3° CONGRESSO NEWMICRO
….The need for speed: il laboratorio di Microbiologia e le Urgenze Infettive

NEWMICRO
Network di Microbiologia e Virologia del Nord Est

Le nuove tecnologie per la gestione dell'urgenza/emergenza in microbiologia:

Diarree acute e infezioni da *Clostridium difficile*

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Microbiologia Clinica e Virologia
AOSMA Pordenone

Acute diarrhea in adults and children: a global perspective

World Gastroenterology Organisation Global Guidelines

February 2012

Table 1 Overview of causative agents in diarrhea

Bacteria	Viruses	Parasites
• Diarrheagenic *Escherichia coli* • *Campylobacter jejuni* • *Vibrio cholerae O1* • *V. cholerae O139** • *Shigella species* • *V. parahaemolyticus* • *Bacteroides fragilis* • *C. coli* • *C. upsaliensis* • Nontyphoidal *Salmonellae* • *Clostridium difficile* • *Yersinia enterocolitica* • *Y. pseudotuberculosis*	• Rotavirus • Norovirus (calicivirus) • Adenovirus (serotype 40/41) • Astrovirus • Cytomegalovirus*	**Protozoan** • *Cryptosporidium parvum* • *Giardia intestinalis* • *Microsporida** • *Entamoeba histolytica* • *Isospora belli** • *Cyclospora cayetanensis* • *Dientamoeba fragilis* • *Blastocystis hominis* ***Helminths*** • *Strongyloides stercoralis* • *Angiostrongylus costaricensis* • *Schistosoma mansoni, S. japonicum*

Acute diarrhea in adults and children: a global perspective

February 2012

World Gastroenterology Organisation Global Guidelines

Table **1** Epidemiology of acute diarrhea: developed versus developing countries.

Per year	Estimated episodes of acute diarrhea	Hospitalizations	Deaths
United States	375 million — 1.4 episodes per person per year	900 000 total	6000 total
	> 1.5 million child outpatient visits	200 000 children	300 children
Worldwide	1.5 billion episodes		1.5–2 million children < 5 y
	In developing countries, children < 3 y have 3 episodes per year		

Definition of acute diarrhoea

3 or more episodes a day, <14d and sample takes shape of pot

- About 20% of the population develop infectious intestinal disease per year
- Most infectious diarrhoea is a self-limited, usually viral illness
- If the diarrhoea has stopped, culture is not indicated, as recovery of pathogen is unlikely

Infectious Diarrhoea
The Role of Microbiological Examination of Faeces
Quick Reference Guide for Primary Care
For consultation and local adaptation

When to send a faecal specimen

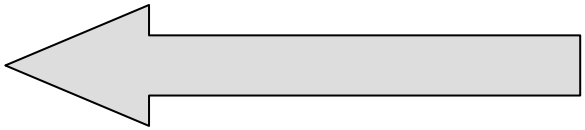
- Persistent diarrhoea
- Blood or pus in stool (in children also consider E. coli O157)
- Post antibiotics or hospitalization
- Diarrhoea after foreign travel
- For reassurance, as diagnosis of infection may exclude other pathologies

Infectious Diarrhoea
The Role of Microbiological Examination of Faeces
Quick Reference Guide for Primary Care
For consultation and local adaptation

History should be included on form to help determine diagnostics methods

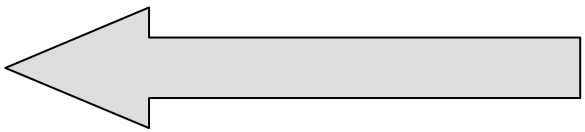
Clinical features:

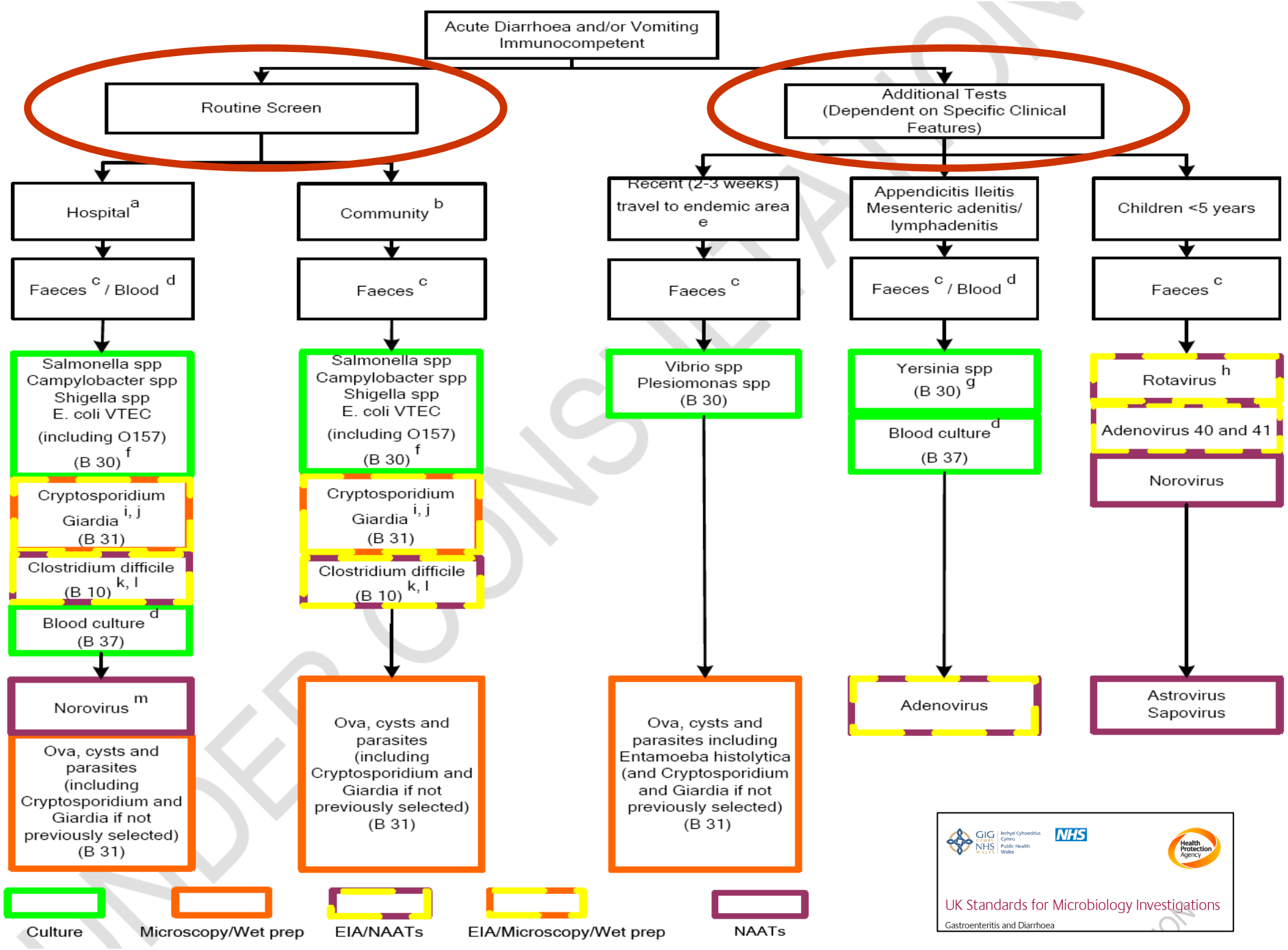
- ❑ Systemic illness, fever, bloody stool
- ❑ Symptoms; duration, recurrent, chronic
- ❑ Severe abdominal pain (Campylobacter)
- ❑ Immunosuppression



Epidemiological setting:

- ❑ Food intake e.g. barbecue; restaurant; eggs; chicken; shellfish
- ❑ Recent foreign travel and to which country
- ❑ Recent antibiotic, PPI or hospitalisation (*C. difficile*)[11,12]
- ❑ Family or nursing home; (Norovirus)
- ❑ Exposure to untreated water (protozoa) or animals
- ❑ Contact with other affected individuals or outbreak




Acute Diarrhoea and/or Vomiting Immunocompetent
Routine Screen
Additional Tests (Dependent on Specific Clinical Features)
Hospital a
Community b
Recent (2-3 weeks) travel to endemic area e
Appendicitis Ileitis Mesenteric adenitis/ lymphadenitis
Children <5 years
Faeces c / Blood d
Faeces c
Faeces c
Faeces c / Blood d
Faeces c
Salmonella spp Campylobacter spp Shigella spp E. coli VTEC (including O157) (B 30) f
Cryptosporidium Giardia i, j (B 31)
Clostridium difficile (B 10) k, l
Blood culture d (B 37)
Norovirus m
Ova, cysts and parasites (including Cryptosporidium and Giardia if not previously selected) (B 31)
Salmonella spp Campylobacter spp Shigella spp E. coli VTEC (including O157) (B 30) f
Cryptosporidium Giardia i, j (B 31)
Clostridium difficile (B 10) k, l
Ova, cysts and parasites (including Cryptosporidium and Giardia if not previously selected) (B 31)
Vibrio spp Plesiomonas spp (B 30)
Ova, cysts and parasites including Entamoeba histolytica (and Cryptosporidium and Giardia if not previously selected) (B 31)
Yersinia spp (B 30) g
Blood culture d (B 37)
Adenovirus
Rotavirus h
Adenovirus 40 and 41
Norovirus
Astrovirus Sapovirus
Culture
Microscopy/Wet prep
EIA/NAATs
EIA/Microscopy/Wet prep
NAATs
UK Standards for Microbiology Investigations
Gastroenteritis and Diarrhoea

Infectious Diarrhoea
The Role of Microbiological Examination of Faeces
Quick Reference Guide for Primary Care
For consultation and local adaptation

BIAM British Infection Association

Health Protection Agency

INTERPRETING THE LABORATORY REPORT

- ❑ A pathogen is found in only 2 – 5% of specimens submitted.[3]
- ❑ A negative report does not mean all pathogens are excluded; the pathogens sought will usually be listed.
  e.g. There are no routine methods for detecting enterotoxigenic *E. coli*, the commonest cause of traveller's diarrhoea.

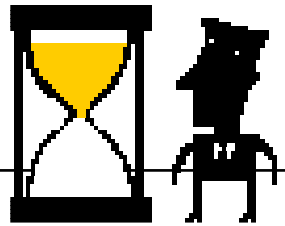
TREATMENT FOLLOWING REPORT

- ❑ Most patients in whom pathogens are detected will NOT require specific treatment unless systemically unwell or treatment is advised by a microbiologist or consultant in communicable disease control.
- ❑ Urgently refer all previously healthy children with acute painful, bloody diarrhoea or confirmed *E. coli* O157.
- ❑ *C. difficile:* Stop unnecessary antibiotics and/or PPIs to re-establish normal flora. Prescribe metronidazole 400 or 500 mg oral tds. 70% of patients respond after 5 days; 94% in 14 days. Monitor >85 year olds as mortality double.[13,14]
  If severe (characterised by T >38.5; WCC >15; rising creatinine or signs/symptoms of severe colitis) or 3rd episode, prescribe vancomycin 125mg oral qds for 10-14 days.
- ❑ Campylobacter: Antibiotic therapy has little effect on duration of symptoms unless given very early in illness course.[15]
- ❑ *G. lamblia* and *E. histolytica* should be treated.[7]
- ❑ Unless symptoms persist, Blastocystis & *Dientamoeba fragilis* do not usually require treatment in otherwise healthy.[16,17]

For maximal clinical impact, the test should ideally be available on-site 24 h/day, 7 days/week, with results available within no more than a few hours. Tests that are not readily available will have limited impact on patient care.

IDSA. CID 2011

Quanto riusciamo ad essere rapidi in caso di diarrea acuta infettiva?

INDAGINI	***IN UN TURNO DI LAVORO***	24-48H	48-72H
•Microscopia a fresco •EIA su campione	***•Uova, cisti, parassiti*** ***•E.coli O157*** ***•Salmonella spp*** ***•Campylobacter spp*** ***•C.difficile*** ***•Virus enterotropi***		
Indagini colturali su terreni selettivi + EIA/ MALDI-TOF su colonia		***•E.coli O157*** ***•Salmonella spp*** ***•Campylobacter spp*** ***•Shigella spp.*** ***•Vibrio spp.*** ***•Yersinia enterocolitica***	
Indagini colturali su terreni selettivi ed identificazione di specie			***•E.coli O157*** ***•Salmonella spp*** ***•Campylobacter spp*** ***•Shigella spp.*** ***•Vibrio spp.*** ***•Yersinia enterocolitica***
NAATs su campione	***•E.coli O157*** ***•Salmonella spp*** ***•Campylobacter spp*** ***•C.difficile*** ***•Virus enterotropi***	***•Multiplex assay platforms***	

***NOROVIRUS*: Enzyme Immunoassays *versus* NAATs**

Rapid commercial assays, such as enzyme immunoassays (EIAs), that detect norovirus antigen have recently been developed. However, these kits have poor sensitivity (50%) and are not recommended for diagnosing norovirus infection in sporadic cases of gastroenteritis. The RIDASCREEN Norovirus 3rd Generation EIA was recently cleared by Food and Drug Administration for preliminary identification of norovirus when testing multiple specimens during outbreaks. However, samples that test negative should be confirmed by a second technique, such as RT-qPCR. Thus, **EIA kits should not replace molecular methods during outbreak investigations.**

http://www.cdc.gov/norovirus/lab-testing/diagnostic.html last reviewed: April 12, 2012

Guidelines for Diagnosis, Treatment, and Prevention of *Clostridium difficile* Infections

Enzyme Immunoassays *versus* NAATs

as 50% (18,23). The sensitivity of GDH antigen detection has led to its use as a screening test as part of CDI testing algorithms, although it should be noted that as many as 10% of patients with toxigenic organisms can be missed by this method. In this approach, GDH is the initial test, and GDH-negative specimens are reported as negative with no further testing done. GDH-positive specimens must undergo additional testing for *C. difficile* either by NAAT or by EIA testing followed by NAAT if the EIA results are discordant (24–27).

Evidence suggests that NAATs for toxigenic *C. difficile* are good stand-alone tests for toxigenic *C. difficile*. There are several Food and Drug Administration (FDA)-approved NAAT's, including

Am J Gastroenterol advance online publication, 26 February 2013; doi:10.1038/ajg.2013.4

Cepheid's SmartNorovirus Rapid and accurate identification of Norovirus infection is a critical part of effective infection control.

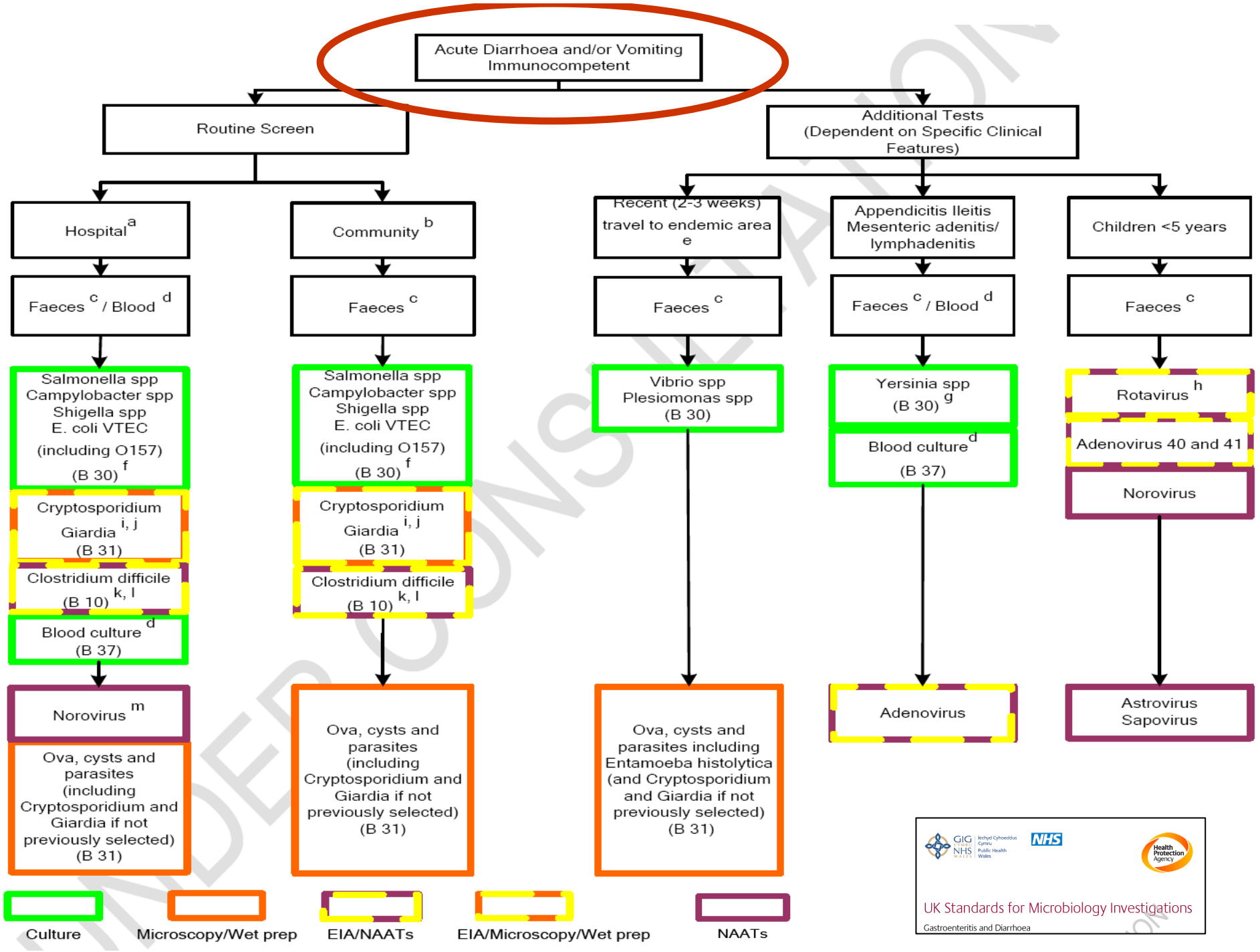
<3h

Xpert C. difficile detects the presence of toxin-producing *Clostridium difficile* (027/NAP/BI).

<1h

illumigene C. difficile Assay

<1h



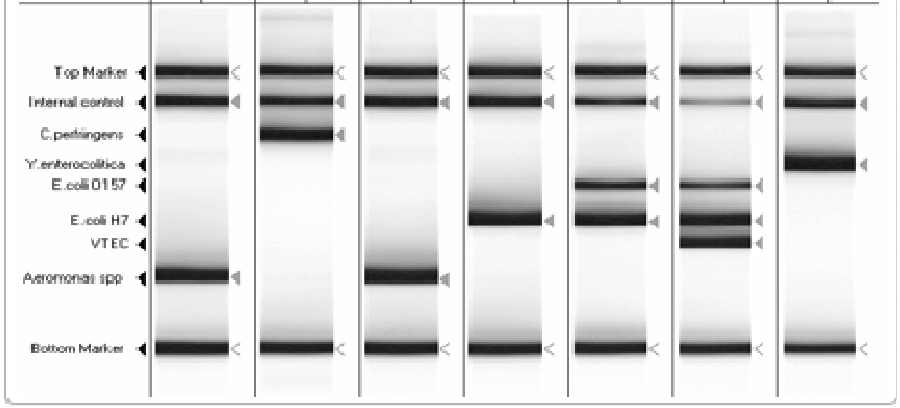
UK Standards for Microbiology Investigations

Gastroenteritis and Diarrhoea

Seeplex® Diarrhoea ACE Detection

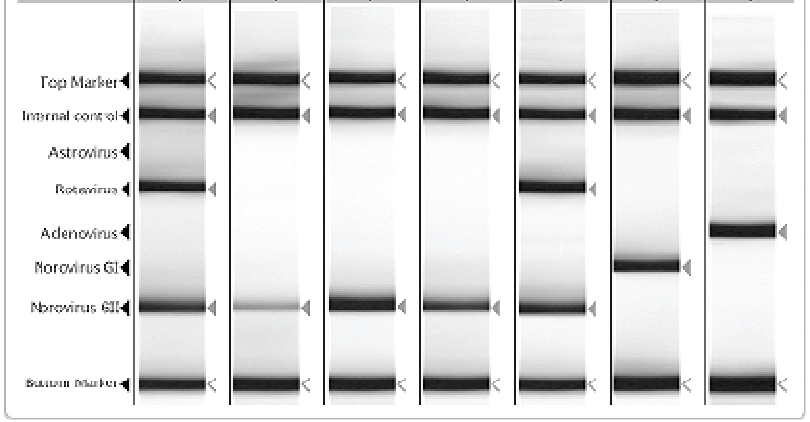
Bacterial panel 1 [Panel B1]

Lane	L2		L3		L4		L5		L6		L7		L8	
Sample ID	1		2		3		4		5		6		7	
Internal control	+	98	+	97	+	101	+	101	+	92	+	49	+	100
C.perfringens	−		+	105	−		−		−		−		−	
Y.enterocolitica	−		−		−		−		−		−		+	100
E.coli O157	−		−		−		−		+	90	+	91	−	
E.coli H7	−		−		−		+	105	+	102	+	102	−	
VTEC	−		−		−		−		−		+	101	−	
Aeromonas spp	+	102	−		+	101	−		−		−		−	
Unidentified	−		−		−		−		−		−		−	



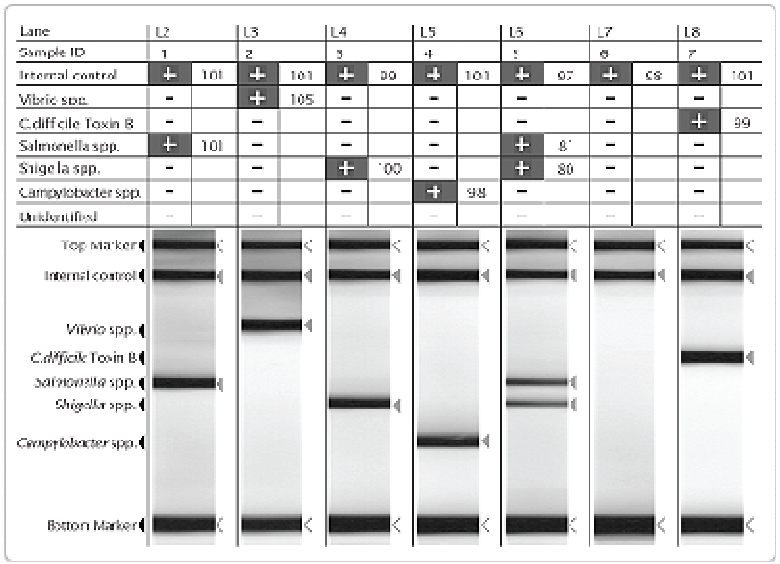
Virus panel [Panel V]

Lane	L2		L3		L4		L5		L6		L7		L8	
Sample ID	1		2		3		4		5		6		7	
Internal control	+	102	+	103	+	102	+	102	+	101	+	98	+	99
Astrovirus	−		−		−		−		−		−		−	
Rotavirus	+	103	−		−		−		+	106	−		−	
Adenovirus	−		−		−		−		−		−		+	100
Norovirus GI	−		−		−		−		−		+	99	−	
Norovirus GII	+	93	+	44	+	100	+	88	+	98	−		−	
Unidentified	−		−		−		−		−		−		−	



Bacterial panel 2 [Panel B2]

Lane	L2		L3		L4		L5		L6		L7		L8	
Sample ID	1		2		3		4		5		6		7	
Internal control	+	101	+	101	+	99	+	101	+	97	+	98	+	101
Vibrio spp.	−		+	105	−		−		−		−		−	
C.difficile Toxin B	−		−		−		−		−		−		+	99
Salmonella spp.	+	101	−		−		−		+	87	−		−	
Shigella spp.	−		−		+	100	−		+	80	−		−	
Campylobacter spp.	−		−		−		+	98	−		−		−	
Unidentified	−		−		−		−		−		−		−	



For Research Use Only

Nanosphere
SIGN UP

Verigene® Enteric Pathogens Test utilizes the Verigene System's multiplexing capabilities and automated workflow to deliver a rapid, easy-to-use, robust, cost-effective alternative to stool culture and other enteric pathogen identification methods.

FilmArray® GI Panel — Currently in Development

For Research Use Only
Not for Use in Diagnostic Procedures

Information Sheet

The FilmArray: User-Friendly Multiplex PCR

250µl di campione

The FilmArray GI Panel tests for a comprehensive panel of common gastrointestinal (GI) pathogens that cause infectious diarrhea. The FilmArray integrates sample preparation, amplification, detection, and analysis into one simple system that requires approximately 5 minutes of hands-on time and has a total run time of 1 hour.

- **Simple:** ~5 minutes of hands-on time
- **Easy:** No precise measuring or pipetting required
- **Fast:** Turnaround time of one hour
- **Comprehensive:** 23 target GI panel

nel
targets:

- *Salmonella*
- *Vibrio*
- *Vibrio cholerae*
- *Yersinia enterocolitica*

lloides

i/Shigella

coli
- *Shigella*/Enteroinvasive *E. coli* (EIEC)
- Enteroaggregative *E. coli* (EAEC)
- *E. coli* O157

E. coli

ing
l/stx2

Viruses
- Adenovirus F40/41
- Human Astrovirus
- Norovirus GI/GII
- Rotavirus A
- Sapovirus

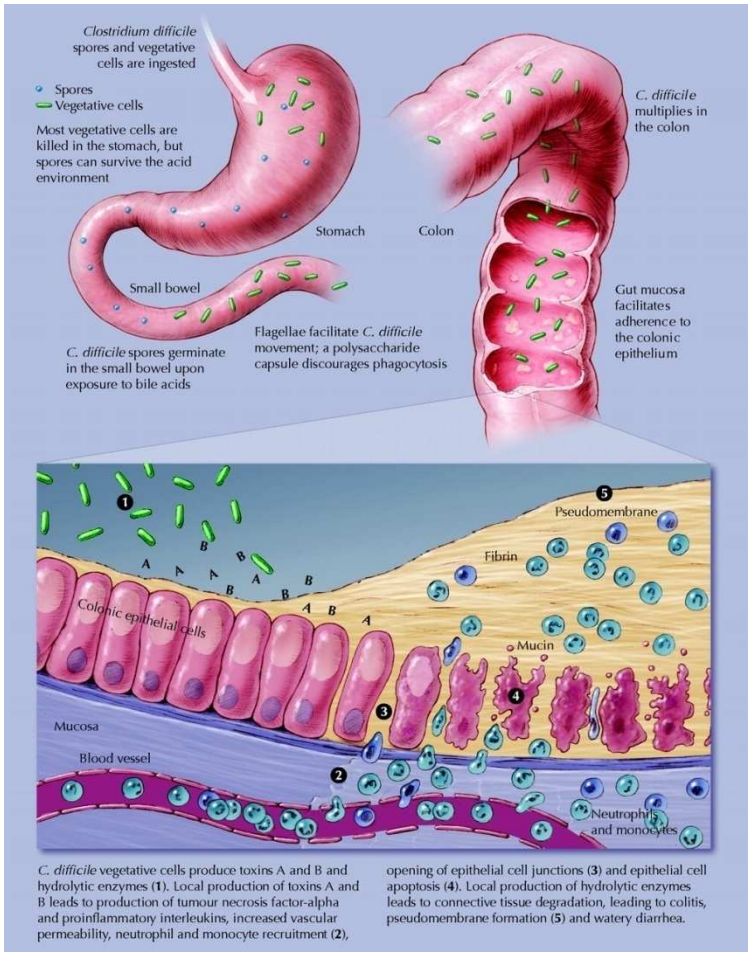
Protozoa
- *Cryptosporidium*
- *Cyclospora cayetanensis*
- *Entamoeba histolytica*
- *Giardia lamblia*

Idaho Technology is now

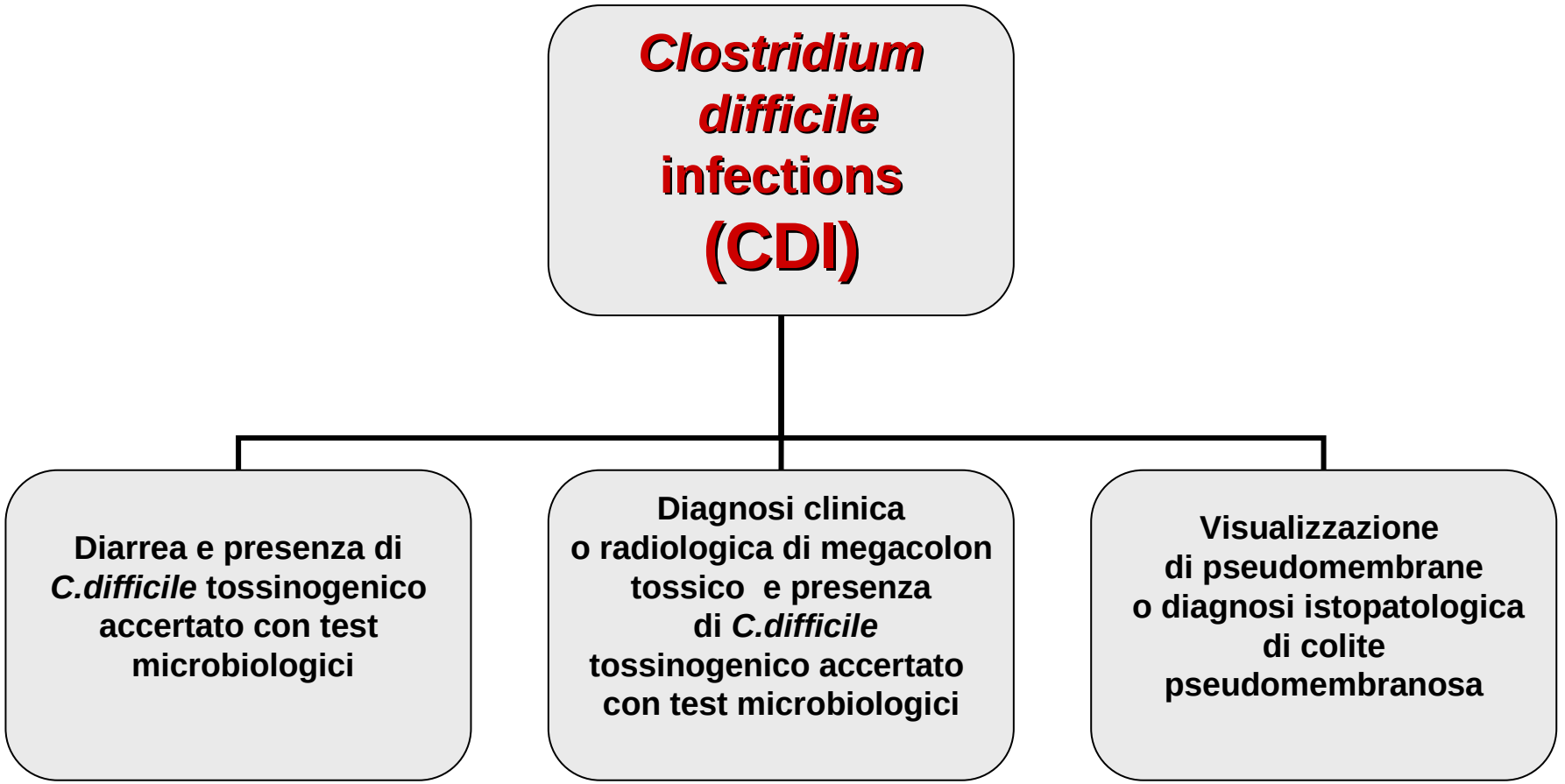
BIOFIRE™ DIAGNOSTICS, INC.

Quanto è difficile questo *Clostridium*?

Clostridium difficile



C. difficile vegetative cells produce toxins A and B and hydrolytic enzymes (**1**). Local production of toxins A and B leads to production of tumour necrosis factor-alpha and proinflammatory interleukins, increased vascular permeability, neutrophil and monocyte recruitment (**2**), opening of epithelial cell junctions (**3**) and epithelial cell apoptosis (**4**). Local production of hydrolytic enzymes leads to connective tissue degradation, leading to colitis, pseudomembrane formation (**5**) and watery diarrhea.


Clostridium difficile infections (CDI)
Diarrea e presenza di *C.difficile* tossinogenico accertato con test microbiologici
Diagnosi clinica o radiologica di megacolon tossico e presenza di *C.difficile* tossinogenico accertato con test microbiologici
Visualizzazione di pseudomembrane o diagnosi istopatologica di colite pseudomembranosa

2001-2003: Quebec >22casi/1000 ricoveri; letalità 6.9%

STATI UNITI

N° diagnosi di CDI nel 2000 134.36

N° diagnosi di CDI nel 2005 **291.303**

INGHILTERRA

N° esami positivi per la tossina di C.difficile <2000 nel 1986/1987

N° esami positivi per la tossina di C.difficile **>12.000 nel 2000/2001**

Cohen, et al. *Clinical Practice Guidelines for Clostridium difficile Infection in Adults: 2010 Update by SHEA and IDSA*
Infection Control and Hospital Epidemiology , Vol. 31, No. 5 (May 2010), pp. 431-455

Risk factors - Antibiotic therapy.

•More than 90% of health-care-associated *C difficile* infections occur after or during antimicrobial therapy.

•Almost **all antimicrobial agents** have been associated with *C.difficile Associated Disease* (CDAD).

•**Broad-spectrum antimicrobial agents**, which have a greater effect on the normal intestinal flora, are more likely to lead to CDAD.

•However, several later studies found fluoroquinolones to be more strongly linked to CDAD than any other antimicrobial agents, including clindamycin and beta-lactam/beta-lactamase.

•The risk is also greater when patients receive multiple antimicrobial agents and undergo a longer course of therapy.

Suneshine, Mc Donald, *Cleveland Clinic Journal of Medicine 73,2 187-197,2006*

Other risk factors (cited in at least three studies) are:

- Age greater than 65 years
- Severe underlying illness
- Nasogastric intubation
- Antiulcer medications.
- Longer hospital stay.

Suneshine, Mc Donald, *Cleveland Clinic Journal of Medicine 73,2 187-197,2006*

A recently identified strain of *C. difficile*, designated **N**orth **A**merican **p**ulsed-field gel electrophoresis type **1** (**NAP 1**) and PCR ribotype 027 (**Type 027**) has caused numerous outbreaks of clinically severe disease in North America and Europe.

NAP 1 produces **16 times more** toxin A and **23 times more** toxin B than other strains, possibly due to a deletion in a negative regulatory gene. In addition, NAP 1 **produces a third toxin**, known as binary toxin, although its significance is unknown.

McDonald LC et al. *N Engl J Med.* 2005;353:2433-2441

First Report of Hypervirulent Strains Polymerase Chain Reaction Ribotypes 027 and 078 Causing Severe *Clostridium difficile* Infection in Italy

Rossella Baldan,[1,a] Paolo Cavallerio,[3,a] Antonella Tuscano,[1] Caterina Parlato,[3] Lucina Fossati,[3] Matteo Moro,[2] Roberto Serra,[3,b] and Daniela M. Cirillo[1,b]

[1]Emerging Bacterial Pathogens Unit and [2]Chief Medical Office, San Raffaele Scientific Institute, Milan, and [3]Microbiology Laboratory, Azienda Ospedaliero–Universitaria San Giovanni Battista, Turin, Italy

Clinical Infectious Diseases 2010; 50:125–6

Soggetti adulti asintomatici:

C.difficile si riscontra nel 3-4% dei casi, circa lo 0,6% produce tossine

Soggetti ospedalizzati:

C.difficile si riscontra nel 7-25% dei casi, circa lo 2-8% produce tossine

n.b: popolazioni a rischio emergenti bambini da **2 a 12 anni e puerpere**. Al di sotto di 1 anno stato di portatore asintomatico per immaturità recettoriale.

Cohen, et al. *Clinical Practice Guidelines for Clostridium difficile Infection in Adults: 2010 Update by SHEA and IDSA*
Infection Control and Hospital Epidemiology , Vol. 31, No. 5 (May 2010), pp. 431-455

CDI: RECURRENCE OR RELAPSE?

Recurrence is one of the most frustrating and challenging complications of CDAD

Be it a reinfection or relapse, 12% to 24% of patient develope a second episode of CDAD within 2 months of the initial diagnosis.

Suneshine, Mc Donald, *Cleveland Clinic Journal of Medicine 73,2 187-197,2006*

C. difficile: impatto economico

Table 4. Cost of antibiotic therapy for *C. difficile* infection

	Cost per dose	Regimen	Cost per 10-day regimen
Metronidazole 500 mg	$0.73	500 mg three times a day	$22.00
Vancomycin 125 mg pills	$17.00	125 mg four times a day	$680.00
Vancomycin 125 mg IV compounded for oral	$2.50–$10.00	125 mg four times a day	$100.00–$400.00
Fidaxomicin 200 mg	$140.00	200 mg twice a day	$2,800.00

IV, intravenous.
Vancomycin IV form can be compounded for oral use as well as used for enema therapy.

Am J Gastroenterol advance online publication, 26 February 2013; doi:10.1038/ajg.2013.4

Since 2006, the **European Centre for Disease Prevention and Control (ECDC)** has been addressing the new CDI situation.

The working group recently published a systematic review of infection control measures to **limit the spread of *C. difficile*** that can be used for the elaboration of evidence-based guidelines in Member States.

Vonberg RP et al. Infection control measures to limit the spread of Clostridium difficile. Clin Microbiol Infect. 2008;14 Suppl 5:2-20.

The paper underlines the **specific difficulties to prevent *C.difficile* transmission** linked to the capacity of *C. difficile* to form spores that survive for months in the environment, may be excreted in large numbers by affected patients, cannot be destroyed by standard alcohol-based hand disinfection and persist despite usual environmental cleaning agents.

Vonberg RP et al. Infection control measures to limit the spread of Clostridium difficile. Clin Microbiol Infect. 2008;14 Suppl 5:2-20.

Reservoir e veicoli di spore di *CD*

CATEGORIE PER L'IMPLEMENTAZIONE NELLA PRATICA CLINICA: Protocollo mnemonico *SIDIT*

S SOSPETTO: in assenza di altra potenziale causa di diarrea, sospettare l'origine infettiva

I ISOLAMENTO: ove possibile isolare il paziente e consultare il CIO

D DPI: usare guanti e sovracamici monouso per tutti i contatti del paziente

I IGIENE delle mani: dopo ogni manovra sul paziente lavaggio con acqua/sapone antisettico

T TEST: eseguire tempestivamente il test per la ricerca di *C.difficile* tossinogenico

DH Department of Health

Update gudance on the diagnosis and reporting of Clostridium difficile DH-NHS March 2012, modificata

AMERICAN SOCIETY FOR MICROBIOLOGY

A practical guidance document for
***Clostridium difficile* toxin laboratory testing**
August 24, 2010

The laboratory diagnosis of *C.difficile* remains a challenge to many microbiology laboratories.

Diagnosi di CDI: protocolli diagnostici

Table 1. Summary and strength of recommendations

Diagnostic tests
1. Only stools from patients with diarrhea should be tested for *Clostridium difficile*. (Strong recommendation, high-quality evidence)
2. Nucleic acid amplification tests (NAAT) for *C. difficile* toxin genes such as PCR are superior to toxins A+B EIA testing as a standard diagnostic test for CDI. (Strong recommendation, moderate-quality evidence)
3. Glutamate dehydrogenase (GDH) screening tests for *C difficile* can be used in two- or three-step screening algorithms with subsequent toxin A and B EIA testing, but the sensitivity of such strategies is lower than NAATs. (Strong recommendation, moderate-quality evidence)
4. Repeat testing should be discouraged. (Strong recommendation, moderate-quality evidence)
5. Testing for cure should not be done. (Strong recommendation, moderate-quality evidence)

Am J Gastroenterol advance online publication, 26 February 2013; doi:10.1038/ajg.2013.4

Diagnosi di CDI: protocolli diagnostici

TABLE 1. Laboratory Tests for *Clostridium difficile* Infection

Test	Substance detected	Time required	Sensitivity,[a] %	Specificity,[a] %	Concerns and comments
Cytotoxin	Toxin B	1–3 days	95	90–95	Long time required and technical demands; rarely used now
Toxin culture	Toxigenic *C. difficile*	3–5 days	>95	80–90	Long time required and technical demands; rarely used in US
EIA toxin A or A/B	Toxin A or A/B	Hours	75–80	97–98	False-negative results, but rapid and not technically difficult; used by most US laboratories in 2008
EIA GDH	*C. difficile*	Hours	95–100	70–80	Some variations in reported sensitivity and nonspecificity
EIA GDH and toxin A/B	*C. difficile* and *C. difficile* toxin	Hours	95–100	97–98	Results depend on the toxin test
RT-PCR	Toxigenic *C. difficile*	Hours	>98	80–99	Nonspecificity attributed to carriers

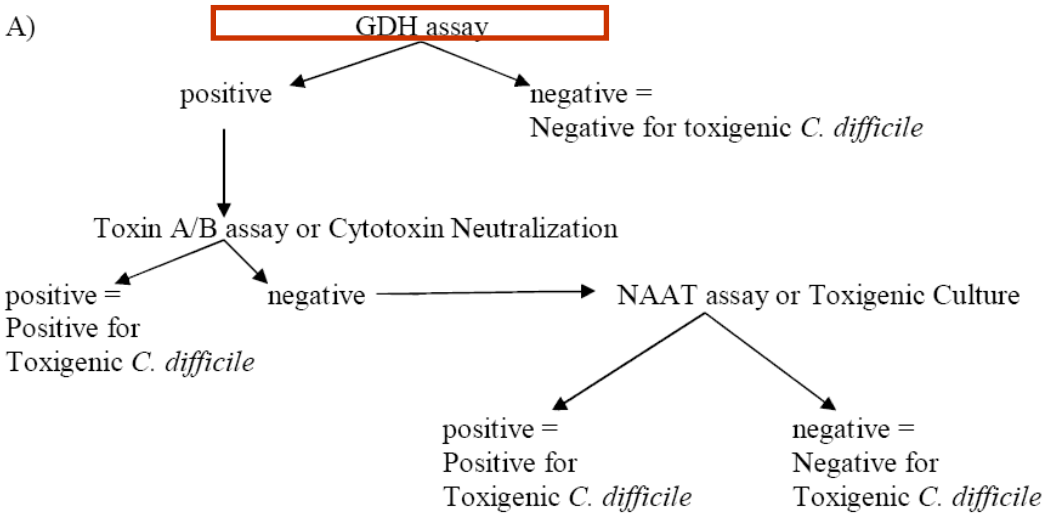
NOTE. EIA, enzyme immunoassay; GDH, glutamine dehyrogenase; RT-PCR, reverse-transcriptase polymerase chain reaction.

[a] Reported data for sensitivity and specificity are based on multiple reports. It is assumed that 20%-30% of hospitalized patients are colonized with *C. difficile* and that 50% of strains are toxigenic. Clinical correlations are always important but are especially important with RT-PCR and EIA tests for GDH.

A significant number of clinical laboratories are currently utilizing **EIA for toxin A/B** testing due to its ease of use. However, there is a wealth of information now available indicating that utilizing this **test alone is not appropriate** for toxigenic *C.difficile* detection.

Bartlett Detection of *Clostridium difficile* Infection Iinfect control and hospital epidemiology 2010, vol. 31, no. s1

A)


GDH assay
positive
negative =
Negative for toxigenic C. difficile
Toxin A/B assay or Cytotoxin Neutralization
positive =
Positive for
Toxigenic C. difficile
negative
NAAT assay or Toxigenic Culture
positive =
Positive for
Toxigenic C. difficile
negative =
Negative for
Toxigenic C. difficile

Diagnosi di CDI: nuove tecnologie a confronto

- *GDH-TOXIN A/B combination*
- *NAATs as stand alone*

GDH-TOXIN A/B combination versus *NAATs:* *CARATTERISTICHE A CONFRONTO*

GDH-TOXIN A/B combination	*NAATs*
1. Test immunocromatografico *on demand* e *all in one* (GDH+TOX)	1. Test molecolare *on demand* e *all in one*
2. Non richiede strumenti dedicati	2. Richiede strumento dedicato
3. Tempi tecnici di esecuzione: circa 30 minuti	3. Tempi tecnici di esecuzione: circa 45 minuti
4. Costo inferiore ai NAATs, ma **necessità di disporre comunque della PCR**	4. Costo elevato, ma coerente con *performance* del test
5. I risultati dubbi (GDH+/TOX-) vanno confermati con PCR	**5. Stand alone test**: il risultato non richiede alcuna ulteriore conferma
6. Necessità di rigorosa selezione del campione per ridurre i costi ed evitare ripetizioni dovute a risultati dissociati	6. Necessità di rigorosa selezione del campione per appropriatezza diagnostica e contenimento dei costi
7. Non Riconosce i ceppi ad alta virulenza e la produzione di tossina binaria (ribotipo 027)	**7. Possibilità di riconoscere i ceppi ad alta virulenza e la produzione di tossina binaria (ribotipo 027)**
8. La conservazione del campione può influire sul risultato delle tossine (per degradazione)	8. Risultato **non influenzato dalla degradazione del campione (tossine)**

Guidelines for Diagnosis, Treatment, and Prevention of *Clostridium difficile* Infections

Table 2. Diagnostic testing for *C. difficile*

Test	Sensitivity	Specificity	Availability	Expensea	Utilization
C. difficile culture	Low	Moderate	Limited	$5–10	No diagnostic use; only toxigenic organisms cause disease
Toxigenic culture	High	High	Limited	$10–30	Reference method Epidemiologic tool Limited diagnostic use
CCNA	High	High	Limited	$15–25	Reference method Limited diagnostic use
GDH	High	Low	Widely	$5–15	Diagnostically as a screening test; must be confirmed
Toxin EIA tests	Low	High	Widely	$5–15	Must detect toxins A+B; inferior sensitivity
NAATs	High	High	Widely	$20–50	Use only in acute disease; false positives of concern

CCNA, *C. difficile* cytotoxin neutralization assay; GDH, glutamate dehydrogenase; EIA, enzyme immunoassay; NAAT, nucleic acid amplification tests.
[a]Cost of goods; does not reflect laboratory changes.

Am J Gastroenterol advance online publication, 26 February 2013; doi:10.1038/ajg.2013.4

COST SAVINGS

- REDUCED ANTIBIOTIC USE
- IMPROVED CLINICAL OUTCOME
- SHORTER HOSPITALIZATION
- USE FEWER OF OTHER DIAGNOSTIC TEST
- PUBLIC BENEFIT (NOT JUST COST)

CID 2010; 52 (Suppl.4): S384-S395

CONTESTUALIZZAZARE LE SCELTE

- Quanto costerebbe l'algoritmo ***GDH-TOXIN A/B + eventuale molecolare?*** Quanto costerebbe la ***PCR stand alone***?

- Dove mandare il campione per il test di conferma sui risultati discrepanti?e i tempi di esecuzione?

- Possibilità di eseguire il test in automazione (se il numero di campioni è notevole)

LAVORARE SULL'APPROPRIATEZZA

- Solo feci formate*
- Nessun controllo dopo precedente positività**
- Scoraggiare ripetizione di un test negativo***

GImPIOS, *vol 1,n° 2 2011*

*In caso di sospetta CDI e in assenza di diarrea la diagnosi si basa essenzialmente su criteri clinici e/o radiologici
**anche in caso di recidive dopo trattamento, ed escludere sempre altre cause di diarrea (counseling)
***In relazione alla sensibilità del metodo utilizzato.

Health Protection Agency

DH Department of Health

Appendix 2:
The Bristol Stool Form Scale (Bristol Stool Chart)

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges (passed easily)
Type 6		Fluffy pieces, a mushy stool
Type 7		Watery, no solid pieces ENTIRELY LIQUID

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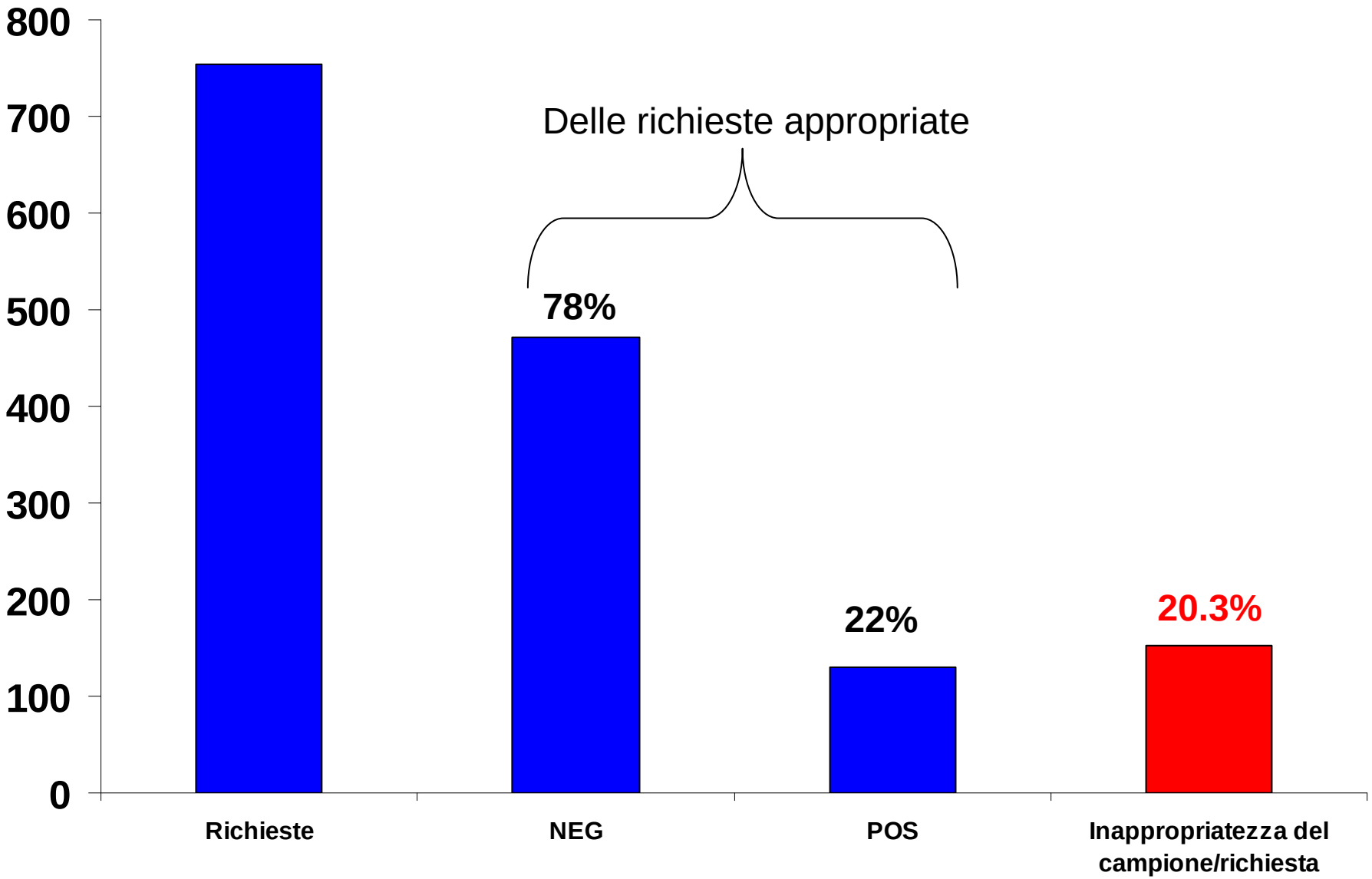
PORDENONE 2012



Appropriateness and costs

Today everyone is used to “cheap microbiology” - every patient can get every test.

In the future, expensive diagnostics will be offered - not every patient can get every test.

Clinical Microbiology Services play a role

Table 1. Early diagnosis

	Recommendations
1	Promptly perform tests for *Clostridium difficile* toxins (± the bacterium) in stool specimens in each case of nosocomial diarrhoea and for individuals who are ad tted with diarrhoea acquired outside the hospital. S ated testing of diarrhoeal stool samples as soon a s been diagnosed. Only when a recur spected, repeat the *C. difficile* testing tial causes of diarrhoea.
2	Perform tests for *C. dif* l (unform t. Testi p
3	Do not r
4	Faeca ily patients ssion to intensive cases), or (b) in an so that typing can be ctively.
	CL d diarrhoea.

TAKE HOME MESSAGES

- **Promptly perform Tests**
- **EIA for toxin A/B** ***NOT STAND ALONE***
- ***Stop* repeated testing**
- ***Not formed* stools**
- **Not *test of cure* after treatment**

Vonberg RP et al. Infection control measures to limit the spread of Clostridium difficile. Clin Microbiol Infect. 2008;14 Suppl 5:2-20.

IMPROVE
PATIENT
MANAGEMENT

RAPID
REPORTING

CLINICALLY
RELEVANT
REPORTING

CONTROL
C.DIFFICILE
SPREADS

3° CONGRESSO NEWMICRO

….The need for speed: il laboratorio di Microbiologia e le Urgenze Infettive

NEWMICRO
Network di Microbiologia e Virologia del Nord Est

GRAZIE

Manuela Avolio
Microbiologia Clinica e Virologia
AOSMA Pordenone