Diagnosi microbiologica delle infezioni gastrointestinali

M. Rita Rossi

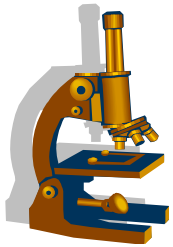
Modulo Dipartimentale di Microbiologia

Azienda Ospedaliera- Universitaria

Nuovo S.Anna Cona Ferrara

Faecies culture examination

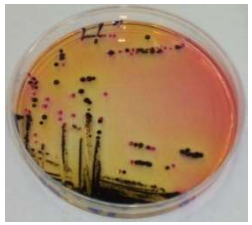
Macroscopic evaluation
&
Microscopic evaluation



Presence of WBC, RBC, phagocytes, bacteria and parasites

Analysis of the cultures

Bacterial isolation



Biochimical/MALDI-TOF
for bacterial identification
and immunoassay

Vitek2

VITEK MS™

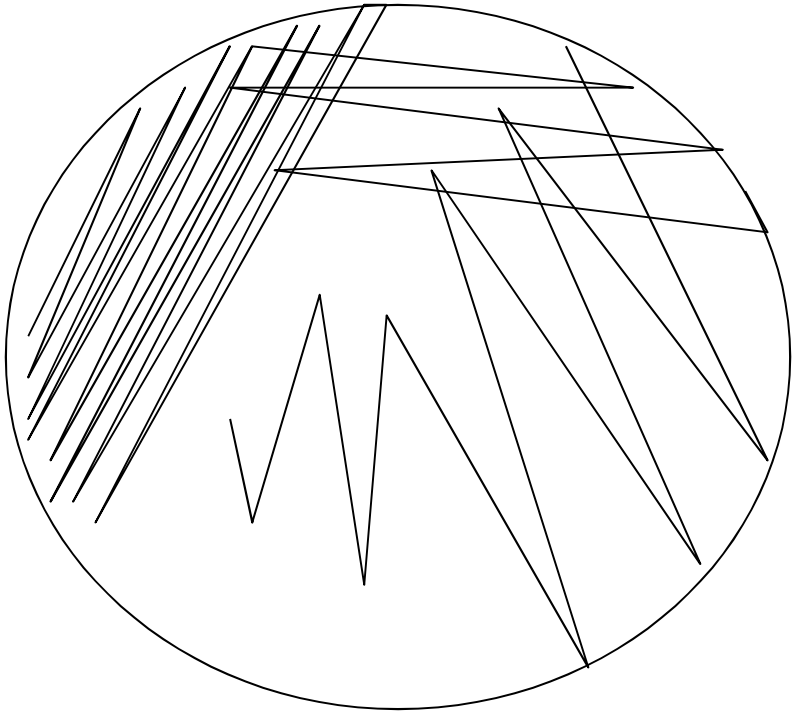
Serological immunophenotype

SEMINA PER ISOLAMENTO

Utilizzare l'ansa da 1 µl

Modalità di semina:

- Con l'ansa si strisciare fittamente a zig-zag su un quarto di piastra
- ruotare l'ansa (analogo cambiare ansa)
- ruotare leggermente la piastra e tracciare 4-5 righe senza mai staccare l'ansa dal terreno toccando prima fino in fondo poi fino a metà e poi l' inizio del settore già seminato
- cambiare ansa
- ripetere la procedura 3 con righe più distanziate e concludendo con una serpentina.



Semina in due differenti terreni di coltura

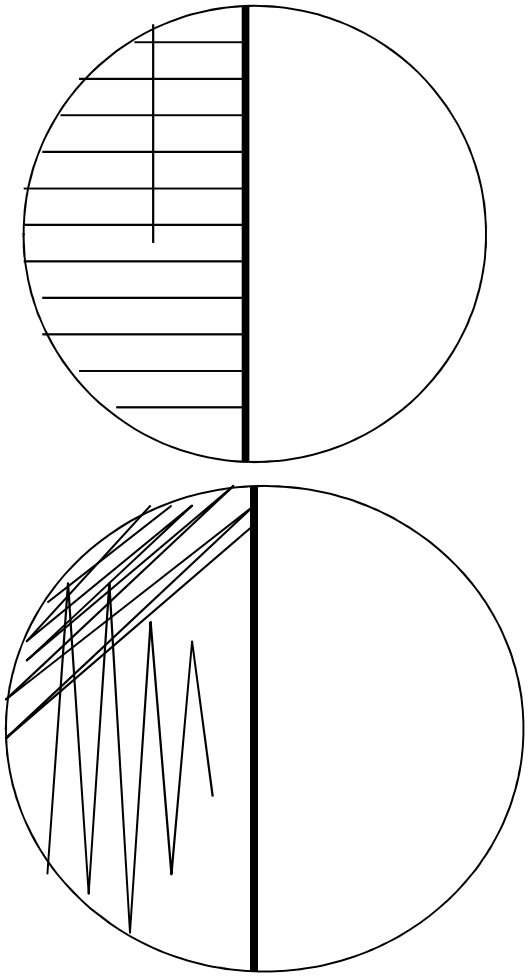
Utilizzare l'ansa da 10µl

Primo metodo

- -tracciare con l' ansa grande una riga parallela alla divisoria fino a metà con la stessa ansa.
- -tracciare delle righe perpendicolari fino alla fine della piastra

Secondo metodo

- -depositare il materiale in un angolo e spargerlo
- -ruotare la piastra e tracciare 2-3 righe (lungo, medio, corto)

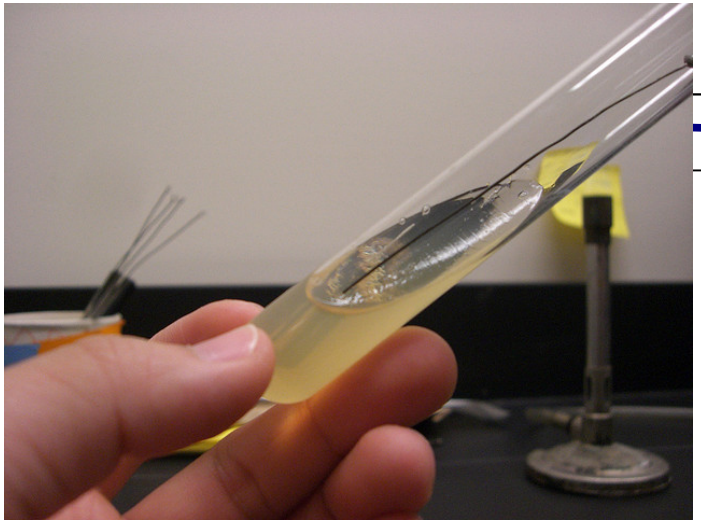


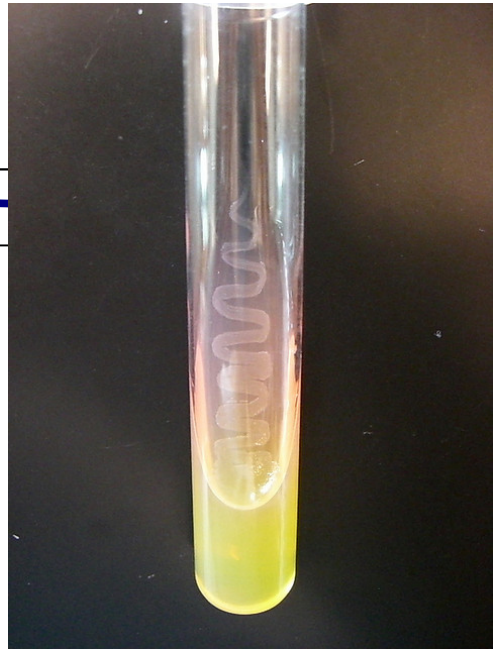
Semina in tubi a becco di clarino

Si utilizza per batteri che hanno tempi lunghi di incubazione

per mantenere vitali nel tempo ceppi batterici

- prelevare 10µl di sospensione batterica
- infiggere l' ansa nel terreno e uscire dal tubo strisciando a zig-zag l'ansa per tutto il terreno.





Selective media for Enterobatteriaceae isolation

Contents **MacConkey**

It contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (which also inhibits certain Gram-positive bacteria),neutral red dye (which stains microbes fermenting lactose), lactose and peptone.

Composition:

Peptone - 17 g Proteose peptone - 3 g Lactose - 10 g
Bile salts - 1.5 g Sodium chloride - 5 g Neutral red - 0.03 g
Agar - 13.5 g
Water - add to make 1 litre; adjust pH to 7.1 +/- 0.2

There are many variations of MacConkey agar depending on the need. If the spreading or swarming of Proteus species is NOT required, sodium chloride is omitted. Crystal violet at a concentration of 0.0001% (0.001 g per litre) is included when needing to check if Gram-positive bacteria are inhibited.

Uses MacConkey

Acting as a visual pH indicator, the agar distinguishes those Gram-negative bacteria that can ferment the sugar lactose (Lac+) from those that cannot (Lac-).
This medium is also known as an "indicator medium" and a "low selective medium".
Absence of electrolytes serves to inhibit swarming by Proteus species.

Lac+

By utilizing the lactose available in the medium, Lac+ bacteria such as *Escherichia coli*, *Enterobacter* and *Klebsiella* will produce acid, which lowers the pH of the agar below 6.8 and results in the appearance of red/pink colonies. The bile salts precipitate in the immediate neighborhood of the colony, causing the medium surrounding the colony to become hazy.

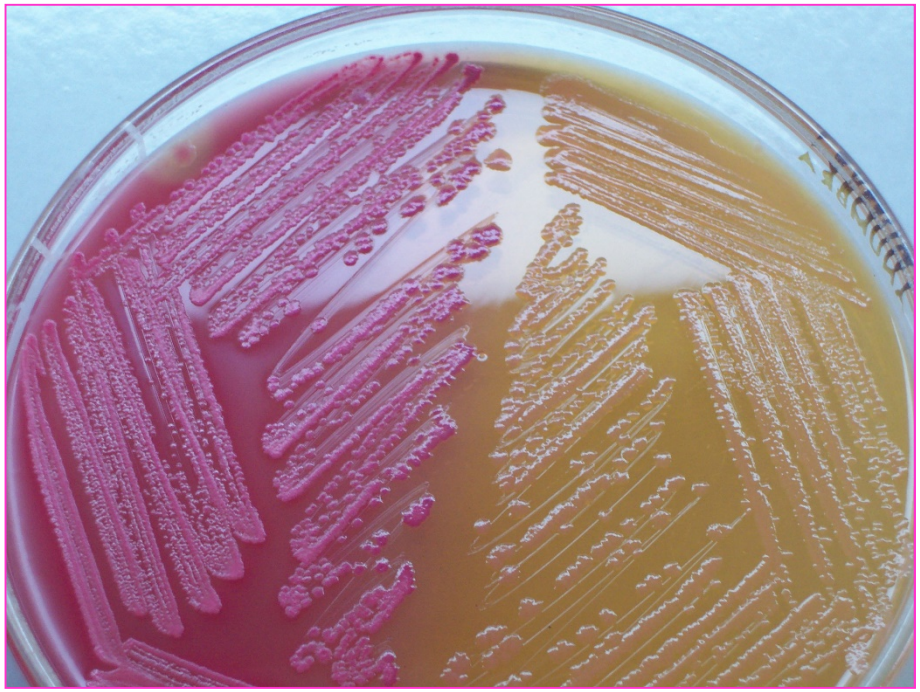
Lac-

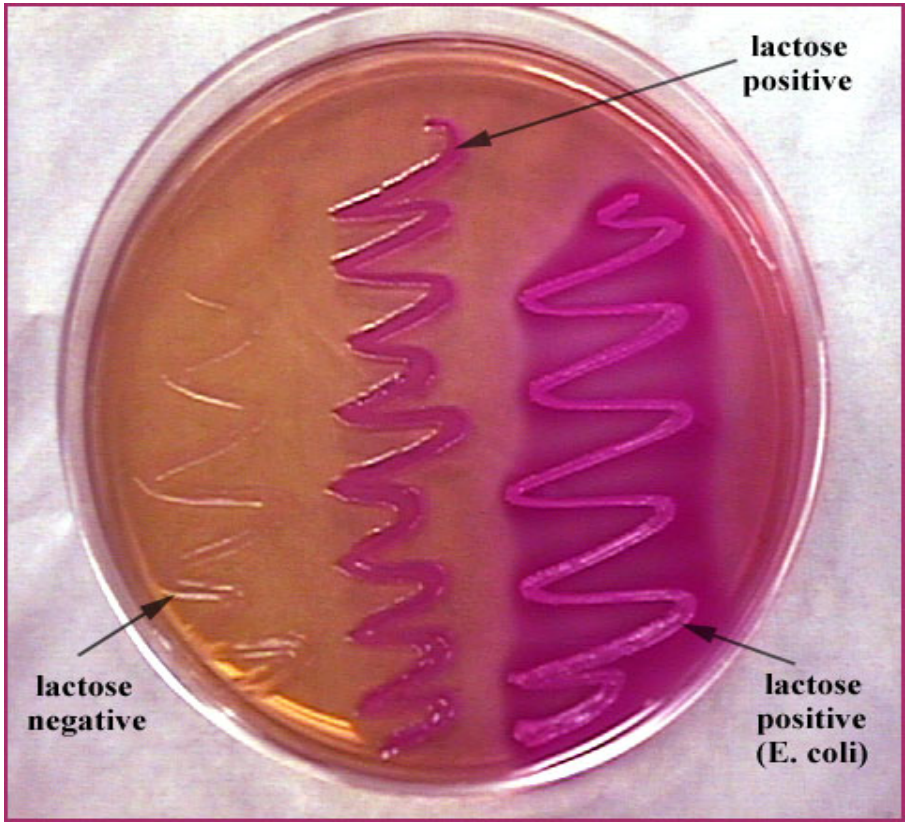
Non-Lactose fermenting bacteria such as *Salmonella*, *Proteus species*, *Pseudomonas aeruginosa* and *Shigella* cannot utilize lactose, and will use peptone instead. This forms ammonia, which raises the pH of the agar, and leads to the formation of white/colorless colonies formed in the plate. They can also look golden to brown with dark centers. They are circular colonies and arranged randomly.

Slow

Some organisms ferment lactose slowly or weakly, and are sometimes put in their own category. These include *Serratia* and *Citrobacter*.

MacConkey agar

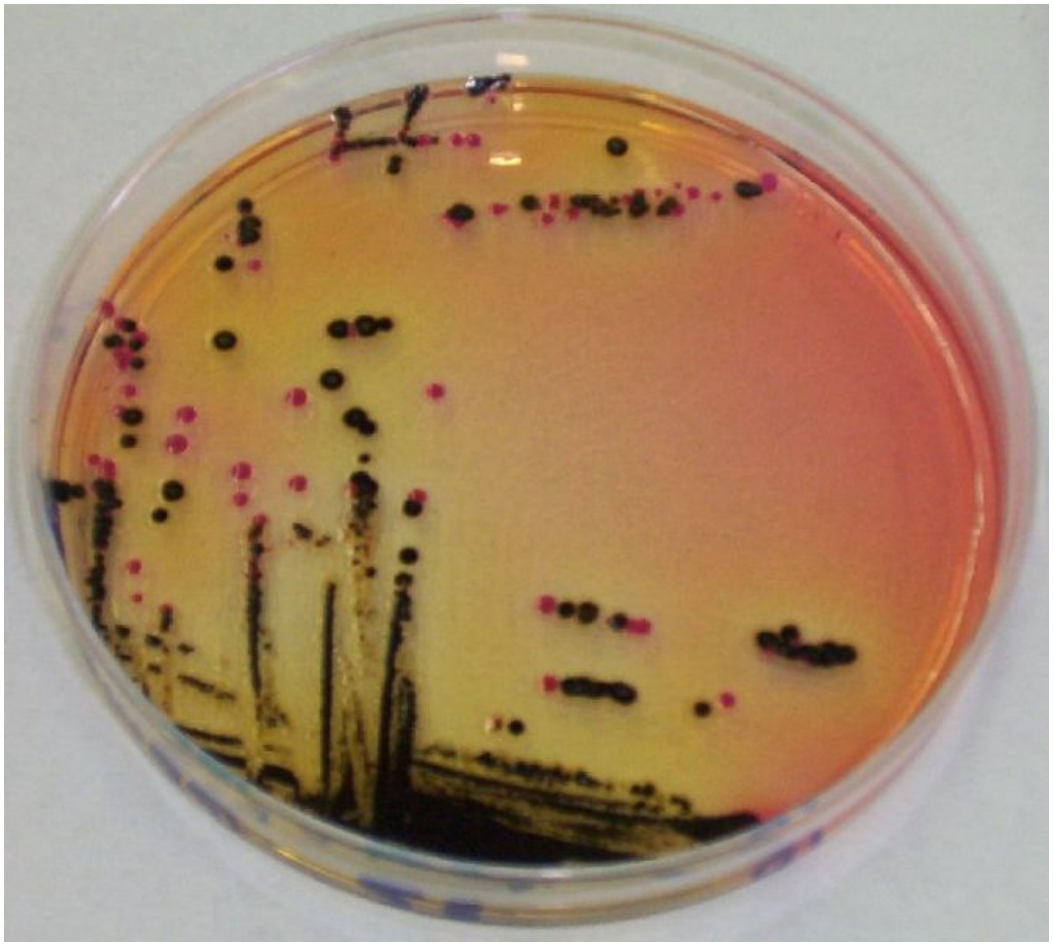




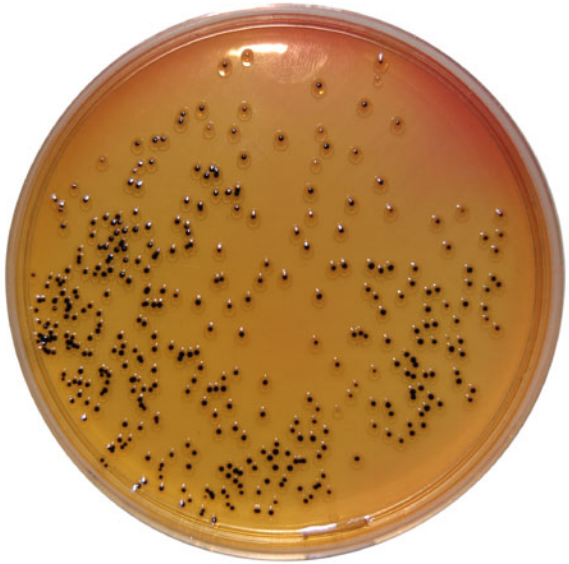
Selective media for Enterobatteriaceae isolation

Medium	Typical composition & Mode of action	Experimental Procedure and Evaluation
Agar Salmonella-Shigella (SS)	Peptones 10.0; lactose 10.0; ox bile 8.5; sodium citrate 10.0; sodium thiosulfate 8.5; ammonium iron(III) citrate 1.0; brilliant green 0.0003; neutral red 0.025; agar-agar 12.0. **Brilliant green, bile and high concentrations of thiosulfate and citrate largely inhibit the accompanying microbial flora. Sulfide production is detected by using thiosulfate and iron ions, the colonies turn black. The presence of coliform bacteria is established by detecting degradation of lactose to acid with the pH indicator neutral red.**	Spread the sample or material from an enrichment culture on the surface of the culture medium. Incubation: 18-24 hours at 35°C aerobically. Lactose-negative colonies are colorless. Lactose-positive colonies are pink to red. Colonies of microorganisms producing H2S have a black centre.

Salmonella-Shigella agar



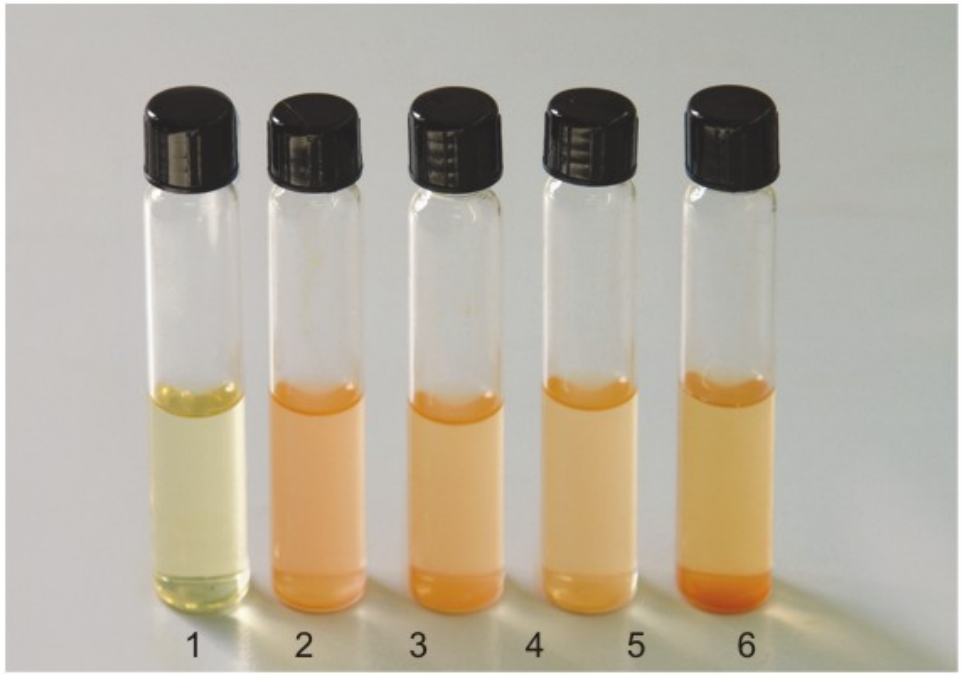
Shigella



Salmonella

Enrichment broth media for the isolation of Enterobatteriaceae

Broth	Typical composition & Mode of action	Experimental Procedure and Evaluation
Selenite broth	*For Salmonella* isolation from urine feces or other.. Selenite broth inhibit the growth of *E.coli* and other coliformes species. Some *Shigella sp.* could be also inhibited	After seeding the growth of coliformes become predominant so that it is better to perform another sub-seeding on agar *Salmonella Shigella* within 6-8 hours.



Fluid Selenite Cystine Medium (Selenite Cystine Medium) (Twin Pack) M025

1. Control
2. Salmonella Typhimurium ATCC 14028
3. Salmonella Choleraesuis ATCC 12011
4. Salmonella Typhi ATCC 6539
5. Escherichia coli ATCC 25922

Salmonella-Shigella

CULTURAL APPROACH

FAECIES

BLOOD

SERUM

DIRECT SEEDING

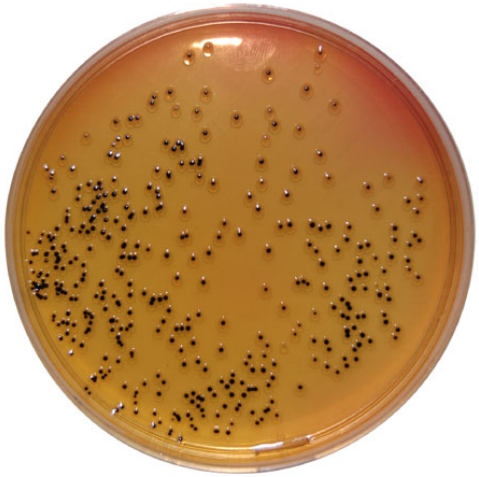
ENRICHMENT

SELECTIVE MEDIUM

SERUM DIAGNOSIS-WIDAL

BLOOD CULTURES

CHROMOGENIC MEDIUM



PHENOTYPE DETECTION

MALDI-TOF

ANTIBIOGRAM

SEROLOGIC CONFIRM SERUM GROUP

Salmonella, 2435 existing species

"maggiori" specifiche per l'uomo, "minori" tutte le altre

Specific for Homo sapiens	Host of specific animals	Ubiquitaris species
•*S. typhi*		•S.*enteritidis*
•*S.paratyphi A*	•*S.gallinarum* (polli)	•*S.typhimurium*
•*S.paratyphi B*	•*S.abortus suis* (suini)	•*S.infantis*
•*S.hirschfeldi* (ex *paratyphi C)*	•*S.equi* (equini)	•*S.wien*
		•….
		•….

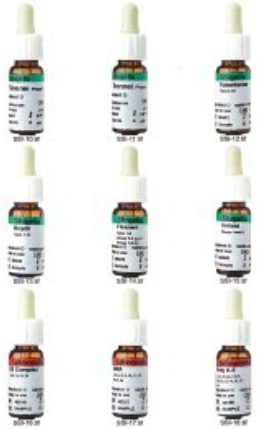
Salmonella, immunophenotyping as indicated by Kauffmann-White

- Antigene somatico o antigene O

  localizzato a livello di parete, è di natura polisaccaridica (50 gruppi indicati con le lettere dell'alfabeto A, B, C, D, E…)
- Antigene flagellare H (fase 1 e 2) di natura proteica (indicato da lettere minuscole e numeri arabi)
- Antigene capsulare o Vi tipico di *Salmonella typhi* e *S.paratyphi* possono inibire l'agglutinazione per antigene somatico e devono essere distrutte con il calore

ESEMPIO STRUTTURA ANTIGENICA:

S.typhimurium 1,4,(5),12:i:1,2



Campylobacter

FAECAL SUSPENSIONS

SELECTIVE MEDIA

Skirrow,Karmali,Blaser
Wang, Butzler,
Preston, Bolton,CCDA

MICROAROFILIA

42°C 48 h

POSITIVE SAMPLES

PHENOTYPE

MICROSCOPY STUDY

MALDI-TOF

Terreni utilizzati per l'isolamento selettivo di Campylobater jejuni

Terreno	Base	Altri componeneti	Incubazione
Butzler	Brodo tioglicollato	Agar, sangue di pecora 10%, bacitracina, novobiocina, colistina, cefalotina, actidione	48 ore a 42°C Atmosfera 5% O_2, 10% CO_2 e 85% di N_2
Skirrow	Blood agar base N° 2	Emolisato di sangue di cavallo (7%), vancomicina, polimixina B, trimetoprim	
Campy BAP	Brucella agar base	Sangue di pecora (10%) vancomicina, trimetoprim, polimixina B, cefalotina, anfotericina B	

Type of colonies for Campylobacter detection
irregular, gray, flat and mucoid colonies

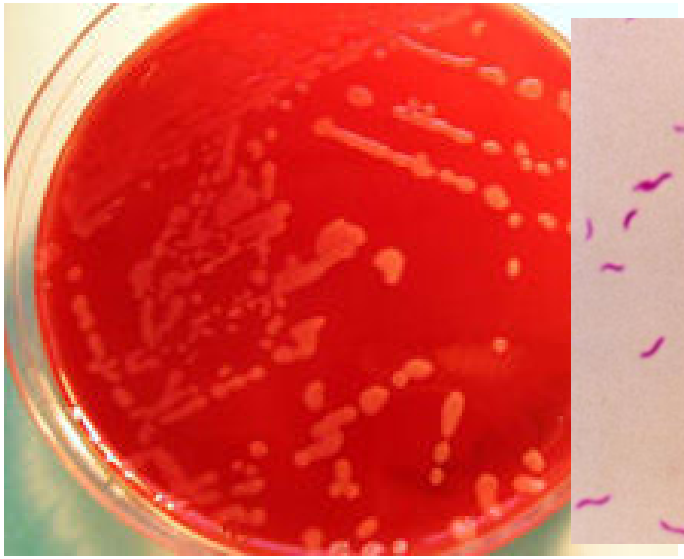
Gram staining

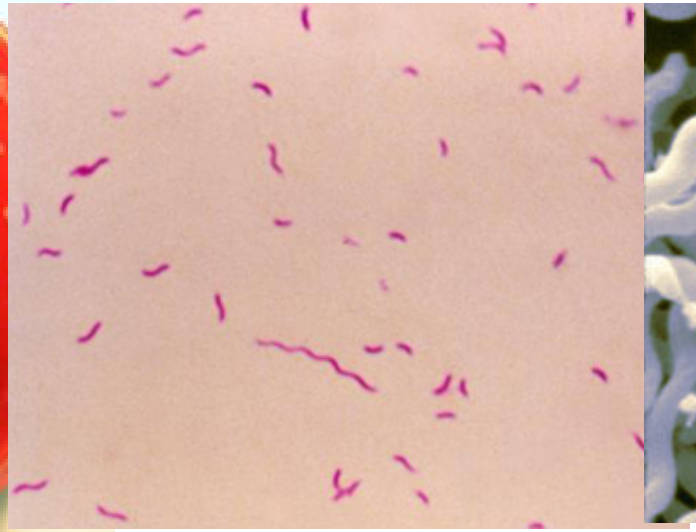
Bastoncelli ricurvi
Gram negative

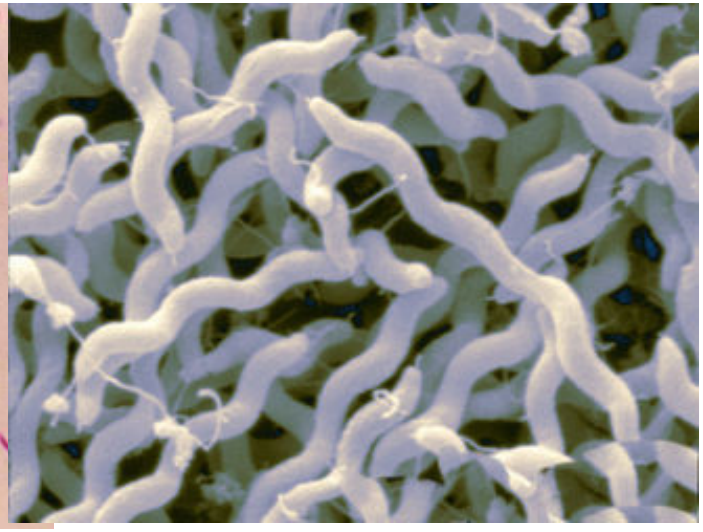
Fresh detection

Rapidly moving

Maldi TOF



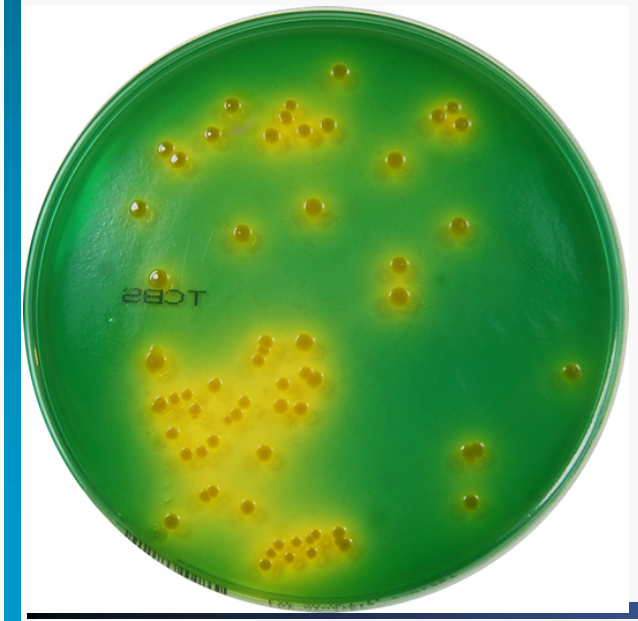




Vibrio cholerae-Aeromonas-Plesiomonas-Vibrio spp

FAECIES

Direct seeding



ENRICHMENT

PEPTONATED WATER

SELECTIVE MEDIUM

TCBS

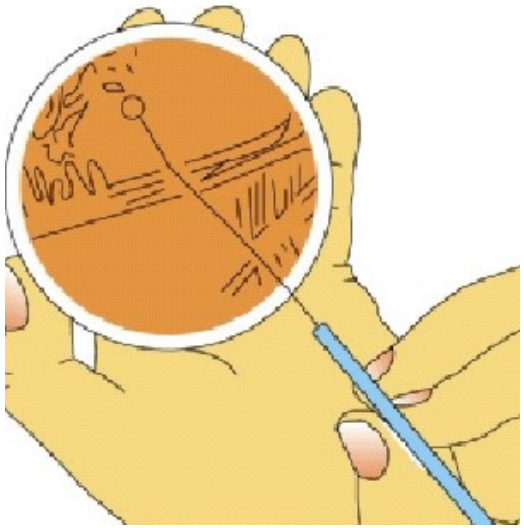
MALDI-TOF

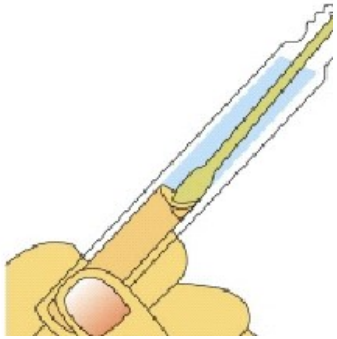
SEROLOGICAL

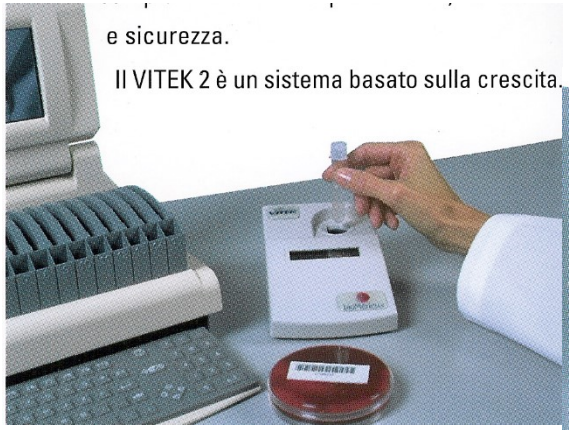
CONFIRM

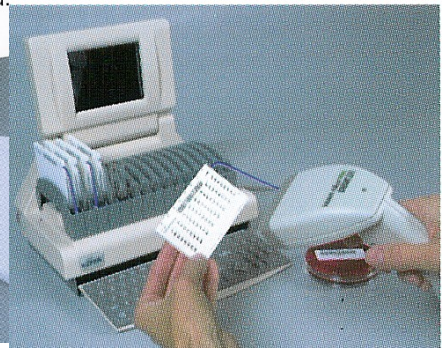
Biochemical detection and antibiogram with Vitek 2

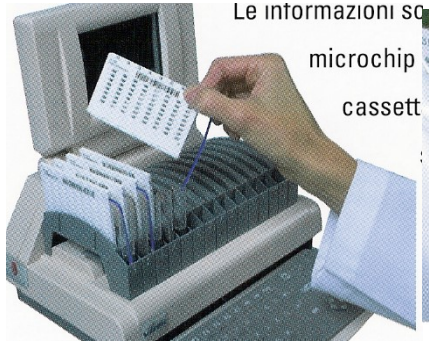
identification cards (ID) and antibiotic susceptibility testing (AST) cards provides testing flexibility







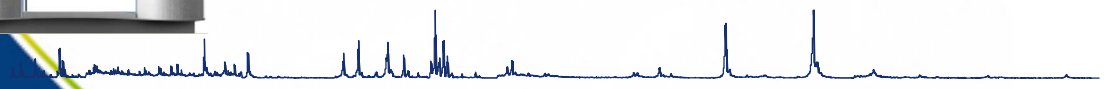




Identificazione biochimica e Antibiogramma in automazione con Vitek 2

VITEK MS™

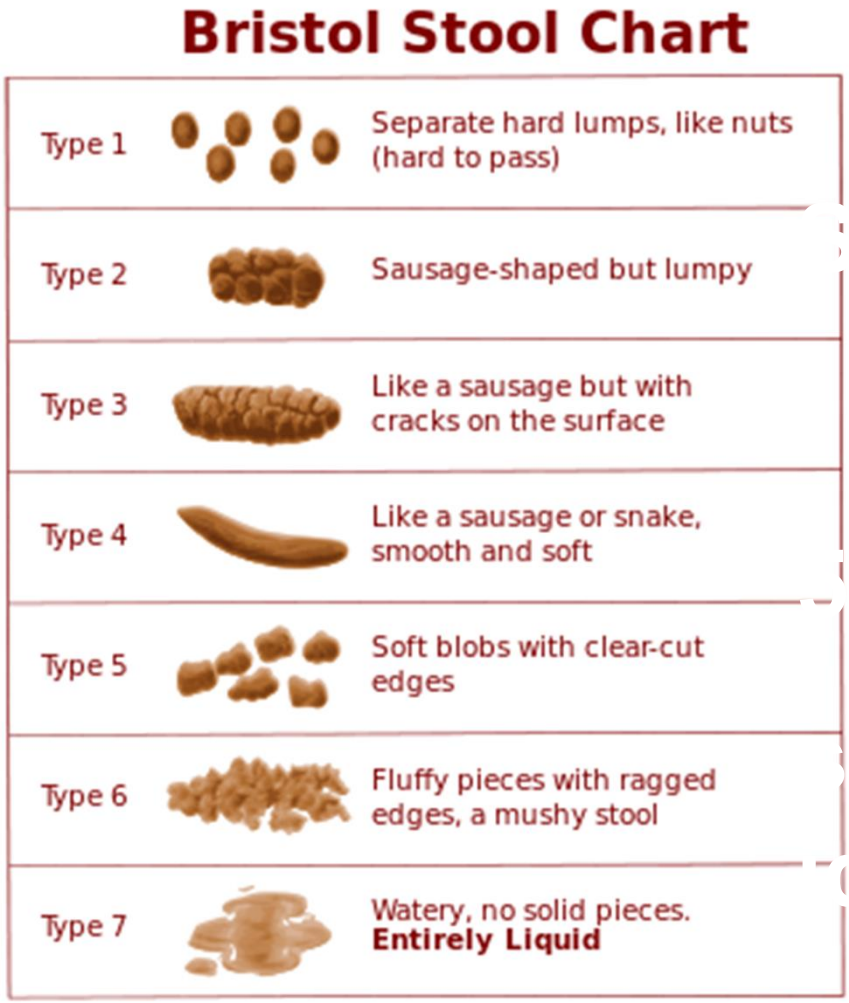
MALDI-TOF for bacterial identification



BIOMÉRIEUX UNIVERSITY

Test rapidi per la rivelazione di antigeni batterici/virali

Idoneità del campione



Xpert® *C. difficile* Testing Procedure

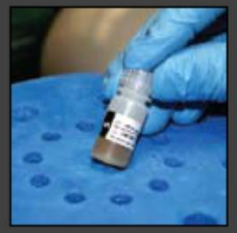
Xpert®
C. difficile

A – Preparing the Cartridge

1. Remove cartridge and reagents from the package.

2. Insert swab with sample into Sample Reagent Vial up to the score.

3. Break swab by bending it over side of vial. Use a gauze to prevent splashing.



4. Recap and vortex Sample Reagent Vial for 10 secs.

5. Open cartridge lid.

6. Draw the contents of the Sample Reagent Vial into a sterile transfer pipette.

7. Dispense contents of transfer pipette into cartridge chamber S.

10. Close the cartridge lid.

Xpert® *C. difficile* Testing Procedure

Xpert®
C. difficile

B – Starting the Test

1. Turn on the computer, and then turn on the GeneXpert Dx Instrument

2. On the Windows® desktop, double-click the GeneXpert Dx shortcut icon.

3. Log on to the GeneXpert Dx System software using your user name and password.

4. In the GeneXpert Dx System window, click Create Test. The Scan Cartridge Barcode dialog box appears.

5. Scan the Barcode on the cartridge. Enter Sample ID. Click Start Test. Wait for the green light to begin flashing.

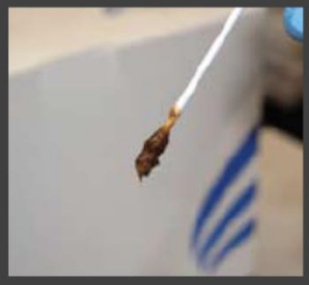
6. Insert cartridge. Close the door.

***C difficile* proper sample loading**

Too Little Sample

Right Amount

Too Much Sample

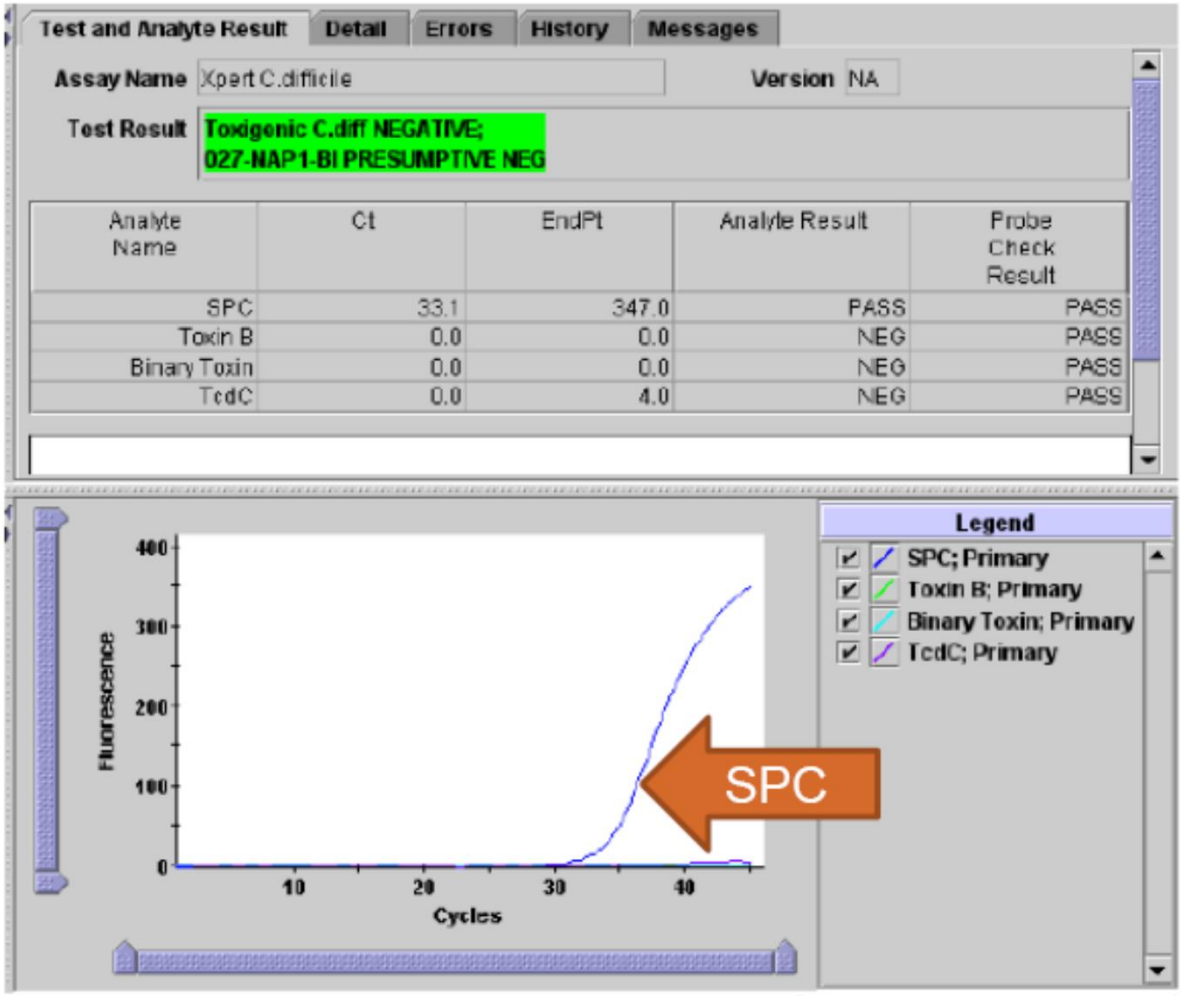


Interpretazione risultati Xpert *C.difficile*

Tox B+/Binary-/tcdC-

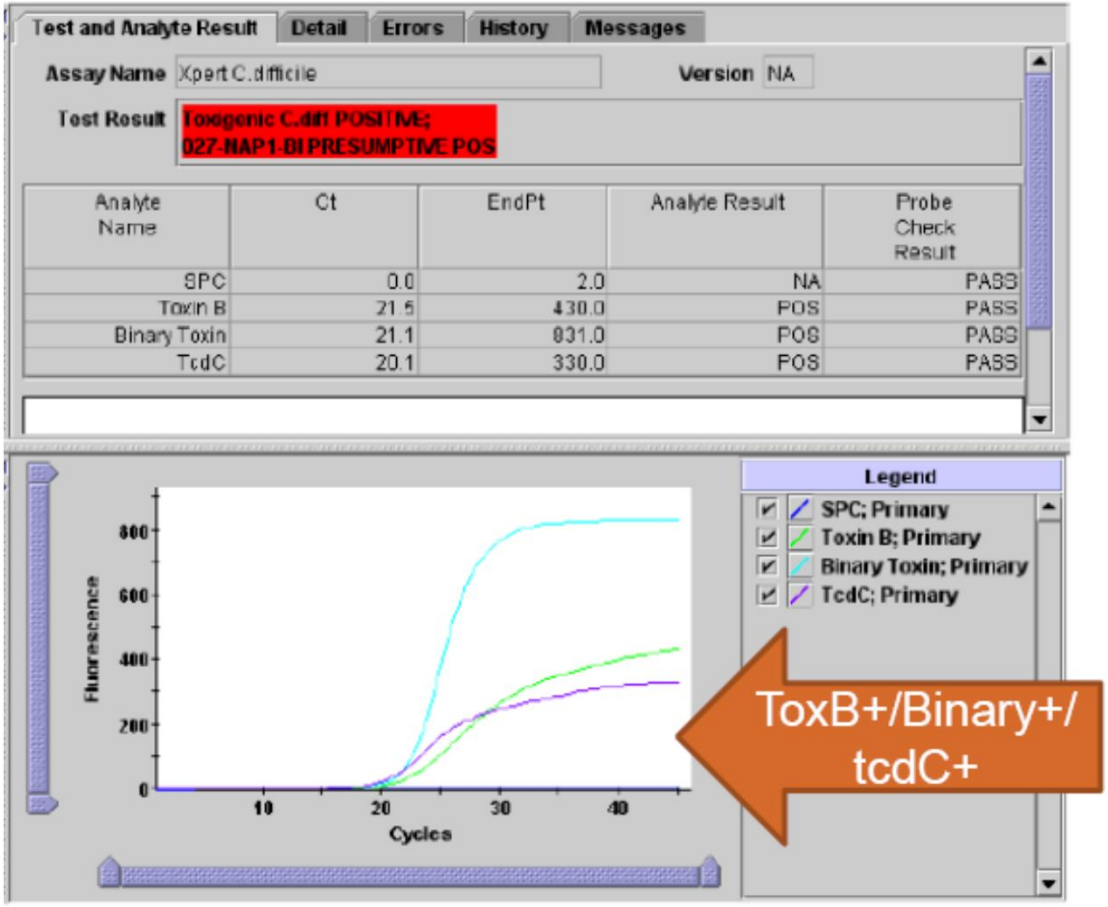
:metronidazolo

Interpretazione risultati Xpert *C.difficile*



C. diff non tossigenico

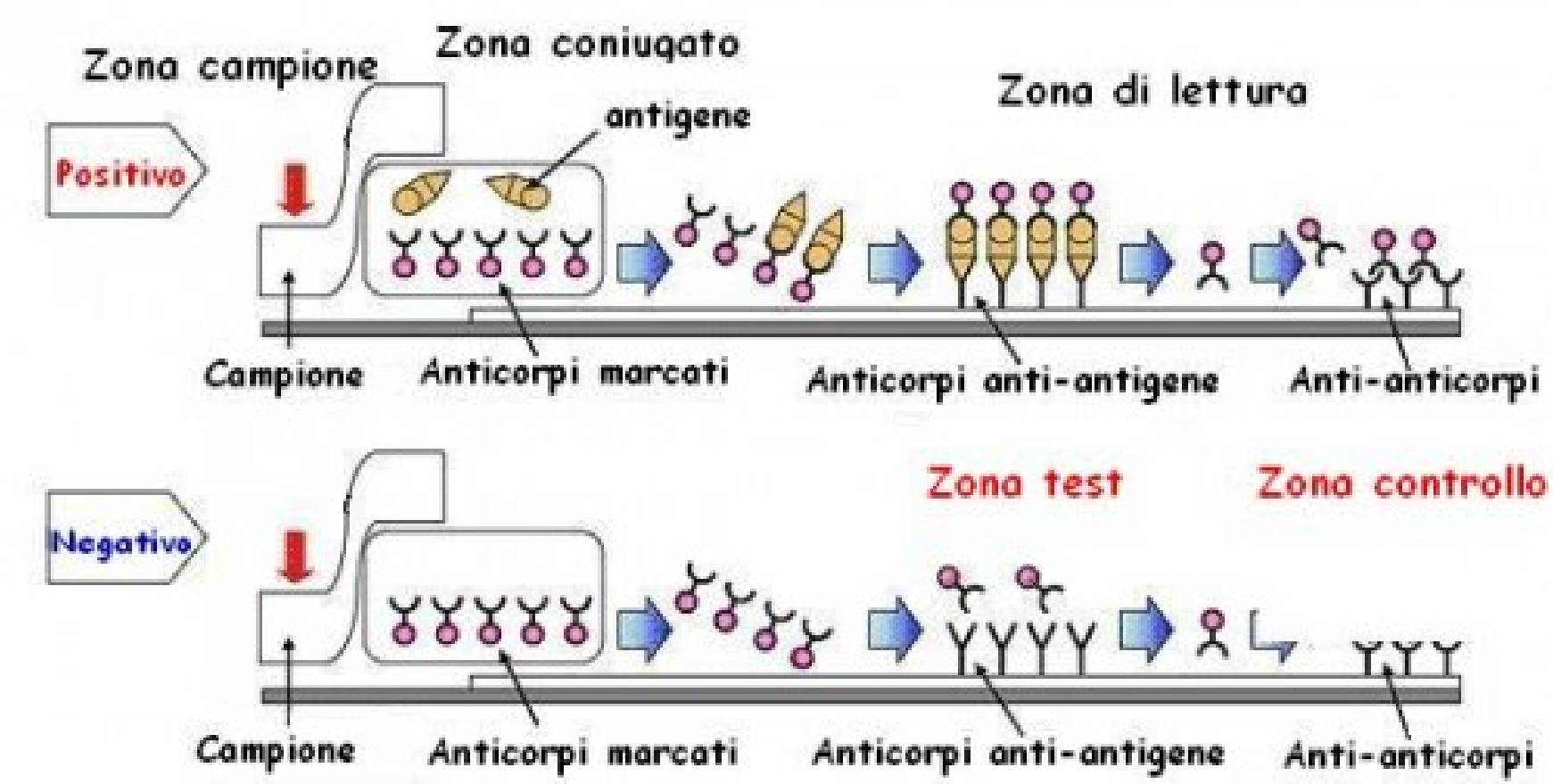
Interpretazione risultati Xpert *C.difficile*



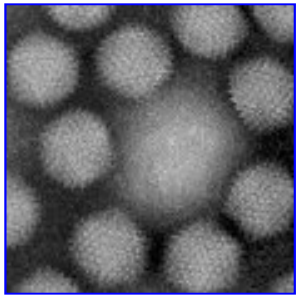
Tox B+/Binary+/tcdC+:

Considerare la vancomicina orale, discontinuare i chinoloni (moxifloxacina)

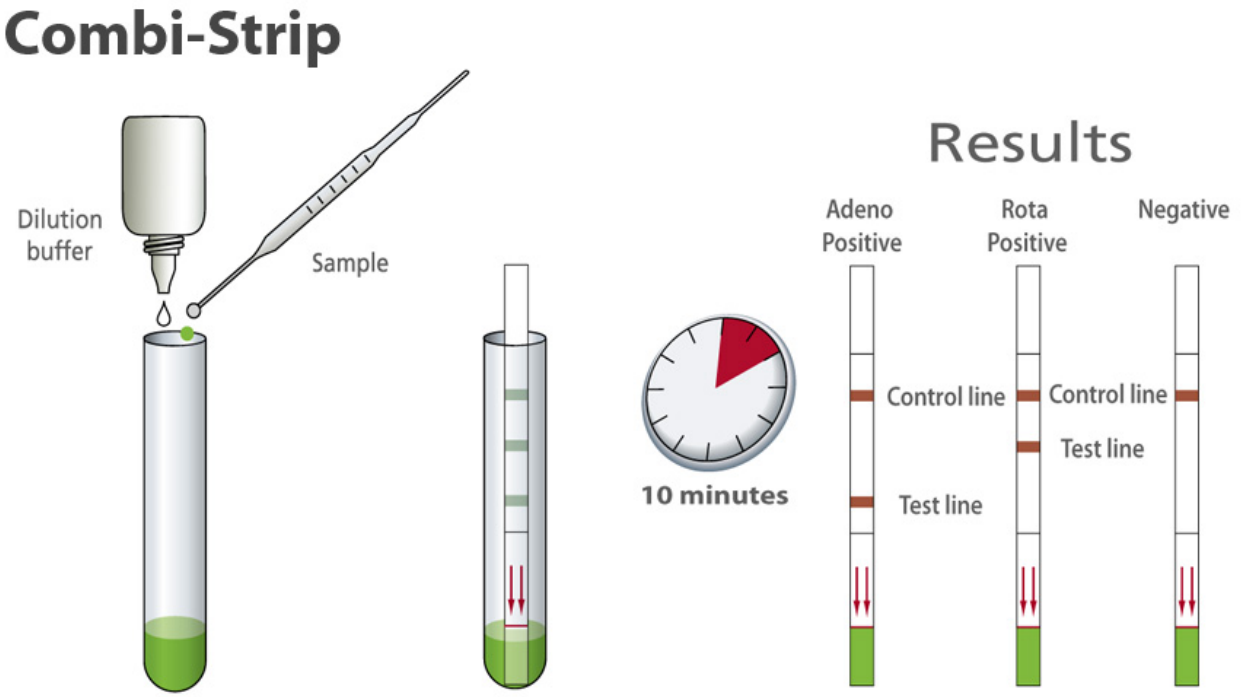
Immunocromatografia a flusso laterale



Detection of Rotavirus/Adenovirus antigens



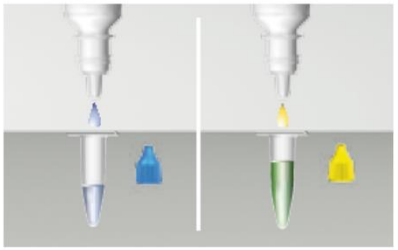
Immunocromatographic assay



Remove the required number of test cassettes after all kit components and stool specimens have reached room temperature (20 - 25°C).

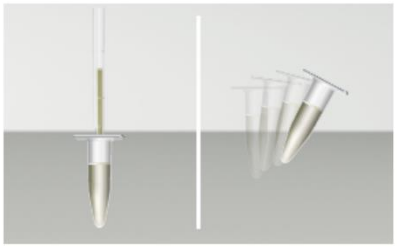
Norovirus

1.



First add 0,5 ml (approx. 12 - 14 drops) of Reagent A (blue) and then 0,5 ml (approx. 12 - 14 drops) of Reagent B (yellow) in the graduated reaction vial.

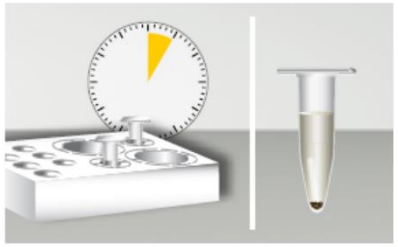
2.



Add 50 mg or 50 µl of stool specimen to the vial using the Pasteur pipette included in the kit.

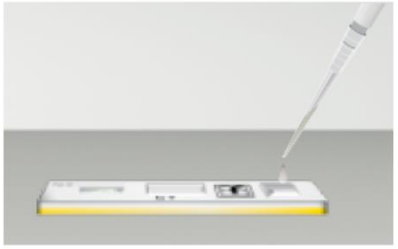
Mix well after sealing the reaction vial tightly.

3.



Place the reaction vial containing the homogenized sample (stool) in the reaction vial holder (reagent tray) and allow the contents to settle for 5 minutes at room temperature.

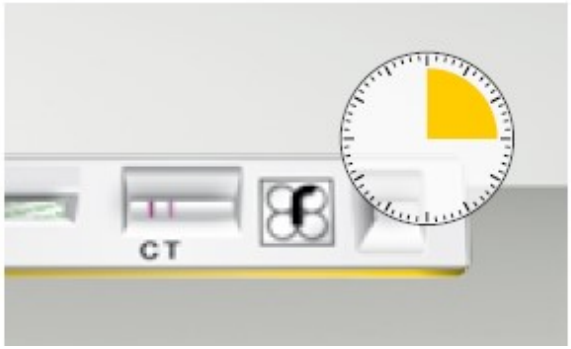
4.



Using the microliter pipette included in the test kit, apply 150 µl of the supernatant to the application field on the test cassette.

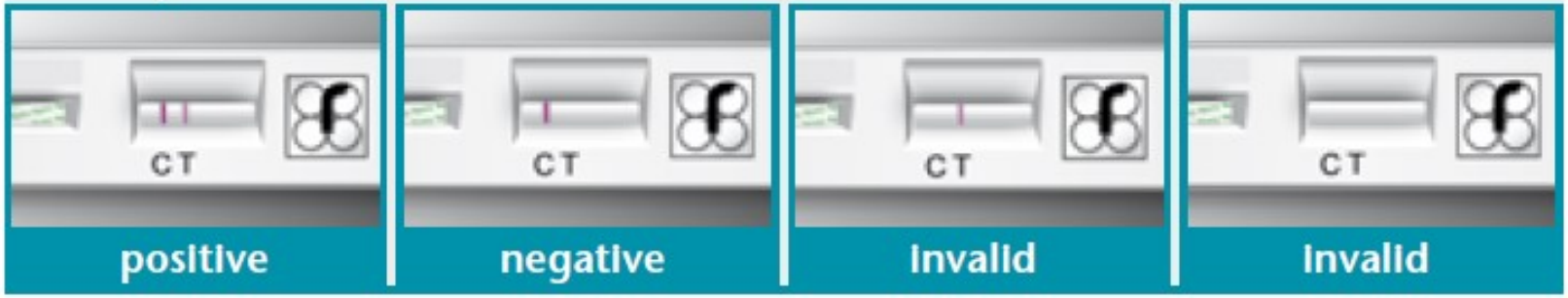
The test cassette should be placed on a horizontal surface for this.

Norovirus

5. 

Read the results after 15 minutes.

Interpretation



Helicobacter pylori

- HePy Stool Card è un metodo immunocromatografico che prevede l'utilizzo di anticorpi specifici per l'H. pylori fissati ad una membrana per cromatografia. L'eventuale presenza di H. pylori nelle feci determina la formazione di un primo legame capace di legare il secondo anticorpo coniugato con oro colloidale. Alla membrana è fissato anche un anticorpo generico capace di legare il secondo anticorpo coniugato, confermando o meno la buona riuscita del test.

E.Coli 0157:H7

Immunocromatographic assay