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Platelet adhesion assay for streptococci

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We use this protocol and it's working

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Disclaimer

We have used this assay for several streptococcal species, but it may need optimizing for different strains.

Abstract

This protocol describes how to perform adhesion assays examining the interaction between streptococci and fixed platelets.

Attachments

adhesion assay templ...

13KB

Guidelines

Planning the layout of your plate prior to starting the experiment is helpful. The platelets are prepared, and the plate coated as described **dx.doi.org/10.17504/protocols.io.n92ld8wb8v5b/v1** in this protocol

Safety warnings

Relevant biosafety protocols must be followed.

Basics

1 The volumes of all washes are 120 µl of PBS

2 Everything is diluted and made in PBS pH 7.4

3 Binding of each strain or condition is performed in triplicate and each strain has a control of binding to BSA.

Inoculum preparation

4 Grow bacteria to OD_{600} 0.5 ±0.005 in Todd Hewitt with yeast extract. Place cultures on ice.

5 Bacteria are diluted 1:50 in PBS, mix well by pipetting the mixture up and down, this is your inoculum, keep it on ice.

6 If you need to add a competitive inhibitor alter the amount of PBS added appropriately. For example, CBM40 is used at a final concentration of 30 µM.

Preparation of plate

7 Aspirate liquid from platelet coated wells

8 If you need to pretreat your platelets with neuraminidase follow steps here. If not proceed to step 6.

8.1 Prepare enough neuraminidase to pre-treat all the necessary wells (50 µl per well).

8.2 Dilute Neuraminidase (5U/mL) 1:50 in PBS

8.3 Add 50 µl to each well to be treated and 50 µl of PBS to wells that will not be treated

8.4 Incubate 30 min at 37^{o}C.

8.5 Wash all wells twice with 120 µl PBS

9 Block all wells with 120 µl of 3% BSA for 60 min at 37 ^{o}C.

10 Aspirate BSA, wash the wells twice 120 µl PBS. **The plate is ready for adherence.** If bacteria are not ready for adherence then leave the wells in PBS until ready to start the assay. However, this time should be minimized. Once inoculums are ready remove the PBS

Adhesion assay

11 Add 50 µl of inoculum to platelet coated wells and BSA controls

12 Cover the plate and place it at 37 ^{o}C in 5% $CO_2$for 1 hour.

13 During the 1-hour incubation set up a dilution plate and perform serial dilutions of the inoculums, usually plating 10^{-1} to 10^{-4} is adequate.

13.1 Dilute one sample at a time and keep the others on ice

13.2 Mix the samples by pipetting or vortexing immediately prior to dilution.

13.3 Perform 10-fold dilutions in PBS in a 96-well plate

13.4 Plate 3 x 10 µl spots of each dilution on tryptic soy agar plates with 5% sheep's blood.

13.5 Incubate plate in 5% CO_2 at 37 ^{o}C overnight

14 After the 1-hour incubation, aspirate the liquid from the wells and wash each well 3x with 120 µl PBS

15 Add 100 µl of 0.25% trypsin/1mM EDTA to each well and incubate the plate in 5% CO_2 at 37 ^{o}C for 15 min.

16 Using a 200 µl pipette mix the contents over the surface of the well a few times and scrape the pipette tip over the surface to loosen adherent bacteria. Transfer the contents in a micro-centrifuge tube. Place the tube on ice.

17 Perform serial dilutions of samples from test and control wells as described above. Typically, 10^{-0} to 10^{-3} is sufficient. Plate the dilutions on TSA Blood agar plates and incubate at in 5% Co 37 ^{o}C overnight.

18 The next day calculate the percentage adherence. Attached is an excel sheet that will help with the calculations - it will need adapting for each experiment. and determine the percent adherence with
respect to the inoculum.

18.1 Count the colonies at appropriate dilutions for each inoculum and well.

18.2 Average the counts for each of the three spots for each inoculum and well. Then calculate the total amount of bacteria in each inoculum and the total number that were bound in each well.

18.3 Average the triplicate wells for each condition and BSA control.

18.4 For each strain subtract the average of the bacteria bound to BSA from the average bound to platelets.

19 Experiments are conducted on three independent occasions and the percentage adherence for each averaged. The statistical significance is measured by one way ANOVA using Tukey's post test. $p<0.05$ is considered significant.