SIMPLE BIOCHEMICAL TEST FOR THE IDENTIFICATION OF BACTERIA IN LABORATORY

Author’s name: 1Aqsa Rehman, 2Hafsa Quraishi and 3Muhammad Muzammal
1,3Gomal Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, KPK, Pakistan
2Department of Biotechnology, BushraMateen Building, Lahore collage For Women University, Lahore
Corresponding E-mail: aqsarehman7576@gmail.com

Abstract
One of the most difficult task for young microbiologists is the isolation and identification of unknown bacteria form different sources in their university or collage laboratory. Most of the time they got succeeded by studying different literature but not all the time and they have to purchase ATCC stains to continue their work but ATCC are expensive for them and it is not easy for every student to buy it. Identification of bacteria is difficult but by using different simple test and proper approach, young researchers can identify bacteria in their laboratory. In this review some of the most commonly used test are mentioned for the isolation of bacteria in small scale university or collage laboratory. In this review Nine simple tests are mentioned and by using these test Young microbiologists can almost identify bacteria without any harden. Although it is just a mini review but it can be very handy for young microbiologists and young researchers of different fields to get the detail in one paper instead of reading different literature.

Keywords
isolation, identification, Bacteria and Test

INTRODUCTION
Researchers of bacteriology and microbiology are working day by day to understand the mechanism of different bacteria and microbes. They are isolating bacteria from different sources to use them in different field’s i.e. antibiotic production, enzyme production and different dairy products. But before that they have to identify these bacteria from huge bulk of different bacterial colonies. So for this reason they need certain biochemical test to identify bacteria (Talaiekhozani et al., 2015; Gajdács et al.,2017; Muzammal et al., 2019). In this review we have mention some of the simple tests, which can be done in the laboratory to identify bacteria. Our review will be handy to the young research related to bacteriology and microbiology and besides going foe expensive ATCC strains, they can use these test to identify bacteria isolated from different sources. There are different tests to identify bacteria but in this review we will discuss some tests that are commonly used in the laboratory. These tests includes the following as mentioned below:

GRAM STAINING

Purpose
Gram staining technique was developed by the Danish physician Christian Gram in 1884. This techniques used to separate bacteria into two groups on the basis of composition of cell wall. On the basis of results we categorize bacteria into gram positive and gram negative bacteria (Sandle, 2004)

Procedure
1. Take a drop on autoclaved distill water on the clean Petri dish.
2. With the help of clean loop touch a bacteria in the petri dish and then mix it with drop of water present on the petri dish.
3. Mix it so the solution become slightly turbid and left for air dry.
4. Slightly heat the slide with the help of sprit lamp (Do not over heat) and allow to cool.
5. Put a 2 - 3ml (Approx.) of crystal violet dye on the bacterial smear and leave it for 1-2 min.
6. After that wash the slide with tap water to remove crystal violet dye.
7. Now apply 2-3ml of Gram’s Iodine solution and left the slide for 1-2 min.
8. Wash the slide with tap water.
9. Now apply decolorizing solution (Alcohol) to decolorize the smear.
10. Again wash with tap water.
11. Apply 2-3ml safranin and left the slide for 1-2 min.
12. Wash the slide with tap water and allow to dry.
13. Examine the result under the microscope.

RESULTS

a) Gram Negative
Cells of gram negative bacteria will be decolorized by applying alcohol (9th step) and appears pink or red when washed with safranin (11th step)(Sandle, 2004).

b) Gram Positive
Cells of gram positive bacteria will not be decolorized (9th step) and remains violet at the end (1st step)(Sandle, 2004).

Some common bacteria and their gram staining results are in figure 1.

![Figure 1: Gram staining result and colony color of different medically important bacteria](image)

QUALITY CONTROL
For Best result and quality control, also use known bacterial specie while performing Gram staining as positive control. Not all bacteria can be separated on the basis of gram staining. There are many medically important bacteria that cannot be separated by Gram staining and their reason is in table 1.

![Quality Control Table](table)

MOBILITY TEST

**Purpose**
Presence of flagella on the bacteria shows its mobility. So mobility test is used to determine whether the bacteria are motile or not (Hemraj et al., 2013).

**Procedure**
This method is known as semi-solid medium method.

1. In this method, any unknown bacteria are inoculated in the semi-solid media in test tube/flask.
2. Incubate for 24 hours.
3. Examine the results by turbidity of media.
RESULTS

**Positive result**
If bacteria are motile then the media become turbid. Clearly seen through naked eyes.

**Negative Results**
If bacteria are non-motile then a turbid line in the middle of the media will be formed where the actual inoculum was done. Clearly seen through naked eyes (Hemraj et al., 2013).

**Quality control**
For best result and quality control, also use known bacterial species while performing semi-solid medium method as positive control.

**CATALASE TEST**

**Purpose**
This test is used to identify bacteria that produce catalyze enzyme. In this test hydrogen peroxide (H$_2$O$_2$) is used. Bacteria that produce catalase will decompose hydrogen per oxide (H$_2$O$_2$ into H$_2$O and O$_2$ (Constantinescu et al., 2001; Reiner, 2010; Fung and Petrishko, 1973).

**Procedure**
1. Make smear of the unknown bacteria on the clear slide with the help of wire loop.
2. Apply one drop on hydrogen per oxide (H$_2$O$_2$) on it.
3. Result can be seen immediately.

**RESULTS**

**Positive Results**
Bacteria are catalase positive if bubble are form (O$_2$) as in figure 2

**Negative Results**
Bacteria are catalase Negative if no bubbles are form.

**Quality control**
For best result and quality control, also use known bacterial species while performing catalase test as positive control.

**Figure 2: Catalase positive bacteria producing O$_2$ after applying H$_2$O$_2$**

**OXIDASE TEST**

**Purpose**
In this test cytochrome oxidase as known as indophenol oxidase, is detected. Bacteria which contain this enzyme will convert colorless reagent into colored product. This test can be performed by many methods i.e. filter paper spot test, filter paper method direct plate method (Tarrand and Gröschel, 1982; Gadeholt, 1964). Today we will discuss filter paper test method.

**Procedure-Filter Paper Test Method**
1. In this test filter paper (Available in laboratory) is soaked in the 1% Kovács oxidase reagent and allow to dry.
2. Now with the help of sterilized wire loop apply a unknown bacterial specie (Present in the form of culture) on the filter paper which is soaked in in the 1% Kovács oxidase reagent.
3. Note for color changes.

**RESULT**

**Positive result**
Bacteria are oxidase positive if color changes into dark purple.

**Negative result**
Bacteria are oxidase negative if no color change is detected.

**Quality control**
For Best result and quality control, also use known bacterial specie, while performing oxidase test as positive control.

**INDOLE TEST**

**Purpose**
The indole test is used for the ability of a bacteria to reduce the amino acid tryptophan and produce indole(Miller and Wright,1982;MacWilliams, 2012)

**Procedure**
1. Small amount of unknown bacterial specie is applied to the tube of tryptone broth with a small amount of a pure culture.
2. Incubate for 24 hours at 37°C.
3. Add 5 drops of Kovác's reagent directly to the tube with the help of dropper.

**RESULTS**

**Positive result**
Red or pink ring will be formed on the surface of media

**Negative result**
No change in the media color (Remains yellow)

**Quality control**
For Best result and quality control, also use known bacterial specie, while performing Indole test as positive control.

**GELATINASE TEST**

**Purpose**
This is another simple test for identification of bacteria. In this test bacteria is identified by production of enzyme known as gelatin as enzyme. This enzyme liquefy gelatin (Calander et al., 2006;Nakayama et al.,2006).

**Procedure**
1. Unknown bacterial species are inoculated into nutrient gelatin media( stab ½ to 1 inch deep into the media) for 24-36 hours at 37°C.
2. Bacteria used in this test are of high growth in the petri dishes.

**RESULTS**

**Positive result**
Media will be liquefying. Try to pour a drop of media on your palm. Dropping of a drop will confirm positive result.

**Negative result**
No liquefaction occurs in media.

**Quality control**
For Best result and quality control, also use known bacterial specie, while performing Gelatinase test as a positive control.

**DECARBOXYLASE TEST**

**Purpose**
This test is used to determine the enzymatic activity of bacteria to decarboxylase any amino acid
especially (Lysine) and convert into amine with resultant alkalinity (Fay and Barry, 1972; Freier et al., 1976).

**Procedure**

1. In this test Light inoculum will be used in lysine and decarboxylase test tubes.
2. Add 1.5 to 2.5 ml oil in each tube contains inoculum.
3. Incubate at 20°C for 24 hours (Prolonged incubation is recommended).

**RESULTS**

Results of Decarboxylase test is in table 2

<table>
<thead>
<tr>
<th>Table 2: Result of Decarboxylase test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test Results</strong></td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td><strong>Positive</strong></td>
</tr>
<tr>
<td><strong>Negative</strong></td>
</tr>
<tr>
<td><strong>Negative</strong></td>
</tr>
</tbody>
</table>

**Quality control**

For Best result and quality control, also use known bacterial specie, while performing Gelatinase test as a positive control.

**MALONATE TEST**

**Purpose**

This test is used to identify those bacteria, which are able to utilize only sodium malonate as its only carbon source (Leifson, 1933; Fleury and Magnolato, 1992)

**Procedure**

1. Inoculate the malonate media with unknown specie of bacteria for 24 to 48 hours at 20°C.
2. Light inoculum will be used.
3. Results will be examined by color change.

**RESULT**

**Positive Results**

Malonate Media will turn deep blue from green.

**Negative Results**

Malonate media will remains green.

**Quality control**

For Best result and quality control, also use known bacterial specie, while performing Malonate test as a positive control.

**ESCULIN TEST**

**Purpose**

This test is used to identify those bacteria which has the ability to hydrolyze the glycoside esculin (aesculin) toesculetin (aesculetin) and glucose in the presence of bile (10 to 40%) (Facklam and Moody, 1970; Qadri and Johnson, 1981).

**Procedure**

1. Prepare slant of bile esculin slant.
2. Inoculate the slant with unknown bacterial specie and incubate for 24 to 36 hours at 20°C.
3. Results will be examined by color change.

**RESULTS**

**Positive result**

Dark Brown or black color form. Confirm the presence of esculin positive bacteria.
Negative result
No change in the color of the slant

Quality control
For Best result and quality control, also use known bacterial specie, while performing Esculin test as a positive control.

For overall result of all the tests see table 3.

**Table 3: Overall results of all the above mention test**

<table>
<thead>
<tr>
<th>S NO</th>
<th>Test Name</th>
<th>Positive Result</th>
<th>Negative Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gram Stain</td>
<td>Purple Color</td>
<td>Pink Color</td>
</tr>
<tr>
<td>2</td>
<td>Motility test</td>
<td>Diffuse growth</td>
<td>Stab Line</td>
</tr>
<tr>
<td>3</td>
<td>Catalase Test</td>
<td>Bubbles formation</td>
<td>Clear or No bubbles</td>
</tr>
<tr>
<td>4</td>
<td>Oxidase</td>
<td>Blue or Purple Color</td>
<td>No change in Color</td>
</tr>
<tr>
<td>5</td>
<td>Gelatinase Test</td>
<td>Liquid or partial liquid tube at 4°C</td>
<td>Solid tube at 4°C</td>
</tr>
<tr>
<td>6</td>
<td>Indole test</td>
<td>Red color</td>
<td>Yellow color</td>
</tr>
<tr>
<td>7</td>
<td>Decarboxylase Test</td>
<td>Purple Color</td>
<td>Yellow Color</td>
</tr>
<tr>
<td>8</td>
<td>Malonate Test</td>
<td>Blue Color</td>
<td>Green Color</td>
</tr>
<tr>
<td>9</td>
<td>Esculin Test</td>
<td>Dark Brown or black</td>
<td>No change</td>
</tr>
</tbody>
</table>

There are number of test available to identify bacteria depending on specie to specie but we hope the Staring from nowhere and after the end of the mentions test, researchers will be able to almost identify the bacterial specie. Step by step tests and end result is in figure 3.

**Figure 3: Step by step tests and end result of tests.**

Below is the detail of some of the commonly used bacteria and their test result in figure 4

**CONCLUSION**
There are lot of biochemical test are performed to identify bacteria. Some of those tests are easy while other is difficult. In this review we tries to highlight some of the common test available in the laboratory to identify bacteria. We hope our effort will be highly appreciated by the young researchers who find difficulty to identify bacteria in their collage research.

**Figure 4: Test result of different bacteria commonly used in university and collage laboratory.**
REFERENCES


