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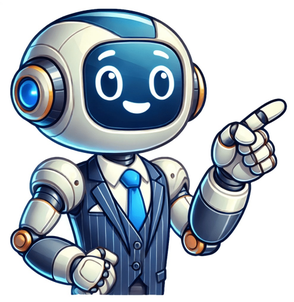

Oxidase test bacteria

Home » Biochemical Test The oxidase test is a biochemical testing module used to assay the ability of bacteria to synthesize cytochrome c oxidase enzymes. This test was first introduced by Gordon and McLeod in 1928 to distinguish Neisseria gonorrhoeae from Staphylococcus spp. and Streptococcus spp. Later it was modified by Kovacs and used Kovacs' oxidase reagent (tetra-methyl-p-phenylenediamine dihydrochloride) for the identification of the cytochrome oxidase enzyme. Again, Gaby and Hadley modified the test and used p-amino dimethylaniline oxalate with α-naphthol as a reagent to detect the cytochrome oxidase enzyme in tube culture. Cytochrome c oxidase is a large transmembrane protein acting as the terminal enzyme in the electron transport chain of aerobic bacterial and mitochondrial respiration system that catalyzes the final electron transfer from cytochrome c to oxygen molecule. Some bacteria contain this enzyme and have the ability to transfer a terminal electron to molecular oxygen; however, some bacteria lack this enzyme and fail to transfer a terminal electron to molecular oxygen or may use different cytochrome to do the job. Differentiating the bacteria that contain and do not contain cytochrome c oxidase enzyme is very important to characterize bacteria and identify them biochemically. To assess the presence of cytochrome c oxidase enzyme within bacterial electron transport chain system To biochemically characterize bacteria and helps in their identification In the presence of molecular oxygen, the cytochrome c oxidase enzyme of bacteria oxidizes phenylenediamine in the colorless reagent to a deep purple to blue-colored compound, indophenol blue. Hence, if the bacteria possess the cytochrome c oxidase, there will be the development of deep purple/blue color, but if the bacteria lack that enzyme, there won't be any color change. While performing the oxidase test following either the disc method or filter paper method or swab method or direct plate method, there is no need for culture media. Bacteria grown in any selective medium (or pure colonies from any media) can be used for the test. However, for the tube method (Gaby and Hadley method) of the oxidase test, nutrient broth medium (or any standard broth medium with low glucose content) is required. (Here, we will use nutrient broth.) Peptone- 5.00 grams HM Peptone B (Beef Extract)- 1.50 grams Yeast Extract- 1.50 grams Sodium Chloride- 5.00 grams Final pH 7.4 ±0.2 at 25°C (References: Nutrient Broth (himedialabs.com)) Measure the appropriate amount of nutrient broth powder (or the media components) and mix in the water of the required volume in a conical flask (or glass bottle) according to the instruction of the manufacturing company. Stir well using a magnetic stirrer or manually and heat to boiling if necessary so that all the components dissolve completely in water. Dispense 5 mL of broth in each test tube and loosely put on the screw cap (or use a cotton plug to cover the opening). Autoclave the tubes with nutrient broth at 1210C and 15 lbs pressure for 15 minutes and let it cool to room temperature before inoculation. 1% N, N, N, N-tetramethyl-p-phenylenediamine dihydrochloride Preparation of Kovacs' oxidase reagent: Dissolve 1.0 grams of N, N, N, N-tetramethyl-p-phenylenediamine dihydrochloride in 100 mL of sterile distilled water and mix well. 1% dimethyl-p-phenylenediamine dihydrochloride Preparation of Gordon and McLeod Reagent Dissolve 1.0 grams of dimethyl-p-phenylenediamine dihydrochloride in 100 mL of sterile distilled water and mix well. Reagent A (1% α-naphthol) Add 1.0 grams of α-naphthol in 100 mL of 98% ethanol. Reagent B (1% p-amino dimethylaniline oxalate) Add 1.0 grams of p-amino dimethylaniline oxalate in 100 mL of distilled water. Petri PlatesWhatman no.1 Filter paper(disc or strip)Weighing MachineAutoclaveBunsen burnerTest TubesDropperInoculating loop(Cotton Swab)PPEOther laboratory materials Positive control: Pseudomonas aeruginosa ATCC 2783 Negative control: E. coli ATCC 25922 (Gordon and McLeod Oxidase Reagent or Kovacs' Oxidase Reagent can be used for oxidase test following the filter paper method, swab method, or direct plate method. Kovacs' oxidase reagent has higher sensitivity than Gordon and McLeod reagent and comparatively gives faster and clearer results. Therefore, Kovacs' oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) is mainly used for the oxidase test. In this procedure, we use Kovacs' oxidase reagent, but if you have Gordon-McLeod oxidase reagent, you can use it.) In a sterile petri plate, place a strip/disc of Whatman no. 1 filter paper. Soak the filter paper with 1% Kovacs' oxidase reagent and let it dry. Using a sterile inoculating loop, pick up a well-isolated colony of test bacteria from a fresh (18 to 24 hours old) culture and make a smear on the reagent-soaked filter paper piece. Observe for color change and note the time required for change in color for up to 60 seconds. Alternatively, Pick a well-isolated colony of test bacteria from fresh culture using a sterile inoculating loop and make a smear over Whatman no. 1 filter paper strip/disc. Add 1 to 2 drops of Kovacs' oxidase reagent over the smear. Observe for color change and note the time required for change in color for up to 60 seconds. Moisten a sterile swab with 1% Kovacs' oxidase reagent. Touch a well-isolated colony from a fresh culture with the swab. Observe the development of color in the swab and note the time required for change in color for up to 60 seconds. Over well-isolated (pure culture) colonies of test bacteria from fresh culture, add a few drops of Kovacs' oxidase reagent. Tilt the plate and shake it gently so that the colonies get exposed to oxygen. Observe for the formation of purple (deep blue) color over the reagent-moistened colonies and note the time required for change in color for up to 60 seconds. Place the impregnated oxidase disc or strip over a clean petri plate (or glass slide) and moisten it with sterile deionized water. (Some discs may not need to be moistened. Look for the manufacturer's instructions.) Using a sterile inoculating loop, pick up a well-isolated colony of test bacteria from fresh culture and make a smear on the oxidase disc/strip. Observe for color change and note the time required for change in color for up to 60 seconds. Inoculate a nutrient broth medium with sample bacteria and incubate aerobically at 35±2°C for 18 to 24 hours. Add 0.2 mL of Gaby-Hadley Reagent A (1% α-naphthol) and add 0.3 mL of Gaby-Hadley Reagent B (1% p-amino dimethylaniline oxalate) and mix well by shaking the medium. Observe for color change and note the time required for change in color for up to 3 minutes. Positive Test Development of purple to deep blue color within 10 to 30 seconds indicates a positive oxidase test. Development of purple to deep blue color within 30 to 60 seconds indicates a weak oxidase positive reaction or delayed oxidase positive. Negative Test No development of purple to deep blue color within 60 seconds. Development of purple to deep blue color after 60 seconds. Oxidase Test Positive Test Development of purple to deep blue color within 15 to 30 seconds indicates a positive oxidase test. Development of purple to deep blue color within 2 to 3 minutes indicates a weak oxidase positive reaction or delayed oxidase positive. Negative Test No development of purple to deep blue color within 3 minutes. Development of purple to deep blue color after 3 minutes. Oxidase Positive Bacteria Neisseria gonorrhoeae, Neisseria spp., Pseudomonas aeruginosa, Aeromonas spp., Vibrio spp., Brucella spp., Moraxella spp., Micrococcus spp., Bordetella pertussis, Campylobacter spp., etc. Oxidase Negative Bacteria E. coli and all Enterobacteriaceae except Plesiomonas shigelloides, Staphylococcus spp., Streptococcus spp., Pseudomonas maltophilia, Mycoplasma spp., Bordetella parapertussis, Listeria spp., etc. Variable Oxidase Result Showing Bacteria Haemophilus spp., Brucella spp., Pasteurella spp. Positive control: Pseudomonas aeruginosa ATCC 2783 rapidly produce deep blue or purple color within 10 to 30 seconds. Negative Control: E. coli ATCC 25922 doesn't result in the formation of deep blue or purple color within 60 seconds. Use fresh culture of bacteria for testing. Don't test bacteria grown on media with dyes (like EMB, MAC medium). Don't test bacteria grown on a glucose-rich culture medium. Don't test strict anaerobes. Use freshly made oxidase reagent. While storing the oxidase reagent, store it in a dark place at – 200C. The sample must be taken from well-isolated colonies. Never perform the direct plate method if the culture is mixed culture. (It is recommended to use cultures from selective media to ensure sample purity.) Don't overflood the plate with an oxidase reagent. Never record the result after 60 seconds while using Kovacs reagent and after 3 minutes while using Gaby-Hadley Reagents. Record time exactly to differentiate rapid oxidase-positive, delayed oxidase-positive, and oxidase-negative bacteria. Do not use nichrome wire loops, as they can give false-positive results. To determine the ability of bacteria to synthesize the cytochrome c oxidase enzyme. Differentiation of Neisseria spp. (oxidase positive cocci) from Staphylococcus spp. and Streptococcus spp.(oxidase negative cocci) Differentiation of Enterobacteriaceae from other Gram-negative bacilli. Differentiation of Pseudomonas aeruginosa from Enterobacteriaceae. It doesn't give a confirmatory result and needs further biochemical tests for complete identification. The reagents must be prepared freshly. The reagents have been shown to auto-oxidize and are photosensitive; hence it is recommended to prepare reagents daily. If the direct plate method is performed, the reagent-soaked colonies will be quickly nonviable, so they need immediate subculture. Bacteria grown on glucose-rich media shows false negative results. Bacteria from older cultures may give false negative results. Commonly used Nichrome or iron inoculating loop may give a false-positive result. Hence, you need either a plastic loop or an expensive platinum loop. Need pure culture or well-isolated colonies for testing. Need precise recording of time taken for the development of color change. Leber, Amy L., editor in chief. (2016). Clinical microbiology procedures handbook (Fourth edition). Washington, DC : ASM Press 1752 N St., N.W., [2016] Tille, P. M., & Forbes, B. A. (2014). Bailey & Scott's diagnostic microbiology (Thirteenth edition.). St. Louis, Missouri: Elsevier. MacFaddin JF, editor. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia:Lippincott Williams and Wilkins; 2000. p. 363-7 Michel H, Behr J, Harrenga A, Kannt A. Cytochrome c oxidase: structure and spectroscopy. Annu Rev Biophys Biomol Struct. 1998;27:329-56. doi: 10.1146/annurev.biophys.27.1.329. PMID: 9646871. Chavan, Dharmappa & Khatoon, Halima & Anokhe, Archana & Kalia, Vinay. (2022). Oxidase test: A biochemical method in bacterial identification. (PDF) Oxidase test: A biochemical method in bacterial identification (researchgate.net) Pseudomonas biochemical tests – BiochemGems Oxidase positive bacteria – BiochemGems Oxidase Test: Principle, Procedure, Results • Microbe Online 27: Oxidase Test – Biology LibreTexts Oxidase Test: Purpose, Principle, Method, Interpretations, Limitations (medicallabscientist.org) What is Oxidase Test ? Principle, Composition, Interpretation of Results – Laboratoryinfo.com oxidase-test-protocol-3229.pdf (asm.org) Oxidase test: Principle, Procedure, Result interpretation and Precautions – Online Biology Notes Oxidase test: Principle , procedure, Result interpretation and various (universe84a.com) Gaby-Hadley Reagent B (himedialabs.com) Gaby-Hadley Reagent A (himedialabs.com) About Author Microbiological and biochemical method for identification The oxidase test is used to determine whether an organism possesses the cytochrome c oxidase enzyme. The test is used as an aid for the differentiation of Neisseria, Moraxella, Campylobacter and Pasteurella species (oxidase positive). It is also used to differentiate pseudomonads from related species.[1] Strains may be either oxidase-positive (OX+) or oxidase-negative (OX-). OX+ normally means the bacterium contains cytochrome c oxidase (also known as Complex IV) and can therefore use oxygen for energy production by converting O2 to H2O2 or H2O with an electron transfer chain. The Pseudomonadaceae are typically OX+.[1] The Gram-negative diplococci Neisseria and Moraxella are oxidase-positive.[2] Many Gram-negative, spiral curved rods are also oxidase-positive, which includes Helicobacter pylori, Vibrio cholerae, and Campylobacter jejuni. Legionella pneumophila may be oxidase-positive.[3] OX− normally means the bacterium does not contain cytochrome c oxidase and, therefore, either cannot use oxygen for energy production with an electron transfer chain or employs a different cytochrome for transferring electrons to oxygen. Enterobacteriaceae are typically OX−.[4] TMPD DMPD The test uses disks impregnated with reagents such as N,N,N′,N′-tetramethyl-p-phenylenediamine, TMPD (or N,N-dimethyl-p-phenylenediamine, DMPD, which is another redox indicator). The reagent is a dark-blue to maroon color when oxidized, and colorless when reduced. Oxidase-positive bacteria possess cytochrome oxidase or indophenol oxidase (an iron-containing hemoprotein).[5] These both catalyze the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen). The test reagent TMPD acts as an artificial electron donor for the enzyme oxidase. The oxidized reagent forms the colored compound indophenol blue. The cytochrome system is usually only present in aerobic organisms that are capable of using oxygen as the terminal electron acceptor. The end-product of this metabolism is either water or hydrogen peroxide (broken down by catalase).[1] Wet each disk with about four inoculating loops of deionized water. Use a loop to aseptically transfer a large mass of pure bacteria to the disk. Observe the disk for up to three minutes. If the area of inoculation turns dark-blue to maroon to almost black, then the result is positive. If a color change does not occur within three minutes, the result is negative. In alternative manner, live bacteria cultivated on trypticase soy agar plates may be prepared using sterile technique with a single-line streak inoculation. The inoculated plates are incubated at 37 °C for 24–48 hours to establish colonies. Fresh bacterial preparations should be used. After colonies have grown on the medium, 2-3 drops of the reagent DMPD are added to the surface of each organism to be tested. A positive test (OX+) will result in a color change violet to purple, within 10–30 seconds. A negative test (OX-) will result in a light-pink or absence of coloration. ^ a b c MacFaddin JF, editor. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia:Lippincott Williams and Wilkins; 2000. p. 363-7 ^ S. T., Cowan; Steel, K.J. (1993). Cowan and Steel's Manual for the Identification of Medical Bacteria (3rd ed.). Cambridge: Cambridge University Press. ISBN 9780511527104. ^ "UK SMI" (PDF). ^ Farmer JJ, Fanning GR, Huntley-Carter GP, et al. (May 1981). "Kluyvera, a new (redefined) genus in the family Enterobacteriaceae: identification of Kluyvera ascorbata sp. nov. and Kluyvera cryocrescens sp. nov. in clinical specimens". J. Clin. Microbiol. 13 (5): 919–33. doi:10.1128/jcm.13.5.919-933.1981. PMC 273917. 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Zanderigo F et al. 2018. [11C]Harmine Binding to Brain Monoamine Oxidase A: Test-Retest Properties and Noninvasive Quantification. Molecular Imaging and Biology. 20, 667–681. Oxidase test video Oxidase Test Procedure Retrieved from " Share — copy and redistribute the material in any medium or format for any purpose, even commercially. Adapt — remix, transform, and build upon the material for any purpose, even commercially. The licensor cannot revoke these freedoms as long as you follow the license terms. Attribution — You must give appropriate credit , provide a link to the license, and indicate if changes were made . You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use. ShareAlike — If you remix, transform, or build upon the material, you must distribute your contributions under the same license as the original. No additional restrictions — You may not apply legal terms or technological measures that legally restrict others from doing anything the license permits. You do not have to comply with the license for elements of the material in the public domain or where your use is permitted by an applicable exception or limitation . No warranties are given. The license may not give you all of the permissions necessary for your intended use. For example, other rights such as publicity, privacy, or moral rights may limit how you use the material. The oxidase test is a technique for detecting the presence of the terminal enzyme system in aerobic respiration called cytochrome C oxidase or cytochrome a3. Usually, the family Enterobacteriaceae gives a negative result, whereas Pseudomonas spp, Aeromonas spp, Vibrio spp, and Neisseria spp give a positive result.The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine dihydrochloride) to indophenols, a purple or dark blue color end product. When the enzyme is not present, the reagent remains reduced and is colorless.Mechanism of the Cytochrome Oxidase ReactionAll bacteria that are oxidase-positive are aerobic and can use oxygen as a terminal electron acceptor in respiration. This does NOT mean that they are strict aerobes. Bacteria that are oxidase-negative may be anaerobic, aerobic, or facultative; the oxidase negative result just means that these organisms do not have the cytochrome c oxidase that oxidizes the test reagent. They may respire using other oxidases in electron transport. Moist filter paper with the substrate (1% tetramethyl-p-phenylenediamine dihydrochloride), or commercially prepared paper disk, wooden wire, or platinum wire.Kovács oxidase reagent (1% tetra-methyl-p-phenylenediamine dihydrochloride, in water). Store refrigerated in a dark bottle for no longer than 1 week.Various types of oxidase test procedureOxidase test can be performed in various ways. These include, but are not limited to, the filter paper test, filter paper spot test, direct plate method, and test tube method.Soak a small piece of filter paper in 1% Kovács oxidase reagent and let it dry.Use a sterile loop to pick a well-isolated colony from a fresh (18- to 24- hour culture) bacterial plate and rub it onto treated filter paper.Observe for color changes.Note: Nickel, steel and other wire loop give false-positive results, so one should use platinum or inert transfer loop. The alternative options are glass rods, wooden sticks, sterile plastic loops, sterile toothpicks and sterile swabs. Oxidase-positive Pseudomonas aeruginosa (left) andoxidase-negative Escherichia coli (right).ResultsOxidase positive: color changes to dark purple within 5 to 10 seconds.Delayed oxidase-positive: color changes to purple within 60 to 90 seconds.Oxidase negative: color does not change or it takes longer than 2 minutes.Use a loop to pick a well-isolated colony from a fresh bacterial plate and rub it onto a small piece of filter paper.Place 1 or 2 drops of 1% Kovács oxidase reagent on the organism smear.Observe for color changes.Oxidase-positive Pseudomonas aeruginosa (left) andoxidase-negative Escherichia coli (right).ResultsOxidase positive: color changes to dark purple within 5 to 10 seconds.Delayed oxidase-positive: color changes to purple within 60 to 90 seconds.Oxidase negative: color does not change or it takes longer than 2 minutes.Grow a fresh culture (18 to 24 hours) of bacteria on nutrient agar or trypticase soy agar using the streak plate method so that well-isolated colonies are present.Place 1 or 2 drops of 1% Kovács oxidase reagent on the organisms.Do not invert or flood plate.Observe for color changes.Oxidase-positive Vibrio cholerae showing purple colonies, and oxidase-negative Escherichia coli with lack of color changeResultsOxidase positive: color changes to dark purple within 5 to 10 seconds.Delayed oxidase-positive: color changes to purple within 60 to 90 seconds.Oxidase negative: color does not change or it takes longer than 2 minutes.Grow a fresh culture (18 to 24 hours) of bacteria in 4.5 ml of nutrient broth (or standard media that does not contain a high concentration of sugar).Add 0.2 ml of 1% α-naphthol, then add 0.3 ml of 1% paminodimethylaniline oxalate (Gaby and Hadley reagents).Observe for color changes.Oxidase positive Neisseria sicca (left) and oxidase negative Staphylococcus aureus (right)ResultsOxidase positive: color changes to blue within 15 to 30 seconds.Delayed oxidase-positive: color changes to purple within 2 to 3 minutes.Oxidase negative: no change in colorOxidase test is most helpful in screening colonies suspected of being a member of the Enterobacteriaceae family; all the members of the Enterobacteriaceae family including E. coli are oxidase negative.To avoid misidentification, perform an oxidase test on all Gram-negative rods. Oxidase test is especially important in separating Aeromonas from Enterobacteriaceae.Note: If you see swarming colonies in a culture media, do not perform oxidase test, as its unique characteristics of Proteus spp, which are oxidase negative.Oxidase test is used as a major characteristic for the identification of Gram-negative rods that are not in the Enterobacteriaceae family. Colonies suspected of belonging to other genera Aeromonas, Pseudomonas, Neisseria, Campylobacter, and Pasteurella are oxidase positive.Gram-negative diplococci give a positive reaction. All members of the genus Neisseria are oxidase positive. Moraxella spp. that are either Gram-negative diplococci or coccobacilli are also oxidase-positive.Bacterial species showing positive and negative reactions should be run as controls at frequent intervals. The following are suggested:A. Oxidase positive: Pseudomonas aeruginosaB. Oxidase negative: Escherichia coliTiming is critical to accurate testing.Use fresh reagents, no older than 1 week, older reagents can autooxidize thus giving erroneous results. Do not use if the reagent or filter paper is purple.Do not test organisms growing on media that contain glucose or dyes (e.g., MacConkey agar or EMB agar).Do not use nickel-base alloy wires containing chromium and iron (nichrome) to pick the colony and make a smear as this may give false-positive results.Bacteria grown on media-containing dyes may give aberrant results.Older cultures are less metabolically active so may give false-negative results within the mentioned observation time.The oxidase test must be performed from 5% sheep blood agar or another medium without fermentable sugar. Fermentation of carbohydrates results in acidification of the medium (e.g., lactose in MacConkey Agar or Sucrose in TCBS), and a false negative oxidase test may result if the surrounding pH is below 5.1. Subinoculation on nutrient agar is required before the oxidase test can be performed.During the identification of suspected Vibrio cholerae isolate, it is not possible to perform an oxidase test directly from a TCBS culture because the acid produced by the sucrose fermenting colonies will inhibit the oxidase reaction.Bacterial genera characterized as oxidase-positive include Neisseria and Pseudomonas etc. Genera of the Enterobacteriaceae family are characterized as oxidase negative. Mneomonics for oxidase positive organisms- PVNCH (It's just an acronym inspired by the famous mnemonic for urease positive organisms-PUNCH)P: Pseudomonas sppV: Vibrio choleraeN: Neisseria sppC: Campylobacter sppH: Helicobacter spp/ Haemophilus spp. Aeromonas sppAlcaligensReferences and further readingsPatricia Shields, Laura Cathcart. 2010. Oxidase test protocol.Clinical Microbiology Procedures Handbook, Fourth Edition. (2016). 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