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Specific Identification of *Staphylococcus aureus* by Staphychrom II, a Rapid Chromogenic Staphylocoagulase Test

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We compared the performance of Staphychrom II (International Microbio, Signes, France), a rapid (2-h) chromogenic staphylocoagulase test that uses human prothrombin and protease inhibitors, with those of the reference tube coagulase test (TCT) and the latex agglutination test (LAT) Slidex Staph Plus for the rapid identification of *S. aureus*. Prospective evaluation with 293 fresh clinical isolates yielded sensitivities, specificities, and predictive and negative predictive values of 98.1, 100, 100, and 95.1%, respectively, for the Staphychrom II test; 98.6, 98.7, 99.6, and 96.3%, respectively, for LAT; and 97.6, 98.7, 99.5, and 93.9%, respectively, for TCT. The perfect specificity of the Staphychrom II test was confirmed by testing 193 collection strains selected because of their potential testing pitfalls. The Staphychrom II test was positive for 90% of the 215 *S. aureus* strains tested after only 1 h of incubation. The Staphychrom II test was as sensitive as the reference TCT and was 100% specific.

Rapid identification of *Staphylococcus aureus* is a major priority for clinical microbiologists. The tube coagulase test (TCT) is the reference method for distinguishing *S. aureus* and rare coagulase-positive non-*S. aureus* staphylococci (e.g., *S. intermedius*) from coagulase-negative staphylococci. However, this test requires 18 to 24 h of incubation and is not perfectly reliable (1, 13, 16, 18). Agglutination tests with either erythrocytes or latex particles coated with various ligands may lack sensitivity, notably, for methicillin-resistant *S. aureus* (MRSA), and may also lack specificity for non-*S. aureus* staphylococci, such as *S. lugdunensis*, *S. schleiferi*, and *S. intermedius* (3, 8, 14, 15).

In the present study we evaluated Staphychrom II (International Microbio, Signes, France), a rapid (2-h) chromogenic staphylocoagulase test which specifically detects *S. aureus*, and compared the results obtained with those obtained by a reference TCT (Becton Dickinson, Le Pont de Claix, France) and the latex agglutination test (LAT) Slidex Staph Plus (bioMérieux, Marcy l'Etoile, France). The tests were compared prospectively with 293 clinical isolates in two clinical laboratories and retrospectively with 193 typical and atypical collection strains chosen because of their potential testing pitfalls.

MATERIALS AND METHODS

Test organisms. From March 2002 to May 2002, a total of 293 *Staphylococcus* isolates were prospectively tested at Bellevue Hospital, Saint Etienne, France ($n = 149$) or Debrousse Hospital, Lyon, France ($n = 144$). They comprised 215 *S. aureus* isolates (43 MRSA strains and 172 methicillin-susceptible strains) and 78 non-*S. aureus* staphylococci. The 78 non-*S. aureus* isolates comprised *S. capitis* ($n = 14$), *S. caprae* ($n = 3$), *S. cohnii* ($n = 1$), *S. epidermidis* ($n = 49$), *S. haemolyticus* ($n = 3$), *S. hominis* ($n = 4$), *S. simulans* ($n = 2$), and *S. warneri* ($n = 2$). The strains were isolated from the following clinical specimens: pus ($n = 85$), respiratory tract ($n = 83$), nose ($n = 32$), blood ($n = 26$), catheter ($n = 22$), urine ($n = 13$), stool ($n = 12$), eye ($n = 7$), ear ($n = 5$), throat ($n = 5$), and cerebrospinal fluid ($n = 3$).

The 193 collection strains (French National Reference Center for Staphylococci) comprised 99 strains of *S. aureus* originating from 25 different French towns ($n = 59$) or from England ($n = 40$) and 94 non-*S. aureus* staphylococci, as follows: *S. auricularis* ($n = 3$), *S. capitis* subsp. *capitis* ($n = 5$), *S. capitis* subsp. *ureolyticus* ($n = 3$), *S. caprae* ($n = 6$), *S. cohnii* ($n = 5$), *S. epidermidis* ($n = 11$), *S. haemolyticus* ($n = 7$), *S. hominis* subsp. *hominis* ($n = 2$), *S. hominis* subsp. *novobiosepticus* ($n = 3$), *S. intermedius* ($n = 8$), *S. lugdunensis* ($n = 5$), *S. pasteuri* ($n = 4$), *S. saprophyticus* subsp. *saprophyticus* ($n = 6$), *S. schleiferi* subsp. *coagulans* ($n = 2$), *S. schleiferi* subsp. *schleiferi* ($n = 7$), *S. sciuri* subsp. *rodentium* ($n = 2$), *S. sciuri* subsp. *sciuri* ($n = 3$), *S. simulans* ($n = 5$), *S. warneri* ($n = 5$), and *S. xylosus* ($n = 2$).

Some of the *Staphylococcus* species listed above were selected to evaluate the specificity of the Staphychrom II test, as they are known either to produce coagulase, pseudocoagulase, or agglutination factors or to possess biochemical characteristics close to those of *S. aureus* (1, 5, 14, 17). Eleven strains were selected for their atypical phenotypic or genetic characteristics. They comprised six *S. aureus* strains and five *S. epidermidis* strains. Among the atypical *S. aureus* strains, one was negative for lactose, trehalose, and mannitol acidification; one was negative by latex agglutination with several reagents; two were negative by TCT (19); and two were negative by the catalase test (A. Le Coustumier, Y. Brun, M. Bes, A. Le Coustumier, J. Fleurette, and J. Etienne, Abstr. 8th Int. Symp. Staphylococci Staphylococcal Infect., abstr. P288, p. 260). The five atypical *S. epidermidis* strains were positive for mannitol acidification.

Conventional identification. All primary isolates were identified on the basis of their morphology, Gram staining, and catalase positivity. Catalase-positive colonies and gram-positive cocci were agglutinated by the Slidex Staph Plus LAT, and coagulase production was detected by the reference TCT described below. All coagulase-negative isolates were identified by using the ID32 Staph gallery (bioMérieux).

Reagents and methodologies. (i) Slidex Staph Plus LAT. The Slidex Staph Plus test (bioMérieux) is an LAT that simultaneously detects clumping factor, protein A, and capsular polysaccharides on the *S. aureus* cell surface (20). It was used according to the recommendations of the manufacturer.

(ii) Reference TCT. For TCT (9), strains were incubated overnight at 37°C in staphylocoagulase broth (Bio-Rad, Marnes la Coquette, France). A 0.05-ml aliquot of broth was then mixed with 0.5 ml of rabbit plasma containing EDTA (Becton Dickinson). The tubes were incubated at 37°C and examined at 4 and 24 h.

(iii) Staphychrom II test. Staphychrom II (International Microbio) is a 2-h staphylocoagulase test based on a chromogenic substrate, human prothrombin, and protease inhibitors [6, 11; A. M. Freydière, M. Bes, M. A. Charveriat, A.

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TABLE 1. Performances of the three methods for *S. aureus* identification with 293 clinical isolates[a]

Assay	S (%)	Sp (%)	PPV (%)	NPV (%)
Staphychrom II	98.1	100	100	95.1
Slidex Staph Plus LAT	98.6	98.7	99.6	96.3
Reference TCT	97.7	98.7	99.5	93.9

[a] Abbreviations: S, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

Carricajo, and C. Ploton, Program Abstr. 12th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P392, 2002, Clin. Microbiol. Infect. **8**(Suppl. 1)**:**57, 2002]. The kit consists of a panel of eight wells containing dehydrated medium suitable for staphylococci and a chromogenic substrate for staphylocoagulase. A freeze-dried coagulase reagent (COAG) containing human prothrombin is extemporaneously reconstituted with 2 ml of sterile distilled water. Two wells are used per test: the control well is filled with the COAG reagent, and the test well is filled with the COAG reagent and the test microorganism.

Two colonies with an identical appearance on the isolation agar were directly inoculated (Presto ABG Inoculator; International Microbio) in the test well containing 0.1 ml (2 drops) of the COAG reagent. After incubation at 37°C for 1 to 2 h, the color was read with the naked eye. The result is valid only if the control well shows no yellow color. Any yellow color of the test well identifies the isolate as *S. aureus*, while a total lack of yellow color after 2 h identifies a non-*S. aureus* staphylococcal species.

(iv) Definite identification. When the results of the three tests disagreed, the isolate was formally identified by using the ID32 Staph gallery (bioMérieux) and/or the Accuprobe test (bioMérieux), which detects rRNA sequences specific to *S. aureus*.

Statistical analysis. Fisher's exact test was used for analysis of the categorical variables. Differences between groups were considered significant for variables with P values <0.05.

RESULTS

The Staphychrom II test was tested with a total of 486 staphylococci, comprising 293 fresh clinical isolates and 193 collection strains.

With the 293 fresh clinical isolates (Table 1), the sensitivities, specificities, and positive and negative predictive values were 98.1, 100, 100, and 95.1%, respectively, by the Staphychrom II test; 98.6, 98.7, 99.6 and 96.3%, respectively, by LAT; and 97.7, 98.7, 99.5, and 93.9%, respectively, by TCT. Statistical analysis of the sensitivity and specificity results showed that the differences between the three methods were not statistically significant ($P = 0.5$). The Staphychrom II test was positive for 90% of the 215 *S. aureus* strains after only 1 h of incubation.

TABLE 2. Comparison of the Staphychrom II test with an LAT (Slidex Staph Plus) and a conventional TCT with 293 clinical isolates

Species	No. of strains	No. of strains with the indicated result by: Staphychrom II test		Slidex Staph Plus LAT		Conventional TCT	
		+	−	+	−	+	−
S. aureus	215	211	4[a]	212	3[b]	210	5[c]
Non-*S. aureus* staphylococci	78	0	78	1[d]	77	1[e]	77
Total	293	211	82	213	80	211	82

[a] These four strains (three MRSA strains and one methicillin-sensitive *S. aureus* strain) yielded a positive result by the Slidex Staph Plus LAT and three yielded a negative result by the conventional TCT.

[b] These three strains (one MRSA strain and two methicillin-sensitive *S. aureus* strains) yielded a positive result by the Staphychrom II test and the conventional TCT.

[c] Among these five strains, two yielded a positive result by both the Staphychrom II test and the Slidex Staph Plus LAT. The other three (see footnote *a*) yielded a negative result by the Staphychrom II test and a positive result by the Slidex Staph Plus LAT.

[d] This false-positive reaction corresponded to *S. hominis*.

[e] This false-positive reaction corresponded to *S. simulans*.

TABLE 3. Comparison of the Staphychrom II test with LAT (Slidex Staph Plus) and a conventional TCT with 193 collection strains

Species	No. of strains	No. of strains with the indicated result by: Staphychrom II test		Slidex Staph Plus LAT		Conventional TCT	
		+	−	+	−	+	−
S. aureus	93	91	2[a]	93	0	93	0
Atypical *S. aureus*	6	4	2[b]	4	2[c]	4	2[b]
Non-*S. aureus* staphyloccci	94	0	94	8[d]	81[e]	10[f]	84
Total	193	95	98	105	83	107	86

[a] Two MRSA strains isolated from two patients admitted to the same unit and showing the same pulsed-field gel electrophoresis pattern.

[b] Two strains giving a false-negative coagulase reaction, even though they possess the coagulase gene (19).

[c] One strain showing negative agglutination with several other latex reagents and one strain yielding negative lactose, trehalose, and mannitol acidification.

[d] Three *S. lugdunensis* strains, three *S. schleiferi* subsp. *schleiferi* strains, 1 *S. schleiferi* subsp. *coagulans* strain, and one *S. cohnii* strain.

[e] Five tests were uninterpretable (agglutination in the control reagent).

[f] Two *S. schleiferi* subsp. *coagulans* strains and eight *S. intermedius* strains yielding a positive reaction by the conventional TCT.

Nine of the 215 *S. aureus* strains yielded false-negative results by one of the tests (4 by the Staphychrom II test, 3 by LAT, and 5 by TCT) (Table 2).

The 78 non-*S. aureus* isolates yielded a negative result by the Staphychrom II test (specificity, 100%). A positive reaction was observed by TCT with one *S. simulans* strain and by LAT with one *S. hominis* strain (Table 2).

The results for the 193 collection strains are summarized in Table 3. Among the 99 *S. aureus* strains tested (including 6 atypical strains), 6 yielded a false-negative reaction by one or more of the three tests (Table 3). Four *S. aureus* strains yielded a false-negative result by the Staphychrom II test: two of them were very weakly positive by TCT after 24 h of incubation, while the other two were TCT negative. The Staphychrom II test was positive for the other four atypical *S. aureus* strains (one was negative for lactose, trehalose, and mannitol acidification; one was negative for latex agglutination with several reagents; and two were catalase negative).

The 94 non-*S. aureus* collection strains were all negative by the Staphychrom II test, yielding a specificity of 100%. In contrast, 8 strains were positive by TCT and 10 strains were positive by LAT (Table 3).

DISCUSSION

We prospectively evaluated the Staphychrom II test with fresh clinical isolates under real conditions of use in two clinical laboratories. The test was also evaluated with a panel of collection strains from the French National Reference Center

for Staphylococci. The prospective evaluation involved staphylococcal isolates of various origins that corresponded to the usual epidemiological patterns seen in the two participating laboratories.

With the 293 fresh clinical isolates, the sensitivity, specificity, and positive and negative predictive values of the Staphychrom II test (98.1, 100, 100, and 95.1%, respectively) were superior to those obtained by TCT (97.7, 98.7, 99.5, and 93.9%, respectively). The Staphychrom II test was also more rapid than TCT (incubation times, 1 to 2 h and 4 to 24 h, respectively).

All 94 non-*S. aureus* staphylococci among the collection strains were negative by the Staphychrom II test, which was therefore perfectly specific. A large proportion of these strains (*S. intermedius*, *S. lugdunensis*, *S. schleiferi*, etc.) were chosen because they expressed coagulase, pseudocoagulase, or surface proteins known to produce false-positive results with several LAT kits. Although *S. intermedius* and *S. schleiferi* are not commonly encountered in clinical samples, several investigators (2, 4, 7, 10, 12, 17) have underlined the need to accurately identify these species in some clinical situations. The excellent specificity of the Staphychrom II test had previously been demonstrated with *S. delphini* and *S. hyicus* [11; Freydière et al., Program Abstr. 12th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P392, 2002, Clin. Microbiol. Infect. **8**(Suppl. 1)**:**57, 2002].

Among the 99 *S. aureus* collection strains, two "typical" isolates from patients admitted to the same unit of an English hospital gave negative results by the Staphychrom II test. These two strains showed the same pulsed-field gel electrophoresis restriction pattern and belonged to the same epidemic clone. They were weakly coagulase positive by the TCT method after 24 h of incubation, suggesting that the quantity of coagulase produced in 2 h might have been too small to be detected by the Staphychrom II test. Only two atypical *S. aureus* collection strains were falsely negative by the Staphychrom II test. These two strains possess the coagulase gene but do not produce coagulase (19).

As the TCT method is known to lack sensitivity and specificity, the use of TCT in combination with an LAT has been strongly recommended. This has the dual advantages of improving specificity and yielding more rapid *S. aureus* identification. In our study, the Staphychrom II test was 100% specific, relatively rapid, and as sensitive as LAT, suggesting that it could serve as a stand-alone test for accurate *S. aureus* identification.

The Staphychrom II test is more expensive than TCT, but it is less time-consuming and does not require additional consumables. It might thus be cost-effective in industrialized countries where technician time is expensive. The Staphychrom II test is clearly cost-effective compared with the cost of the combination of TCT and LAT.

In conclusion, this study, performed with a broad range of staphylococcal species, confirms previous reports that the Staphychrom II test is rapid, reliable, and easy to use. Its perfect specificity makes it suitable as a stand-alone test for accurate *S. aureus* identification in the clinical laboratory.

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