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Working

Protocol for bacterial cultivation of nasal and throat samples in the Fit Futures study

Version 2

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ABSTRACT

Introduction:

The aim of the study is to evaluate prevalence of *Staphylococcus aureus* in a youth population. This samples will be connected to other possible riskfactors for *Staphylococcus aureus* carriage.

Aim:

Samples will be tested for methicillin-sensitive (MSSA) and methicillin-resistant *Staphylococcus aureus*.

Samples:

Microbiological samples from anterior nares and throat from the protocol "Protocol for sampling and transport of nose- and throat samples in the Fit Futures study (DOI: [dx.doi.org/10.17504/protocols.io.za9f2dw](https://doi.org/10.17504/protocols.io.za9f2dw)).

MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾	CAS NUMBER ▾ RRID ▾
Bacto Staphylococcus medium broth	View	Difco	
Blood agar	View	Oxoid Microbiology Products - Thermo Fischer	
CROMagar-plates for S.aureus detection	View		
Staphaurex plus agglutination test	View		
Control ATCC 12228 MSSE	View		
Control ATCC 25923 MSSA	View		
Control ATCC 33591 MRSA	View		
Inoculating loop	View		

Day 1

- 1 Examine testnumber and sort the samples. Label the enrichment broth with identical testnumbers. Separate the nasal samples from the throat samples. Nasal swab 1 and throat swab 1 are used. Nasal swab 2 and throat swab 2 are frozen for microbiome analysis.
- 2 Place the samples in a fume hood, open the Copan medium and place the rayon-tipped swab into the broth. Rotate the swabsample for approximately 1 second and seal the enrichment broth with a plastic cork.

- 3 Incubate the enrichment broths for 18-24 hours at 37 degrees celsius.

Day 2

- 4 For every participant, use one blood plate, one SAID-plate and one MRSA-plate. Mark the plate with testnumber and mark the plate with an vertical pencil stroke to split the plate in two equal parts. Mark the left one "n" for "Nasal swab" and the right "T" for "throat swab". (see picture).
- 5 There should also be one blood plate, one SAID-plate and one MRSA-plate for the controls. These plates should be marked control.
- 6 Place the plates and the enrichment broths in a fume hood. Use a pasteur pipette to mix the enrichment broth and place 1 drop in associated plates. Use a inoculating loop to spread the drop on the agar. First place the broth on the left side from the nasal swab enrichment, and then on the right side from the throat swab enrichment.
The same procedure for the controls, but use the whole plate instead of splitting it in two parts.
- 7 Incubate the controls and the participant plates in 37 degrees celsius until the next day.

Day 3

- 8 After incubation, identify growth on the plates (both control plates and participant plates) and place numbers in tables. 1= growth, 0 = no growth. The growth on the SAID and MRSA plates should be pink.
- 9 If there is growth of pink colonies on the SAID or MRSA-plates, the most dominating colony from both the left and right side should be picked out and spread on a bloodplate. The bloodplate should be marked with testnumber and with an vertical pencil stroke to split the plate in two equal parts. Mark the left one "N" for "Nasal swab" and the right "T" for "throat swab". The dominating colony should be spread out on associating sides of the plates.
- 10 Incubate the remaining tests at 37 degrees celsius for 20-24 hours.

Day 4

- 11 Use the Staphaurex plus agglutination test to verify the presence of *Staphylococcus aureus* on the bloodplates. Note in tables K+ or K- for koagulase positive or koagulase negative test. Koagulase positive is defined as growth of *Staphylococcus aureus*.
- 12 The confirms *Staphylococcus aureus* isolates should then be frozen at -70 degrees celsius in glyserol-containing liquid media for molecular analysis.



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