Measurement of the red blood cell lysis by bacterial hemolysin

Naxin Jiang
National University of Singapore
Nguan Soon Tan
National University of Singapore
Bow Ho
National University of Singapore
Jeak Ling Ding
National University of Singapore

Method Article

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Abstract

Introduction

The lysis of the red blood cells can be monitored by the release of hemoglobin to the extracellular environment. After removing the intact RBC by centrifugation, the absorbance at 404 nm of the supernatant reflects the hemolysis. The RBC lysate obtained by total disruption by 1% Triton- X100, is used as a positive control.

Procedure

The RBC lysis by bacterial hemolysin is measured as described[1] with modification. 1. Collect the RBCs into heparinized tubes. 2. Wash the RBC two times by gentle resuspension with 10 times volume of pyrogen free saline \(0.9% NaCl), and centrifugation at 1000 x g for 10 min at room temperature. 3. Gently resuspend the RBC pellet with pyrogen free saline and dilute the RBC to 0.8% \(v/v). 4. Culture the bacteria under test according to protocol #4, and adjust the population to 10^7 cfu/ml. 5. Incubate the washed RBC with equal volume of bacteria at room temperature under steady rotation at 6 rpm. 6. Set up pyrogen-free saline \(0.9%) as the negative control, and 1% Triton-X100 in saline as the positive control. 7. At time intervals, retrieve aliquots of the reaction mixture and pellet the RBC by centrifuging at 1000 x g for 10 min at room temperature. 8. Transfer the supernatant into 96 well microtitre plate. Monitor the absorbance at 404 nm with ELISA-reader as an indication of the hemoglobin released into the supernatant.

Anticipated Results

Hemolysin producing microbes, such as _S. aureus_, lyses the RBC and the typical A404= 1.2 – 1.6.

References

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Figures

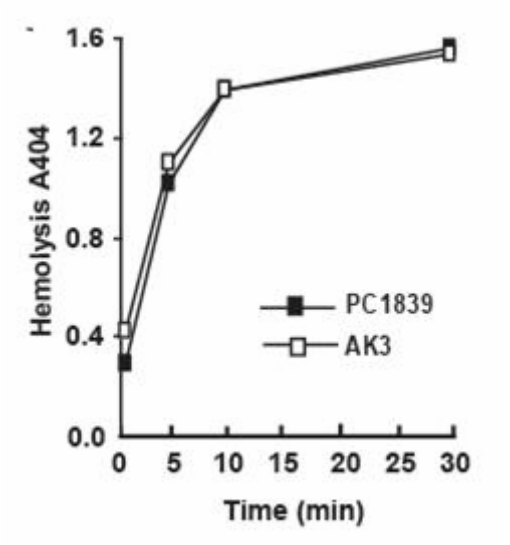

Figure 1

Hemolysis of RBC by S. aureus laboratory strains PC1839 and AK3. _S. aureus_ strains, PC 1839 and AK3, at cell density of 107 cfu/ml, rupture the RBC within 5 min.