Microbiology Focus

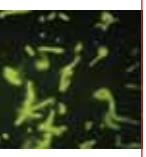
Volume 5.3, 2013

Fluka® Analytical

Fluorescence Assays in Microbiology

Fluorescence assays are interesting tools used for the detection of microorganisms. Fluorogenic substrates and fluorescence labelling of organisms are possible, as are sensitive methods of detection either by using a fluorescence reader, viewing the sample under the fluorescence microscope, or by simply viewing the sample directly with the naked eye.

Image from CDC: Mixed culture of microorganisms stained with fluorescent antibodies (1959)



FluoroSELECT™ Assays

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

FluoroSELECT is a rapid test based on an assay with fluorogenic substrates.

The FluoroSELECT Assay system is a presumptive screen intended for the rapid detection of *E. coli* and Gram-negative bacteria from a surface swab sample using the FluoroSELECT handheld fluorometer and the fluorescent assay. It can also be used as a confirmation test after using a classical or modern method. *E. coli* is commonly found in the lower intestine of warm-blooded organisms and is therefore an indicator for fecal contamination. Most strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination. Gram-negative bacteria are bacteria that are defined by their inability to retain crystal violet dye in the Gram staining protocol. This test was and still is vital for the differentiation and the classification of bacteria. Compared with Gram-positive bacteria, Gram-negative bacteria are more resistant to antibiotics because of their impenetrable cell wall.

FluoroSELECT utilizes specific fluorogenic substrates which are cleaved from characteristic enzymes of *E. coli* or Gram-negative bacteria. During the typical peptide hydrolysis, the specific enzymes hydrolyze the fluorogenic substrates and produce a fluorescence which is read by a portable, low cost fluorometer. The excitation wavelength is at 360 nm (UV), while the emission wavelength is at 460 nm (blue light), the intensity of which is then measured to decide if the sample is positive or negative. It is a rapid test since the assay takes only 30 minutes, although it may require some incubation time. Because the enzymes are specific, the assay is highly specific and robust. In addition, since it is a typical fluorescence assay, it is also highly sensitive and small amounts of the cleaved fluorogen will already have been detected. The detection performance for both tests is immediate detection of 100,000 cfu (colony forming units), while 100 cfu are detected in 8 hours, and 1 cfu is detected within 10 hours of incubation time.

Simplified testing procedures:

1. A cotton swab is used to wipe a particular surface of the detection area. The swab is then inserted into 0.5 mL of distilled water for an immediate test, or into 0.5 mL of incubation solution for an incubation period ranging from 3 to 10 hours at 38.5 °C, before measuring. Add an enzyme inducer after inserting the swab into the water or incubation solution.

2. Then add a lysing agent to the solution and let incubate for 5 minutes.
3. Next, a substrate is added to the solution and allowed to stand for 5 minutes.

4. Afterwards, the solution is transferred into a glass test tube and then inserted into the Fluorometer. The first measurement is taken immediately, and after 20 minutes, another measurement is taken. The findings can then be used to determine a positive or negative result.

Did you know…

Gram-negative bacteria are generally less sensitive to antibiotics?

The outer membrane of Gram-negative bacteria contributes to this intrinsic resistance by acting as an efficient permeability barrier, because the narrow porin channels limit the penetration of hydrophilic solutes and the low fluidity of the lipopolysaccharide leaflet slows down the inward diffusion of lipophilic solutes.

[Source: P. Plésiat, et al., Mol. Microbiol. 1992]

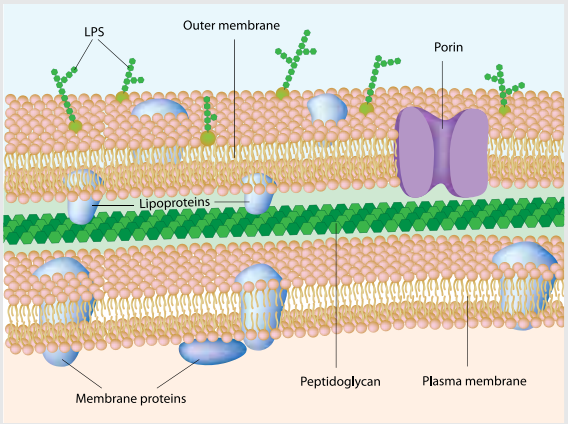


Figure 1: Gram-negative bacteria cell wall

Cat. No.	Description	Qty.
53649	FluoroSELECT *E. coli* Assay Kit	50 Tests
91333	FluoroSELECT Gram-Negative Assay Kit	50 Tests
Z805726	FluoroSELECT single channel fluorometer (λex 360 nm; λem 460 nm)	1 ea.
Z805823	Glass vials for FluoroSELECT fluorometer (O.D. × L 6 mm × 25 mm, volume nominal capacity 200 µL)	100 ea.

Table 1: Product list of microbiology FluoroSELECT kits and equipment

There are more enzymatic tests available for quantitative detection of acetate, ammonia, ascorbic acid, formaldehyde, glycerol and lactose with the FluoroSELECT system. Visit **sigma-aldrich.com/fluoroselect** or refer to **Table 2**.

Cat. No.	Description	Qty.
53659	FluoroSELECT Ammonia Kit	50 Tests
89872	FluoroSELECT Formaldehyde Kit	50 Tests
00254	FluoroSELECT Glycerol Kit*	50 Tests
76691	FluoroSELECT Ascorbic acid Kit*	50 Tests
76786	FluoroSELECT Acetate Assay Kit	50 Tests
91218	FluoroSELECT Lactose Detection Assay*	50 Tests

Table 2: Non microbiology FluoroSELECT assays
*Inquire on availability.

Fluka Analytical

Microbiology Special Catalog

This is the premiere Sigma-Aldrich catalog for microbiology, and it contains over 900 specific microbiology products. The tests, media, reagents, microorganisms and base ingredients are sorted to help microbiologists find the best test, media and additives for the desired microbiological analysis.

Contents:

- Microorganisms Standards (Vitroids™)
- Proficiency Testing
- Molecular Biological, Immunological, Biochemical Tests
- Microscopical Kits and Reagents
- Media for Specific Organism, Special Application, or ISO Guideline
- Supplements
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Reserve your catalog via reply card or place your request online.*
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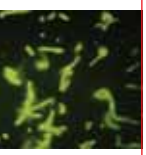
*only available as long as supplies last



Fluorogenic Media

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

Fluorogenic media combine traditional methods with new knowledge for beneficial results.

Traditional validated media versus improved media formulations containing chromogenic and fluorogenic substrates is an important topic in the field of microbiology. The focus behind such developments is the production of media that would make the detection and identification of microorganisms more rapid and more reliable. Diverse chromogenic and fluorogenic substrates such as ONPG, X-Gal, X-Glu MUP or MUG are available and are already combined in today's media formulations. Fluorogenic substrates have the disadvantage of requiring a UV-lamp, but the fluorogenic media are more sensitive and give an earlier detectable result. They can also be combined with a chromogenic system without interference. These reasons give such media additional value, and together with the specified selectivity of the medium and the substrate, new possibilities are available to solve problems in a smart way.

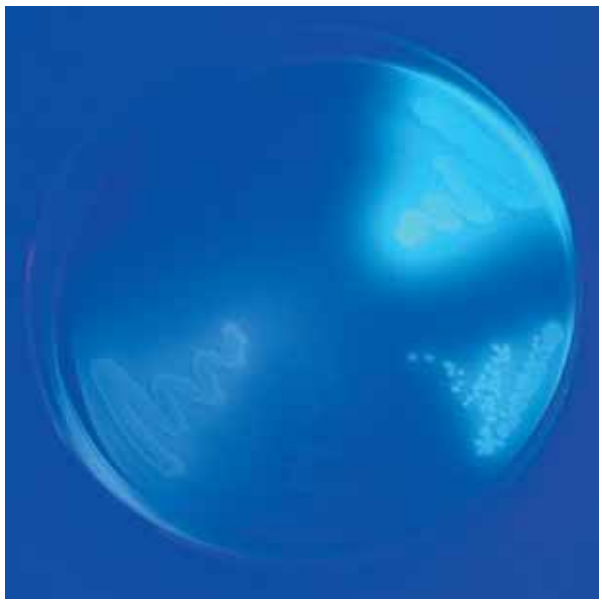


Figure 2: Fluorogenic media containing *E. coli* strains and *Pseudomonas putida* (right at the bottom) and *Lactobacillus* spp. (left).

Did you know…

about upconversion luminescence?

In normal cases, the color of emitted light requires a higher energy light source (in most cases UV light, invisible to the human eye). But there are also some exceptions. Now it is possible to have an upconversion luminescence where infrared is used as the excitation wavelength.

sigma-aldrich.com/upconversion

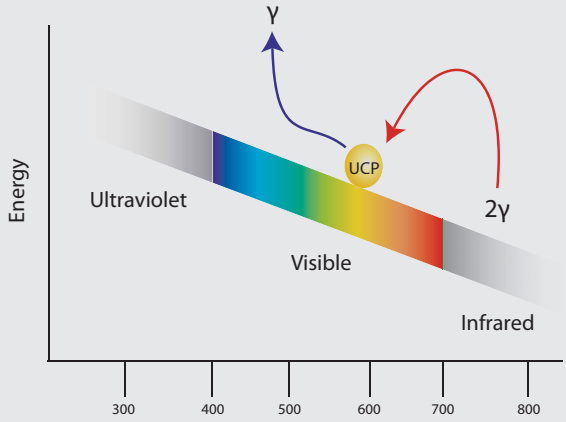


Figure 3: Upconversion Luminescence

The principle behind fluorogenic media is simple - the target organisms are characterized by enzyme systems that metabolize the substrates to release the fluorogen. The fluorogen can then be visually detected by direct observation under UV lamp. Direct confirmation of the target organism without further testing is sometimes possible. Today, it is also possible to detect and differentiate more than one organism on the same plate in combination with chromogenic substrates and the help of selective media.

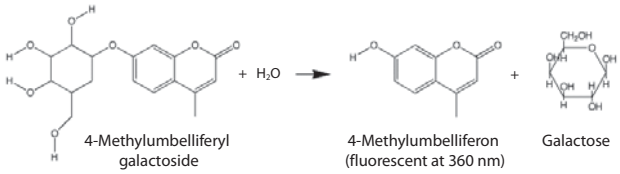


Figure 4: An example of a fluorogenic reaction. In the presence of a β-galactosidase positive organism, the 4-methylumbelliferyl galactoside (MUG) is split and results in the fluorophore and in free galactose.

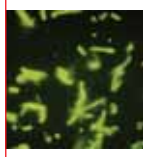
Advantage of fluorogenic media:

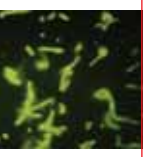
- Faster results (compared to traditional methods)
- Reliable visual detection (often no further testing required)
- Additional testing possible directly from the media

In recent years, great strides have been made in the sector of fluorescence. There are dyes, labels and substrates which have a stronger fluorescence and are more stable. Additional advancements in the knowledge of enzyme and species specificity have also occurred within the past year. These recent gains in the development of selective agents and diverse fluorescence compounds have led to an impressive range of fluorescence tests.

Cat. No.	Medium	Description
51413	Plate Count MUG Agar	For determination of plate count of microorganisms in milk and other dairy products and especially *E. coli* by fluorogenic method.
95273	VRB MUG Agar	Selective medium for the detection and enumeration of coliform bacteria, in particular *E. coli*. Gram-positive accompanying flora are extensively inhibited by crystal violet and bile salts. A color-change to red indicates lactose-positive colonies.
63014	MacConkey MUG Agar	For the isolation of *Salmonella*, *Shigella* and coliform bacteria, in particular *E. coli*, from diverse material. Bile salts and crystal violet extensively inhibit the Gram-positive flora. The presence of lactose and neutral red indicate lactose-positive colonies from which *E. coli* can be identified by fluorescence in the UV.
78996	HiFluoro™ Pseudomonas Agar Base	Used as a selective medium for the isolation of *Pseudomonas aeruginosa* from pus, sputum and drains etc. *Pseudomonas aeruginosa* breaks the fluorogenic compound to release the fluorogen which produces a visible fluorescence under long wave UV light.
62634	LST-MUG Broth	Fluorescent method for the detection of *E. coli*.
16016	BRILA MUG Broth*	Bile and brilliant green extensively inhibit the growth of accompanying flora, in particular Gram-positive microorganisms. The presence of *E. coli* results in fluorescence in the UV.
44782	*E. coli* O157:H7 MUG Agar*	Selective agar for the isolation and differentiation of enterohemorrhagic (EHEC) *E. coli* O157:H7-strains from food and clinical material.
51489	HiCrome™ Rapid Coliform Broth	Used for detection and conformation of *E. coli* and coliforms on the basis of enzyme substrate reaction from water samples, using a combination of chromogenic and fluorogenic substrate.
09142	HiCrome ECD Agar with MUG*	For the detection of *E. coli* in water and food samples by using a combination of chromogenic and fluorogenic substrate.
44657	ECD MUG Agar*	The bile-salt mixture in this *E. coli* Direct Agar extensively inhibits the non-obligatory intestinal accompanying flora. Fluorescence in the UV demonstrates the presence of *E. coli*.
M1678	MUG EC Broth	Used for the detection of *E. coli* by a fluorogenic procedure.
17165	MUG Tryptone Soya Agar	For cultivation of fastidious and nonfastidious microorganisms by fluorogenic method.

Table 3: Fluorogenic media (*not available in U.S.A.)





Vitroids™ – The Quantitative and Qualitative Control Strains

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

Control of test results, or general testing performance, is currently an important issue in microbiology.

Organizations like ISO and UKAS either have regulations or recommendations to regularly verify the accuracy of various test methods. One really important requirement is to use certified microorganism standards, or if not available, at least a reference strain from a recognized culture collection like ATCC, DSMZ and NCTC. The test strains should show all relevant properties used for the applied application.

According to ISO 11133-1, it is possible to culture reference strains one time to produce reference stock cultures which are then controlled for purity and for use in biochemical tests. They should be stored in a freezer or in a freeze-dried form in small aliquots; however, defrosted cultures should not be refrozen. It is preferable for working strain cultures to be made out of stock cultures, and they should not be subcultured again.

In UKAS LAB 31 it is written that to conform with ISO/IEC 17025:2005, it is necessary to use, where possible, a traceable control strain which is certified according to ISO 17025. The strains should be from a recognized national culture collection or from a reference material producer accredited to ISO Guide 34.

For detailed performance testing, organizations such as ISO recommend special protocols, an example being media testing under ISO/TS 11133-2.

Reference Strain

- From an official strain collection (e.g. ATCC, NCTC, DSMZ)
- Defined, described and cataloged, at least down to the genus and species

Stock Culture

Prepared from a reference strain

Working Culture

Produced from a stock culture and used for control

Figure 5: Definition of ISO for control strains

Microorganisms in Discs

Sigma-Aldrich's innovation, small discs called Vitroids, is a solution which supports all of these requirements. The patented technology allows the production of stable and highly reliable certified microorganisms. The discs contain a defined certified number of colony forming units (CFU) from defined bacteria, molds and yeasts from a recognized culture collection like ATCC and NCTC. The discs are easy-to-use since they can be placed directly in water, diluent, broth or even on agar plates.

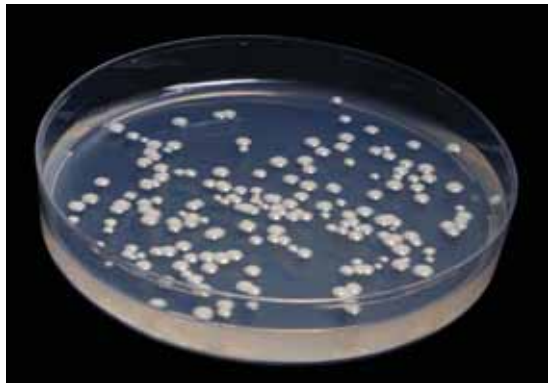


Figure 6: Classical plate count

The Vitroids contain highly viable bacteria, and when placed in contact with media, they dissolve rapidly and start to grow without a lag-phase. The viability of the CFU in a disc is stable for at least one year (for most organisms, more than two years) when kept at -20 °C. It is not a problem if the product is transported at ambient temperature or if it is kept for a short time in the refrigerator. The discs are produced under the ISO guideline 34 and are certified according ISO 17025. Each disc is packed in an individual tube with some desiccant and the tubes are then packed in mylar foil. Each package comes with a comprehensive certificate of analysis reporting the CFU and standard deviation.

All of the above mentioned features help microbiologist to have reliable results, save a lot of time (labor, documentation) and lower costs.

Reference microorganisms in certified and defined colony forming units (CFU)

- Standards in concentrations of 30–50,000 CFU per disc
- Produced acc. ISO Guide 34
- Certified acc. ISO 17025
- Delivered with detailed certificate of analysis
- Reference strains from ATCC, NCTC, etc.
- Minimum one year shelf life at -20 °C (usually two years)
- No lag-phase
- Amazingly low standard deviation (e.g. 100 CFU+/- 4%)

The Vitroids are packed in microvials and are lens shaped. They are colored and can easily be seen on the top of the filter plug. Below the filter plug is a desiccant which should be yellow. Clear (opaque) desiccant indicates the silica gel capacity has been depleted and is no longer effective. The tubes are packed in a resealable aluminium foil bag.

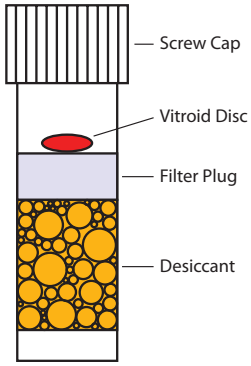


Figure 7: Vitroids in the vial

Preparation

Rehydrate the disc with a common phosphate buffer, or place the disc onto a solid or into a liquid medium. The rehydration process takes approximately 10 minutes. On solid media, the disc forms a droplet that can be spread with a loop. Liquid media may simply be shaken to dissolve the disc. The discs can be rehydrated in as little as 100 µL of water (preferably a buffer) or added into larger volumes, e.g. 100 mL, for general water testing methods (MF, MTF, Quanti-Tray, etc). It is also possible to add the disc to the media for pour plate techniques.

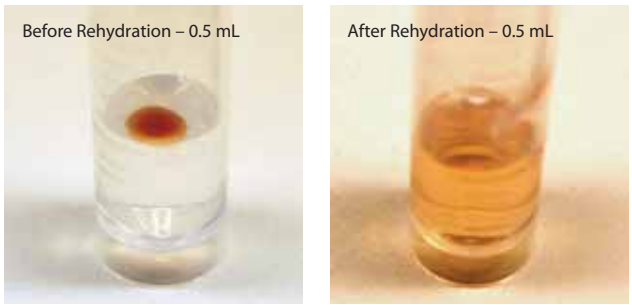


Figure 8: A Vitroid just put into buffer (left) and after 10 minutes it is completely dissolved (right).

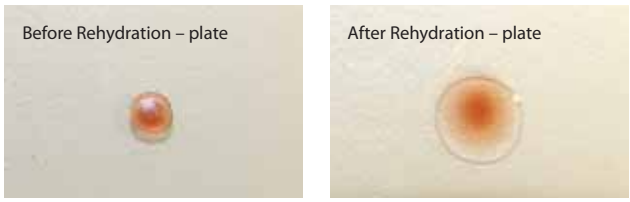


Figure 9: A single Vitroids disc on an agar plate. After about ten minutes on a plate, it is rehydrated automatically and forms a droplet (no water addition is needed). The drop can be spread with a loop.

Vitroids Test Strains	Origin	Strain #	CFU	Cat. No.
Aspergillus brasiliensis	ATCC	16404™	80	RQC15003
Bacillus subtilis	ATCC	6633™	10,000	RQC02258
Bacillus subtilis	ATCC	6633	80	RQC16003
Candida albicans	ATCC	10231™	80	RQC14003
Citrobacter freundii	ATCC	8090	200	RQC02105
Clostridium perfringens	NCTC	10240	30	RQC02351
Clostridium perfringens	NCTC	10240	200	RQC02355
Clostridium perfringens	NCTC	10240	500	RQC20106
Clostridium sporogenes	ATCC	19404™	80	RQC19003
Enterobacter aerogenes	ATCC	13048™	50	RQC01652
Enterobacter aerogenes	ATCC	13048	200	RQC01655
Enterobacter aerogenes	ATCC	13048	1,000	RQC01657
Enterobacter aerogenes	ATCC	13048	10,000	RQC01658
Enterococcus cloacae	ATCC	35030™	50	RQC21102
Enterococcus faecalis	ATCC	19433™	50	RQC01772
Enterococcus faecalis	ATCC	19433	200	RQC01774
Enterococcus faecalis	ATCC	19433	500	RQC01775
Enterococcus faecalis	ATCC	19433	1,000	RQC01777
Escherichia coli	ATCC	11775™	50	RQC01702
Escherichia coli	ATCC	11775	200	RQC01705
Escherichia coli	ATCC	11775	1,000	RQC01707
Escherichia coli	ATCC	11775	10,000	RQC01708
Escherichia coli	ATCC	8739™	80	RQC11003
Heterotrophic Organisms	—	—	100	RQC02504
Legionella bozemanii	NCTC	11368	50,000	RQC02908
Legionella pneumophila (serogroup 1)	NCTC	12821	50,000	RQC02008
Listeria monocytogenes	ATCC	19115™	30	RQC01901
Pseudomonas aeruginosa	ATCC	9027™	30	RQC02202
Pseudomonas aeruginosa	ATCC	9027	100	RQC02204
Pseudomonas aeruginosa	ATCC	9027	50	RQC12002
Pseudomonas aeruginosa	ATCC	9027	200	RQC12005
Pseudomonas aeruginosa	ATCC	9027	1,000	RQC12007
Salmonella enterica subsp. Enterica serovar Abony	NCTC	6017	80	RQC18003
Salmonella enterica subsp. Enterica serovar Typhimurium	ATCC	14028	50	RQC17002
Salmonella goldcoast	NCTC	13175	30	RQC02301
Staphylococcus aureus susp. Aureus	ATCC	6538	50	RQC13002
Staphylococcus aureus susp. Aureus	ATCC	6538	200	RQC13005
Vitroids Blank	—	—	0	RQC0001

Table 4: Vitroids portfolio

ATCC Licensed Derivative

ATCC Licensed Derivative®

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