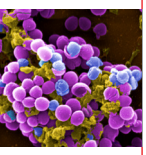
Microbiology Focus

Volume 7.2, 2015

Fluka® Analytical

Fluorescence Rapid Swab Test for MRSA

MRSA is a problematic germ found primarily in hospitals: It forms biofilms which are 3D communities of microbial cells held in association and firmly attached to surfaces via an extracellular polymeric matrix. Growth in biofilms enables bacterial populations to survive better in hostile environments and during host infections (i.e. in the presence of antibiotics), increasing the probability of causing infections. The pseudocolored scanning electron micrograph shows a *Staphylococcus aureus* biofilm disrupted by an antibiotic, showing cell-shape deformation and cell wall breakdown. Bacterial cytoplasm is shown in blue, bacterial cell wall in purple and extracellular matrix in yellow. (Magnification × 30.000; Source: José Ramos Vivas, IFMAV)



Fluorescence Rapid Swab Test for MRSA

Jvo Siegrist, Product Manager Microbiology — *ivo.siegrist@sial.com*

Methicillin resistant *Staphylococcus aureus* is frequently a problem in hospitals. Therefore, rapid detection and hygiene checks can help to protect patients.

S. aureus is commonly found on the skin and within the nostrils of humans. It can occasionally cause minor skin infections such as boils, abscesses, and infection of cuts. These minor skin infections can be treated with antibiotics. More severe symptoms can also be caused by *S. aureus* such as wound infections after surgery where standard antibiotics like methicillin can't help in cases involving a MRSA strain. The consequence of a severe infection can be septicemia, septic shock, osteomyelitis, abscesses, meningitis, pneumonia and endocarditis.

There are several mechanisms that contribute to resistance in *S. aureus*. These include the production of a supplemental penicillin-binding protein (PBP) that is encoded by a chromosomal mecA gene, hyper ß-lactamase production, and the production of modified PBPs, which lowers the organism's affinity for ß-lactam antibiotic [6]. Panton-Valentine Leukocidin (PVL) is a cytotoxic substance (β-pore-forming toxin) produced by some strains of MRSA and is associated with an increased ability to cause severe infection [4,5].

A test usually takes about two working days. If MRSA is identified, the correct antibiotics can be used to treat infection. Current studies report a remarkable increase of Methicillin Resistant *S. aureus* (MRSA) over recent years.

Did you know…

MRSA can remain in your body for your entire life?

Some people carry MRSA for a few hours or days, while others can carry it unaware for their entire lives since they do not have any symptoms.

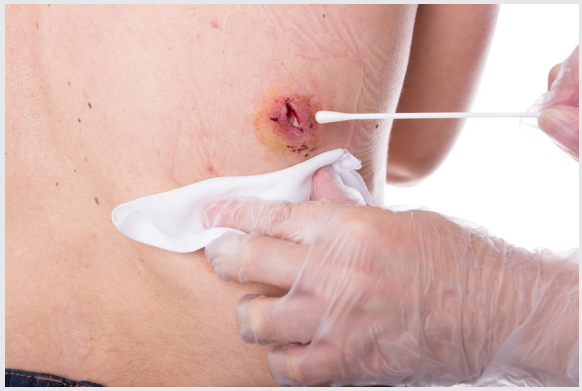


Figure 1: A swab is taken of a fresh, open wound.

S. aureus is a spherical, facultative anaerobic Gram-positive bacterium (see **Figure 2**) that grows by aerobic respiration or by fermentation that yields principally lactic acid. It can build biofilms which sometimes makes it difficult to clean rooms and equipment or provide treatment for the infection. The bacterium is catalase-positive and oxidase-negative. *S. aureus* produces diverse enzymes such as staphylokinase (coagulase), proteases, phosphatase, a lipase, a deoxyribonuclease (DNase) and a fatty acid modifying enzyme (FAME). The majority of clinical isolates of *S. aureus* express special surface polysaccharide and protein A. Differentiation and identification of *S. aureus* can be made based on these biochemical characteristics.

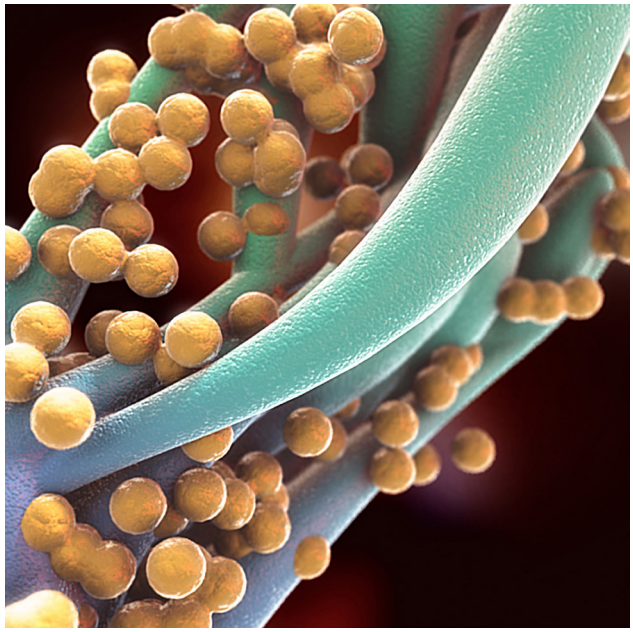


Figure 2: MRSA bacteria.

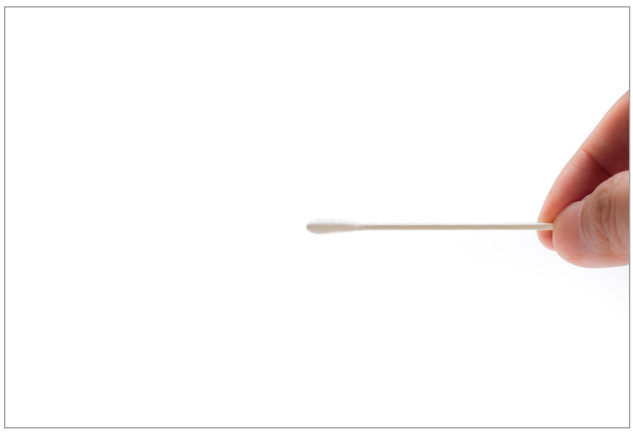
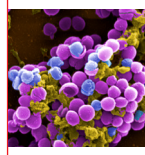


Figure 3: Determination of the presence of MRSA contamination on surfaces by using a swab.



A New and Simple Rapid Test of the Surface within 15 Minutes

The *Fluoro*SELECT™ MRSA Assay Kit is a new swab test for testing surfaces for the presence of MRSA. Our rapid detection system utilizes a fluorogenic substrate which, when hydrolyzed by a specific enzyme (during peptide hydrolysis), produces a fluorescence signal which is read by a fluorometer operating at $480nm_{ex}/530nm_{em}$. This convenient and sensitive method takes about 15 minutes and can be performed anywhere with the portable handheld low cost fluorometer. The guaranteed sensitivity is 1,000 cfu per sampling as this fluorescence method is highly sensitive. The reagents are stable for at least 24 months if kept at 2–8 °C. The surface of a table, floor or equipment can be simply wiped with a moistened swab and then the swab is left to stand for 5 minutes in a buffer. The liquid of the swab is pressed out as well as possible and then the swab is removed and discarded. One drop of inducer is added to the buffer and after two minutes, one drop of substrate is added and gently mixed. In less than 30 seconds after adding the substrate (because the reaction occurs upon adding the substrate), the measurement with the fluorometer should be started and in less than 3 minutes the result is obtained.

Figure 4: High technology fluorometer with stable high performance LED source and touchscreen.

Description	Cat. No.
*Fluoro*SELECT™ MRSA Assay Kit	42779
Components (for 50 assays): • Reagent A (Substrate): 4 mL • Reagent B (Inducer): 4 mL • Reagent C (Buffer): 10 mL × 2 bottles • Large Plastic Vials (Sampling): 50 pcs • Small Plastic Vials (Testing): 50 pcs • Short Sterile Swab: 50 pcs	
*Fluoro*SELECT™ single channel fluorometer (λex 480 nm; λem 530 nm)	Z805602

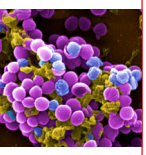
Table 1: Assay and fluorometer.

References

1. Methicillin Resistant Staphylococcus aureus Copyright © 1997-2005 Canadian Centre for Occupational Health and Safety, Sept 19th (2005).
2. Johnson, A. Methicillin resistant Staphylococcus aureus (MRSA) infection. The Support Group for MSRA Sufferers and Dependents, Aug 1st (2005).
3. M. Bassetti, M. Merelli, C. Temperoni, A. Astilean, New antibiotics for bad bugs: where are we?, Annals of clinical microbiology and antimicrobials 12 (2013) 22.
4. S. Szmiegielski, G. Prevost, H. Monteil et al., "Leukocidal toxins of staphylococci". Zentralbl. Bakteriol. 289, (2), 185–201 (1999).
5. J. Kaneko, Y. Kamio, "Bacterial two-component and hetero-heptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes". Biosci. Biotechnol. Biochem. 68 (5), 981–1003 (2004).
6. A.C. Petersson, C.Kamme, H. Miörner, Disk with High Oxacillin Content Discriminates between Methicillin-Resistant and Borderline Methicillin-Susceptible Staphylococcus aureus Strains in Disk Diffusion Assays Using a Low Salt Concentration, J. Clin. Microbiol., 37(6), 2047–2050. (1999).

Related Products for MRSA Detection

Test and Media	Testing Features	Cat. No.
Baird Parker agar base (RPF) Supplements: RPF Supplement (Fluka® 05939)	Detection of the coagulase activity and the ability to reduce tellurite is detected (EN-ISO 6888-2:2000)	79893
Baird Parker Agar Supplements: Egg-Yolk Tellurite Emulsion (Fluka® 75208)	Detection of lipolytic and proteolytic activity, ability to reduce tellurite to metallic tellurium (EN-ISO 6888-1: 1999)	11705
Catalase Test (Hydrogen peroxide 3%)	Testing of catalase production	88597
Coagulase Test (Slide)	Detection of coagulase	75832
Coagulase Test (Tubes)	—	74226
HiCrome™ Aureus Agar Base Supplement: Egg-Yolk Tellurite Emulsion (Fluka® 75208)	Testing for ability to reduce tellurite to metallic tellurium and detection of lipase and protease by chromogenic substrate; brown-black colonies	05662
HiCrome™ MeReSa Agar Base Supplement: MRSA Selective Supplement (Fluka® 51387)	Detection by chromogenic substrate mixture specifically cleaved by *S. aureus*; selective to MRSA; MRSA give bluish green colonies	90923
Staphylo Monotec test kit Plus	Coagulase, capsular protein and protein A can be detected in one step (increased sensitivity and specificity compared to the previous Staphylo Monotec test kit, resulting in increased detection of MRSA)	50448



Cronobacter Rapid Test

Jvo Siegrist, Product Manager Microbiology — *ivo.siegrist@sial.com*

Rapid and reliable testing of infant formula.

Cronobacter is a Gram negative, facultative anaerobic rod-shaped and motile bacterium and belongs to the Enterobacteriaceae family. It is closely related to the *Enterobacter* and *Citrobacter* species. *Cronobacter* was first described as yellow-pigmented Enterobacter cloacae (yellow pigment on a tryptic soy agar at 25 °C). *However*, there are also current studies that have demonstrated that only about 80% of *Cronobacter spp*. produce yellow-pigmented colonies on tryptic soy agar. In the 1980's researchers used DNA-DNA hybridization to show that these strains were a unique taxonomic group and should be recognized as a separate species '*Enterobacter sakazakii*' (named in honor of the Japanese bacteriologist Riichi Sakazaki). The *Cronobacter* genus was first defined in 2007 and revised in 2008 based on studies of both partial 16S rDNA and hsp60 gene sequences, which showed that '*E. sakazakii*' isolates formed at least four distinct genomogroups which could be unique species. Today the genus is composed of *C. sakazakii*, *C. malonaticus*, *C.turicensis*, *C. muytjensii,* and *C. dublinensis*, plus an unnamed sixth species. *Cronobacter spp.* can grow over a wide temperature range. It starts near refrigeration temperature (5.5 °C) and goes up to a growth temperature of 44–47 °C, depending on the strain. The organism is very tolerant of drying steps and can survive for two years desiccated in infant formula and then rapidly grow on reconstitution.

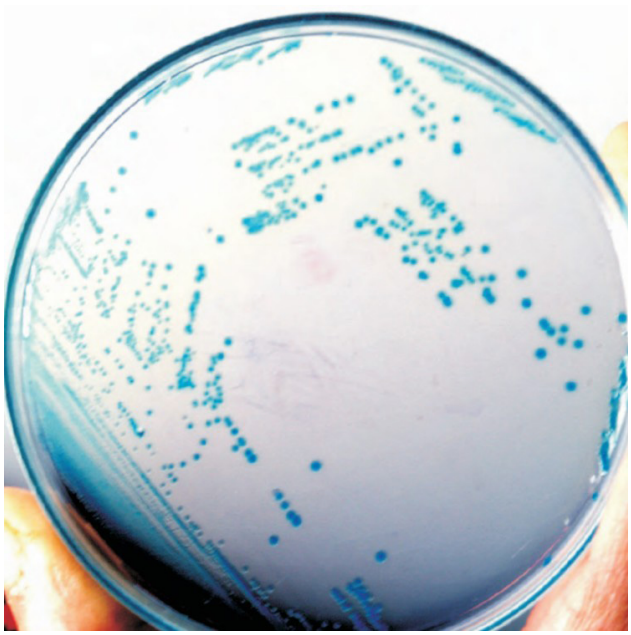


Figure 2: *Cronobacter* on chromogenic media called HiCrome™ *Cronobacter spp.* Agar, Modified (*Enterobacter sakazakii* isolation agar; ESIA).

Did you know…

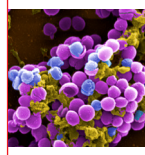
***Cronobacter* can cause different kinds of infections, and symptoms vary with both the site of the infection and the age of the patient?**

In infants, *Cronobacter* usually causes sepsis or severe meningitis, resulting in long-term neurological problems and a high mortality rate. *Cronobacter* can cause wound infections or urinary tract infections in all people.

People who are immunodeficienct and the elderly may also develop bloodstream infections due to *Cronobacter*. *Cronobacter* has also been isolated from the respiratory secretions of people undergoing mechanical ventilation.

Figure 1: Baby drinking infant formula.

The slow recognition of *Cronobacter* as its own genus reflects the laborious, time consuming methods used in this pre-genomic period for bacterial characterization. Therefore, a rapid and simple solution for qualitative and quantitative detection of the *Cronobacter* species was a necessity for the market. The HybriScan™ *D Cronobacter spp.* assay is an *in situ* hybridization method with the target being ribosomal RNA. It is a robust system, unlike PCR, and is therefore well suited for difficult matrices (such as infant formula) in the food industry. As RNA is not stable for long outside of a cell, it also detects only living cells, which is an advantage over PCR. With an enrichment step, 1 cfu per 10 g can be detected.



Benefits of the HybriScan™ System

- Time saving
- Easy handling
- Analysis in 2.5 hours
- Only living cells are detected (rRNA quickly decomposes in a dead cell)
- Sensitive (up to 1 CFU/mL with enrichment step)
  High specificity of the probes (low cross reaction)
- Robust system, not sensitive sample matrix
- Cost-effective analyses (96-well microplate format)

Principle of the Method

The HybriScan™ method is based on the detection of rRNA via hybridization events and specific capture and detection probes. Sandwich hybridization is very sensitive, detecting attomoles of the respective target rRNA molecules. The ideal hybridization target for *Cronobacter* is rRNA, as the cells contain a large number of rRNA-containing ribosomes; a single cell therefore contains several thousand copies of rRNA but only one DNA. Sandwich hybridization also provides sensitivity in crude biological samples because it is not susceptible to matrix interference like PCR.

Specificity is achieved by targeting conserved or unique rRNA sequences. A biotin-labeled capture probe is used to immobilize the target sequence on a solid support plate (streptavidin-coated microtiter plate). A digoxigenin-labeled detection probe provides an enzyme-linked optical signal readout. Detection results from application of anti-DIG-horseradish peroxidase Fab fragments. The bound complex is visualized by horseradish peroxidase substrate TMB (3,3',5,5'-tetramethylbenzidine). Photometric data are measured at 450 nm and compared with standard solutions. The HybriScan™ software enables easy measurement and data analysis.

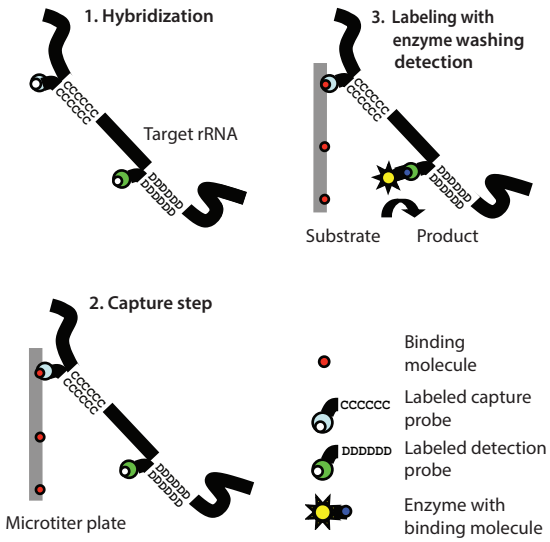


Figure 3: Principle of the HybriScan™ System.

Figure 4 shows the specificity of HybriScan™ *D Cronobacter spp.* Different cell amounts and related Enterobacteriaceae were tested within a validation study. No signals were observed using 2,3 × 108 *Enterobacter* cloacae cells or 7 × 108 *Citrobacter freundii* cells per assay, whereas clear specific signals were detectable using 2,6 × 103, 1,3 × 104, and 2,6 × 104 cells of *Cronobacter* species, respectively. These results demonstrate that the HybriScan™ system is highly specific for *Cronobacter* spp.

The above mentioned data came out of a validation study from HybriScan™ *D Cronobacter* spp., which was performed using two different enrichment procedures:

- Single-step enrichment for 24–26 hours at 37 °C in ESSB broth (*Enterobacter sakazakii* selective broth)
- Two-step enrichment starting with a pre-enrichment for 18–20 hours at 37 °C in buffered peptone water and followed by a selective enrichment for 24–26 hours at 45 °C in mLST selective broth.

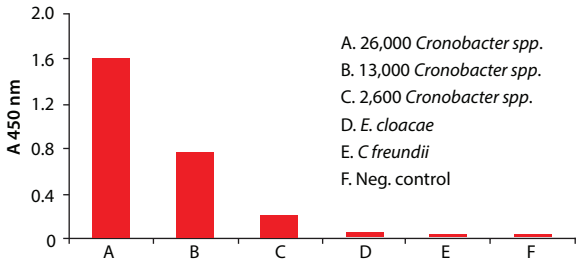
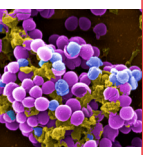


Figure 4: Specificity of HybriScan™ *D Cronobacter spp.* Different cell numbers of *Cronobacter spp.* and related Enterobacteriaceae like *E. cloacae* and *Citrobacter freundii* were tested. Measurement data for HybriScan™ analyses represent absorption at 450 nm.

Ordering Information

Cat. No.	Brand	Name
12838-96TESTS	Fluka	HybriScan™ *D Cronobacter spp.*



Detection of Cyanobacteria

Jvo Siegrist, Product Manager Microbiology — *ivo.siegrist@sial.com*

Cyanobacteria can produce powerful toxins that harm the health of animals and humans.

Cyanobacteria, also known as blue-green algae, is a phylum of bacteria that obtain their energy through photosynthesis. Aquatic cyanobacteria are known for their extensive and highly visible blooms that can form in both freshwater and marine environments. The blooms can have the appearance of blue-green paint or scum. These blooms can be toxic and are then called cyanobacterial harmful algal blooms (CyanoHABs). Freshwater CyanoHABs can use up the oxygen and block the sunlight that other organisms need to live. They also can produce powerful toxins that affect the brain and liver. This frequently leads to the closure of recreational waters when spotted. For these reasons alone, it should be clear that there is an interest in measuring concentrations of cyanobacteria.

Figure 2: The polluted water of Taihu Lake by a cyanobacteria bloom in Jiangsu province of China.

A simple test to check the concentration of cyanobacteria is fluorescence detection. Cyanobacteria contain accessory pigments from the phycobiliprotein family. In fresh water, the primary phycobilin pigment is phycocyanin (PC) that happens to have strong fluorescent signatures that do not interfere with the fluorescence of the chlorophylls. This allows the *in vivo* detection of cyanobacteria without interference from other groups of algae. From this perspective, the fluorometric technique is the most versatile, sensitive, and convenient way to measure the concentrations of cyanobacteria in water. *Fluoro*SELECT™ phycocyanin quantification fluorometer uses sophisticated electronic and optical systems to detect low levels of phycocyanin in water, and generally the fluorescence signal is directly proportional to the PC concentration. Application examples include, but are not limited to, the monitoring of cyanobacteria in natural freshwater environments, reservoirs, water and sewage treatment plants, and aquacultural systems.

Did you know…

Some species of cyanobacteria can also be beneficial?

Arthrospira platensis and *Arthrospira* maxima were originally classified in the genus *Spirulina*, a classification which for historical reasons is still in use today. The dried powder of these cyanobacteria contains about 51–71% protein (containing all essential amino acids), a lot of unsaturated fatty acids, diverse vitamins (B1, B2, B3, B6, B9, C, A, E) and also trace metals like calcium, iron, magnesium, manganese, zinc, etc. In addition, their diverse pigments may be beneficial to the human body, by increasing the bioavailability.

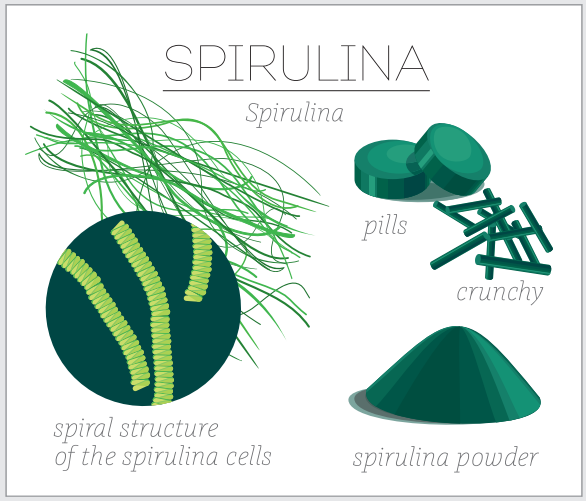


Figure 1: Arthrospira and its dietary supplements.

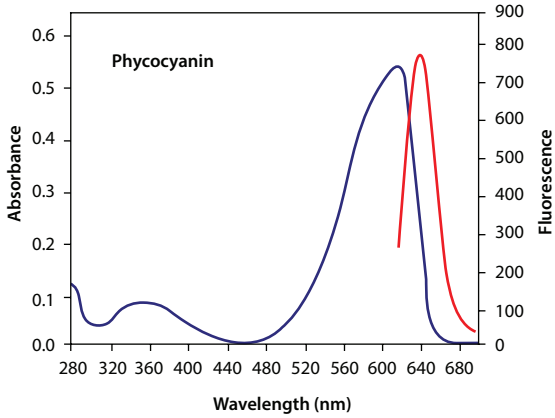
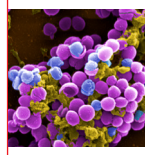


Figure 3: Fluorescence spectrum of phycocyanin.



Fluka® 89463 *Fluoro*SELECT™ Single Channel Fluorometer (wavelengths 600 nmex / 650 nmem)

This fluorometer is well suited for detecting the phycocyanin, particularly the cyanobacteria quantification, but not limited to this application. After a short calibration, measurements can be taken. This convenient, sensitive and rapid method can be performed anywhere with the portable handheld low-cost fluorometer. This fluorometer meets today's highest standards with a stable high performance LED source and touchscreen.

Fluorometer Performance

- Uses mini glass tube or standard 0.2 mL PCR tubes for easy sample collection
- Rapid (5 second reading) and sensitive (<5 ppb)
- Wide measurement range (0–30,000 ppb with proper calibration)
- Simple touch screen calibration. No repeated calibrations needed
- Portable for field operations, and stores up to 3 × 80 data points for computer analysis

Fluorometer comes with a 5 VDC power adapter, a USB cable, and manual/data-management software CD.

The following equipment and material are required but are not included:

- Mini glass tube (Fluka Z805823), or 0.2 mL PCR tubes (Sigma® CLS6571)
- Phycocyanin standard solution (Sigma 52468, Phycocyanin 10 mg/mL)
- Pipettor

For more information, visit
sigma-aldrich.com/fluoroselect

ID Membranes

- Membranes functioning like chromogenic & fluorogenic medium
- Place them on established colonies for 30 seconds, then incubate for 1-4 hours
- Outcome is similar to results on a chromogenic and/or fluorogenic medium

If you are interested to learn more and see the complete range of 19 different ID Membranes, order the new ID Membrane brochure (**code RMQ**).

The New Microbiology Photo Competition 2015

After a record response in 2014, Sigma-Aldrich® has again decided to sponsor a photography competition. The aim was and still is to encourage microbiologists to promote something about their work and their science and combine it with interesting facts and/or humor.

The best photographic entries will win prizes such as an Android™ tablet PC, a Sigma-Aldrich Giant Microbe, a Swiss army knife and a USB stick. The winning images will have the honor of being published in Microbiology Focus, and the best one will be featured on one of the covers.

The competition will be judged by:

Prof. Mohammad Manafi
Medical University of Vienna,
Head of the Department for Food Hygiene

Dr. Lars Fieseler
Zurich University of Applied Sciences – ZHAW,
Supervisor, Department Microbiology

Jvo Siegrist
Sigma-Aldrich,
Product Manager, Microbiology

Method of Entry

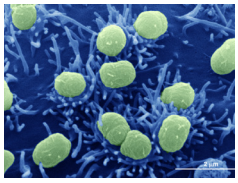
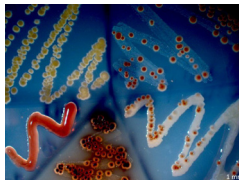
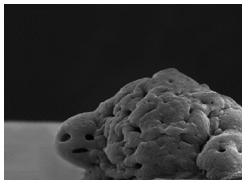
There is no entry fee, but for each entry, an entry form must be completed (a maximum of three entries may be submitted).

Entry form available from
sigma-aldrich.com/mibi-competition

Rules of the Competition and Conditions of Entry

- The competition is open to all residents worldwide
- Entries should illustrate any microorganism (living or dead) or a microbiologist in action at work
- Picture size should be at least 400 dpi and 90 × 120 mm (max 10 MB). The file format must be in jpg, tiff or pdf!
- The entries will be judged on:
  - Clarity of presentation
  - Composition
  - Illumination and contrast
  - Congruency of subject matter and title of photograph
  - Scientific interest and relevance
  - Originality
- Winning entries will be retained by Sigma-Aldrich, who will have sole rights of publication, reproduction and display
- Closing date will be August 31, 2015
- Entries after the closing date will not be considered. Entries that are incomplete, illegible, mutilated, altered or not complying exactly with the instructions and theme may be disqualified
- Decisions of the judges in all matters affecting the competition will be final and legally binding

2014 examples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St. Louis, MO 63103
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