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Protocol for study of the in vitro effect of purified serum anti-anti-anti-SpA antibodies (Ab-3) on cultures of Staphylococcus aureus.

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Protocol status: Working

We use this protocol and it's working

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Abstract

This test shows the ability of Ab-3 antibody to inhibit the growth of *S. aureus* in vitro. The amounts of samples tested were 18 samples from chicks fed with hyper-immune eggs and therefore have the Ab-3, and six samples from chicks fed non-hyperimmune eggs and therefore do not have the Ab-3. Inhibition of the growth of the bacteria plated out in blood agar was observed only when the Ab-3 was added to said culture media, suggesting that the anti-anti-anti-SpA (Ab-3) bound to the *S. aureus* and inhibited their ability to proliferate in culture. On the other hand, bacterial growth was observed when blanks or sera of chicks fed non-hyper-immune eggs were used because these samples lacked the protective Ab-3 [1].

Reference

Justiz Vaillant AA, Akpaka PE, Smikle M, McFarlane-Anderson N (2012) In vitro Inhibition of *Staphylococcus aureus* isolates by anti-antidiotypic antibodies to *Staphylococca* protein (SpA). *J Vaccines Vaccin* 3:127. doi:10.4172/2157-7560.1000127.



Materials

MATERIALS

 Blood agar **Oxoid Microbiology Products - Thermo Fischer**

 Culti-Loops[®]; Staphylococcus aureus subsp. aureus ATCC[®]; 33592[®]; **Thermo Fisher Catalog #R4607020**

Troubleshooting

Safety warnings

-  The risk associated with a particular microbe can be consulted in the following website of the CDC. (<http://www.cdc.gov/biosafety/publications/BiologicalRiskAssessmentWorksheet.pdf>)

- 1 The investigation of the neutralizing ability of purified anti-SpA antibodies is carried out as follows: One ml of brain heart infusion (BHI) broths is placed in 10 sterile test tubes.
- 2 To each tube is added an equal volume of 10 μ l of purified anti-anti-anti-SpA (Ab-3) antibodies in a concentration of 1.25- μ g/ μ l.
- 3 All samples are tested in duplicate.
- 4 An inoculum of an ATCC Staphylococcus aureus strain (ATCC #33592) is prepared to 0.5 commercially prepared McFarland scale standard ($1=300 \times 10^6$ /ml bacteria concentration). Then, ten μ l of this inoculum is added serially from tubes 1 to 10 (prepared for each sample) and the dilutions were reported in this study as a bacterial concentration of 0.1, 0.01, and 0.001.
- 5 The absorbance value of each tube at 600 nm is measured.
- 6 A sample from each tube is plated out on Blood agar and incubated overnight at 35°C.
- 7 Inhibition of the bacterial growth is observed in individual and pooled sera of chicks fed with anti-SpA hyperimmune eggs up to 28 days, 15 days, and eight days period.