DELVOTEST® P 5 PACK

Microbial inhibition test validated for the detection of residues of PENICILLIN G, AMPICILLIN, AMOXICILLIN and CEPHAPIRIN in raw, commingled, bovine milk.

FINISHED MILK

The Delvotest® P 5 PACK has been evaluated by the FDA and has been found to be acceptable for the testing of the following drugs at tolerance/safe level or below in pasteurized, homogenized, white milk and in chocolate milk (0% fortified to 3.5% fat) and in cream: Amoxicillin, Ampicillin, Cephapirin and Penicillin-G. Delvo®Scan has not been evaluated for testing of chocolate milk and cream. Only the visual reading option can be used for testing these products.

Contents

–5 Multiplates (6 x 16 test wells each) containing an agar medium with a standardized number of spores of *Bacillus stearothermophilus var. calidolactis* and a pH indicator.
–1 Bottle with 500+ nutrient tablets and a silicagel capsule (to absorb moisture).
–5 Sheets (3.5" x 5") adhesive tape to cover test wells (whole plate) during incubation.
–5 Sets of 6 strips (3.5" x 0.75") adhesive tape to cover test wells (block of 16 wells) during incubation.

Materials required but not included

– Water bath or dry incubator set at 64.0 ± 2.0°C.
– Calibrated thermometer appropriate for designated use.
– A fixed volume pipettor with disposable tips to dispense 0.1 ±0.005 ml portions of samples.
– Cool space (below 15°C) for storage of test kit, refrigerator and deep freeze for storage of milk samples. Note; Milk samples subjected to NCIMS testing may not be frozen before testing. Store milk samples at 0 - 4.4°C for not more than 72 hours.
– Positive (5 ppb penicillin G) and negative control samples in lyophilized form are commercially available. Call DSM Food Specialties representative; tel. 610-650-8480 to obtain information regarding source of approved control samples.

TEST PERFORMANCE

1. Sensitivity (dose response information Delvotest® P 5 PACK test)[1]

ppb	AMOX*	AMP*	CEPH*	PEN*
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] Percentage positive (full purple color) at each concentration.

Tolerance/Safe Level (ppb)	10	10	20	5
90/95% conc (ppb)	4.6	4.0	8.2	2.1

* The drugs indicated by an asterisk (*) have demonstrated a 90/95% sensitivity of this test kit which is at least 25% less than the tolerance or safe level.

2. Selectivity

60 negative control milk samples were evaluated in an independent laboratory and 0 of these negative samples tested positive with the Delvotest® P 5 PACK test.

DSM Food Specialties USA, Inc
45 Waterview Blvd.
Parsippany, NJ 07054
Phone: 610-650-8480 / 800-662-4478
Fax: 610-650-4005
info.dairy-usa@dsm.com
www.dsm-foodspecialties.com / www.delvotest.com

3. Cross reactivity

Delvotest® P 5 PACK test does not cross-react with the following drugs at level of 100 ppb: sulfadiazine, sulfanilamide, sulfathiazole, sulfamethazine, sulfapyridine, sulfadimethoxine, tetracycline, oxytetracycline, chlortetracycline, doxycycline, gentamycin, neomycin, streptomycin, ivermectin, erythromycin, novobiocin, furosemide, trichlormethiazide, chlorothiazide, oxytocin, phenylbutazone, dexamethasone and dipyrone.
Delvotest® P 5 PACK test does cross-react with the following drugs at a level of 100 ppb or below;

Cross reactivity (dose response information Delvotest® P 5 PACK test)[1]

Concentration	ticarcillin	cloxacillin	dicloxacillin	cefadroxil	ceftiofur
10 ppb	0	0	0	0	0
30 ppb		83			
50 ppb	100	100	100	0	83
70 ppb					100
100 ppb	100		100	100	

[1] Percentage positive (full purple color) at each concentration.

Operating instructions

Before starting the test procedure

1. **Test kit** Take the test kit from storage and let it acclimate or adapt to room temperature. This is important in particular for the bottle with nutrient tablets (see under Storage and precautions).

2. **Incubator** Allow sufficient time for water bath or dry incubator to stabilize at the required temperature of 64°C ± 2°C.

3. **Test kit validation** Testing according to the NCIMS procedure requires a successful completion of the test kit validation on every day that the test kits are used, using a Negative and Positive control sample before any milk samples are evaluated (see section; 'Materials required but not included').

4. **Samples** Mix milk samples before use. When pipetting tilt the milk to avoid the foam; drawing the volume slowly into the pipettor without taking in bubbles. Use a new disposable pipette tip for each milk sample.

5. **Number of samples** The number of milk samples that can be tested at one time with one whole test plate is 96 and 16 with one detached block of test wells.

Testing procedure

1. One should observe the wells before using them to ensure that the wells of agar have the correct volume and are intact. Depending on the number of samples to be tested use a whole plate or cut off the required number of blocks of 16 test wells using a sharp knife or a pair of scissors. Do not tear off blocks, as this may damage the foil of the remaining part of the multiplate and this will cause drying out of the test wells. Any test with a torn foil must be used immediately or discarded.

2. Slowly peel off the aluminium foil.

3. Put one nutrient tablet in each well using the plastic forceps or tablet dispenser. Remove the cap from the bottle of nutrient tablets and place it upside down on the table. Remove the (white) silicagel capsule and (slowly) the piece of foam with the forceps. **Cautiously shake some tablets into the bottle cap.** If a tablet is spilled it should be discarded (each bottle contains a number of spares). Alternatively use a tablet dispenser. Without delay, return the unused tablets to the bottle, and, using the forceps, the piece of foam and the silicagel capsule, and close the bottle well. After opening store bottle with tablets at room temperature.

4. Pipet 0.1 ± 0.005 ml of a negative control milk sample in the first well and pipet 0.1 ±0.005 ml of a positive control in the second well. Negative and Positive control samples in lyophilized form are commercially available (call the DSM Food Specialties representative tel. 610-650-8480 to obtain information regarding source of approved control samples).

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5. Add 0.1 ± 0.005 ml of each milk sample to be tested in a test well. Identify each sample on the plate or well preferably using a template. The figures and letters engraved in the test plate are to be used to facilitate identification of the milk samples.

6. When all samples are introduced, seal the blocks and/or plates carefully with respectively the adhesive strips or sheets, enclosed in the test kit. Gently float the blocks and/or plates upright in a water bath prewarmed at 64.0 ± 2.0°C or place these on a rack in a dry incubator of the same temperature. In the case of the use of a dry incubator leave room between the blocks and/or plates. Record the time or set the timer at 2 hours 30 minutes.

7. After 2 hours 30 minutes remove the blocks and/or plates from the incubator and determine visually the color of the test wells from the bottom side. The color of the agar medium with the Negative control must be yellow. If not, immediately incubate for another 15 minutes. If the color is still not yellow, repeat the test after checking the incubation temperature. The color of the positive control must be purple. Use the enclosed COLOR CHART TEST RESULTS / DELVOTEST® for color references.
Users of Delvo®Scan must follow software instructions for instrumental reader of Delvotest® P 5 pack. Delvo®Scan reader is used after firstly visually inspecting the Negative control sample to be yellow. Negative control sample must read Delvo®Scan 'negative' and Positive control sample must read Delvo®Scan 'positive'.

Interpreting results
(once the control samples give appropriate colors)
- A purple color (Delvo®Scan 'positive') of the whole agar medium indicates the presence of antibiotic residues in the corresponding milk sample at or above the levels indicated in the tables above. Such a result should be regarded as an **INITIAL POSITIVE** result. The procedure to further validate this initial positive result is described below.

- A partly[*] or completely yellow color (Delvo®Scan 'negative') of the agar medium indicates the absence of antibiotic residues below the levels indicated above. Such a result should be regarded as a **NEGATIVE** result (**report as Not Found**).
[* NCIMS requirement for visual reading must follow FDA 2400* form calling for partial yellow wells to be subjected to confirmation/validation. After confirmation/validation then a partial yellow would be reported as NF]

Validation of initial result
If validation is required, the milk sample must be tested according to the NCIMS procedure by repeating the test in duplicate using a sample heat (82 + 2°C) treatment and penicillinase treatment [optional by State] with the same test kit, together with a Positive and Negative control sample. Validation by the NCIMS procedure requires the milk sample to be refrigerated; the samples may not be frozen at any time during storage.
Positive (5 ppb penicillin G) and Negative control samples in lyophilized form are commercially available (Call DSM Food specialties representative; tel. 610-650-8480 to obtain information regarding source of approved control samples). The Positive control sample must give a purple color (Delvo®Scan 'positive') of the agar medium after incubation of the test for 2 hours 30 minutes at 64 + 2°C, the Negative control sample should give a yellow color (Delvo®Scan 'negative').
If one or both of the duplicates give a purple color (Delvo®Scan 'positive') of the agar medium the result is a **PRESUMPTIVE POSITIVE** result.
If both duplicate samples give a partly or completely yellow color (Delvo®Scan 'negative') after incubation the test result is **NEGATIVE (report as Not Found)**.
Certified laboratories are to follow NCIMS procedures for reporting and acting on Positive testing results.

Training
First-time users are advised to practice the test with Negative and Positive control samples.

Requests for training or technical assistance can be directed to the DSM Food specialties representative; tel. 610-650-8480.
First-time Delvo®Scan users must be trained before obtaining/using the Delvo®Scan Reader Software.

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Storage and precautions

1. Store test kit in a dry and cool place (preferably below 15°C (60°F) (do not freeze). Test kits should not be used after the expiration date indicated on the label. Nutrient tablet bottles should be left to acclimate or adapt to room temperature before opening to avoid exhausting of the drying capacity of the silicagel capsule and condensation of moisture on the tablets, which will result in deterioration of essential nutrient components. After opening store bottle with tablets at room temperature.

2. Anything that may come in contact with the milk (hands, pail, receptacle, cup, stirrer etc.) must be cleaned thoroughly, rinsed with tap water and dried with a clean (paper) towel to avoid contamination with inhibiting substances like antibiotics, other drugs, cleaning agents or disinfectants.

3. Collect the milk in a clean pail or other receptacle. Stir the milk well and take a sample with a cup. Label the milk with proper reference to avoid confusion or mistakes.

4. Keep the milk sample(s) in a refrigerator (0-4.4°C for not more than 72 hours). If it is not possible to carry out the test within 72 hours samples may be stored frozen at or below -18°C.
Testing of samples that have been frozen do not meet the NCIMS regulations. Samples that have turned sour cannot be tested.

5. The test can be reliably operated at ambient temperatures, between 15°C and 37°C (60°F - 100°F).

ADDITIONAL INFORMATION

Delvotest® P 5 PACK can be used for the screening of other types of processed or individual milk and/or to detect other antibiotics. Such uses have not been validated by AOAC-RI and are not included under the AOAC-RI performance tested certificate.
Further information, also about the actual detection limits, is available upon request. Call DSM Food Specialties representative tel. 610-650-8480.

The above text has received AOAC approval for this test kit use in North America.

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE

LICENSE NUMBER 930703

"Samples of this test kit model were independently evaluated by the AOAC Research Institute and were found to perform to the producer's specification as stated in the descriptive insert. The producer certifies this test kit conforms in all respects to the specification originally evaluated by the AOAC Research Institute a detailed in the Performance Tested SM Certificate number 930703".

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