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# Identification of staphylococci using commercial kits STAPHYtest 24 and API Staph

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## Abstract

The present study tested identification power of two commercial biochemical systems for staphylococcal identification. The STAPHYtest 24 kit has been appeared as reliable identification tool for staphylococci of human clinical material while the API Staph kit gave less-faithful results probably due to an inappropriate internet identification software api*web*.

## Introduction

Staphylococci represent a substantial part of the surface micro flora of mammals and birds (skin, skin gland and mucous membranes) and some *Staphylococcus* species, mainly *Staphylococcus aureus* subsp. *aureus*, are found frequently as etiological agents of a variety of human and animal infections. The species identification of staphylococci based on biotyping is still commonly performed in many routine clinical laboratories, although a lot of new molecular techniques are applicable for the strain differentiation and identification presently. The aim of presented study was the comparison of species identification of staphylococci by using two commercial biochemical systems and their database identification tools.

## Methods

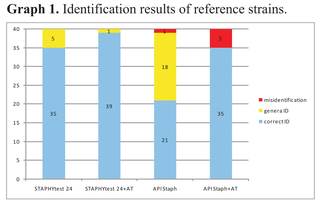
Forty reference staphylococcal cultures and 155 strains of presumptive staphylococci mostly isolated from human clinical material were evaluated by two commercial identification kits in this comparative study. Reference cultures of staphylococci were obtained from the Czech Collection of Microorganisms (CCM) (http://www.sci.muni.cz/ccm) and only most frequent staphylococcal species and subspecies were chosen for testing (*S. aureus* subsp. *aureus, S. saprophyticus* subsp. *saprophyticus, S. epidermidis, S. haemolyticus, S. simulans, S. warneri, S. hominis* subsp. *hominis* and. subsp. *novobiosepticus, S. xylosus, S. lugdunensis, S. schleiferi* subsp. *schleiferi, S. sciuri, S. lentus* and *S. capitis* subsp. *capitis* and subsp. *ureolyticus*). Human clinical isolates of staphylococci were originated from the three different clinical microbiological laboratories in Czech Republic and all of them represented an independent samples of routinely isolated and identified gram-positive catalase positive cocci.

The identification of both reference cultures and isolates was based on STAPHYtest 24 kit (Pliva-Lachema) and API Staph kit (bioMérieux) without any additional tests. Both identification kits were used in accordance with standard recommended procedure of manufacturer's instruction and only manual reading of test results was applied for both identification kits. Evaluation of obtained results was performed with TNW v. 6.5 identification programme (STAPHYtest 24) or using the internet identification tool api*web* (https://apiweb.biomerieux.com) (API Staph). In case of intermediate identification based on kits' results some additional tests (AT) suggested by the identification programme were done. No individual approach for final identification was allowed. All unidentified or discrepantly identified isolates were re-tested in the National Reference Laboratory for Staphylococci based on a large set of tests to provide the reliable identification results.

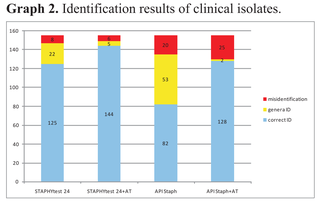
**Table 1.** False identifications of staphylococcal reference cultures and isolates.

| Correct result | Mistake identification on: | | | |
|---|---|---|---|---|
| | STAPHYtest 24 | STAPHYtest 24+AT | API Staph | API Staph+AT |
| *S. hominis* subsp. *hominis* | | | NI | NI |
| *S. hominis* subsp. *hominis* | | | *S. lugdunensis* | |
| *S. hominis* subsp. *hominis* | | | *S. sciuri* | *S. sciuri* |
| *S. hominis* subsp. *hominis* | | | NI | NI |
| *S. hominis* subsp. *hominis* | | | *S. lugdunensis* | |
| *S. hominis* subsp. *novobiosepticus* | | | | *S. saprophyticus* |
| *S. hominis* subsp. *novobiosepticus* | | | | NI |
| *S. hominis* subsp. *novobiosepticus* | | | | NI |
| *S. hominis* subsp. *novobiosepticus* | | | | *S. epidermidis* /*S. hominis* |
| *S. saprophyticus* subsp. *saprophyticus* | | | NI | |
| *S. saprophyticus* subsp. *saprophyticus* | | | NI | |
| *S. saprophyticus* subsp. *saprophyticus* | | | *S. warneri* | *S. warneri* |
| *S. saprophyticus* subsp. *saprophyticus* | | | *S. sciuri* | *S. hominis* /*S. warneri* |
| *S. saprophyticus* subsp. *saprophyticus* | | | | *S. xylosus* |
| *S. intermedius* | | | | *S. aureus* |
| *S. intermedius* | | | | *S. aureus* /*S. hominis* |
| *S. intermedius* | | | | *S. simulans* |
| *S. intermedius* | *S. hyicus* | | *S. xylosus* | *S. xylosus* |
| *S. intermedius* | | | *S. xylosus* | *S. xylosus* |
| *S. epidermidis* | *S. capitis* subsp. *ureolyticus* | | | |
| *S. epidermidis* | | | *S. sciuri* | *S. sciuri* |
| *S. epidermidis* | | | *S. aureus* | *S. aureus* |
| *S. haemolyticus* | | *S. epidermidis* | | *S. hominis* |
| *S. capitis* subsp. *capitis* | *S. auricularis* | | | |
| *S. capitis* subsp. *ureolyticus* | | | | *S. haemolyticus* |
| *S. xylosus* | | | | *S. hominis* |
| *S. xylosus* | NI | NI | *S. lentus* | |
| *S. sciuri* | | | NI | NI |
| *S. sciuri* | | | *S. xylosus* | *S. xylosus* |
| *S. lentus* | | | NI | NI |
| *S. arlettae* | | *S. cohnii* subsp. *cohnii* | | *S. xylosus* |
| *S. cohnii* subsp. *ureafyticum* | | | NI | NI |
| *S. lugdunensis* | | | *S. lentus* | *S. lentus* |
| *S. auricularis* | *Kocuria varians* | | *S. capitis* | *S. capitis* |
| *S. pasteurianus* | | | *S. simulans* | *S. simulans* |
| *Micrococcus luteus* | NI | NI | | |
| *Staphylococcus* sp. | NI | NI | *S. lugdunensis* | NI |
| *Staphylococcus* sp. | *Aerococcus viridans* | NI | | *S. lentus* |

**Graph 1.** Identification results of reference strains.




**Graph 2.** Identification results of clinical isolates.




## Results

A total of 195 gram-positive, catalase positive cocci including 40 reference CCM strains and 155 clinical isolates obtained from three different laboratories were identified with two commercial identification kits. Forty reference strains representing 13 most frequent staphylococci in clinical material were reliable identified by STAPHYtest 24 kit. Any cultures were misidentified with mentioned kit and only at 5 cases the final identification required additional tests. But API Staph kit required additional tests at 19 cases of forty reference strains and even after applying of recommended tests the two cultures were misidentified (*S. hominis* instead of *S. xylosus* CCM 4580 and *S. saprophyticus* instead of *S. hominis* subsp. *novobiosepticus* CCM 4747) and three cultures stayed unidentified after additional testing (CCM 3474, CCM 4748, CCM 4786). These failing identifications revealed 12.5% mistake of api*web* identification tool in case of reference staphylococcal cultures. Generally, the identification of reference cultures was more successful than the identification of clinical isolates – see Graph 1 and Graph 2, especially in case of STAPHYtest 24 testing.

The clinical isolates were composed of common species of staphylococci as *S. aureus* subsp. *aureus* (19 strains), *S. epidermidis* (26), *S. haemolyticus* (20), *S. saprophyticus* subsp. *saprophyticus* (16), *S. hominis* subsp. *hominis* (15); or of less-frequent species *S. simulans* (9), *S. warneri* (7), *S. xylosus* (10), *S. sciuri* (5), *S. intermedius* (5), *S. lugdunensis* (4), *S. capitis* subsp. *capitis* (3), *S. capitis* subsp. *ureolyticus* (4) and *S. lentus* (3). Very rare identification (only per 1 strain) was showed for *S. hominis* subsp. *novobiosepticus, S. auricularis, S. piscifermentans, S. arlettae, S. schleiferi* subsp. *schleiferi* and *S. cohnii* subsp. *urealyticum*. Three isolates of cocci were stayed identified to the genus level only as *Staphylococcus* sp. even if the large set of tests was used for their characterization.

Generally, STAPHYtest 24 kit successfully identified to the species or subspecies level 160 strains (82%) of tested staphylococci. Remaining 27 strains (14%) were identified to the genus level only and 8 strains (4%) stayed unidentified or misidentified. Applying of additional tests recommended by identification software (TNW v. 6.5) improved species identification to 94% when 6 strains (3%) were misidentified and next 6 strains (3%) stayed identified to genus level only. The second tested commercial system API Staph kit surprisingly identified only 103 cultures (53%) to the species level. Great number of strains, 71 of 195 studied (36%), was identified by API Staph just to genus level and 21 strains (11%) were not identified or misidentified. This fading proved insufficiency of API Staph kit for reliable identification of staphylococci when supplementary tests are not used. Applying of additional tests to API Staph results according to internet identification tool api*web* improved correct species identification to 84%. Unfortunately this step also increased false identifications to 30 cases and the high number of misidentified staphylococci represented a serious problem of kit API Staph and/or internet software api*web*.

The false identifications of both kits are summarized in Table 1. It is evident that very important staphylococci (e.g. *S. aureus, S. epidermidis, S. haemolyticus*) have mostly been identified by both systems correctly. Biochemical identification of staphylococci was problematic in case of *S. hominis* subspecies and in a group of several novobiocin resistant staphylococci (e.g. *S. saprophyticus, S. xylosus, S. sciuri* group). The phosphatase test showed problematic reading in both tested kits.

<u>STAPHYtest 24 kit has yielded several advantages in comparison with API Staph system:</u>

✓ STAPHYtest 24 does not require any reagents for results fading what is very comfortable for users (and quicker) in comparison with API Staph kit reading. Reagent free design accelerates the evaluation of results and eliminates the risk of technical errors associated with applying reagents into appropriate wells.

✓ The evaluation of obtained results by TNW programme was more comfortable and user-friendliness in our testing when compared with internet identification tool api*web*. TNW programme enabled subspecies identification of some staphylococci and its database contains almost all validly described staphylococcal species while api*web* tool database was not current. This non-updated matrix in internet identification tool api*web* hardly complicated the species identification. In many cases internet tool api*web* does not identify subspecies of *S. hominis, S. capitis, S. saprophyticus* or *S. cohnii*.

✓ The drawback of internet identification tool api*web* was that some suggested additional test are hardly available (e.g. turanose) in routine laboratories while TNW v. 6.5 suggested more available additional tests for final decision

On the other hand the main disadvantage of STAPHYtest 24 was occurrence of intermediate or doubtful results in several acidification tests (sugars). Acidification of sugars was clear-cut in API Staph kit enabling reading of well distinguishing results of acidification tests. It is evident that pH indicator in API Staph strip is more suitable for sugar tests than one of STAPHYtest 24 kit.

## Conclusions

- Our results proved usefulness of the new identification kit STAPHYtest 24 for efficient and reliable identification of staphylococci. In our view the STAPHYtest 24 is superior to API Staph as regards of identification efficacy as well as user friendliness.
- Both identification kits are suitable for species identification of staphylococci from clinical material and in case of STAPHYtest 24 kit without any additional tests.
- Species identification with STAPHYtest 24 was 82% and improved to 94% after applying additional tests suggested by identification software TNW v. 6.5. Identification to the species level based on API Staph kit was less-successful in our testing when we have obtained only 53% of staphylococci identified to the species or respectively 84% successfulness after performing of additional tests.
- STAPHYtest 24 kit misidentified only 6 strains while API Staph kit misidentified 30 staphylococcal strains. In our opinion this problem is not a mistake of the API Staph kit but demerit of internet identification tool apiweb.
- The less-frequent staphylococci isolated from non-human material required additional tests for species identification; the identification of staphylococci based on kit results only was often insufficient.

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