Intended Use
LIFESIGN-PYR is a rapid colorimetric test for the determination of PYRase activity in streptococcal organisms.

Summary & Explanation
The LIFESIGN-PYR test for the detection of PYRase activity in streptococcal organisms offers a rapid diagnostic alternative to time consuming culture methods such as growth in 6.5% NaCl. PYRase activity is indicative of enterococci or Group A streptococci. In association with serological grouping, this test will provide identification of the enterococci or Group A streptococci. All enterococci are PYRase positive while none of the Group D streptococci (S. bovis, previously called non-enterococci) possess this activity.

Principle
The LIFESIGN-PYR test utilizes test cards impregnated with substrate for the detection of PYRase activity. The enzymatic hydrolysis of this substrate by enterococci or Group A streptococci organisms produces a red color upon the addition of the color developer.

Reagents
Each LIFESIGN-PYR kit contains the following reagents sufficient for 60 tests:
- Test Cards: Sixty test cards each containing filter paper impregnated with L-pyroglutamic acid-2-naphthylamide.
- Buffer: One dropper bottle containing 28 ml of saline solution with 0.05% sodium azide.
- Color Developer: One dropper bottle containing 14 ml of P-Dimethylaminocinnamaldehyde with 0.05% sodium azide.

Warnings and Precautions
For in vitro diagnostic use only.

Do not use the LIFESIGN-PYR reagents past the expiration date.

The buffer in this kit contains 0.05% sodium azide as a preservative. Azides can react with copper and lead used in some plumbing systems to form explosive salts. When disposing of azide-containing materials they should be flushed away with large volumes of water.

Specimen material may contain pathogenic organisms; handle with appropriate precautions.

Used test cards and mixing sticks should be disposed of as biohazardous waste and incinerated or autoclaved for 15 minutes at 121°C.

Avoid skin contact with the Color Developer. Rinse contact area with water.

Do not use test cards if test area is discolored.

Materials Provided
- 60 Test Cards
- Buffer
- Color Developer

Materials Required But Not Provided
- Wooden mixing sticks or microbiological loop
- Watch or timer

Test Procedure
Use one test card for each specimen to be tested.
1. Apply several suspect colonies (0.5mm or larger) onto the test area (enough to make a visible smear).
2. Moisten test area with 3 drops of Buffer.
3. Incubate the inoculated test card at room temperature (15°C - 30°C) for 5 minutes.
4. Dispense 3 drops of Color Developer onto the test area. Development of a red color on and around the colonies within 20 seconds confirms PYRase activity.

Reading and Interpretation of Results
Positive Result
A positive result is indicated by the development of a red color in the inoculated portion of the test area within 20 seconds after adding the color developer.

Negative Result
A negative result is indicated by no color development in the inoculated portion of the test area within 20 seconds after adding the color developer.

Interpretation Chart

<table>
<thead>
<tr>
<th>Organism</th>
<th>Reactivity With</th>
<th>Lancefield Group</th>
<th>LifeSign PYR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pyogenes</td>
<td>A</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>B</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Streptococcus Group C</td>
<td>C</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Streptococcus bovis</td>
<td>D</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>D</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>D</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Streptococcus Group F</td>
<td>F</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Streptococcus Group G</td>
<td>G</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Quality Control Procedure
Each day the kit is used the following control procedures should be performed.

1. **Positive Control** - Use a known PYR positive strain such as *E. faecalis* ATCC #29212 or *S. pyogenes* ATCC #19615. Follow the method given in the Test Procedure. Ensure that a red color forms within 20 seconds.

2. **Negative Control** - Use a known PYR negative strain such as *S. agalactiae* ATCC #13813. Follow the method given in the Test Procedure. Ensure that no red color forms within 20 seconds.

Do not use the reagents if reactions with control organisms are incorrect.

Limitations of the Test
LIFESIGN-PYR is intended for the detection of PYRase activity in gram positive, catalase negative cocci.

Some less commonly encountered isolates of lactococci and aerococci may be PYRase positive. The confirmation of enterococci or Group A streptococci can be achieved by serological grouping with a suitable test, e.g., Streptococcal Grouping Kit (LifeSign, Stock No. 50040).

Reading results after 20 seconds may produce non-specific color reactions.

Performance Characteristics
The LIFESIGN-PYR test was evaluated using 185 strains of streptococci and enterococci: 96 isolates of enterococci, 47 isolates of Group A streptococci and 42 isolates of non-enterococci/non Group A streptococci. Reactions were confirmed with commercially available biochemical and serological assays. The LIFESIGN-PYR kit demonstrated 100% agreement with the other commercial tests.

<table>
<thead>
<tr>
<th>Organisms Identity</th>
<th># of Specimens Tested</th>
<th>PYRase Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>enterococci</td>
<td>96</td>
<td>+</td>
</tr>
<tr>
<td><em>S. pyogenes</em> (Group A streptococci)</td>
<td>47</td>
<td>+</td>
</tr>
<tr>
<td>Non-Group A streptococci and/or Non-enterococci</td>
<td>42</td>
<td>-</td>
</tr>
</tbody>
</table>

References


