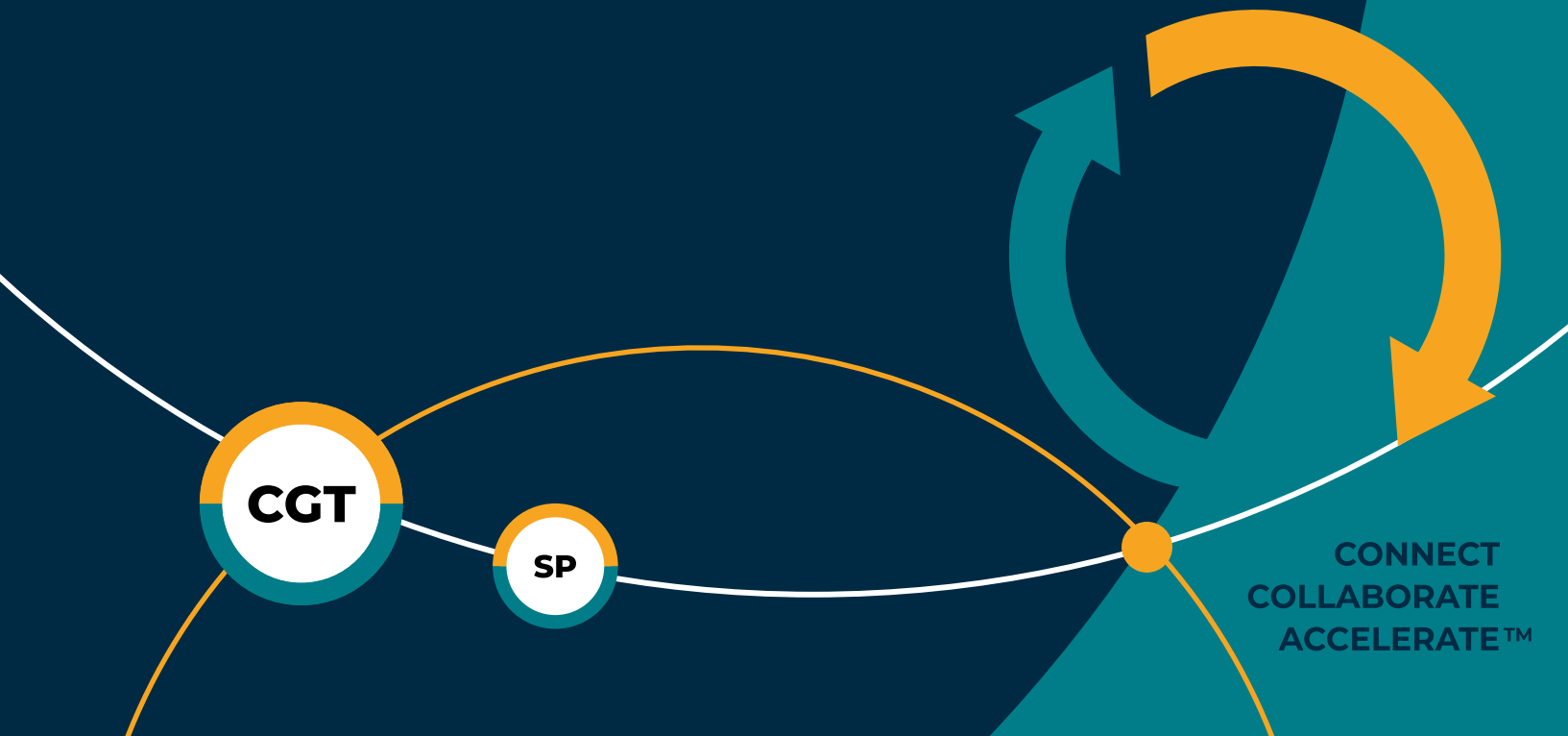
BioPhorum

RAW MATERIALS

A proposal to align release standards for endonucleases used in nucleic acid removal



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About BioPhorum

BioPhorum's mission is to create environments where the global biopharmaceutical and device industry can collaborate and accelerate its rate of progress, for the benefit of all.

Since its inception in 2004, BioPhorum has become the open and trusted environment where senior leaders of the biopharmaceutical industry come together to openly share and discuss the emerging trends and challenges facing their industry.

Growing from an end-user group in 2008, BioPhorum's membership now comprises top leaders and subject matter experts from global biopharmaceutical manufacturers and suppliers, working in both long-established and new Phorums. They articulate the industry's technology roadmap, define the supply partner practices of the future, and develop and adopt best practices in drug substance, fill finish, process development and manufacturing IT.

In each of these Phorums, BioPhorum facilitators bring leaders together to create future visions, mobilize teams of experts on the opportunities, create partnerships that enable change and provide the quickest route to implementation, so that the industry shares, learns and builds the best solutions together.

Contributors

This paper is the work of a combined team of industry experts, with key contributors from the following member companies:

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Abstract

Endonucleases are enzymes which cleave a polynucleotide chain by separating nucleotides. Although they are used ubiquitously in cell and gene therapy (CGT), there is a lack of defined release test criteria throughout the pharmacopeial landscape, particularly for those enzymes used in the nucleic acid removal steps of manufacturing. This bioprocessing step is predominated by commercially available Serratia marcescens-derived endonucleases recombinantly expressed from different host cell systems, marketed and sold in similar formulations under various brands.

Pharmaceutical compendial standards provide guidance and criteria via monographs and general chapters, and are the framework for many ancillary raw material control strategies. This paper proposes to unify the polychotomy of current industry approaches with a single core standard of test methods and criteria necessary for GMP manufacturing. A common testing standard would have multiple benefits including consistency across suppliers, protection of supplier intellectual property (IP), and facilitation of drug development.

1.0

Introduction

Cell and gene therapy (CGT) products are the vanguard of biopharmaceuticals offering groundbreaking opportunities for the treatment of diseases and injuries. Cell-based products and viral vectors in particular hold the promise to move from symptomatic disease management to modifying and eventually curing diseases. However, the novelty of these innovative products is also associated with a lack of maturity that complicates the development of therapies. The BioPhorum CGT raw materials team was created to unite leaders in the biopharmaceutical industry, and to formulate recommendations on best practices when conducting CGT research, clinical or commercial manufacturing.

Endonucleases are used almost universally as an ancillary raw material in the CGT industry. Endonucleases are sold by multiple suppliers, but only a few suppliers provide most of the world's supply of endonuclease that meets the quality requirements for good manufacturing practice (GMP) in the CGT industry. There is a lack of defined release criteria, so a compendial chapter would align testing across the CGT industry. It would have multiple benefits including ensuring consistency across suppliers, protecting supplier intellectual property (IP) and facilitating drug development. This paper proposes standards and methods to bridge the gap between current methods and those required for a pharmacopeia general chapter.

Action requested from the reader: Please comment on the questions in this paper and add points for discussion to support development of an endonucleases testing standard and to facilitate its future use. All information will be used to generate consensus.

Please note that in this paper 'user' means 'CGT company' or 'drug developer'.

Testing outlined in this paper is not intended to supersede the requirement of USP<1043> or ICH Q8. It is intended to be used with qualified materials once the risk-based approach is completed. Suggested test methods could be used for phase I. They could also be the basis for future phases and commercialization, with justification and validation (as testing must be optimized to the product and regulations become more stringent).

2.0

Problem statement

The objective of this paper is to propose a framework for testing and release of non-site-specific recombinant endonucleases used to cleave nucleic acids into small fragments. This is intended to accelerate and streamline the development of CGT products.

Due to a lack of guidance for this type of ancillary raw material, this paper proposes universal standards and release testing for endonucleases.

3.0

Scope

Non-site-specific nucleases such as genetically engineered endonucleases derived from *Serratia marcescens*, described as Enzyme Commission (EC) number 3.1.30.2 present as dimers of approximately 30kDa subunits with two essential disulfide bonds[1,2]. The enzyme degrades ribonucleic and deoxyribonucleic acids by cleavage of 3'- and 5'-phosphodiester bonds to form 5'-phosphorylated oligonucleotides approximately 3 to 5 bases in length[3].

This scope excludes RNase, DNAse used in mRNA vaccine manufacturing and sequence-specific endonucleases, such as CRISPR-Cas9 or restriction enzymes. The scope is specific only to GMP bioprocessing involving the removal of residual nucleic acids and excludes endonucleases when used in assays.

4.0

Proposed universal test methods for release of endonucleases

The BioPhorum team have compared certificates of analysis (CoAs) from multiple suppliers and observed significant differences in how release testing is performed for endonucleases. Differences were observed between suppliers' certificates for identity, total protein, specific activity and purity testing. An example using two blinded suppliers is provided in Table 1.

Table 1: Example supplier testing of endonucleases prior to release

	Example Certificate of Analysis 1		Example Certificate of Analysis 2	
Attribute	**Method**	**Specification**	**Method**	**Specification**
Appearance	Visual	Clear colorless solution	Visual	Clear transparent solution
Activity	Photometric: One unit (U) will digest herring sperm DBA to acid soluble oligonucleotides equivalent to a change in A260 of 1.0 in 30 mins at pH 8.0 (37°C)	≥250 units/µl	Photometric: One unit (U) will digest salmon sperm DBA to acid soluble oligonucleotides equivalent to a change in A260 of 1.0 in 30 mins at pH 8.0 (37°C)	≥250 Units/µl
Purity	SDS-PAGE	99%	SDS-PAGE	99%
Specific activity	Bradford assay	>1.1 x 10^6 U/mg	Photometric at 280nm	>6 x 10^5 U/mg
Total protein (not on CoA)	Bradford assay	Used in Specific Activity Calculation	A280	Used in Specific Activity Calculation
Protease activity	Protease detection assay	Not detected	Protease detection assay	Not detected
Endotoxin level	LAL	<0.25 EU/kU	LAL as per EP 2.6.14	Not detected
Mycoplasma	-	Not detected	-	-
Adventitious agents	-	Not detected	-	-
TMC	TAMC/TYMC	Aerobic bacteria: <10 CFU/100 kU Yeast/molds: <10 CFU/100 kU	TAMC/TYMC (EP 2.6.12)	Aerobic bacteria: <5 CFU/200 uL Yeast/molds: <5 CFU/200 uL
Elemental impurities	ICP-MS	ICH Q3D—no metals above option 1 limit except: Co (≤0.5 ppm) Cr (≤110 ppm) Cu (≤30 ppm) Mo (≤150 ppm) Ni (≤2 ppm)	-	-
Expression host	*E. coli* (Gram negative)		*Bacillus sp.* (Gram positive)	
Buffer composition	50% glycerol containing 20mM Tris HCl, pH 8.0 20mM NaCl 2mM $MgCl_2$ 50% (v/v) Glycerol solution		20mM Tris-HCl pH 8.2 20mM NaCl 2mM $MgCl_2$ 50% (v/v) Glycerol (synthetic)	

A '-' denotes information is unavailable from the supplier as part of CoA.

Table 2 describes a proposed unified testing approach non-site-specific endonucleases to harmonize an industry approach and reduce complexity:

Table 2: Recommended endonucleases chapter

Test method	Proposed acceptance criteria
Appearance/Visual inspection	Clear and colorless
Identification (electrophoresis) (for *Bacillus sp. and E. coli derived endonucleases*)	Co-migrates with reference standard
Identification (activity)	Conforms to specification ≥250 U/µL
Activity (one unit defined as a change of 1.0 absorbance units at 260nm in 30 minutes with a reference nucleic acid substrate at an appropriate pH between 7 and 8.5, and 2mM MgCl2) (for *Bacillus sp. and E. coli derived endonucleases*)	≥250 U/µL
Specific activity (calculated on protein)	≥1100 kU/mg
Purity (SDS-PAGE)	≥99.0%
Protease activity/Protease detection assay	Not detectable
Colony count (aerobic bacteria)	<10 CFU in 100 kU
Colony count (yeasts and molds)	<10 CFU in 100 kU
Endotoxins: (LAL-test)	<0.25 EU in 1000 U
Total protein Bradford assay	**Report specific result in order to calculate Specific Activity**

The tests proposed, and associated test criteria, are representative of a survey of manufacturers of GMP materials used in clinical and commercial programs. These criteria are potentially not all inclusive, but represent the current standards of identity, quality, purity, strength and safety of materials on the market.

5.0

Discussion

There are no clearly defined universal endonuclease release standards. Users that proceed toward phase III drug development are bound by regulatory guidelines to perform nominal appearance and identity testing in earlier phases. In phase III development and beyond, users must also perform a full complement of release testing, guided by a quality risk design. This often necessitates repeating all tests that are listed on a vendor's CoA (subject to risk-based exceptions).

In the case of protein/enzyme ancillary raw materials, simply identifying the material requires biochemistry tools such as immunoassays (e.g. ELISA) or electrophoresis (e.g. SDS-PAGE). Indeed, ELISA kits for detection of endonucleases are commercially available and may be a logical proxy for early-phase identity testing. Validation of such an approach is certain, but is constrained by the supply of proprietary kit materials. Compounding the challenge of testing approach is the reluctance of some enzyme manufacturers to provide their test methods for proprietary reasons, and the reality that even when methods are obtained, GMP validation or transfer must still be done by users. Overall, the outcome is that pharmaceutical companies and contract laboratories both expend time and effort separately developing and validating complex analytical test methods from scratch to solve the same problem.

While generalized guidance exists in EP 5.2.12 and USP <1043> for ancillary materials used in CGT, there is no explicit standard or monograph for testing of endonucleases. There are few pharmacopeia monographs for enzymes, but some precedent exists in monographs such as trypsin, collagenase, pepsin and papain, as well as guidance from general chapters like USP <89>. However, these materials have clinical application in the treatment of diseases, whereas the *Serratia marcescens* endonucleases do not, and the implementation of such a compendial standard is uncharted territory. Although communications with compendia representatives during work leading to this paper showed great interest in the proposed strategy, greater buy-in from industry is still required.

6.0

Conclusion

The absence of a universally accepted standard for the testing and release of non-site-specific endonucleases slows down the product development of CGT and adds unnecessary effort. It is proposed that a universal standard be applied across the industry to harmonize and support CGT product development to ensure higher quality and safety standards prior to commercialization.

For a global adoption, it would be proposed to add these recommendations to dedicated references in the European Pharmacopeia and United States Pharmacopeia.

The outcomes of proofs of concept shared by BioPhorum members show that there is more to implement in this area. Member companies are starting their journeys from any point on the digital maturity scale [DPMM] increasing their maturity level over time. By sharing their learnings, both biopharmaceutical manufacturers and suppliers to the industry are building their knowledge and removing the mystery associated with smart maintenance.

Appendix

Feedback

Readers are invited to comment on the specific standards and tests where possible, in addition to the overall proposed approach. Feedback may be provided by completing the following **form**.

Specification/Testing

1. What experience do you have of supplier's ID methods being unable to discriminate similar molecules in their facility and what have you done to address this?
2. What has your organization developed for an endonuclease identity test?
3. Have you used Raman testing for endonuclease identity?
4. Do you agree with the testing proposed in Table 2? What other testing and/or methodology do you recommend?
5. What release specifications do you use: vendor-specified criteria on CoAs, or user-/process-defined CQAs?
6. What is your experience with endonucleases of other origins?

Regulatory

7. Are there relevant references other than EP 5.2.12 or USP<1043> that should be evaluated from a regulatory perspective that may dictate the use of these endonucleases?
8. Would you be interested in helping to write a pharmacopeia entry for endonuclease?

Responding to the content of the article

The team leveraged multiple discussions and a blinded survey to enable sharing of data and opinions. The final proposals are a combination of thoughts, suggestions and questions. The objective of this paper is to solicit feedback on the proposed universal standards and testing for endonucleases. Table 2 sets out an industry best practice which could be used as a basis for a chapter.

Readers are invited to comment on the specific standards and tests where possible, in addition to the overall proposed approach. Feedback may be provided by completing the following **form**.

The team recognizes that the proposed approach is a work in progress. It understands that many users are doing some innovative work. This is an opportunity for you to use your voice to inform a standardized approach, a baseline of tests and agreed methods that should be followed for the acceptable specification ranges for endonucleases.

Benefits

An agreed framework for endonucleases testing and release has many benefits. It will provide confidence that your actions are aligned with those of your peers. The alignment will bring reliability and consistency to the manufacturing process as everyone is meeting the same standard for a particular material.

It will also mean that manufacturers, suppliers and clients can use the same language and refer to an agreed reference table and testing. Importantly, when you come to file with regulatory authorities, you will have a data pack that covers what they will expect to see and that demonstrates you are managing and controlling that material appropriately.

Nobody has tried to define the endonuclease release testing needed for CGT processes, so the BioPhorum approach is an industry first. With the explosive growth of the CGT industry, the need for these release specifications is loud and clear. What do you think?

If you would like to help shape an important part of the CGT industry, please share your thoughts in BioPhorum group meetings, complete the confidential survey or return the downloadable pdf to **info@biophorum.com**.

Acronyms

Term	Definition
CGT	Cell and gene therapy
CoAs	Certificates of analysis
CQA	Critical quality attribute
EC	Enzyme Commission
ELISA	Enzyme-linked immunosorbent assay
EP	European Pharmacopeia
ICH	International Conference on Harmonization
ID	Identification
IP	Intellectual property
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TMC	Total microbial count—further specified as total aerobic microbial count (TAMC) and the total combined yeasts and molds count (TYMC)
USP	United States Pharmacopeia

Definitions

Term	Definition
EP 5.2.12	Raw materials of biological origin for the production of cell-based and gene therapy products for human use
USP<1043>	Ancillary materials for cell, gene and tissue-engineered products

References

1. NA1 Benedik, M. J., & Strych, U. (1998). *Serratia marcescens and its extracellular nuclease. FEMS Microbiology Letters*, 165(1), 1–13. **https://doi.org/10.1111/j.1574-6968.1998.tb13120.x**
2. Shlyapnikov, S. V., Lunin, V. V., Perbandt, M., Polyakov, K. M., Lunin, V. Y., Levdikov, V. M., Betzel, C., & Mikhailov, A. M. (2000). *Atomic structure of the Serratia marcescens endonuclease at 1.1 Å resolution and the enzyme reaction mechanism. Acta Crystallographica Section D Biological*, 56(5), 567–572. **https://doi.org/10.1107/s090744490000322x**
3. Koziolkiewicz, M., Owczarek, A., Domański, K., Nowak, M., Guga, P., & Stec, W. J. (2001). *Stereochemistry of cleavage of internucleotide bonds by Serratia marcescens endonuclease. Bioorganic & Medicinal Chemistry*, 9(9), 2403–2409. **https://doi.org/10.1016/s0968-0896(01)00214-0**

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