Development of diagnostic kits for selected markers of resistance, virulence and zoonotic transmission among methicillin-resistant *Staphylococcus aureus* strains

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   b) NALFIA
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Q-Bioanalytic GmbH

Existing portfolio

More than 20 Real-Time PCR kits
for food safety and quality testing

Selection of Real-Time PCR Kits

1. QuickBlue MRSA
2. OneCup *Salmonella*
3. OneCup *Listeria* spec.
4. QuickBlue *Listeria monocytogenes*
5. QuickBlue *Staphylococcus aureus*
6. QuickBlue *Cronobacter sakazakii*
7. QuickBlue *Clostridium perfringens*
8. QuickBlue *E. coli*
9. QuickBlue *Campylobacter jejuni*
10. QuickBlue *E. coli*, EHEC, EPEC, EIEC, *Shigella*
11. QuickBlue EHEC (stx1, stx2, eaeA)
12. QuickBlue *Vibrio vulnificus*
13. QuickBlue *Vibrio parahaemolyticus*
14. QuickBlue *Vibrio cholerae*
15. QuickBlue *Vibrio alginolyticus*
16. QuickBlue *Legionella pneumophila*
17. QuickBlue *Legionella spec*.
18. QuickBlue *Pseudomonas aeruginosa*

Q-Bioanalytic GmbH

Existing portfolio in medical microbiology

IVD CE marked Real-Time PCR for MRSA

DNA Purification kit based on magnetic nano-particles

Q-Bioanalytic GmbH

Tasks:

- Development of PCR-based test kits using NALFIA and Real-Time PCR for rapid multianalyte diagnosis of resistance determinants

  a) *mecA* and new *mecC* homologues

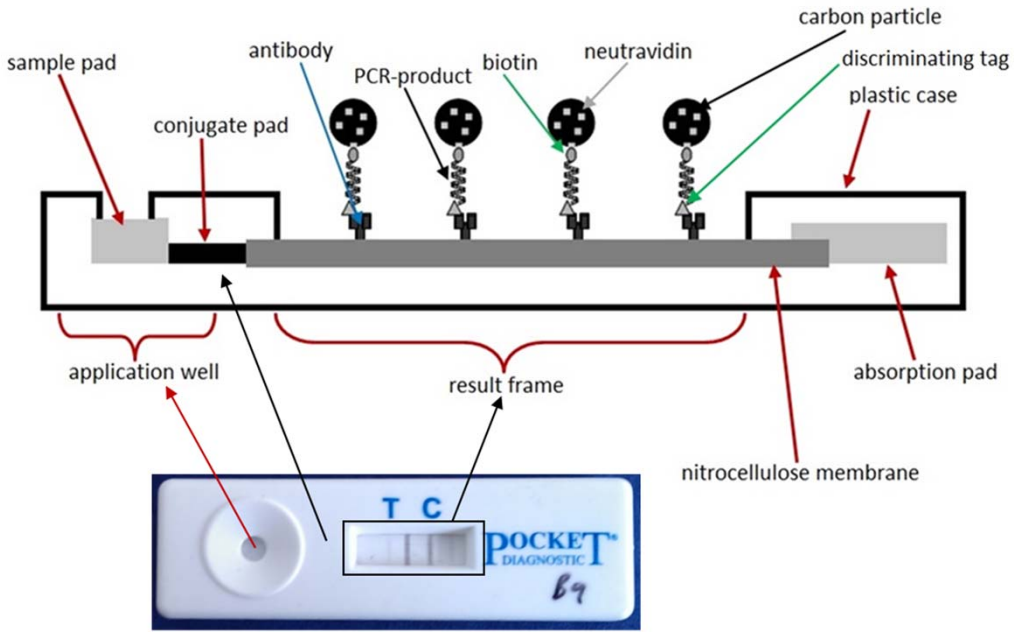
  b) Differentiate between human and livestock-related MRSA lineages such as CC398

  c) Typical relevant virulence or resistance markers

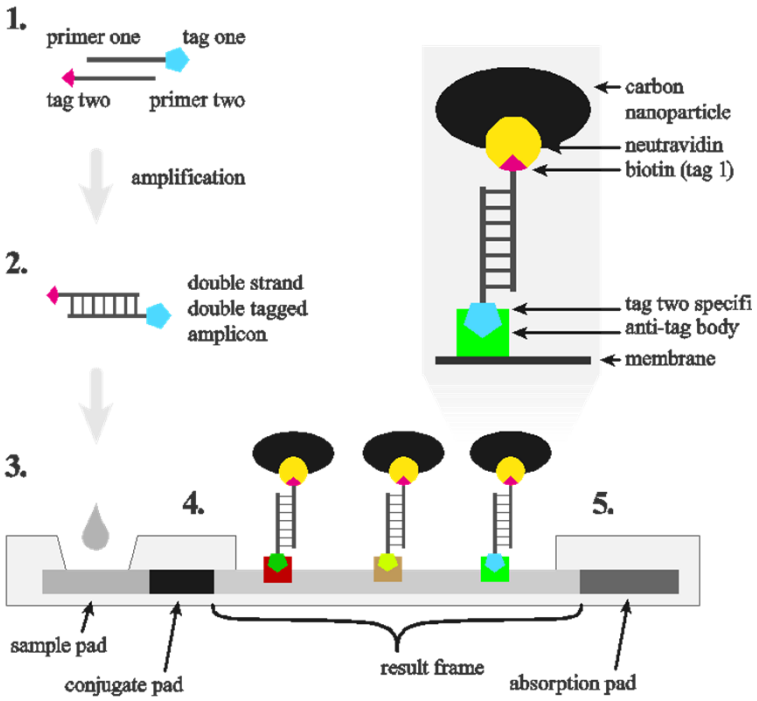
NALFIA

„Nucleic acid lateral flow immuno-assay (NALFIA)“ =

Combining molecular biological principle of detection with immunochemical principle of visualization



NALFIA


1.
primer one
tag one
tag two
primer two
amplification
2.
double strand
double tagged
amplicon
carbon
nanoparticle
neutravidin
biotin (tag 1)
tag two specifi
anti-tag body
membrane
3.
4.
5.
sample pad
conjugate pad
result frame
absorption pad

Why NALFIA?

1. Bringing PCR to environments outside of labs and to Point-of-Care applications
2. Bringing the analytical knowledge to Point-of-Care users through smartphone applications

MedVet-Staph

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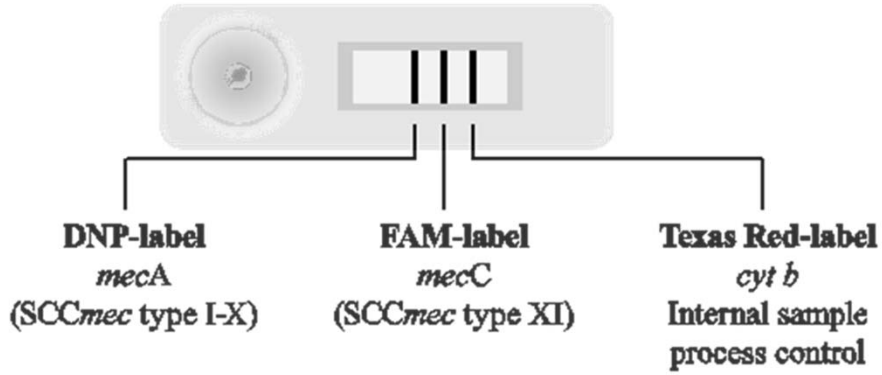
Developments

1. NALFIA test addressing *mecA*, *mecC* and cyt *b*

Detection of *mecA*, *mecC* gene and cyt *b* as internal amplification control (IAC) using NALFIA.

Detection limit: SCC*mec* VI (*mecA*) 1.5 pg, 10-100 cfu
SCC*mec* XI (*mecC*), 15 pg, 100-1000 cfu



Seidel et al. "Development of a nucleic acid lateral flow immunoassay (NALFIA) for reliable, simple and rapid detection of the methicillin resistance genes *mecA* and *mecC*." *Veterinary Microbiology (2016)*.

DNA purification and optimization of sampling

Risk assessment concerning limit of detection in clinical samples revealed:

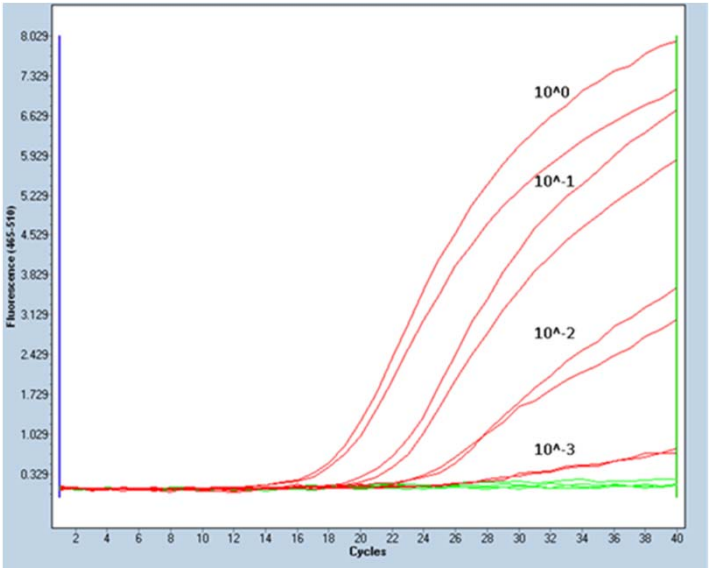
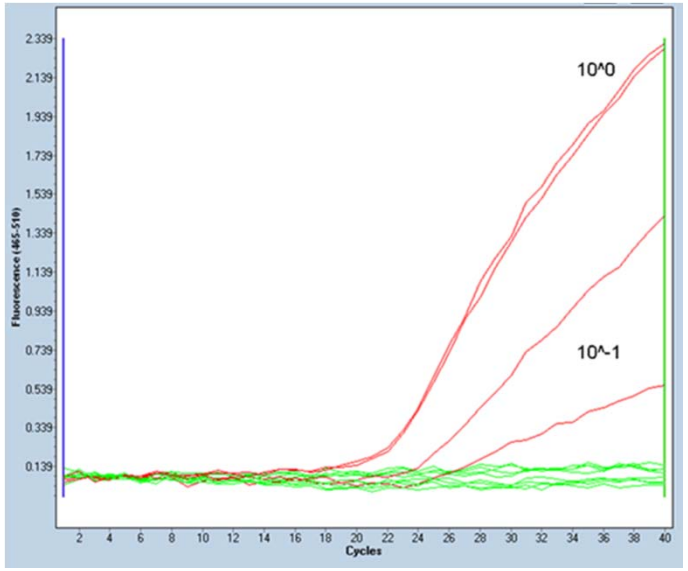
- Insufficient DNA purification can lead to false negative results
- Sampling with certain swabs can lead to insufficient release of the material into the purification reagents
- Time of analysis is a critical factor for acceptance of the methods in clinical settings

Conclusion:

- DNA extraction and/or sampling have to be optimized

Developments

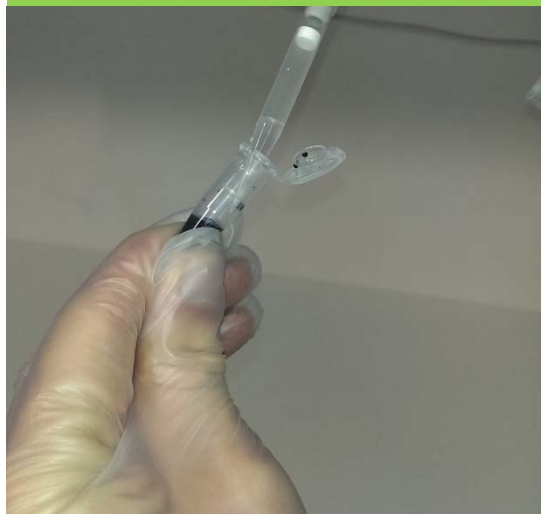
Performance of prior existing DNA purification methods

	QuickBlue DNA extraction kit (QBA):	Extraction using spin column technology :
		
LoD:	300 cfu	3 x 10^4 cfu

Developments

2. New optimized DNA purification procedure

Step 1	Step 2	Step 3
Swab sample transferred into a tube with lysis buffer (thermal lysis)	Supernatant transferred to tube with binding buffer and silica-magnetite nanoparticles	Nano-particles are magnetically immobilized and re-suspended in 100µL elution buffer

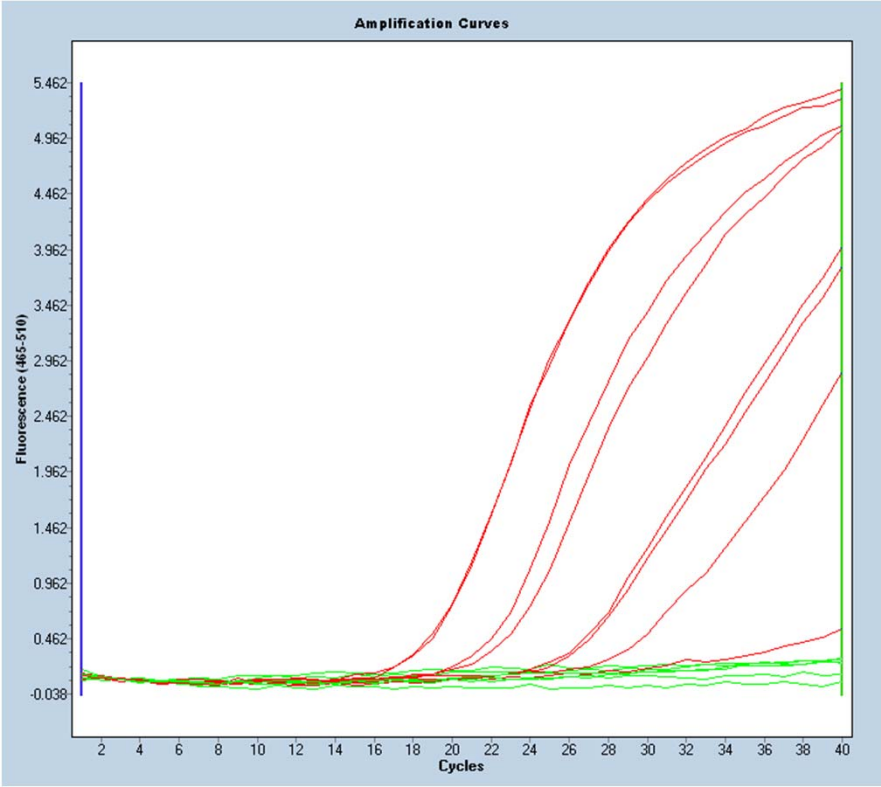


Purification time max 30 min

Developments:

2. New DNA purification

Optimized QuickBlue DNA extraction kit (QBA)



15000 cfu

1500 cfu

150 cfu

15 cfu

Optimization of sampling

Comparison of detection efficiency by Real-Time PCR

Classiq Swabs by COPAN:
Tip wrapped with traditional Polyester fiber.

Flocked Swabs by COPAN:
FLOQSwabs

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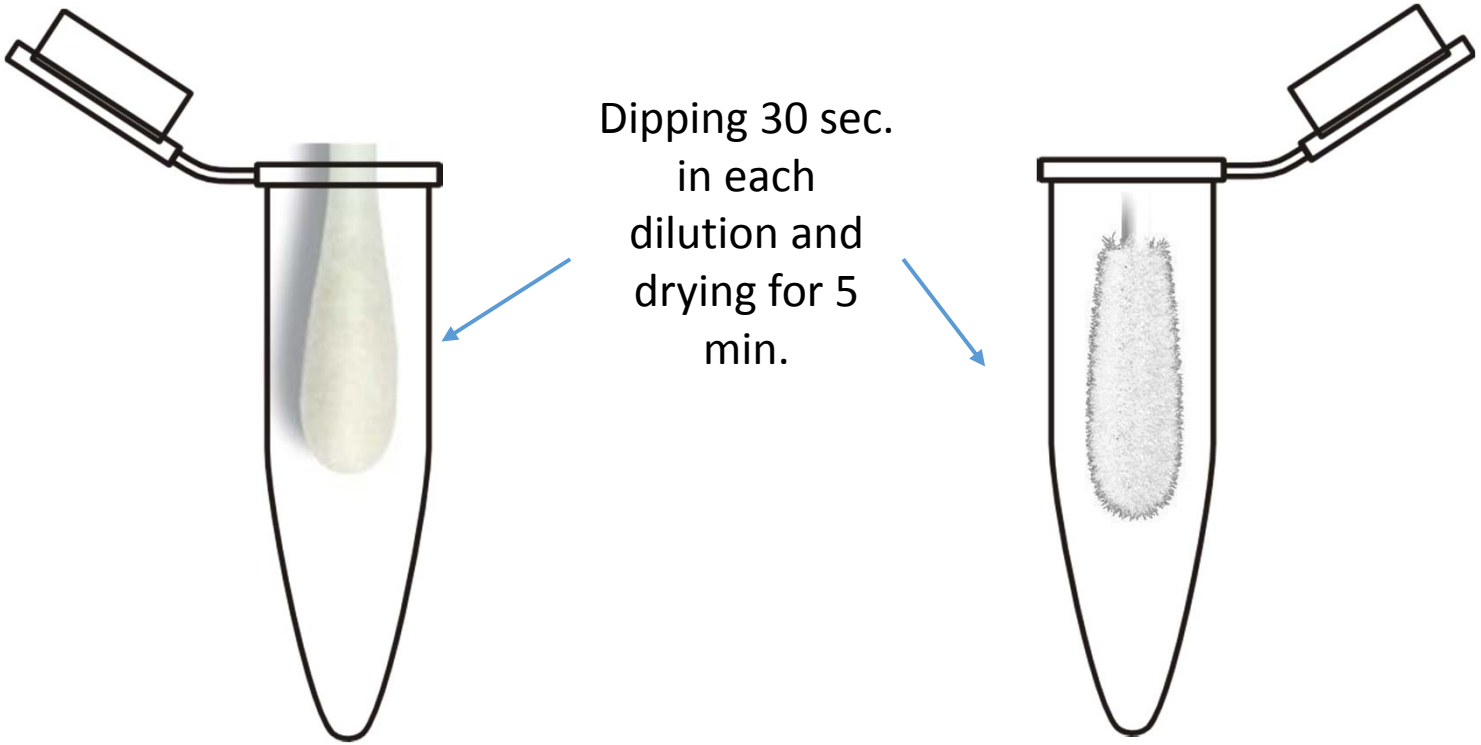
Optimization of sampling,

Testing by dipping swabs in a decadal dilution series. A MRSA strain was grown 48hrs in Giolitti broth. Subsequently the culture was diluted and swabs were dipped into it in the presence and absence of 30µl blood.

Classiq Swabs by COPAN:

Flocked Swabs by COPAN:

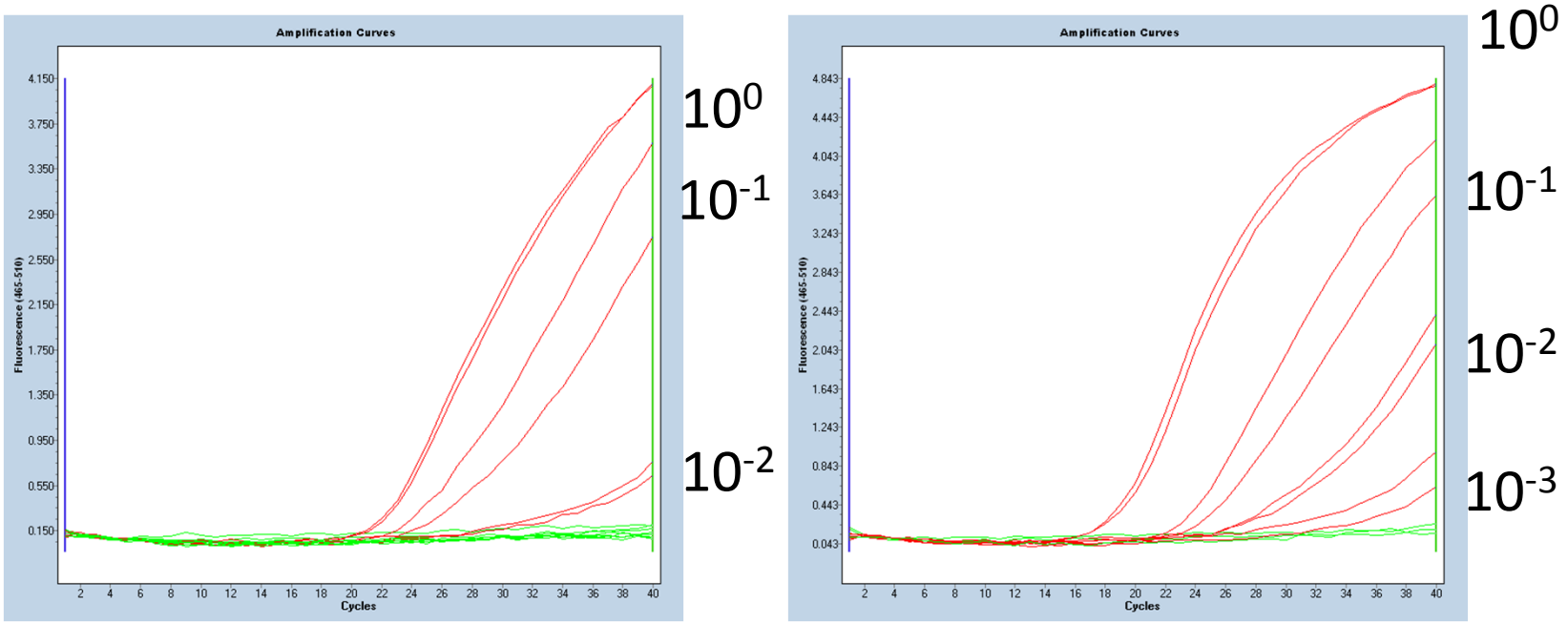


Dipping 30 sec. in each dilution and drying for 5 min.

Optimization of sampling

Classiq® Swabs by COPAN

FLOQSwabs® by COPAN



Results of experiments in the presence of blood

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Results optimization of DNA purification and sampling

1. Risk of false negative results due to insufficient DNA purification could be reduced by reducing the limit of detection from 300 cfu to 15-150 cfu (30 minutes purification time)
2. Recovery rates from swabs in the presence of blood could be enhanced by an order of magnitude through application of FLOQSwabs

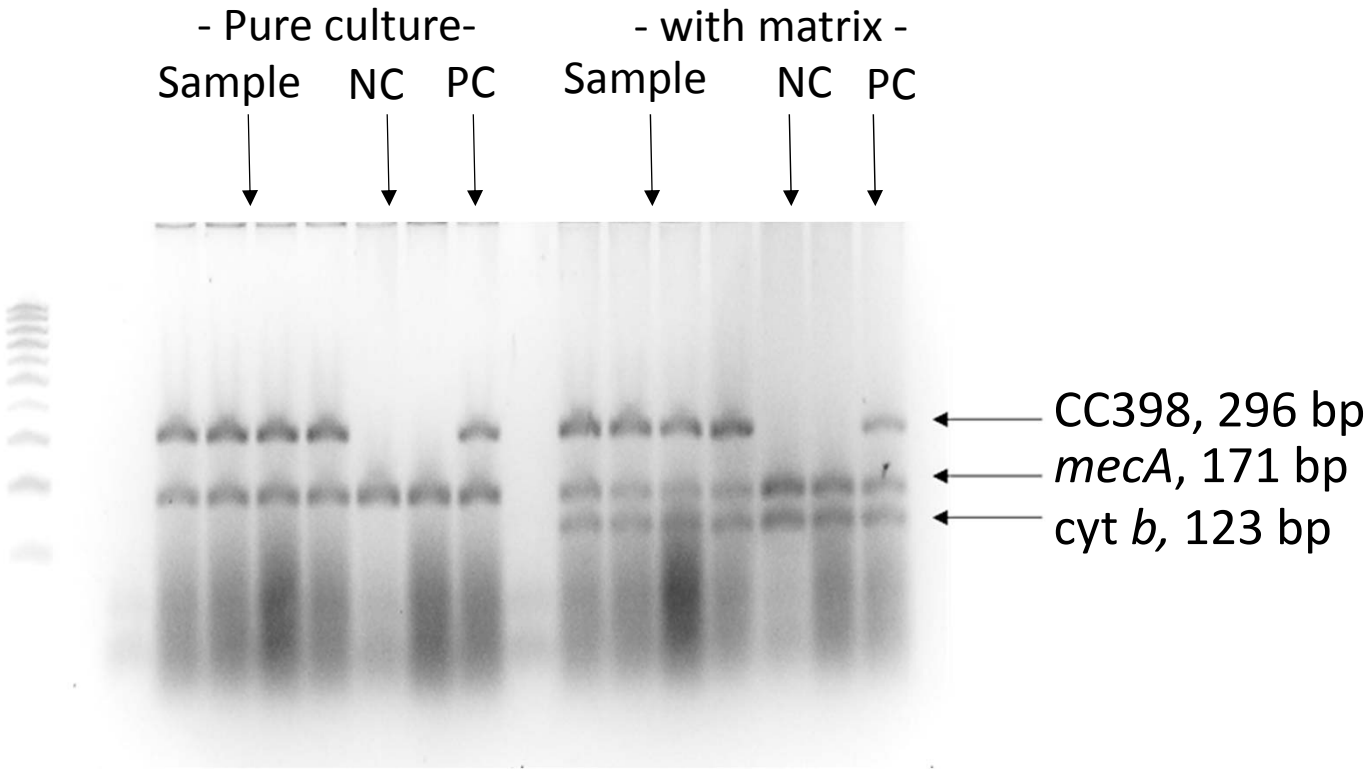
Developments

3. Triplex CC398, mecA and cyt b Real-Time PCR

- Specific primers for LA-MRSA CC398 after Stegger et al. (2011)
- Primers for the detection of mecA and cyt b
- Real-Time PCR with SybrGreen®
- Real-Time PCR with TaqMan® probe

Developments

3. Triplex CC398, mecA and cyt b Real-Time PCR



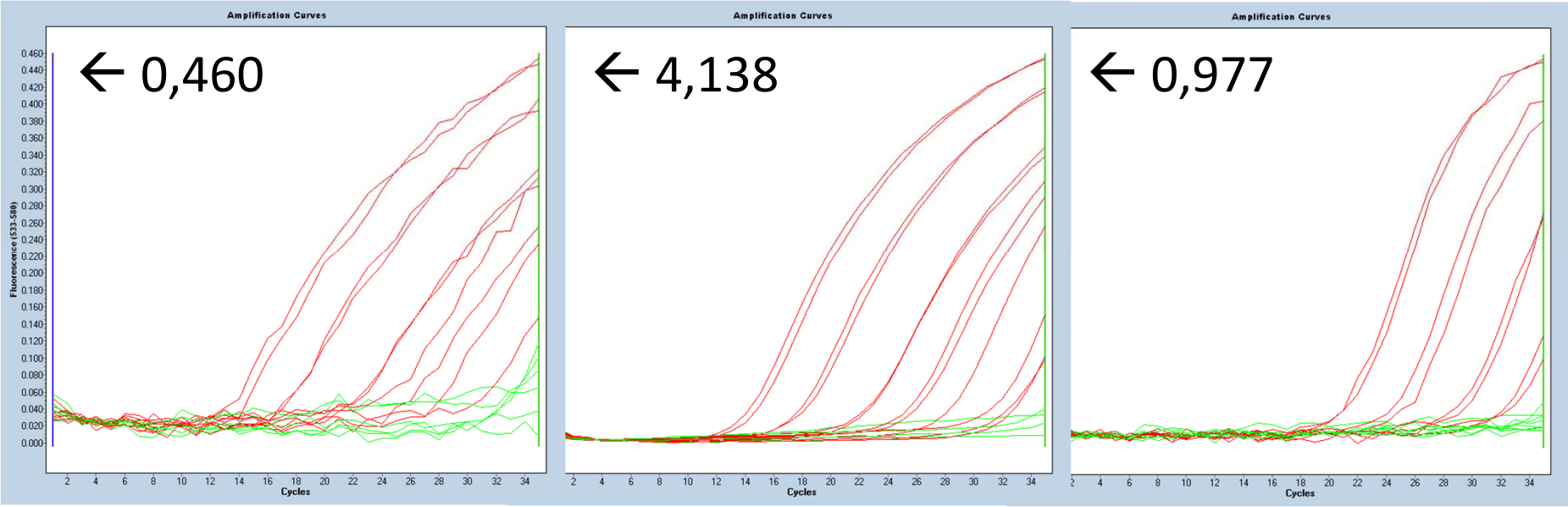
Developments

3. CC398 Real-Time PCR

Detection of cc398 in HEX channel

Detection of *mecA* in Cy5 channel

Detection of cyt *b* in FAM channel



3. Summary

Developments:

a) Multiplex test for *mecA*, *mecC* gene and cyt *b* (IAC) using NALFIA

b) Optimization of magnetic nano-particle-based purification method in combination with FLOQSwabs

c) Triplex test cc398 with Real-Time PCR including internal control

Outlook:

Validation of the CC398 Real-Time PCR

4. Outlook

- Optimization work of the triplex Real-Time PCR system for detection of cc398, *mecA*, and cyt *b*
- Validation of the Real-Time PCR for detection of CC398:
  - Sensitivity
  - Specificity
  - Limit of detection
  - Robustness
- Development of NALFIAs to detect:
  - *mecA/C*, *S. aureus* and IAC
  - other MRSA resistance genes
  - MRSA virulence genes

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