

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

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Title: Inhibition Screen Test for Antimicrobial Drugs		
Revision: 03	Replaces: CLG-ADD 3.02	Effective: 04/26/21

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A. INTRODUCTION

1. Background / Summary of Procedure

The Charm KISTM Test is an antibiotic screen test for bovine, porcine, ovine, caprine, and poultry kidney and for bovine, porcine, ovine, and poultry muscle tissue. The KIS™ test is designed to absorb kidney serum or juice using a swab. Bacteria, cultured in agar with purple pH indicator and tissue swab extract, generate acid that produces a yellow color. If antimicrobial drugs are present, microbial growth in the KIS™ vial is inhibited which prevents a color change to yellow. Thus, positives remain purple.

Note: The KIS™ test has been extended to muscle juice.

Note: This method is not an endorsement by the Food Safety and Inspection Service (FSIS) of the Charm KIS™ Test over other similar commercially available products.

2. Applicability

This method is suitable for the *screening* of the following antimicrobial drugs in bovine, porcine, ovine, caprine and poultry kidney and for bovine, porcine, ovine, and poultry muscle tissue. Kidney analyte sensitivities are listed in Table 1 in Appendix Section J.2

Note: Refer to 21CFR for tolerance values set by FDA and 40CFR for tolerance values set by EPA.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus

- a. Incubator - Digital Incubator Block with dry well and internal timer, Cat. No. 949300S1, Charm Sciences, Inc.
- b. White fluorescent light
- c. Interpretation Card
- d. Test tube rack
- e. Timer

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted.

1. Reagents

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- a. KIS-100K: (Charm Sciences Inc.) KIS™ Test Swab Devices: KIS-100K. Store kit supplies at 2 to 8 °C.
- b. Negative Control - Code: NGKIS-4 (Charm Sciences Inc.) Kidney negative control tablets
- c. Deionized or distilled water

D. STANDARD(S)

Note: Equivalent standards / solutions may be substituted. Purity and counterions are to be taken into account when calculating standard concentrations. In-house prepared standards shall be assigned an expiration date that is no later than the stability stated in the method.

1. Standard Information

- a. Penicillin G – Penicillium G sodium (β -lactam, USP, 100%)

2. Preparation of Standard Solution(s)

- a. Stock standard (1,000 μ g/mL):

Weigh accurately and transfer using deionized water 10 mg of Penicillin G into a 10 mL volumetric flask. Dilute to volume. The standards are stored at < -10 °C and are stable for two months.

- b. Working standard (1.00 μ g/mL):

Add 100 μ L of stock standard (1,000 μ g/mL) and dilute to volume with deionized water in a 100 mL volumetric flask. The standards are stored at < -10 °C and are stable for two weeks.

Note: It is recommended to store stock and working standards in multiple aliquots. Working standards may degrade if thawed and re-frozen several times.

E. SAMPLE RECEIPT AND PREPARATION

No sample preparation is required. Intact kidney or muscle tissue is used for testing.

F. ANALYTICAL PROCEDURE

Note: The following steps are from the manufacturer test kit instructions and may be subject to change. If any discrepancies exist, follow the current manufacturer test kit instructions.

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1. Preparation of Controls

a. Negative Control:

Add one negative control tablet to a screw cap vial. Add 1.0 mL deionized or distilled water, cap, and shake for 10 sec to dissolve tablet. Shake again after 5 min. Store at 2 to 8 °C for up to 5 days (or per manufacturer shelf life instructions).

Note: Alternately, negative control can be prepared by freeze-thawing and squeezing and collecting kidney or muscle juice. Extract must be screened as negative before use. Store extracts in a freezer at < -10 °C.

b. Positive Control (50 ng/mL):

Add 25 µL Penicillin Working Standard (1.00 µg/mL) and 25 µL deionized water into tube containing 450 µL Negative Control Matrix or extract (F.1.a). Mix.

Note: Change volumes proportionately as needed.

2. Extraction Procedure

- a. Remove swab housing from device by pulling swab handle from KIS™ body. Use the exposed end of the KIS™ body like a cookie cutter to make a circular cut in the kidney or muscle tissue that is about 1/2 inch (1 to 2 cm) deep.

Note: For poultry kidney, proceed to step F.2.b

- b. Hold shaft to support the swab and place cotton tip inside the circular cut into the tissue. Twirl and move tip around cut for 30 sec or until the swab is saturated with juice. For poultry kidney, absorb kidney juice from thawed samples. Remove any tissue particulates on the swab. Any whitish appearance in cotton tip of swab indicates more sample absorption is needed. An absorbed swab contains at least 80 µL of sample

- c. For Negative and Positive Controls, place swabs in test tubes containing negative or positive controls for 10 sec.

- d. Replace swabs on device bodies. Hold each device upright and slowly activate swab by engaging cap with body threads. Screw down halfway so that swab pierces through top vial foil seal and goes into top clear liquid only but not through bottom foil seal *.

**Note: If bottom seal is accidentally pierced, screw swab completely down.*

- e. Wait 2 minutes

- f. Completely screw down swab so it is directly above agar in vial bottom. Lightly tap vial bottom ~5 times on hard surface to force any residual liquid down to the bottom of the vial. Fully retract swab, and lightly tap vial bottom ~5 times again.

- g. Incubate tubes at 64 ± 2 °C in the dry well incubator for the prescribed time specified on the KIS™ Test label. After specified incubation time, remove vials to

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cool and interpret results (See Interpretation section G. below).

Note: If auto shut off feature of incubator is used, set time to 15 min less than the specified time. Tests should not be removed from incubator until cooled.

3. Sample Set
 - a. Negative control
 - b. Positive control
 - c. Samples up to a maximum of 100

Note: Specified incubation times are lot dependant. Analyze a negative and positive control for each lot used.

G. DECISION CRITERIA / CALCULATIONS

Compare agar color to interpretation card provided with test kit (See Section K.2 for picture).

1. Color is stable for 16 hours after test has cooled. If auto shut off of incubator is used, incubator will cool and vial color will remain stable in incubator.
2. Read results under cool white fluorescent light. Do not read color under direct sunlight.
 - a. Yellow or yellow/green colors are **negative**.
Note: the manufacturer's negative control may have an orange/brown color
Blue or purple colors are **positive**.
 - b. Yellow or yellow/green in lower half of vial and blue/purple or brown in upper half of the vial are **caution**. These samples should be interpreted as negative.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Personal Protective Equipment — Lab coat, safety glasses, and gloves
2. Hazards

Consult all Safety Data Sheets (SDS) associated with the method
3. Disposal Procedures

Follow federal, state and local regulations.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

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- a. No false positives from negative control (F.1.a).
 - b. No false negatives from positive control (F.1.b).
2. Intralaboratory Check Samples
- a. Acceptability criteria.
Refer to I. 1.
If unacceptable values are obtained, then:
 - i. Investigate following established procedures.
 - ii. Take corrective action as warranted.

J. APPENDIX

1. References

Charm Kidney Inhibition Swab (KIS™) Test for Antimicrobial Drug Detection in Kidney (Manual for Bovine and Porcine Kidney), Charm Sciences, Inc., February 25, 2009

2. Analyte Sensitivities

Table 1: Kidney Analyte Sensitivities Provided by the Kit Manufacturer

Drug	KIS Detection Level In Kidney Tissue (ppb)
Penicillin G	35
Ampicillin	100
Amoxicillin	100
Cloxacillin	300
Ceftiofur*	4000
Cephapirin*	100
Sulfamethazine	500
Sulfadimethoxine	250
Sulfathiazole	250
Oxytetracycline	3000
Chlortetracycline	12000
Tetracycline	1000
Tylosin	400
Erythromycin	500
Pirlimicin*	1000
Tilmicosin	2500
Tulathromycin*	400
Neomycin	4000
Gentamicin	750
Streptomycin	10000

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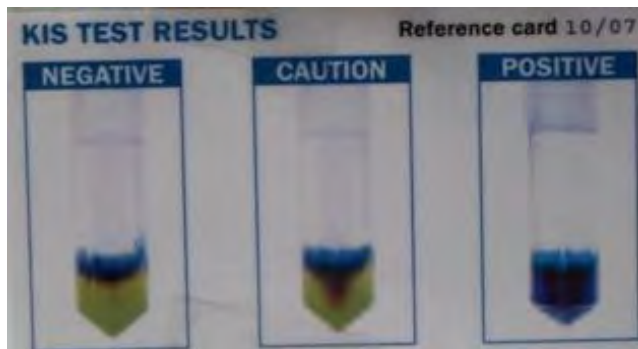
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Drug	KIS Detection Level In Kidney Tissue (ppb)
Dihydrostreptomycin	4000
Florphenicol	10000
Chloramphenicol	50000
Enrofloxacin	25000
Ciprofloxacin	25000
Spectinomycin	10000
Novobiocin	5000
Trimethoprim	1000
Virginiamycin	25000
Bacitracin	10000
FZD (Furazolidone)	20000
FZD AOZ (3-Amino-2-oxazolidinone)	>20000 (detection level not determined)

* This drug is known to metabolize into multiple forms in incurred samples. Fortified drug sensitivity may not accurately reflect incurred sample detection.

Note: Analyte sensitivities in muscle tissue may differ from those listed in the table.

3. Example of Interpretation Card from the kit manufacturer



K. APPROVALS AND AUTHORITIES

1. Approvals on file.
2. Issuing Authority: Director, Laboratory Quality Assurance Staff.