CLG-ADD3.00		Page 1 of 9
Title: Swab Test for Antimicrobial Drug Detection		
Revision: NA	Replaces: NA	Effective: 07/20/2009

Contents

A. INTRODUCTION

The Charm KIS™ Test is an antibiotic detection test for bovine and porcine kidney or muscle tissue.

1. Theory

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

The KIS™ test is an inhibition test for screening broad spectrum antimicrobial drugs in kidney or muscle tissue. Analyte sensitivities are listed in Table 1.

Table 1: 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
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.

B. EQUIPMENT

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

1. Reagents

   Equivalent reagents and solutions may be substituted for the following:

   a. KIS-100K: (Charm Sciences Inc.) KIS™ Test Swab Devices: KIS-100K
   b. Negative Control - Code: NGKIS-4 (Charm Sciences Inc.) Kidney negative control tablets.
   c. Deionized or distilled water

2. Storage of kit supplies

   Store kit supplies at 2 to 8 °C.

D. STANDARDS

Equivalent standards may be substituted for the following:

1. Penicillin G – Penicillium G sodium (β-lactam, USP, 100%), Preparation
   a. Stock standard (1,000 µg/mL):

      Weigh accurately and transfer using deionized water 10 mg of Penicillin G into a 10 mL volumetric flask. Dilute to volume.

   b. Working standard (1.00µg/mL):

      Add 100 µL of stock standard (1,000 µg/mL) and dilute to volume with deionized water in a 100 mL volumetric flask.

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

2. Storage and Stability

   The standards are stored at < -10 °C. The stock standard is stable for two months and, and the working standard is stable for two weeks.

E. SAMPLE 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.

1. Sample Set Requirements

   a. Each sample set will contain a negative control and a 50 ppb Penicillium G positive control and any number of samples that can be accommodated in a set.

2. Preparation of Negative and Positive 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.2.a). Mix.

      Note: Change volumes proportionately as needed.

3. 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.

   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. 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 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.

G. INTERPRETATION

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. Required Protective Equipment — Lab coat, safety glasses, and gloves

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Penicillin G standard	Some individuals may have allergic reactions to certain ß-Lactams.	Wear appropriate personal protective equipment to avoid inhalation or dermal contact.

3. Disposal Procedures

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Used kits/tubes	None	Dispose in accordance with local, State, and Federal regulations.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

   a. No false positives from negative control (F.2.a).

   b. No false negatives from recoveries (F.2.b).

2. Critical Control Points and Specifications

	Record	*Acceptable Control*
a.	F.3.G	Incubate tubes at 64 ± 2 ºC

3. Readiness To Perform

   a. Familiarization

Phase I: Observe a qualified analyst running the method.

Phase II: Demonstrate proper control and sample preparation while under observation of trainer. Run and interpret positive and negative controls while under observation of trainer.

Phase III: Check samples for analyst accreditation.

(a) The analyst will be provided 16 unknown samples (at least 8 will be positive) for phase III qualification.

Note: Prepare unknown negative and positive controls as described in F.2.a and F.2.b.

(b) Report analytical findings to QAM through Supervisor.

(c) Authorization from QAM and Supervisor is required to commence official analysis.

b. Acceptability criteria.

Refer to I. 1.

4. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: One per week per analyst

b. Acceptability criteria.

Refer to I. 1.

If unacceptable values are obtained, then:

i. Stop all official analyses by that analyst.

ii. Take corrective action.

5. Sample Acceptability and Stability

a. Matrix: Intact kidney and muscle tissue

b. Species: Bovine and porcine

c. Sample receipt size: Minimum approximately 25 g of tissue

d. Sample receipt condition: Frozen or cool

e. Sample storage: Store frozen at < - 10 °C.

6. Sample Set

Each sample set must contain:

a. Negative Control

b. Positive Control

c. Samples

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

7. Sensitivity

a. Minimum proficiency level (MPL): See Table1 for test kit detection levels.

J. WORKSHEET

[RESERVED]

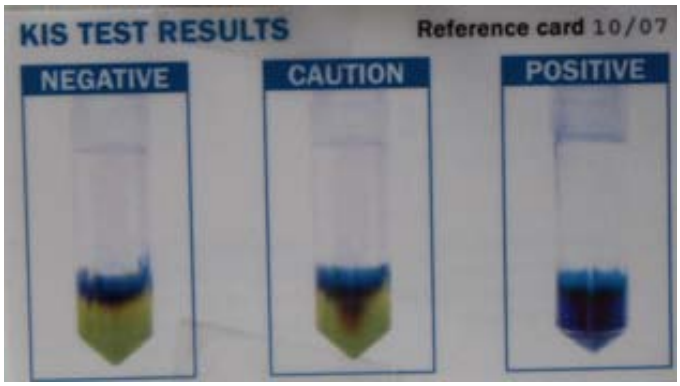
K. APPENDIX

References:

1. 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. Example of Interpretation Card from the kit manufacturer.

Interpretation Card



L. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Division.