

DEMONSTRATION OF CLUMPING FACTOR USING A SCREENING TEST IN STAPHYLOCOCCI ISOLATED FROM ANIMALS

Iulia BUCUR, Oana PETREC, D. MITRICĂ MĂRĂCINE, J. DEGI, L. FLUERAȘU

Faculty of Veterinary Medicine Timișoara, 300645,
C. Aradului Street, No.119, Timișoara, România

Corresponding email: bucur_iulia@ymail.com

Abstract

The clumping factor is a chemical compound found in the bacterial cell wall, which reacts directly with the fibrinogen, without using a plasma factor, causing the aggregation of bacterial cells in clusters with fibrin masses.

The demonstration of clumping factor can be done with rapid kits containing latex particles sensitized with fibrinogen and IgG. Strains that have clumping factor or protein A, brought into contact with the kits ingredients, on a glass slide, produce an agglutination of the mixture.

Research was made in order to detect the clumping factor in the staphylococci strains isolated from animals and, based on biochemical properties, were included in the following species: *S. aureus* ssp. *aureus* (82 strains), *S. intermedius* (114 strains), *S. hycus* (11 strains) and *S. xylosus* (12 strains).

For the staphylococci strains, the clumping factor's presence was tested with a commercial kit and the obtained results are the following:

- *S. aureus* ssp. *aureus* (64 strains possessed clumping factor);
- *S. intermedius* (79 strains possessed clumping factor);
- *S. hycus* (the tested strains didn't possess clumping factor);
- *S. xylosus* (the tested strains didn't possess clumping factor).

The results show that the production of clumping factor is correlated with the species and also with the pathogenicity of the staphylococci strains.

Key words: clumping factor, *S. aureus* subsp. *aureus*, *S. intermedius*.

INTRODUCTION

Pathogenic staphylococci strains develop extracellular enzymes called coagulase, that are active against rabbit and human plasma, being responsible for the coagulation of blood plasma both "in vivo" and "in vitro" (Codiță, 2008; Răpunțean G. and Răpunțean S., 2005). There are two types of staphylococcal coagulase:

- *free coagulase*, similar to prothrombin, from chemically and biologically point of view, which induces the formation of a coagulated plasma sleeve around the germ with anti chemotactic role to phagocytes;
- *bound coagulase*, also known as "clumping factor", is a chemical compound located in the bacterial cell wall, which reacts directly with fibrinogen, without using a plasma factor, causing the aggregation of bacterial cells in clusters with fibrin masses (Codiță, 2008; Răpunțean G. and Răpunțean S., 2005).

In routine diagnosis are used two techniques for revealing staphylococcal coagulase, that allow differentiating the isolated strains of staphylococci from pathological materials. Free diffusible coagulase is revealed by a technique in tubes, while bound coagulase or "clumping factor" is revealed by rapid tests on the blade (Codiță, 2008; Răpunțean G. and Răpunțean S., 2005).

Clumping factor can be revealed with rapid kits containing latex particles sensitized with fibrinogen and IgG. Strains that have bound coagulase brought into contact with the ingredients of such kits produce the agglutination of this mixture (Codiță, 2008).

Research was made in order to detect bound coagulase in staphylococci strains isolated from animals with different diseases.

MATERIALS AND METHODS

The pathological samples for bacteriological examination were taken from animals with different lesions, and primary inseminations were made on agar with 5% sheep

defibrinated blood. The isolated strains were screened based on cultural, morphological and tinctorial characters.

Biochemical identification of the isolated and purified staphylococci strains was made by using API Staph system and, thus, the strains

were included into the following species: *S. aureus ssp. aureus*, *S. intermedius*, *S. hycus* and *S. xylosus*.

The clumping factor was revealed by a rapid kit PROLEX STAPH LATEX KIT, produced by PRO-LAB DIAGNOSTICS (Figure 1).

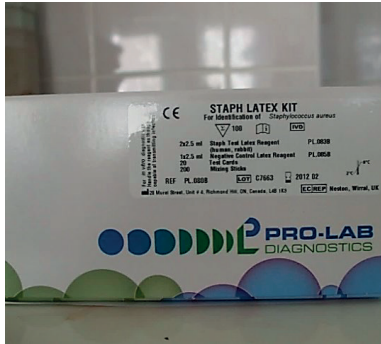


Figure 1. PROLEX STAPH LATEX KIT

The principle of this test consists in agglutination of the mixture made by the staphylococci strains producing bound coagulase (clumping factor) and the latex particles sensitized with fibrinogen and IgG of the kit. Thus, on the test card, was dispensed one drop of each of the two vials, respectively reagent 1 containing blue polystyrene latex particles sensitized with human fibrinogen and immunoglobulins and reagent 2 containing white unsensitized latex particles (control). In every drop of the two reagents was dispensed with a bacteriological loop, one or two young (18-20 h) suspect culture and homogenized until the appearance of small clots indicating the presence of bound coagulase.

219 strains were tested with this kit, as follows:

- *S. aureus ssp. aureus*: 82 strains;
- *S. intermedius*: 114 strains;
- *S. hycus*: 11 strains;
- *S. xylosus*: 12 strains.

RESULTS AND DISCUSSIONS

At the staphylococcal strains, the presence of bound coagulase was revealed with a kit that enables testing the strains as a screening test for the detection of this enzyme.

The positive reaction indicates the presence of the clumping factor by the appearance of

small clots, as a result of precipitation of plasma fibrinogen into fibrin, under the action of this enzyme within 2-3 minutes. The negative reaction is represented by the absence of such clots in the mentioned interval (Figure 2).

For the results accuracy, a strain of *S. aureus ssp. aureus* was used as the positive control and a strain of *S. epidermidis* as a negative control.

The results were the following:

- *S. aureus ssp. aureus*: 64 strains possessed clumping factor (78.04%);
- *S. intermedius*: 79 strains possessed clumping factor (69.29%);
- *S. hycus*: the tested strains did not possess clumping factor;
- *S. xylosus*: the tested strains did not possess clumping factor.

Staphylococcal strains that synthesize bound coagulase, as well as protein A, produced a positive reaction, as this enzyme, into contact with latex particles sensitized with IgG and fibrinogen, agglutinate this mixture. The presence of this enzyme was revealed only in two species of staphylococci, which normally synthesize this enzyme and absent in strains belonging to the species *S. hycus* and *S. xylosus*. These results showed that the strains of *S. aureus ssp. aureus* and *S. intermedius* produced positive reactions, but the

proportion of strains that synthesized bound coagulase was lower than the proportion of positive strains in this test, communicated by other writers (El-Khabaz K. A. et al., 2011; Ganesh V. K. et al., 2011; Schissler, 2009). Demonstration of bound coagulase should be referred at cautiously, due to the nonspecific agglutinative action of immunoglobulin on staphylococci strains, on one hand, or because some strains do not synthesize it, on the other hand. The absence of bound coagulase does not give an indication of the pathogenicity of staphylococci strains, because these have other pathogenicity factors (Codiță, 2008; Ganesh V. K. et al., 2011).

The results of this research recommends using this test as a screening method, the presence of

bound coagulase indicating the pathogenicity of the strains, also representing a rapid test to differentiate the staphylococci strains within the primary identification.

In the case of extensive research, regarding the pathogenicity, the results provided by this screening test must be confirmed on the test in tubes, which reveals the free coagulase. Only the strains producing free coagulase are considered coagulase positive strains.

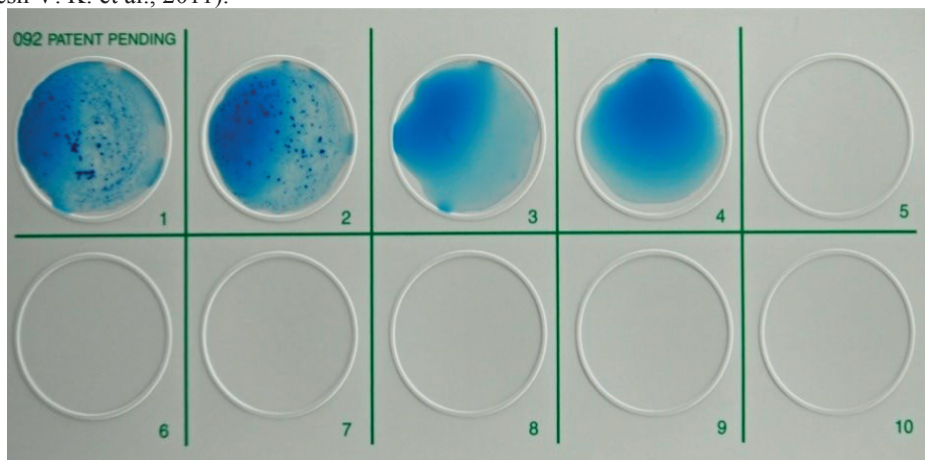


Figure 2. Positive and negative reactions on PROLEX STAPH LATEX KIT

CONCLUSIONS

Using a fast screening kit were tested for the presence of bound coagulase a number of 219 staphylococci strains, included in four species, this enzyme being present in 65,30% of them.

In the strains belonging to the species *S. aureus ssp. aureus* and *S. intermedius*, the bound coagulase was present in a less proportion than the existing data.

This test for detection of clumping factor is recommended only as a screening method for the primary typing of staphylococci strains isolated from the animals.

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