JOURNAL OF UNIVERSITY OF BABYLON
For Pure and Applied Sciences (JUBPAS)

ARTICLE

Vol.29; No.2. May-August | 2021

Biochemical Testing Revision For Identification Several Kinds of Bacteria

Ban Mahmood Shaker AL-Joda [1] Aziz H. Jasim[2]

[1]College of Medicine, University of Babylon, Aljodaban@gmail.com/ Babel, Iraq
[2]College of Medical Technology, Al-Zahrawi University College/ Azizhusain750@yahoo.com, Kerbala, Iraq.

*Corresponding auther email: Aljodaban@gmail.com; Mobile: 07721138921.

Received: 24/5/2021	Accepted: 24/6/2021	Published: 1/7/2021

Abstract:

Bacteria are pathogenic germs that cause a variety of diseases in humans, ranging from minor to life-threatening. Proper detection of the disease-causing bacterial agent is required for proper treatment of patients affected with these disorders.

Bacteria are classified into two groups: Gram Positive Bacteria and Gram Negative Bacteria. Both types of bacteria have a variety of inherited biochemical traits that allow us to distinguish them, check for their presence and absence, and determine whether they are gram negative or gram positive. As a result, the current review focuses on describing many biochemical assays in a single piece.

Conclusion

Gram positive bacteria are identified using biochemical tests such as the catalase test, coagulase test, starch hydrolysis test, and nitrate test, while Gram negative bacteria are identified using biochemical tests such as the oxidase test, urease test, indole test, sulfur test, and methyl red /voges-proskauer test. The analytical profile index test 20E was created to distinguish between Gram-negative Enterbacteriacea and non-Enterbactriacea bacteria. Gram-positive microbes such as Staphylococcus species, Micrococcus species, and other related organisms have also been generated using the API method.

Keywords:

Microorganism, Bacteria, Biochemical Tests

Citation:

Ban Mahmood Shaker AL-Joda [1] & Aziz H. Jasim[2]. **Studying the Efficiency Dependence of CR-39 Detector on the Chamber Height** . Journal of University of Babylon for Pure and applied science (JUBPAS). May-August, 2021. Vol.29; No.2; p:168-176 .

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Vol.29; No.2. May-August | 2021

مراجعة للاختبارات الكيمياحيوية لتشخيص عدة انواع من البكتريا

بان محمود شاكر الجودة[1] عزيز حسين جاسم[2]

[1]كلية الطب,جامعة بابل, aljodaban@gmail.com, بابل, العراق
[2]كلية الزهراوي الجامعة, Azizhusain750@yahoo.com, كربلاء, العراق

Received: 16/12/2021 **Accepted:** 9/2/2021 **Published:** 1/7/2021

الخلاصة:

البكتيريا هي الجراثيم المسببة للأمراض التي تسبب مجموعة متنوعة من الأمراض لدى البشر ، تتراوح من الطفيفة إلى التي تهدد الحياة. مطلوب الاكتشاف المناسب للعامل البكتيري المسبب للمرض من أجل العلاج المناسب للمرضى المصابين بهذه الاضطرابات.

يتم تصنيف البكتيريا إلى مجموعتين: البكتيريا موجبة الجرام والبكتيريا سلبية الجرام. يحتوي كلا النوعين من البكتيريا على مجموعة متنوعة من السمات البيوكيميائية الموروثة التي تسمح لنا بتمييزها ، والتحقق من وجودها وغيابها ، وتحديد ما إذا كانت سالبة الجرام أو موجبة الجرام. نتيجة لذلك ، تركز المراجعة الحالية على وصف العديد من الاختبارات الكيميائية الحيوية في قطعة واحدة.

الاستنتاجات

يتم التعرف على البكتيريا موجبة الجرام باستخدام الاختبارات الكيميائية الحيوية مثل اختبار الكاتلاز ، واختبار تجلط الدم ، واختبار التحلل المائي للنشا ، واختبار النترات ، بينما يتم تحديد البكتيريا سالبة الجرام باستخدام الاختبارات الكيميائية الحيوية مثل اختبار أوكسيديز ، واختبار اليورياز ، واختبار إندول ، واختبار الكبريت ، و اختبار الميثيل الأحمر / voges–proskauer. تم إنشاء اختبار المؤشر التحليلي E20 للتمييز بين بكتيريا Enterbacteriacea سالبة الجرام والبكتيريا غير Enterbactriacea. تم أيضًا إنشاء ميكروبات موجبة الجرام مثل أنواع المكورات العنقودية وأنواع المكورات الدقيقة والكائنات الحية الأخرى ذات الصلة باستخدام طريقة API.

الكلمات الدالة

الكائنات الحية الدقيقة Microorganism ، البكتيريا Bacteria ، الاختبارات البيوكيميائية Biochemical Tests .

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Ban Mahmood Shaker AL-Joda [1] & Aziz H. Jasim[2]. **Studying the Efficiency Dependence of CR-39 Detector on the Chamber Height** . Journal of University of Babylon for Pure and applied science (JUBPAS). May-August, 2021. Vol.29; No.2; p:168-176 .

INTRODUCTION

Different metabolic activities displayed by different species of bacteria are employed in biochemical assays for microbiological identification. The several biochemical tests used to identify gram positive and negative bacteria are mentioned below.

i. Catalase Test
ii. Coagulase Test
iii. Oxidase Test
iv. Indole Test
v. Sulfur Test
vi. Urease Test
vii. Triple sugar iron test
viii. Nitrate Test
ix. Starch Hydrolysis Test
x. Carbohydrate Fermentation Test
xi. Methyl Red Test
xii. Voges-Proskaur Test
xiii. Citric Acid Utilization Test
xiv. Bile Esculin Agar Test
xv. Analytical Profile Index Test

1.1 Catalase Test

The catalase enzyme is produced by bacteria, and this test is used to see if they produce it. The hydrogen peroxide will be neutralized by the catalase enzyme generated by these bacteria, and bubbles will form, indicating a positive test. Obligate aerobes and facultative anaerobic bacteria are the primary producers of catalase enzyme. The test is done on a slide or in a test tube by mixing a colony of bacteria with a few drops of 3 percent H2O2 and looking for bubble formation within 10 seconds [1].

1.2 Coagulase Test

The following test is used to identify microorganisms that can manufacture the coagulase enzyme. Mostly, it aids in the identification of Staphylococcus aureus, which is a coagulase and catalase test positive bacteria.

Coagulase is one of the virulence factors found in S. aureus. During the reaction phase, the coagulase enzyme will coagulate the blood plasma. This test is carried out by combining blood plasma with a bacterial colony. Bacteria generate the coagulase enzyme, which causes the blood plasma to coagulate, indicating a positive reaction [2].

1.3 Oxidase Test

The oxidase test is useful in identifying microorganisms that may manufacture cytochrome oxidase enzyme. The test distinguishes between the oxidase-positive Pseudomonacea and the oxidase-negative Enterobacteriacea groups. Cytochrome oxidase works on the basis of electron transfer from the donor (electron transport chain) to the final acceptor (oxygen), with water as the final acceptor. The electron donor will be oxidized by cytochrome oxidase, and the hue will change to dark purple. This test is carried out by impregnating a filter paper with 1 percent tetra-methyl-p-phenylenediamine dihydrochloride, which acts as an artificial electron donor, and drying it. The bacterial colonies are smeared on a paper strip, and the color change is checked after 10 seconds [3].

1.4 Indole Test

The test below can be used to identify bacteria that have the ability to manufacture tryptophanase enzyme. This enzyme converts the amino acid tryptophan to indole gas. As a result, several reagents, such as Ehrlich's reagent or Kovac's reagent, can be used to check the gas. In isoamyl alcohol and concHCl, Kovac's indicators contain para-dimethyl amino benzaldehyde, but Ehrlich's contain ethanol instead of isoamyl alcohol. Indole gas combines with the reagent, forming the red rosindole dye, indicating a positive result [4].

1.5 Sulfur Test

The sulfur test is useful in identifying microorganisms that can produce the cysteine desulfurase enzyme. This enzyme converts cysteine (an amino acid) to thiosulphate, which then converts sulfur to hydrogen peroxide. The color of the medium turns to black due to the generation of hydrogen sulfide. The organism Proteus mirabilis is sulfur-positive [5].

1.6 Urease Test

The urease test is used to identify bacteria that can produce the urease enzyme. This enzyme belongs to the superfamily of amidohydrolases and phosphodoesterases. Urease is an enzyme that breaks down urea into NH3 and CO2. The creation of ammonia raises the pH of the medium to alkaline, and the color changes to pink at pH 8.1, signifying positive findings. This test is used to identify Helicobacter pylori strains that produce urease. The test is carried out by soaking infected stomach mucosa or bacteria colonies in urea broth. A color shift within 30 minutes indicates a positive test [6].

1.7 Triple Sugar Iron Test

This test is useful for identifying microorganisms that belong to the Enterbacteriaceae family. The test medium is made up of three sugars: 0.1 percent glucose, 1% lactose, and 1% sucrose. Red phenol and ferrous as an indication, sulphate is utilized.

Butt and tilt are used to prepare the media. The glucose is used by the injected bacteria, and glucose intake is kept low in comparison to other sugars. Within 6-8 hours of inoculation, both the slant and butt color will shift to yellow due to acid generation if the bacteria can use glucose in both aerobic and anaerobic circumstances. If the bacteria are able to use sucrose and lactose, acid production will continue and the color of the media will remain yellow. If it can't use sucrose or lactose, the bacteria turns to amino acids, which makes the medium alkaline and turns the color of the medium red owing to phenol red. If the bacteria is a strict aerobe, the color of the butt will not change and the response will only take place in a slant. The reaction will occur in both if the bacteria is a facultative anaerobe. Many kinds of bacteria produce hydrogen peroxide gas by reducing thiosulfate, as indicated by rising or breaking agar material [7].

1.8 Nitrate Test

The nitrate test is useful for identifying microorganisms that can convert nitrate to nitrite by releasing the nitratase enzyme. This test can be used to distinguish between Gram +ve and Gram –ve bacterial species. After adding the bacterial colonies we want to test, the test tubes containing the nitrate broth were incubated. In Durham tubes, the tubes are first incubated and examined for the presence of gas. The nitrate will not be fermented by non-fermenter bacteria, and the nitrate will be converted to nitrogen gas. Gas may be created as a result of the fermentation process, which may necessitate additional testing to ensure nitrate reduction. Add sulfanilic acid (also known as nitrate I) and dimethyl-alpha-napthalamine to this method (also represented as nitrate II).

In the media, the presence of nitrite If nitrite is present in the medium, both reagents I and II will react with it. Red color will form as a result of this reaction, indicating a positive result, but no red color production indicates that the nitrate has not transformed to nitrite or that the nitrite has been transformed to another non-detectable form of nitrogen. For further testing, zinc is added to the broth, which converts any remaining nitrate to nitrite and causes the formation of red color, indicating negative outcomes; however, if no color develops even after the addition of zinc, it indicates that the nitrate has been converted to nitrite and then to other forms, indicating a positive result [8].

1.9 Starch Hydrolysis Test

This test aids in the identification of microorganisms capable of producing the starch hydrolase enzymes alpha amylase and oligo-1,6-glucosidase. Clostridium and Bacillus are frequently distinguished using this term. Because starch is such a big molecule, it is unable to pass through the bacterial cell wall and be used as a carbon source. To transform starch into tiny molecules, enzymes are released that hydrolyze the starch into glucose, which can then be used as an energy source in metabolic pathways. To verify this, iodine is added to the agar medium, which turns a dark brown hue when starch is hydrolyzed, suggesting positive findings [9].

1.10 Carbohydrate Fermentation Test

This test is useful for identifying bacteria that can ferment carbohydrates and those that cannot. This test is based on the principle of producing acid or gas. This test may only employ glucose, sucrose, or lactose as the medium [9].

1.11 Methyl Red Test

As a final product, certain bacteria use glucose to convert to other acids such as lactic acid (LA), acetic acid (AA), and formic acid (FA). They convert glucose to pyruvic acid, which is then converted to other acids depending on the bacteria species. Acid production lowers the pH of the media, changing the color of the methyl red from yellow to red, indicating the bacteria's ability to utilise glucose in the culture medium [10].

1.12 Voges-Proskaur Test

This is an extension of the Methyl red test, which detects microbes that can produce butylene as a byproduct. Acetoin is the reaction's intermediate product, which may be identified using alpha-naphthol and 40 percent KOH. Acetoin is oxidized to diacetyl in the presence of KOH. In the presence of alpha-naphthol, diacetyl will react with the gunidine component of peptone, resulting in the formation of red color,

indicating a positive test. This is accomplished through the use of a magnetic resonance imaging (MRI) test [11].

1.13 Citric Acid Utilization Test

This test is useful in identifying microbes that can use citrate as an energy source. This test is performed on citrate agar, which contains citrate and inorganic ammonium as carbon and nitrogen sources, respectively. The CAU test aids in the identification of microorganisms that generate the enzyme citrate permease, which converts citrate to pyruvate, which then enters the organism's metabolic cycle and creates energy, as well as growth on culture media. When microorganisms consume citrate, NH3 is formed as a result of the conversion of ammonium salts, and the pH of the medium rises. When the pH rises above 7.6, the color of bromothymol blue will change from green to blue. In the CAU test, bromothymol blue is utilized as an indicator [12].

1.14 Bile Esculin Agar Test

In the presence of bile, this test is utilized to identify the microorganisms that hydrolyze the esculin. For the identification of enterococcus, this test is both selective and differential. Bile and sodium azide serve as selective medium, while esculin serves as a differential medium. Except for enterococci and a few species of streptococci, bile inhibits the growth of Gram-positive bacteria, while sodium azide inhibits the growth of Gram-negative bacteria. Bacteria that can hydrolyze esculin in the presence of bile create esculetin, which reacts with ferric citrate in the medium to generate phenolic iron complex, changing the color of the agar from dark brown to black, indicating a positive test, such as E. faecalis. In the event of a negative test, the color will remain unchanged [13].

1.15 Analytical Profile Index Test

The Analytical Profile Index (API) is a method of bacterial classification that uses tests to identify bacteria quickly. Only known microbes can be recognized this way. The AP20E/NE is a fast identification technique for a small number of Gram-negative Enterbacteriaceae and non-Entebactriaceae bacteria. Gram-positive microbes such as Staphylococcus species, Micrococcus species, and other related organisms are handled by the other API system. The AP 20E/NE system consists of 20 tiny reaction mixture wells containing dehydrated substrates for determining bacterial enzymatic activity.

The actions involve inoculation microorganisms fermenting carbohydrates, proteins, and catalyzing amino acids. First, known bacteria colonies are combined with sterile distilled water and added to all wells to rehydrate the wells, after which the strip is incubated for a certain time at a given temperature. Following incubation, the hue of all wells changes. All of the results are combined into a profile number, which is then compared to profile numbers found online or in a commercial codebook for bacterial species identification [14].

Conclusion

Gram positive bacteria are identified using biochemical tests such as the catalase test, coagulase test, starch hydrolysis test, and nitrate test, while Gram negative bacteria are identified using biochemical tests such as the oxidase test, urease test, indole test, sulfur test, and methyl red /voges-proskauer test. The analytical profile index test 20E was created to distinguish between Gram-negative Enterbacteriacea and non-Enterbactriacea bacteria. Gram-positive microbes such as Staphylococcus species, Micrococcus species, and other related organisms have also been generated using the API method.

Conflict of interests.

There are non-conflicts of interest.

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