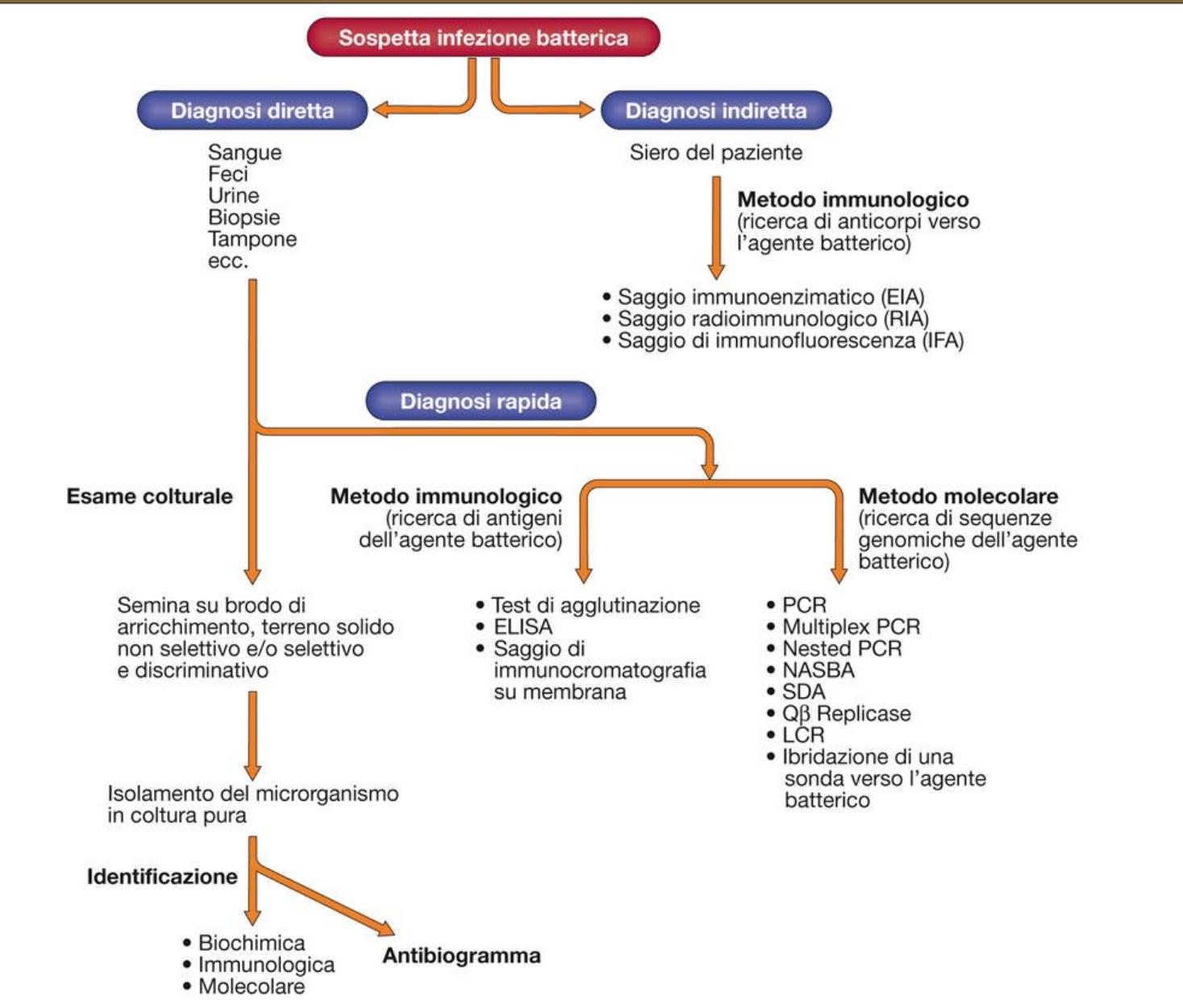




DIAGNOSI BATTERIOLOGICA

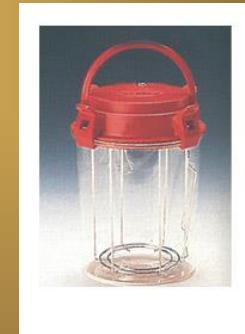




Raccolta e trasporto del campione

L'attendibilità dell'esame batteriologico dipende da vari fattori:

- **ADEGUATA RACCOLTA DEL CAMPIONE**



- **TRASPORTO DEL CAMPIONE:**

terreno semisolido di Stuart o di Amies; Amies con carbone vegetale; contenitori adatti per anaerobi

DIAGNOSI COLTURALE

L'identificazione delle colonie viene eseguita ricorrendo a **colture pure** derivate da ciascun tipo di colonia precedentemente isolata e si basa sullo **studio di alcuni caratteri**:

- ❖ **CARATTERI CULTURALI:** ASPETTO COLONIE
- ❖ **CARATTERI MICROSCOPICI:** (morfologia e caratteristiche tintoriali)
- ❖ **CARATTERI BIOCHIMICI:** (fermentazione zuccheri; produzione di prodotti metabolici peculiari; presenza/assenza di enzimi particolari)
- ❖ **CARATTERI ANTIGENICI**

In base alle proprietà selettive o differenziali
i terreni vengono distinti in:

- 1. non selettivi**
- 2. elettivi**
- 3. selettivi**
- 4. differenziali**



Terreni non selettivi

I substrati non selettivi consentono (almeno in teoria) di coltivare tutti i microrganismi. Sono in genere substrati ricchi, a pH neutro. Esempi:

- **Agar sangue**: al 5% di sangue (di cavallo o montone)
- **Agar cioccolato**: per batteri particolarmente esigenti
- **Plate Count Agar** (Standard) un substrato per la determinazione del tenore in germi in prodotti alimentari
- **Tryptone Soya Broth/Agar**: un substrato ricco per la coltivazione dei batteri con elevate esigenze nutrizionali



Terreni elettivi

I substrati elettivi hanno una composizione che favorisce, anche se non in modo assoluto, un determinato gruppo di microrganismi.

Sono terreni ricchi di nutrienti, spesso con la presenza di sangue di pecora o cavallo, che consentono la crescita di quasi tutte le specie batteriche di interesse medico.

Esempi:

- a. **M17:** un substrato elettivo per gli streptococchi lattici, ricco in nutrienti e tamponato



M17 Agar • M17 Broth

Intended Use

M17 Agar is used for isolating and enumerating lactic streptococci in yogurt, cheese starters and other dairy products.

M17 Broth is used for isolating lactic streptococci from yogurt, cheese starters and other dairy products.

Summary and Explanation

Lactic streptococci are acid-producing bacteria. They are nutritionally fastidious and require complex culture media for optimum growth. One study showed that in a synthetic medium, all strains had an obligate requirement for at least

six amino acids and three vitamins.¹ These homofermentative lactic streptococci produce large amounts of acid and, in a culture medium without an adequate buffering system, the pH decreases and adversely affects growth. Lowrie and Pearce² developed M16 Medium but it lacked a strong buffering system. Terzaghi and Sandine³ worked with M16 Medium and demonstrated that the rapid drop in pH that accompanies lactic streptococcal growth can adversely affect colony size and phage plaque formation. They modified M16 Medium using disodium-β-glycerophosphate as a buffer and called it M17.

Shankar and Davies⁴ found that disodium-β-glycerophosphate in M17 Broth suppressed *Lactobacillus bulgaricus* and *Lactococcus lactis*.

TERRENI SELETTIVI

Consentono la crescita selettiva di determinati microrganismi; vengono utilizzati quando si trattano campioni microbiologici contenenti popolazioni batteriche differenti.

Per poter rendere selettivo un terreno si può operare su vari parametri:

A. CONDIZIONI NUTRIZIONALI

B. PH

C. TEMPERATURA

D. INIBITORI

TERRENI SELETTIVI E DIFFERENZIALI

Selettivi perché favoriscono la crescita di determinate specie batteriche inibendone altre

Differenziali perché permettono la crescita di tipi diversi di microrganismi che però crescendo formano colonie facilmente distinguibili (per esempio in base alla colorazione)

Common Microbiological Media for Aerobic Cultures

Luria
Bertani
Broth (LB)

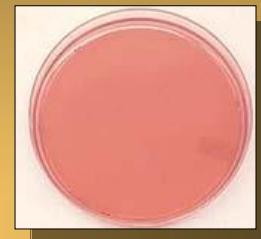
LB-Agar

Sheep's
Blood Agar

Chocolate
Agar

MacConkey
Agar

Hektoen
Enteric Agar



Uses:
Culture of
aerobic
bacterial
species

Uses: Culture
of aerobic
bacterial
species on solid
media

Uses: Non-
selective media,
good for
culturing many
bacterial
species including
Gram negative
and Gram-
positive species
including
Staphylococci
and
Streptococci

Uses: Very
enriched
media with
lysed red
cells, to
release many
nutrients
needed by
fastidious
organisms.

Uses: Selective
media for Gram-
negative,
enteric bacteria
(bile tolerant).
Assess lactose
fermentation

Uses: Selective
media for Gram-
negative enteric
bacteria (higher bile
salt concentrations
than MacConkey).
Used to assess
lactose
fermentation and
ability to produce
hydrogen sulfide
(H₂S). Allows for
distinguishing
among, E. coli,
Salmonella and
other gut pathogens
including Shigella
species

Blood Agar Sheep



This enriched and differential plating medium supports the growth of most medically significant bacteria. It is used for primary plating and for subculturing of colonies and is especially useful for detecting hemolytic activity of bacteria. It is also used for the demonstration of the "satellite phenomenon" by *Haemophilus* and for the CAMP test on b-hemolytic streptococci.

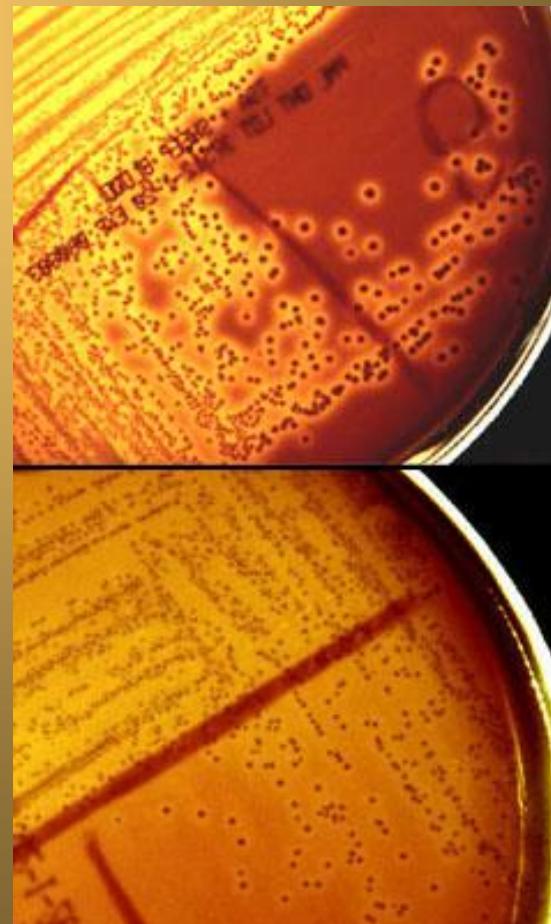
Blood Agar: Hemolytic Reactions

When streaked on Blood Agar, many species of bacteria cause **hemolysis** - i.e., destruction of the erythrocytes (and hemoglobin) in the medium. Hemolytic reactions are generally classified as **alpha**, **beta** or **gamma** according to the appearance of zones around isolated colonies growing on or in the medium:

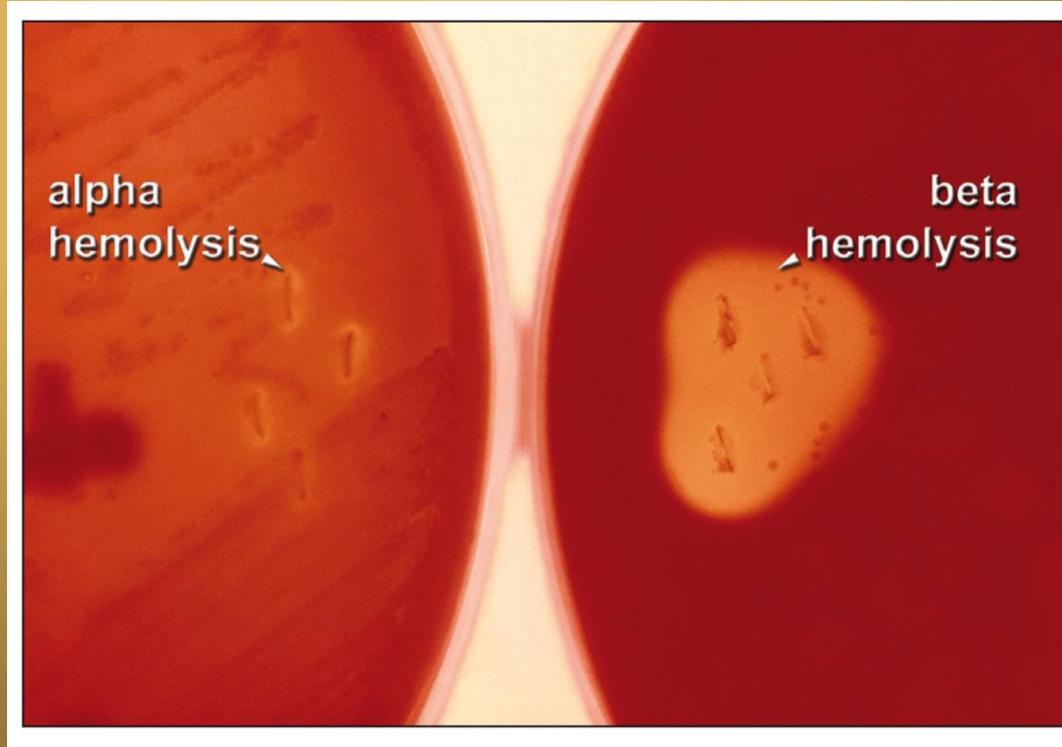
- **Beta hemolysis:** The colony is surrounded by a white or clear zone in which few or no intact erythrocytes are found. This reaction is best seen when the organism is growing under anaerobic conditions. Beta hemolysis is caused by one or more erythrocyte-lysing enzymes called **hemolysins**.
- **Alpha hemolysis:** The colony is surrounded by a zone of intact but discolored erythrocytes that have a greenish color. This appearance is generally due to the action of **peroxide** produced by the bacteria.
- **Gamma hemolysis** is simply a synonym for **negative hemolysis** in which there is no change in the medium surrounding the colony

Blood Agar: Hemolytic Reactions

- **Beta hemolysis:** In the top photo at right which shows *Enterococcus durans* growing on Blood Agar, the light to clear zones can be seen around the colonies. Where growth occurs in the presence of less oxygen the reaction is more pronounced.
- **Alpha hemolysis:** The bottom photo shows *Streptococcus mitis* growing on Blood Agar; some greenish zones of alpha hemolysis are visible.



Blood Agar Sheep

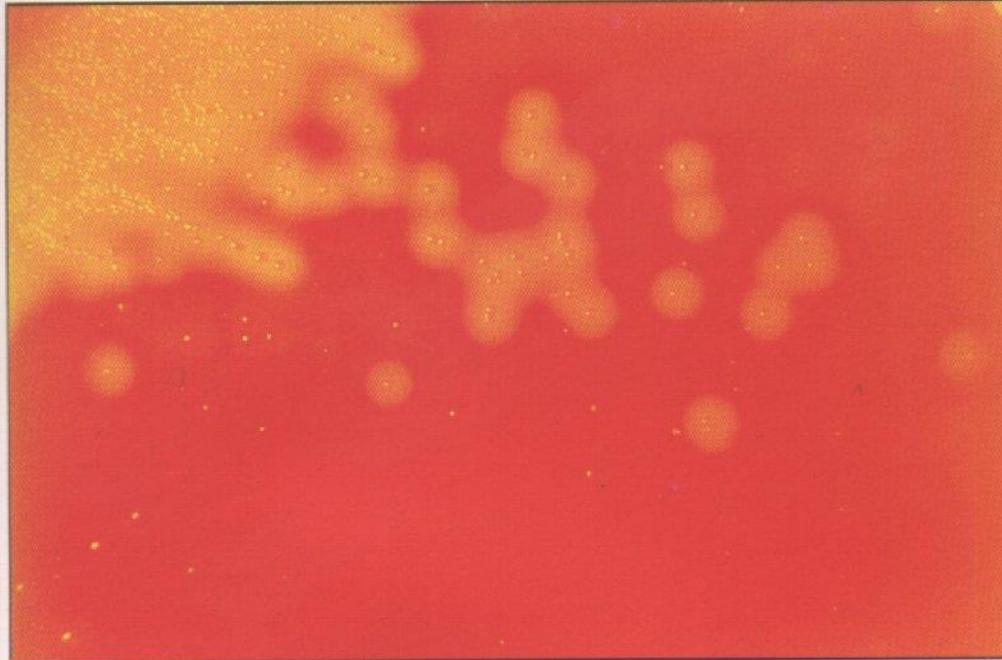


Formazione di zone di emolisi intorno a colonie che producono emolisine su agar sangue.

A sinistra ***Streptococcus mitis***, un batterio **a-emolitico**

A destra ***Streptococcus pyogenes*** di gruppo A (GAS), tipico **b-emolitico**

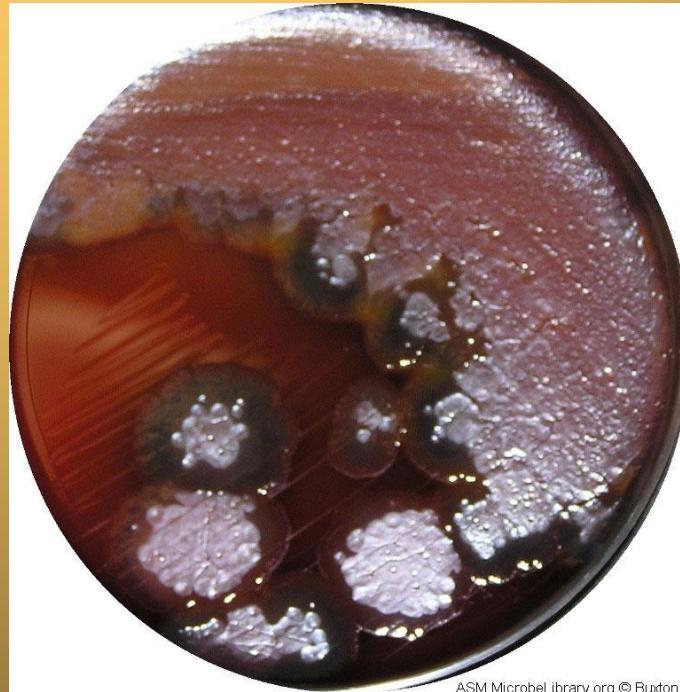
Blood Agar Sheep



7. BLOOD AGAR SHEEP

Colonie di streptococco beta emolitico di gruppo A.

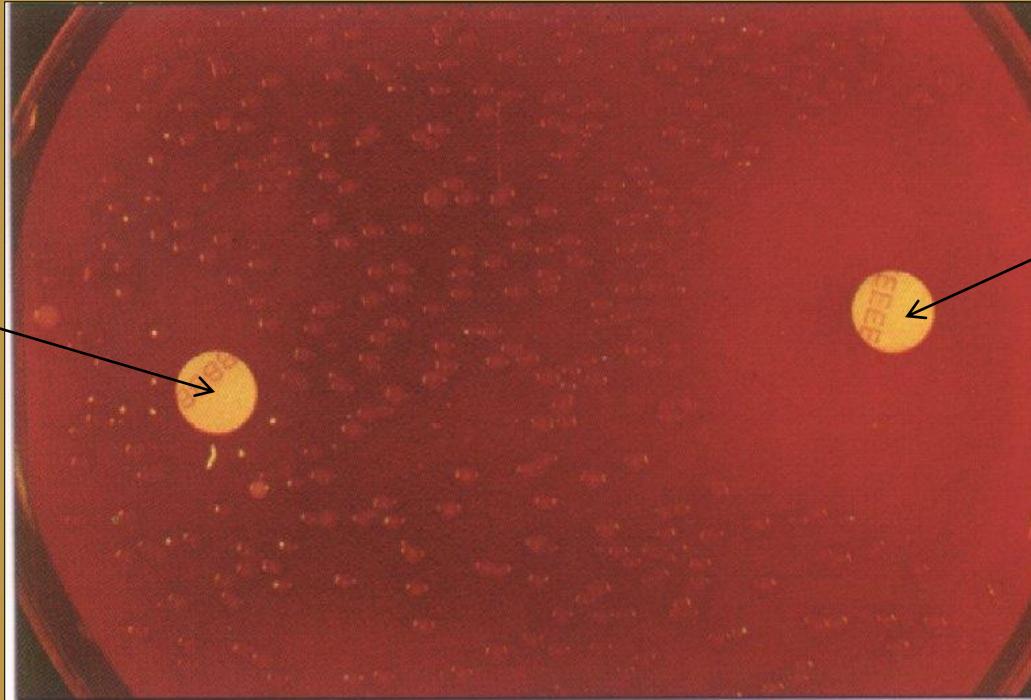
Blood Agar



ASM MicrobeLibrary.org © Buxton

Colonies of *Pseudomonas aeruginosa* typically display beta hemolysis, a metallic sheen, and blue or green pigment.

Blood Agar (with 5% Sheep Blood)



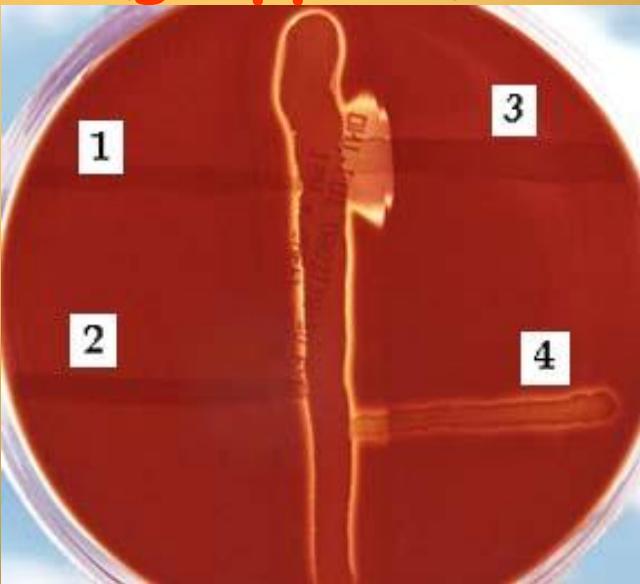
OPTOCHINA

BACITRACINA

8. BLOOD AGAR SHEEP
S.pneumoniae, sensibile all'optochina, resistente alla bacitracina.

CAMP test on b-hemolytic streptococci (gruppo B)

Enterococcus faecalis



Streptococcus agalactiae

Streptococcus salivarius

Enterococcus durans

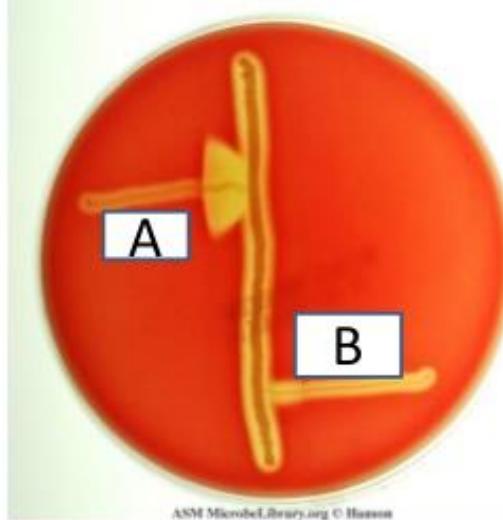
Staphylococcus aureus

S. agalactiae strains produce a diffusible, extracellular compound (CAMP factor) that will, in conjunction with a specific beta-hemolysin of *Staphylococcus aureus*, cause complete lysis of sheep red blood cells in an agar medium.

In this photo, a Blood Agar plate is shown after 24 hours of incubation at 37°C. The vertical streak is a beta-hemolysin-producing strain of *Staphylococcus aureus*, and at right angles to it are streaks of (1)*Enterococcus faecalis*, (2)*Streptococcus salivarius*, (3)*S. agalactiae*, and (4) *E. durans*.

Note the large area of complete lysis where the extracellular compound of *S. agalactiae* encounters the beta-lysin of *S. aureus*.

CAMP test on b-hemolytic streptococci (gruppo B)



ASM MicrobeLibrary.org © Hasenst

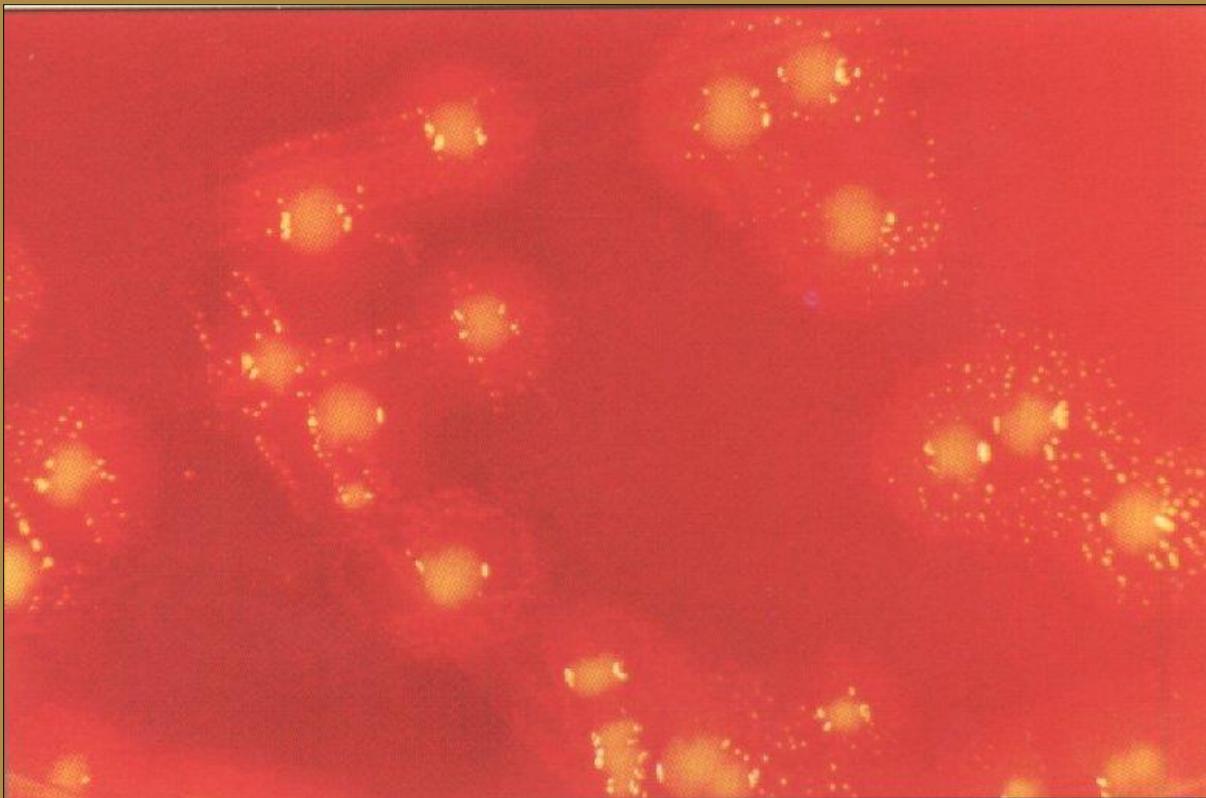
Name of the test: CAMP test

Example A: Positive - Strept agalactiae (Arrow shaped)

Example B: Negative - Strept pyogens

Principle : Strept agalactiae produce CAMP factor (a diffusible extracellular protein) that synergistically acts with the beta-lisin of *Staphylococcus aureus* and enhances the lysis of red blood cells.

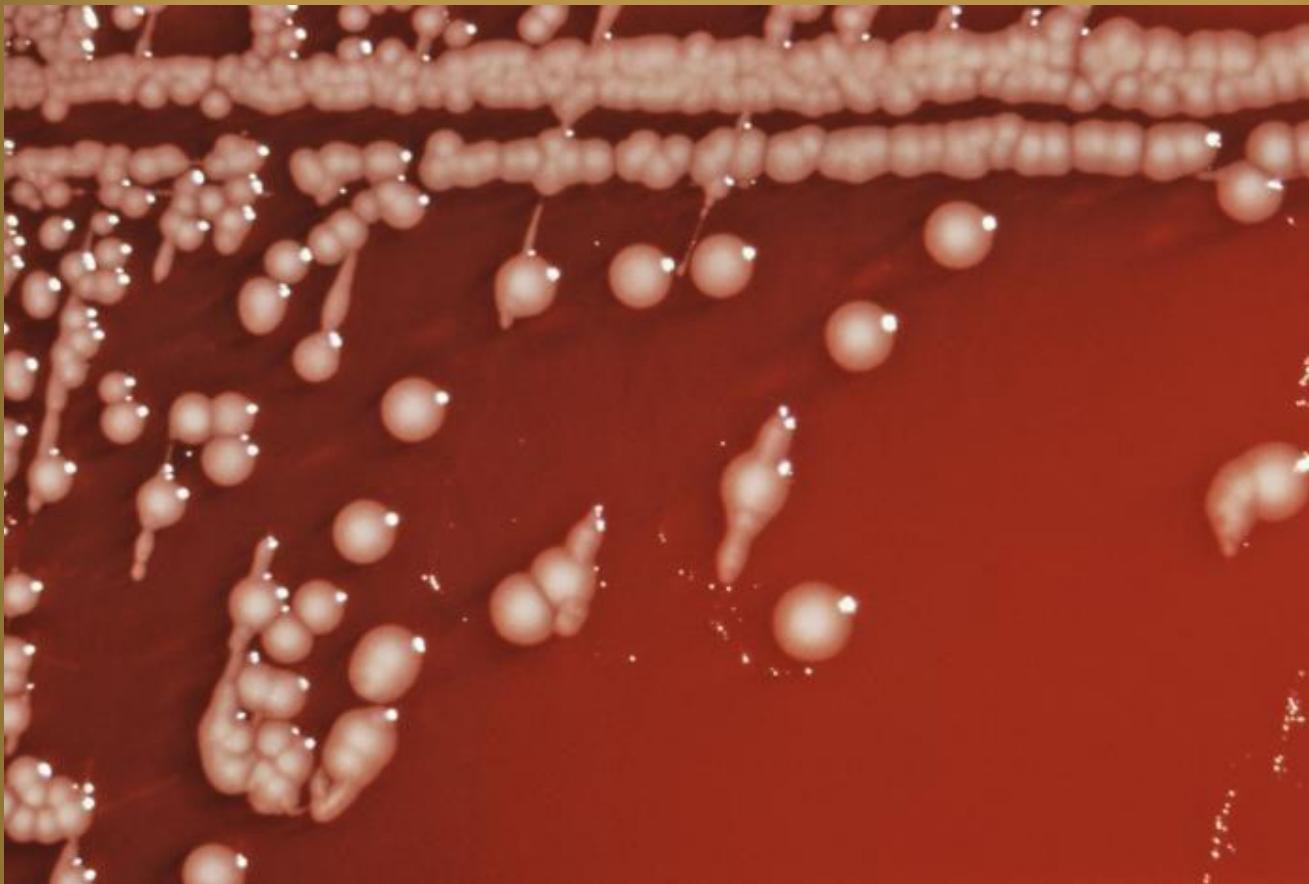
Blood Agar Sheep



9. BLOOD AGAR SHEEP

Fenomeno di satellitismo di *H.influenzae*, attorno a colonie di *S.aureus*

Colonie di *Salmonella choleraesuis* subsp. *arizonaee* su a blood agar



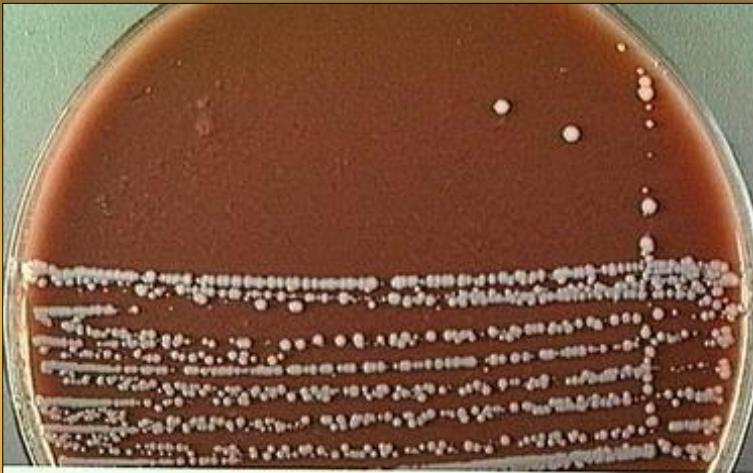
E' conosciuta anche come *Salmonella arizonaee*, batterio zoonosico
che può infettare uomo, uccelli, rettili, ed altri animali.

CHOCOLATE AGAR



Chocolate agar is a nutrient medium which is used in culturing fastidious organisms such as *Haemophilus* species and *Neisseria*. It is comprised of sheep blood that provides the X and V factors necessary for *Haemophilus* growth.

THAYER MARTIN AGAR



Neisseria gonorrhoeae
on Thayer Martin Agar

Medium proposed by THAYER and MARTIN (1964, 1966) for the selective isolation of *Neisseria gonorrhoeae* and *Neisseria meningitidis* from clinical specimens.

Addition of various antibiotics (THAYER-MARTIN Supplement I), and specific growth-promoting agents (THAYER-MARTIN Supplement II) to the basic substrate with or without hemoglobin usually inhibits or totally suppresses the growth of accompanying bacterial flora, while allowing pathogenic *Neisseria* species to grow normally.

THAYER-MARTIN Supplement I: vancomicina, colistina, nistatina e trimetoprim lattato

TRYPTIC SOY BROTH/AGAR



substrato ricco non selettivo per la coltivazione dei batteri con elevate esigenze nutrizionali

Tryptic soy agar and broth is a basic medium used for culturing many kinds of microorganisms. Tryptic soy agar is mainly used as an initial growth medium for the following purposes:

- observe colony morphology
- develop a pure culture
- achieve sufficient growth for further biochemical testing
- culture storage

It can also be used in the determination of bacterial numbers.

Pseudomonas aeruginosa su agar soy trypticase



AGAR SALMONELLA-SHIGELLA

Contiene:

1. Sali biliari, verde brillante e sodio citrato che rendono il terreno selettivo poiché inibiscono la crescita di Gram+
2. Lattosio con indicatore di pH (rosso neutro) che rende il terreno differenziale poiché la Salmonella e la Shigella non fermentano il lattosio a differenza di altri Gram-.

Quindi su questo terreno potranno crescere tutti i Gram-; ma la Salmonella e la Shigella daranno colonie incolori mentre i batteri che fermentano il lattosio daranno colonie di colore rosa.

AGAR SALMONELLA-SHIGELLA



•*Salmonella typhimurium*

Colonie lattosio negative sono incolori

Colonie lattosio positive sono rosa

Colonie di microrganismi che producono H₂S sono nere al centro

(Alcune specie dei generi *Proteus* e *Salmonella* presentano colonie con centro nero; ciò è dovuto alla precipitazione del ferro solfuro, indotta dalla produzione di idrogeno solforato a partire dal sodio tiosolfato presente nel terreno.)

AGAR SALMONELLA-SHIGELLA

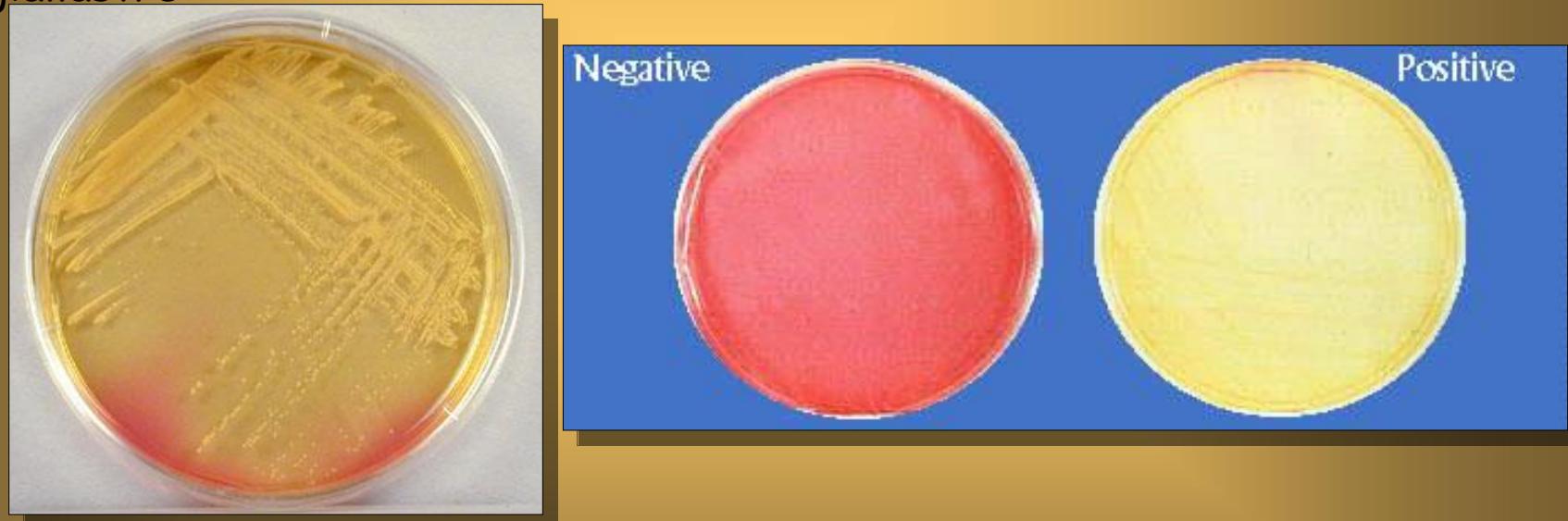


33. SS AGAR
Salmonella sp.

AGAR MS (salato al mannitollo)

E' selettivo perché presenta una elevata concentrazione di NaCl che inibisce la maggior parte dei batteri tranne quelli sale-tolleranti come gli Stafilococchi.

Il **mannitollo** rende il terreno differenziale: questo zucchero infatti viene fermentato dagli Stafilococchi patogeni che daranno origine a colonie giallastre



Utilizzato per isolare Stafilococchi patogeni. L'acido prodotto dalla fermentazione del mannitollo determina il viraggio dell'indicatore di pH (**rosso fenolo**) da rosso (alcalino) a giallo (acido). Gli stafilococchi non patogeni sono capaci di crescere sul mezzo ma non producono acido.

Stafilococchi su AGAR SALE MANNITE

TOLLERANO ELEVATE CONCENTRAZIONI DI NaCl (germi ALOFILI);
LA MAGGIOR PARTE DEI CEPPI PATOGENI FERMENTA LA MANNITE

AGAR SALE MANNITE: terreno utilizzato per l'isolamento di Stafilococchi patogeni:

CONTIENE 7.5% NaCl che inibisce la crescita della maggior parte degli altri microrganismi.

CONTIENE LA MANNITE COME UNICA FONTE DI CARBONIO

S. epidermidis:
colonie alcaline (rosa);
non fermentanti la
mannite



S. aureus:
colonie acidificanti per
fermentazione della
mannite e viraggio al giallo
dell'indicatore rosso fenolo

MACCONKEY AGAR



Sali biliari (1,5%) e
cristal violetto:
inibiscono i Gram +

Indicatore di PH: rosso
neutro

LATTOSIO

MacConkey agar is probably the most popular solid differential medium in the world. It is mainly used in identification of lactose fermenting, Gram-negative enteric pathogens and for inhibiting growth of Gram-positive organisms. Bacterial colonies that can ferment lactose turn the medium red. This red color is due to the pH indicators response to the acidic environment created by fermenting lactose. Organisms that do not ferment lactose do not cause a color change.

MACCONKEY AGAR



a) *Serratia marcescens* su agar MacConkey



Shigella flexneri su agar MacConkey

HEKTOEN ENTERIC (HE) AGAR



Indicatore di PH

Colonie lattosio non fermentanti

REAGENTI

BD Hektoen Enteric Agar

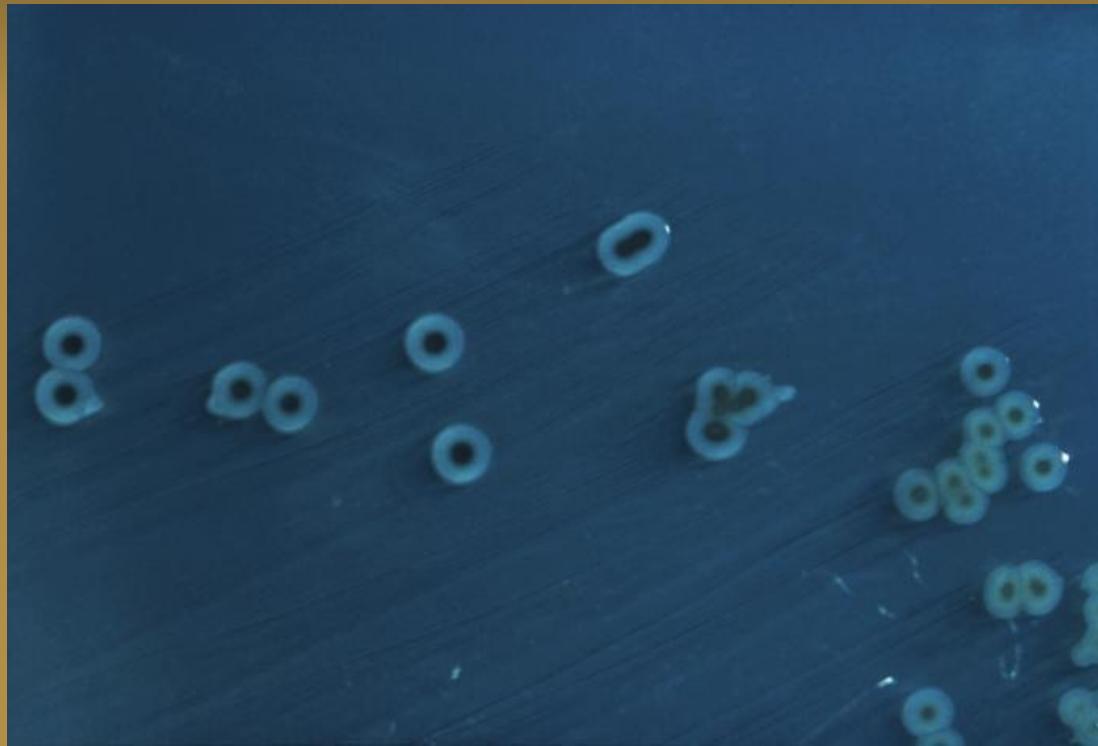
Formula* per litro di acqua purificata

Digerito peptico di tessuto animale	12,0 g	Cloruro di sodio	5,0 g
Estratto di lievito	3,0	Tiosolfato di sodio	5,0
Sali biliari	9,0	Citrato di ammonio ferrico	1,5
Lattosio	12,0	Blu bromotimolo	0,064
Saccarosio	12,0	Fucsina acida	0,1
Salicina	2,0	Agar	13,5

pH 7,6 +/- 0,2

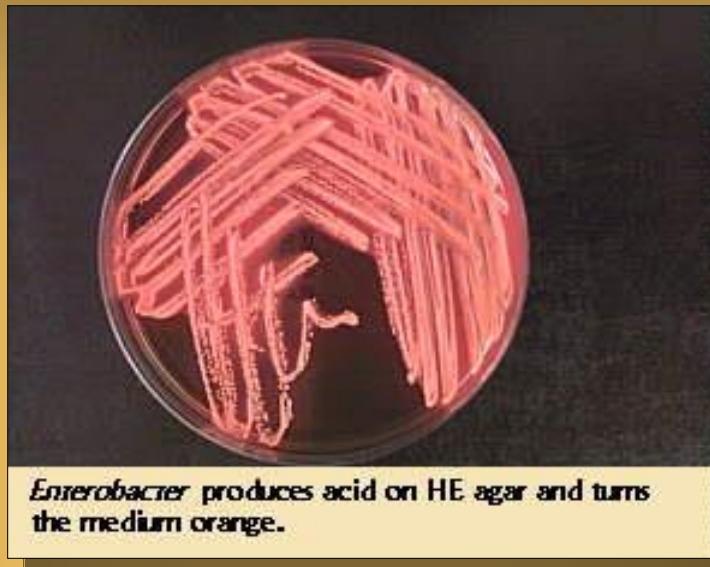
*Compensata e/o corretta per soddisfare i criteri di rendimento.

Colonie di *Salmonella typhimurium* su Hektoen enteric (HE) agar



S. typhimurium su HE agar forma colonie blu-verdi non fermentanti il lattosio, inoltre produce idrogeno solforato (H_2S), come evidenziato dal deposito nero al centro delle colonie.
HE agar è un mezzo utilizzato per isolare i batteri fecali della famiglia delle Enterbacteriaceae.

HEKTOEN ENTERIC (HE) AGAR

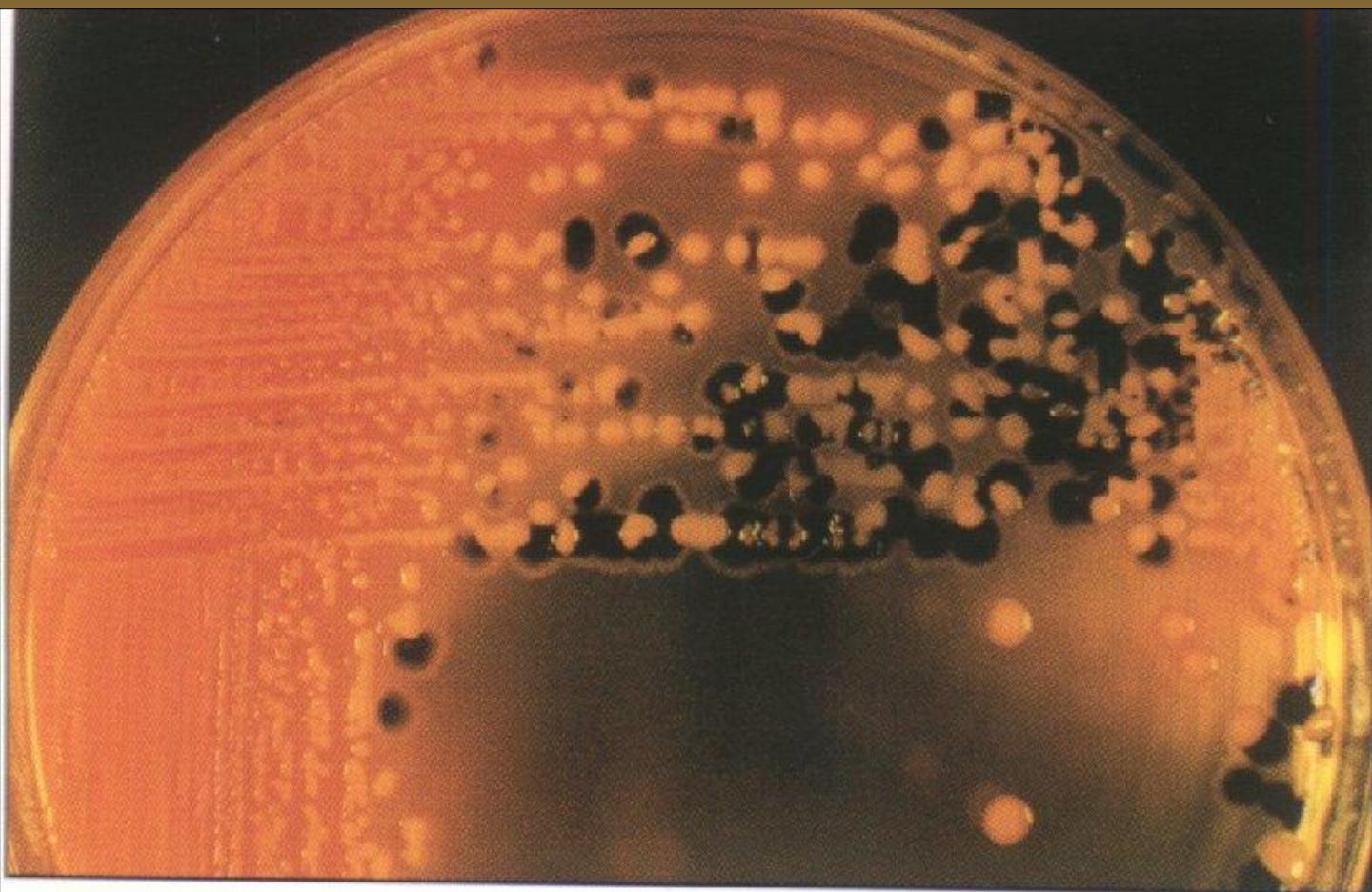


Hektoen enteric agar is a medium designed for the isolation and recovery of fecal specimens belonging to the Enterbacteriaceae family, especially *Salmonella* and *Shigella*. It can differentiate between bacteria that ferment lactose and those that don't. Acid produced from fermenting lactose imparts a yellow-orange color to the medium due to the presence of a pH indicator. Non-lactose fermenters do not significantly change the color of the medium. HE agar can also detect the production of hydrogen sulfide gas, which turns parts of the medium black.

HEKTOEN ENTERIC (HE) AGAR



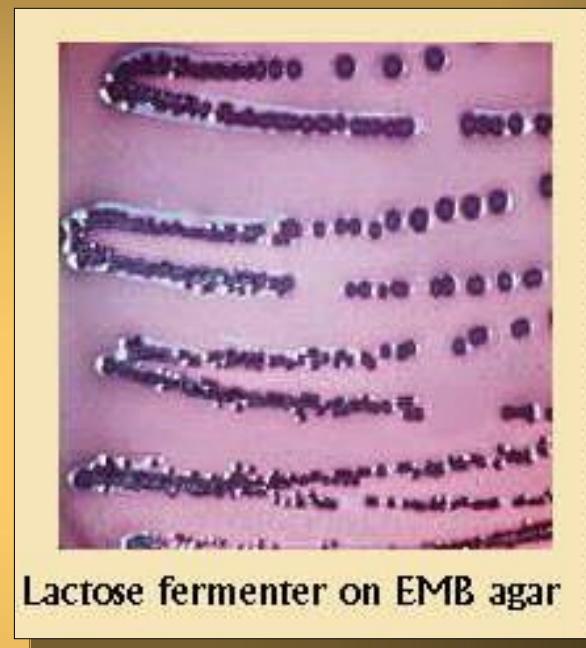
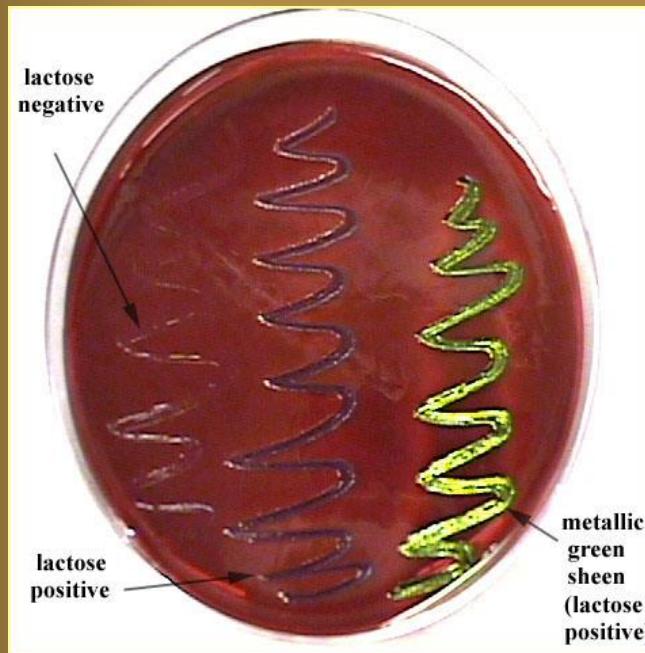
Klebsiella pneumoniae ferments lactose and produces salmon-colored growth. *Micrococcus luteus* does not grow. The white specks are crystals formed by a component of the media that precipitated out of solution when the plates were cooled. They look somewhat like a contaminant, but they do not interfere with the performance of the media.



21. HEKTOEN ENTERIC AGAR

Colonie di *Salmonella* sp. nere e di *Klebsiella* sp. color
salmone.

EOSIN METHYLENE BLUE (EMB) AGAR



Eosin methylene blue agar, as the name suggests, contains the dyes eosin and methylene blue. EMB agar is selective because the aniline dyes in this purple media inhibit growth of Gram-positive organisms. Lactose fermenters metabolize the lactose in the media and produce acid byproducts, causing a color change in the colony. Thus, EMB is also a differential medium. **Strong acid production by organisms such as *E. coli* results in a metallic green sheen.** Weaker fermentation of lactose results in colonies with a pinkish-purple color. Colonies of non lactose fermenters remain colorless, or at least are no darker than the color of the media.

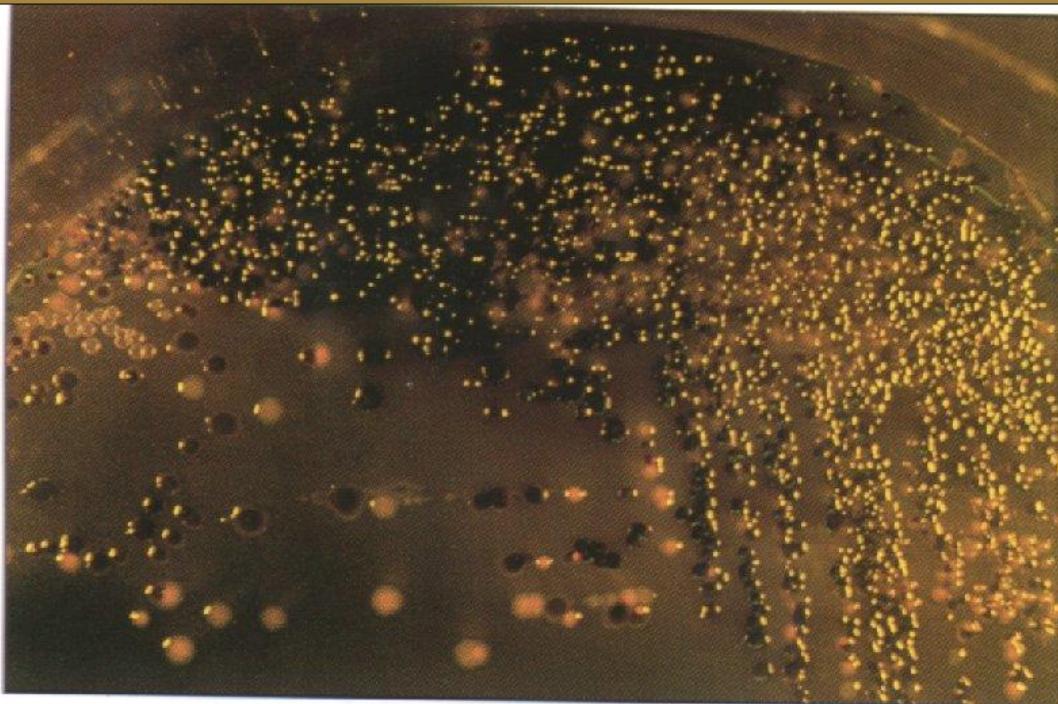
EOSIN METHYLENE BLUE (EMB) AGAR



Klebsiella pneumoniae

Pseudomonas aeruginosa

LEVINE EMB BLUE AGAR

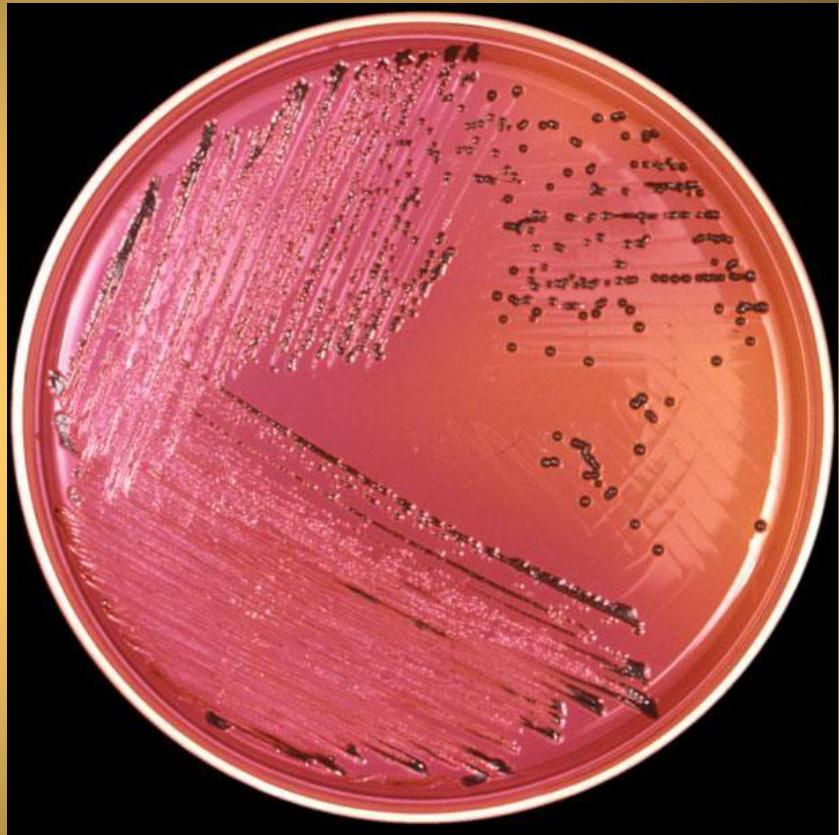


22. LEVINE EMB BLUE AGAR
Flora mista lattosio fermentante e non.

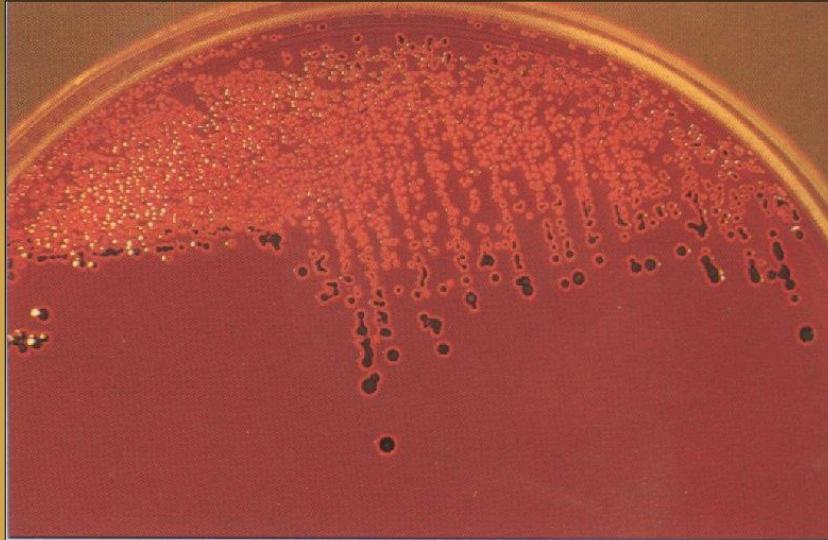
The dyes contained in this medium inhibit the growth of many accompanying Gram-positive microorganisms

Salmonella sp. su XLD agar

L' agar **Xylose Lysine (XL)** è utilizzato per isolare i bacilli enterici Gram-negativi. Quando l' agar XL è supplementato con sodio tiosolfato, citrato ammonio ferrico e sodio deossicolato viene chiamato **agar XLD**, un mezzo estremamente selettivo. La presenza di un area colorata di nero nella colonia indica la produzione di idrogeno solforato.



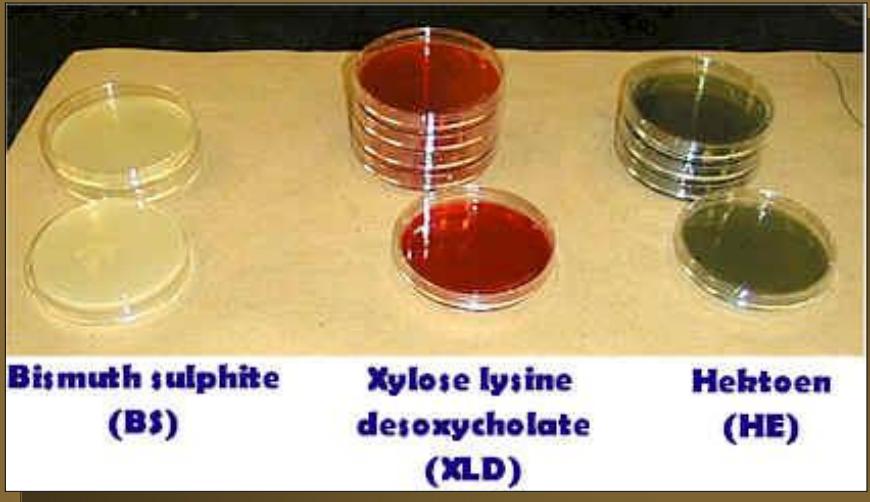
XLD



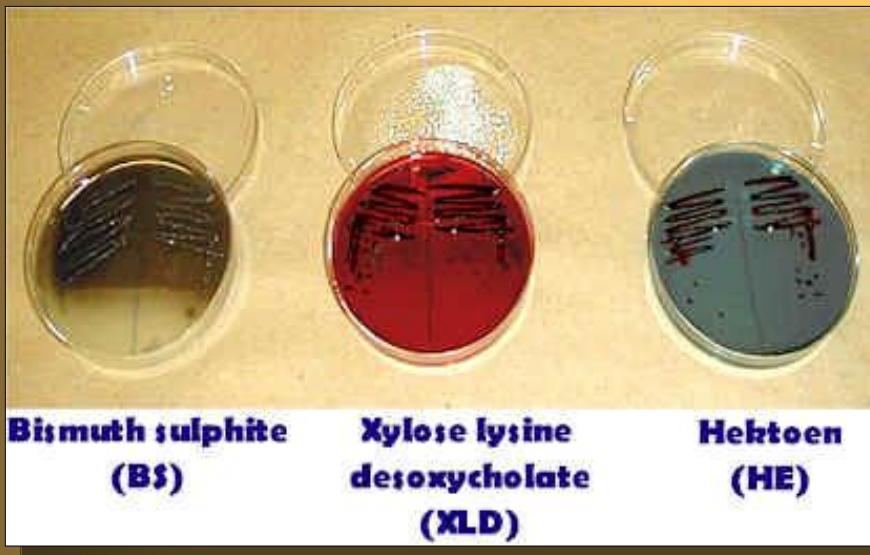
40. XLD AGAR
Salmonella sp.

Xylose lysine deoxycholate (XLD) agar is a highly selective and differential medium for the isolation and identification of *Salmonella* and *Shigella* species. Both *Salmonella* and *Shigella* species can decarboxylate the lysine in the media to produce red colonies. Additionally, *Salmonella* produces a positive hydrogen sulfide production reaction, which colors the centers of the colonies black

*Isolation of *Salmonella* onto selective agar:*



Sterile media (before streaking):



Plates positive for *Salmonella* after incubation:

Note: If the agar media change in color and the colonies are not dark (e.g., yellow), bacteria OTHER than *Salmonella* were isolated

Kligler Iron Agar (KIA) & Triple Sugar Iron (TSI) Agar

source of amino acids which may be deaminated (alkaline rx.)

amino acid added to note its decarboxylation (alkaline rx.)

fermentable sugar(s) (acid rx.)

pH indicator

source from which H_2S may be produced

indicator of H_2S production

peptone,
proteose peptone,
beef extract,
yeast extract

none

lactose (1%),
sucrose (1% - in KIA),
glucose (0.1%)

phenol red:
net acid = yellow,
net alkaline = red

sodium thiosulfate

ferrous sulfate

Lysine Iron Agar (LIA)

peptone,
yeast extract,
lysine

lysine

glucose (0.1%)

brom-cresol purple:
net acid = yellow,
net alkaline = purple

sodium thiosulfate

ferric ammonium citrate

Motility Indole Ornithine (MIO) Medium

peptone,
yeast extract,
ornithine

ornithine

glucose (0.1%)

brom-cresol purple:
net acid = yellow,
net alkaline = purple

none

none

TRIPLE SUGAR IRON (TSI) AGAR

Triple sugar iron (TSI) agar is a medium used in the identification of **Gram-negative enteric rods**.

- The medium measures a bacterium's ability to utilize **three sugars**, glucose, sucrose and lactose.
- A pH indicator included in the medium can detect acid production from fermentation of these carbohydrates.
- A yellow color change indicates acid in the medium while no color change indicates an alkaline surrounding.

TRIPLE SUGAR IRON (TSI) AGAR

Inoculation of the tube is a two step procedure. First, a loop of bacteria is spread across the surface of the agar. Second, a needle of bacteria is inserted (stabbed) into the bottom (butt) of the tube.

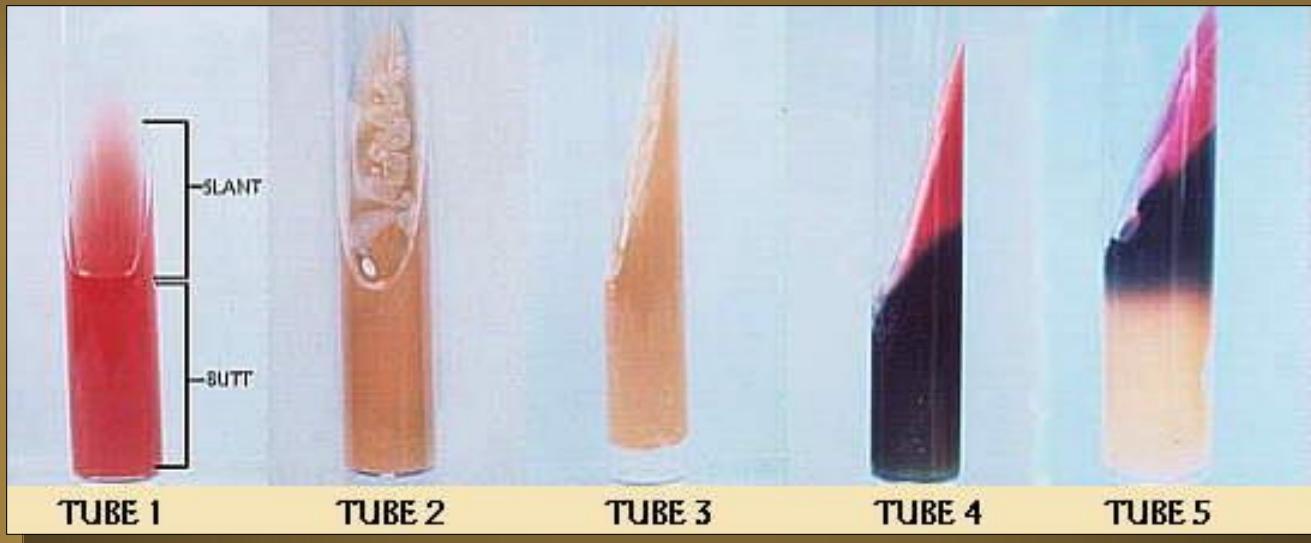
Carbohydrate utilization can be determined through analysis of the extent of acid production.

Acid production limited to only the butt of the tube is indicative of glucose utilization. This is because the concentration of glucose is lower than that of other sugars, thus the acid production is not very extensive.

Acid production in the slant and butt indicates sucrose or lactose fermentation because of the relatively high concentrations of these sugars, thus leading to extensive acid production.

TSI agar can also detect reduction of sodium thiosulfate to hydrogen sulfide. Hydrogen sulfide production will turn parts of the agar black. Production of other gases is marked by cracks in the agar as well as an air gap at the bottom of the test tube.

TRIPLE SUGAR IRON (TSI) AGAR

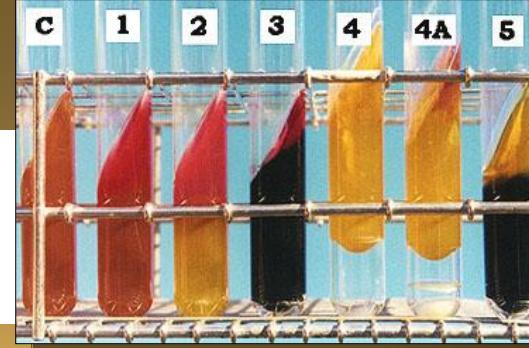


INTERPRETATION OF TUBES ABOVE

	TUBE 1 (UNINOCULATED)	TUBE 2	TUBE 3	TUBE 4	TUBE 5
SLANT	-	A	A	K	K
BUTT	-	A	A	A	A
HYDROGEN SULFIDE	-	-	-	+	+
GAS	-	-	+	-	-

A=Acidic K=Alkaline

TRIPLE SUGAR IRON (TSI) AGAR

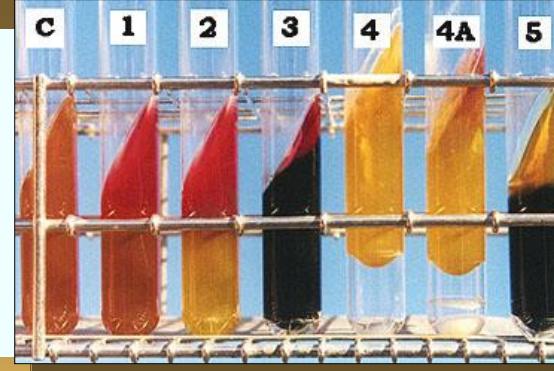


corresponding tube no. above	1	2	3	4*	5
deamination of amino acids (aerobic alkaline rx.)	+	+	+	+	+
glucose fermentation (minor acid rx.)	-	+	+	+	+
lactose and/or sucrose fermentation (major acid rx.)	-	-	-		+
H₂S production (black color)	-	-	+	-	+**
typical examples	<i>Pseudomonas</i> (a non-enteric)	<i>Morganella</i> <i>Providencia</i> <i>Shigella</i>	<i>Citrobacter</i> <i>Salmonella</i> <i>Edwardsiella</i>	<i>E. coli</i> <i>Enterobacter</i> <i>Klebsiella</i>	<i>Proteus</i> <i>E. coli</i> lactose+ <i>Salmonella</i> H2S+

* **Tube 4:** Much **gas** is often seen for this tube, evidenced by cracks in the medium. Also, **methyl red-negative** organisms which ferment lactose and/or sucrose may show a "reversion" toward an alkaline reaction as neutral products are formed from some of the acid. Note the slight orange to red color at the tip of the slant in **tube 4A**. How might such a tube look at two or more days of incubation? (Regarding the methyl red test, recall the activities of enterics in MR-VP Broth which are illustrated [here](#).)

** **Tube 5:** Enough acid can be produced to cause the black iron sulfide precipitate to break down and not be seen. In this case, the tube will look like tube 4.

Kligler Iron Agar (KIA)

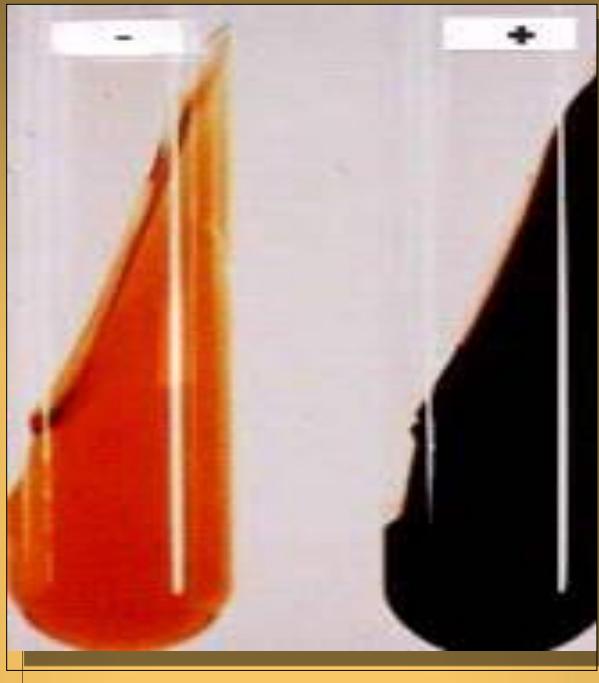


corresponding tube no. above	1	2	3	4*	5
deamination of amino acids (aerobic alkaline rx.)	+	+	+	+	+
glucose fermentation (minor acid rx.)	-	+	+	+	+
lactose fermentation (major acid rx.)	-	-	-	+	+
H₂S production (black color)	-	-	+	-	++*
typical examples	<i>Pseudomonas</i> (a non-enteric)	<i>Morganella</i> <i>Providencia</i> <i>Shigella</i>	<i>Citrobacter</i> <i>Salmonella</i> <i>Proteus</i> <i>Edwardsiella</i>	<i>E. coli</i> <i>Enterobacter</i> <i>Klebsiella</i>	H ₂ S+ <i>Salmonella</i> lactose+ <i>E. coli</i>

* **Tube 4:** Much **gas** is often seen for this tube, evidenced by cracks in the medium. Also, lactose fermenters which are **methyl red-negative** may show a "reversion" toward an alkaline reaction as neutral products are formed from some of the acid. This appears as shown in **Tube 4A** where a slight reddening of the slant occurs as the alkaline deamination reaction becomes no longer over-neutralized by acid from fermentation. How might such a tube appear after two or more days of incubation? (Regarding the methyl red test, recall the activities of enterics in MR-VP Broth, illustrated [here](#).)

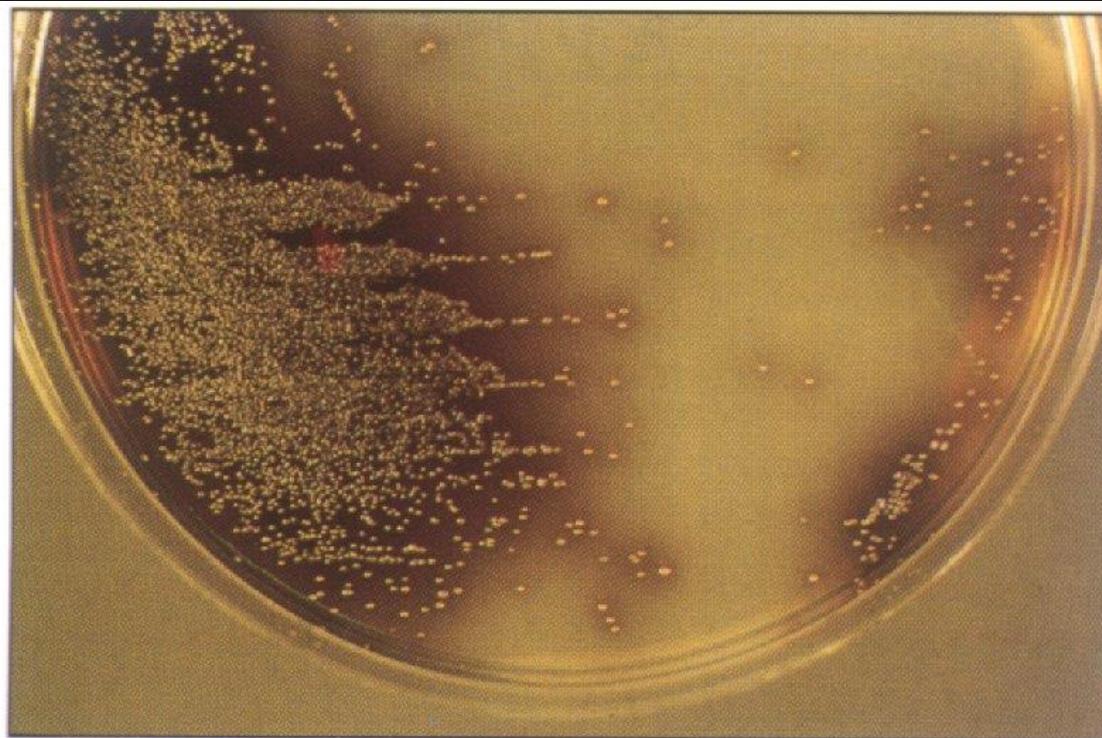
** **Tube 5:** Enough acid can be produced to cause the black iron sulfide precipitate to break down and not be seen. In this case, the tube will look like no. 4.

BILE ESCULIN AGAR



Bile esculin agar is a medium used to identify group D streptococci. This group of bacteria have the ability to grow in the presence of bile. Group D streptococci also have the ability to hydrolyze esculin. This hydrolysis of esculin turns the medium black and denotes a positive test. Other bacteria capable of growing in the presence of bile do not turn the medium black.

BILE ESCULIN AZIDE AGAR



2. AESCULIN BILE AZIDE AGAR
Colonie di *E faecalis*, nere, esculinasi positive.

A variation of bile esculi agar, uses sodium azide to inhibit the growth of all other Gram-positive bacteria and Gram-negative bacteria.

Terreni selettivi per l'isolamento di Listeria

1. Listeria Fraser Broth: brodo di arricchimento selettivo contenente esculina e cloruro di litio. **Supplemento:** contiene citrato ferrico di ammonio e acido nalidixico.

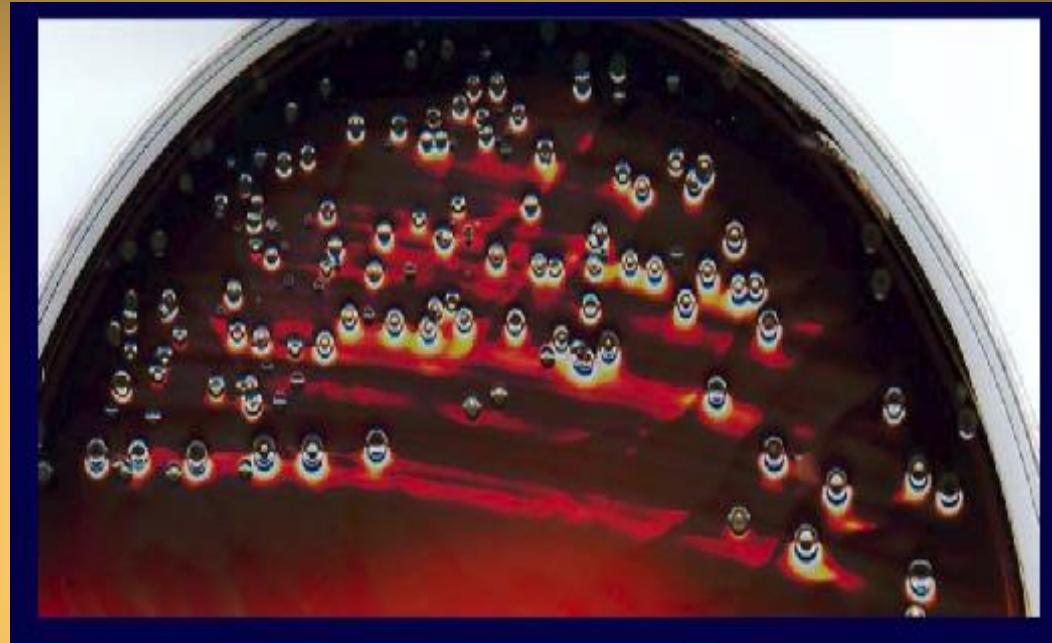
La *Listeria* idrolizza l'esculina ad esucleicina che, reagendo con gli ioni ferrici, causa l'annerimento del brodo. La presenza del cloruro di litio e di acido nalidixico inibiscono la crescita degli enterococchi che, come *Listeria*, idrolizzano l'esculina.

2. Palcam Agar Base: terreno selettivo per la presenza del cloruro di litio. **Supplemento:** selettivo, perché costituito da ceftazidime, polimixina B e acriflavina. Presenta un doppio sistema indicatore:

- **Esculina e ferro ferroso** (*L. monocytogenes* idrolizza l'esculina, producendo colonie con un alone nero)
- **Mannitolo e rosso fenolo** (*L. monocytogenes* non fermenta il mannitolo; enterococchi e stafilococchi fermentano il mannitolo, determinando un viraggio dell'indicatore di pH dal rosso al giallo).



L. monocytogenes
in Fraser Broth



Colonie di *L. monocytogenes*
su Palcam

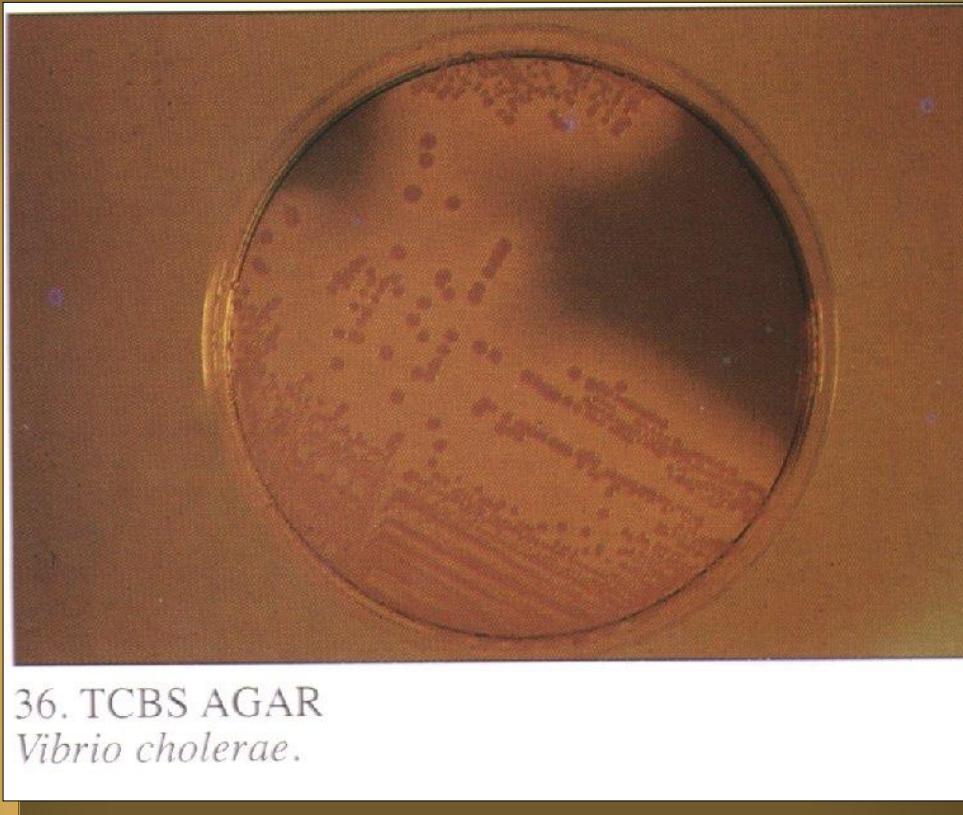
CAMPY-BAP



Campy-BAP medium in a microaerophilic bag

Campy-BAP is a blood agar that is highly selective for the growth of *Campylobacter* species. It contains several antibiotics which inhibit the growth of Gram-positive bacteria as well as most Gram-negative organisms. After the plate is inoculated, it is placed in a microaerophilic environment such as a sealed bag containing high carbon dioxide concentrations and low oxygen concentrations.

TCBS AGAR: Vibrio Selective Agar



36. TCBS AGAR
Vibrio cholerae.

The high concentrations of thiosulfate and citrate and the strong alkalinity of this medium largely inhibit the growth of Enterobacteriaceae. Any coliform bacteria, which may grow, cannot metabolize sucrose. Only a few sucrose-positive *Proteus* strains can grow to form yellow, vibrid-like colonies. The mixed indicator thymol blue-bromothymol blue changes its colour to yellow, when acid is formed, even in this strongly alkaline medium.

Colonie di *V. cholerae* (gialle) su TCBS (tiosolfato-citrato-sali biliari-saccarosio)



TCBS AGAR: Vibrio Selective Agar



Colonie di vibrioni saccarosio negativi su TCBS agar

Terreni per Micobatteri



LOWENSTEIN-JENSEN
(PETRAGANI)

- Tuorlo d'uovo
- Glicerolo

These substances provide fatty acids and protein required for the metabolism of mycobacteria.

- Verde malachite:
inibitore selettivo della crescita altri batteri contaminanti

Terreni per Micobatteri



Uninoculated plate
of Middlebrook 7H11 Agar



Colonie di *M. tuberculosis* su 7-H11

Glycerol is provided as a source of carbon and energy

Malachite green is added as a selective agent, which partially inhibits the growth of other bacteria.

Enrichment contains :

- albumin to protect the tubercle bacilli against toxic agents;
- oleic acid, a fatty acid utilized in the metabolism of the organism;
- sodium chloride to maintain osmotic equilibrium;
- catalase to destroy any toxic peroxides in the medium;
- and dextrose as an energy source.

Terreni per *B.cereus*

PEMBA

- Nutritional component (eg: peptone)
- Mannitol
- Sodium chloride
- Magnesium sulphate
- Disodium hydrogen phosphate
- Potassium dihydrogen phosphate
- Bromothymol blue
- Sodium pyruvate
- Agar

MEYP

- Nutritional components (meat extract, peptone)
- Mannitol
- Sodium chloride
- Phenol red
- Agar

SUPPLEMENTS PEMBA and MEYP

- 5% egg yolk emulsion
- Polymixin B 100,000 IU/l

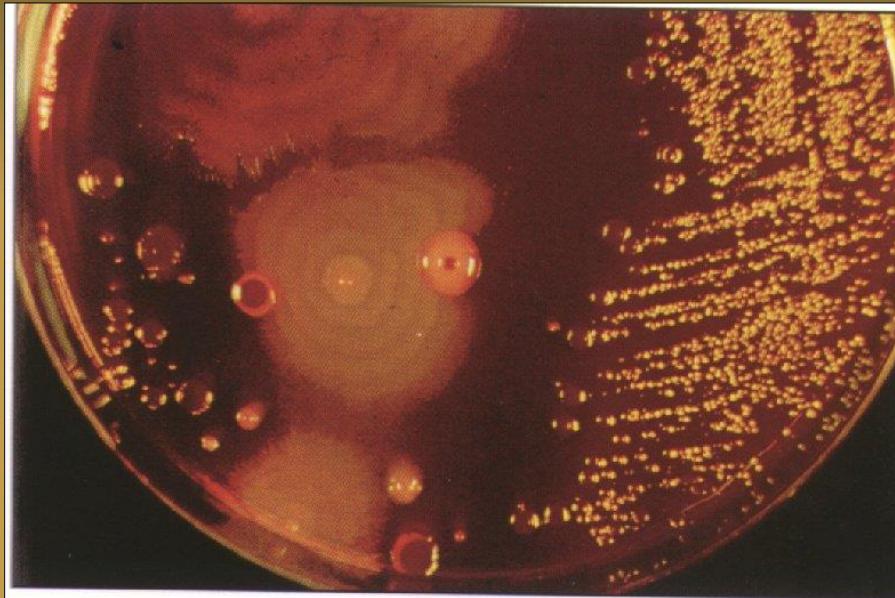
PEMBA



4. BACILLUS CEREUS SELECTIVE AGAR (PMBA)
Colonie di *B.cereus*, blu turchese con alone di precipitazione dell'emulsione d'uovo.

Colonies of *B. cereus* are crenated and approximately 5mm in diameter. They have a distinctive turquoise to peacock blue colour on PEMBA and pink on MEYP surrounded by a zone of egg yolk precipitate.

ENDO AGAR

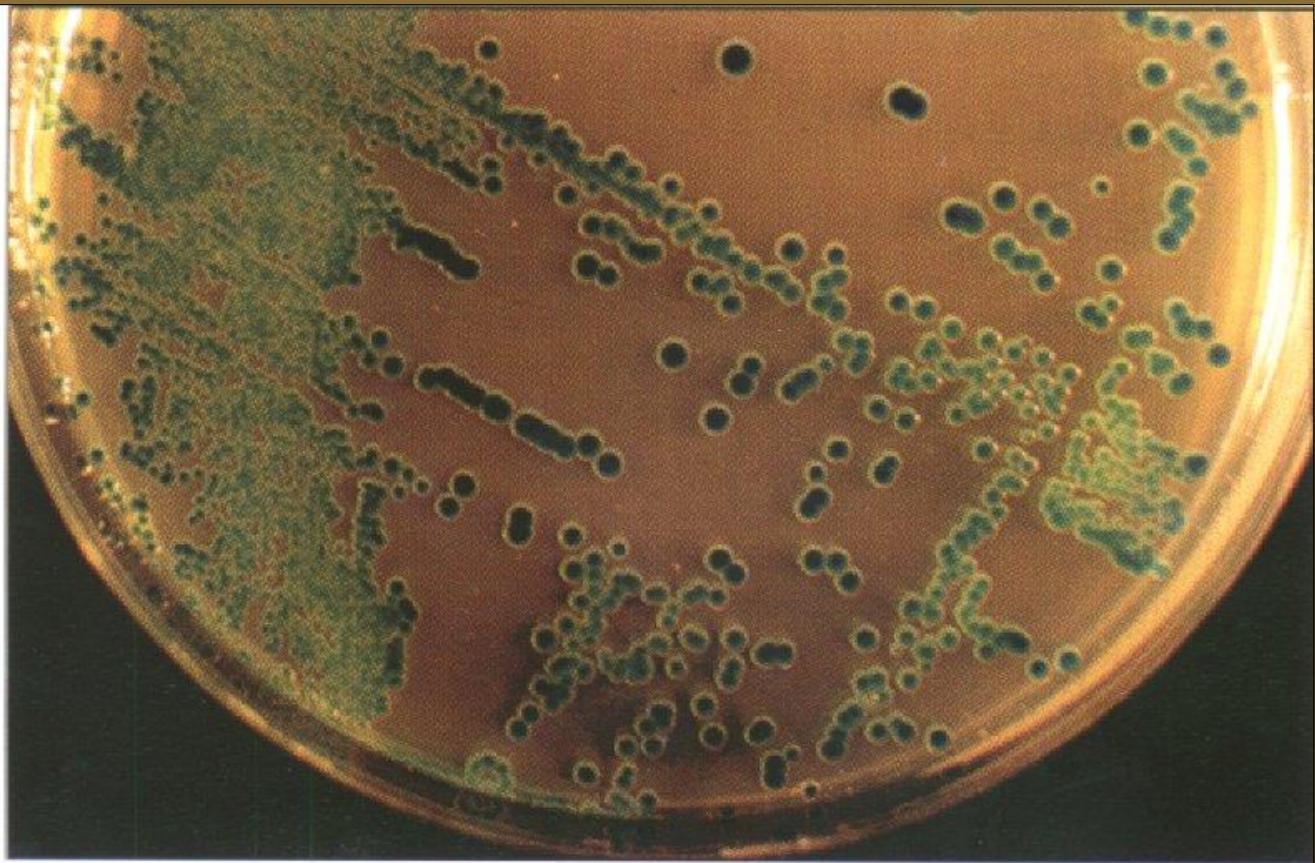


20. ENDO AGAR

Flora mista: *E.coli* con colonie metalliche, *E.aerogenes* con colonie rosse, *P.mirabilis* con colonie incolori sciamanti.

Sodium sulfite and fuchsin inhibit the growth of gram-positive bacteria.

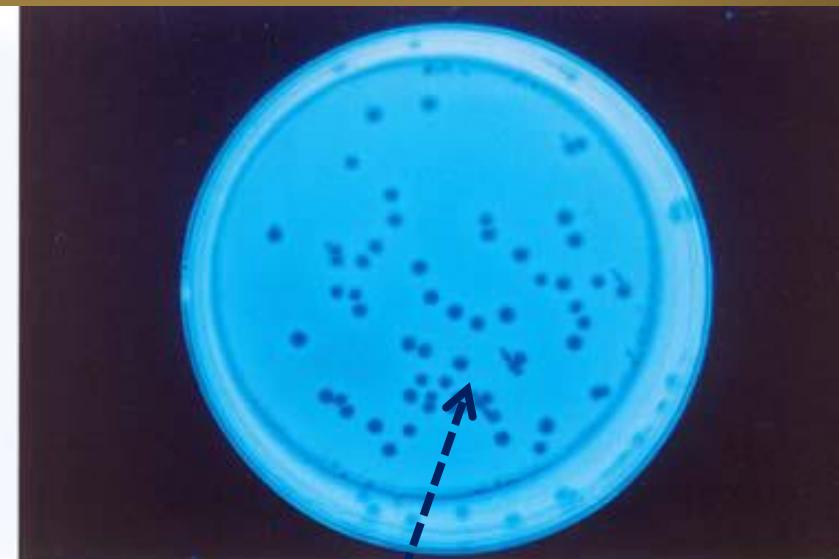
E. coli and coliform bacteria metabolize lactose with the production of aldehyde and acid. The aldehyde liberates fuchsin from the fuchsin-sulfite compound, the fuchsin then colors the colonies red. In the case of *E. coli*, this reaction is so intense that the fuchsin crystallizes out giving the colonies a permanent greenish metallic sheen (fuchsin sheen). Lactose-negative and weakly lactose-positive *E. coli* do not show any fuchsin sheen.



17. CHROMOGENIC E.COLI (EC X-GLUC) AGAR
Colonie di *E.coli* beta glucuronidasi positive (verde-blu).

C - EC AGAR

Substrato selettivo fluorogenico e cromogenico per la determinazione simultanea di E.coli e dei coliformi nelle acque e negli alimenti



Escherichia coli alla luce normale e sotto lampada di Wood.
Si osserva la positività al test dell'indolo e l'intensa fluorescenza azzurra.

Il terreno consente la crescita selettiva degli enterobatteri e di pochi altri batteri Gram negativi, essendo i batteri Gram positivi inibiti dagli agenti selettivi presenti. Tra gli enterobatteri, **i coliformi possiedono l'enzima beta-galattosidasi, idrolizzano il composto X-GAL (5-bromo-4-cloro-3 indolil - beta - D - galattopiranoside) e sviluppano colonie verde-blu.** Tale reazione è resa maggiormente evidente dall'IPTG presente nel terreno. *Escherichia coli*, oltre ad idrolizzare il composto X-GAL, **idrolizza anche il MUG (4 - metilumbelliferil - beta - D – glucuronide)** e sviluppa quindi con **colonie verde blu, fluorescenti alla lampada di Wood** e produce indolo dal triptofano, quindi **positive al test dell'indolo.** Tale test è eseguibile direttamente sulla piastra con una goccia di reattivo di Kovacs che vira al rosso se la prova è positiva.

HAVELAAR



1. AEROMONAS SELECTIVE AGAR (HAVELAAR)
Colonie di *A. hydrophila*, gialle per la fermentazione dell'a destrina.



BTB indicator in acidic, neutral, and alkaline solutions (left to right).

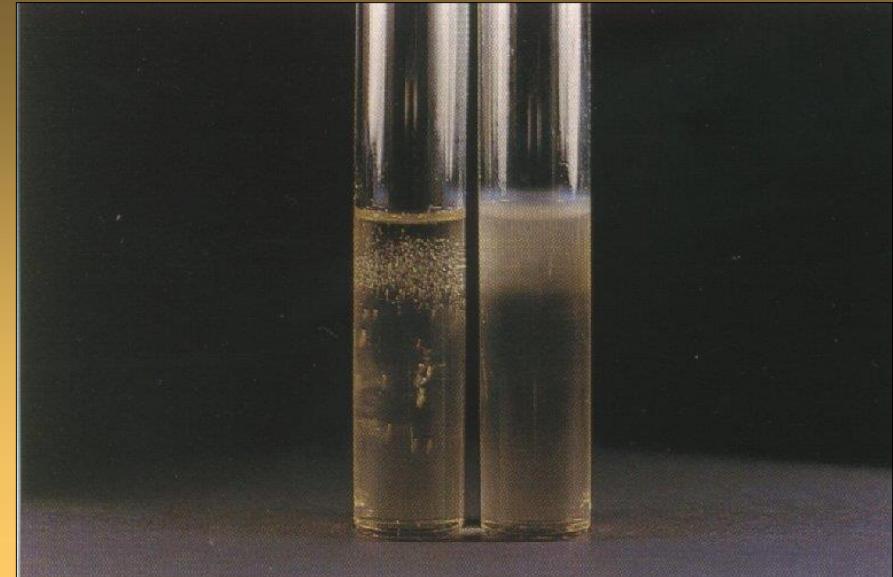
m-Aeromonas Selective Agar enhances growth of nearly all Aeromonades. The supplement Ampicillin and Vancomycin partly inhibits growth of accompanying Gram-positive and Gram-negative organisms.

Aeromonades form acid from Dextrin indicated by a color change from blue to yellow of the pH indicator Bromothymolblue.

Thioglycollate media

Thioglycollate media is used for cultivating anaerobic, microaerophilic and aerobic microorganisms and for detecting the presence of bacteria in normally sterile materials.

Actinomiceti



37. THIOGLYCOLLATE MEDIUM

Crescita di un ceppo aerobio (a destra) ed anaerobio sinistra).

Thioglycollate media, formulated with **yeast extract and casitone or pancreatic digest of casein**, support growth of a wide variety of **fastidious microorganisms** having a range of growth requirements. **Sodium thioglycollate** lowers the oxidation-reduction potential of the media and neutralizes the antibacterial effect of mercurial preservatives in the specimen. **Resazurin** and methylene blue indicate the status of oxidation or aerobiosis. Since methylene blue is toxic to some bacteria, resazurin has replaced it as the **OR indicator**. **Dextrose** is included in some formulations because most organisms show earlier and more vigorous growth in the presence of a carbohydrate. Just prior to inoculation, the medium may be autoclaved to drive off absorbed oxygen it acquired in storage.

CHOPPED MEAT GLUCOSE MEDIUM



Chopped meat broth is used for culturing anaerobic organisms