AG – Triple Sugar Iron Agar Slant - INSTRUCTIONS FOR USE

(Ready to use Media)

- **INTENDED USE:**

In vitro diagnostic. For the differentiation of Enterobacteriaceae, especially Salmonella, based on carbohydrate fermentation and production of hydrogen sulphide.

- **PRINCIPLE:**

Triple Sugar Iron Agar – from left: uninoculated tube, S.Typhimurium, E.coli.

Triple Sugar Iron (TSI) Agar is intended for the differentiation of Enterobacteriaceae, especially Salmonella spp., grown on primary isolation media, based on the fermentation of glucose, lactose and sucrose, with production of acids and gas, and the production of hydrogen sulphide.

The fermentation of the three carbohydrates can take place both on the surface of the slant and in the butt with or without the presence of gas ($CO_2 + H_2$) and 3 reaction models can be registered:
1-fermentation of glucose; 2-fermentation of glucose, lactose and/or sucrose; 3-no fermentation.
In the first case, after 18-24 hours of incubation, an alkaline reaction on the slant and an acid reaction in the butt is observed. The complete consumption of glucose, present at a concentration of 0.1%, on the surface, where aerobic conditions exist, after 18-24 hours induces the oxidative degradation of peptones, with production of ammonia, alkalinity and a red colour change of phenol red (reversal of the acid-alkaline reaction). However, in the anaerobic butt the bacteria metabolize the glucose producing ATP and pyruvate, which is converted into stable acid end-products with a colour change of the indicator to yellow (acid pH).
In the second case, the microorganisms ferment glucose and one or both lactose and sucrose: after 18-24 hours of incubation an acid reaction is recorded on the slant and in the butt. This is due to the high concentration of lactose and sucrose: after 18-24 hours their degradation is not exhausted on the surface and therefore there is no utilisation of peptones and therefore no reversal of the reaction.
In the third model an alkaline reaction is recorded both on the slant and in the butt. This behavior is not typical of Enterobacteriaceae but of some non-enteric non fermenting Gram-negative bacteria that can utilise the peptones for growing (Alcaligenes faecalis, Acinetobacter, Pseudomonas). If the degradation of the peptones is anaerobic the indicator will turn to red (alkaline pH) both on the surface and in the butt, if the degradation is aerobic, there is no colour change of phenol red in the butt.

- **MATERIALS PROVIDED:**

PRODUCT	TYPE	REF	PACK
AG – Triple sugar iron slant	Ready to use Media	AG/TSI/22/01	10 Tubes in a pack

- **MATERIALS REQUIRED BUT NOT PROVIDED:**

Sterile loops, incubator, and laboratory equipment as required.

- **SPECIMENS:**

Triple Sugar Iron Agar Medium is not intended for primary isolation from clinical specimens; it is inoculated with pure colonies from a culture on solid media, isolated from clinical specimens or other materials. Good laboratory practices for collection, storage and transport of the specimens to the Laboratory should be applied.

AG – Triple Sugar Iron Agar Slant - INSTRUCTIONS FOR USE

(Ready to use Media)

- **TEST PROCEDURE, READING AND INTERPRETATION:**
  - Three kinds of data may be obtained from the reactions.
  - Sugar fermentations-
  - Acid (yellow) butt, alkaline (red) slant: glucose fermented, sucrose or lactose not fermented. Acid (yellow) butt, acid (yellow) slant: glucose, lactose and/or sucrose fermented.
  - Alkaline (red) butt, alkaline (red) slant: neither glucose, lactose, nor sucrose fermented.
  - Gas production-
  - Presence of bubbles in the butt. With large amounts of gas, the agar may be cracked and displaced.
  - Hydrogen sulphide production-
  - Hydrogen sulphide production from thiosulfate is indicated by a blackening of the butt as a result of the reaction of H2S with the ferric ions to form black ferrous sulphide. Formation of H2S requires an acidic environment; sometimes the butt will be entirely black; in such a case, it is assumed that butt portion of the tube is acid (yellow colour is masked by H2S production).
  - All combinations of the reactions described above can be observed on Triple Sugar Iron Agar, therefore it is important to record the results of all the reactions (sugar fermentations, gas production, H2S production).
- **USER QUALITY CONTROL:**

All manufactured lots of the product are released for sale after Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for quality control.

CONTROL STRAINS	INCUBATION	EXPECTED RESULTS
Escherichia coli ATCC 25922	35 ±2°C /18-24H-Aerobic	Growth, Yellow slant, yellow butt, No H2S production
Salmonella typhimurium ATCC 14028	35 ±2°C /18-24H-Aerobic	Growth, Red slant, yellow butt, H2S gas production
Shigella flexneri ATCC 12022	35 ±2°C /18-24H-Aerobic	Growth, Red slant, yellow butt, No H2S gas production

Key: ATCC is a trademark of American Type Culture Collection

- **LIMITATIONS OF THE METHOD:**
  - It is necessary to inoculate the medium with a microbiological needle without breaking the agar (do not use loops).
  - Perform the reading between 18 and 24 hours of incubation; early readings can induce false acidity results of the type or there is not enough time for the sugar fermentation with consequent color change of the indicator; delayed readings can give false results due to the use of peptones and alkaline change of the medium
  - H2S production can mask the acid reaction in the butt, however the production of H2S requires acidic conditions therefore the butt must be considered acid when there is blackening.
  - The medium does not contain inhibitors therefore a large variety of microorganisms can grow on it; for this reason, before inoculation, make sure that the organisms are catalase positive, Gram-negative bacilli.
  - . Pure culture is essential when inoculating the medium. If the culture is not pure, irregular results may be obtained.
  - Make sure that the caps are loosened during incubation since for a correct medium performance a free exchange of air is necessary. If the caps are too closed, an acid reaction occurs only on the slant even in the presence of glucose fermentation.
  - It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
  - This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.
- **PRECAUTIONS AND WARNINGS:**
  - This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
  - All laboratory specimens should be considered infectious.
  - The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.

AG – Triple Sugar Iron Agar Slant - INSTRUCTIONS FOR USE

(Ready to use Media)

- Sterilize all biohazard waste before disposal. Dispose of the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.

- The Certificates of Analysis and the Safety Data Sheet of the product are available with AstraGene and can be provided on request.

- **STORAGE CONDITIONS AND SHELF LIFE:**
  - Upon receipt, store at +2 - 8°C away from direct light in a cool, dry place. Storage below 2°C may lead to crystallization of media components due to near freezing temperature. The user is responsible for the storage method (temperature) of the medium.
  - If properly stored, the product may be used up to the expiration date. Do not use it beyond the mentioned expired date.

- **SYMBOLS:**

	Date of manufacture		Use-by-date		Do not use if package is damaged		Manufacturer
LOT	Batch Code		Refer to the instructions	ISO 13485:2016	ISO		GMP
IVD	*In-Vitro* diagnostic Medical devices	CE	Mark of conformity				

AstraGene FZ LLC, Office No. 208 – 209, Dubai Science Park Building, Dubai, United Arab Emirates
+971-4-8781222, contact@astragene.com