bioFILM PA™ ANTIMICROBIAL SUSCEPTIBILITY KIT IMPACTS OUTCOME IN CYSTIC FIBROSIS PATIENTS

1.0 BACKGROUND

For the past two years, the University of Alberta Hospital has been evaluating a new technology from an Alberta biotechnology company, Innovotech Inc. Under the direction of Dr Robert Rennie, Site Chief, Laboratory Medicine and Dr Neil Brown, Director, Adult Cystic Fibrosis (CF) Clinic, bioFILM PA™, a breakthrough in susceptibility diagnostics has been evaluated in cystic fibrosis patients. Innovotech recognized that current susceptibility testing were for free-floating or single bacteria, whereas cystic fibrosis lung infections and other chronic infections were caused by bacteria in a biofilm state which are much more difficult to kill than free-floating bacteria. The company also recognized that doctors regularly used combinations of antibiotics to treat CF lung infections acknowledging the difficulty in killing biofilm bacteria. The result was bioFILM PA™, designed to assist doctors in selecting antibiotics for the treatment of chronic infections caused by the bacteria, *Pseudomonas aeruginosa*.

2.0 CYSTIC FIBROSIS AND BIOFILMS

Cystic fibrosis (CF) is the most common, fatal genetic disease affecting young Canadians. It is estimated that one in every 3,600 children born in Canada has CF. At the present time, approximately 3,500 children, adolescents, and adults in Canada have cystic fibrosis.
CF is a multi-organ disease affecting primarily the lungs and the digestive system. In the lungs, CF causes severe breathing problems. A build-up of thick mucus makes it difficult to clear bacteria resulting in biofilm formation (Figure 1). This leads to cycles of infection and inflammation, which damage the delicate lung tissues. Patients follow a demanding daily routine of physical therapy and antibiotics to keep the lungs free of congestion and infection. In spite of these measures, the bacteria can multiply and overrun the lung airways leading to chronic pneumonia.

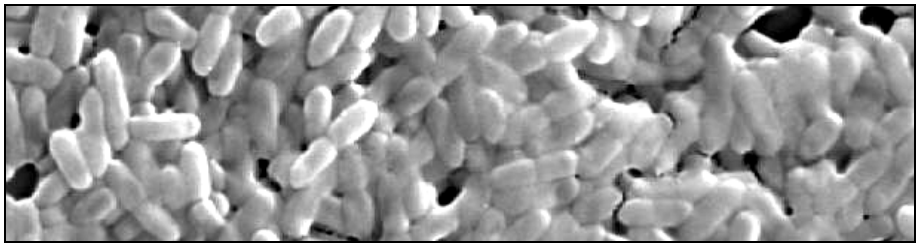

Figure 1: Scanning Electron Micrograph of *Pseudomonas aeruginosa* bacteria isolated from CF patient's lung that is growing as a biofilm. The bacterial cells are growing in a large colony and cells are embedded in polysaccharide (carbohydrate) matrix.

3.0 bioFILM PA™ ANTIMICROBIAL SUSCEPTIBILITY KIT

Current treatment for cystic fibrosis lung infection, involves selection of antibiotics based on traditional susceptibility testing and empirical methods (an educated guess based upon the doctor's experience).
bioFILM PA™ is the world's first biofilm susceptibility test allowing doctors to make scientific based selection of antibiotics for biofilm infections like cystic fibrosis lung disease. In addition, bioFILM PA™ provides guidance on the selection of combination antibiotics recognizing that doctors

routinely use combinations as they know that single antibiotics rarely work on difficult to treat biofilm infections.

4.0 PROCEDURE DESCRIPTION

The bacteria isolated from each patient are identified as *Pseudomonas aeruginosa* prior to bioFILM PA™ being used. Procedurally, the use of bioFILM PA™ is very similar to traditional susceptibility testing with an added front end step that grows the bacteria as a biofilm on a peg lid (Figure 2) prior to susceptibility testing.

Growing bacteria as a biofilm prior to testing more closely represents how the bacteria exist in the CF patient's lungs. The challenge plate in bioFILM PA™ contains 12 single antibiotic and 35 combination antibiotics at achievable blood concentration providing the doctor with a standard SIR (susceptible, intermediate, resistant) profile for each bacteria tested. In simple language, the test will tell doctors which antibiotics the bacteria are resistant to (and therefore not to use these antibiotics) and which antibiotics the bacteria are sensitive to (and therefore to choose from this group of antibiotics). Doctors incorporate this data along with other relevant clinical data (drug allergies, liver function, etc) in their choice of an antibiotic combination to treat the patient. Equally important, bioFILM PA™ will also provide guidance on the antibiotics that the bacteria are resistant to, thereby avoiding a treatment failure and further patient inconvenience and health care costs.

Figure 2: Peg lid for *in vitro* biofilm growth

5.0 CASE HISTORY

The CF clinic at the University Hospital has tested over 100 isolates from patients ranging from 9 to 51 years of age with bioFILM PA™ over the past two years. Data from 4 patients are summarized below:

PATIENT 1:

History:

- Prior to admission for emergency abdominal surgery (unrelated to CF), the patient had experienced ongoing breathing difficulties with variable lung function. Two multi-resistant strains of *Pseudomonas aeruginosa* had been identified at a routine testing.
- Lung function had been slowly improving immediately prior to the admission for abdominal surgery.

- In the post-operative period, lung function had declined by 20%. Another three multi-drug resistant strains of Pseudomonas were subsequently identified. Despite the use of intravenous antibiotics, the patient did not improve and lung function declined a further 20%. Antibiotic treatment based on traditional susceptibility testing had failed to produce a positive response.

Intervention with bioFILM PA™:

- A bioFILM PA™ test was order by the doctor, and based on information provided, therapy was changed to a new combination of antibiotics based on the biofilm PA results.

Outcome:

- After two weeks of new antibiotic treatment the patient improved
- Three of the resistant bacterial strains were eradicated.
- Lung function had improved by 33% from the lowest post operative measurement.
- The patient was discharged from hospital.
- Since the discharge from hospital in March 2008, the patient has had no major issues, exacerbations or hospitalization.

PATIENT 2:

History:

- Patient was on a waiting list for lung transplantation.
- Patient was infected with a multi-drug resistant *Pseudomonas aeruginosa* and was receiving chronic oral and inhaled antibiotic treatment without any improvement for a severe persistent and progressive lung infection.
- It was determined that the patient should be admitted to hospital on a semi-urgent basis in an effort to stabilize the course of the disease and provide a bridge to transplantation.

Intervention with bioFILM PA™:

- A bioFILM PA™ test was order by the doctor, and based on information provided, therapy was changed to a new combination of antibiotics.

Outcome:

- Following antibiotic treatment, donor lungs became available and the patient underwent a successful double lung transplantation (May 2007).
- The patient was kept on the pre-operative intravenous drug regimen as recommended by the bioFILM PA™ test during the recovery from surgery.
- Over the past two years since transplantation, the patient has not required antibiotics for lung infection. The transplant surgeons stated that patients will normally see a reoccurrence of symptoms within 6-8 months post-transplantation and gave credit to the antibiotics received based on the bioFILM PA test in the peri-operative period.

PATIENT 3:

History:

- Patient was on a waiting list for lung transplantation and was infected with two multi-drug resistant strains of *Pseudomonas aeruginosa* while receiving antibiotics based on traditional susceptibility testing,
- Patient was admitted to hospital with a severe lung infection but the transplant team were reluctant to proceed with transplantation based on the patient's poor condition.

Intervention with bioFILM PA™:

- A bioFILM PA™ test was order by the doctor, and based on information provided, therapy was changed to a new combination of antibiotics.

Outcome:

- The infection responded to treatment and the transplant was performed successfully.

- Since that time (**over 2 years**), the patient has had only one recurrence of a lung infection and as it was not severe, the patient was able to be treated as an out-patient.

PATIENT 4:

History:

- Patient was receiving home intravenous antibiotics for a *Pseudomonas aeruginosa* lung infection. The same antibiotics had been successfully used one year earlier for a similar infection.
- Patient continued to deteriorate and was admitted to hospital (June 2008).

Intervention with bioFILM PA™:

- A bioFILM PA™ test was order by the doctor.
- While waiting for the test results from bioFILM PA, the antibiotics were changed based on the traditional MIC test but no improvement was noted.
- When the bioFILM PA™ test results were obtained, an antibiotic not often used in CF lung infections was identified and added to the treatment.

Outcome:

- The patient improved and was released from hospital within two weeks.
- The patient has not had a recurrence of symptoms and has not required antibiotics in over one year.

6.0 CONCLUSION

bioFILM PA™ represents a breakthrough antimicrobial in how chronic infections caused by *Pseudomonas* bacteria are treated. For the first time doctors receive susceptibility information on the biofilm state of the bacteria and as well are provided with guidance on the selection of combination antibiotic drugs.

bioFILM PA™ has received regulatory approval by Health Canada and has meets current standards of the Clinical Laboratory Standards Institute (CLSI) for reproducibility and consistency.

While the data presented in this paper is early stage, it points in a very positive direction to the potential benefits of use:

Potential patient benefits:

- Improvement in quality of life
- Delay in progression of disease
- Improved lung function and breathing

Potential doctor benefits:

- Improved patient outcomes
- Greater confidence in susceptibility testing
- Reduction of treatment failures
- Better guidance on selection of combination antibiotic choices

Potential diagnostic laboratory benefits:

- Reduced susceptibility testing as a result of ongoing treatment failures
- Greater confidence in susceptibility testing

Benefits to the Healthcare system:

- Reduced costs of drug treatment and hospitalization
- Delay in lung transplantation costs
- Reduced resistance development from the use of inappropriate drugs