



SepCheck™

Reliability

Speed

Easy

The sepsis syndrome is one of the leading causes of death in hospitalized patients. Mortality rates vary between 30% and 70%. The vast majority (>90%) of cases of bacteraemia is caused by a limited number of pathogenic bacteria including:

- *Coagulase Negative Staphylococci*
- *Staphylococcus aureus*
- *Enterobacteriaceae*
- *Escherichia coli*
- *Streptococcus spp.*
- *Enterococcus spp.*
- *Klebsiella pneumoniae*
- *Pseudomonas aeruginosa*

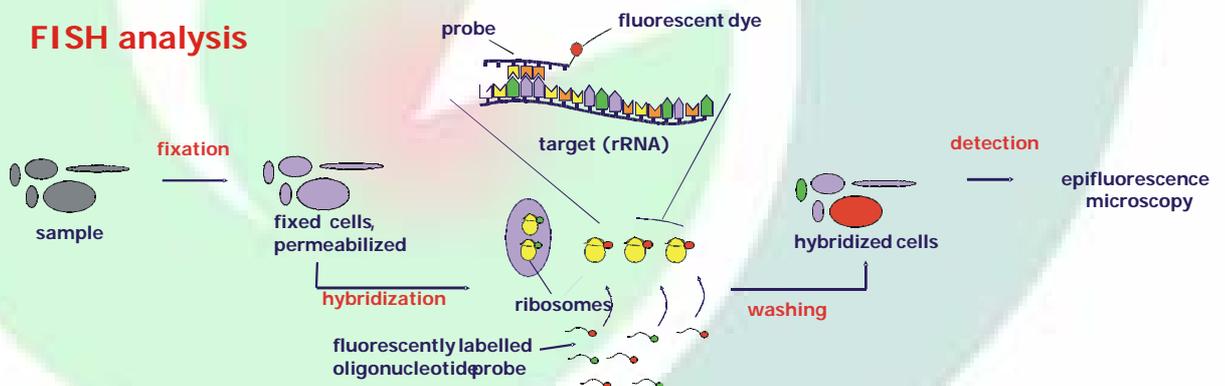
Rapid identification is essential for several reasons:

- **Rapid diagnosis, enabling the clinician to select the appropriate antibiotic.**
- **Reduction of unnecessary broad spectrum antibiotic therapy and of development of antibiotic resistance.**

A positive blood pre-culture is taken as starting point and a Gram stain will be performed. Based on the outcome of the Gram stain the bacteria are identified using standard culture techniques. Depending on the growth of the bacteria this identification will consume valuable time (days). Reduction of the identification time will enable the clinician to select the correct antibiotic therapy. New molecular diagnostic techniques have emerged which can significantly reduce the identification time. Fluorescence In Situ Hybridization (FISH) is a rapid and reliable method for identification of bacteria grown in blood cultures. The SepCheck FISH protocol enables identification of the bacteria within 60-90 minutes – depending on the type of bacterium.

FISH is useful if a polymicrobial infection is suspected or the interpretation of the Gram-stain preparation is difficult.

High speed bacterial diagnosis



SepCheck

The detection of whole-bacterial cells via the labeling of specific nucleic acids with fluorescently labeled oligonucleotide probes is called; Fluorescence In Situ Hybridization (FISH). The rRNA molecule, which exists in multiple copies in the cell (up to 10^4 - 10^5), is an excellent target for fluorescently labeled oligonucleotide probes which are directed against regions on the rRNA molecule specific for a bacterial group, genus or species. The small probes (16-20 nucleotides) cross the bacterial cell wall and hybridize with their complementary target sequence. Evaluation of the test result is done by epifluorescence microscopy.

Molecular diagnostics using FISH requires the following steps;

- 1- Pre-culture of blood sample
- 2- Testing the sample with a Gram stain
- 3- Testing the sample with a FISH probe selected according to the outcome of the Gram stain.

FISH probes are genus or species specific. The test is performed using a very simple protocol involving simple additions and centrifugation steps.

The protocol of the SepCheck FISH assay is simple and does not require complicated or expensive equipment.

Features

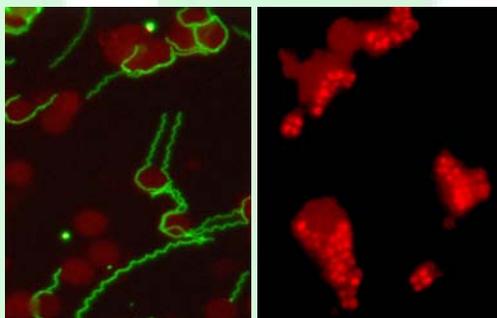
Validation

In a study*, carried out at the University Medical Centre of Groningen, The Netherlands, 182 blood samples which tested positive were processed simultaneously using FISH and accepted culturing methods. The results of the study showed that bacterial identification by whole-cell hybridization dramatically increased the speed of the diagnosis. All probes scored a sensitivity of 1.00. Moreover, it was observed that the specificities of all probes were 1.00.

Performance of the method

Application criterion	Isolated microorganism	n	Correlation coefficient
Each assay	All bacteria	182	1.00
	Negative control	182	1.00
Gram-positive cocci (chains)	<i>Streptococcus spp.</i>	20	1.00
	<i>Enterococcus faecalis</i>	10	1.00
	<i>Enterococcus faecium</i>	7	1.00
Gram-positive cocci (clusters)	<i>Staphylococcus aureus</i>	13	1.00
	Coagulase Negative Staphylococci	73	1.00
Gram-negative rods	<i>Escherichia coli</i>	23	1.00
	<i>Pseudomonas aeruginosa</i>	4	1.00
	Enterobacteriaceae	23	1.00

Using the FISH protocol, a clear-cut positive signal was obtained See (fig 1 and 2). Repeated microscopic evaluation by different observers confirmed the unambiguity of the interpretation of the images obtained by this method. The observation that all strains hybridize with the EUB-probe indicates that the hybridization protocol is applicable for FISH studies with the bacterial species and genera tested in this study. The negative results obtained with the non-EUB-probe indicate the absence of specific interaction between the probe and constituents of the cellular matrix. The speed of the diagnosis (after the blood sample was positive) varied between 25 min (*streptococci/enterococci*) and 2h (*staphylococci*), while routine bacteriological determination would take at least 24h to 48h. Although the species or genus name of a pathogen yields only indirect information on the expected antibiotic sensitivity, this information may be applied by the clinician in order to narrow down the scope of applicable antibiotics. Antimicrobial treatment can be adjusted soon after a positive blood culture e.g. due to the rapid discrimination between *Staphylococcus aureus* and coagulase-negative staphylococci or between *Pseudomonas aeruginosa* and Enterobacteriaceae. Unnecessary broad empirical antimicrobial therapy or inadequate treatment is prevented.



Streptococcus spp.

Fig. 1

Staphylococcus aureus

Fig. 2

Testkit components

- Comprehensive and easy to follow protocol
- Positive and Negative control probes

Ordering information (10 tests per probe)

Cat. No

- 10-MC-B001 *Staphylococcus aureus*
- 10-MC-B002 Coagulase Negative Staphylococcus
- 10-MC-B003 *Staphylococcus aureus* and Coagulase Negative Staphylococcus
- 10-MC-B004 *Streptococcus spp.*, *Enterococcus faecium* and *Enterococcus faecalis*
- 10-MC-B005 *Escherichia coli*, *Pseudomonas aeruginosa* and Enterobacteriaceae

* - G.J. Jansen, M. Mooibroek, J. Idema, H.J.M. Harmsen, G.W. Welling, J.E. Degener. 2000.

Rapid Identification of Bacteria in Blood Cultures by Using Fluorescently Labeled Oligonucleotide Probes.

J. Clin. Microbiol. **38**:814-817.

- unpublished information by G.W. Welling, Department of Medical Microbiology, UMCG, Groningen, the Netherlands.

Manufactured by:

BioVisible BV

Microbial Diagnostics

L.J. Zielstraweg 1

9713 GX Groningen

The Netherlands

Tel. +31 (0)50 526 07 08

Fax. +31 (0)50 526 07 11

E-mail: info@biovisible.com

