Approccio della microbiologia tradizionale delle gastroenteriti infettive

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Gastroenteritis Definition

Gastroenteritis" means "inflammation of the stomach and small intestine." Medically, gastroenteritis is defined as a diarrheal disease, in other words, an increase in bowel movement frequency with or without vomiting, fever, and abdominal pain.

Classification

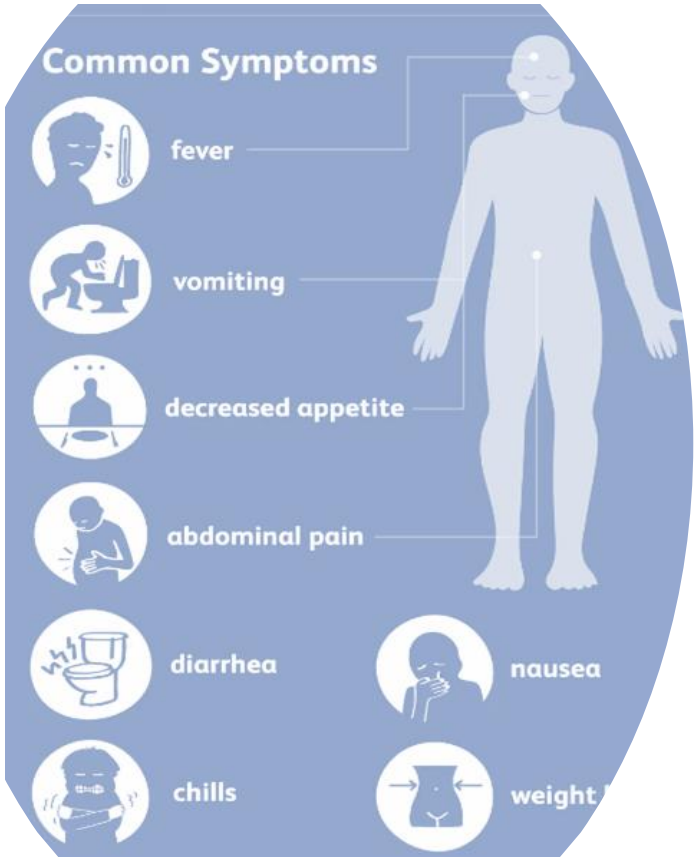
Acute
14 days or fewer than 14 days in duration

Persistent
More than 14 but fewer than 30 days in duration

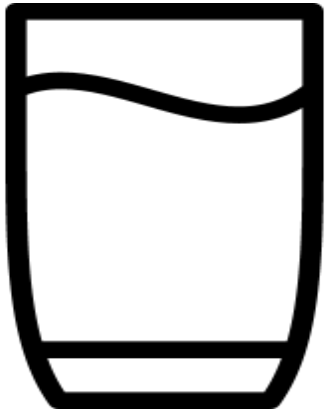
Chronic
More than 30 days in duration

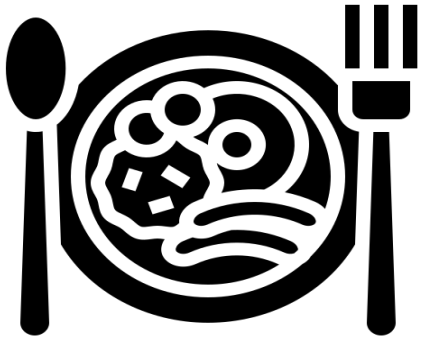
Recurrent
Diarrhea that recurs after 7 days without diarrhea

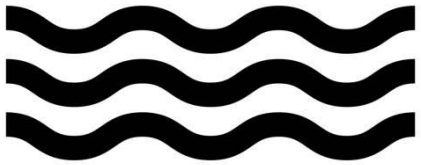
Gastroenteritis Symptoms

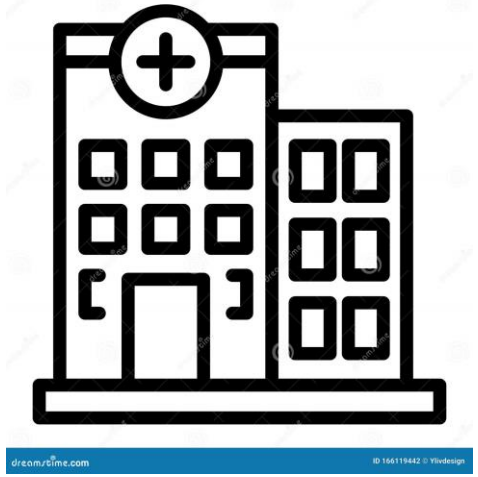


Some Informations









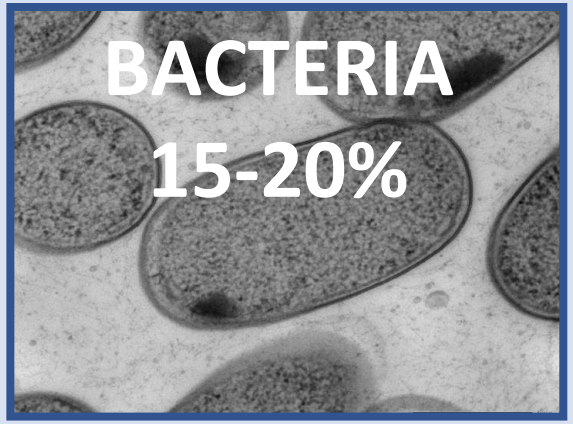
Le sindrome diarroiche tendono, nella maggior parte dei casi ad una risoluzione spontanea

Possono risultare comunque causa rilevante di ospedalizzazione e dar seguito ad importanti sequele

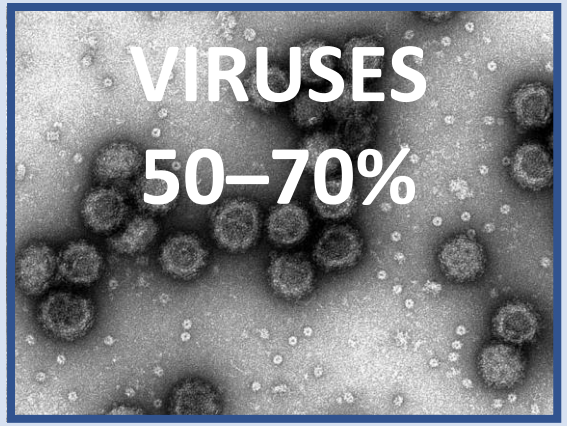
Per la maggior parte dei pazienti con diarrea acuta, i sintomi si risolvono prima dell'esame colturale e in generale il risultato non modifica il trattamento

Nonostante i miglioramenti diagnostici, una percentuale di sindromi diarroiche rimane ancora senza diagnosi o sconosciuta

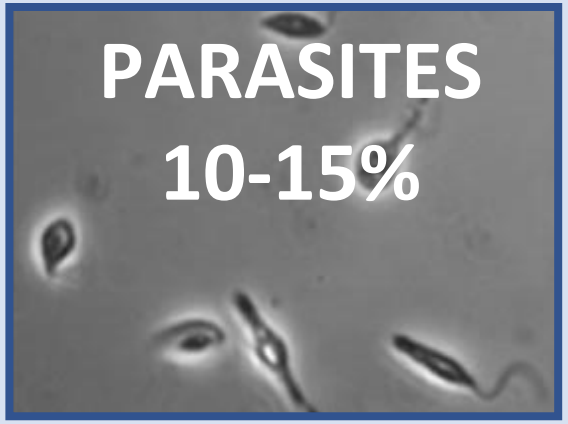
Causative agents of infectious gastroenteritis



Campylobacter jejuni
Campylobacter upsaliensis
Campylobacter coli
Salmonella spp.
Shigella spp.
Escherichia coli (STEC, EIEC, EPEC, ETEC)
Vibrio cholerae (O1, O139)
Vibrio parahaemolyticus
Bacteroides fragilis
Staphylococcus aureus
Bacillus cereus
Clostridium difficile
Yersinia enterocolitica
Helicobacter pylori
Arcobacter spp.



Rotavirus
Adenovirus
Norovirus
Sapovirus
Astrovirus
Cytomegalovirus
Enterovirus



Cryptosporidium parvum
Giardia intestinalis
Microsporidia
Entamoeba histolytica
Cystoisospora belli
Cyclospora cayetanensis
Dientamoeba fragilis
Blastocystis hominis
Strongyloides stercoralis
Angiostrongylus costaricensis
Schistosoma mansoni
Schistosoma japonicum

Luo et al., 2019

Diagnostic approach to infective gastroenteritis

- Cultural methods
- Immunological methods
- Microscopic methods
- Molecular methods
- Immunochromatographic methods

CIDT (Culture Indipendent Diagnostic Test)

Pre-Analytic Phase (When)

Strong Clinical Suspicion of Infectious Diarrhea

Possible Epidemic Event

In the presence of diarrhea, blood and / or mucus

AGE

Immunosuppressed

Clinical and Diagnostic purpose

Immunoassay antigenic and/or molecular systems viral aetiology

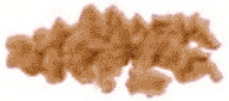
***C.difficile* Toxins**

Structural components STEC and *Campylobacter*

Food poisoning from *B.cereus*, *C.perfingens* and *S. aureus* sent to public health laboratories

Pre-Analytic Phase (HOW)

Scala Bristol

Tipo 1		Grumi duri separati tra loro, come noci (difficili da espellere).
Tipo 2		A forma di salsiccia, ma formata da grumi uniti tra loro.
Tipo 3		Come un salame, ma con crepe sulla sua superficie.
Tipo 4		Come una salsiccia o un serpente, liscia e morbida.
Tipo 5		Pezzi separati morbidi con bordi come tagliati/spezzati; chiara (facile da evacuare).
Tipo 6		Pezzi soffici/flocculari con bordi frastagliati, feci pastose.
Tipo 7		Acquosa, nessun pezzo solido. Completamente liquida.

Pre-Analytic Phase (HOW)

- Quantity (0.5-2 g., 5-10 ml)
- No antimicrobials
- Provide instructions
- Not contaminated with urine, sanitary water or toilet paper

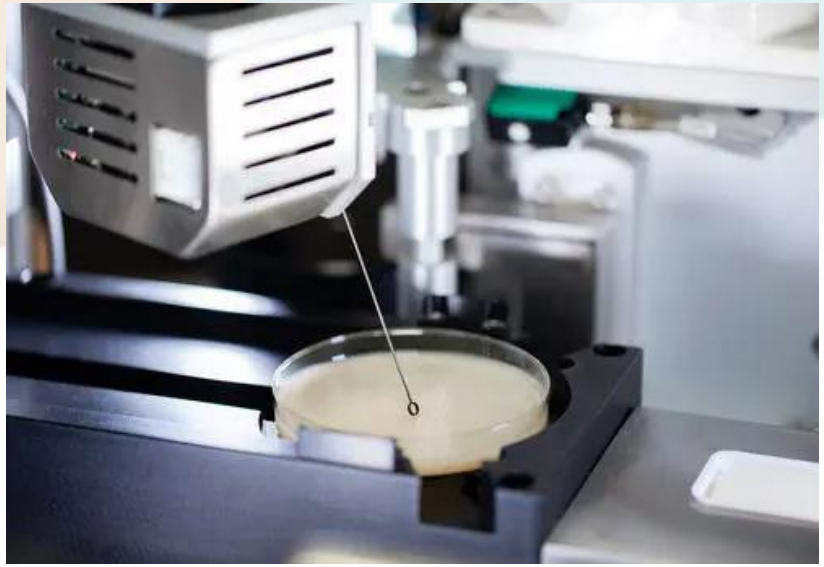
- Fecal swab: Swab with transport medium (Carey-Blair) dipped in the stool. Useful for Shigella, Salmonella, STEC
- For the genus Vibrio, also take the mucus present on the walls of the rectum
- Samples collected with swabs are not to be considered suitable for the detection of toxins and / or antigens or for parasitological research.

Pre-Analytic Phase

- Time < 2h R.T.
- Time > 2h and <24h 4-6°C (buffered saline, Carey-Blair / Stuart / Amies transport medium)
- Time > 24h -70°C

N.B. Vibrio in alkaline pH (Cary-Blair-Alkaline peptone 2% NaCl)

LBM (Liquid Based Microbiology)



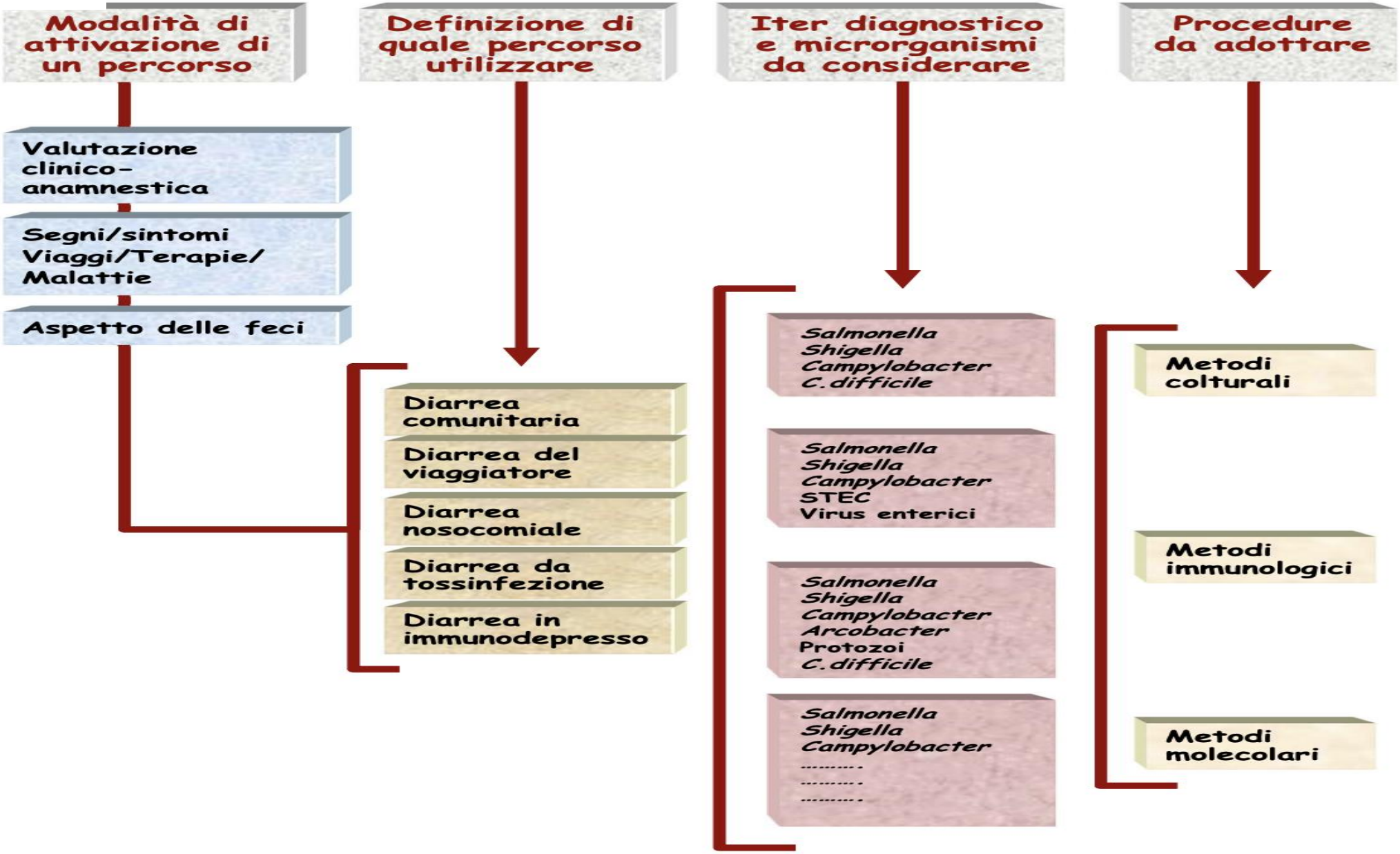
Sonde floccate abbinato ad uno specifico terreno di trasporto/ arricchimento

Capacità di preservare la vitalità dei microrganismi da sottoporre a coltura

Possibilità di standardizzare la semina dei campioni utilizzando un terreno liquido abbinato o meno ad un sistema automatico

Possibilità di utilizzo oltre che per i test colturali anche per la ricerca di AG e NA

Analytic Phase (Classic Flowchart)



Bacterial Analytic Phase (LEVELS)

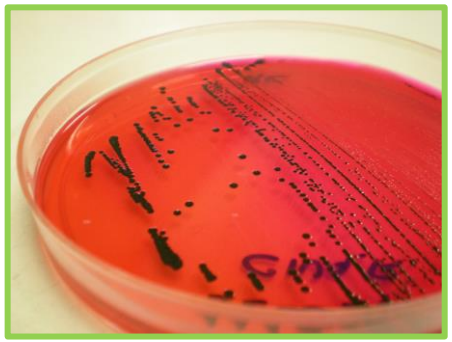
Laboratorio livello 1 → Protocolli standard differenziati

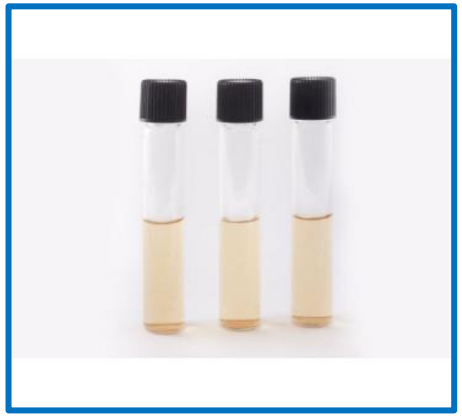
Diarrea autoctona/ epidemica	Diarrea acuta	Ricerca di:	Coltura	
	Diarrea persistente	· Salmonella	Identif. a livello di: · sierogruppo	
	Diarrea protratta	· Shigella	· specie	
Diarrea del viaggiatore		· Campylobacter	· specie	
		· E.coli O157	· specie	
		· Yersinia	· specie	
		· Vibrio	· specie	
		· Aeromonas	· specie	
		· Plesiomonas	· specie	
		· N.gonorrhoeae	· specie	
AAD,AAC,AAPMC		Ricerca di: · Campylobacter · E.coli STEC · C.difficile · Rota/Adeno · Norwalk	EIA · ELISA/IC · ELISA/IC · ELISA/IC · ELISA/IC · ELISA/IC	Molecolare · PCR · NAAT
Diarrea in immunocompromesso		· C.trachomatis	ELISA	PCR

Laboratorio livello 2 → Protocolli standard differenziati

Diarrea autoctona/ epidemica	Diarrea acuta	Ricerca di: · Salmonella · Shigella · Campylobacter · E.coli O157 · Yersinia · Vibrio · Aeromonas · Plesiomonas · C.difficile	Identif. a livello di: · sierotipo/fagotipo · specie/sierotipo · specie · specie/sierotipo · specie · specie/sierotipo · specie · specie · specie/sierotipo	PCR · Multiplex PCR · DNA ibridazione · *eltA, estA, vtx1, vtx2, eae, ipaH ehly, ipaH,bfpA aap, aatA, astA, shf, irp2, pet, set1A*
	Diarrea persistente			
	Diarrea protratta			
Diarrea del viaggiatore				
Diarrea da tossinfezione				
AAD,AAC,AAPMC		Ricerca di: · Campylobacter · E.coli O157 · E.coli STEC · Rota/Adeno · Norwalk	EIA · ELISA/IC · ELISA/IC · ELISA/IC · ELISA/IC · ELISA/IC	Coltura cellulare · PCR · Multiplex PCR · DNA ibridazione
Diarrea in immunocompromesso		Ricerca di: · C.difficile tossina A/B	EIA · ELISA/IC	Coltura cellulare · NAAT
		Ricerca di: · B.cereus · C.perfringens · S.aureus	RPLA	

Bacterial Analytic Phase (Stool Culture)





Basic investigations

- *Salmonella*
- *Shigella*
- *Campylobacter*
- STEC

Modality

Direct

Dilution 1:10 a few drops of fecal suspension are placed on a culture plate, occupying 1/3 of the surface, proceeding with the seeding of the remaining surface.

Enrichment

Faeces is suspended in enrichment broth (ratio 1:5); after incubation, we proceed to subculture by sowing on appropriate solid soils with a sterile loop. The use of an enrichment aims to create favorable conditions for the growth of a given microorganism, based on its nutritional needs, while simultaneously inhibiting the growth of other microorganisms, so that they do not "interfere" with the isolation.

Filter membrane

Faeces is suspended in enrichment broth (ratio 1: 5); after incubation (≤6 hours); 4-5 drops of suspension are placed on a sterile filter membrane (cellulose acetate), placed on a culture plate with non-selective medium. After a short incubation at 37 ° C for 45 ', the filter is removed and the final incubation is carried out for 24-48 hours at 37 ° C in a microaerophilic atmosphere (Campylobacter).

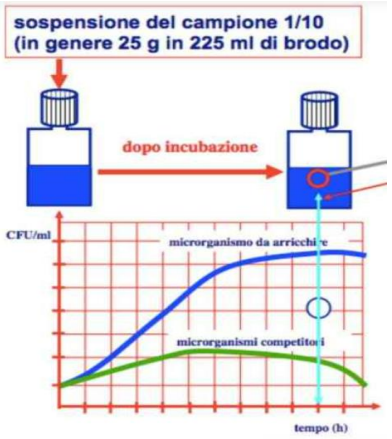
Traditional Analytic Phase (Example 1)

72 h

1 h

Multiplex PCR

37°C



Selenite 1:5 6h

Gn Brth-Tetrathionate 1:5 6h

Salmonella spp.

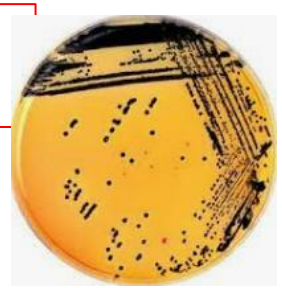
Shigella spp.

Hektoen enteric, Xylose Lysinr, SS agar)

Terreni selettivi

Terreni cromogeni

37°C



Blood culture

Serodiagnosis

Febbre tifoide

Identificazione biochimica o MALDI

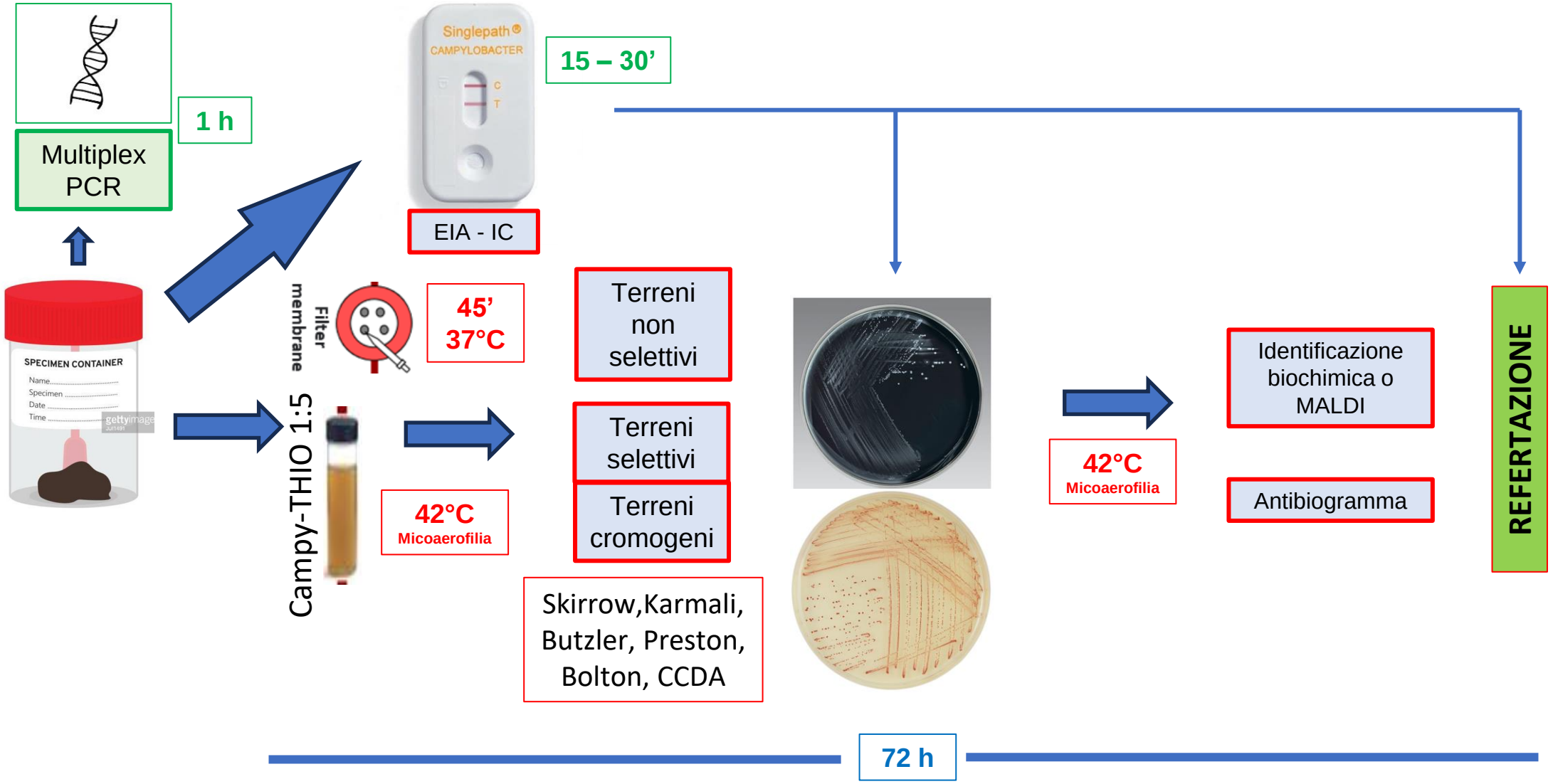
Antibiogramma

Tipizzazione Gruppo

REFERTAZIONE

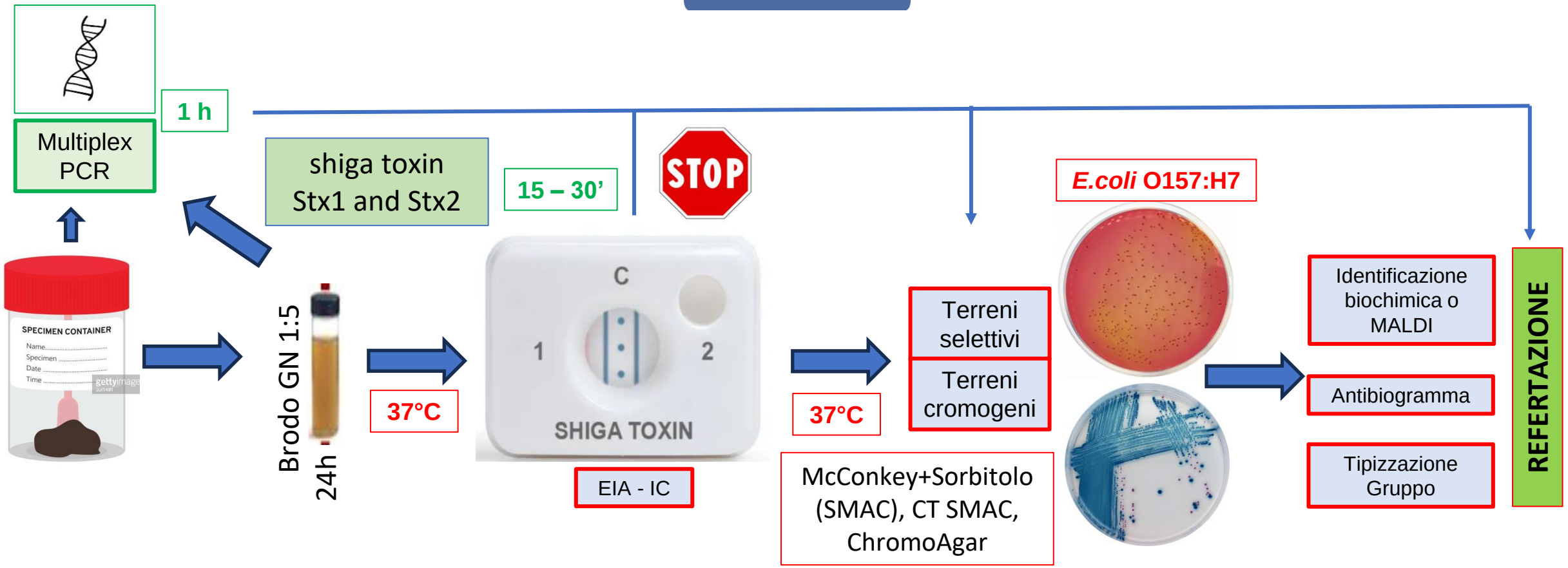
Traditional Analytic Phase (Example 2)

Campylobacter spp.



Traditional Analytic Phase (Example 4)

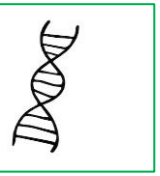
E. coli STEC



Traditional Analytic Phase (Example 4)

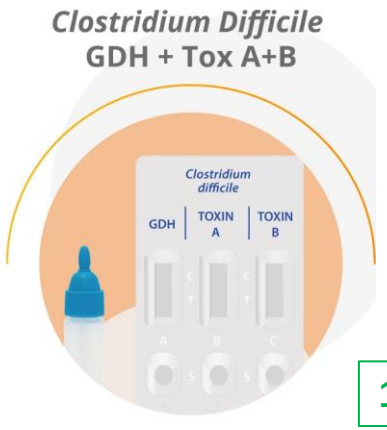
Target 1: glutamate dehydrogenase GHD
Target 2_ Toxin A/B

C. difficile



NAAT

1 h



15 – 30'

Test EIA - IC
GDH/Toxin A-B

37°C

Terreni selettivi

Terreni cromogeni

CCFA48h anaerobic

Identificazione

Antibiogramma

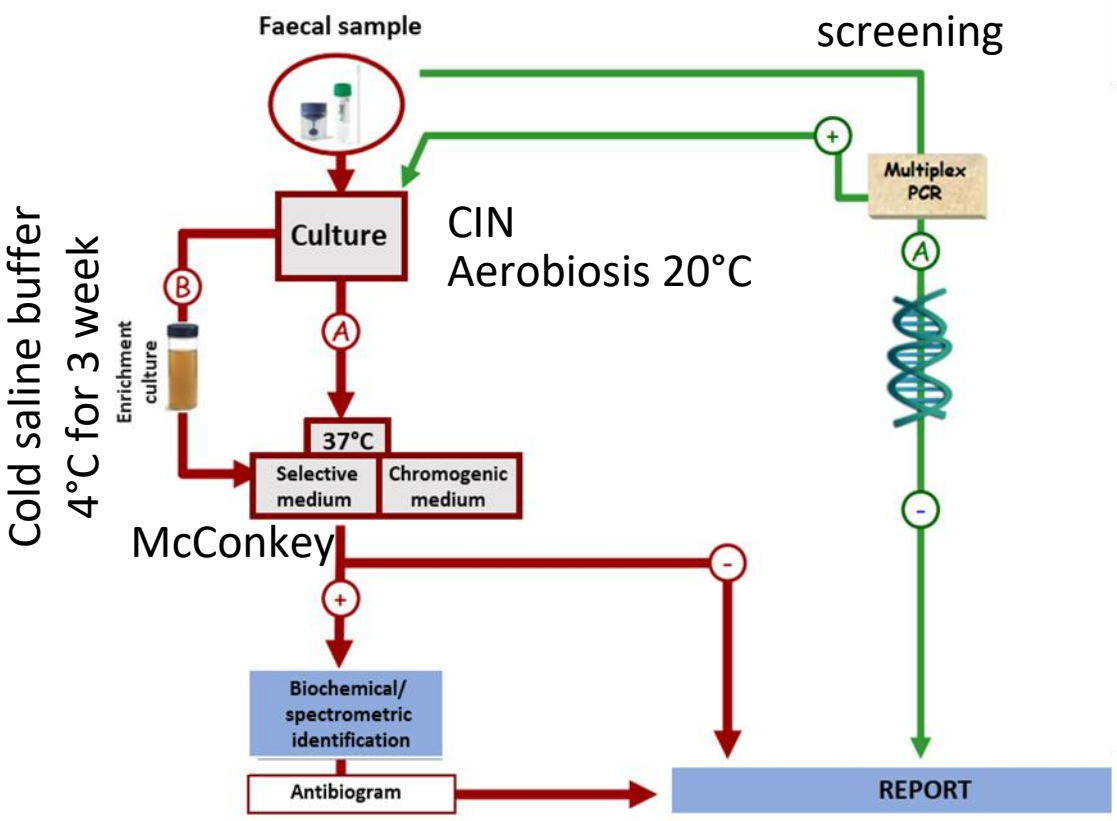
Tipizzazione Ribotipo

REFERTAZIONE

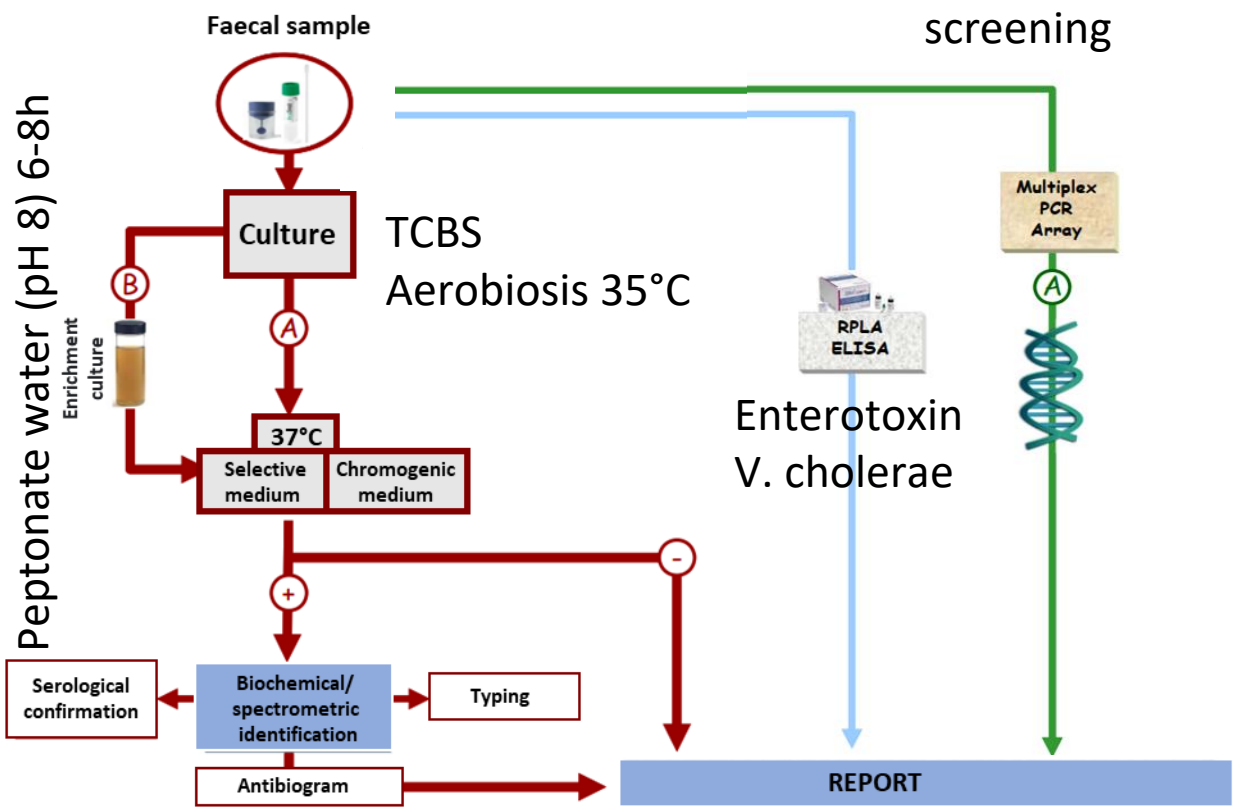
>72 h

Traditional Analytic Phase (Example 3)

Yersinia spp.



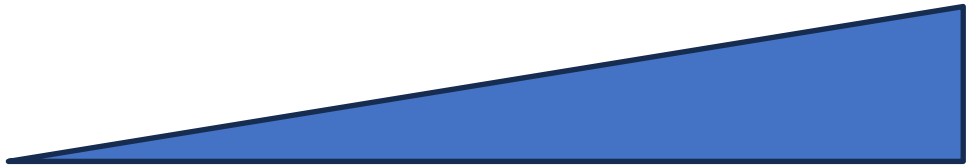
Vibrio spp.



CIDT IMPACT

Test diagnostici indipendenti dalla cultura (CIDT)

EIA - IC



Aumento costante di utilizzo nei laboratori fino ad oggi

La percentuale di diarree da *Campylobacter* diagnosticate solo dai CIDT nei siti FoodNet è aumentata dal 13% nel 2012-2014 al 38% nel 2018.

Multiplex PCR NAAT

Sorveglianza ed Epidemiologia

I CIDT influiscono sulla capacità della sanità pubblica di **rilevare e prevenire le epidemie**. Perdita dell'isolato in coltura pura • Difficoltà di tipizzazione

I CIDT influenzano la capacità di tracciare e contenere la **resistenza agli antibiotici**. Mancanza dei test di sensibilità agli antibiotici.

I CIDT influenzano la capacità di monitorare il trend di una infezione : perdita della capacità di tracciare gli outbreak o spreco di risorse per indagare pseudo-outbreak; • Minore monitoraggio dell'emergenza di sottotipi e serovar.

Vantaggi

1) Rapidità di esecuzione (rapida diagnosi)
2) Possono essere più sensibili della coltura, in particolare quando sono stati somministrati farmaci antimicrobici prima della raccolta dei campioni
3) la relativa facilità di uso può aumentare il numero di pazienti testati
4) Possono individuare infezioni polimicrobiche
5) Favorire la diagnosi dislocata (POCT)

Svantaggi

1) Test antigenici poco sensibili possono produrre falsi-negativi, se poco specifici, falsi-positivi
2) Test molecolari altamente sensibili possono produrre falsi-positivi (non distinguono fra infezione e colonizzazione)
3) Mutazioni molecolari nel microrganismo possono provocare una diminuzione della sensibilità
4) Possono individuare infezioni polimicrobiche
5) Difficoltosa interpretazione di risultati discordanti tra i test molecolari (Ag e NA) e colturali
6) Over-diagnosi e Over-trattamento
7) Abbandono della coltura e ricerca dell'isolato

https://www.cdc.gov/foodsafety/challenges/cidt.html#:~:text=These tests can identify the,cause of a patient's illness.

CIDT IMPACT

Il CDC sta incoraggiando i laboratori clinici a continuare a coltivare e isolare i batteri da persone malate con CIDT positivi.

Il CDC promuove la necessità di Laboratori centrali selezionati per l'esecuzione della coltura di feci con CIDT positivi

Il CDC sta incoraggiando le aziende che producono CIDT a progettare i test in modo da mantenere vivi i batteri in modo che possano essere coltivati se il test è positivo.

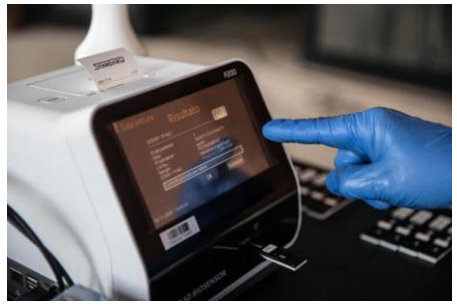
Il CDC sta valutando modi per rendere le colture di follow-up più facili ed economiche per i laboratori clinici

Il CDC sta lavorando per sviluppare nuovi metodi di test avanzati che non richiederanno la necessità di coltivare isolati batterici dalla maggior parte dei pazienti ma possono fornire informazioni sulla resistenza agli antibiotici del germe o sulla probabilità di causare malattie gravi.

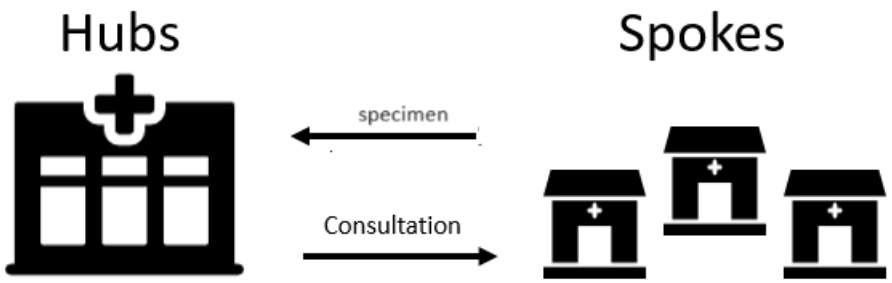
Soluzioni a breve termine

Protocolli specifici

Pazienti sintomatici



TEST FIA microfluidica



Test CIDT

Colture complesse (Batteriche e virali)

Tipizzazione

WGS

Sensibilità antibiotica

Parassitologia

Ampia gamma di CIDT (Batteri, virus, parassiti)

Test Ag

NAAT

Multiplex PCR

Colture semplici (Facoltativo)

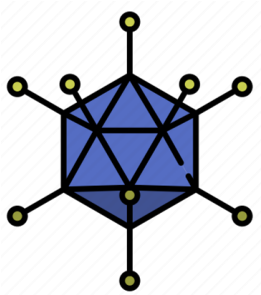
Virus

Rotavirus

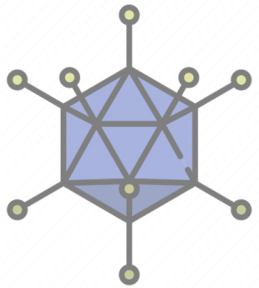
Reoviridae
dsRNA
Double capsid

Vaccine

Adenovirus

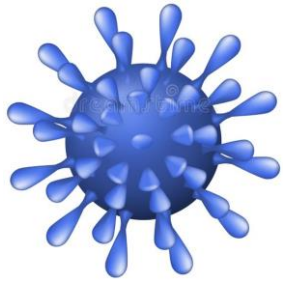


Adenoviridae
dsDNA
Envelop

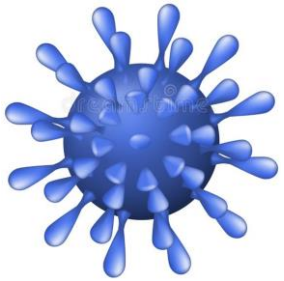


Vaccine
Military

Norovirus

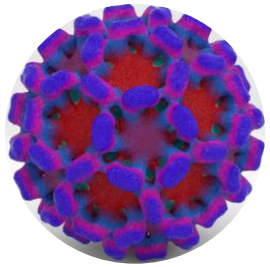


Caliciviridae
ssRNA +
Capsid

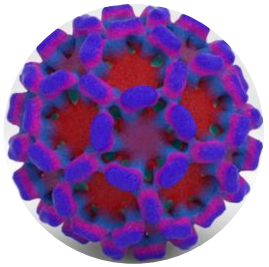


VP1-based VLP
phase2

Sapovirus

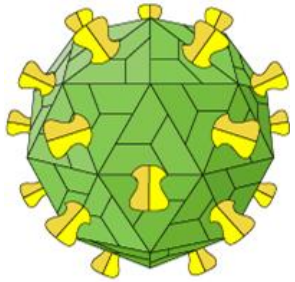


Caliciviridae
ssRNA +
Capsid

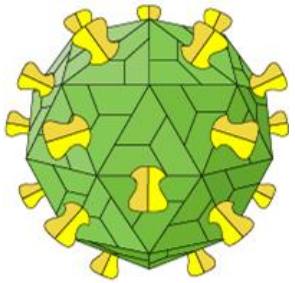


VLP
Preclinical

Astrovirus



Astroviridae
ssRNA +
Capsid



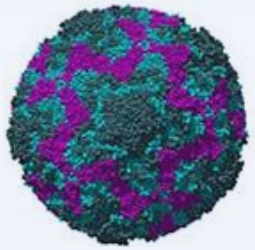
VLP
Preclinical

Citomegalovirus

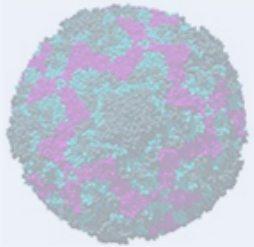
Herpesviridae
dsDNA
Envelop

Triplex
Phase III

Enterovirus



Picornaviridae
ssRNA +
Capsid



4 China
WHO 1 Inact.

Immune-compromised Patients

Virus

frontiers in Microbiology

REVIEW
published: 13 September 2019
doi: 10.3389/fmicb.2019.01957

Advances in Diagnostic Approaches for Viral Etiologies of Diarrhea: From the Lab to the Field

Yashpal Singh Malik[1], Atul Kumar Verma[1], Naveen Kumar[2], Nadia Touil[3], Kumaragurubaran Karthik[4], Ruchi Tiwari[5], Durlav Prasad Bora[6], Kuldeep Dhama[7]*, Souvik Ghosh[8], Maged Gomaa Hemida[9,10], Ahmed S. Abdel-Moneim[11,12], Krisztián Bányai[13], Anastasia N. Vlasova[14], Nobumichi Kobayashi[15] and Raj Kumar Singh[1]*

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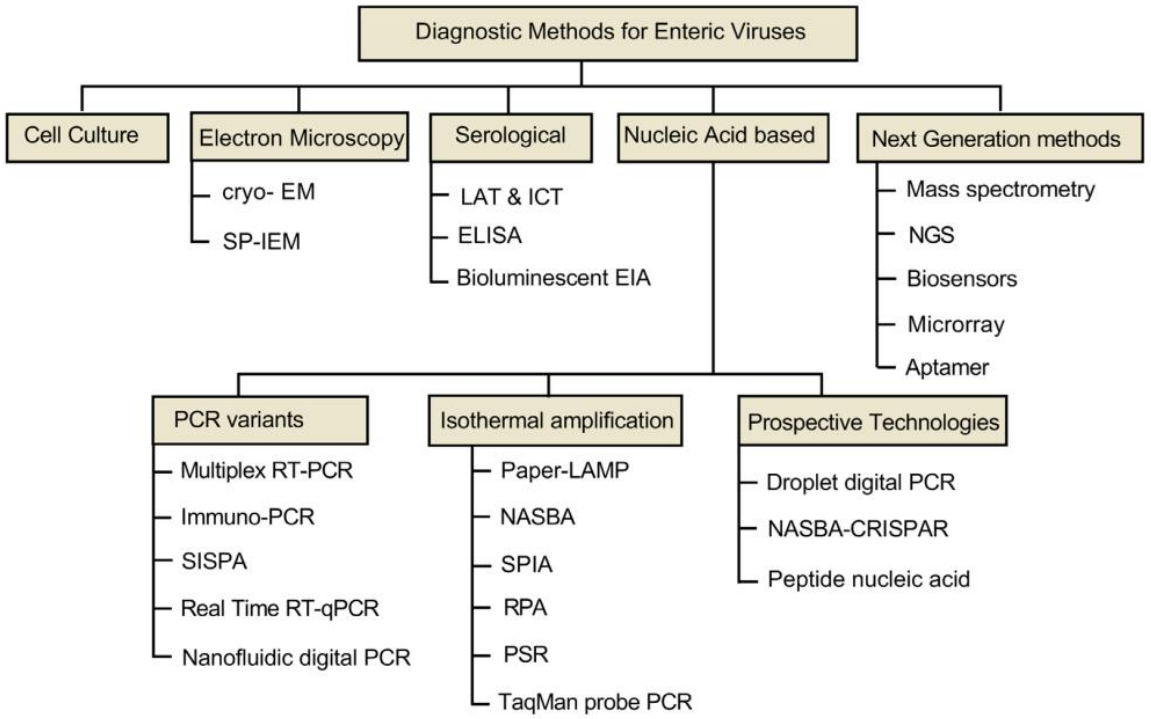
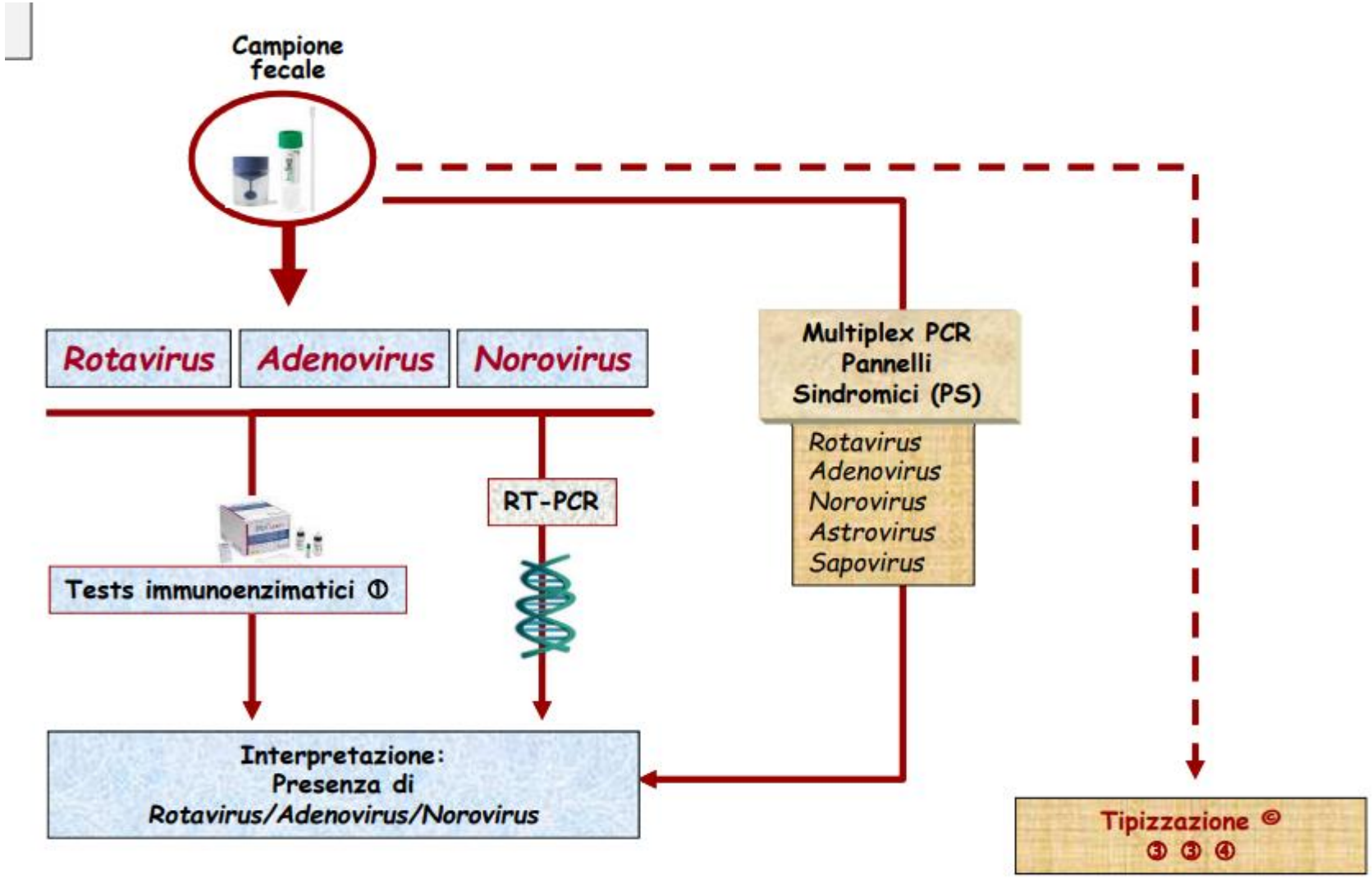
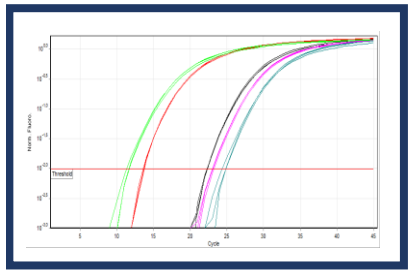


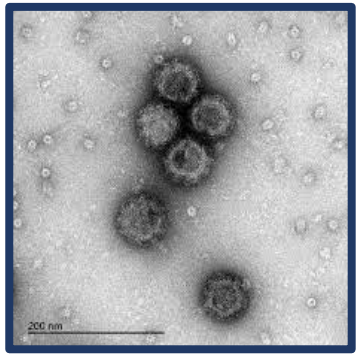
FIGURE 1 | A schematic representation of diagnostic methods for enteric viruses. The diagnostic assays are classified in five major categorizes (i) cell culture, (ii) electron microscopy, (iii) serological methods, (iv) nucleic acid methods, which further have three sub-classifications, and (v) next-generation methods. The techniques are individually dealt under different sections.

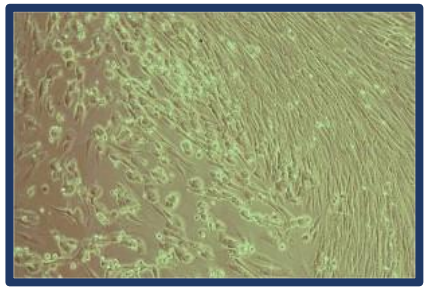
Virus Analytic Phase

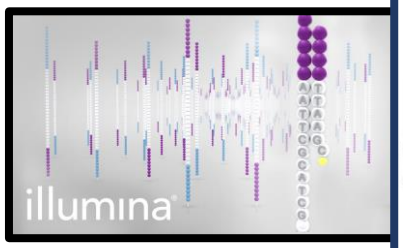
Enteric viruses











eference centers

Virus Analytic Phase (Approaches)

Agent	Biomarker	Detection Method	Brand	Sensitivity	Specificity
Rotavirus	Rotavirus antigen	ELISA	Rota Antigen Test Device, Cambridge	86–98%	92–96%
		EIA	Premier Rotaclone, Meridian Bioscience Inc., Cincinnati, OH, USA	76.8–77.8%	100%
			RIDASCREEN® Rotavirus	82.1–97.8%	99.1–100%
			ProSpect Rotavirus Test, Oxoid Ltd., UK	75%	100%
	Rotavirus RNA	RT-PCR	Primerdesign Genesig® Kit	100%	100%
		Real-time RT-PCR	GeneAmp EZ rTth RNA PCR kit (Applied Biosystems, Inc., Foster City, CA, USA)	98.8–100%	99.7–100%
Norovirus	Norovirus antigen	ICT	RIDA®QUICK Norovirus N1402	72.8–87%	97–99.5%
			QuickNaviTM Norovirus 2	27.5%	97.7%
		ELISA	RIDASCREEN® Norovirus 3rd Generation	84.6–85.7%	>96%
		FIA	AFIAS-Noro	66%	97.6%
	Norovirus RNA	Real-time RT-PCR	Cepheid Xpert® Norovirus	100%	100%
		Real-time PCR	RIDA®GENE Norovirus	98%	98%
Astrovirus	*ORF2* gene	RT-PCR	RT-PCR Luminex Assay	100%	100%
			rRTLAMP Assay	94%	100%
Enteric Adenovirus Serotypes 40 and 41	Adenovirus antigen	ICT	BioNexia RotaAdeno	60%	98.8%
			RIDA Quick Rota-Adeno-Combi R-Biopharm AG	72.7%	98.2%
		CLIA	LIAISON Adenovirus	77%	98.8%

Haziqah Hasan et al., 2021

Virus Analytic Phase (Approaches)

Test rapidi con tecnologia Fluorescent Immuno Assay

I test antigenici ad immunofluorescenza, sono noti anche come test di terza generazione; sarebbero attualmente i migliori test rapidi, con risultati sovrapponibili ai tamponi molecolari.

Products available	• 155047 STANDARD F200 Analyzer • 155046 STANDARD F2400 Analyzer
Method	Fluorescence immunodiagnostics
Sample type	Test dependent
Time to result	10 or 15 minutes
Reading of the result	Instrument read
Storage	2 - 30 °C
Sensitivity	Test dependent

GASTROINTESTINAL DISEASE

STANDARD Q Rotavirus Ag

» Background

STANDARD Q Rotavirus Ag Test is a Colloidal gold-based immunochromatographic assay for the detection of Rotavirus in human fecal sample. The test result can be determined visually and using the STANDARD F analyzers.

STANDARD F Norovirus Ag FIA

» Background

STANDARD F Norovirus Ag FIA is a fluorescent immunoassay for the detection of Norovirus antigen in human fecal sample. The test kit is for *in vitro* use only.

STANDARD F Rota/Adeno Ag FIA

» Background

STANDARD F Rota/Adeno Ag FIA is a fluorescent immunoassay for the qualitative detection of the presence of Rotavirus and/or Adenovirus antigens in fecal specimens. STANDARD F Rota/Adeno Ag FIA should be used with STANDARD F Analyzers manufactured by SD BIOSENSOR.

STANDARD F *C. difficile* GDH FIA

» Background

STANDARD F *C. difficile* GDH FIA qualitatively analyzes *Clostridium difficile* Glutamate dehydrogenase (GDH) antigen in human feces.

STANDARD F *C. difficile* Toxin A/B FIA

» Background

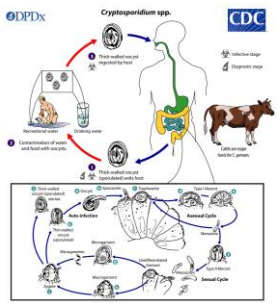
STANDARD F *C. difficile* Toxin A/B FIA qualitatively analyzes *C. difficile* Toxin A & B in human feces.

STANDARD F *H. pylori* Ag FIA

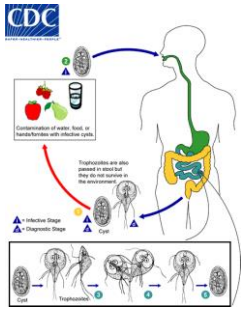
» Background

STANDARD F *H. pylori* Ag FIA is a fluorescent immunoassay for the detection of *H. pylori* antigen in human fecal samples.

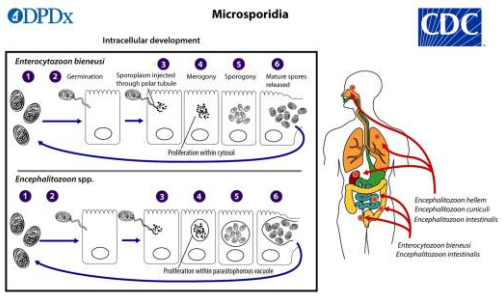
Parasites



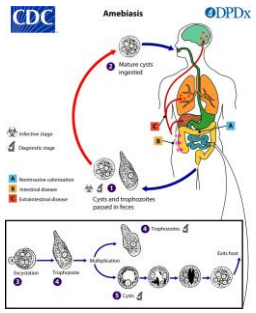
Cryptosporidium parvum



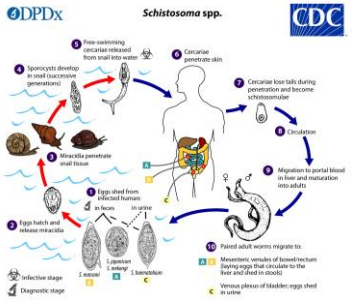
Giardia intestinalis



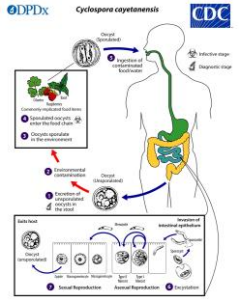
Microsporidia



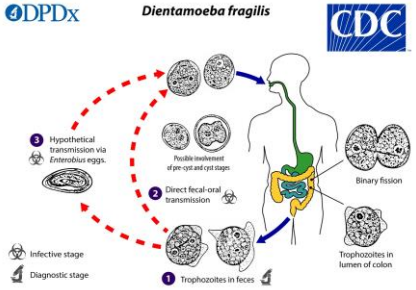
Entamoeba histolytica



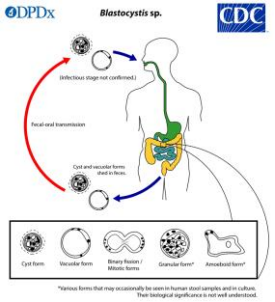
Schistosoma japonicum



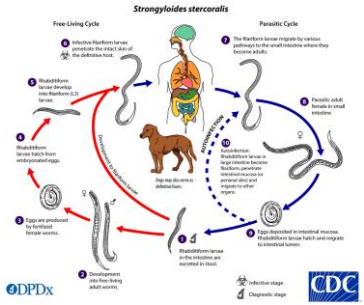
Cyclospora cayetanensis



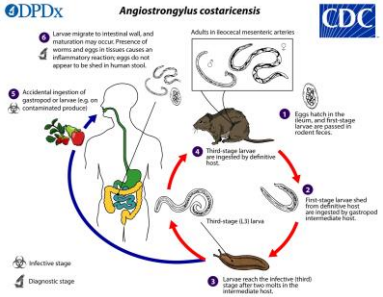
Dientamoeba fragilis



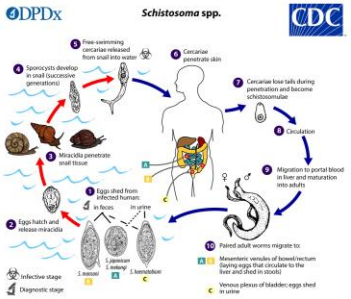
Blastocystis hominis



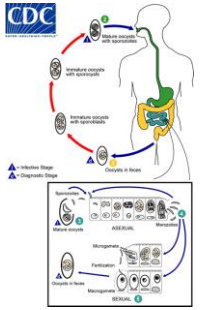
Strongyloides stercoralis



Angiostrongylus costaricensis



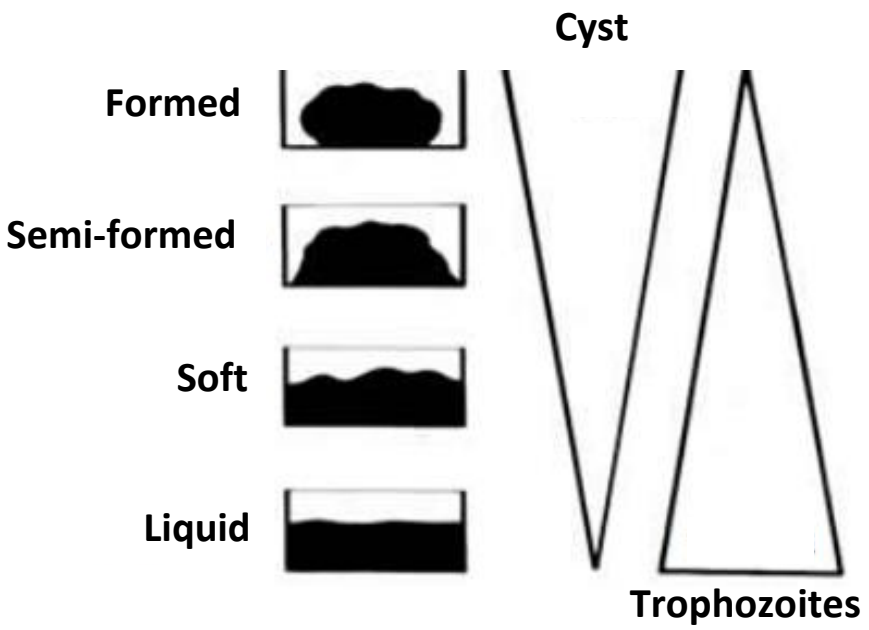
Schistosoma mansoni

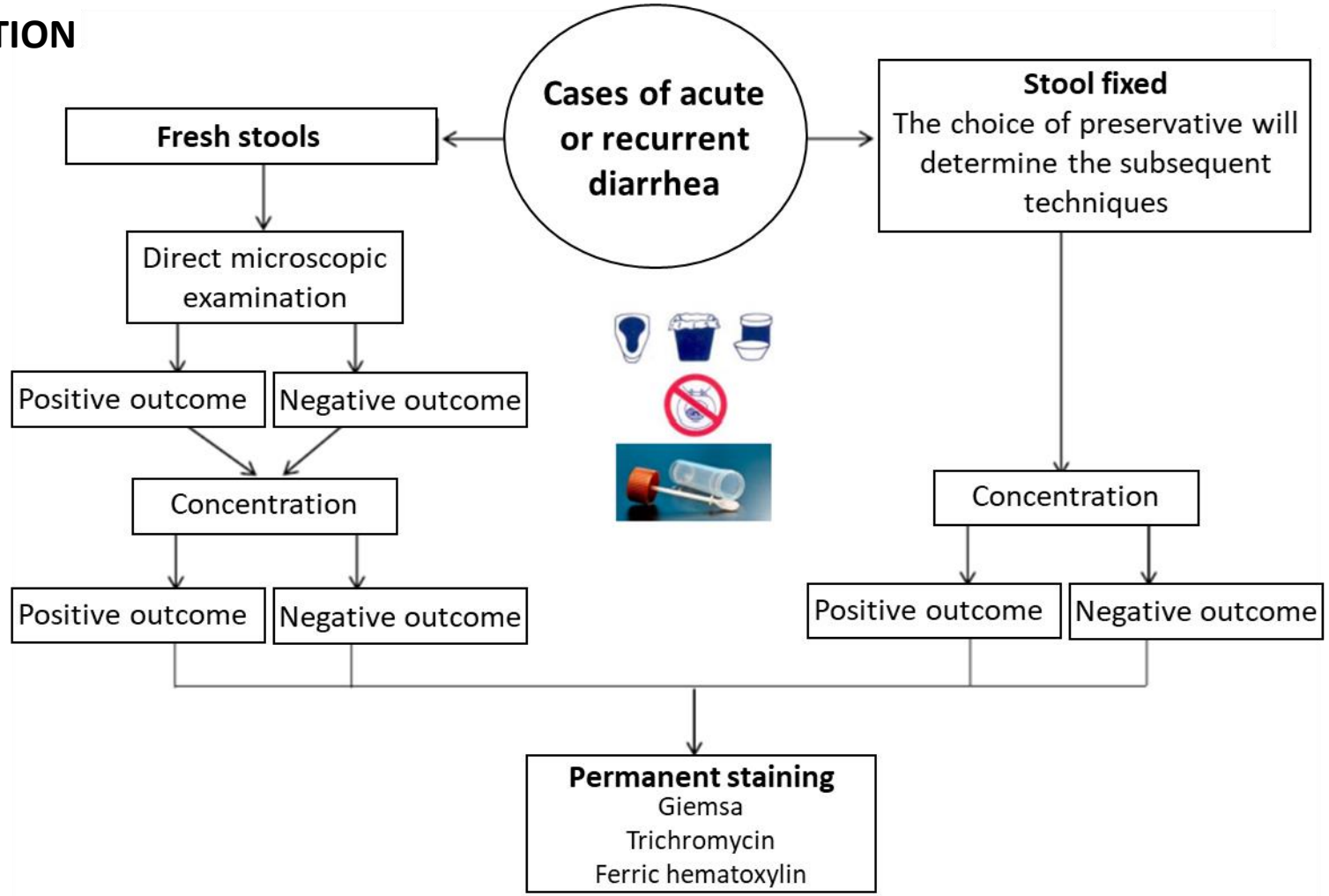


CystoIsospora belli

Parasites

STANDARD COPRO-PARASITOLOGICAL EXAMINATION





From: L.S. Garcia. Diagnostic Medical Parassitology. 2016 (modified)

Parasites

Table 1 Diagnostic approaches for the detection of intestinal parasites

From: Advancements in Parasite Diagnosis and Challenges in the Management of Parasitic Infections: A Mini Review

Helminth	Diagnostic approaches				References
	Microscopy-based	Serology-based	Molecular-based	Proteomics	
Schistosoma species	Using Kato-Katz technique	IHA, ELISA, dipstick	PCR, real-time PCR, multiplex PCR	LC-MS/MS	Katz et al. (1972); van Gool et al. (2002); ten Hove et al. (2008); Cnops et al. (2013); Sousa-Figueiredo et al. (2013); Lodh et al. (2014); Wang et al. (2016)
Soil-transmitted helminths	Using sedimentation or concentration techniques	ELISA	Multiplex real-time PCR	–	Bungiro et al. (2005); Basuni et al. (2011)
Taenia solium	Using sedimentation or concentration techniques	ELISA, Immunoblot	Nested PCR	Mass Spectrometry	Del Brutto et al. (2001); Bueno et al. (2005); Deckers et al. (2008); Mayta et al. (2008)
Protozoa					
C. parvum, C. hominis	Using modified acid-fast staining	DFA, ICT assay kit	PCR, real-time PCR, multiplex real-time PCR, LAMP, Luminex	LC-MS/MS	Weber et al. (1991); Johnson et al. (1995); Johnston et al. (2003); Bandyopadhyah et al. (2007); Karanis et al. (2007); ten Hove et al. (2007); Jothikumar et al. (2008)
Giardia lamblia	Using trichrome or ion hematoxylin staining; Using concentration or sedimentation techniques	DFA, ICT assay kit	Multiplex real-time PCR	–	Danciger et al. (1975); Young et al. (1979); Garcia et al. (1997); ten Hove et al. (2007); Siddiki (2012)
Entamoeba histolytica	Using staining methods	IHA, IIF, ELISA, ICT assay	Multiplex real-time PCR, LAMP	LC-MS/MS	Hira et al. (2001); Gonin and Trudel (2003); Fotedar et al. (2007); Liang et al. (2009); Ali et al. (2012); Luca-Nacar et al. (2016)

C Cryptosporidium, *IHA* indirect hemagglutination, *ELISA* enzyme-linked immunosorbent assay, *PCR* polymerase chain reaction, *LAMP* loop-mediated isothermal amplification, *DFA* direct fluorescent antibody, *ICT* immunochromatograhic, *LC-MS/MS* liquid chromatography-tandem mass spectrometry

Parasites

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Review

Deep learning for microscopic examination of protozoan parasites

Chi Zhang [a], Hao Jiang [a], Hanlin Jiang [a], Hui Xi [a], Baodong Chen [b], Yubing Liu [c], Mario Juhas [d], Junyi Li [e,*], Yang Zhang [a,*]

CONCLUSIONS

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