NCCHTA

30 April 2009

1. PROJECT TITLE: A multicentre, randomised, placebo controlled trial of lactic acid bacteria and bifidobacteria in the prevention of antibiotic-associated diarrhoea (AAD) and *Clostridium difficile* diarrhoea (CDD) in patients aged 65 years and over admitted to hospital and receiving antibiotics (06/39/02)

2. HOW THE PROJECT HAS CHANGED SINCE THE OUTLINE PROPOSAL WAS FIRST SUBMITTED

The following changes to the proposal were made in response to review of the recently published literature and recommendations from the first DMEC meeting held on 22/09/2008:

- Reference to the probiotic bacteria has been modified to "… lactic acid bacteria *and bifidobacteria* …" to more accurately identify the organisms. The description "optimal" has been removed.

- The former Swansea NHS Trust has merged to form the Abertawe Bro Morgannwg (ABM) University NHS Trust, Trust Headquarters, One Talbot Gateway, Baglan Energy Park, Baglan, Port Talbot, SA12 7BR. Tel: (01656) 752752 Fax: (01639) 687675/687676.

- We have extended the exclusion criteria following the publication of the PROPATRIA trial.[1] This RCT evaluated a high dose (10^{10} live bacteria / day) multi-strain (4 lactobacilli and 2 bifidobacteria) probiotic preparation in a total of 296 adult patients with predicted severe acute pancreatitis. Trial interventions were given via naso-jejunal (NJ) tubes together with a fibre-enriched feed (Nutrison Multi Fibre; Nutricia).

  No differences between the two intervention groups was found for the primary outcome (a composite outcome of infectious complications). No probiotic infections were identified. However, mortality was significantly higher in the probiotic than placebo group (24/152 [16%] vs. 9/144 [6%] respectively). All patients who died had multi-organ failure; all 9 cases of bowel ischaemia occurred in the probiotic group and 8 of these patients died. A higher frequency of organ failure before or during the first day of administration of the interventions in the probiotic (13.2%) versus the placebo group (4.9%) may have contributed to the increased mortality.

  The authors and accompanying commentary[2] hypothesise that live bacteria may result in bowel ischaemia in patients with impaired splanchnic circulation. Also, there may be an interaction between the probiotic preparation and the NJ feed.

  These findings in this specific patient population need to be considered alongside the numerous studies that have not identified adverse effects of probiotics (as detailed in the "safety" section of the proposal). However, in light of these potentially concerning findings and to pursue a conservative approach, we suggest 3 modifications to the PLACIDE study:

- Extend exclusion criteria. Patients with the following conditions will be not be recruited to the trial:

  a. Acute pancreatitis (defined as abdominal pain with serum amylase or lipase concentration ≥3 times the institutional upper limit of normal)
  b. Jejunal tube in-situ and/or jejunal feeding (as documented in the clinical / nursing records)
  c. Likely impaired splanchnic perfusion: any past or current abnormality or disease affecting the mesenteric arteries (as documented in the clinical records)
  d. Severe illness requiring care in either a high dependency or intensive care unit (but not planned admission to these facilities for observation only – e.g. after cardiac surgery)

- Withdrawal criteria: patients who require care in either a high dependency or intensive care unit (but not planned admission to these facilities for observation only – e.g. after cardiac surgery) will be withdrawn from the study and the trial intervention discontinued. However, these participants would be included in the intention-to-treat analysis. Patients who develop acute pancreatitis will also be excluded

- The independent statisticians will undertake an unblinded, interim analysis for important safety outcomes including the first 500 participants with complete data and report to the DMEC. Outcomes will include all SUSARs and all serious adverse events (see below) these will be handled in accordance with the EU directive 2001/20/EC.

  We expect that these modifications will result in the exclusion and withdrawal of only a small number of participants. However, the interim analysis will also assess recruitment according to targets and, as detailed in the proposal, additional recruitment sites will be invited to join the study if needed.

  References

  1. Besselink MGH *et al.* Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008; 371:651-59.

  2. Sand J, Nordback I. Probiotics in severe acute pancreatitis. Lancet 2008; 371:634-5.

- The maximum follow-up period will be 3 months from the date of recruitment

- Elderly people may have difficulty in describing stool consistency. Use of the validated Bristol stool chart is likely to be helpful.

- Because of labeling requirements, rather than stick sachets, the investigational medical products will be presented in a labeled bottle, each bottle containing

21 vegetarian capsules of either the probiotic food supplement or the inert placebo.

- The lactic acid bacteria and bifidobacteria have now been deposited in the National Collection of Industrial, Food and Marine Bacteria (NCIMB) and the appropriate identification numbers added to the proposal

- The two outcomes used to generate the sample size have been identified as co-primary outcomes. Other endpoints have been listed as secondary outcomes.

- Safety monitoring. The DMEC discussed at length the most appropriate methods for safety monitoring. Definitions for SUSARs were identified and procedures for the reporting of SUSARs and review of all SAEs determined. Arrangements for an interim analysis to assess safety outcomes were also discussed.

- Participant unblinding. It was agreed that immediate participant unblinding was not necessary as this would not inform clinical management. Unblinding could be undertaken by Dr Duolao as and when necessary in respect of SUSARS and adverse events.

- Although expected soon, final MHRA approval for the study has not been granted. Therefore, no changes have been made to the project timetable at this stage.

3. PLANNED INVESTIGATION

- **Research objectives**

Primary objectives: to determine the effectiveness and cost-effectiveness of an formulation of lactic acid bacteria and bifidobacteria in preventing or ameliorating AAD and CDD in people aged 65 years and over who are representative of patients admitted to secondary care NHS facilities in the UK and are exposed to oral or intravenous antibiotics.

Secondary objectives: to assess the acceptability and adverse effects of the probiotic preparation and the effect of the intervention on quality of life.

- **Existing research**

AAD is diarrhoea occurring in association with antibiotic treatment without an alternative cause (Bartlett JG; 2002). It occurs typically 2-8 weeks after exposure to antibiotics. The frequency of AAD varies markedly between studies according to risk factors such as exposure to broad-spectrum antibiotics, nosocomial infections and host factors such as age, health status, gender (McFarland LV 1998; Bartlett 2002). The following table shows the frequency of AAD in the placebo group of probiotic intervention studies undertaken in adults.

Reference	**Participants**	**No. (%) diarrhoea in placebo group**	
Studies of lactic acid bacteria			
Thomas 2001	18-93 years	40/134	(29.9)
Armuzzi 2001	mean 40 ±2 yrs; Rx *H. pylori*	8/30	(26.7)
Cremonini 2002	18-61 years; Rx *H. pylori*	6/21	(28.6)
Gotz 1979	adults	6/43	(14.0)
Wunderlich 1989	adults	6/22	(27.3)
Orrhage 1994	adults; Rx clindamycin	7/10	(70.0)
Beniwal 2003	adults	23/97	(23.7)
Studies of S. boulardii			
Surawicz 1989	Adults	14/64	(21.9)
McFarland 1995	18-86 years; Rx β lactam	14/96	(14.6)
Lewis 1998	>65 years	5/36	(13.9)
Can 2006	25-50 years	7/80	(8.8)
Overall		**136/633 (21.5%)**	

The major mechanism whereby antibiotics result in diarrhoea is through disruption of the commensal gut flora. This results in changes in carbohydrate, short chain fatty acid and bile acid metabolism and impairs colonization resistance which allows the emergence of a variety of gut pathogens. Some antibiotics also increase gut motility and may have direct effects on the gut mucosa (Bartlett JG, 2002). Although AAD is usually of moderate severity and self-limiting, it is a considerable nuisance to patients, prolongs hospital stay and increases healthcare costs.

C. difficile is an anaerobic bacterium which produces heat- and drying-resistant spores that persist long-term in the environment and make environmental control difficult. Transmission is faecal-oral and in health facilities occurs through contact with colonized patients, contaminated fomites and the hands of health care staff. Acquisition during admission is common (4-21%) and occurs in both endemic and outbreak scenarios (Barbut 2001; Poutanen 2004; Berrington 2004). Since 2003, CDD has become more frequent and associated with more severe illness in North America and Europe attributable to the emergence of a new strain which may produce higher amounts of toxin (Warny 2005; Bartlett JG, 2006). Outbreaks in the UK attract adverse media attention (Independent Newspaper, August 25th, 2005; Hospital Doctor, September 21st, 2006).

Most people who acquire the organism remain asymptomatic. However symptomatic disease has been reported to occur in 3.4-8.4/1000 admissions and to account for 10% of cases of nosocomial diarrhoea (Poutanen 2004). Severity of illness ranges from mild diarrhoea with abdominal discomfort through to pseudomembranous colitis complicated by toxic megacolon that may require colectomy and result in high case fatality. Disease mechanisms of increased mucosal fluid secretion and inflammation are due to exotoxins and both toxins A and B result in disease. The infection usually responds to treatment with metronidazole or vancomycin but 20-25% cases go on to get recurrent disease. The cost to the health services of CDD has been estimated to be £4000/case.

Exposure to antibiotics is the major risk factor for CDD and is associated with >90% cases. CDD accounts for about 25% of cases of AAD and occurs more commonly in health care facilities than in the community. CDD can occur with any antibiotic but the risk is greater with broad spectrum antibiotics (e.g. cephalosporins and β-lactamase resistant penicillins), clindamycin, antibiotic combinations and long treatment courses. CDD may occur from the first day of starting treatment or within 6 weeks or more after treatment. Other well documented risk factors include extremes of age, severity of underlying illness, use of proton-pump inhibitors, gastro-intestinal surgery and naso-gastric catheters.

Probiotics are defined as live microbial organisms which, when administered in adequate numbers, are beneficial to health (Joint FAO/WHO Expert Consultation, 2001). Probiotics are food supplements and are classified by the Food Standards Agency as “generally regarded as safe”. In general, probiotics do not cause adverse effects and have been used in people with a wide variety of different illnesses including many studies in preterm infants and also people with HIV infection. However, lactic acid bacteria have been reported to cause septicaemia in immunocompromised patients and endocarditis in people with artificial heart valves (Hammerman 2006).

In research studies, many different probiotics with varying numbers of organisms and modes of administration have been tested. There is little scientific rationale for selecting a particular strain and dosage of organisms for specific health outcomes. However, a strategy that is likely to maximize gut colonization and, thereby, colonization resistance is to use a combination of different organisms with large viable numbers of each strain.

In view of the central role of colonization resistance in preventing AAD, several double-blind, randomized, placebo-controlled trials of probiotics in the prevention of AAD and CDD in adults have been undertaken. There have also been several systematic reviews and meta-analyses conducted recently.

Antibiotic-associated diarrhoea.

A systematic review assessed studies of *Lactobacillus GG* in the prevention of AAD (Hawrelak 2005) and meta-analyses pooled data from studies of probiotics in the prevention of AAD (McFarland 2006; Sazawal 2006; Cremonini 2002; D’Souza 2002) and of *S. boulardii* in the prevention of ADD (Szajewska 2005).

In a comprehensive meta-analysis, McFarland (2006) pooled data from 25 RCTs (total of 2,810 adults and children) and reported a reduced relative risk of AAD in participants receiving a probiotic (0.43; 95% CI 0.31 – 0.58). A wide range of probiotics were tested in these studies including single strains (including *S. boulardii)*, probiotic mixtures and probiotic and prebiotic mixtures. Dosages (number of organisms) varied markedly between studies. In sub-group analyses, factors associated with greater efficacy in preventing AAD were use of *S. boulardii* or *L. rhamnosus GG*, mixtures of probiotics and preparations with high numbers of organisms. Reported adverse events in these studies were mild but occurred with *S. boulardii* (constipation, increased thirst) and *L. rhamnosus* GG (bloating, gas). This meta-analysis included all of the studies included in reviews undertaken by other researchers.

Sazawal (2006) assessed probiotics in the prevention of acute diarrhoea. In 19 studies of AAD in adults and children which tested a variety of probiotics, the frequency of diarrhoea was reduced in the probiotic group by 0.52 (95% CI 0.35-0.65).

D-Souza (2002) included studies of *S. boulardii*, Lactic acid bacteria and a strain of enterococcus. Three trials used a probiotic combination and two were done in children. In the pooled analysis including data from 9 trials, the odds ratio (OR) in favor of the probiotic preparation over placebo in the occurrence of diarrhoea was 0.37 (95% CI 0.26 to 0.53). Importantly, the efficacy appeared to be similar for the bacterial (5 trials/384 participants; OR 0.34, 0.19 to 0.61) and yeast preparations (4 trials/830 participants; OR 0.39, 0.25 to 0.62).

Cremonini (2002) included trials in which either *Lactobacillus* or *Saccharomyces* spp. had been tested. They identified 7 randomized, placebo-controlled studies where participants had been followed-up for a minimum of 2 weeks. Overall, the relative risk of diarrhoea in the probiotic compared to the placebo group was 0.40 (95% CI 0.27 to 0.57).

Szajewska (2005) pooled data from 5 RCTs of *S. boulardii* (1076 participants including 269 children) and reported a reduction of diarrhoea in the probiotic group by 0.43 (95% CI 0.23-0.78). Although no adverse effects were reported in these studies, the authors noted reports of fungaemia occurring in people receiving *S. boulardii*.

C. difficile diarrhoea

Members of our research group (Plummer *et al*, 2004) assessed the effect of a combination of *L. acidophilus* and *B. bifidum* on CDD in a pilot study in elderly patients receiving antibiotics. Stools were cultured for *C. difficile* as well as tested for toxins A and B. Overall, 30/138 (22%) patients developed diarrhoea with 5/69 in the placebo group and 2/69 in the probiotic group testing positive for *C. difficile* toxin. In this small study, the main effect of the intervention appeared to be neutralisation of the toxin rather than prevention of colonization with *C. difficile*.

We are not aware of any other studies that have assessed probiotics in the prevention of CDD in adults. The meta-analysis by McFarland (2006) included 5 studies, in

addition to our study, but all of these were treatment trials of patients with established or recurrent CDD. Kotowska (2005) reported that *S. boulardii* reduced the risk of CDD in children by 0.3 (95% CI 0.1-0.14).

In summary, a variety of probiotics with different administration regimens appear to reduce the risk of AAD by around 50%. There is insufficient data to assess the effectiveness of probiotics in the prevention of CDD.

- **Research methods**

We will undertake a randomized, placebo-controlled, double blind trial in 5 secondary care hospitals in 2 NHS regions. All clinical, laboratory and research methods will be uniform across the centres involved in the study. To ensure that our participants are generally representative of older patients admitted to NHS hospitals throughout the UK, we will recruit from all wards admitting adult patients in Singleton and Morriston Hospitals (total 1450 beds), ABM University NHS Trust and all Medical and Care of the Elderly wards at the University Hospital of North Durham, Bishop Auckland General Hospital and Darlington Memorial Hospital, County Durham & Darlington Foundation Trust (CDDFT; 598 beds). We aim to recruit people with a wide range and severity of illnesses to ensure that our findings are directly applicable to the general hospital population. In 2005/6 (12 months), 26,692 people aged ≥65 years were admitted in Swansea and 21,676 in CDDFT 30-37% of patients received antibiotics.

Planned inclusion criteria:

- People aged ≥65 years admitted to hospital without diarrhoea and who have been exposed to one or more antibiotics within the last 7 days or are about to start antibiotic treatment

Planned exclusion criteria:

- People with known immunosuppressive disorder, prosthetic heart valve or active inflammatory bowel disease (the latter defined as requiring specific treatment in the past 12 months)
- Acute pancreatitis (defined as abdominal pain with serum amylase or lipase concentration ≥3 times the institutional upper limit of normal)
- Jejunal tube in-situ and/or jejunal feeding (as documented in the clinical / nursing records)
- Likely impaired splanchnic perfusion: any past or current abnormality or disease affecting the mesenteric arteries (as documented in the clinical records)
- Severe illness requiring care in either a high dependency or intensive care unit (but not planned admission to these facilities for observation only – e.g. after cardiac surgery)
- People with a previous history of adverse reactions to probiotics
- Informed consent not granted by patient or their carer(s)

Withdrawal criteria:

- patients who require care in either a high dependency or intensive care unit (but not planned admission to these facilities for observation only – e.g. after cardiac surgery)

- patients who develop acute pancreatitis (defined as abdominal pain with serum amylase or lipase concentration ≥3 times the institutional upper limit of normal)

  The trial intervention would be discontinued for participants who are unable tolerate it and compliance to that time recorded. Their data would be included in the analysis on an intention to treat basis. Patients would continue in follow-up if they were happy to continue in the study.

Recruitment (see participant flow chart; appendix 1)

Dedicated research nurses will visit all wards twice daily, including weekends, to:

- record the total number of admissions
- record the working diagnosis/diagnoses or reason for admission in those aged ≥65 years
- apply the inclusion / exclusion criteria (as above)
- invite eligible patients to participate

The aims and methods of the study will be discussed and an approved information sheet provided. Sufficient time will be given for the participant to consider and discuss with relatives and health care personnel whether or not they wish to participate in the study.

Participants admitted to hospital in the mornings will be revisited later that day and those admitted after midday will be revisited the next morning. The research nurse will take signed, informed consent according to ICH/GCP guidelines. The consent form will be held in the investigator file, with copies filed in the hospital notes and given to the participant. A sticker will be placed on the hospital notes to signify that the patient has joined the study and the GP informed by letter. The reasons for declining to participate, if given, will be recorded.

Demographic and baseline clinical data will be recorded including the type and dose of antibiotics, duration of treatment in those already receiving antibiotics, other risk factors for CDD and episodes of CDD within the last 3 months. Participants will be required to stop any regular usage of probiotic preparations for the duration of the trial.

Generation and concealment of a simple random allocation sequence and participant allocation

A stratified randomisation by centre using blocks of variable sizes will be used to allocate subjects to either placebo or probiotic on a 1:1 basis with an aim to ensure similar numbers of patients in all centres. The randomisation codes will be produced by Dr Duolao Wang at London School of Hygiene and Tropical Medicine, using SAS PROC PLAN Version 9.1. Subjects fulfilling the eligibility criteria will be assigned a randomization code (subject numbers with an unique 11 digit identifier) starting and ending as follows:

- Centre 1, PLACIDE1001 ---- 1800
- Centre 2, PLACIDE2001 ---- 2800

- Centre 3, PLACIDE3001 ---- 3800
- Centre 4, PLACIDE4001 ---- 4800
- Centre 5, PLACIDE5001 ---- 5800

The random allocation sequence will be deposited with the DMEC who will check its reliability. It will not be available to any members of the research team.

Cultech Ltd. will prepare packs of the appropriate trial intervention (probiotic or placebo) labeled with each unique number in the series according to the random sequence. Each hospital will be supplied with a consecutive series of 100 study numbers and corresponding packs and re-supplied as recruitment progresses. Participants will be enrolled strictly sequentially in each centre. The research nurse will allocate each participant to the next unique study number in the sequence and provide the participant with the corresponding trial preparation. If a patient discontinues from the study, the patient number will not be reused, and the patient will not be permitted to re-enter the study.

Administration of intervention and follow-up to determine study outcomes

The participant will be instructed to take the first dose of the trial preparation (probiotic or placebo) on the day of recruitment. Project nurses will review participants daily during admission to identify the onset and determine the duration of diarrhoea and ask about gastrointestinal symptoms (abdominal pain, bloating, flatus, nausea), acceptability and adverse effects of the interventions. All participants will be followed-up for 8 weeks after completing antibiotic treatment. The maximum follow-up period will be 3 months from the date of recruitment Participants will also be asked to complete a quality of life questionnaire at baseline, 3 days post intervention, on hospital discharge and at the end of follow-up. We expect that most participants will have been discharged before completion of the 8 week follow-up. After discharge, follow-up will be weekly by telephone call, postal questionnaire or home visit as appropriate. Participants will be provided with a card with contact details and will have ready access to research staff by telephone throughout the study to notify the onset of diarrhoea or any other adverse events.

Diarrhoea is defined as the occurrence of 3 or more loose stools (loose stools will be identified with the help of the Bristol stool chart: Types 6 and 7) in a 24 hour period. All participants who develop diarrhoea during the study period will be asked to provide a stool sample (collected during a home visit if required). The cause of diarrhoea will be determined by NHS laboratories according to their usual practice. Stools will be analysed for diarrhoeal pathogens (bacterial culture for Salmonella sp, Shigella sp, Campylobacter, E. Coli 0157; wet film for ova, cysts and parasites) and for *C. difficile* toxins A and B using the Biostat EIA test. If a cause of the diarrhoea is not identified, a further stool sample will be collected and tested 2 days later.

Diagnosis of the cause of diarrhoea will be based on stool analysis. AAD is defined as diarrhoea without pathogens detected on routine laboratory analysis and negative for *C. difficile* toxin. CDD is defined as diarrhoea with stools positive for *C. difficile* A or B toxin. For quality control purposes, *C. difficile* culture and confirmation by

immunoassay will be undertaken in 1 in 5 *C. difficile* toxin positive stool samples collected in Swansea.

Participants who develop severe diarrhoea will be investigated and managed according to the current practice of their clinicians who will have access to the Cochrane review of antibiotic treatment of CDD (Bricker *et al* 2005). Investigations other than stool analyses are not part of the research protocol and will not be advised or undertaken solely for the purposes of this project. Information from clinical records regarding investigations and management as undertaken by the usual clinicians caring for the patients (e.g. findings at sigmoidoscopy, colectomy) will be recorded in the participant log.

- **Planned interventions**

Participants will be allocated randomly on a 1:1 basis to receive either:

a. Live bacteria of human origin: 2 strains of *Lactobacillus acidophilus* (CUL60, National Collection of Industrial, Food and Marine Bacteria [NCIMB] 30157 and CUL21, NCIMB 30156), *Bifidobacterium bifidum* (CUL20, NCIMB 30153), *Bifidobacterium lactis* (CUL34, NCIMB 30172). Prepared as 5g lyophilised powder in a capsule containing 6 x 10^{10} organisms/capsule.
b. Identical formulation of inert placebo: maltodextrin 5g

Dosage: 1 capsule/day taken with food for 21 days.

These probiotics are known to survive passage through the upper gut, adhere to intestinal mucosa and have excellent viability at the point of administration.

The rationale for the selection of these organisms is based on our previous work with probiotics in the prevention of CDD (Plummer *et al* 2004) and we have recent evidence that one of the organisms (*L. acidophilus*) neutralises *C. difficile* toxin in an epithelial cell assay *in vitro* (SPUR, Welsh Development Agency Research Grant; submitted April 2004). These probiotic preparations are already commercially available through BioCare UK and Pharmax, USA. We consider that it is important to select human commensal organisms for probiotic interventions in at-risk patients to reduce the possibility of adverse effects such as systemic infection by the probiotic strains. Therefore, we decided not to test organisms that are not part of the normal human commensal flora such as *S. boulardii*.

To ensure the quality of the probiotics, identity will be checked by established molecular techniques and viability by quantitative bacterial culture in a representative sample of the study preparations retrieved from wards on a regular basis throughout the study. This will be done by a laboratory independent of the research team to maintain masking of the allocation sequence. If any deterioration or deviation in the product is detected, including a reduction of >10% in the number of viable organisms of each strain, fresh supplies of trial preparations will be provided and testing repeated.

Outcome measures

- Diarrhoea is defined as 3 or more stools in a 24 hour period. Loose stools will be identified with the help of the Bristol stool chart (Types 6 and 7).
- Severe diarrhoea is defined as diarrhoea that requires treatment (oral or intravenous rehydration therapy for clinical dehydration and/or antibiotics) or investigation beyond stool culture (blood culture for suspected septicaemia, sigmoidoscopy for suspected PMC)
- AAD is defined as diarrhoea occurring in association with antibiotic therapy without an alternative explanation
- CDD is defined as diarrhoea not attributable to another cause and with stools positive for either *C. difficile* toxin A or B as detected by the Biostat EIA
- Pseudomembraneous colitis (PMC) is diagnosed by finding characteristic features at endoscopy and/or mucosal histology

The effect of the probiotic on the following outcomes will be determined:

Primary outcomes

During antibiotic treatment and within 8 weeks of stopping antibiotics:

a. The occurrence of AAD
b. The occurrence of CCD

Secondary outcomes

a. severity and duration of AAD
b. abdominal symptoms (abdominal pain, bloating, flatus, nausea)
c. severity and duration of CDD and incidence of recurrence within the study period
d. incidence of PMC, need for colectomy, death
e. well-being and quality of life
f. duration of hospital stay
g. adverse effects
h. acceptability of the probiotic preparation
i. viability of the probiotic at point of administration
j. risk factors for ADD, CDD and severe disease (PMC, colectomy, death)

Although not part of the main brief, an important issue is whether prevention strategies should be provided to all patients or just those at high risk of severe *C. difficile* infection. We will assess clinical outcomes according to proposed risk factors for severe CDD: age, duration of admission, severity of illness, previous episodes of CDD, specific antibiotics and usage of proton-pump inhibitors (Barbut 2001).

Estimate of likely recruitment rate

We have obtained a favourable response from senior clinicians and the Chief Nursing Officer in each of the participating hospitals to allow us to approach patients. Short presentations will be arranged for medical and nursing staff to invite them to agree to

their patients joining the study. We will announce the study in local media to raise awareness and aid recruitment.

As detailed above, and based on data from 2005/6, we expect to admit about 14,000 patients per year to the study hospitals aged ≥65 years and exposed to antibiotics. Conservatively, we expect to be able to recruit between 1:9 and 1:10 of these patients – 124 patients/month.

This estimate is supported by a limited 2-week pilot study of our recruitment process in 23 wards in Morriston Hospital during September 2005 (Elderly Care, Medical, Gastroenterology, Renal, Cardiology, General Surgery, Urology, Trauma, Burns and Orthopaedic wards). Research nurses visited the wards daily and identified a total 253 admissions aged ≥65 years. The nurses excluded 166 patients (no current or planned exposure to antibiotics – 152 patients; already had diarrhoea – 12 (inc. 1 *C. difficile*); active inflammatory bowel disease -1; previous adverse reaction to probiotic reported -1).

Eighty-seven (34.4%) patients were eligible to participate in the pilot study but 8 of these were excluded because they were either confused or not available (in theatre, undergoing investigations). Further attempts to recruit these patients would be made in an on-going study by follow-up visits and/or seeking assent from relatives. Therefore, the design of the study was then explained to 79 patients (31.2% of total admissions) and 58 (73.4%) patients stated that they would have agreed to participate in this study.

As detailed in our proposal, there were a total of 963 cases of CDD in 2005/6 in the hospitals involved in this study.

As a safeguard, we will monitor closely the number of participants reaching study endpoints in each hospital every 3 months from the beginning of the study so that we can take remedial action if needed. In Swansea, we have already undertaken research in hospitals in neighbouring Trusts and are confident that we could include additional hospitals in our study if required.

- **Ethical arrangements**

The project will be submitted to the Central Office for Research Ethics Committees (COREC) for allocation for review by an Ethics Committee (EC). The PI will report promptly all changes to the study, all unanticipated problems involving risks to participants or others and any protocol deviations which are necessary to eliminate immediate hazards to patients. Serious adverse events will be reported to the EC in accordance with national and local requirements. The Investigator will not make any changes to the study or its conduct without EC approval, except to eliminate a danger. The investigator will submit annual progress reports and a final report to the EC following the study completion or in the event of a premature termination of the study. For essential amendments after the study has started, participants will be informed and invited to sign a revised consent form should they wish to continue in the study.

- **Risks and anticipated benefits for trial participants and society, including how the benefits justify the risks**

Risk of no benefit to participants

The 50% of the participants allocated to the control arm will not derive any direct benefit from the trial intervention.

Risks of adverse effects

- Probiotics are members of the normal gut commensals and were classified in 2002 as "generally regarded as safe" by the US Food and Drug Administration (notice GRN 000049). The European Society of Paediatric Gastroenterology, Hepatitis and Nutrition Committee on Nutrition concluded that probiotics can be considered safe but surveillance for side effects is needed (ESPGHAN 2004; von Wright 2005). A recent review of the safety of lactic acid bacteria found only anecdotal reports of systemic infection that had occurred in people with severe disability, immune deficiency or prosthetic heart valves. In these few cases, it was difficult to differentiate infection caused by administered probiotics from that caused by the endogenous flora. In prospective studies, probiotics have been administered without adverse effects to vulnerable groups such as children and adults with HIV infection and preterm infants (Hammerman 2006; Schlegel 1998). We will exclude patients at high risk of probiotic infections from our study as detailed above.

- In the case of suspected sepsis developing after starting the trial intervention, laboratory staff will be alerted that the patient is enrolled in the study by a sticker attached to the laboratory request forms. They will undertake bacterial culture for the probiotic organisms as well as common bacterial pathogens. Although highly unlikely, any infection attributed to the probiotic organisms would be treated according to their pre-determined antibiotic sensitivity.

- *Participant unblinding.* Arrangements for the immediate unblinding of participant allocation are not necessary as this would not inform clinical management. If required by the DMEC, Safety Monitor or participant's clinician, unblinding could be undertaken by Dr Duolao as and when necessary.

- Research staff will have ready access to senior physicians on a 24 hour basis to discuss adverse events and safety issues as needed.

Anticipated benefits

- Regular follow-up of all participants (including those in the placebo group) for diarrhoea and any adverse events may increase the recognition of morbidity and therefore, improve overall care and outcome.

- Those who receive the active intervention may have a reduced risk of developing AAD and CDD or may develop milder disease.

- If the intervention proves to be successful against CDD, fewer cases will reduce the risk of nosocomial diarrhoea amongst other admissions.

- This large study will also provide further information about frequency and risk factors for AAD and CDD which may allow high risk groups to be better identified.

Informing potential trial participants of possible benefits and known risks of the intervention (or of no intervention or a placebo)

As part of the informed consent process, research staff will strive to ensure that all participants (and their relatives or carers where appropriate) understand that they have a 50% chance of being allocated to the placebo arm of the study and would, in that case, derive no direct benefit from the intervention. They will also explain that the probiotic preparation may not prove to be effective in preventing or ameliorating diarrhoea. Each participant will have frequent contact with a named research nurse who will be available by telephone throughout the study to answer any questions that may arise.

- *Informed consent from participants wherever possible*

Potential participants will be given a verbal and written explanation of the study by one of the study team who is experienced in taking informed consent. Participants will be encouraged to ask questions and every attempt will be made to ensure that they understand the study including that they can withdraw from the study at any time without giving a reason and without it affecting their medical care in any way. They will be given sufficient time to discuss the study with others. Once a participant has decided to enter the study, they will be asked to sign a consent form. Participants will be aware that all information will be anonymous to ensure complete confidentiality and that individual participants will not be identified in any reports or publications.

Proposed action where fully informed consent is not possible (e.g. emergency settings)

Every effort will be made to communicate details of the study to the participant but, in this older population, assent will be required in many cases where the patient is unable to give full, informed consent. Assent will be sought from next of kin, other relative or carer in line with Article 5 of the EU Directive 2001/20/EC (Clinical Trials on incapacitated adults not able to give informed legal consent). The information sheet will be sent to the relative or carer and they will be given the opportunity to ask questions of a member of the research team. We will appoint a senior clinician in each NHS Trust who is independent of the research team to act as an advisor for participants and relatives regarding their involvement in the study if they wish. Participants and relatives will also be encouraged to discuss the study with their General Practitioner. If a participant is later deemed to be able to give informed consent, then this will be sought.

Relatives / carers will be informed that they can withdraw assent at anytime without it affecting the patient's care. The participant would be withdrawn from the study if he/she declines on two consecutive occasions to take the trial preparation.

Retention of relevant trial documentation

Data containing participant's identification details will be retained for 10 years from the termination of the study. This will allow the linking of an individual participant's data with their other health records (e.g. GP record, other hospital records). Beyond this period, all participant identification details (e.g. name, contact details, hospital number) will be removed. This anonymised data set will be retained indefinitely.

Action to comply with EU Directive 2001/20/EC

MHRA are assisting us in the completion of the necessary submission for a Clinical Trial Authorisation for this study.

- **Sample size**

Conservatively, we expect ADD to occur in 20% and CDD in 4% of participants in the placebo group. To detect a 50% reduction in the frequency CDD in the probiotic group (i.e. 2% frequency) with 80% power at the 5% significance level, we will require 2,478 subjects (1,239 in each group; 1:1 allocation). At the 5% significance level, this number of participants would provide a power of >99% to detect a 50% reduction in ADD (i.e. 10% frequency) and a power of 90% to detect a 25% reduction in ADD (i.e. 15% frequency) in the probiotic group. To allow for 10% drop-outs and 10% loss to follow-up due to deaths unrelated to diarrhoea, we will recruit 2,974 participants.
On this basis, we expect 50 cases of CDD in the control group and 25 in the probiotic group over 2 years. Since we observed 963 cases of CDD in one year (2005/6) in the hospitals involved in this study, we would have to recruit less than 1 in 20 cases into our study to reach our recruitment target. We are likely to recruit more cases of CDD than required which would increase the power for all CDD comparisons.

- **Statistical analysis**

Primary outcomes will be analysed with standard methods for a multicentred RCT. Confidence intervals for the odds ratios for ADD and CDD will be estimated from regression models that include the relevant covariates (such as age, gender, specific antibiotic, centre). A similar approach will be taken for the outcomes of severity and PMC. Careful inspection of interaction terms will identify sub-group effects, and these will be interpreted in light of power relative to main effects and supporting evidence of mechanism (Brookes 2001). All analyses will be performed using the R statistical environment (Ihaka & Gentleman, 1996, *J Comp Graph Stat*, 5).

Quality of life (QoL)

There are few tools that are validated for measuring QoL in older people and none specifically targeted at treatment-induced diarrhoea. We will modify existing tools which have been validated to measure QoL in treatment-induced diarrhoea in people with HIV (Thielman 2002) and older patients with faecal incontinence (Rockwood 2000). We will also use the generic measures EQ-5D and the York SF12 (Iglesias

2001) to understand the broader health impact related to treatment-induced diarrhoea and facilitate cost-effectiveness analysis.

Health economic analysis

The health economic evaluation will be undertaken from the perspective of the NHS. Resources utilised by each participant will be logged using appropriate recoding forms and collected as part of the on-going data collection process. The resources utilised will consist of the number and cost of the probiotics, the costs of staff time involved in administering the probiotics, costs of treatments relating to adverse events, costs incurred in the assessment of cases of diarrhoea (stool collection and culture/toxin assay, endoscopy) and costs resulting from dealing with and treatment relating to cases of diarrhoea, such as laundry, antibiotics, increased hospital stay and co-morbidities. Data relating to unit costs will be collected through discussions with relevant clinicians and finance department staff, while published information will also be utilised.

Cost differences between the probiotic and placebo group will be determined and used in conjunction with differences in outcomes between groups in undertaking a cost-consequences analysis, with cost per case averted as the primary outcome measure for the economic evaluation, but with other outcomes considered. Sub-group analyses will also be conducted to determine the relative cost-effectiveness of preventive strategies in different risk groups. In addition, a cost-utility analysis will be undertaken based on the differences in costs between the two groups and differences in QALYs derived from the EQ-5D responses during the course of the investigation.

Given the timescale of the project there will be no discounting of the costs or benefits. Sensitivity analyses will investigate the robustness of the results to changes in estimated costs and outcomes and probabilistic sensitivity analysis will use bootstrap resampling to determine the probability that preventive strategies are within certain thresholds.

The budgetary impact (again from a NHS perspective) of adopting a policy of administering a probiotic preparation containing 4 strains of live bacteria to prevent or ameliorate AAD and CDD in people aged 65 years and over who are admitted to secondary care NHS facilities and receive oral or intravenous antibiotics will also be assessed as part of the health economic evaluation.

- **Research Governance** – see organogram; appendix 2

Trial Steering Committee (TSC)

The chair and members of the TSC will be appointed formally by the HTA. The proposed Chair is Professor Stephen Bain, Director of R&D, ABM University NHS Trust. Patients admitted under Professor Bain's care would be eligible to participate in the study, but he would have no other involvement in the trial. Membership would also include a service user representative, two other independent members, Dr. Steve Allen (PI), Ms. Kathie Wareham (Project Manager). Observers from the HTA and the

trial sponsor (Swansea University) will be invited to all meetings and will also be able to convene additional meetings.

An initial TSC meeting before the trial start will be arranged by the PI to review and agree the trial protocol and establish a DMEC (see below). In advance of subsequent meetings, evidence regarding progress with recruitment based on eligible population, adherence to protocol, loss to follow-up and AEs will be provided. The TSC will also be required to review any new information regarding CDD, AAD and probiotics that may be relevant to the local trial.

Safety

Safety reporting will follow the requirements as described in The Medicines for Human Use (clinical Trials) Regulation 2004: SI 2004/1031.and the EU Directive 2001/20/EC.

Adverse events
All serious adverse events (SAE's) will be reported immediately to the sponsor except for those which are described in the protocol/addendum as not needing immediate reporting. The immediate reports will be followed promptly by detailed, written reports. The reports will follow the guidelines of 4.11.of the ICH Guidelines for Good Clinical Practice.

Suspected unexpected serious adverse reactions (SUSARs)
All relevant information about suspected unexpected serious adverse reactions (SUSAR) which occur during the course of the study and are fatal or life-threatening will be reported immediately/as soon as possible to the MHRA, the competent authority and the relevant Ethics Committee. This will be done within 7 days of first being aware of the reaction. Additional information would be forwarded as soon as possible and within eight days of filing the initial report. In respect of a SUSAR which is not fatal or life threatening it will be reported as soon as possible but not later than 15 days after the Sponsor is first aware of its occurrence.

SUSAR reporting
CIOMS 1 form will be used to inform the MHRA and it will include all relevant information including the EudraCT number, CTA number protocol number and Study name. Reports may be faxed, emailed or sent as electronic documents on disk.

Safety Monitor

A large number of Serious Adverse Events (SAEs) are likely in the course of this study of elderly people and a realistic approach is necessary in AE reporting bearing in mind the excellent safety record of probiotics. SAEs will be defined according to GCP guidelines and assessed by the local research clinicians involved in the project as to their attribution. See appendix 3

In respect of what constituted a SUSAR, it was agreed that this would include the following but not limited to these serious adverse events:

- bacterial infection caused by a probiotic organism (i.e. lactobacillus or bifidobacteria)
- the development of bowel ischaemia not present at recruitment (any past or current abnormality or disease affecting the mesenteric arteries is an exclusion criterion).
- the development of pancreatitis (defined as abdominal pain with serum amylase or lipase concentration ≥3 times the institutional upper limit of normal; pancreatitis present on admission is an exclusion criterion)

These SUSARs will be reported immediately to the Independent Safety Monitor to consider their attribution to the participant's participation in the trial and also to the Ethical Committee/MHRA/ regulatory bodies in accordance with local and national requirements.

A dedicated EXCEL database will record all SAE's and SUSARs and this would be available at any time to the Safety Monitor and the DMEC.

The research team will send the Independent Statistician details of all SAEs every 3 months. The statistician will allocate these to the two intervention groups (but labeled as only "A" or "B") and discuss the findings with the Safety Monitor. These reports will be reviewed at DMEC meetings. The identity of groups "A" and "B" will be provided by the Independent Statistician immediately should either the Safety Monitor or the DMEC have any concerns regarding participant safety

In addition, the Independent Statistician will undertake an unblinded, interim analysis for important safety outcomes including the first 500 participants with complete data and report to the DMEC. Outcomes will include all SUSARs and all serious adverse events.

Data Monitoring and Ethics Committee (DMEC)

The chair and members of the DMEC will be appointed formally by the HTA. Proposed membership includes an independent Chair (Professor JG Williams, Consultant Gastroenterologist, Neath Port Talbot Hospital and Director of Welsh Office Research and Development Programme), Dr. Duolao Wang, Medical Statistics Unit, Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine and 1-2 additional independent members. Patients admitted under the care of Professor Williams would be eligible to participate in the study, but he would have no other involvement in the trial. Dr. Wang will generate and hold the random allocation sequence for the trial but is otherwise independent of the study. Regular meetings will be organized with the PI in association with the DMEC chair. Prior to each meeting, the trial statistician will prepare a report of trial progress.

The DMEC would review data from other related studies and advise as to how this might reflect on the local study. The DMEC would also advise regarding the needs for extended funding should this be requested by either the funding body or the TSC. The DMEC will report to the subsequent TSC meeting.

Trial Management Committee

This group will be based in Swansea and include the PI, the Project Manager, the CDDFT Site Co-ordinator and CDDFT hospital site leads. It will meet frequently prior to commencing the study and at least monthly as the study progresses. It will focus on the day-to-day operation of the trial including mechanisms for the prompt reporting of adverse events.

After initial face-to-face meetings, use of teleconference facilities will help to reduce travel costs.

4. Project timetable and milestones

Key milestones:

- May - June 2008: submission to MREC; staff recruitment and training; pilot testing of patient recruitment, data collection and stool collection and analysis; development of database; preparation of trial interventions; writing Standard Operating Procedures; local meetings with NHS staff; establish TSC, DMEC and local trial management committee.
- July 2008 – June 2010: participant recruitment and laboratory analyses (target 117 participants/month for 24 months)
- July – September 2010: complete participant follow-up
- October 2010 - March 2011: clinical and cost-effectiveness data analysis; report writing, presentation of results at national and international meetings and preparation of publications
- March 2011: completion of study

5. Expertise

We consider that we have a highly experienced and committed team of investigators with strengths in each of the major areas of the study. All of the investigators have contributed to the design of the study, will be closely involved in the trial on a day-to-day basis and will also be involved in data interpretation and the writing of scientific publications. Trial management will be centered in the Clinical Research Unit (CRU) based at Morriston Hospital, Swansea.

Dr. Stephen Allen is a Reader and Honorary Consultant Paediatric Gastroenterologist. He has completed a Cochrane systematic review in probiotics in acute diarrhoea. He has extensive experience of clinical research mainly in economically-poor countries and has led research teams in both hospital and community settings. During a 4 year period based at the MRC Laboratories, The Gambia, he was a member of the Nutrition Research Group and the Scientific Co-ordinating Committee which met monthly to review new research proposals. He is a member of the UK Medicines for Children Research Network Clinical Studies Group for General Paediatrics. As PI of this study, he will provide overall supervision of the conduct of the study, including assessing progress against milestones, supervising data management and financial control. He will report on progress and adverse events to the TSC and take the lead in the writing of trial reports and publications.

Ms. Caroline Bradley has worked as a Clinical Pharmacist within the secondary care environment for over 10 years. She has a special interest in the use of antibiotics and leads on antibiotic policy and use

for County Durham and Darlington Acute Hospitals NHS Trust (CDDAH). CDDAH is an acute Trust providing healthcare across County Durham and Darlington and surrounding areas from three main acute hospitals, at Durham, Darlington and Bishop Auckland alongside other community hospitals. The Trust serves a population of 550,000 people across County Durham and Darlington, and offer services to many patients outside this area.

Recent achievements in antibiotic management are producing and managing the policy for the use of antibiotics across in the Trust, advising and monitoring the use of antibiotics with particular attention to MRSA and *C. difficile* rates. Results include reducing the average duration of IV antibiotic use, halving the rate of IV macrolide use in the Directorate of Medicine and the introduction of automatic stop orders to limit the duration of antibiotic treatment. She will oversee the management of the trial in CDDAH and be the main point of contact with the Swansea research team.

Dr. Anjan Dhar is a Consultant Gastroenterologist in the Directorate of Medicine & Elderly Care at Bishop Auckland General Hospital. He obtained D. M. in Gastroenterology in 1994 at the Postgraduate Institute of Medical Education and Research, Chandigarh, India and won a Commonwealth Fellowship in Gastroenterology, Association of Commonwealth Universities, undertaken with Professor Derek Jewell, Radcliffe Infirmary, University of Oxford, UK between 1998 and 2000. He gained extensive experience in gastroenterology from working in leading clinical and research centres including Middlesex Hospital, University College London Hospitals, The Royal Hospital, Muscat, Oman, the All India Institute of Medical Sciences, New Delhi, and Postgraduate Institute of Medical Education and Research, Chandigarh India. His research has focused on *Helicobacter pylori*, peptic ulcer and inflammatory bowel disease. He will provide supervision of clinical recruitment for the trial in Bishop Auckland General Hospital and also provide expert advice regarding clinical management of patients with ADD and CDD.

Professor Dietrich Mack is Professor of Medical Microbiology and Infectious Diseases and Honorary Consultant Microbiologist. He has extensive research experience of techniques for susceptibility determination in multiresistant nosocomial organisms like ESBL-containing enterobacteria, VRE, and staphylococci and exploring their epidemiology as well as the molecular pathogenesis of biomaterial-related staphylococcal infections. He will supervise all of the laboratory analyses undertaken in the trial including quality assurance for *C. difficile* culture and toxin assays.

Dr. Sue Plummer is the Technical Director of Cultech Ltd., Swansea, a leading manufacturer of specialist nutritional products for the healthcare industry. She leads the development of the human nutritional supplement sector and has a special interest in probiotics. She will ensure a reliable supply of the trial preparations for this study allocated according to a random sequence. She will also supervise quality control of the trial preparations and give expert guidance on new developments in the field of probiotics.

Dr. Wyn Harris is a Consultant Geriatrician with extensive clinical experience of the diagnosis and management of *C. difficile* infection in the elderly. He has completed an audit of antibiotic use in an effort to reduce the incidence of CDD and implemented prescribing guidelines. He will be primarily responsible for the welfare of trial participants in Swansea and be available for expert clinical guidance. He is an active member of the Welsh Branch of the British Geriatrics Society and this will assist in disseminating the results of the study to inform clinical practice.

Dr. Wai Yee Cheung is a Senior Lecturer in Health Services Research with expertise in the development and validation of patient-focused outcome measures. She has led development of condition-specific and systemic quality of life measures for use in many multi-centre studies, including trials funded by the HTA Programme. She will oversee the development and application of quality of life measures in the current study.

Dr. Mike Gravenor is a Reader in Epidemiology and Statistics. His research centres on the application of statistical and mathematical models to practical problems in epidemiology and the link between good data collection and sophisticated analysis techniques. He will supervise data collection and storage and perform the statistical analysis.

Professor Ceri Phillips is a health economist and Head of the Institute for Health Research at the School of Health Science, Swansea University. He has extensive experience of health economic evaluation in many projects, including HTA projects, and will oversee the detailed economic evaluation in this study.

Ms. Kathie Wareham is the Director of the Clinical Research Unit (CRU), ABM University NHS Trust. She has 25 years experience in clinical research, having spent 10 years setting-up and running phase I clinical trials unit at Smith, Kline & French (now Glaxo SmithKline). For the past 15 years, she has been responsible for setting up a research network in Swansea within and outside of the Trust. She was a member of two phase I Ethics Review Committees for 20 years and was recently an external examiner for an MSc in Clinical Research at John Moores University, Liverpool. She has successfully managed projects funded by the Welsh Office of Research and Development, Welsh Assembly Government and these have resulted in publications in leading journals. She will supervise the overall running of the trial both in Swansea and CDDAH.

The CRU has been operational for 16 years with continual growth. A purpose built facility was established in 2000 and now undertakes most of the clinical research projects in ABM University NHS Trust. It has an alliance with The School of Medicine at Swansea University. The unit has been commissioned by a number of blue chip pharmaceutical companies. It undertakes proof of concept studies, phases IIa, IIb and III across a number of disciplines. All staff are trained and updated in Good Clinical Practice guidelines. The Unit sets up unique, password-protected computer databases which are archived regularly off site in a secure location.

Dr. Helga Brown is Consultant Physician and Honorary Clinical Lecturer at University Hospital of North Durham (UHND). Most of her inpatients (850 per annum) are frail elderly. She also provides regular assessment of inpatients in the Orthopaedic and Psychiatry of Old Age departments. Her work brings her into direct contact with the patients most at risk of developing ADD and CDD. In response to an alarming rise in the incidence of CDD in UHND in 2006, Dr Brown undertook an audit of risk factors and revised the hospital guidelines for the management of CDD, in association with colleagues in microbiology. She will be the clinical lead for the study at UHND and be responsible for advising on the clinical assessment and care of trial participants.

Dr. Alwyn Foden is a Consultant Physician with an interest in Respiratory Medicine at Darlington Memorial Hospital, Co Durham. He has extensive experience of clinical trials. He has a special interest in conditions that lead to infections, especially resistant ones, and has experience in trials of anti-infective agents. He has recently completed a formal course on Good Clinical Practice. He is the Acute Care Trust representative on the Darlington Respiratory Team of the Primary Care Trust. In this study, he will be w the clinical lead for the study at Darlington Memorial Hospital and be responsible for advising on the clinical assessment and care of trial participants.

6. Service users

Mr. John Pollock has kindly agreed to represent consumers on the Steering Group. He is a retired businessman and Rotarian. He has been involved as research participant for a number of years and is fully conversant with the procedures for clinical research and issues regarding lay interpretation of consent. He fully understands the needs of participants and how best these can be met. Another service user representative based in CDDAH will be also be invited to join the TSC. We propose to pay the service user representatives for their time spent attending meetings as well as re-imbursement of travel expenses.

7. Justification of support required

Project manager (50% FTE; 1 post in Swansea): Ms. Kathy Wareham, Director of the CRU, will undertake this post. She will take the lead for ensuring close liaison between Swansea and CDDAH. She will draft all Standard Operating Procedures for the conduct of the study. She will assess progress against milestones and take action if targets are not being met. In Swansea, she will be responsible for training the research nurses and motivating the team and will liaise with hospital clinicians and senior nurses throughout the project to maintain their support.

Study Co-ordinators (50% FTE; 1 post in each NHS region): These posts will be pivotal in ensuring efficient working practices and good lines of communication and will report directly to the project manager. They will supervise data collection from the hospital sites and following discharge, data entry, maintenance of participant files, take minutes at local meetings and ensure that interim and final reports are drafted, circulated and finalised by the project manager and PI. They will liaise with laboratory staff in respect of stool samples. The post holders will help to produce a three-monthly newsletter, which will be circulated to hospital medical and nursing staff. They will also provide back-up for patient recruitment during periods of staff leave and sickness.

Administrator (25% FTE; 1 post in Swansea): This post will provide essential administrative and secretarial support to the project teams in both NHS regions.

Research nurses (100% FTE; 3 posts in each NHS region): Six posts are required to ensure flexibility to cover all hospital wards twice daily including at weekends and complete follow-up with adequate coverage for holidays and sickness. They will liaise with ward staff in the identification of eligible participants, recognition of diarrhoea and collection of stool samples. Regular updates and a supportive working relationship with all hospital staff will ensure maximal cooperation. Research nurses will recruit participants, encourage participants to take the trial interventions daily and collect clinical outcome data. Participants will be allocated a named research nurse throughout their involvement, including after discharge.

Research Assistant – Cost-effectiveness analysis (50% FTE; 1 post in Swansea): The research assistant will conduct a detailed and comprehensive assessment involving extensive data collection and analysis in both NHS regions.

Statistician / Data Manager (30% FTE; 1 post in Swansea): This person will help with design of data collection forms, build the databases for the clinical and laboratory data and ensure that reliable data is entered into the database as the study progresses. He/she will also be responsible for the initial data analyses supported by the project statistician.

Laboratory assistant (100% FTE; 1 post in Swansea): This post is required to support NHS staff for the prompt and careful handling and analysis of a large number of stool samples from the participants. The number of stool samples for analysis will increase significantly as a result of the research project.

Data clerk (50% FTE; 1 post in each NHS region): Data entry for both clinical and laboratory data will occur at each site with exchange of records between the data clerks for double data entry and checking for errors.

Consumables: Consumables are limited to the trial preparations, minor laboratory equipment and 4 computers for data entry and maintenance of other trial documentation. The computers should be of sufficient specification to generate quality graphics for reports and to write back-up data CDs. Other consumables include stationery items, paper and stamps.

Travel: We have carefully considered the need for research staff to make regular visits between the two NHS regions to ensure that all study procedures are uniform. In addition, travel expenses will be incurred by research nurses following-up participants in their homes. A nominal fee and travel costs will be paid to the independent members of the TSC for attending meetings.

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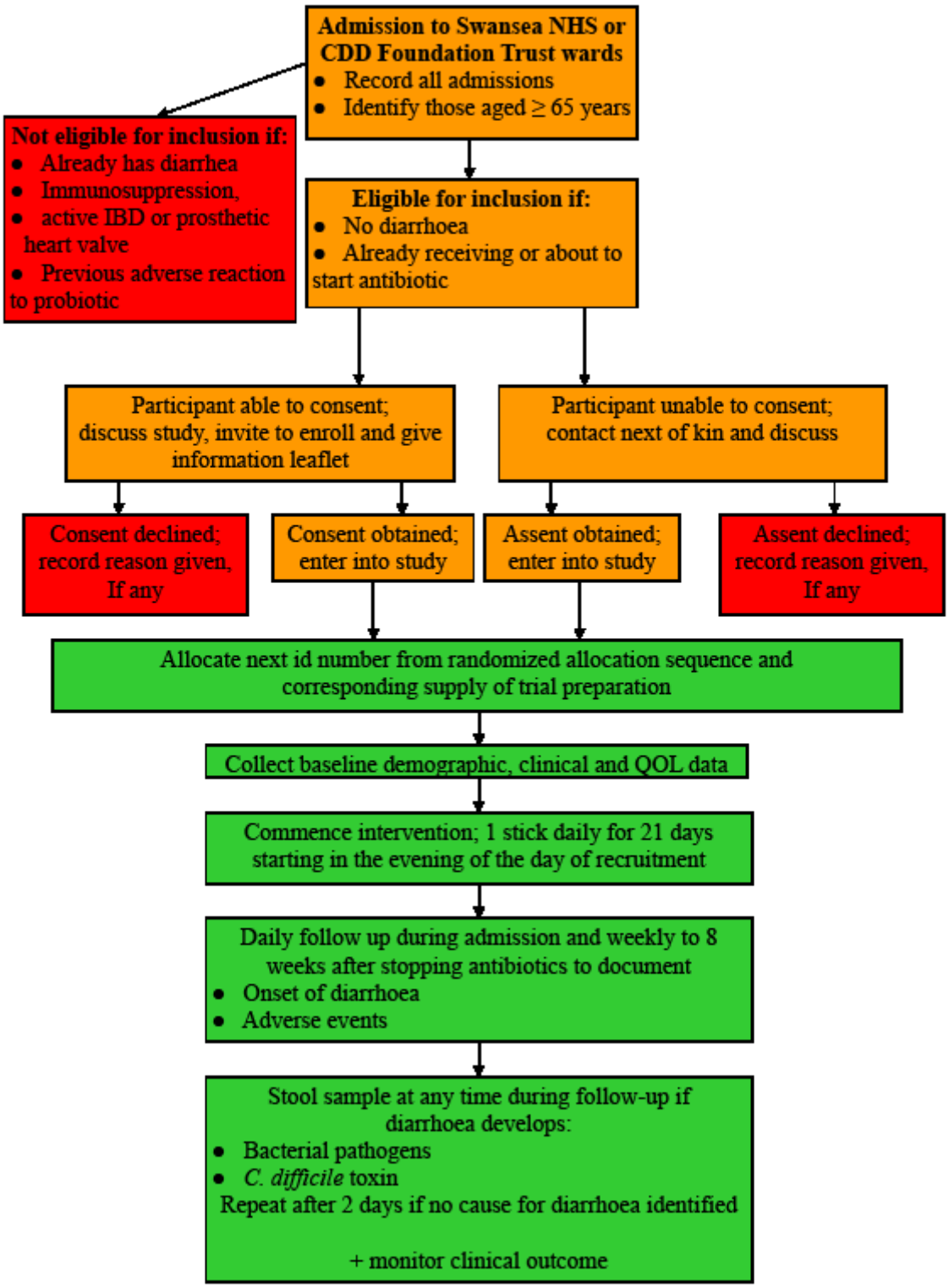
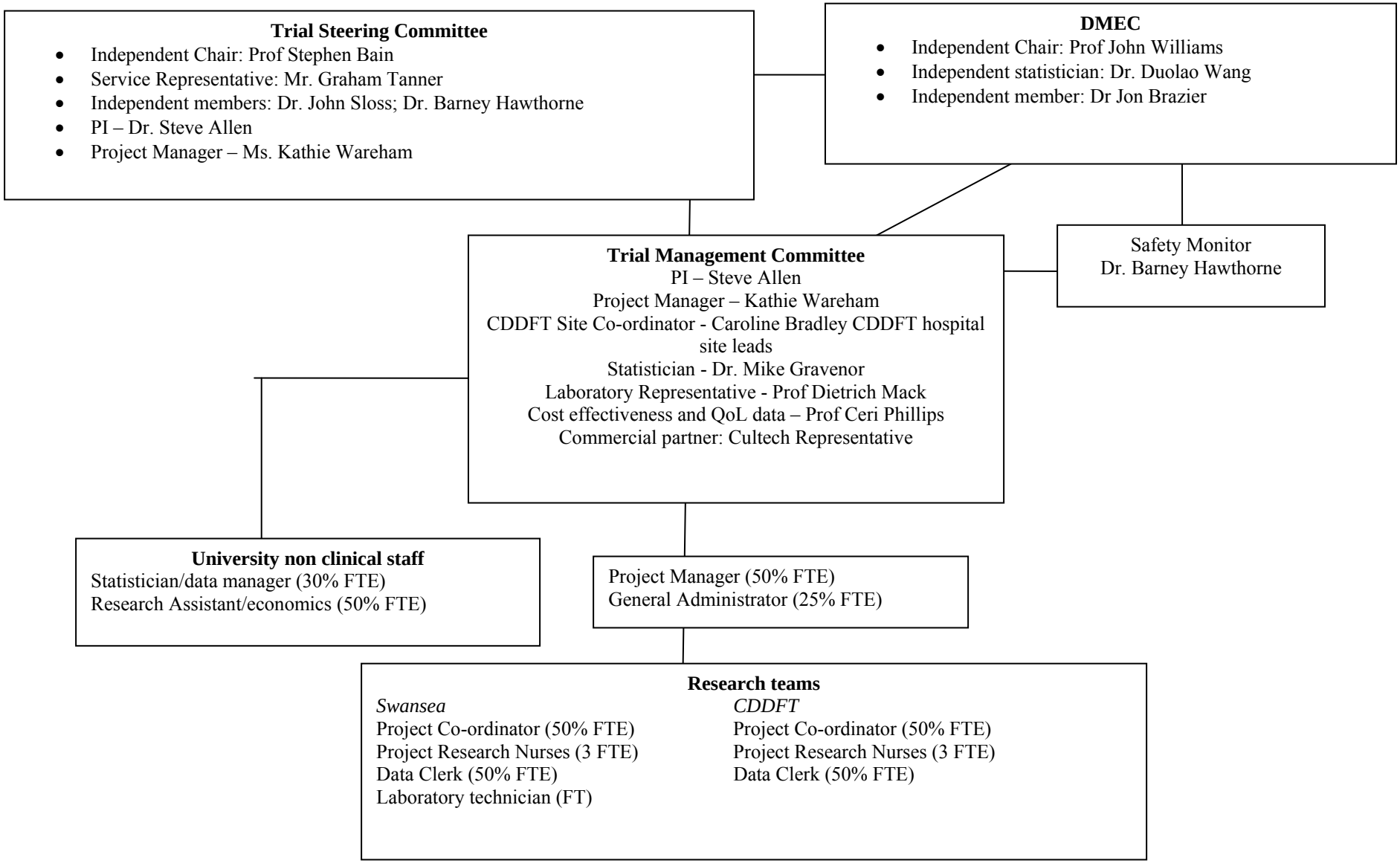
Appendix 1: Participant flow chart



Appendix 2


Trial Steering Committee
Independent Chair: Prof Stephen Bain
Service Representative: Mr. Graham Tanner
Independent members: Dr. John Sloss; Dr. Barney Hawthorne
PI – Dr. Steve Allen
Project Manager – Ms. Kathie Wareham
DMEC
Independent Chair: Prof John Williams
Independent statistician: Dr. Duolao Wang
Independent member: Dr Jon Brazier
Trial Management Committee
PI – Steve Allen
Project Manager – Kathie Wareham
CDDFT Site Co-ordinator - Caroline Bradley CDDFT hospital site leads
Statistician - Dr. Mike Gravenor
Laboratory Representative - Prof Dietrich Mack
Cost effectiveness and QoL data – Prof Ceri Phillips
Commercial partner: Cultech Representative
Safety Monitor
Dr. Barney Hawthorne
University non clinical staff
Statistician/data manager (30% FTE)
Research Assistant/economics (50% FTE)
Project Manager (50% FTE)
General Administrator (25% FTE)
Research teams
Swansea
Project Co-ordinator (50% FTE)
Project Research Nurses (3 FTE)
Data Clerk (50% FTE)
Laboratory technician (FT)
CDDFT
Project Co-ordinator (50% FTE)
Project Research Nurses (3 FTE)
Data Clerk (50% FTE)

1

Appendix 3 : Adverse events summary overview for guidance

All serious adverse events (SAE's) should be reported immediately to the sponsor except for those which are described in the protocol/addendum as not needing immediate reporting. The immediate reports should be followed promptly by detailed, written reports. The reports will follow the guidelines of 4.11.of the ICH Guidelines for Good Clinical Practice and the EU Directive 2001/20/EC.

Adverse Events categories for determining relation to study medication

Description	**Related**			**Unrelated**
	Probable	Possible	Remote	
Clearly due to extraneous causes	-	-	-	+
Reasonable temporal association with drug administration	+	+	-	-
May be produced by patient clinical state etc	-	+	+	+
Known response pattern to suspected drug	+	+	-	-
Disappears or decreases on cessation or reduction in dose	+	-	-	-
Reappears on rechallenge	+	-	-	-

Unrelated:
This category is applicable to those adverse events which are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment etc) and do not meet the criteria for drug relationship listed under remote, possible or probable.

Related

Probable (must have first three)
This category applies to those adverse events that are considered, with a high degree of certainty, to be related to the test drug. An adverse event may be considered probable if:

1 It follows a reasonable temporal sequence from administration of the drug
2 It cannot be reasonably explained by the know characteristics of the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject.
3 It disappears or decreases on cessation or reduction in dose.
4 It follows a known pattern of response to the suspected drug
5 It reappears upon rechallenge

Possible (must have first two)

This category applies to those adverse events in which the connection with the study drug administration appears unlikely, but cannot be ruled out with certainty. An adverse event may be considered possible if or when:It follows a reasonable temporal sequence from administration of the drug

1 It may have been produced by the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject
2 It follows a known pattern of response to the suspected drug

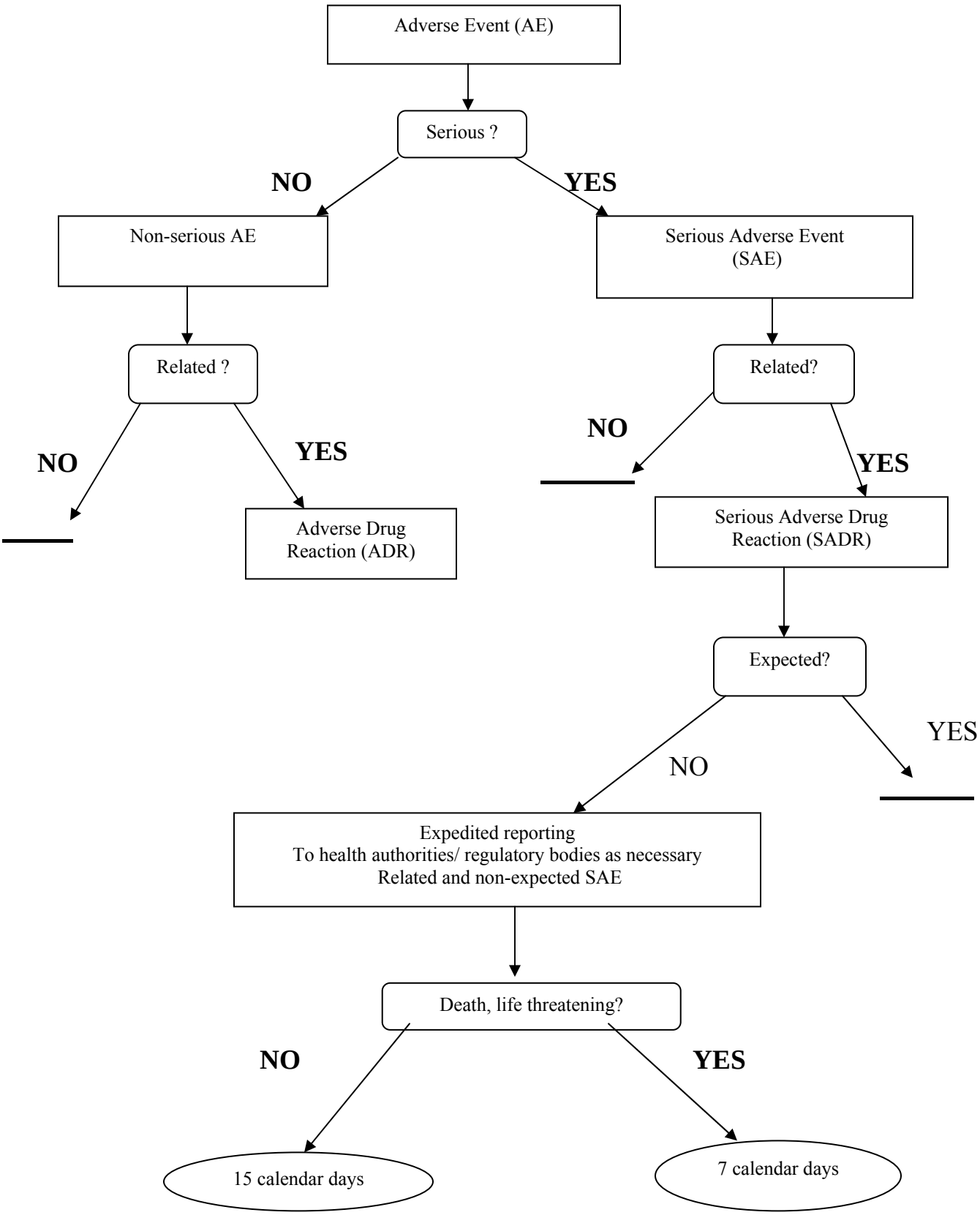
Remote (must have first two)

In general, this category is applicable to an adverse event which meets the following

criteria:

1 It does not follow a reasonable temporal sequence from drug administration
2 It may readily have been produced by the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject.
3 It does not follow a known pattern of response to the suspected drug
4 It does not reappear or worsen when the drug is readministered

Flow Chart of the management of Adverse Events



Appendix 2b (SOP for staff managing Adverse events/SUSARs)

Notification of a serious adverse event

As the study is recruiting patients who are 65 years and older with mixed pathology and disease progression (with no upper age limit) there are expected to be a number of adverse events including death.

With the above expectation it has been decided that the following list (although not conclusive) will be used as a guideline for reporting "sudden unexpected severe adverse reactions" **(SUSARs**) and will be reported to DMEC and other regulatory bodies (MHRA) as required following the guidelines in the EU Directive 2001/20/EC.

1 Bacterial infection caused by a probiotic organism. This would be any manifestation of infection (abscess, bacterial endocarditis, bacteraemia etc.) where a lactobacillus or bifidobacteria is isolated in pathological specimens by the microbiology laboratories.

2 The development of multi- organ failure not present at recruitment (vasopressor administration for circulatory support and multi- organ failure are exclusion criteria)

3 The development of bowel ischaemia not present at recruitment (any past or current abnormality or disease affecting the mesenteric arteries is an exclusion criterion).

These SUSARs will be reported immediately to the Independent Safety Monitor to consider their attribution to the participant's participation in the trial and also to the Ethical Committee in accordance with local and national requirements.

For other serious adverse events, a summary will be provided to the Safety Monitor every 3 months and to the Chair of the DMEC every 6 months.

Procedure
The person who is first aware of the SAE/ SUSAR must notify the project manager / study co-ordinator immediately.

The investigator to be informed and to assist in completing the relevant documents. Where possible the investigator should clarify if the event was related to the trial intervention and assess the severity of the event.

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