Microbiology Focus

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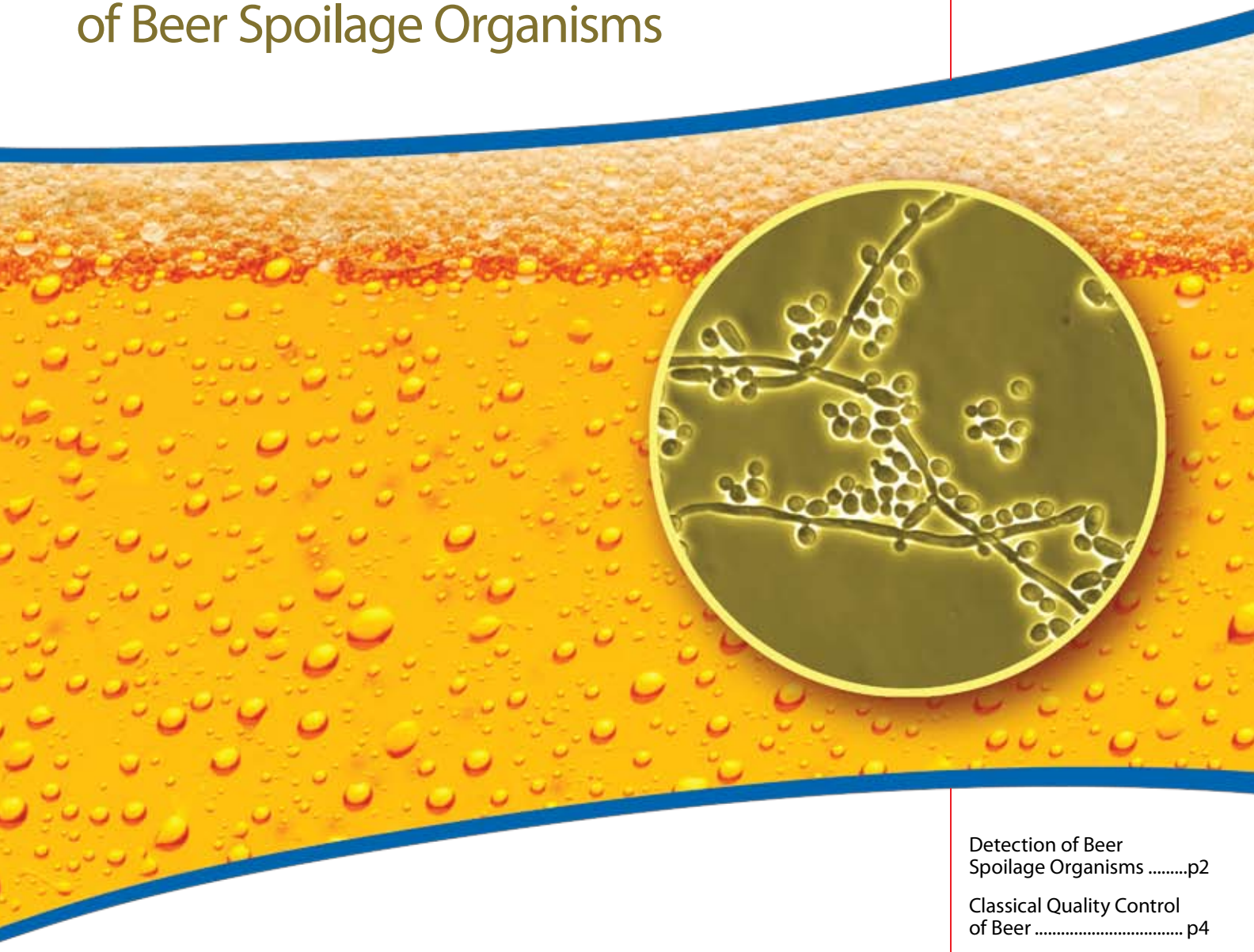
Fluka Analytical

New Fast and Specific Detection of Beer Spoilage Organisms



Yeast used for the beer production

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New Fast and Innovative Detection of Beer Spoilage Organisms

By Manuela Fabienke, Scanbec GmbH and Jvo Siegrist, Product Manager Microbiology, Sigma-Aldrich…. *ivo.siegrist@sial.com*

HybriScan® an innovative screening method for beer spoilage organisms based on the detection of rRNA

The popularity of beer remains high but the quality of beer has to be very high to survive in a competitive market. Beer spoilage organisms are either lactic acid bacteria belonging to the genera *Lactobacillus* and *Pediococcus* or they are obligate anaerobes of the species *Pectinatus* and *Megasphaera* (see **table 1**). Within the species of lactobacilli known to cause spoilage of beer, only certain strains can grow in the beer and are responsible for spoiling (exception: *Lactobacillus lindneri* all strains cause spoilage). *L. brevis* is the most common beer spoilage bacterium followed by *L. lindneri* [1]. Additionally many wild yeasts are responsible for beer spoilage such as *Saccharomyces cerevisiae* and *Candida pelliculosa* [2]. One of the biggest problems is biofilm formation in beer plants, which makes it very difficult to remove spoilage organisms.

Highly skilled lab staff perform microbiological analysis in specific quality control laboratories. Most of the laboratories still use conventional standard based cultivation methods, which are very time consuming and take 3 to 5 days for beer to be released to the market.

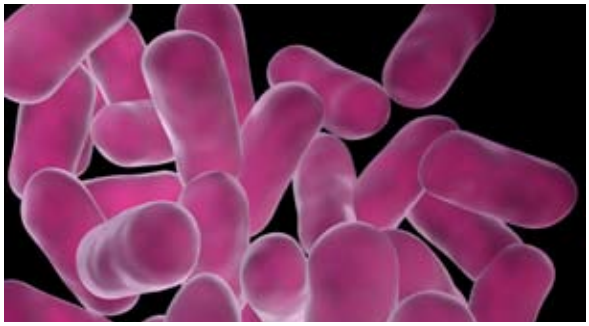


Figure 1: Lactobacilli (beer spoilage organisms)

Figure 2: Vats in a brewery

HybriScan® Beer kit, a rapid test system developed by Scanbec GmbH in collaboration with Sigma-Aldrich, could lead to a faster product release of beer and could act as an alternative for the detection of beer spoilage contaminants. After as little as two hours (pre-enrichment for 24 h, if necessary) the brewery could have the first reliable results.

A variety of applications have been developed for HybriScan including the detection of bacteria and yeast in non-alcoholic beverages. The robustness of the HybriScan assay enables it, in contrast to other rapid test systems, to detect bacterial contamination in brewer's yeast and leads to efficient use of this valuable resource. Furthermore HybriScan test system is a perfect tool for microbiological control of dispensing equipment. The legal standard for sterility control of dispensing equipment is 100,000 cfu/ml; a fast, direct determination of beer spoiling bacteria is possible without pre-enrichment-procedure delivering results within two hours.

Comparison of HybriScan and other rapid test systems: Performing quality control by using the standard cultivation based method takes a long time. In recent years many companies have developed rapid test systems to hasten this procedure. For quality control of beer and beverages three main technologies are available:

- HybriScan (sandwich hybridization)
- PCR (Polymerase Chain Reaction)
- VIT (Vermicon Identification Technology)

Did you know…

that rRNA is decomposed rapidly in dead cells?

Ribosomal RNA disappears in a few hours, unlike DNA, which is quite stable outside of a living cell.

This is the reason why PCR can give false positive results.

Genus *Lactobacillus*:	*Lactobacillus acidophilus*
	Lactobacillus brevis
	Lactobacillus brevisimilis
	Lactobacillus buchneri
	Lactobacillus casei
	Lactobacillus collinoides
	Lactobacillus coryniformis
	Lactobacillus curvatus
	Lactobacillus fermentum
	Lactobacillus fructivorans
	Lactobacillus lindneri
	Lactobacillus malefermentans
	Lactobacillus parabuchneri (frigidus)
	Lactobacillus paracasei
	Lactobacillus paraplantarum
	Lactobacillus plantarum
	Lactobacillus rhamnosus
Genus *Pediococcus:*	*Pediococcus acidilactici*
	Pediococcus claussenii
	Pediococcus damnosus
	Pediococcus inopinatus
	Pediococcus parvulus
	Pediococcus pentosaceus
Genus *Pectinatus:*	*Pectinatus cerevisiiphilus*
	Pectinatus fringensis
Genus *Megasphaera:*	*Megasphaera cerevisiae*

Table 1: Species of beer-spoilage microorganisms that can be detected with the **HybriScan®*D* Beer–Kit (Cat. No. 62533**, 96 assays)

A comparison of these different technologies is given in **table 2**. Comparing HybriScan to PCR or VIT –technology the benefits of this rapid test system are:

- Fast and cost efficient analysis
- Inexpensive read-out technology
- High sensitivity and specificity

Using two different probes for detection of microbial RNA, false-positive results are almost impossible. In **Figure 1** results of quantification of *Lactobacillus buchneri* within a starter culture (silage) of three different samples are presented. Comparison of the HybriScan test with a cultivation based analytical method (MRS agar) displays the equivalent results within the limits of microbiological sample variability.

References:

1. E. StorgArds, M.-L. Suiiiko, Detection and identification of Lactobacillus linderi from Brewery Environments, J. Inst. Brew., Vol. 104, p. 47-54 (1998)
2. Markus Timke, et al., Identity, beer spoiling and biofilm forming potential of yeasts from beer bottling plant associated biofilms, Springer Science+Business Media B.V. (2007)

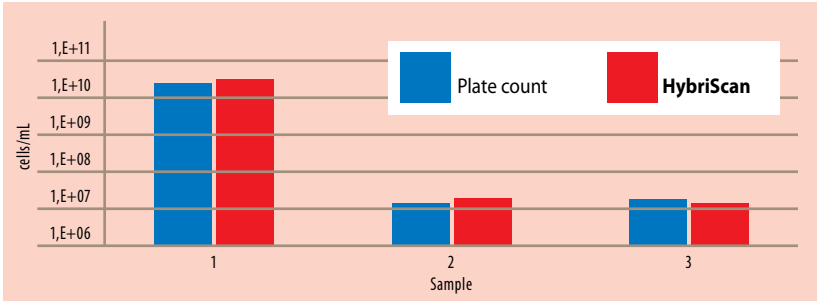


Figure 3: Detection of beer spoilage bacteria can be performed group- or species-specific.

	Cultivation based method	PCR	VIT	HybriScan
Method	cultivation based method with optical or microscopic read out	PCR/real-time PCR	fluorescence microscopy	sandwich hybridization and photometrical signal read out
Detection spectrum	detection and identification of all beer spoilage microorganisms	identification of all relevant Beer spoilage microorganisms possible	*Lactobacillus sp.* and *Pediococcus damnosus, Lactobacillus sp. + L.brevis, Pectinatus + Megasphaera cerevisiae*	identification of all relevant Beer spoilage microorganisms possible
Sample preparation	selective pre-enrichment	enrichment and lysis of bacteria, if necessary pre-enrichment	selective pre-enrichment	enrichment and lysis of bacteria, if necessary pre-enrichment
Time	3 to 7 days	3 hours to 2 days	2 days	3 hours to 2 days
Costs per test	ca. 1 €	12 €	15 €	3 €
Detection limit (cfu)	1	1-5 x 10^3	1 x 10^3	1-5 x 10^3
Devices	None	PCR cycler	fluorescence microscope	microplate reader
Advantages	high sensitivity, relatively cheap	high sensitivity, quantitative analysis	simple detection technology set up, detects only living cells (RNA)	rapid and sensitive, qualitative and quantitative detection of living cells, cost efficient analysis
Disadvantages	time consuming, no detection of non-culturable microbes, labor expensive	expensive devices needed, no discrimination between live and dead cells, not officially accepted	time consuming, low sample throughput, expensive, not automatable, difficult data analysis, not officially accepted	no differentiation of serotypes or subspecies, limited probe design (rRNA target), not officially accepted

Table 2: Comparison of different technologies for detection of beer spoilage bacteria

Overview of HybriScan® Kits

The HybriScan can detect much more than beer spoilage organisms. There are a wide variety of applications for this innovative rapid test system in beverage, food, water and other specimens. E.g. *Legionella* can be detected in water and foods using the HybriScan rapid test system.

GENUS	Lactobacilli, *Legionella, Listeria, Salmonella, Campylobacter, Megasphaera, Leuconostoc*
SPECIES	*E. coli, Legionella pneumophila, Listeria monocytogenes, Microthrix parvicella, Brettanomyces, Candida albicans, Lactobacillus brevis, Lactobacillus buchneri, Lactobacillus lindneri, Pectinatus cerevisiiphilus, Pectinatus frisingensis, Pediococcus damnosus, Enterobacter sakazakii*
GROUPS/APPLICATIONS	Waste Water Index, Beer Spoilage Organisms, Beverage Spoilage, Organisms, Total Bacterial Count, Yeasts

Further information about HybriScan method can be found at ***www.sigma-aldrich.com/hybriscan***

Cultural Method for Quality Control of Beer

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

The classical cultural method for the quality control of beer is still important. Most modern methods use at least a pre-enrichment step and the costs for the analysis are lower.

Traditional methods are based morphology, staining methods, enzyme reactions (metabolism) and diverse media. One of the first steps is the filtration of the beer followed by an incubation of the filter on an agar plate. Suspect colonies are then examined under the microscope followed by staining procedures and biochemical tests. More details of a systematic identification can be seen in **figure 1**.

Listed in **table 1** are the typical media used for the detection of spoilage organisms like lactobacilli, pediococci, *Pectinatus*, *Megasphaera* molds and wild yeasts in the brewery and fermentation industry. Only a few a few genera of bacteria, wild yeasts, and molds cause spoilage of beer and wine because of the alcohol content, low pH, and other ingredients having inhibitory effects. Some of these contaminants can interfere with fermentation or have deleterious effects on flavor and shelf life. Most of these spoilage organisms make the beer acidic or producing substances with a bad taste like diacethyl and tetraden compounds. *Pectinatus* and *Megasphaera* are also dangerous pathogens.

Figure 2: Beers

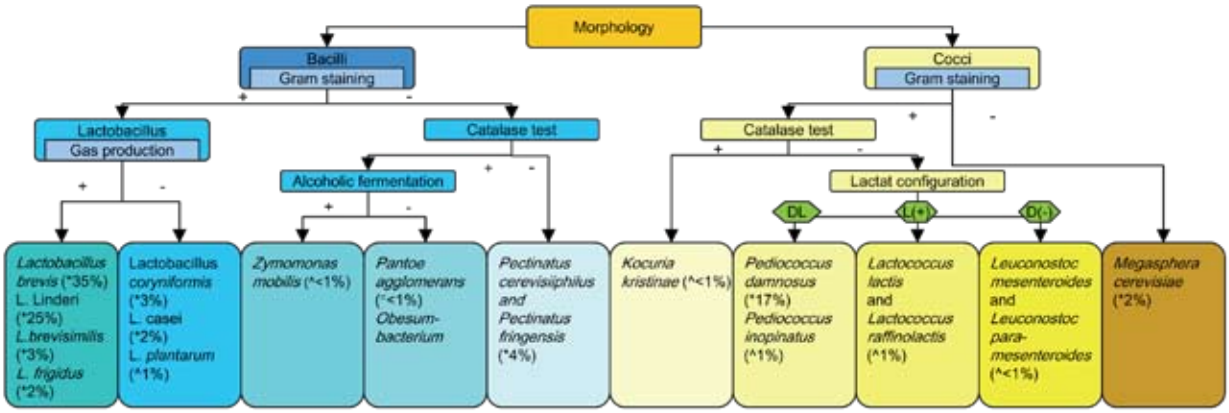


Figure 1: Identification flow chart for beer spoilage bacteria

* obligate beer spoilage. ^ potential beer spoilage. ° indirect beer spoilage. Numbers in % gives the frequency of spoilage organisms from quality complaints for beer (1980-2002) (Source: Handbook of filling techniques: Grundlagen und Praxis für das Abfüllen flüssiger Produkte; S. Blüml, S. Fischer 2004)

Medium	Brand	Cat #
$CaCO_3$ Agar	Fluka	40545
Corn Meal Agar	Fluka	42347
HiCrome™ OGYE Agar Base	Fluka	66481
Lysine Medium	Fluka	L5910
Malt Agar	Fluka	M9802
Malt Extract Agar, modified	Fluka	97218
MRS Agar	Fluka	69964
MRS Agar, original acc. DeMan-Rogosa-Sharpe	Fluka	30912
MRS Agar, Vegitone	Fluka	41782
MRS Broth	Fluka	69966
MRS Broth modified, Vegitone	Fluka	38944
NBB Agar	Coming soon!	
OGY Agar	Fluka	75310
Plate Count Agar	Fluka	70152
Plate Count Agar according to Buchbinder et al.	Fluka	88588
Plate Count Agar, Vegitone	Fluka	19718

Medium	Brand	Cat #
Plate Count MUG Agar	Fluka	51413
Raka Ray Agar, Base	Fluka	02538
Rogosa SL Agar	Fluka	R1148
Rose Bengal Chloramphenicol Agar	Fluka	17211
Schwarz Differential Agar	Coming soon!	
Tomato Juice Agar	Fluka	17216
Universal Beer Agar	Fluka	17226
WL Differential Agar	Fluka	17215
WL Nutrient Agar	Fluka	17222
WL Nutrient Broth	Fluka	W2261
Wort Agar	Fluka	70196
Yeast Carbon Base	Sigma	Y3627
Yeast Malt Agar	Sigma	Y3127
Yeast Nitrogen Base	Fluka	51483
Yeast Nitrogen Base without Amino Acid	Sigma	Y0626
Yeast Nitrogen Base without Amino Acid and Ammonium Sulfate	Sigma	Y1251

Table 1: Typical brewery quality control media

Viable but Nonculturable Bacteria

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

In many specimens, more bacteria are present than we can detect with common cultural methods.

The expression "viable but nonculturable" (VNC) bacteria, describes cells that cannot normally be cultured. However this makes little sense, when one considers that the demonstration of culturability remains the best practically acceptable definition of viability. So a better explanation of the status of these bacteria would be "not immediately culturable". In most cases the non-spore-forming bacteria is in a survival state (e.g., resting, dormancy, quiescence, or debilitation) and the metabolic pathways are still active but the organism are not growing. According to the latest VNC definition, VNC cells are regarded as viable and potentially replicative, but the methods required for resuscitation are beyond our current knowledge. With special media or with certain supplements it has been shown that it is possible to recover them. VNC bacteria have often undergone a treatment like heating, drying, setting under high osmotic pressure (high salt content) or contact with inhibiting chemicals. The end result of the treatment is sensitive cells or sub-lethally damaged cells, which can mean the loss of some ribosomes, damaged enzymes, cell membranes and other problems causing malfunctions in cells.

In the recent years species of *Vibrio cholerae, E. coli, Campylobacter jejuni, Salmonella* spp., *Listeria monocytenenes* and *Yersinia enterocolitica* have been reported to enter the viable but nonculturable (VNC) state [1-10].

Supplementing the pre-enrichment and enrichment broths with ferrioxamine E significantly improved the recovery of *Salmonella, Cronobacter* spp., *Staphylococcus aureus* and *Yersinia enterocolitica* from artificially or naturally contaminated foods [1-3]. A concentration of ferrioxamine E (available from Sigma, see **table 2**) in the range of 5-200 ng/ml supports growth (see **table 1**). Ferrioxamine E provides the essential micro-nutrient iron (III) to the organisms. This leads to a reduced lag-phase in the medium and reactivates damaged bacteria. The ferrioxamine E is often used in Buffered Peptone Water the medium recommended by the ISO-Norms for Enterobacteriacea (see **table 2**). The motility of *Salmonella* is also improved which helps to improve the identification by semisolid selective motility media like MRSV, DIASSALM or SMS. It is recommended when isolating small quantities of cells from dried powders like tea, spices, dried fruits etc.. Ferrioxamine E is semi-selective, as it does not improve growth of *E. coli, Shigella, Proteus, Providencia* and *Morganella* species.

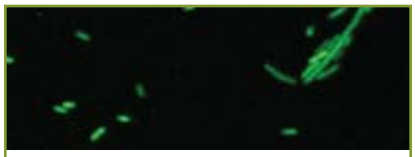
Organisms	[ng/mL]
Salmonella	75
Cronobacter spp.(Enterobacter sakazakii)	150
Yersinia enterocolitica	100

Table 1: Recommended end concentration of Ferrioxamine E

Another application is the fast and selective detection of methicillin-resistant *Staphylococcus aureus* (MRSA) where a combination of Ferrioxamine E and Desferrioxamine B is used in the enrichment media. Desferrioxamine B (available from Sigma, see **table 2**) adsorbs iron traces and thus inhibits growth of concomitant microorganisms and Ferrioxamine E supports *Staphylococcus aureus*, which is able to utilize Ferrioxamine E for iron uptake.

Product	Brand	Cat #
Ferrioxamine E	Fluka	38266
Desferrioxamine B (Deferoxamine mesylate)	Sigma	D9533
Peptone Water, phosphate-buffered with Ferrioxamine E	Fluka	67331
HiCrome™ MeReSa Agar	Fluka	90923
MRSA Selective Supplement	Fluka	51387

Table 2: Products to assist in the detection of VNC



Did you know…

about 99% of bacteria in nature cannot be cultured in a medium?

They are detected with the **FISH** method (**f**luorescence **i**n-**s**itu **h**ybridisation) based on general probes labelled with fluorescence marker.

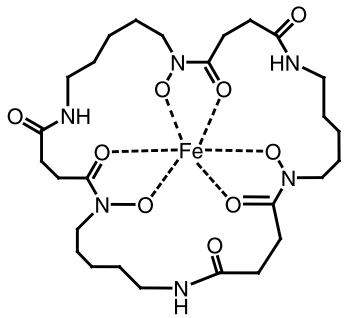


Figure 1: Structure of Ferrioxamine E

References:

1. S. Makino, *et al.*, Does Enterohemorrhagic *Escherichia coli* 0157:H7 Enter the Viable but Nonculturable State in Salted Salmon Roe?, Appl. Environ. Microbiol., 66(12): 5536–5539 (2000)
2. I. Barcina, P. Lebaron, J. Vives-Rego, Survival of allochthonous bacteria in aquatic systems: a biological approach, FEMS Microbiol. Ecol., 23:1–9 (1997)
3. J.C. Choa, S.J. Kim, Viable, but non-culturable, state of a green fluorescence protein-tagged environmental isolate of *Salmonella typhi* in groundwater and pond water, FEMS Microbiol. Lett., 170:257–264 (1999)
4. R.R. Colwell, *et al.*, Viable but non-culturable *Vibrio cholerae* and related pathogens in the environment: implications for release of genetically engineered microorganisms, Bio/Technology., 3:817–820 (1985)
5. D.M. Rollins, R.R. Colwell, Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. Appl. Environ. Microbiol., 52:531–538 (1986)
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8. H.-S. Xu, et al., Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment, Microb. Ecol., 8:313–323 (1982)
9. V. Besnard, *et al.*, Environmental and physico-chemical factors induce VBNC state in *Listeria monocytogenes*, Vet. Res. 33 (4) 359-370 (2002)
10. A. Singh, G.A. McFeters. Survival and virulence of copper- and chlorine-stressed *Yersinia enterocolitica* in experimentally infected mice. Appl Environ Microbiol., 53(8):1768–1774 (1987)
11. R. Reissbrodt, et al., Resuscitation by ferrioxamine E of stressed *Salmonella enterica* serovar *typhimurium* from soil and water microcosms., Appl. Environ. Microbiol., 66(9):4128-30 (2000)
12. R. Reissbrodt et al., Ferrioxamine E-supplemented pre-enrichment and enrichment media improve various isolation methods for Salmonella., Int. J. Food Microbiol., 29(1):81-91(1996)
13. P. Pless, and R. Reissbrodt., Improvement of Salmonella detection on motility enrichment media by ferrioxamine E-supplementation of pre-enrichment culture., Int. J. Food Microbiol., 27:147-159 (1995)

Identification of Microrganisms Based on Color

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

Color is important in our lives as a tool for differentiation. Biochemical tests commonly use a color system.

For microbiologists the most fundamental stain was developed in 1884 by the Danish bacteriologist Hans Christian Gram. The Gram Stain allows the determination of morphology, dividing bacteria into two large groups. Bacteria that are stained purple are called "Gram-positive". Those that stain pink are called "Gram-negative". This staining technique provides information about cell wall structure as Gram-positive organisms have only a grid of peptidoglycan and Gram-negative cells posses an additional lipid bilayer. This information is an important predictor of reaction to antibiotics as many are only effective on Gram-positive bacteria. The Gram Stain remains important for identification of bacteria, and forms the basis for the selection for biochemical tests. (see **table 1** and **figure 1**)

Cat. No.	Product
77730	Gram Staining Kit
94448	Gram's crystal violet solution
90107	Gram's iodine solution
75482	Gram's decolorizer solution
94635	Gram's safranin solution

Table 1: Products for Gram staining

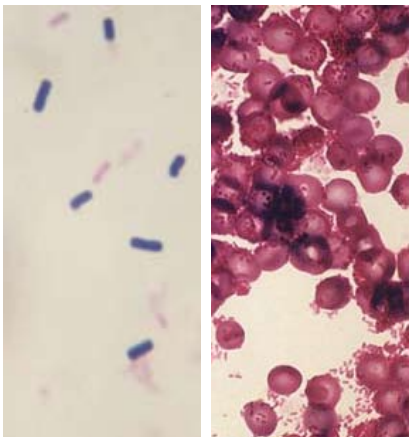

Figure 1: Gram staining (on the left Gram-positive *Bacilus cereus*, on the right Gram-negative *Citrobacter*)

Figure 2: Section of an example ID flow chart

Often a rapid, simple, low cost method is required for confirming or determining the bacteria in water or food control. One such method uses a chromogenic and fluorescent substrate in combination with selective media. Another method is to use biochemical test reagents. All biochemical tests are based on the selective detection of characteristic enzyme activity for the different microorganisms. The targets are to differentiate pathogens, indicate of specific problems and the desired organism present at certain level. In most cases an identification flow chart (**figure 2**) can help to demonstrate a simple way. Within such ID flow charts there are of course other tests that do not use color systems like catalase test (production of air bubbles), grow or inhibition test, microscopic tests etc.

One of the best-known and most used tests is the indole test (Kovac's reagents) where a color complex mechanism is used. The ability of microorganisms to split indole (benzopyrrole) from the tryptophan molecule by tryptophanases is used to differentiate Enterobacteriaceae. Tryptophanase cleaves tryptophan to indole, pyruvate and NH_3. p-Aminobenzaldehyde present in the reagent binds with indole to form a cherry-red complex, soluble in alcohol, ether and chloroform. As isoamylic alcohol or butanol is in the reagent it gives an upper phase, which will be cherry-red in a positive reaction. It is recommended to use a growth media without glucose and a peptone with a high tryptophane content. The Kovac's Reagent is added to a 24-48 hour old culture e.g. incubated in Tryptone Water (Fluka 70194). The incubation time can be reduced to 4 hours by inoculating more cell material in a smaller volume. Slight shaking helps the extraction and within less than a minute a cherry-red coloration should develop to indicate a positive reaction. A negative reaction shows no color change.

Another frequently used method is the determination of an enzyme reaction detected by an indicator. The most commonly used indicators are pH indicators but there are others such as redox. The simplest system is the detection of fermentation from a certain sugar like dextrose, lactose or other carbohydrates. This system is found in many media like Glucose Azide Broth with bromo cresol purple as an indicator (see **figure 4**) and Phenol Red Broth, which is based on the detection of the acid produced in the fermentation process by a pH-indicator that changes color.

Tryptophan
↓ Tryptophanase
Indole + Pyruvate + NH_3
Indole + Kovac's Reagents (red complex)
↓
Confirmation of *E. coli*

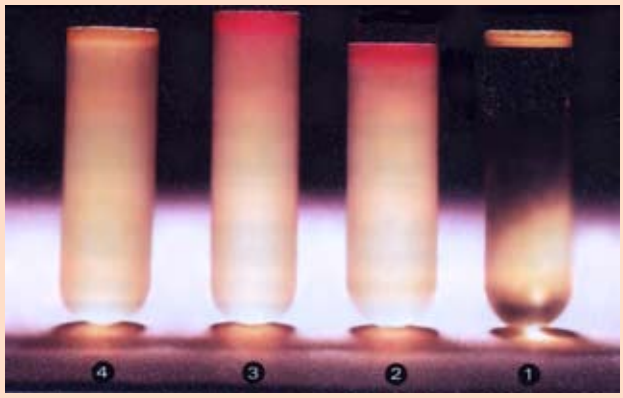

Figure 3: Indole reaction (red upper phase is tryptophanase positive)

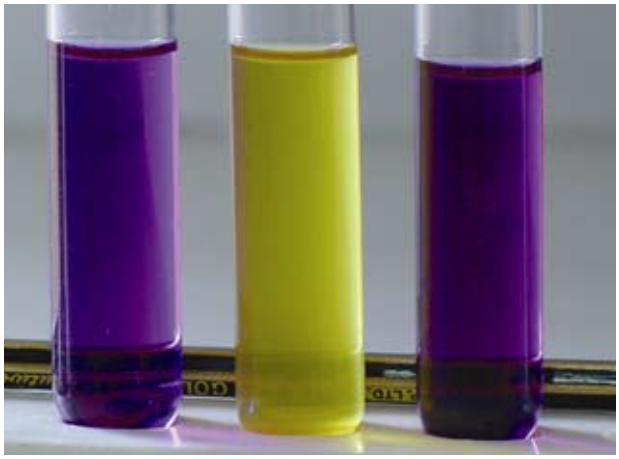

Figure 4: Glucose Azide Broth with bromo cresol purple as indicator (left control, middle positive reaction, right negative reaction)

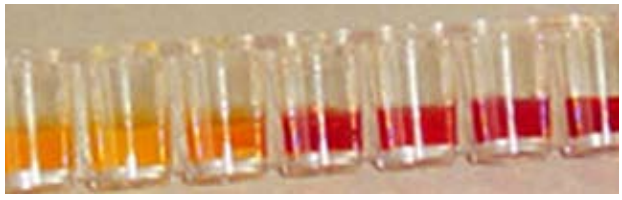

Figure 5: β- Lactamase Test kit (positive reaction is shown by a color change to yellow)

There are other systems based on the production of hydrogen sulphide such as ammonium ferric citrate (media) or lead acetate (test strips). An interesting system is the β-Lactamase Test kit, which is a rapid acidimetric test for detection of β-lactamase activity of microorganisms. It is based on hydrolysis of the β-lactam ring in benzylpenicillin, which results in the production of penicilloic acid. This process causes acidification of the bacterial suspension, and changes the colour of the acid-based indicator. In presence of microorganisms with β-lactamase the solution turns from red to yellow. The result of the reaction is read after 10-30 minutes (see **figure 5**).

There are lot of products where color reactions are used for differentiation and identification and Sigma-Aldrich lists all these products under ***www.sigma-aldrich.com*** under following product groups:

Identification Tests & Reagents

i. Biochemical Reagents
ii. Biochemical Discs and Strips
iii. Test Kits

Media

i. Biochemical Identification Media
ii. Chromogenic Media
iii. Fluorogenic Media

In **table 2** the most important biochemical tests available from Sigma-Aldrich are listed. For further details please refer the Sigma-Aldrich website. (Not included here, the simple carbohydrate discs with pH-indicator)

Detection Target	Cat. No.	Test
Acetate Esterase	04739	Indoxyl Strips
Acetoin production	29333 39442 07689	Barritt's Reagent A Barritt's Reagent B O'Meara's Reagent
Acid production	08714	Methyl Red Solution
Aminopeptidase	75554	Aminopeptidase Test
Catalase	88597	Catalase Test
Coagulase	75832 74226	Coagulase Test (Slide) Coagulase Test (Tubes)
Esculin hydrolysis	80507	Bile Esculin Disks
β-Galactoside Permease, β-Galactosidase	49940	ONPG Disks
Hippurate Hydrolase	40405 01869	Hippurate Disks Hippurate Strips Kit
Lactamase	80489 40561 49862	beta-Lactamase Strips beta-Lactamase Testkit Nitrocefin disks
Lipase	75744	Tributyrin-Strips
Nitrate Reductase	07773 38497 39441 08086 51138 73426	Bacteriuria Test Kit Nitrate Reagent A Nitrate Reagent B Nitrate Reagent Disks Nitrate Reagent Disks Kit Nitrate Reduction Test
Oxidase	07345 07817 18502 40560 70439	Oxidase Reagent acc. Gaby-Hadley A Oxidase Reagent acc. Gaby-Hadley B Oxidase Reagent acc. Gordon-McLeod Oxidase Strips Oxidase Discs
Pyrase	67886	PYRase Test Strips
Requirement for hemin	08482 77148	X + V Factor Disks X-Factor Disks
Requirement for NAD	89788 08482	V-Factor Disks X + V Factor Disks
Sensitivty to Bacitracin	08382	Bacitracin Disks
Sensitivty to Optochin	74042	Optochin Disks
Sulfate Reduction	06728	Hydrogen Sulfide Test Strips
Tryptophan deaminase	80353	TDA Reagent
Tryptophanase	05686 49825 67309 60983 78719	DMACA Indole Disks DMACA Reagent Kovac's Reagent for indole Kovac's Reagent for indole Kovac's Reagent Strips

Table 2: Biochemical tests

Argentina
SIGMA-ALDRICH DE ARGENTINA S.A.
Free Tel: 0810 888 7446
Tel: (+54) 11 4556 1472
Fax: (+54) 11 4552 1698

Australia
SIGMA-ALDRICH PTY LTD.
Free Tel: 1800 800 097
Free Fax: 1800 800 096
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Austria
SIGMA-ALDRICH HANDELS GmbH
Tel: (+43) 1 605 81 10
Fax: (+43) 1 605 81 20

Belgium
SIGMA-ALDRICH NV/S.A.
Free Tel: 0800 14747
Free Fax: 0800 14745
Tel: (+32) 3 899 13 01
Fax: (+32) 3 899 13 11

Brazil
SIGMA-ALDRICH BRASIL LTDA.
Free Tel: 0800 701 7425
Tel: (+55) 11 3732 3100
Fax: (+55) 11 5522 9895

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SIGMA-ALDRICH DENMARK A/S
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Fax: (+358) 9 350 92555

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SIGMA-ALDRICH CHEMIE GmbH
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Tel: (+49) 89 6513 0
Fax: (+49) 89 6513 1160

Greece
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SIGMA-ALDRICH Kft
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Ingyenes fax szám: 06 80 344 344
Tel: (+36) 1 235 9055
Fax: (+36) 1 235 9050

India
SIGMA-ALDRICH CHEMICALS
PRIVATE LIMITED
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Bangalore: (+91) 80 6621 9400
New Delhi: (+91) 11 4358 8000
Mumbai: (+91) 22 2570 2364
Hyderabad: (+91) 40 4015 5488
Kolkata: (+91) 33 4013 8003
Fax
Bangalore: (+91) 80 6621 9650
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SIGMA-ALDRICH JAPAN K.K.
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Fax: (+81) 3 5796 7315

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SIGMA-ALDRICH KOREA
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Fax: (+82) 31 329 9090

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Fax: (+60) 3 5635 4116

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Free Tel: 0800 022 9088
Free Fax: 0800 022 9089
Tel: (+31) 78 620 5411
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Fax: (+61) 2 9841 0500

Norway
SIGMA-ALDRICH NORWAY AS
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Fax: (+47) 23 17 60 10

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SIGMA-ALDRICH Sp. z o.o.
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Fax: (+48) 61 829 01 20

Portugal
SIGMA-ALDRICH QUÍMICA, S.A.
Free Tel: 800 202 180
Free Fax: 800 202 178
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Fax: (+351) 21 924 2610

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Tel: (+7) 495 621 5579
Fax: (+7) 495 621 5923

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SIGMA-ALDRICH PTE. LTD.
Tel: (+65) 6779 1200
Fax: (+65) 6779 1822

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SIGMA-ALDRICH spol. s r. o.
Tel: (+421) 255 571 562
Fax: (+421) 255 571 564

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SIGMA-ALDRICH (PTY) LTD.
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Fax: (+27) 11 979 1119

Spain
SIGMA-ALDRICH QUÍMICA, S.A.
Free Tel: 900 101 376
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Tel: (+34) 91 661 99 77
Fax: (+34) 91 661 96 42

Sweden
SIGMA-ALDRICH SWEDEN AB
Tel: (+46) 8 742 4200
Fax: (+46) 8 742 4243

Switzerland
SIGMA-ALDRICH CHEMIE GmbH
Free Tel: 0800 80 00 80
Free Fax: 0800 80 00 81
Tel: (+41) 81 755 2828
Fax: (+41) 81 755 2815

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SIGMA-ALDRICH COMPANY LTD.
Free Tel: 0800 717 181
Free Fax: 0800 378 785
Tel: (+44) 1747 833 000
Fax: (+44) 1747 833 313

United States
SIGMA-ALDRICH
Toll-Free: 800 325 3010
Toll-Free Fax: 800 325 5052
Tel: (+1) 314 771 5765
Fax: (+1) 314 771 5757

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