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A New User Friendly and Rapid Molecular Test

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Publication date:
2012

Document Version
Early version, also known as pre-print

Link to publication from Aalborg University

Citation for published version (APA):
Detection of Bacterial Gene Expression by mRNA PNA FISH – A New User Friendly and Rapid Molecular Test

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Introduction
• Fluorescence in situ hybridization (FISH) is primarily used for microbial species identification.
• Bacterial mRNA molecules which hold information on expressed functional genes e.g. antibiotic resistance have a much shorter half-life (often only few minutes) than rRNA and are generally much less abundant (1% or less of rRNA abundance).
• Therefore, so far it has only been possible to target and visualize microbial mRNA using very laborious protocols.

Objective
The aim was to develop a PNA FISH protocol to actually visualize the mRNA molecules responsible for the antibiotic resistance (mecA) in methicillin resistant Staphylococcus aureus (MRSA) in an end-user friendly and rapid assay format (2 hrs) and combine this with species identity.

Methods

**Induction in tube – 30 minutes**
Add blood culture from ventilation needle to the tube
Add pre-mixed cefoxitin/TSB solution to the tube. Incubate at 35 ± 2°C for 40 min.

**Fixation – 10 minutes**
Add 10 µl of induced blood culture to the well on the microscope slide.
Add fixation solution to each well on microscope slide and mix gently to emulsify.
Heat slides at minimum 80°C for 2 min. and transfer slides to PNA FISH Workstation pre-heated to 55°C ± 1°C.
Add 100 µl (or fill slides well) 100% MeOH to the slide well. Incubate slides for 5 min. on PNA FISH Workstation at 55°C ± 1°C .

**Hybridization – 30 min.**
Same as current PNA FISH

Rinse, Stringent Wash, Mount and Examination – 35-40 min.
Same as current PNA FISH for Gram-negative rods.

**Validation**
As a benchmark for the mRNA PNA FISH assay reverse transcriptase quantitative PCR (RT-qPCR) measurements of both 16S rRNA and mecA mRNA were used.

Results
• The mecA PNA FISH assay was positive for all strains that exhibited baseline values above 10-15 mecA copies/10.000 16S rRNA copies.
• The assay showed 100% (13/13) sensitivity and 100% specificity (14/14) for identification of MRSA directly from S. aureus-positive blood culture bottles.

Conclusion
• The mRNA PNA FISH technique is a new molecular test that provides a phenotypic antibiotic resistance answer.
• Great potential for coupling species identity to expression of selected genes on single cell level.
• Can shed light on heterogeneities in gene expression for instance in complex and stratified microbial systems.
• The method has great potential in different clinical applications but also in industrial and environmental settings.

Acknowledgements
This work was supported by the Ministry of Science Technology and Innovation. We thank Masumeh Chavoshi, Helle Andersen, Allan Mortensen, Berit Kummerfeldt for valuable technical assistance.

Keywords: PNA FISH, mRNA, mecA, rRNA, MRSA, MSSA, antibiotic resistance, in situ hybridization, measurement of gene expression.