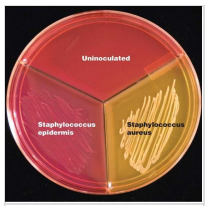


Version 8

Sep 19, 2022

Staphylococcus Aureus Sampling V.8

Mar Roca Cugat[1], Olga Sánchez[2]

[1]Universitat de Girona; [2]Universitat Autònoma de Barcelona

Mar Roca Cugat: Author;

Olga Sánchez: Peer review

1 *Works for me* Share

dx.doi.org/10.17504/protocols.io.81wgb6pk1lpk/v8

Mar Roca Cugat
Universitat de Girona, Universitat Autònoma de Barcelona, In...

DISCLAIMER

ABSTRACT

This protocol is intended to study the affectation of Staphylococcus *Aureus*, including the MRSA, VISA and VRSA variants, even if it makes the test more difficult to perform. It outlines the basic protocol for a multi-subject study, while using basic and minimal resources found in almost every biology lab.

DOI

dx.doi.org/10.17504/protocols.io.81wgb6pk1lpk/v8

PROTOCOL CITATION

Mar Roca Cugat, Olga Sánchez 2022. Staphylococcus Aureus Sampling. **protocols.io**
https://protocols.io/view/staphylococcus-aureus-sampling-cguatwse
Version created by Mar Roca Cugat

KEYWORDS

Microbiology, sampling, swab, staphilococcus aureus

CREATED

Sep 19, 2022

LAST MODIFIED

Sep 19, 2022

PROTOCOL INTEGER ID

70242

GUIDELINES

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

MATERIALS TEXT

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.
- Materials to make LB
- Inactivation buffer
- OD600nm machine

SAFETY WARNINGS

Citation: Mar Roca Cugat, Olga SÃÂ¡nchez Staphylococcus Aureus Sampling
https://dx.doi.org/10.17504/protocols.io.81wgb6pk1lpk/v8

This protocol requires interaction with people, possibly infected with a pathogen (especially in 2022, when this protocol was written and put into practice, as COVID-19 was still going strong). 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 airflow in order for it not to affect the results.

DISCLAIMER:

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 the 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 between the sampler and the subject, but not enough distance as for the sampling to be uncomfortable.
The environment should be comfortable, within the following range of temperatures: 🌡 **20 °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 Using a permanent marker, divide each plate into two to four equal parts. You should help yourself by using a guide, such as a ruler.

Citation: Mar Roca Cugat, Olga SÃÂ¡nchez Staphylococcus Aureus Sampling
https://dx.doi.org/10.17504/protocols.io.81wgb6pk1lpk/v8

Sampling 1d 6h

2 Observe the subject's hands. If their nails are longer than 1-4 mm (the white part of the nail that can overgrow).
Bitten-down nails could lead to invalid results. Too long nails could lead to cross-contamination.
Ask the subject for their identificative and contact information if this has not been done previously.

3 Place a Petri dish on the side of the Bunsen burner. The burner will be the center of our sterile field.

Watch out so as not to break the sterile field

Step 3 includes a Step case.
Nail sampling
Nose sampling

step case

Nail sampling

For cases where the nails are 3-6mm long

4 Ask the subject to wash their hands thoroughly and below the nails with soap and lukewarm water. Note their subject ID on the bottom (agar side) of the Petri dish.

4.1 Bring the subject's hands below the sterile area generated by 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.

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

5  1d

Citation: Mar Roca Cugat, Olga SÃÂ¡nchez Staphylococcus Aureus Sampling
https://dx.doi.org/10.17504/protocols.io.81wgb6pk1lpk/v8

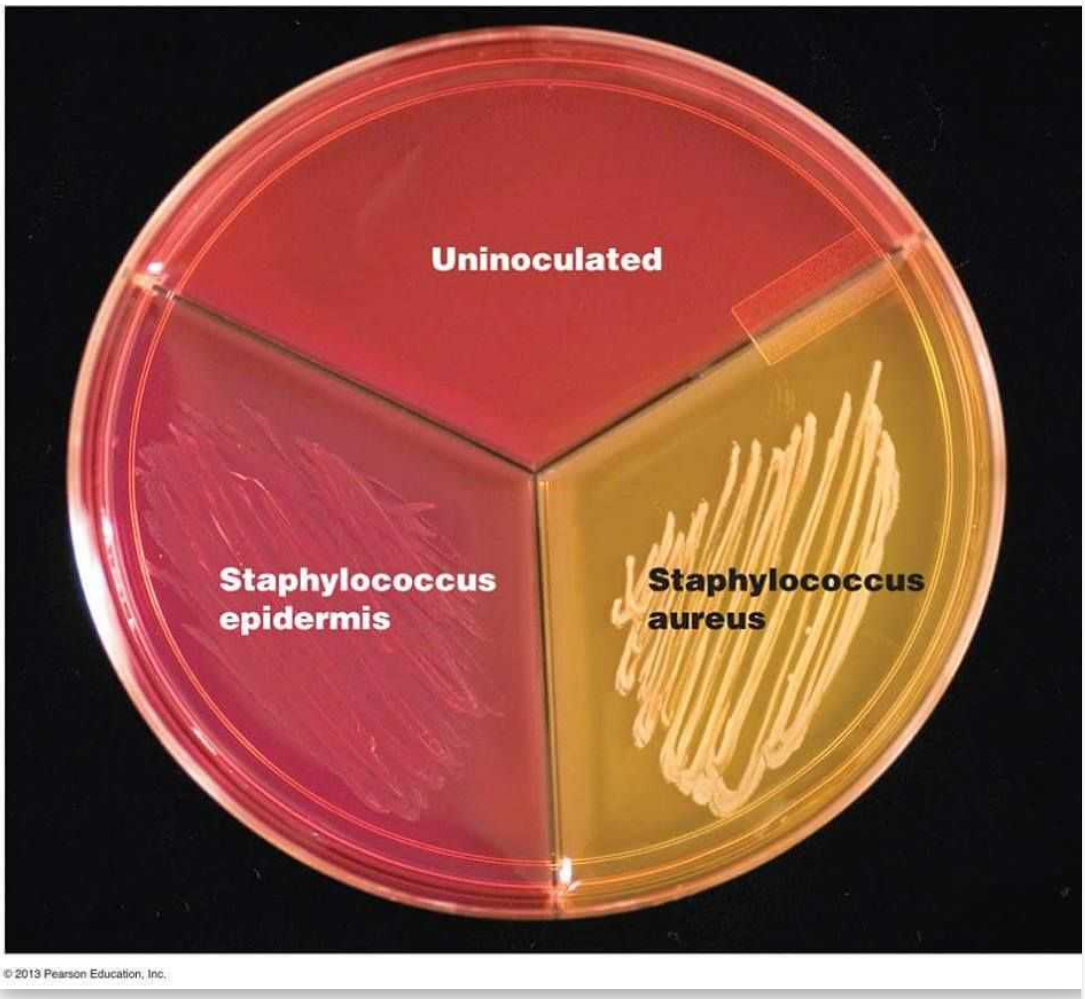
Repeat n times

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** .

Study 1d 2h

6



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 is *Staphylococcus epidermis* present. A faint yellow colour or a bright yellow colour means there is *Staphylococcus aureus* present

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

Citation: Mar Roca Cugat, Olga SÃÂ¡nchez Staphylococcus Aureus Sampling
https://dx.doi.org/10.17504/protocols.io.81wgb6pk1lpk/v8

7

Using one of the extra MSA Agar plates, culture and purify a sample of Staphilococcus Aureus This can then be treated with GRAM tinture in order to observe it under an optical microscope (ideally at x1000-x1200).

Watch out for impurities/contaminations

8

1d

Prepare an LB dillution (**20 mL LB**) with as many pure colonies as possible. Incubate at **37 °C** for **24:00:00** .

Perform an OD600nm. The sample should be diluted to get around **1 OD** in order for it to be accurate. When the result comes in, the solution should be diluted with LB to an OD of **8 OD** to **12 OD** . With the OD600, we can check the result and contrast it to a known source.

Calibration Protocol - Conversion of OD_{600} to Colony Forming Units (CFUs)
by Paul Rutten,
The University of Oxford

PREVIEW RUN

Citation: Mar Roca Cugat, Olga SÃÂ¡nchez Staphylococcus Aureus Sampling
https://dx.doi.org/10.17504/protocols.io.81wgb6pk1lpk/v8