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

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**AdvanDx**

## 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.

## 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.

## 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.

## Results

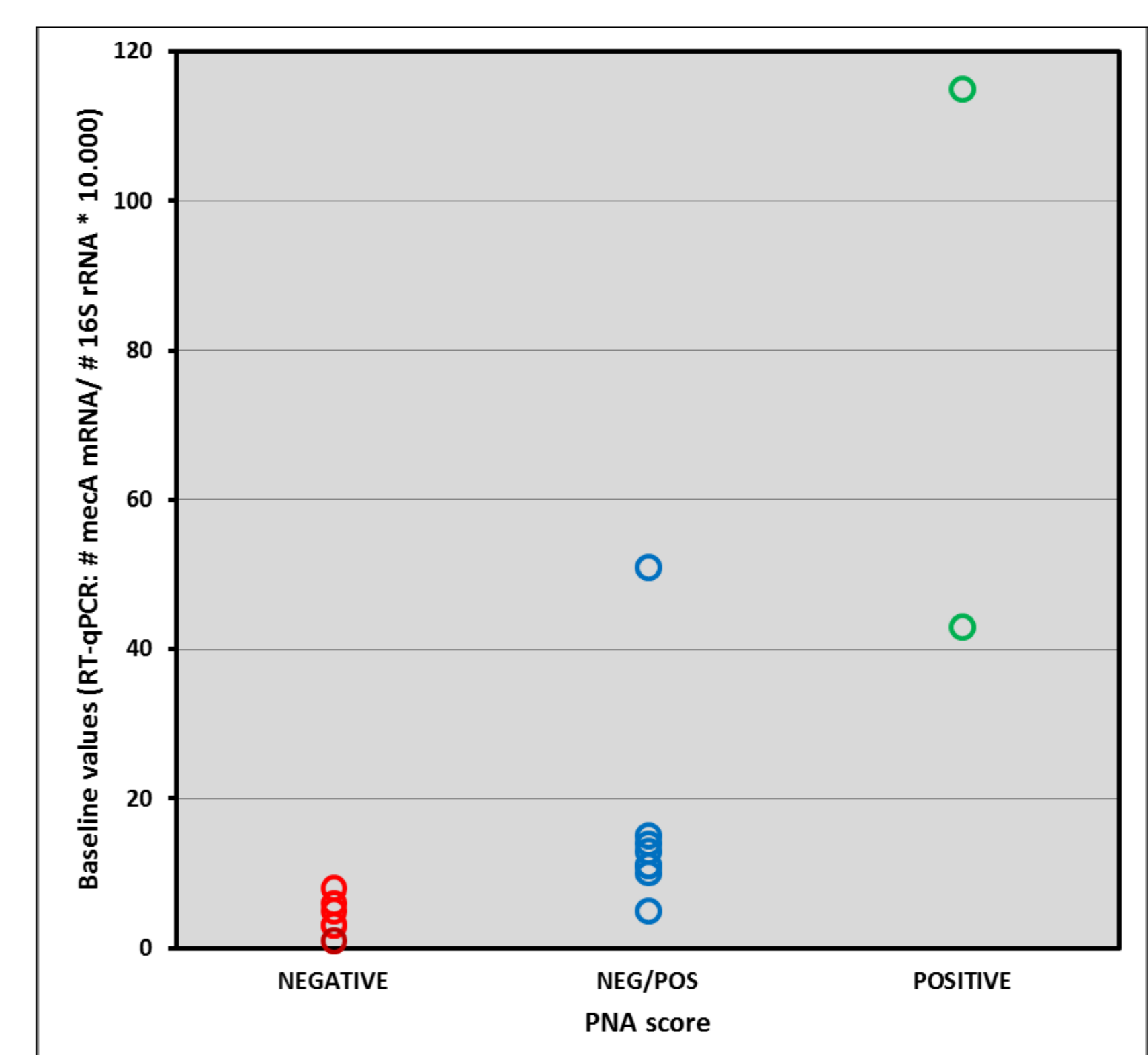
Fig 1: Uninduced MRSA show strain dependent *mecA* expression in PNA FISH.

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 ceftiofur.



## Methods



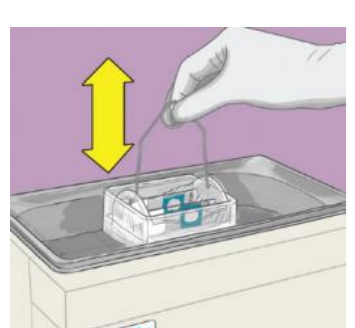
### Induction in tube – 30 minutes

Add blood culture from ventilation needle to the tube  
Add pre-mixed ceftiofur/TSB solution to the tube.  
Incubate at  $35 \pm 2^\circ\text{C}$  for 40 min.



### Fixation – 10 minutes

Add 10  $\mu\text{L}$  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  $80^\circ\text{C}$  for 2 min. and transfer slides to PNA FISH Workstation pre-heated to  $55^\circ\text{C} \pm 1^\circ\text{C}$ .  
Add 100  $\mu\text{L}$  (or fill slides well) 100% MeOH to the slide well.  
Incubate slides for 5 min. on PNA FISH Workstation at  $55^\circ\text{C} \pm 1^\circ\text{C}$ .

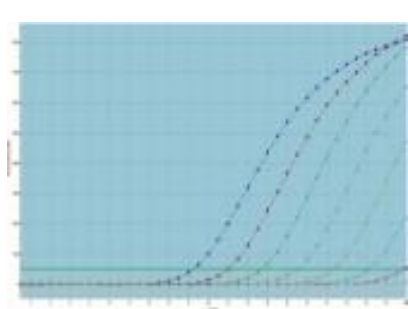


### Hybridization – 30 min.

Same as current PNA FISH

### Rinse, Stringent Wash, Mount and Examination – 35-40 min.

Same as current PNA FISH for Gram-negative rods.



### Validation

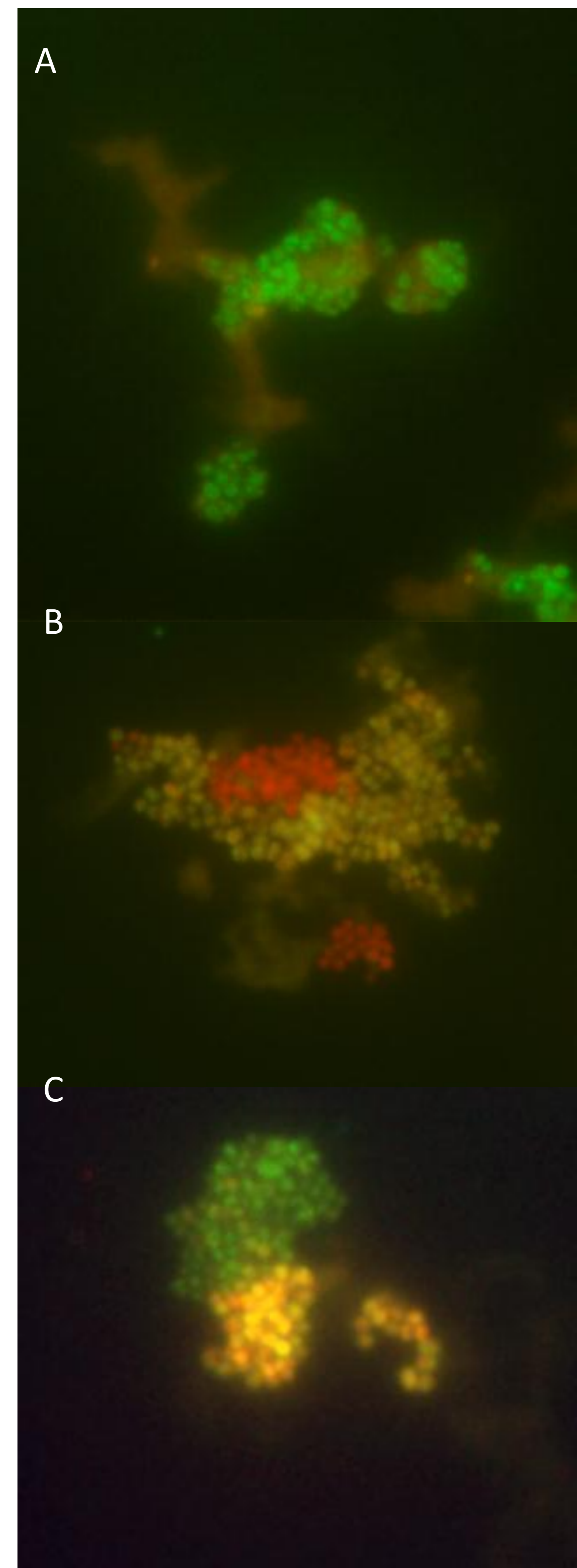
As a benchmark for the mRNA PNA FISH assay reverse transcriptase quantitative PCR (RT-qPCR) measurements of both 16S rRNA and *mecA* mRNA were used.

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 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).



## 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.

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