

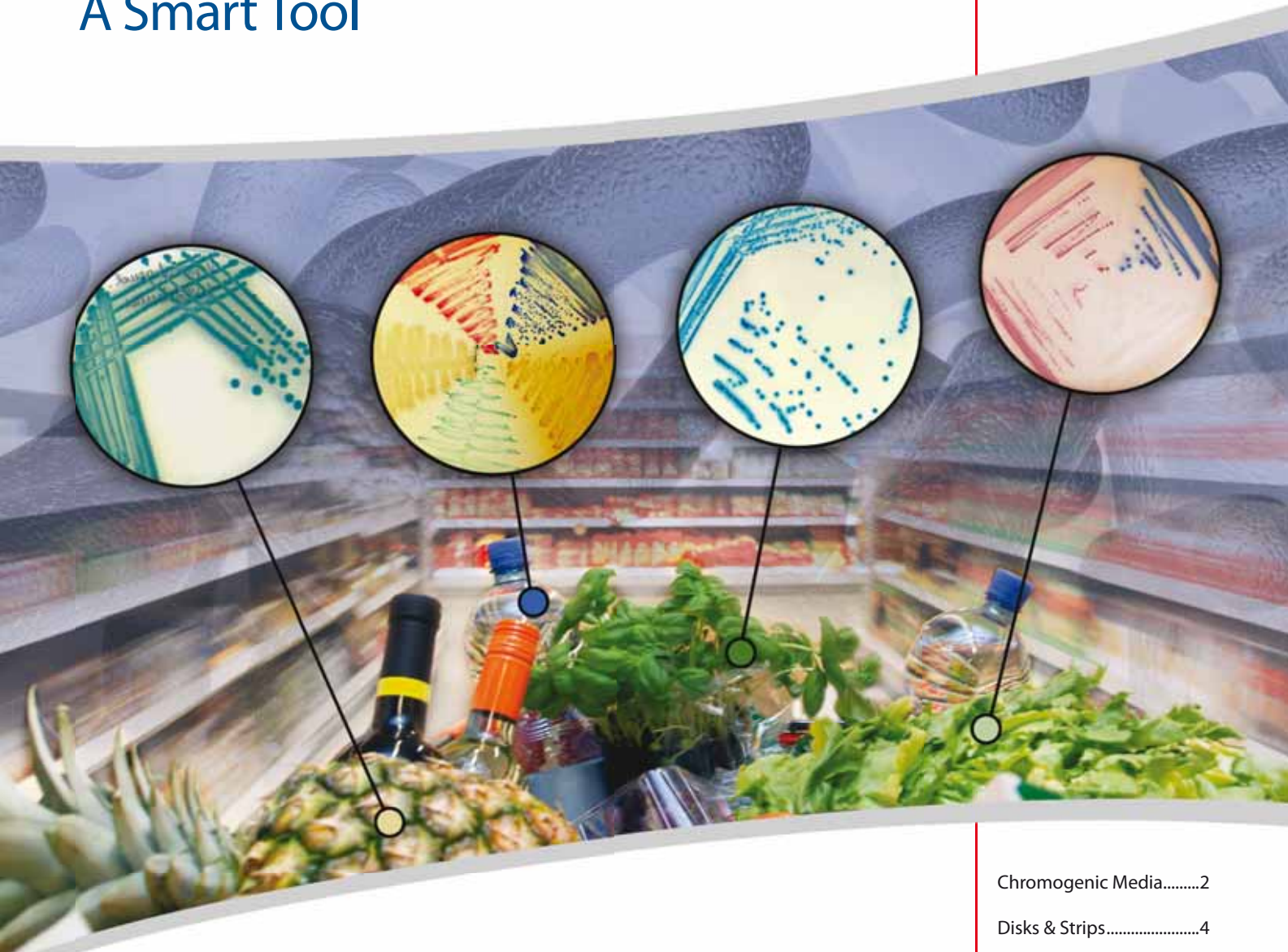


Microbiology Focus

Volume 4.1, 2012



Chromogenic Media: A Smart Tool



Color of Microbiology: Nutrients Tested by Chromogenic Media

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Chromogenic Media: A Smart Tool

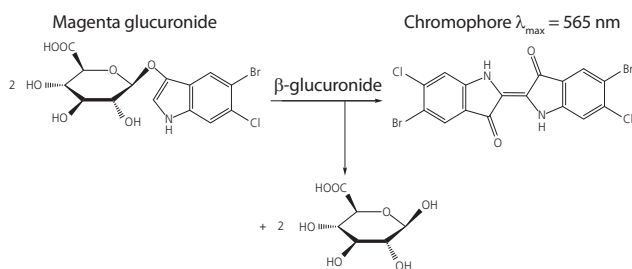
By Jvo Siegrist, Product Manager Microbiology...ivo.siegrist@sial.com

Chromogenic media demonstrate the combination of traditional methods with modern knowledge.

The validation and use of traditional methods versus improved media formulations containing chromogenic substrates is currently 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. Chromogenic substrates such as ONPG, X-Gal, or X-Glu, together with a specified selectivity of the medium, serve as the basis for the simple principle behind chromogenic media. The target organisms are characterized by enzyme systems that metabolize the substrates to release the chromogen. The chromogen can then be visually detected by direct observation of a distinct color change in the medium. 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. With the help of a combination of several chromogenic substrates and adequate selectivity, it is possible to differentiate several species or groups of microorganisms on one plate. In **Table 1** currently known substrates and selective agents are listed and give some ideas about additional possibilities.

Advantage of chromogenic media

Figure 1: An example of a chromogenic reaction. In the presence of a β -glucuronidase positive organism, the Magenta glucuronide is split and results in the chromophore and in free glucose



- 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 chromogenic media. Initial research concentrated on the use of synthetic substrates for the detection of enzymatic microbial activities. Nitrophenol and nitroaniline compounds were used at this time, producing a yellow coloration. The color of nitrophenol, however, is influenced by a pH-change, making it difficult to use reliably in microbiology. Later developments included the use of naphthol or naphthylamine. Today, while diverse modern chromogenic substrates are available, most are based on the indoxyl substrate. The use of different chromophore and

metabolite derivatives then makes it possible to detect diverse enzyme activities all in one assay. The color of the indoxyl substrates can be as follows: blue (5-bromo-4-chloro-3-indoxyl- = X, 3-indoxyl- =Y), magenta (5-bromo-6-chloro-3-indoxyl-), salmon (6-chloro-3-indoxyl-), purple (5-iodo-3-indoxyl-) and green (N-methylindoxyl-). One of the major advantages of the indoxyl substrate and these chromophores is that they remain in the cell, making the characterization of a single cell possible (no diffusion into the media).

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 chromogenic substrates have led toward an impressive range of chromogenic media available to meet our customers' unique analytical emphases (**Tables 1 and 2**).

Figure 2: HiCrome™ UTI Agar, modified (Fluka 16636)

- K. pneumoniae* = blue, mucoid
- E. faecalis* = blue green, small
- E. coli* = purple-magenta

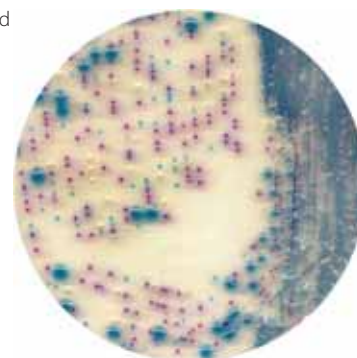


Figure 3: HiCrome™ ECC Selective Agar (Fluka 85927)

- E. aerogenes* = red colonies
- E. coli* = blue colonies

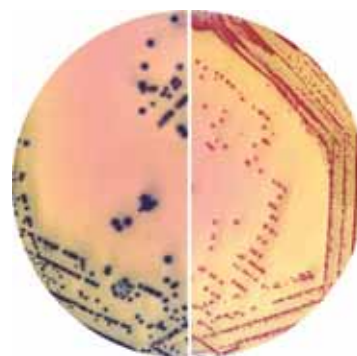




Table 1: Summary of possible enzyme activities, chromogenic substrates, and selectivity system for microorganisms

Species	Enzyme	Substrate	Selective Agents
<i>Bacillus cereus</i>	β -glucosidase, Phosphatidylinositol-specific Phospholipase C	indoxyl- β -glucopyranoside, indoxyl-myo-inositol-1-phosphate	polymyxin B
<i>Campylobacter</i>	n/a	n/a	deoxycholate, cefoperazone, amphotericin B
<i>Candida</i>	β -acetylgalactosaminidase, alkaline phosphatase	indoxyl-N-acetyl- β -D-glucosaminide, indoxyl-phosphate	Chloramphenicol, Gentamicin
<i>Clostridium perfringens</i>	β -glucosidase (plus sucrose fermentation)	indoxyl- β -D-glucoside	D-cycloserine, polymyxin B
Coliforms/ <i>E. coli</i>	β -glucuronidase, β -galactosidase	indoxyl- β -glucuronide, Indoxyl- β -galactoside	bile salts, tergitol 7 [®] , SDS, novobiocin, cefsulodin
<i>Cronobacter (E.sakazakii)</i>	α -glucosidase	indoxyl- α -D-glucoside	deoxycholate, crystal violet, sodium thiosulfate
<i>E. coli</i> O157	β -glucosidase, α -galactosidase	indoxyl- β -D-glucuronide, indoxyl- α -galactoside	bile salts, SDS, crystal violet, potassium tellurite, novobiocin, cefixime
Enterococci	β -D-glucosidase	indoxyl- β -glucoside	sodium azide, polysorbate 80
Extended Spectrum β -Lactamase Enterobacteria (ESBL)	β -D-glucosidase	indoxyl- β -glucoside	cefpodoxime, cefotaxime, ceftazidime
<i>Klebsiella</i>	β -D-ribofuranosidase, β -D-glucosidase	indoxyl- β -D-ribofuranoside, indoxyl- β -D-glucoside	bile salts, SDS, carbenicillin
<i>Listeria spp.</i>	β -glucosidase	indoxyl- β -glucoside	lithium chloride, ceftazidime, amphotericin B, nalidixic acid, polymyxin B
<i>L. monocytogenes</i>	Phosphatidylinositol-specific Phospholipase C, β -glucosidase	indoxyl- β -glucoside, indoxyl-myo-inositol-1-phosphate	lithium chloride, ceftazidime, amphotericin B, nalidixic acid, polymyxin B
<i>Pseudomonas</i>	β -Alanyl arylamidase	7-Amido-1-pentyl-phenoxazin-3-one	cetrimide
<i>Salmonella</i>	α -galactosidase, lipase	indoxyl- α -galactoside, indoxyl-fatty acid ester	sodium deoxycholate
MRSA (Methicillin Resistant <i>Staphylococcus aureus</i>)	α -glucosidase	indoxyl- α -D-glucopyranoside	methicillin, high concentration of sodium chloride
<i>Staphylococcus aureus</i>	α -glucosidase, phosphatase, deoxyribonuclease	indoxyl- α -D-glucoside, phenolphthalein phosphate, indoxyl-phosphate	tellurite, lithium chloride
Streptococci	β -glucuronidase	indoxyl- β -glucuronide	sodium azide
UTI (Urinary Tract Infections)	β -glucosidase, β -galactosidase	indoxyl- β -glucopyranoside, indoxyl- β -galactoside	-
<i>Vibrio</i>	β -glucosidase, β -galactosidase	indoxyl- β -glucopside, indoxyl- β -galactoside, indoxyl- β -galactoside	high concentration of sodium chloride, sodium thiosulphate, sodium citrate, sodium cholate
VRE (Vancomycin Resistant Enterococci)	α -glucosidase, β -glucosidase, β -galactosidase	indoxyl- α -glucopyranoside, indoxyl- β -glucopyranoside, indoxyl- β -galactoside	vancomycin
Yeasts and Moulds	β -N-acetylgalactosaminidase, β -xylosidase	indoxyl-N-acetyl- β -D-glucosaminide, indoxyl- β -D-xyloside	oxytetracycline

Table 2: Sigma-Aldrich's product line of chromogenic media according to organisms detected (Table 2 continued on page 4)
(Fluorogenic media are not listed. Complete product listings are available at sigma-aldrich.com/chromo)

Organisms	Brand	Cat. No.	Media
<i>Bacillus cereus</i>	Fluka	92325	HiCrome™ Bacillus Agar*
<i>Candida albicans</i>	Fluka	94382	Candida Ident Agar, modified
<i>Cl. perfringens</i>	Fluka	12398	CP ChromoSelect Agar
	Fluka	75605	m-CP Agar
<i>E. coli</i>	Fluka	70722	HiCrome™ E. coli Agar B*
	Fluka	09142	HiCrome™ ECD Agar with MUG*
	Fluka	92435	TBX Agar
<i>E. coli</i> & Coliforms	Fluka	81938	HiCrome™ Coliform Agar*
	Fluka	73009	HiCrome™ ECC Agar*
	Fluka	85927	HiCrome™ ECC Selective Agar*
	Fluka	51489	HiCrome™ Rapid Coliform Broth*
	Fluka	39734	Membrane Lactose Glucuronide Agar

Table 2: Sigma-Aldrich's product line of chromogenic media according to organisms detected (Fluorogenic media are not listed. Complete product listings are available at sigma-aldrich.com/chromo)

Organisms	Brand	Cat. No.	Media
<i>E. coli</i> O157:H7	Fluka	39894	HiCrome™ EC O157 Agar*
	Fluka	72557	HiCrome™ EC O157:H7 Selective Agar, Base*
	Fluka	80330	HiCrome™ Enrichment Broth Base for EC O157:H7*
	Fluka	83339	HiCrome™ Mac Conkey Sorbitol Agar*
Thermotolerant <i>E. coli</i>	Fluka	90924	HiCrome™ m-TEC Agar*
<i>Enterobacter sakazakii</i> (<i>Cronobacter</i> spp.)	Fluka	92324	HiCrome™ Cronobacter spp. Agar*
	Fluka	14703	HiCrome™ Cronobacter spp. Agar, modified*
Enterococci	Fluka	52441	HiCrome™ Enterococci Broth*
	Fluka	51759	HiCrome™ Rapid Enterococci Agar*
<i>Enterococcus faecium</i>	Fluka	90919	HiCrome™ Enterococcus faecium Agar Base*
<i>Klebsiella</i>	Fluka	90925	HiCrome™ Klebsiella Selective Agar Base*
<i>Listeria</i>	Fluka	53707	HiCrome™ Listeria Agar Base, modified*
	Fluka	77408	Listeria mono Differential Agar (Base)
<i>Proteus</i> , enteropathogenic gram-positive organisms	Fluka	16636	HiCrome™ UTI Agar, modified*
<i>Salmonella</i>	Fluka	00563	HiCrome™ MM Agar*
	Fluka	90918	HiCrome™ RajHans Medium, Modified*
	Fluka	78419	HiCrome™ Salmonella Agar*
	Fluka	05538	HiCrome™ Salmonella Agar, Improved*
	Fluka	84369	Salmonella Chromogen Agar*
<i>Staphylococcus aureus</i>	Fluka	05662	HiCrome™ Aureus Agar Base*
	Fluka	68879	Phenolphthalein Phosphate Agar
Methicillin-resistant <i>Staph. aureus</i>	Fluka	90923	HiCrome™ MeReSa Agar Base*
<i>Vibrio</i>	Fluka	92323	HiCrome™ Vibrio Agar*
Yeasts and fungi	Fluka	66481	HiCrome™ OGYE Agar Base*

* not sold in USA

Biochemical Test Disks & Strips

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

Disks and strips are very helpful for identification and confirmation of microorganisms or to monitor sterilization. These methods are rapid, easy to prepare, and have very good quality for the price.

The strips and disks are made of a cellulose-based material impregnated with the appropriate chemical reagents or biological material. This converts them into intelligent systems that can be used for the detection of specific abilities and properties of microorganisms, either based on the detection of enzymes using chromogenic substrates, indicators or on complex building reactions. Also, sensitivity to certain inhibitory substances can also be tested. Since no complicated handling is required to use the disks and strips, and there are no additional reagents used, a lot of time and money is saved.

Often a biochemical test serves as confirmation of a presumed colony found on an agar plate, or as one of the first steps to classify the direction of further identification (e.g., aminopeptidase, oxidase, catalase test). It is also possible to do identification of an organism by combining several tests or using differential media. In several microbiological text books and other literature, diverse identification flow paths are described and recommended. In **Table 1** the most useful tests are listed and described with regard to application and mechanisms.

Carbohydrate Differentiation Disks are used to differentiate bacteria on the basis of carbohydrate fermentation abilities. A sugarfree liquid or solid medium base with indicator serves as a base. With the addition of the unknown microorganism and the disks, the test is

Figure 1: β-Lactamase Strips

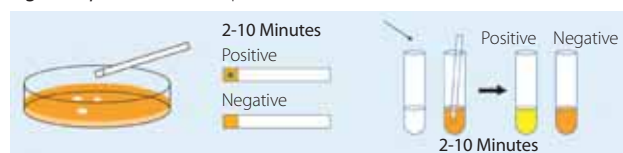


Figure 2: Carbohydrate Disks in base media after incubation (yellow means positive carbohydrate utilization). Gas production can be seen because of the bubbles in the small Durham tubes (inside the tubes).

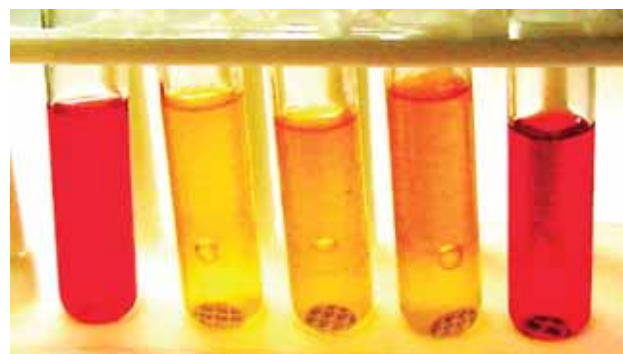



Table 1: Biochemical Tests on Disks and Strips

Cat. No.	Product	Description
75554	Aminopeptidase	For the detection of L-alanine-aminopeptidase in microorganisms. Found almost only in gram-negative microorganisms.
08382	Bacitracin Disks	Used in the presumptive identification of group A β -hemolytic streptococci.
07773	Bacteriuria Test Kit	Used to detect the presence of nitrite in urine.
80489	β -Lactamase Strips	For the rapid acidimetric detection of the β -lactamase activity of microorganisms.
80507	Bile Esculin Disks	For rapid detection of esculin hydrolysis in the presence of bile for differentiating group D streptococci from non-group D streptococci.
75832	Coagulase Disks	For the detection of coagulase-negative or -positive organisms (<i>Staphylococcus aureus</i>).
05686	DMACA Indole Disks	Used for the Indole test to determine the ability of an organism to split tryptophan into indole and α -aminopropionic acid.
40405	Hippurate Disks	Recommended in qualitative procedures to detect organisms possessing the enzyme hippurate hydrolase.
01869	Hippurate Strips Kit	Useful for differentiating β -hemolytic group B streptococci, <i>Gardnerella vaginalis</i> and <i>Campylobacter</i> species.
06728	Hydrogen Sulfide	Used for detection of hydrogen sulfide production by microorganisms.
04739	Indoxyl Strips	For the rapid detection of the acetate esterase activity of microorganisms. Acetate esterase activity is present, e.g., in <i>Campylobacter</i> spp., and in <i>Branhamella catarrhalis</i> .
78719	Kovac's Reagent Strips	Used to determine the ability of microorganisms, primarily <i>Enterobacteriaceae</i> , to split indole from the tryptophan molecule.
51138	Nitrate Reagent	Used to detect an organism's ability to reduce nitrate.
49862	Nitrocefin Disks	Used for the rapid detection of β -lactamase enzymes in isolated colonies of <i>Neisseria gonorrhoeae</i> , <i>Moraxella catarrhalis</i> , <i>Staphylococcus</i> spp., <i>Haemophilus influenzae</i> and anaerobic bacteria.
49940	ONPG Disks	Used to detect the presence of β -galactosidase, an enzyme found in lactose-fermenting organisms.
74042	Optochin Disks	Optochin (ethyl hydrocuprein hydrochloride) is inhibitory for pneumococcal growth whereas other streptococci show good growth or a very small zone of inhibition.
40560	Oxidase Strips	Provide a diagnostic test for the detection of the cytochrome oxidase activity of microorganisms.
70439	Oxidase Disks	
67886	Pyrase Test Strips	Provide a diagnostic test for the rapid differentiation of Enterococci from the group D Streptococci and differentiation of <i>Streptococcus pyogenes</i> from other haemolytic Streptococci.
74146	Sterile Disks	Used to test a variety of antibiotics, carbohydrates, substrates, and antiseptics on bacteria in petri dishes. Soak a disk in a solution or apply some solution on the disks.
75744	Tributyryn-Strips	A diagnostic test for the differentiation between <i>Branhamella</i> and <i>Neisseria</i> .
08482	X + V Factor Disks	<i>Haemophilus</i> species requiring both X and V factors exhibit growth only in the vicinity of the X +V factor disks.
89788	V-Factor Disks	

prepared. After 18 to 48 hours of incubation at 36 °C, the result can be recorded at 18-24 hours and again at 48 hours. Acid production and eventually the gas production (CO₂) are observed. In **Table 2** all usual carbohydrates are listed for a typical metabolic profiling.

Sterility indicators are used to monitor the sterilization process performance. There are the normal tapes which change color when they have been exposed to sufficient heat, but for a real evaluation and to be on the safe side, it is recommended to use spore indicators which undergo the same procedure as the sterile goods. *Bacillus stearothermophilus* is a thermophilic species that can grow at temperatures such as 65 °C and higher. Its spores are an excellent tool to monitor autoclave performance, as they are highly resistant to temperature. This type of indicator is recommended by the United States military specification MIL-S-36586 and meets the GMP requirements of the FDA. For the radiation sterility indicator, the FDA and the US military recommend *Bacillus pumilus* spores due to their resistance to radiation. They are an excellent tool to monitor the efficiency of radiation sterilization.

Table 2: Carbohydrates Differentiation Disks

Cat. No.	Media	Cat. No.	Media
55876	Adonitol disks	94438	Mannitol disks
80372	Arabinose disks	94445	Mannose disks
56481	Cellobiose disks	93196	Melibiose disks
63367	Dextrose disks	94226	Raffinose disks
73044	Dulcitol disks	93999	Rhamnose disks
53901	Fructose disks	92971	Salicin disks
89608	Galactose disks	93998	Sorbitol disks
89614	Inositol disks	94309	Sucrose disks
90058	Inulin disks	92961	Trehalose disks
28816	Lactose disks	07411	Xylose disks
77653	Maltose disks		

Figure 3: Sterility Indicator for Radiation Sterilization Strips and Envelopes




Rhodococcus equi Invasion of Non-Phagocytic Cells

By Sara Remuzgo-Martínez¹, Lilian Pílares Ortega², Jesús Navas², José Ramos Vivas¹.....ifmav,jvivas@fmdv.org

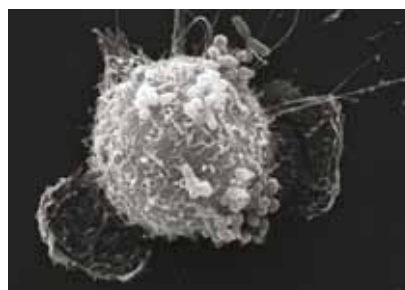
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Rhodococcus equi: A Little Known Potential Pathogen.

Rhodococcus equi is a facultative intracellular bacterium with rod to coccus pleomorphism (Figure 1). Although *R. equi* is primarily a pathogen of foals, it also infects humans and other animal species [1]. In immunocompromised persons, *R. equi* causes tuberculosis-like pneumonia associated with a high case-fatality rate, principally in HIV patients. Endocarditis, brain abscesses, and other infections have also been reported [2]. *R. equi* infections are diagnosed by culture and subsequent phenotypic analysis of samples by means of classical morphological and biochemical tests. In clinical microbiology laboratories, *R. equi* is usually identified using the API Coryne system (bio-Mérieux, France), a commercial multsubstrate kit that includes *R. equi* in its database. However, its reliability for the identification of rhodococcal isolates is limited, resulting in misidentification of *R. equi* as other rhodococcal species or even other actinomycetes [3].

Figure 1: A scanning electron microscopy showing rod and coccoid forms of *R. equi* over a partially detached human epithelial cell



While the interaction of *R. equi* with macrophages has been comprehensively studied, little is known about its interactions with non-phagocytic cells. Epithelial cells play an important role as the interface between the host mucosal surfaces and the surrounding environment and are the initial site of colonization for bacterial pathogens. *R. equi* could enter either epithelial or other types of non-phagocytic cells avoiding the host hostile environment and disseminating more easily like other intracellular pathogens do [4-6]. In a recent work, we have studied the interaction of *R. equi* human isolates with the human lung epithelial cell line A549 [7]. After interaction of bacteria with host cells, we consistently recovered viable intracellular bacteria in almost all the strains, although the invasion rate varied depending on the strain. Differential double immunofluorescent labeling of *R. equi* allowed us to differentiate extracellular from intracellular bacteria (Figure 2a-c).

After four hours of infection (up to 24 hours), we observed vacuoles filled with bacteria, significant bacterial growth for all invasive strains, and in the majority of instances, the bacterial growth was clumped together as microcolonies (Figure 3). These observations under the microscope correlate well with the increase in numbers obtained from quantitative experiments. All together, our data demonstrate that *R. equi* is capable of invading and replicating within non-phagocytic tissue culture cells, indicating that entry and multiplication within epithelial cells during *R. equi* infection may contribute to pathogenesis, spread of bacteria, and/or disease progression. Identification and characterization of the virulence factors required by *R. equi* in the intracellular environment would further elucidate the requirements for survival and spread of this pathogen in tissues.

Figure 2: A Single *R. equi* Bacterium Inside an Epithelial Cell A549 Undergoing Mitosis. Infected cells were fixed and processed for immunofluorescence labeling. To stain intracellular bacteria, cover slips were incubated with antibodies against *R. equi*. Fluorescent-labeled phalloidin, which binds polymerized F-actin, was used to identify actin filaments and fibers (a). After washing, cover slips were mounted on glass slides with mounting medium containing DAPI (b). Merged images (c) show an intracellular bacterium in yellow, polymerized actin in green, and the DAPI-stained nuclei in blue. Micrograph was originally captured at $\times 1000$ magnification using a Zeiss Axiovert 200 Microscope and acquired using a Zeiss AxioCam HRc digital camera.

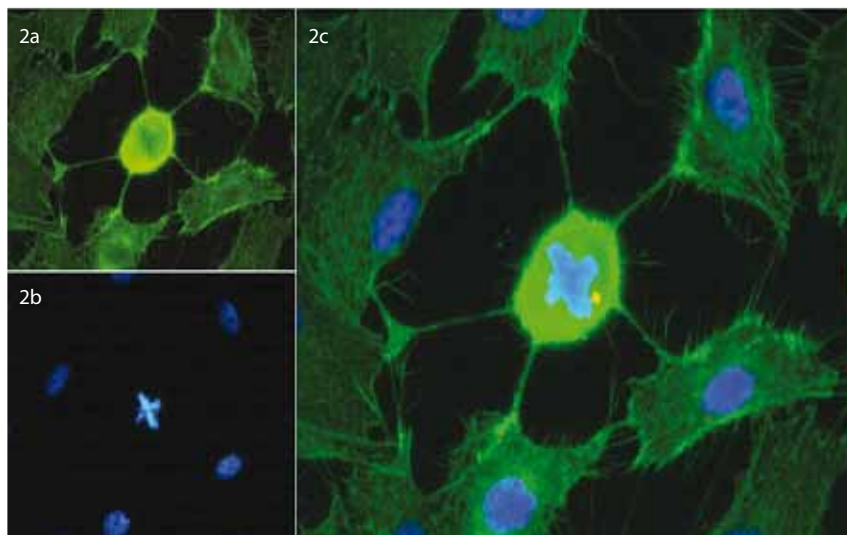


Figure 3: Intracellular Survival and Proliferation of *R. equi*. A549 cells were infected for 24 hours, and after washing, cells were fixed and processed for immunofluorescence labeling. Intracellular bacteria were detected with anti-*R. equi* rabbit antiserum and a secondary antibody. **3a:** grey scale; **3b:** grey scale merged with the fluorescent channel (**3c**), that show bacteria in red; **3d:** microcolonies of bacteria that are magnified from **3b**. Arrowheads indicate bacterial microcolonies emerging from infected cells. Micrographs were captured at x400 magnification. Scale bars, 20 µm.



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Atto Dyes for Superior Fluorescent Imaging

By Monika Bäuml, Product Manager Detection.... monika.baeumle@sial.com

Activated fluorescent dyes are routinely used to tag proteins, nucleic acids, and other biomolecules for use in life science applications including fluorescence microscopy, flow cytometry, fluorescence in situ hybridization (FISH), receptor binding assays, and enzyme assays.

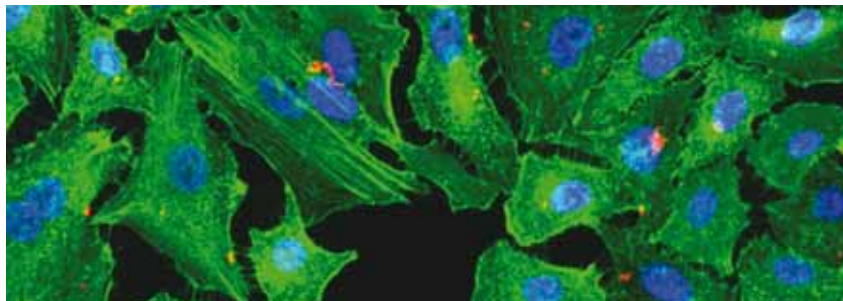
The Atto dyes, a series of fluorescent dyes, meet the critical needs of modern fluorescent technologies:

- **Stability** - Atto 655 and Atto 647N are photostable and highly resistant to ozone degradation.
- **Long Signal Lifetimes** - Signal decay times of 0.6–4.1 nanoseconds allow timegate studies to reduce autofluorescence background and scattering.
- **Reduced Background** - Several Atto dyes employ excitation wavelengths greater than 600 nm, reducing background fluorescence from samples, Rayleigh and Raman scattering.
- **Selection** - Atto dyes have strong fluorescent signals that cover visible and near-IR emission wavelengths.

Atto Phalloidin

Phalloidin is a fungal toxin isolated from the poisonous mushroom *Amanita phalloides*. Its toxicity is attributed to the ability to bind F actin in liver and muscle cells. As a result of binding phalloidin, actin filaments become strongly stabilized. Phalloidin has been found to bind only to polymeric and oligomeric forms of actin, and not to monomeric actin. The dissociation constant of the actin-phalloidin complex has been determined to be on the order of 3×10^{-8} .

Figure 1: Groups of *R. equi* inside A549 cells. The bacteria were stained with antibodies conjugated labels (red). Atto 488 phalloidin, which binds polymerized f-actin, was used to identify actin filaments and fibers (green). DAPI stains the nucleus blue.



Phalloidin differs from amanitin in rapidity of action; at high dose levels, death of mice or rats occurs within 1 or 2 hours. Fluorescent conjugates of phalloidin are used to label actin filaments for histological applications. Some structural features of phalloidin are required for the binding to actin. However, the side chain of amino acid 7 (γ -dihydroxyleucine) is accessible for chemical modifications without appreciable loss of affinity for actin.

Table 1: Other Atto Dye Conjugates

Name	Recommended $\lambda_{ex} / \lambda_{em}$	Cat. No.
Phalloidin–Atto 390 conjugate	390 / 472 nm in 0.1 M phosphate pH 7.0	50556-10NMOL
Phalloidin–Atto 425 conjugate	436 / 484 nm in 0.1 M phosphate pH 7.0	66939-10NMOL
Phalloidin–Atto 488 conjugate	501 / 523 nm in 0.1 M phosphate pH 7.0	49409-10NMOL
Phalloidin–Atto 532 conjugate	532 / 553 nm in 0.1 M phosphate pH 7.0	49429-10NMOL
Phalloidin–Atto 550 conjugate	554 / 574 nm in 0.1 M phosphate pH 7.0	19083-10NMOL
Phalloidin–Atto 565 conjugate	563 / 592 nm in 0.1 M phosphate pH 7.0	94072-10NMOL
Phalloidin–Atto 590 conjugate	594 / 624 nm in 0.1 M phosphate pH 7.0	93042-10NMOL
Phalloidin–Atto 633 conjugate	629 / 651 nm in 0.1 M phosphate pH 7.0	68825-10NMOL
Phalloidin–Atto 647N conjugate	644 / 669 nm in 0.1 M phosphate pH 7.0	65906-10NMOL
Phalloidin–Atto 655 conjugate	663 / 684 nm in 0.1 M phosphate pH 7.0	18846-10NMOL
Phalloidin–Atto 665 conjugate	663 / 684 nm in 0.1 M phosphate pH 7.0	04497 -10MNOL
Phalloidin–Atto Rho6G conjugate	535 / 560 nm in 0.1 M phosphate pH 7.0	55212-10NMOL

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