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5100 Paint Branch Parkway
College Park, MD 20740-3835

M-a-85 (Revision #14)

March 22, 2012

TO: All Regional Food and Drug Directors
Attn: Regional Milk Specialists

FROM: Dairy and Egg Branch (HFS-316)

SUBJECT: Beta Lactam And Other Test Methods For Use Under Appendix N And Section 6 Of The *Grade "A" Pasteurized Milk Ordinance* (PMO)

This coded memorandum replaces and rescinds the previous revision of this coded memorandum (M-a-85 (Revision #13), Issued January 4, 2010).

This revision addresses the following changes**:**

- The acceptance of the Neogen Corporation BetaStar® Plus Beta Lactam Test and concurrent removal of the Neogen BetaStar® US Beta Lactam Test (M-I-11-3, Issued June 3, 2011);
- The acceptance of the Charm Sciences, Inc. Charm® 3 SL3 Beta Lactam Test and the removal of the Charm Sciences, Inc. Charm® SL3 Beta Lactam Test as Charm Sciences is no longer manufacturing the test kit and all lots have reached their expiration date (M-I-10-8, Issued November 4, 2010); and
- The acceptance of the Charm Sciences, Inc. Charm® FLUSLBL Flunixin and Beta Lactam Test (M-I-12-3, Issued February 3, 2102).

The individual Test Tables presented in this revision provide data points that were derived from testing at least thirty (30) samples at each concentration for each drug detected.

The attached information is summarized from the evaluation of data submitted by test sponsors. Information related to the protocol used in this evaluation is available from Dr. David White, FDA's Center for Veterinary Medicine (CVM), (301) 210-4760. Additional information regarding the performance of these Tests may be available from the test kit manufacturers.

Label claims for these new approved Tests were evaluated for use on raw, commingled bovine milk samples. The evaluation protocol did not measure the performance of these Tests in the assay of drug residues in other milk matrices, i.e., pasteurized milk or milk

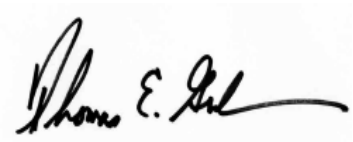
taken from individual cows, although claims for such use are made by some of the manufacturers of these Tests.

> **NOTE:** FDA recognizes that six (6) Beta lactams are widely used in treating disease in lactating dairy cattle and are the most likely to cause a residue in milk if misused. These are penicillin, ceftiofur, cloxacillin, cephapirin, amoxicillin, and ampicillin. While it is preferred that monitoring for Beta lactams include all of these drugs, at this time, the Agency is recommending that methods be utilized that have been shown to detect at least four (4) of the six (6) Beta lactams identified above.
>
> Testing for drug residue(s) in compliance with the provisions of Sections 6 and 7 of the PMO may be accomplished by the use of any accepted Appendix N Test for raw milk or an accepted Section 6 Test for raw and pasteurized milk.

The NCIMS Executive Board has agreed that future updates to M-a-85 that add, delete or revise these Tests will not require a public comment period or follow the protocol established in the Procedures document for the issuance of M-a's.

Copies of this coded memorandum are enclosed for distribution to Regional Milk Specialists, State Milk Regulatory Agencies, State Laboratory Evaluation Officers and State Milk Sanitation Rating Officers in your region. This memorandum should be widely distributed to representatives of the dairy industry, State Veterinarians, State Veterinary and Pharmacy Boards, Veterinarian Professional Organizations and other interested parties and also will be available on the FDA Web site at http:// www.fda.gov at a later date.

If you would like an electronic version of this document prior to it being available on the FDA Web Site, please e-mail your request to Robert.Hennes@.fda.hhs.gov.



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ATTACHMENT TO M-a-85 (REVISION #14)

MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS[1]
Beta lactams

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE OR SAFE LEVEL	10 ppb	10 ppb	100 ppb[2]	20 ppb	10 ppb	5 ppb
SCREENING TEST						
BETASTAR® PLUS BETA LACTAM TEST	5.5	5.2	80	19.0	8.2	4.7
CHARM® *B. stearothermophilus* TABLET DISK ASSAY[4, 5, 6]	7.5	6.7	ND[3]	11.7	50[7]	3.8
CHARM® II TABLET BETA LACTAM TEST (COMPETITIVE ASSAY)[4]	7.5	5.7	47	4.2	70[7]	3.0
CHARM® II TABLET BETA LACTAM TEST (SEQUENTIAL ASSAY)[6]	8.1	6.6	58	4.1	50[7]	3.4
CHARM® II TABLET BETA LACTAM TEST (QUANTITATIVE ASSAY)[8]	8.1	6.6	58	4.1	8.5	3.4
CHARM® II TEST FOR CLOXACILLIN IN MILK (COMPETITIVE ASSAY)[4, 9]	ND[3]	ND[3]	ND[3]	ND[3]	8.5	ND[3]
CHARM® SL BETA LACTAM TEST[10,11,12]	5.6	8.5	77	13.7	50[7]	3.6
CHARM® SL6 BETA LACTAM TEST	7.1	9.6	72	18.7	8.3	4.2
CHARM® 3 SL3 BETA LACTAM TEST	8.4	8.0	79	20.0	8.6	3.8
CHARM® FLUSLBL FLUNIXIN AND BETA LACTAM TEST[13]	5.9	6.8	63	13.4	NA[14]	2.0
DELVOTEST P 5 PACK (READER)[4, 15]	4.6	4.0	ND[3]	8.2	NA[14]	2.1
DELVOTEST P 5 PACK (VISUAL)[4, 5, 16]	4.6	4.0	ND[3]	8.2	NA[14]	2.1
DELVOTEST P/DELVOTEST P MINI[5, 6, 11]	7.7	5.1	NA[14]	7.0	30[7]	3.1
DELVOTEST SP/DELVOTEST SP MINI[11]	6.0	7.9	NA[14]	7.7	33[7]	2.7
NEW SNAP® BETA LACTAM TEST KIT[17]	7.3	5.8	12	11.7	50[7]	3.0

Continued on next page

CONTINUED: MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS[1]
Beta lactams

FOOTNOTES:

1. Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each Test in the following Tables and should be considered when selecting drug residue monitoring tests. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory.
2. The ceftiofur tolerance is based on measuring the sum of ceftiofur and desfuroylceftiofur related metabolites in milk as desfuroylceftiofur. The screening test detection concentrations for ceftiofur were evaluated using milk containing ceftiofur and desfuroylceftiofur related metabolites from treated animals. Due to the approval of "Spectramast", an intramammary ceftiofur product, the safe level of 50 ppb as parent ceftiofur is no longer used.
3. ND indicates "Not Detected" at or below tolerance.
4. This Test is acceptable for use to detect Beta lactam residues when used with bovine pasteurized whole and skim milk.
5. Refer to M-I-01-4, Issued July 2, 2001, for certification requirements to use this visual test.
6. This Test is acceptable for testing raw, commingled goat milk.
7. 90/95% concentrations were not determined for sensitivities significantly above the tolerance/safe level.
8. Test sensitivity when presumptive positive milk samples are verified in accordance with label directions using the Charm® II Tablet Beta Lactam Test (Sequential Assay) and the Charm® II Test for Cloxacillin in Milk (Competitive Assay).
9. For Appendix N bulk milk tanker screening, this Test must be used in combination with other approved screening methods in order to detect at least four (4) of the six (6) targeted Beta lactam drugs.
10. The Charm® SL Beta Lactam Test is acceptable for testing raw, commingled goat milk (M-I-03-3, Issued 2/25/2003).
11. The Charm® SL Beta Lactam Test, Delvotest P/Delvotest P Mini and Delvotest SP/Delvotest SP Mini are acceptable for testing raw, commingled water buffalo milk (M-I-09-6, Issued October 16, 2009).
12. The Charm® SL Beta Lactam Test is acceptable for testing raw, commingled sheep milk (M-I-09-7, Issued 11/3/2009).
13. The Charm® FLUSLBL Flunixin and Beta Lactam Test is a multi-class Test. The information listed here is only for the performance of the test kit in detecting Beta lactam drug residues. For information on flunixin, refer to MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS NSAIDs on page 15.
14. NA indicates "Data Not Available".
15. The DelvoScan Reader option for the Delvotest 5 P Pack has not been validated in fat-free chocolate, whole chocolate, half & half, heavy cream and pasteurized goat milk.
16. The Delvotest 5 P Pack (VISUAL) is acceptable to detect ampicillin, amoxicillin, cephapirin and penicillin residues in bovine fat-free chocolate, whole chocolate, half & half, heavy cream and pasteurized goat milk.
17. The visual reading option is not available with the New Snap® Beta Lactam Test.

BETASTAR® PLUS BETA LACTAM TEST
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**10**	**5**
DRUG CONCENTRATION (ppb)						
1						0
2	0	0			0	0
3						0
4	0	0		0	0	0
5	27	10				100
6	100	100			0	
8	100	100		0	83	
10	100	100			100	
12				0		
15				63		
20			0	100		
40			0			
60			20			
80			90			
100			100			

[1]Percent positive
[2]Based on 30 samples at each concentration
[3] Total parent and desfuroylceftiofur related metabolites

CHARM® *B. stearothermophilus* TABLET DISK ASSAY
DRUG CONCENTRATION RESPONSE [1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**20**	**5**
DRUG CONCENTRATION (ppb)				
1				0
2	0	0	0	0
3				0
4	10	3	0	55
5				100
6	30	67		
8	90	100	0	
10	100	100		
14			100	
20			100	

[1]Percent positive
[2]Based on 30 samples at each concentration

CHARM® II TABLET BETA LACTAM TEST (COMPETITIVE ASSAY)
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**5**
DRUG CONCENTRATION (ppb)					
1					10
2	3	3		30	67
3					97
4	10	43		100	100
5			0		100
6	83	97			
8	100	100		100	
10	100	100	20		
14				100	
20			43	100	
40			100		
60			97		
80			100		
100			100		

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

CHARM® II TABLET BETA LACTAM TEST (SEQUENTIAL ASSAY)
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**5**
DRUG CONCENTRATION (ppb)					
1					0
2	0	0		3	10
3					80
4	20	10		100	100
5			0		100
6	23	83			
8	93	97		100	
10	100	100	0		
14				100	
20			3	100	
40			67		
60			97		
80			100		
100			100		

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

CHARM® II TABLET BETA LACTAM TEST (QUANTITATIVE ASSAY)
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**10**	**5**
DRUG CONCENTRATION (ppb)						
1						0
2	0	0		3	0	10
3						80
4	20	10		100	3	100
5			0			100
6	23	83			17	
8	93	97		100	87	
10	100	100	0		100	
14				100		
20			3	100		
40			67			
60			97			
80			100			
100			100			

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

CHARM® II TEST FOR CLOXACILLIN IN MILK (COMPETITIVE ASSAY)
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	CLOXACILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**
DRUG CONCENTRATION (ppb)	
2	0
4	3
6	17
8	87
10	100

[1]Percent positive
[2]Based on 30 samples at each concentration

CHARM® SL BETA LACTAM TEST
DRUG CONCENTRATION RESPONSE [1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**5**
DRUG CONCENTRATION (ppb)					
1					0
2	3	3			13
3					73
4	70	13		0	100
5			0		100
6	100	83			
8	100	100		50	
10	100	97[4]	0		
12				97	
16				100	
20			0	100	
40			0		
60			23		
80			100		
100			100		

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites
[4] All statistical models used to calculate 90/95 allow for a single negative result at tolerance

CHARM® SL6 BETA LACTAM TEST
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**10**	**5**
DRUG CONCENTRATION (ppb)						
1						7
2	0	0			0	23
3						93
4	7	3		0	40	100
5						100
6	57	17			83	
8	97	77		7	97	
10	100	100			100	
12				53		
16				100		
20			3	100		
40			37			
60			90			
80			97			
100			100			

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

CHARM® 3 SL3 BETA LACTAM TEST
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**10**	**5**
DRUG CONCENTRATION (ppb)						
1						0
2	0	0			0	0
3						13
4	0	0		0	0	97
5						100
6	3	23			13	
8	83	97		0	93	
10	100	100			100	
12				3		
16				83		
20			0	100		
40			0			
60			50			
80			100			
100			100			

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

CHARM® FLUSLBL FLUNIXIN AND BETA LACTAM TEST[1]
DRUG CONCENTRATION RESPONSE[2,3]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**100[4]**	**20**	**5**
DRUG CONCENTRATION (ppb)					
1					0
2	0	0			20
3					97
4	13	10		0	100
5					100
6	90	43			
8	97	97		3	
10	100	100			
12				67	
16				97	
20			0	100	
40			37		
60			97		
80			100		
100			100		

[1]Beta-lactam data only. See separate listing under NSAIDs for flunixin drug concentration response on page 15.
[2] Percent positive
[3] Based on 30 samples at each concentration
[4]Total parent and desfuroylceftiofur related metabolites

DELVOTEST P 5 PACK (VISUAL AND READER)
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**20**	**5**
DRUG CONCENTRATION (ppb)				
1				3
2	10	7	3	60
3				100
4	100	97	100	100
5				100
6	100	100		
8	100	100	100	
10	100	100		
14			100	
20			100	

[1]Percent positive
[2]Based on 30 samples at each concentration

DELVOTEST P/DELVOTEST P MINI
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**20**	**5**
DRUG CONCENTRATION (ppb)				
1				0
2	33	3	0	0
3				100
4	47	70	7	100
5				100
6	93	100		
8	97	100	100	
10	100	97[3]		
14			100	
20			100	

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]All statistical models used to calculate 90/95 allow for a single negative result at tolerance

DELVOTEST SP/DELVOTEST SP MINI
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**20**	**5**
DRUG CONCENTRATION (ppb)				
1				7
2	0	0	3	47
3				100
4	80	10	17	100
5		33		100
6	100			
8	100	100	100	
10	97[3]	100		
14			100	
20			100	

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]All statistical models used to calculate 90/95 allow for a negative result at tolerance

NEW SNAP® BETA LACTAM TEST KIT
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**100[3]**	**20**	**5**
DRUG CONCENTRATION (ppb)					
1					7
2	0	0		0	37
3					93
4	20	37		0	100
5			7		100
6	70	100			
8	100	100		0	
10	100	100	90		
12				100	
20			100	100	
40			100		
60			100		
80			100		
100			100		

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]Total parent and desfuroylceftiofur related metabolites

MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS[1]
NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

DRUG	FLUNIXIN[2]
TOLERANCE/SAFE LEVEL (ppb)	2 ppb
SCREENING TEST	
CHARM® FLUSLBL FLUNIXIN AND BETA LACTAM TEST	1.9

[1]Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each Test in the following Table. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory.
[2]As 5-hydroxyflunixin, the major metabolic form of flunixin and the chemical marker of flunixin in milk.

CHARM® FLUSLBL FLUNIXIN AND BETA LACTAM TEST[1]
DRUG CONCENTRATION RESPONSE[2,3]

DRUG	FLUNIXIN[4]
TOLERANCE/SAFE LEVEL (ppb)	**2**
DRUG CONCENTRATION (ppb)	
0.4	30
0.8	70
1.0	
1.2	97
1.6	97
2.0	100

[1]Flunixin data only. See separate listing under Beta lactams for Beta lactam drug concentration response on page 12.
[2] Percent positive
[3] Based on 30 samples at each concentration
[4]As 5-hydroxyflunixin, the major metabolic form of flunixin and the chemical marker of flunixin in milk.

MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS[1]
SULFONAMIDES

DRUG	SULFADIMETHOXINE	SULFAMETHAZINE	SULFATHIAZOLE	SULFADIAZINE
TOLERANCE/SAFE LEVEL (ppb)	10 ppb	10 ppb	10 ppb	10 ppb
SCREENING TEST				
CHARM® II SULFA DRUG TEST (COMPETITIVE ASSAY)	4.0	9.4	7.3	4.9

[1]Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each test in the following Table. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory.

CHARM® II SULFA DRUG TEST (COMPETITIVE ASSAY)
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	SULFADIMETHOXINE	SULFAMETHAZINE	SULFATHIAZOLE	SULFADIAZINE
TOLERANCE/SAFE LEVEL (ppb)	**10**	**10**	**10**	**10**
DRUG CONCENTRATION (ppb)				
2	97	7	0	40
4	100	80	57	100
6	100	97	100	100
8	100	100	100	100
10	100	100	100	97[3]

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]All statistical models used to calculate 90/95 allow for a single negative result at tolerance

MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS[1]
TETRACYCLINES

DRUG	CHLORTETRACYCLINE	OXYTETRACYCLINE	TETRACYCLINE
TOLERANCE/SAFE LEVEL (ppb)	**300 ppb (Chlortetracycline + Tetracycline + Oxytetracycline)**		
DRUG CONCENTRATION (ppb)			
CHARM® II TETRACYCLINE DRUG TEST (COMPETITIVE ASSAY)	257	119	67

[1]Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each test in the following Table. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory.

CHARM® II TETRACYCLINE DRUG TEST (COMPETITIVE ASSAY)
DRUG CONCENTRATION RESPONSE[1,2]

DRUG	Chlortetracycline	Oxytetracycline	Tetracycline
TOLERANCE/SAFE LEVEL (ppb)	**300 ppb (Chlortetracycline + Tetracycline + Oxytetracycline)**		
DRUG CONCENTRATION (ppb)			
20			0
30			7
40			37
60		13	93
70		37	
90	17		
100		87	
120	20		
150		100	
160	77		
230	93		
300	97[3]	100	100

[1]Percent positive
[2]Based on 30 samples at each concentration
[3]All standard statistical models used to calculate 90/95 allow for a single negative result at tolerance