CEN Workshop Determination of nucleic acid encapsulation efficiency in Lipid Nanoparticles using fluorometry

Workshop description form

- PART A – Workshop Summary
- PART B – Project Plan

PART A – Workshop SUMMARY

1	WS details		
1.1.	**Organization**	☒ CEN ☐ CENELEC ☐ Joint with ☐ CEN lead ☐ CENELEC lead	
1.2.	**Title**	Determination of nucleic acid encapsulation efficiency in Lipid Nanoparticles using fluorometry	
1.3.	**Scope**	The planned Workshop will define a detailed method for the determination of the encapsulation efficiency of DNA or RNA into Lipid Nanoparticles (LNPs) using a fluorometer.	
1.4.	**Does this WS stem from an EU Research project?**	☒ YES Name of the project: Boosting the reduction of the environmental impact of pharmaceutical products throughout their entire life cycle. (ETERNAL) Grant number: 101057668 ☐ End date 31-08-2026 NO	
1.5.	**Financial support**	☒ EU Research project ☐ EC/EFTA Grant reference: Type here ☐ Other Specify, if needed: Type here	
1.6.	**WS Proposer/Proposed Chair**	Name: Organization: Postal address: Email: Phone: Webpage: Contact person (name and email):	Elisabet Rosell/ Iria Naveira Laboratorio Reig Jofre SA Av. de les Flors, 08970 Sant Joan Despí, Barcelona, Spain erosell@reigjofre.com/ inaveira@reigjofre.com +34 93 480 67 10 www.reigjofre.com Elisabet Rosell (erosell@reigjofre.com, alternative mail: elisabet.rosell@upf.edu)/ Iria Naveira (inaveira@reigjofre.com)
1.7.	**WS Secretariat**	Organization: Postal address: Email: Phone: Webpage: WS Secretary name: Email: Phone:	UNE – Spanish association for standardization Calle Génova 6, Madrid (Spain),28004 info@une.org +34 915 294 900 www.une.org Teresa Sanchez Segura tsanchez@une.org +34 650 251 555
1.8.	**CEN and CENELEC Management Centre (CCMC) contact**	Organization: Postal address: Webpage: CCMC Project Manager name: Email: Phone:	CEN and CENELEC Rue de la Science 23B - 1040 Brussels, Belgium https://www.cencenelec.eu/Pages/default.aspx Claire Van Thielen cwa@cencenelec.eu +3225500831 +32478793545
1.9.	**Tentative date and place of the Kick-off Meeting**	Date: 2025-06-04	Place: Online

1.10.	**Does the proposed Workshop fall within the scope of existing CEN and/or CENELEC Technical Bodies?[1]**	☐ ☒	YES Specify: Type here NO
1.11.	**Are there other Technical Bodies or Joint Advisory and Coordination Groups potentially interested in the Workshop? ?[2]**	☒ ☐	YES Specify: CEN/TC 352 – Nanotechnologies; CEN/TC 233 - Biotechnology NO
1.12.	**Are the following aspects affected?**	Safety matters Management system aspects Conformity assessment aspects Security matters	YES[3] ☐ NO ☒ YES[4] ☐ NO[7] ☒ YES[5] ☐ NO ☒ YES[6] ☐ NO ☒ NO[8]
		Add information/explanations if Management System aspects and Conformity Assessment aspects are affected: Type here	
2	**WS Deliverables**		
2.1.	**CWA #1**		
2.1.1	**Title**	☒ ☐	Same as WS title (1.2) Other: Type here
2.1.2	**Scope**		The CWA will define a detailed method for the determination of the encapsulation efficiency of DNA or RNA into Lipid Nanoparticles (LNPs) using a fluorometer.
2.1.3	**Does the proposed CWA conflict with a published EN**	☐ ☒	YES Specify: Type here NO In case the answer is 'yes', the development of the CWA shall be stopped

[1] Part A and Part B of this form shall be sent by the WS secretary to the secretary of the Technical Bodies identified in this section to inform them about the creation of the WS and register any possible objection within 30 days (45 during the holiday period).

[2] Part A and Part B of this form should be sent by the WS secretary to the Bodies identified in this section to inform them about the creation of the WS.

[3] Work on the proposed CEN and/or CENELEC Workshop shall not be initiated.

[4] The CEN and/or CENELEC Workshop proposal shall be submitted to the CEN/CENELEC BT(s) for decision.

[5] CEN-CENELEC Internal Regulations - Part 3, Clause 33 applies.

[6] For projects dealing with security matters the security risk analysis provided in Annex I shall be carried out.

[7] See Note 2 in CEN-CENELEC Guide 29, Clause 3.

[8] See Note 2 in CEN-CENELEC Guide 29, Clause 3.

PART B – Project Plan

Abstract

The encapsulation of RNA and DNA in lipid nanoparticles (LNPs) has emerged as a pivotal technology in the delivery of genetic materials for therapeutic applications, including vaccines and gene therapies. Despite significant advancements, the accurate determination of encapsulation efficiency remains a critical challenge. Traditional methods, such as gel electrophoresis and UV spectroscopy, often suffer from limitations including low sensitivity, interference from free nucleic acids, and the inability to differentiate between encapsulated and non-encapsulated species.

The main objective of the WS is to present a fluorometric method for the precise quantification of RNA and DNA encapsulation efficiency in LNPs. This method leverages the high sensitivity and specificity of fluorescence-based detection, utilizing dyes that selectively bind to nucleic acids. The fluorometric approach addresses the shortcomings of existing techniques by providing a clear distinction between encapsulated and free nucleic acids, thereby enhancing the accuracy of encapsulation efficiency measurements.

The WS aims at establishing a standardized protocol for the fluorometric determination of nucleic acid encapsulation efficiency in LNPs. By detailing the methodological steps and validation procedures, we aim to provide a robust analytical framework that can be adopted across research and clinical laboratories. This standardization is expected to facilitate the consistent and reliable assessment of LNP formulations, ultimately contributing to the optimization of nucleic acid delivery systems.

This CWA will be developed in the framework of ETERNAL European research project.

1 Status of the project plan

Draft project plan for public commenting (Version 1.0)

This draft project plan is intended to inform the public of a new Workshop. Any interested party can take part in this Workshop and/or comment on this draft project plan by sending an email to the WS secretary.

All those who have applied for participation or have commented on the project plan by the deadline will be invited to the kick-off meeting of the Workshop on 2025-06-04.

2 Workshop proposer and potential Workshop participants

This workshop is proposed in the framework of the EU-funded research project ETERNAL (GA 101057668). More information: https://www.eternalproject.eu/

2.1 Workshop proposer

Proposed Chair: Elisabet Rosell. Head of Biological Sciences at Reig Jofre, and Iria Naveira (contact details are provided in Part A of this form).

Founded in 1929 in Barcelona, Spain, REIG JOFRE is a pharmaceutical company listed on the Spanish Stock Exchange market, under the ticker RJF, dedicated to the research, development, manufacture and marketing of pharmaceutical products and food supplements.

2.2 Potential participants

This CWA will be developed in a Workshop (temporary body) that is open to any interested party. The participation of other experts would be helpful and is desired. It is recommended that:

- Academic and research institutions
- Pharma Industry
- Biotechnology Industry
- Standards application

take part in the development of this CWA.

3 Workshop objectives and scope

3.1 Workshop background

The initiation of this Workshop is driven by the need to establish a standardized method for determining the encapsulation efficiency of RNA and DNA in lipid nanoparticles (LNPs) using fluorometry. Current methods, such as gel electrophoresis and UV spectroscopy, face limitations including low sensitivity and interference from free nucleic acids. The Workshop aims to address these issues by developing a robust fluorometric protocol that enhances accuracy and reliability in encapsulation efficiency measurements. The future benefit of the CWA(s) includes providing a consistent analytical framework that can be adopted across research and clinical laboratories, thereby optimizing nucleic acid delivery systems. Explicitly, the CWA(s) will not cover the broader aspects of LNP formulation or therapeutic efficacy, focusing solely on the analytical determination of encapsulation efficiency.

Currently, the market offers several methods for assessing nucleic acid encapsulation efficiency, including gel electrophoresis, UV spectroscopy, and dynamic light scattering. However, these methods often lack the sensitivity and specificity required for accurate measurements. The envisaged CWA(s) will differ by providing a fluorometric approach that leverages fluorescence-based detection for higher sensitivity and specificity. This method will offer a clear distinction between encapsulated and free nucleic acids, addressing the shortcomings of existing techniques.

The resulting CWA will be applicable to manufacturers of lipid nanoparticles in academia and industry. The results will form a valuable basis for design for pharmaceutical and biotechnology companies. It will allow these companies to consider nucleic acid encapsulation efficiency in the early design phases and hence develop more effective and reliable delivery systems. The subject of the planned CWA is not currently covered by any existing standard or Technical Committee.

In the framework of standardization activities within the Eternal European Project, several Technical Committees were contacted with two main goals: firstly, to raise awareness of the project, and secondly, to inform about the specific needs for standardization detected over the course of the project regarding the characterization and testing of lipid nanoparticles for nucleic acid delivery applications. However, the above-mentioned Technical Committees (or any other identified which could have related activities or standards/technical specifications that

could be of interest for the future development of the CWA) will be contacted and invited to participate in the development of the CWA or to provide feedback on it.

No regulations have been identified within the scope of the future CWA.

4 Workshop programme

4.1 General

The kick-off meeting is planned to take place on 2025-06-04, online as virtual meeting, through TEAMS. A draft for public commenting will be published for 30 days.

After the kick-off meeting, the necessary number of Workshop meetings (kick-off meeting and Workshop meetings) and web conferences will be held, during which the content of the CWA will be discussed, agreed and approved. The number of agreed meetings will be arranged as needed in the drafting and agreement process. Online work will be preferred to improve the sustainability of this work.

The working language (language of meetings, minutes, etc.) of the WS will be English. The CWA will be written in English.

4.2 Workshop schedule

A tentative timeline is described in the Figure below.

Table 1: Workshop schedule (preliminary)

CEN/CENELEC Workshop	M01 Abr 25	M02 May 25	M03 Jun 25	M04 Jul 25	M05 Ago 25	M06 Sep 25	M07 Oct 25	M08 Nov 25	M09 Dec 25	M10 Ene 26
Initiation										
1. Workshop description form submission										
2. Open commenting period on draft project plan (mandatory)										
Operation										
3. Kick-off meeting										
4. CWA(s) development										
5. Open commenting period on draft CWA(s) (optional)										
6. CWA(s) finalized and approved by Workshop participants										
Publication										
7. CWA(s) publication										
Dissemination										
Milestones			K	V	V	V	V		A	P D

Legend

K Kick-off

M Workshop meeting

V Virtual Workshop meeting

A Adoption of CWA

P Publication of CWA

D Online distribution of CWA

5 Resource planning

Registration and participation at this CEN Workshop are free of charge, but each participant shall bear his/her own costs for travel, accommodation, and subsistence in the case of on-site meetings (at the moment of writing this document most meetings are planned to take place on-line).

The administrative costs of the CEN Workshop Secretariat will be financed within the framework of a research project: European Union's Horizon Europe Framework Programme (HORIZON) funded project ETERNAL under grant agreement No 101057668.

The copyright of the CWAs shall be with CEN. 8% secretariat costs will be provided by UNE to CCMC to cover the free download of the published CWA.

6 Workshop structure and rules of cooperation

6.1 Participation in the Workshop

The Workshop will be constituted during the kick-off meeting. By approving this project plan, the interested parties declare their willingness to participate in the Workshop and will be formally named as Workshop participants, with the associated rights and duties. Participants at the kick-off meeting who do not approve the project plan are not given the status of a Workshop participant and are thus excluded from further decisions made during the kick-off meeting and from any other decisions regarding the Workshop.

As a rule, the request to participate in the Workshop is closed once it is constituted. The current Workshop participants shall decide whether any additional members will be accepted or not.

Any new participant in the Workshop at a later date is decided on by the participants making up the Workshop at that time. It is particularly important to consider these aspects:

a. expansion would be conducive to shortening the duration of the Workshop or to avoiding or averting an impending delay in the planned duration of the Workshop;
b. the expansion would not result in the Workshop taking longer to complete;
c. the new Workshop participant would not address any new or complementary issues beyond the scope defined and approved in the project plan;
d. the new Workshop participant would bring complementary expertise into the Workshop in order to incorporate the latest scientific findings and state-of-the-art knowledge;
e. the new Workshop participant would actively participate in the drafting of the manuscript by submitting concrete, not abstract, proposals and contributions;
f. the new Workshop participant would ensure wider application of the CWA.

All Workshop participants who approved the publication of the CWA or its draft will be named as authors in the European Foreword, including the organizations which they represent. All Workshop participants who did not approve the publication of the CWA will not be named in the European Foreword.

6.2 Workshop responsibilities

The Workshop Chair is responsible for content management and consensus building. The Workshop Chair is supported by the Workshop Vice-Chair (if any) and the responsible Workshop secretariat, whereby the Workshop secretariat will always remain neutral regarding the content of the CWA(s). Furthermore, the Workshop secretariat shall ensure that CEN-CENELEC's rules of procedure, rules of presentation, and the principles governing the publication of CWA(s) have been observed. Should a Workshop Chair no longer be able to carry out her/his duties, the Workshop secretariat shall initiate the election of a new Workshop Chair. The list below covers the main tasks of the Workshop Chair. It is not intended to be exhaustive.

- Content related contact point for the Workshop
- Presides at Workshop meetings
- Ensures that the development of the CWA respects the principles and content of the adopted project plan
- Manages the consensus building process, assesses when the Workshop participants have reached agreement on the final CWA, on the basis of the comments received
- Ensures due information exchange with the Workshop secretariat
- Represents the Workshop and its results to exterior

The Workshop secretariat, provided by a CEN and/or CENELEC Member, is responsible for organizing and leading the kick-off meeting, in consultation with the Workshop proposer. Further Workshop meetings and/or web conferences shall be organized by the Workshop secretariat in consultation with the Workshop Chair. The list below covers the main tasks of the Workshop secretariat. It is not intended to be exhaustive.

- Administrative and organizational contact point for the Workshop
- Ensures that the development of the CWA respects the principles and content of the adopted project plan and of the requirements of the CEN-CENELEC Guide 29
- Formally registers Workshop participants and maintains record of participating organizations and individuals
- Offers infrastructure and manages documents and their distribution through an electronic platform
- Prepares agenda and distributes information on meetings and meeting minutes as well as follow-up actions of the Workshop
- Initiates and manages CWA approval process upon decision by the Workshop Chair
- Interfaces with CEN-CENELEC Management Centre (CCMC) and Workshop Chair regarding strategic directions, problems arising, and external relationships
- Advises on CEN-CENELEC rules and brings any major problems encountered (if any) in the development of the CWA to the attention of CEN-CENELEC Management Centre (CCMC)
- Administrates the connection with relevant CEN or CENELEC/TCs

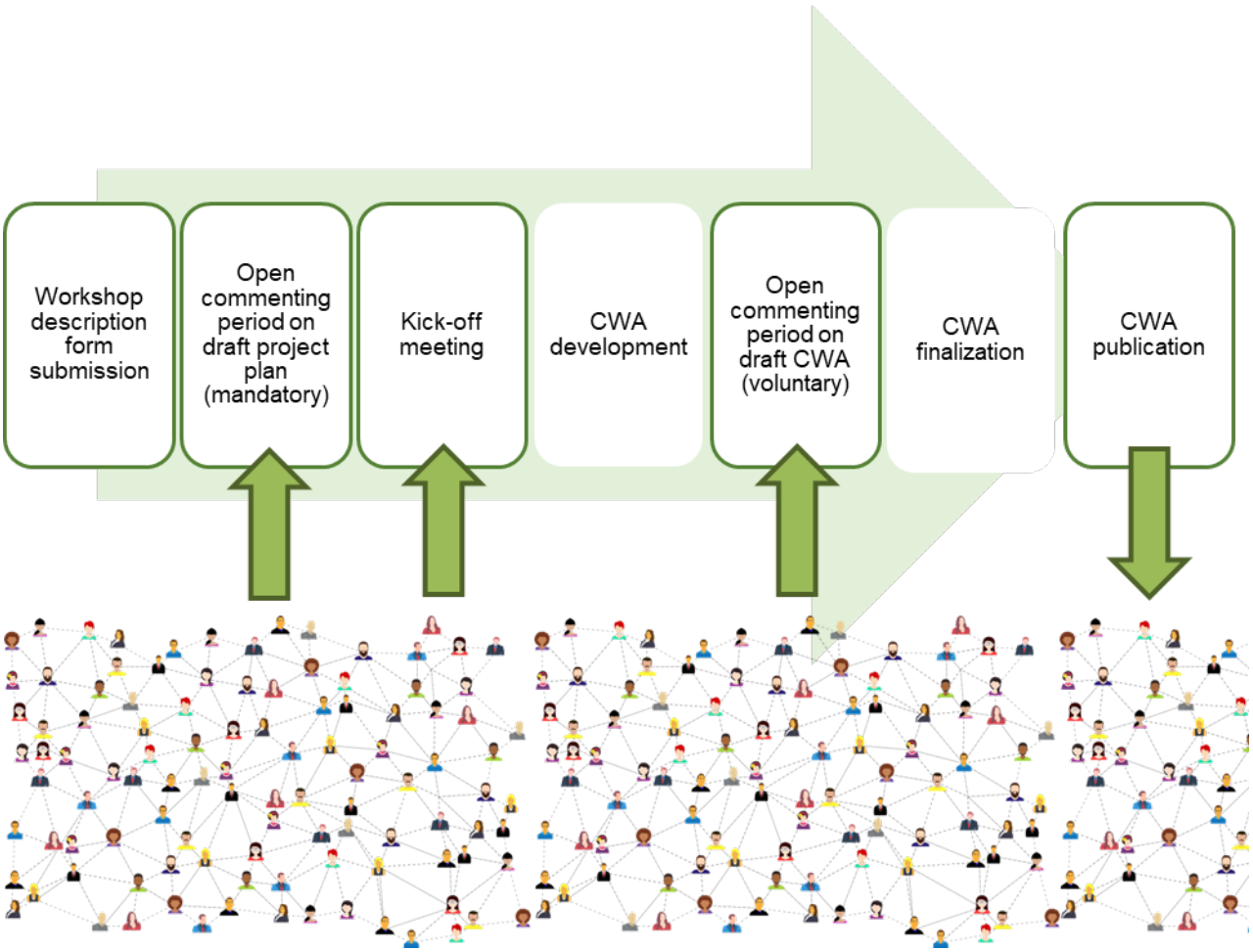
6.3 Decision making process

The CEN and/or CENELEC Workshop Chair is responsible for ensuring that the development of the CWA follows the principles and content of the project plan described in this document and the requirements of CEN-CENELEC Guide 29. The CEN and/or CENELEC Workshop Chair may take decisions on the conduct of the CEN and/or CENELEC Workshop on the basis of the comments expressed by the participants and of CEN-CENELEC Guide 29.

Decisions shall be taken based on consensus of the WS participants.

7 Dissemination and participation strategy

Potential participants identified in section 2.2 and potential interested stakeholders identified in Part A should be informed of the open commenting phase, if any, and of the publication of the CWA.



In addition to the CCMC website, the final CWA might be advertised on:

- sector specific newsletter
- social media, such as
  - web page of European project: www.eternalproject.eu
  - Facebook
  - Instagram
  - LinkedIn
  - X
- Research Gate
- EC Newsroom
- Others