



MYCOPLASMA TISSUE CULTURE NI (MTC-NI)

RAPID DETECTION SYSTEM

INTENDED USE

The GEN-PROBE® MYCOPLASMA TISSUE CULTURE NI (MTC-NI) RAPID DETECTION SYSTEM is a DNA probe test for the detection of mycoplasma contamination in tissue culture.

For laboratory use.

SUMMARY AND EXPLANATION OF THE TEST

Species from the genus *Mycoplasma* are a serious source of contamination in tissue cultures. Mycoplasmas grow to high titers in cell cultures with concentrations of up to 10⁸ colony forming units per milliliters of supernatant medium. Additionally, Mycoplasma may be found attached to the cell membranes (1, 4).

These organisms, which lack cell walls, do not normally produce turbidity in cell cultures and, therefore, cannot be detected visually. Mycoplasmas are resistant to antibiotics that act on the cell wall, making them difficult to eliminate from tissue cultures.

The incidence and effects of mycoplasma contamination of tissue cultures have been described by Barile et al. (2) and McGarrity and Kotani (6). Infections have been demonstrated in up to 15% of cell lines after passage. Therefore, it has been recommended that all cell lines should be screened monthly to properly control spread of the contamination (3, 6, 9).

When present in a culture, mycoplasma interfere with investigational cell parameters. Mycoplasma can induce chromosomal abnormalities and alter the antigenicity of cell membranes. They can affect cellular metabolism by competing for nutrients and influence cell fusion procedures (8).

Six species, from the genera *Mycoplasma* and *Acholeplasma* have been shown to cause 98% of laboratory infections (5). These are:

<i>Acholeplasma laidlawii</i>	<i>Mycoplasma hyorhinis</i>
<i>Mycoplasma orale</i>	<i>Mycoplasma salivarium</i>
<i>Mycoplasma arginini</i>	<i>Mycoplasma hominis</i>

with *Mycoplasma hyorhinis* causing 30-40% of infections in some laboratories.

Early methods for the detection of mycoplasma relied on culture or biochemical procedures. Culturing mycoplasma can take up to three weeks and is difficult due to their requirements for microaerophilic growth conditions and specialized media. In addition, some species cannot be cultivated by routine methods.

The GEN-PROBE MTC-NI RAPID DETECTION SYSTEM employs the principle of nucleic acid hybridization and ribosomal RNA (rRNA) detection (10). It is possible to detect positive samples in 75 minutes. The MTC-NI kit utilizes an all bacterial probe that detects all species of *Mycoplasma* and *Acholeplasma* which commonly infect tissue culture as well as other bacterial species which may be present.

PRINCIPLES OF THE PROCEDURE

Nucleic acid hybridization assays are based on the ability of complementary nucleic acid strands to come together to form stable double-stranded complexes. The GEN-PROBE MTC-NI uses a single-stranded DNA probe with a chemiluminescent label which is complementary to the rRNA of the target organism. After the ribosomal RNA is released from the organism, the labeled DNA probe combines with the target organism's ribosomal RNA to form a stable DNA:RNA hybrid. The Selection Reagent allows for the differentiation of non-hybridized and hybridized probe. The labeled DNA:RNA hybrids are

measured in a GEN-PROBE luminometer (7). A positive result is a luminometer reading equal to or greater than the cut-off. A value below this cut-off is a negative result.

REAGENTS

Reagents for the MTC-NI Detection System are provided in two separate reagent kits:

MTC-NI KIT 50 tests	Cat No. 4573
Reagent 1 (Probe Reagent)	5 x 10 tubes
Positive Control	1 x 2.5 mL buffered RNA solution
Reagent 2 (Hybridization Reagent)	1 x 15 mL buffered solution
Reagent 3 (Selection Reagent)	1 x 20 mL buffered solution

GEN-PROBE® DETECTION REAGENT KIT Cat No. 1791

Detection Reagent I	1 x 240 mL
0.1% hydrogen peroxide in 0.001 N nitric acid	
Detection Reagent II	1 x 240 mL
1N sodium hydroxide	

MATERIALS REQUIRED BUT NOT PROVIDED

Water bath or heating block (60±1°C)
Micropipettes (100 µl, 300 µl)
Re-pipettor (300 µl)
Vortex Mixer
Microcentrifuge (12,000-15,000 x g)
Microcentrifuge tubes (2 mL)

Available from your Gen-Probe distributor

GEN-PROBE® Luminometer (LEADER® 50i, Cat. No. 3100i)
GEN-PROBE® Detection Reagent Kit (Cat. No. 1791)
GEN-PROBE® Heating Block (60±1°C) (Cat. No. 2775)

WARNINGS AND PRECAUTIONS

- For laboratory use only. Not for use in diagnostic procedures.
- Use universal precautions when performing this assay.
- Use for the detection of mycoplasma contamination in tissue cultures.
- Use only supplied or specified disposable laboratory ware.
- Avoid contact of Detection Reagents I and II with skin and mucous membranes. Wash with water if these reagents come into contact with skin. If spills of these reagents occur, dilute with water before wiping dry.

STORAGE AND HANDLING REQUIREMENTS

Probe Reagent Tubes must be stored in the foil pouches at 2° to 8°C. The Probe Reagent Tubes are stable in the unopened pouches until the expiration date indicated. Once opened, the pouch should be resealed, stored at 2° to 8°C, and the tubes should be used within one month and prior to the expiration date.

The positive control should be stored at 2° to 8°C and warmed with gentle mixing at 60°C for 2 minutes prior to use.

Other reagents used in the MTC-NI Kit may be stored between 2° to 25°C and are stable until the expiration date indicated.

MTC-NI reagent labels include the following standardized symbols for storage and handling instructions:

Expiration Date **Y** Lot Number **a**

Temperature Limitation

Symbols are based on European Standard EN980 and ISO/TR15223:1998(E) recommendations.

DO NOT FREEZE THE REAGENTS.

SAMPLE COLLECTION

The GEN-PROBE MTC-NI RAPID DETECTION SYSTEM is designed to detect mycoplasma contamination in tissue culture. Tissue culture cell suspensions may also be assayed using this method. This test may be performed on cultures grown in antibiotic-containing media, but Mycoplasma levels will be reduced due to the presence of the antibiotics. It is recommended that cells be passed twice in the absence of antibiotics before being assayed. Media to be tested should be exposed to cells a minimum of three days. Refer to the PROCEDURAL NOTES for processing specimens that do not meet these criteria. For maximum sensitivity samples should be prepared within 4 hours of collection. If this is not possible the media may be stored at 2° to 8°C for no longer than 3 days before sample preparation. Samples may be archived or stored for later assay by processing them through step 3 of SAMPLE PREPARATION. The hybridization buffer should be added to the pellet and the sample stored at -20°C or below for one month.

NOTE: Samples which are visibly turbid may be contaminated with yeast or other bacteria and should be discarded.

TEST PROCEDURE

A. EQUIPMENT PREPARATION

1. Adjust the heating block or water bath to 60°± 1°C.
2. Prepare the GEN-PROBE luminometer for operation. Make sure there is sufficient volume of Detection Reagents I and II to complete the tests.

B. SAMPLE PREPARATION

1. Pipette 1.5 mL of tissue culture medium into a microcentrifuge tube. Identify and mark the side of the tube on which the pellet will form. For samples containing greater than 1 million eukaryotic cells please refer to PROCEDURAL NOTES.
2. Centrifuge at 12,000-15,000 x g for 10 minutes. Remove all of the supernatant with a Pasteur pipette and discard. The pellet may not be visible and care must be taken to avoid any loss of the pellet when removing the supernatant.
3. Add 100 µl of Hybridization Reagent to the microcentrifuge tube. Vortex to mix thoroughly. (These samples may be stored at -20°C or below for 1 month. Frozen samples should be heated at 60° ± 1°C for 2 minutes and vortexed to achieve homogeneity prior to use in the assay.)

C. HYBRIDIZATION

1. Open the foil pouch by cutting evenly across the top of the pouch. Remove enough Probe Reagent Tubes to test the samples and/or controls. Re-seal the pouch by folding the opened edge over several times and securing with adhesive tape or a clip. Leave the desiccant pillow in the pouch and store the resealed pouch at 2° to 8°C.
2. Label a sufficient number of Probe Reagent Tubes for samples and controls. Remove and retain the caps.
3. Pipette 100 µl Hybridization Reagent into the bottom of the Negative Control Tubes.
4. Pipette 100 µl of Positive Control into the bottom of the Positive Control Tubes.
5. Pipette 100 µl of each sample into the bottom of the appropriately labeled sample tube.
6. Recap all tubes and vortex at moderate speed for 1 to 3 seconds to mix. All liquid should be in the bottom of the tubes during the incubation.
7. Incubate for 45 minutes at 60° ± 1°C in a water bath or heat block.

D. SELECTION

1. Remove the Probe Reagent Tubes from the water bath or heating block. Remove and retain the caps. Immediately pipette 300 µL of Reagent 3 (Selection Reagent) into each tube. Re-cap the tubes and vortex at moderate speed for 1 to 3 seconds to mix. All liquid should be in the bottom of the tubes during the incubation.

2. Incubate the Probe Reagent Tubes for 10 minutes at 60°± 1°C in a water bath or heating block.
3. Remove the Probe Reagent Tubes from the water bath or heating block and leave them at room temperature for at least 5 minutes. Remove and discard the caps. Read the results in the luminometer within 30 minutes after removing from the water bath or heating block.

E. DETECTION

1. Select the appropriate protocol from the menu of the luminometer software.
2. Using a damp tissue or paper towel, wipe each tube to ensure that no residue is present on the outside of the tube and insert the tube into the GEN-PROBE luminometer according to the instrument directions.
3. When the analysis is complete, remove the tube(s) from the luminometer.

PROCEDURAL NOTES

- A. REAGENT: Reagent 2 (Hybridization Reagent) and the Positive Control may precipitate. Warming and mixing the solution at 60° ± 1°C for 2 minutes will dissolve this precipitate.
- B. TEMPERATURE: The Hybridization and Selection reactions are temperature dependent. Therefore, it is imperative that the water bath or heating block is maintained within the specified temperature range during the entire assay.
- C. TIME: The Hybridization and Selection reactions are time dependent. Hybridize at least 45 minutes but no more than 60 minutes. Incubate the Probe Reagent Tubes during the SELECTION step for at least 10 minutes but no more than 12 minutes.
- D. WATER BATH: The level of water in the water bath should be maintained to ensure that the liquid reaction mixture in the Probe Reagent Tube is completely submerged during the reaction.
- E. VORTEXING: It is crucial to have a homogeneous mixture during the SELECTION step, specifically after the addition of Reagent 3. All liquid must be in the bottom of the tube during the incubations.
- F. MEDIA SAMPLES CONTAINING CELLS: If a large number of eukaryotic cells are present in the sample (i. e., cell suspensions), centrifuge at 500 X g for 5 minutes. Remove the supernatant from this low speed spin and use 1.5 mL of it as the sample. Proceed from step 1 in SAMPLE PREPARATION.
- G. PELLETING: When preparing samples, remove as much supernatant as possible from the centrifuged samples with a Pasteur pipette. Remnants of media have proven to cause low false positives. However, be careful not to disturb the Mycoplasma pellet, which may not be visible.
- H. SAMPLES IN REPEAT RANGE:
 1. Growth of cultures in antibiotics may reduce Mycoplasma levels. It is recommended that cells be passed twice in the antibiotic-free media before performing the assay. Media to be tested must be in contact with the tissue culture cells for a minimum of three days.
 2. Preparing a larger sample proportionately increases the sensitivity of the test. Prepare a pellet from a larger sample volume as follows:
 - a. Obtain a centrifuge tube which will contain from 10 to 50 mL and which can be centrifuged at high speed. Carefully mark the position where the pellet will form.
 - b. Add the collected media and centrifuge at 12,000 to 15,000 x g for 15 minutes. Carefully remove and discard the supernatant. The pellet may not be visible and care must be taken to avoid any loss of the pellet during this step.
 - c. Resuspend the pellet in 1.5 mL of sterile saline or cell culture media and proceed to step 1 of the SAMPLE PREPARATION procedure.

RESULTS

A. INTERPRETATION OF RESULTS

The results of the GEN-PROBE MYCOPLASMA TISSUE CULTURE NI (MTC-NI) RAPID DETECTION SYSTEM are based on Relative Light Unit (RLU) cut-off values. Samples producing greater than or equal to the positive cut-off value are considered positive. Signals less than the negative cut-off value are considered negative. Results in repeat range should be repeated. Samples continuing to give values within the repeat range may represent very low level contamination and you may wish to re-test as described in PROCEDURAL NOTES.

	Positive	Negative
Cut-off Values:	>5,000 RLU	<3,000 RLU
Repeat Range:	3,000 to 5,000 RLU	

B. QUALITY CONTROL AND ACCEPTABILITY OF RESULTS

Negative Control and Positive Control should be within the following ranges:

Negative Control: <3,000RLU

Positive Control: 35,000-85,000 RLU

To check machine calibration and functioning please refer to the instrument manual.

LIMITATIONS

Use only for the detection of bacterial contamination in tissue culture samples. Bacteria other than mycoplasma will vary in positivity based on their lysis during the sample preparation.

This test does not distinguish among bacterial species.

A negative result does not exclude the possibility that mycoplasma contamination is present at a level below the sensitivity of the test, which is approximately 100,000 mycoplasma per mL of culture media.

This test will detect certain other bacterial species if they are lysed by the sample preparation procedures, see PERFORMANCE CHARACTERISTICS. In any case, a positive result indicates contamination of the tissue culture sample.

PERFORMANCE CHARACTERISTICS

Hybridization of the probe with a wide range of commonly occurring species of *Acholeplasma*, *Mycoplasma*, *Spiroplasma*, and *Ureaplasma* have yielded positive signals at $>10^5$ organisms.

The following species of Mycoplasma are among those tested with the MYCOPLASMA TISSUE CULTURE NI (MTC-NI) RAPID DETECTION SYSTEM.

<i>Acholeplasma laidlawii</i>	<i>Mycoplasma negroni</i>
<i>Mycoplasma arginini</i>	<i>Mycoplasma neurolyticum</i>
<i>Mycoplasma arthritidis</i>	<i>Mycoplasma orale</i>
<i>Mycoplasma collis</i>	<i>Mycoplasma pirum</i>
<i>Mycoplasma fermentans</i>	<i>Mycoplasma primum</i>
<i>Mycoplasma gallisepticum</i>	<i>Mycoplasma pneumoniae</i>
<i>Mycoplasma genitalium</i>	<i>Mycoplasma pulmonis</i>
<i>Mycoplasma hominis</i>	<i>Mycoplasma sualvi</i>
<i>Mycoplasma hyorhinis</i>	<i>Spiroplasma species</i>
<i>Mycoplasma iowae</i>	<i>Ureaplasma parvum</i>
<i>Mycoplasma muris</i>	<i>Ureaplasma urealyticum</i>

Gram negative organisms from a wide variety of taxa also yield positive signals with the broad spectrum bacterial probe contained in this kit when prepared as outlined in the sample preparation section. Reaction to Gram positive organisms is dependent on the ability of the sample preparation method to lyse the organisms. With appropriate lysis conditions all Gram positive organisms from a wide range of taxa also react with this probe system. No reaction to eukaryotic targets has been observed.

EXPECTED VALUES

A. SAMPLES:

One hundred and fifty samples were tested using MTC-NI and the GEN-PROBE MTC isotopic assay.

RLU values for positive samples ranged from 7,644 to 3,034,700 RLU. RLU values for the negative samples ranged from 490 to 2,963 RLU.

There were no discrepant samples, all samples positive by the isotopic assay were also positive by this non-isotopic assay, all negative samples were determined to be negative in both assays.

B. CONTROLS:

One hundred and fifty controls were tested and gave the following results:

	Number of Observations	RLU Values
Negative Controls	81	273 to 2,484 mean = 903 \pm 371 SD
Positive Controls	69	35,489 to 72,955 mean = 52,092 \pm 8,283 SD

These data were generated by several technicians on different days with multiple lots of reagents using various Gen-Probe Leaders.

TROUBLESHOOTING

LOW POSITIVE CONTROL (BELOW 35,000 RLU)

- Confirm that the positive control tube contains approximately 800 μ L. Low readings may indicate that less than 100 μ L of Positive Control was added to the Positive Control probe tube.
- Check that the temperature of the water bath or heat block is 60 $^{\circ}$ \pm 1 $^{\circ}$ C.
- Ensure that the probe tube is immersed in water bath or heat block so as to completely cover the liquid contents during the reaction. Do not allow any water or other non-sterile solutions to enter the probe tubes during the assay.
- Check luminometer settings to confirm a reading time of 2 seconds.
- Check accuracy of pipetting instruments.

HIGH POSITIVE CONTROL (ABOVE 85,000 RLU)

- Confirm that the positive control tube contains approximately 800 μ L. High readings may indicate that 300 μ L of the selection reagent was not added.
- Check that the temperature of the water bath or heat block is 60 $^{\circ}$ \pm 1 $^{\circ}$ C and that the luminometer settings are correct.
- Ensure that the tubes are incubated for 10 minutes during the Selection step.

HIGH NEGATIVE CONTROL (ABOVE 3000 RLU)

- Confirm that the negative control tube contains approximately 800 μ L. High readings may indicate that 300 μ L of the selection reagent was not added.
- Check that the temperature of water bath or heat block is 60 $^{\circ}$ \pm 1 $^{\circ}$ C and that the luminometer settings are correct.
- Ensure that the tubes are incubated for 10 minutes during the Selection Step.
- DO NOT interchange the caps of positive and negative controls.
- Eliminate static charge by wiping tubes with a damp cloth or tissue just prior to placing in the luminometer.

SAMPLES IN THE REPEAT RANGE (3000-5000 RLU)

- A. Check that the temperature of the incubations is $60^{\circ} \pm 1^{\circ}\text{C}$. An accurate thermometer must be used.
- B. Check that the sample is homogeneous and that no drops remain above the surface of the liquid during either incubation.
- C. Cultures may be contaminated with a very low level of bacteria. Increase the sensitivity of the test as described in PROCEDURAL NOTES - H. Cells may need to be passed at least twice after removal from cryogenic storage before there are sufficient bacteria for detection.
- D. Culture media cannot be frozen before centrifugation, step 2 of SAMPLE PREPARATION.

VARIABLE READINGS

- A. Check accuracy of pipetting instruments.
- B. Eliminate static charge and any residue by wiping tubes with a damp cloth or tissue just prior to placing in the luminometer.
- C. Ensure that the entire sample pellet is homogeneously resuspended and that all of it is transferred to the probe tube.
- D. Check function of luminometer pumps as directed by the instrument manual. Check instrument calibration.
- E. Contamination of tubes or reagents with RNase or bacteria may affect test results. Use aseptic technique when handling all tubes and reagents.

BIBLIOGRAPHY

1. **Barile, M. F.** 1977. Mycoplasma contamination of cell cultures: a status report. p. 291-334. Cell culture and its applications. Academic Press, New York, New York.
2. **Barile, M. F., H. E. Hoops, and M. W. Grabowski.** 1978. Incidence and sources of Mycoplasma contamination: a brief review. p.35-45. *In* G. J. McGarrity, D. G. Murphy and W. W. Nicholes (ed.), Mycoplasma infection of cell culture. Plenum Press, New York, New York.
3. **Lincoln C. K., and M. G. Gabridge.** 1998. Methods Cell Biol, **57**:49-65.
4. **Maniloff C. J., R. N. McElhaney, L. R. Finch, and J. B. Baseman,** ed. 1992. Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
5. **McGarrity, G. J.** 1982. Detection of Mycoplasmal infection of cell cultures. p. 99-131. Advances in cell culture, Vol. 2. Academic Press, New York, New York.
6. **McGarrity, G. J., and H. Kotani.** 1985. Cell culture Mycoplasmas. *In* S. Razin, and M. F. Barile (ed.). The Mycoplasmas Vol. IV, Mycoplasma pathogenicity. Academic Press, New York, New York.
7. **Nelson N. C., M. A. Reynolds, and L. J. Arnold, Jr.** 1995. Detection of acridinium esters by chemiluminescence p. 391-428. *In* Kricka, L. J. (ed.), Non isotopic probing, plotting and sequencing. Academic Press, Inc. San Diego, CA.
8. **Rottem, S. and M. F. Barile.** 1993. Beware of mycoplasmas. TIBTECH, **11**:143-151.
9. **Stacey A., and A. Doyle.** 1997. Routine testing of cell cultures and their products for mycoplasma contamination. Methods in Molecular Biol, **75**:305-11.
10. **Weisburg, W. G., J. G. Tully, D. L. Rose, P. Petzel, H. Oyaizu, D. Yang, L. Mandelco, J. Sechrest, T. G. Lawrence, J. Van Etten, J. Maniloff, and C. R. Woese.** 1989. A phylogenetic analysis of the mycoplasmas: basis for their classification. J Bacteriol., **171**:6455-6467.

Gen-Probe Incorporated
San Diego, California 92121 USA

Customer and Technical Services
(858) 410-8000; (800) 523-5001;
(800) 342-7441 (Canada)

©1999–2011 Gen-Probe Incorporated
104574 Rev. D
2011-03