MORANTEL AND PYRANTEL DRUG-RELATED METABOLITES

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DETERMINATIVE METHOD

A. INTRODUCTION

Theory

This assay is designed to detect and estimate morantel ([E]-1, 4, 5, 6-tetrahydro-1-methyl-2-[2-(3methyl-2-thienyl)vinyllpyrimidine and drug-related metabolites that are hydrolyzable to N-methyl-1, 3-propanediamine in bovine liver and muscle. Calibration curves are constructed to determine morantel residues in the range of 0.2 to 0.8 ppm in muscle and 0.4 to 1.2 in liver. It is also designed to detect and estimate pyrantel (trans-1, 4, 5, 6-tetrahydro-1-methyl-2-[2-(2-thienyl)vinyllpyrimidine) and drug-related metabolites that are hydrolyzable to N-methyl-1, 3-propanediamine in swine liver and muscle. A calibration curve is constructed to determine pyrantel residues in the range of 0.4 to 1.2 ppm in muscle and 6 to 12 ppm in liver. Tissue containing morantel and metabolites or tissue containing pyrantel and metabolites are hydrolyzed in aqueous potassium hydroxide to yield N-methyl-1, 3-propanediamine, a product common to drug-related residues. N-ethyl, 3-propanediamine dihydrochloride is added as an internal standard to the sample before the hydrolysis step at a concentration equivalent to 0.4 ppm morantel or 4 ppm pyrantel in tissue. Following hydrolysis, both diamines are extracted into toluene, back-extracted into dilute aqueous acid, and converted to their bis-2-nitro-4-trifluoromethylphenyl derivatives with 4-fluoro-3-nitrobenzotrifluoride in a borax-buffered solution. The diamine derivatives are separated from the reaction mixture by extraction into n-hexane and purified by thin-layer chromatography prior to evaluation by pulsed electron capture gas-liquid chromatography.

Accurate results are obtained by referring the relative peak heights of the diamine derivatives of the pyrantel-related compound and the internal standard to a standard curve. This curve is constructed by relating, in a linear manner, the relative peak height response of calibration solutions of the diamine derivatives to a weight ratio of each. The concentrations of the standards are selected to optimize the working range of the GLC and to reflect residue levels ranging from one-half to two times the action level. A broader dynamic range may be employed, but the recommended range simplifies GLC calibration and assay of tissue extracts. From the established relationship of the relative response of the two diamines to weight ratio, and knowing the amount of internal standard added to the samples, the unknown concentration of morantel or pyrantel can be obtained by a graphical extrapolation or a linear regression calculation.
DETERMINATIVE METHOD

B. EQUIPMENT

1. Apparatus

a. Precoated thin-layer plates: 20 x 20 cm, 250 μ thickness, LK6DF (channeled plate, preadsorbant area, silica gel, fluorescent indicator).

b. Desega multiplate developing tank: for ten 5 x 20 cm TLC plates, distributed by Brinkmann Instruments, Inc., Westbury, NY, or equivalent.

c. Centrifuge tubes, heavy-duty: 50 mL graduated (60 mL capacity), equipped with glass stoppers, Lab Glass, Inc., or equivalent.

d. Centrifuge tubes: 15 and 50 mL graduated, equipped with glass stoppers.

e. Volumetric flasks: 50, 100, 200, and 250 mL capacity, glass-stoppered.

f. Glassine paper.

g. Pipets, automatic transfer: 25 mL delivery volume.

h. Pipets, measuring: 0.5 mL delivery volume.

i. Pipet: ultramicro, 0.100 mL capacity, Scientific Glass Apparatus Co., Inc., JM-6840, or Medical Laboratory Automation, Mt. Vernon, NY.

j. Aluminum foil.

k. Electronic calculator equipped with a linear regression program.

l. Pipets, volumetric: 1, 2, 3, 4, and 5 mL delivery volume.

m. Pipets, serological: 10 mL delivery volume.

n. Pipets, Pasteur disposable.

o. Propipet bulb.


q. Hamilton syringe: 10 mL capacity.

r. Screw-cap test tubes with Teflon liners, A. H. Thomas, #9447-E50, or equivalent.

s. Crystallizing dish: 190 mm (diameter) x 100 mm (height), for oil bath.

t. Scalpel.

u. Test tube rack.

v. Test tube mixer: Vortex mixer, or equivalent.

w. Water bath.

x. Thermo-stir hot plate.
DETERMINATIVE METHOD

B. EQUIPMENT (Continued)

y. Magnetic stirrer bar (Teflon).

z. Thermometer, Celsius, 0-150° C range.

aa. Knife, for cutting frozen tissue.

bb. Sartorius electronic balance, model 3716, or equivalent.

c. Cahn electrobalance, model G-2, or equivalent.

d. Centrifuge, international size 2: model K, or equivalent.

2. Instrumentation

Gas liquid chromatograph: Hewlett Packard 5880, or equivalent, equipped with electron capture detector. Column 6 ft x 4 mm id packed with 3% OV-17 on Gas Chromosorb Q 100/120 mesh. Septum: high-temperature type (HT-13), Applied Science Laboratories, Inc., or equivalent.
DETERMINATIVE METHOD

C. REAGENTS AND SOLUTIONS

<table>
<thead>
<tr>
<th>Reagent and Solution List</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. N-hexane, glass distilled, Burdick and Jackson, or equivalent.</td>
</tr>
<tr>
<td>b. Toluene, glass distilled, Burdick and Jackson, or equivalent.</td>
</tr>
<tr>
<td>c. Ethyl acetate, glass distilled, Burdick and Jackson, or equivalent.</td>
</tr>
<tr>
<td>d. Acetone, glass distilled, Burdick and Jackson, or equivalent.</td>
</tr>
<tr>
<td>e. Potassium hydroxide, pellets, 85%, reagent, J. T. Baker, or equivalent.</td>
</tr>
<tr>
<td>f. Sodium hydroxide, pellets, reagent grade.</td>
</tr>
<tr>
<td>g. Sodium tetraborate decahydrate, reagent grade.</td>
</tr>
<tr>
<td>h. Boric acid, reagent grade.</td>
</tr>
<tr>
<td>i. 4-Fluoro-3-nitrobenzotrifluoride, INC Life Sciences Group (K &amp; K), Plainview, NY.</td>
</tr>
<tr>
<td>j. Thymol blue indicator; Matheson, Coleman, and Bell; or equivalent.</td>
</tr>
<tr>
<td>k. 0.2N Hydrochloric acid.</td>
</tr>
<tr>
<td>l. 4M Potassium hydroxide; dissolve 264 g of 85% KOH in distilled water, dilute to 1 L.</td>
</tr>
<tr>
<td>m. 1M Sodium hydroxide.</td>
</tr>
<tr>
<td>n. 0.1M Sodium hydroxide.</td>
</tr>
<tr>
<td>o. 0.1M Sodium tetraborate.</td>
</tr>
<tr>
<td>p. 0.2M Boric acid.</td>
</tr>
<tr>
<td>q. 0.2% 4-Fluoro-3-nitrobenzotrifluoride: Dilute 0.135 mL (200 mg) of 4-fluoro-3-nitrobenzotrifluoride to 100 mL with distilled acetone. Fresh solutions are prepared. Use pipet control device (Adams, or equivalent) to deliver reagent.</td>
</tr>
<tr>
<td>r. 0.1% Thymol blue: Dissolve 100 mg of thymol blue in 100 mL of 0.1M sodium hydroxide.</td>
</tr>
<tr>
<td>s. Toluene-ethyl acetate (85:15): Dilute 150 mL of ethyl acetate to 1 L with toluene.</td>
</tr>
</tbody>
</table>
DETERMINATIVE METHOD

D. STANDARDS

1. Source

Morantel tartrate; pyrantel tartrate; N-ethyl-1, 3-propanediamine dihydrochloride; N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-ethyl-1, 3-propanediamine; N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-methyl-1, 3-propanediamine
—Pfizer, Inc.

2. Preparation of Standards

a. N-Ethyl-1, 3-propanediamine dihydrochloride internal standard solutions.

i. Stock solution A: Dissolve 42.5 mg reference standard N-ethyl-1, 3-propanediamine dihydrochloride in distilled water to dilute to 100 mL.

ii. Working standard solution B: Dilute 1.0 mL stock solution A to 25.0 mL with distilled water (conc. 17.0 µg/mL; 0.097 µmoles/mL).

iii. Working standard solution C: Dilute 5.0 mL of working standard solution B to 50.0 mL with distilled water (conc. 1.79 µg/mL; 0.097 µmoles/mL).

b. Morantel tartrate solutions.

i. Stock solution A: Dissolve 105.07 mg reference standard morantel tartrate (equivalent to 62.5 mg morantel) in distilled water and dilute to 100.0 mL (conc. 625 µg/mL as morantel; 2.84 µmoles/mL).

ii. Working standard solution B: Dilute 4.0 mL of stock solution A to 50.0 mL with distilled water (conc. 50.0 µg/mL as morantel; 0.227 µmoles/mL).

iii. Working standard solutions C, D, and E: Dilute 1.0, 2.0, and 4.0 mL of working standard solution B to 50 mL with distilled water (conc. 1.0, 2.0, and 4.0 µg/mL as morantel; 0.0045, 0.0091, and 0.018 µmoles/mL).

c. Pyrantel tartrate solutions.

i. Stock solution A: Dissolve 107.96 mg reference standard pyrantel tartrate (equivalent to 62.5 mg pyrantel) in distilled water and dilute to 100.0 mL (conc. 625.0 µg/mL as pyrantel; 3.034 µmoles/mL).

ii. Working standard solution B: Dilute 4.0 mL of stock solution A to 50.0 mL with distilled water (conc. 50 µg/mL as pyrantel; 0.2427 µmoles/mL).

iii. Working standard solution C: Dilute 20 mL of stock solution A to 50.0 mL with distilled water (conc. 50 µg/mL as pyrantel; 0.1214 µmoles/mL).

iv. Working standard solution D: Dilute 5 mL of working standard solution B to 50 mL with distilled water (conc. 5.0 µg/mL as pyrantel; 0.0243 µmoles/mL).

v. Working standard solution E: Dilute 5 mL of working standard solution C to 50 mL with distilled water (conc. 2.5 µg/mL as pyrantel; 0.0121 µmoles/mL).
d. N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-ethyl-1, 3-propanediamine.
i. Stock solution A: Dissolve 5.15 mg of reference standard N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-ethyl-1, 3-propanediamine in 200 mL toluene (stable if protected from light).

ii. Working standard solution B: Dilute 10.0 mL of stock solution A to 100.0 mL with toluene (conc. 2.58 µg/mL).

e. N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-methyl-1, 3-propanediamine.
i. Stock solution A: Dissolve 5.00 mg of reference standard N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-methyl-1, 3-propanediamine in 200 mL toluene (stable if protected from light).

ii. Working standard solution B: Dilute 10.0 mL of stock solution A to 100.0 mL with toluene (conc. 2.50 µg/mL).

f. Gas-Liquid chromatography calibration standard solutions for morantel in liver and pyrantel in muscle. Dilute 6.0, 5.0, 4.0, 3.0, and 2.0 mL aliquots respectively of N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-methyl-1, 3-propanediamine working standard solution B with 2.0 mL of N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-ethyl-1, 3-propanediamine working standard solution B and adjust each solution to 250.0 mL with toluene. Cover flasks with aluminum foil. The concentrations of these derivatives are:

<table>
<thead>
<tr>
<th>Solution Number</th>
<th>N-methyl-1, 3-propane diamine derivative</th>
<th>N-ethyl-1, 3 propane diamine derivative</th>
<th>Molar ratio</th>
<th>Equivalent ppm morantel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0600 1.29 × 10^{-4}</td>
<td>0.206 4.29 × 10^{-3}</td>
<td>3.0</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>0.0500 1.07 × 10^{-4}</td>
<td>2.5 1.0</td>
<td>2.0</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>0.0400 8.58 × 10^{-5}</td>
<td>1.5 0.6</td>
<td>1.0</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>0.0300 6.43 × 10^{-5}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.0200 4.29 × 10^{-5}</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DETERMINATIVE METHOD

D. STANDARDS (Continued)

<table>
<thead>
<tr>
<th>Solution Number</th>
<th>N-methyl-1, 3-propane diamine derivative† µg/mL</th>
<th>N-ethyl-1, 3 propane diamine derivative‡ µg/mL</th>
<th>Molar ratio: Equivalent ppm</th>
<th>Equivalent ppm pyrantel§</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0600</td>
<td>0.206</td>
<td>3.0</td>
<td>12.0</td>
</tr>
<tr>
<td>2</td>
<td>0.0500</td>
<td>1.07 x 10⁻⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.0400</td>
<td>8.58 x 10⁻⁴</td>
<td>2.0</td>
<td>8.0</td>
</tr>
<tr>
<td>4</td>
<td>0.0300</td>
<td>6.43 x 10⁻⁴</td>
<td>1.5</td>
<td>6.0</td>
</tr>
</tbody>
</table>

† = N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-methyl-1, 3-propanediamine.
‡ = N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-ethyl-1, 3-propanediamine.
§ = When the internal standard is added to tissue at a level equivalent to 0.4 ppm pyrantel.

3. Storage Conditions

Stock and working solutions of morantel tartrate, pyrantel tartrate, and N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-ethyl-1, 3-propanediamine should be stored in the refrigerator.

4. Shelf Life Stability

Stock and working solutions of morantel tartrate, pyrantel tartrate, and N, N'-bis-(2-nitro-4-trifluoromethylphenyl)-N-ethyl-1, 3-propanediamine are stable for at least one month when properly stored.
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E. EXTRACTION PROCEDURE

1. Sample Preparation
   Slice frozen tissue into small pieces, keeping it frozen during weighing.

2. Sample Extraction
   a. Transfer 5.0 g of sliced tissue to a screw-cap test tube. Add 1.0 mL N-ethyl-1, 3-propanediamine dihydrochloride working internal standard solution B and 10 mL 4M potassium hydroxide solution. Cap the tube.

   NOTE: To estimate morantel recoveries at the 0.2, 0.4, and 0.8 ppm levels, 5.0 g control sliced tissue is fortified with 1.0 mL morantel tartrate working standard solutions C, D, and E (1.0, 2.0, and 4.0 μg/mL as morantel base). To estimate pyrantel recoveries at the 1.88, 3.74, and 7.5 ppm levels, 5.0 g control sliced tissue is fortified with 1.0 mL pyrantel tartrate working standard solutions C, D, and E.

   b. Immerse the tube in an oil bath at 110° C so that the contents of the tube are just below the oil level. Digest the samples for 16-18 hours (overnight). Cool the hydrolysates in an ice bath.

   c. Pour the hydrolysate into a 50 mL centrifuge tube and add 6-7 g of potassium hydroxide pellets. Stopper, cool the sample, and dissolve the pellets by mixing on a test tube mixer. Return the tube to the ice bath.

   d. Wash the test tube used for hydrolysis with 20 mL toluene and transfer the solvent to the centrifuge tube containing the hydrolysate. Stopper and shake gently for about 15 sec. Centrifuge the mixture at 2000-2500 rpm for 5 min to clarify the phases. Recover the toluene layer using a blow-out pipet equipped with a propipet bulb and transfer this extract to a 50 mL centrifuge tube.

   e. Re-extract hydrolysate with an additional 20 mL toluene, centrifuge to clarify the extract, and combine the extracts. Discard the aqueous layer.

   f. Add 5.0 mL of 0.2N HCl to the combined toluene extracts, shake for 10-15 sec, and centrifuge at 2000 rpm.

   g. Aspirate the toluene layer and discard. Pipet 40 mL of the aqueous layer into a 50 mL centrifuge tube and add 1 drop of the thymol blue indicator. (Solution should be pink.) Add 0.5 mL of 0.2M boric acid and 1N sodium hydroxide until the color of the solution changes from pink to blue. Add 1 mL of 0.1M sodium tetraborate solution.

   h. Add 5.0 mL of 0.2% 4-fluoro-3-nitro-benzotrifluoride solution, stopper, mix by gentle hand shaking, and place in a water bath set at 50-55° C for 16 hours (overnight).

   i. Cool solution to room temperature. Add 5.0 mL hexane and 25 mL water. Mix on test tube mixer and centrifuge at 2000 rpm.

3. Thin-Layer Chromatography
   a. Apply 50 μL of each of the hexane extracts to individual channels. Also prepare control channels by applying 50 μL of one of the sample hexane extracts. Let dry and overlay with 50 μL of chromatography marker solution. (Avoid developing plates in areas subject to drafts.)
DETERMINATIVE METHOD

E. EXTRACTION PROCEDURE (Continued)

b. Prior to chromatographic development, place an edge (ca 5 mm deep) of the thin-layer plate into a beaker of ethyl acetate so that the solvent will rise through the applied sample zones to form them into narrow bands 1 cm above their origins. Air-dry the plate in a hood before chromatographic development.

c. Place the prepared plate in a chromatographic chamber lined with blotting paper and saturated with toluene-ethyl acetate (85:15). Develop until the solvent front reaches the top of the plate. Air-dry the developed plate in a fume hood to remove toluene.

d. Examine the plate under short wavelength ultraviolet light (254 nm) and locate on the control channel the zone containing the N, N’-bis-(2-nitro-4-trifluoromethylphenyl) derivatives of N-methyl-1, 3-propanediamine and N-ethyl-1, 3-propanediamine (R,’s ca 0.4 and 0.5 respectively).

e. Line up the tissue-related bands on the sample and control channels. Mark out a zone of silica gel on the sample channel encompassing an area containing each derivative.

NOTE: Allow for irregularities in the chromatographic migration of sample bands in zones to be removed by scraping along their periphery. Extracts of the derivative may be stored at room temperature for replication of the TLC step.

f. Using a 5 3/4” Pasteur pipet with a glass wool plug, connect the narrow end to a vacuum supply and, with low vacuum, scrape and aspirate the selected zone up to the plug. Pour the powder into a 15 mL centrifuge tube and add 1.0 mL toluene. Drain the solvent into a centrifuge tube. Mix thoroughly and centrifuge. Examine the supernatant by gas-liquid chromatography.
DETERMINATIVE METHOD

F. ANALYTICAL QUANTITATION

1. Instrumental Settings and Conditions
   a. Temperature.
      i. Column—260° C.
      ii. Injection port—280° C.
      iii. Detector—300° C.
   b. Carrier gas: argon/methane at 60 mL/min.

2. General Operation
   Inject into the gas-liquid chromatograph equal μL aliquots of samples and GLC calibration solutions. The peak height of the "EAPA" component of these standards should approximate 30-40% of full-scale deflection. The retention times of the "MAPA" and "EAPA" components, under the recommended GLC conditions, are 4.7 and 3.8 min respectively.
DETERMINATIVE METHOD

G. CALCULATIONS

1. Procedure

a. Compute the peak height (mm) ratio of the N-methyl to N-ethyl derivatives in each GLC calibration solution. Fit the data to the best straight line. Calculate the slope and the intercept for the standard curve using the peak height ratio as the "Y" coordinate and the equivalent ppm morantel or pyrantel level of the standards as the "X" coordinate.

b. From the standard slope and intercept values and the observed peak height ratio of the bis-(2-nitro-4-trifluoromethylphenyl) derivatives of N-methyl-1,3-propanediamine and N-ethyl-1,3-propanediamine in the sample, determine the ppm of morantel in the tissue sample by the following equation:

\[
\text{ppm morantel in tissue} = \frac{\text{Peak height ratio of MAPA to EAPA in sample}-\text{intercept (Std)}}{\text{Slope (Std)}}
\]

Determine the ppm of pyrantel in the tissue sample by the following equation.

\[
\text{ppm pyrantel in tissue} = \frac{\text{Peak height ratio of MAPA to EAPA in sample}-\text{intercept (Std)}}{\text{Slope (Std)}}
\]

2. References


b. NADA 92-444, 93-903, and 43-290, Pfizer.
DETERMINATIVE METHOD

J. QUALITY ASSURANCE PLAN

1. Performance Standard

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Analytical Range (ppm)</th>
<th>Acceptable Recovery (%)</th>
<th>Repeatability %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morantel</td>
<td>0.2-0.8 (muscle) 0.4-1.2 (liver)</td>
<td>80-110</td>
<td>≤ 10</td>
</tr>
<tr>
<td>Pyrantel</td>
<td>0.4-1.2 (muscle) 6-12 (liver)</td>
<td>80-110</td>
<td>≤ 10</td>
</tr>
</tbody>
</table>

2. Critical Control Points and Specifications

<table>
<thead>
<tr>
<th>Record</th>
<th>Acceptable Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Weight of sample</td>
<td>5.0 g ± 0.01 g</td>
</tr>
<tr>
<td>b. Weight of KOH</td>
<td>6.0-7.0 g</td>
</tr>
<tr>
<td>c. Temperature of water bath</td>
<td>50-55 °C</td>
</tr>
<tr>
<td>d. Final volume of toluene</td>
<td>1.0 mL</td>
</tr>
</tbody>
</table>

3. Readiness To Perform

a. Familiarization.
   i. Phase I: Standards—5 levels, 3 replicates each (each compound).
      (a) Morantel: 0.4, 0.6, 0.8, 1.0, and 1.2 ppm.
      (b) Pyrantel: 4.0, 6.0, 8.0, 10.0, and 12.0 ppm.
   ii. Phase II: Fortified samples—5 levels, 3 replicates each (each compound) over a minimum of 3 days (30 total samples).
      NOTE: Phase I and Phase II may be performed concurrently.
   iii. Phase III: Check samples for analyst accreditation.
      (a) 14 samples from FSIS Western Laboratory (samples submitted by supervisor if only one laboratory is performing this test).
      (b) Report analytical findings to Chemistry Division.

   Letter from Chemistry Division is required to commence official analysis.

b. Acceptability criteria.
   Refer to section J.1 above.

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DETERMINATIVE METHOD

J. QUALITY ASSURANCE PLAN (Continued)

4. Intralaboratory Check Samples
   a. System, minimum contents.
      i. Frequency: 1 per week per analyst, or 20% of official samples analyzed (whichever is smaller).
      ii. Records are to be maintained by the analyst and reviewed by the supervisor and Laboratory QA Officer for:
          (a) All replicate findings.
          (b) Running average difference between replicates.
          (c) All % recoveries.
          (d) Running average, standard deviation, and CV for recoveries.
          (e) Appropriate CUSUM charts.
   b. Acceptability criteria.
      If unacceptable values are obtained, then:
      i. Stop all official analyses by that analyst.
      ii. Investigate and identify probable cause.
      iii. Take corrective action.
      iv. Repeat Phase III of section J.3 above if cause was analyst-related.

5. Sample Acceptability and Stability
   a. Matrices.
      i. Morantel—Bovine liver and muscle.
      ii. Pyrantel—Swine liver and muscle.
   b. Sample receipt size, minimum: Varied; enough to obtain matrix for all required screening, quantitation, and confirmation tests, and reserve sample.
   c. Condition upon receipt: Frozen.
   d. Sample storage:
      i. Time: Not yet determined.
      ii. Condition: Frozen.

MORANTEL AND PYRANTEL
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J. QUALITY ASSURANCE PLAN (Continued)

6. Sensitivity

   a. Lowest detectable level (LDL):
   
   b. Lowest reliable quantitation (LRQ):
   
   c. Minimum proficiency level (MPL): 0.5 ppm.