SUMMARY AND EXPLANATION

API 20 Strep is a standardized system combining 20 biochemical tests that offer widespread capabilities. It enables group or species identification of most streptococci and enterococci, and those most common related organisms. The complete list of those organisms that it is possible to identify with this system is given in the Identification Table at the end of this package insert.

PRINCIPLE

The API 20 Strep strip consists of 20 microtubes containing dehydrated substrates for the demonstration of enzymatic activity or the fermentation of sugars. The enzymatic tests are inoculated with a dense suspension of organisms, made from a pure culture, which is used to reconstitute the enzymatic substrates. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. The fermentation tests are inoculated with an enriched medium which rehydrates the sugar substrates. Fermentation of carbohydrates is detected by a shift in the pH indicator. The reactions are read according to the Reading Table and the identification is obtained by referring to the Analytical Profile Index or using the identification software.

CONTENT OF THE KIT (Kit for 25 tests)
- 25 API 20 Strep strips
- 25 incubation boxes
- 25 ampules of API GP Medium
- 25 result sheets
- 1 package insert

COMPOSITION

Strip
The composition of the API 20 Strep strip is given in the Reading Table of this package insert.

Medium

<table>
<thead>
<tr>
<th>API GP Medium</th>
<th>L-cystine</th>
<th>Tryptone (bovine/porcine origin)</th>
<th>Sodium chloride</th>
<th>Sodium sulfite</th>
<th>Phenol red</th>
<th>Demineralized water to make 1000 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 ml</td>
<td>0.5 g</td>
<td>20 g</td>
<td>5 g</td>
<td>0.5 g</td>
<td>0.17 g</td>
<td>pH : 7.4 - 7.6</td>
</tr>
</tbody>
</table>

The quantities indicated may be adjusted depending on the titer of the raw materials used.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagents / Instrumentation
- API® Suspension Medium, 2 ml (Ref. 70 700)
- Reagents : NIN (Ref. 70 491)
  - VP 1 + VP 2 (Ref. 70 422)
  - ZYM A (Ref. 70 494)
  - ZYM B (Ref. 70 493)
- Mineral oil (Ref. 70 100)
- McFarland Standard (Ref. 70 900) point 4 on the scale or DENSIMAT (Ref. 99 234)
- API 20 Strep Analytical Profile Index (Ref. 20 690)
  - AGPS identification software (Ref. 40 011)
  - Columbia blood agar plates (Ref. 43 041)
  - Schaedler broth (optional)

Material
- Swabs
- Pipettes or PSIpettes
- Ampule rack
- Ampule protector
- Anaerobic jar
- General microbiology laboratory equipment

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use and microbiological control.
- For professional use only.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to "CLSI® M29-A, Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline - Current revision". For additional handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories - CDC/NIH - Latest edition", or to the regulations currently in use in each country.
- Do not use reagents past the expiry date.
- Before use, check that the packaging and components are intact.
- Do not use strips which have been damaged: cupules deformed, desiccant sachet open, etc.
- It is recommended to perform a quality control test when a new ampule of ZYM B reagent is opened.
- Open ampules carefully as follows :
  - Place the ampule in the ampule protector.
  - Hold the protected ampule in one hand in a vertical position (white plastic cap uppermost).
  - Press the cap down as far as possible.
  - Position the thumb tip on the striated part of the cap and press forward to snap off the top of the ampule.
  - Take the ampule out of the ampule protector and put the protector aside for subsequent use.
  - Carefully remove the cap.

The performance data presented were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.
STORAGE CONDITIONS
The strips and media should be stored at 2-8°C until the expiry date indicated on the packaging.

SPECIMENS (COLLECTION AND PREPARATION)
API 20 Strep is not for use directly with clinical or other specimens. The microorganisms to be identified must first be isolated on a suitable culture medium according to standard microbiological techniques.

INSTRUCTIONS FOR USE

Selection of colonies
Once the microorganism to be identified has been isolated and verified to be a member of the family Streptococcaceae (Gram, catalase test):
- Note the type of hemolysis on the result sheet (21st test).
- Pick a well-isolated colony (Note 1) and suspend it in 0.3 ml of sterile water. Homogenize well.
- Flood a Columbia sheep blood agar plate (Note 2) with this suspension (or aseptically swab the entire surface of the agar).
- Incubate the plate for 24 hours (± 2 hours) at 36°C ± 2°C in anaerobic conditions.

NOTE 1: β-hemolytic streptococci and enterococci produce sufficiently large colonies after 24 hours of incubation. For other streptococci, it is preferable to select a colony after 48 hours of incubation. For fastidious strains (producing minute colonies after 48 hours), the following procedure is recommended:
- Culture the colony in 1 ml of Schaedler broth at 36°C ± 2°C for 5 hours.
- Flood a Columbia sheep blood agar plate with the entire culture. Remove any excess liquid.
- Incubate the plate for 18-24 hours at 36°C ± 2°C in anaerobic conditions.

NOTE 2: In the case of suspected pneumococci, it is advisable to prepare 2 agar plates in order to obtain sufficient growth.

Preparation of the strip
- Prepare an incubation box (tray and lid) and distribute about 5 ml of distilled water or demineralized water [or any water without additives or chemicals which may release gases (e.g. Cl₂, CO₂, etc.)] into the honey-combed wells of the tray to create a humid atmosphere.
- Record the strain reference on the elongated flap of the tray. (Do not record the reference on the lid as it may be misplaced during the procedure).
- Remove the strip from its individual packaging.
- Place the strip in the incubation box.

Preparation of the inoculum
- Open an ampule of API Suspension Medium (2 ml) as indicated in the paragraph "Warnings and Precautions" or use any tube containing 2 ml of distilled water without additives.
- Using a swab, harvest all the culture from the previously prepared subculture plate.

- Make a dense suspension with a turbidity greater than 4 McFarland. This suspension must be used immediately after preparation.

Inoculation of the strip
- In the first half of the strip (tests VP to ADH), distribute this suspension, avoiding the formation of bubbles (tilt the strip slightly forwards and place the tip of the pipette or PSipette against the side of the cupule):
  - For the tests VP to LAP: distribute approximately 100 µl into each cupule.
  - For the ADH test: fill the tube only.
- In the second half of the strip (tests RIB to GLYG):
  - Open an ampule of API GP Medium as indicated in the paragraph "Warnings and Precautions" and transfer the rest of the suspension into it (appr. 0.5 ml). Mix well.
  - Distribute this new suspension into the tubes only.
- Fill the cupule of the underlined tests (ADH to GLYG) with mineral oil to form a convex meniscus.
- Place the lid on the tray.
- Incubate at 36°C ± 2°C in aerobic conditions for 4 - 4 ½ hours to obtain a first reading and for 24 hours (± 2 hours) to obtain a second reading if required.

READING AND INTERPRETATION

Reading the strip
After 4 hours of incubation:
- Add the reagents:
  - VP test: 1 drop of each of VP 1 and VP 2.
  - HIP test: 2 drops of NIN.
  - PYRA, αGAL, βGUR, βGAL, PAL and LAP tests: 1 drop of each of ZYM A and ZYM B (*).
- Wait 10 minutes, then read the reactions by referring to the Reading Table. If necessary, expose the strip to a strong light (10 seconds with a 1000 W lamp) to decolorize any excess reagents in tubes PYRA to LAP.

Reincubation is necessary in the following cases:
- low discrimination;
- unacceptable or doubtful profile;
- or if the following comment is given for the profile:
  IDENTIFICATION NOT VALID BEFORE 24 HOURS OF INCUBATION

In this case, after 24 hours, reread the reactions ESC, ADH, and RIB to GLYG. Do not reread the enzymatic reactions (HIP, PYRA, αGAL, βGUR, βGAL, PAL, LAP) and VP. Record all the reactions on the result sheet.

Interpretation
Identification is obtained with the numerical profile.
- Determination of the numerical profile:
  - On the result sheet, the tests are separated into groups of 3 and a value of 1, 2 or 4 is indicated for each. By adding together the values corresponding to positive reactions within each group, a 7-digit profile number is obtained.
• Identification:
  This is performed using the database (V 7.0)
  * with the Analytical Profile Index:
    - Look up the numerical profile in the list of profiles.
  * with the apiweb™ identification software:
    - Enter the 7-digit numerical profile manually via the keyboard.

NOTE: The hemolytic reaction constitutes the 21st test: β-hemolysis is considered as positive with a numerical value of 4. All other hemolytic reactions are considered as negative with a numerical value of 0. Nevertheless, this test may be of discriminant value for the identification of certain species.

QUALITY CONTROL

The media, strips and reagents are systematically quality controlled at various stages of their manufacture.

Streamlined quality control may be used to confirm acceptable performance of the API 20 Strep system after shipping-storage. This methodology may be performed by following the instructions above for testing and meeting the criteria stated in CLSI® M50-A Quality Control for Commercial Microbial Identification Systems.

Testing may be conducted using Streptococcus equi spp zooepidemicus ATCC® 700400 to evaluate the performance of the ARA test. Testing performed by bioMérieux has shown that the ARA test is the most labile on the API 20 Strep strip. When testing the strip, Streptococcus equi spp zooepidemicus ATCC 700400 can be used to detect degradation.

For those users who are required to perform comprehensive quality control testing with the strip, the following two strains should be tested to demonstrate positive and negative reactivity for most of the API 20 Strep tests.

1. Streptococcus equi spp zooepidemicus ATCC 700400
2. Streptococcus uberis ATCC 700407

ATCC : American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA.

<table>
<thead>
<tr>
<th></th>
<th>VP</th>
<th>HIP</th>
<th>ESC</th>
<th>PYRA</th>
<th>αGAL</th>
<th>βGUR</th>
<th>βGAL</th>
<th>PAL</th>
<th>LAP</th>
<th>ADH</th>
<th>RIB</th>
<th>ARA</th>
<th>MAN</th>
<th>SOR</th>
<th>LAC</th>
<th>TRE</th>
<th>INU</th>
<th>RAF</th>
<th>AMD</th>
<th>GLYG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>V</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* This result may vary depending on the culture medium used.
* Inoculum adjusted to between 4.5 and 5.5 McF using DENSIMAT.
* Profiles obtained after:
  - 4 hours of incubation for tests VP to LAP
  - 24 hours of incubation for tests ADH to GLYG.
* Strains cultured on Columbia sheep blood agar.
### READING TABLE

<table>
<thead>
<tr>
<th>TESTS</th>
<th>ACTIVE INGREDIENTS</th>
<th>QTY (mg/cup.)</th>
<th>REACTIONS/ENZYMES</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP</td>
<td>sodium pyruvate</td>
<td>1.9</td>
<td>acetoin production (Voges Proskauer)</td>
<td>NEGATIVE: Colorless, POSITIVE: Pink-Red</td>
</tr>
<tr>
<td>HIP</td>
<td>hippuric acid</td>
<td>0.4</td>
<td>hydrolysis (HIPpuric acid)</td>
<td>NEGATIVE: Colorless, POSITIVE: Colorless/Pale blue to Dark blue/Violet</td>
</tr>
<tr>
<td>ESC</td>
<td>esculin, feric citrate</td>
<td>1.16, 0.152</td>
<td>8-glucosidase hydrolysis (ESculin)</td>
<td>NEGATIVE: Colorless Pale yellow, POSITIVE: Colorless Pale yellow to Black/Grey/Black</td>
</tr>
<tr>
<td>PYRA</td>
<td>pyroglutamic acid, 6-naphthylamide</td>
<td>0.0256</td>
<td>PYRroldionyl Arylamidase</td>
<td>NEGATIVE: Colorless/Pale blue to Orange</td>
</tr>
<tr>
<td>αGAL</td>
<td>6-bromo-2-naphthyl-α-D-galactopyranoside</td>
<td>0.0376</td>
<td>α-Galactosidase</td>
<td>NEGATIVE: Colorless, POSITIVE: Violet</td>
</tr>
<tr>
<td>βGUR</td>
<td>naphthol ASBI-glucuronic acid</td>
<td>0.0537</td>
<td>β-Glucuronidase</td>
<td>NEGATIVE: Colorless, POSITIVE: Blue</td>
</tr>
<tr>
<td>βGAL</td>
<td>2-naphthyl-β-D-galactopyranoside</td>
<td>0.0306</td>
<td>β-Galactosidase</td>
<td>NEGATIVE: Colorless or Very pale violet, POSITIVE: Violet</td>
</tr>
<tr>
<td>PAL</td>
<td>2-naphthyl phosphate</td>
<td>0.0244</td>
<td>ALkaline Phosphatase</td>
<td>NEGATIVE: Colorless or Very pale violet, POSITIVE: Violet</td>
</tr>
<tr>
<td>LAP</td>
<td>L-leucine-6-naphthylamide</td>
<td>0.0256</td>
<td>Leucine AminoPeptidase</td>
<td>NEGATIVE: Colorless, POSITIVE: Orange</td>
</tr>
<tr>
<td>ADH</td>
<td>L-arginine</td>
<td>1.9</td>
<td>Arginine DiHydrolase</td>
<td>NEGATIVE: Colorless or Very pale orange, POSITIVE: Orange</td>
</tr>
<tr>
<td>RIB</td>
<td>D-ribose</td>
<td>1.4</td>
<td>acidification (RIBose)</td>
<td>NEGATIVE: Red, POSITIVE: Orange/Yellow to Yellow</td>
</tr>
<tr>
<td>ARA</td>
<td>L-arabinose</td>
<td>1.4</td>
<td>acidification (ARArinose)</td>
<td>NEGATIVE: Red, POSITIVE: Orange/Yellow to Yellow</td>
</tr>
<tr>
<td>MAN</td>
<td>D-mannitol</td>
<td>1.36</td>
<td>acidification (MANnitol)</td>
<td>NEGATIVE: Orange/Yellow to Yellow, POSITIVE: Orange/Yellow</td>
</tr>
<tr>
<td>SOR</td>
<td>D-sorbitol</td>
<td>1.36</td>
<td>acidification (SORbitol)</td>
<td>NEGATIVE: Orange/Yellow, POSITIVE: Orange/Yellow to Yellow</td>
</tr>
<tr>
<td>LAC</td>
<td>D-lactose (bovine origin)</td>
<td>1.4</td>
<td>acidification (LAClose)</td>
<td>NEGATIVE: Red, POSITIVE: Orange/Yellow</td>
</tr>
<tr>
<td>TRE</td>
<td>D-trehalose</td>
<td>1.32</td>
<td>acidification (TREhalose)</td>
<td>NEGATIVE: Red, POSITIVE: Orange/Yellow</td>
</tr>
<tr>
<td>INU</td>
<td>inulin</td>
<td>5.12</td>
<td>acidification (INUlin)</td>
<td>NEGATIVE: Red, POSITIVE: Orange/Yellow</td>
</tr>
<tr>
<td>RAF</td>
<td>D-raffinose</td>
<td>3.12</td>
<td>acidification (RAFfinose)</td>
<td>NEGATIVE: Red, POSITIVE: Orange/Yellow</td>
</tr>
<tr>
<td>AMD</td>
<td>starch (2)</td>
<td>2.56</td>
<td>acidification (AmiDon)</td>
<td>NEGATIVE: Red, POSITIVE: Orange/Yellow</td>
</tr>
<tr>
<td>GLYG</td>
<td>glycogen</td>
<td>1.28</td>
<td>acidification (GLYcoGen)</td>
<td>NEGATIVE: Red or Orange, POSITIVE: Bright yellow</td>
</tr>
</tbody>
</table>

(1) During a second reading after 24 hours of incubation, a deposit may be noticed in the tubes where the ZYM A and ZYM B reagents have been added. This phenomenon is normal and should not be taken into consideration.
(2) The acidification of starch is frequently weaker than that of other sugars.
(3) A pale pink color obtained after 10 minutes should be considered negative.
- The quantities indicated may be adjusted depending on the titer of the raw materials used.
- Certain cupules contain products of animal origin, notably peptones.

**PROCEDURE** p. I  **LITERATURE REFERENCES** p. III
**IDENTIFICATION TABLE** p. II  **INDEX OF SYMBOLS** p. IV
METHODOLOGIE / PROCEDURE / METHODIK / TECNICA / PROCEDIMENTO / ΔΙΑΔΙΚΑΣΙΑ / METOD / METODE / METODYKA

Gélose au sang / Blood agar / Blutagar /
Agar con sangre / Agar al sangue / Gelose de sangue / Αιματούχο δύοπ / Blodagar / Agar krwawy

Gélose Columbia au sang / Columbia blood agar / Columbia Blutagar / Agar Columbia con sangre / Agar Columbia al sangue / Gelose Columbia de sangue / Αιματούχο αγαρ Columbia / Columbia blodagar / Agar Columbia z krwią

24:00 ± 2:00  36°C ± 2°C

API Suspension Medium 2 ml

~ 500 µl

API GP Medium

4:00 – 4:30  36°C ± 2°C

24:00 ± 2:00  36°C ± 2°C

API 20 Strep

+ - + - + - 

- Cocc / Kokken / Cocos / Cocchi / Κόκκοι / Kocker / Kokker / Ziarniaki
- Gram +
- Catalase / Katalase / Catalas / Καταλάση / Katalas / Katalase / Katalaza –

> 4 McF

VP ➔ ADH
ADH

RIB ➔ GLYG

VP : VP 1 + VP 2
HIP : NIN
PYRA ➔ LAP : ZYM A + ZYM B

bioMérieux SA
| Streptococcus uberis | 99 | 98 | 97 | 96 | 95 | 94 | 93 | 92 | 91 | 90 | 89 | 88 | 87 | 86 | 85 | 84 | 83 | 82 | 81 | 80 | 79 | 78 | 77 | 76 | 75 | 74 | 73 | 72 | 71 | 70 | 69 | 68 | 67 | 66 | 65 | 64 | 63 | 62 | 61 | 60 | 59 | 58 | 57 | 56 | 55 | 54 | 53 | 52 | 51 | 50 | 49 | 48 | 47 | 46 | 45 | 44 | 43 | 42 | 41 | 40 | 39 | 38 | 37 | 36 | 35 | 34 | 33 | 32 | 31 | 30 | 29 | 28 | 27 | 26 | 25 | 24 | 23 | 22 | 21 | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |}

**TABELLA DI IDENTIFICAZIONE / IDENTIFICATION TABLE / PROZENTTABELLE / TABLA DE IDENTIFICACION / TABELLA DI IDENTIFICAZIONE / QUADRO DE IDENTIFICAÇÃO / IDENTIFIERINGSTABELL / IDENTIFIKATIONSTABEL / TABELA IDENTYFIKACYJNA**

- **% de réactions positives après 4/24 h à 36°C ± 2°C / % of reactions positive after 4/24 hrs. at 36°C ± 2°C / % der positiven Reaktionen nach 4/24 h bei 36°C ± 2°C / % de las reacciones positivas después de 4/24 h a 36°C ± 2°C / % de реакции положительные после 4/24 ч o 36°C ± 2°C / % das reações positivas após 4/24 h a 36°C ± 2°C / % relatives reactionen po 4/24 godzinach w 36°C ± 2°C /
- **% des réactions positives après 4/24 h à 36°C ± 2°C / % of reactions positive after 4/24 hrs. at 36°C ± 2°C / % der positiven Reaktionen nach 4/24 h bei 36°C ± 2°C / % de las reacciones positivas después de 4/24 h a 36°C ± 2°C / % des реакции положительные после 4/24 ч о 36°C ± 2°C / % das reações positivas após 4/24 h a 36°C ± 2°C / % relatives reactionen po 4/24 godzinach w 36°C ± 2°C /

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**Voir § Limites du test / See § Limitations of the method / Siehe § Limitierungen / Ver § Limites del método / Veder § Limitet del metode / Consultar § Limites do teste / Вижте § Поръчвания Методът / See annex "Methoden begränsningar" / Se § Methodens begränsningar / Patrz § Ograniczenia testu**
1. APPELBAUM P.C., CHAURUSHIYA P.S., JACOBS M.R., DUFFETT A.
   Evaluation of the Rapid Strep System for Species Identification of Streptococci.

2. BALL L.C., COLMAN G.
   A Comparison of Conventional Methods and API Galleries for the Identification of Streptococci.

3. BANNISTER M.F., BENSON C.E. and SWEENEY C.R.
   Rapid Species Identification of Group C Streptococci Isolated from Horses.

4. COLMAN G., BALL L.C.
   Identification of Streptococci in a Medical Laboratory.

5. FACKLAM R.R., RHODEN D.L., SMITH P.B.
   Evaluation of the Rapid Strep System for the Identification of Clinical Isolates of Streptococcus Species.

6. HUMAN R.P. and TILLOTSON G.S.
   Identification of Gardnerella vaginalis with the API 20 Strep System.

7. KLOOSTERMAN R.E., CULLEN K.D., McCLATCHEY K.D.
   Comparison of Two Commercial Systems for the Rapid Identification of Streptococci.

8. MacGOWAN A.P., MARSHALL R.J., REEVES D.S.
   Evaluation of API 20 STREP System for Identifying Listeria Species.

9. RUOFF K.L., KUNZ L.J.
   Use of the Rapid STREP System for Identification of Viridans Streptococcal Species.

10. TILLOTSON G.S.
    An Evaluation of the API 20 Strep System.

<table>
<thead>
<tr>
<th>Symbole / Symbol</th>
<th>Signification / Meaning / Bedeutung</th>
<th>Référence du catalogue / Catalogue number (GB) / Catalog number (US)</th>
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<tr>
<td>REF</td>
<td>Dispositif médical de diagnostic in vitro / In Vitro Diagnostic Medical Device / In Vitro Diagnostikum</td>
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<td>Producto sanitario para diagnóstico in vitro / Dispositivo medico-diagnostico in vitro / Producto sanitario para diagnóstico in vitro</td>
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<tr>
<td></td>
<td>Code du lot / Batch code / Code del lote / Código de lote / Αριθμός Παρτίδας / Lot number / Lotnummer / Kod partii</td>
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<tr>
<td></td>
<td>Consulter les instructions d'utilisation / Consult Instructions for Use / Gebrauchsanweisung beachten / Consulte las instrucciones de uso</td>
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</tr>
<tr>
<td></td>
<td>Contenu suffisant pour &quot;n&quot; tests / Contains sufficient for &lt;n&gt; tests / Inhalts ausreichend für &lt;n&gt; Prüfungen</td>
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<tr>
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<td>Wystarczy na wykonanie &lt;n&gt; testów / Wystarczy na wykonanie &quot;n&quot; testów</td>
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</tbody>
</table>

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