



STAPHYCHROM II

Identification of *Staphylococcus aureus* from isolated colonies

64 Tests (Cat. N° 22709)

GB-2006-11

1 - AIM

The STAPHYCHROM II test is designed to differentiate easily and quickly, from isolated colonies, *Staphylococcus aureus*, the main pathogen species, from other staphylococci species.

A kit contains 64 tests, including negative controls. Each kit allows the testing of a minimum of 32 samples (urinary tests) to a maximum of 63 samples (serial tests).

2 - INTRODUCTION

Staphylococci are a serious problem in hospital epidemiology; *Staphylococcus aureus* is the main pathogenic species and is responsible for septicemias and nosocomial infections. Coagulase-Negative Staphylococci (CNS) are most frequently considered to be opportunistic pathogens.

Multi-drug resistance poses considerable therapeutic problems, in particular which staphylococci resistant to methicillin. The rapid differentiation of *Staphylococcus aureus* from other species of staphylococci is therefore a necessity in microbiological diagnosis.

The STAPHYCHROM II test can be performed rapidly and is just as sensitive as the standard *S. aureus* identification tests. Its excellent specificity means that a rapid *S. aureus* identification can be reliably performed (1, 4).

3 - PRINCIPLE

The principle of the STAPHYCHROM II test is based upon the detection of the characteristic *S. aureus* staphylocoagulase enzyme, with the aid of prothrombin (3, 5) and a synthetic chromogenic substrate specific for the enzyme (6). The release of a paranitroaniline group leads to the appearance of a yellow coloration of the reaction medium. After 1 to 2 hours of incubation at 37°C, the appearance of a yellow coloration reveals the presence of *S. aureus*.

4 - REAGENTS

Description	Amount
STAPHYCHROM II Tray : Microplate divided into 8 wells containing a staphylococci specific dehydrated culture medium as well as a staphylocoagulase chromogenic substrate	8
COAG Reagent : Lyophilisate containing purified human prothrombin and NaCl. To be reconstituted with 2 ml of sterile distilled water. Drop-counter included.	4
4-adhesif bands	8

The reaction medium contains 26.8 mg/L of human prothrombin, 0.5 g/L of chromogenic substrate and 20 g/L of NaCl.

5 - PRECAUTIONS

The reagents are intended solely for *in vitro* use and must be handled by authorised personnel.

The patient samples and inoculated reagents are potentially infectious; they must be handled with caution, in observance of hygiene rules and the current regulations for this type of product in the country of use.

The reagents contain raw materials of animal origin.

The COAG reagent contains human prothrombin which has been screened for and found not to contain anti-HIV 1 and 2 antibodies, anti-HCV antibodies and HBSAg. Nonetheless it must be handled as a potentially infectious product.

Do not use reagents after the expiry date.

Do not use reagents that have been damaged before use or that have been poorly conserved after opening (§7).

Ensure that the dessicant is present in the aluminium sachet of the STAPHYCHROM II tray.

6 - SAMPLE COLLECTION

Use colonies of Gram positive cocci grouped in clumps and of identical appearance. The colonies should be freshly isolated (18-24h at 37°C), grown in trypticase soy agar with the addition or otherwise of 5% sheep blood or in the following media: Chaparran, Columbia blood, Baird-parker, URICHROM II or other chromogenic media (2). The sample taking must be carried out from a pure culture, in compliance with good laboratory practice.

7 - CONSERVATION AND PREPARATION OF REAGENTS

Reagents conserved at 2-8°C, in their original state, are stable up to the expiry date indicated on the box.

After reconstitution of the lyophilisate using 2 ml of sterile distilled water, the COAG reagent, sealed with its drop counter, is stable for 4 months at 2-8°C. It must not be frozen.

The unused wells of the STAPHYCHROM II kit are stable for 4 weeks and must be conserved at 2-8°C in the closed aluminium sachet with the dessicant.

8 - MATERIAL REQUIRED BUT NOT SUPPLIED

- PRESTO inoculators (#22204, INTERNATIONAL MICROBIO)
- Sterile distilled water
- Incubator at 37°C

9 - METHOD

Allow the reagents to reach room temperature (18-25°C) before use.

9.1 Inoculation of the STAPHYCHROM tray

Cut out the tray according to the number of tests to be performed. Identify the Control well (C) and one or several Assay wells (A).

Note: A Control well is needed for a urinary test or for a series of tests incubated at the same time.

Check for the presence of a light yellow reagent at the bottom of the well.

Distribute 2 drops of COAG reagent with the help of the drop-counter into each of the wells (C) and (A).

Inoculate a well (A) with 2 colonies taken imperatively with the PRESTO inoculator, proceeding as follows:

- Introduce the inoculator vertically into the center of a colony (min. diameter 1mm) pushing it through the full depth of the agar until lightly touching the base of the dish, ensuring that the inoculator remains perpendicular to the agar at all times.

- Gently withdraw the inoculator with a single movement, maintaining it vertically at all times.

- Repeat the operation a second time with the same inoculator.

For further sampling, inoculate a fresh well (A) with another PRESTO inoculator and proceed as above.

Note : For correct colony sampling, do not touch the end of the PRESTO inoculator prior to use and do not flame sterilise it.

9.2 Incubation - Reading

To avoid spilling of the contents of the wells during incubation and reading of the tray, it is recommended to place an adhesif band under the wells (C) and (A).

Incubate the tray at 37°C.

- After 1 to 2 hours incubation, read the appearance of a yellow coloration in well (A) in comparing it to that of well (C). Do not read after more than 2 hours.

- To confirm a negative result, it is essential to read after 2 hours.

9.3 Interpretation of results

The appearance of a yellow coloration in well (A) of greater intensity than that of well (C) signifies the presence of *Staphylococcus aureus*. If no difference in colour is visible after 2 hours of incubation, the staphylococcus is other than *S. aureus*.

Note: The interpretation of the results should not be affected in any way by the possible apparition of cloudiness in (A) wells.

10 - QUALITY CONTROL

Recommended strains: *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 14990.

For each strain to be tested, isolate in trypticase soy agar (TSA) with the addition of 5% sheep blood and incubate for 18-24 hours at 37°C. For both strains proceed as described in §9.

Read the results after 2 hours by comparison with a control well.

- *Staphylococcus aureus* ATCC 25923:
Appearance of yellow coloration

- *Staphylococcus epidermidis* ATCC 14990:

Coloration identical to the control well

11 - CAUSES OF ERROR

Respect the reconstitution and conservation conditions of the reagents (§7). Respect the procedure for the inoculation of the tray with the PRESTO inoculator, as well as the incubation times (§9).

12 - TEST LIMITS

Sampling must be carried out from freshly isolated colonies of no more than 18-24 hours old.

A negative result cannot be validated before 2 hours of incubation has elapsed.

The appearance of yellow coloration after 2 hours of incubation should not be taken into account.

13 - PERFORMANCE

Clinical Strains

A study performed on 193 reference strains (Centre National de Référence des Staphylococcus (National Staphylococcus Reference Centre)) (99 *S. aureus* strains and 94 other *Staphylococcus* strains representing 15 species) revealed **100% specificity** with the STAPHYCHROM II test. The result is higher than that obtained with the reference method (determination of free coagulase by the coagulation in tube of rabbit plasma) (specificity 89,3%) as well as that obtained with the latex agglutination test (specificity of 92% for the interpretable results) (1).

Clinical Isolates

A study performed on 193 clinical strains (144 *S. aureus* strains, 106 of which were methicillin resistant, and 49 other *Staphylococcus* strains) demonstrated **99,3% sensitivity and 100% specificity** with the STAPHYCHROM II test. The results are comparable to the reference method (determination of free coagulase by the coagulation in tube of rabbit plasma) and higher than the results obtained with the latex agglutination test for which sensitivity was 98,6% and specificity 98% (1).

14 - WASTE ELIMINATION

Waste should be disposed of in accordance with the hygiene rules and current regulations for this kind of product in the country of use.

15 - BIBLIOGRAPHY

- 1-Bes M., N. Fonsale, C. Mazellier, A. Carricajo, C. Ploton, A.M. Freydière, G. Aubert, J. Etienne. 2002, "Evaluation d'un nouveau test chromogène STAPHYCHROM II pour la détection de la staphylocoagulase" Poster 222 P2. RICAI. Paris. Décembre 2002.
 - 2-Carricajo A., A. Treny, N. Fonsale, M. Bes, M.E. Reverdy, Y. Gille, G. Aubert, A.M. Freydière. 2001, "Performance of the Chromogenic Medium CHROMagar Staph Aureus and the Staphychrom Coagulase Test in the Detection and Identification of *Staphylococcus aureus* in Clinical Specimens. J. Clin. Microbiol., **39** (7) : 2581-2583.
 - 3-Engels W., M.A. Kamps, C.P. Van Boven. 1981, "Rapid and direct staphycoagulase assay that uses a chromogenic substrate for identification of *Staphylococcus aureus*". J. Clin. Microbiol., **14** : 496-500.
 - 4-Fonsale N., M. Bes, I. Verdier, A. Carricajo, C. Ploton, G. Aubert, J. Etienne, F. Vandenesch and A.M. Freydière. 2004, "Specific Identification of *Staphylococcus aureus* by Staphychrom II, a Rapid Chromogenic Staphylocoagulase Test". J. Clin. Microbiol., **42** (5) : 1962-1964.
 - 5-Kawabata S., T. Morita, S. Iwanaga, H.J. Igarashi. 1985, "Enzymatic properties of staphylochromin, an active molecular complex formed between staphylocoagulase and human prothrombin". Biochem. **98** : 1603-1614.
 - 6-Langlet S., G. Quentin, G. Contant, J.C. Ghnassia. 1999, "Méthode chromogénique d'identification rapide de *Staphylococcus aureus*". Ann. Biol. Clin., **57** : 191-196.
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