

Kit Information

Introduction

Charm CowSide II Test detects antibiotics in raw, pasteurized, or ultra-pasteurized cow milk and milk powder. Bacteria, cultured in a vial with milk, generate acid and turn a pH indicator from purple to yellow. Dairy samples that prevent a color change are considered positive for antibiotics.

Kit Contents and Materials Needed

Supplied with Kits	Disposables	Equipment
<ul style="list-style-type: none"> Charm CowSide II Test Vials 100 µL Poly-Pipets Interpretation/Procedure Card Pipet Tip for Puncturing Seal Operator's Manual 	<ul style="list-style-type: none"> 4 ppb Penicillin G Standard, 10 mL 5 ppb Penicillin G Standard, 10 mL Zero Control Standard, 100 mL 	<ul style="list-style-type: none"> Charm CowSide Incubator Timer

Charm CowSide equipment available only from Charm Sciences Inc.

For details and ordering information see **Order Codes and Kit Contents**.

Sensitivity and Selectivity

Selectivity - Antimicrobial drug-free samples should yield 90% negative results with 95% confidence.

Sensitivity - Table 1: Antimicrobial drugs detected as positive compared to regulatory levels.

Antimicrobial Drug ¹	Concentration ² (ppb ³)	US Safe Level/Tolerance (ppb ³)	EU / CODEX MRL ⁴ (ug/kg)	Antimicrobial Drug ¹	Concentration ² (ppb ³)	US Safe Level/Tolerance (ppb ³)	EU / CODEX MRL ⁴ (ug/kg)
Amoxicillin	3 to 4	10	4 / 4	Chlortetracycline	200 to 300	300	100 / 100
Ampicillin	3 to 4	10	4	Doxycycline	25 to 75	None	0 ⁶
Cefacetile	10 to 15	None	125	Oxytetracycline	75 to 100	300	100 / 100
Cefalexin	75 to 100	None	100	Tetracycline	50 to 100	300	100 / 100
Cefalonium	15 to 20	None	20	Gentamicin	75 to 150	30	100 / 200
Cefazolin	6 to 10	None	50	Neomycin	100 to 150	150	1500 / 1500
Cefoperazone	20 to 30	None	50	Sulfadiazine	40 to 60	10	100
Cefquinome	40 to 60	None	20	Sulfadimethoxine	25 to 50	10	100
Ceftiofur & Metabolites ⁵	50 to 100	100	100 / 100	Sulfamethazine (Sulfadimidine)	75 to 125	10	100 / 25
Cefuroxime	20 to 25	None	None	Dapsone	1 to 2	None	0 ⁶
Cephapirin	8 to 10	20	60	Trimethoprim	200 to 300	None	50
Cloxacillin	10 to 25	10	30	Erythromycin	75 to 100	50	40
Dicloxacillin	5 to 10	None	30	Lincomycin	75 to 150	None	150
Nafcillin	5 to 10	None	30	Pirlimycin	25 to 50	None	100 / 100
Oxacillin	5 to 10	None	30	Spiramycin	300 to 400	None	200 / 200
Penethamate ⁷	2 to 3	None	4	Tilmicosin	25 to 35	None	50
Penicillin G	2 to 3	5	4 / 4	Tylosin	20 to 30	50	50 / 100

¹ Antimicrobial drugs listed are representative of their respective drug families. Other drugs will be detected at different levels.

² Positive 90% of the time with 95% confidence

³ Parts per billion or µg/L

⁴ Maximum Residue Limit

⁵ Total parent and metabolites concentration

⁶ Not for use in animals from which milk is produced for human consumption

⁷ Penethamate is rapidly converted to benzylpenicillin, the marker residue in milk

Storage

Store CowSide II Test vials and standards refrigerated (defined as 0 to 7°C or 0 to 4.5°C for US certified labs). See **Reagents and Storage** for details.

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Interferences and Cross Reactivity

The Charm CowSide II Test is a broad-spectrum antibiotic screening test.

The following animal drugs, at 100 ppb, show no interference; amphenicols, fluoroquinolones and nitrofurans, or chlorothiazide, dexamethasone, dipyrone, flunixin, furosemide, ivermectin, novobiocin, oxytocin, phenylbutazone, and trichlormethiazide. Other antimicrobials are detected. There are no interferences from high fat (up to 6%), somatic cells at 5×10^5 SCC/mL, or bacteria at 3×10^5 cfu/mL.

Training

- Equipment setup and use is simple and can be self-taught from the manual.
- For questions contact your local representative or Charm Sciences at +1.978.687.9200 or support@charm.com.

Reagents and Storage

CowSide II Test Vials

- Store refrigerated. Store vials upright. **Do not freeze.**

Negative Control

- Use a known negative raw, pasteurized, or ultra-pasteurized cow milk sample for negative control.
- Use refrigerated for up to 3 days.
- Zero Control Standard, a known negative milk powder, may be purchased separately for use as a negative control.

4 and 5 ppb Penicillin G Standards

- 4 ppb and 5 ppb Penicillin G Standard may be purchased separately for use as a positive control appropriate to EU, CODEX or US regulatory levels.
- As directed on the label, reconstitute with negative control (known negative) raw, pasteurized, or ultra-pasteurized milk (10.0 mL for 4 ppb standard and 8.0 mL for 5 ppb standard). Shake reconstituted standard well. Allow to stand refrigerated or on ice for 15 minutes before use.
- Store dry standard refrigerated. While in use, reconstituted standard may be held refrigerated or on ice for up to 48 hours.

Long-Term Sample and Control Storage

- Mix sample and freeze (at least 0.5 ml) aliquots in clean vials.
- Controls should be frozen within 6 hours of preparation.
- Freeze aliquots at -15°C or below for up to 2 months.
- Thaw slowly (overnight in refrigerator or with cool water) and shake well. Thawed sample is stable for 24 hours refrigerated. Noticeable protein precipitation indicates an unsuitable sample. Discard remaining thawed sample. Do not refreeze.

Sample and Test Information

- For raw, pasteurized, or ultra-pasteurized (UHT, ultra-high temperature) cow milk and reconstituted milk powder (10% solids). For individual cow samples, avoid foremilk and pool milk from all 4 quarters. Test milk at 0 to 15°C or cool to 0 to 7°C and test within 5 days of milking. Freeze samples at -15°C or below for longer storage. Thaw with lukewarm water and shake samples well before testing. Noticeable precipitation from frozen/thawed milk indicates poor quality sample for testing.
- Abnormal milk may yield incorrect results due to natural inhibitory substances. Milk with high somatic cells, colostrum and/or bacterial levels may interfere with test performance.

Equipment



- Charm CowSide Incubator (4 place, shown, order code: INC-COWSIDE), Digital Incubator Block (20 place, order code: INC-110-20 or INC-220-20) or Delvotest P/SP ampule block heater set at $64.0 \pm 0.5^{\circ}\text{C}$
- Timer



Performance Check

Negative controls and positive controls (sold separately) are recommended for equipment and reagent performance check.

Procedure – CowSide II

Preparation

1. Plug in dry well incubator (Charm® CowSide Incubator, Charm Inctronic 1, Digital Incubator Block, or Delvotest® P/SP ampule block heater) with temperature set at $64.0 \pm 0.5^\circ\text{C}$.
2. Allow temperature to stabilize.

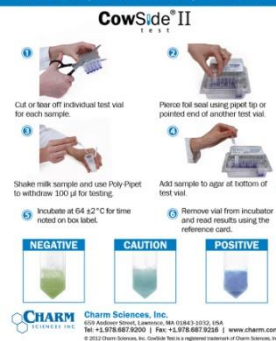
	<p>Step 1</p> <ul style="list-style-type: none">• Cut or tear off individual test vials (one vial per test sample).
	<p>Step 2</p> <ul style="list-style-type: none">• Place vial in holes in box. Pierce foil seal with pipet tip or pointed end of unused vial.
	<p>Step 3</p> <ul style="list-style-type: none">• Shake milk sample and withdraw 100 µL of milk (at 0 to 15°C) using unused poly-pipet. Hold poly-pipet vertically as shown and squeeze top bulb. Insert tube in sample (approximately 1 cm below foam/bubbles to avoid air bubbles) and release bulb slowly. Stop releasing bulb when sample starts to overflow into lower bulb. Remove tube from milk sample.• First time users: The 100 µL poly-pipet is a single-use disposable pipet to deliver 100 µL. Do not reuse poly-pipets.
	<p>Step 4</p> <ul style="list-style-type: none">• Dispense 100 µL milk sample to purple agar at bottom of vial by slowly squeezing top bulb of poly-pipet (excess sample should remain in lower bulb). Transfer vial to incubator (Charm CowSide Incubator, Charm Inctronic 1, Digital Incubator Block, or Delvotest P/SP ampule block heater).• First time users: A negative control should also be tested to check CowSide II Test operation. Test 100 µL of milk known to be antibiotic free in another vial.
	<p>Step 5</p> <ul style="list-style-type: none">• Set timer for time specified on kit label for raw or ultra-pasteurized milk and incubate. (Note: Incubation time printed on kit label is approximately 3 hours.)• After incubation, negative control should be a negative color as shown in Interpretation section. If not, incubate up to an additional 15 minutes. After incubation, remove vials and interpret results. See Interpretation.

Interpretation

Interpret results within 30 minutes of removing tests from incubator. Observe color on the bottom of tubes (the lower 2/3 of agar). Read results under cool white fluorescent light. Compare agar color to interpretation card provided with test kit.

The color results are stable for 16 hours if left in incubator after automatic shut off.

Refer to Current Operator's Manual for Complete Test Procedure



- **NEGATIVE** - Yellow or yellow/green colors are negative. Report as "Not Found".
- **CAUTION** - Blue/green colors are initial **positive**. Sample should be retested. See **Retest of Initial Positive**.
- **POSITIVE** - Blue/purple colors are initial **positive**. Sample should be retested. See **Retest of Initial Positive**.

Retest of Initial Positive

- In a test tube, heat 500 ± 100 µL initial positive milk sample to 82 to 100°C for 3 minutes.
- Immediately cool to 0 to 15°C and shake well.
- Run heated sample in duplicate along with a negative control, a positive control (4 or 5 ppb penicillin G standard if available), and unheated sample as milk sample in **Procedure**.
- The negative control must be negative result and positive control must be positive result. See **Interpretation**.
- If sample is positive after heat treatment and unheated, sample is a "CowSide II Test positive."
- If sample is negative after heat treatment and unheated sample is positive, sample may contain a non-antibiotic (heat sensitive) inhibitor.

Order Codes and Kit Contents

KITS		NOT SUPPLIED WITH KIT
<p>MI-COWSIDE-II-20:</p> <ul style="list-style-type: none"> • 20 Charm CowSide II Test Vials • 20 100 µL Poly-Pipets • Interpretation/Procedure Card • Pipet Tip for Puncturing Seal • Operator's Manual 		<p>PG4: 4 ppb Penicillin G Standard, 10 mL bottle</p> <p>PG5: 5 ppb Penicillin G Standard, 10 mL bottle</p> <p>ZCS: Zero Control Standard, 100 mL bottle</p>
<p>MI-COWSIDE-II-100:</p> <ul style="list-style-type: none"> • 100 Charm CowSide II Test Vials • 100 100 µL Poly-Pipets • Interpretation/Procedure Card • Pipet Tip for Puncturing Seal • Operator's Manual 		

Warranty Information

Charm Sciences, Inc. ("Charm") warrants each reagent product, including but not limited to test kits, to be free from defects in materials and workmanship and to be free from deviations from the specifications and descriptions of Charm's reagent products appearing in Charm's product literature, when stored under appropriate conditions and given normal, proper and intended usage, until the expiration of such reagent product's stated shelf life, or, if none is stated, for one year from the date of delivery of such reagent product to the end-user purchaser. **THIS WARRANTY IS IN LIEU OF ALL OTHER WARRANTIES, WHETHER STATUTORY, EXPRESS, IMPLIED (INCLUDING WARRANTIES OF TITLE, NON-INFRINGEMENT, MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE AND ALL WARRANTIES ARISING FROM COURSE OF DEALING OR USAGE OF TRADE).** The warranty provided herein may not be altered except by express written agreement signed by an officer of Charm. Representations, oral or written, which are inconsistent with this warranty are not authorized and if given, should not be relied upon. In the event of a breach of the foregoing warranty, Charm's sole obligation shall be to replace any reagent product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Charm promptly of any such defect prior to the expiration of said warranty period. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Charm is willing to replace any nonconforming reagent product or part. **Charm shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damages sustained by any customer from the use of its reagent products.** Except for Charm's obligation set forth above to replace any reagent product that proves defective within the warranty period, Charm shall not be liable for any damages of any kind arising out of or caused by any incorrect or erroneous test results obtained while using any such reagent product, whether or not caused by a defect in such reagent product.