Standard Operating Procedure

for

Detection of Chloramphenicol in Tissue by Charm-II (OM-354-009)

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Issued to	**Lab Manager (LM)**

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1. INTRODUCTION

The Charm II Chloramphenicol Test for Tissue is a rapid radioimmunoreceptor assay for the detection of chloramphenicol in tissue. Chloramphenicol is part of the amphenicol family of antibiotics, and is reserved for certain serious bacterial infections when other drugs are ineffective in humans. Because of its dangerous potential side effects, this potent compound has been banned for use in food producing animals in many countries.

The sensitivity of the Charm II Chloramphenicol Test for Tissue is set to detect chloramphenicol at the EU and U.S. MRPL (minimum residue performance limit) of 0.3 µg/kg or ppb (parts per billion). This test is routinely quality controlled for use in muscle tissue from beef cattle, poultry, salmon, and shrimp. Pork muscle tissue often yields initial positive results during the initial test, so this tissue requires use of the Verification Procedure for Tissue. This test is designed for use by veterinary, laboratory, field, and regulatory personnel.

2. SCOPE

These standard Operating Procedures (SOP) describe the procedure for determination/ screening of chloramphenicol in tissue by Charm II that is rapid radioimmunoreceptor assay.

3. OBJECTIVE/PURPOSE

To ensure the accuracy, quality and reproducibility of the procedure / protocol.

4. DEFINITIONS AND ABBREVIATIONS

4.1. CAP: Chloramphenicol

4.2. SOP: Standard Operating Procedures

4.3. ppb: parts per billion

4.4. HPLC: High-Performance Liquid Chromatography

4.5. MRPL: Minimum residue performance limit

4.6. TCA: Trichloroacetic acid

4.7. MeOH: Methanol

5. PRINCIPLE OF THE METHOD

The Charm II Chloramphenicol Test uses a binding reagent with specific receptor sites that bind chloramphenicol. The binder is added to a sample extract along with an exempt amount of [3H] labeled chloramphenicol (tracer). Any chloramphenicol in the sample competes for the binding sites with this tracer. The amount of tracer that binds to the receptor sites is measured and compared to a previously determined Control

Point. The Control Point is the cutoff point between a negative or positive sample. The greater the amount of tracer measured, the lower the chloramphenicol concentration in the sample. The smaller the amount of tracer measured the greater the chloramphenicol concentration in the sample.

6. SAFETY CONSIDERATIONS AND PRECAUTIONS

The chemicals used in these procedure can be highly toxic and may constitute a serious health risk for the laboratory workers. Further, TCs are considered a possible carcinogen to humans.Suitable gloves must be worn whenchemicals/TCs standards are handled, and the work shall be performed in a fume hood at all possible time.

7. MATERIALS AND EQUIPMENT

7.1. WATER

Unless stated otherwise, use only ultrapure water double distilled water or water of equivalent purity.

7.2. CHEMICALS

Unless stated otherwise, use only the reagents of recognized analytical grade

7.2.1.Scintillation fluid

7.2.2.ATBL Tablet Reagents

7.2.3.10 ppb chloramphenicol Standard

7.2.4.Tissue performance negative concentrate

7.2.5.MSU Extraction Buffer

7.2.6.M2 Buffer

7.2.7.Double Distilled water (Fistreem International LTD, WSC044.MH3.7)

7.3. EQUIPMENT / APPARATUS

7.3.1.Bench top Centrifuge 5430 R (Eppendorf, Germany)

7.3.2.CHARM II Analyzer (Liquid scintillation counter)

7.3.3.Water distillation unit (Fistreem International LTD, WSC044.MH3.7)

7.3.4.Electric Balance (Shimazdu, ATX224)

7.3.5.Vortex mixer (Wisd Vortex Mixer VM-10)

7.3.6.Water bath (Buchi B-480 Water Bat)

7.3.7.Tube shaker (Dream SI-300)

7.3.8.Homogenizer (Wisd HG-15D)

7.3.9.pH meter (Horiba, 502-S)

7.3.10.pH Strips

7.3.11.Fume Hood (Abaseen Fume Hood, 25/A)

7.3.12.Freezer (Sanyo Biomedical Freezer, MDF U-333)

7.3.13.Graduated cylinder (50, 250 and 1000 mL, glass), Borosil.

7.3.14.Separating funnel (100mL, glass), Pyrex Japan

7.3.15.petri dishes (90×20 mm, glass)

7.3.16.Beakers (50-1000mL, glass), Pyrex Indonesia

7.3.17.Centrifuge tubes with caps (50ml) , VWR USA

7.3.18.Test tubes (13 x 99, round bottom) , Charm Sciences INC USA

7.3.19.Conical flasks (Erlenmeyer , Pyrex)

7.3.20.Micropipettes (100-1000µL) Eppendorf Reasearch Plus, Germany

7.3.21.Screw cap tubes (15mL)

7.3.22.Spatula

7.3.23.Gloves

7.3.24.Scalpel, blades

7.3.25.Eppendorf tubes (l.5 mL) Eppendorf AG, Germany

8. REAGENTS/SOLUTIONS

8.1. ATBL Tablet Reagents

Store tablet reagents at -15°C or below. Remove from freezer the number of tablets to be used in one day. Tablets may be kept at room temperature during daily use for up to 12 hours. Discard unused tablets after room temperature storage. Each green tablet contains less than 3.0 kilobecquerels (kBq) of [3H]-chloramphenicol.

8.2. 10 ppb Chloramphenicol Standard

Reconstitute with 10.0 ml deionized or distilled water. Shake well. Allow Chloramphenicol Standard to stand refrigerated or on ice for 15 minutes. Mix before use. Reconstituted Chloramphenicol Standard contains 10 ppb chloramphenicol.
Store dry Chloramphenicol Standard refrigerated. While in use, reconstituted Chloramphenicol Standard may be held refrigerated or on ice for up to 48 hours. Reconstituted Chloramphenicol Standard may be stored up to 2 months at -15°C or below. Store thawed Chloramphenicol Standard on ice or refrigerated for up to 24 hours. Do not refreeze.

8.3. Tissue Performance Negative Concentrate (TPNC)

Reconstitute with 10.0 ml deionized or distilled water. Shake well. Allow TPNC to stand refrigerated or on ice for 15 minutes. Mix before use. Store dry TPNC refrigerated. While in use, reconstituted TPNC may be held refrigerated or on ice for up to 48 hours. Reconstituted TPNC may be stored up to 2 months at -15°C or below. Store thawed TPNC refrigerated for up to 24 hours. Do not refreeze.

8.4. MSU Extraction Buffer (MSU-EB)

Reconstitute with 1000 ml of deionized or distilled water in a clean container. Mix well. Allow MSU-EB to reach room temperature before use. Store dry MSU-EB refrigerated. Reconstituted MSU-EB may be refrigerated for up to 2 months. Do not freeze.

8.5. M2 Buffer

Reconstitute with 50.0 ml deionized or distilled water. Mix well. Store dry M2 Buffer refrigerated. Reconstituted M2 Buffer may be refrigerated for up to 2 months. Do not freeze.

8.6. Opti-Fluor® Scintillation Fluid (Purchased Separately)

Charm II tests use Opti-Fluor scintillation fluid specifically manufactured for Charm Sciences. A Material Safety Data Sheet is available at http://las.perkinelmer.com/ catalog/MSDSSearch.htm and certificate of analysis is available at http:// las.perkinelmer.com/catalog/COASearch.htm. Other scintillation fluids may yield erroneous results. Dispose of waste or used scintillation fluid by flushing down a sink drain with at least 25 times the volume of water, or follow applicable regulations. Store at room temperature. Expires 2 years from date of manufacture or 6 months after opening.

8.7. Standard Solutions

1. Negative Control

Add 2.0 ml Tissue Performance Negative Concentrate to 6.0 ml MSU Extraction Buffer to make the Negative Control. Mix well before use. Use at room temperature

(15 to 25°C) for up to 6 hours. Use 5.0 ml Negative Control in Step 3 of **Charm II Chloramphenicol Test - Procedure**.

2. Positive Control

Add 300 µl (0.3 ml) of 10 ppb Chloramphenicol Standard to 4.7 ml Tissue Performance Negative Concentrate. Mix well. Dilute 2.0 ml of this mixture with 6.0 ml of MSU Extraction Buffer to make the Positive Control. Mix well before running assay. Use at room temperature (15 to 25°C) for up to 6 hours. Use 5.0 ml Positive Control in Step 3 of **Charm II Chloramphenicol Test - Procedure**.

9. PROCEDURE

9.1. Sample Extraction

Label 50 ml centrifuge tube.

Pour MSU Extraction Buffer into centrifuge tube to the 20 ml mark.

Trim excess fat from tissue. Cut and add pieces of tissue (20 g) to centrifuge tube until liquid level reaches 40 ml mark.

To fortify tissue (for Control Point determination only): Add 0.6 ml of 10 ppb Chloramphenicol standard.

Homogenize sample:

If using a food processor - Pour buffer and tissue pieces into food processor. Homogenize for 30 to 60 seconds. If processing more than one sample, clean food processor thoroughly after each sample to avoid cross-contamination.

If using a stomacher - Pour buffer and tissue pieces into stomacher bag and homogenize for 2 minutes or as needed to break up tissue.

If using a tissue press - Pour buffer and tissue pieces into Whirl-Pak bag. Fold bag over 4 to 5 times, removing air, and seal with attached wire. Stamp bag with press 60 times or as needed to break up tissue.

Return all of the homogenate to the same centrifuge tube.

Place centrifuge tube in 80 ± 2°C large bore incubator for 45 minutes. (For larger volume testing an 80 ± 2°C water bath may be used in place of large bore incubator.)

Place centrifuge tube in ice water bath for 10 minutes.

Centrifuge tubes 10 minutes at 1750 G (setting 7 on IEC Clinical centrifuge, 3.3 x 1000 rpm on IEC Centra CL-2 centrifuge, or 33 x 100 rpm on Hettich Rotofix 32 centrifuge).

Pour off top layer into a clean centrifuge tube for testing. Avoid decanting any floating fat particles with supernatant. Run through filter paper or cheese cloth if necessary.

Check pH with pH indicator strips; pH of extract should be equivalent to 7.5 on pH strip. If pH is low, add 300 µl M2 Buffer, mix and retest pH. If pH is still low, slowly add M2 Buffer dropwise, mix, and retest pH until desired pH is reached.

NOTE: If pH is high, add 300 µl 0.1M HCl, mix, and retest. If pH is still high, add 0.1M HCl drop-wise, mix, and retest. Chemical grade HCl is not provided with test kits, as almost all sample extractions are slightly acidic or neutral.

Run extracts on same day as they are prepared. Allow extract to reach room temperature immediately prior to running assay.

9.2. TEST PROCEDURE

Note: Up to six tests may be run at a time. Allow extract to reach room temperature immediately prior to running assay. pH of extract should be equivalent to 7.5 on a pH strip.

Step 1

Add white tablet to the empty test tube.

Step 2

Add 300 ± 100 µl water.

Mix 10 seconds to break up tablet. Take additional time if required to be sure tablet is broken up.

Step 3

Add 5.0 ± 0.25 ml extract or control.

Use a new tip for each sample.

Immediately mix by swirling sample up and down 15 times for 15 seconds.

Step 4

Incubate at 50 ± 2°C for 3 minutes.

Step 5

Remove test tube from incubator and mix by swirling sample up and down 15 times for 15 seconds.

Step 6

Incubate at 50 ± 2°C for 3 minutes.

Step 7

Add green tablet.

Immediately mix by swirling sample up and down 15 times for 15 seconds.

Tablet addition and mixing of all samples should be completed within 40 seconds.

Step 8

Incubate at 50 ± 2°C for 3 minutes.

Step 9

Centrifuge for 10 minutes at 1750 G (setting 7 on IEC Clinical centrifuge, 3.3 x 1000 rpm on IEC Centra CL-2 centrifuge, or 33 x 100 rpm on Hettich Rotofix 32 centrifuge).

Step 10

Immediately pour off extract. (Any delay from time centrifuge stops may cause pellet to slide out of test tube with extract.)

Blot edge of test tube on absorbent towel.

Step 11

Add 300 ± 100 µl water.

Mix thoroughly to break up pellet.

Pellet must be suspended in water before adding scintillation fluid.

Step 12

Add 3.0 ± 0.5 ml scintillation fluid.

Cap and invert (or shake) until mixture has a uniform cloudy appearance.

Step 13

Immediately count in Analyzer for 60 seconds. Read cpm (count per minute) on [3H] channel. Count within 10 minutes of adding scintillation fluid.

Recount if greater than and within 50 cpm of the Control Point.

10. REFERENCES

AOAC Official Methods of Analysis. (2012). Appendix F: Guidelines for standard method performance requirements.

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