin	300-500
Oxytetracycline	100-150
Tylosin	40-50

Additional Comments

None

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	Delvotest® MCS
Supplier	Gist-Brocades B.V. PO Box 1, 2600 MA Delft, The Netherlands
UK Agent	Dairy Ingredients Group - UK, Brent House, Brent, Tame Valley Industrial Estate, Tamworth Staffs. B77 5DF
Expert Technical Contact	Jan H.P.M. Kerkhof (email jan.kerkhof@gist-brocades.infonet.com)
Recommended application	Diffusion test for detection of antibiotic and sulphonamide residues in raw commingled bovine milk.
Principle of test	The test detects inhibition of growth of bacteria. A dye is present in the growth medium which changes colour when bacterial growth occurs. If growth is inhibited, no colour change occurs.
Cross-reactivity	Test detects a wide range of microbial inhibitors
Interference	Caution should be exercised to avoid contamination by antibiotics or other drugs, cleaning agents or disinfectants.
Apparatus supplied	Adhesive covers for plates
Extra apparatus required	Water bath ; syringe (0.1 ml) with disposable tips
Reagents supplied	96 well plates containing detection media
Extra reagents required	Negative control milk
Time (set-up)	5 minutes
Time per test	<2½ hours, 96 samples including negative controls may be conveniently run at the same time
Operator skill level	Easy; some skill in pipetting required
Instructions	Concise and clear
Safety advice	No known hazards.
Reporting method	Positive or negative based on reference to colour chart. Samples with any trace of yellow colour were deemed negative i.e., only samples with entirely purple colour were positive
Ease of use	Requires minimal training (Ease of use = 5)

Test results (antibiotic concentration in $\mu g\ kg^{-1}$)

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	30	0	0	0	30	0
1.0	0.9	30	0	10	9.1	30	0
2.0	1.9	30	30	15	13.6	30	30
3.0	2.8	30	30	20	18.2	30	30
4	3.8	30	30	30	27.2	30	30
				50	45.4	30	30

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity* ($\mu g\ kg^{-1}$)
Penicillin G	2-4
Sulphadiazine	25-100

Additional Comments

None

DDM/NIV March 29, 1999 (Final revision)

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	Delvotest® P Mini
Supplier	Gist-Brocades B.V. PO Box 1, 2600 MA Delft, The Netherlands
UK Agent	Dairy Ingredients Group - UK, Brent House, Brent, Tame Valley Industrial Estate, Tamworth Staffs. B77 5DF
Expert Technical Contact	Jan H.P.M. Kerkhof (email jan.kerkhof@gist-brocades.infonet.com)
Recommended application	Diffusion test for detection of antibiotic residues in raw bovine milk. (From individual animals and commingled milk.)
Principle of test	The test detects inhibition of growth of bacteria. A dye is present in the growth medium which changes colour when bacterial growth occurs. If growth is inhibited, no colour change occurs.
Cross-reactivity	Test detects a wide range of microbial inhibitors
Interference	Caution should be exercised to avoid contamination by antibiotics or other drugs, cleaning agents or disinfectants. Samples that have soured should not be tested.
Apparatus supplied	Tweezers; syringe (0.1 ml)with disposable tips
Extra apparatus required	Delvo Incubator
Reagents supplied	Ampoules with B.stearothermophilus var. calidolactis in a solid agar medium; nutrient tablets.
Extra reagents required	Optional: Penicillinase
Time (set-up)	10 minutes
Time per test	<2½ hours, 40 test samples may be conveniently run at the same time
Operator skill level	Easy; some skill in pipetting required
Instructions	Clear
Safety advice	No known hazards.
Reporting method	Positive or negative based on reference to colour chart. If any trace of yellow colour was detected, the sample was reported as negative i.e., completely purple.
Ease of use	Requires minimal training (Ease of use = 5)

Test results (antibiotic concentration in µg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	30	0	0	0	30	0
1.0	0.9	30	0	20	18.2	30	0
2.0	1.9	30	0	30	27.2	30	0
3.0	2.8	30	30	40	36.3	30	30
4	3.8	30	30	50	45.4	30	30
5	4.5	30	30	70	63.6	30	30

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Minimum detection levels of DELVOTEST® P (ppb)

Antibiotic	Control time	2½ hours
Penicillin G	2.5	3
Cloxacillin	15-25	25
Dicloxacillin	15	20
Oxacillin	10	10-15
Nafcillin	5-10	10
Ampicillin	3-4	5
Amoxicillin	4	5-6
Cephapirin	5	8-10
Cefalonium	10-15	15-20
Cefalexin	40-60	60-100
Cefacetril	20-30	30-40
Cefaperazon	40-60	80
Tetracycline	50-100	200-300
Oxytetracycline	50-200	300
Chlotetracycline	50-100	250-300
Tylosin	40-150	100-300
Erythromicin	300-1000	500-1500
Lincomycin	100-300	400-1000
Spiramycin	1000-5000	5000+
Gentamycin	200-800	1000-4000
Neomycin	300-1000	1000-5000
Dihyrostreptomycin	2000-5000	10000+
Kanamycin	7500+	10000+
Chloramphenicol	2500-5000	5000-7500
Sulfamethazine	10000+	10000+
Sulfadimethoxine	10000+	10000+
Sulfathiazole	10000+	10000+
Sulfadiazine	10000+	10000+
Dapsone	10000+	10000+
Trimethoprim	200-500	5000-15000

* Control time = time at which blank control has just changed to yellow; based on yellow/purple colour

Additional Comments

None

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	Delvotest® SP Mini
Supplier	Gist-Brocades B.V. PO Box 1, 2600 MA Delft, The Netherlands
UK Agent	Dairy Ingredients Group - UK, Brent House, Brent, Tame Valley Industrial Estate, Tamworth Staffs. B77 5DF
Expert Technical Contact	Jan H.P.M. Kerkhof (email jan.kerkhof@gist-brocades.infonet.com)
Recommended application	Diffusion test for detection of antibiotic residues and sulphonamides in raw bovine milk. (From individual animals and commingled.)
Principle of test	The test detects inhibition of growth of bacteria. A dye is present in the growth medium which changes colour when bacterial growth occurs. If growth is inhibited, no colour change occurs.
Cross-reactivity	Test detects a wide range of microbial inhibitors
Interference	Caution should be exercised to avoid contamination by antibiotics or other drugs, cleaning agents or disinfectants. Samples that have soured should not be tested.
Apparatus supplied	Tweezers; syringe (0.1 ml)with disposable tips
Extra apparatus required	Delvo Incubator
Reagents supplied	Ampoules with B.stearothermophilus var. calidolactis in a solid agar medium; nutrient tablets.
Extra reagents required	None
Time (set-up)	10 minutes
Time per test	<3 hours, 40 test samples may be conveniently run at the same time
Operator skill level	Easy; some skill in pipetting required
Instructions	Clear
Safety advice	No known hazards.
Reporting method	Positive or negative based on reference to colour chart. Samples containing any yellow colour were deemed negative i.e., samples which were completely purple were positive
Ease of use	Requires minimal training (Ease of use = 5)

Test results (antibiotic concentration in µg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	30	0	0	0	30	0
1.0	0.9	30	0	10	9.1	30	0
2.0	1.9	30	0	15	13.6	30	0
3.0	2.8	30	30	20	18.2	30	30
4	3.8	30	30	30	27.2	30	30
				50	45.4	30	30

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Minimum detection levels of DELVOTEST® SP (ppb)

Antibiotic	Control time	2¾ hours	3 hours
Penicillin G	2	2.5	2.5
Cloxacillin	15	15-25	20-25
Dicloxacillin	10	10-15	10-15
Oxacillin	5	10	10
Nafcillin	5	5-8	10
Ampicillin	2-3	3-4	3-5
Amoxicillin	2	3-4	3-5
Cephapirin	5	5-8	5-10
Cefalonium	5-10	10-20	15-25
Cefalexin	40-60	60-100	60-100
Cefacetril	20	20-40	20-40
Cefaperazon	40	60-100	60-100
Tetracycline	100	200-400	300-600
Oxytetracycline	100	200-400	40-50
Chlotetracycline	100-150	200-400	300-600
Tylosin	10-20	30-50	100
Erythromicin	50	100-150	250
Lincomycin	100	200	300-400
Spiramycin	200	350-750	10000+
Gentamycin	100-300	200-400	400-500
Neomycin	100-200	300-1000	400-2000
Dihyrostreptomycin	300-500	1500-3000	2500-10000
Kanamycin	2500	7500	10000+
Chloramphenicol	2500	7500	75000-10000
Sulfamethazine	25	50-100	100-200
Sulfadimethoxine	50	50-100	100
Sulfathiazole	50	50-100	100-150
Sulfadiazine	50	50-100	100
Dapsone	1	1-4	4-8
Trimethoprim	50	100-300	500

* Control time = time at which blank control has just changed to yellow

Additional Comments

None

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	Delvo-X-PRESS® L-II test for beta lactam residues
Supplier	Gist-Brocades B.V. PO Box 1, 2600 MA Delft, The Netherlands
UK Agent	Dairy Ingredients Group - UK, Brent House, Brent, Tame Valley Industrial Estate, Tamworth Staffs. B77 5DF
Expert Technical Contact	Jan H.P.M. Kerkhof (email jan.kerkhof@gist-brocades.infonet.com)
Recommended application	Rapid test for detection of beta lactam residues in commingled bovine milk. Milk samples can be frozen but should not be thawed more than twice.
Principle of test	The test is a qualitative competitive receptor-enzyme assay .
Cross-reactivity	With other beta-lactams
Interference	Caution should be exercised to avoid contamination by antibiotics.
Apparatus supplied	None
Extra apparatus required	ISR(P) workstation; micropipette (0.2 ml) plus disposable tips; tube racks; 250 ml polythene wash bottle; 5 ml polythene measuring beaker; 1 ml multipette plus disposable tips
Reagents supplied	Reagent tubes, Tracer, Standard and diluent, Colour developer, wash solution.
Extra reagents required	Deionised or distilled water
Time (set-up)	30 minutes
Time per test	8 minutes (5 samples may be tested simultaneously)
Operator skill level	Modest, training required by a Gist-Brocades representative.
Instructions	Clear and concise
Safety advice	Detailed.
Reporting method	Positive or negative based instrument reading. samples which are negative have reader units < 00.
Ease of use	Requires some training (Ease of use = 10)

Test results (antibiotic concentration in μg kg^{-1})

Note: these results are provisional and should be regarded as indicative only

Pen G*	Pen G**	No. trials	No. positive
0	0	50	2
3.0	2.8	20	13
4.0	3.8	20	18
5.0	4.5	20	19
6.0	5.4	20	20

* Nominal concentration, * Concentration as free base.

Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity* (μg kg^{-1})
Penicillin G	2-4
Ceftiofur	4-8
Cephapirin	4-8
Ampicillin	4-8
Amoxicillin	4-8
Cloxacillin	30-40

Additional Comments

Workstation displays instructions for each stage of test sequence.

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	LacTek™ Penicillins Kit
Supplier	IDEXX Laboratories Inc., One IDEXX Drive, Westbrook, ME 04092, USA
UK Agent	Guildhay Ltd., 6 Riverside Business Centre, Walnut tree Close, Guildford, Surrey GU1 4UG
Expert Technical Contact	Kalpna Raval (Kalpna.Raval @guildhay.co.uk)
Recommended application	Qualitative test for rapid detection of penicillins in raw (individual and comingled) and pasteurised milk.
Principle of test	The LacTek test is an enzyme-linked immunoabsorbant assay. Beta-lactams in milk compete with an enzyme tracer for antibody binding sites coated on an analysis tube. The presence of penicillins in the milk reduces the amount of tracer bound. When a reagent for colour development is introduced, the ultimate intensity of colour development is less than for a control sample. The colour measurement is carried out in a spectrophotometer.
Cross-reactivity	The test cross reacts with amoxicillin, ampicillin , cloxacillin. And dicloxacillin
Interference	No known interference
Apparatus supplied	None
Extra apparatus required	Timer, shaker, Reader/printer, micropipette (250 l) and disposable tips, dispensers (250 & 500 l),JetWasher.
Reagents supplied	Reaction tubes, Beta-lactam standard, diluent for standard, Tracer solution, diluent for tracer, colour developer, stop solution, wash concentrate, reconstitution labels, product insert.
Extra reagents required	De-ionised, distilled or RO water
Time (set-up)	Preparation of reagents , equilibration of test kit to room temperature.
Time per test	10 minutes (5 samples may be tested at a time; time per test 2 minutes)
Operator skill level	Training is required by a LacTek representative
Instructions	Detailed and unambiguous.
Safety advice	No special precautions are required.
Reporting method	Reader/printer automatically displays and records results as positive or negative
Ease of use	Easy to use (rating = 11)

Test results (antibiotic concentration in μg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive	Clox*	Clox**	No. trials	No. positive
0	0	20	0	0	0	20	0
2.0	1.9	20	0	5.0	4.5	20	1
2.5	2.3	20	1	7.5	6.8	20	20
3.0	2.8	20	6	10.0	9.1	20	20
3.5	3.3	20	20	12.5	11.4	20	20
5.0	4.7	20	20	15.0	13.6	20	20

* Nominal concentration; ** Corrected to free base

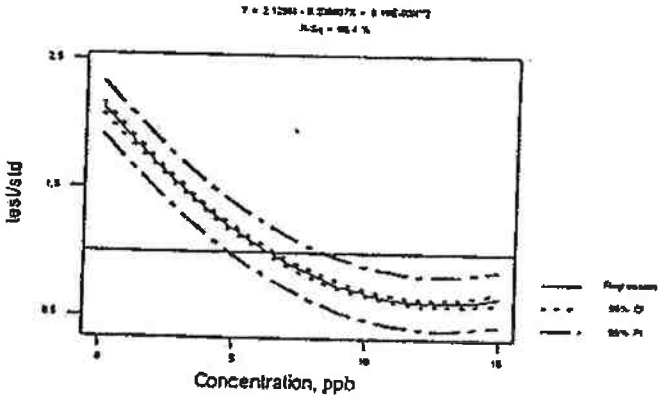
Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity (μg kg^{-1})
Penicillin G	At or below 4.8
Cloxacillin	90/95% sensitivity at 6.25

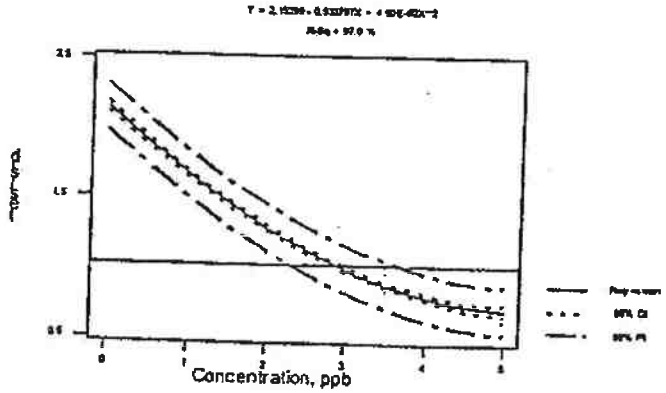
Additional Comments

Provision of dispensers facilitates liquid transfers and washing station is a helpful piece of ancillary equipment. In addition to the printout of the results, optical density readings are also provided which may be used to determine the sensitivity of the test. Test curves are shown below:

Lactek - response curve for cloxacillin



LACTEK - response curve for penicillin G



Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	Penzym® 100
Supplier	UCB Bioproducts, Braine-L'Alleud, Belgium
UK Agent	Axient, Axient Laboratories, Unit 20, Goldthorpe, Rotherham S63 9BL
Expert Technical Contact	Jacques Degelaen (jacques.degelaen@ucb-group.com)
Recommended application	Qualitative test for betam-lactam antibiotics, including penicillin G, ampicillin, amoxicillin, cloxacillin, cephapirin, oxacillin, cephalexin, cephuroxime, cephalonium, cephalosporin C, cephalothin, cephaloridine, cephadroxyl and ceftiofur. Penzym 100 is designed for isloated or occasional use with milk e.g. on the milk tanker.
Principle of test	The Penzym test employs an enzyme called DD-carboxypeptidase. DD-carboxypeptidase specifically hydrolyses substrates of the R-D-alanine-D-alanine type with liberation of D-alanine. The alanine is detected by a coupled enzyme reaction that changes a dye from yellow to pink orange. Beta-lactam antibiotics specifically and quantitatively inhibit the enzyme. When they are present in milk the colour change does not occur.
Cross-reactivity	The following drugs do not cross react at levels of 100ppb: sulfadiazine, sulfanilamide, sulfathiazole, sulfamethazine, sulfapyridine, sulfadimethoxine, tetracycline, oxytetracycline, chloretetracycline, doxycycline, gentamicin, neomycin, streptomycin, ivermectin, erythromycin, novobiocin, furosemide, trichlomethiazide, chlorothiazide, oxytocin, phenylbutazone, dexamethasone, dipyrone and dicloxacillin
Interference	Operator should not be under medical treatment by beta lactam antibiotics
Apparatus supplied	Tweezers, colour chart.
Extra apparatus required	Dry incubator or water bath at 47°C, syringe and needle, micropipettes (10 & 50 µl), Eppendorf type vials (1.5 ml)
Reagents supplied	Enzyme vials, colour generating tablets
Extra reagents required	De-ionised water
Time (set-up)	Warm-up time of dry incubator or water bath
Time per test	Negative in 13 minutes, < 20 minutes for positive. (8 samples may be tested simultaneously.)
Operator skill level	Minimal
Instructions	Detailed and unambiguous.
Safety advice	No specific hazards
Reporting method	In 3 categories according to reference colour chart: yellow = positive,; between peach and peach yellow = positive , peach = caution, pink orange = negative
Ease of use	Relatively easy to use (rating = 10)

Test results (antibiotic concentration in µg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive (caution)	Clox*	Clox**	No. trials	No. positive
0	0	20	0	0	0	20	0
2	1.9	20	0	40	36	20	(20)
3	2.8	20	(20)	60	54	20	(20)
4	3.7	20	(20)	80	73	20	20
5	4.7	20	(20)	100	91	20	20
6	5.6	20	20				
8	7.5	20	20				

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity (µg kg^{-1})
Penicillin G	4-6
Amoxicillin	4-6
Ampicillin	4-7
Cephapirin	5-7
Oxacillin	30-50
Cloxacillin	60-100
Cephalexin	20-40
Cephuroxime	50-100
Cephalonium	10-15
Cephalosporin C	8-10
Cephalothin	4-6
Cephaloridine	6-8
Cephadroxyl	25-50
Ceftiofur	40-70

Additional Comments

None

Dairy Industry Federation Survey - Report on Antibiotic Test Kit

Name:	Penzym® 20
Supplier	UCB Bioproducts, Braine-L'Alleud, Belgium
UK Agent	Axient, Axient Laboratories, Unit 20, Goldthorpe, Rotherham S63 9BL
Expert Technical Contact	Jacques Degelaen (jacques.degelaen@ucb-group.com)
Recommended application	Qualitative test for betam-lactam antibiotics, including penicillin G, ampicillin, amoxicillin, cloxacillin, cephapirin, oxacillin, cephalexin, cephuroxime, cephalonium, cephalosporin C, cephalothin, cephaloridine, cephadroxyl and ceftiofur. Penzym 20 is designed for isloated or occasional use with milk e.g. on the milk tanker.
Principle of test	The Penzym test employs an enzyme called DD-carboxypeptidase. DD-carboxypeptidase specifically hydrolyses substrates of the R-D-alanine-D-alanine type with liberation of D-alanine. The alanine is detected by a coupled enzyme reaction that changes a dye from yellow to pink orange. Beta-lactam antibiotics specifically and quantitatively inhibit the enzyme. When they are present in milk the colour change does not occur.
Cross-reactivity	The following drugs do not cross react at levels of 100ppb: sulfadiazine, sulfanilamide, sulfathiazole, sulfamethazine, sulfapyridine, sulfadimethoxine, tetracycline, oxytetracycline, chloretetracycline, doxycycline, gentamicin, neomycin, streptomycin, ivermectin, erythromycin, novobiocin, furosemide, trichlomethiazide, chlorothiazide, oxytocin, phenylbutazone, dexamethasone, dipyrone and dicloxacillin
Interference	Operator should not be under medical treatment by beta lactam antibiotics
Apparatus supplied	Tweezers, syringe and 20 tips, colour chart.
Extra apparatus required	Dry incubator or water bath at 47°C
Reagents supplied	Enzyme vials, colour generating tablets
Extra reagents required	None
Time (set-up)	Warm-up time of dry incubator or water bath
Time per test	Negative in 13 minutes, < 20 minutes for positive. (8 samples may be tested simultaneously.)
Operator skill level	Minimal
Instructions	Detailed and unambiguous.
Safety advice	No specific hazards
Reporting method	In 3 categories according to reference colour chart: yellow = positive,; between peach and peach yellow = positive , peach = caution, pink orange = negative
Ease of use	Very easy to use (rating = 6)

Test results (antibiotic concentration in µg kg^{-1})

Pen G*	Pen G**	No. trials	No. positive (caution)	Clox*	Clox**	No. trials	No. positive
0	0	20	0	0	0	20	0
2	1.9	20	0	40	36	20	(20)
3	2.8	20	(20)	60	54	20	(20)
4	3.7	20	(20)	80	73	20	20
5	4.7	20	20	100	91	20	20
6	5.6	20	20				
8	7.5	20	20				

* Nominal concentration; ** Corrected to free base

Manufacturer's Sensitivity Declaration

Antibiotic	Sensitivity (µg kg^{-1})
Penicillin G	4-6
Amoxicillin	4-6
Ampicillin	4-7
Cephapirin	5-7
Oxacillin	30-50
Cloxacillin	60-100
Cephalexin	20-40
Cephuroxime	50-100
Cephalonium	10-15
Cephalosporin C	8-10
Cephalothin	4-6
Cephaloridine	6-8
Cephadroxyl	25-50
Ceftiofur	40-70

Additional Comments

None