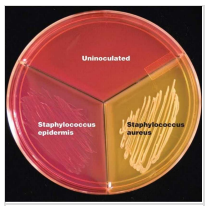

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Jan 12, 2022

Staphylococcus Aureus Sampling V.2

Pol Roca Cugat

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dx.doi.org/10.17504/protocols.io.b3q2qmye

Pol Roca Cugat

This protocol is intended to study the affectation of *Staphilococcus Aureus*, including the MRSA variant. It outlines the basic protocol for a multi-subject study.

DOI

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Pol Roca Cugat 2022. Staphilococcus Aureus Sampling. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b3q2qmye Pol Roca Cugat

Ventilation no longer mandatory.

Microbiology, sampling, swab, staphilococcus aureus

protocol ,

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This protocol is intended to study the affectation of *Staphilococcus Aureus*, which is a Biosecurity Level 2 bacteria. As such, the laboratory should be adequate to those standards, or take measures in order to prevent infection, cross-contamination or leaks.

PPE

- Face shield or protective goggles
- FFP2/KN95 or higher-rated mask
- Rubber, non-powdered gloves
- Lab coat

Sampling material

- Clean and sterile cotton swabs (n+n/10 being n the number of tests required)
- MSA Agar Petri dishes (n/3 being n the number of tests required)
- Sterile Ringer solution
- Permanent marker

Support material

- Bunsen burner
- Incubator
- Ethanol: >80%
- Bleach solution at $50\%_{V}$ in water.

This protocol requires the interaction with people, possibly infected with a pathogen (especially in 2022, when this protocol was written and put into practice). As such, there is a risk of infection which can be reduced with proper PPE use.
Proper ventilation is recommended at all times, even when the pandemic situation is over. However, the sterile field must be preserved at all costs, so try to direct the air flow in order for it not to affect the results.

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this protocol or any of our Sites/Apps and Services.

Preparation 1h

10m

1 ⚠

Wash your hands with soap. Put on your lab coat, your mask and your goggles or face shield. Make sure your mask is airtight and air cannot escape through the sides.

1.1 Prepare the area where you are going to work. Disinfect the surfaces with bleach solution.
The subjects should not be able to walk behind you, only to the side or to the front. Make sure to leave enough distance betweeen the sampler and the subject, but not enough distance as for the sampling to be uncomfortable.
The environment should be comfortable, within this range of temperatures:
🌡 **10 °C** -- 🌡 **35 °C**
You should have a plastic, sealable box to your side or on the table to store the sampled Petri dishes.
The bunsen burner should be to the front of you, within a hand of distance.
The fresh swabs and Petri dishes should never be accessible by the subjects.

1.2 Grab a permanent marker and divide each plate in three equal parts. You can help yourself with a guide.

Sampling 1d 6h

2 Observe the subject. If their nails are longer than 3-6mm (white part that can be accessed). Bitten-down nails could lead to invalid results. Too long nails could lead to cross-contamination.
Ask the subject for his identificative information if this has not been done previously.

3 Place a Petri dish on the side of the Bunsen burner. Open the swab below the Bunsen burner.

⚠ Watch out as not to break the sterile field

Step 3 includes a Step case.

Nail sampling

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Nose sampling

step case

Nail sampling

For cases where the nails are 3-6mm long

4 A random positive sample can then have its DNA isolated and loaded onto a DNA/RNA Shield **Zymo Research Catalog #R1100-50** in order for it to be sent to sequence. The DNA sequence can then be translated into an aminoacid sequence and folded.

Watch out for impurities/contaminations

5 Ask the subject to wash their hands thoroughly and below the nails with soap and lukewarm water. Note their subject ID on the bottom (non--removable part) of the Petri dish.

5.1 Bring the subject's hands below the effect area of the Bunsen burner. Open the Ringer solution and soak the swab. Proceed by swabbing below every nail in both hands. Once done, the subject can be dismissed.

5.2 "Paint" one third of the Petri dish with the swab, softly as not to break the agar but firmly as to get the sample to transfer to the plate.

6 1d

Repeat n times

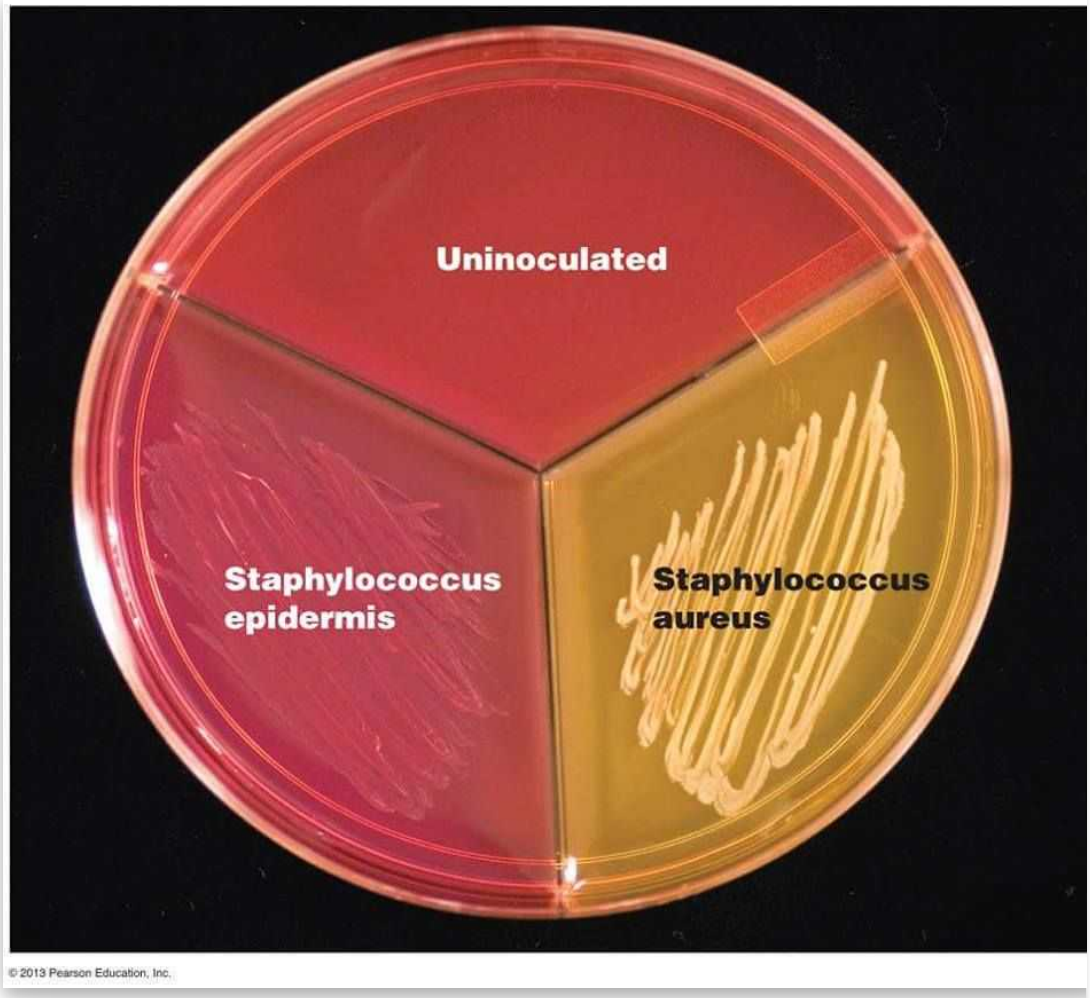
Once the plate has 3 samples, place it in the full plates box.
Once there are 20 plates in the box, group them together with tape, write an identificative group number and place it in the incubator.

The incubator should be set to **37 °C** and left to incubate for **24:00:00** .

Citation: Pol Roca Cugat Staphilococcus Aureus Sampling https://dx.doi.org/10.17504/protocols.io.b3q2qmye

Study 1d 2h

7



It is expected to one of these results. A bright red colour means the sample was uninoculated. A pinkish colour with translucent streaks means there was *Staphylococcus epidermis* present. A faint yelow colour or a bright yellow colour means there was *Staphylocooccus aureus* present

The samples should be taken out of the incubator, the results introoduced into the database and comunicated to the subject.

Using one of the extra MSA Agar plates, culture a sample of Staphilococcus Aureus (incubate at **37 °C** for **24:00:00**). This can then be treated with GRAM tinture in order to observe it under an optical microscope (ideally at x1000-x1200).

8 1d

Using one of the extra MSA Agar plates, culture a sample of Staphilococcus Aureus (incubate

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Watch out for impurities/contaminations

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