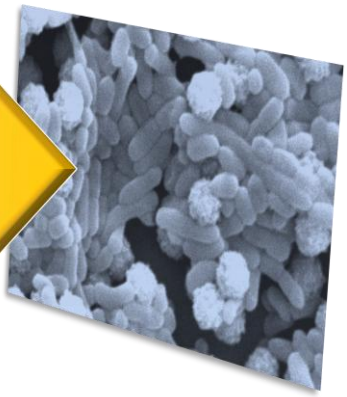
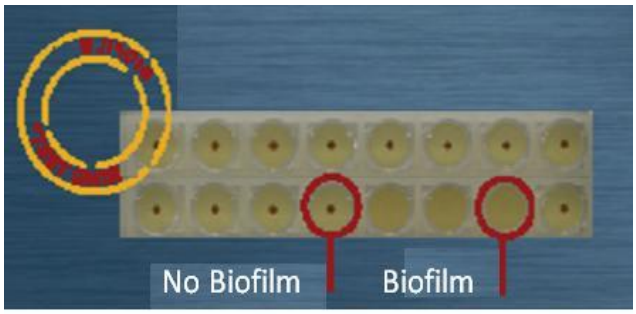
BioFilm Control®

Become part of the biofilm success story





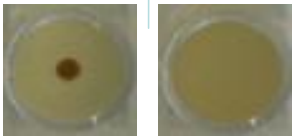
A new technology

contact@biofilmcontrol.com

The BioFilm *Ring Test*® is a fast and convenient new approach for studying biofilm formation

Straightforward data interpretation

Absence of Biofilm	Presence of Biofilm
NO	YES

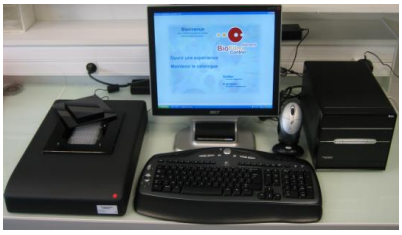


This unique and proprietary approach monitors how paramagnetic beads are immobilized during the formation of the biofilm:

- ⇨ A magnet is used to assemble the remaining non-immobilized beads into a macroscopic spot.
- ⇨ The resulting spot is quantified through specialized image algorithms.

<u>This technology is protected worldwide by several patents.</u>

The **Biofilm *Ring Test*** ® is operated through the **BioFilm *Scan M*** using ready-to-use kits provided exclusively by **BioFilm Control**.



The **BioFilm *Scan M*** includes:

- A precision 96 well magnetic plate holder,
- A specialized scanner,
- A ready-to-use high quality desktop computer,
- The data-acquisition and analysis software already installed and ready to run.

Each kit contains

- 10 microtiter plates or 120 strip-wells in SBS format specially designed and manufactured for biofilm applications.
- Negatively or positively charged paramagnetic beads.
- A special anti-reflective solution used at image acquisition time to allow reliable image quantification.

The procedure is extremely simple and fast to operate

a) Add the paramagnetic beads to the bacterial culture solution, mix and dispense it into the wells
b) Incubate the desired time at the desired temperature.
c) Add the anti-reflective solution
d) Make a first reading using the **BioFilm *Scan M***,
e) Place the wells on the magnetic holder
f) Make a second reading using the **BioFilm *Scan M***,
g) Let the software analyze the images and quantify the extend of immobilized beads
h) Proceed further with data analysis: data is provided directly in a standard spreadsheet file

The **BioFilm *Ring Test***® specific time **overhead is of the order of 5 minutes**, even for a full 96 well micro-titer plate.

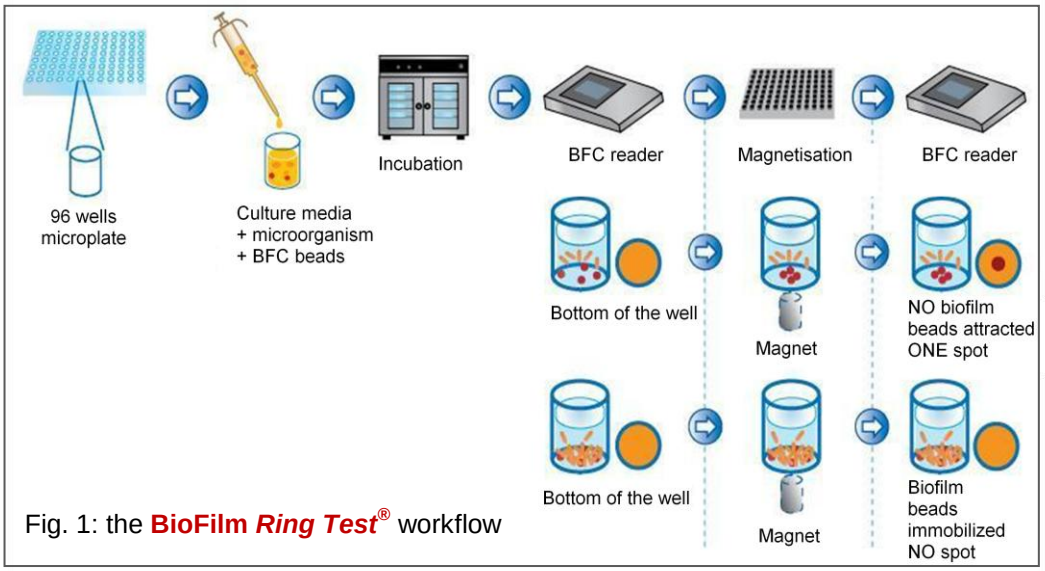


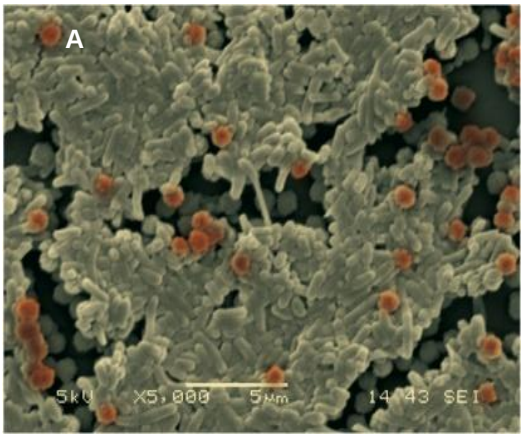
Fig. 1: the **BioFilm *Ring Test***® workflow

BioFilm Control®

The BioFilm *Ring Test* ® has been thoroughly validated

The proof of concept has been conducted in collaboration with a group of INRA (the French national research institute for agronomic research) and have been published in a peer-reviewed journal: Chavant *et al.*, Journal of Microbiological Methods (2007) **68**, 605-12.

For instance, scanning electron microscopy clearly shows that the beads are immobilized by the biofilm matrix of *Listeria monocytogenes* (Fig. 2.A) and *Staphylococcus xylosus (Fig. 2B), which are respectively low and high EPS producers.*



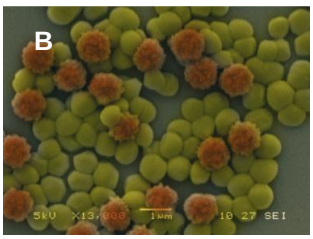


Fig. 2: scanning electron microscopy images(false colors)showing paramagnetic beads (red-brown spheres) immobilized on *Listeria monocytogenes (grey sticks, A)* and *Staphylococcus xylosus* (yellow spheres, B) biofilms.

The technology has already been applied on a wide variety of **bacterial and algal micro-organisms**:

- ✓ ***Escherichia coli***
- ✓ ***Staphylococcus***
- ✓ ***Pseudomonas***
- ✓ ***Lactobacillus***
- ✓ ***Salmonella***
- ✓ ***Streptococcus***
- ✓ ***Arthrospira***
- ✓ ***Rhodella***
- ✓ ***Listeria***
- ✓ ***…***

The key strengths of the BioFilm *Ring Test* ®

The Cristal Violet has been for many years the reference method used for studying biofilm formation. Compared to this reference method (Fig. : 3), the **BioFilm *Ring Test* ®**:

- ⇨ Gives the **same qualitative results**
- ⇨ Is **significantly more sensitive**: bacterial adhesion and the initial steps of **bioflm formation can be detected and scored already after 15 minutes incubation**
- ⇨ Requires **much less manipulations**
- ⇨ Can be **fully automated using high-throughput robots**
- ⇨ Is **much faster to operate**, **overhead is of the order of 5 minutes**, even for a full 96 well plate
- ⇨ **Use only reactive safe for the user**
- ⇨ Gives information about the **early stage of biofilm formation, including surface conditioning and primo adhesion.**

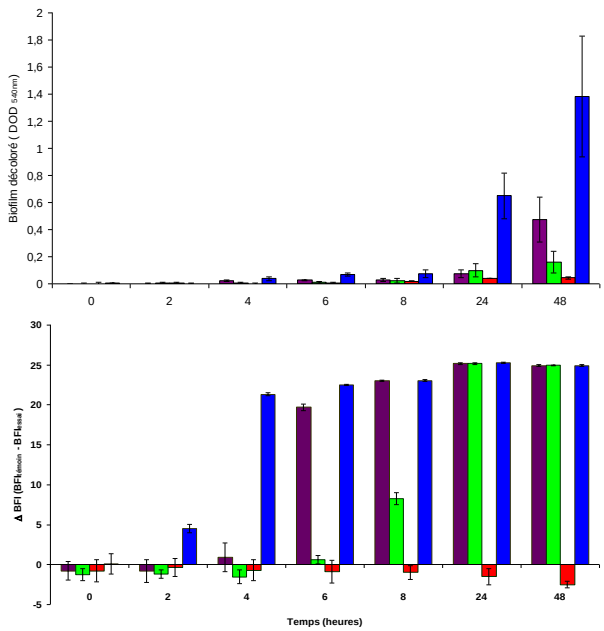


Fig. 3 : biofilm formation of *E.c* DH5α (violet), *S.c* TM300 (red) *S.x* DSM 20267 (blue) and *L.m* EGDe (green) monitored using the Cristal Violet method (top) and the **BioFilm *Ring Test* ®** (bottom).

BioFilm Control®

The BioFilm *Ring Test* ® has already been used for a wide variety of applications:

- ⇨ High-throughput screening of **curative and preventive antibacterial agents and antibiotics**
- ⇨ Characterization of **anti-biofilm coating agents**
- ⇨ Research of **pathogenicity/virulence signatures** of micro-organsims
- ⇨ Study of the **effect of enzymatic activity on biofilm formation and degradation**, for instance the action of dextranases, **proteases** and **DNAses**
- ⇨ Study of the **degradation of purified exo-polysacharides by enzymes**
- ⇨ Study of **molecules secreted by micro-organisms** (when such molecules induce the immobilization of the paramagnetic beads to the surface of the well)
- ⇨ Measuring the **adherence of the biofilm** on the surface…

The BioFilm *Ring Test* ® is also available as a service

- ⇨ We offer **several years of expertise and innovation** in biofilm analysis,
- ⇨ We operate routinely in a **class P2 laboratory,** and can access to a partner's **P3 class laboratory** when required
- ⇨ We can perform **complementary analyses** such as Cristal Violet, fluorescence microscopy, …
- ⇨ We have developed a **unique high-throughput robotic system** performing the **BioFilm *Ring Test* ®** in a fully automated manner on large number of samples and numerous conditions.
- ⇨ BioFilm control is on the track of becoming **ISO9001:2008 certified** in January 2010.

The BioFilm *Ring Test* ® is leading to success

This method is used by a growing number of industrial and academic groups. It has generated already 7 peer-reviewed articles (see attached list for full details) and numerous posters presented at international conferences (full details and copies on request):

- *Journal of Applied Microbiology (2009)*
- *J Mol Microbiol Biotechnol. (2009)*
- *PLoS One. (2008)*
- *Int J Antimicrob Agents. (2009)*
- *Int J Food Microbiol. (2009)*
- *Appl Biochem Biotechnol. (2008)*
- *J Microbiol Methods. (2007)*
- *Invited presentation at CHRO2009 (2009, Japan)*
- *Posters at: Eurobiofilm2009 (2009, Italy), 2nd Meeting of IADR Pan Asian Pacific Federation (2009, China), 4th IDF Dairy Science & Technology Week, (2009, France) , 3rd International Conference Biofilm III (2008, Germany), …*

Become part of the biofilm success story…Contact us:

contact@biofilmcontrol.com

+33 (0) 473 333 980