(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 November 2015 (12.11.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/169451 Al

(51) International Patent Classification:
A61K 31/7048 (2006.01) *A61P 31/04* (2006.01)

(21) International Application Number:
PCT/EP20 15/000965

(22) International Filing Date:
11 May 2015 (11.05.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
14 075 031.6 9 May 2014 (09.05.2014) EP

(71) Applicant: **ASTELLAS PHARMA EUROPE LTD** [GB/GB]; 2000 Hillswood Drive, Chertsey, Surrey KT16 0RS (GB).

(72) Inventors: **KARAS, Andreas Johannis;** c/o Astellas BV, Sylviusweg 62, NL-2333 BE Leiden (NL). **LONGSHAW, Christopher Mark;** c/o Astellas BV, Sylviusweg 62, NL-2333 BE Leiden (NL).

(74) Agent: **OLTHOFF, Margaretha;** Astellas BV, P.O. Box 344, 2300 AH Leiden (NL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2015/169451 A1

(54) Title: TREATMENT REGIMEN TIACUMICIN COMPOUND

(57) Abstract: A tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, and a pharmaceutical composition, containing a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, are provided for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CD AD) in a patient in accordance with a dosage regimen selected from the group consisting of: i. Administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and ii. Administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days. Further, a method is provided for recovering of gut Bifidobacteria population in a patient, suffering from Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CD AD) and receiving oral treatment with a tiacumicin compound, to 50 to 90 % of the gut Bifidobacteria population prior to administering the tiacumicin compound during days 15-45 after start of the treatment by orally administering the tiacumicin compound to the patient according to a dosage regimen, which is selected from the above-mentioned group.

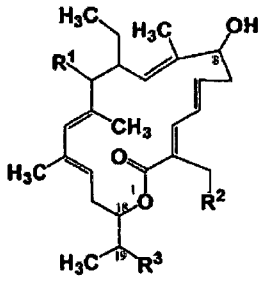
TREATMENT REGIMEN TIACUMICIN COMPOUND

The present invention relates to a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea/disease (CDAD) in a patient.

BACKGROUND OF THE INVENTION

Tiacumicin compounds are naturally occurring compounds with an antibiotic activity that can be obtained by cultivating various microorganisms belonging to the Actinoplanes family (especially the genus Dactylosporangium aurantiacum, subspecies hamdenensis) in a suitable nutrient medium at a suitable temperature and isolating the compounds having antibiotic activity against a variety of microorganisms (tiacumicins A-F; US Patent 4,918,174). Especially tiacumicins B and C turned out to possess antibiotic activity against a number of Gram-positive bacteria in vitro including strains resistant to therapeutic antibiotics, used at the time. US patent 5,583,115 discloses dialkyltiacumicin compounds, which are derivatives of the above-mentioned tiacumicin compounds A-F, were found to have in vitro activity against a variety of bacterial pathogens and in particular against Clostridium species. US Patent 5,767,096 discloses bromotiacumicin compounds, which are also derivatives of tiacumicin compounds A-F, which were found to have in vitro activity against some bacterial pathogens and in particular against Clostridium species.

From a chemical point of view the tiacumicins share an 18-membered macrocyclic ring, which is glycosidically attached to one or two optionally substituted sugar molecules (US patent 4,918,174 and WO 2004/014295) as follows:



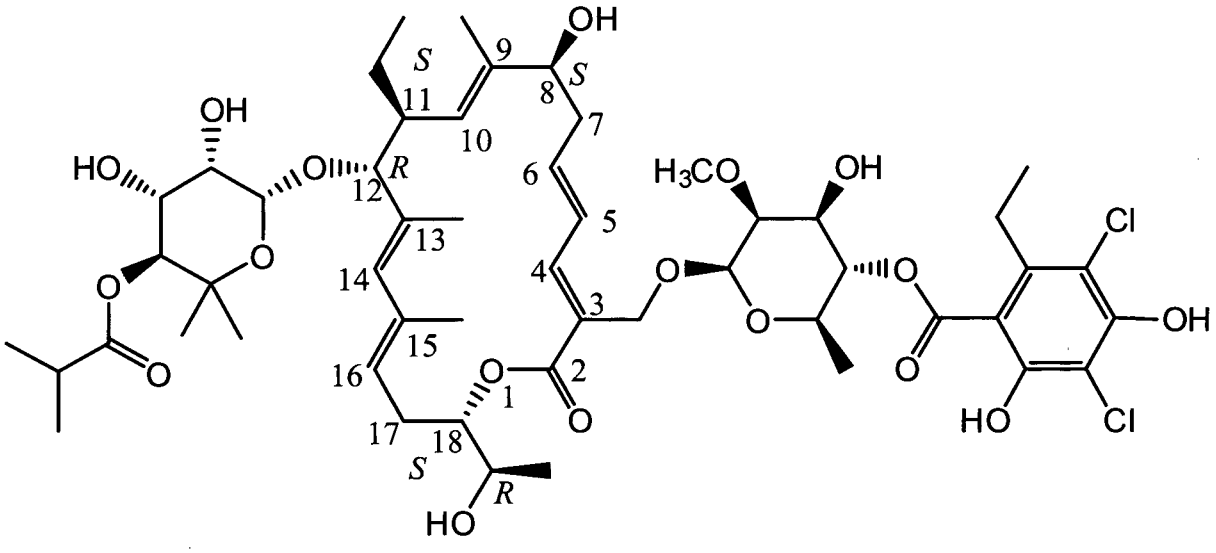
Formula I

WO 2004/014295 describes substantially pure R-tiacumicins, obtained by submerged aerobic fermentation of Dactylosporangium aurantiacum hamdenensis. WO 2006/085838

discloses pharmaceutical compositions containing R-tiacumicins and especially R-tiacumicin B, which contains an R-hydroxy-group at CI9, which shows surprisingly lower MIC values when tested in vitro against Clostridium species than the optically pure S-isomer of tiacumicin B and other tiacumicin related compounds.

Chinese patent applications having publication numbers 102030791 and 102219815 respectively and S. Niu et al. (201 1) in ChemBioChem 12: page 1740-1748 describe 11 new tiacumicin analogues all lacking the 2'-0-methyl group on the internal rhamnose moiety. Two of those analogues have shown to have improved antibacterial properties.

R-tiacumicin B is also known under the name fidaxomicin (3-[[[6-deoxy-4 -*O*-(3,5-dichloro-2-ethyl-4,6-dihydroxybenzoyl)-2-0-methyl -**P**-**D**-mannopyranosyl]oxy] methyl]-12(i?)-[[6-deoxy-5-C-methyl-4-0-(2-methyl-l-oxopropyl)-**P**-**D**-lyxo-hexopyranosyl]oxy]-ll(5)-ethyl-8(5)-hydroxy- 18(5)-(1*(R)*-hydroxyethyl)-9, 13,1 5-trimethyloxacyclooctadeca-3,5,9, 13,15-pentaene-2-one or oxacyclooctadeca-3,5,9,13,15-pentaen-2-one, 3-[[[6-deoxy-4 -*O*-(3,5-dichloro-2-ethyl-4,6-dihydroxybenzoyl)-2 -*O*-methyl-β-D-mannopyranosyl]oxy]methyl]-12-[[6-deoxy-5-C-methyl-4 -*O*-(2-methyl-l-oxopropyl)-β-D-/);xo-hexopyranosyl]oxy]-l l-ethyl-8-hydroxy- 18-[(l *R)*- 1-hydroxyethyl]-9, 13,1 5-trimethyl-, (3*E*,5*E*,85,9*E*, 1 *S*,\2*R*,\3*E*,\5*E*,*18S*)). It is a compound that has a narrow antimicrobial spectrum, with activity against Clostridium difficile and most strains of staphylococci and enterococci but negligible activity against gram-negative organisms and fungi. It is obtained by fermentation of Dactylosporangium aurantiacum and corresponds to the following formula **(II):**



II

According to an in vitro BCS study, fidaxomicin is a BCS (Biopharmaceutics Classification System) Class IV compound (low solubility, low permeability). Upon oral administration fidaxomicin is poorly absorbed from the intestinal tract and is therefore associated with a low incidence of systemic side effects.

Tablets containing 200 mg fidaxomicin are commercially available in Europe (under the trademark Dificlir) and in the USA (under the trademark Dificin).

Not pre-published international patent application PCT/EP20 14/000091 discloses compositions containing a tiacumicin compound in admixture with an excipient, selected from the group consisting of xanthan gum, carrageenan, sodium alginate, guar gum, water dispersible cellulose (microcrystalline cellulose and sodium carboxymethylcellulose) and mixtures thereof, which is used as an anti-foaming agent for the preparation of stabilised suspension formulations.

Fidaxomicin is indicated for the treatment of *Clostridium difficile* infections (CDI) also known as *C. difficile-associated* diarrhea or disease (CDAD) and prevention of recurrences. CDI is a major burden on healthcare facilities worldwide (Wiegand P.N., Nathwani D., Wilcox M.H. et al. in J. Hosp Infect of 10 April 2012; Ghantoji S.S., Sail, K. Lairson D.R. (2010) in J. Hosp. Infect. 74: 309-318). These infections are normally caused by changes in the composition and function of the intestinal flora following the use of antimicrobials and are called antibiotic-associated diarrhea (AAD).

A *Clostridium difficile* infection is a type of bacterial infection that can affect the digestive system. It most commonly affects people who have been treated with antibiotics. The symptoms of a C. difficile infection can range from mild to severe and include diarrhea, a high temperature (fever) of above 38°C and painful abdominal cramps. A *C. difficile* infection can also lead to life-threatening complications such as severe swelling of the bowel from a build-up of gas (toxic megacolon). *Clostridium difficile* infections (CDI) also known as C. *difficile-associated* disease (CDAD) refers to a wide spectrum of diarrheal illnesses caused by the toxins produced by this organism, including cases of severe colitis with or without the presence of pseudomembranes. The occurrence of AAD varies greatly and is influenced by a number of factors, including nosocomial outbreaks, patterns of antimicrobial prescription, and individual susceptibility. It is estimated that 10% to 15% of all hospitalized patients treated with antibiotics will develop AAD. Most important, twice as many will become asymptomatic carriers. Risk factors include compromised immune status, advanced age, abdominal surgery, comorbidity, types and prolonged use of antibiotics, reduced gastric acid,

and the length of hospitalization. For example, infection rates for *C. difficile* are reported to be around 10% after 2 weeks of hospitalization but may reach 50% after 4 or more weeks (McFarland LV. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. Dig Dis 1998; 16:292-307). All groups of antibiotics may cause AAD, but those with broad-spectrum coverage - in particular cephalosporins, fluoroquinolones, extended-coverage penicillins, and clindamycin - are the most common culprits (Wistrom J, Norrby SR, Myhre E, et al. Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalized patients: a prospective study. J Antimicrob Chemother 2001; 47:43-50).

Treatment options are limited and are associated with effects on gut microflora recovery of the patients and high rates of recurrence.

Therefore it remains a need for improved treatment options and dosage regimens. Along with its narrow antimicrobial spectrum, fidaxomicin also has a prolonged postantibiotic effect against C. difficile. Besides the obvious benefit to the patient, the prevention of recurrence would eliminate the costs of treating additional episodes of C. difficile infection and should reduce the rate of person-to-person transmission. The currently recommended treatment regimen for adults and elderly people (65 years and older) is 200 mg administered twice daily (ql2h) for 10 days.

This is an effective treatment for CDI, and is associated with reduced rates of recurrence as compared with vancomycin. However, this treatment/dosing regimen was not optimised for recovery of microflora but chosen based on existing practice for vancomycin and metronidazole. Both vancomycin and metronidazole disrupt microflora and so on recovery cannot start until after treatment has been removed.

In two Phase III randomised, double-blind, clnical trials, fidaxomicin demonstrated non-inferiority to vancomycin for initial clinical cure of CDI, but superiority in reduction of recurrence and sustained clinical response (Crook et al. (2012) in Clin. Infect. Dis. 55(Suppl 2): S93-103).

In phase III clinical trials the risk of fidaxomicin or vancomycin treatment failure doubled for each treatment day less than 10 days (T. Louie et al. Poster presented at 22nd European Congress of Clinical Microbiology & Infectious Diseases, March 31 - April 3, 2012, London). The relatively low impact of fidaxomicin on gut microflora may allow better recovery of bacteria during prolonged treatment periods, so reducing risk of CDI recurrence (T.J. Louie et al. (2012) in Clin. Infect. Dis. 55(S2) S132-142 ; Tannock in Microbiology (2010), 156, 3354-3359 (Phase II trials)).

The management of *C. difficile* infections (CDI), thus, is complicated by high recurrence rates with over 50% of second episodes experiencing a recurrence (RCDI). Guidelines recommend managing multiple recurrences with a vancomycin taper. No clear recommendation is available for patients failing this approach. In a recent case series report (Soriano et al in Exp Rev Antiinf Ther 2013;1 1:767-776), patients with multiple RCDI that were refractory to vancomycin taper therapy were given either fidaxomicin 200mg BID for 10 days (FID-TX), or a repeat of CDI treatment followed by either a 10-day fidaxomicin regimen as a chaser (FID-CH), or a taper as 200mg daily for 7 days, followed by 200mg QOD for 7-26 days (FID-TP). Demographic information, CDI history, treatment outcomes, and symptom-free interval (SFI) were collected from patient records. Treatment success was considered if symptoms resolved by the end of therapy and no additional antibiotic was needed. RCDI was defined by the onset of CDI symptoms following successful treatment for a previous episode. 14 patients received 18 courses of fidaxomicin for RCDI (mean age of 60, mean of 4.6 previous CDI episodes, mean of 2.3 previous vancomycin taper courses). All 18 courses resulted in treatment success (3 courses as FID-TX, 8 as FID-CH, and 7 as FID-TP). Of 3 FID-TX courses, there were 2 RCDI episodes (66%). When excluding RCDI due to antimicrobial exposure, there were 2 RCDI (25%) observed after the 8 FID-CH courses and no RCDI following the 7 FID-TP courses. The average SFI following a vancomycin taper was 37 days. The average SFI following FID-TX, FID-CH, and FID-TP was 73, 240, and 150 days, respectively. Patients with RCDI that failed multiple vancomycin tapers had symptom resolution following fidaxomicin therapy. All 3 regimens provided a greater SFI compared to a vancomycin taper. No patient experienced RCDI following FID-TP. FID-CH had the longest SFI, yet follow-up time with FID-TP was shorter given more recent adoption of this regimen. These results suggest the utility of using fidaxomicin to treat RCDI. (M.M. Soriano et al. Abstract 42591 ; presentation No. 1410; IDWeek, 5 October 2013).

The use of fidaxomicin for the treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in an adult patient wherein the dosage regime is selected from the group consisting of:

a. 200 mg of fidaxomicin BID (Latin: bis in die; which means twice a day) for 20 days (reference example Model A)

b. 200 mg of fidaxomicin BID for 5 days followed by 5 days of rest and then 200 mg BID for a further further 5 days (double pulse) (reference example Model B)

was mentioned by C.H. Chilton during the ICAAC congress in September 2013 (reference example; C.H. Chilton et al. (2013) in J. Antimicrobial Chemotherapy Advance Access Sept 2013 and C.H. Chilton et al. , abstract 23rd European Congress of Clinical microbiology & Infectious Disease, April 27-30, 2013, Berlin).

However, there still is a need to find a modified dosing regimen for tiacumicin compounds and in particular for fidaxomicin that combines efficacy, recovery of gut micro flora or a reduced effect on gut microflora, a reduction of recurrence, and a low chemical burden to the patients with cost-effectiveness.

SUMMARY OF THE INVENTION

After having carried out detailed investigations, the present inventors have been able to provide one or more of a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, as well as a pharmaceutical composition, comprising one or more of a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease in a patient, according to a dosage regimen which is selected from the group consisting of:

i. 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and

ii. 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days.

Further, the inventors have provided a method for recovering of gut Bifidobacteria population in loglOcfu/ml in a patient, suffering from Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) and receiving oral treatment with a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, to 50 to 90 % of the gut Bifidobacteria population in loglOcfu/ml prior to administering the tiacumicin compound during days 15-45 after start of the treatment by orally administering the tiacumicin compound to the patient according to a dosage regimen, which is selected from the group consisting of:

i. Administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and

ii. Administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1/12 represents the three-stage compound continuous culture system used in example 1, as described by Macfarlane et al. (Microbial Ecology (1998), 35: 180-187).

Fig. 2/12 provides a graphical representation of the treatment regimens for the reference example (A and B) and example 1 (C and D; treatment regimens i and ii).

Fig. 3/12 provides the experimental design for the reference example (A and B).

Fig. 4A/12 provides a graphical representation of the total counts (diamonds/rhombi), spore counts (squares) and cytotoxin titre (triangles) versus time in days for the reference example A.

Fig. 4B/12 provides a graphical representation of the total anaerobes level versus time in days for the reference example A.

Fig. 4C/12 provides a graphical representation of the Bacteroides level versus time in days for the reference example A.

Fig. 4D/12 provides a graphical representation of the Bifidobacteria level versus time in days for the reference example A.

Fig. 4E/12 provides a graphical representation of the Lactobacilli level versus time in days for the reference example A.

Fig. 4F/12 provides a graphical representation of the Enterococci level (triangles) versus time in days for the reference example A.

Fig. 4G/12 provides a graphical representation of the Lactose fermenters level (circles) versus time in days for the reference example A.

Fig. 4H/12 provides a graphical representation of the total Clostridia level (crosses) versus time in days for the reference example A.

Fig. 41/12 provides a graphical representation of the facultative anaerobes level (diamonds/rhombi) versus time in days for the reference example A.

Fig. 5A/12 provides a graphical representation of the total counts (diamonds/rhombi), spore counts (squares) and cytotoxin titre (triangles) versus time in days for the reference example B.

Fig. 5B/12 provides a graphical representation of the total anaerobes level versus time in days for the reference example B.

Fig. 5C/12 provides a graphical representation of the Bacteroides level versus time in days for the reference example B.

Fig. 5D/12 provides a graphical representation of the Bifidobacteria level versus time in days for the reference example B.

Fig. 5E/12 provides a graphical representation of the Lactobacilli level versus time in days for the reference example B.

Fig. 5F/12 provides a graphical representation of the Enterococci level (triangles) versus time in days for the reference example B.

Fig. 5G/12 provides a graphical representation of the Lactose fermenters level (circles) versus time in days for the reference example B.

Fig. 5H/12 provides a graphical representation of the total Clostridia level (crosses) versus time in days for the reference example B.

Fig. 51/12 provides a graphical representation of the facultative anaerobes level (diamonds/rhombi) versus time in days for the reference example B.

Fig. 6/12 provides a graphical representation of the antimicrobial concentrations achieved in the In Vitro Gut Model (IVGM) versus days (after 200 mg fidaxomicin BID for 7 days).

Fig. 7A/12 provides a graphical representation of the antimicrobial concentrations achieved in IVGM versus days (after 200 mg fidaxomicin BID for 20 days).

Fig. 7B/12 provides a graphical representation of the antimicrobial concentrations achieved in IVGM versus days (after 200 mg fidaxomicin 2x5 days pulse).

Fig. 8A/12 provides the experimental design for the example 1 (C and D; treatment regimens i and ii).

Fig. 8B/12 provides an alternative representation of the experimental design for the example 1 (C and D; treatment regimens i and ii).

Fig. 9A/12 provides a graphical representation of the total counts (diamonds/rhombi), spore counts (squares) and cytotoxin titre (triangles) versus time in days for the example C.

Fig. 9B/12 provides a graphical representation of the total anaerobes level versus time in days for the example C.

Fig. 9C/12 provides a graphical representation of the Bacteroides level versus time in days for the example C.

Fig. 9D/12 provides a graphical representation of the Bifidobacteria level versus time in days for the example C.

Fig. 9E/12 provides a graphical representation of the Lactobacilli level versus time in days for the example C.

Fig. 9F/12 provides a graphical representation of the Enterococci level (triangles) versus time in days for the example C.

Fig. 9G/12 provides a graphical representation of the Lactose fermenters level (circles) versus time in days for the example C.

Fig. 9H/12 **provides** a **graphical representation of the total Clostridia level (crosses) versus time in days for the example** C.

Fig. 91/12 provides a graphical representation of the facultative anaerobes level (diamonds/rhombi) versus time in days for the example C.

Fig. 1OA/12 provides a graphical representation of the total counts (diamonds/rhombi), spore counts (squares) and cytotoxin titre (triangles) versus time in days for the example D.

Fig. 10B/12 provides a graphical representation of the total anaerobes level versus time in days for the example D.

Fig. IOC/12 provides a graphical representation of the Bacteroides level versus time in days for the example D.

Fig. 10D/12 provides a graphical representation of the Bifidobacteria level versus time in days for the example D.

Fig. 10E/12 provides a graphical representation of the Lactobacilli level versus time in days for the example D.

Fig. 10F/12 provides a graphical representation of the Enterococci level (triangles) versus time in days for the example D.

Fig. 10G/12 provides a graphical representation of the Lactose fermenters level (circles) versus time in days for the example D.

Fig. 10H/12 **provides** a **graphical representation of the total Clostridia level (crosses) versus time in days for the example** D.

Fig. 101/12 provides a graphical representation of the facultative anaerobes level (diamonds/rhombi) versus time in days for the example D.

Fig. 11A/12 provides a graphical representation of the antimicrobial concentrations achieved in IVGM versus days for the example C.

Fig. 11B/12 provides a graphical representation of the antimicrobial concentrations achieved in IVGM versus days for the example D.

Fig. 12A/12 shows mean C. difficile PCR ribotype 027 total viable counts (diamonds/rhombi) and spore counts ($\log_{10}$ cfu/mL) (squares) and cytotoxin titres (relative units, RU) (triangles) in vessel 3 of Model C (dosing regimen i). Horizontal dotted line indicates the limit of detection. Letters A-H refer to the different stages in the treatment as shown in Fig. 8B/12.

Fig. 12B/12 shows the antimicrobial concentration (mg/L) in vessel 3 of Model C (dosing regimen i).

Fig. 12C/12 shows mean C. difficile PCR ribotype 027 total viable counts (diamonds/rhombi) and spore counts ($\log_{10}$ cfu/mL) (squares) and cytotoxin titres (relative units, RU) (triangles) in vessel 3 of Model Model D (dosing regimen ii). Horizontal dotted line indicates the limit of detection. Letters A-G refer to the different stages in the treatment as shown in Fig. 8B/12.

Fig. 12D/12 shows the antimicrobial concentration (mg/L) in vessel 3 of Model D (dosing regimen ii).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in an adult patient acoording to a dosage regimen, which is selected from the group consisting of:

i. 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and
ii. 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days.

In a first embodiment the present invention relates to a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regimen which

consists of administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days.

In a second embodiment the present invention relates to a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regime which involves administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days.

In a third embodiment the present invention relates to a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regimen which consists of administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days, wherein the CDI is refractory CDI or recurrence CDI.

In a fourth embodiment the present invention relates to a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regime which involves administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days, wherein the CDI is refractory CDI or recurrence CDI.

The expression "stereo-isomer thereof refers to isomers of identical constitution that differ in the arrangement of their atoms in space. Enantiomers and diastereomers are examples of stereoisomers. The term "enantiomer" refers to one of a pair of molecular species that are mirror images of each other and are not superimposable. The term "diastereomer" refers to stereoisomers that are not mirror images. The term "racemate" or "racemic mixture" refers to a composition composed of equimolar quantities of two enantiomeric species, wherein the composition is devoid of optical activity. The symbols "R" and "S" represent the configuration of substituents around a chiral carbon atom. The isomeric descriptors "R" and "S" are used as described herein for indicating atom configuration relative to a core molecule and are intended to be used as defined in the literature (IUPAC Recommendations 1996, Pure & Applied Chemistry 68: 2193-2222).

The expression "polymorph thereof describes any alternative crystalline form having different physical properties as a result of the different Order of the molecule in a crystal lattice. More specifically, polymorphs such as disclosed in WO2008/09 1554 are included.

The expression "pharmaceutically acceptable solvate thereof ' describes any pharmaceutically acceptable solvate that, administered to a patient (directly or indirectly) provides a tiacumicin compound. Preferably, the solvate is a hydrate, a solvate with an alcohol such as methanol, ethanol, propanol, or isopropanol, a solvate with an ester such as ethyl acetate, a solvate with an ether such as methyl ether, ethyl ether or THF (tetrahydrofuran) or a solvate with DMF (dimethylformamide), of which a hydrate or a solvate with an alcohol such as ethanol is more preferred. A solvent for constituting the solvate is preferably a pharmaceutically acceptable solvent.

The tiacumicin compound according to the present invention, has an 18-membered macrocyclic glycoside structure and is a compound as disclosed in US Patents 4,918,174; 5,583,1 15; 5,767,096; and in Chinese patent applications 201010526416.9 and 201 110104051.5, herein incorporated by reference. Preferably, the active ingredient is selected from the group consisting of tiacumicin A, tiacumicin B and analogues thereof, (dialkyltiacumicins and bromotiacumicins), tiacumicin C, tiacumicin D, tiacumicin E, tiacumicin F and lipiarmycin. Though all tiacumicin compounds have in common that they are insoluble or almost insoluble in water, more preferably, the active ingredient is lipiarmycin or tiacumicin B or a stereo-isomer thereof or a polymorph thereof. Most preferably R-tiacumicin B (also known as fidaxomicin, OPT-80, or PAR-101) is used as the active ingredient.

In a further embodiment the present invention relates to a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease(CDAD) in a patient according to a dosage regimen which consists of administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days, wherein the tiacumicin compound is fidaxomicin.

In yet a further embodiment the present invention relates to a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regimen which

consists of administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days, whereby the tiacumicin compound is fidaxomicin.

In yet a further embodiment the present invention relates to a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regimen which consists of administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days, wherein the CDI is refractory CDI or recurrence CDI and whereby the tiacumicin compound is fidaxomicin.

In yet a further embodiment the present invention relates to a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regime which involves administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days, wherein the CDI is refractory CDI or recurrence CDI and whereby the tiacumicin compound is fidaxomicin.

A further embodiment is a pharmaceutical composition, comprising a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regimen selected from the group consisting of:

i. Administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and
ii. Administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days.

Yet another embodiment relates to a pharmaceutical composition, comprising a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regimen selected from the group consisting of:

i. Administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and

ii. Administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days

wherein the tiacumicin compound is fidaxomicin.

And yet another embodiment relates to a pharmaceutical composition, comprising a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regimen selected from the group consisting of:

i. Administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and

ii. Administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days

wherein the tiacumicin compound is fidaxomicin and is administered in a film-coated tablet.

The compositions to be used in the dosage regimen according to the invention may be an aqueous suspension, a dry powder for an aqueous suspension, a dry granulate for an aqueous suspension or a dispersible tablet, a capsule, a tablet, optionally film-coated. A preferred composition for oral administration is a tablet, in particular a film-coated tablet. A further preferred composition is an aqueous suspension. The aqueous suspension can be administered as such or prepared by adding a sufficient amount of water to a dry powder for an aqueous suspension, a dry granulate for an aqueous suspension or a dispersible tablet.

The term tablet also comprises fast-disintegrating tablets, amongst which are dispersible tablets and effervescent tablets.

The most commonly used methods of tablet preparation are direct compression, dry granulation and wet granulation. Direct compression involves compressing a mixture containing the active ingredient(s) and the excipient(s) on a tablet press (L. Lachman et al., in: The Theory and Practice of Industrial Pharmacy, 3rd ed., 1986). The mixture to be compressed must possess both good flow and compression properties in order to produce tablets having a uniform content of the active ingredient(s). Good flow properties cannot always be achieved by adding appropriate excipients, such as lubricants, anti-adhesive agents and flow-promoters to the mixture. Hence frequently the mixture is granulated prior to compression.

Granulation is a process by which sphere-like or regularly shaped aggregates called granules are formed out of the powder mixture. This can be achieved by dry granulation

methods and wet granulation methods. Granulation is also used for converting a mixture of powders with poor cohesion into aggregates, which when compressed result in tablets that have good cohesion properties.

In the case of fast-disintegrating tablets, the active ingredient(s), optionally in admixture with one or more excipients, is (are) advantageously provided with a coating in order to mask the taste of such ingredient(s) and/or to protect the same against possible harmful effects by light and/or moisture and in the case of bendamustine to protect the mucosa in the mouth against the harmful effects exerted by the active compound. For that purpose a granulate preferably is prepared and processed as further outlined below.

The expression "granulate" refers to aggregates of particles, sometimes called granules. A granulate in general is prepared by compaction and/or compression techniques (dry granulation) or by wet granulation techniques, using a liquid in which optionally a wet granulation binding agent is dissolved (Remington's Pharmaceutical Sciences 18th ed. 1990, page 1641). Wet granulation techniques also include extrusion techniques. Accordingly the term granulate also comprises pellets, spherules, and extrudates, of which pellets preferably are used as examples of a granulate.

A pellet may be described as a small particle of approximately 1.0 - 1.6 mm in diameter and having a certain density, which particle is prepared by application of the pharmaceutical processes of extrusion and spheronisation to powder mixtures.

The active ingredient(s), optionally in admixture with one or more excipients, may be advantageously provided with a coating in order to mask the taste of such ingredient and/or to protect the same against possible harmful effects by light and/or moisture and/or to protect the mucosa in the mouth against the harmful effects exerted by the active compound.

Preferably the dosage forms to be used in accordance with the dosage regimens according to the invention are prepared by dry compaction techniques. Suitable techniques are for example described in Remington's Pharmaceutical Science 18th. ed. 1990, page 1644. They comprise dry granulation, roller compaction and direct compression. When tablets are prepared by these techniques, it is even more advantageous to use direct compression.

The dosage forms to be used in accordance with the ttreatment regimen according to the present invention are preferably provided with a coating. The coating has different purposes: it may serve for masking the taste of the active ingredient(s) used in the

composition, whilst at the same time it is protecting the active ingredient against possible harmful effects by light and/or moisture such as oxidation, degradation, etc. Furthermore, the coating layer may prevent the subject from damage of the oral mucosa by the active ingredient.

The coating layer can be applied to the dosage forms by techniques well-known in the art such as spray-coating and microencapsulation. For tablets it can be in the form of a film-coating, a saccharide-coating or a compression coating. Preferably a film-coating process is used (Remington's Pharmaceutical Sciences 18th ed. 1990, page 1666). In case an active ingredient requires the application of a coating for fast-disintegrating tablets the individual granules can suitably be provided with a coating prior to compression into tablets.

Preferably it also contains a filler or diluents agent. Examples of such suitable compounds are:

- sugars, which may be selected from the group consisting of sucrose, fructose, sorbitol, xylitol, maltitol, aspartame, erythritol, isomalt, trehalose, maltose, mannose, sorbose, xylose, dextran, dextrin, pullulan, mannitol and lactose;
- microcrystalline cellulose or microfine cellulose;
- starch, a soluble starch or a starch derivative, such as a hydroxyethyl starch;
- calcium carbonate, sodium chloride, calcium phosphate, calcium hydrogen phosphate, calcium sulfate, sodium phosphate, carmellose potassium, carmellose calcium, carmellose sodium, synthetic aluminum silicate, etc.

Most preferred are microcrystalline cellulose and a sugar, selected from the group consisting of D-mannitol, erythritol, isomalt and trehalose. However, there is a preference for the use of microcrystalline cellulose, in view of stability of the composition containing fidaxomicin and xanthan gum, under a variety of storage conditions. On top of that for certain groups of patients who should not take sugar-containing compositions, the use of microcrystalline cellulose is advantageous.

The amount of microcrystalline cellulose should be as low as possible, but does not seem to be critical. The same is true when a sugar is used.

The granulate may further contain one or more of a disintegrant, since it is important that the fidaxomicin is quickly and uniformly dispersed, both in in vitro and in vivo situations. Suitable disintegrating agents are corn starch, potato starch, partly pregelatinized starch, but also the so-called super-disintegrants can be used; examples of which are crosscarmellose calcium, crosscarmellose sodium, crospovidone, sodium starch glycolate, low-substituted

hydroxypropylcellulose and Amberlite IRP 88. A preferred disintegrant is sodium starch glycolate, which is commercially available under the trademark Primojel®. This disintegrant has shown that it is effective in compositions which contain either microcrystalline cellulose or a sugar as the diluents. Further it has shown that it contributes to an easy manufacturing of a granulate composition. Optionally a second disintegrant can be used, such as partly pregelatinised starch.

The composition to be used in accordance with the treatment regimen according to the invention can be an aqueous suspension, preferably in admixture with excipients, such as buffering agents, preservatives, flavouring agents, sweetening agents and viscosity increasing agents. Most preferably the compositions contain flavouring and sweetening agents to mask the taste of the tiacumicin compounds.

Examples of buffering agents are hydrochloric acid, diluted hydrochloric acid, sulfuric acid, adipic acid and its salt, citric acid and its salt, gluconic acid and its salt, succinic acid and its salt, ascorbic acid and its salt, glacial acetic acid and its salt, acetic acid and its salt, tartaric acid and its salt, fumaric acid and its salt, maleic acid and its salt, lactic acid and its salt, malic acid and its salt, phosphoric acid, and its salt, glycine, sodium hydrogencarbonate, sodium carbonate, sodium hydroxide, magnesium hydroxide etc. and combinations of the afore¬mentioned agents.

Examples of preservatives are benzoic acid and its salt, an edetate acid and its salt, salicylic acid and its salt, dibutylhydroxytoluene, sorbic acid and its salt, a sodium dehydroacetate, para-hydroxybenzoic acid, and its salt, methylparaben, propylparaben, etc. and combinations of the afore-mentioned preservatives.

Examples of flavouring agents are orange essence, an orange oil, caramel, camphor, cinnamon oil, a spearmint oil, strawberry essence, chocolate essence, a cherry flavor, oil of bitter orange, pine|pineapple oil, mentha oil, a vanilla flavor, bitter essence, a fruits flavor, peppermint essence, a mix flavor, a mint flavor, menthol, lemon powder, a lemon oil, a rose oil etc. and combinations of the afore-mentioned flavouring agents.

Examples of sweetening agents are sucralose, aspartame, fructose, xylitol, glycyrrhizinic acid and its salt, saccharin and its salt, stevia, sucrose, sorbitol, glucose, hydrogenated maltose starch syrup, maltitol, maltose, etc. and combinations of the afore¬mentioned sweetening agents.

Examples of viscosity enhancing agents are celluloses such as methylcellulose, ethylcellulose, hydroxypropylcellulose, hydroxyethylcellulose,

hydroxypropylmethylcellulose, carboxymethylcellulose; gums such as xanthan gum, guar gum, gellan gum, dextran, carrageenan; polyvinylpyrrolidone; specially treated microcrystalline celluloses, such as water dispersible celluloses (microcrystalline cellulose and sodium carboxymethylcellulose); and combinations of the afore-mentioned viscosity enhancing agents.

Alternatively, the granulate to be used in accordance with the treatment regimen according to the invention in admixture with extragranular excipients can be used for the preparation of dispersible tablets.

Both dosing regimens i and ii according to the invention rapidly reduced ***C. difficile*** counts to below the level of detection. Spores continued to be detected sporadically, but no signs of recurrence of vegetative growth or toxin production were observed. Resolution of CDI was comparable with previously investigated dosing regimens. Effects of fidaxomicin on gut microflora populations such as total anaerobes, Bacteroides, total Clostridia, Lactobacilli, lactose fermenters and facultative anaerobes were modest, with only Bifidobacteria and Enterococci populations declining. Although Bifidobacteria declined to below the level of detection, they recovered to near pre-installation counts, which means to concentrations that were almost as high as before treatment with the tiacumicin compound. Effects of fidaxomicin on bifidobacteria levels in previous models have varied, likely due to variation in the composition of bifidobacteria species in the faecal samples of volunteers. Persistence of fidaxomicin at supra MIC level was noted (2-5 mg/L) but to a lesser extent than seen with some previous fidaxomicin dosing regimens (20 mg/L). Persistence of antimicrobial may prevent recrudescence of CDI spores for longer, whilst allowing recovery of gut microflora and hence the recovery of colonisation resistance.

Another embodiment is therefore directed to a method for recovering of gut Bifidobacteria population in logl Ocfu/ml in a patient, suffering from Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) and receiving oral treatment with a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, to 50 to 90 % of the gut Bifidobacteria population in logl Ocfu/ml prior to administering the tiacumicin compound during days 15-45 after start of the treatment by orally administering the tiacumicin compound to the patient according to a dosage regimen, which is selected from the group consisting of:

i. Administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and

ii. Administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days.

Yet another embodiment is directed to a method for recovering of gut Bifidobacteria population in loglOcfu/ml in a patient, suffering from Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) and receiving oral treatment with a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, to 50 to 90 % of the gut Bifidobacteria population in loglOcfu/ml prior to administering the tiacumicin compound during days 15-45 after start of the treatment by orally administering the tiacumicin compound to the patient according to a dosage regimen, which is selected from the group consisting of:

i. Administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and

ii. Administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days,

wherein the tiacumicin compound is fidaxomicin.

A further embodiment is directed to a method for recovering of gut Bifidobacteria population in logl Ocfu/ml in a patient, suffering from Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) and receiving oral treatment with a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, to 50 to 90 % of the gut Bifidobacteria population in logl Ocfu/ml prior to administering the tiacumicin compound during days 15-45 after start of the treatment by orally administering the tiacumicin compound to the patient according to a dosage regimen, which is selected from the group consisting of:

i. Administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and

ii. Administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days,

wherein the tiacumicin compound is fidaxomicin and is administered to the patient in the form of a film-coated tablet.

A further embodiment relates to a method for maintaining in a patient, suffering from Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD), having taken 200 mg of a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, BID for 5 days and as a consequence thereof having a concentration of the tiacumicin compound, 2-7% of the concentration of the tiacumicin compound, as measured on day 5, by orally administering the tiacumicin compound to the patient according to a follow-up dosage regimen, which is selected from the group consisting of:

i. 5 days of rest and then 200 mg of the tiacumicin compound once daily for a further 10 days and

ii. a single 200 mg of the tiacumicin compound every other day for 20 days,

for at least 20 days after completing the follow-up dosage regimen.

The following example further illustrates the invention. It will be apparent to the skilled person that these examples are solely for illustrative purposes and must not be considered to limit the invention.

EXAMPLES

Reference example

Comparison of Extended Duration fidaxomicin dosing regimens for treatment of *Clostridium difficile* infection (CDI) in an in vitro gut model

The effectiveness of long (Model A: 200 mg BID during 20 days) vs short pulsed (Model B: 200 mg BID during 5 days, rest during 5 days and 5 days 200 mg BID) course fidaxomicin using a validated CDI model was investigated. Results are available for this model (C.H. Chilton et al. (2013) in J. Antimicrobial Chemotherapy Advance Access Sept 2013 and C.H. Chilton et al. , abstract 23rd European Congress of Clinical microbiology & Infectious Disease, April 27-30, 2013, Berlin). The description of the model is provided in example 1 (see there).

Two 3-stage chemostat gut models were inoculated with pooled faeces (n=5). 107cfu CD ribotype 027 (NAPl/BI) spores were added, and then clindamycin (CL, 33.9 mg/L qid, 7d) was instilled to induce CDI i.e. germination and toxin production. Models were then treated with fidaxomicin (200 mg/L bd) for 20 or 5 d to achieve in vivo gut levels. 5 days post treatment in the short course model, a further 5 days fidaxomicin pulse was given. CD total viable counts (TVC), spore counts (SP), toxin titres (CYT), and gut bacteria were measured throughout. Data were also compared with results for FDX given for 7 days (model 7).

RESULTS:

CL induced CD germination and high level toxin production (>3 RU). In the model A, CD TVC and CYT reduced to the limit of detection (LOD) by day 5 & 7 (Fig. 4/12), respectively, with no evidence of recurrence. In the model B CD TVCs were reduced markedly (~4 $\log_{10}$ cfu/mL), but were still detectable on day 5 of treatment. The second 5 days fidaxomicin pulse, decreased TVC and SP to LOD (Fig. 4/12).

Both dosing regimens had limited effect on gut microflora, except bifidobacteria, which decreased -6-8 logio cfu/mL to below LOD and did not recover (Fig 5/12).

CONCLUSIONS:

In an in vitro gut model, 5 days fidaxomicin was less effective than 20 days (or 7 days) fidaxomicin in reducing CD TVC, SP and CYT, but a further 5 days fidaxomicin pulse increased efficacy, and overall was comparable to other dosing regimens (model B and model 7).

Effects on gut flora were similarly modest in model A and model B (and model 7).

5 days fidaxomicin alone may be sub-optimal for CDI treatment, however model A (a 5 days + 5 days pulsed dosing regimen may be as effective as model B (20 days fidaxomicin, extending the total length of coverage to 15 days).

Example 1

Pilot study of extended dosing regimens for treatment of *C, difficile* infection in an *in vitro* gut model

Two tests in a validated three-stage compound continuous culture system (Freeman J, O'Neill FJ, Wilcox MH. The effects of cefotaxime and desacetylcefotaxime upon Clostridium difficile proliferation and toxin production in a triple-stage chemostat model of the human gut. J Antimicrob Chemother 2003; 52: 96-102; Baines SD, Freeman J, Wilcox MH. Effects of piperacillin/tazobactam on Clostridium difficile growth and toxin production in a human gut model. J Antimicrob Chemother 2005; 55: 974-82) were run in parallel. Models were inoculated with pooled faecal slurry (10% in anaerobic distilled water) from healthy volunteers (n=5, age >60yrs).

The continuous culture system consisted of three vessels, V I, V2, and V3, with respective operating volumes of 0.22, 0.32, and 0.32 L (Fig. 1/12). Temperature (37°C) and pH were automatically controlled to reflect the proximal-distal colon. Culture pH in the three vessels was 5.5, 6.2, and 6.8, respectively. Each fermentor was magnetically stirred and maintained under an atmosphere of C02. The growth medium was continuously sparged with 0_2-free N_2 and fed by peristaltic pump to V I. V I sequentially supplied V2 and V3 via a series of weirs. The culture medium consisted of the following constituents (g liter—1) in distilled water: starch (BDH Ltd.), 5.0; pectin (citrus), 2.0; guar gum, 1.0; mucin (porcine gastric type III), 4.0; xylan (oatspelt), 2.0; arabinogalactan (larch wood), 2.0; inulin, 1.0; casein (BDH Ltd.), 3.0; peptone water, 5.0; tryptone, 5.0; bile salts No. 3, 0.4; yeast extract, 4.5; FeS04 z 7H20, 0.005; NaCl, 4.5; KCl, 4.5; KH2PO4, 0.5; MgS04 z 7H20, 1.25; CaC12 z 6H20, 0.15; NaHC03, 1.5; cysteine, 0.8; hemin, 0.05; Tween 80, 1.0 The system was initially operated at a retention time *(R)* of 27.1 h (experiment 1), followed by an increase to R = 66.7 h (experiment 2). Retention time was calculated as the reciprocal of dilution rate. System retention constitutes the sum of individual R values in each fermentor. Minimum doubling times of bacteria were calculated as 0.693/D, where D is the dilution rate (h-1) for each culture vessel. Each fermentor was inoculated with 100 ml of a fresh 10% (w/y) fecal slurry from a healthy, nonmethane producing donor. The

fermentation system was allowed to equilibrate for 2 weeks before the medium pump was started at a flow rate of 13.2 ml/hr (System retention time of 67 hr) , and was run for at least 336 h at each retention time to establish steady-state conditions, before material was taken for analysis. Steady-state conditions were assessed by monitoring short-chain fatty acid (SCFA) formation. Two samples were taken 48 h apart at each steady state. Once gut microbiota populations stabilised, models were spiked with 107 PCR ribotype 027 *C. difficile* spores, and simulated CDI was induced by clindamycin instillation (33.9mg/L, QDS). Once high level toxin production was observed, fidaxomicin treatment commenced. Model C was instilled with 200mg/L fidaxomicin BID for 5 days, followed by five days rest then 200mg/L fidaxomicin once daily for a further 10 days (dosing regimen i). Model D was instilled with 200mg/L fidaxomicin BID for 5 days followed by a single 200mg/L fidaxomicin dose every other day for 20 days (dosing regimen ii). The model was left without further intervention for 2 1 days post treatment.

- Measurements:
  - — *C. difficile* Total viable count and spore count (CFU/ml)
  - — Toxin concentration (Vero cell cytotoxin neutralisation assay)
  - — Microflora composition by selective culture
  - - Resistance emergence
  - - Antimicrobial concentration (bioassay)

Both tapered dosing regimens rapidly (<3 days) reduced *C. difficile* viable counts (~6 $\log_{10}$ cfu/mL), spore counts (~4 $\log_{10}$ cfu/mL) and toxin titres (3 RU) to below the level of detection. Vegetative cells and toxin remained below the level of detection for the remainder of the experiment. Spores were detected sporadically, at the limit of detection, in all three vessels of model C, but only intermittently from vessel 3 in model D. Fidaxomicin concentrations peaked at ~100mg/L in both models. Persistence of fidaxomicin activity was slightly greater in model D (5mg/L) (see Fig. 1IB/12) than model C (2-5 mg/L) (see Fig. 11A/12), and remained at supra-MIC (0.25mg/L) level for the duration of the experiment in both models. The effects of both dosing regimen on gut microflora were similarly limited, with declines in enterococci (2-5 $\log_{10}$ cfu/mL) and Bifidobacteria (6-8 $\log_{10}$ cfu/mL to limit of detection).

Bifidobacteria populations recovered to close to pre-fidaxomicin levels in both models by the end of the experiment.

- 5 days fidaxomicin sufficient to end toxin production

- but, less effective than Model A or model 7 at reducing *C. difficile* total counts and spores
- however, a further pulse of 5 days fidaxomicin reduced *C. difficile* counts further (comparable to model A or model 7)
- Effects of fidaxomicin pulse vs extended dosing on gut flora similarly modest
- Persistence of active fidaxomicin greater in extended and pulsed dosing regimens
- Pulsed dosing regimen may increase fidaxomicin persistence
- Further studies required to determine optimal dosing regimen to minimise recurrence

Conclusions: Both evaluated tapered dosing regimens were effective for rapid resolution of simulated CDI in an in vitro gut model, and were comparable to previously evaluated standard and pulsed dosing regimen. Persistence of antimicrobial activity and some suppression of *C. difficile* spore recovery was observed. Tapered dosing regimens may help to suppress *C. difficile* spore germination for long periods of time, whilst allowing recovery of the indigenous gut microflora.

INDUSTRIAL APPLICABILITY

The treatment regimens with fidaxomicin compositions according to the present invention shows many advantages.

The extension of the treatment duration period out from 10 to 20 or 25 days allows additional time for recovery of the patients colonic microflora which provides colonisation resistance against subsequent CDI relapse/recurrence without using additional medication. Therefore the clear benefit of the dosing regimens according to the present invention over the 20 day twice daily regimen is that it provides equivalent efficacy in terms of reduction of C. difficile cells, spores and toxin while allowing recovery of the bowel flora which is expected to translate into a further reduction in the recurrence rate over the existing dose (200 mg BID during 10 days), but it does this using the standard 10 day pack of fidaxomicin tablets (DIFICLIR™) rather than having to use 2 packs.

Therefore by changing the dosing frequency it is expected that the sustained clinical cure achieved using 1 pack of fidaxomicin tablets (DIFICLIR™) may be increased from around 14% to <5%. However also other fidaxomicm-containing compositions, such as a suspension, will have the same effect.

If the proposed clinical study based on the results of the in vitro test will be successful then it will be obvious that where possible, the recommended dosing regimen will be changed

from the twice daily 200 mg for 10 days regimen to the dosing regimens according to the present invention. The expected benefit to patients, doctors and society would be that reducing the recurrence to below 5% would significantly alter the cost effectiveness argument in fidaxomicin's favour.

5

CLAIMS

1. A tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regimen selected from the group consisting of:
   i. Administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and
   ii. Administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days.

2. The tiacumicin compound for use according to claim 1, characterized in that the tiacumicin compound is selected from the group consisting of tiacumicin A, tiacumicin B and analogues thereof, (dialkyltiacumicins and bromotiacumicins), tiacumicin C, tiacumicin D, tiacumicin E, tiacumicin F and lipiarmycin.

3. The tiacumicin compound for use according to each of claims 1-2, characterized in that the tiacumicin compound is lipiarmycin or tiacumicin B or a stereo-isomer thereof.

4. The tiacumicin compound for use according to each of claims 1-3, characterized in that the tiacumicin compound is tiacumicin B or a polymorph thereof.

5. The tiacumicin compound for use according to each of claims 1-4, characterized in that the tiacumicin compound is R-tiacumicin B (fidaxomicin).

6. The tiacumicin compound for use according to each of claims 1-5, characterized in that a tablet, a suspension, a dry powder for an aqueous suspension, a dry granulate for an aqueous suspension, a film-coated tablet or a dispersible tablet is used.

7. The tiacumicin compound for use according to each of claims 1-6, characterized in that a film-coated tablet is used.

8. A pharmaceutical composition, comprising a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, for use in the oral treatment of Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) in a patient according to a dosage regimen selected from the group consisting of:

iii. Administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and

iv. Administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days.

9. The pharmaceutical composition, comprising the tiacumicin compound, for use according to claim 8, characterized in that the tiacumicin compound is selected from the group consisting of tiacumicin A, tiacumicin B and analogues thereof, (dialkyltiacumicins and bromotiacumicins), tiacumicin C, tiacumicin D, tiacumicin E, tiacumicin F and lipiarmycin.

10. The pharmaceutical composition, comprising the tiacumicin compound, for use according to each of claims 8-9, characterized in that the tiacumicin compound is lipiarmycin or tiacumicin B or a stereo-isomer thereof.

11. The pharmaceutical composition, comprising the tiacumicin compound, for use according to each of claims 8-10, characterized in that the tiacumicin compound is tiacumicin B or a polymorph thereof.

12. The pharmaceutical composition, comprising the tiacumicin compound, for use according to each of claims 8-11, characterized in that the tiacumicin compound is R-tiacumicin B (fidaxomicin).

13. The pharmaceutical composition, comprising the tiacumicin compound, for use according to each of claims 8-12, characterized in that the composition is a tablet, a suspension, a dry powder for an aqueous suspension, a dry granulate for an aqueous suspension, a film-coated tablet or a dispersible tablet.

14. The pharmaceutical composition, comprising the tiacumicin compound, for use according to each of claims 8-13, characterized in that the composition is a film-coated tablet.

15. Method for recovering of gut Bifidobacteria population in logl Ocfu/mL in a patient, suffering from Clostridium difficile infections (CDI) or Clostridium difficile associated diarrhea or disease (CDAD) and receiving oral treatment with a tiacumicin compound, a stereo-isomer thereof, a polymorph thereof or a pharmaceutically acceptable solvate thereof, to 50 to 90 % of the gut Bifidobacteria population in loglOcfu/ml prior to administering the tiacumicin compound during days 15-45 after start of the treatment, by orally administering the tiacumicin compound to the patient according to a dosage regimen, which is selected from the group consisting of:

i. Administering 200 mg of the tiacumicin compound BID for 5 days followed by 5 days of rest and then 200 mg once daily for a further 10 days and
ii. Administering 200 mg of the tiacumicin compound BID for 5 days followed by a single 200 mg every other day for 20 days.

16. The method for recovering according to claim 15, characterized in that the tiacumicin compound is selected from the group consisting of tiacumicin A, tiacumicin B and analogues thereof, (dialkyltiacumicins and bromotiacumicins), tiacumicin C, tiacumicin D, tiacumicin E, tiacumicin F and lipiarmycin.

17. The method for recovering according to each of claims 15-16, characterized in that the tiacumicin compound is lipiarmycin or tiacumicin B or a stereo-isomer thereof.

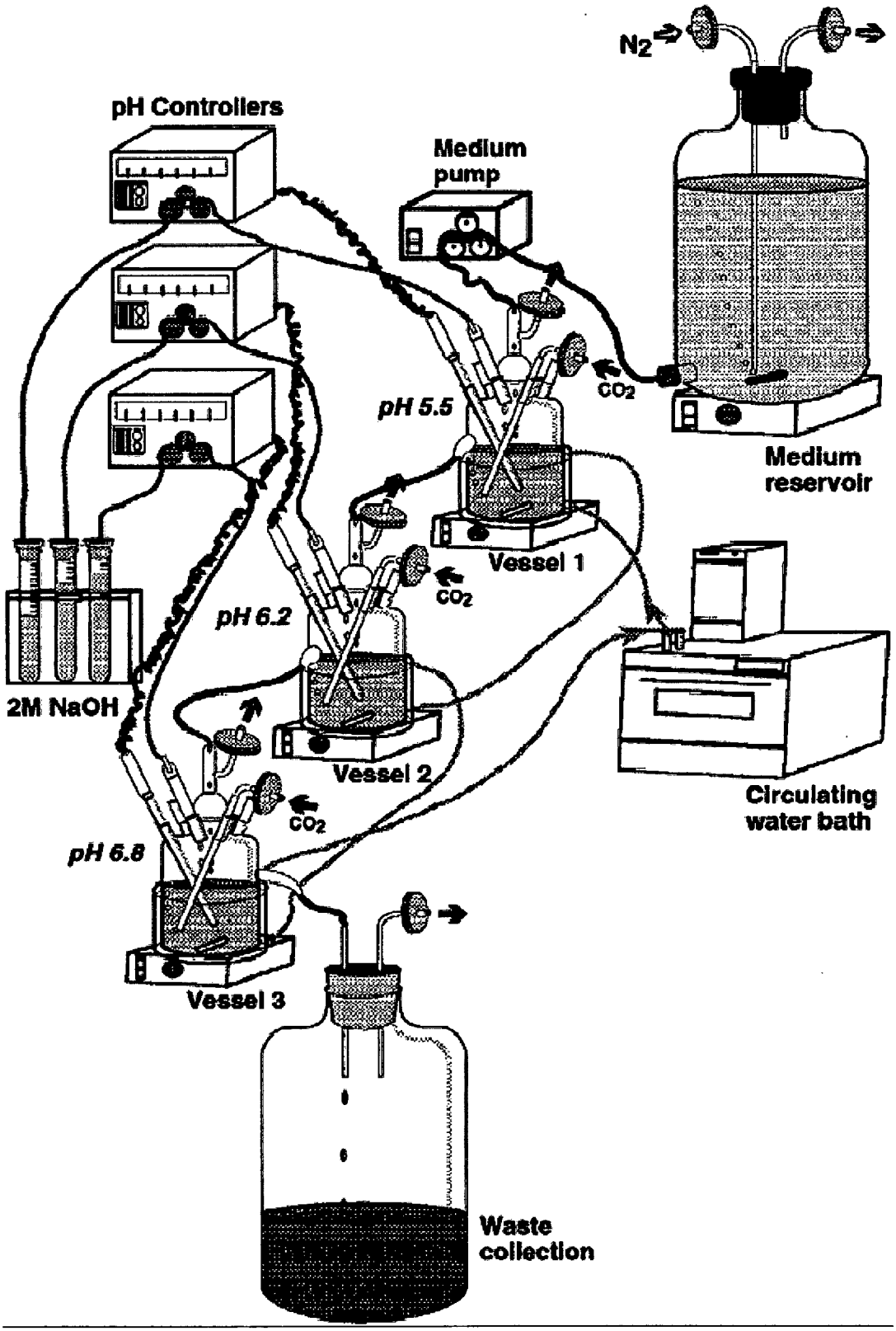
18. The method for recovering according to each of claims 15-17, characterized in that the tiacumicin compound is tiacumicin B or a polymorph thereof.

19. The method of recovering according to each of claims 15-18, characterized in that the tiacumicin compound is R-tiacumicin B (fidaxomicin).

20. The method of recovering according to each of claims 15-19, characterized in that a tablet, a suspension, a dry powder for an aqueous suspension, a dry granulate for an aqueous suspension, a film-coated tablet or a dispersible tablet is used.

21. The method of recovery according to each of claims 15-20, characterized in that a film-coated tablet is used.

Fig. 1/12


N2
pH Controllers
Medium pump
pH 5.5
CO2
Medium reservoir
Vessel 1
pH 6.2
CO2
2M NaOH
Vessel 2
Circulating water bath
CO2
pH 6.8
Vessel 3
Waste collection

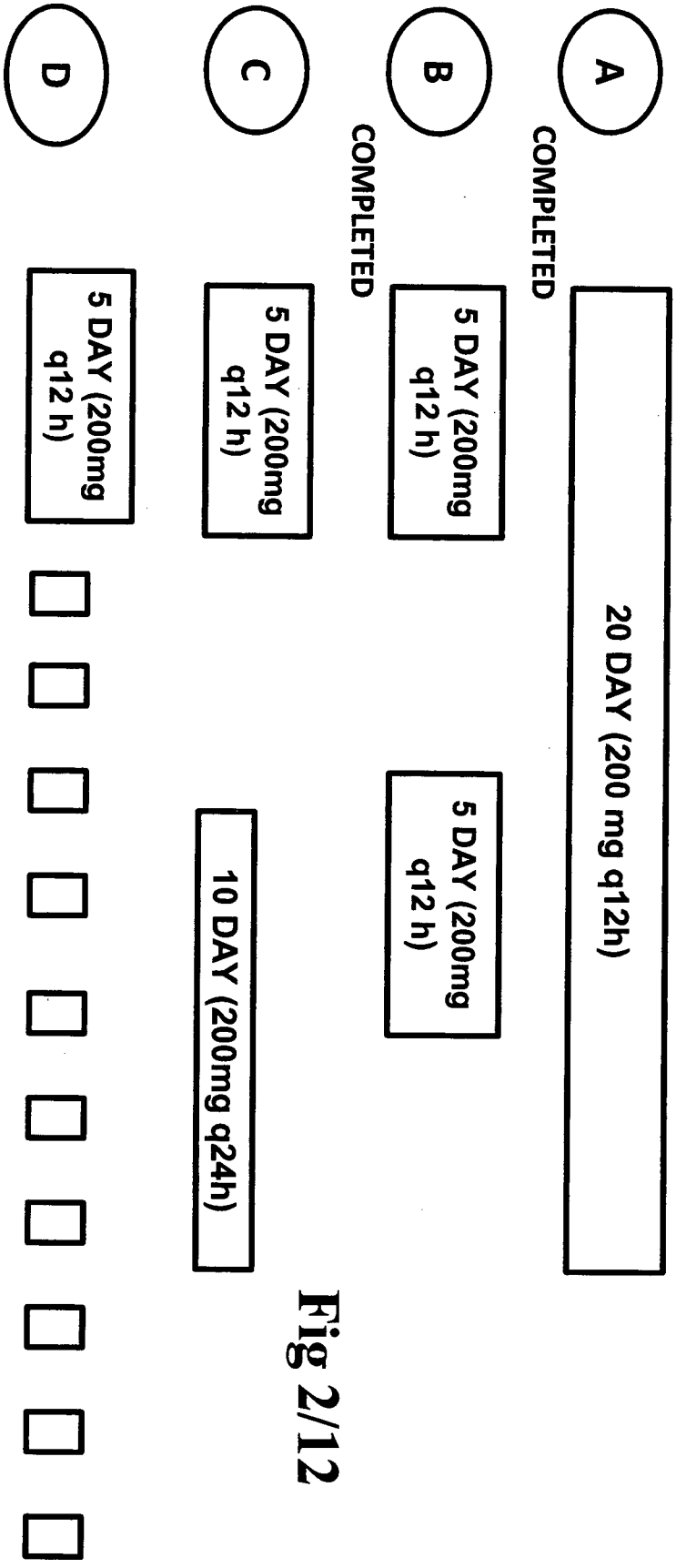

A
B
C
D
COMPLETED
COMPLETED
20 DAY (200 mg q12h)
5 DAY (200mg q12 h)
5 DAY (200mg q12 h)
5 DAY (200mg q12 h)
5 DAY (200mg q12 h)
10 DAY (200mg q24h)

Fig 2/12

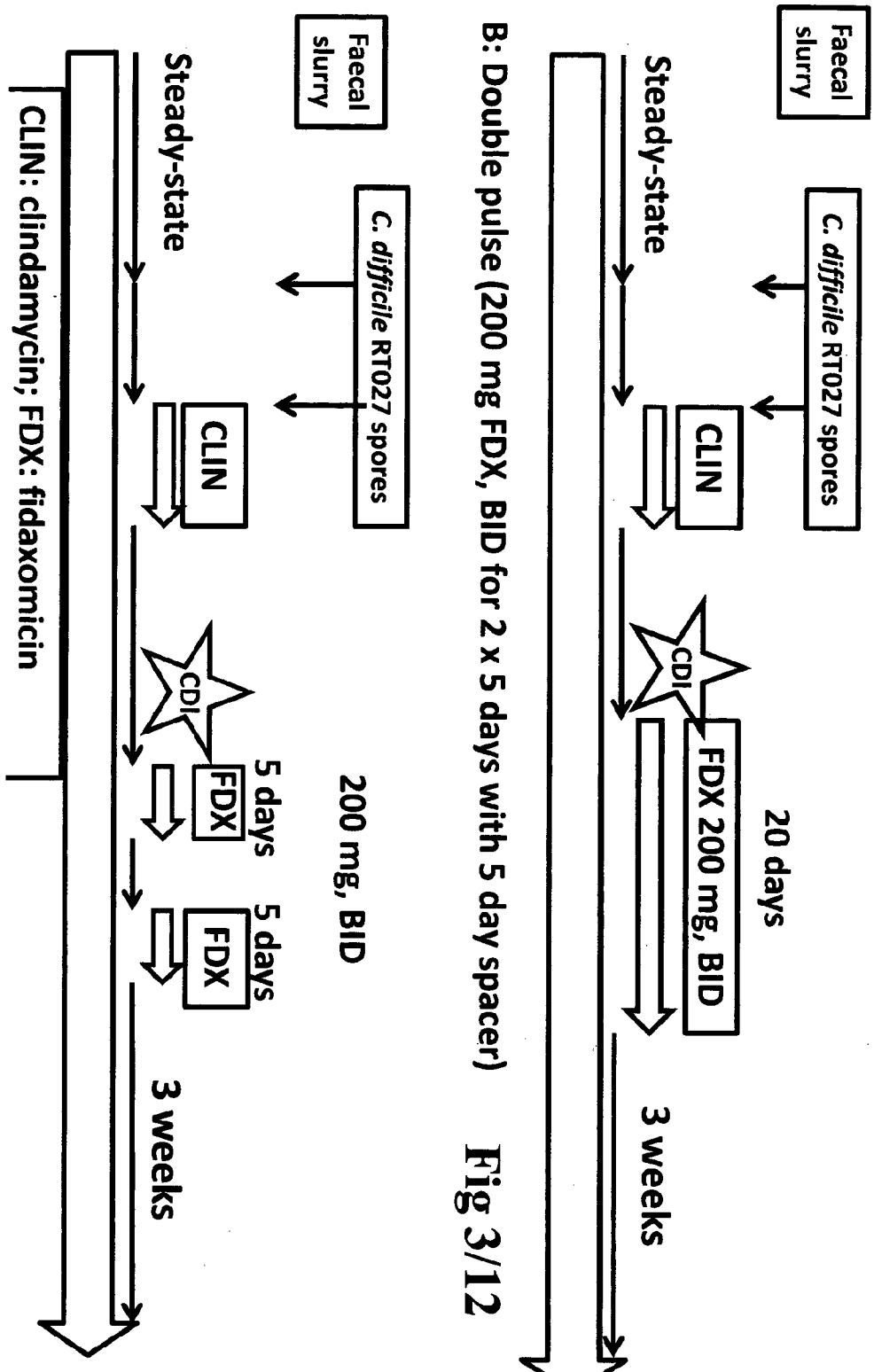

Experimental Design
A: 20 day dose (200 mg FDX, BID for 20 days)
Faecal slurry
C. difficile RT027 spores
CLIN
Steady-state
CDI
FDX 200 mg, BID
20 days
3 weeks
B: Double pulse (200 mg FDX, BID for 2 x 5 days with 5 day spacer)
Faecal slurry
C. difficile RT027 spores
CLIN
Steady-state
CDI
FDX
5 days
200 mg, BID
5 days
FDX
3 weeks
CLIN: clindamycin; FDX: fidaxomicin

Fig 3/12

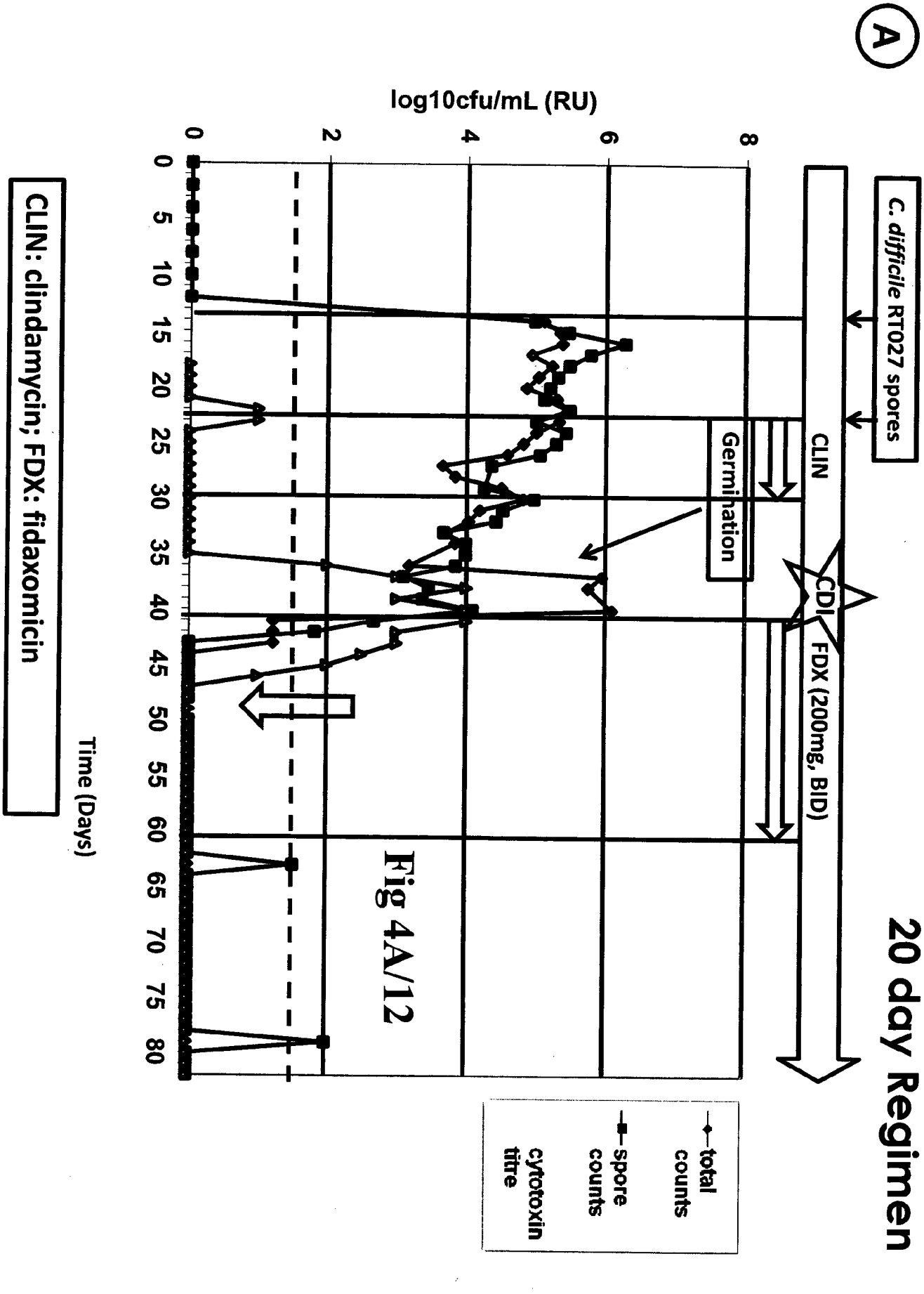

A
20 day Regimen
C. difficile RT027 spores
CLIN
CDI
FDX (200mg, BID)
Germination
log10cfu/mL (RU)
0
2
4
6
8
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Time (Days)
total counts
spore counts
cytotoxin titre
CLIN: clindamycin; FDX: fidaxomicin

Fig 4A/12

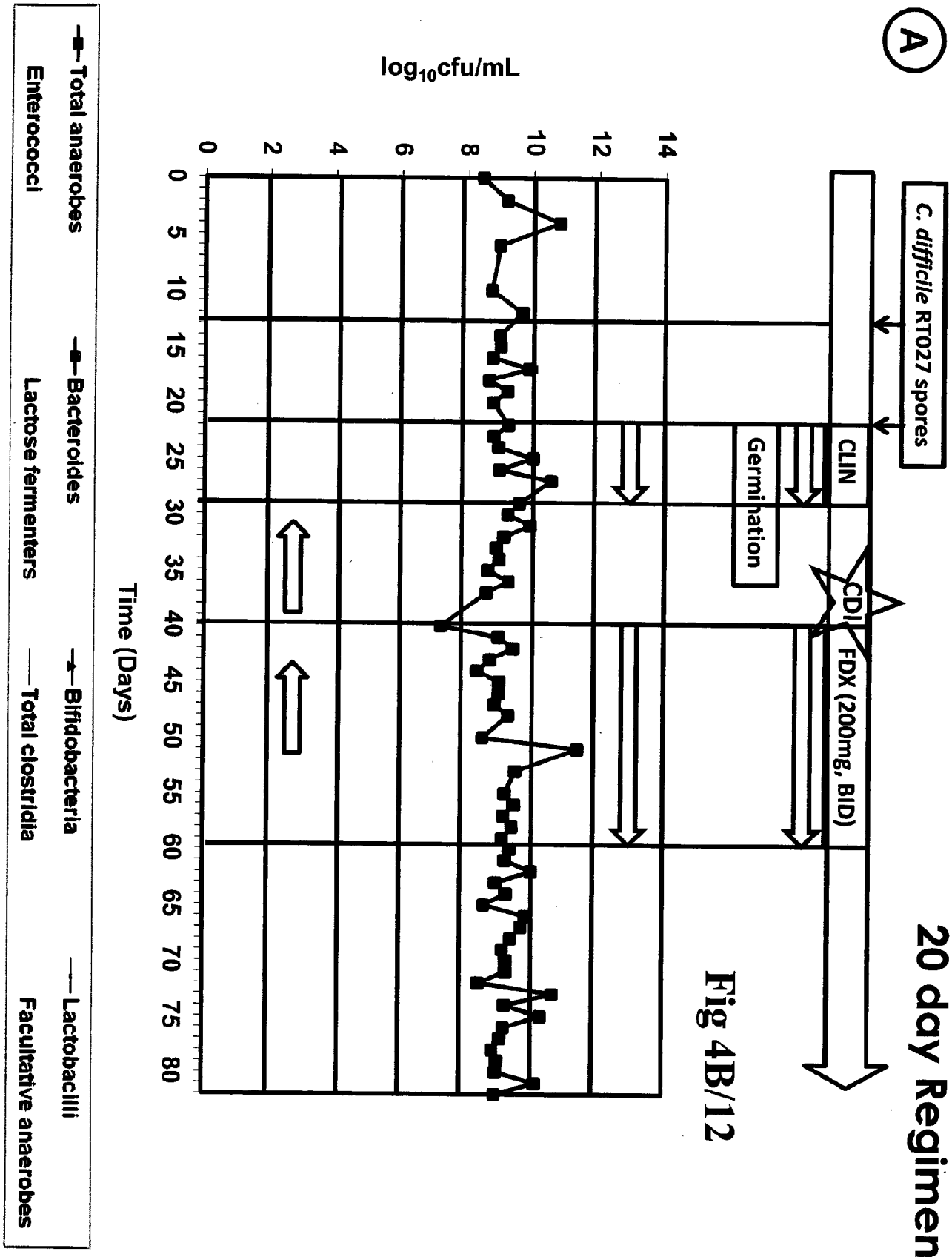

A
20 day Regimen
C. difficile RT027 spores
CLIN
Germination
CDI
FDX (200mg, BID)
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 4B/12

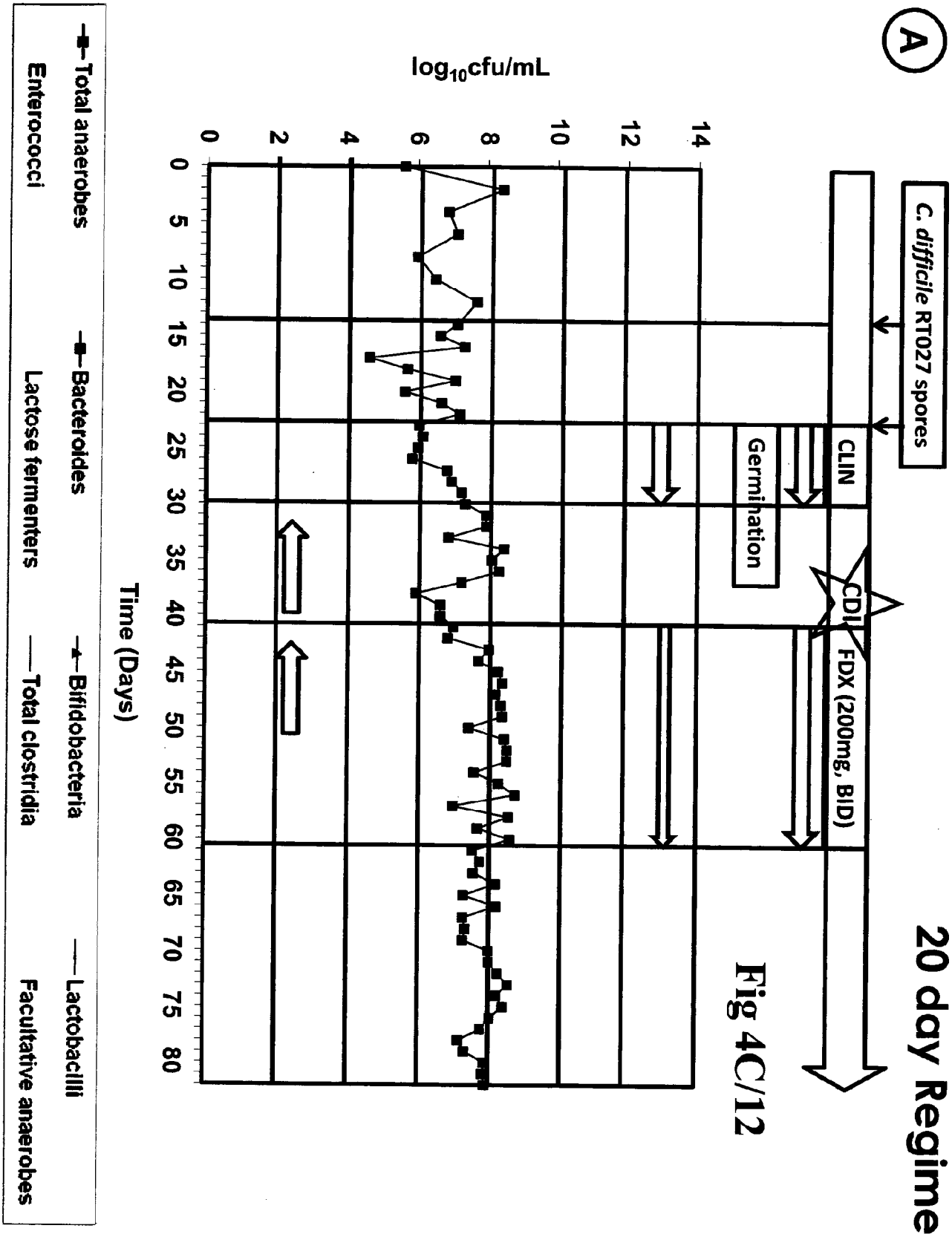

A
20 day Regimen
C. difficile RT027 spores
CLIN
Germination
CDI
FDX (200mg, BID)
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 4C/12

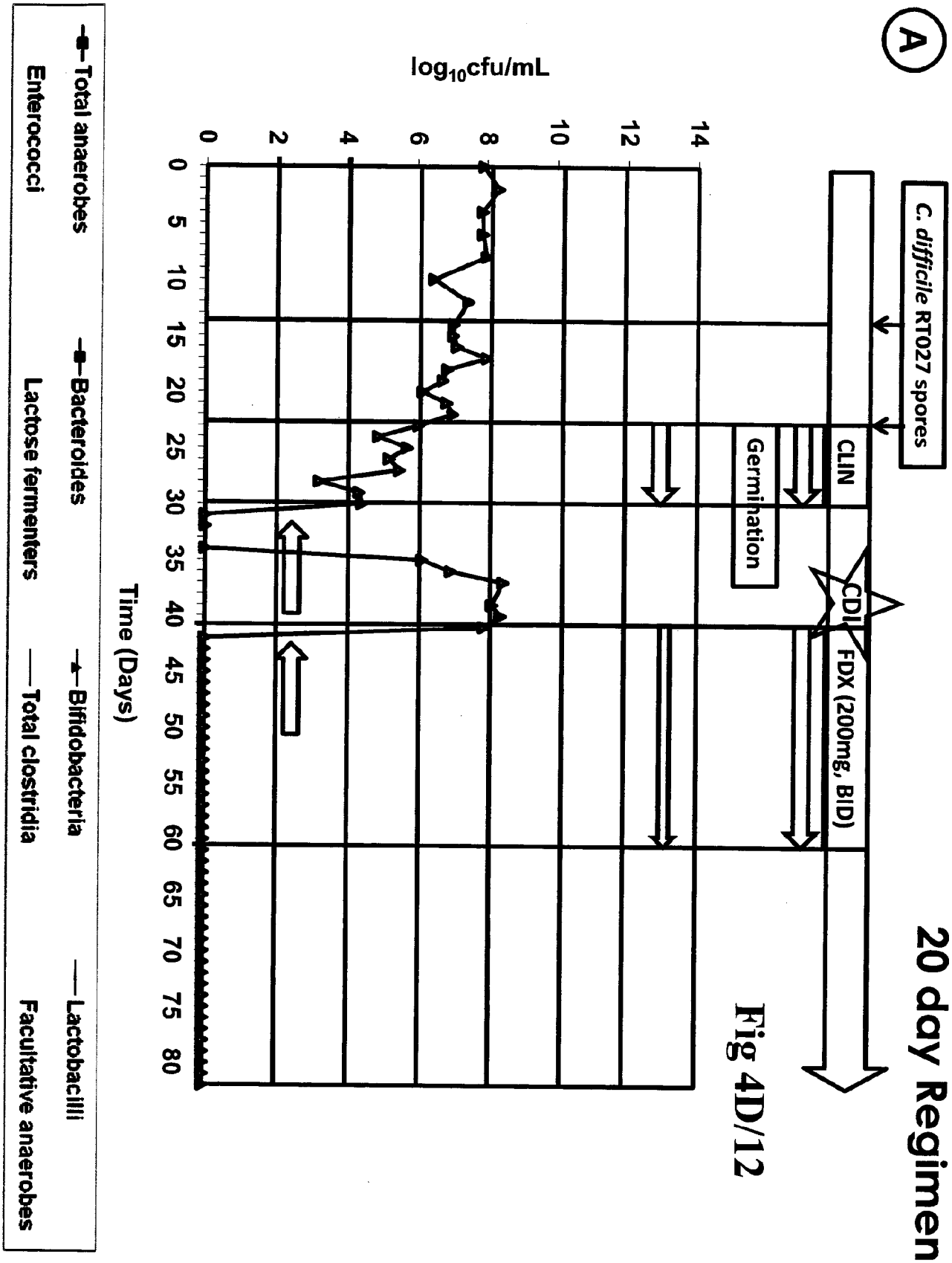

A
20 day Regimen
C. difficile RT027 spores
CLIN
Germination
CDI
FDX (200mg, BID)
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 4D/12

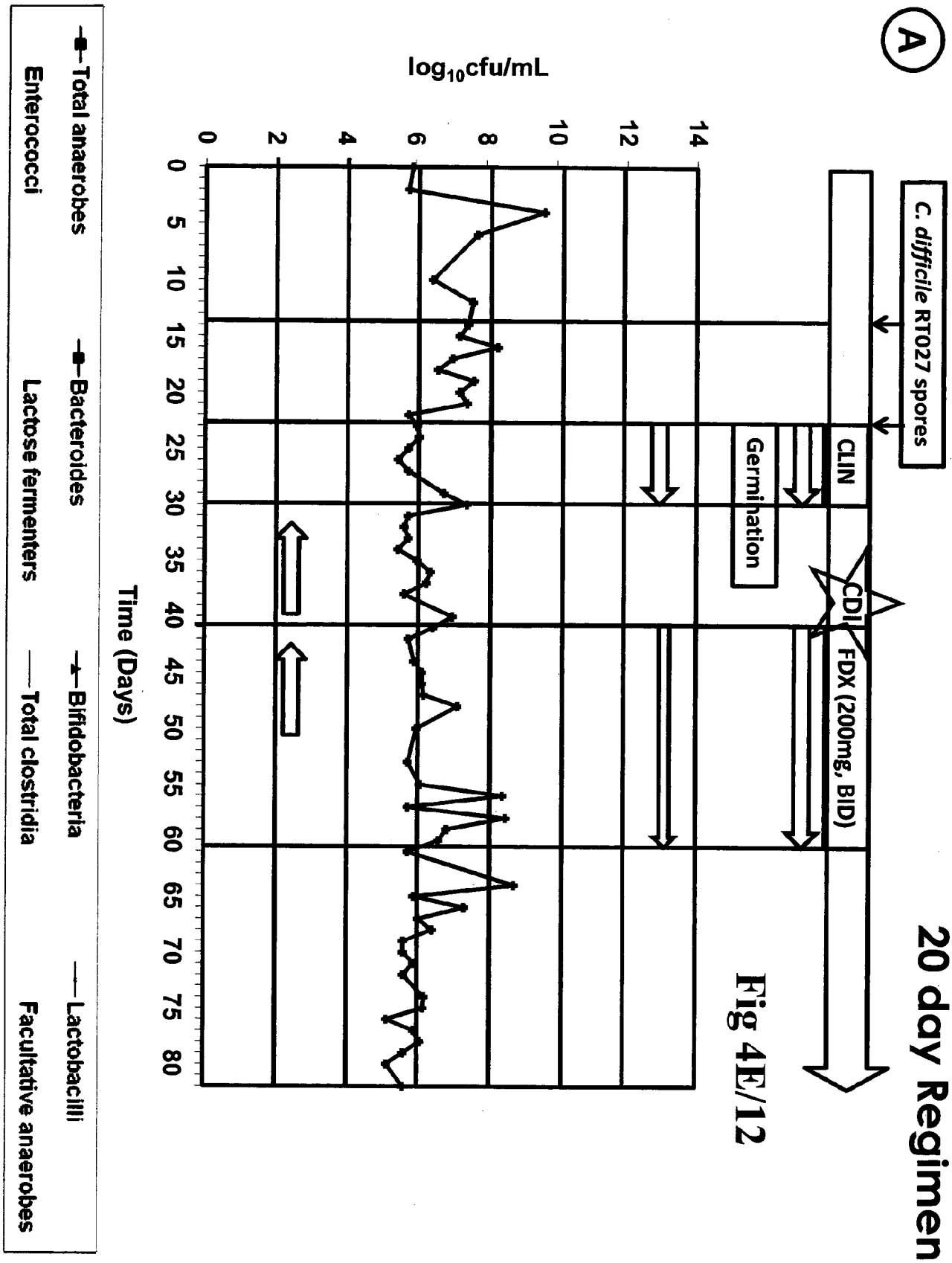

A
20 day Regimen
C. difficile RT027 spores
CLIN
CDI
FDX (200mg, BID)
Germination
log10cfu/mL
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 4E/12

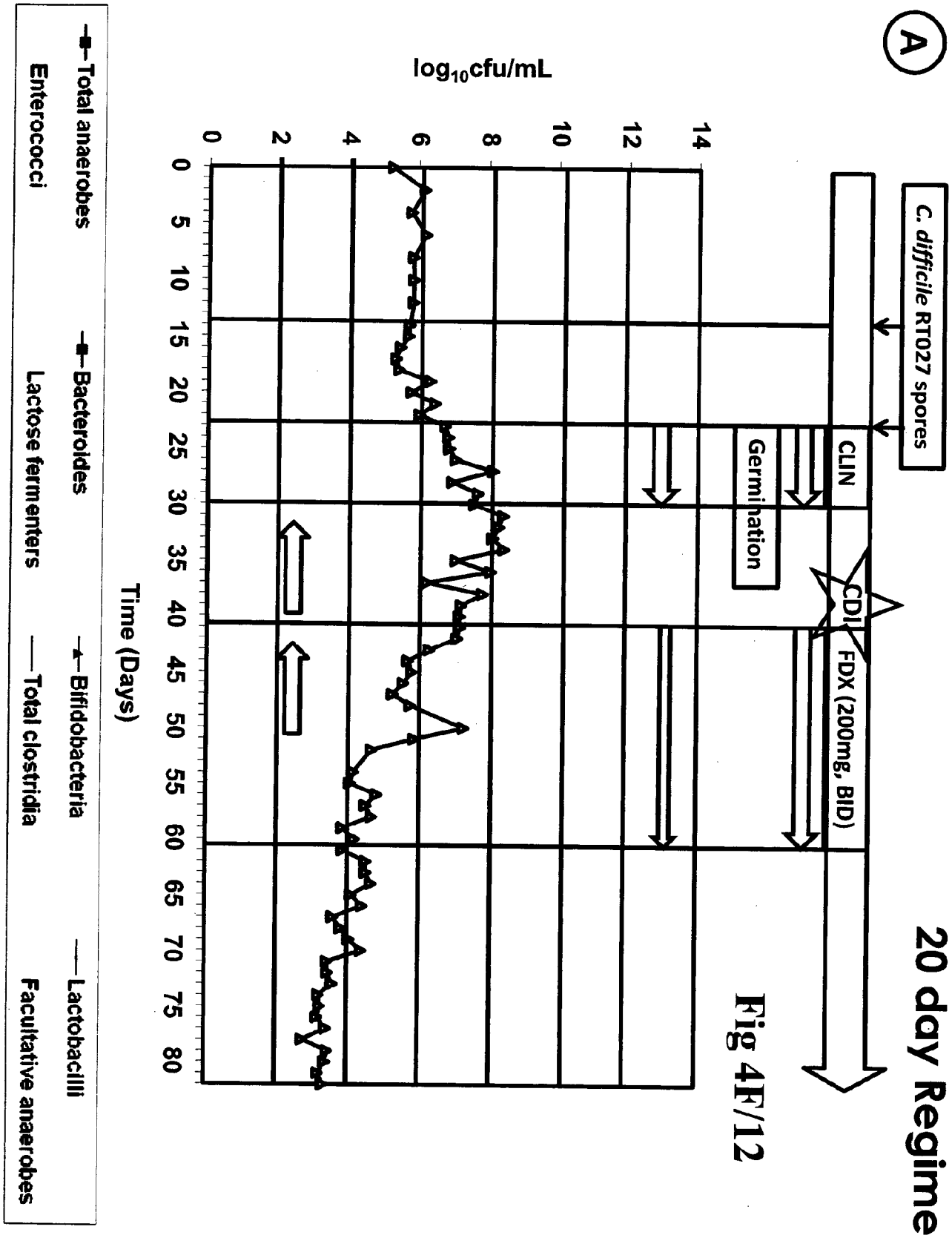

A
20 day Regimen
C. difficile RT027 spores
CLIN
CDI
FDX (200mg, BID)
Germination
log10cfu/mL
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 4F/12

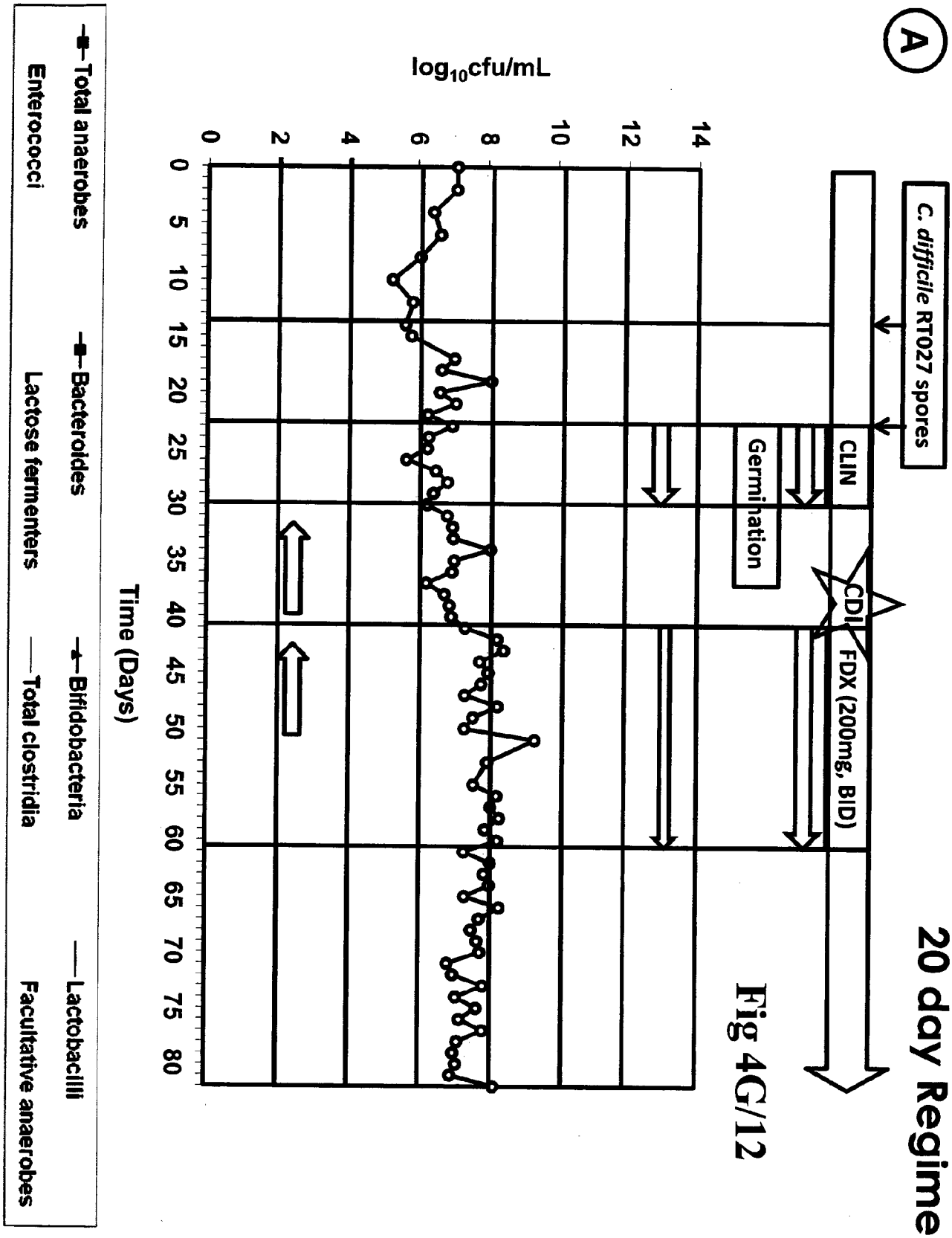

A
20 day Regimen
C. difficile RT027 spores
CLIN
Germination
CDI
FDX (200mg, BID)
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 4G/12

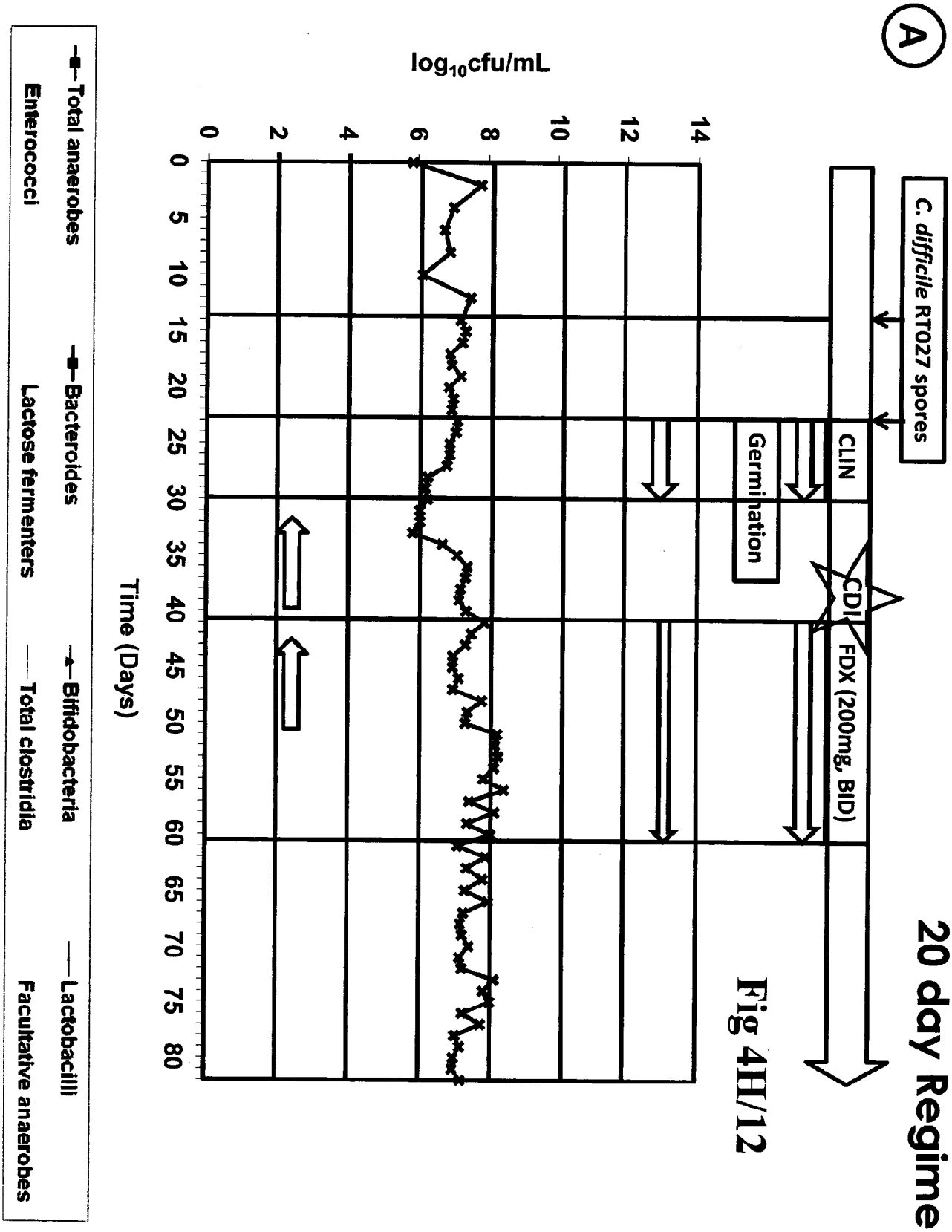

A
20 day Regimen
C. difficile RT027 spores
CLIN
CDI
FDX (200mg, BID)
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 4H/12

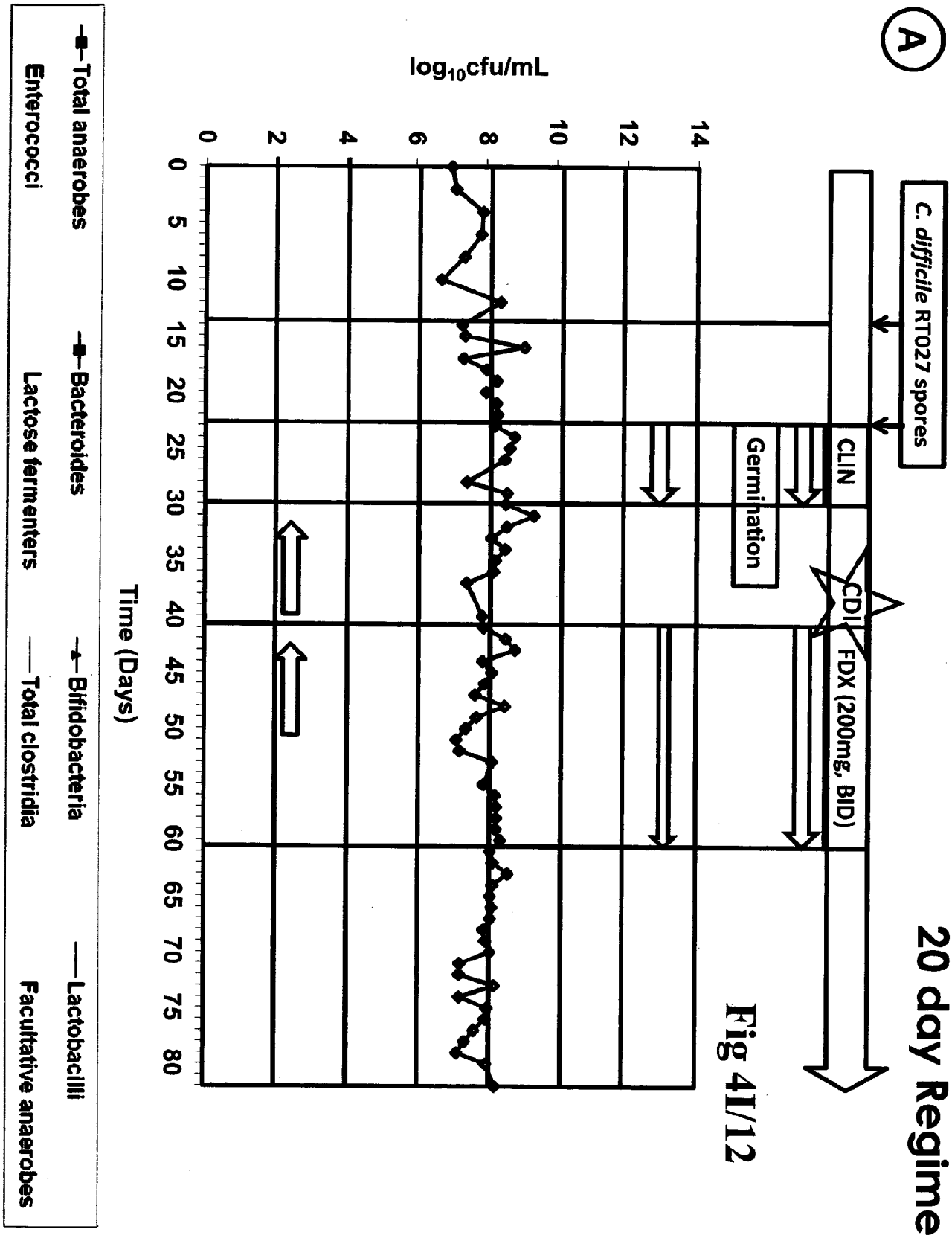

A
20 day Regimen
C. difficile RT027 spores
CLIN
Germination
CDI
FDX (200mg, BID)
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 4I/12

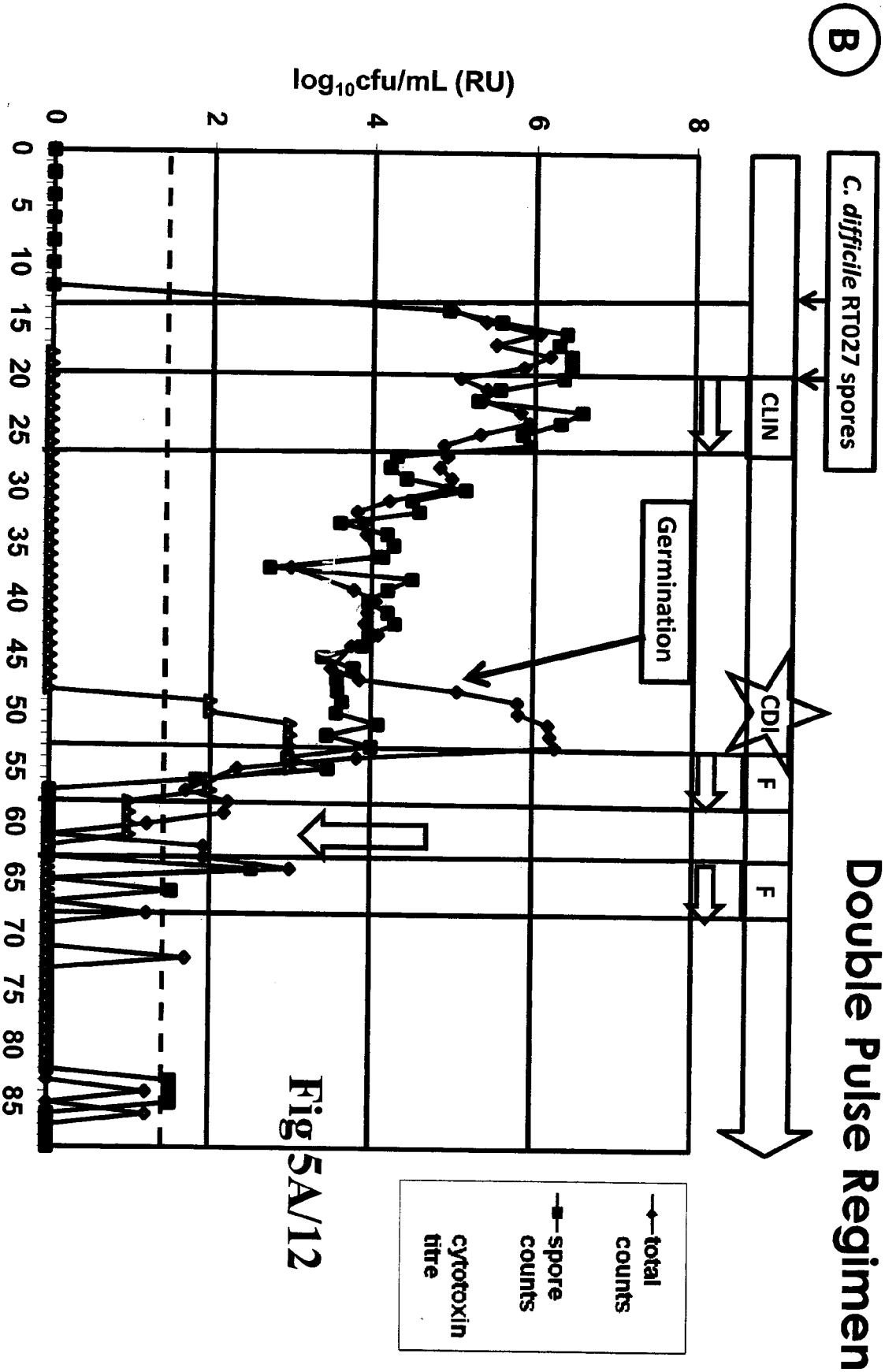

B
Double Pulse Regimen
C. difficile RT027 spores
CLIN
Germination
CDI
F
F
log10cfu/mL (RU)
0
2
4
6
8
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Time (Days)
total counts
spore counts
cytotoxin titre

Fig 5A/12

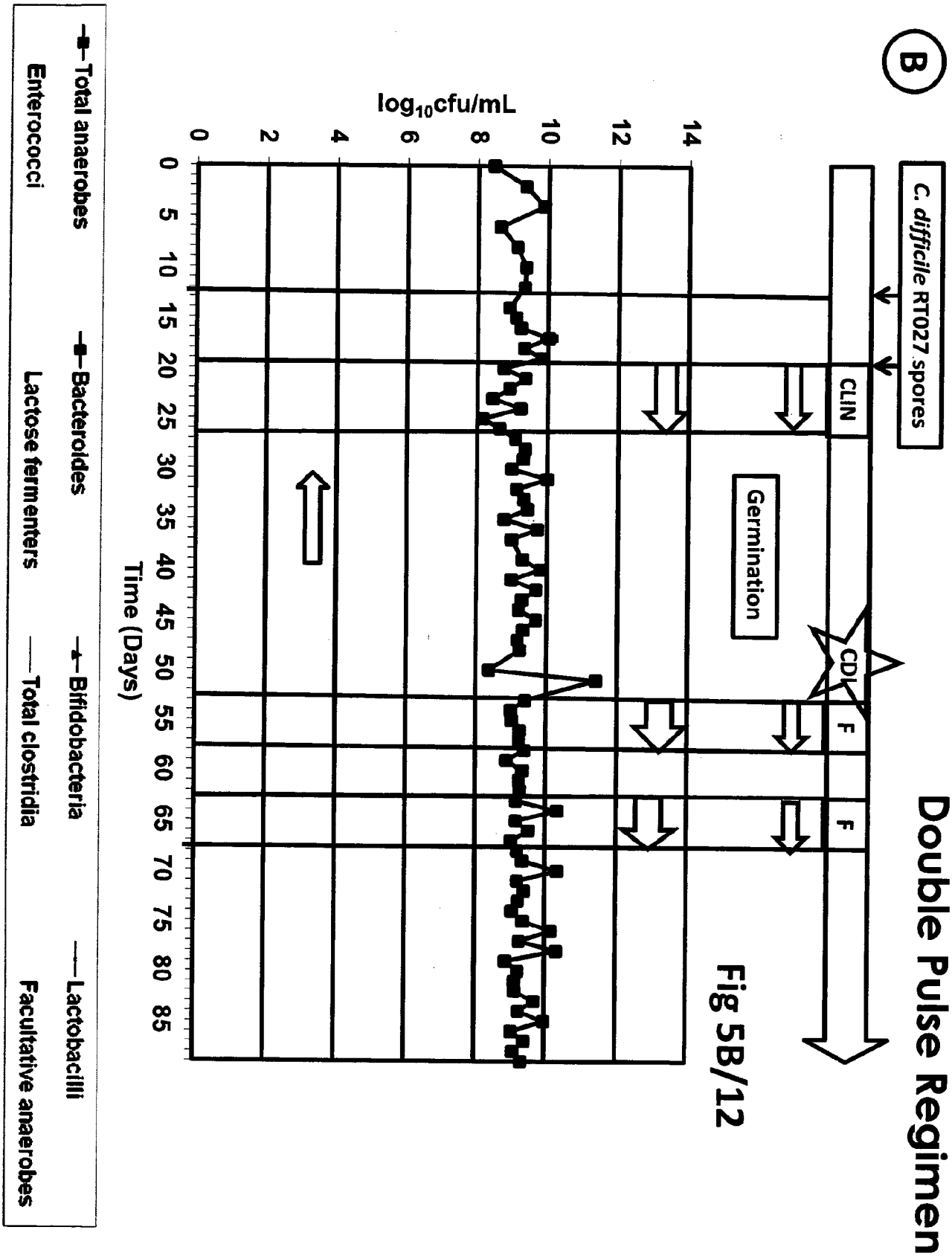

B
Double Pulse Regimen
C. difficile RT027 spores
CLIN
CDI
F
F
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
Time (Days)
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 5B/12

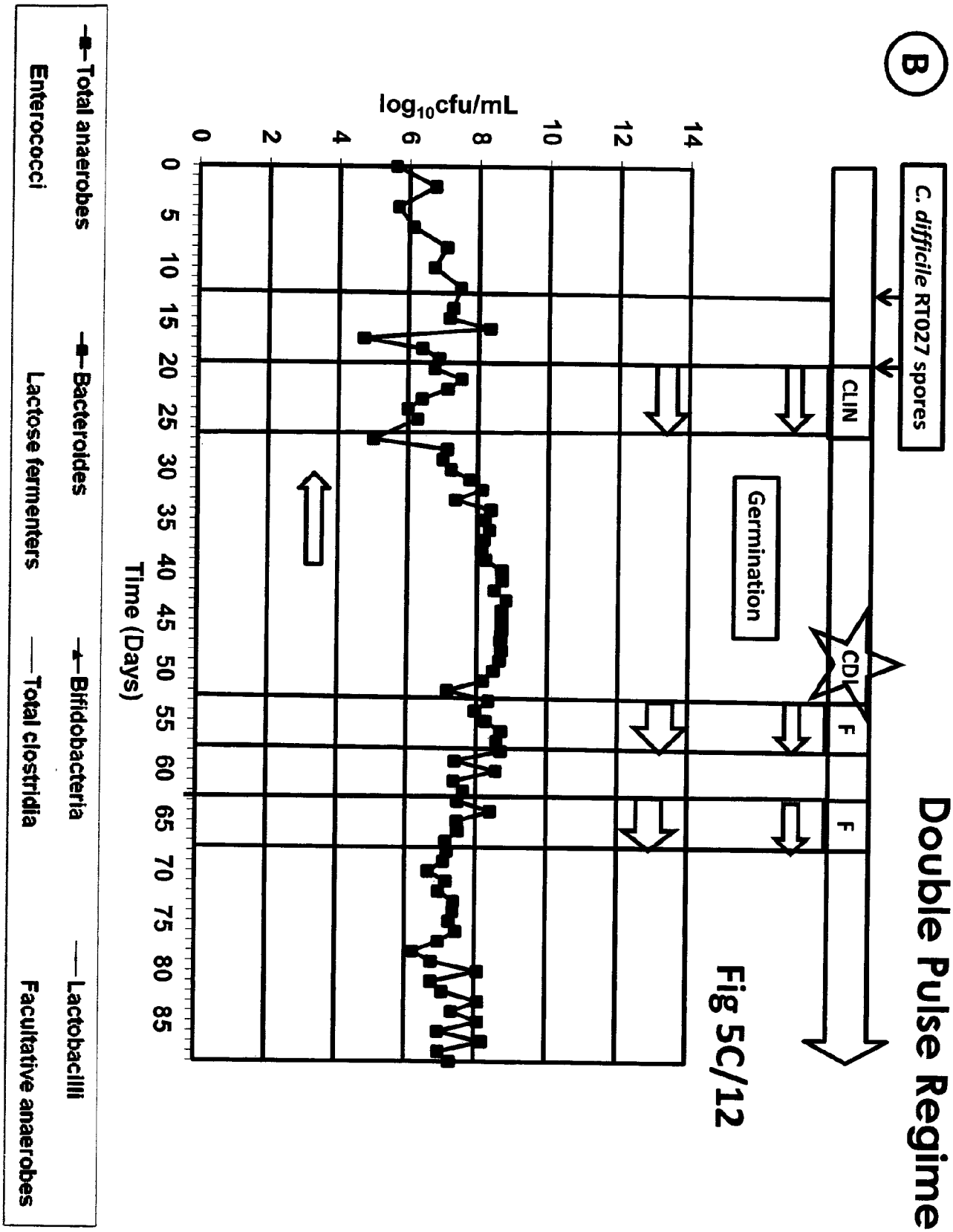

B
Double Pulse Regimen
C. difficile RT027 spores
CLIN
CDI
F
F
Germination
log10cfu/mL
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 5C/12

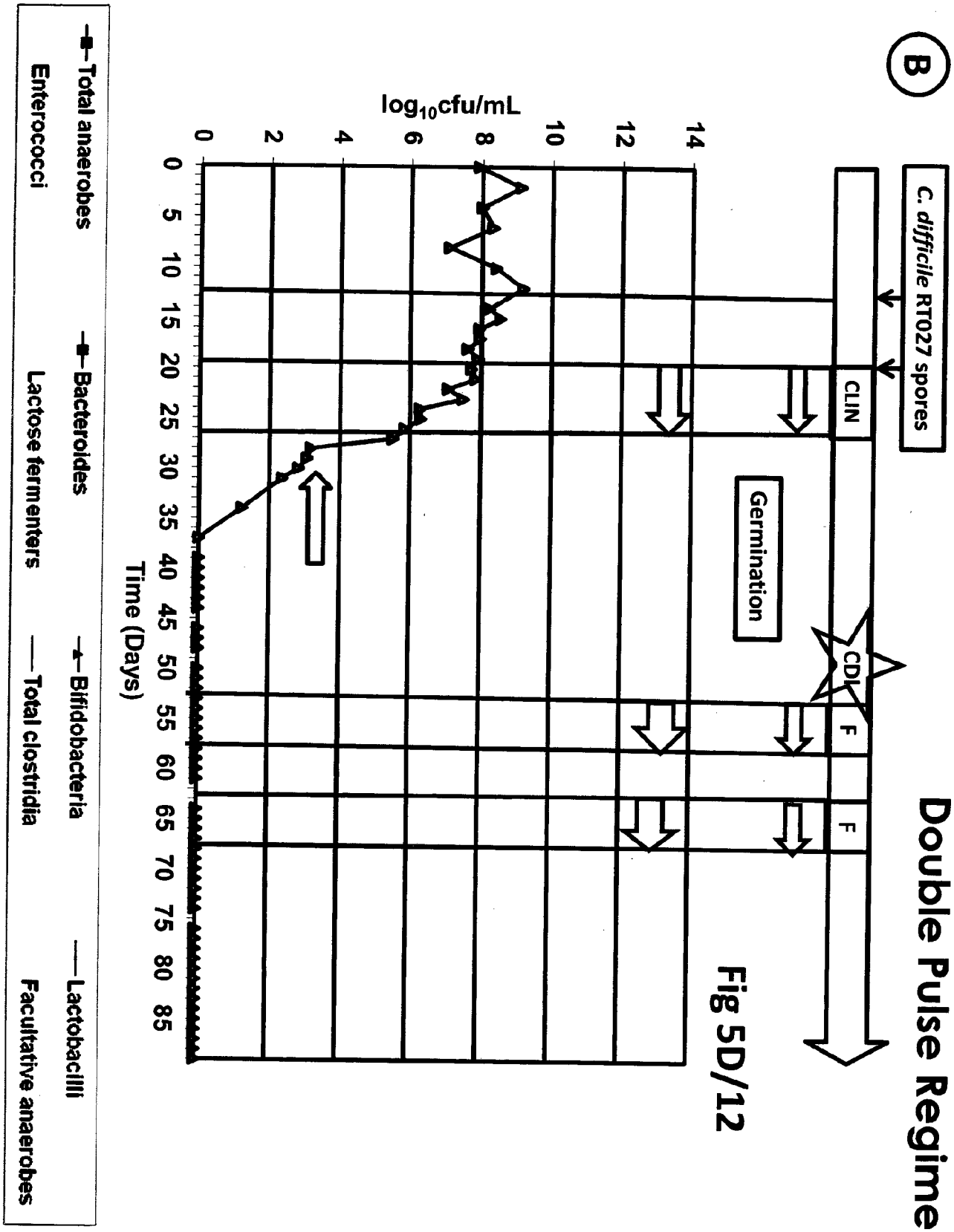

B
Double Pulse Regimen
C. difficile RT027 spores
CLIN
CDI
F
F
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 5D/12

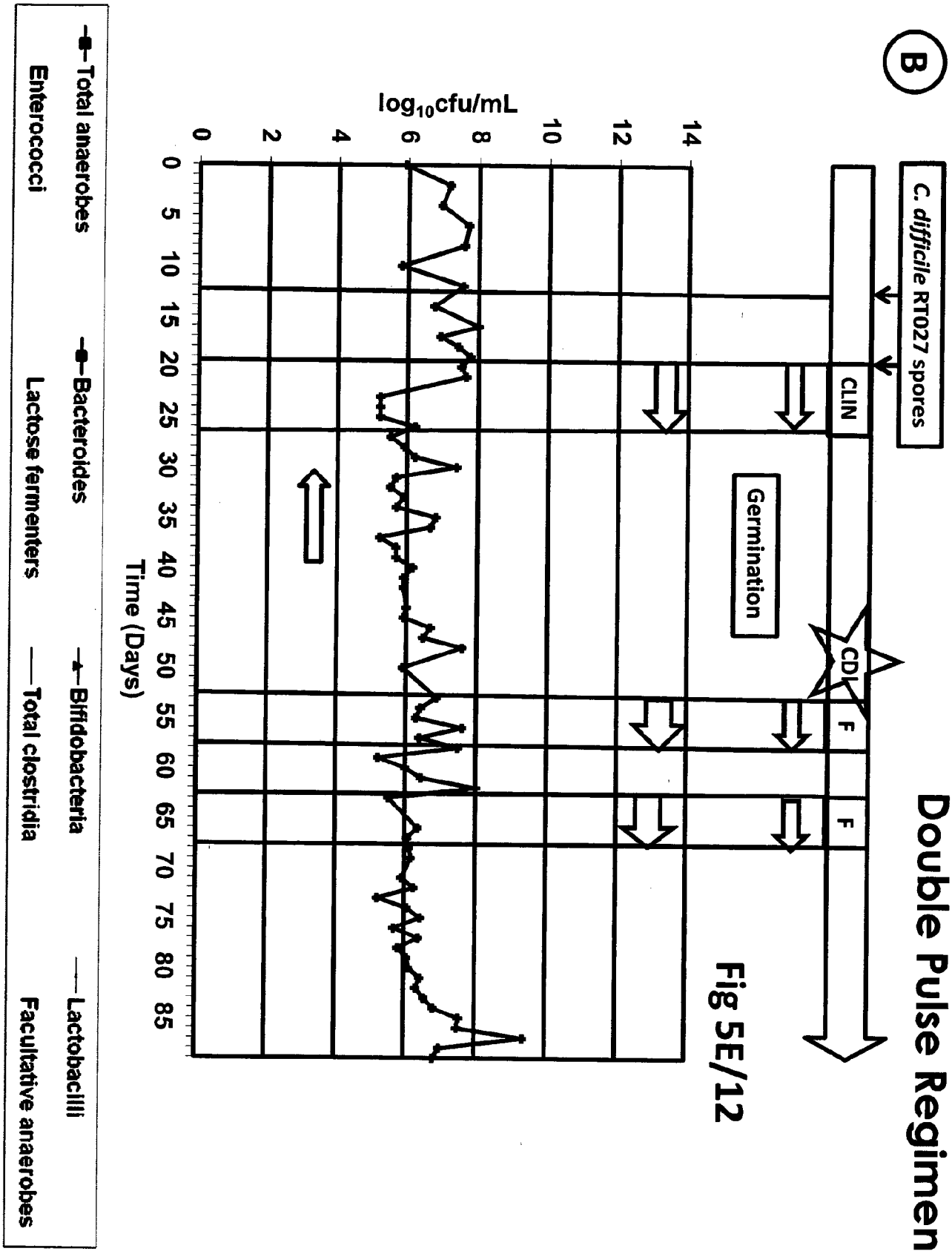

B
Double Pulse Regimen
C. difficile RT027 spores
CLIN
CDI
F
F
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
Time (Days)
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 5E/12

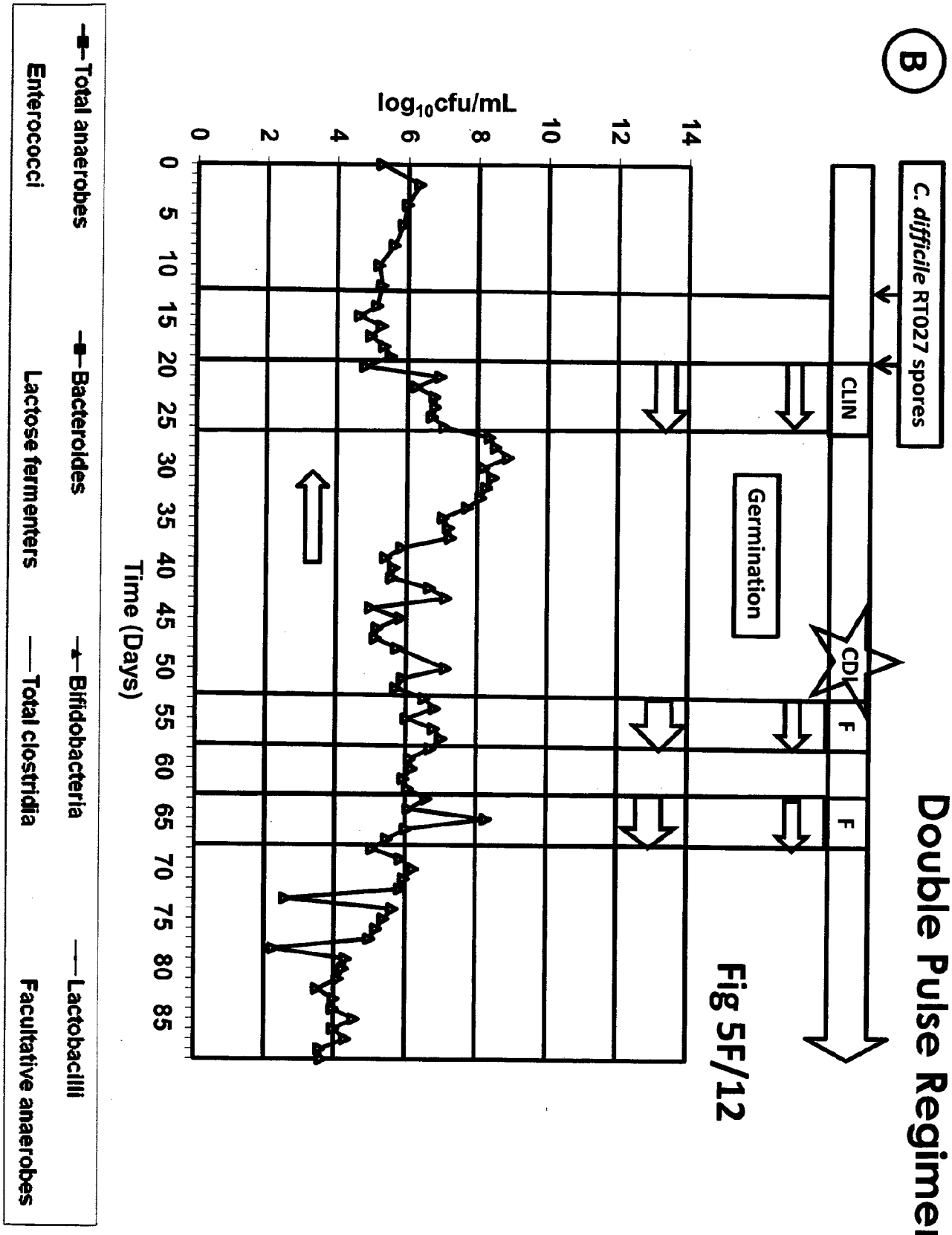

B
Double Pulse Regimen
C. difficile RT027 spores
CLIN
CDI
F
F
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
Time (Days)
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 5F/12

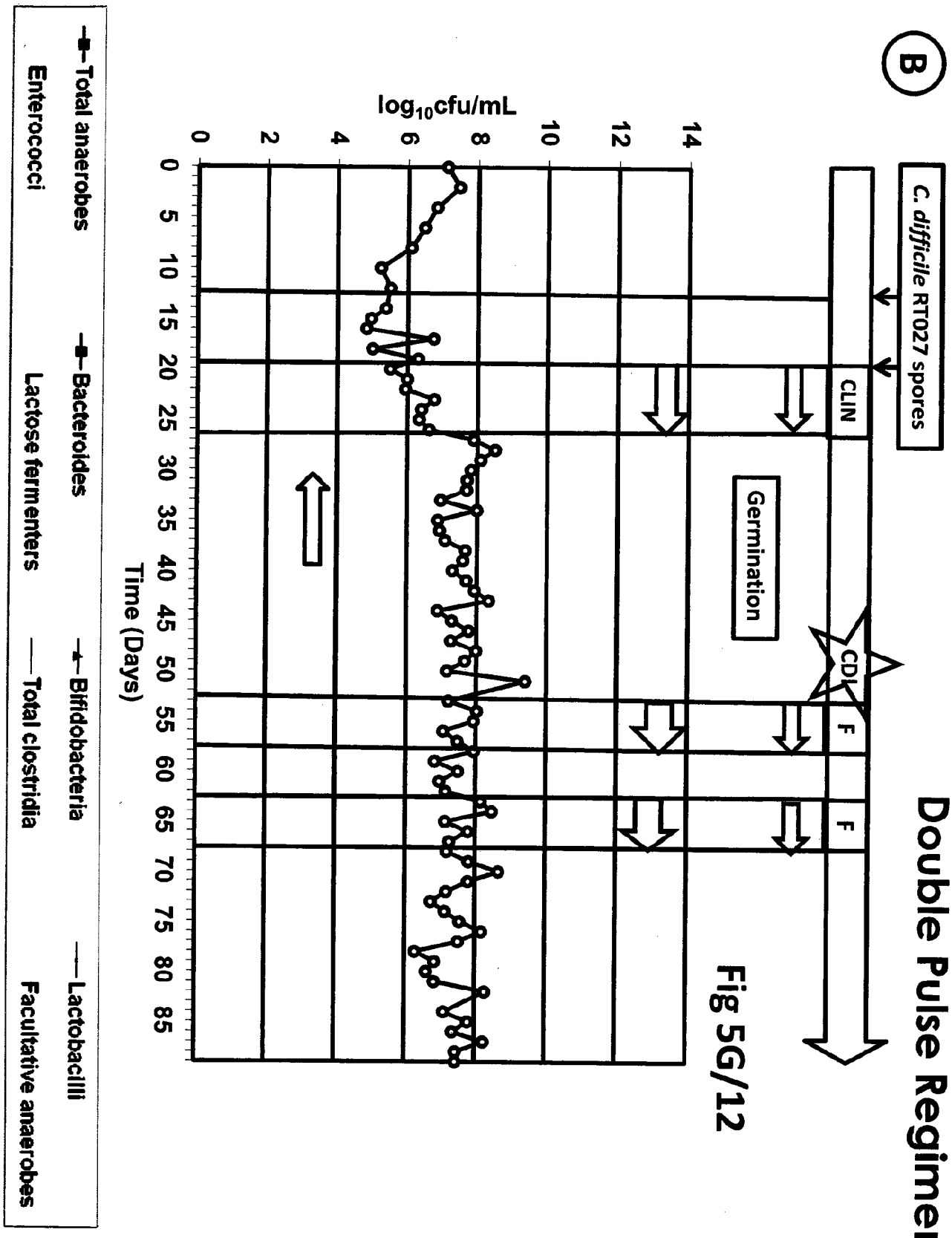

B
Double Pulse Regimen
C. difficile RT027 spores
CLIN
CDK
F
F
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 5G/12

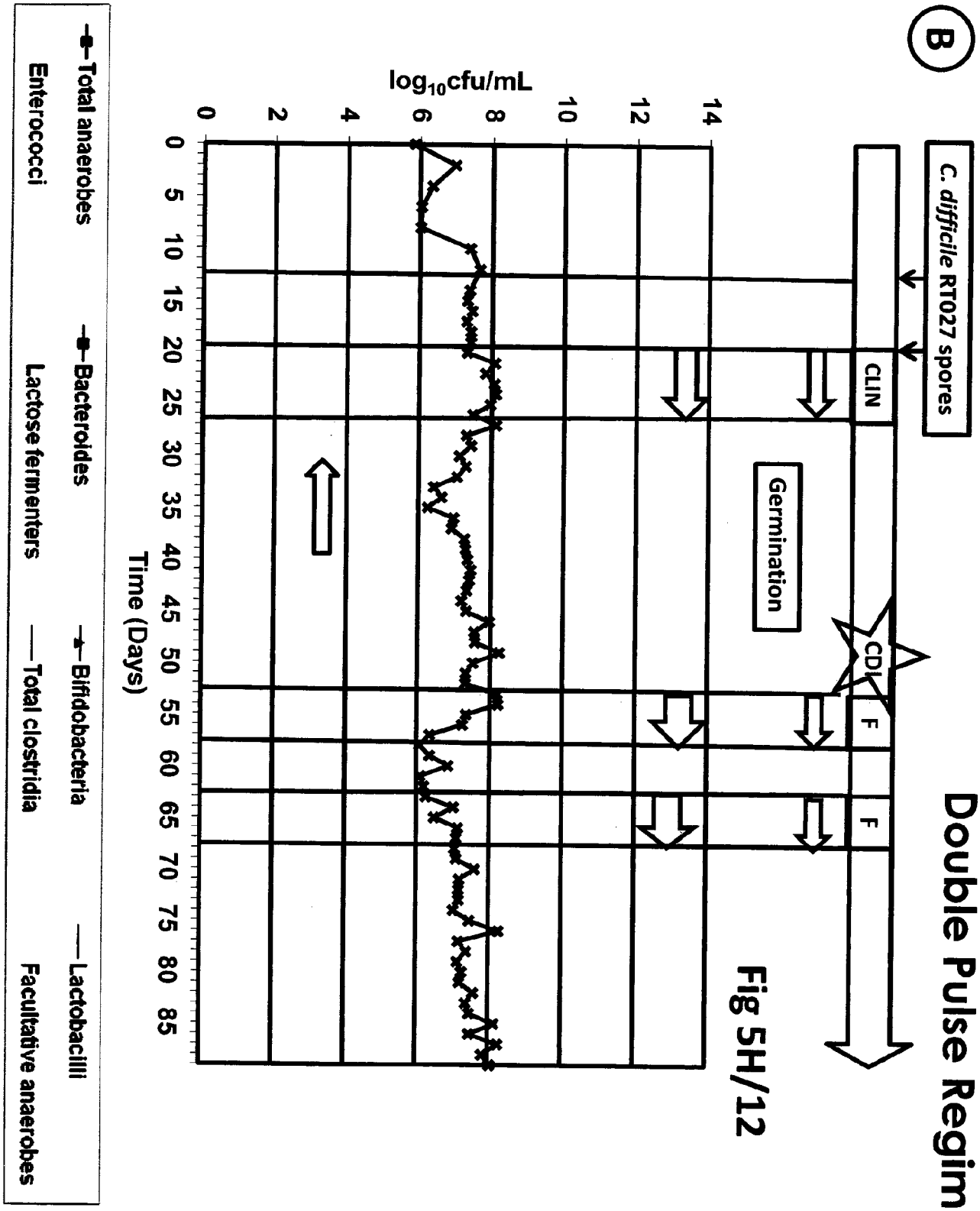

B
Double Pulse Regimen
C. difficile RT027 spores
CLIN
CDI
F
F
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
Time (Days)
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 5H/12

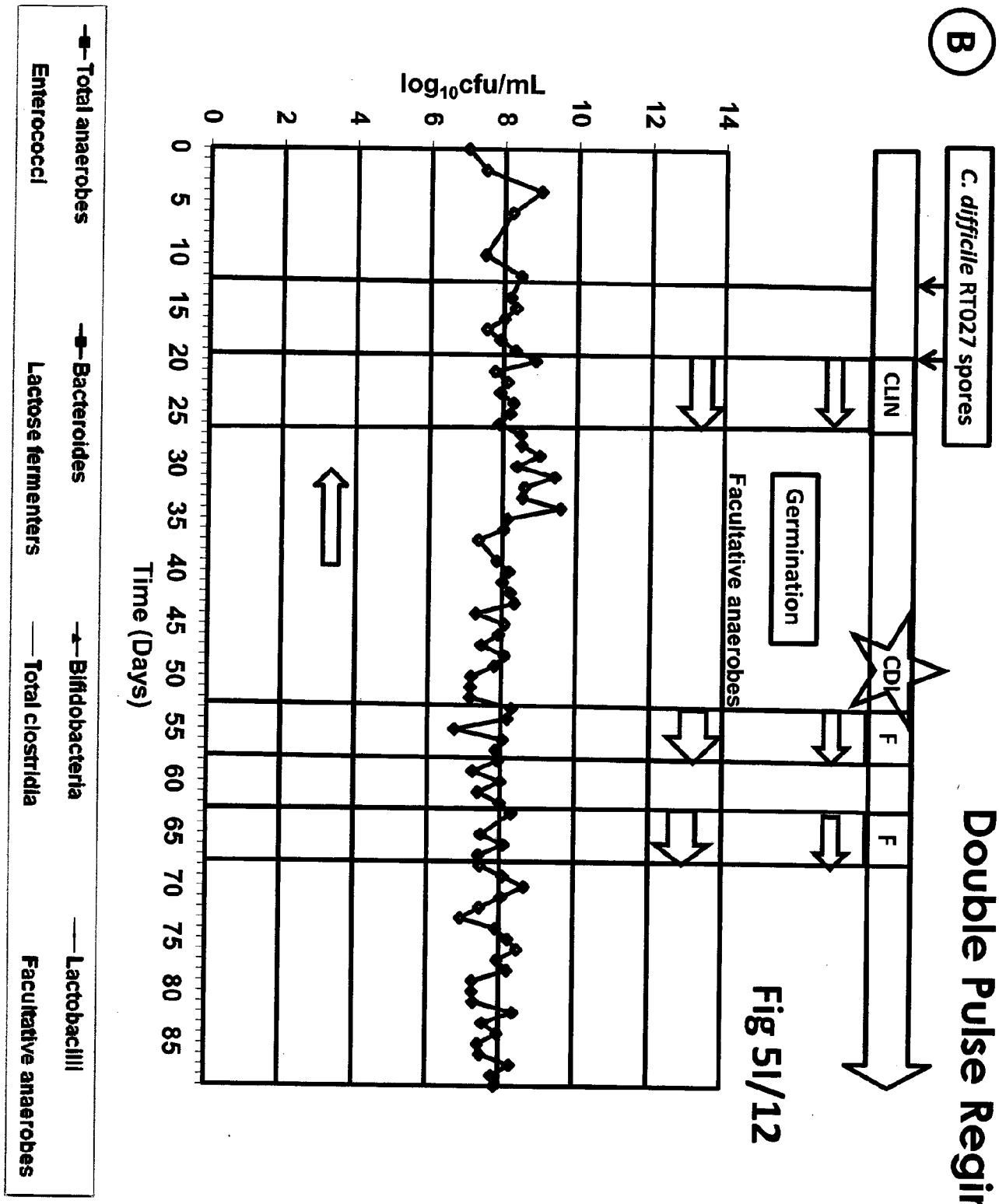
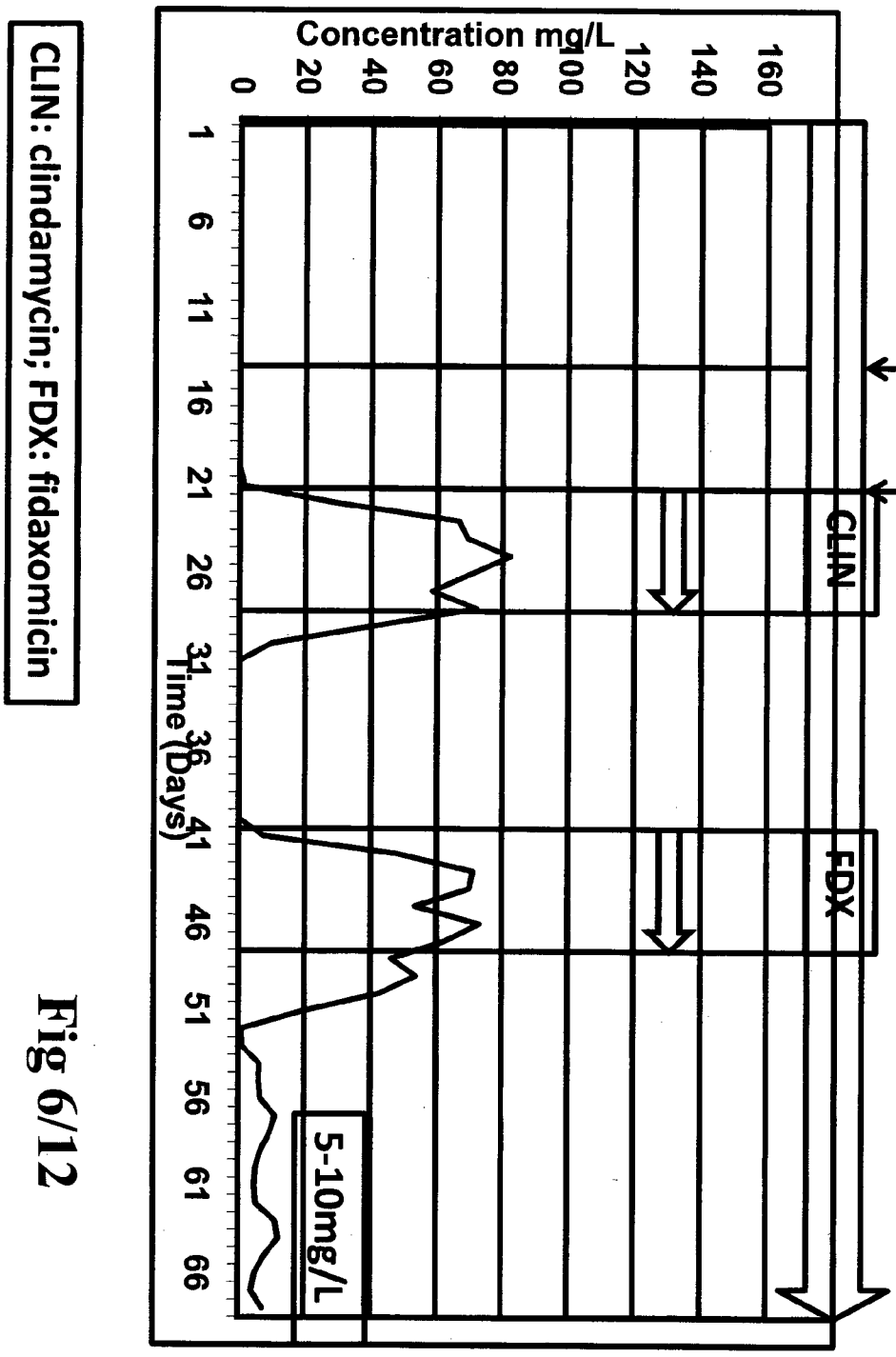

B
Double Pulse Regimen
C. difficile RT027 spores
CLIN
CDI
F
F
Germination
Facultative anaerobes
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Time (Days)
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 5I/12



CLIN: clindamycin; FDX: fidaxomicin

Fig 6/12

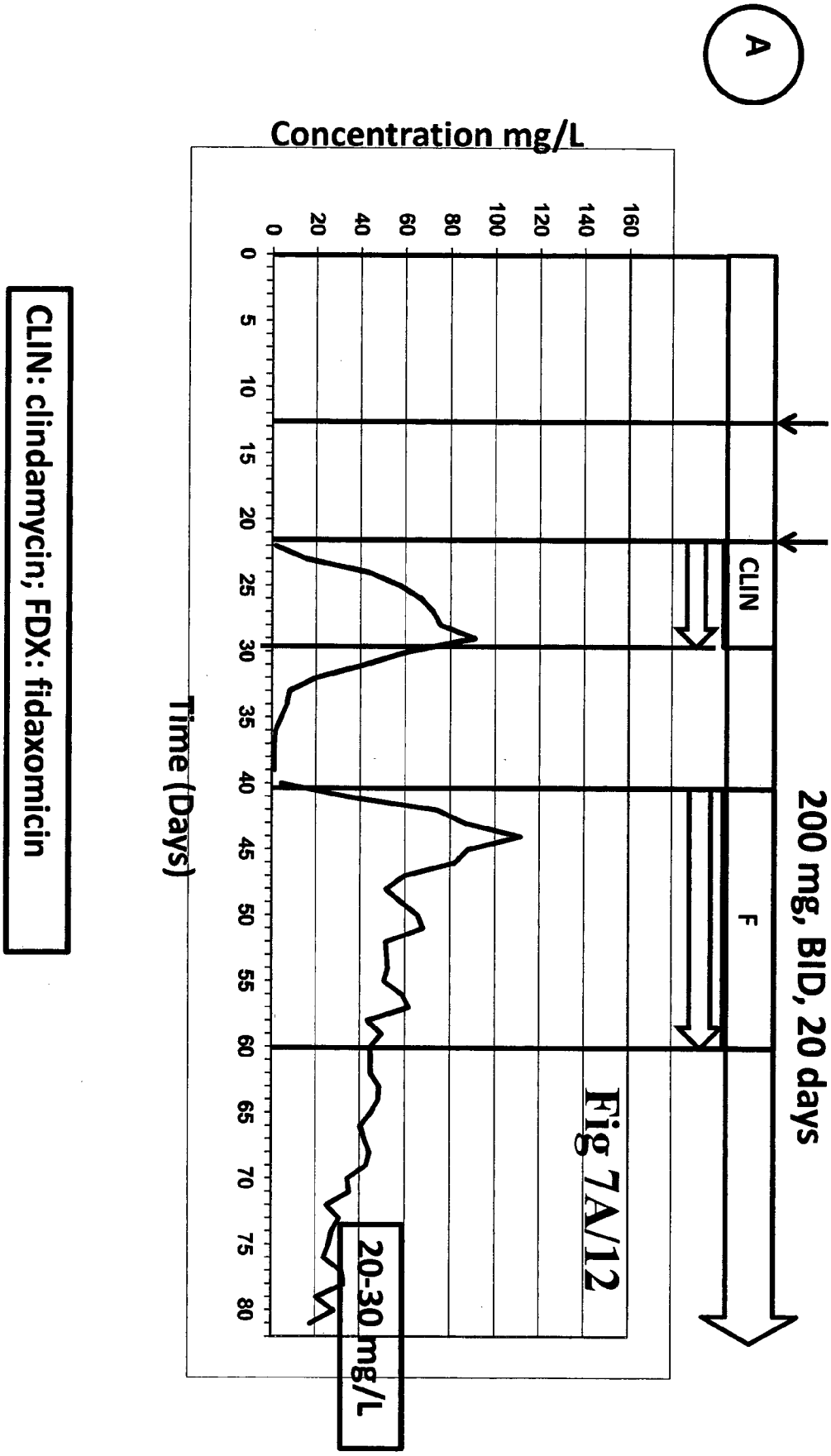

A
Concentration mg/L
0
20
40
60
80
100
120
140
160
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
Time (Days)
CLIN
F
200 mg, BID, 20 days
20-30 mg/L
CLIN: clindamycin; FDX: fidaxomicin

Fig 7A/12

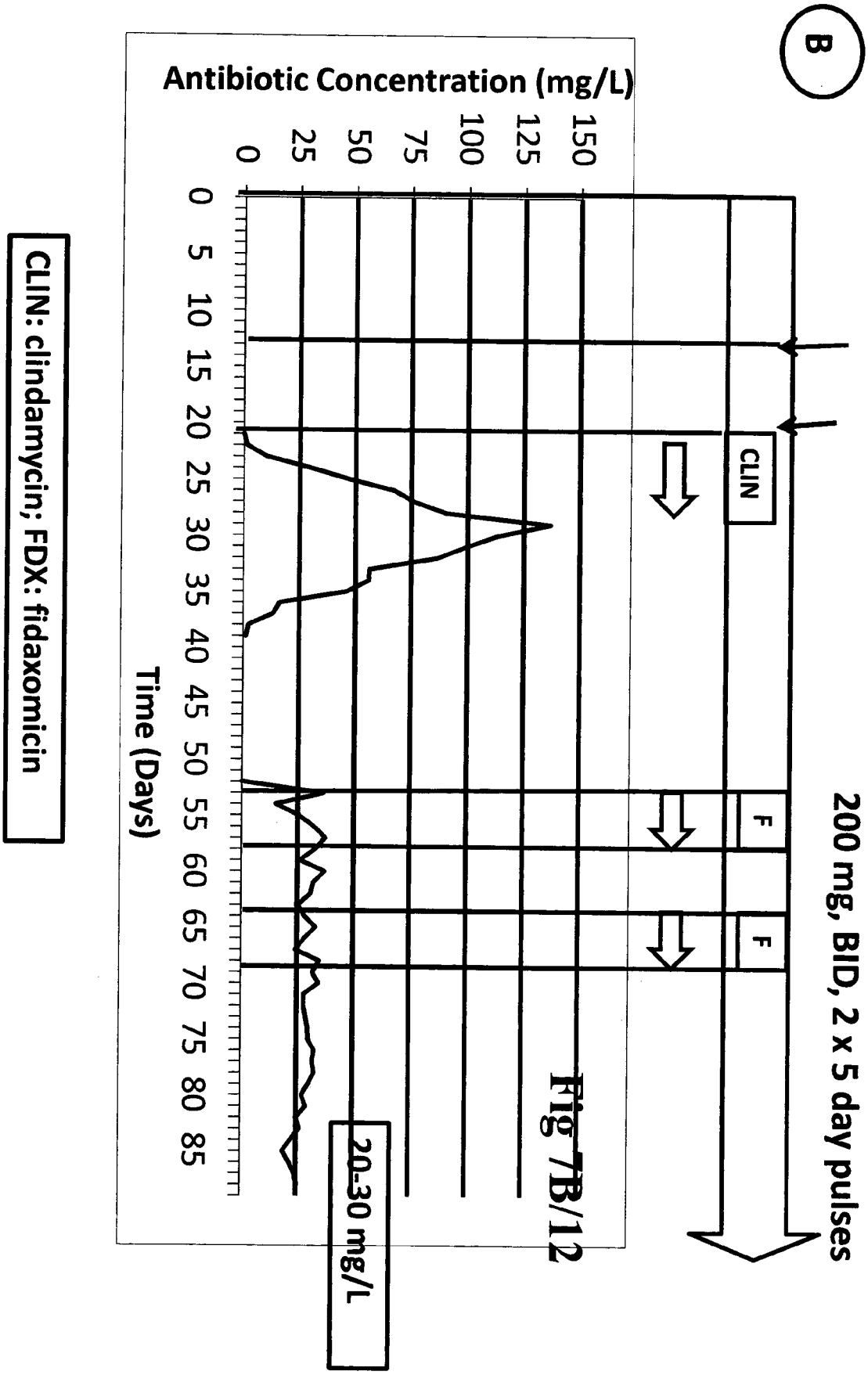

B
Antibiotic Concentration (mg/L)
0
25
50
75
100
125
150
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Time (Days)
CLIN
F
F
200 mg, BID, 2 x 5 day pulses
20-30 mg/L
CLIN: clindamycin; FDX: fidaxomicin

Fig 7B/12

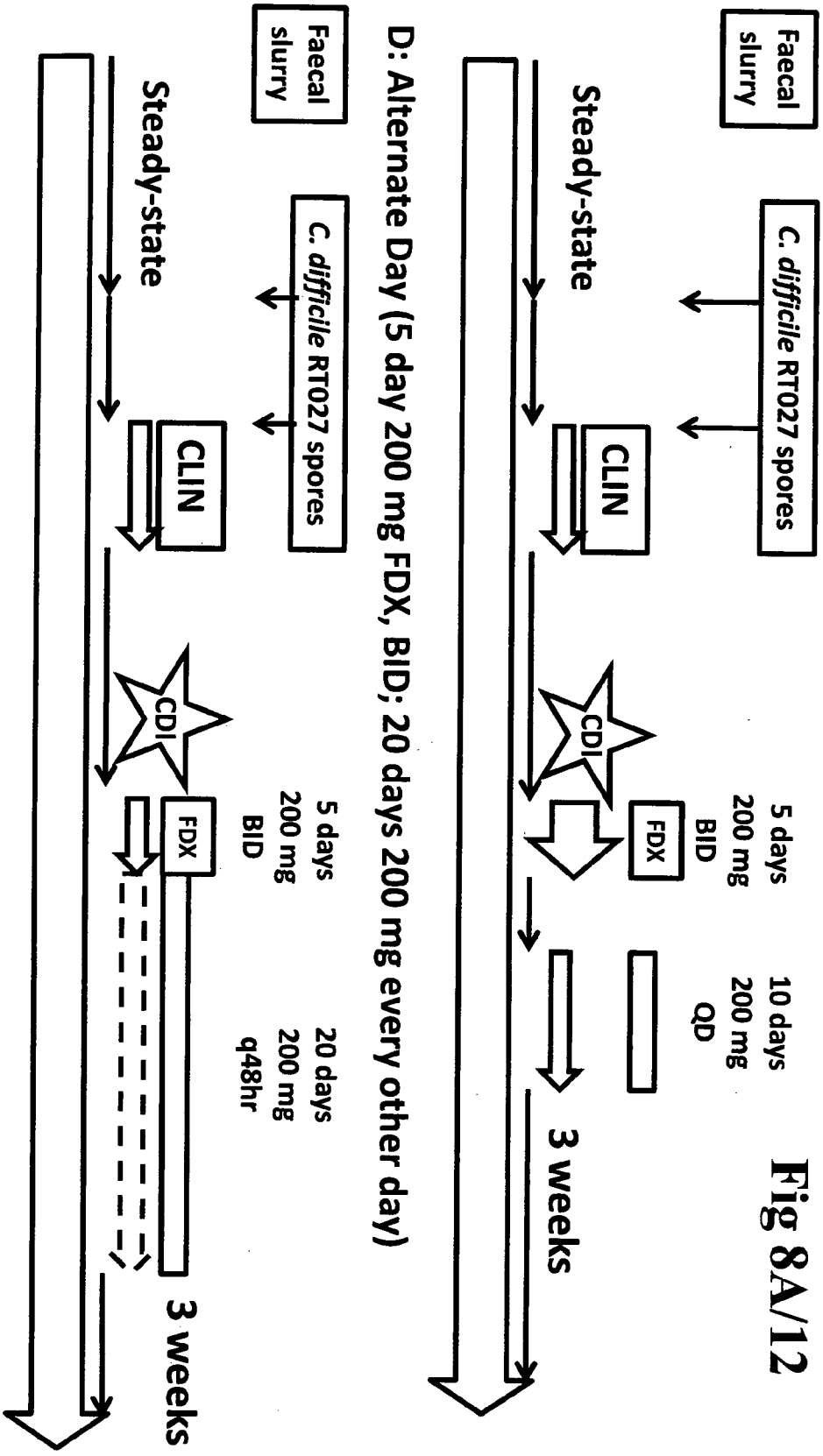

Experimental Design
C: Pulse Taper (5 days 200 mg FDX, BID; 5 day spacer; 10 days 200 mg FDX, QD)
Faecal slurry
C. difficile RT027 spores
Steady-state
CLIN
CDI
FDX
5 days 200 mg BID
10 days 200 mg QD
3 weeks
D: Alternate Day (5 day 200 mg FDX, BID; 20 days 200 mg every other day)
Faecal slurry
C. difficile RT027 spores
Steady-state
CLIN
CDI
FDX
5 days 200 mg BID
20 days 200 mg q48hr
3 weeks

Fig 8A/12

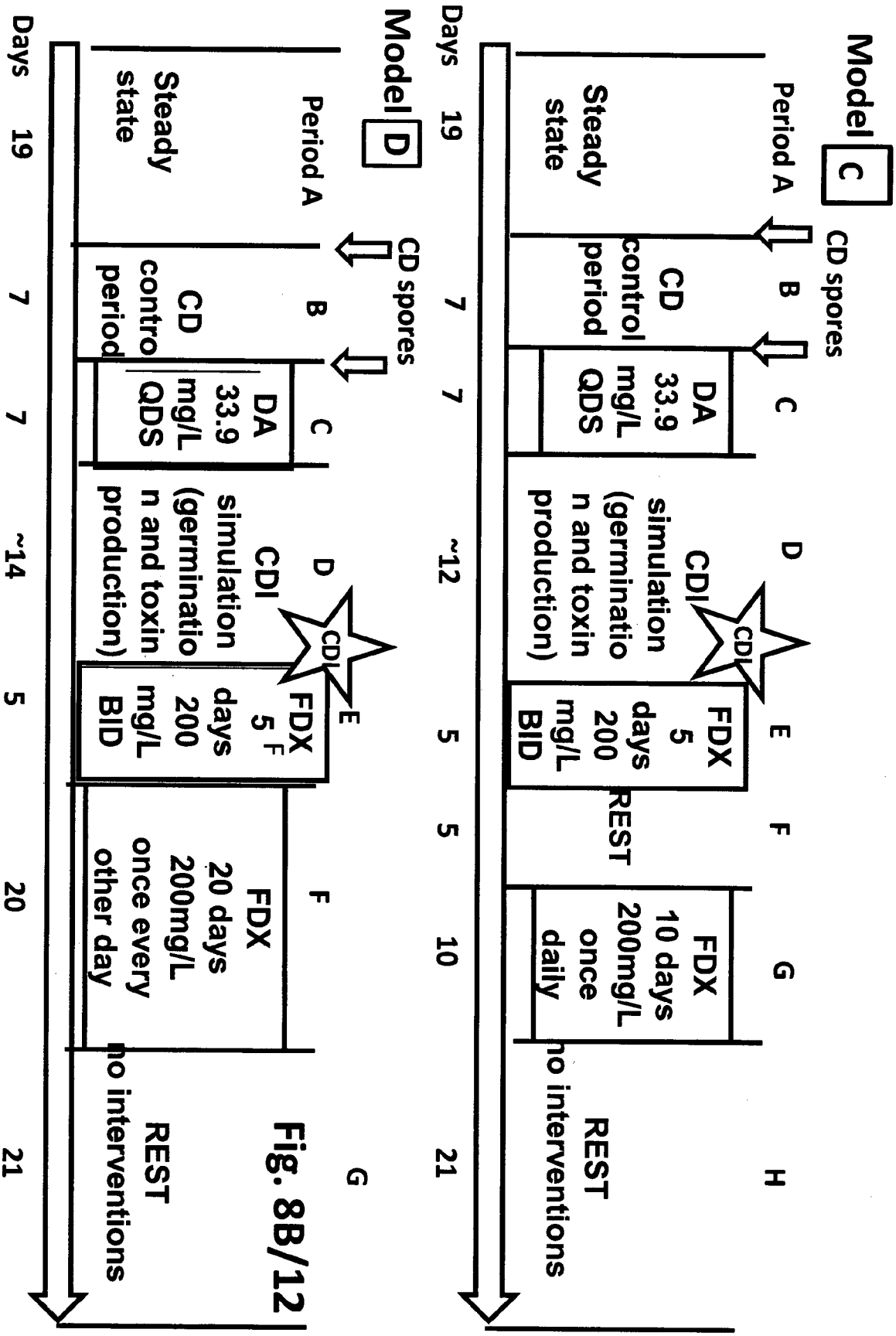

Model C
Period A
Steady state
CD spores
B
CD control period
C
DA 33.9 mg/L QDS
D
CDI simulation (germination and toxin production)
CDI
E
FDX 5 days 200 mg/L BID
F
REST
G
FDX 10 days 200mg/L once daily
H
REST
no interventions
Days 19 7 7 ~12 5 5 10 21
Model D
Period A
Steady state
CD spores
B
CD control period
C
DA 33.9 mg/L QDS
D
CDI simulation (germination and toxin production)
CDI
E
FDX 5 days 200 mg/L BID
F
FDX 20 days 200mg/L once every other day
G
REST
no interventions
Days 19 7 7 ~14 5 20 21
Experimental design of the two models
DA = clindamycin, FDX -= fidaxomicin

Fig. 8B/12

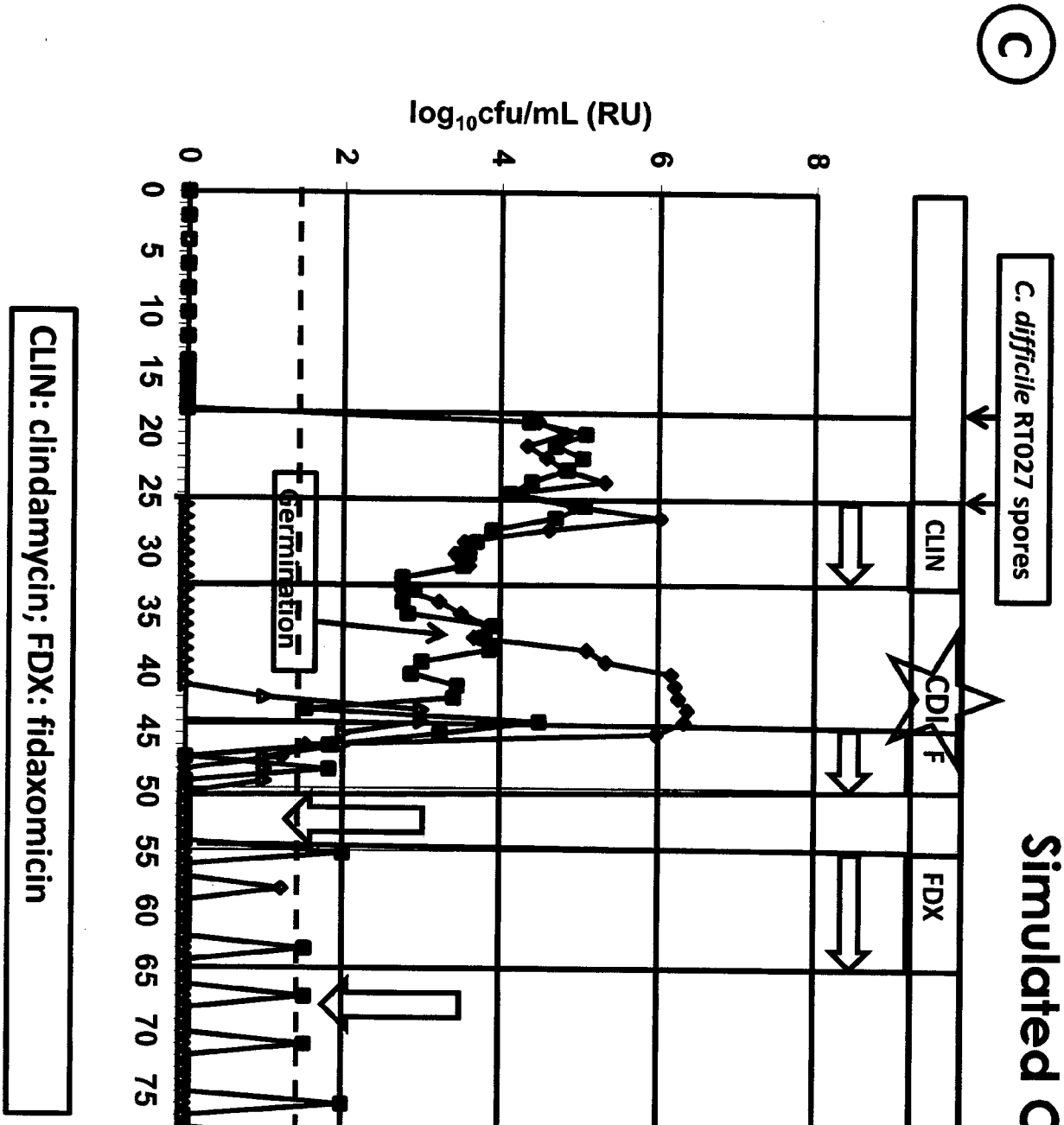

C
Simulated CDI – Pulse Taper
C. difficile RT027 spores
CLIN
CDI
FDX
log10cfu/mL (RU)
germination
total counts
spore counts
cytotoxin titre
CLIN: clindamycin; FDX: fidaxomicin

Fig 9A/12

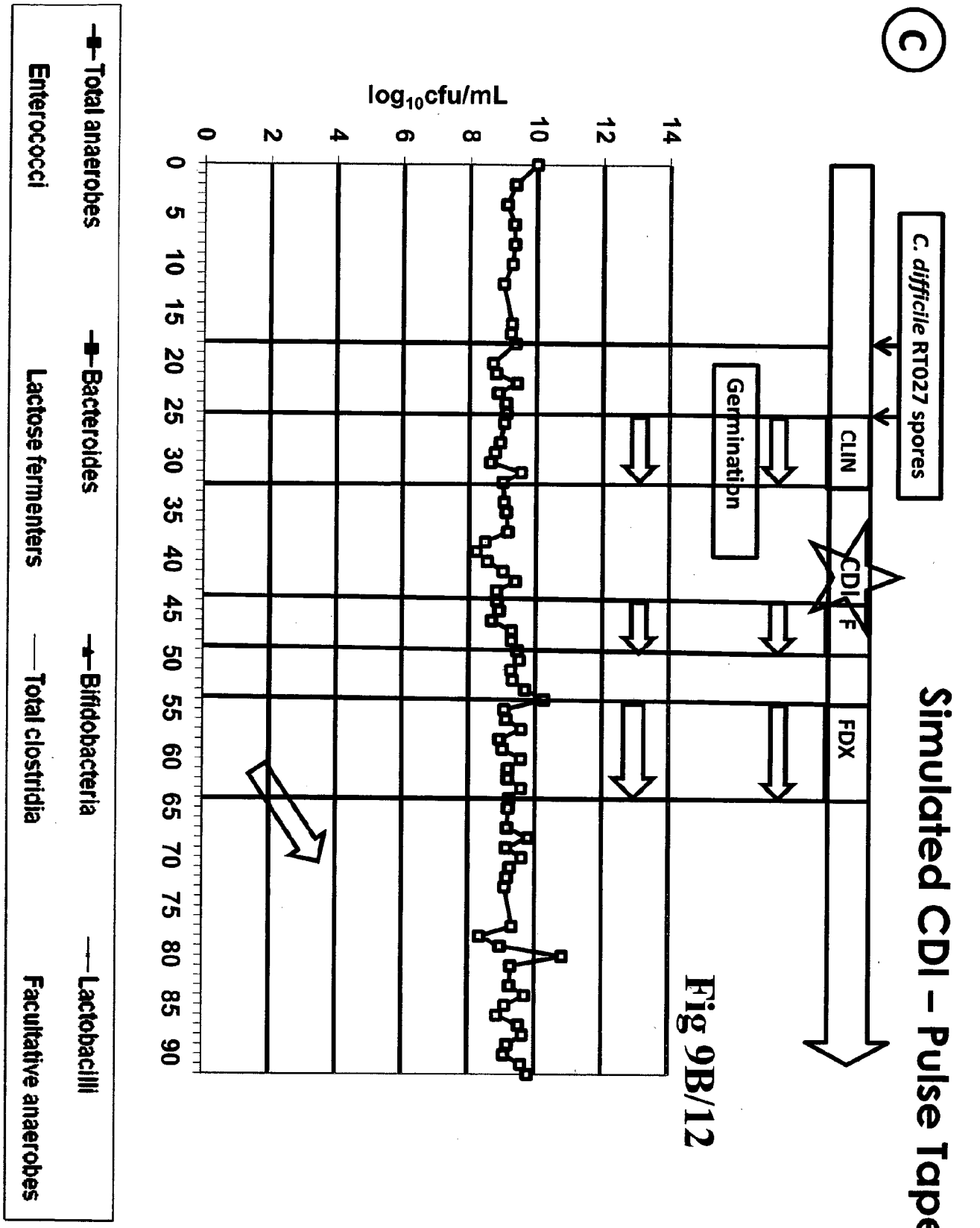

C
Simulated CDI – Pulse Taper
C. difficile RT027 spores
CLIN
CDI
F
FDX
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 9B/12

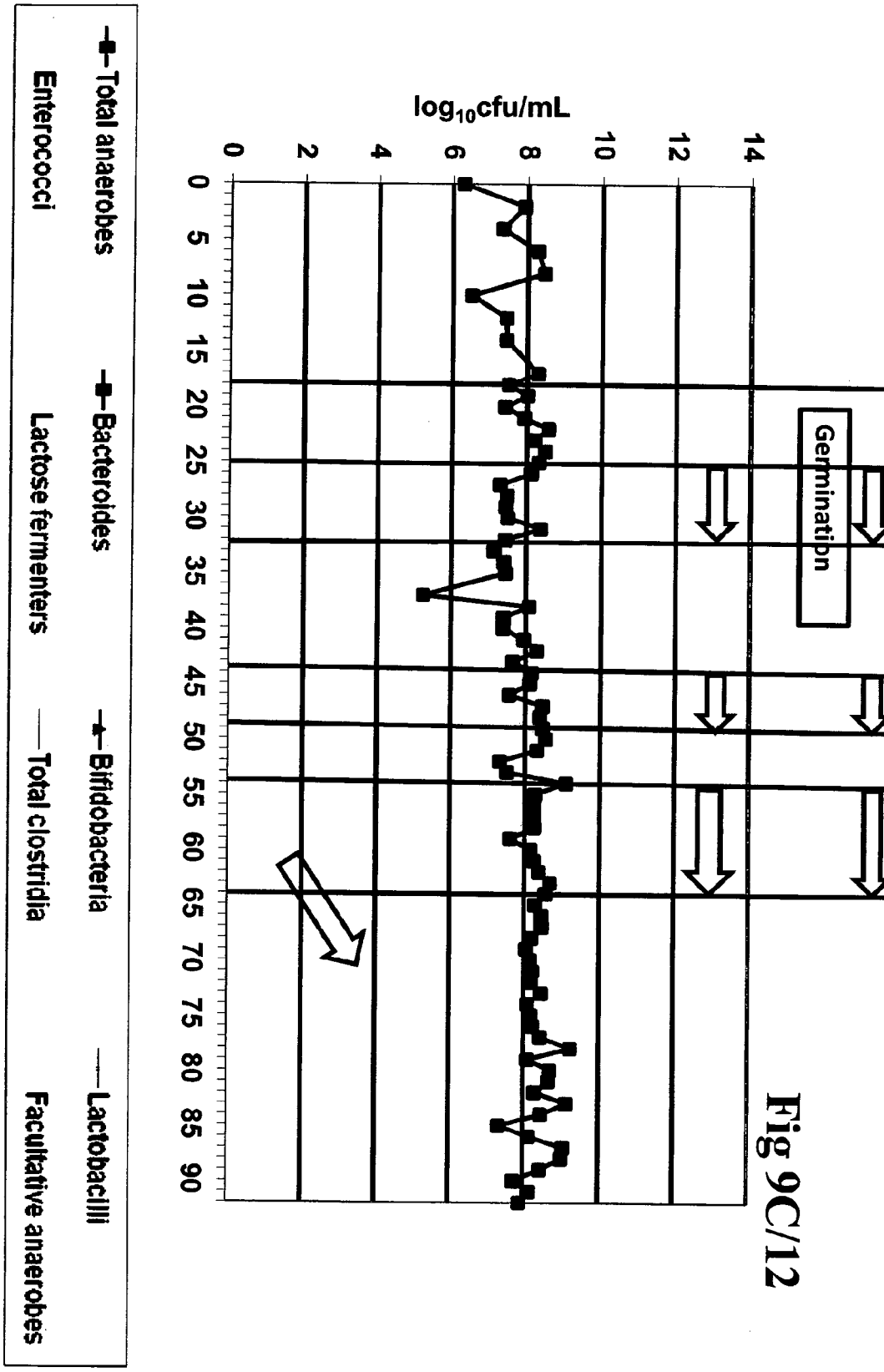

C
Simulated CDI – Pulse Taper
C. difficile RT027 spores
CLIN
CDI
F
FDX
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 9C/12

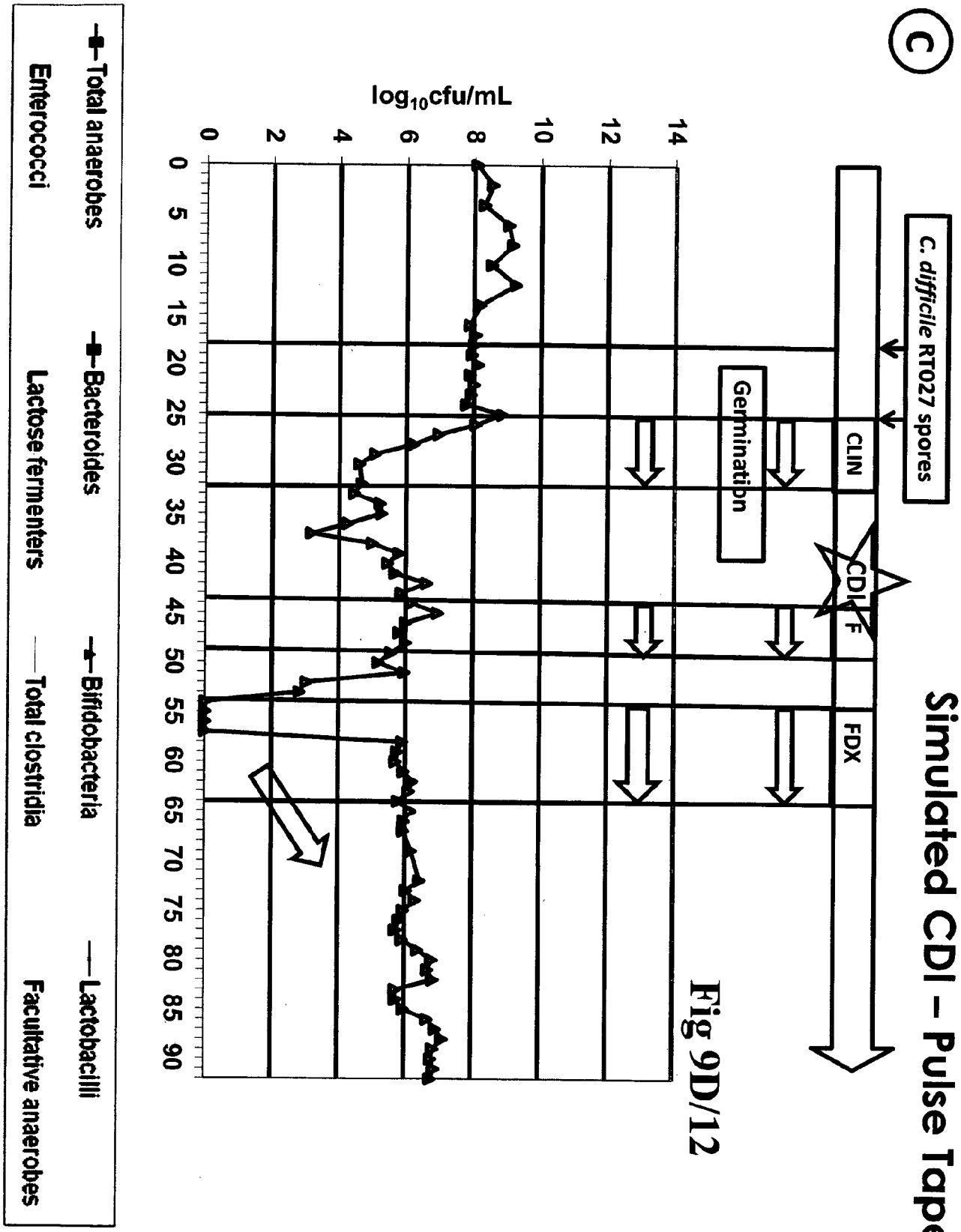

C
Simulated CDI – Pulse Taper
C. difficile RT027 spores
CLIN
CDI
F
FDX
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 9D/12

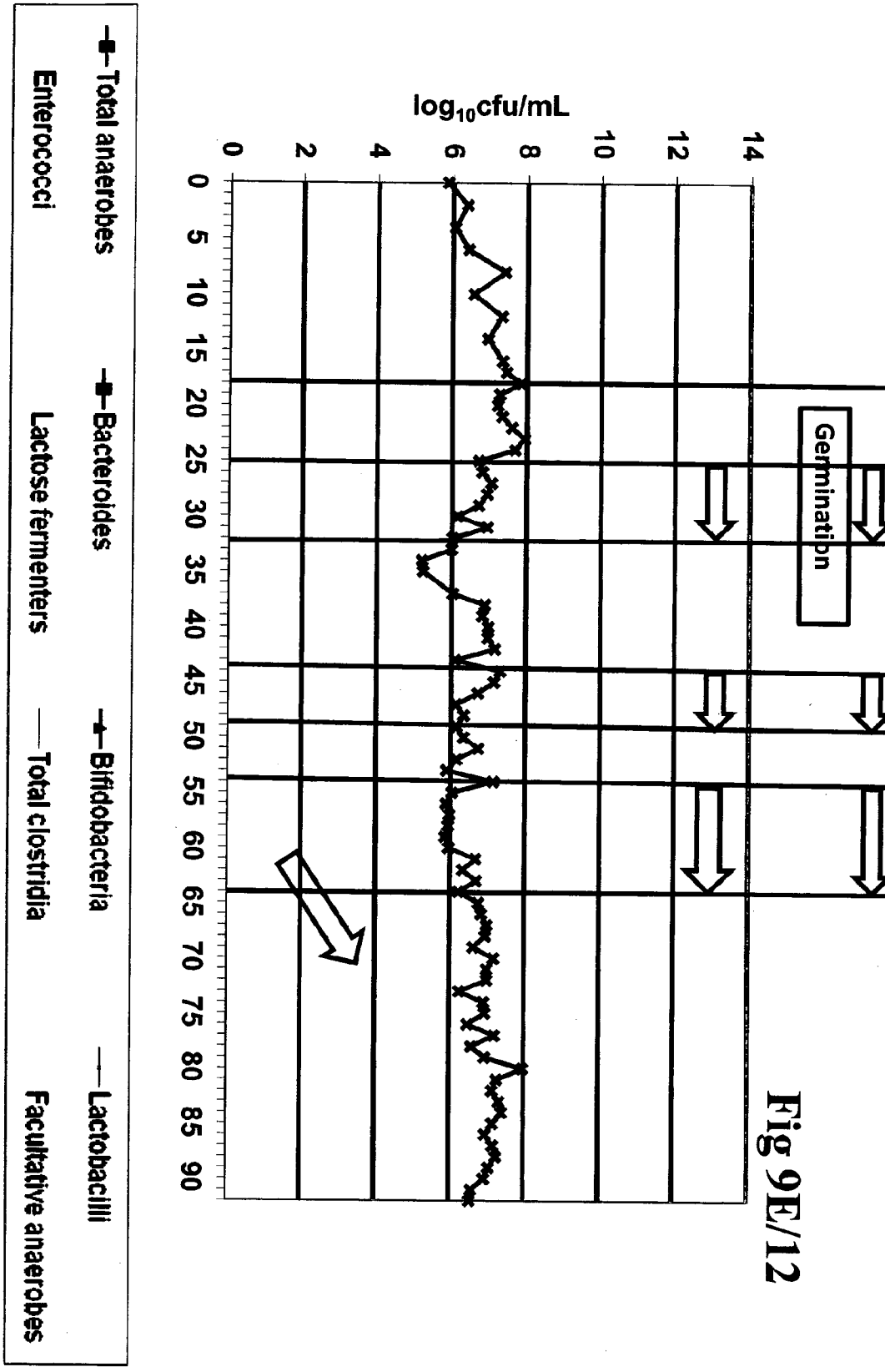

C
Simulated CDI – Pulse Taper
C. difficile RT027 spores
CLIN
CDI
F
FDX
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 9E/12

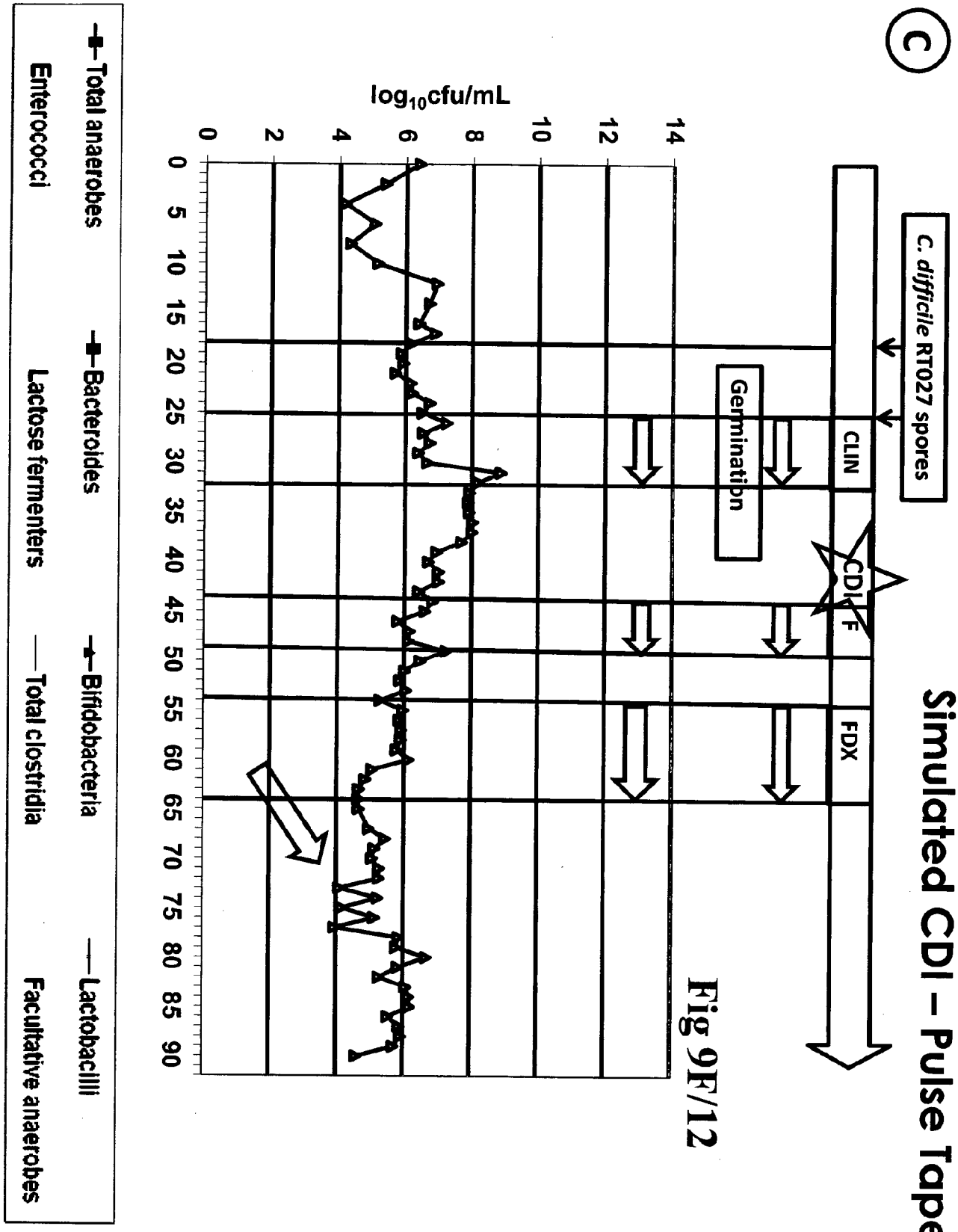

C
Simulated CDI – Pulse Taper
C. difficile RT027 spores
CLIN
CDI
F
FDX
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 9F/12

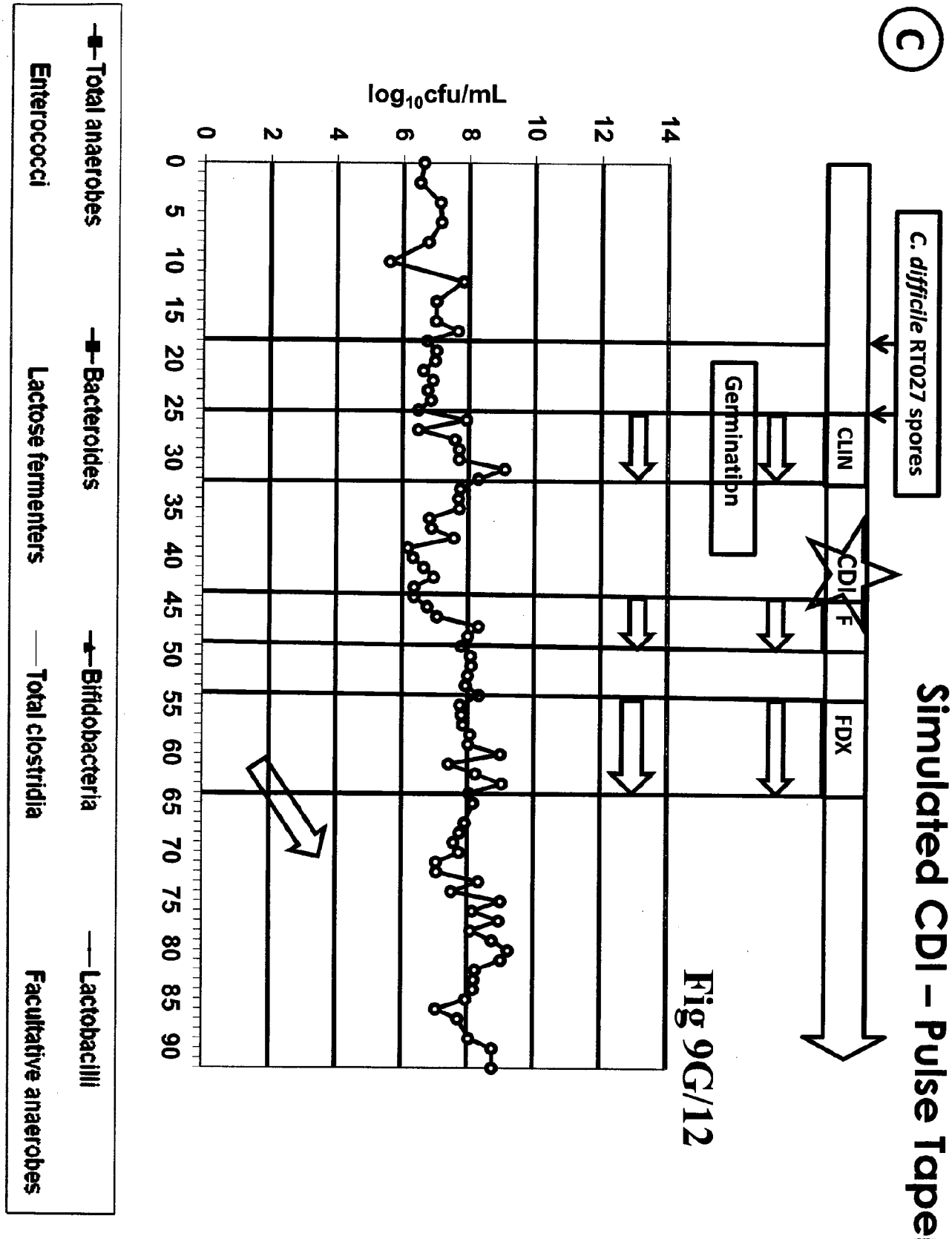

C
Simulated CDI – Pulse Taper
C. difficile RT027 spores
CLIN
CDI
F
FDX
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 9G/12

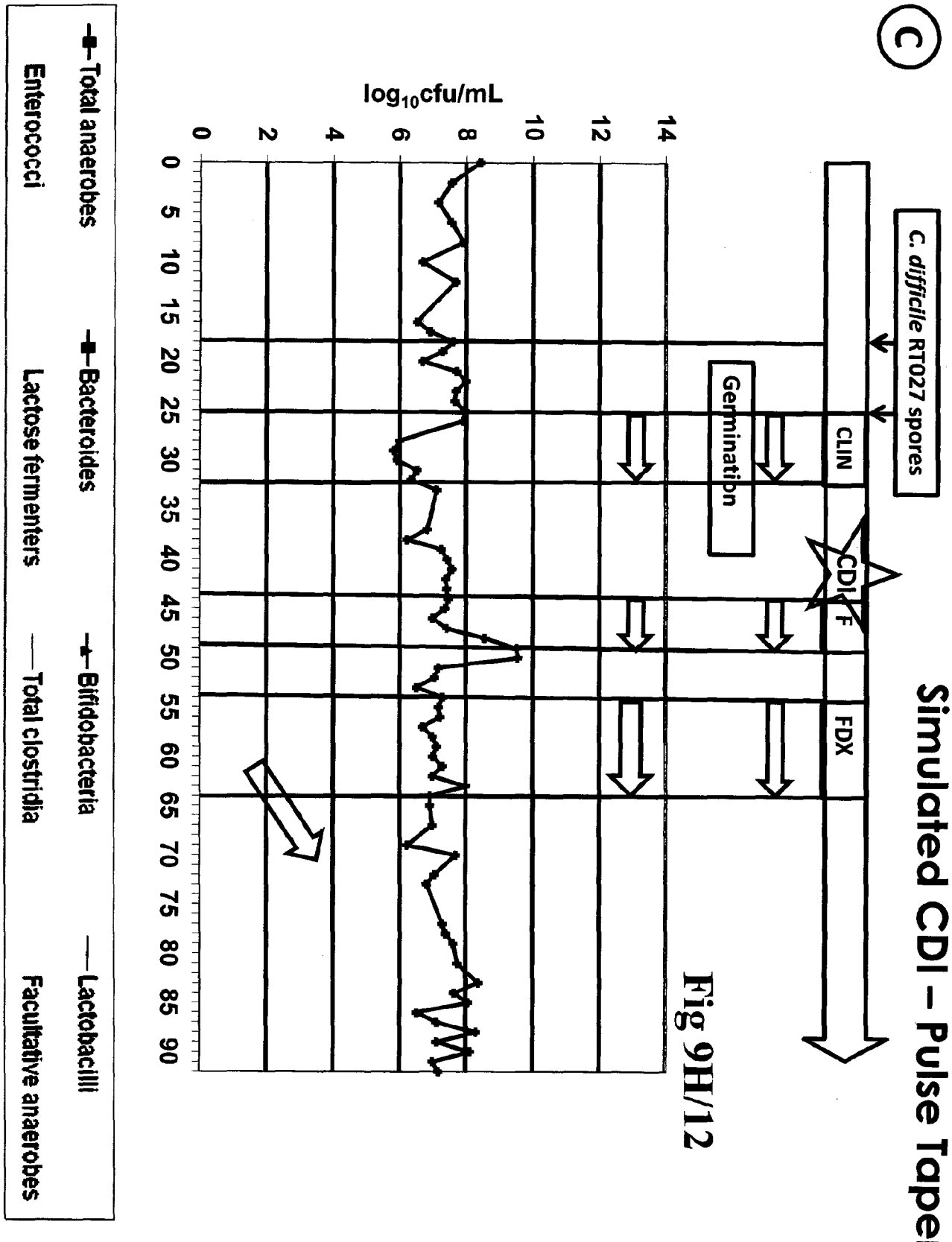

C
Simulated CDI – Pulse Taper
C. difficile RT027 spores
CLIN
CDI
F
FDX
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 9H/12

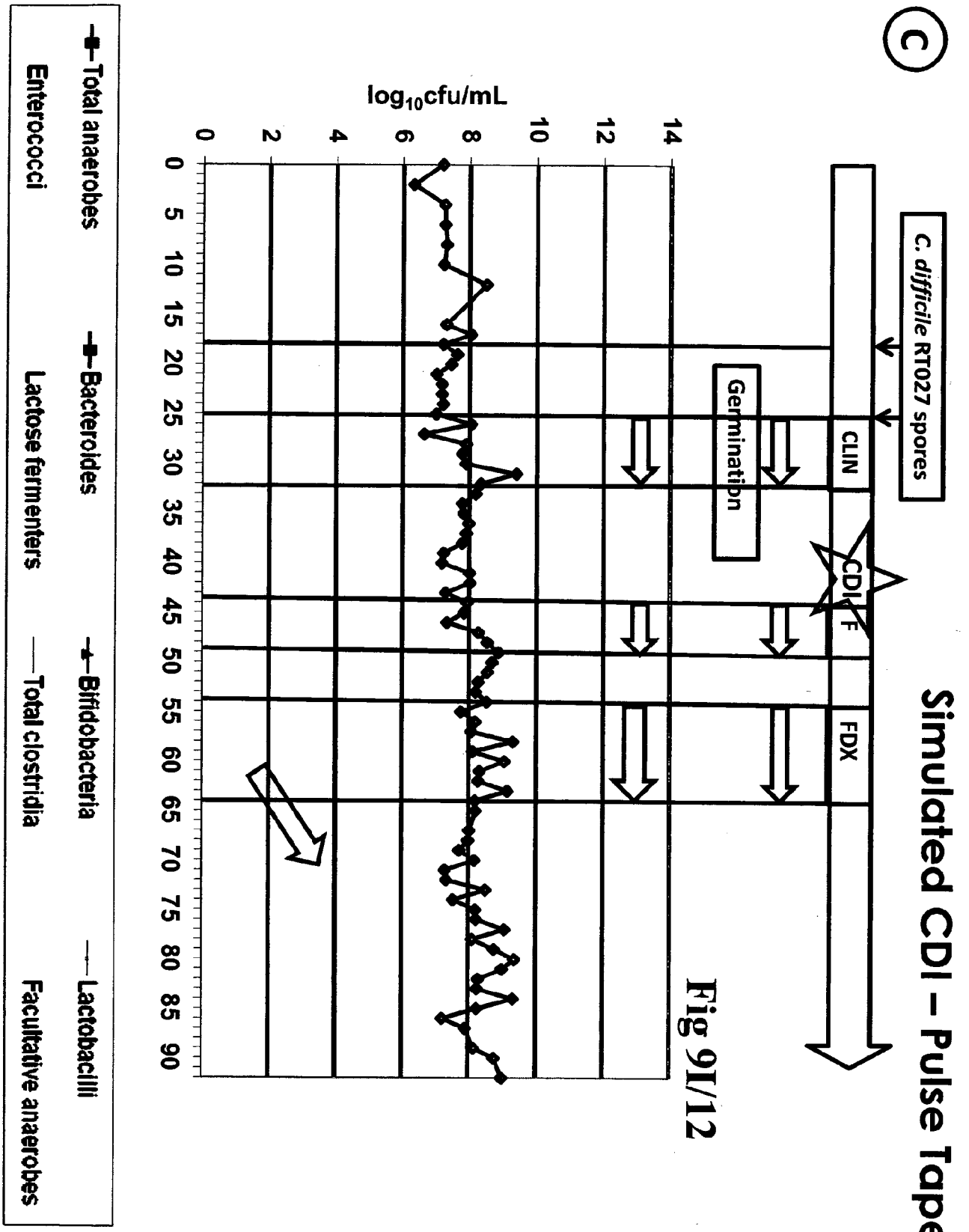

C
Simulated CDI – Pulse Taper
C. difficile RT027 spores
CLIN
CDI
F
FDX
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 9I/12

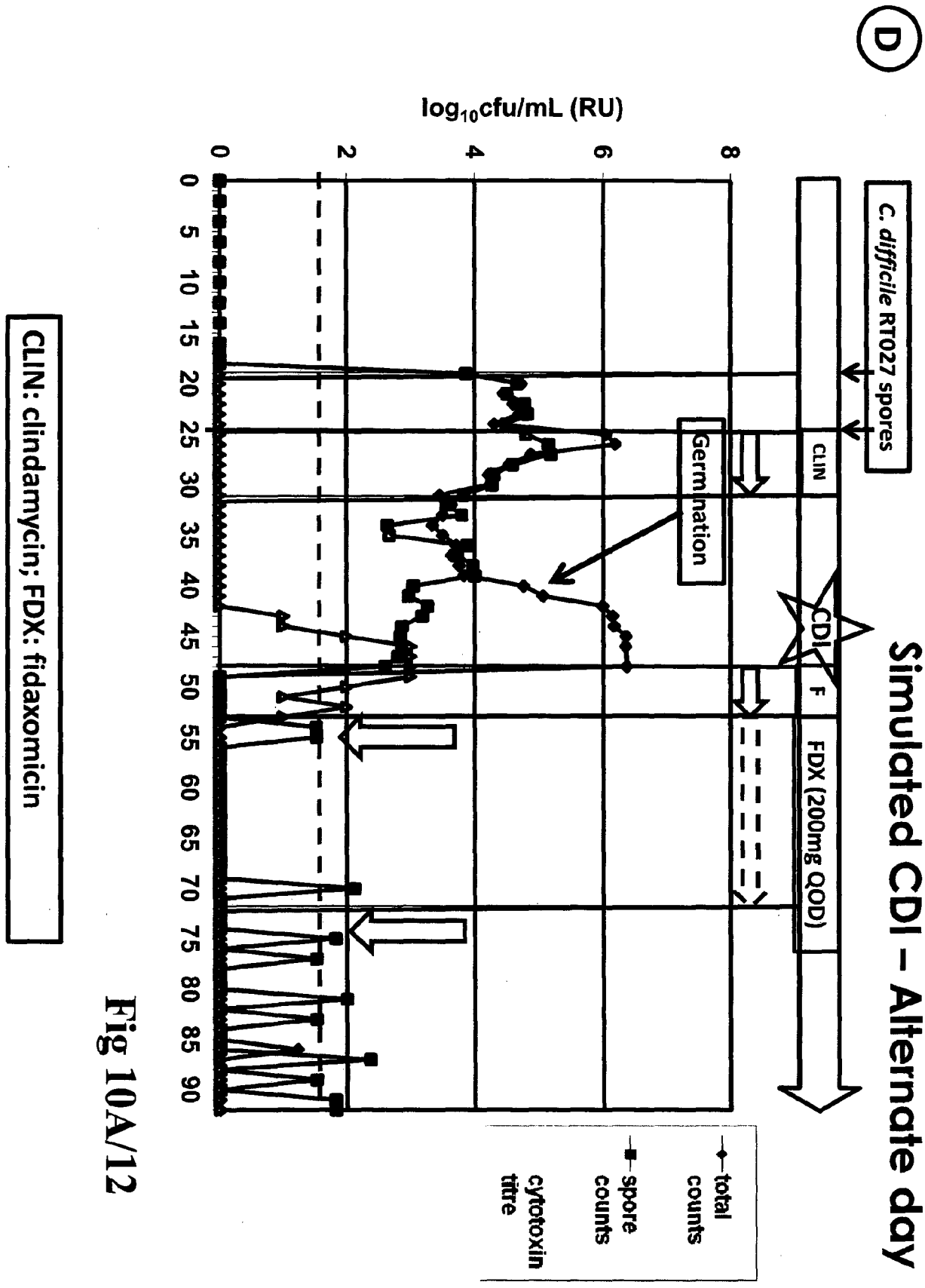

D
Simulated CDI – Alternate day
C. difficile RT027 spores
CLIN
CDI
F
FDX (200mg QOD)
Germination
log10cfu/mL (RU)
0
2
4
6
8
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
total counts
spore counts
cytotoxin titre
CLIN: clindamycin; FDX: fidaxomicin

Fig 10A/12

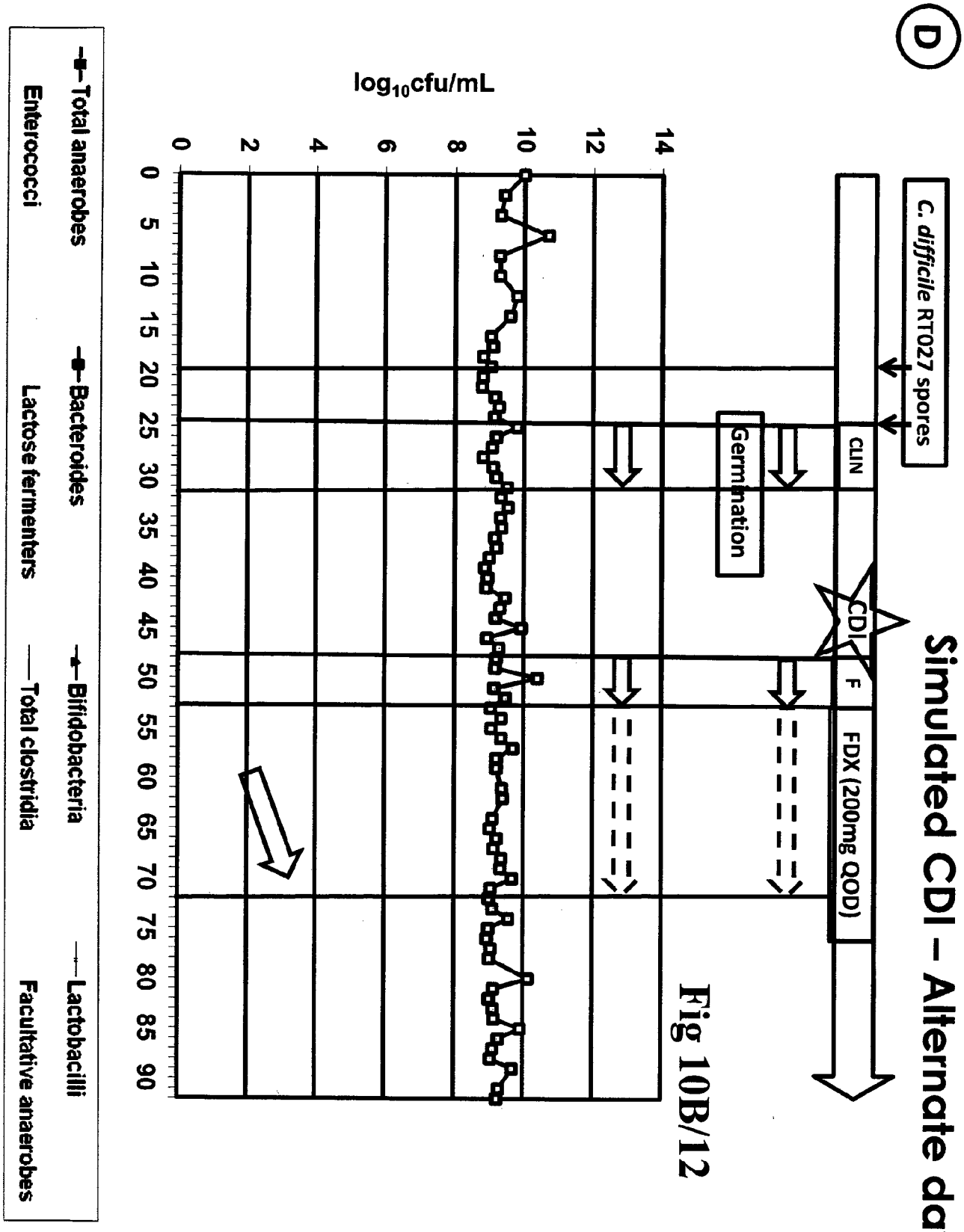

D
Simulated CDI – Alternate day
C. difficile RT027 spores
CLIN
CDI
F
FDX (200mg QOD)
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 10B/12

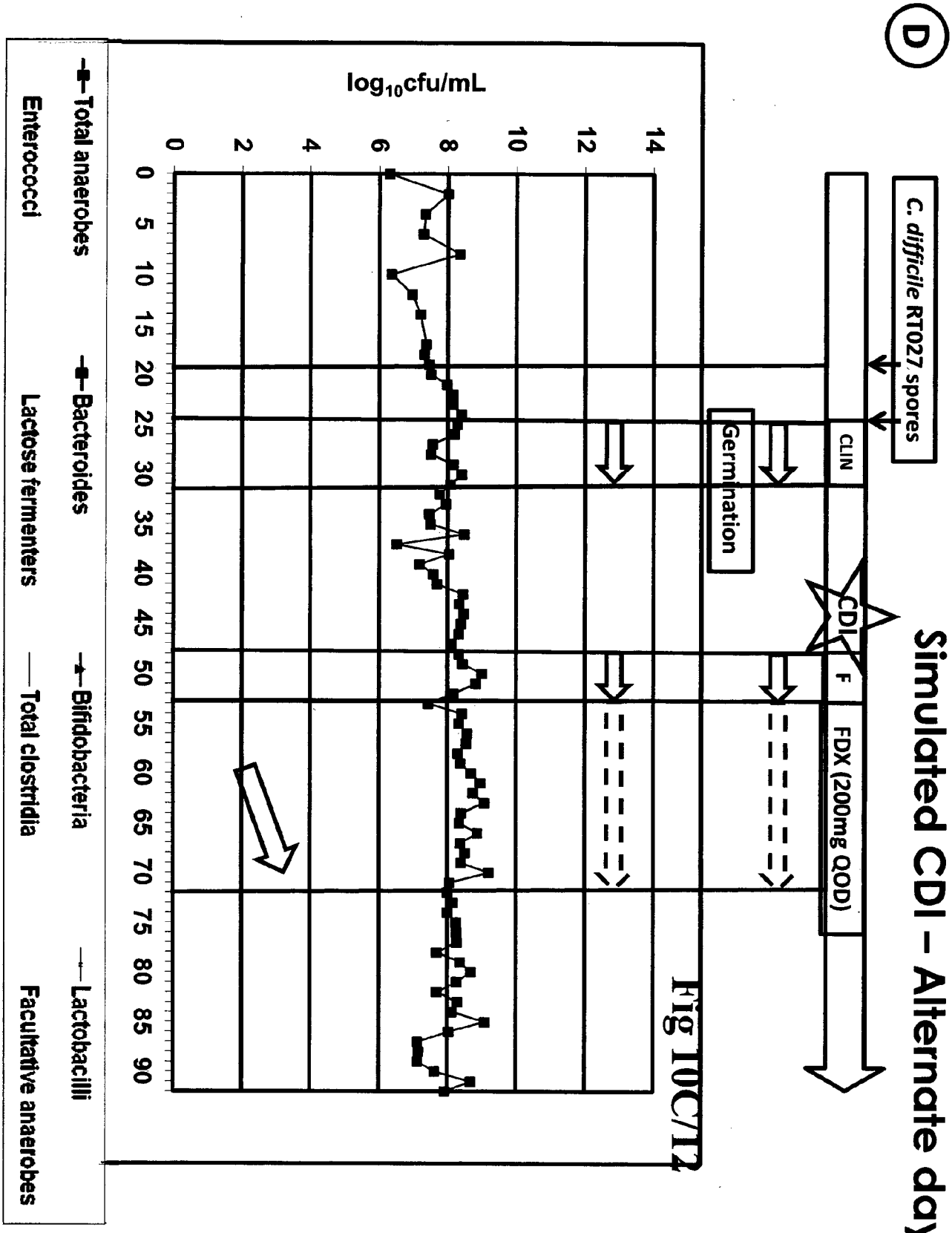

D
Simulated CDI – Alternate day
C. difficile RT027 spores
CLIN
CDI
F
FDX (200mg QOD)
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 10C/12

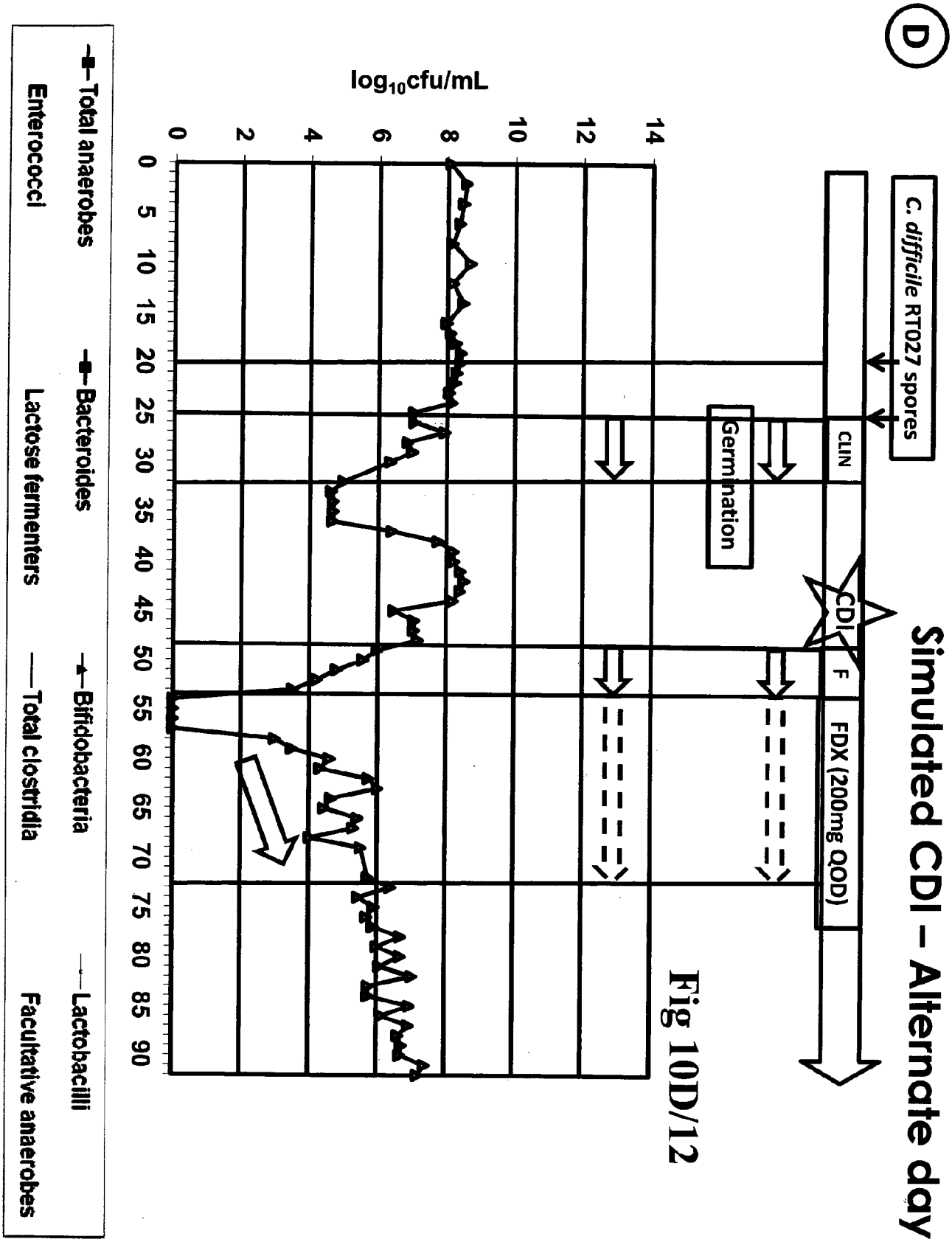

D
Simulated CDI – Alternate day
C. difficile RT027 spores
CLIN
CDI
F
FDX (200mg QOD)
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 10D/12

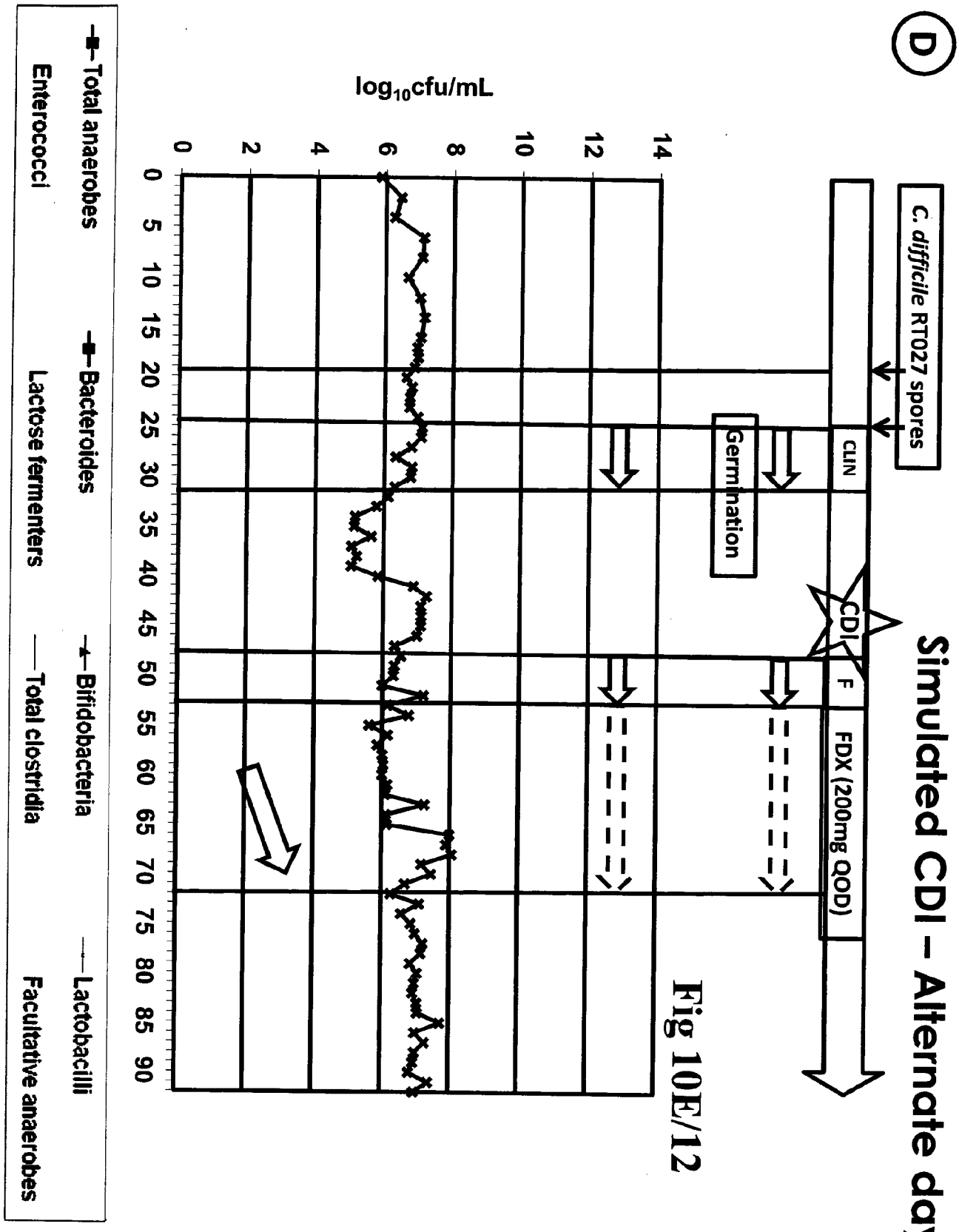

D
Simulated CDI – Alternate day
C. difficile RT027 spores
CLIN
Germination
CDI
F
FDX (200mg QOD)
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 10E/12

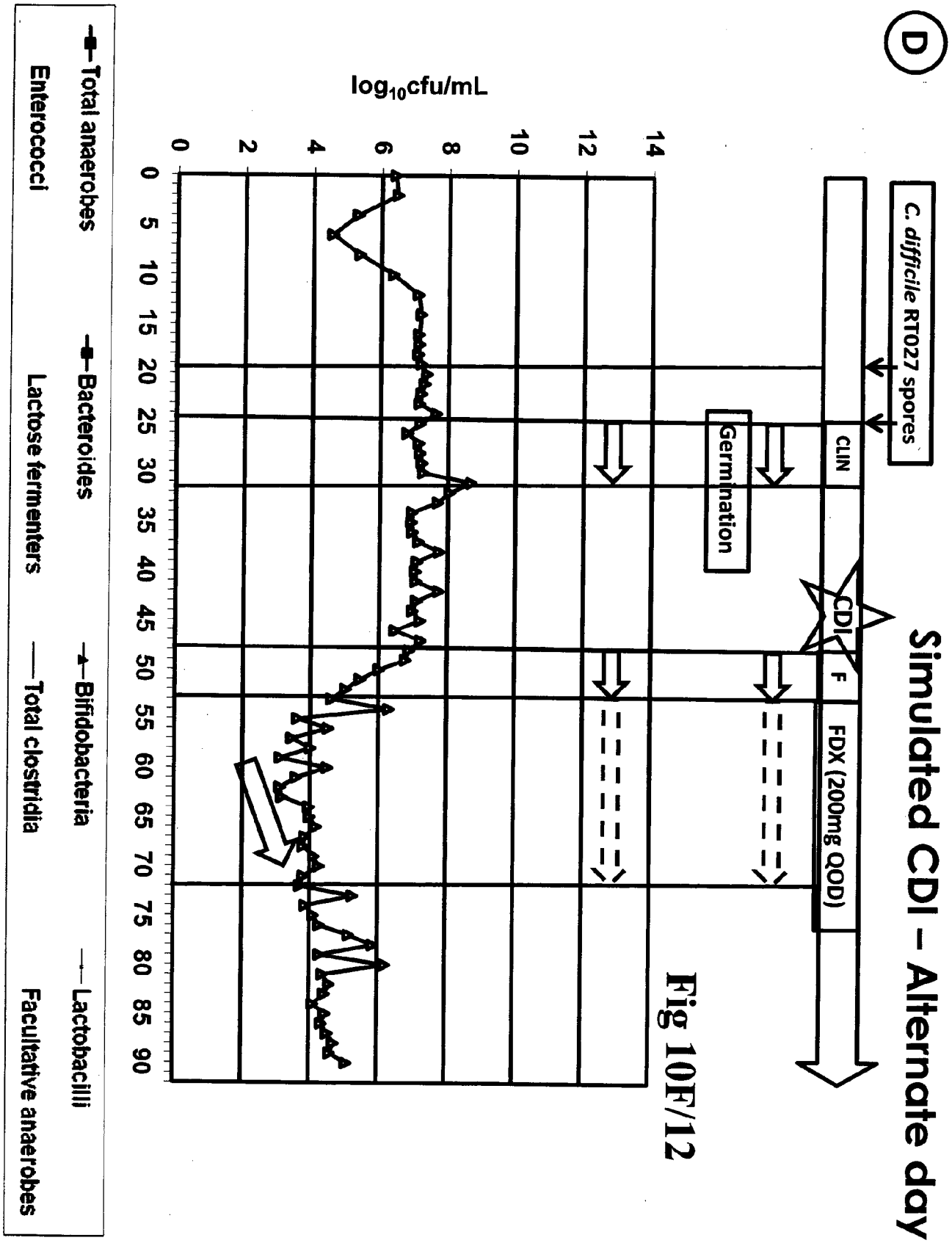

D
Simulated CDI – Alternate day
C. difficile RT027 spores
CLIN
CDI
F
FDX (200mg QOD)
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 10F/12

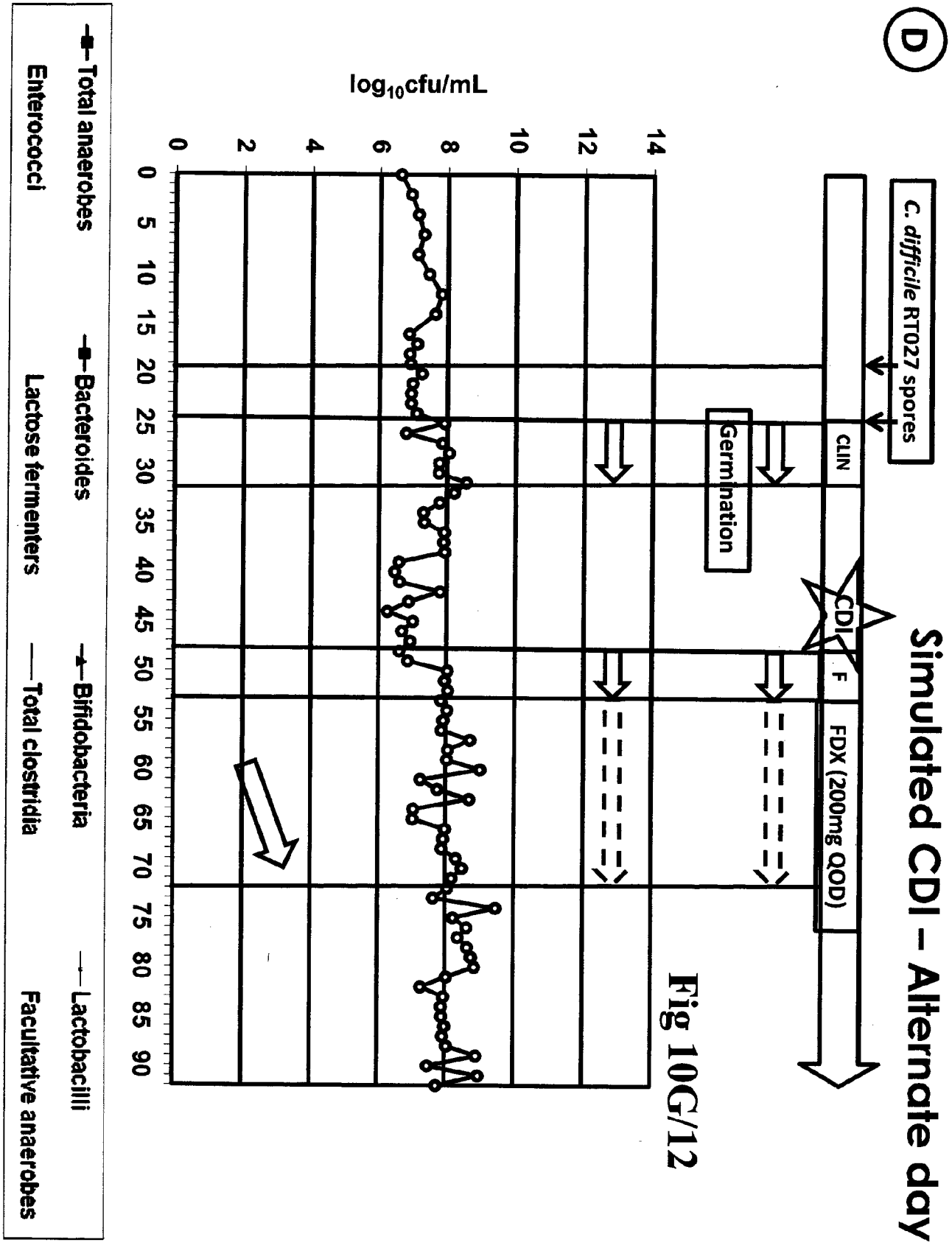

D
Simulated CDI – Alternate day
C. difficile RT027 spores
CLIN
CDI
F
FDX (200mg QOD)
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 10G/12

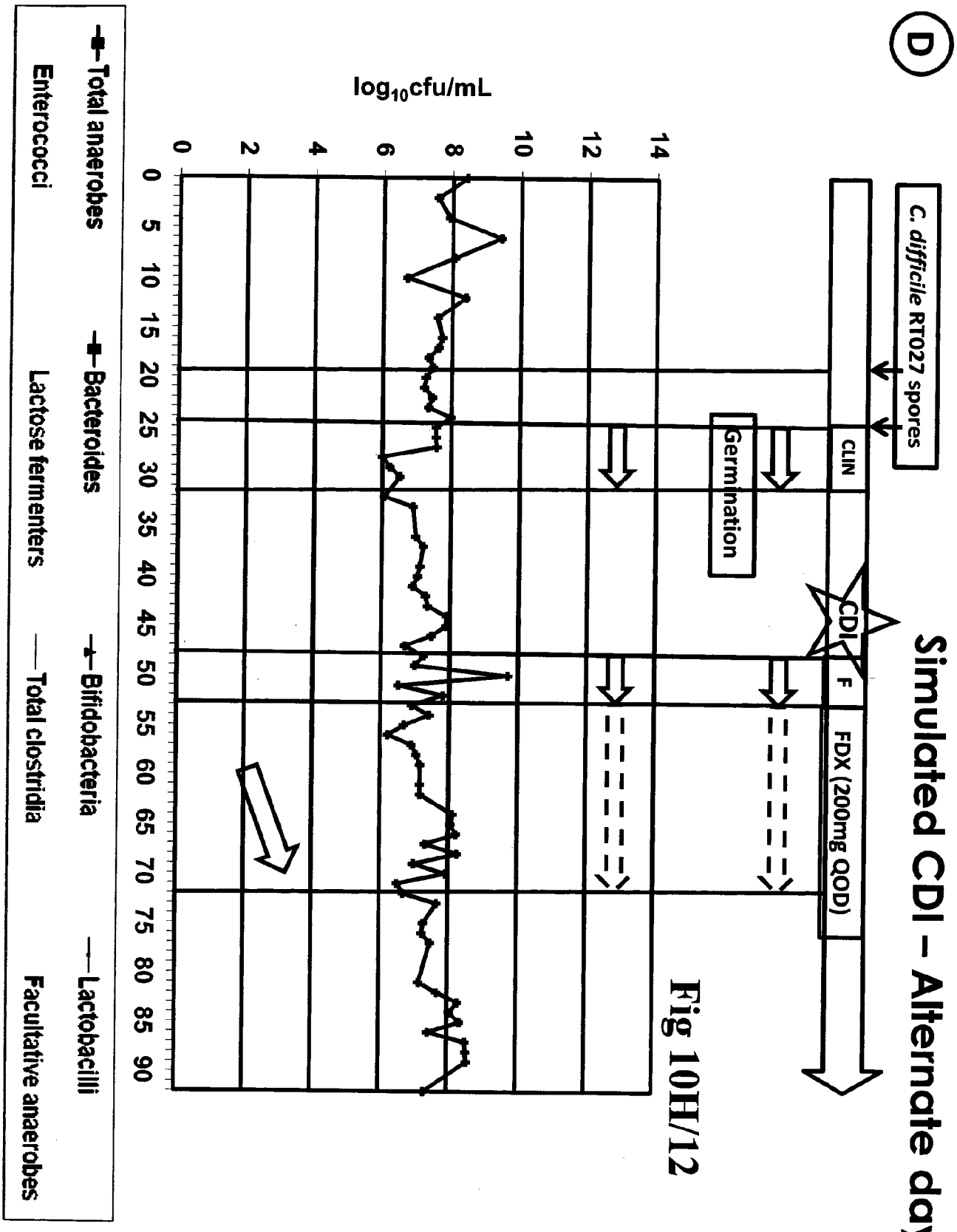

D
Simulated CDI – Alternate day
C. difficile RT027 spores
CLIN
CDI
F
FDX (200mg QOD)
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 10H/12

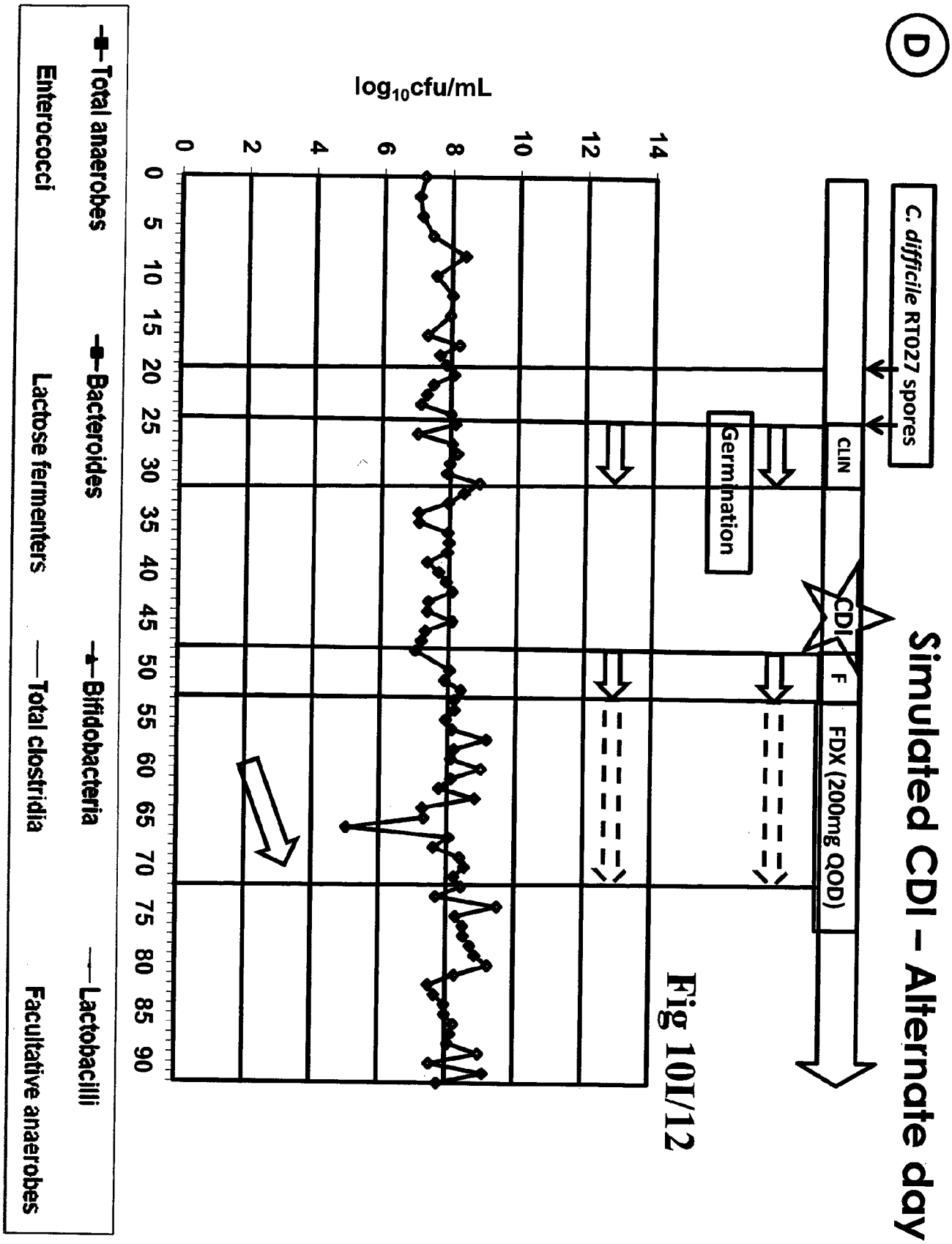

D
Simulated CDI – Alternate day
C. difficile RT027 spores
CLIN
CDI
F
FDX (200mg QOD)
Germination
log10cfu/mL
0
2
4
6
8
10
12
14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Total anaerobes
Bacteroides
Bifidobacteria
Lactobacilli
Enterococci
Lactose fermenters
Total clostridia
Facultative anaerobes

Fig 10I/12

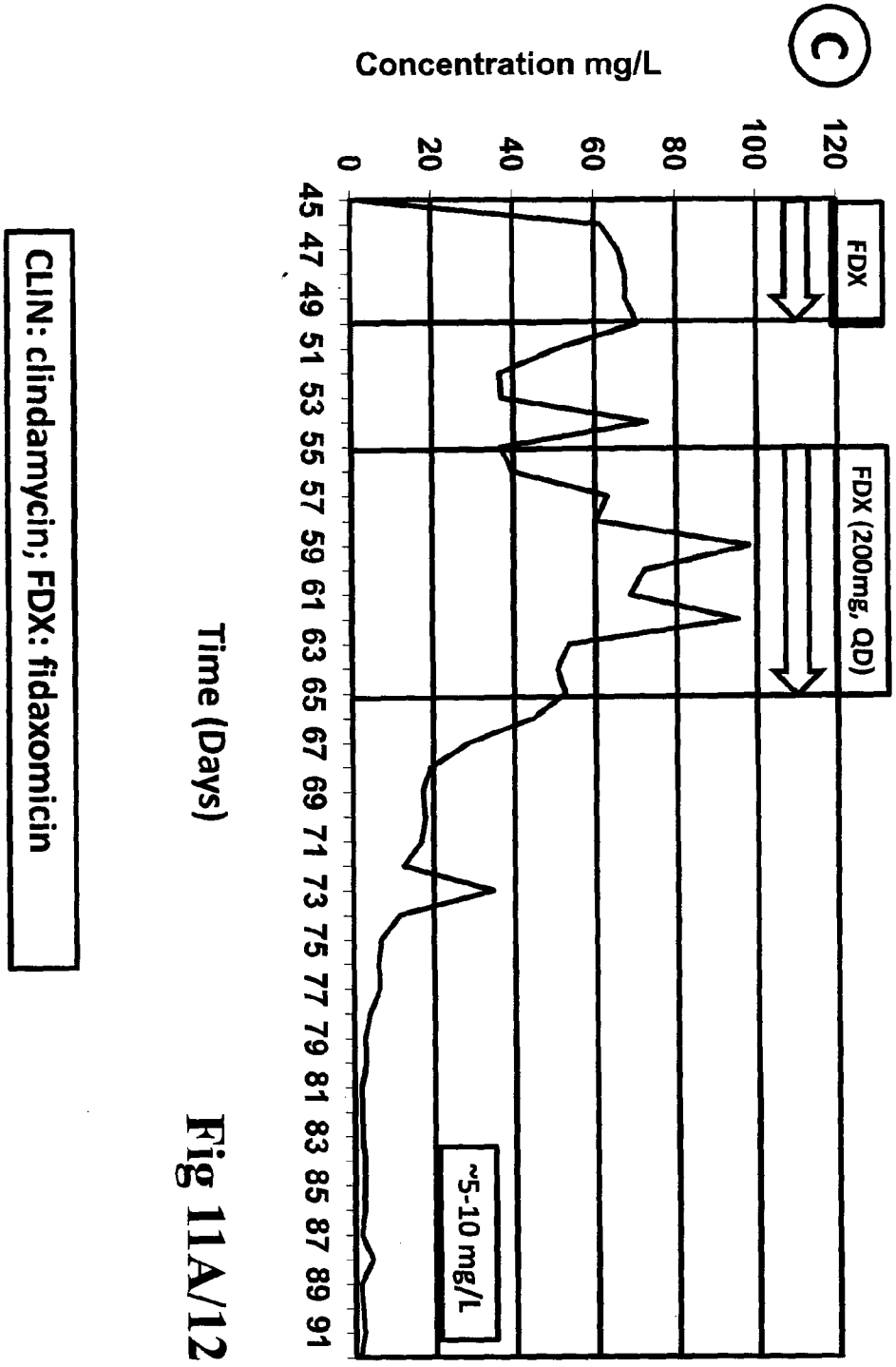


Fig 11A/12

CLIN: clindamycin; FDX: fidaxomicin

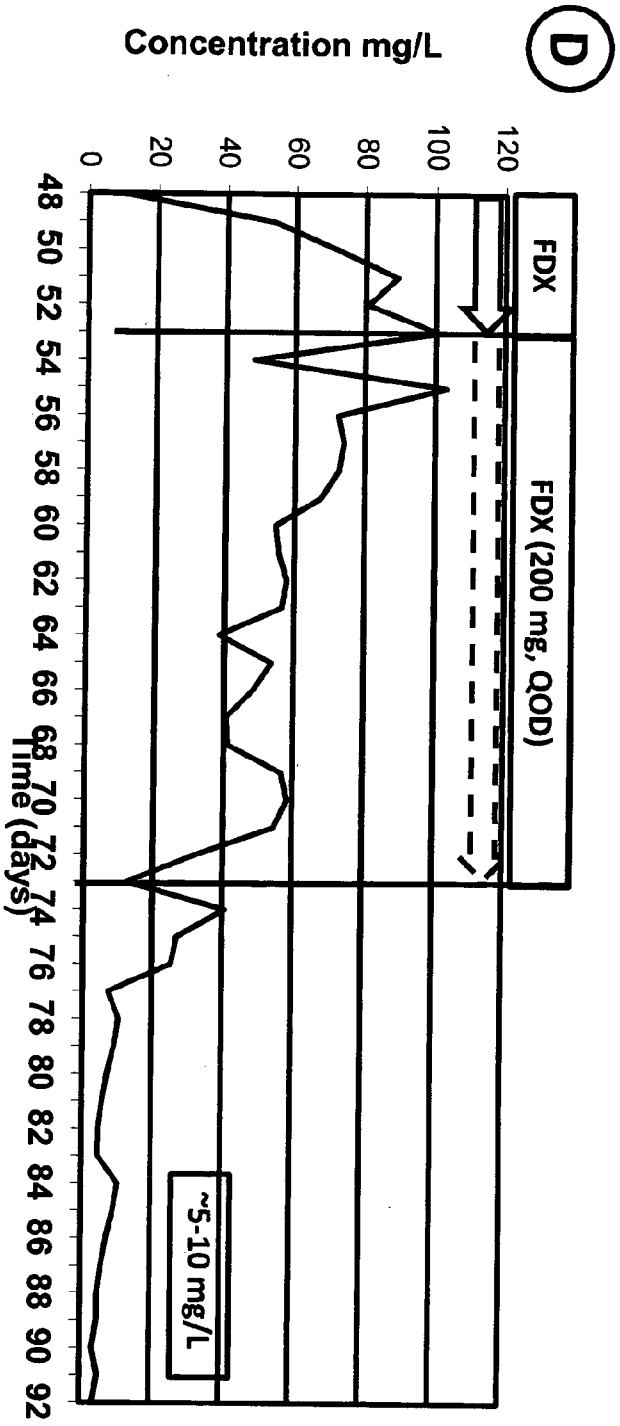

D
Concentration mg/L
0
20
40
60
80
100
120
FDX
FDX (200 mg, QOD)
~5-10 mg/L
48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80 82 84 86 88 90 92
Time (days)

Fig 11B/12

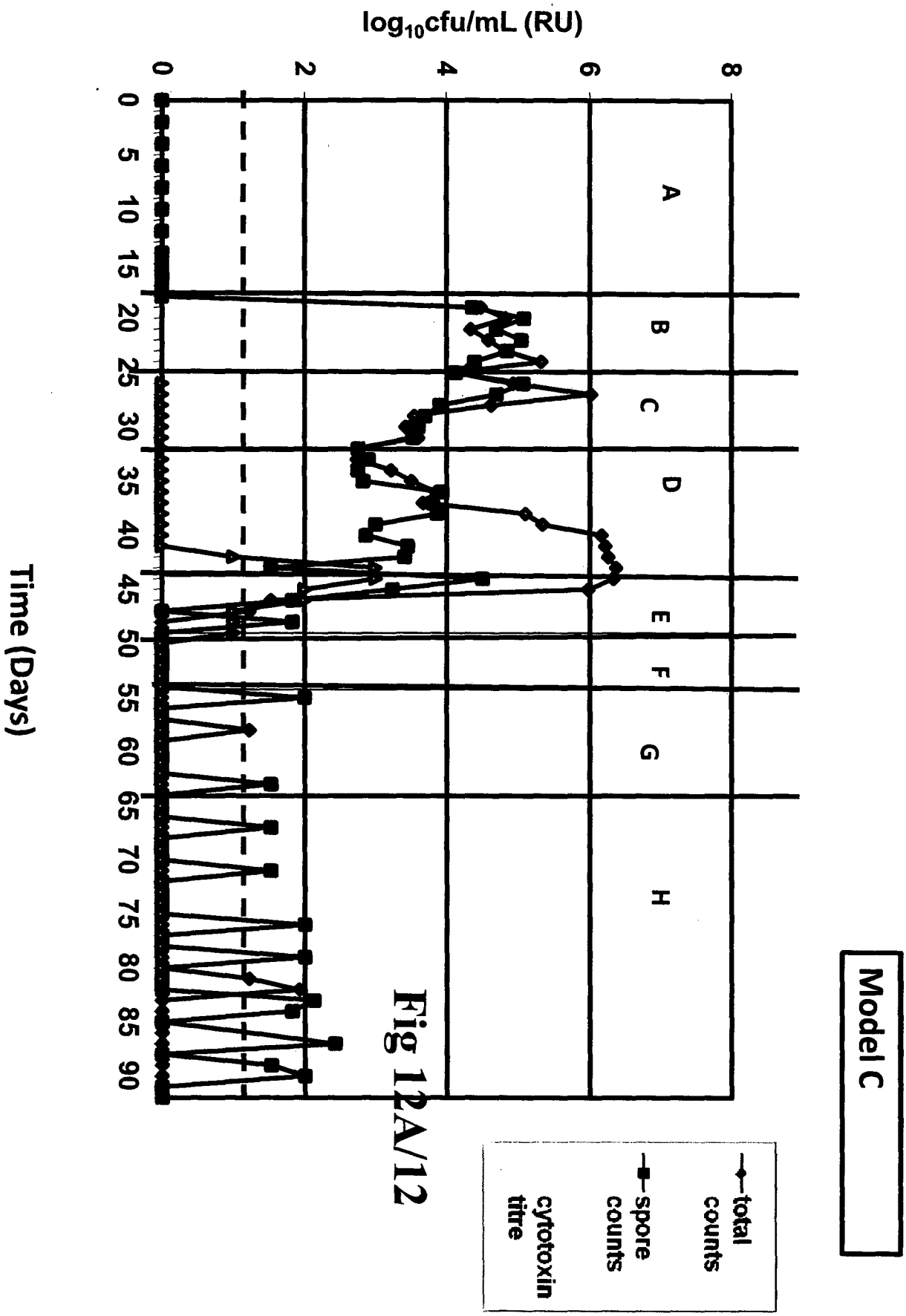

Model C
log10cfu/mL (RU)
0
2
4
6
8
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Time (Days)
A
B
C
D
E
F
G
H
total counts
spore counts
cytotoxin titre

Fig 12A/12

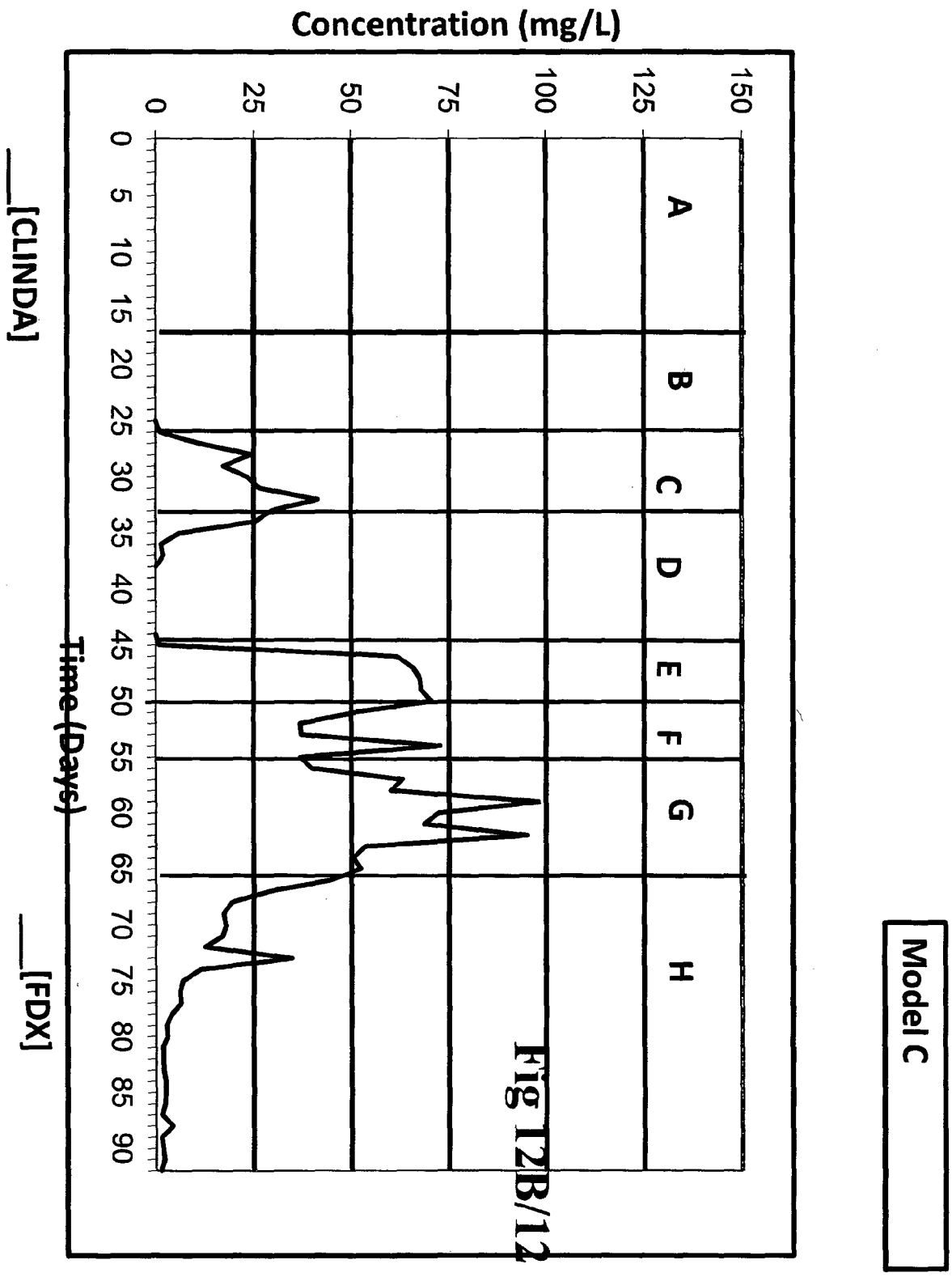

Concentration (mg/L)
0
25
50
75
100
125
150
A
B
C
D
E
F
G
H
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Time (Days)
[CLINDA]
[FDX]
Model C

Fig 12B/12

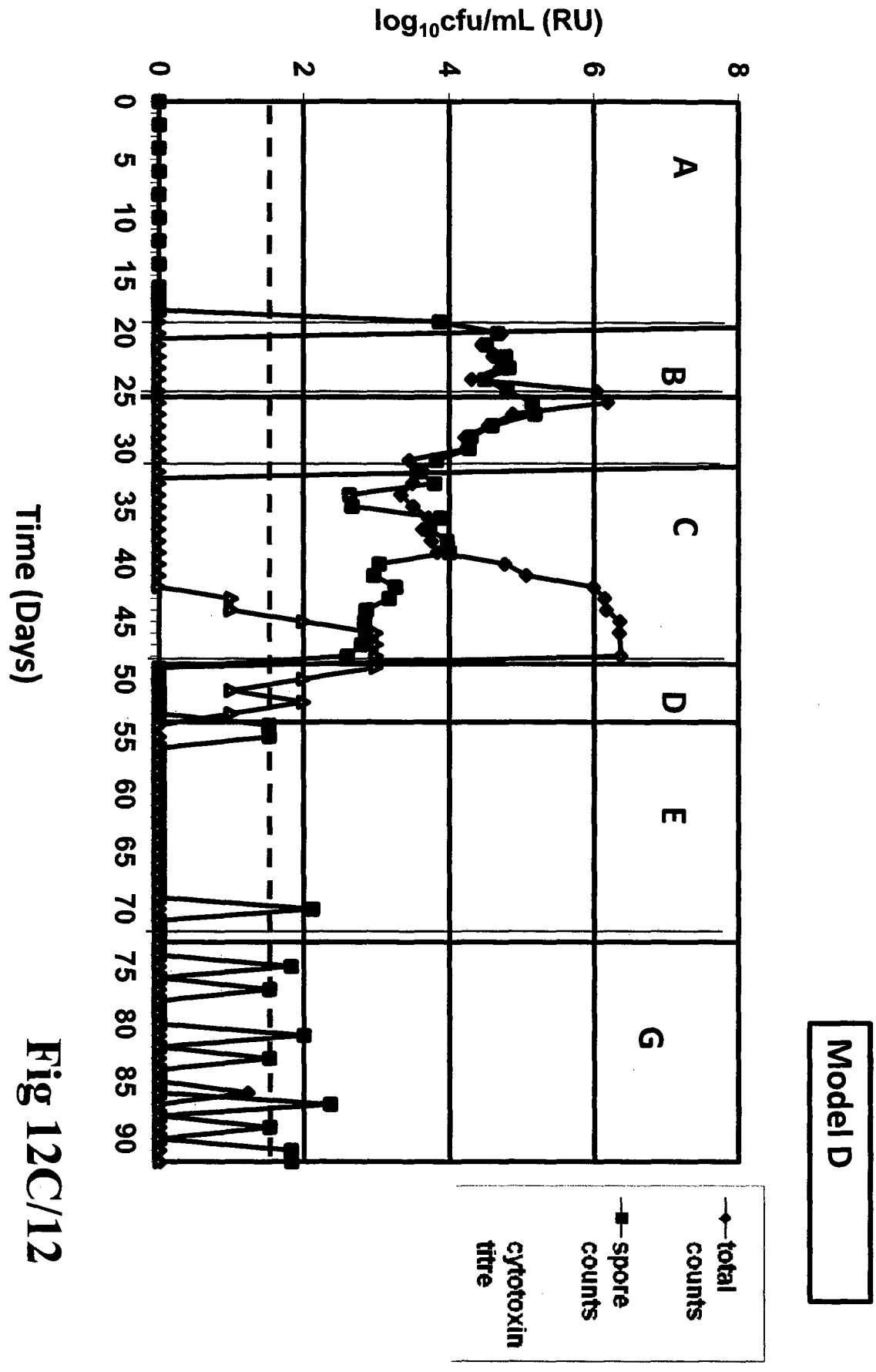

log10cfu/mL (RU)
0
2
4
6
8
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Time (Days)
A
B
C
D
E
G
Model D
total counts
spore counts
cytotoxin titre

Fig 12C/12

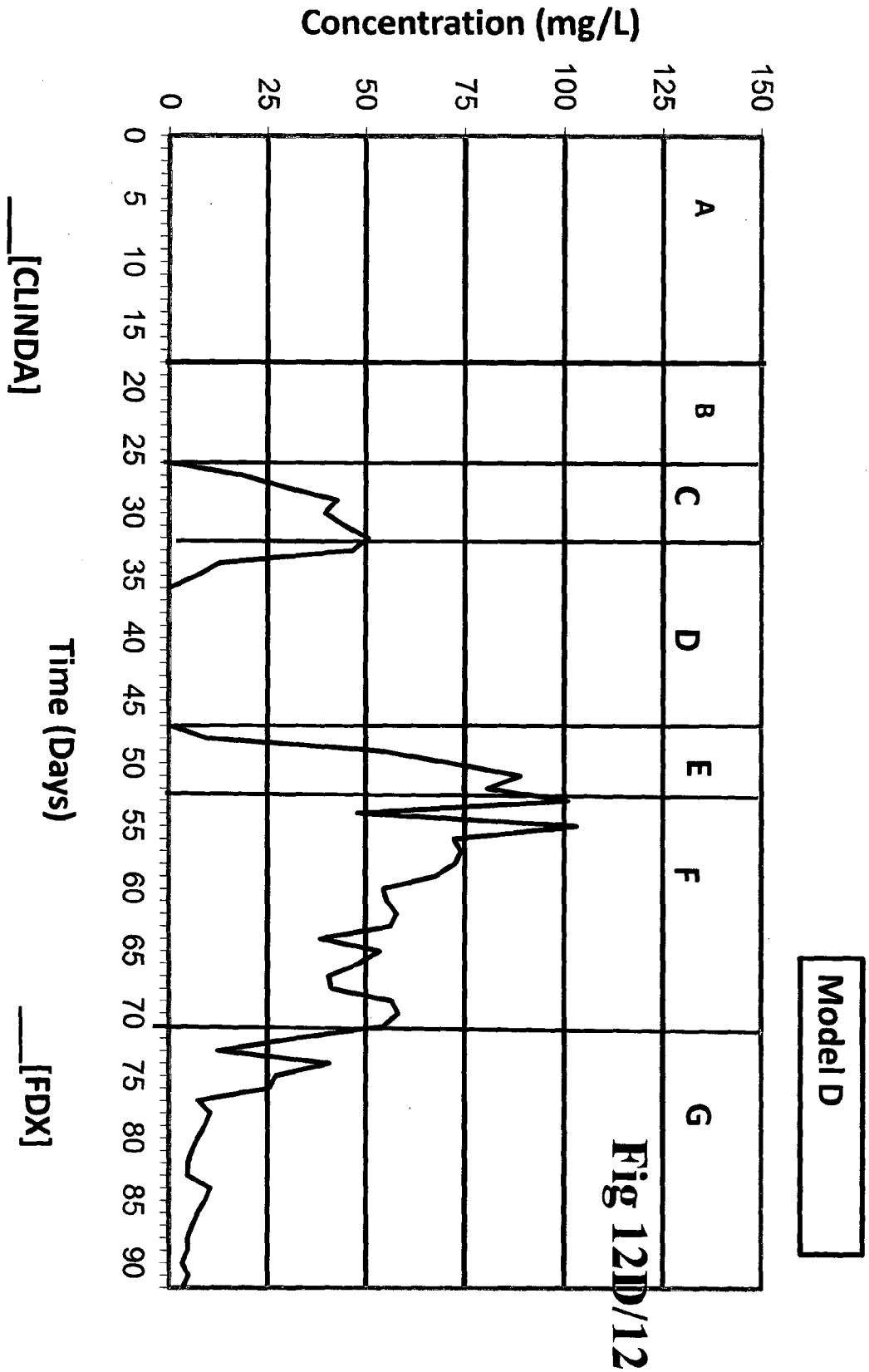

Concentration (mg/L)
0
25
50
75
100
125
150
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Time (Days)
A
B
C
D
E
F
G
Model D
[CLINDA]
[FDX]

Fig 12D/12

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/000965

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/7048 A61P31/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Sori ano et al.: "Abstract: The use of a fidaxomicin chaser as effecti ve sal vage therapy for vancomyci n-refractory, recurrent Clostri dium diffi cile infecti ons (IDWeek 2013) ", , 5 October 2013 (2013-10-05) , XP055182724, Retri eved from the Internet: URL: https ://i dsa.conf ex.com/i dsa/2013/webp rogram/Paper42591 .html [retri eved on 2015-04-14] cited in the appl i cati on the whol e document -/--	1-21

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" documentwhich may throw doubts on priority claim(s) orwhich is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 July 2015	10/08/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Steendij k, Marti n

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-& Melinda M Sori ano ET AL: "The Use of a Fi daxomi cin Taper/Pul sed Chaser as Effecti ve Sal vage Therapy for Vancomyci n-Refractory, Recurrent Cl ostri di um diffi cile Infecti ons" , , 5 October 2013 (2013-10-05) , XP055182720, Retri eved from the Internet: URL: https ://i dsa.conf ex.com/i dsa/2013/webp rogram/Paper42591 .html [retri eved on 2015-04-14]	
Y	C. Chilton ET AL: "2013 : K-336. Compari son of Extended Durati on Fi daxomi cin Dosing Regimens for Treatment of Cl ostri di um diffi cile Infecti on (CDI) in an in vitro Gut Model" , , September 2013 (2013-09) , XP055182841 , Retri eved from the Internet: URL: http: //www. icaacon 1ine. com/php/i caac20 13abstracts/data/papers/2013/K/2013_K-336. htm [retri eved on 2015-04-14] cited in the appl icati on the whol e document	1-21
Y	C. H. CHILTON ET AL: "Successful treatment of simul ated Cl ostri di um diffi cile infecti on in a human gut model by fidaxomicin first line and after vancomyci n or metroni dazol e fai l ure" , JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY, vol . 69, no. 2, 3 September 2013 (2013-09-03) , pages 451-462 , XP055182651 , ISSN: 0305-7453 , D0I: 10. 1093/jac/dkt347 cited in the appl icati on abstract; figures 2,5	1-21
A	S. JOHNSON ET AL: "Fidaxomicin "Chaser" Regimen Foll owing Vancomyci n for Pati ents Wi th Mul tiple Cl ostri di um diffi cile Recurrences" , CLINICAL INFECTIOUS DISEASES, vol . 56, no. 2, 28 September 2012 (2012-09-28) , pages 309-310, XP055182579 , ISSN: 1058-4838, D0I: 10. 1093/ci d/ci s833 page 2, right-hand col umn	1-21

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Ther Adv: "Therapeuti c Advances i n Gastroenterol ogy Revi ew", , 2012 , pages 395-402 , XP055182638, Retri eved from the Internet: URL: http://www.ncbi .nlm.ni h.gov/pmc/arti cl es/PMC3491684/pdf/10. 1177_1756283X12461294 .pdf page 400	1-21
A	WO 2013/138748 Al (OPTIMER PHARMACEUTICALS INC [US]) 19 September 2013 (2013-09-19) page 12	1-21
A	WO 2005/112990 A2 (OPTIMER PHARMACEUTICALS INC [US]; SHUE Y0UE-K0NG [US]; BABAKHANI FARAH) 1 December 2005 (2005-12-01) page 19	1-21
Y	G. W. TANN0CK ET AL: "A new macrocycl i c anti bioti c, fidaxomi cin (OPT-80) , causes l ess al terati on to the bowel mi crobi ota of Cl ostri dium diffi cile-i nfected pati ents than does vancomyci n", MICROBIOLOGY, vol . 156, no. 11, 19 August 2010 (2010-08-19) , pages 3354-3359 , XP055182699 , ISSN: 1350-0872 , D0I: 10. 1099/mi c.0.042010-0 page 3358	1-21
Y	T. J. LOUI E ET AL: "Fidaxomi cin Preserves the Intesti nal Mi crobi ome Duri ng and After Treatment of Cl ostri dium diffi cile Infecti on (CDI) and Reduces Both Toxi n Reexpressi on and Recurrence of CDI", CLINICAL INFECTIOUS DISEASES, vol . 55, no. suppl 2, 2 July 2012 (2012-07-02) , pages S132-S142 , XP055205254, ISSN: 1058-4838, D0I: 10. 1093/ci d/ci s338 page sl37	1-21
A, P	WO 2014/111254 Al (ASTELLAS PHARMA EUR0P LTD [GB]) 24 July 2014 (2014-07-24) abstract	1-21

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	"Effi cacy of tapered fidaxomicin dosing regimens to treat simulated Clostridium difficile infection (CDI) in an in vitro gut model", , 14 May 2014 (2014-05-14) , XP055182784, Retrieved from the Internet: URL: http://www.researchgate.net/profile/Chris_Longshaw/publication/262292381_Efficacy_of_tapered_fidaxomicin_dosing_regimens_to_treat_simulated_Clostridium_difficile_infection_(CDI)_in_an_in_vitro_gut_model/links/02e7e53737f70bb807000000.pdf?origin=publication_detail [retrieved on 2015-04-14] the whole document	1-21

Y,P	M. M. SORIANO ET AL: "Novel Fidaxomicin Treatment Regimens for Patients With Multiple Clostridium difficile Infection Recurrences That Are Refractory to Standard Therapies", OPEN FORUM INFECTIOUS DISEASES, vol. 1, no. 2, 1 August 2014 (2014-08-01) , pages ofu069-ofu069 , XP055182559 , DOI: 10.1093/ofid/ofu069 pages 5-6	1-21

T	C. H. CHILTON ET AL: "Efficacy of alternative fidaxomicin dosing regimens for treatment of simulated Clostridium difficile infection in an in vitro human gut model", JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY, 14 June 2015 (2015-06-14) , XP055205152 , ISSN: 0305-7453 , DOI: 10.1093/jac/dkv156 page 9	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/000965

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013138748 A1	19-09-2013	US 2015157653 A1	11-06-2015
		WO 2013138748 A1	19-09-2013
WO 2005112990 A2	01-12-2005	AT 516024 T	15-07-2011
		BR PI0511119 A	27-11-2007
		CA 2566687 A1	01-12-2005
		CA 2776682 A1	01-12-2005
		EP 1765312 A2	28-03-2007
		EP 2070530 A1	17-06-2009
		EP 2305244 A1	06-04-2011
		EP 2305245 A1	06-04-2011
		ES 2395404 T3	12-02-2013
		JP 5734545 B2	17-06-2015
		JP 2007537290 A	20-12-2007
		JP 2012197309 A	18-10-2012
		US 2007173462 A1	26-07-2007
		US 2013274175 A1	17-10-2013
		WO 2005112990 A2	01-12-2005
WO 2014111254 A1	24-07-2014	TW 201440775 A	01-11-2014
		WO 2014111254 A1	24-07-2014