Nuclease-Free Handling of Test Article and Tissues in Rodent and Nonhuman Primate Inhalation Toxicology Studies – A Case Study

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Abstract

A nonclinical program of a readily degraded oligonucleotide test material in rodents and nonhuman primates (NHPs) required ribonuclease (RNase)-free handling in compliance with detailed Study Partner-defined preparation, aerosol generation and sample processing procedures. Dose administration required several hours of aerosol generation with Test Article stability specified by the Study Partner as 60 minutes from the start of preparation to last use. The aerosol generation mesh micropump platform was also specified by the Study Partner. Where practical, the work environment and all materials that contacted aerosol generation, sampling, sample processing and necropsy equipment required pretreatment with RNase*Zap*™ and then washing with ultrapure water before contact with the test article or tissues. Disposable sterile and RNase-free instruments and containers were used where practical and available. Overall, the program was successfully completed using these special procedures to produce quality data.

Introduction

A program of IND enabling work placed at the Labcorp Early Development Laboratories Somerset, NJ facility required the exposure of both rodents and NHPs to aerosols generated from a readily degraded oligonucleotide test article. Due to concern regarding RNase-free handling of their Test Article, the Study Partner provided detailed protocols for the preparation and handling of formulation and for aerosol generation and sample processing procedures. Test Article stability after initial formulation was specified by the Partner as 60 minutes from the start of preparation to time of last use. The mesh micropump aerosol generation platform was also specified by the Study Partner.

Where practical, the work environment and all materials used for that contacted aerosol generation, sampling and processing equipment had to be pretreated with RNase*Zap*™ (ThermoFisher) then washed with ultrapure water before use with the Test Article. Necropsy instruments and surfaces were treated with RNase*Zap*™ and allowed to dry between both animals and individual tissues, and disposable instruments were used where practical and available.

Problem Statements

- Test Article required preparation and use under RNase-free conditions.
- The Test Article as formulated for use in the Labcorp Somerset Pharmacy had a Partner-defined stability of 60 minutes from the start of preparation to final use. Formulated Test Article had to be transferred from the pharmacy preparation area to the inhalation exposure laboratories on foot (five minutes transit time).
- Exposure durations were six hours for the rodents and two hours for the NHPs.
- Aerosol concentration targets and system airflows required the combined output from three mesh micropump nebulizers to each exposure system. Nebulizers had to be emptied and refilled for each new formulation batch without interrupting the animal exposures.
- Formulation of the Test Article in ultrapure water was associated with high surface tension and separation of the prepared formulation from the mesh micropump pores. This resulted in frequent periods of reverse pumping with air movement from the underside of the mesh into the reservoir with concomitant failure of the nebulizers to deliver aerosol.
- Samples had to be collected into chilled glassware and eluted into RNase-free, ultrapure water then analyzed within 60 minutes of collection to allow separate determinations of the concentrations of the free and encapsulated mRNA presented in the exposure system breathing zone.
- Tissue collections at the end of the treatment period required RNase-free handling at necropsy followed by collection and snap freezing of selected tissues.

Methods

Conduct of the IND-enabling studies required method review and coordination between multiple Labcorp Somerset departments with additional formulation and handling instruction provided by the Study Partner. Daily exposures required the coordinated involvement of the pharmacy, inhalation, animal operations and bioanalytical departments, terminal procedures were managed by the necropsy department. All procedures were overseen and approved by the safety assessment department assigned Study Director.

Animal care and use was conducted in alignment with animal welfare regulatory requirements in an AAALAC-accredited facility.

Methods 1 – Delivery of the Test Article Aerosol Under RNase-Free Conditions

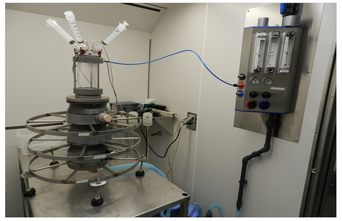
All staff involved in the programs received detailed instruction in the ubiquitous nature of RNase active sources of contamination and in the use of RNase*Zap*™ together with subsequent maintenance of RNase minimizing conditions. All work areas and items of equipment including nebulizers and sample collection glassware were sprayed with RNase*Zap*™ solution, dried with RNase-free wipes, and rinsed with ethanol before three separate washes with ultrapure water.

Methods 2 – Limited Formulation Stability

After preparation of an RNase-free work area, an aliquot of Test Article formulation for the first interval of aerosol generation was prepared by the pharmacy department. The formulation procedure required approximately 2 minutes with a further 2 minutes to complete the GLP documentation. The Test Article formulation was then transferred to the inhalation department and loaded into RNase-free syringes for use. The transfer, receipt documentation and syringe fill time totaled approximately 10 minutes. Once syringes were prepared, the changeover of Test Article was initiated and reliably completed in a further 5 minutes. Based on these logistics, a nominal maximum aerosol generation period of 40 minutes was assigned to each formulation preparation. Due to the staggered start of exposure groups, this required the preparation of 10 separate formulations on each exposure day for rodent studies and five formulation occasions per day for each NHP exposure occasion. Removal of Test Article aliquots from deep-frozen storage was coordinated between the inhalation department and pharmacy to ensure fully thawed test Article was available for dilution and issue at the necessary intervals.

Methods 3 – Use of Multiple Mesh Micropump Nebulizers on Each System

Test Item syringes were directly filled from the transfer container and an RNase-free environment for the formulations maintained by directly connecting filled syringes to each of three vibrating mesh micropump nebulization platforms on each exposure system. Emptying the nebulizers at the end of the defined acceptable stability period was achieved by inverting the nebulizer and drawing up the reservoir residues directly into the already attached syringe.

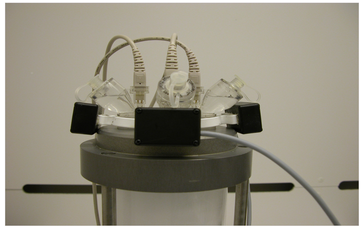


Rodent Exposure Tower with Pre-Chamber

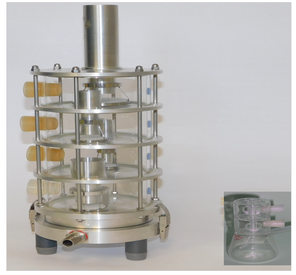
Nonhuman Primate Aerosol Distribution Manifold

Methods 4 – Failures of Aerosol Generation Due to Formulation in Ultrapure Water

Test Article contact with mesh nebulizer screens required intermittent agitation of the reservoir contents by shaker mechanisms. The shaker units were designed and manufactured by Labcorp Inhalation Engineering Services based at the Huntingdon, UK facility. Shakers were found to provide optimal fluid contact with the palladium nebulizer screens using a 12-second cycle of 4 seconds of vibration followed by an 8-second pause. Even with the agitation devices attached, nebulizers required constant monitoring and frequent replacement to address aerosol generation failures. The evolution of the vibration system is the subject of a separate Case Study Poster. Although still requiring continuous observation, the use of the vibration units provided acceptable continuity of delivery for the required daily periods of aerosol generation.



Nebulizer Reservoir Shaker Mechanisms (Black Box)



Multistage Liquid Impingers

Methods 5 – Sample Collection - Concentration

Aerosol samples could not be collected using standard techniques as the Test Article was readily degraded by contact with all collection media types other than ultrapure water and RNase*Zap*™ and ultrapure water-rinsed glassware. Consequently, aerosol samples were collected by direct jet impingement onto glassware surfaces that had been subjected to rigorous cleaning with ethanol followed by RNase*Zap*™ and a minimum of three washes with RNase-free water. To optimize sampling efficiency, impinger traps were maintained in a water ice, water and sodium chloride mix throughout each collection to provide a glassware temperature of -5°C. Two traps in series were employed to minimize aerosol losses due to sampling breakthrough. After collection, the samples were eluted with RNase-free water and adjusted to the required precise volume for subsequent analytical determination of Test Article concentration. Analysis was carried out using a Nanodrop™ A280 analyzer or determination using the Ribogreen method.

The delivered aerosol droplet size was determined by collection of aerosol samples directly into the multistage liquid impinger and the extracted samples were analyzed as indicated for the concentration samples.

Methods 6 – Necropsy and Tissue Collection Using RNase-Free Techniques

Necropsy methods were especially challenging due to the ubiquitous presence of RNase active materials in the environment and intrinsic RNase activity in all tissues. Procedures were designed to eliminate the variability that might be associated with work area contact, necropsy tool contamination, exhaled air contamination from the prosectors and contamination with RNases during organ weight data collections. Each prosector wore a full Tyvek® hooded suit, nitrile gloves and N95 masks throughout all necropsy procedures. The use of the powered air purifying respiratory (PAPR) protection normally used by staff in the necropsy suite area was rejected because the PAPR hoods exhaust under the operator's chin and were considered to potentially increase tissue contamination from exhaled air.

Method for Collection of Frozen Tissues for Toxicokinetic Evaluation (at Necropsy)

1. Tissue collection area cleaned as follows:
   a. Wipe down surfaces (recommend stainless steel tray for containment and ease of cleaning) and spray with 70% isopropanol to clean.
   b. Change gloves and spray with RNase*Zap*™.
   c. Spray down entire work area with RNase*Zap*™ or use RNase*Zap*™ wipes. Allow contact time of approximately 1 minute.
   d. Change gloves and spray with RNase*Zap*™.
   e. Clean with RNase-free water.
2. Animals were exsanguinated away from the clean tray (above) and tissues were exposed in the usual manner. Once each organ or tissue to be collected was exposed/free of the animal, it was placed on the 'clean' tray, gloves were changed and the new gloves were treated with RNase*Zap*™ as above. Technician face masks were not removed during entire procedure.
   Conversation in the lab was minimized to the extent possible to reduce respiratory airflow in the vicinity of harvested tissues.
3. Once a tissue was on the clean tray, all dissection instruments used in subsequent procedures were DNase/RNase free and single use only. Where used, weighing boats were cleaned as described above.
4. For non-disposable equipment (scissors, etc.), equipment had to be visibly free of debris, sprayed with RNase*Zap*™, allowed to stand for a minimum 5-minute contact time, then rinsed in ethanol between each tissue collection.
5. The steps were repeated for each animal and tissue.
6. For each prosector, a clean area (stainless steel tray) was maintained for the frozen tissue collection and cleaned as indicated above for each animal.
7. All of the steps described above were documented on manual records. Paper records could not be RNase free; however, pens were subject to the cleaning procedures and gloves were changed and subject to RNase*Zap*™ procedures in the event of any contact with untreated surfaces.

Conclusion

The procedures adopted permitted the successful conduct of two IND-enabling programs with mRNA-based pharmaceutical candidates. Formulation handling, aerosol generation and aerosol sampling were, while challenging, maintained over long animal exposure intervals despite the stability limitations of the two Test Articles.

Overall, RNase contamination was concluded to have been minimal through the rigid adherence to laboratory management policies that included continuous oversight for every procedural stage. Larger studies requiring the combined output from four nebulizers are planned in 2022.

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