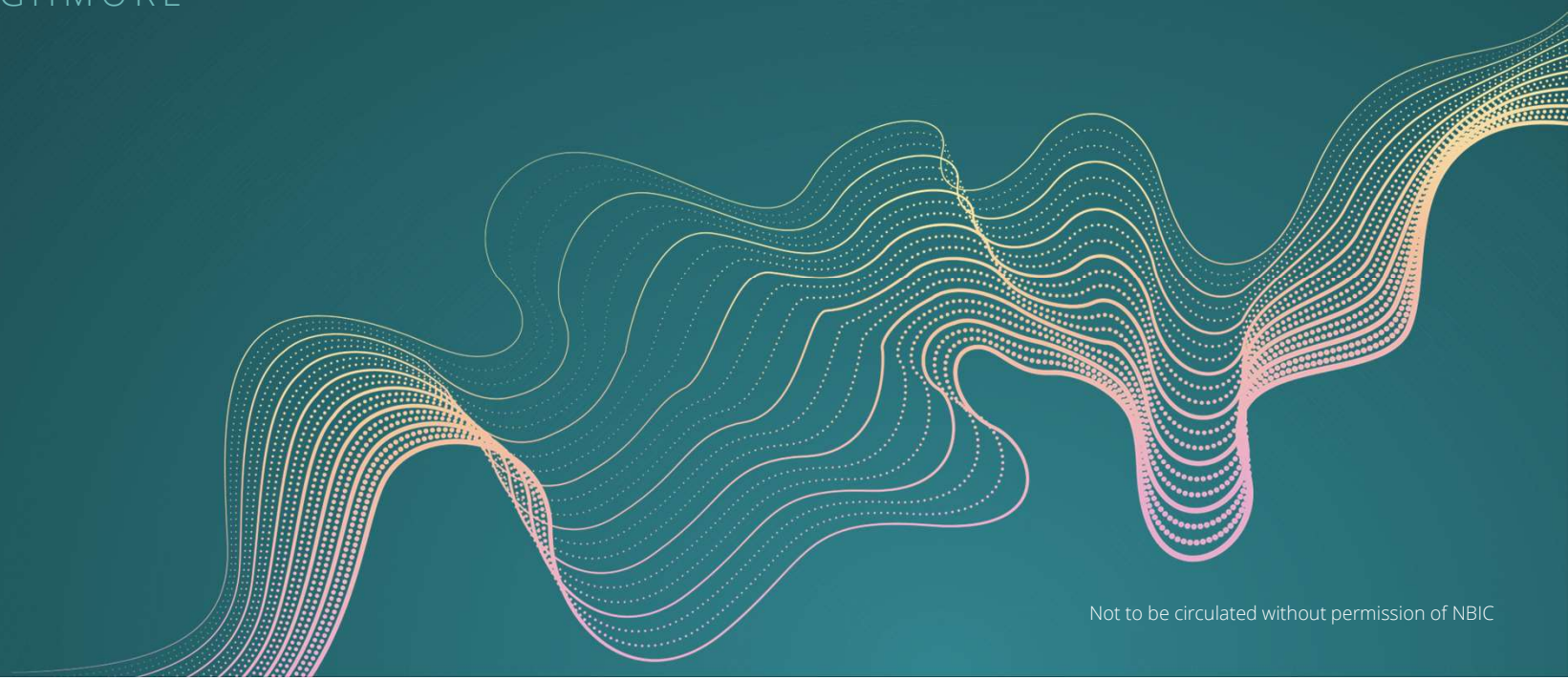
National Biofilms Innovation Centre

Biofilm and Stressed Foodborne Pathogens – A Comparative Study using Bactiscan™ for Real-time Pathogen Detection

DR CALLUM HIGHMORE

25.10.2023



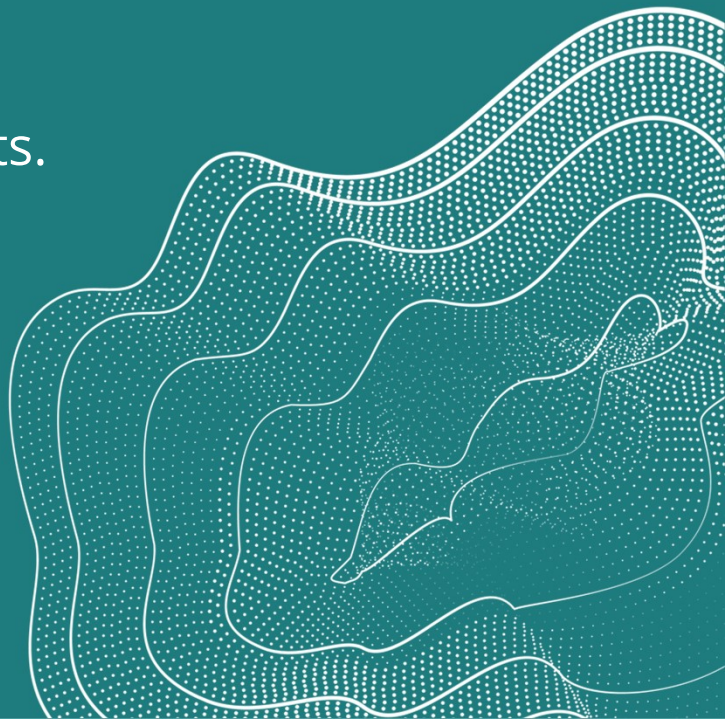
@ukbiofilms

biofilms.ac.uk

Background

THE CHALLENGE OF FOODBORNE CONTAMINATION

- Foodborne disease is a huge and preventable global challenge.
- Bacterial states such as biofilm prevent effective decontamination of food processing environments.
- The FSA estimate that 60% of foodborne disease cases have an unknown cause.

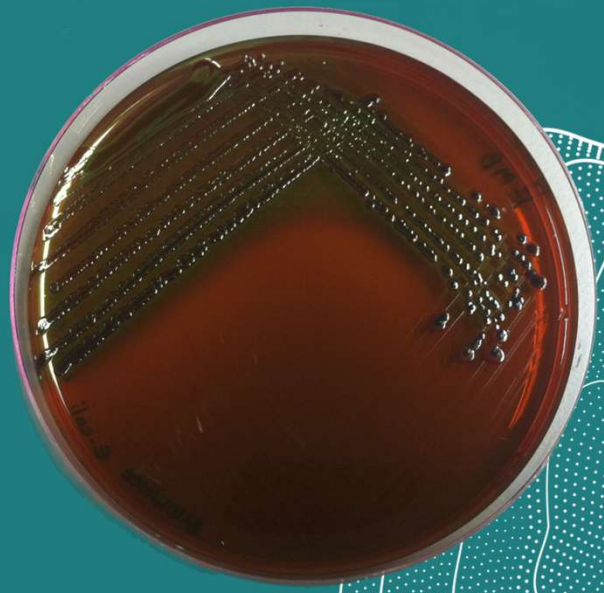


NBIC EIT Preventative Controls & Detection Systems

Background

CONTAMINATION DETECTION

- ATP test swabs
- Culture techniques



Bactiscan

MACRO-SCALE, REAGENTLESS DETECTION

- Bactiscan is a high intensity UV torch designed for microbial detection.
- It uses specific UV wavelengths to excite the S-layer of the bacterial cell wall.
- Rapid, reagentless, and cost effective.

NBIC EIT Preventative Controls & Detection Systems

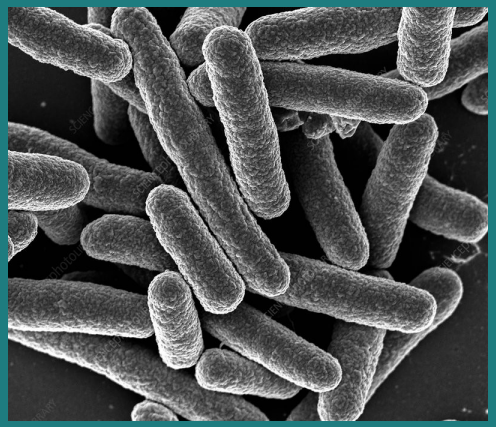
Study Aims

- To measure sensitivity of Bactiscan detection.
- To assess Bactiscan detection efficacy on different modes of bacterial life.
- To compare the utility of Bactiscan against other bacterial detection methods.
- To trial Bactiscan contamination detection on real food samples and characterise their microbial communities.

NBIC EIT Preventative Controls & Detection Systems

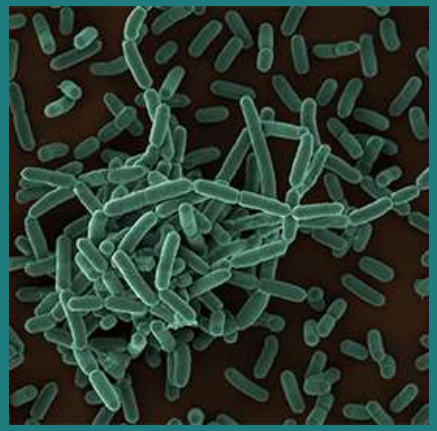
Foodborne pathogens used in the study

ESCHERICHIA COLI



Gram-negative

LISTERIA MONOCYTOGENES

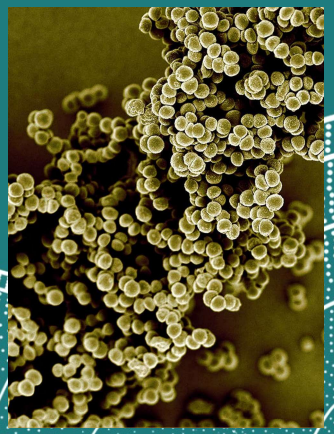


Gram-positive

SALMONELLA ENTERICA

Gram-negative

STAPHYLOCOCCUS AUREUS

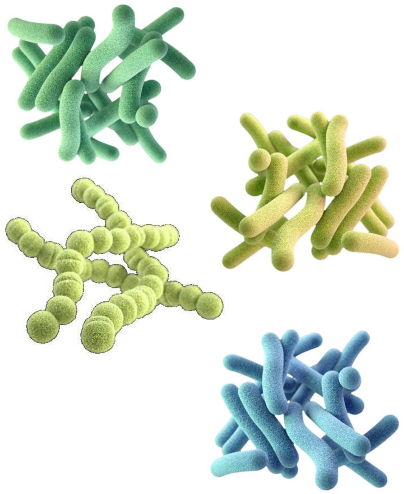


Gram-positive

NBIC EIT Preventative Controls & Detection Systems

Study design

MEASURING VISUALISATION



4 x bacterial species

% Observation

5 x technical repeats

3 x observers

3 x biological repeats

NBIC EIT Preventative Controls & Detection Systems

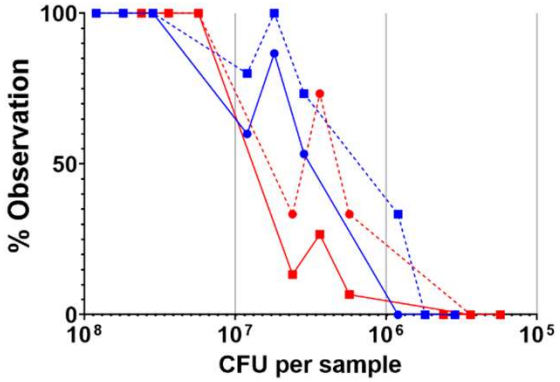
Bactiscan and pathogens

DETECTION LIMITS

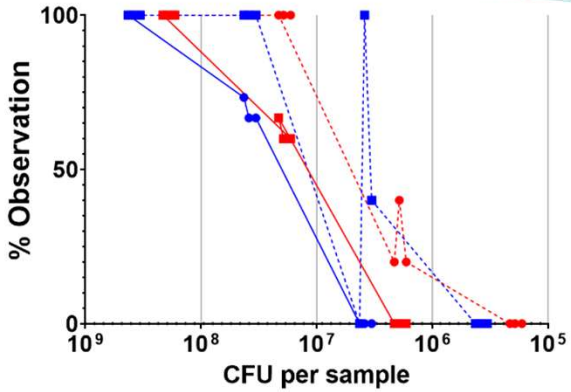
- Detection depends on volume and bacterial concentration of sample.
- Detection at lower bacterial concentrations is subjective to the observer.

	Detection limit (CFU)	
Bacterial species	**Light**	**Dark**
E. coli	$3.33*10^6$	$1.94*10^6$
L. monocytogenes	$1.65*10^7$	$9.32*10^6$
S. enterica	$5.46*10^6$	$1.20*10^6$
S. aureus	$5.37*10^7$	$9.02*10^6$

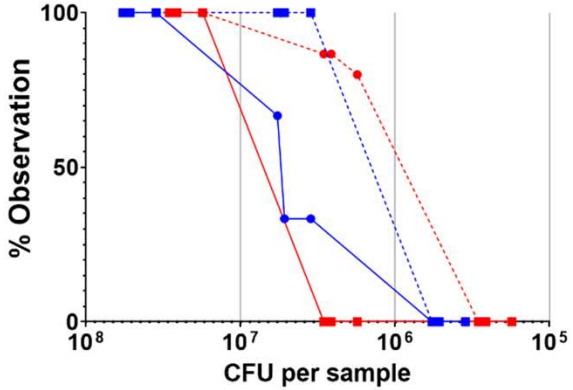
E. coli



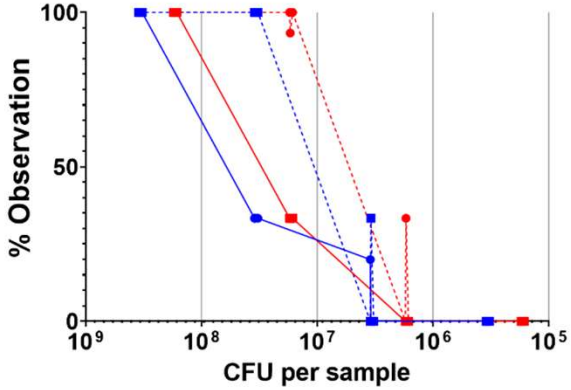
L. monocytogenes



S. enterica



S. aureus

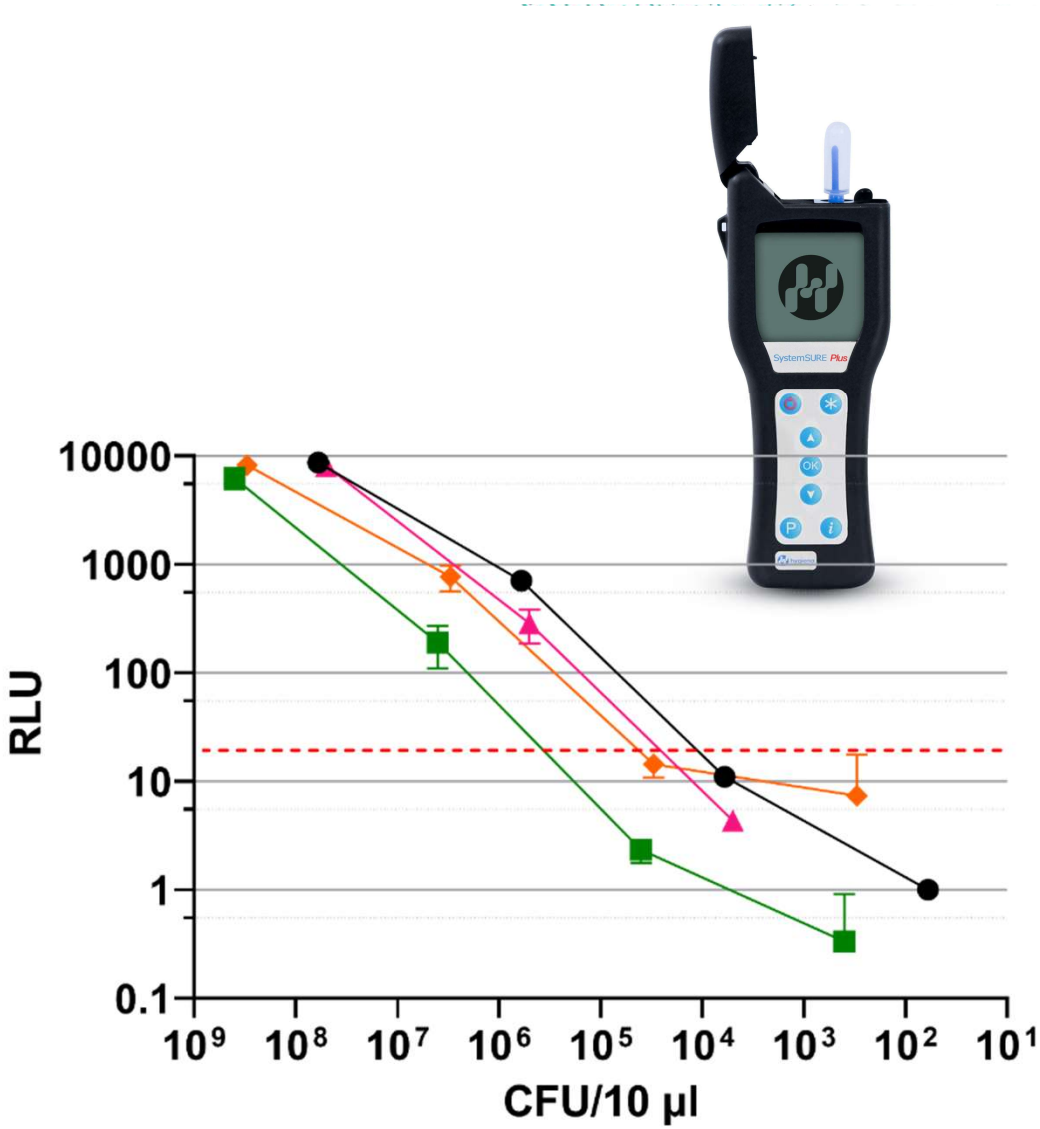


NBIC EIT Preventative Controls & Detection Systems

Bactiscan and pathogens

SENSITIVIY OF ATP TESTS

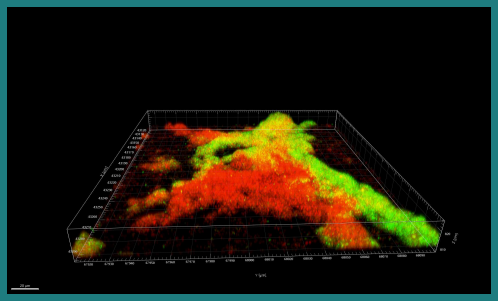
- The sensitivity of ATP swab tests was tested for each of the pathogens.
- ATP swab testing gave a lower detection limit than Bactiscan.
- However, experiments were optimised for use of ATP swabs.



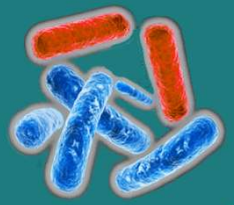
Biofilms and stressed cells

BETTER MODELS FOR INDUSTRY

Biofilms



Stressed cells

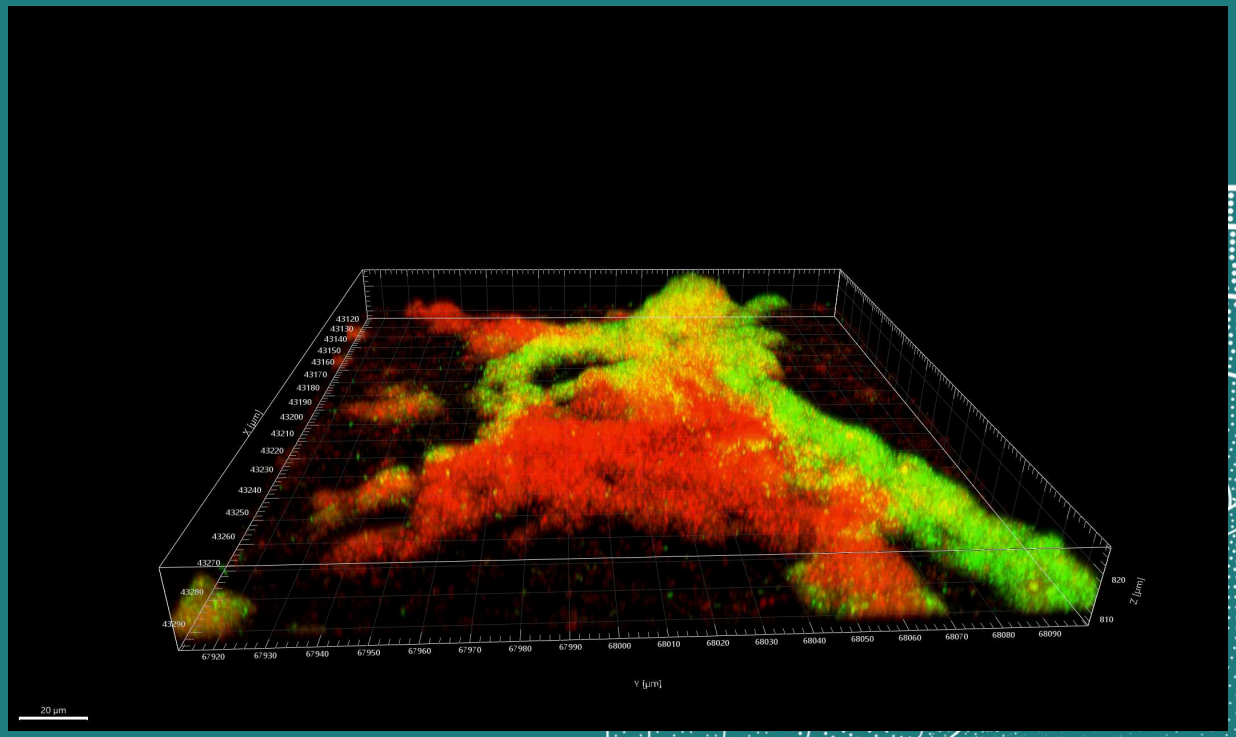


NBIC EIT Preventative Controls & Detection Systems

Biofilms

KEY POINTS

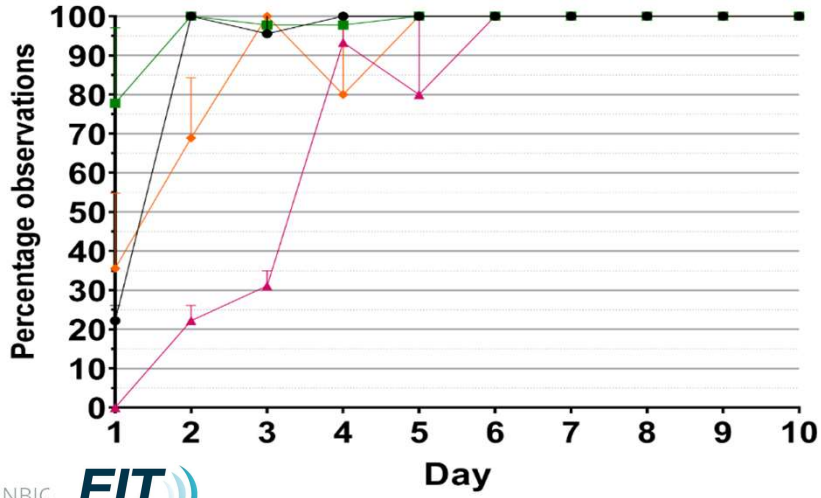
- Biofilms are microbial communities.
- **Resistant** to decontamination practices.
- Cause **persistent** and **recurrent** contamination.

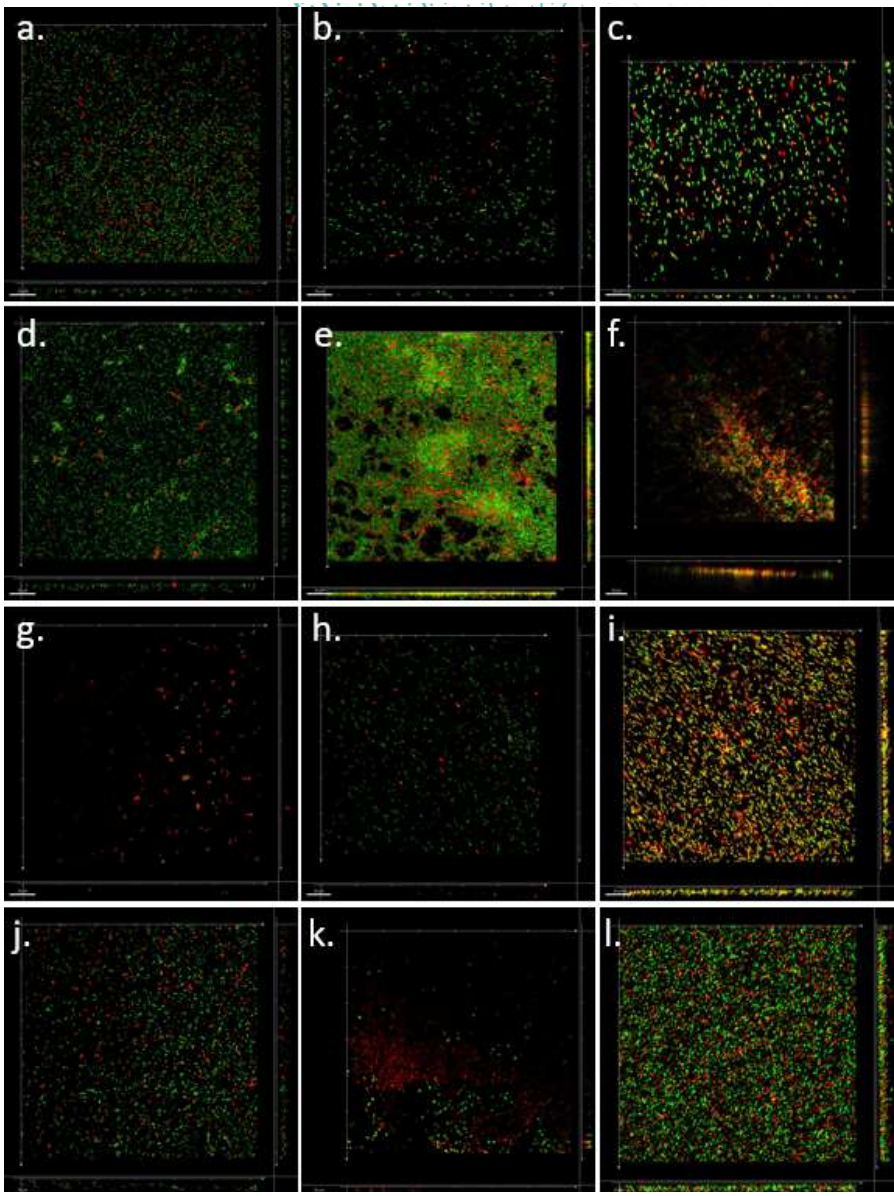


NBIC EIT Preventative Controls & Detection Systems

Bactiscan detects biofilms

- Biofilms of each pathogen were grown over 10 days.
- By day 2, *E. coli* and *L. monocytogenes* biofilms were consistently visible.
- By day 4, all biofilms were consistently visible.

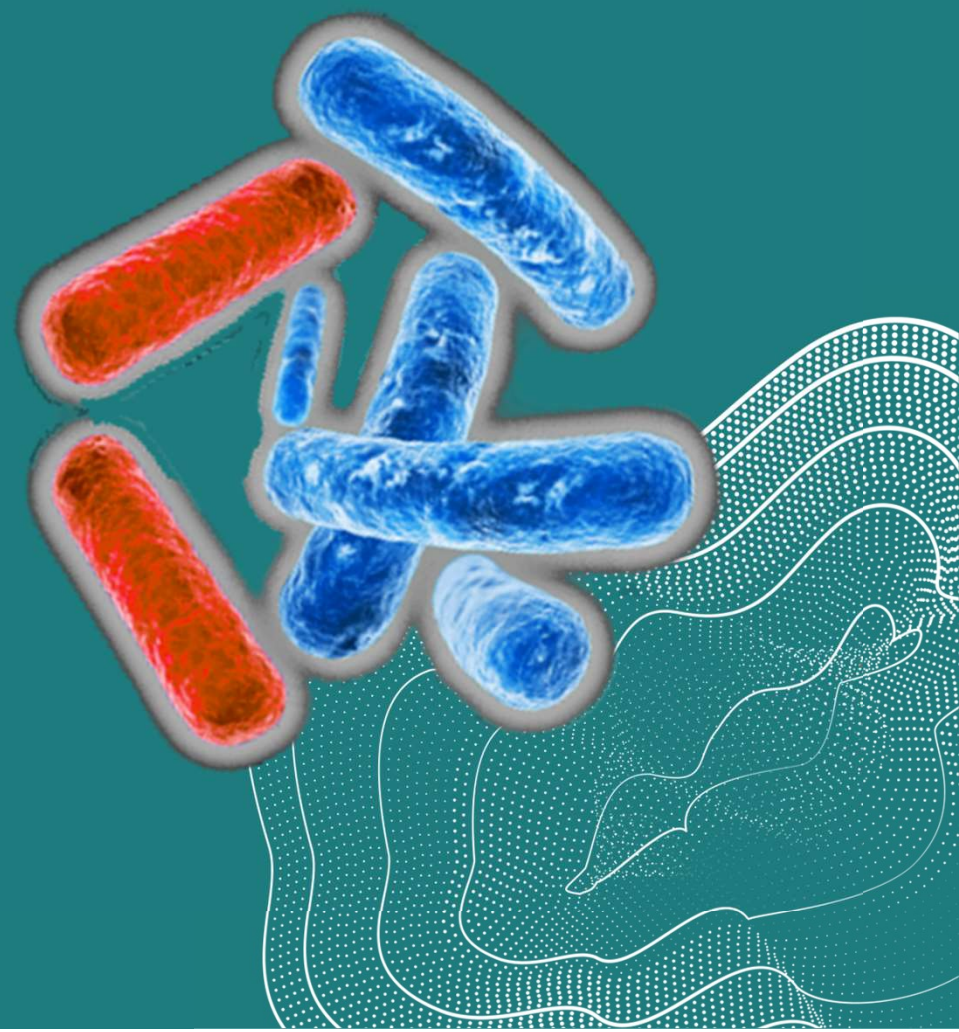




Stressed bacterial cells

KEY POINTS

- Stressed bacteria alter their physiology to survive.
- Reduced metabolism makes them **harder to kill**.
- They can **evade detection**.

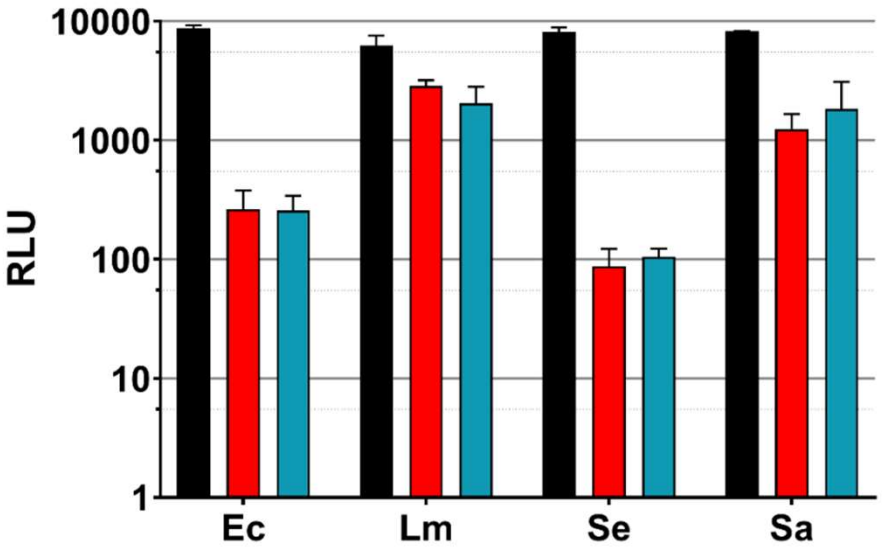


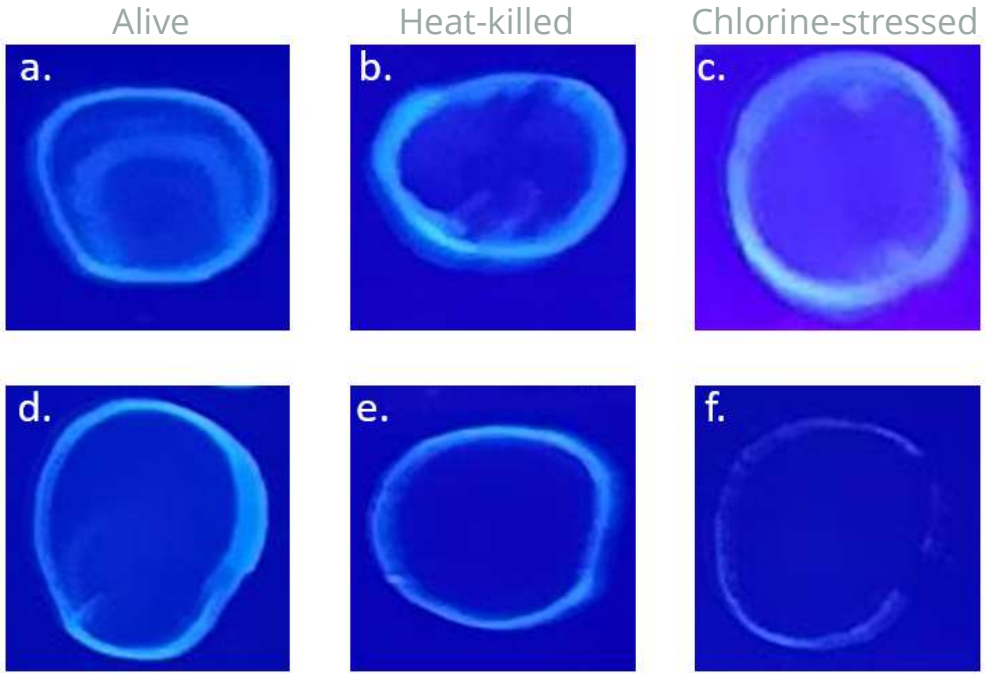
NBIC EIT Preventative Controls & Detection Systems

Bactiscan detects stressed cells

- Droplets of dead and stressed cells were visible by Bactiscan.

- The detection capability of ATP test swabs was reduced by >90% for stressed *E. coli* and *S. enterica.*





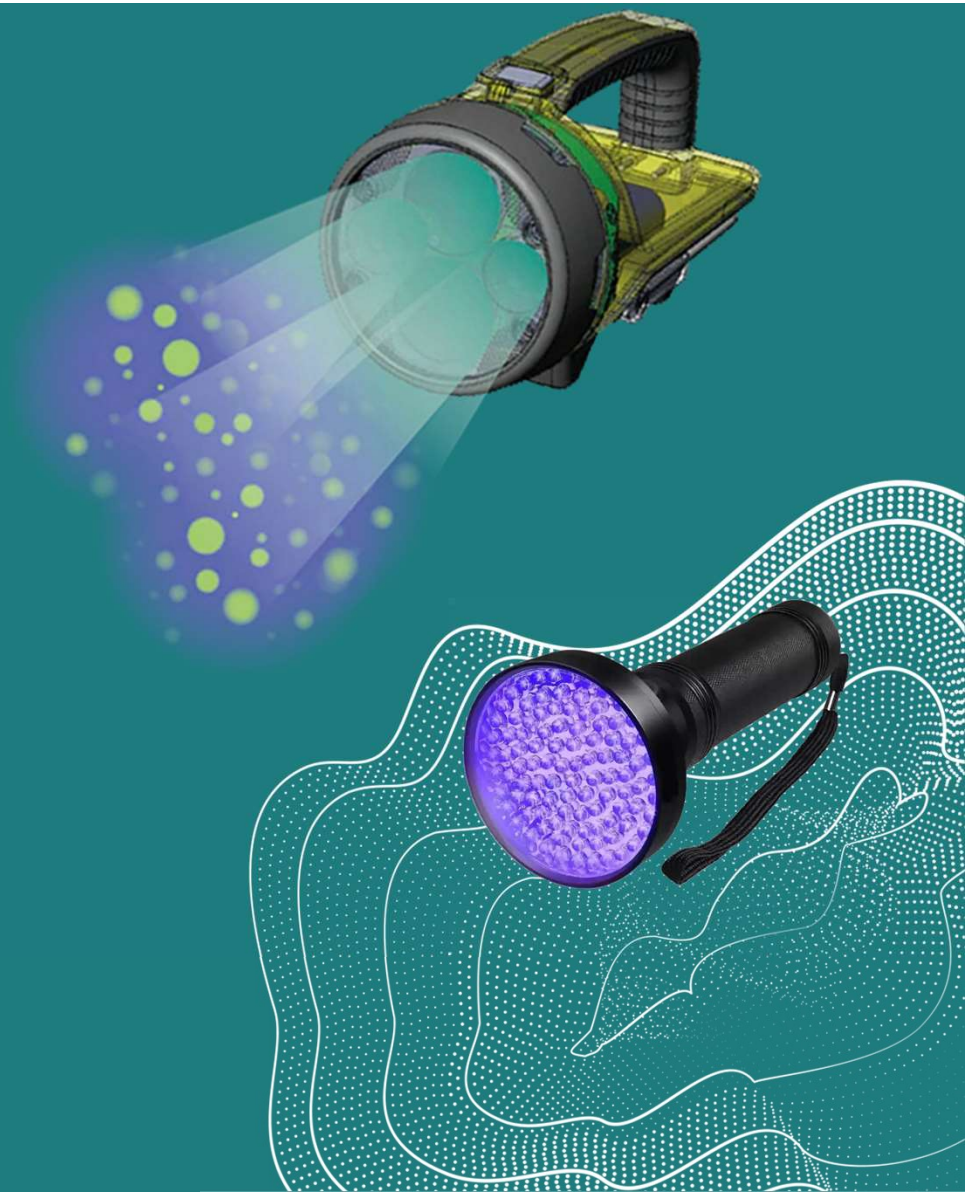
NBIC EIT Preventative Controls & Detection Systems

Complementary use with ATP swabs

NBIC EIT Preventative Controls & Detection Systems

Real world contamination detection

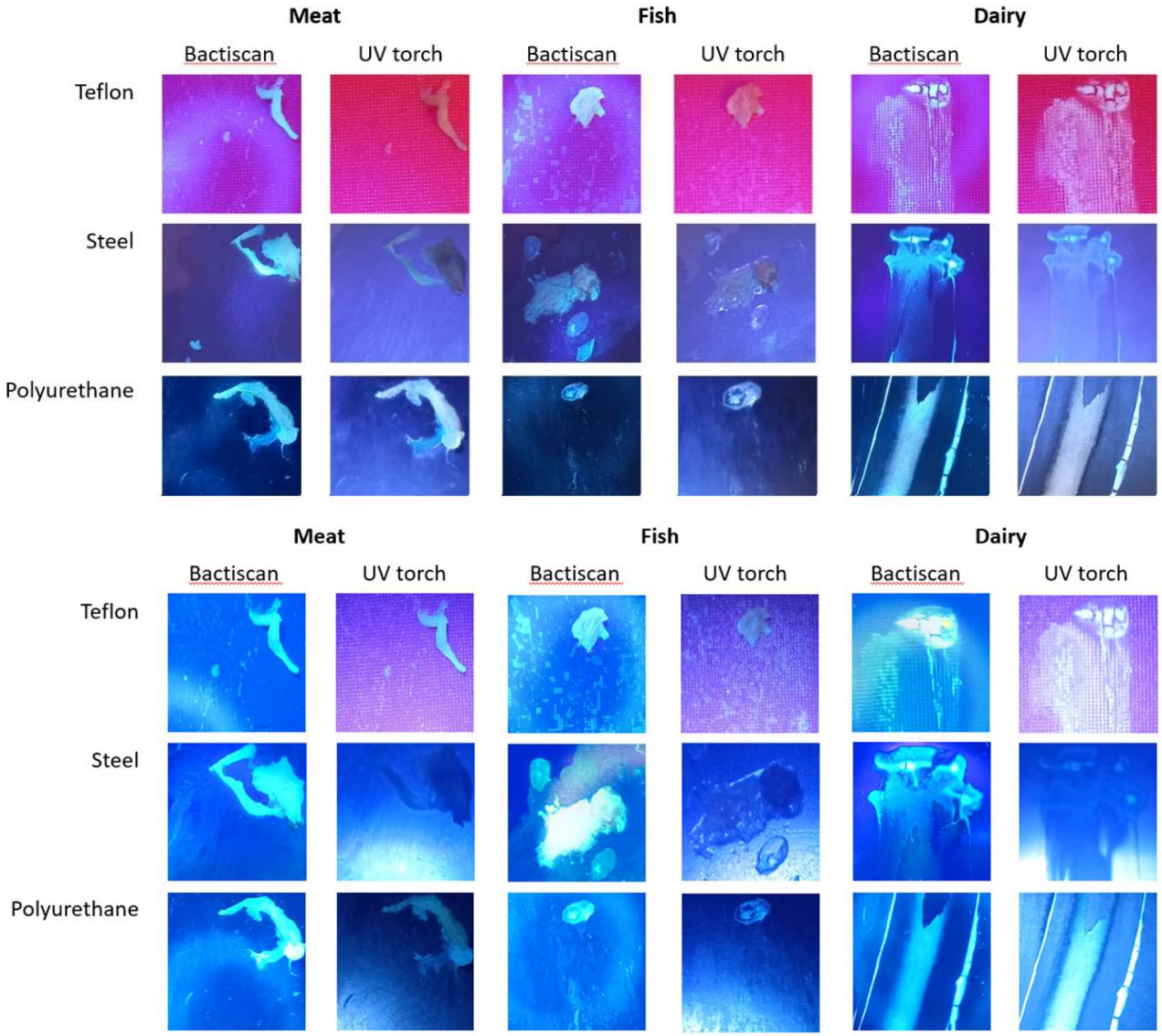
- To test the detection capability of Bactiscan on real food samples, a model system was established.
- Meat, fish, and dairy samples were incubated on surfaces and illuminated with Bactiscan to assess colour and brightness of fluorescence.
- A budget UV torch was used to compare against Bactiscan.



NBIC EIT Preventative Controls & Detection Systems

Bactiscan detects real contamination on food

- Bactiscan illuminates bacterial contamination with bright green-turquoise fluorescence.
- The budget UV torch caused dim fluorescence and illuminated the surfaces, particularly steel.
- Bactiscan was more effective at discriminating between food matter and bacterial contamination.



NBIC EIT Preventative Controls & Detection Systems

Bactiscan in healthcare settings

NBIC EIT Preventative Controls & Detection Systems

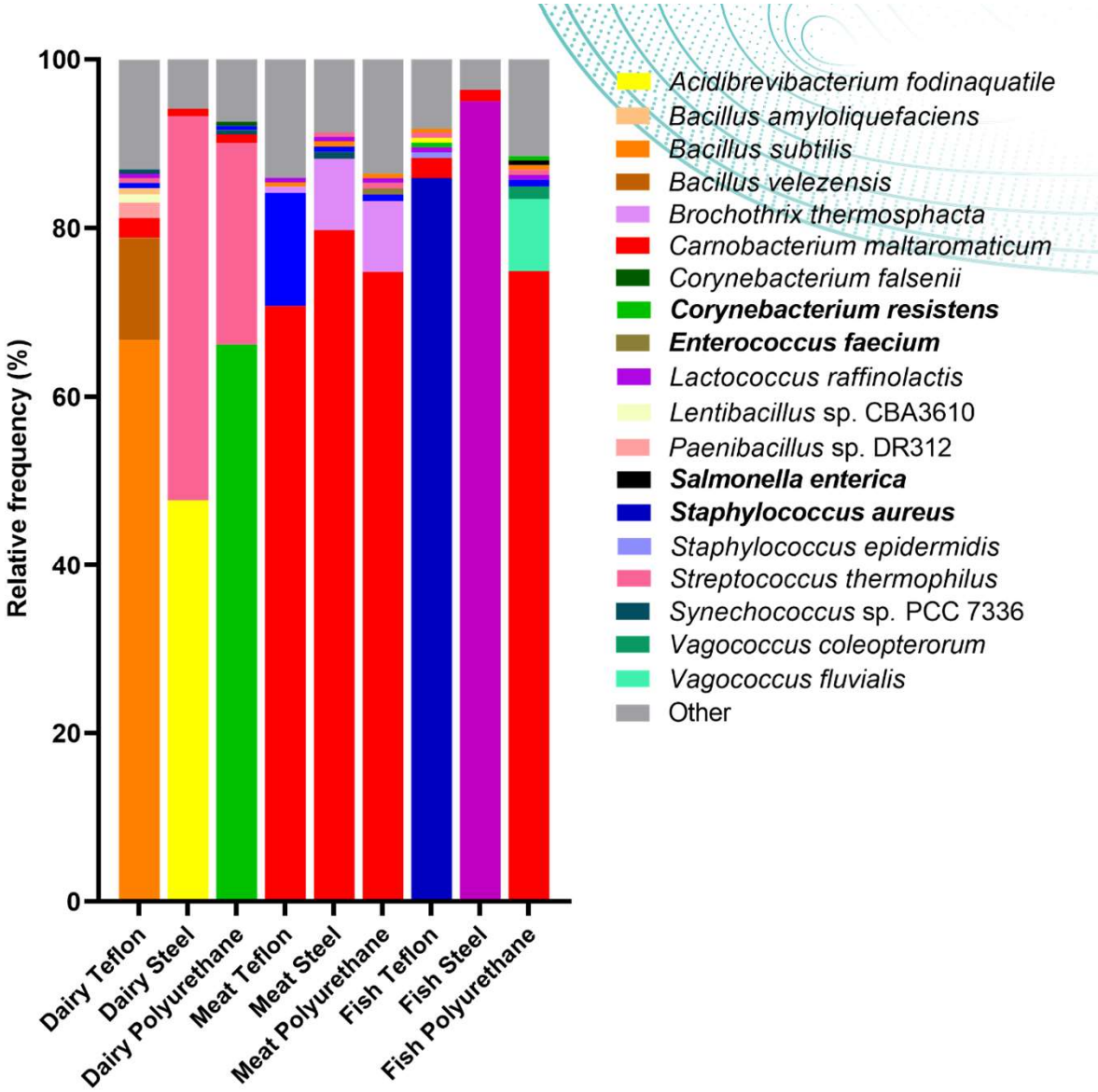
What can Bactiscan detect in the real world?

METAGENOME SEQUENCING

The contaminated food samples underwent metagenomic sequencing to determine what the Bactiscan device was detecting.

- Typical microflora such as *Carnobacterium* dominated most food samples.

- Foodborne pathogens such as *S. aureus* and *Salmonella enterica* were present.



NBIC EIT Preventative Controls & Detection Systems

Summary

- Bactiscan can detect foodborne pathogen biofilms at the macro-scale.
- The detection capability of Bactiscan is unaffected by dead or stressed bacteria.
- Bacterial contamination was detected on food samples without using chemical stains.
- Bactiscan can be used with ATP swab tests to enhance industrial cleaning practices.

NBIC EIT Preventative Controls & Detection Systems

Acknowledgements

UNIVERSITY OF SOUTHAMPTON

Dr. Joe Parker

Kirsty Cooper

Prof. Jeremy Webb

EIT INTERNATIONAL

Dr. Roberto Castangia

Joshua Robinson