

# Using Modified Kelsey Sykes Test Option to Ascertain the Efficacy of Legionella-X Viral Spray Insect Repellent Against Pseudomonas Aeruginosa, Escherichia Coli, Staphylococcus Aureus and Proteus Vulgaris Bacteria.

Nelson Cheng<sup>1</sup>, Patrick Moe<sup>2</sup>,

<sup>1</sup> Magna International Pte Ltd, 10H Enterprise Road, Singapore 629834

Frederick Cheng<sup>2</sup>

Cambridge University, Sidney Sussex College *Sidney Street, Cambridge, CB2 3HU, UK*

## Abstract

This article relates the test method used to ascertain the efficacy of Legionella-X-Viral-Spray Insects Repellent against gram-negative and gram-positive bacteria, some of these included; Escherichia Coli, Proteus Vulgaris, Pseudomonas Aeruginosa and Staphylococcus Aureus bacteria using the Modified Kelsey Sykes Capacity Test Option for Hospital Grade Disinfectant under dirty conditions.

## Keywords:

Disinfectant, Pseudomonas Aeruginosa, Escherichia Coli, Proteus Vulgaris and Staphylococcus Pathogenicity, Modified Kelsey Sykes Test

## Introduction

A number of diseases that impact humans are caused by bacteria. Bacteria that caused disease are called pathogenic bacteria, and they do so by producing poisonous substances called endotoxins and exotoxins [1]. These substances are responsible for the symptoms that occur with bacteria related diseases. The symptoms may range from mild to serious, and some can be deadly. Endotoxins are mostly found in the outer membrane of Gram-negative bacteria. Endotoxins are part of the outer membrane of the cell wall of Gram-negative bacteria. Although the term "endotoxin" is occasionally used to refer to any cell-associated bacterial toxin, in bacteriology it is properly reserved to refer to the lipopolysaccharide complex associated with the outer membrane of Gram-negative

pathogens such as *Escherichia coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, *Haemophilus influenzae*, *Bordetella pertussis* and *Vibrio cholerae*. [2]

Necrotizing fasciitis is a serious infection most often caused by *Streptococcus pyogenes* bacteria (*S. pyogenes*). Other types of bacteria that can also cause necrotizing fasciitis include *Escherichia Coli* and *Staphylococcus aureus*.

Bacteria, viruses and parasites can all cause meningitis. Bacterial meningitis can be caused by a number of bacteria. In newborns, the most common causes of bacterial meningitis are Group B *Streptococcus*, *Escherichia coli*, and *Listeria monocytogenes*.

***Pseudomonas aeruginosa*** has emerged as an important pathogen during the past two decades. It causes between 10% and 20% of infections in most hospitals [3]. It causes urinary tract infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections, and a variety of systemic infections, particularly in patients with severe burns and in cancer [4]. *Pseudomonas aeruginosa* is an opportunistic pathogen commonly found in the environment mainly in soil and water but is also regularly found on plants and sometimes on animals, including humans and widely in the environment. *Pseudomonas aeruginosa* can be a dangerous bacterium. It is one of the main causes of serious hospital-acquired infection in the UK. It is also a leading cause of death among people with cystic fibrosis[5].

The sensitivity and resistance to the following antibiotics were tested against *Pseudomonas aeruginosa*, piperacillin, tazobactam, cefepime, ceftazidime, imipenem, meropenem, ciprofloxacin, levofloxacin, gentamicin, tobramycin, amikacin, Fosfomycin and colistin.

*Pseudomonas aeruginosa* is a common cause of community-acquired and nosocomial-acquired pneumonia[6]. The development of resistance of *P. aeruginosa* to antibiotics is increasing globally due to the overuse of antibiotics[7].

***Staphylococcus Aureus*** can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis *Aureus*.[8]

***E. coli*** are a large and diverse group of bacteria. One of the most frequent causes of many bacterial infections, including cholecystitis, bacteremia and cholangitis. Although most strains of *E. coli* are harmless, others can make you sick. Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses[9].

***Proteus vulgaris*** is an aerobic, rod-shaped, Gram-negative bacterium in the *Enterobacteriaceae* family. In recent years, the resistances to many antibiotic classes (also

beta-lactams) has significantly increased. The main transmission path is direct or indirect contact with contaminated persons or objects. It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs), and bacteremia. The most common infection involving *Proteus mirabilis* occurs when the bacteria moves to the urethra and urinary bladder. Although *Proteus mirabilis* mostly known to cause urinary tract infections, the majority of urinary tract infections are due to *E. coli* [10].

## The method and test procedures are herein described.

The Modified Kelsey Sykes Capacity Test Option for Hospital Grade Disinfectant under dirty conditions was adopted.

- 1) Four test organisms, *Escherichia Coli* NCTC 8196, *Proteus Vulgaris* NCTC 4635, *Pseudomonas aeruginosa* NCTC 6749 and *Staphylococcus* NCTC 4163 were used.
- 2) The inoculum size of each of the test organisms was not less than  $2 \times 10^8$  or more than  $2 \times 10^9$  organisms introduced into the individual test samples of the Legionella-X Viral Off disinfectant solution. The disinfectant sample was tested neat.
- 3) The method is essentially that given by Kelsey & Maurer (Kelsey, J.C and Maurer Isobel, M. *Pharmaceutical Journal* (UK) 213:528-230, (1974). The said disinfectant is tested at the use-concentration.
- 4) The test consists of challenging the waterless Legionella-X Viral-Spray Insects Repellent with bacterial inoculum, withdrawing a sample after a given time (8 minutes) and culturing the sample in a suitable recovery culture medium.
- 5) After this sampling, the mixture is again challenged by a second inoculum and after a second interval (18 minutes) is again samples for culturing.
- 6) The sample is passed or failed according to the extent of growth shown in the two cultures sampled.

Appended below are the observation parameter and test result based on the Modified Kelsey Sykes Capacity Test Option for Hospital Grade Disinfectant under dirty condition [11].

**Observation Parameter and Test Result of Legionella-X Viral Spray  
Insects Repellent**

<b>Escherichia Coli NCTC 8196</b>				
Incubation Time	Positive/Negative Controls	Sampling/Exposure Time		Remarks
		8 minutes	18 minutes	
24 Hours	+ + / - -	-----	-----	
48 Hours	+ + / - -	-----	-----	Pass
<b>Proteus Vulgaris NCTC 4635</b>				
Incubation Time	Positive/Negative Controls	Sampling/Exposure Time		Remarks
		8 minutes	18 minutes	
24 Hours	+ + / - -	-----	-----	
48 Hours	+ + / - -	-----	-----	Pass
<b>Pseudomonas Aeruginosa NCTC 6749</b>				
Incubation Time	Positive/Negative Controls	Sampling/Exposure Time		Remarks
		8 minutes	18 minutes	
24 Hours	+ + / - -	-----	-----	
48 Hours	+ + / - -	-----	-----	Pass
<b>Staphylococcus Aureus NCTC 4163</b>				
Incubation Time	Positive/Negative Controls	Sampling/Exposure Time		Remarks
		8 minutes	18 minutes	
24 Hours	+ + / - -	-----	-----	
48 Hours	+ + / - -	-----	-----	Pass

**Note** + : Growth in one tube of recovery broth  
 - : No growth in one tube of recovery broth.

## Conclusion

Based on the observation parameter and result of the Modified Kelsey Sykes Test Capacity Test Option, it can be ascertained that Legionella-X Viral-Spray Insects Repellent effectively disinfects Escherichia Coli, Proteus Vulgaris, Pseudomonas Aeruginosa and Staphylococcus Aureus bacteria.

## Reference

- [1] Difference Between Endotoxins and Exotoxins  
<http://www.differencebetween.net/science/difference-between-endotoxins-and-exotoxins/>
- [2] Bacterial Endotoxin Definition  
[www.biologicscorp.com/blog/bacterial-endotoxin-definition/#.WzNWKdIzbIU](http://www.biologicscorp.com/blog/bacterial-endotoxin-definition/#.WzNWKdIzbIU)
- [3] Infections caused by Pseudomonas aeruginosa. Bodey GP, Bolivar R, Fainstein V, Jadeja L.  
<https://www.ncbi.nlm.nih.gov/pubmed/6405475>
- [4] Pseudomonas aeruginosa infection in cancer patients. Rolston KV, Bodey GP.  
<https://www.ncbi.nlm.nih.gov/pubmed/1735012>
- [5] Eradication of early Pseudomonas aeruginosa infection. Høiby N, Frederiksen B, Pressler T.  
<https://www.ncbi.nlm.nih.gov/pubmed/16023416>
- [6] Pseudomonas aeruginosa susceptibility in a university hospital: recognition and treatment. Rotschafer JC, Shikuma LR  
<https://www.ncbi.nlm.nih.gov/pubmed/3091346>
- [7] Antibiotic susceptibility patterns of Pseudomonas aeruginosa to available antipseudomonal drugs in Ibadan, Nigeria. Ogbolu DO, Ogunledun A, Adebisi OE, Daini OA, Alli AO.  
<https://www.ncbi.nlm.nih.gov/pubmed/19301711>
- [8] <http://nordichug.com/about-microbes/>
- [9] Escherichia coli (E coli) Infections, Updated: May 18, 2017, Author: Tarun Madappa, MD, MPH; Chief Editor: Michael Stuart Bronze, MD .  
<https://emedicine.medscape.com/article/217485-overview>

[10] Proteus mirabilis and Urinary Tract Infections, Jessica N. Schaffer and Melanie M. Pearson.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4638163/>

[11] Method for Estimation of Concentration of Disinfectants Used in 'Dirty' Conditions in Hospitals by the Modified Kelsey-Sykes Test: Volume 6905 of B.S. (Series)

[https://books.google.com.sg/books/about/Method\\_for\\_Estimation\\_of\\_Concentration\\_o.html?id=6qjWPQAACAAJ&redir\\_esc=y](https://books.google.com.sg/books/about/Method_for_Estimation_of_Concentration_o.html?id=6qjWPQAACAAJ&redir_esc=y)

## About the Authors

**NELSON CHENG** PhD (Honoris Causa) is the founder and chairman of Magna Group, consisting of Magna International; Magna F.E. Chemical Pte., Ltd.; Magna Chemical Canada, Ltd.; Magna Australia Pvt., Ltd.; and Lupromax International Pte., Ltd.

Nelson Cheng received a Doctor honoris causa from the Universidad Autonoma de Baja California, Mexico. He graduated as a marine engineer under the United Nations Development Program Scholarship.

He is recognized as Singapore's leading inventor and the Singaporean with highest number of patents from the Intellectual Property Office of Singapore. He is the inventor of several technologies for corrosion protection including, Vapro VCI (Vapor Corrosion Inhibitors), Vapro VBCI (Vapor Bio Corrosion Inhibitor) and Vapro CRI (Concrete Rebar Inhibitor), Molecular Reaction Surface Technology (MRST), Colloidal corrosion inhibitors (CCI) and Heat Activated Technology (HAT).

He is a member of Society of Tribologists and Lubrication Engineers (STLE), American Chemical Society (ACS) World Corrosion Organization (WCO) and European Federation of Corrosion (EFC).

## **PATRICK MOE**

Patrick Moe is the senior technical manager of Magna International Pte. Ltd. He has a BSc in Industrial Chemistry, Grad. Dip and MSc in Environmental Engineering.

His key responsibilities at Magna International as follows: assisting the CEO in research and development of new products, finding out customers' needs and develop customized

new products, helping in synthesizing new compounds by making appropriate modifications of known methods, recommending and implementing methods to increase the quality of products and service, management of hazardous raw materials.

He is a member of National Association Corrosion Engineers (NACE) and World Corrosion Association (WCA).

## **FREDERICK CHENG**

He is currently studying Law at Cambridge University. He graduated with an International Baccalaureate Diploma and was awarded a Certificate of Academic Excellence by Anglo Chinese School Independent for scoring 44 points out of the perfect score of 45. He was a Ministry of Education Singapore Scholar for his International Baccalaureate Diploma Program. He was also in the School Dean's list and underwent the National Scholars Program Mentorship Attachment at the National University of Singapore organized by Gifted Education, Ministry of Education.

He was awarded several accolades including; 2014 Economic Development Board Award for winning the Excellent of Economic Content and Best Technical Quality Award in National Economics Short Film Competition, Certificate of Distinction for Best Delegate USA (Human Rights Council) on 8<sup>th</sup> Integrated Program Symposium Model United Nations Conference 2011 and Certificate of Distinction for Best Delegation, Model United Nations Certificate of Best Delegation representing Republic of South Korea 2012, Certificate of Honorary Mention, Nanyang Technological University Model United Nations Conference 2011, Certificate of Distinction Young Diplomat 2012.

He is currently the publicity secretary of Cambridge University Law Society, treasurer of the Sidney Sussex College Law Society and an event officer of the Association of British and Chinese University Students (**ABACUS**) at the University of Cambridge.

He is also the co-inventor and co-patent owner for WO2015012761A Chemical composition of a low-mammalian toxicity insecticide  
Priority 2013-07-23 • Filing 2013-07-23 • Publication 2015-01-29