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Detection of Bacterial Gene Expression by mRNA PNA FISH – A New User Friendly and Rapid Molecular Test

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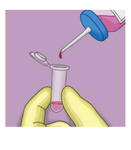
Introduction

- 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

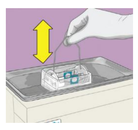
The aim was to develop a PNA FISH protocol to actually visualize the mRNA molecules responsible for the antibiotic resistance (*mecA*) in methicillin resistant *Staphylococcus aureus* (MRSA) in an end-user friendly and rapid assay format (2 hrs) and combine this with species identity.

Methods



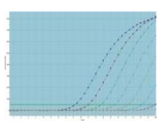
Induction in tube – 30 minutes
Add blood culture from ventilation needle to the tube
Add pre-mixed cefoxitin/TSB solution to the tube.
Incubate at 35±2°C for 40 min.

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

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- 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.

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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.

Results

Fig 1: Uninduced MRSA show strain dependent *mecA* expression in PNA FISH.
Red (negative): Strains that do not express the *mecA* gene without induction.
Green (positive): Strains that express the *mecA* gene constitutively.
Blue (neg/pos): Strains that both show individual cells with and without *mecA* expression.
All strains were *mecA* positive upon induction with the antibiotic cefoxitin.

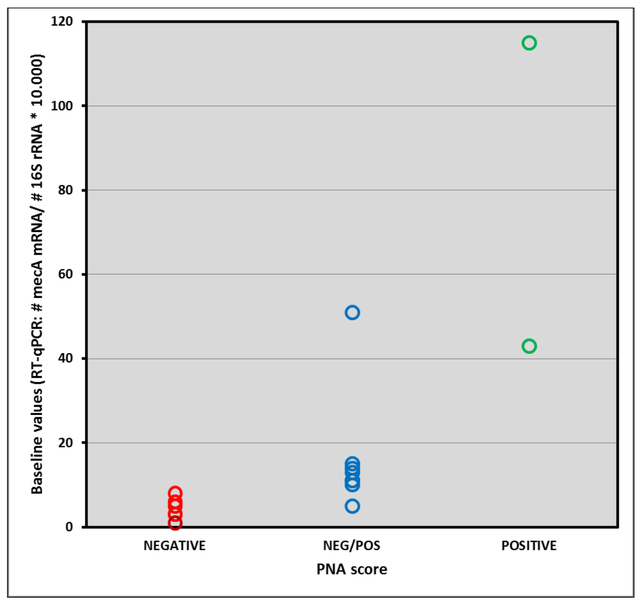

Fig 2: Simultaneous determination of species and gene expression. The 16S rRNA of *S. aureus* was targeted with ~~a~~ red probes and *mecA* mRNA with green probes.

Panel A: *mecA* mRNA in a sample with only methicillin resistant *S. aureus* (MRSA).

Panel B: Simultaneous detection ~~o~~in a mixture of MRSA and methicillin susceptible *S. aureus* (MSSA) using both probes. MRSA appear yellow, because MRSA bind both the red probes for *S. aureus* and the green probes for *mecA*, while MSSA appear red, because these only bind the red species identification probe.

Panel C: Both types of probes are applied, but in this instance on a mixture of MRSA (yellow) and methicillin resistant coagulase negative staphylococci (green). The latter expresses the *mecA*-gene (green probe) but because they are not *S. aureus* they do not bind the red species identification probe (16S rRNA).