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# Screening for MRSA

## -aspects on enrichment and molecular screening

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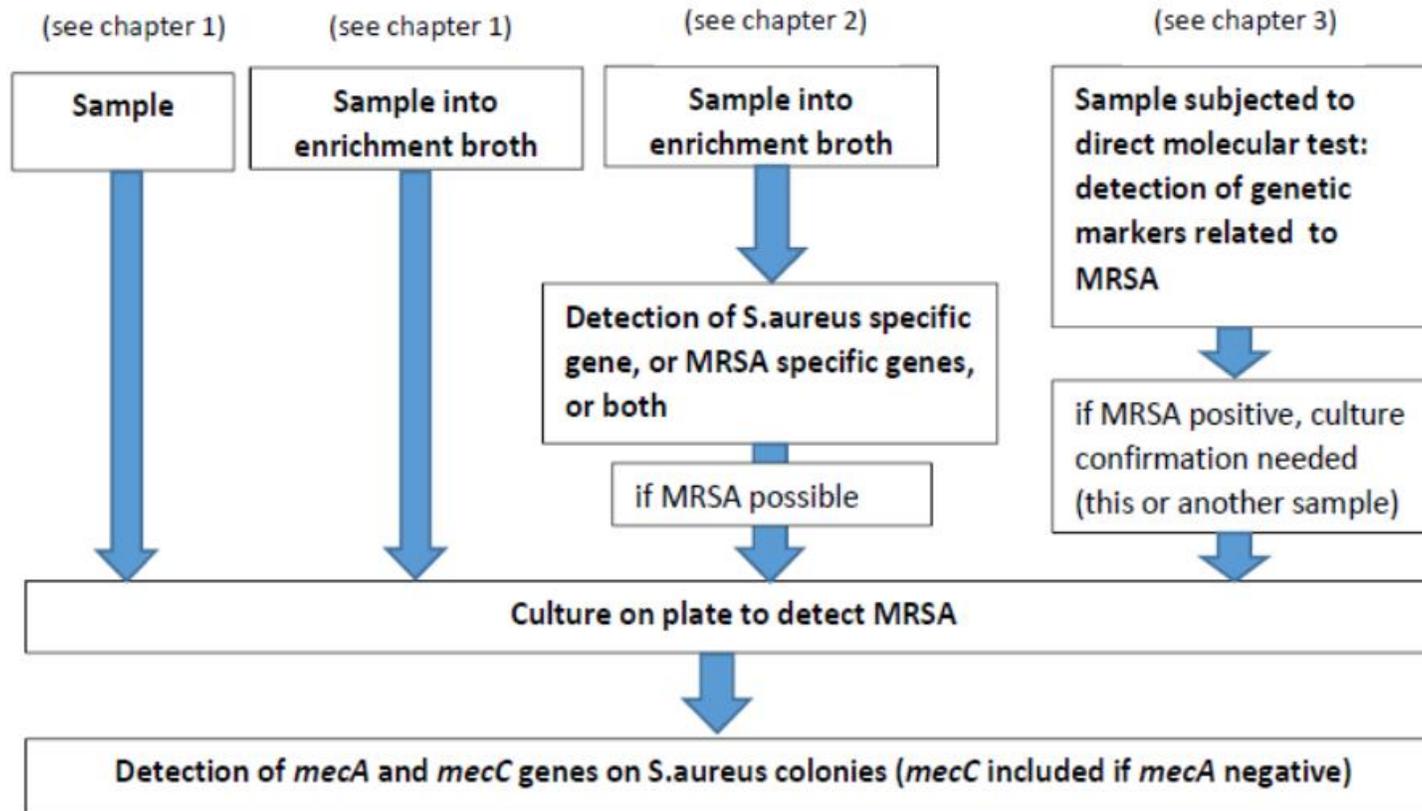
NordicAST workshop

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## Laboratory screening strategies

There are several possible approaches to MRSA screening within the laboratory. The trade-off between sensitivity and specificity varies between strategies for screening. The strategy chosen should serve the local circumstances and context for screening, for example, to produce quick negative results if needed, to ensure optimal sensitivity, or to adjust the process to the amount of screening samples. Molecular tests with variable genetic targets (*S. aureus* specific genes/*mecA* and *mecC* genes/gene sequences located at SCC*mec* junction) are widely available commercially.



Broth enrichment improves the sensitivity of culture method by 15-20% BUT increases the turnaround time

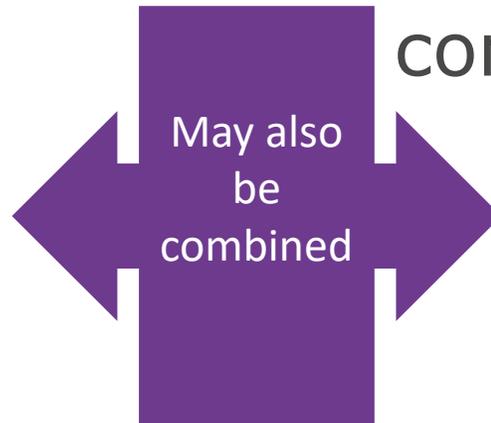
# Selection of MRSA (and MSSA) in broth

## Antibiotic-based selection

- Cefoxitin (3.5 mg/L) – aztreonam (20 mg/L)
- Only MRSA (strains tolerating the cefoxitin concentration) are enriched

## Salt-based selection

- 2.5% - 7.5% NaCl
- Both MRSA and MSSA are enriched (strains tolerating the salt concentration)



# Simplifying MRSA screening by sampling directly to the enrichment broth

Wanted (in 2017):

A transport-enrichment tube which would allow

- sampling directly to the enrichment broth
- pooling samples (nose+throat; perineum separately)
- incubation while waiting for transport (if feasible)
- use of same tube for pre-operative screening of *S. aureus* carriage

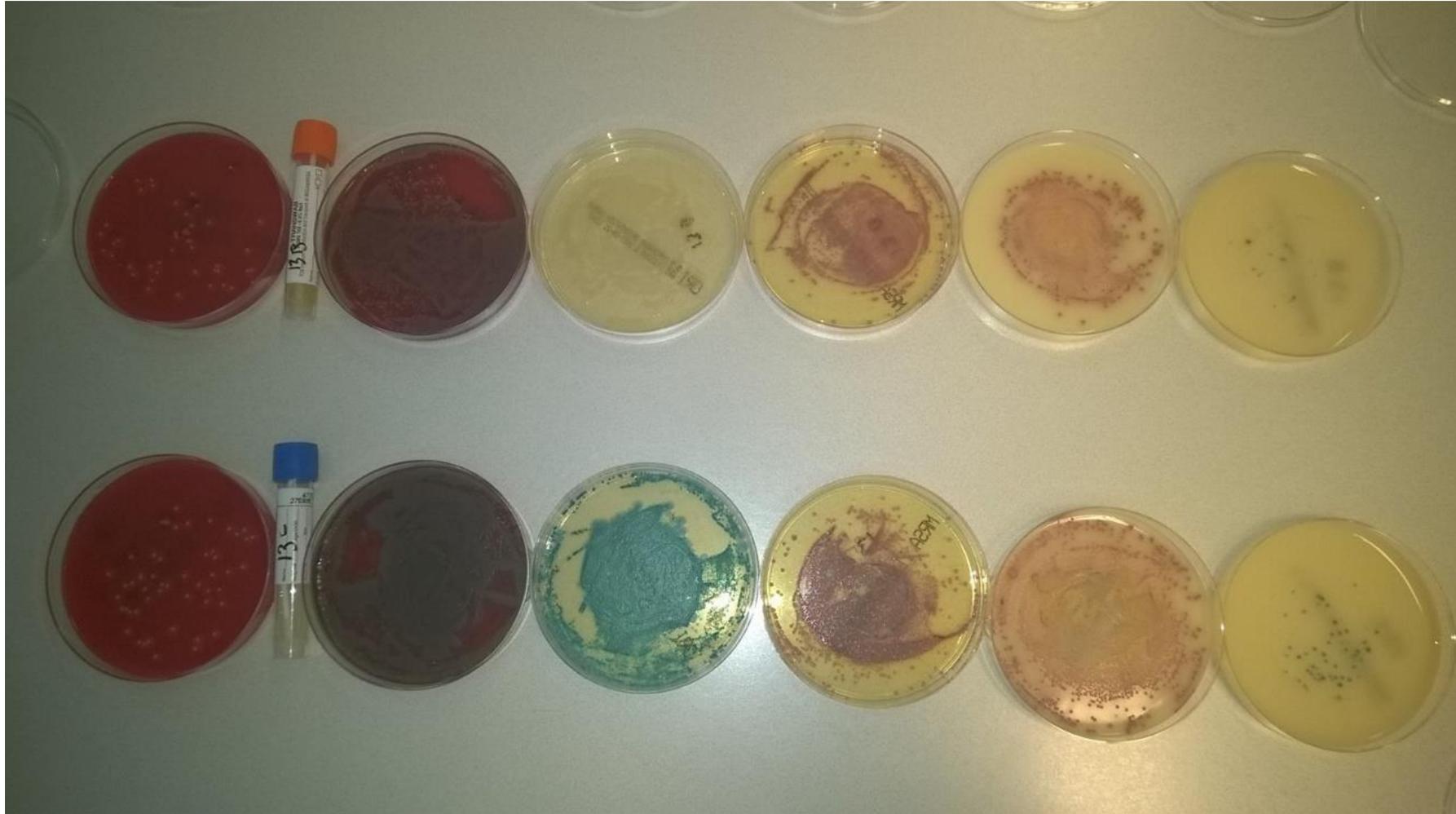


From left: Thermo Contrast MRSA (7.5% NaCl) (negative), Contrast MRSA (positive), Sigma TSB (6,5% NaCl), Copan TSB Salt (2,5% NaCl)

# Testing the broths' ability to enrich

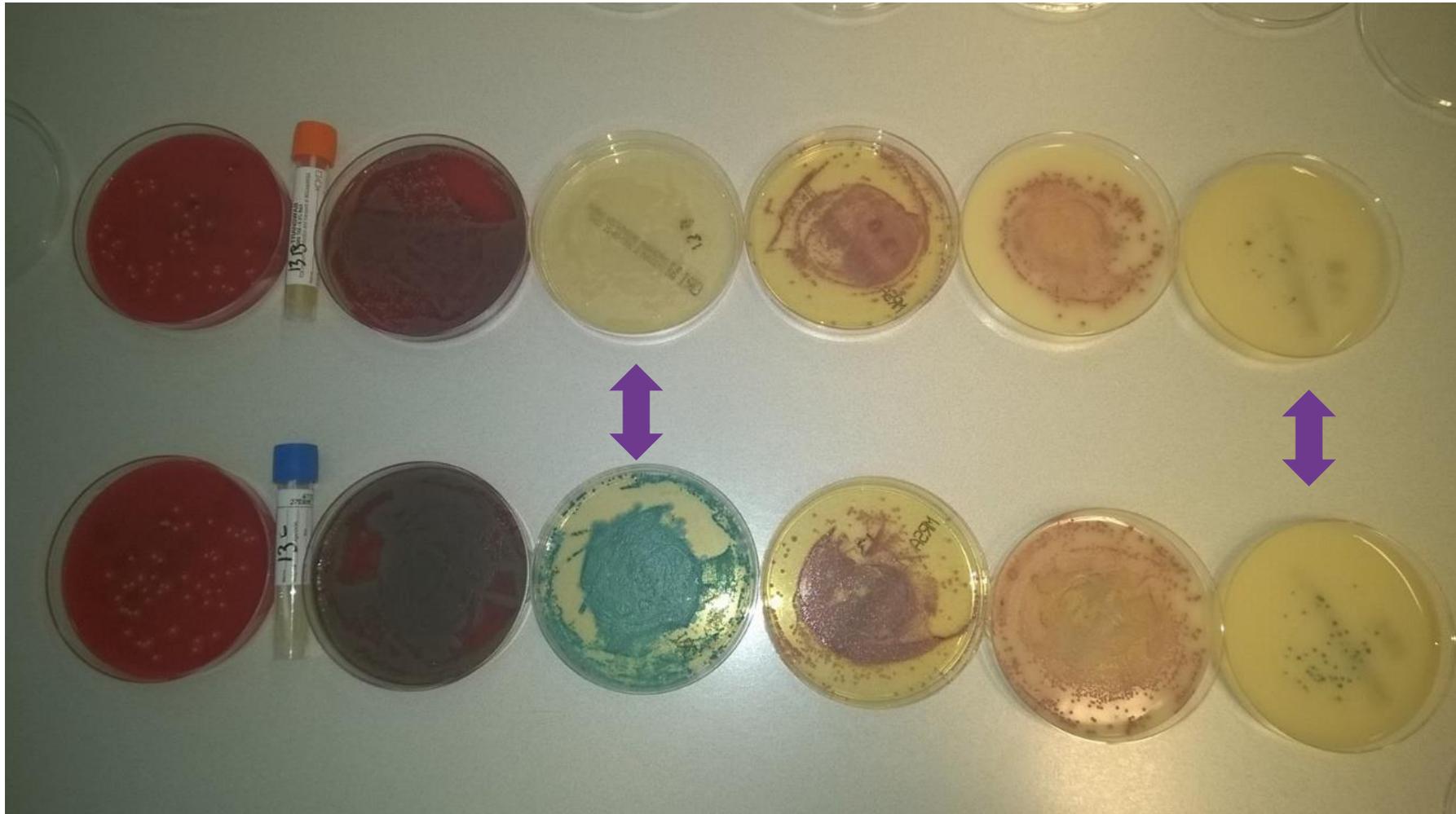
- Broths were tested with 5 MSSA strains and 16 MRSA strains (QC strains, routine strains of which some MDR HA-MRSA strains and some with low oxacillin-MIC only)
- McF 0.5 → 1:100,
  - Day 0: 10 µl to each broth tube and direct inoculation to blood agar and ChromID MRSA Smart (bioMerieux), Chromagar MRSA II (BD) and Brilliance MRSA (Thermo)
  - Day 1: 10 µl from each broth tube onto agars
  - A 100-fold increase in number of colonies after o/n enrichment expected
- Contrast MRSA failed to enrich 2/5 MSSA and 1/15 MRSA strains (and would have been difficult to be sent to and used by clients). The other two broths enriched well all tested strains.

**Also chromagars differed with regards to 1) growth of certain strains and 2) their selectivity against enriched MSSA and mixed axilla-groin flora added**



Strain #13 (MRSA): BA before enrichment, enrichment broth, BA after enrichment, Chromagar Orientation, Chromagar MRSA II, ChromID MRSA Smart and Brilliance MRSA

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# Testing was continued with some UKNeqas strains with different AST profiles (and also a shorter enrichment)

Strain	Growth on blood agar					
	Before enrichment		After 6h enrichment + 13 h/18 h on agar		After 22 h enrichment+ o/n on agar	
	Sigma TSB	TSB Salt	Sigma TSB	TSB Salt	Sigma TSB	TSB Salt
1	≈ 100	≈ 90	9 col/13 col	++	++	+++
2	>100	≈80	+	++		
3	≈ 60	≈ 40	+ /20 col	+++		
4	≈ 50	≈50	++	++		
5	≈50	≈ 50	+ /50 col	++		
6	≈ 50	≈ 50	+	±		
7	≈ 50	≈ 50	10 col	+++	++	+++
8	≈ 50	≈50	10 col	+++	++	+++
9	≈50	≈ 50	+	+++		
10	≈ 50	≈ 50	- / 2 col	+++	++	+++



Different growth at 6 hours!

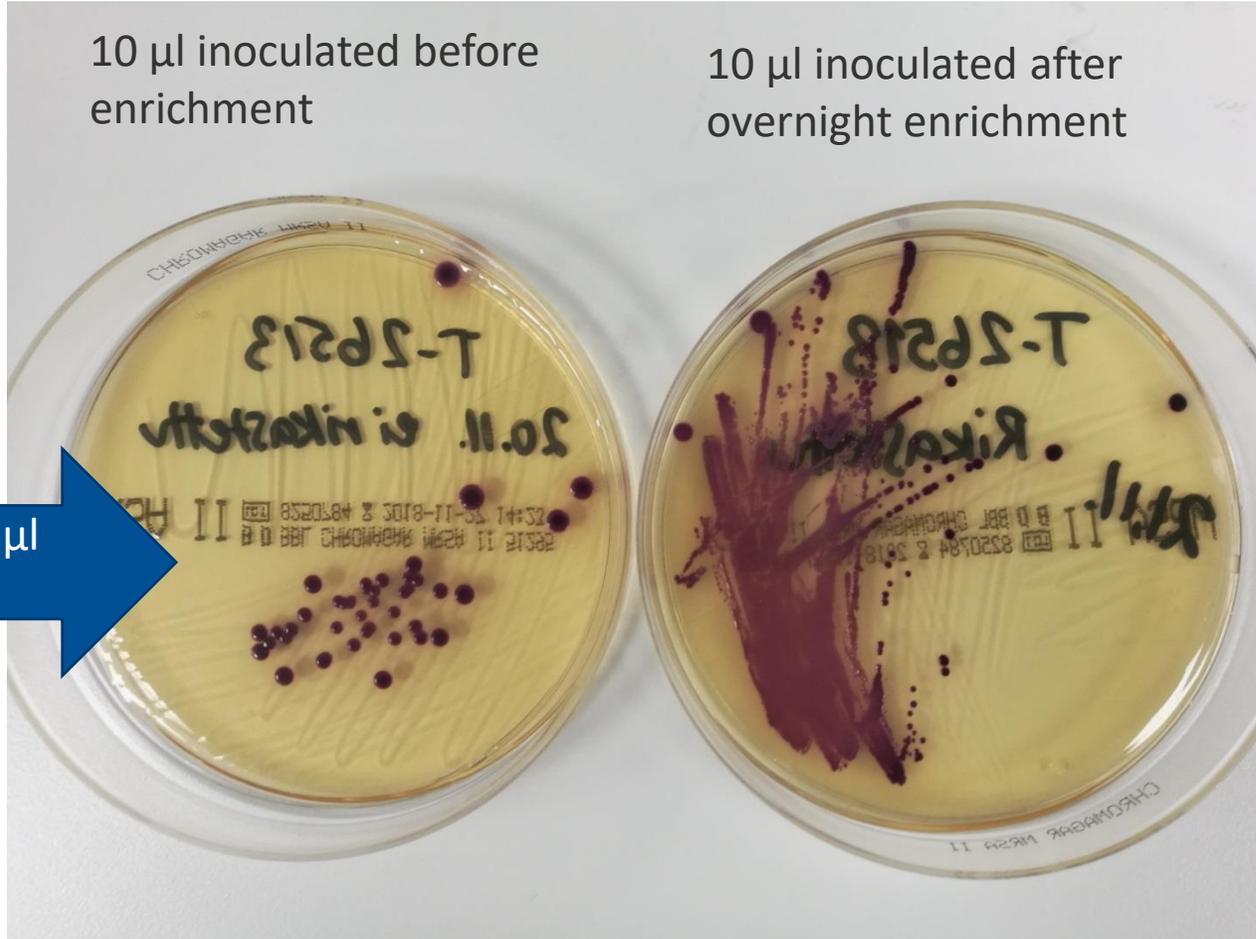
# A MRSA sample before and after enrichment



About 30 CFU/ 10  $\mu$ l

10  $\mu$ l inoculated before enrichment

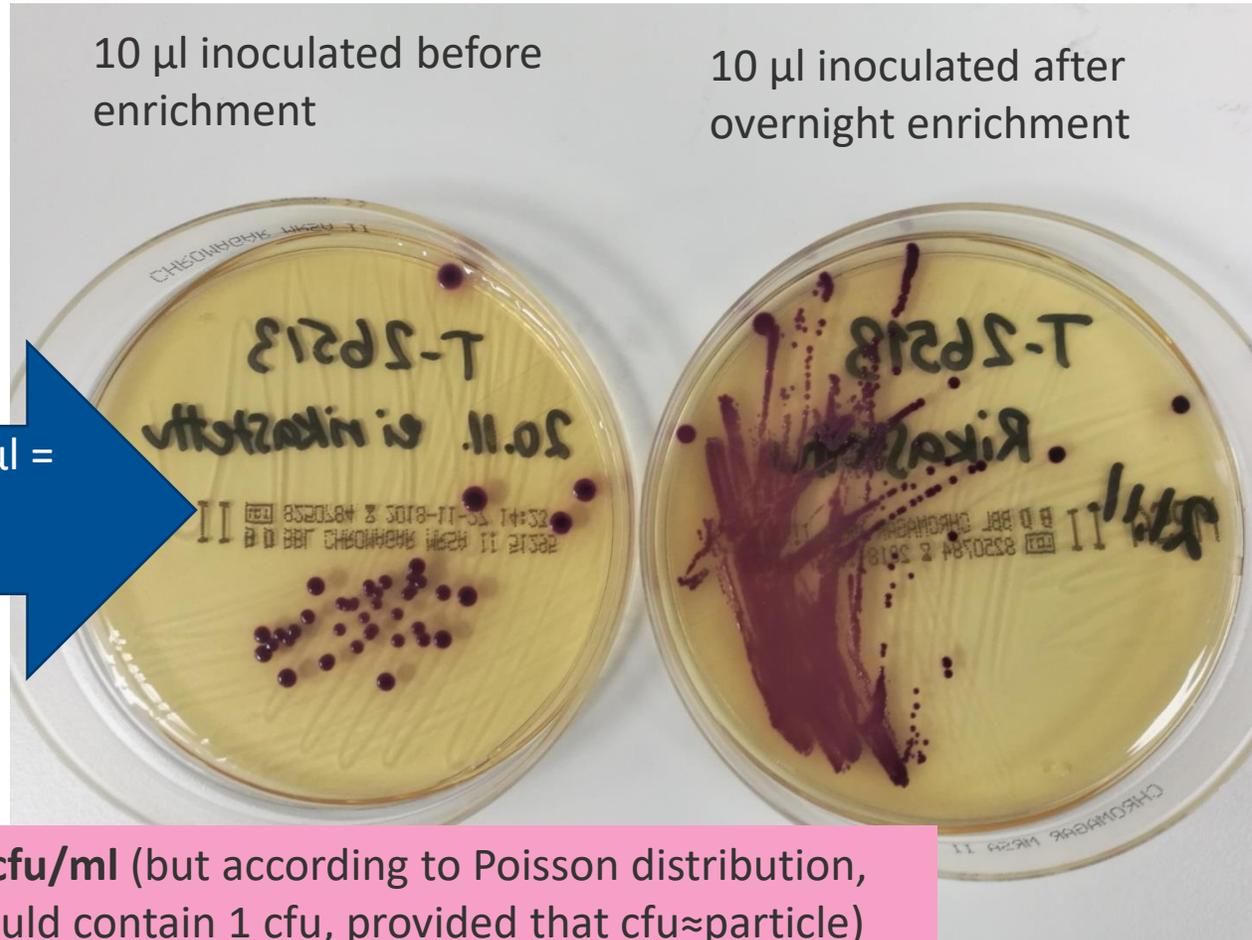
10  $\mu$ l inoculated after overnight enrichment



# A MRSA sample before and after enrichment



About 30 CFU/ 10  $\mu$ l =  
3000 CFU/ml



**Limit of detection (LoD) 100 cfu/ml** (but according to Poisson distribution, only 63% of 10- $\mu$ l aliquots would contain 1 cfu, provided that cfu $\approx$ particle)

# LoDs of some PCR assays

TABLE 2. Sensitivities and limits of detection for the two assays tested on pure strains and their defined mixtures at various concentrations

Sample group (no. of samples)	Assay	LoD range (CFU/ml)	Avg LoD (95% CI)		Sensitivity (%) at indicated MRSA concn (no. of positive samples)		
			CFU/ml	CFU/swab <sup>a</sup>	10 <sup>2</sup> CFU/ml	10 <sup>3</sup> CFU/ml	10 <sup>4</sup> CFU/ml
MRSA strains (27)	GeneOhm	1.4 × 10 <sup>2</sup> –4.1 × 10 <sup>4</sup>	4.3 × 10 <sup>3</sup> (1.7 × 10 <sup>2</sup> –3.2 × 10 <sup>4</sup> )	4.3 × 10 <sup>2</sup> (1.7 × 10 <sup>1</sup> –3.2 × 10 <sup>3</sup> )	33.30 (9)	92.30 (25)	100 (27)
	Xpert	1.4 × 10 <sup>2</sup> –2.0 × 10 <sup>4</sup>	3.3 × 10 <sup>3</sup> (1.6 × 10 <sup>2</sup> –1.1 × 10 <sup>4</sup> )	2.5 × 10 <sup>2</sup> (1.2 × 10 <sup>1</sup> –8.4 × 10 <sup>2</sup> )	14.80 (4)	96.30 (26)	100 (27)
MRSA/non-MRSA mixtures (21)	GeneOhm	5.4 × 10 <sup>0</sup> –5.1 × 10 <sup>3</sup>	2.0 × 10 <sup>3</sup> (4.5 × 10 <sup>1</sup> –4.9 × 10 <sup>3</sup> )	2.0 × 10 <sup>2</sup> (4.5 × 10 <sup>0</sup> –4.9 × 10 <sup>2</sup> )	42.90 (9)	100 (21)	ND <sup>b</sup>
	Xpert	2.7 × 10 <sup>1</sup> –5.1 × 10 <sup>3</sup>	2.4 × 10 <sup>3</sup> (3.7 × 10 <sup>1</sup> –5.0 × 10 <sup>3</sup> )	1.8 × 10 <sup>2</sup> (2.7 × 10 <sup>0</sup> –3.7 × 10 <sup>2</sup> )	38.10 (8)	100 (21)	ND

<sup>a</sup> Calculated for 100-μl and 75-μl sample inputs for GeneOhm and Xpert, respectively.

<sup>b</sup> ND, not determined, as all MRSA-positive mixtures were detectable at the preceding lower concentration.

Malhotra-Kumar et al 2010

Hologic Fusion: 1183-2383 CFU/ml dependent on the isolate tested  
(95% probit analysis) Bartels et al 2021

# The amount of MRSA in a nostril

- 135 subjects with a prior positive MRSA sample (no decolonization process nor antibiotic treatment) were sampled with a rayon swab (one nostril with dry and one with moistened, 100µl plated, results combined)

CFU	<10	10-100	100-1 000	10 <sup>3</sup> -10 <sup>4</sup>	10 <sup>4</sup> -10 <sup>5</sup>	10 <sup>5</sup> -10 <sup>6</sup>	10 <sup>6</sup> -10 <sup>7</sup>	>10 <sup>7</sup>
n(=270)	56	21	27	13	54	46	50	3
%	20.7%	7,8%	10%	4,8%	20%	17%	18,5%	1%

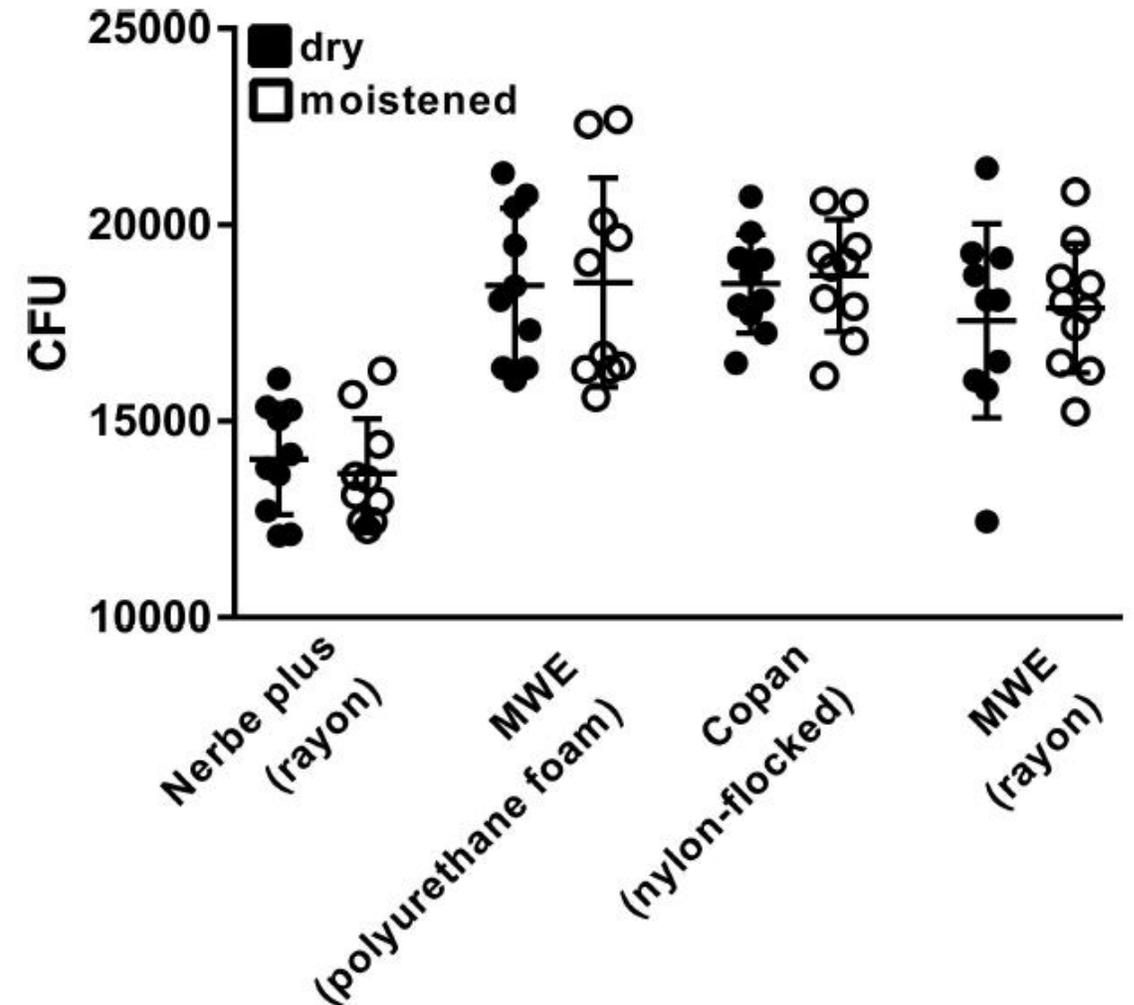
30-40% below or close to LoD of enrichment culture & PCR

May be detected with enrichment

Warnke et al PLOS 2016

# Swabs differ

- Artificial nose models were applied with  $2 \times 10^4$  colony forming units (CFU) MRSA (adjusted to the median bacterial quantities recovered from patients) and  $1 \times 10^5$  CFU *Staphylococcus epidermidis* (to simulate nasal flora)
- Different swabs were used (dry and moistened) for sampling and the recovery of MRSA was assessed by plating  $100 \mu\text{l}$  of 1:10 serial dilutions and counting CFUs



**Fig 2. Recovered MRSA quantities from the artificial nose model utilizing dry or moist rayon, polyurethane foam and nylon-flocked swabs.** Nose models were swabbed utilizing either dry or moistened rayon, polyurethane foam or nylon-flocked swabs. Data results from  $n = 10$  swabs for each setting. p-value (comparing dry and moist swabs within one swab-type) = 0.62 (Nerbe plus, rayon); 0.90 (MWE, polyurethane); 0.72 (Copan, nylon-flocked); 0.96 (MWE, rayon) (Wilcoxon-Mann-Whitney U-test).

# 3 generations of Xpert MRSA tests at ICU

(Jacqmin et al EJCMI 2017)

Table 1 Performance evaluation of the three generations of Xpert MRSA assays

		Reference method + (ICU high-risk, N=§)	Reference method - (ICU high-risk, N=§)	Sensitivity	Specificity	NPV (ICU high-risk, N=§)	PPV (ICU high-risk, N=§)	MRSA Prevalence (ICU high-risk, N=§)
<i>Xpert MRSA 2<sup>nd</sup> Gen.</i> (01/01/2014 - 02/03/2015) N = 2243	PCR +	43 (27)	25 (16)	64.2%	98.9%	98.9%	62.8%	3.2%
	PCR -	24 (13)	2151 (1212)					
<i>Xpert MRSA 3<sup>rd</sup> Gen.</i> (03/03/2015 - 17/04/2016) N = 2771	PCR +	53 (31)	56 (34)	86.9%	97.9%	99.7%	47.7%	2.3%
	PCR -	8 (4)	2654 (1463)					
<i>Xpert MRSA NxG</i> (18/04/2016 - 30/11/2016) N = 1459	PCR +	14 (10)	12 (11)	77.8%	99.2%*	99.9%	47.6%	1.2%
	PCR -	4 (1)	1429 (878)					

\* Statistically highly significant ( $p < 0,01$ )

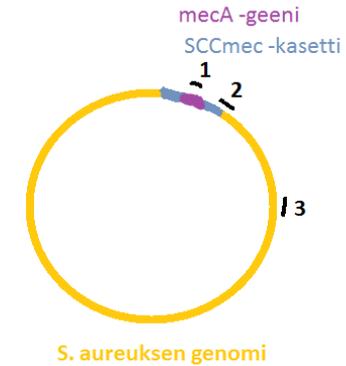
§The numbers in the brackets refer to the number of assessments carried out for the ICU high-risk group

Pre-emptive isolation of high-risk patients until negative molecular results

During MRSA NxG prevalence in high-risk vs low-risk patients 1.2% vs 0.74%)

NB sensitivity, NPV and PPV

# Molecular epidemiology



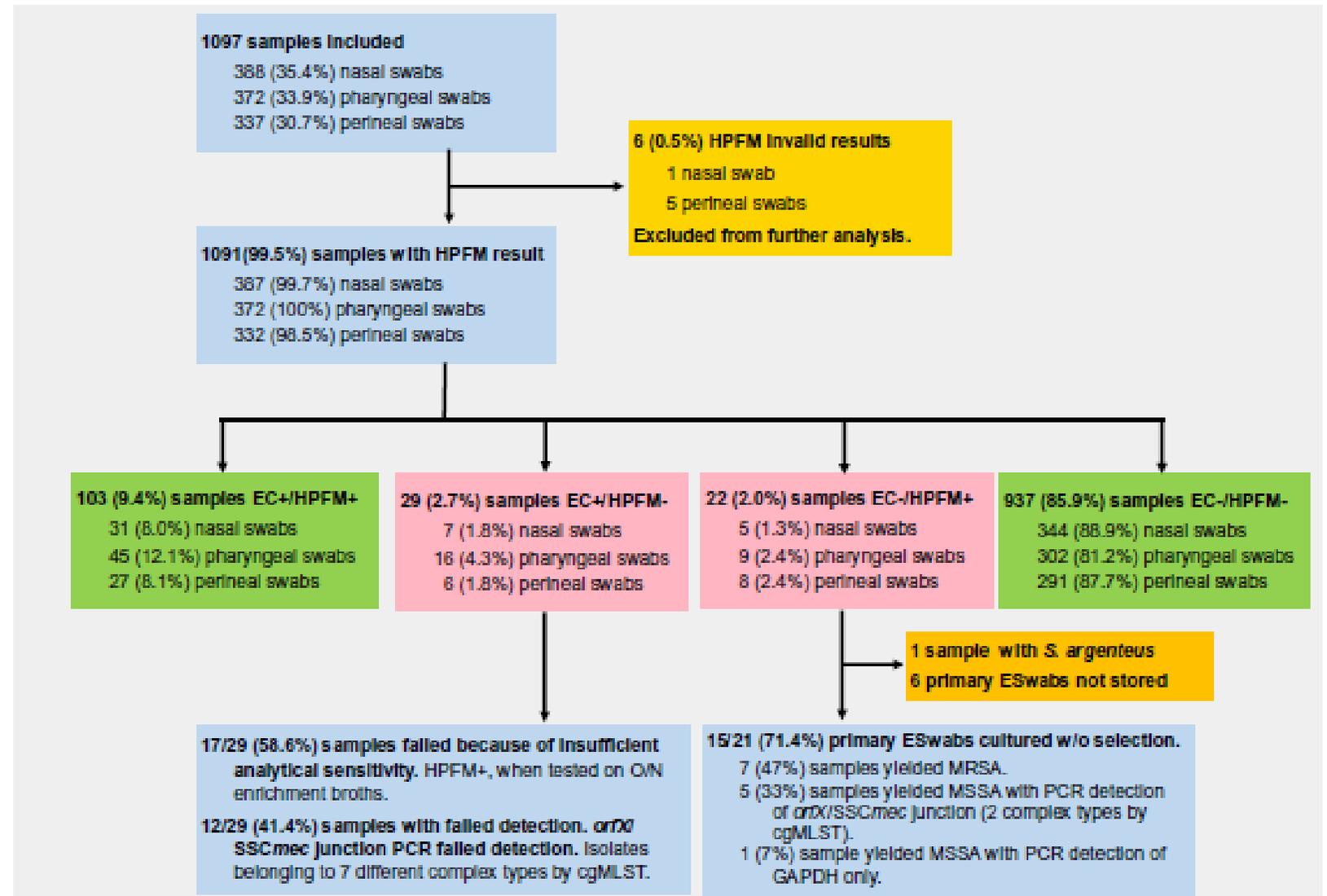
- Typical targets in direct MRSA PCR
1. *mecA*(/*mecC*)
  2. *SCCmec* junction
  3. *S. aureus* – specific target

- In addition to the LoD, the sensitivity of any direct MRSA-PCR depends on prevalence of strains with an *orfA-SCCmec* junction (target 2) which is amplifiable by the assay

- Especially strains with *SCCmec* cassette type IV and V are difficult for many commercial assays
- When testing several commercial PCRs, our "most difficult strains" belonged to *spa* types t688 and t002

# Evaluation of the Hologic Panther Fusion MRSA Assay for the detection of MRSA in eSwab Specimens obtained from nose, throat and perineum

(Bartels et al 2021)



**Fig. 1** Analysis and results of samples included in the study. Flow showing the results of EC and HPFM for all samples. In the green and pink boxes, percentages are of the total number of the swab type and add up to 100% horizontally. EC+, enrichment culture positive; EC-

enrichment culture negative; HPFM+, positive on HPFM when testing the ESwab Amies medium; HPFM-, negative on HPFM when testing the ESwab Amies medium; O/N, overnight; w/o, without

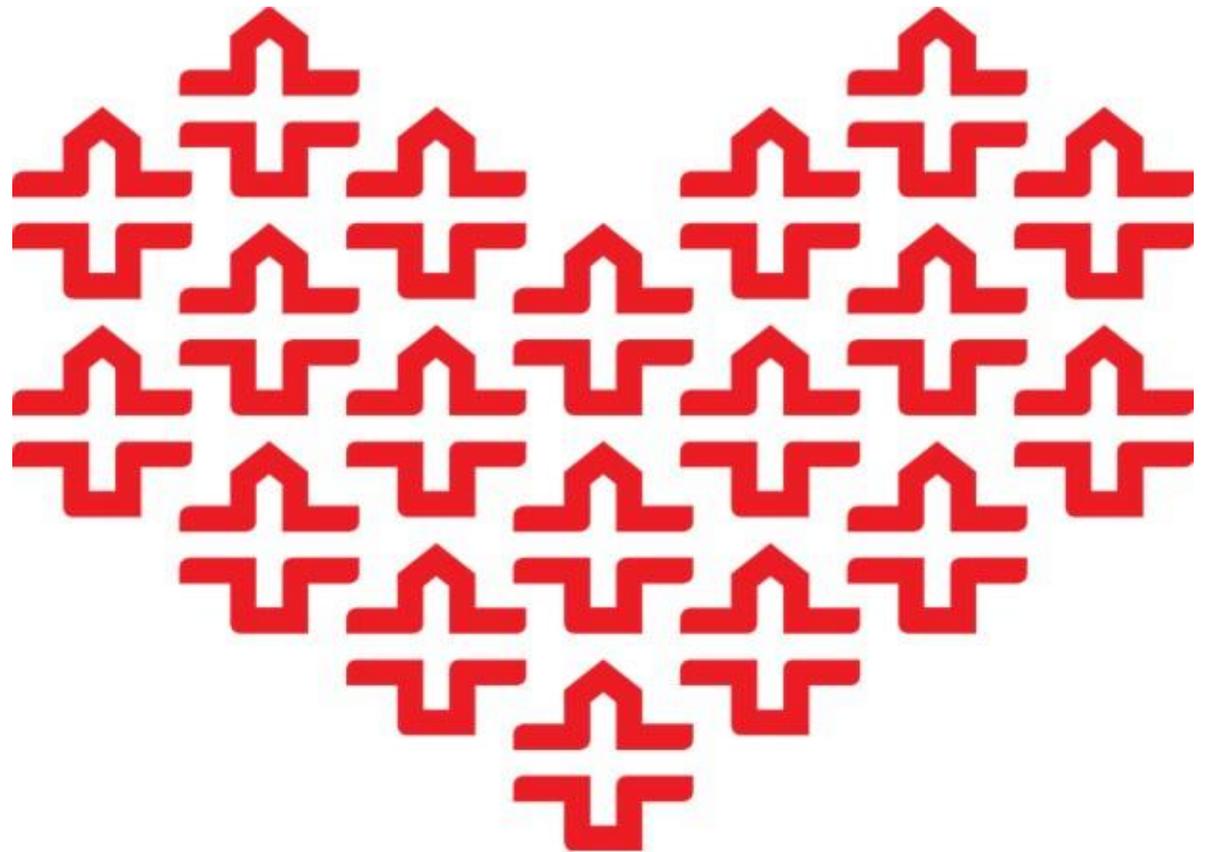
# Panther Fusion MRSA

- Panther test had overall sensitivity 78% as compared to enrichment broth (EB)+ in-house PCR screening (+culture of positives)
- When rerun from EB, 17/29 were positive (sensitivity → 91%)
- 12 cultured isolates were not at all or poorly detected by the Panther assay (SCC mec types IVa or V, spa types t127, t688, t084, t002, t174)
- PCR found 7 samples that were MRSA positive but failed to enrich in cefoxitin
- PCR found 5 MSSA with an empty cassette and one with only SA target

# Take home messages

- A remarkable portion of MRSA-positive specimens contain (very) low amounts of MRSA
- Samples with low amounts give variable results in repetitive testing
- Enrichment improves the sensitivity of both culture and molecular detection. Also a 6 to 8-h enrichment may be useful.
- However, all methods fail to detect some strains
- The trade-off between sensitivity and turnaround time needs to be done according to risk assessment in different patient populations
  - High- vs low-level positivity with regard to infection control?
  - The ultimate goal is to prevent infections caused by MRSA

# Thank you!



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