Triple Sugar Iron Agar Protocols

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Author

- Donald Lehman

Information

History

In 1911, Russell described the use of an agar medium with two sugars to aid in the identification of intestinal gram-negative bacilli. Because the ability of bacteria to produce hydrogen sulfide was recognized as a valuable characteristic, lead or iron salts were added to Russell's Double Sugar medium by other investigators. Kliger added lead acetate and iron salts to detect hydrogen sulfide production and used phenol red as a pH indicator producing Kigler's Iron Agar.

In 1917, Krunweide and Kohn modified Russell's Double Sugar agar by adding a third sugar—sucrose. The addition of sucrose permitted the earlier detection of coliform bacteria that ferment sucrose more rapidly than lactose. Adding sucrose also aided the identification of certain gram-negative bacteria that could ferment sucrose but not lactose.

In 1940, Difco Laboratories, Sulkin and Willet, and Hajna described a similar triple sugar ferrous sulfate medium for the identification of enteric bacilli. The current formulation of triple sugar iron medium is essentially the same as Sulkin and Willet except that phenol red is used as the pH indicator instead of brom thymol blue, Tryptone has been replaced by a combination of Bacto Peptone and Proteose Peptone, and yeast extract has been added.

Purpose

Triple sugar iron (TSI) agar is a tubed differential medium used in determining carbohydrate fermentation and H_2S production. Gas from carbohydrate metabolism can also be detected. Bacteria can metabolize carbohydrates aerobically (with oxygen) or fementatively (without oxygen). TSI differentiates bacteria based on their fermentation of lactose, glucose and sucrose and on the production of hydrogen sulfide. TSI is most frequently used in the identification of the*Enterobacteriaceae*, although it is useful for other gram-negative bacteria.

Theory

TSI contains three carbohydrates: glucose (0.1%), sucrose (1%), and lactose (1%). TSI is similar to Kligler's iron agar, except that Kligler's iron agar contains only two carbohydrates: glucose (0.1%) and lactose (1%). Besides the carbohydrates mentioned, the medium also contains

beef extract, yeast extract, and peptones which are the sources of nitrogen, vitamins and minerals. Phenol red is the pH indicator, and agar is used to solidify the medium. During preparation, tubes containing molten agar are angled. The slant of the medium is aerobic, while the deep (or butt) is anaerobic.

When any of the carbohydrates are fermented, the drop in pH will cause the medium to change from reddish-orange (the original color) to yellow. A deep red color indicates alkalization of the peptones. Sodium thiosulfate in the medium is reduced by some bacteria to hydrogen sulfide (H_2S), a colorless gas. The hydrogen sulfide will react with ferric ions in the medium to produce iron sulfide, a black insoluble precipitate.

Based on carbohydrate utilization and hydrogen sulfide production, a TSI slant can be interpretted in several ways:

Glucose Fermenter. The tube reaction is alkaline over acid (K/A) signifying that only glucose is metabolized. The bacteria quickly metabolized the glucose, initially producing an acid slant and an acid butt (acid over acid; A/A) in a few hours. The Emben-Meyerhof-Parnas pathway was used both aerobically (on the slant) and anerobically (in the butt) to produce ATP and pyruvate. On the slant, the pyruvate was further metabolized to CO_2, H_2O, and energy. After further incubation (18 hours) the glucose was consumed, and because the bacteria could not use lactose or sucrose, the peptones (amino acids) were utilized as an energy source aerobically, on the slant. Utilization of peptones causes the release of ammonia (NH_3) increasing the pH resulting in the pH indicator, phenol red, turning from yellow to red. In the anerobic butt, the bacteria use the Embden-Meyerhof-Parnas pathway to metabolize the glucose producing ATP and pyruvate, which is converted into stable acid endproducts, thus the butt remains acidic. The results would be recorded as alkaline over acid (K/A). Bacteria producing a K/A reaction with or without gas include: *Citrobacter freundii** , *Citrobacter koseri**, and *Morganella morganii**.
* = variable reactions

Glucose, Lactose and/or Sucrose Fermenter. The tube reaction is acid over acid (A/A) indicating that glucose, lactose and/or sucrose have been metabolized. The bacteria quickly metabolized the glucose, producing an acid slant and an acid butt in a few hours. The Emben-Meyerhof-Parnas pathway is used both aerobically (on the slant) and anerobically (in the butt) to produce ATP and pyruvate. On the slant, the pyruvate is further metabolized to CO_2, H_2O, and energy. After further incubation (18 hours) the glucose was consumed, and then the bacteria utilized lactose and/or sucrose, maintaining an acid slant. The results are recorded as acid over acid (A/A). If the medium were incubated longer, over 48 hours, the lactose and sucrose would be depleted, and the slant would revert to an alkaline pH due to metabolism of the peptones. In the anerobic butt, the bacteria convert pyruvate into stable acid endproducts, thus the butt remains acidic. The bacteria commonly producing an A/A reaction with or without gas include: *Enterobacter aerogenes, E. cloacae, Escherichia coli, Klebsiella oxytoca,* and *K. pneumoniae*.

Glucose, Lactose and Sucrose Nonfermenters. The tube reaction is either alkaline over alkaline (K/K) or alkaline over no change (K/NC) indicating that all three sugars have not been metabolized. The difference between K/K and K/NC) is subtle. Some nonenteric bacteria, such as the pseudomonads, are unable to ferment glucose, lactose, or sucrose. These bacteria derive energy from peptones either aerobically or anaerobically. Utilization of peptones causes the release of ammonia (NH_3) resulting in the pH indicator, phenol red, turning from pink to red. Nonglucose fermenters can produce two possible reactions. If the bacteria can metabolize peptones both aerobically and anaerobically, the slant and butt will be red (alkaline over alkaline; K/K). If peptones can only be metabolized aerobically, the slant will be red and the butt will exhibit no change (K/NC). Bacteria producing K/K or K/NC include: *Acinetobacter* spp. and *Pseudomonas* spp.

Gas Production. Gas production (CO_2 and O_2) is detected by splitting of the agar. In some cases, so much gas is produced that the agar is pushed to the top of the tube. Bacteria commonly producing an A/A reaction with gas include: *Enterobacter aerogenes, E. cloacae, Escherichia coli, Klebsiella oxytoca,* and *K. pneumoniae*. However, some strains do not produce gas.

Glucose Fermenter and Hydrogen Sulfide Production. The tube reaction is alkaline over acid (K/A) with black precipitate. The bacteria quickly metabolized the glucose, initially producing an acid slant and an acid butt (acid over acid; A/A) in a few hours. The Emben-Meyerhof-Parnas pathway is used both aerobically (on the slant) and anerobically (in the butt) to produce ATP and pyruvate. On the slant, the pyruvate is further metabolized to CO_2, H_2O, and energy. After further incubation (18 hours) the glucose was consumed, and because the bacteria could not use lactose or sucrose, the peptones (amino acids) were utilized as an energy source aerobically, on the slant. Utilization of peptones causes the release of ammonia (NH_3) resulting in the pH indicator, phenol red, turning from yellow to red. In the anerobic butt, the bacteria use the Embden-Meyerhof-Parnas pathway to metabolized the glucose producing ATP and pyruvate, which is converted into stable acid endproducts, thus the butt remains acidic.

The black precipitate indicates that the bacteria were able to produce hydrogen sulfide (H_2S) from sodium thiosulfate. Because H_2S is colorless, ferric ammonium citrate is used as an indicator resulting in the formation of insoluble ferrous sulfide. Formation of H_2S requires an acidic environment; even though a yellow butt cannot be seen because of the black precipitate, the butt is acidic. The results would be recorded as alkaline over acid (K/A), H_2S positive. Bacteria producing a K/A with H_2S include: *Citrobacter freundii*, Edwardsiella tarda, Proteus mirabilis*,* and *Salmonella*spp*. Bacteria commonly producing an A/A with H_2S include: *Citrobacter freundii*, Proteus mirabilis*,* and *P. vulgaris**.
* = variable reactions

Glucose, Lactose and/or Sucrose Fermenter and Hydrogen Sulfide Producer. The tube reaction is acid over acid (A/A) with black

precipitate. The bacteria quickly metabolized the glucose, producing an acid slant and an acid butt (acid over acid; A/A) in a few hours. The Emben-Meyerhof-Parnas pathway is used both aerobically (on the slant) and anerobically (in the butt) to produce ATP and pyruvate. On the slant, the pyruvate is further metabolized to CO_2, H_2O, and energy. After further incubation (18 hours) the glucose was consumed, and then the bacteria utilized lactose and/or sucrose, maintaining an acid slant. The results are recorded as acid over acid (A/A). If the medium were incubated longer, over 48 hours, the lactose and sucrose would be depleted, and the slant would revert to an alkaline pH due to metabolism of the peptones. In the anerobic butt, the bacteria convert pyruvate into stable acid endproducts, thus the butt remains acidic.

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* = variable reactions

Glucose Nonfermenter Hydrogen Sulfide Producer. The tube appears as alkaline over no change (K/NC) with a black precipitate (H_2S). The reduction of thiosulfate in KIA and TSIA requires H+. Nonfermenters cannot produce an acid environment from the fermenation of the carbohydrates. Cysteine and perhaps other organic sulfate molecules are metabolized to pyruvic acid, ammonia, and H_2S. Nonfermentative H_2S positive reaction is strongly suggestive of members of the genus*Shewenella.*

RECIPE

Pancreatic digest of casein USP (see Note)	10.0 g
Peptic digest of animal tissue USP (see Note)	10.0 g
Glucose	1.0 g
Lactose	10.0 g
Sucrose	10.0 g
Ferrous sulfate or ferrous ammonium sulfate	0.2 g
NaCl	5.0 g
Sodium thiosulfate	0.3 g
Phenol red	0.024 g
Agar	13.0 g
Distilled water	1,000 mL

Note: The following combination of ingredients can substitute for the first two components listed: beef extract, 3.0 g; yeast extract, 3.0 g; and peptone, 20.0 g.

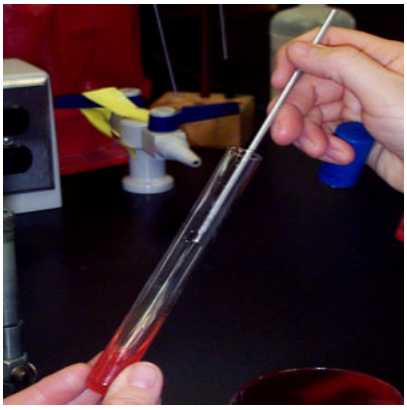
Combine ingredients, and adjust the pH to 7.3. Boil to dissolve the agar, and dispense into tubes. Sterilize by autoclaving at 121°C for 15 min. Cool in a slanted position to give a 2.5-cm butt and a 3.8-cm slant.

TSI agar is also available commercially.

PROTOCOL

Use a straight inoculating needle to pickup an isolated colony.

Inoculate the TSI slant by first stabbing the butt down to the bottom, withdraw the needle, and then streak the surface of the slant. Use a loosely fitting closure to permit access of air.



Read results after incubation at 37°C for 18 to 24 h. Three kinds of data may be obtained from the reactions.

Sugar fermentations
Acid butt, alkaline slant (yellow butt, red slant): glucose has been fermented but not sucrose or lactose.
Acid butt, acid slant (yellow butt, yellow slant): lactose and/or sucrose has been fermented.
Alkaline butt, alkaline slant (red butt, red slant): neither glucose, lactose, nor sucrose has been fermented.

Gas production

Indicated by bubbles in the butt. With large amounts of gas, the agar may be broken or pushed upward.

Hydrogen sulfide production

Hydrogen sulfide production from thiosulfate is indicated by a blackening of the butt as a result of the reaction of H_2S with the ferrous ammonium sulfate to form black ferrous sulfide.

The black precipitate indicates that the bacteria were able to produce hydrogen sulfide (H_2S) from sodium thiosulfate. Because H_2S is colorless, ferric ammonium citrate is used as an indicator resulting in the formation of insoluble ferrous sulfide. Formation of H_2S requires an acidic environment; even though a yellow butt cannot be seen because of the black precipitate, the butt is acidic. The results would be recorded as acid over acid (A/A), H_2S positive.

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

COMMENTS AND TIPS

This section is to evolve as feedback on the protocol is discussed at ASMCUE. Please contact the project manager for further information.

REFERENCE

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REVIEWERS

This resource was peer-reviewed at ASM Conference for Undergraduate Educators 2005.

Participating reviewers:

Sue Merkel
Cornell University, Ithaca, NY

Patty Shields
University of Maryland, College Park, MD