Protocol

Study Title:	NeoCLEAR: **Neo**natal **C**hampagne **L**umbar punctures **E**very time – **A**n **R**CT. A multicentre, randomised controlled 2x2 factorial trial to investigate techniques to increase lumbar puncture success
Short title:	NeoCLEAR
Sponsor:	University of Oxford
Funder:	National Institute of Health Research – Health Technology Assessment (NIHR – HTA CET 15/188/106)
Clinical Trials Unit:	NPEU Clinical Trials Unit
Chief Investigator:	Prof Charles Roehr Academic Consultant Neonatologist, Oxford University Hospitals NHS Foundation Trust, Neonatal Unit, John Radcliffe Hospital, Oxford. OX3 9DU, and Associate Professor, University of Oxford, Department of Paediatrics Clinical Director, Clinical Trials Unit, National Perinatal Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Richard Doll Building, Old Road Campus, Oxford OX3 7LF
Identifiers:	REC Ref: 18/SC/0222 ISRCTN: 14040914

CI Signature:	Charles C Roehr Charles C Roehr (Jul 30, 2020 11:46 GMT+1)	Jul 30, 2020
Statistician Signature:	llinsell llinsell (Jul 30, 2020 12:00 GMT+1)	Jul 30, 2020

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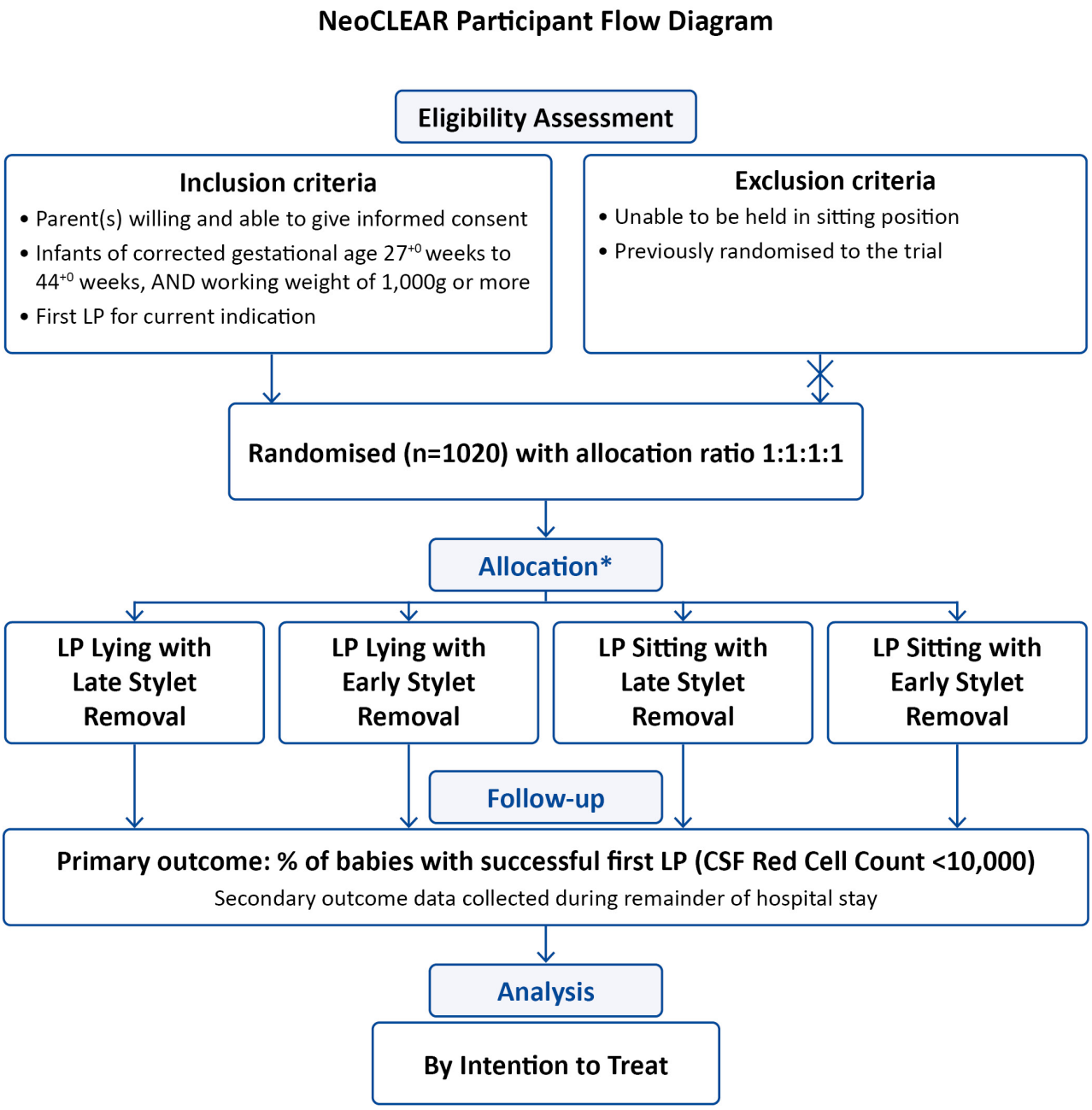


* The 4 groups form a 2x2 design comparing 2 modifications to LP technique: (i) Lying Vs Sitting; (ii) 'Late' Vs 'Early Stylet Removal'. The latter refers to the stylet being removed (from inside the needle) either: after reaching the expected CSF space (late); or soon after passing through the subcutaneous tissues (early) before advancement into the CSF space.

CSF = CerebroSpinal Fluid

2. SYNOPSIS

Study Title	NeoCLEAR: Neonatal Champagne Lumbar punctures Every time – An RCT. A multicentre, randomised controlled 2x2 factorial trial to investigate techniques to increase lumbar puncture success.
Short title	NeoCLEAR
Study Design	Multicentre 2x2 factorial RCT
Study Participants	Neonates and infants, of 27^{+0} weeks to 44^{+0} weeks CGA and 1,000 g or more, in neonatal units and their maternity wards who are requiring an LP and, for the pilot phase, a parent/guardian.
Intervention	Randomisation 1:1:1:1 into one of four LP techniques: (1) Sitting position and 'early stylet removal' (2) Sitting position and 'late stylet removal' (3) Lying position and 'early stylet removal' (4) Lying position and 'late stylet removal'
Planned Sample Size	≥1,020 infants
Award Time Period	1 September 2017 – 28 February 2021
Planned Study Period	1 June 2018 – 28 February 2021, including internal pilot and end of study analysis
Planned Recruitment Period	26 July 2018 – approximately 31 August 2020
Primary Objectives and Outcome Measures	To examine the optimal position of the infant, and timing of stylet removal, comparing the proportion of infants with successful LPs (RBC count fewer than 10,000/mm^3 in CSF on first procedure) when: • Sitting position is adopted versus lying position • ESR is performed versus LSR
Secondary Objectives and Outcome Measures	To investigate the effect of the sitting position compared to the lying position, and ESR compared to LSR on short-term clinical, resource, and safety outcomes, in terms of: - The proportion of infants with: o No CSF obtained, or pure blood/clotted, or blood-stained, or clear o CSF obtained and RBC count <500, <5,000, <10,000, or <25,000 /mm^3, or any RBC count o A CSF white cell count not requiring a correction (whatever the RBC count) - Total number of procedures and attempts performed per infant - Proportion of infants diagnosed (by WBC count criteria, culture, Gram stain, and/or clinically) via CSF with: o Meningitis: WBC count 20 or more in CSF, or a true positive culture/PCR (if RBC count is ≥500, the WBC count will be reduced by 1 for every 500 RBC counts to give a 'corrected' WBC count.)

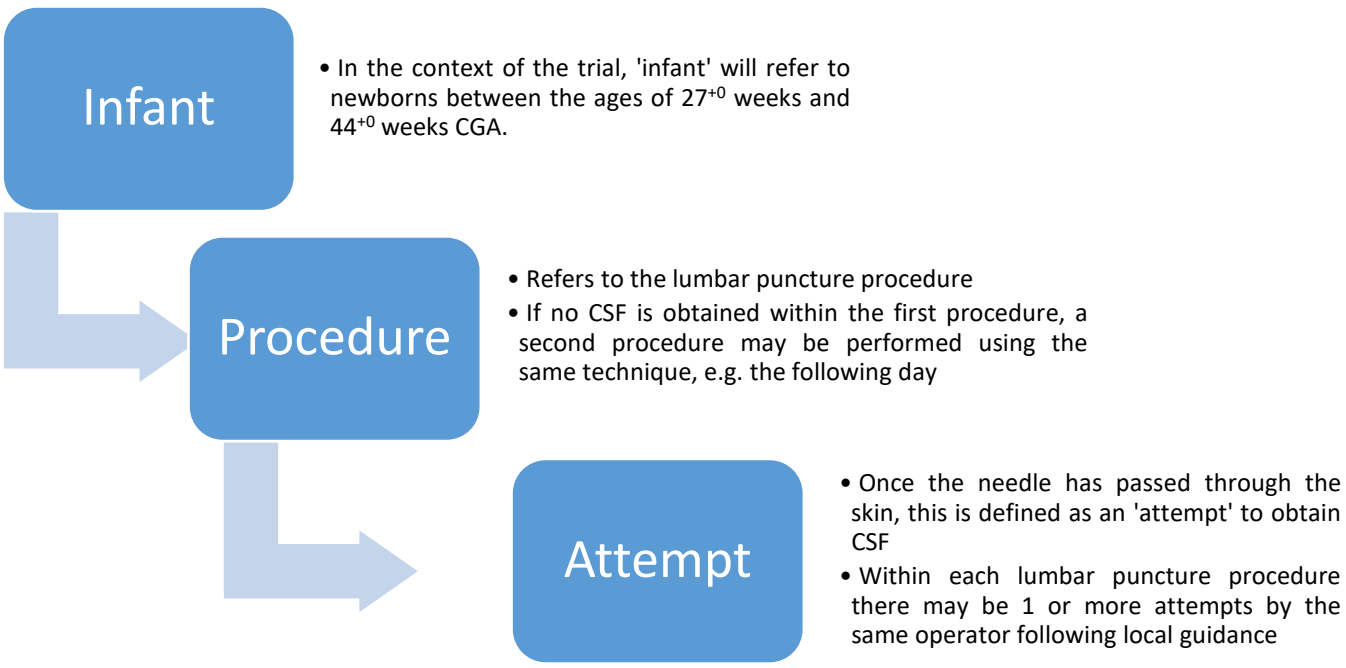
	○ Equivocal: WBC count (or corrected WBC) <20, AND negative (or contaminated/incidental) culture and PCR with: - Either PMN > 2 (and RBC count < 500) OR - Organism found on Gram stain ○ Negative: WBC (or corrected WBC) < 20, PMN ≤ 2 (if RBC < 500), and negative (or contaminated/incidental) cultures, PCR, and Gram stain ○ Uninterpretable: No CSF obtained, or clotted, or CSF so bloody or insufficient that a cell count was impossible - CSF WBC, RBC, corrected WBC counts, PMNs and lymphocytes from the clearest sample - Time taken on first procedure from start of cleaning skin to removing needle at end of all attempts - Infant movement on first procedure using a basic 4-point scale **Outcomes Related to Cost and Safety** - In all infants, according to CSF-defined and clinically-defined diagnostic criteria ○ Duration of the antibiotic course ○ Length of stay in surviving infants ○ Immediate complications related to LP - For the pilot phase: parental anxiety (STAI-S Questionnaire)

3. GLOSSARY

AE	Adverse Event
BPM	Beats Per Minute
CGA	Corrected Gestational Age
CI	Chief Investigator
(e)CRF	(electronic) Case Report Form
CSF	Cerebrospinal Fluid
CTRG	Clinical Trials and Research Governance, University of Oxford
CTU	Clinical Trials Unit
DMC	Data Monitoring Committee
ESR	Early Stylet Removal
GCP	Good Clinical Practice
HRA	Health Research Authority
LP	Lumbar Puncture

LSR	Late Stylet Removal
NPEU	National Perinatal Epidemiology Unit
NRES	National Research Ethics Service
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PMG	Project Management Group
PMNs	Polymorphonuclear Leukocytes
PPI	Patient/parent and Public Involvement
R&D	NHS Trust Research and Development Department
RBC	Red Blood Cells
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
STAI-S	State-Trait Anxiety Inventory Subscale
SSNAP	Support for Sick Newborns and their Parents
TSC	Trial Steering Committee
WBC	White Blood Cells

The following definitions apply within the context of the NeoCLEAR Trial:



4. BACKGROUND AND RATIONALE

4.1. Background and Scientific Justification

Each year 15,000–30,000 UK newborns undergo LP which is an essential diagnostic test for meningitis [2]. LP techniques vary in current practice, with no Level 1 evidence to determine the best approach. Thousands of infants have unsuccessful LPs requiring repeat procedures, causing distress to infants and parents, and often necessitating prolonged courses of antibiotics and hospitalisation for the mother and infant.

Neonatal meningitis has a high mortality and morbidity [1]. Symptoms and signs are subtle, and the diagnosis can only be confirmed by obtaining CSF via LP. At least 15,000 UK newborns per year require an LP. The procedure involves obtaining CSF from the lower spine for laboratory analysis to confirm the presence/absence of meningitis and to identify the causative organism. Infants with meningitis typically require 14–21 days of inpatient intravenous antibiotics, incurring significant financial costs, and often receive hospital follow-up due to the risk of long-term neurological sequelae [3]. Prolonged antibiotic use is associated with significant complications, such as necrotising enterocolitis [4] and a potential for the development of antibiotic resistance [5]. If meningitis can be excluded, antibiotics are usually stopped after five days, allowing discharge with no further follow-up.

Definitions of successful LP vary, but usually describe the acquisition of 'clear' CSF (colloquial medical term: a 'champagne clear tap'). However, in neonates CSF samples are usually pink/red due to RBCs sampled unintentionally from nearby blood vessels. Significant numbers of RBCs hinder CSF interpretation and the presence/absence of meningitis cannot be confirmed. Hence, the LP often needs repeating, and many infants are treated with extended courses of antibiotics because meningitis cannot be excluded. Repeated procedures and concern about meningitis understandably lead to heightened parental anxiety [6].

LP success rates are much lower in neonates (50–60%) [7,8] than older children (78–87%) [9,10]. Modifications to 'traditional' LP technique have been studied, but most data are observational with high risk of bias, so no improvement has been incorporated into routine practice.

An improved LP technique would result in:

- Fewer uninterpretable samples
- Fewer repeated attempts and procedures
- Reduced distress for infants and families
- Decreased antibiotic use and risk of antibiotic resistance
- Reduced NHS costs due to fewer procedures, reduced length of stay, shorter antibiotic courses, and minimised antibiotic-associated complications

This study is particularly timely, since recent NICE guidance [11], whilst aiming to avoid delays in diagnosing meningitis, has led many units to perform more LPs than previously [2,11]. This has consequently increased antibiotic use, at a time of global escalating concerns about antimicrobial

resistance caused by unnecessary antibiotics. Optimising the technique will therefore mitigate the impact of this change in practice on NHS resources. Secondly, as the dangers of antibiotic resistance become ever more pressing, technologies which have the capacity to reduce hospital antibiotic use, are invaluable in preventing antibiotic resistance problems in the future. Thirdly, it also follows the independent publication of a systematic review, highlighting the need for RCTs to investigate improved LP techniques [35].

If we demonstrate an improved LP technique, we anticipate its incorporation into clinical practice across the UK. Based on local cohort data [12], we expect a 10% improvement in LP success across the UK to translate, each year, into: 1,600 fewer infants having repeat procedures; 14,400 fewer doses of IV antibiotics (with fewer complications); and 2,680 fewer bed days for mothers and infants. Parental anxiety would be reduced, as would healthcare costs through reduced hospitalisation, antibiotic use, and improved efficiency of neonatal services. Finally, we would expect to limit the ongoing spread of antibiotic-resistant pathogens.

This will be an area of sustained interest to the NHS for decades to come. LP is the only diagnostic test for confirming meningitis, thus remaining essential in the foreseeable future of medical practice. This would be the first appropriately-powered RCT investigating neonatal LP technique and would therefore make a significant contribution to current knowledge.

4.2. Main research question

What is the optimal technique for lumbar puncture in newborns?

We will determine this by evaluating the success rate, short-term clinical, resource, and safety outcomes of two categories of LP technique; infant position and timing of stylet removal. Both have the capacity to reduce the number of procedures on newborns, enhance current diagnostics for neonatal meningitis, reduce unnecessary antibiotic use, minimise hospital length of stay, and limit stress and anxiety for infants and their families, thereby bringing broad benefits to the NHS immediately and for the foreseeable future.

4.3. Brief description of the intervention

The techniques used in this trial are modifications to 'traditional' lumbar puncture technique; lying position with LSR. There are several modifications in routine use, as detailed in the literature review below, but few have good evidence of benefit. Of all modifications identified, two appeared the most promising and amenable to investigation in an RCT:

- Sitting position, in which the infant is held in a sitting position compared to lying ('lateral decubitus') position
- ESR, which is the removal of the stylet from the hollow LP needle shaft once it has penetrated the subcutaneous tissue before advancing the needle into the CSF, compared to LSR, which is removal of the stylet once it has been inserted into the expected CSF space

4.4. Summary of findings from previous studies

There were no relevant systematic reviews or meta-analyses in children or neonates, before publication of a limited review in August 2016 [35]. Following recent NICE guidance which has

increased the frequency of neonatal LP nationally [2], the imperative to optimise this technique is now stronger than ever. The review published in August 2016 examined sitting position in both children and neonates and concluded that current evidence suggests, “Positions other than the lateral decubitus may be equal or superior in terms of lumbar puncture success” and, “Positions other than the lateral decubitus appear as safe”. The authors concluded, “A large-scale prospective clinical trial directly addressing LP success and safety in different positions would clarify the need to change current practice”. We had previously conducted a systematic review in neonates and children (summarised below), comparing ANY method for improving LP success rate.

Methods: Electronic databases were searched via Ovid: Medline (1946–present), Embase (1974–present) and Global Health (1973–present) on 1 February 2016. The search strategy included the keywords [[neonat* OR newborn OR pediatri* OR paediatr* OR infan*] AND “lumbar puncture”], and generated 56 records. Abstracts were screened for any studies comparing factors relating to LP technique, finding four of relevance. Outcomes included success rates or those predicting success, for example, number of attempts, anatomical benefits, and safety outcomes. Searching the bibliographies of the studies identified by the electronic search strategy, identified 21 further studies.

Results: Eight studies were RCTs and 17 were observational. Interventions/factors with no consistent evidence of significant benefit were: training in LP [13,14]; seniority of practitioner [9,10,15-18]; sedation [15,19]; local anaesthetic [9,10,14,17,20,21]; formulae for needle insertion depth (except certain subgroup analyses) [22]; and ultrasound assistance [23-25]. Sitting was as safe as lying [26-28], with increased space for LP needles [27,29-31] and CSF availability [32]. It was associated with a 25% higher chance of a successful first LP attempt in infants under 90 days (p=0.03) [33]. The hollow LP needle shaft contains a ‘stylet’. Most practitioners aim to insert the needle into the CSF space, then remove the stylet (LSR). If the needle has advanced too far, unintentional blood vessel puncture causes RBC contamination. With ESR the stylet is removed after passing through the subcutaneous tissues, and the needle slowly advanced until CSF flows. Two studies [9,10] found that early stylet removal compared with late stylet removal was associated with increased LP success (OR 2.4 (95%CI 1.1–5.2) and OR 1.3 (1.04–1.7), respectively).

Updated Search: The above search was repeated most recently on 7th May 2017. Three RCTs had been published; one [36] compared sitting and lying position in a paediatric A&E setting but recruited only 167 infants, and a statistically significant difference was not detected, leading the authors to conclude: “further studies are needed to establish stronger statistical power”. Our trial meets that recommendation, being appropriately powered, and complements this study by investigating a similar population (neonates), in whom the need for high quality evidence is greater, due to lower baseline success rates [7-10]. Two studies [41,42] with small sample sizes and/or wide confidence intervals found that ultrasound assistance was associated with increased LP success.

Other trials: The International Clinical Trials Registry was searched (last updated on 25th July 2017) with “lumbar puncture”, and screened as above. Trials listed were investigating: local anaesthetic (7 trials); ultrasound assistance (5); pressure transduction (2); restraint (1); sedation (1). None overlap with our proposal.

Conclusions: None of the currently practiced LP techniques are backed by adequately-powered, high quality evidence. Those warranting further investigation, which can be studied most efficiently and

reliably with an RCT, are: (1) sitting position and (2) early stylet removal. Both are free, 'existing technologies', already used by some practitioners. Previous evidence for these techniques has not changed routine clinical practice. Randomised evidence is now necessary to provide robust and convincing data. If either technique is beneficial, it would be free and easy to introduce nationwide.

4.5. Participant risks and benefits

All infants in the trial will be having LP as part of routine clinical care. All techniques are in current use and none is proven to be more or less safe than another. Therefore, no infants are expected to be placed at risk as a result of being in the trial. Serious complications of LP are rare, and are primarily reported in adults or older children. They include: infection; bleeding; backache; headache; transient paraesthesia; permanent nerve damage; brain herniation (even less likely in neonates due to a non-fixed intracranial volume). Some complications can be prevented by avoiding LPs in infants with contraindications such as coagulopathy; or mitigated, e.g. with sterile technique. Potential benefits of trial participation include: an optimised analgesia protocol; all infants will have heart rate and oxygen saturations monitored during the procedure; if one technique is more successful than another, infants randomised to that technique, are more likely to need fewer procedures, shorter courses of antibiotics and shorter duration of stay.

Consenting parents/guardians will be asked to complete the STAI-S questionnaire. The questionnaire will ask them about their feelings and levels of anxiety which are sensitive and upsetting topics. Our PPI group and previous research has indicated that parents in stressful situations, such as these will be, benefit from being given the opportunity to express their feelings. Completion of the questionnaire will not be pursued if a parent becomes distressed when completing it.

4.6. Setting and Target population

UK Local Neonatal Units and Neonatal Intensive Care Units and their associated maternity and postnatal wards.

Newborn infants who are having an LP and, for the pilot phase, a parent/guardian. Some of these infants may be older than 28 days (outside the strict definition of a 'neonate'). There are various indications for LP, but the most common is suspected/confirmed infection, usually in the first three days of life. Most of the infants will be born at term.

5. OBJECTIVES AND OUTCOME MEASURES

Objectives	Outcome Measures
Co-Primary Objectives • To compare the proportion of infants with successful LPs: o When sitting position is adopted compared to lying position	Proportion of infants with CSF obtained and RBC count < 10,000/mm^3 on the first LP procedure.

○ When ESR is performed compared to LSR	
Secondary Objectives To investigate the effect of sitting position compared to lying position, and ESR compared to LSR on short-term clinical, and safety outcomes	**Clinical outcomes:** • The proportion of infants with: ○ No CSF obtained, or pure blood/clotted, or blood-stained, or clear ○ CSF obtained and RBC count <500, <5,000, <10000, or <25000 /mm^3, or any RBC count ○ A CSF white cell count not requiring a correction (whatever the RBC count) • Total number of procedures and attempts performed per infant • Proportion of infants diagnosed (by WBC count criteria, culture, Gram stain, and/or clinically) via CSF with: ○ Meningitis: WBC count 20 or more in CSF, or a true positive culture/PCR if RBC count is ≥500, the WBC count will be reduced by 1 for every 500 RBC counts to give a 'corrected' WBC count). ○ Equivocal: WBC count (or corrected WBC) <20, AND negative (or contaminated/incidental) culture and PCR with: - Either PMN >2 (and RBC count <500) OR - Organism found on Gram stain ○ Negative: WBC (or corrected WBC) <20, PMN ≤2 (if RBC < 500), and negative (or contaminated/incidental) cultures, PCR, and Gram stain. ○ Uninterpretable: No CSF obtained, or clotted, or CSF so bloody or insufficient that a cell count was impossible • CSF WBC, RBC, corrected WBC counts, PMNs, and lymphocytes from the clearest sample

	• Time taken on first procedure from start of cleaning skin to removing needle at end of all attempts • Infant movement assessed using a basic 4-point scale, as utilised previously in another trial investigating neonatal LP success rates [18] **Outcomes related to cost** • In all infants, according to CSF-defined and clinically-defined diagnostic criteria: o Duration of the antibiotic course from trial entry to discharge home o Length of stay in hospital (for surviving infants) from trial entry until discharge home **Outcomes related to safety** • Immediate complications related to LP: o Cardiovascular instability peri-LP, including oxygen saturations and heart rate o Respiratory deterioration (requirement for escalating respiratory support) post-LP
Internal Pilot • To demonstrate trial processes for approaching parents, gaining consent, randomising, treating and assessing outcomes are optimal, and to implement improvements as required • To assess the feasibility of recruitment rates • To determine other attrition and feasibility metrics, including adherence to the protocol by practitioners, and uptake rate of eligible participants	• Survey responses of parents and clinicians, regarding inclusion/exclusion criteria, consent, randomisation, procedures, and assessing outcomes • Recruitment and attrition rate • Adherence to the protocol by practitioners, and other reasons for attrition • Uptake rate of parents of eligible infants • Parental anxiety (STAI-S)

6. STUDY DESIGN

This is a pragmatic, multicentre, 2x2 factorial RCT. Different units choose different criteria for requiring an LP, different practitioners perform LPs using slightly different techniques, and interpretations of the LP results can be variable. The trial is therefore pragmatic so that its results will be applicable to a wide range

of settings. It will also allow units to continue with their usual practice in certain aspects (such as criteria for who needs an LP) whilst standardising other aspects which could potentially affect/bias the outcomes by providing training and guidance documents for the LP procedure.

6.1. Internal pilot

The internal pilot will be based in all centres recruiting approximately the first 250 randomised infant participants in the first eight months. The purpose of the internal pilot is to optimise study processes around recruitment, intervention delivery, training, and outcome assessments. The TSC will review pilot data and make recommendations regarding continuation.

6.2. Sequence of study events

- All clinicians will receive training in the LP techniques to be used. A delegation log will identify which practitioners are suitably qualified to complete the first and second LPs.
- Once a decision is made to perform an LP by the clinical care team as part of routine treatment, parents of eligible infant participants will be approached to discuss consent to the trial.

Please note: the term parent will include legal guardian and apply for the remainder of this protocol.

- If consent to the trial is given, and the parents and clinical team are ready to proceed with the lumbar puncture, randomisation will proceed. The first LP should be performed in 1–2 attempts.
    - If a second LP is required due to an unsuccessful first procedure, the same technique will be employed in 1–2 attempts.
    - The need for any further procedures, as well as personnel and timing, will be determined by the consultant.
- Data will be recorded on trial specific (e)CRFs.

6.3. Data Collection

Baseline and follow up trial data will be collected in the form of (e)CRFs which research staff will enter onto the online database at site. Information for these (e)CRFs will be obtained from hospital administrative databases and clinical records.

All samples will be sent to the laboratory as per the usual procedure at site. Laboratory data will be analysed on site and the relevant information from the laboratory reports will also be entered into (e)CRFs.

7. PARTICIPANT IDENTIFICATION

7.1. Study Participants

Neonates and infants, of 27^{+0} to 44^{+0} weeks CGA and 1000 g or more, in neonatal units and their maternity wards who are having an LP and, for the pilot phase, one parent/guardian.

7.2. Inclusion Criteria

- Parent(s) willing and able to give informed consent
- Infants of corrected gestational age from 27^{+0} weeks to 44^{+0} weeks, AND working weight of 1,000 g or more
- First LP for current indication

7.3. Exclusion Criteria

- Unable to be held in sitting position (including infants intubated and mechanically-ventilated) or other clinical condition which is likely, in the opinion of the treating clinician, to make sitting difficult, or which is likely to be compromised by sitting (e.g. open gastroschisis).
- Previously randomised to the trial

8. STUDY PROCEDURES

8.1. SCHEDULE OF STUDY PROCEDURES

Tasks	Visits			
	Screening	First LP	Further LPs (if needed)	Discharge
Eligibility confirmed	X			
Informed consent	X			
Randomisation	X			
STAI-S (Parent Questionnaire)*	X	X		
Data collection to (e)CRF	X	X	X	X
Serious/Adverse Event assessments		X	X	

*Internal pilot sites to complete following consent (before first LP) and within 48hrs after first LP.

8.2. Screening and Eligibility Assessment

Infants with suspected infection who are having an LP, or for whom an LP is planned for other reasons, would be screened for eligibility. Screened infants will be recorded on Screening Logs at site. Anonymised screening data will be sent to the Coordinating Centre in Oxford to review rates of ineligibility and participant uptake rates. Eligibility will be further confirmed by the research team at

randomisation.

8.3. Recruitment

Parents of infants who have been identified as eligible for the trial would be provided with both verbal and written information in the form of a Parent Information Leaflet. Parents would be given this information by the clinical team when reviewing or treating the infant. Parents will have the opportunity to discuss the trial and ask any questions they may have.

8.4. Informed Consent

Parent(s) with legal parental responsibility of eligible infants would be approached to discuss the trial further and to request consent. Parent(s) will have as much time as they need to consider the information, and the opportunity to question the research team, or other independent parties to decide whether they will participate in the study. Informed consent for the study will be obtained by a suitably qualified member of the study team. A parent must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed. For parents completing the STAI-S, they will also be asked to sign and date the consent form to agree to participate themselves.

Ideally, consent will be given at such time to allow randomisation and enable the clinical team to prepare for that procedure. However, in instances where this is not possible, the LP will not be delayed if, in the opinion of the infant's clinician, any delay would be deemed clinically unsound: in such cases, the infant would not be recruited to the trial. The original signed form will be returned to the Trial Coordinating Centre at the National Perinatal Epidemiology Unit Clinical Trials Unit (NPEU CTU) within the University of Oxford.

8.5. Randomisation and Blinding

Infants will be randomised 1:1:1:1 to one of the four arms: (1) Sitting position and ESR, (2) Sitting position and LSR, (3) Lying position and ESR or (4) Lying (lateral decubitus) position and LSR, using a 24/7 secure web-based randomisation facility hosted by the NPEU CTU which will ensure balance between the groups. A telephone back-up system will be available 24 hours a day (365 days per year).

Stratified block randomisation will be used to ensure balance between the groups with respect to the collaborating hospital and corrected gestational age (CGA) at trial entry (4 groups: 27^{+0}–31^{+6}/32^{+0}–36^{+6}/37^{+0}–40^{+6}/41+ weeks). If repeat LPs are warranted for the same infant for the same indication after an initial unsuccessful attempt or procedure, they will receive the same allocated technique. Infants who have more than one indication for LP during the trial recruitment period will not be re-randomised. Multiples (twins etc.) will be randomised separately with their study ID numbers linked on the database prior to analysis.

A statistician independent of the trial at the NPEU CTU will generate the randomisation schedule and the Senior Trials Programmer will write the web-based randomisation program; both will be independently validated. The implementation of the randomisation procedure will be monitored by

the Senior Trials Programmer and independent statistician throughout the trial and reports will be provided to the Data Monitoring Committee.

This is an open-label trial as blinding of the practitioner and nursing staff to the allocated technique is not possible. The assessment of the primary outcome and major secondary outcomes will be based on laboratory tests. Parents will not usually be told which technique their infant has been allocated, and are not routinely present for the procedure, however if they request this will be shared with them.

8.6. Baseline Assessments

For eligible infants, clinical details will be collected at trial entry. This will include details to confirm eligibility and confirmation of parental written consent. During the pilot phase, the parent will be asked to complete a STAI-S questionnaire after consent but before the first LP: Consenting parents will be asked to report on a 5-point Likert scale 'How have you felt physically during the last couple of days?'. They will also be asked to complete The State Trait Anxiety Inventory State Subscale [43]. STAI-S is a well validated measure made up of 20 questions that identify how stressed/anxious someone is feeling at the time of assessment. Example items include "I am tense; I am worried" and "I feel calm; I feel secure." All items are rated on a 4-point scale (e.g. from "Not at all" to "Very much so"). Higher scores indicate greater anxiety. The STAI-S mean score will be used in analyses. Studies have shown that the STAI-S is a sensitive predictor of caregiver distress over time, and that it can vary with changes in support systems, health, and other individual characteristics [44]. Continuation of the STAI-S will be determined by the TSC based on a review of the pilot.

8.7. Subsequent Data Collection

Most outcome data for this trial are routinely recorded clinical items that can be obtained from the clinical notes or local laboratory records. Data which is not routinely collected includes procedural details such as the time taken and level of infant movement. Oxygen saturation and heart rate during the procedure will also be recorded.

No additional blood or tissue samples are required for this trial.

All data will be collected using trial specific data collection forms. Outcome data will be collected until the infant is discharged home.

In addition, parents in the internal pilot phase will be asked to complete a second short version STAI-S within 48 hours following the first LP.

8.8. Sample Handling

As per best practice, all samples from any LP attempt should be sent for microbiology, even if bloody or only 1–3 drops obtained, as culture may be informative. Standard lab analyses will be conducted.

8.9. Description of procedure

LP is the only test for diagnosing meningitis. It involves taking a small amount of spinal fluid from the lower back using a small needle. Analysis of the fluid confirms or excludes meningitis, enabling the best treatment to be determined.

All infants in this trial will require LP as part of routine care, but the trial will simply specify which randomised technique should be used, as well as attempting to standardise other potential confounders, including needle type and analgesia. Practitioners will be trained and given written best practice guidance on LP technique. This information will provide a recommendation for LP variables, however it will not be mandatory for all LP procedures to adhere to this. Adherence to guidance will be captured in the trial (e)CRFs.

The first LP should be performed within the same shift as randomisation where possible, to minimise bias, in 1–2 attempts. If a second LP is required due to an unsuccessful first procedure, the same technique should be employed, in 1–2 attempts. The need for any further procedures, as well as personnel and timing, will be determined by the consultant.

8.10. Discontinuation/Withdrawal of Participants from Study

Parents will have the right to withdraw their infant from the study at any time.

Parents who wish to discontinue with the study intervention will be asked for permission for the study team to complete data collection and follow-up. They may withdraw consent for any aspect of the study including future procedures and data collection. In addition, the treating clinician may discontinue a participant from the study at any time if they consider it to be in the best interests of the infant's health and wellbeing. Parents who have consented to both themselves and their infant's participation will be able to withdraw either or both consent.

8.11. Definition of End of Study

The end of the trial will be defined as the date when the trial database is locked. An end of trial declaration will be made to the approving REC.

9. SAFETY REPORTING

An independent DMC will be established to review the study data and outcomes including safety reports of SAEs. The DMC will ensure the safety and wellbeing of the trial participants and, if appropriate, make recommendations to the TSC regarding continuance of the study or modification of the protocol. The TSC will have ultimate responsibility for deciding whether the trial should be stopped on safety grounds. SAEs will be collected until the infant is discharged home, as SAEs occurring after this time point will not relate to the trial intervention. As parental participation is limited to the STAI-S questionnaire, no AE/SAE recording or reporting will be conducted for this group.

9.1. Adverse Events

An AE is any untoward medical occurrence observed in a participant, which may not have a causal relationship with the trial intervention. Due to the high incidence of AEs routinely expected in this infant patient population AEs will not be recorded for this trial.

9.2. Serious Adverse Events

An SAE is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect.

Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

9.3. Reportable Serious Adverse Events

The following are known, but rare, complications of LP. If they occur following the LP until the infant is discharged home they **are to be reported** as SAEs:

- Iatrogenic meningitis
- Iatrogenic haemorrhage: spinal haematoma (symptomatic), intraventricular, intracerebral and subarachnoid haemorrhage
- Cerebral herniation
- Nerve damage

9.4. Expected Serious Adverse Events

The following are serious adverse events that could be reasonably expected to occur in this population of infants during the course of the trial or form part of outcome data. They do not require reporting by trial centres as SAEs unless considered that they may be causally related to the trial procedure:

- Anaemia
- Clinically significant intracranial abnormality on cranial ultrasound scan – intracranial haemorrhage or white matter injury
- Chronic lung disease / Broncho pulmonary dysplasia
- Coagulopathy requiring treatment

- Death (unless related to LP technique)
- Difficulty establishing enteral feeding
- Failed LP resulting in prolonged hospitalisation
- Hyperbilirubinemia (jaundice)
- Hypoglycaemia
- Hypotension
- Hypoxic ischaemic encephalopathy
- Low sodium level/hyponatremia
- Non-iatrogenic meningitis
- Necrotising enterocolitis
- Patent ductus arteriosus
- Pneumothorax or air leaks
- Pneumonia
- Pulmonary haemorrhage
- Pulmonary hypertension requiring treatment
- Respiratory failure
- Retinopathy of prematurity
- Seizures
- Sepsis / infection
- Thrombocytopenia

Only if these events are thought to be causally related to the trial procedures would they require urgent reporting to the trial coordinating centre as SAEs, as outlined in section 9.6.

9.5. Unforeseeable Serious Adverse Events

SAEs which are not included in section 8.3 are regarded as unforeseeable. Unforeseeable SAEs which occur after consent until the infant is discharged home must be reported.

9.6. Reporting Procedures for Serious Adverse Events

Unforeseen SAEs and the SAEs associated with LP (section 9.3) must be reported immediately as soon as study staff become aware of the event. Study staff may use one of the following methods:

- Paper forms, with instructions, will be provided with the trial documentation to enable anyone to report an SAE. The completed SAE form must be emailed or faxed to NPEU CTU.

- Staff with access to the trial electronic database should complete the SAE form online. An automatic email notification to the NPEU CTU staff will be triggered for SAEs reported electronically. Once completed online, forms will be printed for the local PI to complete a causality review.

- Where the above routes are not possible, then the unforeseeable SAE may be reported to NPEU CTU by telephone and the SAE form will be completed by NPEU CTU staff.

If following the reporting of an SAE additional information becomes available, a new SAE form should

be completed with the details and emailed/faxed to NPEU CTU.

The NPEU CTU will forward a copy of the SAE form to the CI as soon as possible on receipt. The CI will assess whether the SAE was as a result of trial related activities (related). SAE reports assessed to be as a result of trial related activities will be sent by the NPEU CTU to the Sponsor and the DMC. All related unforeseeable SAEs should be submitted to the REC that gave a favourable opinion of the study within 15 working days of the CI becoming aware of the event, using the HRA report of serious adverse event form (see HRA website).

10. STATISTICS AND ANALYSIS

10.1. The Number of Participants

483 infants are required for each arm of the main comparison (sitting vs lying position and ESR vs LSR), to detect a 10% absolute difference (from 59% to 69%) in the proportion of infants with successful LPs, with 90% power, a 5% two-sided significance level and assuming (based on expert opinion and the lack of external evidence) no interaction effect between infant position and timing of stylet removal. Allowing for 5% attrition and replacement of infants who are randomised but do not receive an LP, the required recruitment target is a minimum of 1,020 (255 in each of the four groups). The 59% control event rate was derived from local prospective cohort data [12] and corroborated by published UK data [22]. We estimate 50% parental uptake rate from previous CTU and local trial experiences. We estimate minimal 'loss-to-follow-up' as all data will be collected pre-discharge. Very few infants will be transferred to a different hospital post-LP, and those hospitals may also be involved in trial as continuing care sites. Either way, the research nurses would contact the receiving hospital to find out details relating to the secondary outcomes.

Modelling suggests we will need 10 sites to recruit enough infants within 24 months, with a recruitment rate of around 5 infants per centre per month. Assumptions: (i) recruitment window lasting 24 months; (ii) staggered starts – training up two centres per month; (iii) each centre takes four months to reach stable recruitment rate. We have chosen Local Neonatal Units and Neonatal Intensive Care Units as they are more likely to have the required numbers of practitioners and patients to ensure reasonable recruitment. PMG will implement recruitment initiatives and increase the number of recruiting centres as required, based on actual recruitment.

Our inclusion/exclusion criteria are designed to minimise protocol deviations by minimising the number of infants included who cannot be sat up, or with whom there could be clinical reservations about sitting up. Only sparse safety data is available for LPs (in any position) under 27 weeks and under 1,000g, hence these infants will be excluded from our trial. Limited anonymised information on eligible infants not included in the trial will be collected using screening logs, to assess the representativeness of the trial population.

10.2. Description of Statistical Methods

Outcomes for participants will be analysed in the groups to which they are assigned regardless of deviation from the protocol or allocation received, but will be excluded from the analysis if no LP

procedure was received (modified intention-to-treat analysis). To assess the effect of sitting/lying position we will compare groups (1–Lying/LSR) plus (3–Lying/ESR) with groups (2–Sitting/LSR) plus (4–Sitting/ESR), and to assess the effect of the timing of stylet removal we will compare groups (1) plus (2) with groups (3) plus (4). We will calculate the risk ratio (95% CI) for the primary outcome (and all other dichotomous outcomes), the mean difference (95% CI) for normally distributed continuous outcomes or the median difference (95% CI) for skewed continuous variables. Absolute risk difference and confidence intervals will also be calculated for tested dichotomous clinical outcomes (to be presented in a supplementary appendix). Groups will be compared using regression analysis, adjusting for the stratification variables used at randomisation. Both crude and adjusted estimates will be presented but the primary inference will be based on the adjusted risk ratio analyses. Adjusted risk ratios will be estimated using log-binomial regression, or a Poisson regression model with a robust variance estimator in the event of non-convergence. Linear regression will be used for normally distributed outcomes and quantile regression for skewed continuous variables.

Due to the multiple number of procedures and attempts performed for each infant, and correlation between some outcomes, statistical inference will be restricted to a predefined list of tested outcomes. Summary data by trial arm will be provided for all other outcomes but statistical tests (or the calculation of confidence intervals) will not be performed.

10.2.1 Tested Outcomes

Clinical:

- Proportion of infants with CSF obtained and RBC count < 10,000/mm^3 on the first LP procedure
- Proportion of infants with:
    - No CSF obtained, or pure blood/clotted, or blood-stained, or clear CSF from clearest sample of the first procedure – any attempt
    - CSF obtained with any RBC count on first procedure – any attempt
    - CSF obtained with WBC count not requiring correction on first procedure – from any attempt (WBC count < 20 whatever the RBC count, or RBC count < 500)
- Proportion of infants diagnosed by the clinical team at discharge – in relation to their LP(s) - with:
    - Definite/probable meningitis
    - Possible meningitis or equivocal CSF result
    - Negative CSF result
    - Uninterpretable CSF result (e.g. very high RCC or clotted CSF)
    - No CSF obtained
- WBC count, RBC count, corrected WBC count, PMN, and lymphocytes from clearest CSF sample
- Total number of procedures performed per infant
- Total number of attempts performed per infant
- Time taken to complete the first procedure, from start of cleaning skin to removing needle at end of all attempts
- Level of infant struggling movement on first attempt of first procedure

Cost:

- Duration of the antibiotic course from trial entry to discharge home
- Length of stay in hospital in surviving infants from trial entry until discharge home

Safety:

- Immediate complications related to first procedure:
    - Procedure abandoned due to cardiovascular deterioration
    - Infant's lowest oxygen saturation (%)
    - Infant's lowest heart rate (BPM)
    - Infant's highest heart rate (BPM)
    - Respiratory deterioration post-LP (requirement for escalating respiratory support within 1 hour of the LP)

10.2.2 Untested Outcomes

Clinical:

- For the first attempt of the first procedure; first procedure (if not in 'tested' outcomes); first or second procedure:
    - CSF appearance (Clear CSF/Blood-stained/Pure blood or clotted/No sample obtained)
    - CSF obtained and any RBC count
    - CSF obtained and RBC count < 500/mm^3
    - CSF obtained and RBC count < 5,000/mm^3
    - CSF obtained and RBC count < 10,000/mm^3
    - CSF obtained and RBC count < 25,000/mm^3
    - CSF obtained with WBC count not requiring correction (WBC count < 20 whatever the RBC count or RBC count < 500)
- Number of attempts for first and second procedure per infant
- Proportion of infants diagnosed by CSF from first two procedures with:
    - Meningitis: WBC count 20 or more in CSF, or a true positive culture/PCR (if RBC count is ≥ 500, the WBC count will be reduced by 1 for every 500 RBC counts to give a 'corrected' WBC count)
    - Equivocal: WBC count (or corrected WBC) < 20, AND negative (or contaminated/ incidental) culture and PCR with:
        - either PMN > 2 (and RBC count < 500)
        - OR organism found on Gram stain
    - Negative: WBC (or corrected WBC) < 20, PMN ≤ 2 (if RBC < 500), and negative (or contaminated/ incidental) cultures, PCR, and Gram stain
    - Uninterpretable: No CSF obtained, or clotted, or CSF so bloody or insufficient that a cell count was impossible

Safety:

- Immediate complications related to second procedure:
    - Procedure abandoned due to cardiovascular deterioration
    - Respiratory deterioration post-LP (requirement for escalating respiratory support within 1 hour of the LP)

10.2.3 Subgroup Analysis

The consistency of the effect of position and timing of stylet removal on the primary outcome will be assessed across specific subgroups of infants using the statistical test of interaction or test of trend if

indicated.

The subgroup categories are:

- Working weight (g) at trial entry
  - <2500
  - 2500–3500
  - >3500
- Day of life
  - < 3 days
  - ≥ 3 days
- Corrected gestational age at randomisation
  - $27^{+0} – 31^{+6}$ weeks
  - $32^{+0} – 36^{+6}$ weeks
  - $37^{+0} – 40^{+6}$ weeks
  - ≥41 weeks

10.2.4 Interaction Testing

The interaction between sitting/lying position and the timing of stylet removal will be investigated for the primary outcome. Position and timing will be fitted as main effects and an interaction term between position and timing will be added to the model and tested using the likelihood ratio test. The estimated size of the interaction with 95% confidence interval will be reported. A descriptive multi-arm analysis will also be presented for the primary outcome, other tested outcomes, and baseline characteristics (i.e. for each of the four trial arms) as supplementary information [45]. We acknowledge that the trial is not powered to detect an interaction effect.

10.2.5 Interim Data Monitoring

Interim data monitoring will be carried out by the DMC at least annually. Further details about timings of reviews, contents of the reports and any stopping guidelines will be detailed in the DMC Charter.

11. DATA MANAGEMENT

11.1. Access to Data

Direct access to the study data, source data and medical records will be granted to authorised representatives from the NPEU CTU, Sponsor and host institution for the purposes of monitoring, audit, or inspection of the study to ensure regulatory compliance.

Site staff will have authenticated and restricted access to the clinical database ensuring they are only able to see data on participants recruited at their Trust. Access to the electronic data is strictly controlled using individual passwords for all staff accessing the electronic databases.

Source data documents are where data are first recorded, and from which participants' (e)CRF data are obtained, whether electronic or paper records. (e)CRF entries will be considered source data if the

(e)CRF is the site of the original recording (e.g. there is no other written or electronic record of data).

11.2. Data Handling and Record Keeping

Trial data will be collected using (e)CRFs and automatically transferred for storage in the secure clinical database (OpenClinica). The individual participant data will be identified by a study participant number only, parent and infant participants will share a study number to allow linking of the STAI-S questionnaire. Consent forms containing the infant and parent's names will be sent securely, via nhs.net email or pre-addressed envelopes, to NPEU CTU. All data will be processed in line with the NPEU CTU Data Management SOPs. The Sponsor has delegated the responsibility for ensuring confidentiality of participant information to the NPEU CTU.

Archiving will follow the completion of the study and publication of results as detailed in NPEU Standard Operating Procedures (SOPs) and in line with NHS guidelines for a minimum of 25 years. At this point, the requirements to continue to archive these data will be reviewed in line with the applicable data protection guidelines. Electronic files will be stored on a restricted access (named individuals) server held in a secure location. In line with the NPEU CTU security policy, authorised access to the NPEU CTU is via an electronic tag entry system and individual rooms are kept locked when unoccupied. Authorised staff will process data via a secure network which requires individual login name and password (changed regularly). No data are stored on individual workstations. The data is backed up automatically overnight to an offsite storage area accessed by authorised personnel via electronic tag and key-pad systems.

All paper and electronic data will be stored securely in strict compliance with current data protection regulations.

12. QUALITY ASSURANCE PROCEDURES

12.1. Monitoring

The PI will be responsible for the running of the trial at their site. This will include ensuring successful recruitment, staff education and training, and study data completeness and quality.

The NPEU CTU will develop a central monitoring plan for the trial, based on the risk assessment. Recruitment patterns at sites and within the data will be monitored. Any unexpected patterns, issues, or outlier data will be investigated and may trigger 'for cause' site monitoring. No other routine monitoring or auditing will be conducted unless the central monitoring triggers cause to do so.

12.2. Site Initiation and Training

Initiation visits at each participating neonatal unit will be performed by the trial Clinical Research Fellow, Clinical Lead or Advanced Neonatal Nurse Practitioner, and the Clinical Trial Manager or delegate, once HRA Approval has been obtained for that centre. Site staff will be trained on trial procedures, which will be recorded on Site Training Logs.

12.3. Risk Assessment

The trial will undergo a risk assessment prior to starting, which will be reviewed at regular intervals. This trial falls outside the MHRA remit as it is a comparison of standard procedures for LP and any drugs used in the course of the study are as part of standard care.

12.4. Project Management

The study is sponsored by the University of Oxford. The trial will be run by the NPEU CTU, based at the University of Oxford and the CI. On a day-to-day basis, the trial will be run by a PMG according to NPEU CTU SOPs and will be subject to audit and inspection. The core PMG will meet every month, either remotely or face-to-face. An extended PMG (Co-Investigator Group) will meet regularly to troubleshoot, review progress and forward plan. The PMG reports to the TSC.

The trial will be overseen by the TSC which will have ultimate responsibility for considering and, as appropriate, acting on the recommendations of the DMC. The TSC will include an independent chair, at least one clinician, statistician and PPI representative, and the CI. The TSC will meet at least annually and review the progress of the trial.

The DMC will be independent of the study and the TSC. The DMC will review the progress of the trial and interim analysis at least annually, and make recommendations on the conduct of the trial to the TSC.

13. ETHICAL AND REGULATORY CONSIDERATIONS

13.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

13.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

13.3. Approvals

The protocol, informed consent form, parent information leaflet and any proposed advertising material will be submitted and approval will be obtained from an NHS Research Ethics Committee (REC), through the Health Research Authority (HRA) approval system. In addition, Trust Confirmation of Capacity and Capability will be obtained prior to any trial activity at that site.

Where necessary, approvals will be obtained from the above parties for all substantial amendments to the original approved documents.

13.4. Reporting

The CI or delegate will submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required), host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

13.5. Participant Confidentiality

The infants' and parents' names will be shared with NPEU CTU via the consent form. Parents of infants participating in the trial will be informed of, and provide consent to this. No other personal identifiable information will be shared outside of the site.

Overall responsibility for ensuring that each participant's information is kept confidential will lie with the trial Sponsor. All paper documents will be stored securely and kept in strict confidence in compliance with current data regulations. Data collected on the (e)CRFs will be stored in an electronic database held by the Trial Co-ordinating Centre in which the participant will be identified only by a trial specific number.

After the trial has been completed and the reports published, the data will be archived in a secure physical or electronic location with controlled access.

13.6. Participant remuneration

No financial or material incentive or compensation will be provided to parents for enrolling their infant in this trial.

13.7. Other Ethical Considerations

Currently, infants are subjected to a variety of LP techniques without any particular technique being backed by high quality evidence. This trial will help ensure that LP technique is optimised in the future, potentially reducing the number of repeat attempts, reducing the amount of antibiotics prescribed, and reducing distress for infants and families.

- Any infants taking part in this trial should be receiving a standard of care equal or better than that currently provided, based on current evidence.
- There is equipoise about the best LP technique. Previous evidence for sitting and early stylet removal is largely observational and has not changed routine clinical practice.
- Only infants who would be having LPs as part of their routine care would be included in the trial. Practitioners already employ a variety of techniques, often without formal training – therefore none of the study procedures deviate from what would be regarded as routine clinical care.

- There is no evidence suggesting that either sitting/lying position or early/late stylet removal is less safe, or more distressing for infants. Potentially, if position or timing of stylet removal can improve the success rate of LP, it would actually reduce distress for infants due to not requiring as many repeat procedures.

- This trial will only involve non-urgent LP, so parents will not be rushed to consent. LPs are often delayed as part of routine practice for reasons of infant stability or clinical workload. When there is no significant change to clinical care required, LPs in the trial can similarly be delayed, giving parents enough time to consider consent, and clinicians enough time to take informed consent and perform randomisation.

- Parents who lack capacity to consent will not be approached about the trial.

- Training in the trial techniques will be provided to all practitioners.

- The analgesia recommended as part of the trial is in line with current best practice guidance.

14. FINANCE AND INSURANCE

14.1. Funding

This trial is funded by the National Institute of Health Research (NIHR) Health Technology Assessment (HTA) programme (ref: 15/188/106).

14.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

15. PUBLICATION POLICY

The success of the trial depends on a large number of neonatal nurses, neonatologists, and parents. Credit for the trial findings will be given to all who have collaborated and participated in the trial including all local co-ordinators and collaborators, members of the trial committees, the NeoCLEAR Co-ordinating Centre and trial staff. Authorship at the head of the primary results paper will take the form "[name], [name] and [name] on behalf of the 'The NeoCLEAR Collaborative Group'". The drafting of the paper will be the responsibility of a writing committee. All contributors to the trial will be listed at the end of the main paper, with their contribution identified. It is the intention of the NeoCLEAR Collaborative Group to publish the protocol and peer-reviewed articles including the analysis of key outcomes.

16. CONFLICTS OF INTEREST

Dr Manish Sadarangani, grant co-applicant and NeoCLEAR Investigator, was a co-investigator on an investigator-initiated research grant from Pfizer (2012–15).
There are no further conflicts of interest to declare.

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18. APPENDIX A: AMENDMENT HISTORY

Substantial Amendment No.	Updated Protocol Version No.	Date issued	Author(s) of changes	Summary of Changes Made
2	4	04/07/2019	Changes made on behalf of PMG.	Amendment of study timelines to align with the terms of the grant. Clarification of protocol to confirm that the Parent Questionnaire will not continue into the main trial. Consent section updated to clarify that only parents with legal parental responsibility will consent for their infants to participate in the

				trial. Addition of Appendix A: Amendment History
3	6	13/01/2020	Changes made on behalf of PMG	Clarification of wording. Update to CSF-based diagnostic criteria in line with definitions used in concurrent ChiMES study (www.encephuk.org/studies/ukchimes.aspx). Reference to 'short-version STAI' corrected to 'STAI-S'. Continuation of STAI-S following pilot determined by TSC, not DMC. Further detail and clarification in description of statistical method. Addition of table listing tested and untested outcomes.
4	7	30/07/2020	Changes made on behalf of PMG	Recruitment period and overall study award period extended. Updates throughout to reflect target recruitment of 1,020 as minimum rather than absolute total.