

## Appendix A. Technical Expert Panel Members and Affiliation

<b>TEP Member</b>	<b>Affiliation</b>
Marcel Salive, MD, MPH	Division of Medical and Surgical Services Centers for Medicare and Medicaid Services Baltimore, Maryland
Erik Dubberke, MD, SHEA	School of Medicine Washington University St. Louis, Missouri
Christina Surawicz, MD	Past President American College of Gastroenterology Seattle, Washington
Dale Gerding, MD	Associate Chief of Staff Hines VA Hospital Hines, Illinois
Michael Wilson, MD	Department of Pathology & Laboratory Services Denver Health Medical Center Denver, Colorado

## Appendix B. Search Strategies

### Search string for C Difficile (general)

Database: Ovid MEDLINE(R)

Search Strategy:

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- 1 difficile.mp.
- 2 limit 1 to (english language and humans)
- 3 limit 2 to ("all adult (19 plus years)" or "young adult (19 to 24 years)" or "adult (19 to 44 years)" or "young adult and adult (19-24 and 19-44)" or "middle age (45 to 64 years)" or "middle aged (45 plus years)" or "all aged (65 and over)" or "aged (80 and over)")
- 4 randomized controlled trial.pt.
- 5 controlled clinical trial.pt.
- 6 randomized.ab.
- 7 placebo.ab.
- 8 drug therapy.fs.
- 9 randomly.ab.
- 10 trial.ab.
- 11 groups.ab.
- 12 or/4-11
- 13 (animals not (humans and animals)).sh.
- 14 12 not 13
- 15 3 and 14
- 16 limit 15 to (addresses or bibliography or biography or dictionary or directory or duplicate publication or editorial or interview or introductory journal article or lectures or legal cases or legislation or letter or news or newspaper article or patient education handout or portraits)
- 17 15 not 16
- 18 Cohort studies/ or comparative study/ or follow-up studies/ or prospective studies/ or risk factors/ or cohort.mp. or compared.mp. or groups.mp. or multivariate.mp.
- 19 limit 18 to (comment or editorial or historical article or interview or letter)
- 20 18 not 19
- 21 3 and 20
- 22 17 or 21

### Search string for C Difficile (Diagnostic)

Database: Ovid MEDLINE(R)

Search Strategy:

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- 1 difficile.mp.
- 2 diagnostic accuracy.mp.
- 3 (enzyme adj2 immunoassay\$.mp.
- 4 Immunoenzyme techniques/
- 5 enzyme linked immunosorbent assay/
- 6 feces/
- 7 faeces analysis.mp.
- 8 fecal.mp.
- 9 stool culture.mp.
- 10 exp "Sensitivity and Specificity"/
- 11 cytotoxicity test, immunologic/
- 12 cell cytotoxicity assay.mp.
- 13 pcr.mp. or polymerase chain reaction/
- 14 immunochromatography.mp.
- 15 or/2-14
- 16 1 and 15
- 17 limit 16 to (english language and humans and ("young adult (19 to 24 years)" or "adult (19 to 44 years)" or "young adult and adult (19-24 and 19-44)" or "middle age (45 to 64 years)" or "middle aged (45 plus years)" or "all aged (65 and over)" or "aged (80 and over)"))

**Appendix KQ1. Summary of matched comparisons of select† assays for *C. difficile* toxins (continued)**

- 18 limit 17 to (addresses or bibliography or biography or dictionary or directory or duplicate publication or editorial or interactive tutorial or interview or introductory journal article or lectures or legal cases or legislation or letter or news or newspaper article or patient education handout or portraits)
- 19 limit 17 to in vitro
- 20 17 not (18 or 19)

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**Appendix Table C1. Summary of matched comparisons of select† assays for *C. difficile* toxins**

Study	Patients/ Site	Tested Specimens	Reference Standard/ % Positive in Sample	Tests Compared	True Positive/ False Positive‡	PPV/ NPV
Kvach, 2010 <sup>1</sup>	n=341 in hospital with suspected CDI; Yale-New Haven Hospital, CT ?spectrum ?selection	n=400 fresh liquid or semisolid that were either GDH positive or negative; Some frozen at -20° C for < week after two-step GDH/CTA to do other tests; Excluded if patient being treated for CDI or retested within 7 days; ?blinded	If not all positive or negative on 3 tests, then toxigenic culture using Premier Toxin A/B test of cultured organisms  26.2%	GeneOhm <i>C. difficile</i> (tcdB), Becton Dickinson	96/105=91.4% 0/295=0%	96/96=100% 295/304=97.0%
				Tox A/B II, Techlab	70/105=66.7% 1/295=0.3%	70/71=98.6% 294/329=89.4%
				<i>C. Diff</i> Chek-60 (GDH), TechLab, and CTA if positive	87/105=82.9% 1/295=0.3%	87/88=98.9% 294/312=94.2%
Eastwood, 2009 <sup>2</sup>	?n CDI suspected with some previously diagnosed CDI; Leeds Teaching Hospitals, London, UK ?spectrum ?selection	n=600 only 558 for GeneOhm test; fresh, unformed refrigerated except for GeneOHm that were frozen at -20° C for < 8 months; ?blinded;	CTA of specimen and cultured organisms when stool CTA was negative  20.8%	GeneOhm <i>C. difficile</i> (tcdB), Becton Dickinson	92/103=89.3% 16/449=3.6%	92/108=85.2% 433/444=97.5%
				Premier Toxin A&B, Meridian	101/125=80.8% 12/475=2.5%	101/113=89.4% 463/487=95.1%
				Premier ImmunoCard Stat Toxins A&B, Meridian	86/115=74.8% 2/444=0.4%	86/88=97.7% 442/471=93.8%
				Tox A/B II, Techlab	100/125=80.0% 19/475=4.0%	100/119=84.0% 456/481=94.8%
				Tox A/B Quik Chek, Techlab	93/125=74.4% 3/473=0.6%	93/96=96.9% 470/502=93.6%
				ProSpecT Toxin A/B, Remel	102/125=81.6% 32/475=6.7%	102/134=76.1% 443/466=92.9%
				Xpect Toxin A/B, Remel	86/117=73.5% 3/475=0.6%	86/89=96.6% 472/503=93.8%
				VIDAS <i>C. difficile</i> Tox A/B	100/116=86.2% 2/464=0.4%	100/102=98.0% 462/478=96.6%

Appendix Table C1. Summary of matched comparisons of select† assays for *C. difficile* toxins (continued)

Study	Patients/ Site	Tested Specimens	Reference Standard/ % Positive in Sample	Tests Compared	True Positive/ False Positive‡	PPV/ NPV
Novak-Weekley, 2009 <sup>3</sup>	n = 432 suspected CDI; patients under 2 years old excluded; Southern CA Permanente Medical Labs; ?spectrum ?selection	n = 432 ?fresh Unformed, refrigerated; ?blinded;	Toxigenic culture using CTA on cultured organisms  16.7%	GeneXpert <i>C. difficile</i> (tcdB), Cepheid	68/72=94.4% 13/356=3.7%	68/81=84.0% 343/347=98.8%
				Premier Toxins A&B, Meridian	42/72=58.3% 19/360=5.3%	42/61=68.9% 341/371=91.9%
				1 <sup>st</sup> <i>C. difficile</i> CHEK- 60 (GDH), Techlab; if positive, then Premier Toxins A&B	40/72=55.6% 6/360=1.7%	40/46=87.0% 354/386=91.7%
Alcala, 2008 <sup>4</sup>	n=305 mixture of suspected CDI and positive samples by CTA; Hospital General Universitario Gregorio Maranon, Madrid, Spain ?spectrum ?selection	n=367 fresh refrigerated; ?consistency ?blinded ?indeterminate results	CTA of specimen and cultured organisms when direct CTA was negative  27.8%	ImmunoCard Toxins A&B, Meridian	68/102=66.7% 13/265=4.9%	68/81=83.9% 252/265=88.1%
				Xpect <i>C. diff.</i> toxin A/B, Remel	50/102=49.0% 11/265=4.2%	50/61=81.9% 254/306=83.0%
				TOX A/B QUIK CHEK, Techlab	56/102=54.9% 12/265=4.5%	56/68=82.4% 253/299=84.6%
Miendje Deyi, 2008 <sup>5</sup>	n=91 Age 65-99 avg. 81 yrs; suspected CDI; 2 university hospitals in Brussels, Belgium; 1 hospital had recent outbreak of <i>C. difficile</i> ; ?spectrum ?selection	n=100 frozen at -70° C; ?consistency tested blindly on same day in same lab ?indeterminate results	CTA  23.0%	ImmunoCard Toxins A&B, Meridian	21/23=91.3% 0/77=0%	21/21=100% 77/79=97.5%
				Biostar OIA CdTOX AB, Biostar	20/23=87% 0/77=0%	20/20=100% 77/80=96.3%
				Xpect <i>C. diff.</i> toxin A/B, Remel	21/23=91.3% 0/77=0%	21/21=100% 77/79=97.5%
				TOX A/B QUIK CHEK, Techlab	22/23=95.7% 0/77=0%	22/22=100% 77/78=98.7%

Appendix Table C1. Summary of matched comparisons of select† assays for *C. difficile* toxins (continued)

Study	Patients/ Site	Tested Specimens	Reference Standard/ % Positive in Sample	Tests Compared	True Positive/ False Positive‡	PPV/ NPV
Samra, 2008 <sup>6</sup>	n=200 hospitalized patients with diarrhea; Rabin Medical Center, Israel ?spectrum ?selection	n= 200 fresh or refrigerated diarrhea; randomly selected from positive and negative results;  ?blinded ?indeterminate results	In-house PCR for toxin B gene  47.0%	Tox A/B II, Techlab	88/94=93.6% 6/106=5.7%	88/94=93.6% 100/106=94.3%
				Tox A/B Quik Chek, Techlab	89/94=94.7% 3/106=2.8%	89/92=96.7% 103/108=95.4%
				Immunocard Toxin A&B, Meridian	89/94=94.7% 3/106=2.8%	89/92=96.7% 103/108=95.4%
Sloan, 2008 <sup>7</sup>	n=200 suspected CDI; Mayo Clinic, Rochester, MN ?spectrum ?selection	n=200 soft or liquid; fresh or frozen < 48 hrs. ?blinded; ?indeterminate results	Toxigenic culture using toxin A and B gene detection for cultured organisms  22.0%	Premier Toxin A&B, Meridian	21/44=47.7% 3/156=1.9%	21/24=87.5% 153/176=86.9%
				ImmunoCard Toxin A&B, Meridian	21/44=47.7% 2/156=1.3%	21/23=91.3% 154/177=87.0%
				Xpect <i>C. diff.</i> toxin A/B, Remel	21/44=47.7% 25/156=16.0%	21/46=45.6% 131/154=85.1%
				Triage <i>C. difficile</i> (GDH & toxin A), Biosite	14/44=31.8% 0/156=0%	14/14=100% 156/186=83.9%
Musher, 2007 <sup>8</sup>	?n inpatients suspected CDI; Michael E. DeBakey VA Medical Center, Houston, TX ?spectrum ?selection	n=446 ?fresh ?consistency ?blinded ?indeterminate results  <i>Part 2</i> ..... n=131 Convenience sample; Fresh; ?consistency ?blinded ?indeterminate results	CTA  17.0%  CTA  41.2%	Premier Toxin A&B, Meridian	75/76=98.7% 10/370=2.7%	75/85=88.2% 360/361=99.7%
				ImmunoCard Toxin A & B, Meridian	73/76=96.1% 4/370=1.1%	73/77=94.8% 366/369=99.2%
				Premier Toxin A&B, Meridian	52/54=96.3% 5/77=6.5%	52/57=91.2% 72/74=97.3%
				<i>C. difficile</i> TOX A/B II, TechLab	52/54=96.3% 10/77=13.0%	52/62=83.9% 67/69=97.1%
				ProSpecT Clostridium difficile toxin A/B, Remel	49/54=90.7% 2/77=2.6%	49/51=96.1% 75/80=93.8%

Appendix Table C1. Summary of matched comparisons of select† assays for *C. difficile* toxins (continued)

Study	Patients/ Site	Tested Specimens	Reference Standard/ % Positive in Sample	Tests Compared	True Positive/ False Positive‡	PPV/ NPV
van den Berg, 2007 <sup>9</sup>	n=450 all with diarrhea, some suspected CDI others not but inpatients for at least 72 hours; from 4 medical centers in The Netherlands; ?spectrum ?selection	n=547 diarrhea frozen at - 20° C; ?blinded	CTA  5.7%	Premier Toxins A&B, Meridian  VIDAS <i>C. difficile</i> Tox A II, bioMerieux Vitek	30/31=96.8% 29/509=5.7%  26/31=83.8% 15/509=2.9%	30/59=50.8% 480/481=99.8%  26/41=63.4% 494/499=99.0%
Lemee, 2004 <sup>10</sup>	n=80 subset of suspected CDI with positive or negative cultures; Rouen, France ?spectrum ?selection	n=80 ?consistency ?interim time ?storage ?blinded ?indeterminate results	CTA  25.0%	Premier Toxins A&B, Meridian  Triage <i>C. difficile</i> (GDH & toxin A), Biosite	16/20=80% 10/60=16.7%  14/20=70.0% 0/60=0%	16/26=61.5% 50/54=92.6%  14/14=100% 80/86=93.0%
Turgeon, 2003 <sup>11</sup>	n=1003 Consecutive samples, all suspected CDI; Childrens, university & cancer centers in Seattle, WA; 45% stem cell transplant patients ?spectrum ?selection	n=1003 any consistency; fresh for CTA, rest frozen at -20° C; ?interim time ?blinded ?indeterminate results	CTA  10.1%	Premier Cytoclone A/B, Meridian  <i>C. diff</i> Tox A/B, Techlab  Immunocard panel for GDH and toxin A, Meridian  Triage <i>C. difficile</i> (GDH & toxin A), Biosite  VIDAS <i>C. difficile</i> Tox A II, bioMerieux Vitek	74/101=73.3% 8/898=0.9%  78/101=77.2% 5/902=0.6%  56/101=55.4% 70/902=7.8%  60/101=59.4% 93/902=10.3%  69/99=69.7% 10/895=1.1%	74/82=90.2% 890/917=97.1%  78/83=94.0% 897/902=99.4%  56/126=44.4% 832/877=94.8%  60/153=39.2% 808/849=95.2%  69/79=87.3% 885/915=96.7%
Massey, 2003 <sup>12</sup>	n=557 Adult inpatients, CDI suspected; London, Ontario ?spectrum ?selection	n=557 fresh unformed ?blinded ?indeterminate results	CTA  25.7%	<i>C. diff</i> Tox A/B II, TechLab  Triage Micro <i>C.</i> <i>difficile</i> (GDH & toxin A), Biosite	107/143=74.8% 9/414=3.2%  100/143=69.9% 3/414=0.7%	107/116=92.2% 405/441=91.8%  100/103=97.1% 411/454=90.5%

Appendix Table C1. Summary of matched comparisons of select† assays for *C. difficile* toxins (continued)

Study	Patients/ Site	Tested Specimens	Reference Standard/ % Positive in Sample	Tests Compared	True Positive/ False Positive‡	PPV/ NPV
Alfa, 2002 <sup>13</sup>	?n Suspected CDI; St. Boniface General hospital, Winnipeg, Manitoba; ?spectrum ?selection	n=400 fresh, unformed refrigerated; all tests done on same day; ?blinded ?indeterminate results	CTA  14.5%	<i>C. diff</i> Tox A/B, Techlab  Triage Micro <i>C.</i> <i>difficile</i> (GDH & toxin A), Biosite	38/58=65.5% 3/342=0.9%  30/58=51.7% 0/342=0%	38/41=92.7% 339/359=94.4%  30/30=100% 342/370=92.4%
O'Connor, 2001 <sup>14</sup>	n=133 Adults, consecutive samples, CDI suspected; Multiple health centers in Galway County area of Ireland ?spectrum ?selection	n=200 92% liquid or unformed; -20° C for CTA, then frozen at 84° C; ?blinded	CTA  30.5%	Premier Toxins A&B, Meridian  <i>C. diff</i> Tox A/B II, Techlab  ImmunoCard Toxin A, Meridian	50/61=82.0% 1/139=0.7%  49/61=80.3% 1/139=0.7%  32/61=52.4% 0/139=0.0%	50/51=98.0% 138/149=92.6%  49/50=98.0% 138/150=92.0%  32/32=100% 139/168=82.7%
Langley, 1995 <sup>15</sup>	n=170 patients with watery diarrhea who had been receiving broad spectrum antibiotics; Manchester, UK ?spectrum ?selection	n=200 watery, refrigerated and tested within 48 hours; indeterminate results called negative; ?blinded	CTA  19.5%	Cytoclone A+B, Cambridge Biotech  Premier <i>C. difficile</i> toxin A, Meridian Diagnostics	34/39=87.2% 6/161=3.7%  34/39=87.2% 6/161=3.7%	34/40=85.0% 155/160=96.9%  34/40=85.0% 155/160=96.9%
Merz, 1994 <sup>16</sup>	n=329 Inpatients & clinic patients; All suspected CDI; Johns Hopkins Hospital, Baltimore, MD ?spectrum ?selection	n=700 refrigerated and concurrently tested within 24 hours except frozen aliquots used for culture; ?consistency ?blinded	CTA and culture with toxin test  8.7%	Bartels Prima System <i>C. difficile</i> Toxin A, Baxter  Cytoclone A+B, Cambridge  Premier <i>C. difficile</i> Toxin A, Meridian  <i>C. difficile</i> Tox-A, TechLab	51/61=83.6% 20/636=3.1%  50/59=84.7% 2/635=0.3%  48/61=78.7% 12/638=1.9%  49/61=80.3% 28/638=4.4%	51/71=71.8% 616/626=98.4%  50/52=96.2% 633/642=98.6%  48/60=80.0% 626/639=98.0%  49/77=63.6% 610/622=98.1%

Appendix Table C1. Summary of matched comparisons of select† assays for *C. difficile* toxins (continued)

Study	Patients/ Site	Tested Specimens	Reference Standard/ % Positive in Sample	Tests Compared	True Positive/ False Positive‡	PPV/ NPV
Arrow, 1994 <sup>17</sup>	n –not reported Inpatients and unspecified others; Sir Charles Gairdner Hospital, Nedlands, Western Australia ?spectrum ?selection	n=160 fresh for CTA then frozen at -20° C; ?consistency ?blinded ?indeterminate results	CTA  32.5%	Premier <i>C. difficile</i> Toxin A, Meridian	44/52=84.6% 1/108=0.9%	44/45=97.8% 107/115=93.0%
				Cytoclone Toxin A+B, Cambridge	50/52=96.2% 7/108=6.5%	50/57=87.7% 101/103=98.0%
Barbut, 1993 <sup>18</sup>	n=285 consecutive stool samples from suspected cases of CDI; Hospital St.-Antoine, Paris, Fr	n=285 refrigerated for up to 72 hrs; ?consistency ?blinded	CTA  17.9%	Premier <i>C. difficile</i> Toxin A, Meridian	37/51=72.5% 1/233=0.4%	37/38=97.4% 232/246=94.3%
				Cytoclone A+B, Cambridge	39/49=79.6% 6/227=2.6%	39/45=86.7% 221/231=95.7%
				VIDAS <i>C. difficile</i> Tox A, bioMerieux Vitek	32/48=66.7% 0/230=0%	32/32=100% 230/246=93.5%
Mattia, 1993 <sup>19</sup>	?n Patients suspected of CDI Massachusetts General Hospital & University of Massachusetts Medical Center; ?spectrum ?selection	n=945 fresh refrigerated; ?consistency ?blinded, were analyzed simultaneously	CTA  12.9%	Cytoclone A+B, Cambridge	100/119=84.0% 42/805=5.2%	100/142=70.4% 763/782=97.6%
				VIDAS <i>C. difficile</i> Tox A, bioMerieux Vitek	87/119=73.1% 12/805=1.5%	87/99=87.9% 793/825=96.1%
Doern, 1992 <sup>20</sup>	n=262 inpatients and outpatients in Worcester, MA ?spectrum ?selection	n=320 fresh refrigerated; ?consistency ?blinded	CTA  22.8%	<i>C. difficile</i> toxins A&B, Cambridge	60/66=90.9% 0/247=0%	60/60=100% 247/260=95.0%
				<i>C. difficile</i> toxin A, Meridian	49/73=67.1% 0/247=0%	49/49=100% 247/271=91.1%

† presumably available and used in the United States, and at least one comparator is an immunoassay for toxins A and B. ? indicates issues identified by the quality assessment of diagnostic accuracy studies (QUADAS) criteria. CDI – *C. difficile* infection. CTA – cytotoxicity assay using cultured test cells. True + is sensitivity/ False + is 1 – specificity. PPV/NPV – positive/negative predictive value based on prevalence of *C. difficile* in tested sample. GDH - glutamate dehydrogenase. ‡ Varying numbers of indeterminate results are excluded from estimates of true and false positives when possible, thus denominators are not constant for all methods compared within a study.

**Appendix Table C2 . Grade of evidence for comparisons of diagnostic tests for toxigenic C. difficile**

Comparison	Difference in Sensitivity (True Positives)		Difference in False Positives (1 – Specificity)	
	Ratings†	Overall Evidence Grade	Ratings†	Overall Evidence Grade
<b>Immunoassays for Toxins A &amp; B</b>				
Premier Toxin A&B, Meridian Tox A/B II, TechLab	Consistent, Imprecise	Low	Inconsistent, Precise	Low
Tox A/B QUIK CHEK, TechLab ImmunoCard A&B, Meridian	Consistent, Imprecise	Low	Consistent, Precise	Moderate
Premier Toxin A&B, Meridian ImmunoCard A&B, Meridian	Consistent, Imprecise	Low	Consistent, Precise	Moderate
Tox A/B QUIK CHEK, TechLab Xpect Toxin A/B, Remel	Consistent, Imprecise	Low	Consistent, Precise	Moderate
Tox A/B QUIK CHEK, TechLab Tox A/B II, TechLab	Consistent, Imprecise	Low	Consistent, Imprecise	Low
Premier Toxin A&B, Meridian ProSpecT Toxin A/B, Remel	Consistent, Imprecise	Low	Inconsistent, Imprecise	Low
Premier Toxin A&B, Meridian Xpect Toxin A/B, Remel	Consistent, Imprecise	Low	Inconsistent, Imprecise	Low
Tox A/B II, TechLab ImmunoCard A&B, Meridian	Consistent, Imprecise	Low	Consistent, Imprecise	Low
Premier Toxin A&B, Meridian C. diff Tox A/B, VIDAS	Single study, Imprecise	Low	Single study, Precise	Low
Premier Toxin A&B, Meridian Tox A/B QUIK CHEK, TechLab	Single study, Imprecise	Low	Single study, Precise	Low
Tox A/B QUIK CHEK, TechLab ProSpecT Toxin A/B, Remel	Single study, Imprecise	Low	Single study, Imprecise	Low
Tox A/B QUIK CHEK, TechLab C. diff Tox A/B, VIDAS	Single study, Imprecise	Low	Single study, Precise	Low
Xpect Toxin A/B, Remel ProSpecT Toxin A/B, Remel	Single study, Imprecise	Low	Single study, Imprecise	Low
Xpect Toxin A/B, Remel C. diff Tox A/B, VIDAS	Single study, Imprecise	Low	Single study, Precise	Low
ProSpecT Toxin A/B, Remel C. diff Tox A/B, VIDAS	Single study, Imprecise	Low	Single study, Imprecise	Low
Premier Toxin A&B, Meridian 2-Stage test using CHEK-60 for GDH, then if positive Premier Toxin A&B, Meridian	Single study, Imprecise	Low	Single study, Imprecise	Low
<b>Gene Detection Tests vs. Immunoassays for Toxins A &amp; B</b>				
GeneOhm, Becton Dickinson Tox A/B II, TechLab	Inconsistent, Imprecise	Low	Consistent, Precise	Moderate
GeneOhm, Becton Dickinson Premier Toxin A&B, Meridian	Single study, Imprecise	Low	Single study, Imprecise	Low
GeneOhm, Becton Dickinson ImmunoCard A&B, Meridian	Single study, Imprecise	Low	Single study, Imprecise	Low
GeneOhm, Becton Dickinson Tox A/B QUIK CHEK, TechLab	Single study, Imprecise	Low	Single study, Imprecise	Low
GeneOhm, Becton Dickinson ProSpecT Toxin A/B, Remel	Single study, Imprecise	Low	Single study, Imprecise	Low
GeneOhm, Becton Dickinson Xpect Toxin A/B, Remel	Single study, Imprecise	Low	Single study, Imprecise	Low
GeneOhm, Becton Dickinson C. diff Tox A/B, VIDAS	Single study, Imprecise	Low	Single study, Imprecise	Low
GeneXpert, Cepheid Premier Toxin A&B, Meridian	Single study, Imprecise	Low	Single study, Imprecise	Low

**Appendix Table C2. Grade of evidence for comparisons of diagnostic tests for toxigenic *C. difficile* (continued)**

Comparison	Difference in Sensitivity (True Positives)		Difference in False Positives (1 – Specificity)	
	Ratings†	Overall Evidence Grade	Ratings†	Overall Evidence Grade
GeneXpert, Cepheid 2-Stage test using CHEK-60 for GDH, then if positive Premier Toxin A&B, Meridian	Single study, Imprecise	Low	Single study, Imprecise	Low

† Consistency refers to the variation between estimates from different studies. Precision refers to the width of the overall confidence interval. The risk of bias was considered to be low for all comparisons. All the evidence is only indirectly related to clinical decisions and the effect of differences on patient health outcomes is not known.

**Appendix Table C3. Description of studies evaluating risk factors for *Clostridium difficile* associated diarrhea (CDAD)**

Study/Origin	Study Type	Objective	Population	Methods	Results
Sundram, 2009 <sup>21</sup>  United Kingdom	Case control study	Investigates risk factors for onset of CDAD	Hospital inpatients from a single hospital.  Cases (n=97) Mean age 81 years; Female 45%  Controls (n=97) Mean age 80 years; Female 56%	CDAD defined as diarrhea (>1 loose stool per day for ≥2 days) occurring >48 hours after admission plus <i>C. difficile</i> toxin. Controls did not have diarrhea at the time of study and had never tested positive for CDAD before. Each control was matched to a case by sex, age, ward, ASA score and length of stay.  Toxin assay: enzyme immune-sorbent (Meridian Premier, Meridian Bioscience Inc., Cincinnati, OH, USA).  Analysis: conditional logistic regression analysis	Risk factor for CDAD: ciprofloxacin usage for >7 days (adjusted OR 3.72; 95% CI: 1.38 to 10.02)
Walbrown, 2008 <sup>22</sup>  United States	Prospective observational, multicenter, cohort study	Evaluate formulary change of levofloxacin to gatifloxacin as the primary quinolone in 10 VA hospitals was associated with increased CDAD rates.	505 inpatients and outpatients from 10 VA hospitals were identified using the VA's Pharmacy Benefits Management database. Mean age 69 years; Male gender 97%	Total number of days of antibiotic therapy was determined 6 months before and 6 months after the change in the preferred oral fluoroquinolone from levofloxacin to gatifloxacin for the VA health system and electronic medical records of patients with an entry for a positive <i>C. difficile</i> toxin were reviewed.  Analysis: exact Poisson tests were used to compare incidence rates of CDAD (number of CDAD cases per 1,000 days of antibiotic treatment) for antibiotics (overall, quinolones as a group, non-fluoroquinolone antibiotics, individual quinolones), comparing the 6-month time periods before versus after the addition of	Among antibiotic users, incidence rates of CDAD in the pre-change period were 2.3 cases per 1,000 days of antibiotics versus 3.4 cases per 1,000 days of antibiotics in the post-change period ( $P < 0.001$ ; RR 1.5 95%CI 1.2 to 1.8). Quinolones accounted for 54.8% of the CDAD cases in the pre-change period and 67.2% in the post-change period, representing a 22.6% relative increase in the percentage of CDAD cases that were associated with fluoroquinolone use. The CDAD incidence rates per 1,000 days of fluoroquinolone therapy were 3.7 in the pre-change period versus 7.0 in the post-change period ( $P < 0.001$ ; RR 1.9 95%CI 1.5 to

Appendix Table C3. Description of studies evaluating risk factors for *Clostridium difficile* associated diarrhea (CDAD) (continued)

Study/Origin	Study Type	Objective	Population	Methods	Results
				gatifloxacin.	2.5). Overall incidence of CDAD increased following change to gatifloxacin and the incidence rate increased among patients receiving any of the quinolones. In the post-change period, incidence rates for gatifloxacin, ciprofloxacin, and levofloxacin were not significantly different, and levofloxacin was the only quinolone associated with a significant increase in the rate of CDAD between the pre-change and post-change periods.  Results suggest that the increased incidence of CDAD was unrelated to the gatifloxacin formulary change.
Munoz, 2007 <sup>23</sup>  Spain	Prospective cohort series	Risk factors, including hypogammaglobulinemia, of CDAD after heart transplant.	235 patients who underwent heart transplant. Mean age 53 years; Male 84% CDAD was detected in 35 patients (15%).	Diarrhea defined as $\geq 3$ unformed stools for $\geq 2$ consecutive days after hospitalization was considered to be nosocomial. CDAD was defined as diarrhea not attributable to any other cause and was associated with a positive stool cytotoxin test for <i>C. difficile</i> .  Toxin assay: cell-culture cytotoxin test in a culture of human fibroblasts.  Analysis: Stepwise logistic and Cox regression models.	Post-transplant hypogammaglobulinemia is independently associated with an increased risk of CDAD (RR 5.8 95% CI: 1.05 to 32.1)
Peled, 2007 <sup>24</sup>  Israel	Prospective cohort study	Compare the clinical characteristics of patients who developed CDAD versus patients with a negative stool	217 patients with ADD. <i>C. difficile</i> toxin positive (n=52): n=52; mean age 72 years; Male-female ratio 1:26  <i>C. difficile</i> toxin negative (n=165):	Diarrhea was defined as the passage of $\geq 3$ unformed stools for $\geq 2$ consecutive days.  Toxin assay: enzyme immunoassay for <i>C. difficile</i> toxin	Significant factors for CDAD: watery diarrhea (OR=17.1, p=0.000), functional capacity score of 2 or 3 (requiring assistance in daily activities or bedridden) (OR=9.14, p=0.000),

Appendix Table C3. Description of studies evaluating risk factors for *Clostridium difficile* associated diarrhea (CDAD) (continued)

Study/Origin	Study Type	Objective	Population	Methods	Results
		assay for <i>C. difficile</i> toxin	mean age 66 (p=0.21 vs. toxin pos.); Male-female ratio 1:11	A/B (TechLab).  Analysis (controlling for confounding): Stepwise logistic regression	use of a proton pump inhibitor (OR=6.1, p=0.024), hypoalbuminemia (OR=3.8, p=0.001), histamine blocker (OR=3.1, p=0.024) leukocytosis (OR=2.7, p=0.004). Stepwise logistic regression analysis predicted a positive result for <i>C. difficile</i> toxin with 95% specificity and 68% sensitivity.
Samore, 2006 <sup>25</sup>  United States	Prospective case series	Analyze <i>C. difficile</i> susceptibility results and genotypes in relation to antibiotic exposures that precipitated CDAD	83 patients with nosocomial CDAD. Mean age 66 years; female 43%  Median length of stay before onset of CDAD was 10 days (range, 2–95). <i>C. difficile</i> isolates were recovered from patients in 10 different hospital wards and 3 intensive care units. The wards with the largest number of cases were vascular surgery (n=15) and general surgery/liver transplantation (n =14).	Prospective surveillance and collection of stool isolates. Isolates were genotyped by pulsed-field gel electrophoresis and restriction enzyme analysis.  Analysis: multivariable logistic regression	Clindamycin exposure was strongly associated with CDAD caused by isolates that exhibited multiple resistance to clindamycin, erythromycin, and trovafloxacin (prevalence OR 4.2; 95%CI: 1.1 to 16.8)
Yearsley, 2006 <sup>26</sup>  United Kingdom	Prospective case-control	Association between acid suppression therapy and risk of CDAD	N=308 hospital inpatients. CDAD group (n=155): Mean age 79 years (range 37–102); Female 61% Received antibiotics: 92% Received PPI: 40% Received acid suppression: 41%  Control group (n=153): Mean age 79 years (range 43–99); female 55% Received antibiotics: 50%, p<001 (vs. case) Received PPI: 25%, p=0.004 Received acid suppression: 26%, p=0.005	Cases with CDAD were mostly recruited from general medical wards. Control was chosen as a person on the same ward whose birthday was closest to that of the index patient.  Analysis: Logistic regression	CDAD was independently associated with: antibiotic use (OR 13.1, 95%CI: 6.6 to 26.1); acid suppression therapy (OR 1.90, 95%CI: 1.10 to 3.29); and female gender (OR 1.79, 95%CI: 1.06 to 3.04).

Appendix Table C3. Description of studies evaluating risk factors for *Clostridium difficile* associated diarrhea (CDAD) (continued)

Study/Origin	Study Type	Objective	Population	Methods	Results
Vesta, 2005 <sup>27</sup>  United States	Prospective observational case control, multicenter, study	Risk factors associated with the development of nosocomial CDAD, particularly with the use of antibiotics	144 hospitalized patients with diarrhea requiring a <i>C. difficile</i> toxin test as part of their routine clinical workup, Cases (n=72) Mean age 56 years; Female 43%  Controls (n=72) Mean age 56 years; Female 43%	Case patients had nosocomial diarrhea and positive <i>C. difficile</i> toxin tests. Control were patients with stool negative for <i>C. difficile</i> toxin and were individually matched with cases based on hospital, sex, age (within 4 years), and duration of hospital stay up to the time of stool sampling (within 4 days).  Analysis: multivariate logistic regression analysis to identify independent risk factors for the development of CDAD (not performed)	There were no significant differences in antibiotic use between cases and controls. Patient severity, classified by Horn's Index, was significantly different between cases and controls (p=0.0022).
Kyne, 2002 <sup>28</sup>  United States	Prospective cohort series	Determine the diagnostic accuracy of an index of underlying disease severity (Horn's index) in identifying patients with a high probability of having nosocomial CDAD as a complication of antimicrobial therapy	252 inpatients and receiving antibiotics. Mean age 74 years; female 60%;  Disease severity (Horn's index) 1 (mild) 30% (n=76) 2 (moderate) 37% (n=93) 3 (severe) 22% (n=55) 4 (extremely severe) 11% (n=28)  28 (11%) of the patients had CDAD	CDAD defined as diarrhea ( $\geq 3$ unformed stools for $\geq 2$ days) not attributed to any other cause that occurred in association with a positive stool test for <i>C. difficile</i> .  Horn's index as a measure of the severity of underlying disease at the time of admission to the hospital, rated as follows: mild=1; moderate =2 (more severe disease but uncomplicated recovery expected); severe (major illness or complications or multiple conditions requiring treatment) =3; extremely severe (catastrophic illness that may lead to death) =4.  Analysis: stepwise multivariable logistic regression	Extremely severe underlying disease was associated with CDAD (OR 17.6 95%CI: 5.8 to 53.5).  Sensitivity, specificity, and positive and negative predictive values of a Horn's index score of 3 or more (severe to extremely severe disease) as a predictor of nosocomial <i>C. difficile</i> diarrhea were 79%, 73%, 27%, and 96%, respectively.
Mody, 2001 <sup>29</sup>  United States	Prospective case control	Evaluate risk factors and clustering of CDAD cases over 2 years	252 patients from a Veterans Affairs Medical Center with unformed stools and positive stool <i>C. difficile</i> cytotoxin assays over the 24-month period; 98	Cases were patients with CDAD. Controls were patients with unformed stools and <i>C. difficile</i> negative toxin test.	Third-generation cephalosporins were the antibiotics most strongly associated with CDAD (OR 3.63 95%CI 1.56 to 9.80). The association of third-generation

Appendix Table C3. Description of studies evaluating risk factors for *Clostridium difficile* associated diarrhea (CDAD) (continued)

Study/Origin	Study Type	Objective	Population	Methods	Results
			patients served as control.  No information on age. 45 cases (17.8%) and 19 controls (19.4%) were HIV-infected.	Stools for cytotoxin assays were frozen and sent on ice to a reference laboratory.  Analysis: logistic regression	cephalosporin use was particularly striking in HIV-infected patients (p=0.0004 when HIV status was included in the model). 34 (76%) of 45 HIV-infected patients with CDAD died during their hospitalization.
Winston, 2001 <sup>30</sup>  United States and Canada	Randomized, double-blind, multicenter trial	Comparing clinafloxacin with imipenem as empirical monotherapy for febrile granulocytopenic patients	541 febrile granulocytopenic patients Mean age 47 years (18 to 86); Female 52% 56% had received bone marrow transplant.  About 40% of the febrile episodes in each treatment group were caused by either a microbiologically documented infection (bacteremia or localized bacterial infection) or a clinically documented infection.	Patients were randomized to receive either intravenous clinafloxacin 200 mg every 12 h (n=272) or intravenous imipenem (n=269).  Median duration of therapy with study drug was 10 days in each treatment group.  Analysis: All patients were included in both the efficacy and safety analyses (intent-to-treat analysis).	CDAD more common with imipenem (3% vs. 8%; p=0.02 )
Schwaber, 2000 <sup>31</sup>  Israel	Prospective case control	Determine factors associated with the development of nosocomial diarrhea and the acquisition of <i>C. difficile</i> -associated disease	136 hospital inpatients, 98 with nosocomial diarrhea and 38 controls. 59.9 ±17.5 years, whereas that of the controls was 56.3 ±19.9 years  <i>Clostridium difficile</i> toxin B was identified in the stool of 13 cases.	Diarrhea defined as ≥3 loose or watery stools in a 24 h, lasting for ≥ 3 days, beginning ≥ 2 days after admission.  Toxin assay: cell-culture cytotoxin test in a culture of human fibroblasts.  Analysis: No multivariate analyses reported.	Factors associated with the presence of <i>C. difficile</i> toxin B as compared to other causes of nosocomial diarrhea were: greater number of individual antibiotics used during hospitalization (p=0.02); cephalosporin use (p=0.03), more specifically, a third generation cephalosporin (p=0.02). Among patients with nosocomial diarrhea, those who <i>C. difficile</i> toxin positive had a significantly higher total antibiotic burden (as antibiotic days) than those with diarrhea due to other causes (p=0.01).

**Appendix Table C3. Description of studies evaluating risk factors for *Clostridium difficile* associated diarrhea (CDAD) (continued)**

Study/Origin	Study Type	Objective	Population	Methods	Results
Katz, 1997 <sup>32</sup>	Prospective case series	Develop predictors for diagnosis of CDAD	609 adult inpatients tested for <i>C. difficile</i> cytotoxin	Relevant clinical symptoms, signs, and antibiotic exposure were recorded before reporting of assay results.	Potential contributing causes of diarrhea (toxin+ vs. toxin-)
United States			<i>C. difficile</i> toxin positive (n=49) Mean age 58 years; Female 57%	Toxin assay: procedure by Chang	Antibiotic use past 30 days: 98% vs. 84% (p=0.009) Cephalosporin use: 73% vs. 49% (p=0.001)
			<i>C. difficile</i> toxin negative (n=49) Mean age 58 Female 57%	Analysis: logistic regression	Antibiotic use prior to admission/transfer: 51% vs. 32% (p=0.009) Antacid use: 20% vs. 10%, p=0.04.
					Prior antibiotic use and significant diarrhea were significantly greater in <i>C. difficile</i> toxin positive patients.

ADD = antibiotic-associated diarrhea; ASA = American Society of Anesthesiologists; HIV = Human immunodeficiency virus; OR = odds ratio; PPI = proton pump inhibitor

Appendix Table C4. Evidence table for standard antibiotic treatments

Study / Region / Funding Source	Population / Age or Age Range / % Women / Ethnicity / Inclusion Criteria	Sample Size (N) / Intervention(s) / Control (s) / Study Duration	Outcomes Evaluated	Study Quality
<b>1. Newly identified trials</b>				
Musher, 2009 <sup>33</sup>  Region: USA  Funding source: Department of Veterans Affairs	Population: Mild or severe symptomatic inpatient adults with comorbid conditions  Mean age: 63 % women: 35 Ethnicity: White 69%; black 31% (45% in nitazoxanide group, 19% in vancomycin group)  Inclusion criteria: EIA results positive for <i>C. difficile</i> toxin (Premier Toxins A & B; Meridian Bioscience), ≥3 loose stools within 24 h and ≥1 of the following additional findings: fever (temperature, 138.37C), abdominal pain, and/or leukocytosis.  Severity: patients with ≥2 points were considered to have severe CDAD based on an assessment score developed for this study. One point each was given for age ≥60 years, >7 stools/day, temperature >38.3 C, albumin level <2.5 mg/dL, or peripheral WBC count >15,000 cells/mm <sup>3</sup> .	N=50 (severe 41%, n=20)  Intervention 1: Vancomycin 125 mg 4 times/day (n=27)  Intervention 2: Nitazoxanide 500 mg 2 times/day + placebo pill (n=23)  Treatment duration: 10 days Followup period: 21 days	a. End-of-treatment response (cure), # of patients (defined as complete resolution of all symptoms and signs attributable to CDI during the 3 days after completion of therapy) b. Relapse, # of patients (defined as a return of symptoms after an initial response but within 31 days after the onset of treatment with <i>C. difficile</i> toxin detected in stool by EIA or patient was re-treated empirically for CDI and responded to treatment. c. All-cause mortality d. Adverse events	Allocation concealment: adequate (sequentially numbered identical packages)  Blinding: double  Intention-to-treat analysis (all subjects randomized included in the analyses): partially, one subject was found to have IBD (an exclusion criteria) and was removed  Withdrawals and dropouts reported: 9 (18%)
Zar, 2007 <sup>34</sup>  Region: USA  Funding source: none stated	Population: Mild or severe symptomatic inpatient adults with comorbid conditions  Mean age: 58 (47% <60 years) % women: 45  Inclusion criteria: <i>Clostridium difficile</i> -associated diarrhea (CDAD), testing positive for <i>C. difficile</i> cytotoxin  Severity: patients with ≥2 points were	N=172 (mild 54%, severe 46% based on 150 patients completing trial)  Intervention 1: Vancomycin (liquid) 125 mg 4 times/day + placebo pill (n=82)  Intervention 2: Metronidazole (oral) 250 mg 4 times/day plus placebo liquid (n=90)	a. Cure, # of patients (defined as resolution of diarrhea by day 6 of treatment and a negative result of a <i>C. difficile</i> toxin A assay at days 6 and 10 of treatment) b. Relapse, # of patients (defined as recurrence of <i>C. difficile</i> toxin A-positive diarrhea by day 21 after initial cure)	Allocation concealment: adequate (controlled by pharmacy)  Blinding: double  Intention-to-treat analysis: no, completers only  Withdrawals and dropouts reported: 22 (13%)

Appendix Table C4. Evidence table for standard antibiotic treatments (continued)

Study / Region / Funding Source	Population / Age or Age Range / % Women / Ethnicity / Inclusion Criteria	Sample Size (N) / Intervention(s) / Control (s) / Study Duration	Outcomes Evaluated	Study Quality
	considered to have severe CDAD based on an assessment score developed for this study. One point each was given for age >60 years, temperature >38.3 C, albumin level <2.5 mg/dL, or peripheral WBC count >15,000 cells/mm <sup>3</sup> within 48 h of enrollment. Two points were given for endoscopic evidence of pseudo-membranous colitis or treatment in the intensive care. All patients had received antimicrobial treatment prior to onset of CDAD (>90% within 14 days).	Treatment duration: 10 days Followup period: 21 days	c. All-cause mortality	
<b>2. Trials included in Cochrane systematic review<sup>35</sup></b>				
Lagrotteria, 2006 <sup>36</sup>	Population: Symptomatic adults (95% inpatients and 5% outpatients)	N=39	a. Clinical improvement (cure) at study day 10, # (%) of patients (defined as becoming asymptomatic during the treatment course. Failure defined as persistent symptoms and signs after 10 days of antimicrobial therapy)	Allocation concealment: unclear (numbered packages)
Region: Canada	Mean age: 69 years women: 59%	Intervention 1: Metronidazole 500 mg 3 times/day (n=20)	b. Experienced relapse by study day 40, # (%) of patients (defined as recurrence of diarrhea in the followup period for those patients who initially experienced a clinical cure)	Blinding: single (study staff)
Funding source: The Physicians' Services Incorporated Foundation	Inclusion criteria: diagnosis of CDAD on the basis of the Society for Healthcare Epidemiology of America definition, laboratory confirmation of the presence of <i>C. difficile</i> toxins A and B using an enzyme immunoassay, and no other etiology for diarrhea.	Intervention 2: Metronidazole 500 mg 3 times/day and rifampin 300 mg 2 times/day (n=19)	c. Laboratory-confirmed relapse by study day 40, # of patients	Intention-to-treat analysis: yes
		Treatment duration: 10 days Followup period: 30 days	d. Time to clinical improvement (days)	Withdrawals and dropouts reported: 7 (18%)
			e. Time to relapse (days)	
			f. All-cause mortality	
			g. Adverse events	
Musher, 2006 <sup>37</sup>	Population: Symptomatic adults, a substantial proportion had severe, comorbid conditions	N=142	a. Response to therapy, assessed 3 ways: (1) time to resolution of symptoms	Allocation concealment: not defined
Region: USA		Intervention 1: Metronidazole		

Appendix Table C4. Evidence table for standard antibiotic treatments (continued)

Study / Region / Funding Source	Population / Age or Age Range / % Women / Ethnicity / Inclusion Criteria	Sample Size (N) / Intervention(s) / Control (s) / Study Duration	Outcomes Evaluated	Study Quality
Funding source: Romark Pharmaceuticals	Mean age: 68 women: 24% Ethnicity: White 77%; black 17%; Hispanic 6%  Inclusion criteria: inpatients >18 years of age with diarrhea (defined as $\geq 3$ unformed stools within a 24-h period), an enzyme immunoassay result positive for <i>C. difficile</i> toxin, and $\geq 1$ of the following findings: fever, abdominal pain, or leukocytosis.	250 mg 4 times/day (n=44)  Intervention 2: Nitazoxanide 500 mg 2 times/day for 7 days (n=49)  Intervention 3: Nitazoxanide 500 mg 2 times/day (n=49)  Treatment duration: 10 days unless noted Followup period: 31 days	of colitis; (2) complete clinical response at the end of 7 days of treatment, defined as return of normal stool pattern and absence of fever, abdominal pain, or leukocytosis, unless some other explanation was apparent; and (3) sustained clinical response 31 days after the beginning of treatment b. All-cause mortality c. Adverse events	Blinding: double  Intention-to-treat analysis: no  Withdrawals and dropouts reported: 32 (23%)
Wullt, 2004 <sup>38</sup> Noren 2006 <sup>39</sup>  Region: Sweden  Funding source: Region Skåne and the Scandinavian Society of Antimicrobial Chemotherapy, and Leo Pharma AB	Population: Symptomatic adult inpatients (51%) or outpatients (49%) on enrollment  Mean age: 59 % women: 39  Inclusion criteria: age >18 years, lack of hypersensitivity to fusidic acid or metronidazole, a positive <i>C. difficile</i> toxin assay from feces within 6 days before enrolment, and a history of ongoing diarrhea (diarrhea defined as three or more loose stools per day for at least 2 days).	N=131  Intervention 1: Metronidazole 400 mg 3 times/day (n=64)  Intervention 2: Fusidic acid 250 mg 3 times/day (n=67)  Treatment duration: 7 days Followup period: 33 days	a. Clinical cure (defined as cessation of diarrhea within 5–8 days of initiating treatment, and clinical failure as persistence of diarrhea on days 5–8) b. Clinical recurrence, defined as the reappearance of diarrhea on days 8–40 in clinically cured patients who had completed 7 days of treatment c. Adverse events	Allocation concealment: adequate (coded containers of identical appearance)  Blinding: double  Intention-to-treat analysis: no  Withdrawals and dropouts reported: 17 (13%)
Wenisch, 1996 <sup>40</sup>  Region: Austria  Funding source: none stated	Population: Symptomatic adults hospitalized for a minimum of 5 days  Mean age: 42 % women: 48  Inclusion criteria: age of >18 years and the presence of CDAD. Diarrhea was defined as >3 loose stools per day. CDAD was diagnosed on the basis of the results of a <i>C. difficile</i> toxin assay and/or endoscopic evidence of typical	N=126  Intervention 1: Metronidazole 500 mg 3 times/day (n=31)  Intervention 2: Fusidic acid 500 mg 3 times/day (n=29)  Intervention 3: Vancomycin 500 mg 3 times/day (n=31)  Intervention 4: Teicoplanin	a. Clinical cure, # of patients (defined as no loose stools, gastrointestinal symptoms, or fever and normalization of serum levels of C-reactive protein and leukocyte counts) b. Clinical failure (defined as persistence of diarrhea after 6 days of treatment c. Clinical relapse (defined	Allocation concealment: not defined  Blinding: none stated, teicoplanin administered as an injection, the other drugs orally  Intention-to-treat analysis: no  Withdrawals and dropouts reported: 7 (6%)

Appendix Table C4. Evidence table for standard antibiotic treatments (continued)

Study / Region / Funding Source	Population / Age or Age Range / % Women / Ethnicity / Inclusion Criteria	Sample Size (N) / Intervention(s) / Control (s) / Study Duration	Outcomes Evaluated	Study Quality
	colitis, with the finding of granulocytes in stools	(injection) 400 mg 2 times/day (n=28)  Treatment duration: 10 days Followup period: 30 days	as the reappearance of CDAD and other symptoms during the follow-up period) d. Adverse events	
de Lalla, 1992 <sup>41</sup>  Region: Italy  Funding source: none stated	Population: Symptomatic adult inpatients  Mean age (range): 47 (18 to 83) % women: 70  Inclusion criteria: age of >18 years, presence of symptoms (diarrhea, sometimes combined with fever and abdominal pain), and stool culture and/or a rapid diagnostic test positive for <i>C difficile</i> and/or colonoscopic demonstration of the typical endoscopic picture of pseudomembranous colitis	N=51  Intervention 1: Vancomycin 500 mg 4 times/day (n=24)  Intervention 2: Teicoplanin 100 mg 2 times/day (n=27)  Study duration: 10 days  Followup period: 30 days	a. Cure, # of patients (defined as elimination of symptoms and signs were) b. Failure, # patients (defined persistence of diarrhea after 6 days of treatment) c. Relapse (defined as reappearance of diarrhea and other symptoms in the 1-month follow-up period) d. All-cause mortality e. Adverse events	Allocation concealment: not defined  Blinding: none stated  Intention-to-treat analysis: no  Withdrawals and dropouts reported: 5 (10%)
Fekety, 1989 <sup>42</sup>  Region: USA  Funding source: NIH and Upjohn Company	Population: Moderately or severely ill symptomatic inpatients adults (plus one infant)  Mean age/range: 54 (1 to 76) % women: gender not reported  Inclusion criteria: antibiotic associated diarrhea plus at least one stool specimen that demonstrated both <i>C difficile</i> and its cytotoxin. All patients were moderately or severely ill, or unresponsive to supportive therapy (patients with mild illness as judged physicians were treated supportively, and not entered into the study)	N=56  Intervention 1: Vancomycin 500 mg 4 times/day (n=22)  Intervention 2: Vancomycin 125 mg 4 times/day (n=24)  Study duration: 10 days  Followup period: up to 6 weeks after treatment	a. Treatment response (cure) based diarrhea resolution (defined as patients stating their bowel function is normal, or when they were having ≤3 movements a day and their stools were semi-formed) Patients whose diarrhea ceased within seven days after treatment were considered to have a good response; patients whose diarrhea ceased but after seven days of treatment were considered simply to have responded b. Mean duration of symptoms, days c. Adverse events	Allocation concealment: not defined  Blinding: physicians were blinded to treatment assignment  Intention-to-treat analysis: no  Withdrawals and dropouts reported: 10 (18%)

Appendix Table C4. Evidence table for standard antibiotic treatments (continued)

Study / Region / Funding Source	Population / Age or Age Range / % Women / Ethnicity / Inclusion Criteria	Sample Size (N) / Intervention(s) / Control (s) / Study Duration	Outcomes Evaluated	Study Quality
Dudley, 1986 <sup>43</sup> Region: USA Funding source: Upjohn Company	Population: Symptomatic adult inpatients Mean age: 69 % women: 60 (evaluable subjects (n=30) only for age and gender) Inclusion criteria: antibiotic associated diarrhea (≥4 loose stools were passed for ≥2 consecutive days, signs and symptoms of <i>C difficile</i> -induced diarrhea and its cytotoxin.	N=62 Intervention 1: Vancomycin 500 mg 4 times/day (n=31) Intervention 2: Bacitracin 25,000 mg 4 times/day (n=31) Study duration: 10 days Followup period: up to 60 days	a. Treatment response (cure) based diarrhea resolution (defined as ≤4 loose stools were passed for ≥2 consecutive days) b. Treatment failure (defined as diarrhea and other symptoms worsened and were crossed over to the alternative drug in a blinded manner. Patients worsening after 5 days of the crossed over therapy were considered failures and removed from the study) c. All-cause mortality d. Adverse events	Allocation concealment: adequate (coded amber bottles prepared by pharmacy) Blinding: double Intention-to-treat analysis: no Withdrawals and dropouts reported: 32 (52%)
Young, 1985 <sup>44</sup> Region: Australia Funding source: Upjohn Company and the McGauran Trust	Population: Symptomatic adult inpatients Mean age: 62 (gender not reported) Inclusion criteria: antibiotic associated diarrhea (≥4 loose stools were passed for ≥2 consecutive days, signs and symptoms of <i>C difficile</i> -induced diarrhea and its cytotoxin.	N=42 Intervention 1: Vancomycin 125 mg 4 times/day (n=21) Intervention 2: Bacitracin 20,000 mg 4 times/day (n=21) Study duration: 7 days Followup period: 28 days	a. Treatment response (cure) based diarrhea resolution (defined as <3 times/day by the time the last capsule was given. Day of resolution defined as first day of <3 stools, provide frequency did not go above >2) b. Treatment relapse c. Mean days to 50% improvement	Allocation concealment: adequate (identical red capsules and sealed codes held in pharmacy) Blinding: double Intention-to-treat analysis: yes for initial therapy Withdrawals and dropouts reported: all completed initial treatment
Teasley, 1983 <sup>45</sup> Region: USA Funding source: Veterans Affairs and Searle Laboratories	Population: Symptomatic inpatient adults Mean age: 65 % women: 1 Inclusion criteria: <i>C difficile</i> -associated diarrhea and its cytotoxin. All patients had received antimicrobial treatment 14-55 days prior to diarrhea.	N=101 Intervention 1: Vancomycin 500 mg 4 times/day (n=56) Intervention 2: Metronidazole 250 mg 4 times/day (n=45) Study duration: 10 days	a. Cure (defined as diarrhea resolved within 6 days of treatment, toleration of complete treatment course, and no relapse in the 21 day followup period) b. Treatment response based diarrhea resolution (defined as <2 stools	Allocation concealment: not defined Blinding: none stated Intention-to-treat analysis: no Withdrawals and dropouts reported: 7 (7%)

Appendix Table C4. Evidence table for standard antibiotic treatments (continued)

Study / Region / Funding Source	Population / Age or Age Range / % Women / Ethnicity / Inclusion Criteria	Sample Size (N) / Intervention(s) / Control (s) / Study Duration	Outcomes Evaluated	Study Quality
Keighley, 1978 <sup>46</sup> Region: UK Funding source: none stated	Population: Symptomatic adult inpatients. Subjects with evidence of cytotoxins separated with from subjects with <i>C difficile</i> on culture  Age and gender not reported  Inclusion criteria: postoperative diarrhea ( $\geq 3$ loose stools/day or colostomy output $> 1$ liter/day. All patients had received antimicrobial treatment prior to diarrhea.	N=44  Intervention: Vancomycin 125 mg 4 times/day (n=22)  Control: Placebo (n=22)  Study duration: 5 days  Followup period: unclear, up to 29 days in the control group	formed /day) c. Treatment failure (defined as $\leq 4$ loose stools/day after 6 days of treatment. d. Treatment relapse (defined as recurrence with 21 days of diarrhea with $\leq 4$ loose stools/day for a minimum of 2 days)  a. Treatment response based diarrhea resolution (defined as normal stool, improved, same, or worse. Normal was defined as 1 solid stool/day, the others were not described) b. Adverse events	Allocation concealment: adequate (identical looking placebo and based code held in pharmacy)  Blinding: unclear if double (“identical looking placebo”)  Intention-to-treat analysis: yes  Withdrawals and dropouts reported: all completed initial treatment

**Appendix Table C5. Assessment of study quality of individual metronidazole trials**

<b>Study</b>	<b>Allocation Concealment</b>	<b>Blinding</b>	<b>Intention-to-Treat Analysis</b>	<b>Withdrawals and Dropouts Reported</b>	<b>Study Quality Good, Fair, or Poor</b>
<b><i>Versus vancomycin</i></b>					
Zar 2007 <sup>34</sup> (n=172), subset with severe disease (46% based on 150 completing trial)	Adequate	Double	Completers only	22 (13%)	Fair
Wenisch 1996 <sup>40</sup> (n=62)	Not defined	None stated	No	7 (6%)*	Poor
Teasley 1983 <sup>45</sup> (n=101)	Not defined	None stated	No	7 (7%)	Poor
<b><i>Versus nitazoxanide</i></b>					
Musher 2006 <sup>37</sup> (n=142)	Not defined	Double	No	32 (23%)	Poor
<b><i>Versus metronidazole plus rifampin</i></b>					
Lagrotteria (n=39)	Unclear (numbered packages but no further detail)	Single (study staff)	Yes	7 (18%)	Fair

\* based on all subjects, 4-arm trial

**Appendix Table C6. Assessment of study quality of individual vancomycin trials**

<b>Study</b>	<b>Allocation Concealment</b>	<b>Blinding</b>	<b>Intention-to-Treat Analysis</b>	<b>Withdrawals and Dropouts Reported</b>	<b>Study Quality Good, Fair, or Poor</b>
<b><i>Versus metronidazole</i></b>					
Zar, 2007 <sup>34</sup> (n=172), subset with severe disease (46% based on 150 completing trial)	Adequate	Double	No, completers only	22 (13%)	Fair
Wenisch, 1996 <sup>40</sup> (n=62)	Not defined	None stated	No	7 (6%)*	Poor
Teasley, 1983 <sup>45</sup> (n=101)	Not defined	None stated	No	7 (7%)	Poor
<b><i>Versus nitazoxanide</i></b>					
Musher, 2009 <sup>33</sup> (n=50), subset of 20 with severe disease	Adequate	Double	Partially, one subject removed	9 (18%)	Good
<b><i>Versus bacitracin</i></b>					
Dudley, 1986 <sup>43</sup> (n=62)	Adequate	Double	No	32 (52%)	Fair
Young, 1985 <sup>44</sup> (n=42)	Adequate	Double	Yes for initial therapy	None	Good
<b><i>Versus placebo</i></b>					
Keighley, 1978 <sup>46</sup> (n=44)	Adequate	Unclear ("identical placebo")	Yes	All completed initial treatment	Good

\* based on all subjects, 4-arm trial

**Appendix Table C7. Summary of strength of evidence for *C. difficile*—Key Question 3c: Vancomycin studies**

<b>Key Question, # Studies (# Participants)</b>	<b>Study Design</b>	<b>Risk of Bias</b>	<b>Consistency</b>	<b>Directness</b>	<b>Precision</b>	<b>Overall Grade/ Conclusion</b>
<b><i>Versus metronidazole</i></b>						
Initial clinical cure; 3 (335)	RCT	High	Consistent	Direct	Imprecise	Low
Clinical recurrence; 3 (283)	RCT	High	Consistent	Direct	Imprecise	Low
Initial clinical cure, severe disease; 1 (69)	RCT	Medium	Unknown	Direct	Precise	Low
Clinical recurrence, severe disease; 1 (59)	RCT	Medium	Unknown	Direct	Imprecise	Insufficient
<b><i>Versus nitazoxanide</i></b>						
Initial clinical cure; 1 (50)	RCT	Low	Unknown	Direct	Imprecise	Low
Clinical recurrence; 1 (37)	RCT	Low	Unknown	Direct	Imprecise	Low
Initial clinical cure, severe disease; 1 (20)	RCT	Low	Unknown	Direct	Imprecise	Insufficient
Clinical recurrence, severe disease; 1 (15)	RCT	Low	Unknown	Direct	Imprecise	Insufficient
<b><i>Versus bacitracin</i></b>						
Initial clinical cure; 2 (81)	RCT	Low	Consistent	Direct	Imprecise	Low
Clinical recurrence; 2 (37)	RCT	Low	Consistent	Direct	Imprecise	Low
<b><i>Versus placebo</i></b>						
Initial clinical cure; 1 (21)	RCT	Low	Unknown	Direct	Precise	Moderate

**Appendix Table C8. Summary of strength of evidence for *C. difficile*—Key Question 3c: Metronidazole studies**

<b>Key Question, # Studies (# Participants)</b>	<b>Study Design</b>	<b>Risk of Bias</b>	<b>Consistency</b>	<b>Directness</b>	<b>Precision</b>	<b>Overall Grade/ Conclusion</b>
<b><i>Versus vancomycin</i></b>						
Initial clinical cure; 3 (335)	RCT	High	Consistent	Direct	Imprecise	Low
Clinical recurrence; 3 (283)	RCT	High	Consistent	Direct	Imprecise	Low
Initial clinical cure, severe disease; 1 (69)	RCT	Medium	Unknown	Direct	Precise	Low
Clinical recurrence, severe disease; 1 (59)	RCT	Medium	Unknown	Direct	Imprecise	Insufficient
<b><i>Versus nitazoxanide</i></b>						
Initial clinical cure; 1 (142)	RCT	High	Unknown	Direct	Imprecise	Low
Clinical recurrence; 1 (97)	RCT	High	Unknown	Direct	Imprecise	Low
<b><i>Versus metronidazole plus rifampin</i></b>						
Initial clinical cure; 1 (142)	RCT	Medium	Unknown	Direct	Imprecise	Low
Clinical recurrence; 1 (97)	RCT	Medium	Unknown	Direct	Imprecise	Low

Appendix Table C9. Assessment of study quality of individual nonantibiotic treatment trials

Study	Allocation Concealment	Blinding	Intention-to-Treat Analysis	Withdrawals and Dropouts Adequately Described	Study Quality Good, Fair or Poor
<b><i>Adjuvant probiotics (with standard therapy) versus placebo (with standard therapy)</i></b>					
Wullt, 2003 <sup>47</sup> (n=29)	Not defined	Double	No	Yes	Fair
Surawicz, 2000 <sup>48</sup> (n=168, 32 with recurrent CDAD)	Adequate	Double	Yes	Yes (none reported)	Good
McFarland, 1994 <sup>49</sup> (n=124)	Adequate	Double	Yes	Yes	Good
<b><i>Tolvemar (C. difficile toxin binder) versus active control (vancomycin)</i></b>					
Louie, 2006 <sup>50</sup> (n=289)	Not defined	Double	No	Yes	Fair
<b><i>C. difficile immune whey versus active control (metronidazole)</i></b>					
Mattila, 2008 <sup>51</sup> (n=40)	Not defined	Double	No	Yes	Fair
<b><i>Absorptive resin versus active placebo</i></b>					
Mogg, 1982 <sup>52</sup> (n=48)	Possibly adequate	None stated	No	Yes	Fair

Appendix Table C10. Summary of evidence for *C. difficile*—Key Question 4

Key Question, # studies (# participants)	Study Design	Risk of Bias	Consistency	Directness	Precision	Overall Grade/ Conclusion
<b><i>Probiotics and prebiotics (adjuvant to standard care) versus placebo and standard care</i></b>						
Resolution of CDAD; 3 (185*)	RCT	Low	Consistent	Direct	Imprecise	Low
Prevention of CDAD; 8 (1756)	RCT	Low	Consistent	Direct	Imprecise	Low
Prevention of recurrence of CDAD; 3 (339)	RCT	Medium	Consistent	Direct	Imprecise	Low
<b><i>Monoclonal antibodies (adjuvant to standard care) versus placebo and standard care</i></b>						
Prevention of recurrence of CDAD; 1 (200)	RCT	Medium	Unknown	Direct	Imprecise	Low
<b><i>Tolevemar (high-molecular weight, anionic polymer) versus standard care (vancomycin)</i></b>						
Resolution of CDAD; 1 (289)	RCT	Medium	Unknown	Direct	Imprecise	Low
<b><i>C. difficile immune whey versus standard care (metronidazole)</i></b>						
<b>Resolution of CDAD; 1 (n=40)</b>	<b>RCT</b>	<b>Medium</b>	<b>Unknown</b>	<b>Direct</b>	<b>Imprecise</b>	<b>Low</b>
<b><i>Colestipol (an absorptive resin) versus placebo</i></b>						
Resolution of CDAD; 1 (n=48)	RCT	Medium	Unknown	Direct	Imprecise	Low

\* Includes only patients with *C. difficile* positive stools. Some trials, particularly the prevention studies, enrolled patients who were negative for *C. difficile*.

**Appendix Table C11. Reviews and meta-analyses**

Study	Title/Question Patient Population	# of Studies Patient N	Comparators	Outcomes Followup	Results
<b>Diagnosis</b>					
Planche, 2008 <sup>53</sup> Searched 1994 to Nov 2007	Diagnosis of Clostridium difficile infection by toxin detection kits. Toxins A & B All inpatients	18 trials N=62 to 2,891 Meta-analysis	ELISA (Meridian, Techlab), Rapid antigen capture (Techlab), Rapid CI (Remel), EIA (BioMerieux) Rapid EIA (Meridian) compared to cell culture w/ neutralisable toxin	Sensitivity, specificity	No test met acceptable criteria (sensitivity IQR >90%, and false positivity below 3%). No difference in diagnostic performance of commercially available tests. Most had higher specificity than sensitivity. Differences between tests likely due to assay threshold cut-off.
<b>Prevention</b>					
Garey, 2008 <sup>54</sup> Searched 1966 to Aug 2007	Assess risk factors for recurrent CDI Adult inpatients	3 RCT, 9 observational Meta-analysis	Patients with recurrent vs. patients with one episode only.	Studies generally 1 to 3 months	Continued use of non-C. diff antibiotics after CDI diagnosis: OR 4.23 (2.10-8.55), use of antacid medication: OR 2.15 (1.13- 4.08), older age: OR 1.62 (1.11 – 2.36). (Many risk factors not included in analysis due to limited literature.)
Bignardi, 1998 <sup>55</sup> Searched to March 1996	Assess risk factors for CDI NR	49 studies	C. diff cases vs. individuals without diarrhea	CDAD, C. diff carrier	Risk factors with “substantive” evidence: age, severity of underlying diseases, nonsurgical gastrointestinal procedures, nasogastric tube, anti-ulcer medications, ICU, LoS, duration of antibiotic course, multiple antibiotics
Leonard, 2007 <sup>56</sup> Searched 1966 thru 2005	Risk of enteric infection in patients taking acid suppression Primarily inpatient	25 observational studies N=1,382 Meta-analysis	Use of PPI or H2RA vs. multiple control group types	Presence of enteric infection	PPI: OR 2.05 (1.47-2.85); H2RA: OR 1.48 (1.06 – 2.06); Overall: OR 1.95 (1.48- 2.58). Significant heterogeneity between studies. ORs for other enteric infections were even greater. Index cases?
Kramer, 2006 <sup>57</sup> Searched 1966 through 2005	How long do nosocomial pathogens persist on inanimate surfaces	NR number Experimental data		Range of reported duration of persistence	CDAD spores: 5 months. Overall, high inoculum in cold rooms with higher humidity persist longest. No quality check.
Thomas, 2001 <sup>58</sup> Searched 1966 to 2001	Antibiotics and hospital- acquired C.difficile- associated diarrhoea Adult inpatients	48 observational studies	Use of antibiotics vs. multiple control group types	Study quality	General study quality precludes meta- analysis of observational studies for relationships between antibiotics and C. diff. 2 studies provide valid evidence for cephalosporin, penicillin, and clindamycin.
Davey, 2005 <sup>59</sup> Cochrane Searched Jan 1980 thru Jul 2005	Interventions to improve antibiotic prescribing practices for hospital inpatients Hospitals/units for all	66 studies, RCT to time series	60 interventions to improve prescribing practices vs. usual processes	Presence of Gram negative-resistant bacteria, CDAD, vancomycin- resistant	Both persuasive and restrictive interventions were effective overall.

Appendix Table C28. Reviews and meta-analyses (continued)

Study	Title/Question Patient Population	# of Studies Patient N	Comparators	Outcomes Followup	Results
	inpatients			enterococci, MRSA	
<b>Treatment</b>					
Koo, 2009 <sup>60</sup> Searched thru Dec 2007	Antimotility agents for CDI treatment Adult inpatients	1 retrospective 19 case reports/series N=55	With or without antibiotic use	Adverse events, clinical resolution	All patients with documented complications or mortality received antimotility drugs alone initially. 23 patients who received concurrent antibiotics did not experience complications. (Use of antimotility did not appear to shorten disease course in the 23 patients.)
Pillai, 2008 <sup>61</sup> Cochrane Searched 1966 thru Oct 2007	Probiotics for treatment of C. difficile-associated colitis in adults Adults with recurrent CDAD	4 trials	Use of probiotics, multiple forms, vs. placebo	Resolution of diarrhea, negative stool for toxin assay or culture	Insufficient evidence to support use. Studies were small and lacked power.
Eddins, 2008 <sup>62</sup> Jan 1996 thru Sept 2007	Probiotic or symbiotics for ADD, CDAD, or radiation- induced diarrhea All patients	CDAD:1 systematic review, 6 trials	Narrative		Sparse evidence may reduce risk for CDAD or recurrence.
Segarra- Newnham, 2007 <sup>63</sup> Searched 1970 thru March 2007	Probiotics for C. difficile- associated diarrhea: focus on Lactobacillus rhamnosus GG and Saccharomyces boulardii	7 articles, care report to blinded trials	Narrative		Sparse evidence. Risks may outweigh benefits for debilitated and immunosuppressed patients, which are those most at risk for recurrent CDAD.
McFarland, 2006 <sup>64</sup> Searched 1977 to 2005	Probiotics for prevention of antibiotic associated diarrhea and treatment of C. difficile disease Primarily inpatient	31 trials; ADD 25, CDAD 6 N=3,164 Meta-analysis	Use of probiotics, multiple forms, vs. placebo	New diarrhea episode associated with positive culture or toxin assay w/in 1 month of antibiotic exposure	ADD prevention: RR 0.43 (0.31 – 0.58). CDAD treatment: RR 0.59 (0.41 – 0.85) Most benefit in CDAD seen in treatment of patients with recurrent CDAD, S. boulardii was effective agent.
Dendukuri, 2005 <sup>65</sup> Searched up thru Mar 2005	Probiotic therapy for the prevention and treatment of C.difficile-associated diarrhea Adult inpatients	1 prevention trial, 3 treatment	Probiotics vs. placebo; L. acidophilus, L. plantarum, L. GG, Bifidocaterium bifidum, S. boulardii (5)	Prevention of AAD 11 days to 8 weeks	No differences between groups for prevention. Only one found improvement for treatment. Subgroup analysis suggests limited to recurrent CDAD. Dose was same as used in pediatric studies with positive results. Variability in CDAD definition.
Nelson, 2007 <sup>35</sup> Cochrane Searched 1966 thru April 2007	Antibiotic treatment for C. difficile-associated diarrhea (and need for stopping causative) in adults	12 trials Meta-analysis	8 antibiotics, one placebo controlled	Resolution/ negative tests, recurrence/ positive tests,	No single antibiotic clearly superior; teicoplanin showed some benefits over vancomycin, fusidic acid, metronidazole. Mild cases may be self-limiting without

**Appendix Table C28. Reviews and meta-analyses (continued)**

<b>Study</b>	<b>Title/Question Patient Population</b>	<b># of Studies Patient N</b>	<b>Comparators</b>	<b>Outcomes Followup</b>	<b>Results</b>
	Inpatients			surgery, death	treatment. For prevention of spread, teicoplanin showed best bacteriologic cure.
Zimmerman, 1997 <sup>66</sup> Searched 1978 thru 1996	Antibiotic treatment of C. difficile infection	9 trials Meta-analysis	5 antibiotics, two placebo controlled	Clinical resolution of diarrhea, relapse, negative test for toxin Average 1 month	Colestipol no better than placebo, but of other 4, no significant differences between types or doses of antibiotics for clinical resolution. Teicoplanin better than fusidic acid for relapse. Unclear if higher dose of teicoplanin reduces relapse.

## References for Appendix Tables

Note that reference numbers for evidence tables in this appendix are different from those in the text of the report.

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## Appendix D. List of Excluded Studies

### Excluded References – C Difficile (General Search)

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