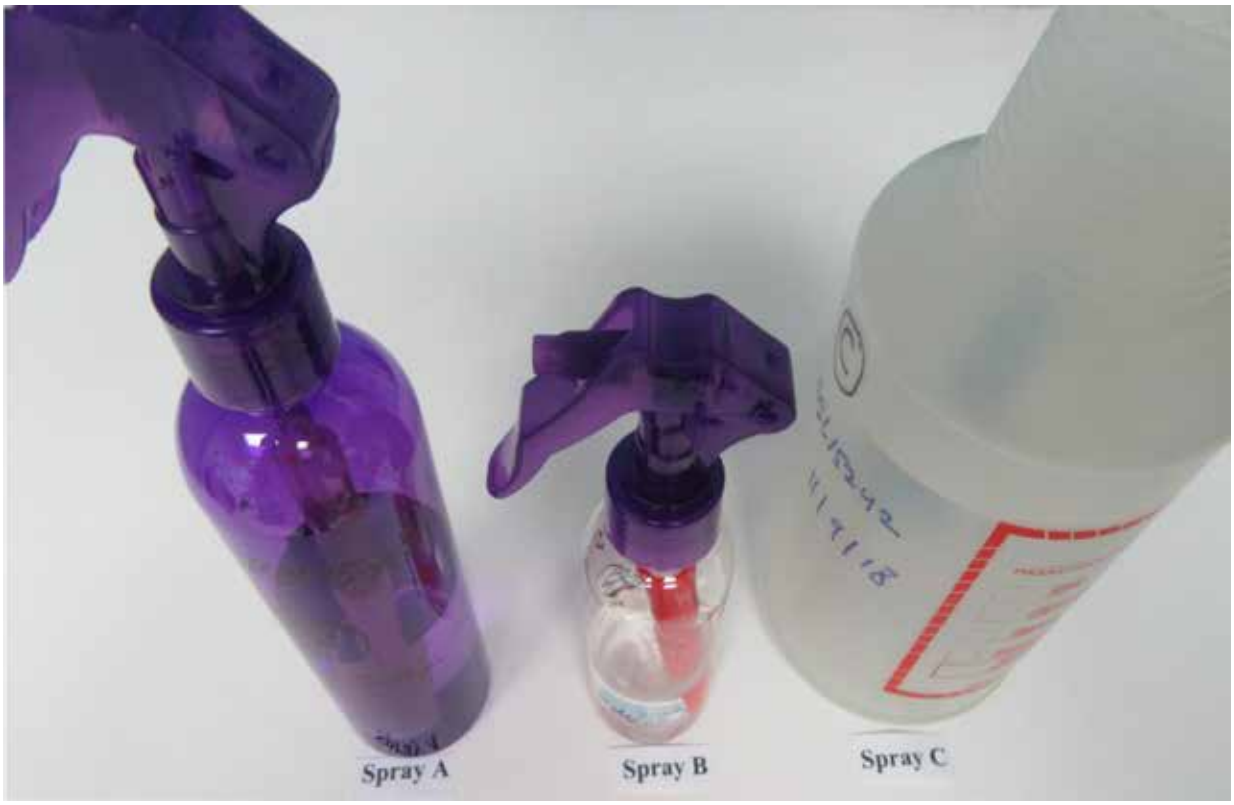
IOTEX™

Anti-Infection Products Inc.

Health Canada NPN 80106491 • November 2020
Canadian & U.S. Patent Pending for the IOTEX™ Spray # 16/906670 filed June 19, 2020

The IOTEX™ Spray eliminates antibiotic resistant pathogenic bacteria, viruses & fungi Without toxicity



Part 1

GAPLAB Environmental Microbiology

X™ Products Inc.

1020 Hargrieve Road, Unit 14
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GAP Project: A14208b

Mar 13, 2020

Iotex Anti-infection Products Inc.
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Barrie, ON L4N 5M3
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ASTM E2315 – Assessment of Antimicrobial Activity Using a Time-Kill Procedure

ASTM Assessment of Antimicrobial Activity Using a Time-Kill Procedure (E2315) was employed to test the Iotex Spray bottle and its chemistry. The bottle was filled with laboratory de-ionized water and the spray effluent was discharged into a sterile container and then tested against the ASTM E2315 method for its antimicrobial properties. More specifically, the spray effluent was brought in contact with a known population of microorganisms at room temperature for a variety of contact times (see below), then neutralized at 1:10 concentration with D/E Neutralization broth. After neutralization, the surviving microorganisms were enumerated using the procedures outlined under "Microbial Population Quantification" below. The log and percent reductions were calculated using the microbial concentrations recovered from a phosphate buffered water control test. The results can be found on the following pages.

Challenge organism:

Clostridium difficile ATCC 43598

Time points:

1 minute ± 5 seconds
5 minutes ± 10 seconds
30 minutes ± 30 seconds

Test materials:

Spray effluent from a bottle insert, bottle was filled with sterile de-ionized water. See Figure 1 below for an image of the tested bottle.

Microbial Population Quantification:

Enumeration of the surviving microbial population was achieved by spread plating the samples onto TSA. The plates were incubated anaerobically at 35±0.5°C for 48 hours.

Antimicrobial Activity Calculation:

The antimicrobial activity (R-value) of the test agent to each microbe was calculated according to the following calculation:

$$R = A_0 - A_t$$

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Where

R is the value of antimicrobial activity, or log reduction of the test agent.

A_0 is the logarithm of the number of viable bacteria, in CFU/mL, initially (T=0) recovered from the phosphate buffered water control.

A_t is the logarithm of the number of viable bacteria, in CFU/mL, recovered from the treated test agent after the specified contact time.

Quality Control:

All conditions were met for a valid test and all media passed quality control checks. The neutralization test results can be found in Table 1 below and are within ±0.5 logs of the initial microbial count. The control test results can be found in Table 2 below; this shows that the initial microbial population counts are within ±0.5 logs of the counts at each test point.

Results:

Results for each test article can be found in Table 2 through Table 3 below, with a summary of the results in Table 4. The ASTM E2315 procedure dose not allow the testing laboratory to specify what is considered a successful test, however the Iotex spray bottle and its chemistry produced an R value of greater than 4 at 1 minute of exposure; this is equivalent to more than a 99.99% reduction of the *C. difficile* used in this test.

Figure 1: The spray bottle with insert used throughout testing. The bottle was filled with de-ionized water and the spray effluent was collected for use in testing.

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Neutralization Test Results:

Table 1. Neutralization Test Results – Inoculum suspensions in Antimicrobial Agent plus D/E Neutralization broth at a 1/10 concentration in contact for 1 hour. All results were within the allowable limits for the method.

	PBW Control	Neutralization 1:10
Rep1 (pfu/mL)	2.10x10^5	1.30x10^5
Rep2 (pfu/mL)	1.30x10^5	1.50x10^5
Log Rep A	5.32	5.11
Log Rep B	5.11	5.18
Average	5.22	5.15
A_0	5.22	
A_t		5.15
$R=A_0-A_t$		0.27

PFU= Plaque Forming Unit, PBW= Phosphate Buffered Water (Lab water control)

Control Test Results:

Table 2. Control (PBW) Test Results – This is used for the A_0 value in the R (antimicrobial activity) calculation for all test articles and to ensure that no external factors are influencing the reduction of the test microbe over the duration of the test.

Exposure Time	T=0	T=1 min	T=5 min	T=30 min
Rep1 (pfu/mL)	2.55x10^5	2.60x10^5	1.70x10^5	1.30x10^5
Rep2 (pfu/mL)	2.60x10^5	2.15x10^5	1.70x10^5	1.80x10^5
Log Rep A	5.41	5.41	5.23	5.11
Log Rep B	5.41	5.33	5.23	5.26
Average	5.41	5.37	5.23	5.18
A_0	5.41			
A_t		5.37	5.23	5.18
$R=A_0-A_t$		0.04	0.18	0.23

PFU= Plaque Forming Unit, PBW= Phosphate Buffered Water (Lab water control)

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Test Results:

Table 3. Test Results for the spray bottle insert. The R value indicates the log reduction measured at a given contact time.

Exposure Time	T=1 min	T=5 min	T=30 min
Rep1 (pfu/mL)	<1.00x10^1	<1.00x10^1	<1.00x10^1
Rep2 (pfu/mL)	<1.00x10^1	<1.00x10^1	<1.00x10^1
Log Rep A	<1.00	<1.00	<1.00
Log Rep B	<1.00	<1.00	<1.00
Average	<1.00	<1.00	<1.00
A_0	5.37	5.23	5.18
A_t	<1.00	<1.00	<1.00
$R=A_0-A_t$	>4.37	>4.23	>4.18

PFU= Plaque Forming Unit, PBW= Phosphate Buffered Water (Lab water control)

Table 4. Summary of the R-value and % reduction for each test article.

Exposure Time	A_0	A_t	$R=A_0-A_t$	% Reduction
1 minute	5.37	<1.00	>4.37	>99.99
5 minutes	5.23	<1.00	>4.23	>99.99
30 minutes	5.18	<1.00	>4.18	>99.99

PFU= Plaque Forming Unit, PBW= Phosphate Buffered Water (Lab water control)

Analyst: J. Patterson (Lab Manager)

Signature: JPatterson

Approved by: C. Odegaard (Technical Manager)

Signature: CJ

These test results relate only to the samples submitted and the analyses requested.
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IOTEX™

Anti-Infection Products Inc.

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Lab reference: MBL15769ET February 25, 2019

Iotex Anti-infection Products Inc.
111-19 Hart Drive
Barrie, ON L4N 5M3
(Attn: Larry Miller or Ron Diamond)

Dear Larry and Ron,

Re: Testing of Iotex Sprays type A, B and C against *the Bacterial mixture*

As per your request, Mold & Bacteria Consulting Laboratories tested the Iotex Sprays type A, B and C for control of the growth of a mixed culture of the following bacteria: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

The Iotex Sprays were effective in the control of the tested bacteria mixture at the concentrations used for the test.

The test procedure and the findings are presented in the next pages.

Sincerely,

Jackson Kung'u, PhD
Principal Microbiologist
Mold & Bacteria Consulting Laboratories (MBL) Inc.

Tested by: Ali Asgharian, M.Sc. Reviewed by: Dr. Jackson Kung'u, PhD.

Efficacy testing for Iotex Sprays against a mixture Bacteria.

Purpose for the Test

The purpose for the test was to determine the efficacy of Iotex Sprays type A, B and C against the growth of a mixed population of the following bacteria: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

1. Materials and Methods

1.1 Test products

- Iotex Sprays; Type A, B and C (See figure 1)

1.2 Bacteria Test Strains

- *Escherichia coli* (ATCC 25922)
- *Staphylococcus aureus* (ATCC 25923)
- *Pseudomonas aeruginosa* (ATCC 15442)
- *Klebsiella pneumoniae* (ATCC 4352)

2. Procedure

The test was conducted as instructed by Iotex Anti-infection Products Inc.

2.1 Preparation of Bacterial Cell Suspension

The challenge bacteria were grown at 35°C for 24 hours and a mixed population of the test bacteria was prepared aseptically. The cell density of each type of bacteria in the mixture was approximately 1.5×10^8 cells per ml as previously determined using McFarland Latex Turbidity Standards.

2.2 Iotex Spray Testing

Into three sterile containers, 1.5 ml of the bacterial suspension was added and then spayed with the Iotex sprays type A, B and C. The spraying for spray A, B and C was achieved by pressing the pump and then releasing after 15, 20 and 5 seconds, respectively. The mixed bacteria population was exposed to the Iotex sprays for 1 and 2 minutes. After the exposure time, 20 ml of D/E neutralizing broth (Oxoid, Lot# 927698) was added and shaken thoroughly. After mixing, 0.1 ml of the broth was plated on TSA plates (Oxoid, Lot# 2470479) in duplicate. Controls were not sprayed with Iotex sprays.

2.3 Calculation of Log Reduction Value

Log Reduction Value (LRV) was calculated using the formula below.

$LRV = \log_{10} (N_0/N)$

N_0 = The initial number of organisms before treatment

N= The final number of organisms after treatment.

3. Results

The LRV results for all Iotex sprays indicated in the table below.

Spray Type	Exposure time: 1 min		Exposure time: 2 min	
	LRV	Percent Reduction	LRV	Percent Reduction
Spray A	5.5	> 99.0%	8.2	>99.0%
Spray B	3.8	> 99.0%	5.5	> 99.0%
Spray C	5.8	>99.0%	8.2	>99.0%

The results indicate that spray C was the most effective in inhibiting the growth of the bacteria mixture after 1 and 2 minutes exposure. Also, spray A and C were more effective against the growth of the bacteria mixture than spray B, at both exposure times 1 and 2 minutes (see figures 2-8). Spray type A and C were completely effective in inhibiting the growth of the bacteria mixture of at exposure time of 2 minutes.

4. Discussion and Conclusion

The Iotex spray was effective in inhibiting the growth of the bacteria mixture. The Iotex sprays (A, B and C) were able to reduce the bacteria count by more than 99%, at both exposure times 1 and 2 minutes.

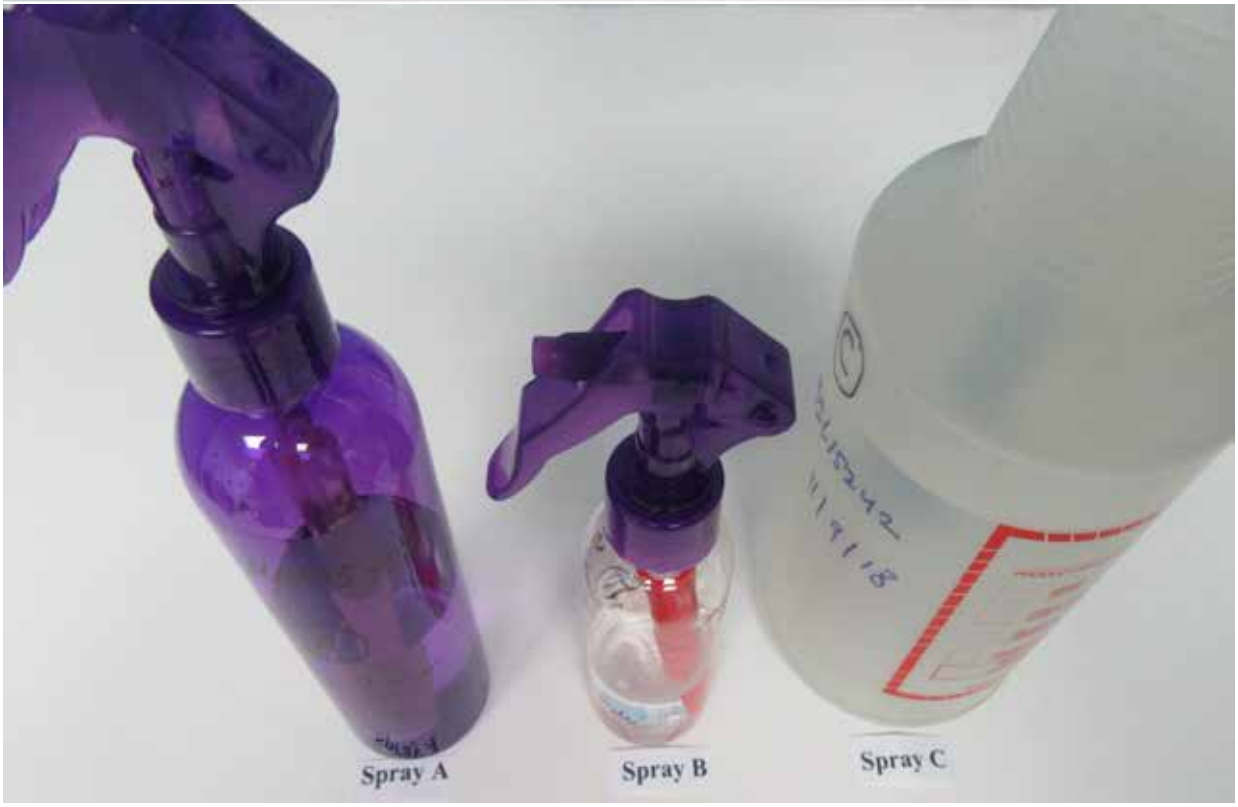

Fig 1: Spray A, B and C

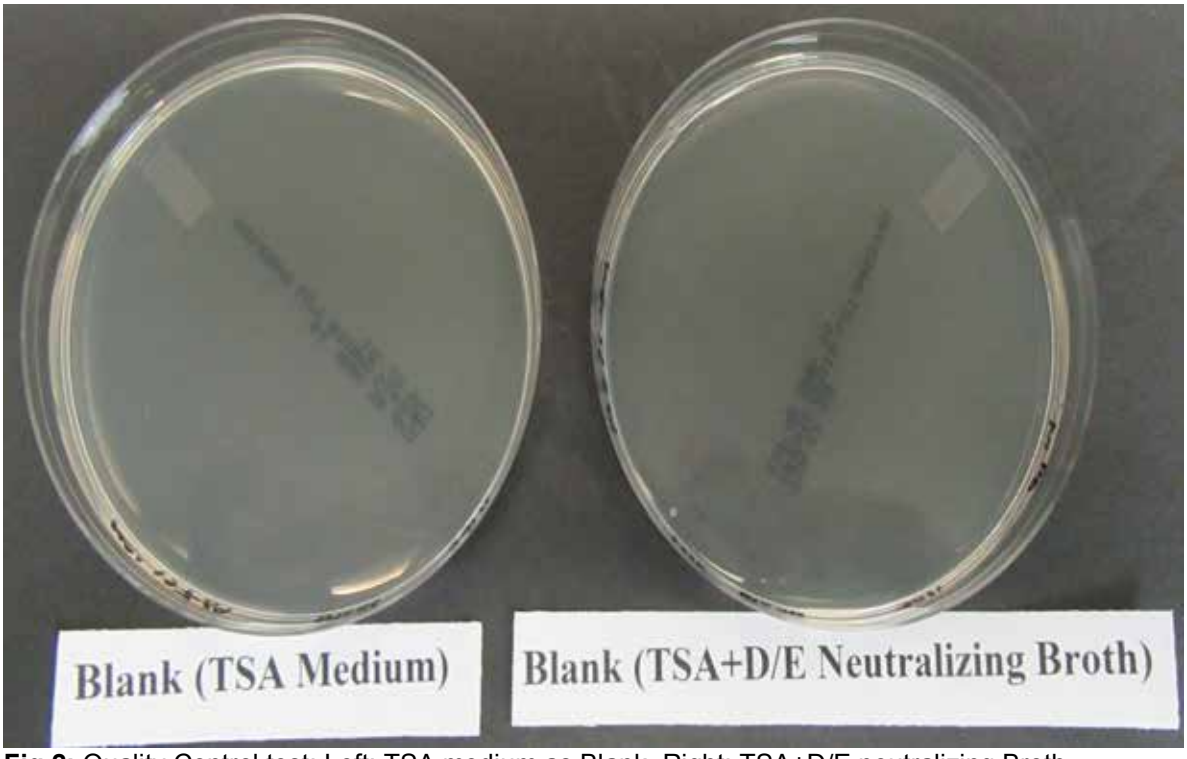

Fig 2: Quality Control test: Left; TSA medium as Blank, Right: TSA+D/E neutralizing Broth

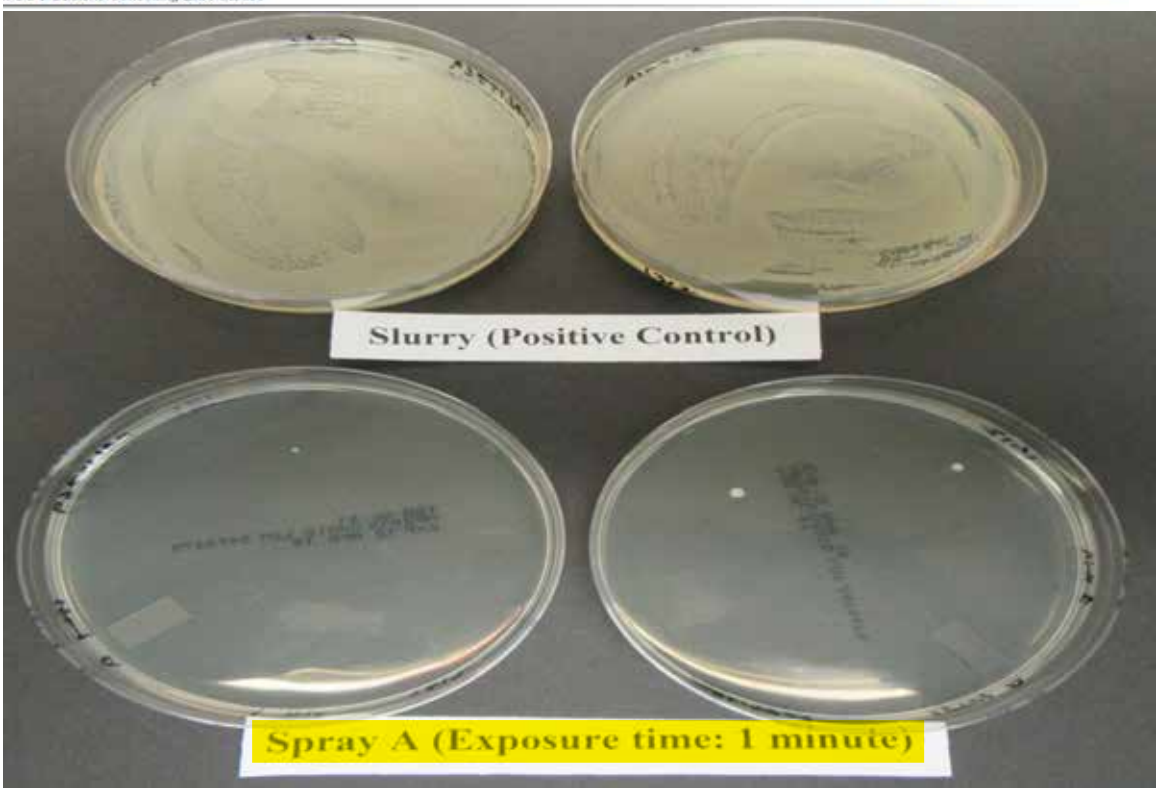

Fig 3: Spray A against bacteria mixture (slurry). Top (positive control); Bottom (treated with the spray A for 1 minute).

Fig 4: Spray A against bacteria mixture (slurry). Top (positive control); Bottom (treated with the spray A for 2 minutes).

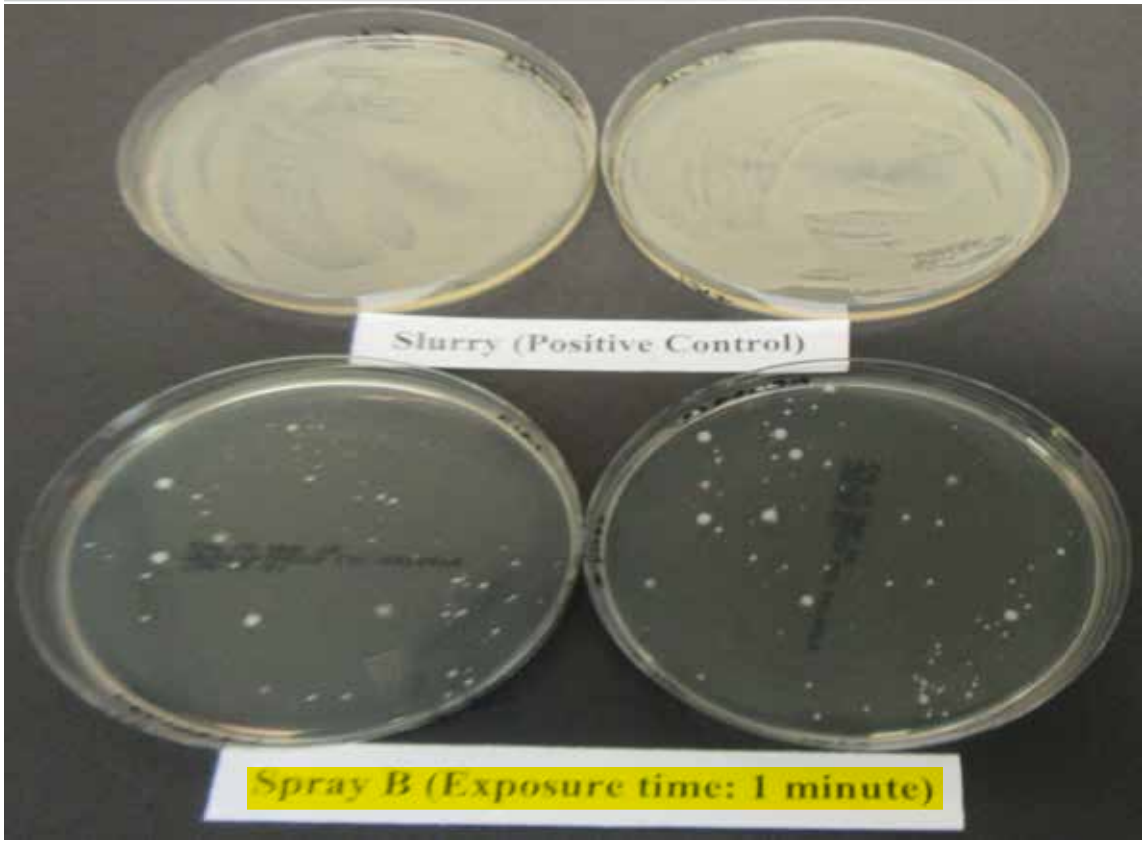

Fig 5: Spray B against bacteria mixture (slurry). Top (positive control); Bottom (treated with the spray B for 1 minutes).

Fig 6: Spray B against bacteria mixture (slurry). Top (positive control); Bottom (treated with the spray B for 2 minutes).

Fig 7: Spray C against bacteria mixture (slurry). Top (positive control); Bottom (treated with the spray C for 1 minute).

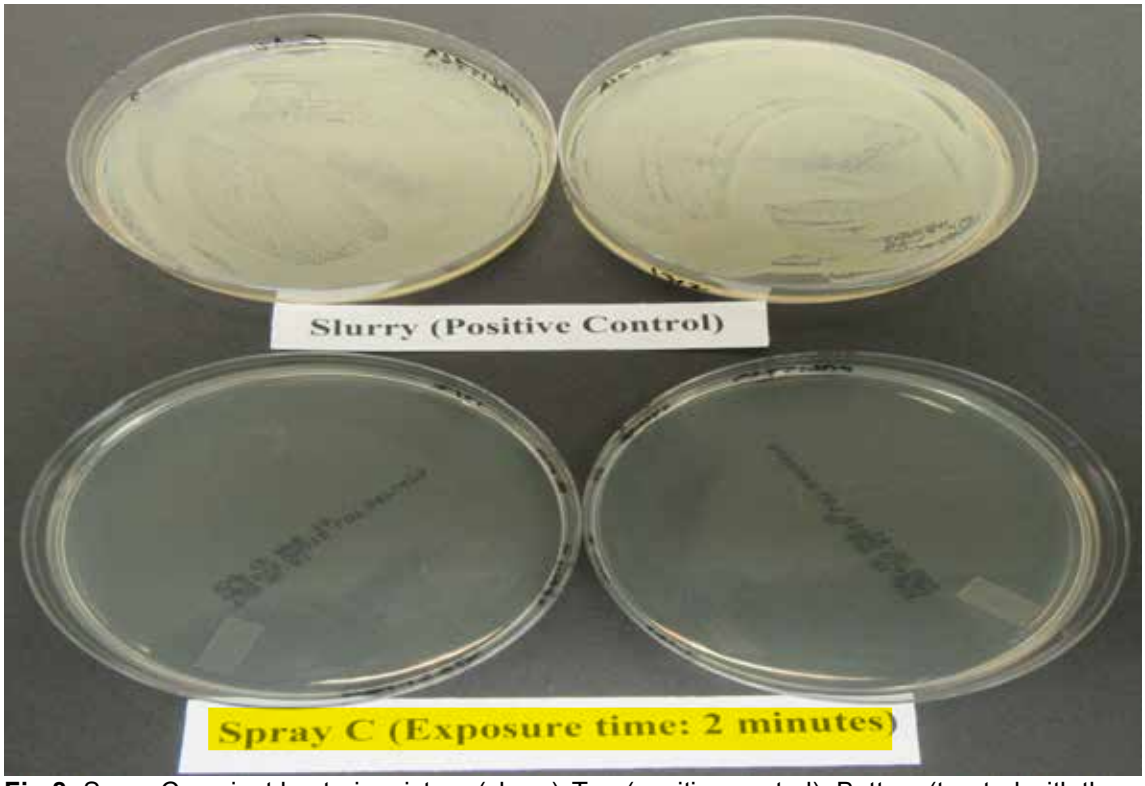

Fig 8: Spray C against bacteria mixture (slurry). Top (positive control); Bottom (treated with the spray C for 2 minutes).

5. References

1- Bergey's Manual of Determinative Bacteriology: John G. Holt (Editor), Noel R. Krieg (Editor), Peter H.A. Sneath (Editor), James T.Staley (Editor), Stanley T. Williams (Editor). Lippincott Williams & Wilkins Ninth Edition, 1994.

2- M. Balouiri, M. Sadiki, SK Ibnsouda, Methods for *in vitro* evaluating antimicrobial activity: A review, In Journal of Pharmaceutical Analysis, Volume 6, Issue 2, 2016, Pages 71-79.

3- USP 31 <1072> Disinfectants and Antiseptics.

Efficacy Testing for Iotex Anti-infection Sprays

1. Purpose for the Test

The purpose for the test was to determine the efficacy of Iotex anti-infection hot and regular sprays against bacterial growth.

2. Materials and Methods

2.1 Test products

a) Iotex anti-infection regular formulation spray
b) Iotex anti-infection hot formulation spray
c) Iotex anti-infection wound treatment

2.2 Bacteria Test Strains

a) *Pseudomonas aeruginosa* (ATCC 15442),
b) *Staphylococcus aureus* (ATCC 6538),
c) *Streptococcus pneumonia* (ATCC 49619) and
d) *Escherichia coli* (ATCC 25922).

3. Procedure

The test was conducted as instructed by Iotex Anti-infection Products Inc.

3.1 Preparation of Bacterial Cell Suspension

The bacteria test strains were grown for 24 hours and a bacterial cell suspension of each strain prepared. The suspension was adjusted to 1.5×10^6 cells per ml using McFarland Latex Turbidity Standards.

3.2 Iotex Anti-infection Hot and Regular formulation Spray Testing

One gram (1 gm) of regular and hot dry formulation was added in separate spray pens. The pens were then filled with sterile distilled water. The mixture was shaken vigorously and allowed to settle for 2-3 min. 0.1 ml of the bacterial suspension was added in a sterile container and then spayed with the hot or regular spray. The spraying was achieved by pressing the pump for about second and then releasing. This was repeated 5 times. The bacteria were exposed to the sprays for 10, 30 and 60 minutes. After the exposure time, 20 ml of neutralizing broth (letheen broth) was added and shaken thoroughly. After mixing, 0.1 ml of the broth was then taken and plated on TSA plates. Controls were not sprayed. The plates were incubated at 35 ºC for 48 hours.

4. Results

4.1 Iotex Anti-infection Hot and Regular Formulation Spray Test Results

Both the regular and hot formulation of the Iotex Anti-infection Spray were equally effective against the tested bacteria at 10 minutes exposure (see figures 1-4). No bacterium was capable of growing after 10 minutes exposure to the sprays.

5. Discussion and Conclusion

The hot and regular formulation of the spray were effective in inhibiting the growth of the tested bacteria.

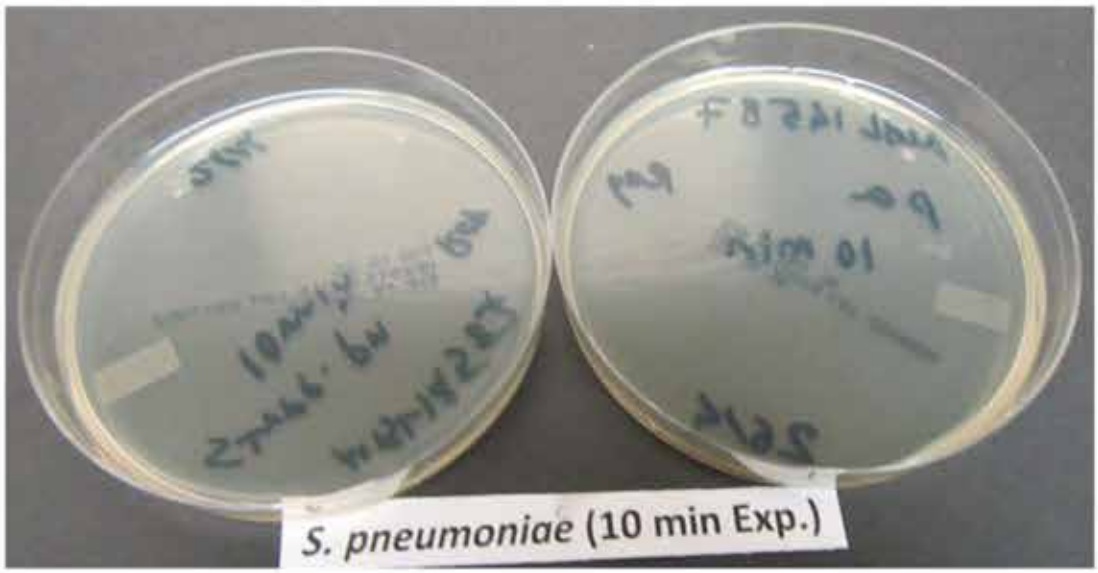

Fig 4: *Streptococcus pneumonia.* Top (positive control); bottom (treated with the spray for 10 minutes).

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Anti-Infection Products Inc.

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Lab reference: MBL16208ET June 06, 2019

Iotex Anti-infection Products Inc.
111-19 Hart Drive
Barrie, ON L4N 5M3
(Attn: Larry Miller or Ron Diamond)

Dear Larry and Ron,

Re: Testing of Iotex Spray A and C against *Acinetobacter baumannii*

As per your request, Mold & Bacteria Consulting Laboratories tested the Iotex Spray A and C for control of *Acinetobacter baumannii* growth.

Both Iotex Spray A and C were effective in the control of *Acinetobacter baumannii* at the concentrations used for the test.

The test procedure and the findings are presented in the next pages.

Sincerely,

Jackson Kung'u, PhD
Principal Microbiologist
Mold & Bacteria Consulting Laboratories (MBL) Inc.

Tested by: Ali Asgharian, M.Sc. Reviewed by: Dr. Jackson Kung'u, PhD.

MBL
Mold & Bacteria Consulting Laboratories
...more than just lab results

Efficacy testing for Iotex Spray A and C against *Acinetobacter baumannii.*

1- Purpose for the Test

The purpose for the test was to determine the efficacy of Iotex Spray A and C against the growth of *Acinetobacter baumannii*. The bacterium selected for the test was carbapenem-resistant that is difficult to treat

2- Materials and Methods

2.1 Test products

Iotex Spray A and C (Figure 1)

2.1 Bacterial Test Strain

Acinetobacter baumannii (ATCC® BAA-1605™). This strain is carbapenem-resistant.

3- Procedure

The test was conducted as instructed by Iotex Anti-infection Products Inc.

3.1 Preparation of Bacterium Cell Suspension

Acinetobacter baumannii was grown for 24 hours at 35°C and bacterial cell suspension was prepared and adjusted to 1.5×10^6 cells per ml using McFarland Latex Turbidity Standards.

3.2 Iotex Spray Testing

0.1 ml of the bacterial suspension was added in a sterile container and then spayed with the Iotex spray A and C. The spraying for spray A and C was achieved by pressing the pump and then releasing after 15 and 5 seconds, respectively. The bacterium was exposed to the both Iotex Sprays for 1 minute. After the exposure time, 20 ml of D/E neutralizing broth (Oxoid, Lot# 927698) was added and shaken thoroughly. After mixing, 0.1 ml of the broth was plated on TSA plates (Oxoid, Lot#2524473) in duplicates. Controls were not sprayed with Iotex Sprays.

3.3 Calculation of Log Reduction Value

Log Reduction Value (LRV) was calculated using the formula below.

$LRV = \log_{10} (N_0/N)$

N_0 = The initial number of colonies without treatment

N= The final number of colonies with treatment.

4- Results

The Iotex Spray A and C were equally effective against the *Acinetobacter baumannii* at the exposure time of 1 minute after 48 hours incubation at 35°C (see figures 2-5). The LRV for both Iotex spray (A and C) was 5.3 which is equivalent to more than 99% reduction (Table 1).

Table1: Effectiveness of spray (A and C) against *Acinetobacter baumannii*

Spray Type	Exposure time: 1 min	
	Long Reduction Value (LRV)	Percent Reduction
Spray A	5.3	> 99.0%
Spray C	5.3	>99.0%

5- Discussion and Conclusion

It is concluded that the Iotex spray A and C were effective in inhibiting the growth of *Acinetobacter baumannii*. Based on the LRV of 5.3, the Iotex spray A and C were able to reduce the bacterial count by more than 99%.

Fig 1: Spray A and C

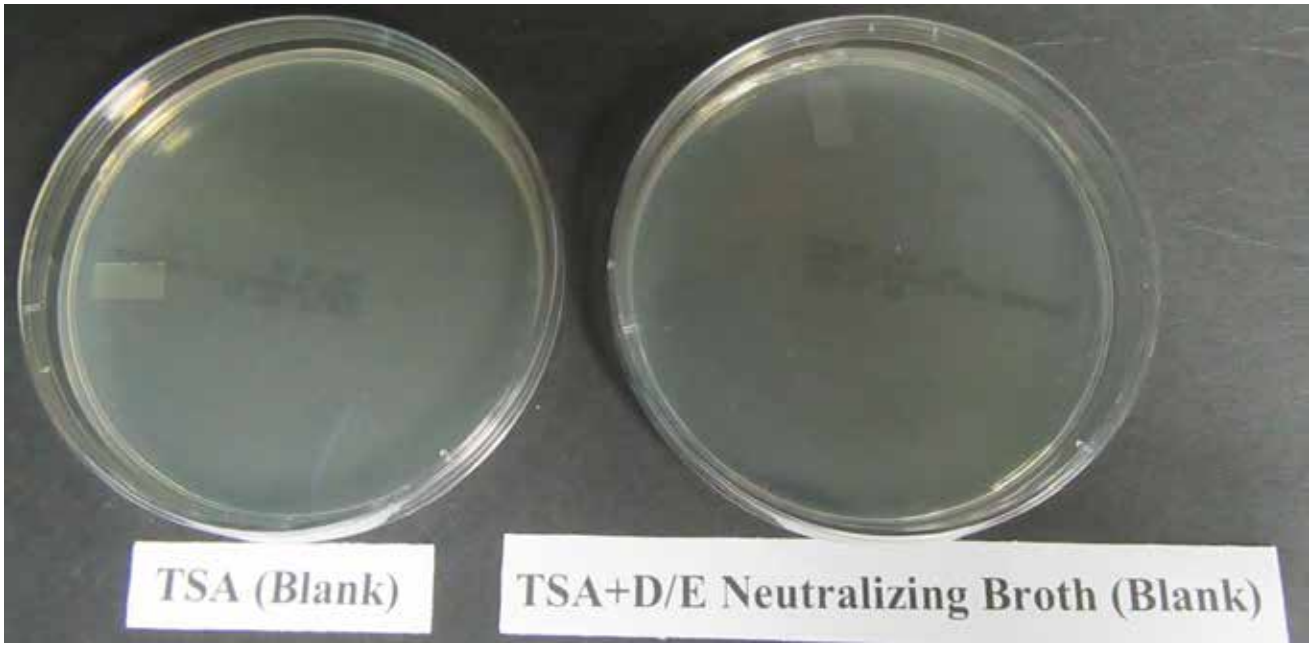

Fig 2: Quality Control test: Left; TSA medium as a Blank, Right: TSA+D/E neutralizing Broth.

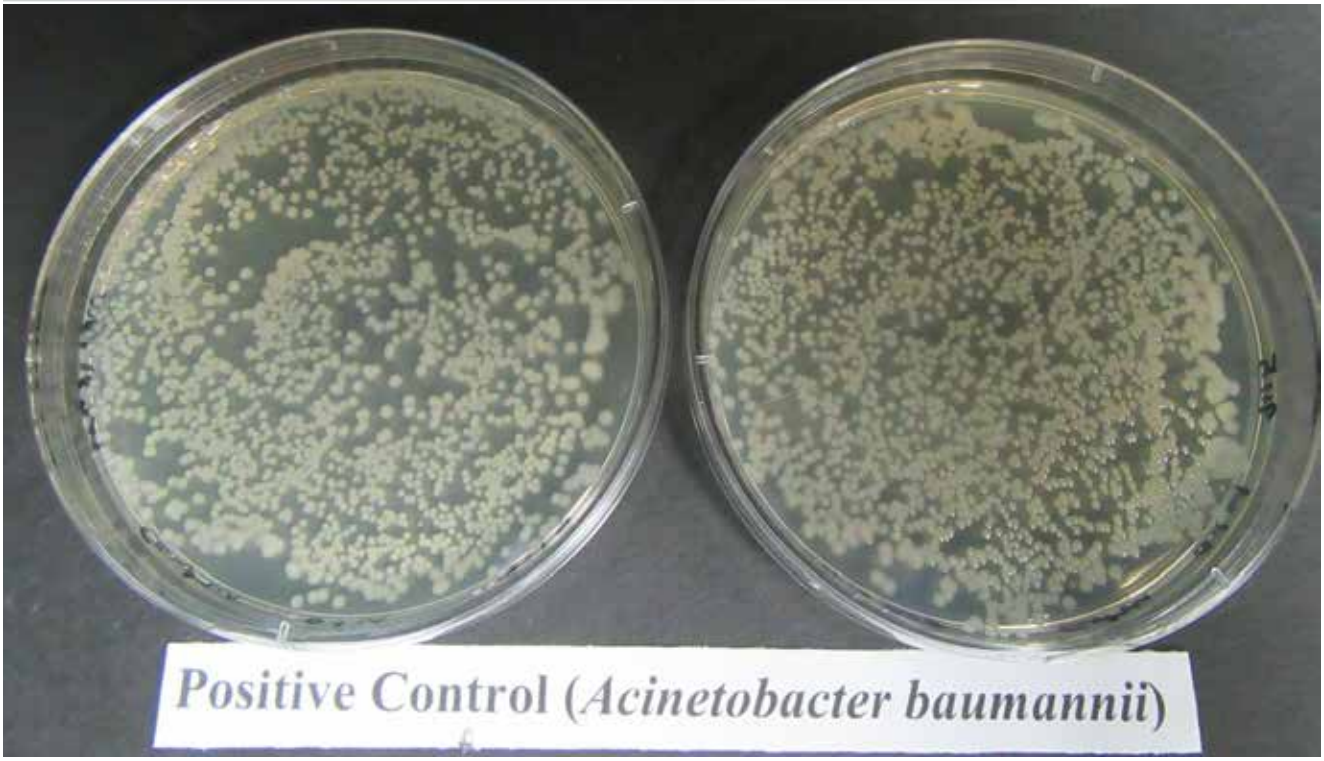

Fig 3: Positive control of *Acinetobacter baumannii.*

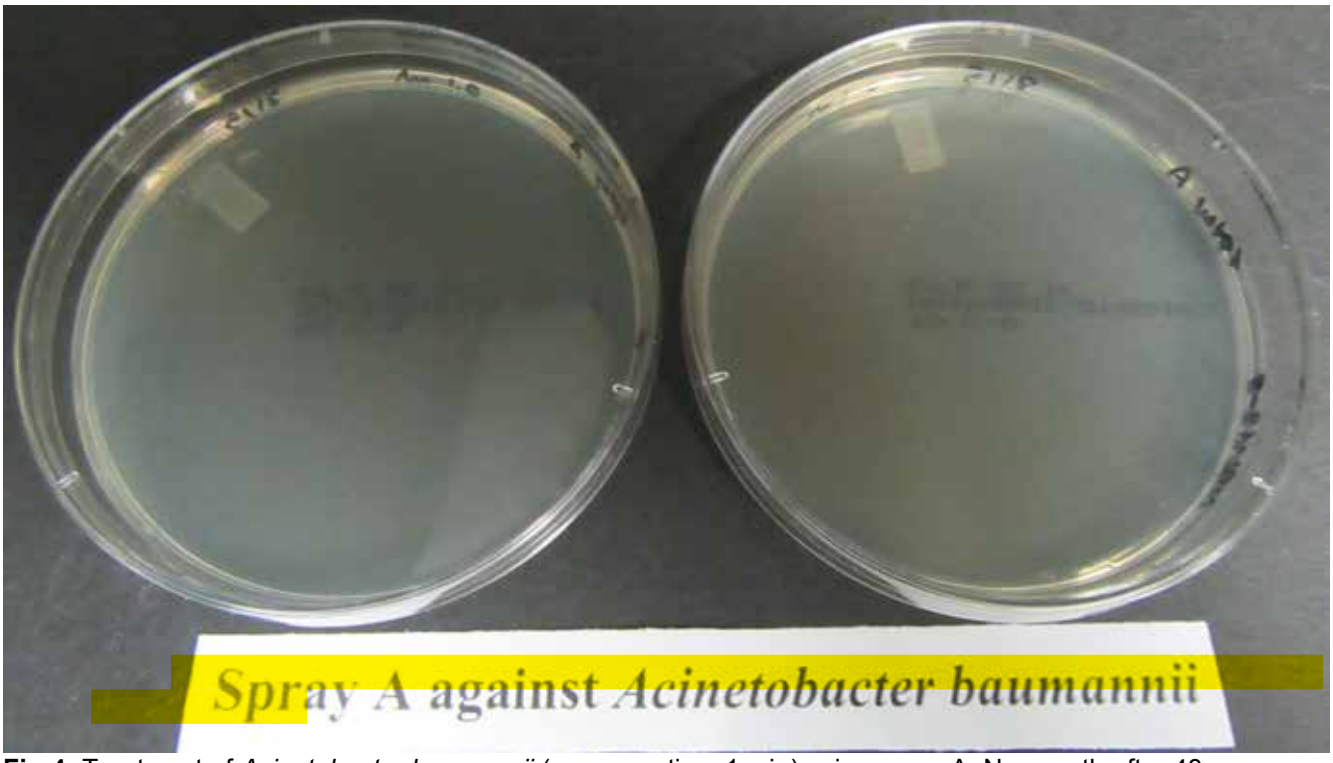

Fig 4: Treatment of *Acinetobacter baumannii* (exposure time:1 min) using spray A. No growth after 48 hours incubation.

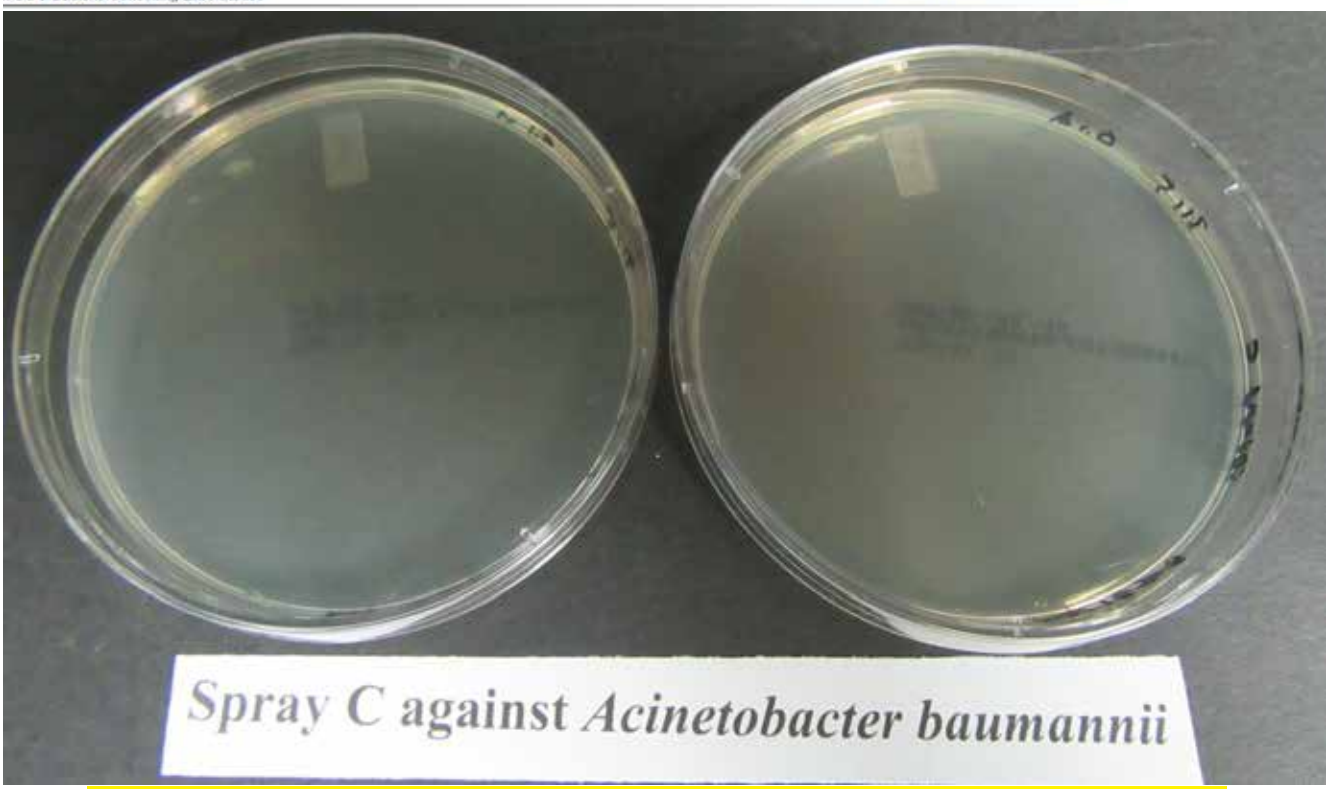

Fig 5: Treatment of *Acinetobacter baumannii* (exposure time:1 min) using spray C. No growth after 48 hours incubation.

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Lab reference: MBL17560ET January 18, 2021

Iotex Anti-infection Products Inc.
3555 Atwater Av. # 209
Montreal, QC H3H1Y3
(Attn: Ron Diamond)

Dear Ron,

Re: Testing the Efficacy of the New Iotex Power Spray Against a Mixed Population of Bacteria

As per your request, Mold & Bacteria Consulting Laboratories tested the efficacy of the New Iotex Power Spray against a mixed population of the following bacteria: *Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae, Staphylococcus aureus and Enterococcus faecium*.

The New Iotex Power Spray was effective in the control of the growth of tested bacteria mixture at the concentrations used for the test.

The test procedure and the findings are presented in the next pages.

Sincerely,

Jackson Kung'u, PhD
Principal Microbiologist
Mold & Bacteria Consulting Laboratories (MBL) Inc.

Tested by: Georget Shamoon, PhD. Reviewed by: Dr. Jackson Kung'u, PhD.

Efficacy Testing for the New Iotex Power Spray Against a Mixed Population of Bacteria.

Purpose of the test

The purpose of the test was to determine the efficacy of the New Iotex Power Spray against the growth of a mixed population of the following bacteria: *Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae, Staphylococcus aureus and Enterococcus faecium*

Materials and Methods

1.1 Test products

New Iotex Power Spray (See figure 1)

1.2 Bacteria Test Strains

- *Escherichia coli* (ATCC 25922)
- *Acinetobacter baumannii* (ATCC 1605)
- *Klebsiella pneumoniae* (ATCC 1705)
- *Staphylococcus aureus* (ATCC 25923)
- *Enterococcus faecium* (ATCC 700221)

1. Procedure

The test was conducted as instructed by the client (Iotex Anti-infection Products Inc.)

2.1 Preparation of Bacterial Cell Suspension

The test bacteria were grown at 35°C for 24 hours and a mixed population of the bacteria was prepared aseptically. The cell density of each type of bacteria in the mixture was approximately 1.5 x 10^8 cells per ml as previously determined using McFarland Latex Turbidity Standards.

2.2 New Iotex Power Spray Testing

The New IOTEX Power Spray containing the active ingredient was filled with 1 litre of sterile distilled water and shaken thoroughly.

Into sterile containers, 1.0 ml of the mixed bacterial suspension was added and then sprayed with the solution in the New Iotex Power Spray. The spraying was achieved by pressing the pump and then releasing after 2-5 seconds. One container of the mixed bacterial population was exposed to the Iotex spray for 1 minute and the other container for 2 minutes. After the exposure time, 20 ml of D/E neutralizing broth (Oxoid, Lot# 927698) was added and shaken thoroughly. After mixing the treated and control sample with the D/E broth, 0.1 ml of the broth was plated on TSA plates (Oxoid, Lot# 3188051) in duplicate. Controls were not sprayed with Iotex spray.

2.3 Calculation of Log Reduction Value

Log Reduction Value (LRV) was calculated using the formula below.

$LRV=\log_{10}(N_0/N)$

N_0 = The initial number of organisms before treatment

N= The final number of organisms after treatment.

2. Results

A Log Reduction Value (LRV) of 1 and 5 were obtained for the 1 minute and 2 minutes exposure respectively as indicated below.

Spray Type	Exposure time: 1 min		Exposure time: 2 min	
	LRV	Percent Reduction	LRV	Percent Reduction
New Power Spray	1	90.000%	5	99.999%

3. Discussion and Conclusion

The results indicate the New Iotex Power Spray was effective in inhibiting the growth of the bacteria mixture after the 1 minute and 2 minutes exposure (see figures 3 and 4). The best control was achieved at 2 minutes exposure with a 99.999% reduction of bacteria.

Fig 1: New Iotex Power Sprayer

Fig 2: Control Test- No spray

Fig 3: Effect of New IOTEX Power Spray against a mixed population of bacteria- 1 minute exposure.

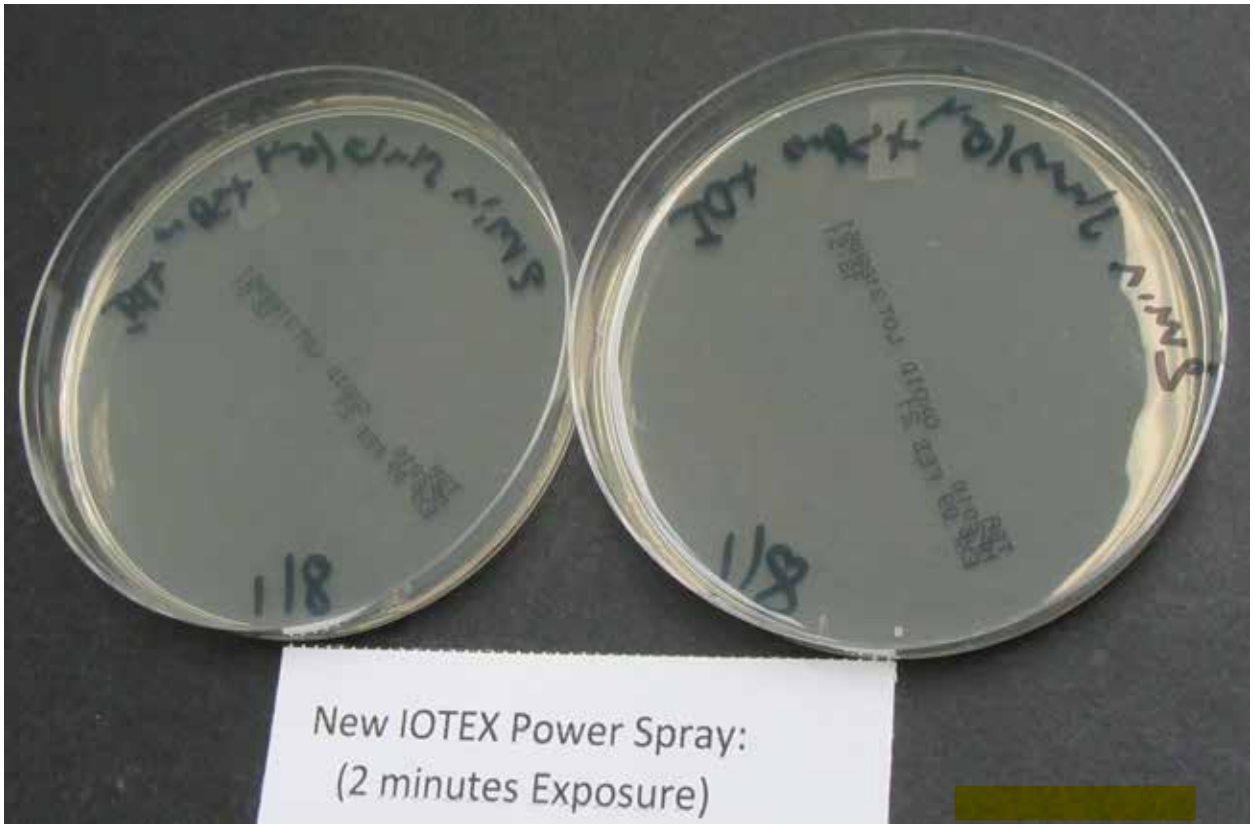


Fig 4: Effect of New IOTEX Power Spray against a mixed population of bacteria- 2 minutes exposure.

References

1- Bergey's Manual of Determinative Bacteriology: John G. Holt (Editor), Noel R. Krieg (Editor),
2- Peter H.A. Sneath (Editor), James T.Staley (Editor), Stanley T. Williams (Editor). Lippincott Williams & Wilkins Ninth Edition, 1994.

2- M. Balouiri, M. Sadiki, SK Ibnsouda, Methods for *in vitro* evaluating antimicrobial activity: A review, In Journal of Pharmaceutical Analysis, Volume 6, Issue 2, 2016, Pages 71-79.

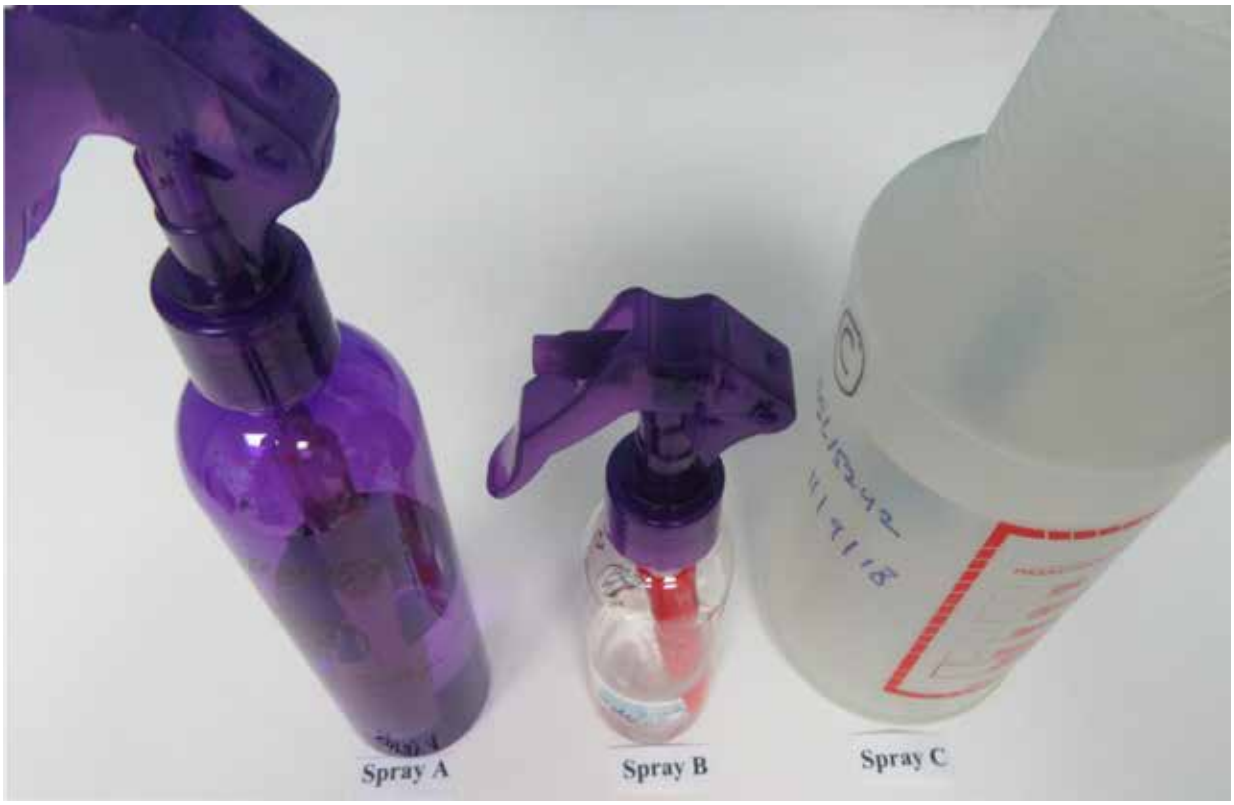
3- USP 31 <1072> Disinfectants and Antiseptics.

IOTEX™

Anti-Infection Products Inc.

Health Canada NPN 80106491 • November 2020
Canadian & U.S. Patent Pending for the IOTEX™ Spray # 16/906670 filed June 19, 2020

The IOTEX™ Spray eliminates antibiotic resistant pathogenic bacteria, viruses & fungi Without toxicity



Part 2

IOTEX™

Anti-Infection Products Inc.

1020 Hargrieve Road, Unit 14
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Email: info@gaplab.com
Website: www.gaplab.com

GAP Project: A14208a

February 24, 2020

Iotex Anti-infection Products Inc.
Attn: Larry Miller, Chairman
19 Hart Dr. Unit 111
Barrie, ON L4N 5M3
Tel: 705-458-9800
Email: larrysmiller@rogers.com

ASTM E2315 – Assessment of Antimicrobial Activity Using a Time-Kill Procedure

ASTM Assessment of Antimicrobial Activity Using a Time-Kill Procedure (E2315) was employed to test the Iotex Spray bottle and its chemistry. The bottle was filled with laboratory de-ionized water and the spray effluent was discharged into a sterile container and then tested against the ASTM E2315 method for its antimicrobial properties. More specifically, the spray effluent was brought in contact with a known population of microorganisms at room temperature for a variety of contact times (see below), then neutralized at 1:10 concentration with D/E Neutralization broth. After neutralization, the surviving microorganisms were enumerated using the procedures outlined under "Microbial Population Quantification" below. The log and percent reductions were calculated using the microbial concentrations recovered from a phosphate buffered water control test. The results can be found on the following pages.

Challenge organism:

MS2 Bacteriophage (ATCC 15597-B1)

The MS2 Bacteriophage was prepared at GAP using a proprietary procedure, identified internally as SOP #40: Preparation and Storage of Concentrated Bacteriophage. In short, a host *E. coli* was grown in Tryptic Soy Broth to log phase in a shaking incubator. A small volume of MS2 (1 transfer removed from ATCC) was added at this time, and the broth mixture remained shaking overnight. The *E. coli* was subsequently removed via centrifugation, leaving the MS2 in solution.

Time points:

1 minute ± 5 seconds
5 minutes ± 10 seconds
30 minutes ± 30 seconds

Test materials:

Spray effluent from a bottle insert, bottle was filled with de-ionized water. See Figure 1 below for an image of the tested bottle.

Microbial Population Quantification:

Enumeration of the surviving microbial population was achieved using GAP method BACTPHAGE-0001: Quantitative Recovery of Bacteriophage Used for Disinfection Equipment Validation. The media used was Tryptone Yeast Extract Glucose agar (TYGA) containing triphenyl tetrazolium chloride (TTC) and incubation was 35±0.5°C.

GAPLAB Environmental Microbiology

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Antimicrobial Activity Calculation:

The antimicrobial activity (R-value) of the test agent to each microbe was calculated according to the following calculation:

$$R = A_0 - A_t$$

Where

R is the value of antimicrobial activity, or log reduction of the test agent.

A_0 is the logarithm of the number of viable bacteria, in CFU/mL, initially (T=0) recovered from the phosphate buffered water control.

A_t is the logarithm of the number of viable bacteria, in CFU/mL, recovered from the treated test agent after the specified contact time.

Quality Control:

All conditions were met for a valid test and all media passed quality control checks. The neutralization test results can be found in **Table 1** below and are within ±0.5 logs of the initial microbial count. The control test results can be found in **Table 2** below; this shows that the initial microbial population counts are within ±0.5 logs of the counts at each test point.

Results:

Results for each test article can be found in **Table 2** through **Table 3** below, with a summary of the results in **Table 4**. The ASTM E2315 procedure dose not allow the testing laboratory to specify what is considered a successful test, but the Iotex spray bottle and its chemistry produced an R value of greater than 6.09 at 5 minutes exposure; this is equivalent to more than a 99.9999% reduction of the MS2-bacteriophage used in this test. As an example, a concentration of 1,000,000 pfu/mL would be reduced to <1 pfu/mL at 5 minutes exposure.

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Figure 1: The spray bottle with insert used throughout testing. The bottle was filled with de-ionized water and the spray effluent was collected for use in testing.

Neutralization Test Results:

Table 1. Neutralization Test Results – Inoculum suspensions in Antimicrobial Agent plus D/E Neutralization broth at a 1/10 concentration in contact for 1 hour. All results were within the allowable limits for the method.

	PBW Control	Neutralization 1:10
Rep1 (pfu/mL)	5.00x10^6	5.50x10^6
Rep2 (pfu/mL)	3.50x10^6	6.50x10^6
Log Rep A	6.70	6.74
Log Rep B	6.54	6.81
Average	6.71	6.84
A_0	6.71	
A_t		6.84
$R=A_0-A_t$		-0.13

PFU= Plaque Forming Unit, PBW= Phosphate Buffered Water (Lab water control)

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Control Test Results:

Table 2. Control (PBW) Test Results – This is used for the A_0 value in the R (antimicrobial activity) calculation for all test articles and to ensure that no external factors are influencing the reduction of the test microbe over the duration of the test.

Exposure Time	T=0	T=1 min	T=5 min	T=30 min
Rep1 (pfu/mL)	5.25x10^6	5.65x10^6	7.35x10^6	8.85x10^6
Rep2 (pfu/mL)	5.00x10^6	5.15x10^6	5.10x10^6	1.03x10^7
Log Rep A	6.72	6.75	6.87	6.95
Log Rep B	6.70	6.71	6.71	7.01
Average	6.71	6.73	6.79	6.98
A_0	6.71			
A_t		6.73	6.79	6.98
$R=A_0-A_t$		-0.02	-0.08	-0.27

PFU= Plaque Forming Unit, PBW= Phosphate Buffered Water (Lab water control)

Test Results:

Table 3. Test Results for the spray bottle insert. The R value indicates the log reduction measured at a given contact time.

Exposure Time	T=1 min	T=5 min	T=30 min
Rep1 (pfu/mL)	7.15x10^3	<5.00x10^0	<5.00x10^0
Rep2 (pfu/mL)	1.06x10^4	<5.00x10^0	≤5.00x10^0
Log Rep A	3.85	<0.70	<0.70
Log Rep B	4.03	<0.70	≤0.70
Average	3.94	<0.70	≤0.70
A_0	6.73	6.79	6.98
A_t	3.94	<0.70	≤0.70
$R=A_0-A_t$	2.79	>6.09	≥6.28

PFU= Plaque Forming Unit, PBW= Phosphate Buffered Water (Lab water control)

Table 4. Summary of the R-value and % reduction for each test article.

Exposure Time	A_0	A_t	$R=A_0-A_t$	% Reduction
1 minute	6.73	3.97	2.79	99.8386
5 minutes	6.79	<0.70	>6.09	>99.9999
30 minutes	6.98	≤0.70	≥6.28	>99.9999

PFU= Plaque Forming Unit, PBW= Phosphate Buffered Water (Lab water control)

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Analyst: S. Verhoeven (Technical Manager)

Approved by: J. Patterson (Lab Manager)

Signature: S. Verh

Signature: J Patterson

These test results relate only to the samples submitted and the analyses requested.
This test report cannot be reproduced except in full, without written permission from GAP.

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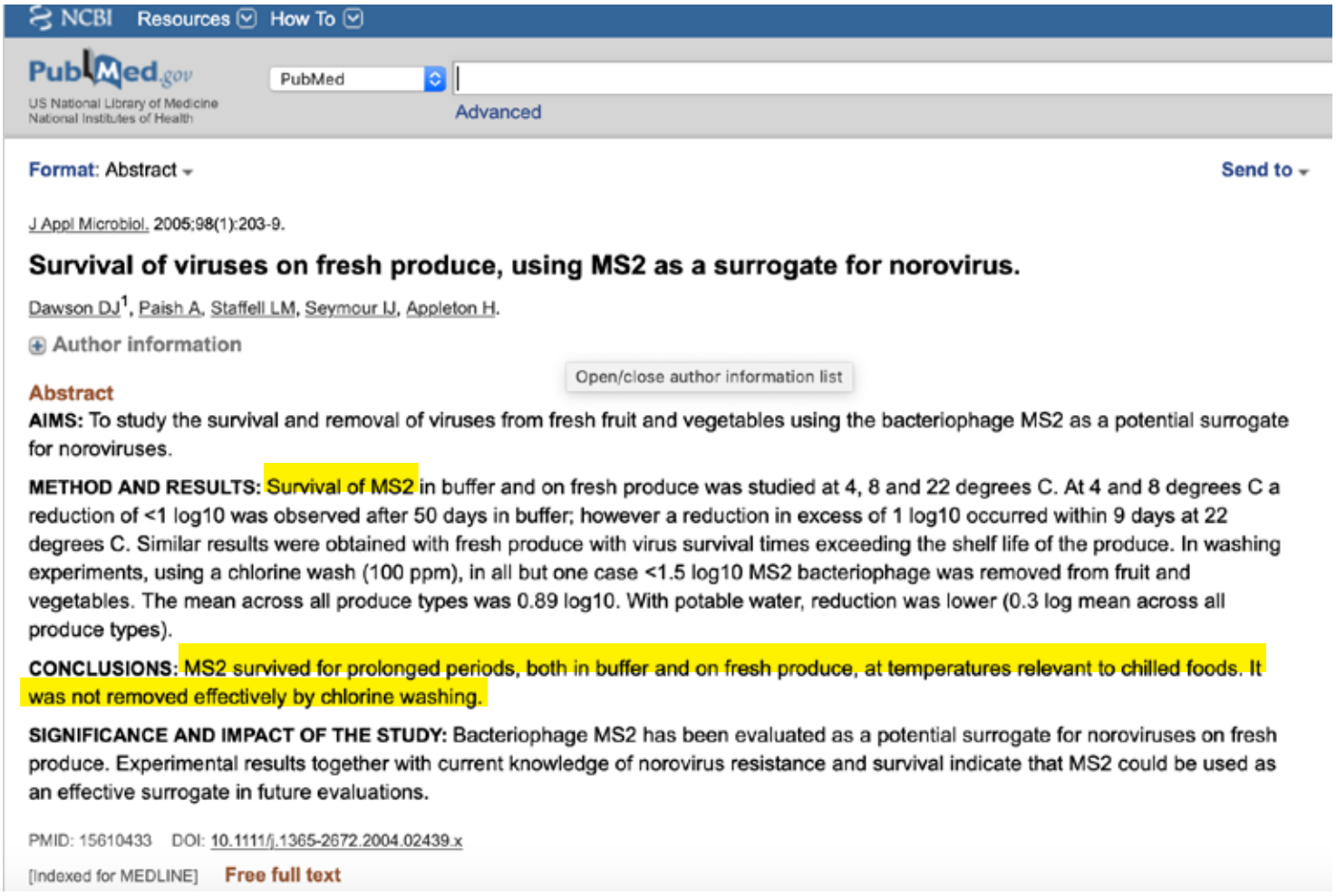

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J Appl Microbiol. 2005;98(1):203-9.

Survival of viruses on fresh produce, using MS2 as a surrogate for norovirus.

Dawson DJ[1], Paish A, Staffell LM, Seymour IJ, Appleton H.

Author information

Open/close author information list

Abstract

AIMS: To study the survival and removal of viruses from fresh fruit and vegetables using the bacteriophage MS2 as a potential surrogate for noroviruses.

METHOD AND RESULTS: Survival of MS2 in buffer and on fresh produce was studied at 4, 8 and 22 degrees C. At 4 and 8 degrees C a reduction of <1 log10 was observed after 50 days in buffer; however a reduction in excess of 1 log10 occurred within 9 days at 22 degrees C. Similar results were obtained with fresh produce with virus survival times exceeding the shelf life of the produce. In washing experiments, using a chlorine wash (100 ppm), in all but one case <1.5 log10 MS2 bacteriophage was removed from fruit and vegetables. The mean across all produce types was 0.89 log10. With potable water, reduction was lower (0.3 log mean across all produce types).

CONCLUSIONS: MS2 survived for prolonged periods, both in buffer and on fresh produce, at temperatures relevant to chilled foods. It was not removed effectively by chlorine washing.

SIGNIFICANCE AND IMPACT OF THE STUDY: Bacteriophage MS2 has been evaluated as a potential surrogate for noroviruses on fresh produce. Experimental results together with current knowledge of norovirus resistance and survival indicate that MS2 could be used as an effective surrogate in future evaluations.

PMID: 15610433 DOI: 10.1111/j.1365-2672.2004.02439.x

[Indexed for MEDLINE] Free full text

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Anti-Infection Products Inc.

MBL Mold & Bacteria Consulting Laboratories

...more than just lab results

Lab reference: MBL16308ET- Spray A July 09, 2019

Iotex Anti-infection Products Inc.
111-19 Hart Drive
Barrie, ON L4N 5M3
(Attn: Larry Miller or Ron Diamond)

Dear Larry and Ron,

Re: Testing of Iotex Spray A against *Candida auris*

As per your request, Mold & Bacteria Consulting Laboratories tested the Iotex Spray A for control of *Candida auris* growth.

The Iotex Spray A was effective in the control of the tested fungus *(Candida auris*) after 7 days of incubation at the concentrations used for the test.

The test procedure and the findings are presented in the next pages.

Sincerely,

Jackson Kung'u, PhD
Principal Microbiologist
Mold & Bacteria Consulting Laboratories (MBL) Inc.

Tested by: Ali Asgharian, M.Sc. Reviewed by: Dr. Jackson Kung'u, PhD.

Efficacy testing for Iotex Spray A against *Candida auris.*

1. Purpose for the Test

The purpose for the test was to determine the efficacy of Iotex Spray A against the growth of *Candida auris* after 7 days incubation. According to the US Centers for Disease Control and Prevention (CDC), *Candida auris* is an emerging fungus that presents a serious global health threat. It is often multidrug-resistant and has caused outbreaks in healthcare settings. *Candida auris* is difficult to identify using standard laboratory methods.

2. Materials and Methods

2.1 Test products

Iotex Spray A (Figure 1)

2.2 Fungal Test Strain

Candida auris (Reference Number: CDC B11903)

3. Procedure

The test was conducted as instructed by Iotex Anti-infection Products Inc.

3.1 Preparation of Fungal Cell Suspension

Candida auris was grown for 3 days at 25°C and a fungal cell suspension prepared. The cell concentration was adjusted to 1.0 x 10^7cells per ml using McFarland Latex Turbidity Standards.

3.2 Iotex Spray Testing

0.1 ml of the fungal suspension (see 3.1 above) was added in a sterile container and then spayed with the Iotex spray A. The spraying for spray A was achieved by pressing the pump and then releasing after about 15 seconds. The fungus was exposed to the Iotex Spray A for 1 minute. After the exposure time, 20 ml of D/E neutralizing broth (Oxoid, Lot# 927698) was added and shaken thoroughly. After mixing, 1.0 ml of the broth was plated on MEA plates (Oxoid, Lot# 2548025) in duplicate. Control was not sprayed with Iotex Spray. All tested plates were incubated at 25°C for 7 days.

3.3 Calculation of Log Reduction Value

Log Reduction Value (LRV) was calculated using the formula below.

$LRV=\log_{10}(N_0/N)$

N_0 = The initial number of colonies without treatment

N= The final number of colonies with treatment.

4. Results

The LRV for the Iotex spray A was 6.0, which was equal to 99.9999% reduction (Figures 2-4).

5. Discussion and Conclusion

It is concluded that the Iotex spray A was effective in inhibiting the growth of *Candida auris* after 7 days of incubation. Based on the LRV of 6.0, the Iotex spray A was able to reduce the fungal count by more than 99.0%.

Fig 1: Spray A

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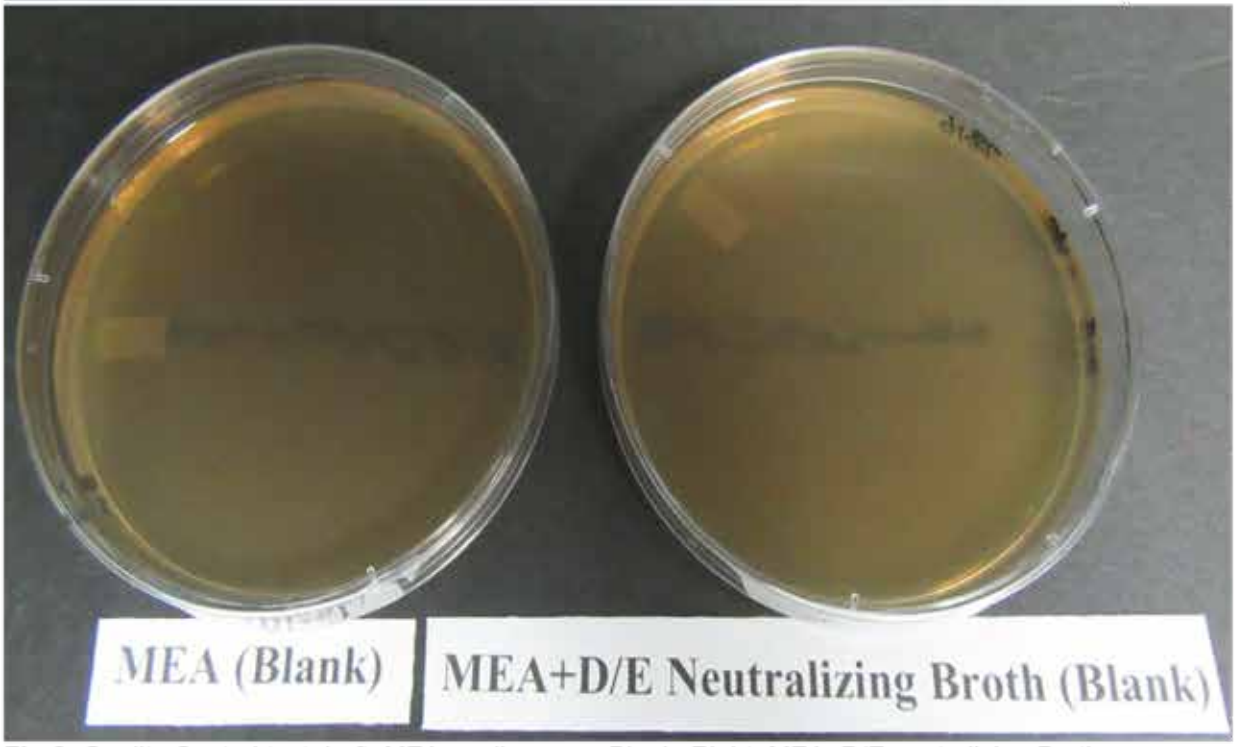

Fig 2: Quality Control test: Left; MEA medium as a Blank, Right: MEA+D/E neutralizing Broth.

Fig 3: Positive control of *Candida auris*.

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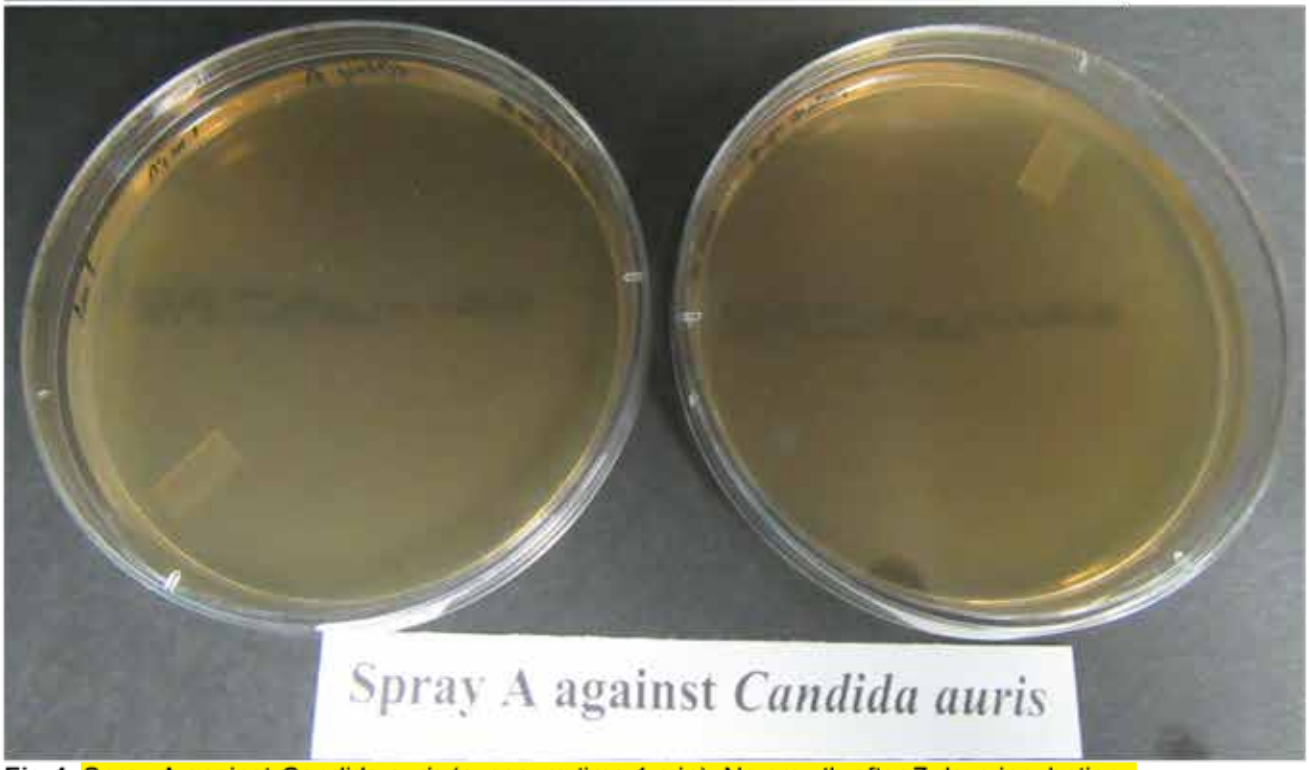

Fig 4: Spray A against *Candida auris* (exposure time:1 min). No growth after 7 days incubation.

6. References

1- M. Balouiri, M. Sadiki, SK Ibnsouda, Methods for *in vitro* evaluating antimicrobial activity: A review, In Journal of Pharmaceutical Analysis, Volume 6, Issue 2, 2016, Pages 71-79.

2- USP 31 <1072> Disinfectants and Antiseptics.

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Anti-Infection Products Inc.

MBL Mold & Bacteria Consulting Laboratories

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Lab reference: MBL16446ET- Spray D August 21, 2019

Iotex Anti-infection Products Inc.
111-19 Hart Drive
Barrie, ON L4N 5M3
(Attn: Larry Miller or Ron Diamond)

Dear Larry and Ron,

Re: Testing of Iotex Spray D against *Candida auris*

As per your request, Mold & Bacteria Consulting Laboratories tested the Iotex Spray D for control of *Candida auris* growth.

The Iotex Spray D was effective in the control of the tested fungus *(Candida auris)* after 7 days of incubation at the concentrations used for the test.

The test procedure and the findings are presented in the next pages.

Sincerely,

Jackson Kung'u, PhD
Principal Microbiologist
Mold & Bacteria Consulting Laboratories (MBL) Inc.

Tested by: Ali Asgharian, M.Sc. Reviewed by: Dr. Jackson Kung'u, PhD.

Efficacy testing for Iotex Spray D against *Candida auris.*

1. Purpose for the Test

The purpose for the test was to determine the efficacy of Iotex Spray D against the growth of *Candida auris* after 7 days incubation. According to the US Centers for Disease Control and Prevention (CDC), *Candida auris* is an emerging fungus that presents a serious global health threat. It is often multidrug-resistant and has caused outbreaks in healthcare settings. *Candida auris* is difficult to identify using standard laboratory methods.

2. Materials and Methods

2.1 Test products

Iotex Spray D (new) (Figure 1)

2.2 Fungal Test Strain

Candida auris (Reference Number: CDC B11903)

3. Procedure

The test was conducted as instructed by Iotex Anti-infection Products Inc.

3.1 Preparation of Fungal Cell Suspension

Candida auris was grown for 3 days at 25 °C and a fungal cell suspension was prepared. The concentration of the suspension was adjusted to 5.0×10^7 cells per ml using McFarland Latex Turbidity Standards.

3.2 Iotex Spray Testing

0.1 ml of the fungal suspension (see 3.1 above) was added in a sterile container and then spayed with the Iotex spray D. The spraying for spray D was achieved by pressing the pump and then releasing after about 5 seconds. The fungus was exposed to the Iotex Spray for 1 minute. After the exposure time, 20 ml of D/E neutralizing broth (Oxoid, Lot# 927698) was added and shaken thoroughly. After mixing, 1.0 ml of the broth was plated on MEA plates (Oxoid, Lot# 2832595) in duplicate. Control was not sprayed with Iotex Sprays. All tested plates were incubated at 25 °C for 7 days.

3.3 Calculation of Log Reduction Value

Log Reduction Value (LRV) was calculated using the formula below.

$LRV = \log_{10} (N_0/N)$

N_0 = The initial number of colonies without treatment

N= The final number of colonies with treatment.

4. Results

The LRV for the Iotex spray D is shown in table 1. An LRV of 6.7 is equivalent to a percent reduction of the fungus of 99.9999% (Figures 2-4).

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Table1: Effectiveness of spray D against *Candida auris*.

Spray Type	Incubation time: 7 days, Exposure time: 1 min	
	LRV	Percent Reduction
Spray D	6.7	99.9999%

5. Discussion and Conclusion

It is concluded that the Iotex spray D was effective in inhibiting the growth of *Candida auris* after 7 days of incubation. Based on the LRV of 6.7, the Iotex spray D was able to reduce the fungal colony count by more than 99.9%.

Fig 1: Spray D

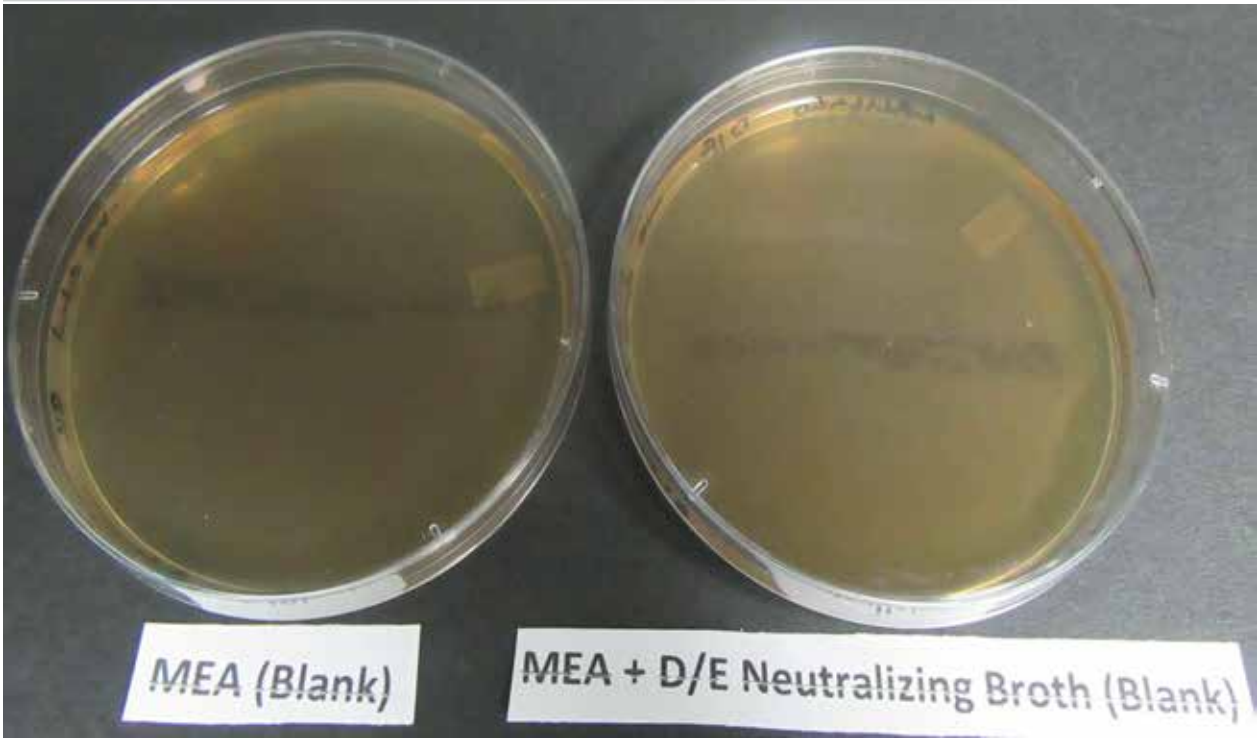

Fig 2: Quality Control test: Left; MEA medium as a Blank, Right: MEA+D/E neutralizing Broth.

Fig 3: Positive control of *Candida auris.*

Fig 4: Spray D against *Candida auris* (exposure time:1 min).

6. References

1- M. Balouiri, M. Sadiki, SK Ibnsouda, Methods for *in vitro* evaluating antimicrobial activity: A review, In Journal of Pharmaceutical Analysis, Volume 6, Issue 2, 2016, Pages 71-79.

2- USP 31 <1072> Disinfectants and Antiseptics.

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MBL Mold & Bacteria Consulting Laboratories

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Lab reference: MBL17047ET- Spray D February 13, 2020

Iotex Anti-infection Products Inc.
111-19 Hart Drive
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(Attn: Larry Miller or Ron Diamond)

Dear Larry and Ron,

Re: Testing of Iotex Spray D against *Malassezia pachydermatis* ★

As per your request, Mold & Bacteria Consulting Laboratories tested the Iotex Spray D for control of *Malassezia pachydermatis* growth.

The Iotex Spray D was effective in the control of the tested fungus *(Malassezia pachydermatis*) after 5 days of incubation at 35ºC at the concentrations used for the test.

The test procedure and the findings are presented in the next pages.

Sincerely,

Jackson Kung'u, PhD
Principal Microbiologist
Mold & Bacteria Consulting Laboratories (MBL) Inc.

Tested by: Ali Asgharian, M.Sc. Reviewed by: Dr. Jackson Kung'u, PhD.

★The Malassezia fungus is a key co-factor in the development of pancreatic cancers: Dr George Miller, NYU-School of Medicine, Oct 2019.

Efficacy testing for Iotex Spray D against *Malassezia pachydermatis*.

1. Purpose for the Test

The purpose for the test was to determine the efficacy of Iotex Spray D against the growth of *Malassezia pachydermatis* after 5 days incubation at 35°C. According to the US Centers for Disease Control and Prevention (CDC), dogs are a natural host of *Malassezia pachydermatis*. Zoonotic transfer has been documented from dogs to immunocompromised patients by healthcare workers who own dogs.

2. Materials and Methods

2.1 Test products

Iotex Spray D (new) (Figure 1)

2.2 Fungal Test Strain

Malassezia pachydermatis (ATCC 14522™)

3. Procedure

The test was conducted as instructed by Iotex Anti-infection Products Inc.

3.1 Preparation of Fungal Cell Suspension

Malassezia pachydermatis was grown for 3 days at 35 °C and a fungal cell suspension was prepared. The concentration of the suspension was adjusted to 0.5×10^8 cells per ml.

3.2 Iotex Spray Testing

0.1 ml of the fungal suspension (see 3.1 above) was added in a sterile container and then spayed with the Iotex spray D. The spraying for spray D was achieved by pressing the pump and then releasing after about 5 seconds. The fungus was exposed to the Iotex spray for 1 minute. After the exposure time, 20 ml of D/E neutralizing broth (Oxoid, Lot# 927698) was added and shaken thoroughly. After mixing, 1.0 ml of the broth was plated on MEA plates (Oxoid, Lot# 2918204) in duplicate. Control was not sprayed with Iotex spray. All tested plates were incubated at 35 °C for 5 days.

3.3 Calculation of Log Reduction Value

Log Reduction Value (LRV) was calculated using the formula below.

$LRV = \log_{10} (N_0/N)$

N_0 = The initial number of colonies without treatment

N= The final number of colonies with treatment.

4. Results

The LRV for the Iotex spray D for *Malassezia pachydermatis* is shown in table 1. An LRV of 3.3 is equivalent to a percent reduction of the fungus of 99.9% (Figures 1-4).

Table1: Effectiveness of spray D against *Malassezia pachydermatis.*

Spray Type	Incubation time: 5 days, Exposure time: 1 min	
	LRV	Percent Reduction
Spray D	3.3	99.9%

5. Discussion and Conclusion

It is concluded that the Iotex spray D was effective in inhibiting the growth of *Malassezia pachydermatis* after 5 days of incubation. Based on the LRV of 3.3, the Iotex spray D was able to reduce the fungal colony count by more than 99.0%.

Fig 1: Spray D

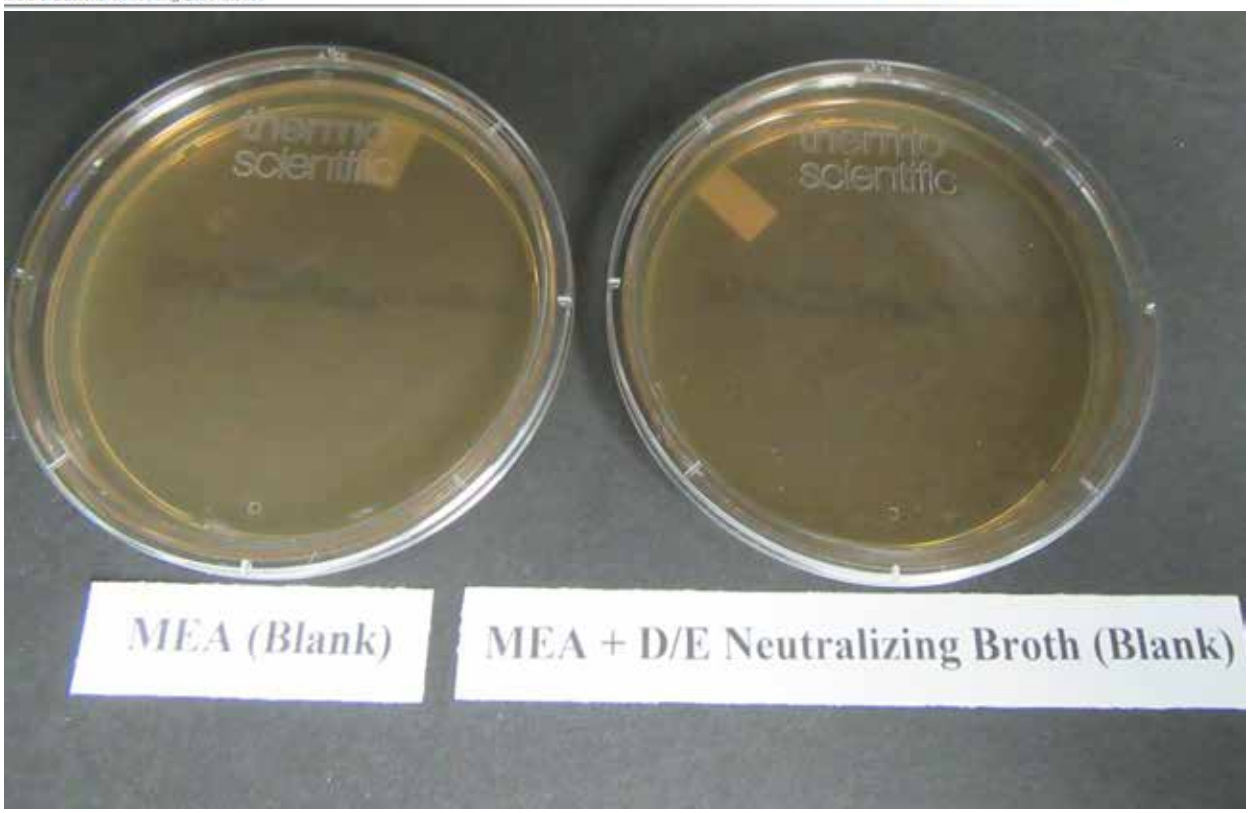

Fig 2: Quality Control test: Left; MEA medium as a Blank, Right: MEA+D/E neutralizing Broth.

Fig 3: Positive control of *Malassezia pachydermatis.*

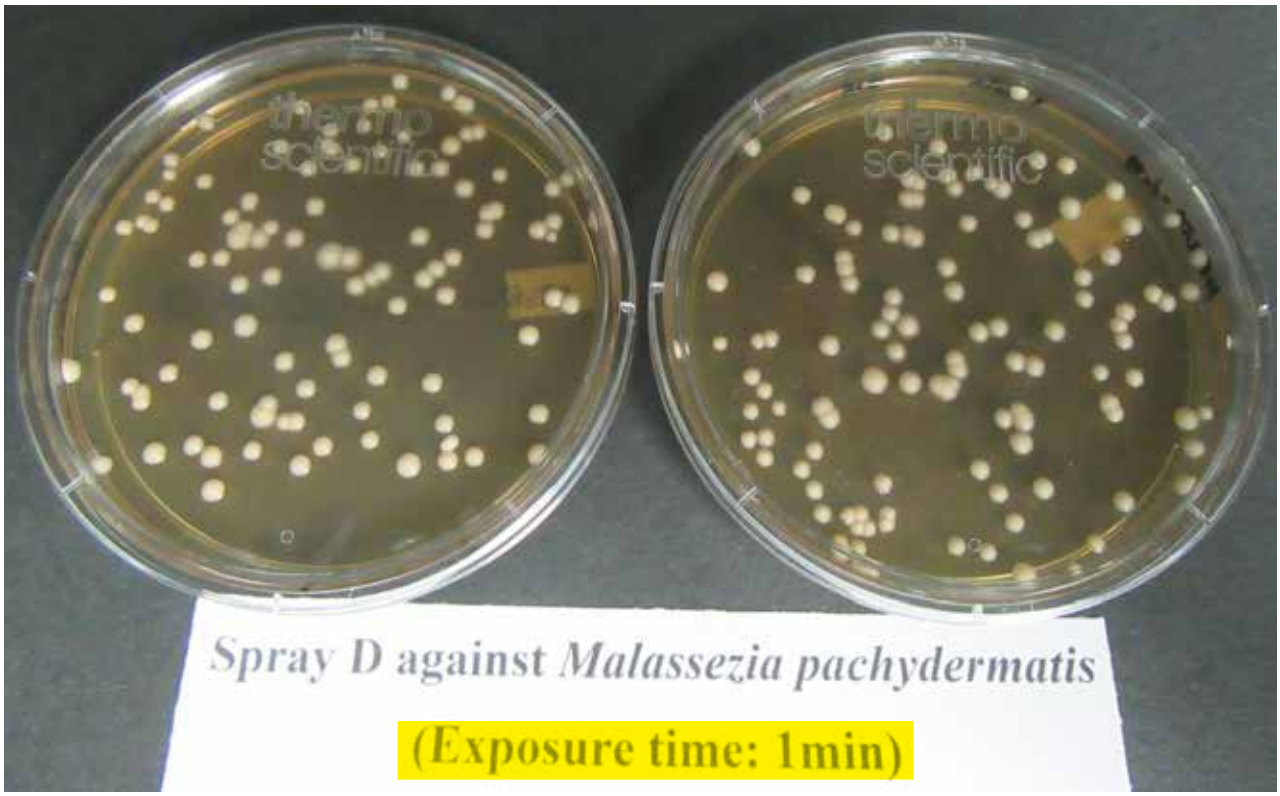

Fig 4: Spray D against *Malassezia pachydermatis* (exposure time:1 min).

6. References

1- USP 35 <1072> Disinfectants and Antiseptics.

2- Daniel O. Morris, Kathleen O'Shea, Frances S. Shofer, and Shelley Rankin. *Malassezia pachydermatis* Carriage in Dog Owners. Emerging Infectious Diseases. www.cdc.gov/eid. Vol. 11, No. 1, January 2005. P: 83- 88