National Clostridium difficile Standards Group

Report to the Department of Health

February 2003
## National _Clostridium difficile_ Standards Group Report to the Department of Health February 2003

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Summary of Recommendations

- Standardisation of the diagnosis and reporting of *C. difficile* Associated Disease (CDAD) is desirable, achievable, and a pre-requisite for any attempt to compare its impact across different populations.

- Clinicians and General Practitioners should send faecal specimens for microbiological analysis whenever patients present with diarrhoea of potentially infectious aetiology.

- For surveillance purposes, microbiology laboratories should test diarrhoeal specimens for evidence of CDAD from all patients over 65 years old who have not been diagnosed with CDAD in the preceding four weeks. Apart from age, this recommendation is made regardless of the presence or absence of any specific risk factors.

- The four-week recommendation is intended to avoid bias caused by the difficulties in distinguishing relapse from re-infection. Laboratories must formulate their own strategies for repeat testing, or otherwise, within this period. However, we take the view that repeated testing early in the course of the infection is usually unhelpful.

- Diarrhoeal stools are defined as those that take the shape of their container. Non-diarrhoeal stools should not be tested.

- The surveillance system envisaged will not be extended to patients under 65 years old, and laboratories must formulate their own testing strategies for younger age groups. However, we recommend that children below the age of one year, in whom *C. difficile* can be regarded as a commensal organism, should not be tested.

- Laboratories should test specimens for *C. difficile* toxin using either an immunoassay detecting both toxin A and toxin B, or a neutralised cell cytotoxicity assay. The method used should be subject to appropriate quality assurance.

- Positive toxin results should be reported to CDSC via CoSurv. Results will be filtered at destination, so there is no requirement to remove those pertaining to patients outside the surveillance population.

- The surveillance system envisaged will also encompass strain typing and antimicrobial sensitivity data. Specimens associated with outbreaks, plus a representative sample of other specimens, will be cultured at designated regional facilities and isolates sent for typing at a designated national facility. The details of this scheme remain to be finalised.
1. Introduction and remit

The Communicable Disease Surveillance Centre (CDSC), via its Division of Healthcare-associated Infection & Antimicrobial Resistance together with the Regional Epidemiology Service, has been charged with the delivery of the major part of a service level agreement (SLA) between the Department of Health (DH) and the Public Health Laboratory Service (PHLS) on developing the surveillance of healthcare-associated infection. The agreement with the PHLS transfers to the Health Protection Agency on 1 April 2003. This SLA is largely derived from the recommendations of the Healthcare-associated Infection Surveillance Steering Group (HAISSG) of the DH.

Accordingly, from April 2003 NHS Trusts in England will be required to report cases of Clostridium difficile associated disease (CDAD) as part of a systematic, national, alert organism, laboratory reporting system. This will represent an extension of the national healthcare-associated infection surveillance initiative, which includes the mandatory surveillance of Staphylococcus aureus bacteraemia instituted in April 2001. The existing CDR laboratory reporting system will be used as the source for these data.

The National C. difficile Standards Group (membership at appendix 6) was established at the request of the DH to review evidence pertaining to the diagnosis and control of CDAD, and to make recommendations for the development of the surveillance system. This report is presented in the form of an executive summary. In-depth appraisal of the evidence can be found in the appendices.

The history, epidemiology and pathogenic mechanisms of CDAD are described in appendix 1. It is clear that CDAD has emerged as a healthcare-associated infection of great clinical and economic significance, and attempts to improve our understanding of its impact are to be welcomed. However it is a prerequisite that any national reporting system collect data that are both standardised and consistent. The laboratory diagnosis of CDAD in England has evolved in a haphazard manner, and we emphasise that the
establishment of the national surveillance system must go hand in hand with improved standardisation of the diagnostic approach.

It is recognised that this may not be immediately achievable, but it is the opinion of Group that investment in complying with its recommendations will be more than compensated by improvements in our understanding, and hence the control, of this complex clinical problem.

2. Laboratory diagnosis of CDAD

The laboratory diagnosis of CDAD is reviewed in appendix 2. The important points to draw from this are as follows:

1. The diagnosis of CDAD requires the detection of *C. difficile* toxins (CDT) in diarrhoeal stool specimens. The only exception is pseudomembranous colitis, which can be confirmed by endoscopy or histopathology.

2. Toxin detection is most reliably achieved by two methods, the neutralised cell cytotoxicity assay, or an immunoassay that detects both toxin A and toxin B. The cell cytotoxicity assay is the only assay to detect toxin by means of its biological properties, and remains the standard by which other tests are judged, but positive results must be confirmed by neutralisation with antitoxin to ensure adequate specificity. Commercial immunoassays are available in several formats and from several manufacturers, but assays detecting both toxins tend to perform better overall than those detecting toxin A alone. The latter also fail to detect the increasingly important toxin A-negative strains.

3. Although not recommended for diagnosis, bacterial culture is necessary to provide strains for epidemiological typing and antimicrobial susceptibility testing.

4. Until now, there has been little consensus as to which specimens should be tested for *C. difficile*. Various criteria derived from proven or perceived risk factors for CDAD (for example old age, previous antibiotic exposure, hospital rather than community origin) have been applied in attempts to
screen out those specimens that are more likely to be negative. While this approach reduces costs by limiting the numbers of tests performed, it restricts diagnosis and hampers understanding of the epidemiology of the condition.

3. **Interventions for the control of CDAD**

Interventions for the control of CDAD can be conveniently divided into three groups: infection control practices, antibiotic manipulations, and novel therapies. These are reviewed in depth in appendix 4, but the main points of emphasis are as follows:

1. *C. difficile* is transmitted between patients, health care workers and the environment. Robust infection control practices are of crucial importance in reducing the incidence of CDAD, and have the additional benefit of effectiveness against other nosocomial infections with risk factors in common. Good hand hygiene by health care workers is paramount, and one of the on-going challenges of infection control is to improve compliance with this simple intervention.

2. Although it can be difficult to distinguish cause and effect, in most studies correlation can be shown between environmental contamination and infection rates. New builds and refurbishments in the healthcare sector should incorporate infection control expertise from the earliest stages of planning, and the highest standards of cleaning should be demanded.

3. A major risk factor for the development of CDAD is prior antibiotic exposure, with cephalosporins being particularly implicated. Restrictive antibiotic policies, to reduce inappropriate administration and to promote the use of agents that carry a lower risk, have proven effectiveness in reducing the incidence of CDAD. Prescriber education is also important.

4. It has become evident that host immunity is an important determinant of CDAD, in particular that there are patients who are particularly prone to developing clinical disease following acquisition of the organism. This insight may lead to improved targeting of infection control interventions,
and perhaps also the development of novel preventative therapies such as vaccination.

4. **Recommendations of the Group**

**Definitions:**

A PHLS/Department of Health joint working group proposed case and outbreak definitions for CDAD in 1994. Despite the advent of new diagnostic methods, in particular the development of molecular methods that permit the detection of *C. difficile* toxin gene sequences rather than toxin itself, the group sees no reason to revise these.

**Case** - diarrhoea not attributable to another cause, occurring at the same time as a positive toxin assay and/or endoscopic evidence of pseudomembranous colitis.

**Outbreak** - occurrence of two or more related cases over a defined period agreed locally taking account of the background rate.

There has been debate about recurrent cases, particularly the distinction between relapse and reinfection. This distinction is beyond the scope of a practical surveillance system, but the Group has decided that for surveillance purposes data analysis should be restricted to cases affecting patients who have not fulfilled the case definition within the previous four weeks. This time-span is necessarily arbitrary, but should provide consistency and practicality.
Specimen collection:

Stool specimens should be submitted for microbiological analysis from all patients in whom a diagnosis of gastrointestinal infection is suspected.

The Group proposes that routine national surveillance of CDAD should be confined to patients over the age of 65 (among whom the available evidence shows it to be most prevalent), but should not be further restricted by inconsistently-supplied clinical information nor by the location of the patient. To comply with the case definition of CDAD, toxin testing should NOT be performed on non-diarrhoeal specimens. However, laboratories will be asked to test ALL diarrhoeal specimens submitted from patients over the age of 65 who have not fulfilled the case definition within the preceding four weeks, regardless of whether they are in the community or in hospital.

Diarrhoeal stools are defined pragmatically as those that take up the shape of their container. Inevitably there will be a grey area between what does and does not constitute a diarrhoeal specimen, but it is important that the surveillance data are not biased by the inclusion of positive toxin assays derived from non-diarrhoeal specimens.

The recommendation to test out-patient as well as in-patient specimens generated much debate during the draft consultation exercise, but after careful consideration we remain firmly of the opinion that this approach is justified. It is likely that for most laboratories the recommendations made here will lead to an increase in numbers of tests performed, although the selection algorithm will be simpler. While there will be an associated economic cost, this should be set against the anticipated benefits of improved patient diagnosis and population epidemiology. The Group recommends that those responsible for funding microbiology services acknowledge the cost implications of this improvement.

Apart from the recommendation that formed stools are not investigated, it is emphasised that the intention is not to discourage individual laboratories from
testing specimens from patients outside the surveillance population, merely that data analysis for routine national surveillance purposes will be confined to results derived from patients over 65 who present with diarrhoea.

**Laboratory methods:**

Specimens should be tested by toxin detection, using either a neutralised cell cytotoxicity assay or an immunoassay that detects both toxins A and B.

Many laboratories have developed their own cell cytotoxicity assays, and at the time of writing there is no accepted standard method, but this is not of concern provided the assay is subject to appropriate quality assurance. Similarly, we see no reason to express a preference for one immunoassay over another, provided that the assay used is reputable, detects both toxin A and toxin B, and is performed according to the manufacturer’s instructions. It is acknowledged that this recommendation will entail a change in practice for those laboratories that currently use assays that detect only toxin A, but the Group believes that for the purposes of optimal surveillance this is justified by the evidence.

**Reporting:**

All positive *C. difficile* toxin results should be reported to the Regional CDSC via CoSurv. Reports will be filtered at destination to remove unwanted data. Provided laboratories make efforts to test only diarrhoeal stools, the resulting data should represent an achievable proxy for CDAD.

These data will include source information for the specimen (ie. hospital, community etc), providing a means of distinguishing hospital from community-acquired cases. The limitations of this are acknowledged. Appropriate denominators have yet to be determined, but might include hospital bed days, and community census data.
Referral:

Suspected outbreaks or clusters of cases should be reported to the Regional CDSC as currently. If further epidemiological information is deemed desirable, specimens should be submitted for culture to a designated regional facility where culture and identification of isolates should be performed according to a national SOP (appendix 3). The regional Health Protection Agency (HPA) laboratories may be best placed to perform this function. Isolates should then be submitted to a single national centre for typing and/or antimicrobial susceptibility testing.

On-going surveillance of strain distribution and antimicrobial susceptibility will also be required. These demand particular expertise and consistency, and again it is recommended that a single institution perform this function. The Group envisages that a consistent subset of toxin-positive specimens will be submitted to the regional facilities for culture, and that the isolates derived will then be sent on to the national centre for typing and susceptibility testing. Such a system would require careful planning to ensure that a representative sample of specimens were characterised, and must await the development of a reliable sub-typing scheme: work is in progress to deliver this. The requirements of the national centre might best be fulfilled by the National Anaerobe Reference Laboratory in Cardiff.

Prevention and control:

Health care providers (both hospital and community based) should be encouraged to promote practices known to reduce the incidence of CDAD. These fall into two broad groups, the first being cleanliness and hygiene (including environmental cleaning and hand washing), and the second being a restrictive approach to antibiotic prescribing. These simple interventions could have a major impact on the impact of CDAD.
5. **Current practice and implications for microbiology laboratories**

The likely implications of these recommendations can be inferred from the results of a questionnaire survey addressed to laboratories in England, Wales and Northern Ireland during the preparation of this report. This survey is presented in appendix 5, but the important points are as follows:

1. Currently, very few laboratories test all submitted specimens for *C. difficile* toxin: most apply selective criteria in the interests of economy. However specimen selection is highly variable between laboratories, with the result that different laboratories test widely different subsets of specimens. For surveillance data to be meaningful it behoves us to minimise these discrepancies. We recommend (in the surveillance population at least) that specimen selection according to location or clinical information should no longer be applied.

2. Despite acknowledgement that the presence of toxin is only of clinical relevance in the presence of diarrhoea, some laboratories continue to test formed stools. This is both wasteful and a source of bias. We recommend that non-diarrhoeal stools should not be tested.

3. All laboratories in the survey that perform testing for CDAD do so by toxin detection, so our recommendations do not require the introduction of unfamiliar testing methodologies. However, of 179 laboratories undertaking toxin detection, 42 (23.5%) use immunoassays that detect toxin A alone. Encouragingly, this proportion is lower than when surveyed in 1998 (Brazier J, personal communication). We recommend that laboratories continue to move towards assays that detect both toxins A and B. These assays operate on identical principles to those detecting toxin A alone, and do not carry a significant cost premium.

4. Only about two thirds of laboratories currently partake in CDR/CoSurv reporting to CDSC, and of these most do not distinguish asymptomatic colonisation from symptomatic infection. It is a criticism of laboratory reporting systems that they reflect test results rather than clinical diagnoses, but our recommendation that only diarrhoeal specimens are tested should redress this without requiring extra work from hard-pressed
laboratory staff. We intend to carry out separate work to determine the true clinical impact of *C. difficile*, i.e. the spectrum of disease in patients with diarrheal stools who are toxin positive.

5. Of those laboratories that report results to CDSC, there is wide variation in the handling of repeatedly positive results from individual patients. Our decision that repeat positives will be excluded from analysis within four weeks of first diagnosis is based on experience rather than published evidence, but will at least permit consistency of interpretation. Laboratories themselves may wish to apply this rule in order to reduce unnecessary testing, but will not be required to do so, and the point is taken that a negative result during this period can sometimes be helpful. Reports will be filtered at destination to eliminate duplicated data.

6. **Conclusions**

NHS Trusts in England and Wales will soon be required to report cases of *Clostridium difficile* associated disease. The National *C. difficile* Standards Group was established to make recommendations to ensure that the data generated by the reporting system will be of value. Our recommendations are designed to promote consistency of specimen selection, accuracy of laboratory analysis, and rational reporting.

For most laboratories this will require review of specimen selection algorithms. In most cases the approach recommended will be a simplification, although it is likely that more specimens than currently will require testing. It is unlikely that laboratories will have to radically change their methods, although those that use immunoassays detecting toxin A only are recommended to move to assays that detect both toxin A and toxin B.

Reporting will be to regional CDSC departments via the existing CoSurv network. This is not expected to add to the workload of busy diagnostic laboratories.
7. **Appendices**

Appendix 1: *C. difficile* and *C. difficile*-associated disease

Appendix 2: Diagnosis of *C. difficile*-associated disease

Appendix 3: Standard Operating Procedure for *C. difficile* isolation and identification

Appendix 4: Prevention and control of *C. difficile*-associated disease

Appendix 5: National survey of current practice

Appendix 6: Results of the draft report consultation exercise

Appendix 7: Membership of the National *C. difficile* Standards Group

Appendix 8: References
Appendix 1 – C. difficile and C. difficile-associated disease

Introduction:

The genus Clostridium comprises a group of Gram-positive, anaerobic or aero-tolerant bacilli, widely distributed in soil and in the intestinal tracts of animals. Its vegetative cells are capable of forming spores, which confer resistance to heating, drying, and chemical agents, and permit the organism to survive harsh environments. The genus includes the causal agents of tetanus (*Clostridium tetani*), botulism (*Clostridium botulinum*) and gas gangrene (predominantly *Clostridium perfringens*). *Clostridium difficile* was described during the 1930s (Hall & O'Toole, 1935).

Pseudomembranous colitis (PMC) was first reported in 1893 (Finney, 1893), but came to prominence during the 1950s following the widespread introduction of antibiotics into clinical practice (Bartlett, 1993). It was not until the 1970s, however, that toxigenic *Clostridium difficile* was identified as the cause of PMC in man (Bartlett et al, 1978; George et al, 1978a; George et al, 1978b; Larson et al, 1978). The organism is now thought to be responsible for a spectrum of largely but not exclusively hospital-acquired disease, ranging from asymptomatic colonisation, to diarrhoea of varying severity, to life-threatening colitis, often as a consequence of antibiotic exposure. This spectrum has become known as *C. difficile*-associated disease (CDAD) (McFarland & Stamm, 1986).

Diarrhoea is a frequent side effect of antibiotic administration. In most cases its pathogenesis cannot be conclusively attributed, but is presumed to reflect alterations in colonic flora in conjunction with effects on gut motility. *C. difficile* is thought to cause about a quarter of cases of antibiotic-associated diarrhoea overall, but accounts for a greater proportion of more severe disease. For instance, toxin assays are positive in more than half of those with antibiotic-associated colitis, and in almost all of those with antibiotic-associated PMC (Bartlett, 1990).
CDAD also imposes a significant financial burden on health care services. Sources of excess costs attributable to nosocomially-acquired CDAD include prolonged hospital stay, the requirement for isolation and more intensive nursing, treatment costs, laboratory costs and infection control costs. A hospital outbreak in Manchester in 1992 affected 175 patients and was estimated to have cost £74,000 (Cartmill et al, 1994). This figure did not include the costs of increased length of stay: in 1996 the total cost of CDAD was estimated at over £4000 per case (Wilcox et al, 1996).

**Epidemiology:**

Currently, surveillance of *C. difficile* infection in England and Wales is performed by the Public Health Laboratory Service Communicable Disease Surveillance Centre (CDSC) through a voluntary laboratory reporting system. Although provisional data suggest it has tailed off more recently, the number of positive *C. difficile* toxin tests rose steadily throughout the 1990s (figure 1). It is unclear how much of this increase reflected rising ascertainment, and how much a true increase in incidence, and it should also be remembered that these were laboratory rather than clinical reports. Nevertheless the trend is dramatic.

Figure 1: *C. difficile* laboratory reports for England and Wales (provisional data)
During the first month of life up to two thirds of infants become colonised with *C. difficile*. This probably reflects acquisition from the hospital environment, but for reasons that remain unclear most colonised neonates are asymptomatic carriers even when toxin production can be demonstrated. As childhood progresses, carriage rates decline to adult levels, while both sporadic and outbreak CDAD begin to appear (Burgner et al 1997; McFarland et al, 2000).

Presence of *C. difficile* in faeces can be demonstrated in up to 3% of healthy adults (Knoop et al, 1993). Rates of colonisation and infection increase markedly beyond the age of 65 (figure 2), such that for England and Wales *C. difficile* is the predominant enteric pathogen among people in this age group (Djuretic et al, 1999). Asymptomatic presence has been reported in about 7% in residents of long-term care facilities (Walker et al, 1993), 14% of hospitalised elderly patients on acute medical wards, and 20% of elderly patients on chronic care wards (Rudensky et al, 1993), in whom it is between three and five times more common than symptomatic disease (Samore 1993; McFarland et al, 1989).

Figure 2: Age-specific *C. difficile* laboratory reports, England and Wales 2001
Apart from age, the main risk factors are antibiotic administration (particularly third generation cephalosporins, although virtually all antibiotics have been implicated) (de Lalla et al, 1989; Schwaber et al, 2000; al-Eidan et al, 2000), and underlying morbidity such as abdominal surgery, cancer, chronic renal disease, and tube feeding (Bliss et al, 1998).

Thanks to the presence of susceptible individuals, plus opportunities for transmission of the organism, CDAD is primarily a nosocomial disease. *C. difficile* may be transmitted from symptomatic excretors, either directly or via the hands of health care workers (Kroker et al, 2001). Patients with asymptomatic colonisation are not thought to represent a significant risk, or to need treatment (PHLS/DoH Working Group Report, 1994). Attempts to eradicate carriage have been unsuccessful (Johnson et al, 1992).

Physical proximity to a symptomatic case has been reported as important for transmission with an attributable risk of 12% (Chang & Nelson, 2000). Mechanisms postulated include the contamination of the near-patient environment, and the movement between patients of contaminated fomites such as commodes. Transfer of patients between wards or between institutions has also been implicated (Safdar & Maki, 2002).

The role of the environment is controversial (Fawley & Wilcox, 2001). In most studies there is good correlation between infection rates and environmental contamination, but it is difficult to distinguish cause from effect. Nevertheless it is plausible that patients might also acquire *C. difficile* from the environment, and important that high standards of environmental cleaning are maintained.

However, community acquired CDAD does occur, and is currently under-diagnosed in the UK because many laboratories do not routinely look for the bacterium or its toxin(s) in general practice faecal specimens. Data from Sweden indicate that 42% of cases of *C. difficile* infection present in the community, half of whom do not have a history of hospitalisation within the previous month (Karlstrom et al, 1998). In Ireland, 11% of cases presenting with cytotoxin positive *C. difficile* diarrhoea had no hospitalisation within the
previous 60 days (Kyne et al, 1998). The Intestinal Infectious Disease (IID) Survey in England identified *C. difficile* as the third most common cause of IID in patients aged >75 years seen by GPs (HMSO, 1999).

More recently, the incidence of community *C. difficile* diarrhoea detected in teaching (urban) and semi-rural district general hospital microbiology laboratories in England was the same (2.1%) when randomly selected faecal samples submitted from general practice were examined for cytotoxin (Wilcox et al, 2001). Risk factor data were collected for 56 community *C. difficile* diarrhoea cases and 182 age- and sex-matched controls: these showed that 50% of cases but only 11% of controls had received antibiotic therapy in the month before diarrhoea ($P < 0.001$), and that 32% of cases and 15% of controls had been hospitalised in the 6 months before diarrhoea ($P < 0.01$). However, a large proportion of cases (39%) had neither of these risk factors. In any case, it is likely that the distinction between nosocomial and community-acquired CDAD is likely to become blurred as trends towards earlier hospital discharge and more intensive community nursing continue.

**Pathogenesis:**

The accepted model of the pathogenesis of CDAD involves disruption to the host defences mediated by the indigenous microflora of the bowel (Wilson, 1993). Healthy adults carry at least 500 recognised bacterial species in the colon, over 90% of them anaerobes. Within an individual this complex population remains stable over time, and has an inhibitory effect on incoming, non-indigenous species. This phenomenon has been called ‘colonisation resistance’. *In vitro* and animal models have demonstrated that vegetative *C. difficile* cells become non-viable when exposed to normal colonic populations. The mechanism is not fully understood, but probably reflects competition for nutrients and adhesion sites, together with suppression by local metabolic products such as H$_2$S and volatile fatty acids.
Colonisation resistance is so effective that exogenous pathogens such as *Salmonella* or *Campylobacter* require specialised mechanisms to circumvent it. However, if the normal flora is disrupted, colonisation resistance is lost and organisms such as *C. difficile* may seize their opportunity. The most frequent reason for this is exposure to antimicrobial agents, and it is noteworthy that in general the antibiotics that carry the greatest risk are those which exert the greatest effect on colonic bacteria (Bignardi, 1998). Moreover, colonic populations of bifidobacteria, which are thought to be protective, are known to decline naturally with advancing age (Hopkins et al, 2001). There is also increasing evidence that humoral immunity is important in defence against progression to disease, if not prior colonisation (Mulligan et al, 1993; Kyne et al, 2000; Kyne et al, 2001), and this observation is likely to have a profound influence on our understanding of the disease (Starr & Campbell, 2001).

The existence of specific outbreak strains implies that bacterial as well as host factors are important. Proposed virulence factors include an antiphagocytic capsule, fimbriae, hydrolytic enzymes, adhesins, and flagella (Borriello, 1998), but our understanding of the relationship between virulence factors and pathogenesis is rudimentary.

The accepted view, then, is that CDAD results when a pathogenic strain of *C. difficile* first colonises, then causes disease in patients rendered susceptible by exposure to antibiotics, old age, or other factors. Once established, however, CDAD is a toxin-mediated condition. Most pathogenic strains of *C. difficile* are capable of expressing two major virulence factors designated toxin A and toxin B. Although highly homologous and encoded contiguously, the two toxins have very different characteristics (Bongaerts & Lyerly, 1994). Toxin A is cytotoxic and also a potent enterotoxin, and in animal models of CDAD is responsible for the intestinal damage and intraluminal fluid accumulation observed. Toxin B is almost devoid of enterotoxic activity in animals but is a potent cytotoxin. This has led some to question the role of toxin B, but there is evidence that it enhances the activity of toxin A, and furthermore recent years have seen the emergence of undoubtedly pathogenic strains which do not express toxin A (Johnson et al, 2001).
Appendix 2 – Diagnosis of C. difficile-associated disease

Approaches to the diagnosis of CDAD have been reviewed periodically in the literature (Brazier et al, 1993; Delmée, 2001), but merit reassessment in the context of the development of a national surveillance system. What follows will address two questions fundamental to consistent data collection: how should CDAD be diagnosed, and which patients should be tested?

Despite the advent of molecular technologies, the first of these is still contested largely between those who advocate detection of toxin in stool specimens, and those who advocate detection of the organism by culture or other methods.

The importance of the second question is under-estimated, and there is no doubt that variability in specimen selection confounds attempts to compare or amalgamate data from different laboratories.

Laboratory diagnosis

For the purposes of discussion, diagnostic methods will be considered according to the following classification:

1. Non-microbiological methods: Clinical assessment
   Endoscopy
   Faecal leukocytes and lactoferrin

2. Detection of C. difficile products: Glutamate dehydrogenase
   Volatile fatty acids
   Toxins

3. Detection of C. difficile genes: 16s rRNA
   Toxin genes

4. Isolation and typing of C. difficile: Culture and identification
   Typing and toxin testing
   Antibiotic sensitivity testing
1. Non-microbiological methods:

**Clinical assessment**: clinical manifestations of CDAD include abdominal pain, profuse, foul-smelling, soft stools, and fever. These features are not specific, and it seems unlikely that the clinical picture can contribute greatly to diagnosis (Watson et al, 1986). A history of antibiotic administration might be a useful pointer, but antibiotic-induced CDAD may occur at any time up to two months after exposure so the association may go unrecognised. The clinical picture is also complicated by the fact that diarrhoea may be completely absent, a presentation associated with the serious complication of toxic megacolon. It has been suggested that leuckocytosis is particularly prominent in CDAD, but again this alone is inadequate for diagnosis (Bulusu et al, 2000; Wanahita et al, 2002). Nor are imaging investigations particularly helpful. The plain abdominal radiograph is usually normal even in PMC (Boland et al, 1994a), while the main CT finding is a thickened bowel wall, which is both insensitive and non-specific (Fishman et al, 1991; Boland et al, 1994b).

**Endoscopy**: pseudomembranous colitis *per se* is a pathological diagnosis and may be confirmed at endoscopy. Pseudomembranes are raised, yellowish nodules, sometimes coalescing to form plaques, which overlie the inflamed mucosa but are easily dislodged from it. The appearance may be enough for macroscopic diagnosis, but biopsy allows histological confirmation of the ‘summit’ or ‘volcano lesions’ typical of PMC. In small series it has been shown that flexible endoscopy is more sensitive than rigid endoscopy, and colonoscopy more sensitive than sigmoidoscopy (Seppala et al, 1981). However, if it is accepted that pseudomembranous colitis is an extreme manifestation of the CDAD syndrome, then by definition endoscopy will fail to provide an aetiological diagnosis in both *C. difficile*-associated non-specific colitis and non-colitic CDAD. Moreover, a small proportion of PMC may not be associated with *C. difficile*. Most authorities believe that endoscopy is of subsidiary value, for instance when clinical suspicion is not confirmed by less invasive means, but that diagnosis generally requires laboratory testing.
**Faecal leukocytes and lactoferrin:** it has been suggested that detection of faecal leukocytes, traditionally by methylene blue staining, can be helpful in the distinction between inflammatory and non-inflammatory causes of diarrhoea (Guerrant et al, 1985). In fact there is only limited published evidence to support this (Manabe et al, 1995), and some reports that it is positively unhelpful (Savola et al, 2001) perhaps because leukocytes degrade with time following specimen collection. Faecal lactoferrin, which can be detected using a latex agglutination test, has been validated as a stable marker of faecal leukocytes (Guerrant et al, 1992), but although there is evidence that this can be useful in the investigation of diarrhoeal diseases, its role in CDAD remains to be defined (Yong et al, 1994; Schleupner et al, 1995; Silletti et al, 1996; Steiner et al, 1997).

2. **Detection of *C. difficile* products:**

**Glutamate dehydrogenase:** a commercial latex agglutination test, Culturette Brand (Marion), was initially claimed to detect toxin A and seemed to yield promising results (Peterson et al, 1986). Its credibility was diminished when this claim was refuted (Lyerly & Wilkins, 1986), but it was later shown to detect the enzyme glutamate dehydrogenase (GDH) (Lyerly et al, 1991), which is a moderately specific marker for *C. difficile*. Re-evaluation suggested that the test might have a role when combined with other techniques, but that it was not sufficiently accurate to replace the prevailing cell cytotoxicity assay (Kelly et al, 1987; Peterson et al, 1988). Culturette Brand continues in production, now promoted as a screening test for detection of the organism rather than its toxin. This position is based on older data that seemed to demonstrate a good negative predictive value (Sherman et al, 1988), but more recent reports are less impressive (Fille et al, 1998; Vanpoucke et al, 2001). Meritec (Meridian), a competitor that utilises the same principle, has undergone only limited evaluation (Kelly et al, 1992).

The same GDH marker is targeted by two more recently developed tests, the ImmunoCard *C. difficile* test (Meridian), which employs the membrane
immunochromatography format, and the Triage *C. difficile* Panel (Biosite), an enzyme immunoassay that also targets toxin A. The ImmunoCard system appears to be superior to the GDH latex test and has performed well in comparison with the cell cytotoxicity assay (Barbut et al, 1994; Staneck et al, 1996), but concerns remain that it might detect carriers and non-toxigenic strains. Triage is discussed below.

**Volatile fatty acids:** in vitro, *C. difficile* exhibits a fairly characteristic pattern of volatile fatty acid production, particularly isocaproic acid, isovaleric acid, and para-cresol. During the 1980s attempts were made to correlate conventional testing methods with the results of gas-liquid chromatography (GLC) performed directly on stool samples. Initial results suggested that GLC might have sufficient negative predictive value to be useful as a screening test (Pepersack et al, 1983; Gianfrilli et al, 1985), but contradictory results (Levett, 1984) and the limitations of the technology have brought this line of research to an end.

**Toxins:** *C. difficile* toxins may be detected either by virtue of their biological properties (the cell cytotoxicity assay) or by immunological methods (latex agglutination, counterimmunoelectrophoresis, or immunoassay). These will be discussed in turn.

**Cell cytotoxicity assay:** recognition that the stools of patients with antibiotic-associated colitis were toxic to cultured cell lines predated the discovery of *C. difficile* as the causative agent of this disease (Larson et al, 1977), and the cell cytotoxicity assay remains the standard by which other tests are measured. The assay is performed by exposing cell monolayers to stool filtrates, and observing the cells for evidence of cytopathic effect (CPE). The specificity of any effect is tested by neutralising with the cross-reacting *C. sordelli* antitoxin (Rifkin et al, 1977). Any CPE that can be neutralised like this is presumed to reflect the presence of *C. difficile* toxins, both of which contribute to the cell damage.
The cell cytotoxicity assay remains the only practicable means of detecting *C. difficile* toxin by means of its biological properties, but it suffers from a number of drawbacks. It requires a supply of cultured cell monolayers, and its results are known to vary according to methodological details such as the cell line applied, dilution factors, reagents used and specimen storage conditions (Chang et al, 1979). Although commercial variants such as the Tox-B Test (TechLab) and the Cytotoxi Test (Advanced Clinical Diagnostics) are available, most laboratories use locally developed methodologies which have not been standardised. At the time of writing there is no nationally agreed Standard Operating Procedure. Its turnaround time is slow, typically 24 hours to demonstrate cytotoxic activity, and a further 24 hours to demonstrate neutralisation by specific antitoxin. Finally, some specimens contain non-specific cytotoxicity that is not neutralised by *C. sordelli* antitoxin and cannot be further assessed.

Despite its limitations, the cell cytotoxicity assay remains the standard by which other methods are judged, and its results are entirely suitable for surveillance purposes provided positive results are confirmed by neutralisation, and appropriate quality assurance is in place.

**Latex agglutination for toxin A:** development of the first rapid agglutination test for *C. difficile* was reported in 1984, using latex particles coated with a commercial antitoxin that could be shown to react with toxin A as well as other antigens that remained undefined (Shahrabadi et al, 1984). In comparison with the cell cytotoxicity assay, the latex test had a good negative predictive value but suffered from a high rate of false positive reactions. This assay has not been taken forward.

**Counterimmunoelectrophoresis:** this technique, by which toxins in faecal specimens can be detected as toxin-antitoxin precipitation lines in agarose gel, was investigated in the early 1980s (Wu & Fung, 1983; Rennie et al, 1984). Although relatively easy to perform, the technique suffered from lack of standardisation, and poorly defined antitoxins meant that it was never clear
whether the assay was detecting toxin A, toxin B, both, or something else. Interest in CIE waned with the advent of immunoassays.

**Immunoassay:** two types of immunoassay have been developed, the traditional enzyme immunoassay (in various formats), and the single-use membrane immunochromatography type. The forerunner of the membrane assays was the dot immunobinding assay (C. diff-CUBE, Difco) (Woods & Iwen, 1990), but this has been superseded and will not be considered further.

The first EIA to detect *C. difficile* toxin was described in the 1980s (Lyerly et al, 1983), since when a large number have been developed, some detecting toxin A, others detecting both toxin A and toxin B (Table 1). Most are marketed as kits but the principle has been adapted for automated equipment such as the Vidas system (Shanholtzer et al, 1992). Triage, an EIA for the simultaneous (but distinct) detection of toxin A and GDH, has been shown to have a very high negative predictive value (Barbut et al, 2000; Landry et al, 2001), suggesting that it might be useful as a screening test (Alfa et al, 2002), but this awaits confirmation.

There have been numerous publications comparing the performance of different kits with each other and with other methods, but no meta-analysis has been performed in an attempt to demonstrate the superiority of any particular test. In general, immunoassays perform reasonably well in comparison to the cell cytotoxicity assay, with sensitivities and specificities of the order of 85-95%. Some investigators have found that the membrane type assays are inferior to the more traditional micro-well EIAs (O'Connor, 2001), others the reverse (Vanpoucke et al, 2001). The principle advantage of the membrane immunochromatography assays is speed, since results can usually be obtained within an hour.

The Group takes the view that for surveillance purposes immunoassays provide satisfactory sensitivity and specificity, provided that an assay detecting both toxin A and toxin B is used. This recommendation was not met with universal approval during the draft consultation exercise, but it has been
made for two reasons. First, the toxin A + B assays tend to perform better than the assays detecting toxin A alone. Second, there is an increasing awareness of the importance of toxin A negative/toxin B positive strains, which are undetectable using assays that detect toxin A alone. The epidemiology of such strains in the UK is poorly understood, but data from elsewhere demonstrate their ability to spread widely and to cause outbreaks (Kuijper et al, 2001; Samra et al, 2002; Barbut et al, 2002; Pituch et al, 2001). Moreover, their distribution is more likely to be patchy than uniform, and there is a risk that the use of assays detecting toxin A alone will lead to significant under-diagnosis in some populations. Kits detecting both toxins do not carry a significant cost premium over those that detect toxin A alone. It is unfortunate that the VIDAS system, which being automated is otherwise convenient for busy laboratories, does not currently support testing for both toxins, and we urge the manufacturers to address this deficiency.

Table 1: Some *C. difficile* toxin immunoassays available in England and Wales

<table>
<thead>
<tr>
<th>Type</th>
<th>Assay</th>
<th>Supplier</th>
</tr>
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<tbody>
<tr>
<td>Well – type EIAs</td>
<td>Premier Toxin A</td>
<td>Meridian</td>
</tr>
<tr>
<td></td>
<td>Premier Toxin A+B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tox-A-Test</td>
<td>Techlab</td>
</tr>
<tr>
<td></td>
<td>Toxin A/B II</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Culturette Brand Toxin CD</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td></td>
<td>Ridascreen Clostridium toxin A</td>
<td>Biopharm</td>
</tr>
<tr>
<td></td>
<td>Ridascreen toxin A/B</td>
<td></td>
</tr>
<tr>
<td>Automated EIAs</td>
<td>Vidas (toxin A)</td>
<td>Vitek</td>
</tr>
<tr>
<td>Membrane assays</td>
<td>Immunocard Toxin A</td>
<td>Meridian</td>
</tr>
<tr>
<td></td>
<td>Oxoid toxin A</td>
<td>Oxoid</td>
</tr>
<tr>
<td></td>
<td>Clearview <em>C. difficile</em> A</td>
<td>Unipath</td>
</tr>
<tr>
<td></td>
<td>Triage (toxin A + GDH)</td>
<td>BioSite</td>
</tr>
</tbody>
</table>
3. Detection of *C. difficile* gene sequences:

**16s rRNA:** primers targeting the *C. difficile* 16s rRNA gene have been used to detect the organism in faecal samples (Kuhl et al, 1993). This detects non-toxigenic as well as toxigenic *C. difficile*, and for specificity requires a second PCR to detect gene sequences. While interesting, there seems to be no advantage in clinical practice over direct detection of toxin genes.

**Toxin genes:** use of PCR to amplify parts of the toxin A gene from stool was first reported in 1993, and yielded results that were in complete accord with the cell cytotoxicity assay (Kato et al, 1993). Stool toxin B PCR was first reported in the same year (Gumerlock et al, 1993). Toxin B sequences were amplified from each of 18 specimens positive by the cell cytotoxicity assay, and also from two of 18 specimens in which cytotoxic activity was not demonstrable: these two were from patients with a clinical history compatible with CDAD. The authors went on to demonstrate concordance between the results of toxin B and toxin A PCRs (Tang et al, 1994).

Although accurate, the major disadvantage of these methodologies was a complicated and technically demanding DNA extraction step necessitated by the presence of PCR inhibitors in faecal specimens. Recently however, the advent of easy-to-use commercial DNA extraction kits has prompted re-evaluation of the diagnostic possibilities of stool PCR (Alonso et al, 1999; Guilbault et al, 2002). So far only applied to toxin B, the method appears to be both sensitive and specific, but it is expensive, remains available only to those laboratories with expertise in molecular methods, and does not confirm that toxins have been expressed.

4. Isolation and typing of *C. difficile*:

**Culture methods:** the first satisfactory medium for the culture of *C. difficile* was cefoxitin-cycloserine fructose agar (CCFA) (George et al, 1979). This has been extensively used since, although there has been debate about the
optimal concentration of cycloserine (Levett, 1985). A pre-inoculation process of heat or alcohol shock has been shown to enhance the isolation of \textit{C. difficile}. It has also been suggested that the medium should be anaerobically reduced before specimen inoculation (Peterson et al, 1996). Egg yolk agar has been employed in an attempt to distinguish \textit{C. difficile} (which is lecithinase and lipase negative) from other common colonic clostridia, but there are concerns that it can hamper the detection of fluorescence (see below) and it is not universally used. Some authorities recommend a broth enrichment step, which gives enhanced isolation of \textit{C. difficile}, but it is debatable whether this is of clinical relevance.

**Identification and toxin testing:** following culture, \textit{C. difficile} can be identified by its characteristic smell, yellow-green fluorescence under long wave ultraviolet light, and/or by a latex slide agglutination test which reacts with cell wall antigens. Identification according to p-cresol production on CCFA (Phillips & Rogers, 1981), and biochemical profile (Aspinall & Dealler, 1992), have been described but have not become widely accepted.

It is also possible to determine whether or not the isolate is a toxin producer. This process has been called ‘toxigenic culture’. Toxin production can be demonstrated by putting broth culture filtrates through the same EIAs used for faecal specimens, and also by the cell cytotoxicity assay (Bouza et al, 2001). Alternative methods reported include reversed passive latex agglutination (applied to toxin A) (Toma et al, 1999), colony blot probe-hybridisation (applied to toxin B) (Wolfhagen et al, 1993), and PCR (toxins A and B) (Karasawa et al, 1999). These so-called ‘second-look’ cytotoxicity assays can be shown to detect toxigenic \textit{C. difficile} in diarrhoeal specimens negative in the stool cytotoxicity assay, but the clinical significance of this finding requires confirmation.

**Antibiotic sensitivity testing:** antibiotic sensitivity testing of anaerobes is a specialised task, currently carried out by the UK Anaerobe Reference Unit, Cardiff. It is of the utmost importance that surveillance of the antibiotic sensitivity of \textit{C. difficile} is maintained, but for surveillance and reference
purposes this is best done by a single national centre and the techniques will not be discussed further.

**Typing:** Strain typing is important for outbreak investigation and descriptive epidemiology. The ideal system is easy, quick and cheap to perform, discriminatory (but not overly so), and is reproducible within and between laboratories such that types may be defined and compared.

As with other organisms, typing of *C. difficile* can be conveniently divided into phenotypic methods and genotypic methods.

**Phenotypic:**
- Phage typing
- Serotyping (Delmée et al, 1985)
- Protein analysis by SDS-PAGE (Costas et al, 1994)
- Immunoblotting (Kato et al, 1993)
- Pyrolysis mass spectrometry (Magee et al, 1993)

**Genotypic:**
- Restriction endonuclease analysis (Kuijper et al, 1987)
- Restriction Fragment Length Polymorphism (Bowman et al, 2001)
- Arbitrarily primed PCR (Wilks & Tabaqchali, 1994)
- Random amplified polymorphic DNA (Barbut et al, 1994)
- PCR ribotyping (Collier et al, 1996)
- Pulsed Field Gel Electrophoresis (Kato et al, 1994)

It is accepted that molecular methods are superior to the phenotypic methods, but debate continues about which format is best. RAPD is probably the most discriminatory, but random primer methods require very strict standardisation if they are to be reproducible. PCR ribotyping is a robust and practical compromise (Bidet et al, 2000), and is the method currently offered by the UK Anaerobe Reference Unit (Brazier, 1998b; Brazier, 2001), but might be insufficiently discriminatory for the purposes of sampling matrix surveillance.
Although UK strains have been allocated to over 100 distinct ribotypes, only 16 of these account for 90% of referrals, and type 1 strains account for 60% of hospital isolates submitted to the Anaerobe Reference Unit. Development of an improved typing scheme must be regarded as a research and development priority.

**Specimen selection strategy**

If every specimen were tested for *C. difficile* toxin using a perfectly sensitive method, then self-evidently all patients with CDAD would be diagnosed. However, the available tests are too expensive to apply indiscriminately, and in practice laboratories employ screening procedures to reduce the numbers of tests performed.

Screening procedures are highly variable. They may be based on the nature of the specimen (for instance a rule that only liquid stools are processed), the epidemiology of the disease (for instance a rule that stools are not processed from patients younger than 50), the clinical details on the request form (for instance a rule that stools are only processed if a history of antibiotic use is given), or pathological criteria (for instance a rule that stools are not processed unless they contain abnormal numbers of leukocytes on staining). Nevertheless, even if just a set of administrative procedures, the screening step may be viewed as a test, and as such will have a sensitivity and specificity associated with it. As well as being easy, quick, and cheap to perform, a good screening method must have a high negative predictive value (so toxin positive specimens are not eliminated as likely negatives without being tested), and fair specificity (in order to eliminate as many of the true negatives as possible, and hence reduce the numbers of CDT tests required).

In the absence of accepted standards different laboratories apply very different selection criteria, and this presents great difficulties in comparing results: a laboratory that tests for CDT only on request is likely to detect fewer CDT positive patients than a laboratory that tests all specimens. Similarly, a
laboratory that tests all liquid specimens from elderly patients with a history of antibiotic use will test a very different subset from a laboratory that tests all liquid specimens, all specimens from elderly patients, and all specimens from patients with a history of antibiotic use.

Plainly, the issue of specimen selection is of great importance in the strategic understanding and day-to-day management of CDAD, yet it has been little studied, and escapes mention in practice guidelines (Fekety, 1997). If standardisation is to be applied here, it would seem sensible to begin with the assumption that the presence of CDT is only of clinical relevance in patients with diarrhoea, and to restrict testing to diarrhoeal stools; a diarrhoeal stool is conventionally defined as a specimen that takes up the shape of its container. Furthermore, if it is accepted that CDAD (as distinct from colonisation) rarely if ever occurs in children below the age of two years, then stools from these patients need not be tested.

Beyond this there is little evidence on which to base a more selective testing strategy. It has been proposed that the most practicable set of selection criteria would be to test only unformed specimens from patients with a history of prior antibiotic use (Gerding, 1996), but this has not been formally tested, and it presupposes a diligence in filling in request forms that is probably unattainable. Selection based on epidemiological parameters such as older age cut-offs, or in-patient vs. out-patient origin, merits debate but cannot be recommended currently.

The ‘three day rule’ deserves special mention. It was reported in 1990 that community acquired enteric pathogens such as Campylobacter were rarely if ever cultured from specimens submitted more than three days after admission to hospital, while C. difficile was the predominant identifiable cause of diarrhoea beyond this point (Siegel et al, 1990). It was recommended that routine stool culture and parasite detection should be confined to specimens submitted within three days of admission in order to save time and money, an outcome that has been independently confirmed (Morris et al, 1996; Ozerek & Gopal Rao, 1999). In contrast, there was a consistent yield of positive CDT
results from outpatients and inpatients within three days of admission, and it was recommended that CDT testing should not take account of admission status. Others have made similar observations and reached similar conclusions (Rohner et al, 1997; Bourgault et al, 1999). Anecdotally, some laboratories have interpreted the ‘three day rule’ to imply that stools do not merit CDT testing unless the patient has been in hospital more than three days, but this policy is not supported by the data.
Appendix 3 - Standard Operating Procedure for the Isolation and Identification of *Clostridium difficile* from Stool Specimens

1. **Culture:**

**Alcohol shock:** make an approximate 1:1 suspension of stool sample in methylated spirit in a suitable glass container. Mix by vortexing and leave to settle at room temperature for 30min. The advantage of using alcohol shock for selection of *C. difficile* is that only spores should survive this process, thus eliminating the growth of non-sporing faecal organisms (Borriello & Honour, 1981). With a disposable pipette inoculate two drops (approx 50-75ul) of the deposit onto an appropriate selective agar medium and streak for single colonies. Non-selective blood agar medium can also be used (Borriello & Honour, 1981).

**Media and incubation:** the recommended selective medium is cefoxitin cycloserine agar (Brazier, 1993; Brazier, 1998a), with or without egg yolk (which allows the detection of lecithinase production) according to local preference, prepared as follows:

Either: BioConnections *C. difficile* CCEY Agar Base (order code: BC2 160R) with selective supplements of cefoxitin 8mg/L and cycloserine 250mg/L (order code: S2 093) supplemented with either 5% defibrinated horse blood or 7% egg yolk suspension (order code: A0686).

Or: Oxoid *C. difficile* Agar Base (order code CM 601) supplemented with the same concentrations of cefoxitin and cycloserine (order code: SR 96).

Incubate plates anaerobically at 36-37°C for 48-72 hrs. If using an anaerobic chamber, cultures may be examined after overnight incubation but should not be removed from the chamber.
2. Identification:

Colonies of *C. difficile* can vary in their morphology from smooth to rough versions and various sizes may be present. *C. difficile* can be recognised by the following characteristics:

**Odour:** reminiscent of horse manure, and enhanced due to *p*-cresol production on selective media containing *p*-hydroxyphenylacetic acid

**Green-yellow fluorescence under long-wave UV light:** colonies should be exposed to long wave ultra-violet light (365nm) in a darkened room or light box, held closely to the UV source and viewed by reflection. Colonies of *C. difficile* may vary in the intensity of fluorescence but will appear as a green-yellow or chartreuse colour. Fluorescence is poorly developed on some agar bases and is strongest on FAA or blood based agars. Fluorescence of older cultures on non-selective media is diminished.

**Agglutination with *C. difficile* somatic antigen latex reagent:** Microscreen *C. difficile* somatic antigen latex agglutination, Launch Diagnostics Ltd. Emulsify one colony in a drop of saline on a glass slide or on the card provided, add 20µl of latex reagent, and mix by rotation for two minutes. Agglutination of latex particles indicates a positive reaction (refer to positive control). Cross-reactions with this reagent are known to occur with *C. bifermentans/sordellii* and *C. glycolicum*.

**Absence of lecithinase activity:** on egg-yolk based media, *C. difficile* colonies are not surrounded by a zone of opacity, unlike those of the lecithinase-producing species *C. bifermentans, C. perfringens* and *C. sordelli*. 
From isolates submitted to the ARU previously, it has become apparent that other clostridial species are commonly mistaken for *C. difficile*. These include *C. innocuum*, *C. glycolicum* and *C. sordelli/bifermentans*, which can be differentiated according to the criteria listed in Table 1. In addition, unlike *C. difficile*, *C. innocuum* is glassy green under normal light.

Table 1. Differential tests for recognition of colonies of *C. difficile*

<table>
<thead>
<tr>
<th></th>
<th><em>C. difficile</em></th>
<th><em>C. innocuum</em></th>
<th><em>C. glycolicum</em></th>
<th><em>C. sordelli/bifermentans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UV fluorescence</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Latex</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lecithinase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

3. Submission of isolates to ARU:

Typing and sensitivity testing will be carried out at the UK Anaerobe Reference Unit, Cardiff.

From a non-selective medium pick one colony into a cooked meat broth and incubate for 48hrs. Cultures should be sealed and sent to ARU in accordance with postal regulations governing infectious materials and accompanied by relevant details.

Local risk assessments should be prepared for this SOP
Appendix 4 – Prevention and control of *C. difficile*-associated disease

Prevention of *C. difficile* infection relies on preventing as far as possible patients' exposure to the organism, and ensuring that they do not become susceptible through disruption of their normal gut flora. Thus interventions for the control of *C. difficile infection* can be divided into infection control measures and antibiotic manipulations.

Both strategies need to be applied together and, particularly in the case of infection control, generic approaches that address issues for many/all infectious agents are more likely to be successful. (Safdar & Maki, 2002; Lai et al, 1997; Cartmill et al, 1992). There are also novel therapies for prevention and control of infection that will briefly be considered.

The prevention and control of *C. difficile infection* was addressed by a Department of Health / PHLS joint working group in 1994.

**Infection control measures**

The transmission of *C. difficile* can be patient-to-patient, via the contaminated hands of health care workers, or via environmental (including healthcare equipment) contamination.

**Hand washing:**

One of the key interventions shown to be effective in both general terms of preventing healthcare associated infection and specifically against the spread of *C. difficile* associated diarrhoea (CDAD) is hand washing (Jones & MacGowan, 1998). Much of the evidence has been observational, but there have been some higher quality studies that have demonstrated the
effectiveness of gloves (Johnson et al, 1990), of antiseptic soaps and of alcohol-based hand-rub solutions (Bettin et al, 1994; Lucet et al, 2002).

Despite being one of the simplest interventions, many observational studies have demonstrated the lack of compliance with hand washing measures – both in studies relating to C. difficile (Johnson et al, 1990) and in more generic infection control programmes (Pittet, 2001; Lucet et al, 2002; Salemi et al, 2002, Pittet et al, 2000). Factors identified as influencing compliance include access to hand washing materials, using cleaning agents that protect rather than irritate the skin, wearing gloves, ‘being too busy’ and ‘not thinking about it’ (Pittet, 2001).

Methods to overcome this poor compliance include multidisciplinary campaigns to improve access to hand washing materials (Pittet, 2001), instigating hospital–wide educational programmes using posters, educational meetings and performance feedback linked with improving access to hand washing materials [including providing bedside and pocket-size bottles of hand-rub (Pittet et al, 2000)]. Work specifically with physicians (reported to have lowest levels of compliance with hand hygiene measures) indicated that the most effective way of improving compliance was personal discussion with an Infectious Diseases physician, and that educational sessions were more effective than sending e-mail reminders of good practice (Salemi et al, 2002).

**Environmental hygiene:**

Contamination of healthcare workers’ hands can lead to and result from contamination of the environment. It has been demonstrated that the level of healthcare worker hand contamination is proportional to the level of environmental contamination (Samore et al, 1996; Fawley & Wilcox, 2001), although demonstrating the cause and effect of this relationship is difficult.

Environmental contamination with C. difficile spores, often widely dispersed, has been demonstrated in 34-58% of sites in hospital wards (Fawley &
Wilcox, 2001; Samore et al, 1996), including after detergent based cleaning (Verity et al, 2001). Commodes, bed frames, sluice rooms and toilet floors were the most frequently contaminated sites and the floor areas showed heaviest contamination. It is noted that CDAD has emerged as a major problem at a time when there is a general perception that standards of cleaning in hospitals have been declining. Carpets in hospital wards have also been found to be heavily contaminated (Skoutelis et al, 1994), although the link between this contamination and levels of pseudomembranous colitis (PMC) are not clear. However, during outbreaks there is strong evidence of exogenous acquisition of infection, and as such the contaminated carpet may act as a reservoir for infection. Other sources of environmental contamination include nurses’ uniforms (Perry et al, 2001), blood pressure cuffs (Manian et al, 1996), and thermometers (Brooks et al, 1998; Jernigan et al, 1998).

Another procedure that has been associated with increased risk of C. difficile infection is tube feeding. Bliss et al identified tube-feeding, particularly post-pyloric feeding, as an independent risk factor for C. difficile acquisition (20% tube-fed vs. 9% non-tube fed) and CDAD (9% vs. 2%) with an odds ratio of 3.1 (95% CI 1.1-8.7) (Bliss et al, 1998). They suggest that handling of equipment by contaminated healthcare workers may be one explanation for this (other explanations being contaminated formula, or that tube feeding renders the intestinal environment more conducive to growth of C. difficile). However, Itou et al felt that the elemental diet formula used, rich in amino acids and short-chain fatty acids, provided a good medium for C. difficile growth as cleaning equipment with chlorine-based solution had little effect on rates of C. difficile infection (Itou et al, 2000).

In view of these levels of environmental contamination, appropriate and adequate cleaning of the hospital environment is an important part of any infection control measure. Studies have investigated the efficacy of various hospital cleaning solutions. Mayfield et al compared the effectiveness of a quaternary ammonium solution with a hypochlorite solution in three hospital areas: a bone marrow transplant (BMT) unit, an ITU and a general medical ward (Mayfield et al, 2000). They reported a reduction in CDAD rates in the
BMT unit after use of the hypochlorite solution compared with the normal ammonium solution – although no reduction on the other wards studied. When the use of ammonium solution was restarted, rates of CDAD rose suggesting that hypochlorite solution is effective in reducing risk of infection in high-risk clinical areas. However, environmental *C. difficile* prevalence was not measured, and antibiotic use altered during the study period. Wilcox & Fawley report that in fact some non-chlorine based hospital cleaning agents may lead to an increase in sporulation, and hence the incorrect use of environmental cleaning agents may in fact increase the persistence of organisms and lead to increased risk of infection (Wilcox & Fawley, 2000).

**Outbreak control:**

Infection control is especially important in the control of transmission in the outbreak situation. For optimal control of an outbreak there must be an alerting mechanism in place, rapid and reliable diagnosis, together with restriction of movement of patients, cohorting of patients and staff where necessary and reinforcement of all generic infection control measures (Rao, 1995; Cartmill et al, 1994; Peterson & Kelly, 1993). Linking surveillance of sporadic cases of *C. difficile* infection with infection control measures has been shown to reduce the incidence of nosocomial infection by up to 70% (Struelans et al, 1991) and allows early treatment to be instigated, thus reducing the burden of disease.

The joint DH/PHLS working group strongly recommended the isolation of patients with suspected *C. difficile* infection, and where isolation facilities were not available, patients should be cohort ed (DH/PHLS, 1994).

One further approach suggested to reduce transmission of infection has been to treat asymptomatic carriers in an attempt to reduce the potential reservoir of infection. However, Johnson et al reported that asymptomatic carriage is usually transitory, that treating carriers with metronidazole was not effective,
and that treatment with vancomycin was only temporarily effective (Johnson et al, 1992). Thus treatment of asymptomatic carriers was not recommended.

**Antibiotic restriction**

**Cephalosporins:**

One of the most commonly implicated groups of antibiotics is the cephalosporins (Yip et al, 2001; Zadik & Moore, 1998; Job & Jacob, 1997). As well as being implicated in the development of CDAD, cephalosporins have been demonstrated to increase shedding (both asymptomatic and symptomatic) of *C. difficile* in healthy adults (Chachaty et al, 1993).

Older patients are known to be at increased risk of developing *C. difficile* infection, and the use of broad-spectrum antibiotics to cover all possible infections has been identified as a particular area of concern. Cefotaxime, for example, has been shown to increase the risk of CDAD in the elderly by up to seven-fold (Impallomeni et al, 1995). Increased bile / GI tract excretion, and hence more effect on GI flora, due to renal impairment (the normal major route of excretion) has been suggested as one mechanism by which elderly are more susceptible. Cefotaxime may also be associated with prolonged excretion of spores (Starr & Rogers, 1997).

Comparisons of restrictive antibiotic formularies (piperacillin or benzyl penicillin, trimethoprim and, where necessary, gentamicin) with cefotaxime have demonstrated increased incidence of *C. difficile* colonisation and of CDAD in patients treated with cefotaxime (Settle et al, 1998), and reduction in rates of CDAD (McNulty et al, 1997). Ludlam et al describe similar results (*C. difficile* infection had dropped by 50%) when implementing a formulary recommending penicillin and ciprofloxacin for suspected infection with overall costs relating to CDAD reduced (Ludlam et al, 1999). Prophylactic use of cephalosporins in surgical patients has also been implicated in CDAD (Crabtree et al, 1999).
Most studies of antibiotic use have been in hospital in-patients, but Levy et al studied the use of oral antibiotics in ambulatory care patients in a retrospective study of CDAD over a 2-year period. Increased use of cephealexin and cefixime were associated with CDAD. The authors comment that although the rates of disease were lower than for in-patients it is a problem that ought to be identified, and suggest that in fact it may be under-reported in the ambulatory-care / out-patient setting (Levy et al, 2000).

**Clindamycin:**

Clindamycin is active against Gram-positive cocci, however its use is limited by its link with the development of CDAD (British National Formulary, 2002). There have been several reports of outbreaks associated with clindamycin-resistant strains of *C. difficile* where restricted of the use of clindamycin has been associated with a reduction in cases of CDAD (Climo et al, 1998; Pear et al, 1994; Johnson et al, 1999).

There have also been case reports of CDAD associated with short courses of both oral and topical clindamycin (Boaz, 2000; Vikenes, 1999).

**Other antibiotics:**

Concerns have been raised about the links between clarithromycin (Guyot et al, 2000), penicillins and macrolides (Shek et al, 2000) and *C. difficile* infection, although the methodology of some of these studies has been questioned (Wilcox, 2001). Co-amoxiclav, popular for its broad spectrum and for ease of administration, has been strongly associated with CDAD (Bignardi, 1998).

There have also been case reports of CDAD associated with levofloxacin (a quinolone) in elderly patients (Ozawa & Valadez, 2002), and lincomycin associated with a paediatric outbreak of CDAD (Ferroni, 1997).
Prescribing behaviour and formularies:

There are numerous examples of restrictive antibiotic policies associated with reduction in rates of CDAD (Climo et al, 1998; Ludlam et al, 1999; Pear et al, 1994; McNulty, 1997). As with infection control measures, the key to success will be ensuring compliance with these guidelines. Despite the apparent increased awareness of CDAD and its link with antibiotic use, levels of infection are still rising and prescribing behaviour needs to be addressed (Gorbach, 1999). While prescriber education must be the corner-stone of these efforts, it is unlikely to be sufficient on its own. Ward pharmacists play an important role in monitoring and advising on antibiotic use, and initiatives such as automatic stop dates on antibiotic prescriptions are valuable. Many hospitals are moving towards electronic prescribing, which provides opportunities not only to monitor prescribing pattern in real time, but also to link prescriptions to specific prescribing advice.

Antibiotic guidelines need to be clear and simple (Freeman & Wilcox, 2001) with clear distinction between classes of antibiotics, and not using broad inaccurate ‘catch all’ descriptions of classes of drugs, and should also include an awareness of the risk of CDAD following the use of short courses of antibiotics, including for surgical prophylaxis.

There are many different formularies and prescribing guidelines for many different conditions, and it is important to have an overall view of prescribing. A particularly pertinent example of this is the British Thoracic Society (BTS) guidelines for treatment of community-acquired pneumonia, which recommend amoxicillin, or clarithromycin for mild to moderate disease, and co-amoxiclav or cefuroxime or cefotaxime or ceftriaxone plus erythromycin or clarithromycin for severe infection (British Thoracic Society, 2001). Several studies into CDAD found prior treatment of respiratory infection to be a risk factor for developing CDAD, and this may be linked to use of antibiotics as described above. Wilcox (Wilcox & Fawley, 2000) calls for a need for an overall perspective on antibiotic prescribing guidelines, with adequate audit
and validation of effectiveness together with review of the impact of the guidelines.

**Novel therapies**

Several novel therapies either to prevent *C. difficile* infection or to limit the extent of disease have been proposed or are in development.

**Probiotics:**

Probiotics – live organisms that improve the microbiological balance of the host – have been proposed as one way of preventing CDAD. Studies of the use of the yeast (*Saccharomyces boulardii*) have indicated an inhibitory effect on *C. difficile* toxins in human colonic mucosa (Castagliuolo et al, 1999), lower relative risks of recurrence of CDAD (McFarland et al, 1994) and decreased duration of diarrhoea in critically ill patients (Bleichner et al, 1997). A recent meta-analysis of the use of probiotics in the prevention of antibiotic associated diarrhoea, including *Saccharomyces boulardii*, lactobacilli and a strain of enterococcus producing lactic acid suggested a protective effect (D'Souza et al, 2002). However, it commented that the efficacy of probiotics in treating antibiotic associated diarrhoea was yet to be proved. The proposal of using live organisms in the prevention of a condition that predominantly effects the elderly and immunocompromised has also been criticised (Beckley & Lewis, 2002; Wilcox, 2002), the authors arguing that the focus should be directed on those measures known to work which are not widely implemented.

**Vaccines:**

Vaccines are being explored as an alternative means of protecting high-risk individuals against *C. difficile* infection. The aim of vaccines is to produce neutralising antibodies to toxins A and B, and hence prevent symptomatic infection. The C-terminal repeat domain of *C. difficile* toxin A has toxin-
neutralising epitopes and has been used in development of vaccines. Ward et al demonstrated significant anti-toxin A serum responses in animal models using intra-nasal, intra-gastric and subcutaneous boosters (Ward et al, 1999). Torres et al found similar results in hamsters following intranasal, intraperitoneal and subcutaneous immunisation, with intranasal immunisation plus an intraperitoneal booster leading to greatest protection against diarrhoea and death (Torres et al, 1995).

Kotloff et al studied the safety and immunogenicity of C. difficile toxoid A & B vaccine (intra-muscular injection) in healthy adult humans, and found it to be both safe and immunogenic, leading to a vigorous serum antibody response to both toxins in over 90% subjects (Kotloff et al, 2001).

**Antibodies / immunoglobulins:**

Animal studies have investigated the role of antibodies against toxin A and B in the protection against C. difficile disease. Kink & Williams used avian antibodies against toxin A & B in a hamster model and found that antibodies to both toxins were required to prevent morbidity and mortality from C. difficile infection, but that this combination also prevented against relapse and re-infection, in contrast to standard vancomycin treatment (Kink & Williams, 1998). Kelly and colleagues measured serial antibody levels in hospitalised patients who were receiving antibiotics (Kyne et al, 2000). After colonisation by C. difficile, those who became asymptomatic carriers had significantly greater increases in serum IgG anti-toxin A than did patients who had C. difficile diarrhoea (P<0.001). In a separate study significantly higher levels of both serum IgM and IgG anti-toxin A were measured in patients who had one as opposed to multiple episodes of C. difficile diarrhoea (Kyne et al, 2001).

Salcedo et al report the use of intravenous immunoglobulin in two cases of pseudomembranous colitis not responding to standard anti-microbial therapy (metronidazole and vancomycin) (Salcedo et al, 1997). The patients were treated with pooled human immunoglobulin and both had rapid clinical
responses suggesting anti-C. difficile activity was present. The authors suggest that in elderly patients, lower levels of anti-toxin in their serum may contribute to more severe or prolonged disease, and that passive immunotherapy with pooled human IgG may be an effective treatment.

Comment on evidence for interventions

The quality of evidence for the various interventions described is variable. There have been some randomised trials of interventions, but more often are of observational studies. Furthermore, many of the interventions are introduced as measures to curtail outbreaks, and are not tested in routine or non-outbreak settings: it could be questioned whether chance fluctuations in the level of disease may have meant that the reduction in levels of infection attributed to the interventions may have occurred anyway. There are also possible biases in what is reported about outbreaks in terms of perceived success or otherwise in infection control.

Regarding the use of antibiotics and their association with CDAD, questions have also been raised about whether the antibiotics identified in studies are merely those in common use – and any antibiotic in common use might be found to have similar association.

Consideration should also be given to the consequences of restricting the use of antibiotics on the levels of other infections. Just as we have stressed how guidelines on the management of clinical conditions should consider the risk of CDAD, we should be aware of the implications of our recommendations.
Conclusions

- Robust infection control practices are of crucial importance in reducing CDAD and have the additional benefit of being effective against other nosocomial infections.
- Good hand hygiene by health care workers – using antiseptic soap or alcohol-based hand-rub – is essential.
- Environmental cleaning – of floors, furniture and equipment is essential. In cases of outbreaks, disposable equipment should be used where possible to reduce the spread of infection via contaminated surfaces and hands.
- In outbreak situations, patients should be isolated or cohort nursed.
- Reducing inappropriate use of broad-spectrum antibiotics / using more narrow spectrum drugs can reduce risk of CDAD.
### Table 1: Evidence for interventions in *C. difficile* infection: infection control

<table>
<thead>
<tr>
<th>Paper</th>
<th>Study design</th>
<th>Setting / no of patients</th>
<th>Intervention</th>
<th>Comments</th>
<th>Outbreak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson et al, 1990</td>
<td>Prospective controlled trial</td>
<td>4 wards – 2 with intervention 2 without</td>
<td>Use of <strong>vinyl gloves</strong> for all body substance contact</td>
<td>Decrease in incidence of <em>C. difficile</em> diarrhoea from 7.7/1000 pt discharges to 1.5/1000 pt discharges.</td>
<td>R</td>
</tr>
<tr>
<td>Betin et al, 1994</td>
<td>Crossover study</td>
<td>10 volunteers</td>
<td><strong>Liquid soap vs. chlorhexidine</strong> for hand washing; bare hands and gloved hands</td>
<td>Bare hands – soap and chlorhexidine did not differ in residual bacterial counts on fingers/thumbs. Gloved hands – soap more effective than chlorhexidine on fingers and palms, but not on fingertips. Residual counts lower on gloved hands than bare hands.</td>
<td>R</td>
</tr>
<tr>
<td>Lucet et al, 2002</td>
<td>Randomised clinical study</td>
<td>43 H/care workers</td>
<td>Compared <strong>hand washing</strong> with non-antiseptic soap, hand washing with antiseptic soap and alcohol-based hand rubs</td>
<td>Both antiseptic soap and alcohol-based hand rub showed significantly better reduction in fingertip contamination than non-antiseptic soap. 4% (11/256) of pre-hand hygiene specimens had pathogenic bacteria, of which 2/11 remained positive after hand hygiene (both with non-antiseptic soap).</td>
<td>R</td>
</tr>
<tr>
<td>Study: Pittet et al, 2000</td>
<td>Type: Observational study</td>
<td>Setting: Large acute-care teaching hospital</td>
<td>Intervention: Hand hygiene Promotion programme – visual poster display performance feedback, individual bottles of hand rub solution to wards and h/c workers, bedside hand rub holders</td>
<td>Results: Compliance improved over three years from 47% - 66%. Frequency of hand washing remained stable, whilst hand disinfection with alcohol-based hand rub increased. Rates of nosocomial infection decreased from 16.9% in 1994 to 9.9% in 1998. MRSA transmission rates decreased from 2.16 to 0.93 / 10 000 pt-days. Provision of bedside alcohol-based antiseptic hand rubs largely contributed to improved compliance.</td>
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<tr>
<td>Study: Salemi et al, 2002</td>
<td>Type: Observational study</td>
<td>Setting: 450-bed hospital</td>
<td>Intervention: Programme to increase physician compliance with hand washing</td>
<td>Results: Physician compliance with hand washing ranged from 19-85%. Personal meeting with ID consultant and videotaped presentations had greatest impact on physician compliance, e-mailed newsletter had least impact.</td>
<td>R</td>
</tr>
<tr>
<td>Study: Fawley &amp; Wilcox, 2001</td>
<td>Type: Observational study</td>
<td>Setting: 2 Elderly medicine hospital wards</td>
<td>Intervention: Monitoring of environmental contamination and rates of CDAD</td>
<td>Results: C. difficile not recoverable from either ward at time of opening, but was found within 1-3 weeks, with level of contamination rising markedly during first six months of study period. C. difficile infection correlated with level of environmental contamination on one ward but not on the other. Difficult to determine whether infected patients or contaminated environment</td>
<td>R</td>
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</tbody>
</table>
was primary source of infection. In general, routine cleaning with detergent is unsuccessful at removing *C. difficile* from the environment.

<table>
<thead>
<tr>
<th>Wilcox &amp; Fawley, 2000</th>
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<tbody>
<tr>
<td>Compared sporulation levels of three strains of <em>C. difficile</em> in sub-inhibitory concentrations of hospital cleaning agents.</td>
</tr>
<tr>
<td>In faecal emulsion, strain p24 produced greater % sporulation than non-epidemic or environmental strains. All three strains showed greater sporulation in sub-inhibitory concentrations of cleaning agents than in faecal emulsion alone. Strain p24 (UK epidemic strain) showed significantly greater sporulation than other strains when exposed to non-chlorine based solutions. Choice of cleaning agent may have substantial effect of spore persistence in hospital.</td>
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</table>

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<th>Mayfield et al, 2000</th>
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<tr>
<td>Before and after intervention study</td>
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<tr>
<td>Bone marrow transplant unit (293 pts), neuro-ITU (1278 pts) &amp; general medical unit (2881 pts).</td>
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<td><strong>Environmental cleaning</strong> with quaternary ammonium solution vs. hypochlorite solution</td>
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<tr>
<td>Incidence of CDAD in bone marrow transplant patients decreased from 8.6 to 3.3/1000 patient-days after switching to hypochlorite solution. The rate increased back to 8.1/1000 pt-days when quaternary solution was re-introduced. No reduction in rates in ITU or general medical units.</td>
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<tr>
<td>Study Authors</td>
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<tr>
<td>Verity et al, 2001</td>
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<tr>
<td>Samore et al, 1996</td>
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<td>Zafar et al, 1998</td>
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<tr>
<td>Study Title</td>
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<td>Manian et al, 1996</td>
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<td>Perry, 2001</td>
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<td>Jernigan et al, 1998</td>
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<td>Brooks et al, 1998</td>
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<td>Boone et al, 1998</td>
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</table>
32 patients isolated on admission – 7 tested positive (used 86 isolation days). 25 tested negative or were discharged without result (107 isolation days). Policy required interdisciplinary co-operation between departments. Recommend outpatient clearance testing for patients with one episode of CDAD who are known to require readmission.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Type</th>
<th>Program Type</th>
<th>Program Description</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Struelens et al, 1991</td>
<td>Observational study</td>
<td>Hospital-wide program</td>
<td>Introduction of hospital-wide CDAD surveillance and control program – initially screening of in-patient stools, enteric isolation precautions, cohorting and terminal room disinfection</td>
<td>Despite intervention quarterly incidence of CDAD remained unchanged (1.5/1000 admissions) and six new clusters occurred. <em>C. difficile</em> spores recovered from 36.7% of surfaces in case rooms vs. 6.7% in control rooms. Further control measures introduced – culture screening of stools, early therapy, enteric precautions and daily room disinfection for each sporadic case. Surface contamination reduced x4; no further clusters in 12/12, incidence of NCDD fell to 0.3/1000 admissions.</td>
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</table>
Table 2: Effective interventions in *C. difficile*: antibiotic restriction

<table>
<thead>
<tr>
<th>Paper</th>
<th>Study design</th>
<th>Setting / no of patients</th>
<th>Intervention</th>
<th>Comments</th>
<th>Outbreak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chacaty et al, 1993</td>
<td>Clinical trial</td>
<td>51 healthy volunteers</td>
<td>Monitoring effect of 8 day course of <strong>cefixime</strong> on shedding of <em>C. difficile</em></td>
<td>Proportion of subjects shedding <em>C. difficile</em> rose from 6% to 57%. 25% subjects were symptomatic</td>
<td>R</td>
</tr>
<tr>
<td>Levy et al, 2000</td>
<td>Retrospective cohort study</td>
<td>Ambulatory care – prevalence of CDD</td>
<td>Identify risks associated with commonly used oral antibiotics</td>
<td>CDAD present in ambulatory setting, but at lower levels than hospitalised patients. Low levels of testing mean that this may be underestimated in ambulatory setting. <strong>Cephalexin</strong> and <strong>Cefixime</strong> associated with CDAD</td>
<td>R</td>
</tr>
<tr>
<td>Ludlam et al, 1999</td>
<td>Before &amp; After observational study</td>
<td>4 geriatric wards at 900-bed teaching hospital</td>
<td>Restriction of antibiotic prescribing – third generation <strong>cephalosporins</strong></td>
<td>2157 pts admitted before; 2037 admitted after. Non-Geriatric wards acted as controls – no alteration to antibiotic policy made. On geriatric wards – 98 cases CDAD before; 45 cases after. Other (control) wards – 213 cases before; 253 cases after. Costs of injectable antibiotics increase but may be offset against ↓ morbidity, mortality and bed occupancy</td>
<td>R</td>
</tr>
<tr>
<td>Settle et al, 1998</td>
<td>Prospective cross-over</td>
<td>2 care of the elderly wards –</td>
<td>Compare rates of colonisation and diarrhoea</td>
<td>Highly significantly increased incidence of colonisation and diarrhoea in patients who</td>
<td>R</td>
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<tr>
<td>Study</td>
<td>Design</td>
<td>Setting</td>
<td>Relevant Information</td>
<td>Outcome</td>
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<tr>
<td>Yip et al, 2001</td>
<td>Case control study</td>
<td>300 bed hospital. 3/12 study period</td>
<td>Identifiable risk factors for nosocomial CD infection</td>
<td>Cephalosporins and Ciprofloxacin use identified as risk factors for nosocomial CDAD.</td>
<td></td>
</tr>
<tr>
<td>Zadik &amp; Moore, 1998</td>
<td>Case-note review</td>
<td>Renal, medical and geriatric wards</td>
<td>Antimicrobial associations with CDAD</td>
<td>Drugs associated with CDAD were cefotaxime (RR=16.9), ceftriaxone (RR=8.6), cefuroxime (RR=5.3) and ceftazadime (RR=4.8).</td>
<td></td>
</tr>
<tr>
<td>Crabtree et al, 2000</td>
<td>Case series</td>
<td>Cases of CDAD in surgical inpatients</td>
<td>Risk factors associated with CDAD</td>
<td>Ciprofloxacin and Cefoxitin most commonly prescribed antibiotics prior to developing CDAD. 16% of cases developed CDAD after receiving prophylactic antibiotics only.</td>
<td></td>
</tr>
<tr>
<td>Impallomeni et al, 1995</td>
<td>Case-note review</td>
<td>46 bed geriatric unit – case notes reviewed for 18/12 period.</td>
<td>Link between Cefotaxime prescribing and C. difficile diarrhoea</td>
<td>After introduction of BTS guidelines use of Cefotaxime increased 20-fold. 43/1037 pts developed CD diarrhoea after antibiotic treatment. Monthly incidence of CDAD strongly related to monthly expenditure on Cefotaxime. Cefotaxime had highest RR for developing CDAD (RR = 7.2 (95%CI 3.9-13.2).</td>
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R (Following change to BTS Comm-acqd pneumonia guidelines)
<table>
<thead>
<tr>
<th>Study</th>
<th>Study Type</th>
<th>Participants</th>
<th>Characteristics</th>
<th>Findings</th>
</tr>
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<tbody>
<tr>
<td>Starr et al, 1997</td>
<td>Observational study</td>
<td>Outbreak associated with Cefotaxime</td>
<td></td>
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</tr>
<tr>
<td>Guyot et al, 2000</td>
<td>Case control study</td>
<td>20 Elderly care wards – 64 pts with CDAD + 64 controls (subjects with non-CD diarrhoea)</td>
<td>Antibiotic use in elderly</td>
<td>Clarithromycin given one month prior to diarrhoea identified as single risk factor for CDAD – risk increased in those treated for respiratory tract infection. (NB controls not matched for location / specific age / duration of admission prior to diarrhoea)</td>
</tr>
<tr>
<td>Climo et al, 1998</td>
<td>Observational cohort study</td>
<td>Hospitalised patients with symptomatic diarrhoea</td>
<td>Restriction of use of Clindamycin</td>
<td>Use of Clindamycin required approval by infectious disease consultant. Led to an overall reduction in clindamycin use and a sustained reduction in CDAD (from 11.5 cases / month to 3.3/mth). Parallel increase in use of other antibiotics – although overall cost saving due to reduced CDAD</td>
</tr>
<tr>
<td>Johnson et al. 1999</td>
<td>Case-control study</td>
<td>3 large hospitals that had had large outbreaks of CDAD between 1989-92.</td>
<td>Association between clindamycin and CDAD due to epidemic strain</td>
<td>Cases = pts with CDAD with epidemic strain. Controls = pts with CDAD with non-epidemic strain. Overall, 35/83 cases associated with epidemic strain had used clindamycin vs. 12/99 controls with non-epidemic strain. Pooled OR for association between clindamycin and diarrhoea due to epidemic strain = 4.35 (95%CI 2.02-9.38).</td>
</tr>
<tr>
<td>Authors, Year</td>
<td>Study Type</td>
<td>Setting</td>
<td>Methodology</td>
<td>Outcomes</td>
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<tr>
<td>Pear et al, 1994</td>
<td>Case-control study then Observational study – before and after</td>
<td>Large university-affiliated veterans affairs medical centre</td>
<td>Control of outbreak by restricting clindamycin use</td>
<td>Case-control study (cases= cytotoxin+ve; control = non-infected diarrhoea pts) identified association with clindamycin use. Clindamycin use restricted – within 3/12 incidence of CDAD dropped from 7.7 infections/ month to 1.9 / month.</td>
</tr>
<tr>
<td>Boaz et al, 2000</td>
<td>Case report</td>
<td>1 female patient</td>
<td>Use of oral clindamycin</td>
<td>Oral clindamycin prescribed for dental infection. Developed severe CDAD 10/7 after antibiotics.</td>
</tr>
<tr>
<td>Vikenes et al, 1999</td>
<td>Case report</td>
<td>32 yr old woman</td>
<td>Clindamycin topical cream</td>
<td>Development of CDAD following administration of topical clindamycin</td>
</tr>
<tr>
<td>Ferroni et al, 1997</td>
<td>Retrospective cohort study</td>
<td>37 patients on paediatric ward.</td>
<td>Risk factors for CDAD</td>
<td>Multivariate analysis identified lincomycin for &gt;3/7 as only significant risk factor</td>
</tr>
<tr>
<td>McNulty et al, 1997</td>
<td>Observational study – before and after</td>
<td>Elderly Care unit 252 pts before / 234 pts after restriction</td>
<td>Control of CDAD through use of restrictive antibiotic policy</td>
<td>Restricted use of broad-spectrum antibiotics in attempt to control outbreak. Used benzyl penicillin, trimethoprim and IV gentamicin where required. <em>C. difficile</em> infection rates fell – 37 cases before, 16 cases after.</td>
</tr>
<tr>
<td>Ozawa &amp; Valadez, 2002</td>
<td>Case series</td>
<td>Nursing Home. 9 cases observed in 6/12 period</td>
<td>Use of antibiotics</td>
<td>Levofloxacin identified in 6 cases.</td>
</tr>
<tr>
<td>Yee et al, 1991</td>
<td>Observation study</td>
<td>Surgical dept with ^ rate of CDAD (50 patients)</td>
<td>Comment on the influence of prophylactic antibiotics</td>
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</table>
Appendix 5 – National survey of current practice

This questionnaire survey was undertaken in order to update our knowledge of *Clostridium difficile* diagnosis and reporting before the inclusion of *Clostridium difficile* as part of the national, systematic, alert organism, laboratory-reporting system. The survey focuses on two areas – the laboratory diagnosis of *Clostridium difficile* and the reporting of subsequent diagnoses.

In August 2002, questionnaires were sent out to 223 laboratories in England, Wales and Northern Ireland. The response rate was 93.3% (208/223 laboratories).

1. **Laboratory Diagnosis**

- 197 laboratories (94.7%) process faecal specimens
- Of those, 183 laboratories (92.9%) process specimens for *C. difficile* (culture, toxin detection, or both)

The following data refer to the 183 laboratories that perform *C. difficile* testing:

**Specimen Selection Criteria:**

- Three laboratories (1.6%) examine all submitted faecal specimens for *C. difficile* (culture, toxin or both)
- 180 laboratories (98.4%) examine only selected submitted faecal specimens for *C. difficile* (culture, toxin or both)
The following data refer to the 180 laboratories that apply selection criteria in the decision to test a specimen for *C. difficile*:

- 159 laboratories (88.3%) apply selection criteria to specimens from hospital in-patients
  - Of these, and amongst other criteria:
    - 66 (41.5%) use the patient’s age
    - 102 (64.1%) use stool consistency
    - 124 (78%) would always test on specific request

- 170 laboratories (94.4%) apply selection criteria to specimens from patients in the community
  - Of these, and amongst other criteria:
    - 30 (17.6%) use the patient’s age
    - 40 (23.5%) use stool consistency
    - 154 (90.6%) would always test on specific request

**Toxin Testing:**
- 179 laboratories (97.8%) undertake *C. difficile* toxin detection
  - Of these:
    - 42 (23.5%) use a commercial kit detecting **toxin A only**
    - 89 (49.7%) use a commercial kit detecting **toxins A and B**
    - 38 (21.2%) use a **cell cytotoxicity assay**
    - 6 (3.4%) use a cytotoxicity assay in conjunction with a commercial kit detecting toxin A only
    - 4 (2.2%) use a cytotoxicity assay in conjunction with a commercial kit detecting toxins A and B

**Culture:**
- 46 laboratories (25.1%) will undertake *C. difficile* culture
2. **Reporting** (derived from responses from all 208 laboratories):

- 141 laboratories (67.8%) report their *C. difficile* results via CDR/CoSurv
- Of these:
  - 133 (94.32%) report a **positive toxin** result
  - 4 (2.84%) report **either** a positive toxin or a positive culture
  - 4 (2.84%) require **both** a positive toxin and positive culture to report
  - 32 (22.7%) report only when the patient is **symptomatic**
  - 81 (57.4%) report **whether or not** the patient is symptomatic
  - 39 (27.7%) report **all** such episodes
  - 41 (29.1%) report **recurrences and re-infections**
  - 37 (26.2%) report **re-infections but not recurrences**

3. **Infection Control** (derived from responses from all 208 laboratories):

- 94 (45.2%) sites (laboratories or associated infection control teams) stated that an assessment was made as to whether cases were hospital acquired
- Of these:
  - 52 (55.3%) systematically **record** this information
  - 14 (14.9%) systematically **report** this information via CDR/CoSurv
- 126 sites (60.6%) routinely report CDAD outbreaks to the **CCDC**
- 72 sites (34.6%) routinely report CDAD outbreaks to **CDSC**
- Regarding contact screening in the event of an outbreak:
  - 8 (3.8%) would **always** advise this
  - 77 (37%) would **sometimes** advise this
  - 111 (53.4%) would **never** advise this

NB: where figures do not add up to the total this reflects data not supplied
### Appendix 6: Results of the draft report consultation exercise

**Person A**

The insistence to look for both toxin A and B will greatly inconvenience at very short notice (if surveillance to be introduced in April 2003) those laboratories which use automated technology (BioMerieux VIDAS EIA in our case) for which an EIA detecting both toxins is not available. We currently look only for toxin B as most C. diff are picked up this way with A non-B strains accounting for a small minority (<1%) of infections. I'm sure BioMerieux might have something to say about unfair trading practices if they were to lose custom because of surveillance requirements.

We are not insisting that toxin A & B are detected. We strongly suggest that a method should be used that detects toxin B given that in at least one UK center 10% of *C. difficile* isolates are toxin A –ve, toxin B +ve. Other centres may have a higher prevalence of such strains but as yet remain unaware of this.

**Person B**

All very laudable. I agree with the standards. I am getting someone to look at the workload increases and the cost increase of extra staff time and additional specimen testing for this lab. I think the metaphorical piece of string will snap soon. We can barely manage the existing workload here, let alone be asked to significantly increase our testing of the existing numbers of specimens. I am sorry to say this, but at first sight the group does not appear to have anyone from a DGH NHS type lab.? Not that we shouldn’t aim to improve standards. But could we possibly get some investment before we push existing staff further? As I said, the standards are evidence based and probably reasonable. The operational issue within this organisation is the staffing levels. The challenge is when the additional work requirement comes before the investment in staff numbers, repeatedly. The politics are that success is rewarded. I do not believe at this point in time that by failing to deliver the surveillance outcome, I will improve our chances of getting the staffing level we need.

It is for the DoH to decide whether to implement the group’s recommendations and indeed to pronounce on funding issues. Our calculations indicate relatively modest increased testing (primarily of some community derived faeces i.e. from those aged ≥ 65 years).
**Person C**

My understanding is that the rate of *C. difficile* associated diarrhoea is going to be logged for patients over 65. What denominator is going to be used? Is it the number of beds within the hospital, if so is it beds for those over 65, or number of bed days of patients who happen to be over 65? Or is the denominator the number of samples received by a lab or some other denominator, e.g. population served by lab. My reading is that all samples from patients over 65 will be tested whether from in-patients or the community. This makes getting the denominator correct very difficult. I don't know that most Trusts could tell you how many patient days they have for patients over 65. It would make a huge difference if you have a geriatric unit for instance.

The other query is not testing samples from patients without diarrhoea. In practice most labs put up whatever they are sent, regardless of whether the sample is solid/liquid etc. Once a sample is put up it will not be possible to pick out those that are from diarrhoeal samples and those that are not.

**Person D**

This all seems very reasonable. If we have to report certain NIs then at least we should try and test for them in a standardised manner. (Will we be able to indicate which cases were acquired in the community vs. HAI when reporting?)

**KHO3 data will be used for hospital-associated cases.** Regional Epidemiologists are discussing an appropriate indicator denominator for community-associated cases.

We know from the recent survey of *C. difficile* protocols that in fact many laboratories use specimen consistency as a determinant of whether or not to test. The group believes it is not appropriate to test non-diarrhoeal samples (i.e. formed faeces) for *C. difficile* toxin.

**CoSurv** reporting does not allow for community and hospital acquisition to be differentiated for faecal *C. difficile* reports. However, the source code of the specimen (e.g. a hospital ward or a GP practice) which is part of the CoSurv dataset could be used as a proxy for setting of acquisition.
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<th><strong>Person E</strong></th>
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<td>I thought the document was good. One or two details might be adjusted if felt necessary.</td>
<td>Agreed. Insert reference.</td>
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<tr>
<td>p.12: in the last paragraph, the statement (....toxin assays are positive in more than half of those with antibiotic-associated colitis, and in almost all of those with antibiotic-associated PMC.) might be attributed to (Bartlett, 1990) - BARTLET, J.G. (1990) Clostridium difficile: clinical considerations. Rev Infect Dis 12 Suppl 2: S243-51.</td>
<td>Agreed. Change text to ‘1-3%’ instead of 5%.</td>
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<tr>
<td>p.14 first paragraph, several of the ‘et al ‘ designations are omitted from references: (de Lalla, 1989; Schwaber, 2000; al-Eidan, 2000), (Bliss, 1998)</td>
<td>Authors will check this and correct as necessary.</td>
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<td>p.24 Two different spellings of Meridien are listed are they two different companies?</td>
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Agreed. Insert reference.

Agreed. Change text to ‘1-3%’ instead of 5%.

Typos to correct.

Authors will check this and correct as necessary.
**Person F**

1. cover you say this document does not ‘impose diagnostic standards’ - but goes on to do just that!

2.1 testing for both A&B toxins routinely will have a major impact on staffing here. At present we use VIDAS (biomerieux) who does not have a toxin B test. VIDAS is walkaway & convenient out of hours & at weekends. cytotoxicity assays- many labs have given up on these very useful tests. Would like to know who does them in NE region.

2.3 submission of specimens for culture to Regional Ref Lab. When would this start? Who pays? I presume this would be outbreaks only.

2.4 this relates to surveillance although the section is headed ‘Laboratory diagnosis of CDAD ‘ testing for toxin B is going to pick up a group of patients whose symptoms are not due to CD & this may be quite misleading & lead to inappropriate treatment. At present we limit further tests - for toxin B other toxins & other pathogen when symptoms are severe or persistent. In most elderly patients the cause is iatrogenic- often due to laxatives!

4. the 4 week rule is fine as a case definition but inappropriate as far as lab testing is concerned. Patients who have apparent resolution of symptoms should have specimens submitted for testing if symptoms recur within 4 weeks - a negative test at least may be relevant.

5.1 anticipated benefits -is there evidence that present protocols for CD tests has resulted in adverse outcomes?

A fact that should be highlighted is that toxin may be detected in the asymptomatic hence a positive test does not exclude an alternative diagnosis.

Implementing the 3 day rule is increasingly difficult - pts may go home over the weekend. may have food brought in. We cannot afford to miss a hospital acquired salmonella which is still a possibility.

---

**We do not impose diagnostic standards – we are recommending an approach to testing as opposed to an SOP.**

Can use EIAs or cytotoxicity test to detect toxin B – see answer for ‘Person A ‘.

These issues have yet to be determined by DoH/Regions.

We are going to carry out surveillance to inform on this important issue.

We are not saying do not retest before week 4. Surveillance data will only be collected for new positives after week 4 (simply to standardize the definition of a new episode).

No, but they are not standardised – this cannot be desirable.

This point will be acknowledged.

No comment.
Person G
No standard documentation of diarrhoea at clinical level is problematic (note does not seem to have been any nurse input on standards group)!
As likely to find C dif toxins in formed specimens from pts with history of diarrhoea. - ?easier for staff to collect the formed part of any specimen.

Need to develop quality assurance scheme

Culture not difficult and should be achievable at local level (many labs now reviewing 7 day service arrangements) - problem is defining outbreak given rapid patient movement and if not done immediately need to agree how store specimens to go back to at later date?

Don't currently look at all specimens from pts over 65 in community - ? pilot and pool results for limited period.

Present info we pass on to you does not reflect clinical illness only pos toxin A and B result. Will be piloting use of standard comment asking recipients of reports to contact Control of Infection Nurse or medical microbiologist prior to commencing treatment in order to improve local information.

We are going to carry out surveillance to inform on this important issue.

Not sure what this comment means.

Survey findings suggest the opposite is true.

The group stands by its recommendations. These could of course be altered in the future.

We are going to carry out surveillance to inform on this important issue.

Person H
Appendix 4
page 3, first paragraph - is it worth mentioning poor standards of daily cleaning

page 3, second paragraph - should glove wearing during manipulation of tube feeding and preparation of feeds in pharmacy be mentioned as a possible solutions to this 'problem area'

page 3, second paragraph, final sentence - could this discourage the use of hypochlorite, is it worth deleting the end of this sentence

Agreed.

While this may be useful, the review aims to describe studies of evidence-based practice.

As this was an important finding in this study, it would be inappropriate to omit this sentence.
Person I
This will undoubtedly increase our testing for C diff toxin so there will be a funding element. I assume that no money has been allocated centrally for this.

We may well end up testing all stools received from patients over 65 as sorting out the formed from the unformed could well be time consuming. In addition stools are not normally received from patients who are asymptomatic, most will have had diarrhoea recently and many formed stools have ‘diarrhoea last 5/7’ or similar for clinical information. Not sure that the rationale of only testing unformed stools from the over 65s stands up clinically or even perhaps medicolegally.

| It is for the DoH to decide whether to implement the group’s recommendations and indeed to pronounce on funding issues. Our calculations indicate relatively modest increased testing (primarily of some community derived faeces i.e. from those aged ≥ 65 years. |
| This is the group’s recommendation. Please see answer for ‘Person C’. |
**Person J**
This does seem to be a reasonable pragmatic approach to giving some consistency to reporting but there are two specific points other than the obvious issue around increasing workload (at a time when the promised new money is not getting through to those delivering NHS care)

1. Ascertainment will vary because of the differing referral practices, especially amongst GPs. This was recognised in *S. aureus* bacteraemia surveillance and although it has seen little use yet labs were required to give data on the numbers of samples cultured. It might be worthwhile looking at this for C diff (relatively easy data to collect) otherwise Trusts will be lambasted for high rates when it simply represents more samples submitted.

2. There is no agreement on the exact method for toxin detection (the PHLS SOP committee has started to look at this but it will be some time before a standard emerges because there has been little work on the variables (sample size, preparation, inoculum, neutralisation, etc,etc) I understand that work from Rod Warren's Group in the Midlands shows that these variables might have a significant effect on the result reported.

The above make it especially important that 'health warnings' are issued about simplistic interpretation of the figures as markers of infection control performance.

**Regional epidemiologists will consider this potentially important issue.**

The group was not tasked with defining an SOP. Please see ‘Person F,1’.

Agreed.

**Person K**
I thought I should just express formally my concerns about including community specimens in the specification. Given that I think the surveillance data will be attributed to trusts, as are the data for MRSA, I think we should only include hospital specimens in the specification.

Further rationale on the need to look for *C.difficile* in community-derived faces will be included in the recommendations.
Person L
Welcome standardised testing and case definitions; any National Surveillance must be standardised. Data produced in this way will force trusts to take notice.

Funding may be at risk as PHLS transfer to NHS & unless existing NHS labs are adequately funded. With devolution of budgets supporting clinical microbiology in ex PHLs to PCTs this work may be at risk re other 'local priorities'.

Few labs now do tissue culture; immunoassays are expensive so labs tend to be selective in those they test. Also some restrict testing for ‘Community Pathogens’ e.g. Salmonella in hospital in-patients.

I personally don’t agree with restricting test range on inaccurate clinical information but labs may be using this as a cost saving measure. Rigorous rejection of formed stools would help.

Of course hygiene and antibiotic policies are vital in controlling C. difficile, but outbreaks are very difficult to control in the face of the extremely high bed occupancy and turnover, which combine with waiting list targets to make effective isolation of infected patients impossible.

The general public has latched on to MRSA as a risk and potential source for litigation. They may well soon perceive C. difficile in the same way and the availability of National data is more likely to ensure trusts address the problem rather than deny that it is any thing to do with the way the hospital is managed.

These points have been addressed above.

We are not suggesting this should happen. We are recommending who should be surveyed (as opposed to tested) for evidence of C. difficile infection.

No comment.

Agreed.
**Person M**

We completed a survey of testing practices for C diff and had the same results as you, roughly. I would be very interested to see a copy of your final standards, ie document after consultation and would appreciate a copy.

Given the expertise on the group that drafted those it would be interesting to see whether we can implement the same standards in Scotland. This would be useful for UK wide comparability of data.

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**Person N**

I have two comments to make:

1) I am not convinced that examining specimens from community patients is valuable. I can see the argument that with early discharge more people will develop their symptoms after discharge, but unless that information is collected (difficult from the lab) then there is no means of assessing whether hospital or community acquired. Perhaps it is worth looking at nursing home specimens - we usually do have a patient address and so can easily achieve that.

2) My major concern is that only diarrhoeal specimens which take the shape of the container should be reported on. This introduces a degree of subjectivity which is undesirable. Most of our recent CDT+s were assessed in the lab as ‘semi-formed’ ie one could easily exclude them if one wished. It is very rare to receive a specimen from a patient who has not had a recent history of diarrhoea, but often the symptoms are improving a bit by the time a specimen is collected. My view is that all specimens from over 65s should be reported. The number who have not had symptoms will be very small and it will avoid any subjective assessment in the lab.

| Further rationale has been included to support this recommendation. |
| Please see answers above, esp. to ‘Person C – second point‘. |
**Person O**  
These are my initial thoughts after reading the first dozen pages:

1. Can one assume that the *C. difficile* Standards Group was established at the request of whatever body will be overseeing the compulsory CDAD surveillance scheme and will therefore accept its final recommendations?

2. We currently process all faeces sample from patients over 70 years for CDT so are used to dealing with an age-based selection protocol.

3. However, the idea that diarrhoeal and non-diarrhoeal specimens can be easily separated is not as straightforward as it sounds. Also, it may not be easy to convince clinicians that non-diarrhoeal specimens can be discarded untested (I have had this argument in the past).

4. On the subject of lab diagnosis, I am informed that a reliable supply of neutralising serum is not currently available and also that the most widely used commercial automated elisa (Vidas) detects only toxn A and that available combined toxin A & B elisas are manual.

5. Will all HPA labs be set up &/or willing to provide a CDT culture service for other labs?

6. If (as stated) that existing CDR lab reporting systems are used as the source data in the first instance, will the Regional Office be able or willing to refine the data in respect of duplicate reports, positives from under 65s etc?

7. The view from Oxford is that we are worried by the idea of testing all diarrhoeal stools from the elderly for the toxin because of the low specificity of toxin for disease. We know that many patients followed prospectively will have toxin but will not manifest disease. We know that many patients who have had a positive toxin result remain toxin positive for many weeks. Subsequent episodes of diarrhoea may be due to other causes. We therefore test only on request. This improves the specificity of a positive test by enriching the population tested for disease. Surveillance should be based on a clinical diagnoses followed by a request for an appropriate test. We should be making the laboratory data as close a surrogate for disease as we can. If we base surveillance on indiscriminate screening, when the clinicians, epidemiologists and members of the public ask what the data means we will have a hard time explaining, when all we are doing is surveying positive tests!

| **I do not understand this comment.** |  |
| **No comment.** |  |
| **Please see answer to ‘Person C – second point’.** |  |
| **Please see answers above.** |  |
| **This has yet to be determined.** |  |
| **Such sorting should be possible** |  |
| **Please see answer to ‘Person F, 2.4’** |  |
**Person P**

Specimens to be tested - Would prefer ‘all liquid specimens’ to ‘all those over 65 years’.

Toxin Detection Methodology - There is no current agreement on the exact methodology for toxin detection. Standards need to be set on this.

For some, practice is to test all abnormal faeces from adult in-patients, nursing home residents and when antibiotics are mentioned on the request form. To expand this to include all abnormal specimens from the over 65s would increase the testing by about one third. Are PCTs willing to pick up this cost?

The word ‘liquid’ would be preferred – rather than ‘specimen that takes the shape of the bottle’

Why >65 only what is the evidence?

| The group stands by its recommendations. |
| This was not in the group’s remit. |
| Please see answer to ‘Person B’. |
| Please see above answers/comments. |
| CDSC data on age distribution of C. difficile laboratory reports. We will refer to this in the report. |}

**Person Q**

My understanding is that these standards were considered necessary because of the new HCAI surveillance agenda. This means that C.diff, like MRSA rates will be attributed to trusts. It is therefore not a good idea to include data on community-acquired infection in the dataset. If there is a recommendation to test all diarrhoea specimens from the over 65s in the community there needs to be an agreement not to report them or separate them from the hospital-associated infections. I’m not sure that such a mechanism currently exists in labs when reporting or CDSC when receiving the reports. These guidelines, although essential for comparable surveillance data, will need financing in some labs.

| Please see answers above. |
| We cannot ignore Community Cases. This point is under further discussion. |
| Please see answer to ‘Person B’. |
| **Person R** | Rather a voluminous document but seems quite sensible to me. I am intrigued that they are concerned about toxin A neg B positive stools as I thought these were very rare. | See answer to ‘Person A ‘. |
| **Person S** | I think it is very comprehensive and well written. I think it would benefit from a decision-making tree/algorithm*. Also while the authors make reference in passing to this, I think the potential resource/time implications for those not currently following the proposed investigation pathway needs to be pointed out. All this enhanced surveillance will contribute to our knowledge and management of some of these difficult problems, but if we are to get good compliance, laboratories need to be resourced to investigate in the most appropriate manner, and to provide the data. | * Uncertain what is envisaged. |
| **Person T** | This is obviously a comprehensive report, and there is a great deal of useful information contained therein. The aims of the group in terms of attempting to standardise surveillance are also laudable. However, as they themselves acknowledge in the report, the net result from the laboratory perspective will be an increase in testing for C.diff. Although the benefits that may accrue might offset this, I am not so sure that this will be reflected in laboratory budgets. If new resources are not forthcoming something else will have to pay for this. | See above answers. Uncertain what is envisaged. |
| **Person U** | This is an interesting and comprehensive report, but it would be useful to have some bullet points at the front to say what they want diagnostic labs to do. At present this seems to be buried around pages 4-5. It would also help to have some explanation of the evidence grading system (range of I-VII) used in the tables. | The introduction will highlight the main points. A systematic review has not been performed, and as such we believe that use of such a grading system may be misleading. |
**Person V**

A front page with an index is required. There should an Executive Summary with key action points. These points should be linked to an evidence grading system. I would prefer the references to be in the Vancouver style.

Why was an age cut off 65 years chosen as opposed to 60 years for routine testing of stool samples. It would be helpful if the evidence was provided.

More data on why all community diarrhoea specimens in patients over 65 years should be tested for C. difficile toxin (CDT) should be given.

Similarly the case against the routine testing of all inpatients diarrhoea stool samples should be more detailed.

Will these standards be subject to audit and review? If so when will a follow up report be prepared? If laboratories increase the number of CDT tests performed, where will the monies come from.

An opportunity to highlight the value of ward-based pharmacists to monitor antibiotic policies has been missed. These staff are very valuable but in short supply due to pay. Similarly the value of automatic stop dates on prescription charts and electronic prescribing to identify inappropriate prescribing is not mentioned.

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<tr>
<td>In view of CDSC data. These data have now been included.</td>
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<td>This point will be addressed.</td>
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<tr>
<td>We do not state this. We are suggesting which infections should be surveilled as opposed to which patients should be tested.</td>
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<tr>
<td>These are issues for the DoH. Please see answers above, especially 'Person B'.</td>
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<td>These are not evidence-based options, but will be mentioned in the text.</td>
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**Person W**

Please note in particular, the importance of integrating the control of *C. difficile* within the overall Clinical Governance process, thus linking in with the 'Learning Organisation'. We welcome standardised testing and case definitions, associated with standardised National Surveillance. Data produced in this way will force trusts (acute and community) to take notice of the *C. difficile* problem.

We are concerned about the 'blanket' testing of all stool samples over 65 years of age and unclear as to the evidence for this.

We would prefer to test all 'liquid' faeces from adult in-patients, nursing home residents and when antibiotics are mentioned on the request form. To expand this to include all abnormal specimens from all those over 65 would increase the testing by about one third. Are PCTs willing to pick up this cost? There is also the issue of someone having to workout the age of the patient submitting a stool sample (assuming the date of birth is given).

We endorse the rigorous rejection of formed stools, whatever the age of the patient and the word 'liquid' would be greatly preferred to ‘specimen that takes the shape of the bottle’.

Few labs now do tissue culture and immunoassays are expensive.

There is no current agreement on the exact methodology for toxin detection. We would welcome standard setting on this.

Funding may become an issue as PHL laboratories transfer to the NHS, with budget devolution to PCTs and competing priorities. Adequate funding for current NHS laboratories is also an issue.

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<tr>
<td>No comment.</td>
<td>What is suggested instead?</td>
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Person W continued…
Whilst hygiene and antibiotic policies are vital in controlling C. difficile, outbreaks are very difficult to control in the face of the extremely high bed occupancy and turnover, which combined with management pressure on waiting list targets makes effective isolation of infected patients very difficult. Comprehensive cleaning practice is also a vital and under-rated area.
If we are to have any impact on seriously controlling Cl. difficile, we need to move the control process into the Clinical Governance arena, in other words, within the context of the Learning Organisation. When cases occur, they should be reported as clinical incidents, with an expectation that the relevant clinical team review their practice to minimise the chance of the problem occurring again. The team might, for example, find that all their patients had been on a cephalosporin. Review of their antibiotic policy, in conjunction with standard infection control procedures, is likely to improve the situation. There would however, need to be formal audit to test whether change in practice made a difference to clinical outcome/quality of patient care.

The general public has latched onto MRSA as a risk and potential source for litigation. They may soon perceive C. difficile in the same way and the availability of National data is more likely to ensure that Trusts address this problem within the context of Clinical Governance, rather than deny that it is any thing to do with the way the hospital is managed.
**Person X**
The main problem we have is associated with the need to perform C.diff culture. As one of the regional HPA labs, we will undoubtedly be asked to provide this service. In itself, this is not difficult, as we used to culture regularly & the procedures are not too difficult. However, since PHLS grouping, we have been forbidden to perform cultures in order to achieve cost savings & would need resourcing anew to be able to restart.

Secondly, I think the document should specify the circumstances where culturing would be indicated. The document leaves it very open-ended & we need to be able to plan a little more specifically if we are going to avoid excessive demands on this service, both regionally & nationally, as, it has already been identified, that there are widely differing practices around the country.

We suspect that C.diff is hugely under-tested & under-reported. Finally, I would be grateful if you could clarify for us whether or not there needs to be a difference in how many colonies are picked for sending to the national reference labs for typing. For routine surveillance of antibiotic susceptibility, one colony per specimen would seem fine. However, we have been advised, in times past, that several strains may be involved in outbreak situations, and, in these cases, we would pick several colonies from each positive culture in order to identify the predominant strains involved. This would have major implications for the reference labs, but would be a change from what we have been asked to do previously.

| This is an issue for the DoH/HPA. | This is an issue best determined by local circumstances/HPA. | This is an SOP issue which will be addressed elsewhere. |
The recommendations of the group were generally agreed; there was particular welcome given to the recommendation that *C difficile* testing should only be carried out on patients with diarrhoea, that there should be no routine retesting of positive patients and that new cases are defined as occurring not less than four weeks after previous case affecting the same patient; the approach of focusing on patients over the age of 65 was agreed and is the general criterion used where an age based policy is in place in this region. Some laboratories which test all ages do so because such a policy is easier to implement than a selective policy. Whilst this report and recommendations are carried out under the auspices of healthcare associated infection surveillance, the group had no problem with surveillance directed to patients over the age of 65, whether they were hospital or community patients.

This may not be a matter for the *C difficile* Standards Group but we were not clear whether CDR reporting guidelines would be amended to request reports only on patients over the age of 65 or whether the age filter should be applied at regional or national level.

The group were surprised that there was no mention of the cost implications between EIA and culture (we understand there to be a 15-fold difference in consumables).

Thank you.

This issue will be addressed by Regional Epidemiologists.

This point is unclear given that culture has **not** been recommended by the group for routine use.
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| The comments received raise a number of interesting, and frequently common, points.  
1.1 I have raised previously the risk of missing a substantial number of cases if testing is restricted to 65+. This is a difficult one, and I don't have a definitive answer. A solution is to test all samples where it is requested (other than age two years or less) and all those 65 and over with diarrhoea. | Please see above answers. The group has not recommended testing only those aged ≥65 years. |
| 1.2 Which testing method is used is an issue. However, all the evidence is that kits which detect both toxins are better than toxin A alone kits. This is more than simply an issue of missing a few A - B+ cases. | Please see above answers. |
| 1.3 Resource for 'extra' testing would be released from not testing stools from patients who do not have diarrhoea. | No comment. |
| 1.4 I was disturbed by the number of comments about toxin being commonly present in asymptomatic adults. You will get some where this represents stools tested soon after resolution of symptoms. But so what; we are not advocating analysis of formed stools. Also, if all appropriate specimens are tested it is highly likely that the patient would have had a lab diagnosis for C. difficile diarrhoea from analysis of a previous specimen. Andrew Stacey (response 15) at his point 7 states that ‘we know that many patients who have had a positive toxin result remain toxin positive for many weeks’. This is news to me, other than for patients who are not treated or where treatment fails and the patient remains symptomatic. | 25% of patients whose symptoms resolve following treatment remain toxin positive – the precise period of time is not well defined. |
| 1.5 Another common issue was that of community versus hospital. Not an easy one. The relative merits of the arguments depend on the question being posed. If, as you imply, the two groups can be identified, then the issue is to ensure that Trust hospital figures are corrected. It is of course the case that some community cases will represent hospital acquired cases detected post discharge either as a first episode, a relapse, and in a few rare cases I know of, discharged while symptomatic. However, no general system is perfect and addressing these issues should be the subject of special R&D studies. | Please see above answers. |
Person Z contd..

2.1 The term 'designated regional reference facility' is used. This should simply be 'designated regional facility'. Happy to explain if anyone feels strongly about this. Put simply, a lab that receives referred specimens is not necessarily a reference lab. Specimens can be referred for reasons of convenience, economics etc. Culture of C. difficile is certainly neither specialised or difficult.

2.2 At 5.2 of the report we need to give more emphasis on the increased sensitivity of A+ B+ detection kits. As written it looks like the only case for their use is detection of uncommon A- B+ strains/cases.

2.3 Appendix 1, introduction: The original reference for the term CDAD should be given.

2.4 Appendix 2, cell cytotoxicity assay: Church and Fazio 1985 are quoted as showing a correlation between toxin titre and disease severity. I haven't had time to re-read this paper, but I thought that overall in the literature that there was not a clear cut correlation. Happy to be told I'm wrong.

2.5 Appendix 2, Table 1: Under the assay column it should be stated for each kit which toxin(s) is detected.

2.6 Appendix 3, culture: A reference for the alcohol shock method should be given (Borriello SP, Honour P, 1981, J. Clin Pathol 34 : 1124-7). I can't send title on request. This is a general comment for appendix 3 ie give references. A more important comment is that the key point of this method has been missed. It was adopted as a simple, inexpensive method for routine labs to isolate C. difficile. Culture following alcohol shock was on conventional blood agar plates, with all manipulations performed on the open bench. It is true that culture onto selective agar increases the selectivity and simplifies the resultant flora further, but is not necessary.

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**Person AA**  
Standardisation in selection of cases for reporting is obviously essential. At present I would not have the clinical information required to permit my reporting cases according to the case definition given: resource to collect and collate.

Standardisation of selection of samples to test is again essential and again my laboratory does not test all diarrhoeic samples from over 65 yr olds: resource to test additional samples from the community.

Standardisation of laboratory testing is also essential and again my laboratory currently uses a toxin A only kit. Resource to change to a kit which tests both A&B toxin – equipment, BMS time in performing the test (little difference in the reagent price).

| These issues have largely been covered above. | The toxin A and B kit(s) are similar in cost to toxin A only kits. |
Appendix 7 – Membership of the National C. difficile Standards Group

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