

Antimicrobial Efficacy Test: Direct and Tube Inoculation Method
Product: Path-Away Protectene 5%

1. Purpose

This test method is used for the assessment of antibacterial activity of the product on immediate contact with the microorganism.

2. Principle

Direct inoculation method:

A known volume of antimicrobial product is added to a culture plate of specific fungi. After appropriate incubation time, the inactivation zones are compared to the control plates.

Tube method:

The specific volume of the antimicrobial product is added to inoculum containing defined fungal colonies. After appropriate contact time, the colony count is determined and compared to the control with no antimicrobial product.

3. Material

Sterile test tubes
Sterile pipette
Sterile loops
Incubator (25°C)
Sterile water

3.1 Fungal-Test organisms

Verticillium lecanii ATCC 36967

4. Media

Path-Away Protectene 5%
Malt extract Agar (MEA)
Potato Dextrose Agar (PDA)

5. Procedure

5.1 Preparation of pre-cultures

The fungi are grown on Malt extract agar (MEA) for 5 days at 25°C. Fungal mycelial mat are removed using sterile forceps and then mixed in sterile water to a final concentration of 10⁶ cfu/ml of McFarland 0.5 (increased inoculum ~10⁷ cfu / ml).

5.2 Determination of inoculum cell count

The cultures are diluted in steps of 1:10 in sterile water as described in 5.1. 0.01 ml of the 10⁶ dilution of the fungal culture are plated out onto Malt extract agar (MEA) plates. After 5 days incubation at 25°C, the colonies are counted and converted into colony forming units per ml [cfu/ml].

6. Inoculation

Direct inoculation method:

1ml of the fungal culture inoculum is plated on Malt extract agar (MEA) and Potato dextrose Agar (PDA). The plates are incubated 25°C for 5 days. 0.02ml of the antimicrobial sample is added to the fungal plates and incubated for 25°C for 5 days.

Tube method:

To 5 ml of fungal inoculum culture, 0.02ml of the antimicrobial sample is added. 1ml of fungal inoculum is plated on Malt extract agar and Potato dextrose Agar (PDA) after specified contact time of 0hr, 8hr and 24hr and incubated at 25°C for 5 days.

7. Interpretation of results

The number of fungal colonies [cfu/sample] on the samples equipped with antimicrobial properties is compared to the fungal colony counts from untreated control samples after incubation for an appropriate time. The difference between these two values provides information about the efficacy of the tested antimicrobial solution.

After incubation, the plates are checked for fungal growth at 5 days.

5.2 Determination of inoculum cell count

The cultures are diluted in steps of 1:10 in sterile water as described in 5.1. 0.01 ml of the 10^6 dilution of the fungal culture are plated out onto Malt extract agar (MEA) plates. After 5 days incubation at 25°C, the colonies are counted and converted into colony forming units per ml [cfu/ml].

6. Inoculation

Tube method:

To 5 ml of fungal inoculum culture, 0.02ml of the antimicrobial sample is added. 1ml of fungal inoculum is plated on Malt extract agar and Potato dextrose Agar (PDA) after specified contact time of 0hr, 1hr, 4hr and 6hr and incubated at 25°C for 5 days.

7. Interpretation of results

The number of fungal colonies [cfu/sample] on the samples equipped with antimicrobial properties is compared to the fungal colony counts from untreated control samples after incubation for an appropriate time. The difference between these two values provides information about the efficacy of the tested antimicrobial solution.

After incubation, the plates are checked for fungal growth at 5 days.

Tube Method:

The tubes are checked for turbidity. 1ml from the inoculum tubes at various contact times are plated and checked for growth of fungi at 5 days.

Written by: Manju Pradeep

Reviewed by: Suzanne Blevins

Approved by:



Test done: 02.25.14.

Test reported: 03.04.14

PROJECT # 14003326

PATH-AWAY PROTECTENE

Method: **DIRECT INOCULATION METHOD**

Organism: *Verticillium lecanii* ATCC 36967

Sample: Path-Away Protectene (5%)

Contact time: Immediate (0 min)

Inoculum concentration: 10^6 cfu/ml

Amount of inoculum used: 1 ml

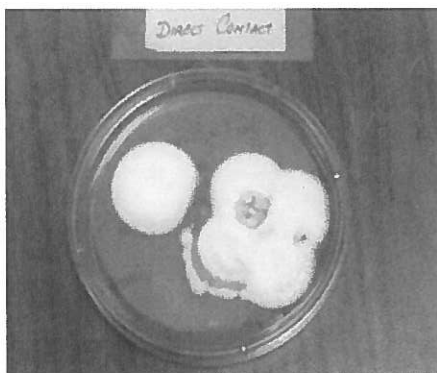
Volume sample used: 0.02ml

Volume plated: 1ml

Incubation time: 5days @ 25°C

Results (Growth/No growth):

Concentration	SAMPLE	
	CONTROL	PATH-AWAY
10^6 cfu/ml	Growth	A small inactivation zone seen after 5 days of incubation at 25°C.



Method: Direct Inoculation method (tube method)
Organism: Verticillium lecanii ATCC 36967
Sample: Path-Away/ Protectene (5%)
Contact time: Immediate (0 min), 8hr and 24hr
Inoculum concentration: 10⁶ cfu/ml
Amount of inoculum used: 5 ml
Volume sample used: 0.02ml
Volume plated: 1ml
Incubation time: 5 days @ 25°C

Results (Growth/No growth):

Contact time	Sample	
	Control	Path-Away/ Protectene
Baseline (0hr)	Growth	Growth
8hr	Growth	No Growth
24hr	Growth	No growth



Analyst: Manju pradeep

Date read: 03.04.14

Antimicrobial Efficacy Test: Tube Inoculation Method
Product: Path-Away Protectene 5%

1. Purpose

This test method is used for the assessment of antibacterial activity of the product on immediate contact with the microorganism.

2. Principle

Tube method:

The specific volume of the antimicrobial product is added to inoculum containing defined fungal colonies. After appropriate contact time, the colony count is determined and compared to the control with no antimicrobial product.

3. Material

Sterile test tubes
Sterile pipette
Sterile loops
Incubator (25°C)
Sterile water

3.1 Fungal-Test organisms

Verticillium lecanii ATCC 36967

4. Media

Path-Away Protectene 5%
Malt extract Agar (MEA)
Potato Dextrose Agar (PDA)

5. Procedure

5.1 Preparation of pre-cultures

The fungi are grown on Malt extract agar (MEA) for 5 days at 25°C. Fungal mycelial mat are removed using sterile forceps and then mixed in sterile water to a final concentration of 10^6 cfu/ml of McFarland 0.5 (increased inoculum $\sim 10^7$ cfu / ml).

Direct inoculation:

The plates are checked for visual zones of inactivation (Growth or No Growth) of the target fungal compared to control plates (Growth).

Tube Method:

The tubes are checked for turbidity at 48 hrs. 1ml from the inoculum tubes at various contact times are plated and checked for growth of fungi at 5 days.

Written by: Manju Pradeep

Reviewed by: Suzanne Blevins

Approved by:



Test done: 03.04.14.

Test reported: 03.10.14

PROJECT # 14003326

PATH-AWAY PROTECTENE

Method: **Direct Inoculation method (tube method)**

Organism: **Verticillium lecanii ATCC 36967**

Sample: **Path-Away/ Protectene (5%)**

Contact time: **Immediate (0 min), 8hr and 24hr**

Inoculum concentration: **10⁶ cfu/ml**

Amount of inoculum used: **5 ml**

Volume sample used: **0.02ml**

Volume plated: **1ml**

Incubation time: **5 days @ 25°C**

Results (Growth/No growth):

Contact time	Sample	
	Control	Path-Away/ Protectene
Baseline (0hr)	Growth	Growth
1hr	Growth	No Growth
4hr	Growth	No Growth
6hr	Growth	No Growth

Analyst: Manju pradeep

Date read: 03.04.14