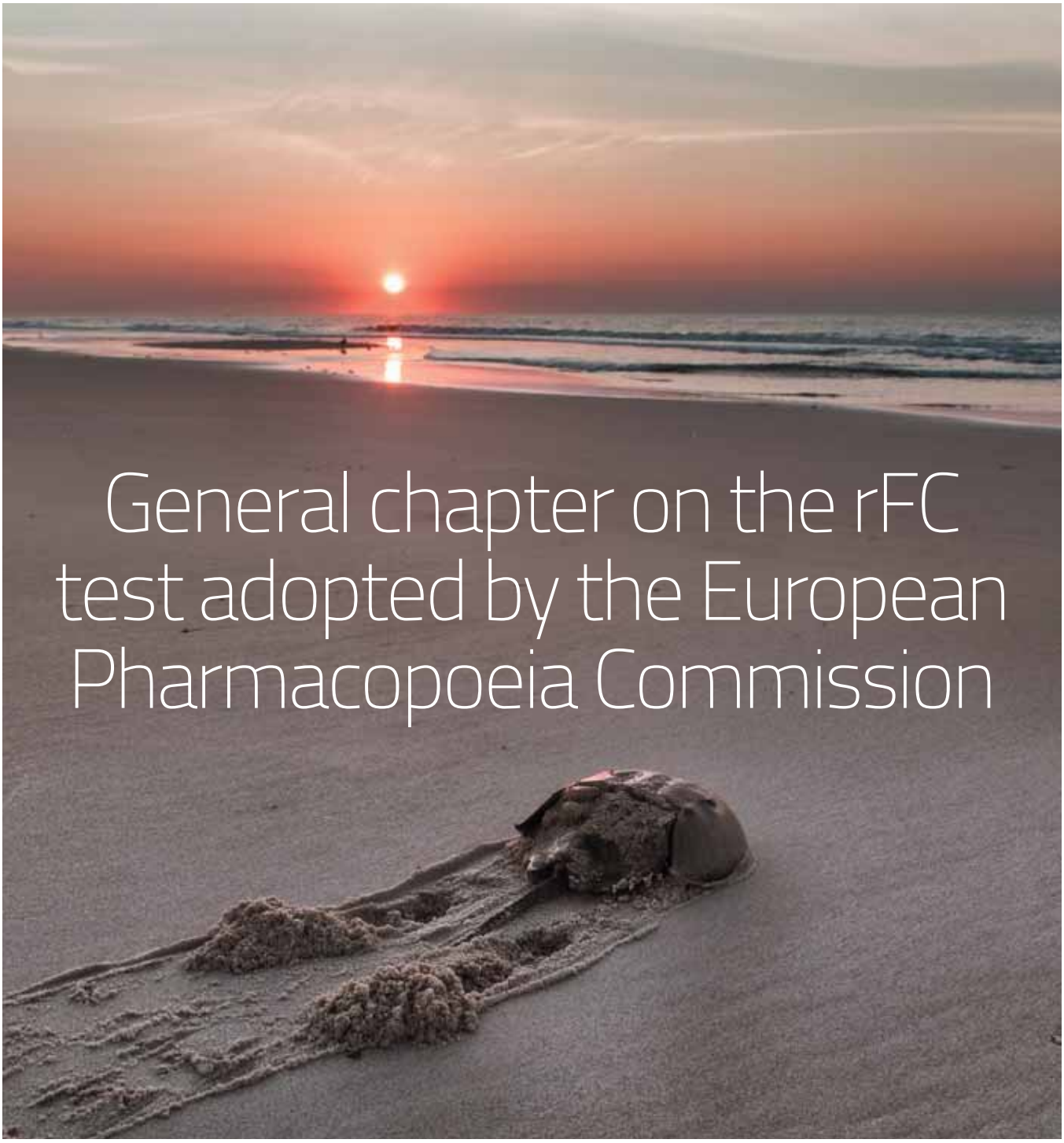

General chapter on the rFC test adopted by the European Pharmacopoeia Commission

The recombinant Factor C (rFC) assay has been developed to help alleviate the pressures of a growing demand for the *Limulus* amoebocyte lysate (LAL) assay. Here, Sven Deutschmann and Johannes Reich discuss the advantages of rFC and its recent recognition by the European Pharmacopoeia as an alternative endotoxin test.

IN NOVEMBER 2019, the European Pharmacopoeia (Ph. Eur.) Commission adopted 13 new monographs and four new general chapters. Among the new chapters is Chapter 2.6.32, titled '*Test for bacterial endotoxins using recombinant factor C (2.6.32)*'. This will be published in the coming weeks in Ph. Eur. Supplement 10.3 and available on the European Directorate for the Quality of Medicines (EDQM) website. The chapter will be effective at the start of next year (1 January 2021).

In January 2019, the Ph. Eur. launched a public consultation for preparing the new general chapter and was the first pharmacopoeia to »

"The benefits from switching from LAL to rFC-based bacterial endotoxin tests has been clearly identified by different regulatory bodies"

refer to the rFC test, which they did in chapter 5.1.10, '*Guidelines for using the test for bacterial endotoxins.*'

"*As stated in the General Notices, the test methods given in monographs and general chapters have been validated in accordance with accepted scientific practice and current recommendations on analytical validation. In consequence, the methods described in general chapters 2.6.14. Bacterial endotoxins, 2.6.30. Monocyte-activation test and 2.6.32. Test for bacterial endotoxins using recombinant factor C therefore do not have to be re-validated per se, other than in consideration of their use for a specific substance or product in a specific analytical environment.*"

Thus, allowing the recombinant protein to be used as an alternative to the *Limulus* amoebocyte lysate assay (LAL assay). For now, only tests based on the fluorometric test method are described in the new chapter as these are the rFC kits currently available on the European market and most of the available scientific data are based on this method.

rFC advantages – improvements on the LAL paradigm

The advantages of the rFC test revolve around the newest paradigm change; the difficulty associated with sustaining and improving modern test methods in light of horseshoe crab population diminishment.

The rFC test is recognised as possessing several advantages to the LAL assay, including (a) reproducibility/quality, (b) sustainability/availability and (c) specificity. These advantages will be briefly outlined.

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rFC as a continuation of historical LAL paradigms

Any envisioning of 'the future' of testing contains elements extrapolated from the past. There are three main elements that predict a positive outcome for recombinant Factor C (rFC) that require very little extrapolation:

(i) the advent of biotechnology and the replacement of animal proteins via the cloning of the necessary animal genes (insulin, growth hormone, etc) and production in bioreactors via single-celled organisms has given us advanced medicines and cures for many diseases and thus serves as a successful paradigm for replacement

(ii) the specificity, sensitivity and expanded utility of using rFC methods has already been demonstrated

(iii) the continued use of horseshoe crabs as harvested from limited geographical locations is not sustainable, and pharmaceutical manufacturers will want to prepare for such a change.

It is important to view rFC as a continuation of the LAL testing paradigm. Since the initial exploratory use of LAL for radiopharmaceutical testing,[1] the past 50 years have established several widespread paradigms including:

(a) use of the LAL test to supplant the rabbit pyrogen test (RPT) as a method of precluding fever reactions in patients subject to injectable drug treatments. The detection of "all pyrogens" was not a particularly sensitive or relevant target for injectable drugs as drugs are a product of water-based manufacturing where GNB are the predominant contaminant.

(b) initially, quality control consisted of the end-product testing of finished drugs only. With the advent of a convenient *in vitro* test, LAL has rapidly taken over the task of testing; over the years testing has expanded from end-point only to raw material, in-process, purified waters as well as end-point release testing. This increased test coverage of the entire manufacturing process has brought about a vast increase in the microbiological safety of drug products.

(c) the rise of test volume (both domestically and globally) has been paralleled by a demise in the reagent source. Horseshoe crabs on the US eastern seaboard (*Limulus*) are listed as "vulnerable" and those in Asia (*Tachypleus*) are now listed as officially "endangered".

While most agree that biomedical bleeding has not been the cause of such a demise, industry must still address the fact that sustainable methods are needed to replace LAL testing.

A new paradigm change that has occurred most recently is the advent of "ease of use" testing. The LAL test began as a fairly user-intensive effort that included manually inverting reaction tubes for gel clot testing and has transitioned to first, semi-automated kinetic testing where absorbance is monitored over time without further user intervention, and finally, to configurations in which test standards and reagents are combined in a single prepackaged unit (cartridge and precoated plate testing). Each paradigm change has aided the pharmaceutical industry by making product contamination events rare.[2]

Today, there is an expectation that, for sustainability reasons, global pharmaceutical companies should explore the use of rFC in routine testing of purified water, raw materials, components and finished drug products. The US Food and Drug Administration (FDA) has been receptive to such a change, as seen in the recent approval of the first drug to be released using rFC testing. Many global companies also continue to pursue raw material, water and component testing using rFC.

There are some in industry that worry about the legacy of LAL; however, the uptake of recombinant methods should be viewed as a fulfillment of that legacy rather than an affront to it, just as the change from animal-sourced medicinal proteins, such as recombinant human insulin, was not an affront to early efforts (animal-derived insulin) to treat disease. Indeed, the biotechnological revolution is a direct response to the recognised utility of natural proteins.

References

1. Cooper, Levin, Wagner. New rapid in vitro test for pyrogen in short-lived radiopharmaceuticals. J. Nucl. Med. 1970; 11:310.
2. Endotoxin-like reactions associated with intravenous gentamicin- California, Morb. Mortal. Weekly Report, 1998, 47(41); p. 877-880.

Reproducibility/quality

Classical LAL assays are sourced from horseshoe crabs and thus underlie natural variability from lot to lot. Production lot rFC to production lot rFC has been shown to maintain its quality attributes in terms of standard curve values and parameters obtained test after test. The biotechnological production of rFC allows users to source a consistent product that is not subject to the variables of naturally harvested proteins. This is important for reproducible and reliable testing. Product variability that occurs with pooled batches of harvested animals includes age, gender, size, environment, etc. Global companies prefer uniformity and standardised platforms as applied across global supply chains. Thus, a recombinant produced product can meet this criterion.

Sustainability/availability

rFC is a product of biotechnology; thus, as opposed to being harvested from a sea creature that is undergoing survival pressures, it can be produced "at will" in cell culture. The genus *Limulus* is listed as "vulnerable" in the US and in Asia the genus *Tachypleus* is listed as "endangered" on the IUCN Red List (www.iucnredlist.org).

The global availability of LAL has been met to date in a supply chain that depends upon geographically isolated production and subsequent transatlantic export of LAL from the US east coast. On a smaller scale, the use of tachypleus amebocyte lysate (TAL) produced locally in Asia has also been used. The "at will" production of a recombinant reagent can help meet the logistical demands of global supply chains.

Specificity

In contrast to LAL, rFC does not contain Factor G, which is the beta-glucan zymogen (biosensor for beta-glucan). Beta-glucan has been found commonly as a breakdown product of cellulosic filters in manufacturing processes, as well as a ubiquitous contaminant of natural waters (lakes, rivers and sewers) as a by-product of plant and fungal growth. The activation of LAL by beta-glucans is referred to as a "false positive". Even when masked with beta-glucan blocking buffer, it has been shown to affect the variability of LAL assay results. The lack of false positive results from rFC testing is another benefit and removes a confounding element of LAL detection.

Over recent decades, countless scientific articles have been published showing reliable and sustainable detection of bacterial endotoxin using rFC-based assays. In comparison, the performance of equivalency of the rFC and LAL methods has been widely demonstrated and different pharmacopoeia are beginning to include the rFC in their official texts.

Taken together, the benefits from switching from LAL to rFC-based bacterial endotoxin tests has been clearly identified by the different regulatory bodies. The European Pharmacopoeia will publish a new General Chapter, titled: *Test for bacterial endotoxins using recombinant factor C (2.6.32)* in July 2020. Today, in the US, a draft revision of the USP <85> '*Bacterial Endotoxin Test*' including the description of both methods, rFC and LAL, was included to solicit user comments until December 2019. In Asia Pacific, the Japanese and Chinese pharmacopoeia have begun the same approach to include the rFC in the next release.

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