CAT
Critically Appraised Topic

Titel: Screening for toxin-producing *Clostridium difficile* in neonates and infants

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CLINICAL BOTTOM LINE

Clostridium difficile is the most common cause of antimicrobial-associated diarrhea and is a common health care-associated pathogen. Clinical symptoms vary widely, from asymptomatic colonization to pseudomembranous colitis with bloody diarrhea, fever and severe abdominal pain. The diagnosis of *Clostridium Difficile* Associated Diarrhoea (CDAD) requires the detection of *C. difficile* toxins (CDT) in diarrhoeal stool specimens. The only exception is pseudomembranous colitis, which can be confirmed by endoscopy or histopathology Published guidelines for managing Clostridium difficile infection (CDI) in adults affirm that there are gaps in the knowledge surrounding CDIs in infants and children. While the incidence of CDI in children has been Increasing (just like adults), the severity of cases has not increased. In sharp contrast to adult data, no significant positive trends in mortality, rate of colectomy or hospital days have been reported. Most studies have failed to show any relationship between *C.difficile* and common forms of diarrheal illness in neonates and infants. Testing for *C. difficile* should be performed only for children who meet the criteria for clinically significant diarrhea and that test results for infants less 1 year of age can be difficult to interpret due to high rates of asymptomatic colonization. Test of cure is not recommended.

CLINICAL/DIAGNOSTIC SCENARIO

Introduction

Clostridium difficile is a spore-forming, obligate anaerobic, Gram-positive bacillus and is acquired from the environment or by the fecal-oral route. C difficile is the most common cause of antimicrobial-associated diarrhea and is a common health care-associated pathogen. Clinical symptoms vary widely, from asymptomatic colonization to pseudomembranous colitis with bloody diarrhea, fever, and severe abdominal pain (Berrington et al., 2004; Policy Statement AAP, 2013). *Clostridium difficile* infection is defined as the acute onset of diarrhea with documented toxigenic *C. difficile* or its toxin and no other documented cause for diarrhea (Cohen et al., 2010; Surawicz et al., 2013)
Toxins A and B are responsible for intestinal disease. Toxin A is an enterotoxin (active in intestinal loop models, as well as in tissue culture), whereas toxin B is a cytotoxin (active only in tissue culture). The toxins bind to proposed receptors on intestinal epithelial cells, leading to cell death, inflammation, and diarrhea . Toxin B appears to be the more critical of the two toxins, since occasional strains that lack toxin A can cause disease similar in severity to strains having both toxins.
A hypervirulent strain of *C. difficile* (BI/NAP1/027) has been responsible for outbreaks worldwide (Duleba et al., 2014). This strain produces increased amounts of toxins A and B, possesses a third toxin called binary toxin, and is largely quinolone resistant (McDonald et al., 2005; CDC 2005-2008; Cooperstock, 2015). Current age-specific epidemiplogy of CDI

among children poorly sudied. Reports suggest the prevalence of BI/NAP1/027 between 10%-38% in pediatric CDI population (Sun et al,. 2008; Toltzis et al., 2009; Zilberberg et al., 2010).

Laboratory diagnosis of CDAD (*Clostridium Difficile* Associated Diarrhoea)

The laboratory diagnosis of *Clostridium difficile* disease has evolved significantly over the last several years, and many tests that may be used to assist with the detection of *C. difficile* infection are now available. These assays include enzyme immunoassays (EIAs), PCR assays, tissue culture cytotoxicity neutralization tests, and toxigenic culture (Sharp et al., 2010). However, there is a lack of validation of these tests in the population of young children (Bryantet al., 2009).

Toxin detection

The diagnosis of CDAD requires the detection of *C. difficile* toxins in diarrhoeal stool specimens. The only exception is pseudomembranous colitis, which can be confirmed by endoscopy or histopathology (Berrington et al., 2004).
Toxin detection is achieved by different methods: the neutralised cell cytotoxicity assay, or an immunoassay that detects both toxin A and toxin B. The cell cytotoxicity assay (CCCA) is the only assay to detect toxin by means of its biological properties, and remains the standard by which other tests are judged, but positive results must be confirmed by neutralisation with antitoxin to ensure adequate specificity (Berrington et al., 2004). Commercial immunoassays are available in several formats and from several manufacturers. Mean test sensitivities range from 60% to 82%, with mean specificities of 97% to 98%, compared with the CCCA (Crobach et al., 2009; Sharp et al., 2010; Policy Statement AAP, 2013). Some investigators have found that the membrane type assays are inferior to the more traditional microwell EIAs,(O'Connor et al., 2001) others the reverse (Vanpoucke et al., 2001). The principle advantage of the membrane immunochromatography assays is speed, since results can usually be obtained within an hour (Berrington et al., 2004).

Glutamate dehydrogenase (GDH) antigen assays

GDH is a metabolic enzyme encoded by *gluD*. This antigen is produced at high levels in all isolates of *C. difficile,* including both toxigenic and nontoxigenic strains. GDH represents a screening test for *C. difficile,* and positive assays must be followed up with a confirmatory test, such as a toxin EIA or a molecular test for detection of toxin genes. Overall, the studies performed to date suggest that GDH exhibits high sensitivity ans specificity (Table 1) as a screening test for *C. difficile.* Similar to toxin EIAs, GDH assays are available in microwell EIA and lateral flow immunochromatographic formats (Burnham et al., 2013).

Combination testing—GDH detection and toxin EIA

Some of the studies evaluating these combination assays are reviewed by Burnham et al., 2013 (Table 2).
These combination assays are relatively rapid, and the cost per test is less than that of the molecular methods. The GDH component of these assays appears to have a sensitivity similar to those of stand-alone GDH assays, but the toxin EIA component appears to perform with

low sensitivity (Chapin et al., 2011; Novak-Weekley et al.,2010, Sharp et al.,2010, Selvaraju et al.,2011; Reviewed by Burnham et al., 2013) .

In general, samples that are GDH and toxin negative by these assays can be reported as negative with relatively high confidence, and GDH- and toxin-positive samples can be reported as positive.

Samples that are GDH positive but toxin negative should have confirmatory testing performed (such as CCCNA or a molecular assay) to rule out *C. difficile* disease (by Burnham et al., 2013).

The C. DIFF QUIK CHEK COMPLETE® (Techlab, inc, USA)test is currently used in medical laboratory of UZ Gasthuisberg for the detection of CDI. This is a rapid membrane enzyme immunoassay for the simultaneous detection of *Clostridium difficile* glutamate dehydrogenase antigen and toxins A and B in a single reaction well. The test detects *C. difficile* antigen, glutamate dehydrogenase, as a screen for the presence of *C. difficile* and confirms the presence of toxigenic *C. difficile* by detecting toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease.

C. Diff Quik Chek Complete assay evaluated by Sharp et al., (2010) for its ability to diagnose *C. difficile* disease. The results of this assay were compared to those of both PCR and toxigenic culture. The results showed that this assay allows 88% of specimens to be accurately screened as either positive (both tests positive) or negative (both tests negative) for the presence of toxigenic *C. difficile* in less than 30 min and with minimal hands-on time. Use of a random-access PCR for the analysis of specimens with discrepant results (one test positive and the other negative) allows the easy, rapid, and highly sensitive (100%; 95% confidence interval [CI], 89.6 to 100%) and specific (99.6%; 95% CI, 97.3 to 99.9%) diagnosis of *C. difficile* disease (Table 1). (Sharp et al.,2010).

This Algorithm is currently used in medical laboratory of UZ Gasthuisberg, however, do not differentiate between active infection and asymptomatic carriage.

Table 1: Sensitivity, specificity, hands-on time, material costs, and reimbursements for *C. difficile* assays (Sharp et al., 2010).

Assay	% sensitivity (95% CI[a])	% specificity (95% CI)	Hands-on time (min)	Turnaround time (min)	Material cost ($)/test	Medicare/Medicaid reimbursement ($)
PCR[b]	100 (89.6-100)	99.6 (97.3-99.9)	5[c]	60[c]	33.38	50.27
GDH	100 (89.6-100)	94.2 (90.3-96.7)	18[d]	70[d]	7.35	17.18
LF-TOX	59.5 (43.3-74.0)	99.2 (96.7-99.9)	9[c]	34[c]	5.50	17.18
C.Diff Complete	60.0 (43.4-74.7)	99.6 (97.3-99.9)	9[c]	34[c]	11.50	$34.36
Algorithm[e]	100 (89.6-100)	99.6 (97.3-99.9)	14[c]	94[c]	11.50, 44.88[f]	34.36, 50.27[f,g]

[a]CI, confidence interval.
[b]The FDA-cleared sensitivity of the Xpert *C. difficile* PCR assay is 93.5% compared to the results of broth-enriched toxigenic culture for *C. difficile*. The 100% sensitivity used in this table is based on our results and reasonable assumptions that were made, as discussed in the Materials and Methods and the Discussion sections.
[c]Hands-on time and turnaround time are based on testing of a single specimen.
[d]Hands-on time and turnaround time are based on batch testing of 20 specimens.
[e]The algorithm comprised the C.Diff Quik Chek Complete assay and testing of samples with discrepant results by PCR.
[f]Data are for 88%, 12% of specimens.
[g]Reimbursement for samples with discrepant results by the C.Diff Quik Chek Complete assay that are then tested by PCR consists of only that for the PCR component.

Table 2: Performance characteristics of EIAs combining GDH detection and toxin EIA for *C. difficile* detection (Reviewed by Burnham et al., 2013

Assay	Format	Gold standard	No. of samples	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Reference
TechLab C. Diff Quik Check Complete	Lateral flow EIA	Detection of *tcdB* by ≥2 molecular methods	81	61.5	100	100	84.6	*Chapin et al., 2011*
TechLab C. Diff Quik Chek Complete	Lateral flow EIA	Toxigenic culture	200 (all from pediatric patients)	70.8	97.4	89.5	91.4	Selvaraju et al.,2011
TechLab C. Diff Quik Chek Complete	Lateral flow EIA	Aggregate of EIA and toxigenic culture	284	60.0	99.6	Not given	Not given	Sharp etal.2010
C. Diff Quik Chek plus Meridian Premier Toxins A & B	Lateral flow EIA/microwell EIA	Detection of *tcdB* by ≥2 molecular methods	81	42.3	100	100	78.6	*Chapin et al., 2011*
C. Diff Chek-60 with Meridian Premier Toxins A & B	Both are microwell EIAs	Toxigenic culture	432	55.6	98.3	87	91.7	Novak-Weekley et al.,2010

Molecular platforms for direct detection of *Clostridium difficile* in clinical specimens

Currently available moleculair assays for the detection of *C. difficile* in fecal samples reviewed in Table 3 (Burnham et al., 2013). Cepheid GeneXpert Assay (Cepheid Inc., Sunnyvale, CA) is currently used in medical laboratory of UZ Gasthuisberg for confirmatory testing in samples that are GDH positive but toxin negative. Cepheid GeneXpert Assay detects *tcdB, cdtA, tcdC* nucleotide 117 deletion by using Multiplex qPCR. Moreover, Detection of not only *tcdB* but also the binary toxin genes and the deletion at nucleotide 117 on *tcdC* (_117) as surrogate markers for presumptive identification of 027/NAP1/BI strains are unique features of the Xpert *C. difficile* Epi assay (Cepheid Inc., Sunnyvale, CA). However, these assays do not solve the diagnostic uncertainty surrounding *C. difficile*, as detection of *C. difficile* in a fecal specimen does not automatically imply disease Burnham et al., 2013. *C. difficile*-associated disease is a clinical diagnosis supported by laboratory

findings; this diagnosis continues to be a challenge for clinicians and laboratories alike (Burnham et al., 2013).

Table 3: Published performance characteristics of currently available NAATs[c] (*Reviewed* by Burnham et al., 2013)

Assay	Sensitivity (%) (range)	Specificity (%) (range)	NPV (%) (range)	PPV (%) (range)
BD GeneOhm	82.1–100	90.6–99.2	96.7.0–100	58.6–94.4
BD Max Cdiff	97.7	99.7	99.7	97.7
Prodesse ProGastro Cd	77.3–100	93.4–99.2	95.9–100	82.8–94.4
Cepheid GeneXpert	94.4–100	93.0–99.2	99.3–100	78.9–94.7
Meridian Illumigene	86.7–98.1	98–100	98.1–99.5	91.8–98.5
Focus Technologies Simplexa[b]	90.1/79.6[a]	93/95.8[a]	NA	NA
Great Basin Portrait[b]	79.6–90.1	93.0–95.8	95.3–98.4	66–81.4
Quidel AmpliVue *C. difficile* Assay	93.6	94.1	NA	NA
Nanosphere Verigene	98.7/91.8[a]	87.6/92.5[a]	99.9/98.5[a]	42.1/67.3[a]

[a]The first result represents comparison to direct toxigenic culture, and the second represents comparison to enriched toxigenic culture.
[b]No publications to date on the FDA platform; data are from the package insert.

Adjunctive tests Inflammatory biomarkers

PCR detection are highly sensitive for *C. difficile* detection, but do not differentiate between active infection and asymptomatic carriage. Inflammatory biomarkers of fecal specimens may be an area to be pursued in future studies with respect to assessing the severity of infection and potentially to resolve whether a detection of toxogenic *C. difficile* is associated with disease or colonization and to monitor a patient's response to treatment (Burbham et al., 2013)

Cytokine analysis
There are no commercial assays available for measuring cytokine levels in fecal specimens. Some investigators have shown the correlation of elevated levels of IL-1_ and IL-8 in active disease, particularly in cases of moderate to severe infection (Steiner et al., 1997; Enocksson et al., 2004; Burbham et al., 2013), with subsequent decreases in levels of these cytokines in stool when the patient recovers from acute infection (Enocksson et al., 2004; Burbham et al., 2013).

Fecal lactoferrin
Lactoferrin is an iron binding glycoprotein found in neutrophils and in secretions such as breast milk, and therefore, this marker may be present in feces of children who are breast fed, reducing its utility as an enticing marker for bacterial
enteritis in children 2 years of age (Ashraf et al., 2007; Burbham et al., 2013). This protein is released following neutrophil activation, and the concentrations in stool and other fluids are proportional to the number of neutrophils recruited (Sherwood RA. 2012; Burbham et al., 2013).

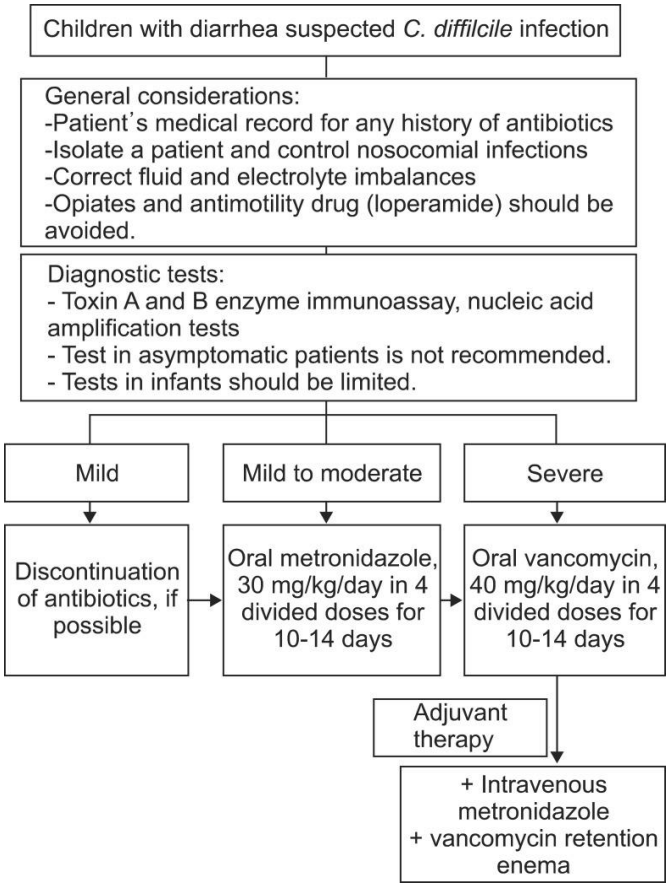
Fecal calprotectin
Calprotectin is a calcium binding protein found within the cytosol of neutrophils, where it accounts for
approximately 60% of their cytoplasmic protein content (Sherwood, 2012). Under inflammatory conditions of the intestinal tract where neutrophils accumulate, calprotectin is excreted in stool and is resistant to bacterial degradation (Sherwood, 2012).

In summary, both fecal lactoferrin and calprotectin are nonspecific markers of intestinal inflammation, and while studies demonstrate that levels of these markers may be significantly elevated in patients with *C. difficile* disease, the sensitivity is too low in most studies to recommend their routine use for screening of patients (Burbham et al., 2013).

Treatment

In 2010, the Society for Healthcare Epidemiology of America and the Infectious Diseases Society of America published clinical guidelines for CDI in adults (Cohen et al., 2009). They recommended metronidazole in mild to moderate disease, and vancomycin as a first-line therapy in severe disease. However, there are sparse data and guidelines for treatment of pediatric CDI (Shim, 2014). The American Association of Pediatrics recommends discontinuation of antibiotics as the first step in treating CDI, which may suffice in most instances (Policy Statement AAP, 2013).
Algorithm for CDI in children is suggested in Fig. 1.
Newer antibiotics such as fidaxomicin appear promising especially for the treatment of recurrent infection.
In recurrent or severe CDI, fecal microbiota transplantation might be effective. Donor-acquired feces are implanted into the gastrointestinal tract of the patient via a nasoduodenal catheters, retention enema, duodenoscopy or colonoscopy. Van Nood et al., (2013) reported 81% resolution of recurrent CDI after the first infusion of feces in a small group of adult patients. Normal bowel flora serve as a defense mechanism against pathogenic organisms and may result in the elimination of *C. difficile* spores (Shim et al., 2014).

Fig 1. Algorithm for CDI in children (Policy Statement AAP, 2013)

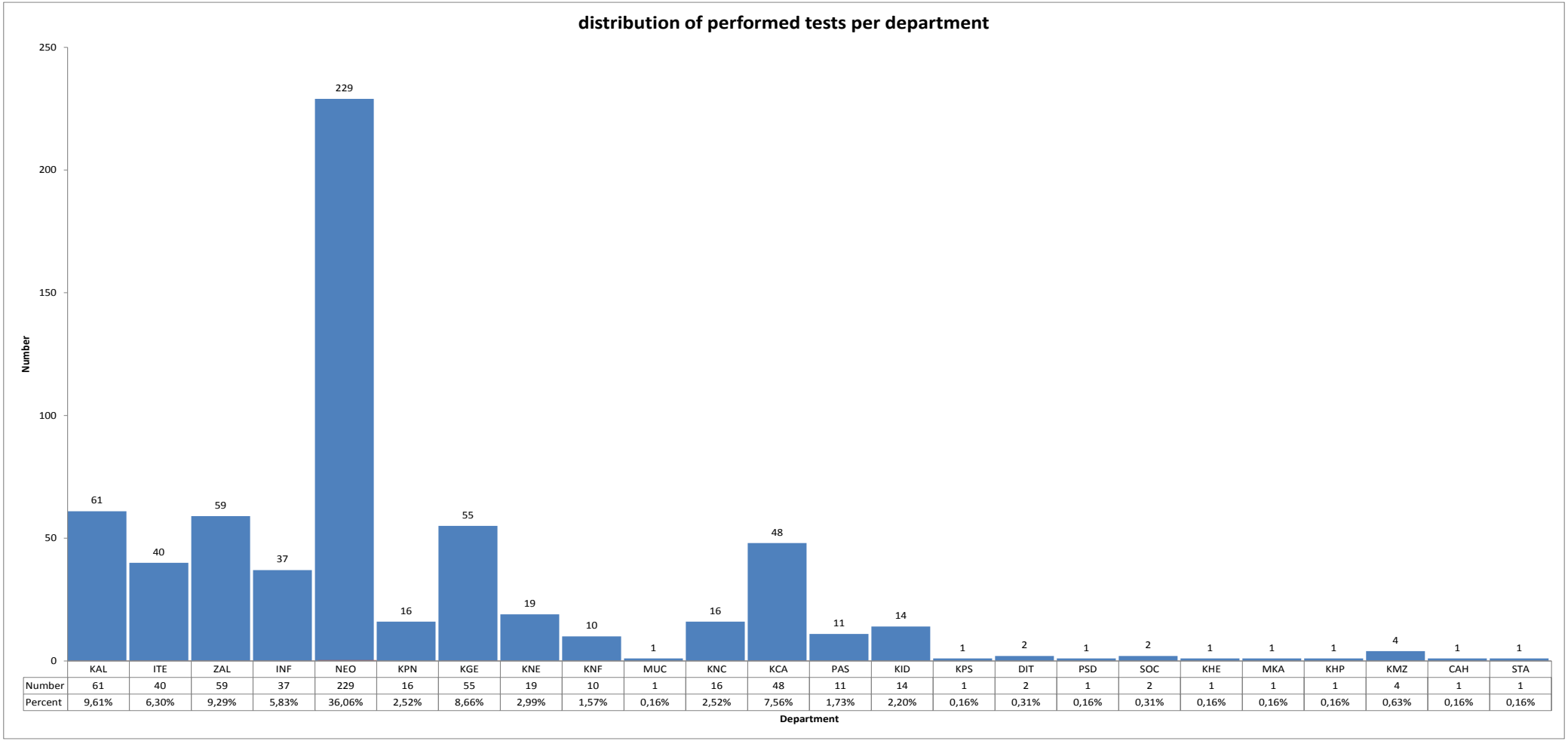


Situation in Gasthuisberg

LAG data

In UZ Gasthuisber all stool samples with a request for detection of *C. difficile* are tested, no selection criteria (such as age-related) are used. We also receive regular follow-up samples for ” test of cure”.
7901 tests for the detection of toxin-producing *C. difficile* were performed in LAG UZ Gasthuisberg during the observation perion between 01/10/2013 and 01/03/2015.
635 tests (8%) have been performped in the group of 342 neonates and infants (between 0-12 months).
Figure 2 illustrates the distribution of performed tests (in the group of neonates en infants) per medical department. The highest number of tests (229 tests of 36%) were performed in the patients from the neonatology department.

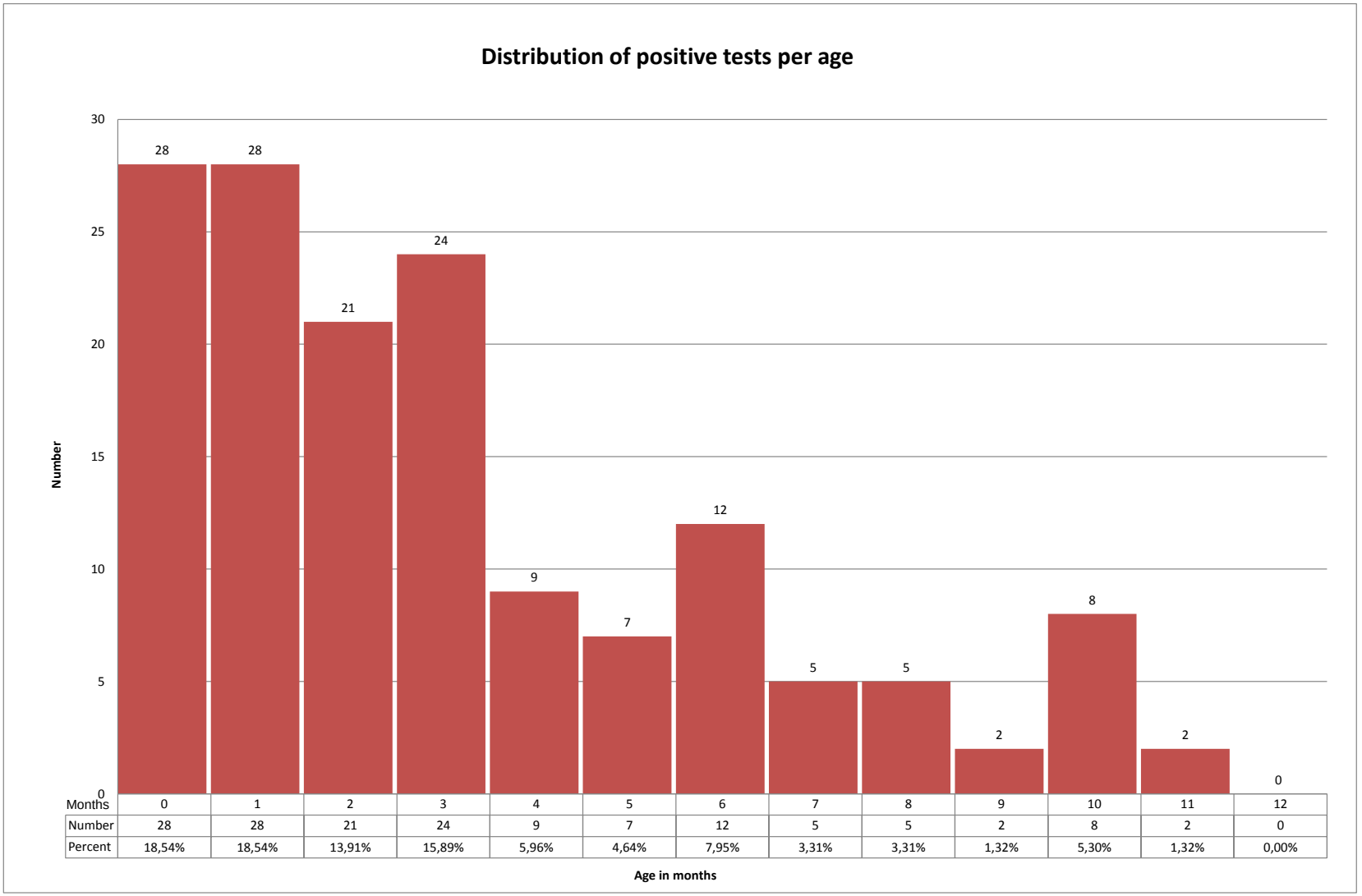
Toxin-producing *C. difficile* has been detected (at least 1 time) in 99 (29%) neonates and infants.
Figure 3 illustrates the distribution of positive tests per age. The highest rate of positive tests for toxin-producing *C. difficile* was revealed among neonates and infants between 1-3 months.
From total amount of 635 tests performed in group of neonates and infants (between 0-12 months) only 151 tests (23.8%) were positive for toxin-producing *C. difficile*.
The methods of toxin detection are distributed as follows: 93 (61.6%) by EIA and 58 (38.4%) by PCR.



	KAL	ITE	ZAL	INF	NEO	KPN	KGE	KNE	KNF	MUC	KNC	KCA	PAS	KID	KPS	DIT	PSD	SOC	KHE	MKA	KHP	KMZ	CAH	STA
Number	61	40	59	37	229	16	55	19	10	1	16	48	11	14	1	2	1	2	1	1	1	4	1	1
Percent	9,61%	6,30%	9,29%	5,83%	36,06%	2,52%	8,66%	2,99%	1,57%	0,16%	2,52%	7,56%	1,73%	2,20%	0,16%	0,31%	0,16%	0,31%	0,16%	0,16%	0,16%	0,63%	0,16%	0,16%

Figure 2

KAL: algemene kindergeneeskunde
ITE: intensieve geneeskunde
ZAL: zuigelingen algemeen
INF: infectieziekten
NEO: neonatologie
KPN: kinderpneumologie
KGE: kindergastro-enterologie
KNE: kinderneurologie
MUC: mucoviscidose
KNC: kinderoncologie
KCA: kindercardiologie
PAS: pasgeborenen
KID: kinder-immuundeficienties
KPS: kinderpsychiatrie
DIT: dietiste
PSD: pastorale dienst
SOD: sociaal werk
KHE: kinderhematologie
MKA: mond-kaak-aangezichtschirurgie
KHP: kinderhepatologie
KMZ: metabole ziekten
CAH: cardiale heelkunde
STA: staalname



Months	0	1	2	3	4	5	6	7	8	9	10	11	12
Number	28	28	21	24	9	7	12	5	5	2	8	2	0
Percent	18,54%	18,54%	13,91%	15,89%	5,96%	4,64%	7,95%	3,31%	3,31%	1,32%	5,30%	1,32%	0,00%

Figure 3

UZ Gasthuisberg Data: *C.difficile* positive Pediatric population
(Data kindly provided by Professor Dr. Veerle Cossey)

Cases, positive for toxin-producing *C. difficile: 61*
Neonates and infants<1 year: 43 (70.5%)
Exposure to antibiotics during 2 months preceding *C.difficile* positive test: 32 (52.5%)
Treatment: Metronidazole : 35
Vancomycine : 18

Retrospective analysis of clinical case records

Retrospective analysis of 9 clinical records of children with positive test for toxin-producing *C. difficile* has been performed to evaluate the symptoms, exposure to antibiotics, treatment and the clinical implications of follow-up samples (See Attachment 1 for Clinical data).
The following observations have been noticed:
Reason of screening for toxin producing *C. difficile* in children <1 year: loose or watery stools.
In some cases test for toxin-producing *C. difficile* has been performed in asymptomatic children (Patient 5 (Attachment 1) contact with *C. difficile* positive sister).
Regular follow-up tests have been requested for " test of cure". False positive "test of cure" may complicate clinical care and result in additional courses of inappropriate anti-*C. difficile* therapy.

QUESTION(S)

1) Usefulness of screening for toxin-producing *Clostridium difficile* in neonates and infants.

2) Usefulness of performing the follow-up "Test of Cure" for toxin-producing *Clostridium difficile*.

SEARCH TERMS

1) MeSH Database (PubMed): MeSH term: "Clostridium difficile, Clostridium Difficile Associated Diarrhoea (CDAD), C. difficile toxins (CDT) AND neonates and infants" PubMed Clinical Queries (from 1966; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi): Systematic Reviews; Clinical Queries using Research Methodology Filters (diagnosis + specific, diagnosis + sensitive, prognosis + specific)

2) Pubmed (Medline; from 1966), SUMSearch (http://sumsearch.uthscsa.edu/), National Guideline Clearinghouse (http://www.ngc.org/), Institute for Clinical Systems Improvement (http://www.icsi.org), The National Institute for Clinical Excellence (http://www.nice.org.uk/), Cochrane (http://www.update-software.com/cochrane, Health Technology Assessment Database (http://www.york.ac.uk/inst/crd/htahp.htm)

3) National Committee for Clinical Laboratory Standards (NCCLS; http://www.nccls.org/), International Federation of Clinical Chemistry (IFCC; http://www.ifcc.org/ifcc.asp), American Diabetes Association (ADA; http://www.diabetes.org/home.jsp), National Diabetes Information Clearinghouse (NDIC; http://diabetes.niddk.nih.gov/), Westgard QC (http://www.westgard.com), Clinical Laboratory Improvement Amendments (CLIA; http://www.cms.hhs.gov/clia/)

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RELEVANT EVIDENCE/REFERENCES

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APPRAISAL

1. Usefulness of the screening for toxin-producing *Clostridium difficile* in neonates and infants
    1.1. Literature review: Colonisation vs *C. difficile*-associated infection?

C. difficile-associated infection has been much less common in children than adults (Bryant et al., 2009). The intestine of the newborn infant is sterile, but by 12 months of age, an infant's intestine has flora similar to that of an adult (Jangi et al., 2010; Policy Statement AAP, 2013). C difficile carriage rates average 37% for infants 0 to 1 month of age and 30% between 1 and 6 months of age (Fig 4) (Jangi et al., 2010). At 6 to 12 months of age, approximately 14% of children are colonized with *C. difficile*, and by 3 years of age, the rate is similar to that of nonhospitalized adults (0% to 3%) (Jangi et al., 2010; Policy Statement AAP, 2013).

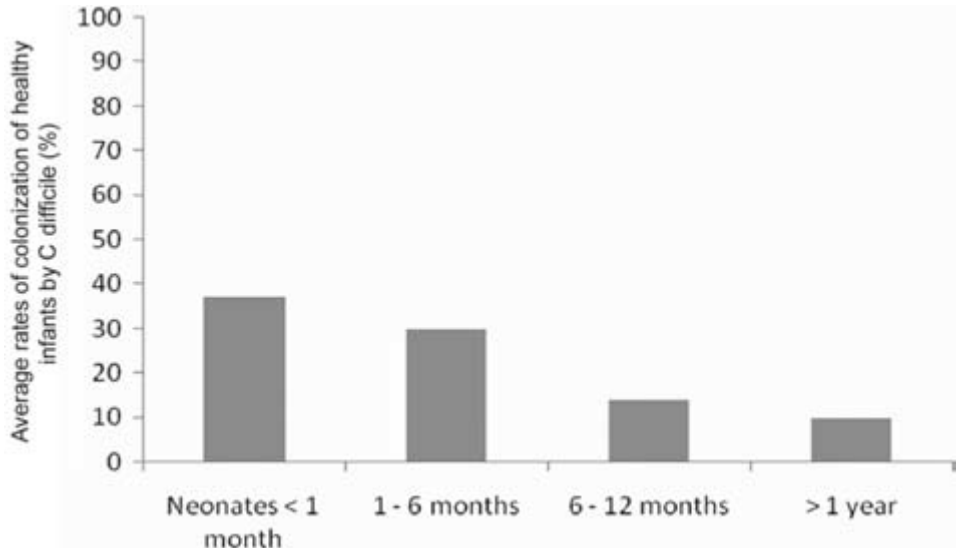

Fig. 4 *C. difficile* colonization stratified by age (Jangi et al., 2010).

While the incidence of CDI in children has been Increasing (just like adults), the severity of cases has not increased. In sharp contrast to adult data, United States Healthcare Cost and Utilization Project Kids' Inpatient data reported no significant positive trends in mortality, rate of colectomy, or hospital days (Nylund et al., 2011). Most studies have failed to show any relationship between C.difficile and common forms of diarrheal illness in neonates and infants (Bryant et al., 2009). For example *C. difficile* was isolated with equal frequency in healthy children between 1 week and 1 year of age (17%) and children <6 years of age with diarrhea (18%) (Svedhem et al., 1982; Bryant et al., 2009). In a study of outpatient children, *C. difficile* was isolated from 7% of patients with diarrhea and 14.8% of healthy controls. Children with *C. difficile* were younger than children without the organism ($P < 0.05$); prior antibiotic exposure was noted in only 22% (Boenning et al., 1982; Bryant et al., 2009). In another study, toxin B was identified in 4.2% of 618 children with diarrhea and in an equivalent proportion of healthy controls (Cerquett et al., 1995; Bryant et al., 2009). In contrast, Tullus et al., observed all-cause diarrhea to be more frequent in children colonized with *C. difficile* after the age of 6 months (Tullus et al., 1998; Bryant et al., 2009). Similar findings have been noted in most controlled studies of NICU patients. *C. difficile* toxin was recovered from the stools of 55% of patients in one NICU but signs of enteric disease, including necrotizing enterocolitis, occurred with equal frequency in both toxin-positive and toxin-negative infants (Donta et al., 1998; Bryant et al., 2009). The causes of this apparent resistance to illness are unknown. Data from juvenile rabbits suggest that the lack of disease in colonized human infants may be related to the relative absence of receptors for toxin A on immature enterocytes (Eglow et al., 1992).

Potential mechanisms of resistance to CDAD in Infants (Jangi et al., 2010):

- Absent machinery for processing of Clostridium toxin
- Local gut environment (depressed fecal pH, poor amino acid availability)
- Competition from maturing flora (nutritional competition, bacteriocin production)
- Neutralization by secretory IgA and oligosaccharides from breast milk

However, sporadic case reports suggest that severe CDI occasionally occurs in infants, especially those with underlying intestinal pathology. For example, *C. difficile* pseudomembranous colitis has been identified at autopsy in infants with Hirshprung's disease (Qualman et al., 1990). Fatal *C. difficile*-associated pseudomembranous colitis has also been described in a premature infant with necrotizing enterocolitis (Singer DB, et al., 1986; Bryant et al., 2009). However, it is impossible to know definitively whethever C.difficile was causal or incidental finding due to the high frequency of toxigenic C.difficile carriage in this age group.

The American Academy of Pediatrics has recently released a policy statement on *C. difficile* infection in infants and children (Policy Statement AAP, 2013). The guidelines caution that testing for *C. difficile* should be performed only for children who meet the criteria for clinically significant diarrhea and that test results for infants less 1 year of age can be difficult to interpret due to high rates of asymptomatic colonization (Policy Statement AAP, 2013).

1.2. Risk factors and predisposing conditions

The American Academy of Pediatrics recommends that the testing of infants should be limited to those with Hirschsprung disease or other severe motility disorders or in an outbreak situation (Policy Statement AAP, 2013).
Children with Hirschsprung disease may have alterations in mucosal defense, predisposing them to *C. difficile* infection (Qualman et al., 1990; Cooperstock et al., 2014).
Other risk factors include gastrostomy or jejunostomy tube; elemental nutrition via gastric or small bowel tube feeding and immune compromise (Chen et al., 2012)
Any antibiotic may predispose to *C. difficile* infection (Shannon-Lowe et al., 2010; Cooperstock et al., 2014). However, penicillins, cephalosporins, clindamycin, macrolides, and fluoroquinolones are most frequently implicated (Cooperstock et al., 2014).

Antimicrobial agents that may induce Clostridium difficile diarrhea and colitis

Frequently associated	Occasionally associated	Rarely associated
Fluoroquinolones	Macrolides	Aminoglycosides
Clindamycin	Trimethoprim	Tetracyclines
Penicillins (broad spectrum)	Sulfonamides	Chloramphenicol
Cephalosporins (broad spectrum)		Metronidazole
		Vancomycin

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In a case-control studyof Kim et al., (2012), exposure to multiple classes of antibiotics in the preceding 30 days was associated with severe *C. difficile* infection.
Proton pump inhibitors have been shown to predispose to *C. difficile* infection in adults. This has also been reported in children (odds ratio 4.5, 95% CI, 1.4-14.4) (Turco et al., 2010; Cooperstock et al., 2014).
Predisposing conditions such as Immunodeficiency – (undiagnosed immunodeficiency is possible in children with *C. difficile* disease; for example, transient hypogammaglobulinemia of infancy and other types of hypogammaglobulinemia (Perlmutter et al., 1985; Gryboski et al., 1991; Cooperstock et al., 2014).
Cancer-related or solid-organ transplant-related immunosuppression – A limited number of observational studies suggest that both *C. difficile* colonization and infection are common in pediatric oncology patients and children who have undergone solid organ transplants (Burgner et al., 1997; Brunetto et al. 1988; Murabata et al., 2008; Castagnola et al., 2009; Simon et al., 2008; Tai et al., 2011). Although *C. difficile* may be an incidental finding, *C. difficile* should be considered in those presenting with more severe intestinal illness (Cooperstock et al., 2014).
Infant botulism – *C. difficile* may be superimposed on the intestinal immobility induced by botulinum toxin (Thompson et al., 1983; Schechter et al., 1999; Cooperstock et al., 2014).

Interleukin-8 AA polymorphism is a reported risk factor in adults (Jiang et al., 2007; Cooperstock et al., 2014).

Inflammatory bowel disease – *C. difficile* infection may be the initial mode of presentation for previously unsuspected inflammatory bowel disease (IBD). At least two studies of IBD in children and adolescents demonstrate an association between IBD exacerbation and fecal *C. difficile* toxin (Gryboski et al., 1991; Pascarella et al., 2009; Cooperstock et al., 2014). Moreover, fecal *C. difficile* toxin was detected in 44 percent of children and adolescents with IBD exacerbation; Pascarella et al., 2009; Cooperstock et al., 2014).

Cystic fibrosis – In studies from the 1980s, asymptomatic colonization with toxigenic C. difficile was reported in 14 to 43 percent of pediatric and young adult patients with cystic fibrosis (CF) who had received recent or continuous antimicrobial therapy (Wu et al., 1983; Welkon et al., 1985; Peach et al., 1986; Cooperstock et al., 2014). In a more recent study, using enzyme immunoassay, fecal toxins were detected in 47 percent of 30 CF patients (Yahav et al., 2006; Cooperstock et al., 2014). However, as with other groups of children noted to have frequent asymptomatic *C. difficile* colonization, observed associations between fecal toxin and intestinal disease in patients with CF may be incidental (Cooperstock et al., 2014).

Breast-fed infants are reported to have lower rates of *C. difficile* colonization compared with formula-fed infants (Fig.5 Jangi et al., 2010). *C.difficile* was nearly 2-fold higher in formula-fed infants (30%), compared with breast –fed infants (14%) (Penders et al., 2005), suggesting that a factor in human breast milk may inhibit the growth of *C.difficile* (Benno et al,.1984; Jangi et al., 2010).

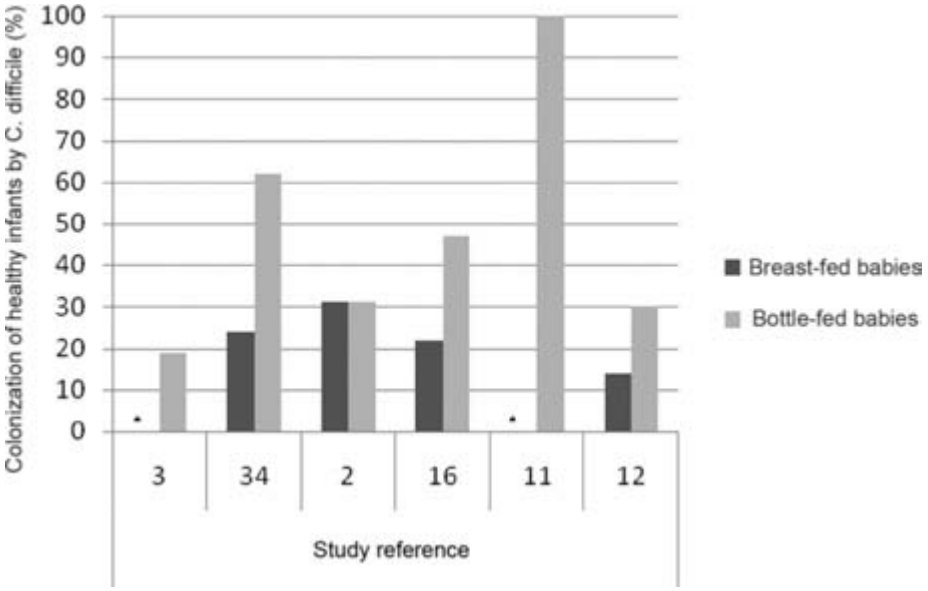

Fig.5 Effects of breast-feeding versus bottle-feeding on *C difficile* recovery from healthy infants. Six studies examined the effect of breast-feeding versus bottle-feeding on *C difficile* recovery rates. A total of 1156 infants were examined. The (*) represents 0% infants colonized by *C difficile*. Studies are arranged in order of year of publication, with the most recent study on the far right of the x-axis (Jangi et al., 2010).

1.3. Diagnostic criteria for the CDI

Pai et al., 2012 proposed the following Diagnostic criteria to help differentiatie between *C.difficile* colonisation and infection for children:

Fulfils likely diagnosis of CDI if – Diarrhoea (Bristol stool chart 5–7) and one or more of the following:

- Significant co-morbidities – Haematology/oncology, Gastrointestinal disease
- Stay in hospital for >2 days
- Antibiotic use in the last 1 month

However, Pai et al (2012) included children ranging in age from 0 to 16 yrs, with a mean age of 2.97 years. There are gaps in the knowledge surrounding CDIs in infants and children. (Policy Statement AAP, 2013; Cohen et al., 2010) .

2. Usefulness of the performing the follow-up "Test of Cure" for toxin-producing *Clostridium difficile*

The American Academy of Pediatrics does not recommend to use EIAs and NAATs as tests of cure after treatment of CDIs. *C difficile*, its toxins, and genome are shed for long periods after resolution of diarrheal symptoms.
None of the assays are licensed or recommended for tests of cure. Excretion of toxin approximates 13% to 24% at 2 weeks and 6% at 4 weeks after therapy (Wenisch et al., 1996; Wullt et al., 2004). Given that NAAT testing is more sensitive than toxin assays, an interval greater than 4 weeks since last testing should be used for testing with a recurrence (Policy Statement AAP, 2013). Test of cure is not recommended (Policy Statement AAP, 2013).

COMMENTS

TO DO/ACTIONS

1) Determination of risico factors in multidisciplinary discussion for the detection of *C.difficile* in population <1 year.
2) Test of Cure is not recommended, however, neonatology department uses the follow-up test for *C. difficile* to reduce time of quarantine, it was agreed that periode of min 7 days will be applied before the next *C. difficile* test will be allowed indien *C.difficile* test was positive.

ATTACHMENTS

Attachment 1

	Eadnr	voorzienAfnameTijdstip	Leeftijd (mnd)	Toxine detectie	Symptomen	Behandeling	Resultaten follow up stalen	Comorbiditeit	AB laatste 4 weken voor diarree
1	60498173	1/10/2013 17:53	0	EIA	Diarree, bloederig stoelgang, pneumatosis intestinalis; NEC	Metronidazol IV 5 dagen, dan Vancomycine per os 5 dagen	EIA +, PCR+, negatief	Prematuriteit (PML 35 weken 1 dag). AB breedspectrum ikv PPROM (Preterm Premature Rupture of the Outer Membranes)	Amoxicilline+ Amukin
		6/10/2013 13:20	0	EIA					
		10/10/2013 18:35	0	PCR					
		14/10/2013 11:02	0	negatief					
		22/10/2013 14:08	1	negatief					
		22/05/2014 17:40	8	negatief					
2	60566781	16/01/2014 20:36	0	EIA	Groene felreikende stoelgang, geen koorts	Vancomycine per os 18/01/2014-27/01/2014	EIA+, PCR+	Prematuriteit	Geen
		23/01/2014 0:03	0	EIA					
		26/01/2014 17:59	1	PCR					
3	60599410	24/02/2014 18:25	0	EIA	Slechtruikende stoelgang, braken , geen koorts	Vancomycine per os 10 dagen	PCR+, PCR+, negatief	Prematuriteit	Amoxicilline+ Amukin
		1/03/2014 6:20	0	PCR					
		5/03/2014 11:59	0	PCR					
		7/03/2014 17:36	0	negatief					
4	60611906	20/04/2014 6:44	1	negatief	Slechtruikende stoelgang, aanwezigheid van slijmen			Methylmalonzuuracidemie	Clamoxyl, Claforan, dan Dalacin
		26/05/2014 3:20	2	EIA	Waterig stoelgang	Metronidazol 26/05/2014-03/06/2014 dan , "gezien blijvend positieve stoelgangsstalen werd geswitcht naar vancomycine per os": 04/06/2014-16/06/2014 , "hiermee negativeerden de stoelgangskweken"	EIA+, negatief		
		2/06/2014 9:08	3	EIA					
		10/06/2014 10:36	3	negatief					
5	60542208	1ste episode:	3	EIA	1ste episode: geen diarree (C.Diff test owv zus die + voor C. diff)	Vancomycine 10 dagen	negatief	Prematuriteit 29w; Coarctatio aortae, VSD	Geen
		31/03/2014 16:04	4	negatief					
		24/04/2014 15:28	5	negatief					
		2de episode:	5	EIA	2de episode: reden van test C,Diff? Geen diarree, ter controle ?"				Augmentin, Glazidim (UWI met E coli en PSAE)
		21/05/2014 1:33	6	EIA					
		28/05/2014 15:33	6	PCR					
		28/05/2014 18:17	6	negatief					
		12/06/2014 17:58	6	PCR					
		26/06/2014 11:49	7	PCR		Vancomycine: zie Verslag raadpleging neonatologie op 25/09/2014: "laatste kuur Vanco voor Clostridium tem 28/8"			
		25/09/2014 16:05	10	PCR		Advies consult 25/09:AB dient herstart worden			
6	60343918	8/10/2013 12:54	8	PCR	KWS: Stoelgang is altijd vrij plaat en ruikt fel, zurig	Metronidazol 10 dagen	negatief		"Amoxicilline 9 dagen ter protectie , gezien zijn tweelingbroer ziek was"
		16/10/2013 9:11	8						
7	60595209	29/03/2014 20:58	8	negatief	waterig stoelgang				
		14/04/2014 6:45	8	negatief	waterig stoelgang				
		26/04/2014 10:51	9	negatief	waterig stoelgang				
		2/06/2014 7:57	10	EIA	waterig stoelgang	Metronidazol 10 dagen	negatief	Osteopetrosis, Immunosupressanten, Stamceltransplantatie op 11/03/14	Meronem (13/05-13/06) koorts, steriel kweken
		16/06/2014 9:16	10	negatief			negatief		
8	60536065	15/11/2013 5:21	10	EIA	Waterig stoelgang 14/11; Slijmen in stoelgang 15/11	Metronidazol 10 dagen	negatief	geen	Claforan: 11/11/2013-14/11/2013
		20/11/2013 11:50	10						
9	60427016	7/04/2014 15:44	10	PCR	Waterig stoelgang 2 dagen	Metronidazol 10 dagen	negatief	Prematuritas in voorgescheidenis (33w); Extrofie van de blaas caecum, anale atresie	Tazocin (08/03/2014-21/03/2014)
		14/04/2014 19:22	10	negatief					

Attachment 2

Attachment 3

Revisie 140224: geen wijzigingen