STUDY PROTOCOL PLAN

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1. GENERAL INFORMATION

1.1. Identification of the CIP

1.1.1. Title of the clinical investigation

Comparative analysis of aEEG recording performed with commercial electrodes and with the aCUP-E (advanced cup electrode) electrode in newborns at risk of developing encephalopathy.

1.1.2. Version control of the CIP

Reason for revision	Date	Version
First version	25/10/2024	001
Second version	08/07/2025	002
Third version	17/07/2026	003
Fourth version	27/07/2026	004
Fifth version	07/09/2026	005

1.1.3. Amendment revision history

Reason for revision	Date	Version
Modifications proposed by the Committee in Version 1, resulting in Version 2 1. It is necessary to draft the beginning of the Information Sheet so that it is clear that the intervention is performed on the baby. The current wording does not clearly specify who is included in the study and must be adapted considering that consent must be signed by the legal guardian. 2. The informed consent states that the results could be shared with companies; in this case, explicit consent should be obtained for this point, for example, through an additional checkbox that the participant can tick.	08/07/2025	002

3. It should be specified that the study will also be conducted at Hospital Joan XXIII. 4. If the study is conducted at both centres, the corresponding Information Sheet and Informed Consent for HJ23 must be attached. 5. The Participant Information Sheet does not indicate who is responsible for data processing. Each centre must be responsible for the data of its patients, or it must be stated that they are joint controllers, especially considering that the investigator responsible for the study is a physician at both centres. 6. The data retention period must be 25 years, since this is a clinical trial involving a medical device. 7. In the protocol, in the section Residual Risks Associated with the Investigational Product, the pending information must be completed.		
Changes in the following chapters of Version 2 in response to the request to remedy deficiencies in Clinical Investigation No. 168/26/EC-R received from AEMPS on 29/06/2026, resulting in Version 3.4 • TABLE OF CONTENTS: pagination is updated due to the modifications made. • 3: the justification of the clinical investigation design is better explained. An Annex (Annex 25) is also added with information and results from preclinical trials. Sections are also removed and modified to ensure consistency between this chapter and what is indicated in	17/07/2026	003

Chapters 3 and 4 of Annex 8, Investigator's Manual. • 4: the expected clinical benefits of the product are better explained. The wording is also improved to facilitate better understanding. • 6.1: more information is added on the study design. • 6.2.1: some technical and functional characteristics are corrected, and the number of participating subjects is corrected. • 6.4: more information is added on the procedures. • 6.5: more information is provided on the monitoring plan. • 7: the statistical considerations are improved. • 9: more information is provided on amendments to the CIP, with reference to Article 75 of MDR. • 10: information on deviations from the CIP is clarified. • 12: a statement of compliance with the requirements for clinical investigations under MDR, Royal Decree and ISO 14155 is added. • 13: more information is provided on the informed consent process. • 14: more information is provided on adverse events, adverse device reactions and device deficiencies, particularly regarding the reporting process with reference to Article 80 of MDR. • 15: more information is provided on the vulnerable population. • 16: more information is provided on the completion, suspension or termination of the trial, with reference to Article 77 of MDR. • 17: the reference to the repealed Royal Decree 223/2004 is corrected.		
Changes in the following chapters of Version 3 in response to the request to remedy	27/07/26	004

deficiencies in Clinical Investigation No. 168/26/EC-R received from AEMPS on 23/07/2026, resulting in Version 4.4 • TABLE OF CONTENTS: pagination is updated due to the modifications made. • 6.1: the section Impact of redistribution on the scientific validity of the study is added to the study design information section.		
Changes to the following chapters of Version 4 in response to the request to remedy deficiencies in Clinical Investigation No. 1648/26/EC-R received from the AEMPS on 19/08/2026, resulting in the generation of Version 5. · **TABLE OF CONTENTS:** the pagination has been updated due to the modifications made. **6.1:** Section 6.1 has been updated to explicitly indicate the minimum number of patients in Cohort 2 in the event of redistribution of patients between cohorts, as well as the criteria applicable to the redistribution of patients between cohorts.	07/09/26	005

1.2. Sponsor

Name:

Salut Sant Joan de Reus – Baix Camp

Address:

Avinguda del Doctor Josep Laporte, 2, 43204 Reus, Tarragona

Telephone: 977 31 03 00 FAX: NO e-mail: vicenc.pascual@salutsantjoan.cat

Contact person designated by the sponsor:

Name:

Vicenç Pascual Rubio

Address:

Avinguda del Doctor Josep Laporte, 2, 43204 Reus

Telephone: 620 75 33 81 FAX: NO e-mail: vicenc.pascual@salutsantjoan.cat

1.3. Principal investigator, coordinating investigator and investigation site(s)

a) Principal investigator

Vicenç Pascual Rubio Clinical Neurophysiology Salut Sant Joan de Reus – Baix Camp Tel: 977310300
e-mail: vicenc.pascual@salutsantjoan.cat

- The investigator undertakes to conduct the clinical investigation in accordance with the ethical principles originating from the Declaration of Helsinki.
- The principal investigator also undertakes to comply with this international standard and any applicable regional or national regulations.
- The principal investigator undertakes not to commence the clinical investigation until approval/favourable opinion has been obtained from the EC and the required regulatory authority.
- The principal investigator declares that any additional requirements imposed by the EC or regulatory authority, if applicable, will be complied with.

The Curriculum Vitae of the Principal Investigator, as well as those of the other collaborating investigators, can be consulted in Annex 23.

b) Investigation site

- Name: Salut Sant Joan de Reus – Baix Camp
- Address: Avinguda del Doctor Josep Laporte, 2, 43204 Reus
-Telephone, Fax, E-mail: 620753381, vicenc.pascual@salutsantjoan.cat

- Name: Hospital Universitari de Tarragona Joan XXIII
- Address: Calle Doctor Mallafre Guasch 4, 43005 Tarragona
-Telephone, Fax, E-mail: 620753381, vicenc.pascual@salutsantjoan.cat

1.4. Funding method

The clinical investigation is funded by Salut Sant Joan de Reus – Baix Camp, which acts as the sponsor. The hospital has funded the expenses arising from the Ethics Committee, insurance policy and personnel.

1.5. Global synopsis

Title	Comparative analysis of aEEG recording performed with commercial electrodes and with the aCUP-E (advanced cup electrode) electrode in newborns at risk of developing encephalopathy.
Objectives	To compare the new aCUP-E electrode, specifically designed for amplitude-integrated electroencephalography (aEEG) in the Neonatal Intensive Care Unit (NICU), with the electrodes currently used in clinical practice. This study is based on published results demonstrating that aCUP-E provides superior recording in neurologically healthy newborns and aims to assess whether these benefits are maintained in prolonged recordings of newborns at risk of encephalopathy. In addition, monitoring these newborns will allow possible subclinical abnormalities that may initially have gone unnoticed to be detected, thereby contributing to early diagnosis and improved neurological management.

Description and study design	This multicentre, observational, prospective and comparative study aims to validate the use of the new aCUP-E electrode for aEEG monitoring in newborns admitted to the NICU, comparing it with conventional electrodes currently used at two hospitals: Salut Sant Joan de Reus – Baix Camp and Hospital Universitari de Tarragona Joan XXIII. A total of 35 newborns at risk of encephalopathy will be recruited. During aEEG monitoring, one type of electrode will be placed on each cerebral hemisphere: on one hemisphere, the standard electrode normally used according to each hospital's protocol, and on the other, the new aCUP-E electrode. This design allows direct comparison under similar conditions to assess recording quality, reliability and ease of use. The multicentre methodology also facilitates a more representative and diverse sample, increasing the robustness and generalisability of the results. This study seeks to determine whether aCUP-E can replace conventional electrodes, offering a safe and effective alternative that improves the quality of neonatal neurological monitoring and adapts to different clinical contexts.
Inclusion - exclusion criteria	Inclusion criteria: For participant selection, the inclusion criteria will be as follows: ● Preterm or term newborns admitted to the NICU who are at risk of encephalopathy due to conditions arising from the clinical context. For example, prematurity, maternal infection (e.g. maternal infections such as fever during labour, urinary tract infection, etc.), maternal history of gestational diabetes, gestational hypertension or preeclampsia, history of delivery complications such as placental displacement or placentation.

	● Informed consent obtained from the parents or legal guardians of the newborns. ● Availability of aEEG monitoring equipment and aCUP-E electrodes during the study period. Exclusion criteria: The exclusion criteria are established to avoid monitoring complications or obtaining inconsistent data: ● Newborns with evident signs of encephalopathy or any other documented neurological abnormality. ● Newborns with critical or unstable medical conditions that interfere with monitoring, such as the need for continuous mechanical ventilation or significant haemodynamic instability. ● Newborns receiving medication that may alter brain activity and interfere with aEEG results. ● Newborns with cranial or skin malformations that prevent proper electrode placement. ● Situations requiring urgent interventions that may interrupt monitoring or affect the quality of the data collected. ● Lack of informed consent from the parents or legal guardians of the newborns to participate in the study.

2. IDENTIFICATION AND DESCRIPTION OF THE PRODUCT

a) Summary description of the product and its intended purpose

The aCUP-E® advanced cup electrode has been specifically designed to capture superficial cerebral bioelectrical activity during long-term recordings with monitoring equipment in the neonatal intensive care unit (NICU), such as amplitude-integrated electroencephalography (aEEG). Its intended use is particularly indicated when good adherence to the baby's scalp, easy replacement of conductive gel, maintenance of low and stable impedance, and more comfortable handling of the newborn by medical staff are required.

The new aCUP-E electrode has the same safety and bioelectrical signal quality compared with liquid-gel electrodes. Its advantages include good adherence, maintenance of low and stable impedance (reducing the number of artefacts), more comfortable handling of the newborn by healthcare staff, and replacement of the conductive gel.

b) Details about the manufacturer

The product was developed by a university:

Corporate name: Universitat Rovira i Virgili
Tax identification number: Q9350003A
Telephone: 977558000
Registered office: Calle Escorxador, S/N, Tarragona, 43003, Tarragona
Email: info@urv.cat
Website: www.urv.cat
Corporate purpose: University education
Legal form: Other public bodies
Activity: Higher education
CNAE activity: 8543 - University education

c) Model/type name or number, including accessories, if any.

Name	Model number	Accessories
aCUP-E	aCUP-E_v2	5 electrode connection cables to a universal monitor (0.7/1.5 mm touch-proof)

d) Description of traceability during and after the clinical investigation (e.g. through assignment of batch or serial numbers).

Product traceability will be carried out using a batch and serial number assignment system.

Each batch refers to a set of identical products sharing certain production characteristics, such as material, time, date and place of production.

The serial number is unique to each product and makes it possible to distinguish it from identical products generated for the same batch.

e) Populations and intended indications for the investigational product.

The population targeted by the product consists of patients in the neonatal intensive care unit (NICU). The intended indications for the product are brain studies in newborns, such as amplitude-integrated electroencephalography (aEEG). Its intended use is particularly indicated when good adherence to the baby's scalp, easy replacement of conductive gel, maintenance of low and stable impedance, and more comfortable handling of the newborn by medical staff are required.

f) Description of the investigational product, including any material that will be in contact with tissues or bodily fluids.

Two materials that will be in contact with the body have been identified: (1) Adhesive between the electrode and the skin, (2) Gel that ensures the electroconductivity of the bioelectrical signal between the adhesive and the skin.

The adhesive has various safety studies conducted by the manufacturer, as well as details of its composition. Regarding the conductive gel, one of the brands that may be used is ECI. Information on its composition is also provided in Annex 14.

The materials used that will be in contact with the skin are intended for medical use and comply with biological safety and biocompatibility requirements. All documentation relating to biocompatibility, technical data sheets and safety studies can be found in Annex 14. Further details of the product can be found in section 2.

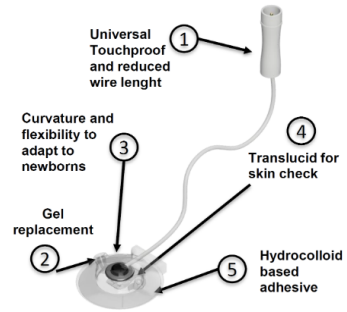
g) Summary of the training and experience required to use the investigational product.

The product has been designed to meet unmet needs and clinical challenges associated with existing electrodes, as experienced by Dr. Vicenç Pascual and the clinical staff at the hospital with commercial solutions. The training required to use it is the same as that required for the use of commercial electrodes currently used in clinical practice. This requires a healthcare professional with clinical training and experience in correctly placing these electrodes.

h) Description of the specific medical procedures used with the investigational product.

No surgical procedures are used with the product. The medical procedure followed consists of the following steps, which can be found in the User Manual in Annex 12:

1. Put on gloves. The use of gloves is mandatory.
2. Clean the application areas with mild soap and water and dry them completely.
3. Correct electrode placement is normally determined by the hospital/physician and/or local protocol/procedures.
4. Protective liners may have sharp corners, and care must be taken when removing the electrodes from the pouch. Hold the electrode in your hand for 1 minute to warm the adhesive and achieve better adhesion. Remove the electrode from the protective liner and place it on the skin. Discard the protective liner immediately after removing the electrodes.
5. Place the electrode (Fig. 1) on the skin by gently pressing around the adhesive edge (Fig. 1, Part 4) with a gentle, firm circular motion.
6. Connect the lead wire (Fig. 1, Part 1) to the EEG monitoring system.
7. Fill the electrode cup with gel through the opening (Fig. 1, Part 3).
8. The skin can be inspected through the transparent housing (Fig. 1, Part 4).
9. Electrode removal is performed using the usual clinical procedure, which may be carried out using the spray method with a clinically certified removal agent.
10. Dispose of the electrodes in the biohazard container, as well as the paper towels used to remove any remaining gel residue.



1. Universal touch-proof connector capable of connecting to all devices.
2. Wire coil to facilitate movement without detachment.
3. The conductive gel fills the opening without detaching the electrode.
4. Hydrocolloid-based adhesive for delicate skin, long recordings and good adherence.

Figure 1. aCUP-E electrode

3. JUSTIFICATION OF THE CLINICAL INVESTIGATION DESIGN

3.1. Evaluation of preclinical trials/studies

3.1.1. Design calculation results

The clinical investigation is based on the evaluation of the safety, usability and comparison of the recording obtained between an electrode (aCUP-E) developed by the research group and commercial electrodes (surface and subdermal needle). The development of the aCUP-E electrode was driven by unmet needs and clinical challenges associated with existing electrodes, as experienced by Dr. Vicenç Pascual and the clinical staff at the hospital with commercial solutions. There are also numerous publications describing the difficulty of performing aEEG due to problems with electrodes:

1. Foreman, SW, Thorngate, L., Burr, RL, & Thomas, KA (2011). Electrode Challenges in Amplitude-Integrated Electroencephalography (AEEG): Research Application of a Novel Noninvasive Measure of Brain Function in Preterm Infants. Biological Research For Nursing, 13 (3), 251-259. https://doi.org/10.1177/1099800411403468https://doi.org/10.1177/1099800411403468
2. El Ters, NM, Mathur, AM, Jain, S., Vesoul, ZA, & Zempel, JM (2018). Long term electroencephalography in preterm neonates: Safety and quality of electrode types. Clinical Neurophysiology, 129 (7), 1.366 to 1.371. https://doi.org/10.1016/j.clinph.2018.02.129https://doi.org/10.1016/j.clinph.2018.02.129
3. Cordeiro, M., Peinado, H., Montes, MT, & Valverde, E. (2020). Evaluation of the

suitability and clinical applicability of different electrodes for AEEG / CEEG monitoring in the extremely premature newborn. Anales de Pediatría. https://doi.org/10.1016/j.anpedi.2020.09.009https://doi.org/10.1016/j.anpedi.2020.09.009

4. Lloyd, R., Goulding, R., Filan, P., & Boylan, G. (2015). Overcoming the practical challenges of electroencephalography for very preterm newborns in the neonatal intensive care unit. Acta Paediatric, 104 (2), 152-157. https://doi.org/10.1111/apa.12869https://doi.org/10.1111/apa.12869

In single-use commercial adhesive electrodes, the gel cannot be replaced, and in most cases, adhesion is inadequate due to their rigidity and the curvature of a newborn's cranial surface. Consequently, recording is interrupted or has insufficient quality for clinical evaluation of the newborn's brain activity. To achieve even minimal monitoring with commercial surface electrodes, they are periodically replaced. This involves greater handling and possible changes in recording conditions (location, impedance, etc.). Our design allows the gel to be safely refilled in a way that also makes it possible for staff to handle the baby's position easily.

On the other hand, commercial needle electrodes are invasive and cause wounds to the baby's skin; therefore, they are also not the best option for monitoring neonatal brain activity.

The aCUP-E electrode was developed through preclinical laboratory testing and an initial clinical trial at HUSJR (“AMPLITUDE-INTEGRATED ELECTROENCEPHALOGRAPHY (AEEG): COMPARATIVE ANALYSIS BETWEEN LIQUID-GEL ELECTRODES AND A NEW CUP ELECTRODE, ADVANCED CUP ELECTRODE (ACUP-E).”, file no. 918/21/EC-R). The initial results were published in Frontiers in Pediatrics: Analysis of electrode performance on amplitude integrated electroencephalography in neonates: evaluation of a new electrode aCUP-E vs. liquid gel electrodes. Frontiers in Pediatrics. Volume 12 - 2024. https://www.frontiersin.org/journals/pediatrics/articles/10.3389/fped.2024.1452862. DOI 10.3389/fped.2024.1452862. ISSN=2296-2360

Annex 25 includes the results of the preclinical studies, in which different medical adhesives were mainly evaluated to select the most suitable one for aCUP-E, and measurements of the recorded signal were also performed to evaluate the electrode design. In a subsequent step, skin-electrode impedance was analysed. Among the studies carried out, the Biomedical Engineering final degree project entitled “Characterization of an electrode (aCUP-E) adhesion system to perform aEEG in newborns”; the paper presented at the mechanical engineering conference entitled

"Comparative analysis between commercial electrodes and a new electrode for newborns"; and the Biomedical Engineering final degree project entitled "ANALYSIS OF THE SKIN-ELECTRODE IMPEDANCE FOR NEONATAL aEEG" are included.

The aCUP-E® advanced cup electrode has been specifically designed to capture superficial cerebral bioelectrical activity during long-term recordings with monitoring equipment in the neonatal intensive care unit (NICU), such as amplitude-integrated electroencephalography (aEEG). Its intended use is particularly indicated when good adherence to the baby's scalp, easy replacement of conductive gel, maintenance of low and stable impedance, and more comfortable handling of the newborn by medical staff are required.

The brain function monitor to which the electrode is connected allows us to determine the functional state of the brain from the time recording of bioelectrical activity originating in brain neurons. For this recording, electrodes are placed on the surface of the neonate's scalp. The electrodes detect the electrical activity and transmit it to the amplifiers located in the CFM. The test allows established brain abnormalities or abnormalities that may appear during the neonatal period to be detected.

3.1.2. Animal safety study

The product did not require an animal safety study, since the parts that will be in contact with the body already exist commercially and have not been modified. In addition, the product only records the electrical activity generated by the body itself in a non-invasive manner, and it does not perform any type of stimulation or have an associated power source for its operation.

3.1.3. Biological safety and biocompatibility

Two materials that will be in contact with the body have been identified: (1) Adhesive between the electrode and the skin, (2) Gel that ensures the electroconductivity of the bioelectrical signal between the adhesive and the skin.

The adhesive has a medical-use certificate and various safety studies conducted by the manufacturer, as well as details of its composition. Regarding the conductive gel, the gel most commonly used in clinical neurophysiology laboratories is used and has been widely used. Documentation for the materials can be found in Annex 14.

The materials used that will be in contact with the skin are intended for medical use and comply with biological safety and biocompatibility requirements. All documentation relating to biocompatibility, technical data sheets and safety studies can be found in Annex 14.

3.2. Evaluation of clinical data

A clinical validation approved by AEMPS has been carried out at Salut Sant Joan de Reus – Baix Camp, sponsor of the clinical investigation "AMPLITUDE-INTEGRATED ELECTROENCEPHALOGRAPHY (AEEG): COMPARATIVE ANALYSIS BETWEEN LIQUID-GEL ELECTRODES AND A NEW CUP ELECTRODE, ADVANCED CUP ELECTRODE (ACUP-E).", file number: 918/21/EC-R.

The results have also been published in the following Frontiers article demonstrating the feasibility of aCUP-E:

> *Analysis of electrode performance on amplitude integrated electroencephalography in neonates: evaluation of a new electrode aCUP-E vs. liquid gel electrodes. Frontiers in Pediatrics. Volume 12 - 2024.*
>
> Fabregat-Sanjuan Albert, Rodríguez-Ballabriga Ángel, Rigo-Vidal Agnès, Pàmies-Vilà Rosa, Larrosa-Capaces Susana, Rius-Costa Vanesa, Pascual-Rubio Vicenç.
>
> https://www.frontiersin.org/journals/pediatrics/articles/10.3389/fped.2024.1452862. DOI 10.3389/fped.2024.1452862. ISSN=2296-2360

4. RISKS AND BENEFITS

Unlike current cup electrodes, the new cup electrode (aCUP-E) is elastic, with firm and secure adherence to the scalp, and features that allow comfortable handling of the newborn by healthcare staff.

Expected clinical benefits

1. Excellent signal quality: This was demonstrated in the previous study (AEMPS Ref.: 918/21/EC-R).

2. Reduction of artefacts: It allows good assessment of HIE. It helps to assess whether therapy is still appropriate, such as antiepileptic treatments. It captures a higher-quality signal, and monitors could therefore achieve better results.

3. Improved detection of skin irritation: The translucent electrode allows irritation to be assessed without removing the electrodes.

4. Improved recognition: Better recognition of pathological electroencephalographic patterns (epileptic discharges) due to improved signal quality.

5. Less intervention on the baby: Removal and repositioning of electrodes is not necessary compared with conventional electrodes (liquid gel and subdermal needle).

Expected adverse effects of the product

A possible adverse event associated with the use of surface electrodes is skin irritation, skin redness, skin itching and small blisters.

Residual risks associated with the investigational product

A risk analysis has been performed, and measures have been taken to control or mitigate the risks.

For each residual risk, a benefit-risk analysis is performed:

OBJECT/ FUNCTION	HAZARD AND HAZARDOUS SITUATION	BENEFIT-RISK ANALYSIS (Y/N)
1	Liquid entering the signal monitoring equipment	The residual risk is accepted, and the risk management team accepts that the benefits that may be obtained by using the electrode outweigh the possible risks that may be encountered. Likewise, as actions to minimise risks, it is decided to add: • Safety information, • Verify, through a usability study in accordance with the applicable regulations, the additional control measures implemented.
2	Deterioration of the electrode's electrical insulation	The residual risk is accepted, and the risk management team accepts that the benefits that may be obtained by using the electrode outweigh the possible risks that may be encountered. Likewise, as actions to minimise risks, it is decided to add: • Protective measures in the product, and • Verify, through a usability study, its implementation in accordance with the applicable regulations and the additional control measures implemented.

3	Creation of electromagnetic fields by the signal monitoring equipment	The residual risk is accepted, and the risk management team accepts that the benefits that may be obtained by using the electrode outweigh the possible risks that may be encountered. Likewise, as actions to minimise risks, it is decided to add: • Safety information, • Protective measures in the product, and Verify, through a usability study, its implementation in accordance with the applicable regulations and the additional control measures implemented.
4	The electrode becomes contaminated when handled by clinical staff and may be a source of infection	The residual risk is accepted, and the risk management team accepts that the benefits that may be obtained by using the electrode outweigh the possible risks that may be encountered. Likewise, as actions to minimise risks, it is decided to add: ● Safety information, ● Carry out PMS and PMCF activities under real-use conditions.
5	An allergic reaction occurs in the patient because of the electrode contact material being in contact with the patient	The residual risk is accepted, and the risk management team accepts that the benefits that may be obtained by using the electrode outweigh the possible risks that may be encountered. Likewise, as actions to minimise risks, it is decided to add: • Protective measures in the product, and • Verify, through a usability study, its implementation in accordance with the applicable regulations and the additional control measures implemented.
6	Redness and irritation of the patient's skin occur	The residual risk is accepted, and the risk management team accepts that the benefits that may be obtained by using the electrode outweigh the possible risks that may be encountered. Likewise, as actions to minimise risks, it is decided to add: ● Safety information, ● Verify, through a usability study in accordance with the applicable regulations, the additional control measures implemented.
7	Liquid entering the electrode	The residual risk is accepted, and the risk management team accepts that the benefits that may be obtained by using the electrode outweigh the possible risks that may be

		encountered. Likewise, as actions to minimise risks, it is decided to add: ● Safety information, ● Carry out PMS and PMCF activities under real-use conditions.
8	High temperature may cause the electrodes to malfunction	The residual risk is accepted, and the risk management team accepts that the benefits that may be obtained by using the electrode outweigh the possible risks that may be encountered. Likewise, as actions to minimise risks, it is decided to add: ● Safety information, ● Carry out PMS and PMCF activities under real-use conditions.
9	The clinician does not know how to use the electrodes correctly	The residual risk is accepted, and the risk management team accepts that the benefits that may be obtained by using the electrode outweigh the possible risks that may be encountered. Likewise, as actions to minimise risks, it is decided to add: ● Safety information, ● Verify, through a usability study in accordance with the applicable regulations, the additional control measures implemented.
10	Skin ulcers are created when the electrodes are removed from the patient at the end of the test	The residual risk is accepted, and the risk management team accepts that the benefits that may be obtained by using the electrode outweigh the possible risks that may be encountered. Likewise, as actions to minimise risks, it is decided to add: ● Safety information, ● Verify, through a usability study in accordance with the applicable regulations, the additional control measures implemented.

Justification of the positive benefit-risk balance

After performing a benefit-risk analysis for each of the risks identified with the risk team, it is concluded that:

1. No risks have been identified that are considered “unacceptable”.
2. No risk has been identified in which the risk exceeds the benefit.

Therefore, the risk management team concludes that the aCUP-E product is safe overall and that monitoring will be carried out to detect and analyse potential emerging risks, as

well as to evaluate the verifications performed during the clinical trial through a usability study in accordance with the applicable regulations.

5. OBJECTIVES AND HYPOTHESES

5.1. Objectives

General objective:

To evaluate the efficacy of the new aCUP-E electrode for long-term aEEG monitoring in newborns at risk of encephalopathy. To achieve this objective, the performance of aCUP-E will be compared with the electrodes currently used in two different clinical settings, in order to validate its applicability and benefits.

Specific objectives:

1. To compare the overall recording quality of aCUP-E with that of commercial electrodes. This analysis will be performed in preterm and term newborns at risk of encephalopathy, selecting segments with low impedance and absence of artefacts to determine whether aCUP-E provides equal or superior recording quality.

2. To evaluate the clinical feasibility of aCUP-E

It will be determined whether aCUP-E facilitates the identification of subclinical abnormalities in newborns without evident signs of encephalopathy through analysis of brain maturation with aEEG. A survey of clinical staff will also be conducted to assess the adaptability of the device in different hospital settings, considering aspects such as electrode placement and removal, as well as qualitative observations regarding its implementation.

3. To evaluate the comfort and safety of aCUP-E

To assess the ease of use of aCUP-E by healthcare staff and the risk of associated complications, using the skin irritation scale to assess newborn comfort and safety during electrode application. The Neonatal Skin Condition Score (NSCS) is used to measure the following factors: skin dryness, erythema, and skin breakdown or damage.

4. To evaluate the reliability of aCUP-E recordings

The reliability of recordings obtained with aCUP-E will be determined using Bland-Altman analysis to assess data correlations. The signal-to-noise ratio (SNR) in the 2-15 Hz frequency band will also be calculated. Impedance variability will be quantified using the standard deviation of 30-minute segments, using a 1-minute moving average.

5. To evaluate electrode survival and durability

To determine the functional duration of aCUP-E electrodes, using Kaplan-Meier curves to analyse electrode survival.

5.2.Hypothesis

The aCUP-E electrode, specifically designed for newborns, provides benefits compared with conventional electrodes in long-term recordings obtained in newborns at risk of encephalopathy. Despite not showing signs or symptoms of encephalopathy, these patients will benefit from detailed brain function monitoring that facilitates the early detection of possible abnormalities or even subclinical abnormalities that may currently go unnoticed, allowing early and appropriate management. This study is intended as a decisive step towards establishing aCUP-E as a standard in aEEG monitoring, expanding its use and validating its benefits in a population that may require advanced neurological surveillance.

5.3. Applications and intended performance to be verified

The comparison includes assessing:

1. Ease of electrode placement and removal; and comfort when handling the newborn during recording.
2. Number of electrode repositioning.
3. Number of times conductive gel is applied to aCUP-E.
4. Recording quality.
5. Skin safety.

5.4. Expected risks and adverse effects

Electrode placement could cause some skin irritation.

6. CLINICAL INVESTIGATION DESIGN

6.1. General information

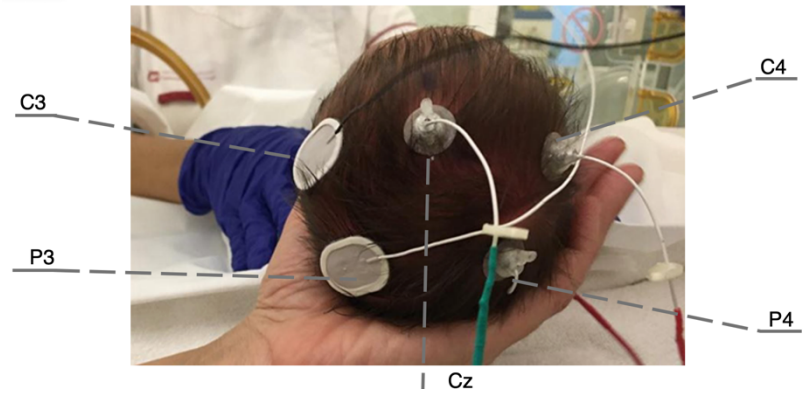
This is an observational, prospective, comparative and multicentre study designed to evaluate the quality of aEEG recording in newborns who do not show evident signs of encephalopathy. The study will be conducted at two hospitals, Salut Sant Joan de Reus – Baix Camp and Hospital Universitari de Tarragona Joan XXIII, to obtain a broader and more representative sample, as well as to evaluate the efficacy of aCUP-E in clinical settings with different practices.

The study preceding the current one was conducted exclusively at Salut Sant Joan de Reus – Baix Camp in neurologically healthy newborns; now, this multicentre design will allow the inclusion of a second hospital, HJ23, to analyse the use of aCUP-E in a more diverse population, thereby increasing the robustness and generalisability of the results.

A total of 35 newborns at risk of encephalopathy will be included. Participants will be selected consecutively during the study period, according to the established inclusion and exclusion criteria.

This design will allow direct comparison of the efficacy of the new aCUP-E electrode with usual standards under similar conditions. During each monitoring session, one type of electrode will be placed on each cerebral hemisphere to ensure an accurate and reliable comparison:

- On one cerebral hemisphere, the aCUP-E electrode will be applied.
- On the other hemisphere, the conventional electrode will be applied.



In the previous study conducted at HUSJR, the results of which were published in Frontiers in Pediatrics, no effect attributable to the location of the electrodes on one hemisphere or the other was identified when comparing the recordings obtained.

The only potential effect related to the hemisphere of placement could result from a failure in the electrode connections to the amplifier, the amplifier itself, or the brain activity monitor, causing a systematic difference in the signal acquired by electrodes on one hemisphere compared with the other. For this reason, alternation of the hemisphere on which the aCUP-E electrodes are placed is contemplated, in order to detect this type of technical bias and prevent it from being confused with differences attributable to the type of electrode used.

However, to detect a possible defect in the connections, amplifier or monitor, it is not necessary to use a strict randomisation method that guarantees an exactly balanced distribution of the number of patients in whom the aCUP-E system is placed on the right or left hemisphere. Since the objective is not to evaluate a possible physiological effect of the recording hemisphere—an effect that was not observed in the previous study—but rather to identify the possible existence of technical bias associated with the acquisition system, an approximately balanced distribution is sufficient for this purpose. Consequently, 1:1 randomisation would not provide an additional methodological benefit and, on the contrary, would introduce greater operational complexity into routine NICU care without improving the scientific validity of the study.

The established procedure consists of NICU nursing staff selecting the hemisphere for placement of the aCUP-E electrodes randomly, although a periodic check will be performed every 10 newborns included to review the distribution of the hemispheres used. If at that time the number of newborns in whom the aCUP-E system has been placed on the right hemisphere is less than 3, placement on that hemisphere will be prioritised in subsequent inclusions until a more balanced distribution between hemispheres is achieved that allows a possible technical bias associated with the recording hemisphere to be detected.

This procedure constitutes a study quality-control measure, compatible with usual clinical practice in the NICU, which allows verification of the absence of technical bias related to the recording hemisphere, without imposing strict randomisation that is not scientifically necessary to address the study objective.

Study development:

Unlike the initial study, this protocol is structured into three independent cohorts, each designed to answer a different clinical question.

Cohort 1. Comparison between aCUP-E and liquid-gel electrodes

The first cohort will include 10 neonates, in whom an intra-individual comparison between aCUP-E and conventional liquid-gel electrodes will be performed.

The main objective of this cohort is to externally validate the results obtained in the previous study authorised by the Spanish Agency of Medicines and Medical Devices (AEMPS), file 918/21/EC-R, the results of which were subsequently published by Fabregat-Sanjuan et al. (Frontiers in Pediatrics, 2024).

Therefore, the results obtained in these ten new patients will be compared with the results previously obtained in the initial study, with the aim of verifying the reproducibility of aCUP-E performance at a second hospital and confirming that its performance does not depend on the participating centre or the staff responsible for electrode placement.

This cohort is not intended to fully repeat the previous study or to demonstrate the superiority of the device again, but rather to confirm the consistency of the results previously obtained under different care conditions. The paired design, in which each neonate serves as their own control, reduces inter-individual variability and increases the statistical efficiency of the comparison; therefore, the inclusion of 10 new neonates is considered sufficient to achieve this inter-centre validation objective.

Cohort 2. Comparison between aCUP-E and needle electrodes

The second cohort will include 15 neonates, all recruited at Hospital Universitari de Tarragona Joan XXIII (HUJ23).

In this cohort, a simultaneous comparison between aCUP-E and the needle electrodes routinely used in clinical practice will be performed. This is the first specific clinical evaluation between the two recording systems.

In a paired intra-individual design, a cohort of approximately 10 neonates would allow an initial assessment of comparative performance to detect large intra-individual differences.

However, it was decided to expand this cohort to 15 neonates in order to increase the precision of the estimates, reduce the width of the confidence intervals and improve the ability to detect smaller differences.

This decision is also based on the experience obtained during the study authorised by AEMPS (file 918/21/EC-R), in which a sample of similar size allowed statistically significant differences to be detected between aCUP-E and liquid-gel electrodes.

Although the comparator will be different in the present study, previous experience provides a reliable estimate of the expected variability and of the ability of the paired design to assess device performance.

Cohort 3. Monitoring exclusively using aCUP-E

The third cohort will include 10 neonates monitored exclusively using aCUP-E electrodes.

This cohort is expected to be distributed evenly between the two participating centres, including approximately 5 neonates at Hospital Universitari de Tarragona Joan XXIII (HUJ23) and 5 neonates at Hospital Universitari Sant Joan de Reus (HUSJR), provided that the recruitment rate allows it.

The main objective of this cohort is not to compare different types of electrodes, but to evaluate the technical feasibility, functional stability and safety of exclusive use of aCUP-E during complete monitoring sessions.

Likewise, this cohort will allow assessment of functional concordance between both cerebral hemispheres by recording both sides using the same type of electrode, avoiding the possible confounding effect arising from the simultaneous use of different technologies.

The detection of Quiet Sleep phases in both hemispheres and the magnitude of the physiological differences observed between them will be specifically analysed.

Subsequently, these differences will be compared with the interhemispheric variability observed in routine clinical recordings obtained using needle electrodes and surface electrodes, with the aim of determining whether the differences detected correspond to normal physiological recording variability or can be attributed to the type of electrode used.

If commercial liquid-gel electrodes do not allow a recording of minimum quality to be obtained for a continuous period of at least 6 hours, it may be decided to use a setup with the Advanced Cup Electrode (aCUP-E) on both hemispheres. This option will be considered provided that clinical staff deem it appropriate and the use of subdermal

electrodes is ruled out, especially in situations such as premature infants.

The initially planned distribution of the three cohorts responds to the specific scientific objectives of the study. However, since the comparison between aCUP-E and needle electrodes presents greater technical complexity than the other cohorts, the possibility of modifying the sample distribution during the course of the study is predefined.

A minimum of **10 patients** is established for Cohort 2, comparing **aCUP-E and needle electrodes**. The minimum of 10 patients allows the scientific objectives of the cohort to be preserved and enables an initial assessment of comparative performance to detect large differences within individuals. If the minimum of 10 patients is not reached in Cohort 2, this will be reported to the AEMPS as a modification to the study.

The redistribution of the **5 patients initially planned for Cohort 2** (in the event that only 10 of the 15 patients initially planned are recruited) to the other cohorts may only be carried out when there are **objective recruitment or technical execution difficulties** that prevent the initially planned cohort of **15 neonates** from being completed. These situations include:

- inability to recruit a sufficient number of neonates meeting the inclusion criteria during the study period.
- difficulty obtaining informed consent before the clinically indicated start of monitoring.
- organisational or care-related limitations of the Neonatal Intensive Care Unit that prevent simultaneous placement of both types of electrodes.
- insufficient availability of trained staff for placement and maintenance of needle electrodes.
- technical difficulties arising from the use of needle electrodes that prevent monitoring from being completed adequately.
- any other care-related circumstance that, in the opinion of the principal investigator, may compromise the safety of the neonate or significantly interfere with usual clinical practice.

If any of these circumstances occur, patients who cannot be included in the cohort comparing aCUP-E and needle electrodes may be redistributed among the other two study cohorts, increasing the number of participants in:

- the cohort comparing aCUP-E and liquid-gel electrodes, and/or
- the cohort involving monitoring exclusively with aCUP-E.

The final distribution between the two cohorts will be determined according to the

recruitment rate at each centre, patient availability and the scientific needs of the study, seeking to maintain, whenever possible, a balanced distribution between hospitals.

The decision to redistribute participants will be made by the principal investigator, in agreement with the investigators responsible at each centre, and must be documented before patient inclusion closes.

Under no circumstances will redistribution depend on preliminary results obtained during the study or on differences observed in recording quality, impedance, signal-to-noise ratio, Quiet Sleep detection or any other outcome variable. Redistribution will respond exclusively to feasibility, practicability and safety criteria, avoiding the introduction of methodological biases into the interpretation of the results.

Impact of redistribution on the scientific validity of the study

Participant redistribution is a predefined contingency measure intended exclusively to ensure study feasibility in the event of possible objective recruitment or technical execution difficulties in the cohort comparing aCUP-E and needle electrodes. Under no circumstances will redistribution depend on the results obtained during the study or on any of the assessment variables.
The three study cohorts address different scientific objectives and have specific hypotheses and statistical analyses. Consequently, redistribution only changes the number of participants assigned to each cohort, without altering the overall scientific objectives of the study or compromising the validity of the analysis corresponding to the remaining cohorts.

The planned objective for the cohort comparing aCUP-E and needle electrodes is to recruit 15 neonates, in order to achieve adequate precision in the estimates and sufficient power to detect differences between the two types of electrodes.

However, due to the technical and clinical difficulties inherent in the use of needle electrodes in newborns, it is possible that this sample size may not be reached. In such a case, the minimum of 10 patients will ensure that the scientific objectives of the cohort are preserved and will allow an initial assessment of comparative performance to detect large within-subject differences. Participants not recruited into this cohort may be redistributed among the remaining cohorts, while maintaining the total study sample size at 35 neonates.

This redistribution **does not affect the main hypotheses or the statistical validity of the cohorts comparing aCUP-E with liquid-gel electrodes or involving monitoring**

exclusively with aCUP-E, as their sample sizes are increased and therefore the precision of the estimates planned for those cohorts is maintained or even increased.

If the cohort comparing aCUP-E with needle electrodes does not reach the initially planned sample size, the results obtained will be considered a **preliminary clinical evaluation of viability and feasibility**, allowing the comparative performance of both recording systems to be described and generating evidence for future studies. However, if it is not possible to recruit the minimum of 10 patients, this will be reported to the AEMPS as a modification to the study, and the results will not be used to draw confirmatory conclusions regarding differences between the two types of electrodes.

1. Salut Sant Joan de Reus – Baix Camp:

aCUP-E will be compared with the non-invasive liquid-gel electrodes routinely used at this hospital, applied to newborns without evident signs of encephalopathy who will nevertheless be monitored to detect possible subclinical abnormalities. The objective is to assess whether aCUP-E maintains the advantages previously observed, such as recording quality and reduction of artefacts, while facilitating the identification of possible neurological abnormalities in this apparently healthy population.

2. Hospital Universitari de Tarragona Joan XXIII:

In preterm newborns, monitoring will be performed with aCUP-E and non-invasive liquid-gel electrodes, following the same procedure as at Salut Sant Joan de Reus – Baix Camp. In term newborns, the use of aCUP-E may be compared with subdermal needle electrodes and liquid-gel electrodes. This choice is because this hospital routinely uses needle electrodes for recordings in term newborns and does not have a monitoring protocol using non-invasive electrodes in preterm newborns. This will make it possible to assess the feasibility and efficacy of aCUP-E as a non-invasive alternative for monitoring preterm newborns.

6.2. Product

6.2.1. Technical and functional characteristics

The new cup electrode (aCUP-E) is flexible, with firm and secure adherence to the scalp, and features that allow comfortable handling of the newborn by healthcare staff.

The electrode body is 7 mm high, with a larger diameter of 21 mm and a smaller diameter of 11 mm. The electrical cable is 100 mm long. The electrode plate has a diameter of 7 mm and a thickness of 0.7 mm. The adhesive in contact with the skin is ring-shaped, with a larger diameter of 20 mm and a smaller diameter of 10 mm. The adhesive dimensions have been chosen so that the electrode body does not come into contact with the skin (although the material used for the electrode body (EPU46) is suitable for skin contact). The electrode plate has an Ag/AgCl coating and is used together with the electrical cable from a commercial electrode (MT-ASC from Almevan). The electrode body is manufactured by 3D printing using Carbon technology with a translucent material (allowing the skin, electrode plate and conductive gel to be seen), EPU46 Extra Soft from Carbon, with a Shore 46A hardness that allows it to adapt perfectly to the curvature of neonates' heads.

Replacement of the conductive gel has been tested for more than three weeks, with gel replacement every 24 to 48 h. Validation of the signal acquired by the electrode was performed in the laboratory using a brain monitoring system and by comparison with hydrogel and liquid-gel electrodes. The results demonstrated electrode safety and recordings showing the same traces with aCUP-E as with hydrogel and liquid-gel electrodes. Correct impedance values similar to those of commercial electrodes were also obtained.

Different adhesives will be tested in the clinical trial (preclinical laboratory tests have been performed to select the most suitable ones), all already certified for medical use. Acrylic and hydrocolloid adhesives will be tested. The acrylic adhesives are from 3M and have references 1510 and 4076. The hydrocolloids are 3M 9943 and HT (High Tack) and VHT (Very High Tack) from Amparo Medical. The adhesive expected to be used most frequently is HT (High Tack from Amparo Medical), and only in some cases where adequate adhesion is not achieved due to neonatal characteristics such as high scalp density (this did not occur in any of the 20 patients in the first clinical trial), another medical adhesive may be used to assess whether better adhesion is achieved.

The results of these functional tests are included in Annex 25.

Number of investigational products to be used, together with justification.

The number of products to be used is 3 electrodes for each patient undergoing comparison with commercial electrodes and 5 electrodes for each patient when only aCUP-E is used. The trial is expected to perform aEEG in 35 patients, but the number of patients could be reduced depending on the results.

List of any other medical device or medication to be used during the clinical investigation

The conductive gel used may be any gel with suitable viscosity to flow through the space between the encapsulation of the electrode plate and the electrode body. The most common and preferred gel for the clinical trial will be ELECTRO-GEL from ECI.

The blunt needle used for injection/replacement of the conductive gel will be the same as that used for cup electrodes.

Preparation of the skin area for electrode placement will be the usual preparation for surface electrodes. The area will be prepared with a swab and Nuprep abrasive paste and then cleaned and allowed to dry.

To remove the electrode in cases of strong adhesion, an adhesive remover (Niltac from Convatec or Brava from Coloplast) will be used.

6.2.2. Comparator product

Description of exposure to the comparator product

The investigational product (aCUP-E electrode) can be compared with any surface electrode, whether hydrogel, liquid gel or cup. Regarding hydrogel electrodes, the product that provides the best comparison is the MT-ASC electrode from Almevan, since this is the electrode from which the electrode plate and connection cable are obtained. Regarding liquid-gel electrodes, the comparator electrode is the Ambu 720, and regarding cup electrodes, it can be compared with any cup electrode.

Justification for the selection of hydrogel/liquid-gel electrodes

The justification for selecting hydrogel/liquid-gel electrodes to compare functionality with the aCUP-E electrode in the clinical trial is based on tests performed in clinical practice by Clinical Neurophysiologist Vicenç Pascual over the last 7 years and by previous investigators in other clinical trials. Specifically, it is based on two publications. The

publication *Long term electroencephalography in preterm neonates: Safety and quality of electrode types, published in Clinical Neurophysiology Volume 129, Issue 7, July 2018, Pages 1366-1371*, demonstrates that hydrogel electrodes are similar to cup electrodes. The publication Evaluation of the suitability and clinical applicability of different electrodes for aEEG/cEEG monitoring in the extremely premature infant, published in *Anales de Pediatría in October 2020*, studies different types of surface electrodes and considers the Ambu 720 liquid-gel electrode to be the best.

6.3.Subjects

6.3.1. Inclusion/exclusion criteria

Inclusion criteria:

For participant selection, the inclusion criteria will be as follows:

- Preterm or term newborns admitted to the NICU who are at risk of encephalopathy due to conditions arising from the clinical context. For example, maternal infection (e.g. maternal infections such as fever during labour, urinary tract infection, etc.), maternal history of gestational diabetes, gestational hypertension or preeclampsia, history of delivery complications such as placental displacement or abnormal placentation.
- Informed consent obtained from the parents or legal guardians of the newborns.
- Availability of aEEG monitoring equipment and aCUP-E electrodes during the study period.

Exclusion criteria:

The exclusion criteria are established to avoid monitoring complications or obtaining inconsistent data:

- Newborns with signs or symptoms of encephalopathy or any other evident neurological abnormality.
- Newborns with critical or unstable medical conditions that interfere with monitoring, such as the need for continuous mechanical ventilation or significant haemodynamic instability.
- Newborns receiving medication that may alter brain activity and interfere with aEEG results.
- Newborns with cranial or skin malformations that prevent proper electrode placement.

- Situations requiring urgent interventions that may interrupt monitoring or affect the quality of the data collected.
- Lack of informed consent from the parents or legal guardians of the newborns to participate in the study.

6.3.2. Criteria for subject withdrawal

Once the study has started, subjects may withdraw at any time according to these criteria:

- Deterioration of the baby's health requiring other medical interventions and making continued monitoring unfeasible.
- Refusal or withdrawal of consent by the parents or legal guardians.
- Persistent difficulty in placing or maintaining the aCUP-E electrode or any other comparator electrode, affecting recording quality.
- Development of an allergic reaction or skin inflammation due to, or related to, placement of the recording electrodes.
- Repeated interference in the aEEG recording preventing reliable data from being obtained.

6.3.3. Time of inclusion

Newborns admitted to the NICU for non-neurological reasons will be selected.

6.3.4. Expected duration of the clinical investigation

The total expected duration of the clinical investigation is 24 months.

Study schedule:

- M1-M12: Data collection.
- M13 - M15: Analysis of results.
- M16 - M17: Article drafting.
- M18 - M24: Publication of results.

In the previous study, patients had to meet the specific criterion of being neurologically healthy, whereas in this case newborns without evident signs of neurological pathology are included, which simplifies the selection process.

6.3.5. Expected duration of each subject's participation

Subject participation will depend on the length of stay in the NICU.

6.3.6. Number of subjects

35 subjects.

6.3.7. Inclusion period

The inclusion period will continue until the determined sample size is reached.

6.4. Procedures

aEEG recording is part of routine clinical practice in NICUs [6], [7], [8]. This test is indicated in newborns with suspected encephalopathy and/or epilepsy, as well as in cases where there is a potential risk of brain injury, such as extreme moderate prematurity, hyperglycaemia, meningoencephalitis, and newborns requiring sedation. aEEG recording is a non-invasive and harmless procedure for the baby that amplifies the small bioelectrical activity generated by brain neurons, thus allowing continuous monitoring of brain function.

Electrode placement may cause skin irritation; therefore, the baby's skin condition will be regularly monitored by the nursing team using the NSCS scale [5]. If the NSCS is greater than 5, the electrodes will be removed, and the baby will be excluded from the study for safety reasons. As a preventive measure, if any of the first five babies scores 5 or higher, the procedures will be reviewed to ensure that electrode application is optimal, and the study will be temporarily stopped to introduce improvements if necessary.

The procedure will consist of placing the aEEG electrodes and periodically checking impedance, following the protocol specifications. Nursing staff will record the interventions performed.

Every 24 h, the neurophysiologist will review the study performed and the notes made by the nursing staff.

Once the recording period has ended, depending on the length of the newborn's stay in the NICU, the study data are extracted in European Data Format (EDF+) with the patient's data encrypted and assigned a numerical code.

The data are stored in the investigator's study file in fully anonymised form and under the custody of the principal investigator.

Electrode application

1. Non-invasive electrodes (liquid gel):

Non-invasive electrodes will be placed following a modification of the method of Lloyd et al. to minimise the time the baby is handled inside the incubator and ensure systematic and safe electrode application [1], [9].

First, the ends of each electrode will be labelled to identify its position, and then all cables

will be grouped in a tubular sleeve (Tubinet®) to prevent them from becoming tangled with other cables or tubing, thereby reducing interference.

Once placement is complete, the cables from these electrodes will be connected according to this arrangement to ensure the baby's safety during monitoring.

2. aCUP-E electrodes:

The aCUP-E electrodes will be placed using the colour code and integrated fastening system, which facilitates quick and safe placement. This unique fastening system does not require adhesive dressings or other fixation materials, reducing the risk of pressure points and skin injuries.

aCUP-E adheres securely to the baby's scalp even in active or moving babies, allowing comfortable handling of the baby by healthcare staff.

The duration of aEEG monitoring will depend on the duration of the baby's stay in the NICU (minimum 24 h). The electrodes will be applied as soon as possible during the admission period and will be removed if signs of skin deterioration occur or at the discretion of the clinical team. No subsequent follow-up of the baby is required once monitoring has been completed. The absence of follow-up after completion of the study is justified by the nature of the procedure being evaluated and by the monitoring measures already incorporated during its development. aEEG recording is a continuous, non-invasive monitoring technique integrated into routine clinical practice, and the additional study intervention is limited exclusively to the type of electrode used, without introducing therapeutic modifications or additional experimental procedures. In this respect, the associated risks are minimal and transient, the main potential adverse event being the occurrence of mild skin alterations related to electrode application.

However, in line with the recommendations of the Spanish Agency of Medicines and Medical Devices (AEMPS), the protocol includes a systematic assessment of skin condition at the time of electrode removal. This assessment is performed immediately by healthcare staff through visual inspection and using validated tools such as the Neonatal Skin Condition Score, with the aim of identifying early any sign of irritation, erythema, pruritus or superficial skin injury. Skin condition is also monitored periodically throughout the recording period, allowing any incipient alteration to be detected before the procedure ends. In addition, unlike conventional electrodes, aCUP-E electrodes are transparent and therefore would allow early detection of hypothetical skin irritation.

Since possible electrode-related injuries are, for the most part, mild, self-limiting and evident at the time they appear or when the electrodes are removed, it is not considered necessary to establish specific follow-up outside the usual care setting. If any skin

alteration is detected at the time of removal, it will be managed in accordance with standard clinical practice and recorded as an adverse event, always ensuring patient safety.

Therefore, the combination of continuous monitoring during the study, a thorough assessment at the time of electrode placement and removal, and the mild and self-limiting nature of possible adverse effects adequately justifies the absence of specific subsequent follow-up, without compromising the safety or quality of care of the newborns included in the study.

These procedures will ensure that the study is conducted under conditions of maximum safety and comfort for the baby, providing accurate and reliable data on the quality of aEEG recording with the different types of electrodes.

6.5. Monitoring plan

Clinical investigation oversight will be carried out by the designated supervisor/monitor. The key aspects of the monitoring plan are detailed below:

Scope and nature of monitoring:

Monitoring will be comprehensive, covering all stages of the clinical trial, from participant selection to data analysis.

Processes that need to be monitored:

Special emphasis will be placed on obtaining informed consent from all participants and on the accurate collection of data relevant to the study objectives.

Monitoring methods:

Regular visits to the investigation site will be conducted to review procedures, documentation and the quality of the data collected.

Scheduled meetings with the principal investigator will be held to discuss progress, resolve concerns, review results obtained and address any problems identified.

Responsibilities:

The supervisor/monitor will be responsible for assessing compliance with the protocol (CIP), data accuracy and participant safety.

The principal investigator will collaborate by providing access to all records and cooperating during monitoring visits.

Documentation and communication of results:

Regular communication between the supervisor/monitor and the principal investigator will ensure a rapid response to any problem identified.

Process for intensifying monitoring:

In cases of ongoing non-compliance or serious problems, a defined process for intensifying monitoring will be followed, which may include more frequent visits or more detailed audits.

Items requiring special attention:

Special attention will be paid to critical aspects of the investigation that could compromise subject protection or data integrity.

This plan ensures comprehensive and proactive oversight of the clinical investigation, ensuring data quality and integrity, as well as participant protection. The flexibility of the plan will allow adjustments as necessary in response to the evolution of the study.

7. STATISTICAL CONSIDERATIONS

7.1 Sample size calculation and/or justification

The objective of this study is to evaluate the technical and functional equivalence of the aCUP-E electrode compared with the electrodes routinely used for monitoring by amplitude-integrated electroencephalography (aEEG) in neonates at risk of encephalopathy. The evaluation will include variables related to electrode stability, electroencephalographic recording quality, impedance evolution, signal-to-noise ratio, the ability to identify Quiet Sleep phases, skin safety and ease of device use by healthcare staff.

Unlike the initial study, this protocol is structured into three independent cohorts, each designed to answer a different clinical question. Consequently, the sample size is justified specifically for each cohort according to its scientific objectives, avoiding the application of a single overall calculation to methodologically different hypotheses.

Cohort 1. Comparison between aCUP-E and liquid-gel electrodes

The first cohort will include **10 neonates**, in whom an intra-individual comparison between aCUP-E and conventional liquid-gel electrodes will be performed.

The main objective of this cohort is to externally validate the results obtained in the previous study authorised by **the Spanish Agency of Medicines and Medical Devices (AEMPS),** file **918/21/EC-R**, the results of which were subsequently published by **Fabregat-Sanjuan et al.** (*Frontiers in Pediatrics, 2024*).

Therefore, the results obtained in these ten new patients will be compared **with the results previously obtained** in the initial study, with the aim of verifying the reproducibility of aCUP-E performance at a second hospital and confirming that its performance does not depend on the participating centre or the staff responsible for electrode placement.

This cohort is not intended to fully repeat the previous study or to demonstrate the superiority of the device again, but rather to confirm the consistency of the results previously obtained under different care conditions. The paired design, in which each neonate serves as their own control, reduces inter-individual variability and increases the statistical efficiency of the comparison; therefore, the inclusion of 10 new neonates is considered sufficient to achieve this inter-centre validation objective.

Cohort 2. Comparison between aCUP-E and needle electrodes

The second cohort will include **15 neonates**, all recruited at **Hospital Universitari de Tarragona Joan XXIII (HUJ23).**

In this cohort, a simultaneous comparison between aCUP-E and the needle electrodes routinely used in clinical practice will be performed.

This is the first specific clinical evaluation between the two recording systems.

In a paired intra-individual design, a cohort of approximately 10 neonates would allow an initial assessment of comparative performance to detect large intra-individual differences.

However, it was decided to expand this cohort to 15 neonates in order to increase the precision of the estimates, reduce the width of the confidence intervals and improve the ability to detect smaller differences.

This decision is also based on the experience obtained during the study authorised by AEMPS (file 918/21/EC-R), in which a sample of similar size allowed statistically significant differences to be detected between aCUP-E and liquid-gel electrodes.

Although the comparator will be different in the present study, previous experience provides a reliable estimate of the expected variability and of the ability of the paired design to assess device performance.

Cohort 3. Monitoring exclusively using aCUP-E

The third cohort will include **10 neonates** monitored exclusively using aCUP-E electrodes.

This cohort is expected to be distributed evenly between the two participating centres, including approximately **five neonates at Hospital Universitari de Tarragona Joan XXIII** (HUJ23) and **five neonates at Hospital Universitari Sant Joan de Reus** (HUSJR), provided that the recruitment rate allows it.

The main objective of this cohort is not to compare different types of electrodes, but to evaluate the technical feasibility, functional stability and safety of exclusive use of aCUP-E during complete monitoring sessions.

Likewise, this cohort will allow assessment of functional concordance between both cerebral hemispheres by recording both sides using the same type of electrode, avoiding the possible confounding effect arising from the simultaneous use of different technologies.

The detection of Quiet Sleep phases in both hemispheres and the magnitude of the physiological differences observed between them will be specifically analysed.

Subsequently, these differences will be compared with the interhemispheric variability observed in routine clinical recordings obtained using needle electrodes and surface electrodes, with the aim of determining whether the differences detected correspond to normal physiological recording variability or can be attributed to the type of electrode used.

Justification based on recording duration

Each neonate will be monitored for a minimum period of 24 hours, a duration corresponding to usual clinical practice in neurological monitoring of neonates at risk of

encephalopathy and allowing several complete sleep-wake cycles to be recorded, including multiple Quiet Sleep episodes, which are essential for functional assessment of the recording.

The EEG signal will be acquired at a sampling frequency of **200 Hz**, while electrode impedance will be recorded at **100 Hz**, following the configuration used in the previous study authorised by AEMPS.

To assess the evolution of the upper and lower margins of the aEEG, the recordings will be summarized in **consecutive 160-second windows**, resulting in approximately **540 analysis windows per patient and per variable** over 24 hours of recording.

Each window will contain approximately **32,000 EEG samples and 16,000 impedance samples**, providing a high volume of longitudinal information to characterise the temporal evolution of electrode performance.

Consequently, each cohort will generate approximately:

Cohort	No. of neonates	Analysis windows
aCUP-E vs liquid gel	10	~5,400
aCUP-E vs needle	15	~8,100
aCUP-E only	10	~5,400

Although these windows do not constitute statistically independent observations, their analysis using repeated-measures models will make it possible to characterise with high precision the temporal evolution of technical variables such as impedance, signal quality, signal-to-noise ratio and detection of Quiet Sleep phases.

Conversely, for variables whose outcome is defined at patient level—such as successful completion of the recording, need for electrode repositioning or occurrence of skin incidents—the experimental unit will remain the neonate.

Overall, the combination of an intra-individual design and a high number of longitudinal observations make it possible to obtain high precision in estimating the temporal behaviour of the device without unnecessarily increasing the number of participants.

Planned sample redistribution

The initially planned distribution of the three cohorts responds to the specific scientific objectives of the study. However, since the comparison between aCUP-E and needle electrodes presents greater technical complexity than the other cohorts, the possibility of modifying the sample distribution during the course of the study is predefined.

Redistribution may be carried out exclusively when there are objective recruitment or technical execution difficulties that prevent completion of the initially planned cohort of 15 neonates. These situations include:

- inability to recruit a sufficient number of neonates meeting the inclusion criteria during the study period.
- difficulty obtaining informed consent before the clinically indicated start of monitoring.
- organisational or care-related limitations of the Neonatal Intensive Care Unit that prevent simultaneous placement of both types of electrodes.
- insufficient availability of trained staff for placement and maintenance of needle electrodes.

- technical difficulties arising from the use of needle electrodes that prevent monitoring from being completed adequately.
- any other care-related circumstance that, in the opinion of the principal investigator, may compromise the safety of the neonate or significantly interfere with usual clinical practice.

If any of these circumstances occur, patients who cannot be included in the cohort comparing **aCUP-E and needle electrodes** may be redistributed among the other two study cohorts, increasing the number of participants in:

- the cohort comparing **aCUP-E and liquid-gel electrodes**, and/or
- the cohort involving **monitoring exclusively with aCUP-E**.

The final distribution between the two cohorts will be determined according to the recruitment rate at each centre, patient availability and the scientific needs of the study, seeking to maintain, whenever possible, a balanced distribution between hospitals.

The decision to redistribute participants will be made by the principal investigator, in agreement with the investigators responsible at each centre, and must be documented before patient inclusion closes.

Under no circumstances will redistribution depend on preliminary results obtained during the study or on differences observed in recording quality, impedance, signal-to-noise ratio, Quiet Sleep detection or any other outcome variable. Redistribution will respond exclusively to feasibility, practicability and safety criteria, avoiding the introduction of methodological biases into the interpretation of the results.

Overall sample justification

Each of the three cohorts has been sized according to its specific objective and the information available from the previous study authorised by the **Spanish Agency of Medicines and Medical Devices (AEMPS, file 918/21/EC-R)** and subsequently published by **Fabregat-Sanjuan et al.** *(Frontiers in Pediatrics, 2024*).

The cohort comparing liquid-gel electrodes is designed as an **external validation** of the results previously obtained. The cohort comparing needle electrodes constitutes a **specific comparative evaluation** between the two recording systems. In accordance with methodological recommendations for pilot studies and validation studies of medical devices with a paired design, a cohort of approximately **10 neonates** provides sufficient information for an initial assessment of comparative performance. However, it was

decided to expand this cohort to **15 neonates** to increase the precision of the estimates, reduce the width of the confidence intervals and improve the ability to detect smaller differences. This decision is also supported by the experience obtained in the previous study authorised by AEMPS, in which a sample of similar size allowed statistically significant differences to be detected between aCUP-E and liquid-gel electrodes.

Finally, the cohort involving monitoring exclusively with aCUP-E will allow evaluation of **technical feasibility**, **functional stability** of the device and **interhemispheric physiological variability**, using the same type of electrode on both hemispheres. Subsequent comparison with routine clinical recordings obtained using needle electrodes and surface electrodes will allow the observed differences to be contextualised and determine whether they correspond to normal physiological variability of neonatal recording.

Overall, the three cohorts together involve the inclusion of a **maximum of 35 neonates**, a number considered adequate to achieve the technical, functional and clinical objectives of the study, while maintaining a balance between methodological robustness, recruitment feasibility and minimisation of the care burden on an especially vulnerable population.

7.2 Statistical analyses

Statistical analysis will be performed according to the specific objectives of each of the three study cohorts. The methodology used for the comparison between **aCUP-E** and conventional electrodes will follow, whenever possible, that used in the study previously authorised by the **Spanish Agency of Medicines and Medical Devices (AEMPS, file 918/21/EC-R)** and subsequently published by **Fabregat-Sanjuan et al.** (*Frontiers in Pediatrics, 2024*), in order to facilitate comparison between the two studies and externally validate the results obtained.

The new objectives incorporated into this protocol, such as comparison with needle electrodes, monitoring exclusively using aCUP-E and evaluation of Quiet Sleep phase detection, will be analysed using additional methodologies adapted to these new hypotheses.

Continuous variables will be summarised using mean and standard deviation when they have an approximately normal distribution, or median and interquartile range when the assumptions of normality are not met. Categorical variables will be described using absolute frequencies and percentages.

Whenever possible, the analysis will use an intra-individual design, in which each neonate will act as their own control. This approach reduces inter-individual variability and increases the statistical efficiency of the comparisons.

All tests will be two-sided and **a p-value < 0.05** will be considered statistically significant.

Interpretation of the results will not be based exclusively on p-values, but also on the magnitude of the observed effect, its 95% confidence intervals and its clinical relevance.

7.2.1 Descriptive analysis

The demographic and clinical characteristics of the patients included in each cohort and at each participating centre will be described.

The variables described will include:

- gestational age;
- postnatal age;
- sex;
- clinical indication for recording;
- total monitoring duration;
- duration of interpretable recording;
- technical incidents;
- need for electrode repositioning or replacement;
- skin alterations.

When relevant, a comparative description between the two participating centres will be performed with the aim of identifying possible differences in the profile of the patients included.

7.2.2 Electrode functional duration

Following the methodology used in the previously published study, electrode functional duration will be analysed using **Kaplan-Meier survival curves**.

Electrode loss of functionality will be considered an event, defined by the sustained appearance of impedance above the clinically established threshold, persistent loss of signal or the need to replace the electrode to continue monitoring. Losses attributable exclusively to initial placement errors or protocol non-compliance will not be considered events.

The curves corresponding to each electrode type will be compared using the **Tarone-Ware test**, following the methodology described in the study by Fabregat-Sanjuan et al.

If the number of events allows, additional exploratory analyses may be performed using Cox proportional hazards models adjusted for possible clinical covariates such as gestational age, participating centre or planned monitoring duration.

7.2.3 Impedance evolution

The impedance analysis will initially follow the methodology used in the previously published study.

Impedance values will be smoothed using a **one-minute moving average** and the **standard deviation will then be calculated in consecutive 30-minute windows with 50%** overlap, obtaining a measure of the temporal stability of each electrode.

Differences between electrode types will initially be compared using the same strategy employed in the previous study to ensure comparability of the results.

Additionally, since the present study incorporates a multicentre design and a greater number of longitudinal recordings, the temporal evolution of impedance may be analysed using linear mixed models, considering electrode type, time, participating centre and the interaction between time and electrode type as fixed effects, and including the neonate as a random effect to account for correlation between repeated measurements.

7.2.4 Recording quality and agreement between methods

Recording quality will be assessed following the methodology described in the previous study.

For this purpose, segments of optimal brain activity (Optimal Brain Activity) will first be selected, defined by:

- absence of relevant artefacts;
- low impedance;
- recording stability;
- adequate clinical interpretability.

From these segments, the upper and lower margins of the aEEG tracing will be calculated, normalised with respect to the mean of the analysed segment.

Agreement between the two recording systems will be assessed using the Bland-Altman method, calculating the mean bias, 95% limits of agreement and their corresponding confidence intervals.

Correlation coefficients will be presented only as supplementary information and not as the primary measure of agreement between methods.

When the same patient provides multiple optimal analysis segments, an adaptation of the Bland-Altman method for repeated measures will be used.

7.2.5 Signal-to-noise ratio (SNR)

Following the previously published methodology, the signal-to-noise ratio will be calculated for consecutive one-minute segments.

The results will be represented graphically as a function of the impedance recorded for each electrode.

Different mathematical models (linear, polynomial, exponential and power) will be fitted, comparing their goodness of fit using the adjusted coefficient of determination (adjusted R^2) and the root mean square error (RMSE), following the strategy used in the previous study.

Additionally, the present study will allow a longitudinal analysis using mixed models to evaluate the independent effect of time, impedance and electrode type on the signal-to-noise ratio.

7.2.6 Detection of Quiet Sleep phases

Assessment of Quiet Sleep phases constitutes an additional objective of the present study and expands the previously published methodology.

Identification of Quiet Sleep episodes will be performed by interpretation of the aEEG tracing by investigators experienced in neonatal neurophysiology, following criteria routinely used in clinical practice and, whenever possible, blinded to the type of electrode used.

In the comparative cohorts, agreement between both systems will be analysed using:

- percentage agreement;

- Cohen's kappa coefficient;
- median Quiet Sleep duration;
- percentage of total time classified as Quiet Sleep.

When the same patient provides multiple observations, appropriate mixed models for longitudinal data will be used.

7.2.7 Interhemispheric comparison

Comparison between both hemispheres recorded exclusively using aCUP-E is also a new objective of the present study.

Using the same type of electrode on both hemispheres will make it possible to estimate interhemispheric physiological variability without the possible confounding effect arising from the simultaneous use of different technologies.

The following will be assessed:

- temporal coincidence of Quiet Sleep phases;
- cumulative Quiet Sleep duration;
- percentage of discordant windows;
- differences in quantitative aEEG parameters between both hemispheres.

Agreement will be quantified using percentage agreement, kappa coefficient, intraclass correlation coefficient and Bland-Altman analysis, as appropriate.

Subsequently, these differences will be compared with the variability observed in routine clinical recordings obtained using needle electrodes and surface electrodes, with the aim of determining whether the differences observed with aCUP-E are compatible with the physiological variability described for currently used recording systems.

This analysis will be exploratory, since the reference recordings will come from different patients and not from simultaneous comparisons.

7.2.9 Assessment by healthcare staff

At the end of monitoring, a structured survey will be administered to healthcare staff.

Aspects related to the following will be assessed:

- ease of placement;
- ease of removal;
- electrode stability;
- ease of handling the neonate;
- skin safety;
- overall assessment of the device.

Ordinal variables will be described using median and interquartile range and, where appropriate, compared using non-parametric tests for paired data.

7.2.10 Significance level

A p-value < 0.05 will be considered statistically significant. Given the exploratory nature of some of the secondary objectives, especially those related to Quiet Sleep detection and interhemispheric comparison, the results will be interpreted together with effect sizes, 95% confidence intervals and clinical relevance, avoiding basing conclusions solely on statistical significance.

8. DATA MANAGEMENT

This study will be conducted in accordance with the General Data Protection Regulation 2016/679 and Organic Law 3/2018 of 5 December on the protection of personal data and guarantee of digital rights.

Study data will be collected in the investigator's study files, where the patient will only be identified by a code. All personal and biometric variables (electroencephalographic recordings and skin tests) collected in the study files will be transcribed into a coded database under the custody of the principal investigator. These already coded data will be securely stored in REDCap. Access to REDCap will be restricted to the principal investigator and study-authorised personnel.

Data confidentiality and patient identity will be maintained during the study and after its completion. Only the principal investigator and authorised study personnel will have access to these confidential records.

No data used in the analysis and subsequent dissemination of the study results will contain any identifiable reference to patient names.

Once the study has been completed, the results will be communicated to the competent authorities, as appropriate, in accordance with local legislation.

9. AMENDMENTS TO THE CIP

As indicated in UNE-EN ISO 14155:2021, the CIP may be modified as necessary during the clinical investigation. The changes made and their justification must be described and, as applicable, the potential impact on clinical performance, efficacy and safety, and identification of other affected documents.

Changes to the CIP will be prepared by the sponsor and agreed and accepted between the sponsor and the principal investigator. The changes will be recorded with a justification for each one.

In accordance with Article 75 of MDR 2017/745, for modifications that may have a substantial impact on the safety, health or rights of subjects, or on the robustness or reliability of the clinical data generated by the investigation, all National Competent Authorities (NCAs) in which the clinical investigation is being conducted will be notified. In this case, AEMPS and the CEIC.

The substantial modification must be notified within one week (from the time the relevant documents have been generated and updated), together with the reasons and nature of

the modification. The template published in MDCG 2021-28 “Substantial modification of clinical investigation under Medical Device Regulation” may be used.

Such substantial modification may not be implemented without the prior favourable opinion of the CEIC and authorisation from AEMPS. Depending on the documentation to be evaluated, only the favourable opinion of the CEIC or the authorisation of AEMPS may be required.

Until EUDAMED is available and fully operational, modifications will be submitted by the same means used for the clinical investigation application.

10. DEVIATIONS FROM THE CIP

The principal investigator must conduct the trial in accordance with the protocol (CIP) agreed with the sponsor, authorised by the regulatory authority (AEMPS) and accepted by favourable opinion from the CEIC.

The principal investigator must not deviate from or make any modification to the protocol without the prior permission and review of the sponsor and the favourable written opinion of the CEIC on the modification, except when the modification involves only logistical or administrative aspects (e.g. change of monitor or telephone number).

In emergency circumstances (section 4.5.4.b) of UNE-EN ISO 14155:2021), the principal investigator may proceed without prior approval from the sponsor and CEIC to reduce an imminent risk to trial subjects and protect their rights, safety and well-being.

The principal investigator, or a person designated by them, must document and explain any deviation from the approved protocol. The sponsor will analyse the deviations and their potential impact and, depending on this, will adopt the necessary corrective and/or preventive measures.

The principal investigator undertakes not to deviate from the protocol except where a modification or change to the protocol must be made, without prior favourable opinion from the CEIC, in order to eliminate imminent risks to trial subjects and protect their rights, safety and well-being. As soon as possible, the deviation or modification made, its justification and, where applicable, the proposed protocol modification must be submitted:

a) to the CEIC for review and favourable opinion,

b) to the sponsor for approval, and

c) to the competent authorities

11. PRODUCT ACCOUNTABILITY

The sponsor will maintain records documenting the physical location of all investigational products from their shipment to the investigation sites until their return or disposal.

The principal investigator or an authorised designee will maintain records documenting the receipt, use, return and disposal of investigational products. The record will include:

a. the date of receipt;
b. identification of each investigational product (batch number, serial number or unique code);
c. the expiry date, if applicable;
d. the date or dates of use;
e. subject identification;
f. the date on which the investigational product was removed from the subject, if applicable;
g. the date of return of investigational products that were unused, expired or malfunctioned, if applicable.

12. COMPLIANCE STATEMENTS

The study will be conducted in accordance with the ethical principles derived from the Declaration of Helsinki (Fortaleza, Brazil, October 2013). In addition, the study will be conducted in accordance with Good Clinical Practice (GCP) principles under the guidelines of the International Council for Harmonisation (ICH) and the regulatory requirements for participating institutions.

In addition, the study will comply with the applicable regulatory requirements for clinical investigations. That is, with harmonised standard UNE-EN ISO 14155:2021 (which establishes good clinical practice standards in Chapter 4), European Regulation MDR 2017/745 (Chapter VI and Annex XV) and Royal Decree 192/2023 (Chapter VII), which regulates medical devices. The statement specifying compliance with the general safety and performance requirements under MDR can be found in Annex 19.

This study will not begin until a favourable opinion has been obtained from the ethics committee and the required regulatory authority (AEMPS), and it must comply with any additional requirements imposed by them.

The study will be conducted according to the protocol (CIP) approved by the CEIm of IISPV. The benefits of the study are considered to outweigh the residual risks; and the rights and well-being of the subjects will be respected.

Participating subjects must sign informed consent in order to be recruited into the study. Due to the subjects' inability to provide consent, as they are newborns, legally designated representatives (parents or legal guardians) will be required to sign the informed consent in accordance with vulnerable population requirements.

Recruitment will be carried out by neonatologists from the NICU, and no financial compensation for participants is planned, nor are public dissemination activities to promote participant recruitment planned.

The Sponsor's civil liability is protected by an insurance policy taken out by Salut Sant Joan de Reus – Baix Camp and another insurance policy taken out by Hospital Universitari de Tarragona Joan XXIII. The centre management's approval (Annex 20) and the insurance certificate (Annex 22) are attached.

13.INFORMED CONSENT PROCESS

Due to the inability of the participating subjects to provide consent, as they are newborns, the legally designated representatives (parents or legal guardians) will be required to sign the informed consent in accordance with vulnerable population requirements.

The parents or legal guardians must provide written consent before the start of the clinical investigation and after having understood, through a prior interview with the neonatologist, the objectives of the investigation, its risks, inconveniences and benefits, as well as the conditions under which it will be conducted, and after having been informed of the subjects' right to withdraw from the investigation at any time without giving a reason and without this causing any liability or prejudice.

The neonatologist, who will identify the subjects for the study, will discuss the study with the legal representative and provide information objectively, without coercion or influence and without offering any inappropriate or undue incentive. The neonatologist will use non-technical language in the legal representative's native language for better understanding and will provide sufficient time to read and understand the information.

Each legal representative will document their provision of informed consent by signing and dating the informed consent form. Each signed and dated consent form will be retained by the neonatologist, and a copy of the informed consent will be provided to the legal representative.

If important new information arises that could be related to the subject's willingness to continue participating in the clinical investigation, it will be provided to the legal representative. If necessary, their continuing informed consent will be confirmed in writing.

The patient and legal representative information sheet, as well as the informed consent document, can be found in Annex 16.

14. ADVERSE EVENTS, ADVERSE DEVICE REACTIONS AND DEVICE DEFICIENCIES

Definition of adverse events and adverse device reactions

In accordance with UNE-EN ISO 14155:2021, an Adverse Device Reaction (ADR) is an adverse event related to the use of an investigational medical device. In turn, an Adverse Event (AE) is any untoward medical occurrence, unintended disease or injury, or undesirable clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational product and whether anticipated or unanticipated.

A possible ADR associated with the use of surface electrodes is skin irritation, skin redness, skin itching and small blisters.

Definition of device deficiencies

In accordance with UNE-EN ISO 14155:2021, a Device Deficiency (DD) is an inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance.

Device deficiencies may include:

- **Incorrect dimensions due to 3D printing defects.** In this case, a DD may be the inability to inject/replace the conductive gel and would not pose any risk, only requiring replacement of the electrode with a correct one. Another DD could be poor electrode functionality, which would result in a poor recording, but would likewise pose no risk, only requiring replacement of the electrode with a correct one.
- **Errors in electrode assembly.** In this case, a DD would be poor electrode functionality, which would result in a poor recording, but would pose no risk, only requiring replacement of the electrode with a correct one.
- **Drying of the conductive gel.** In this case, a DD would be poor electrode functionality because the gel could not be replaced, which would result in a poor recording, but would pose no risk, only requiring replacement of the electrode with a correct one.

- **Errors in electrode adhesion.** In this case, a DD would occur if the electrode became completely detached. This could imply a potential risk that the newborn might interact with the electrode, but since it is passive and has no parts that can easily become detached, it would not entail any risk or would entail the same risks as the commercial electrodes used.
- **Spillage of conductive gel during the conductive gel injection/replacement operation**. In this case there is no DD and it would only require cleaning the area to prevent the gel from drying on the electrode surface or skin.

Definition of serious adverse events, serious adverse device reactions and serious unexpected adverse device reactions.

In accordance with UNE-EN ISO 14155:2021, a Serious Adverse Device Reaction (SADR) is an ADR that has produced any consequence characteristic of a serious adverse event. In turn, a Serious Adverse Event (SAE) is an AE that resulted in any of the following: death, serious deterioration of the subject's, users' or other persons' health, or foetal distress, foetal death, congenital anomaly or birth defect. Finally, a Serious Unexpected Adverse Device Reaction (SUADR) is an SADR that, by its nature, incidence, intensity or consequences, has not been identified in the updated risk determination.

No possible SADRs have been detected in relation to the use of surface electrodes beyond those indicated above: skin irritation, skin redness, skin itching and/or small blisters.

Allergic reactions could occur due to contact with the adhesive or conductive gel. This would be highly unlikely because the only part that comes into contact with the patient is a biocompatible adhesive indicated for the same application in the clinical setting.

Information on the reporting process.

Any SADR, DD or finding related to the above will be duly documented and reported in accordance with Article 80 of MDR 2017/745 and following the provisions of document MDCG 2020-10/1 rev.1 “Safety reporting in clinical investigations of medical devices under the Regulation (EU) 2017/745”.

In the situations detailed in the preceding paragraph, the principal investigator must notify the sponsor immediately (but no later than 3 days after becoming aware of the event). The sponsor will in turn use the form published as an annex to that document, MDCG 2020-10/2 rev.1 “Clinical Investigation Summary Safety Report Form”.

This form must be completed or updated for each reportable event or for new findings or updates to events already reported. The sponsor may submit an incomplete initial report

followed by a complete one. It will be transmitted at the same time to all NCAs in which the clinical investigation is being conducted. In this case, AEMPS:

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Spanish Agency of Medicines and Medical Devices
C/ Campezo 1, Edificio 8, ES - 28022 Madrid
E-mail: psinvclinic@aemps.es - Web sitepsinvclinic@aemps.esWeb site

Once EUDAMED is available and fully operational, the obligations and requirements related to the submission of safety reports through EUDAMED will apply from six months after the date of publication of the notice in the Official Journal of the European Union.

The sponsor's reporting period to the NCA(s) will be immediately (but no later than 2 days after becoming aware of the event) for reportable events involving an imminent risk of death, serious injury or serious illness and requiring immediate corrective action, or new findings or updates to related events. For all other reportable events or new findings or updates, the reporting period will be immediately (but no later than 7 days after becoming aware of the event).

Likewise, as indicated in UNE-EN ISO 14155:2021, the CEIC must be notified of SAEs if the CEIC so requires.

Foreseeable adverse events and expected adverse device reactions, together with their incidence, mitigation or possible treatment.

In the event of reactions such as skin irritation, skin redness, skin itching and/or small blisters, participant care will be immediate.

The baby's skin condition will be regularly monitored by the nursing team using the NSCS scale [5]. If the NSCS is greater than 5, the electrodes will be removed and the baby will be excluded from the study for safety reasons. As a preventive measure, if any of the first five babies scores 5 or higher, the procedures will be reviewed to ensure that electrode application is optimal and the study will be temporarily stopped to introduce improvements if necessary.

On the other hand, although this is a highly unlikely situation, in the event of an allergic reaction to the contact adhesive or conductive gel, participant care will be immediate because the participant is in the neonatal intensive care unit, where severe allergy or anaphylactic reaction cases are treated. It will therefore be managed according to the NICU protocols of Salut Sant Joan de Reus – Baix Camp and Hospital Universitari de Tarragona Joan XXIII.

since the only part that comes into contact with the patient is a biocompatible adhesive indicated for the same application in the clinical setting.

Furthermore, a risk analysis has been carried out based on the applicable regulations and, as a mitigation measure, the use of a biocompatible adhesive indicated for the same application in the clinical setting has been selected.

Emergency contact information for reporting serious adverse events and serious adverse device reactions.

The Principal Investigator's contact details are added:
Name: Vicenç Pascual Rubio
Workplace address: Avinguda del Doctor Josep Laporte, 2, 43204 Reus
Telephone: 620753381
E-mail: vicenc.pascual@salutsantjoan.cat

Contact for CEIm Institut Investigació Sanitària Pere Virgili

Name: TORRES PAISAL, MERITXELL
Telephone: 977779946
E-mail: ceim@iispv.cat
Website: http://www.iispv.cat/plataformes_de_suport/

15. VULNERABLE POPULATION

Description of the vulnerable population

The inclusion in this study of newborns admitted to the Neonatal Intensive Care Unit (NICU) who are at risk of encephalopathy is fully justified by a specific and non-substitutable scientific need. The aCUP-E electrode has previously demonstrated, in studies conducted in healthy neonates, superior recording quality compared with conventional electrodes, with greater signal stability and fewer artefacts. However, to confirm its true clinical utility, it is essential to validate these results in the target population: newborns at risk of brain dysfunction who require continuous neurological monitoring.

This validation can only be carried out in neonates, since both brain physiology and monitoring conditions in the NICU are specific to this stage and cannot be extrapolated to other populations. In addition, aEEG recording in these patients often requires prolonged monitoring, frequently exceeding 24 hours, a context in which conventional electrodes have relevant limitations in terms of adherence and signal quality. In this setting, aCUP-E is proposed as the only alternative capable of maintaining stable and reliable recording over long periods, which is essential for detecting subclinical abnormalities and for adequate assessment of brain function.

Regarding the benefit-risk relationship, this is considered favourable. aEEG is a non-invasive technique integrated into routine clinical practice. The main risk is the possible occurrence of mild skin irritation, which is controllable and monitorable. Against this, patients may benefit from more precise brain monitoring, with potential impact on the early detection of neurological abnormalities.

The study includes strict measures to ensure the protection of this vulnerable population: obtaining informed consent from the parents, exclusion criteria to avoid inclusion of clinically unstable patients, continuous monitoring of skin condition and immediate removal in the event of any sign of risk, as well as oversight by an ethics committee and compliance with current regulations. Participation in the study also does not modify routine medical care or require additional interventions after completion.

Overall, conducting the study in newborns is essential, since it is the only way to validate a device that may significantly improve neonatal brain monitoring in at-risk situations, while maintaining an adequate safety profile and complying with the ethical principles of clinical research.

As moderately preterm infants are a vulnerable population, one of the inclusion factors is the absence of the following signs:

- Absence of identified congenital anomalies or genetic syndromes.
- Absence of signs suggestive of neurological injury: 5-minute Apgar test, <5; first pH <7.
- No encephalopathy.
- No abnormalities on cerebral ultrasound.

Description of the specific informed consent process

The neonatologist will be responsible for explaining the clinical investigation being conducted to the parents or legal guardians of the newborns and, where applicable, will be responsible for collecting and storing the signed informed consent. It can be found in Annex 16.

Description of the specific responsibility of the EC

It will be responsible for assessing any risk and modification of the study protocol through notifications, and for ensuring that the consent is properly described.

16. SUSPENSION OR EARLY TERMINATION OF THE CLINICAL INVESTIGATION

Criteria and preparations for suspension or early termination of the complete clinical investigation or of the clinical investigation at one or more investigation sites

As indicated in UNE-EN ISO 14155:2021, the sponsor may suspend or terminate the clinical investigation early for significant and documented reasons. These are:

- If an unacceptable risk is suspected during the clinical investigation, including a serious threat to the health of subjects. The investigation must be suspended while the risk is determined.
- If an unacceptable risk is confirmed that cannot be controlled.
- Due to the impossibility of including a minimum number of subjects that allows the final assessment of the study within a reasonable period according to the characteristics of the study.
- When instructed by the CEIC or the required regulatory authority (AEMPS).
- Due to non-compliance with the obligations assumed in the contract by any of the contracting parties.
- By mutual agreement between the parties, expressed in writing.
- At the will of one of the parties, expressed in writing with at least one month's notice.

In the event of suspension or early termination of the clinical investigation, the sponsor will inform AEMPS and the CEIC. The sponsor must provide the resources necessary to comply with the CIP obligations and existing agreements. The principal investigator must inform the subjects if this is stipulated in the agreement between the sponsor and the investigation site.

If the clinical investigation resumes, the sponsor must inform the principal investigator, the CEIC and, where applicable, AEMPS. Approval from the CEIC and, where applicable, AEMPS will be required for such resumption. The principal investigator must inform the subjects.

As mentioned, the temporary suspension or early termination of the clinical investigation will be justified and notified to the NCA of each Member State. In this case, AEMPS. In accordance with Article 77(1) of MDR 2017/745, this must be reported within 15 days or within 24 hours if based on safety grounds.

In addition, a Clinical Investigation Report (CIR) must be submitted to the NCAs of each Member State where the clinical investigation has been conducted (in this case, AEMPS) within one year of completion of the clinical investigation or within three months of early termination or temporary suspension, in accordance with Article 77(5) of MDR. The CIR will be accompanied by a summary written in language that is easily understood by the intended user.

Until EUDAMED is available and fully operational, notifications and submission of documentation (both the CIR and the summary) will be made through the same means used for the clinical investigation application, as indicated in MDCG 2024-15 “Guidance on the publication of clinical investigation reports and their summaries in the absence of EUDAMED”.

Criteria for accessing and breaking the blind/masking code in the event of suspension or early termination of the clinical investigation, if the clinical investigation involves a blinded/masked trial technique

Double blinding is not possible, but the data are analysed by the monitor and free of subjectivity.

Requirements for subject follow-up after completion, temporary suspension or early termination of an investigation

No specific follow-up is established after completion or interruption of the study. Participation ends with removal of the electrodes, at which time an immediate assessment is performed, including skin examination. Thereafter, safety is ensured by the continuous clinical monitoring inherent to admission to the NICU.

Requirements for follow-up of a subject who has withdrawn consent

No specific study follow-up is applicable. The subject ceases participation and remains under routine NICU clinical care, where any incident will be detected and managed according to standard care practice.

Procedures for subjects who cannot be followed up

No specific follow-up is planned after completion of the study. If necessary, contact may be made through the sponsor's telephone number provided. Subject safety is ensured by the continuous clinical surveillance inherent to the NICU.

17. PUBLICATION POLICY

This clinical investigation will be made public in accordance with Article 35 of the Declaration of Helsinki and section 5.4 of UNE-EN ISO 14155:2021.

As mentioned, after final closure of the clinical investigation, a CIR will be drafted, even if it has been suspended or terminated early. The CIR must be provided to AEMPS and, if required, to the CEIC. The CIR will be accompanied by a summary written in language that is easily understood by the intended user.

AEMPS will transmit both documents to the European Commission without undue delay after receiving them from the sponsor. The Commission will publish this information in the specific CIRCABC directory (Communication and Information Resource Centre for Administrations, Businesses and Citizens) one year after its submission to AEMPS. In the event of early termination or temporary suspension, the CIR and the summary will be publicly accessible immediately after submission. The documents will be stored in a specific public CIRCABC directory called “Clinical Investigation Reports on MDR and their summaries”.CIRCABC

In addition, the results obtained (positive, inconclusive or negative) will be submitted to specialist Clinical Neurophysiology journals in the form of a research article. Three journals to which the article should be submitted, in order, are: Clinical Neurophysiology, American Journal of Neurophysiology and Revista de Neurología. The anonymity of the subjects participating in the clinical investigation will be maintained at all times.

18. LIST OF TECHNICAL AND FUNCTIONAL CHARACTERISTICS OF THE PRODUCT

A list of the technical and functional characteristics of the product is detailed below:

1. Material used and its hardness: adapted so as not to produce stress concentrations that cause skin injury.
2. Appropriate dimensions and shape: ability to adapt to large curvatures, including in newborns at 23 weeks.
3. Translucency: to allow rapid detection of skin irritation, inspection of the conductive gel, checking the position of the electrode plate and checking the gel needle used to inject the conductive gel.
4. Wire direction: to minimise adhesion problems, the wire is perpendicular to the skin, avoiding contact with the skin.
5. Good adherence to the scalp: the electrode adhesive is easy to keep adhered and easy to remove.
6. Sealing: the electrode contains a part that can be clicked into place to prevent the gel from drying.
7. Flexibility: the electrode has sufficient flexibility to adapt to the baby's scalp.
8. Smaller contact diameter: 15 mm.
9. Material used and its hardness: adapted so as not to produce stress concentrations that cause skin injury.

19. REFERENCES

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