



## **ENDOTOXIN TESTING OF BIOPHARMACEUTICALS: COMPARISON OF LAL AND RECOMBINANT FACTOR C ASSAYS**

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### **1. INTRODUCTION**

Every injectable medicine, every infusion fluid and many medical devices must be shown to be free of endotoxin, the lipopolysaccharide shed from the outer membrane of Gram-negative bacteria. Even small amounts given by injection can provoke fever, hypotension and, at larger doses, shock, because lipid A is recognised with great sensitivity by the innate immune system through Toll-like receptor 4 [1, 2, 3]. Endotoxin is heat stable, survives ordinary sterilisation and can pass sterilising filters

### **Abstract**

Endotoxin control is a release requirement for every parenteral medicine, and the Limulus amebocyte lysate (LAL) test has dominated it for decades. Recombinant factor C (rFC) assays now offer an animal-free alternative. This short review, which reports no new data, compares the two approaches using the published literature. It describes the cascade chemistry behind each reagent, assay formats, interference and low endotoxin recovery, glucan response, reagent supply, and regulatory status, and considers what validation a laboratory needs before changing method. Published comparisons show close agreement for standards and for most products, with advantages for rFC in specificity and sustainability, but independent multi-site studies on complex biologics remain limited.

when bound to other material, so freedom from viable bacteria says little about freedom from endotoxin [4, 5]. For decades the

standard answer has been the bacterial endotoxins test (BET), built on the clotting reaction of blood cells from horseshoe crabs. A recombinant alternative has now entered pharmacopoeias. This short review sets out how the two approaches compare in the published literature and what the comparison implies for laboratories that release biological medicines. It reports no new experimental data.

The history explains much about current debates. In the early 1960s Levin and Bang observed that bacterial endotoxin made the blood of the Atlantic horseshoe crab, *Limulus polyphemus*, clot [6, 7]. Cooper and colleagues later showed that the amebocyte lysate responded to endotoxin quantitatively and that results tracked the rabbit fever response, which opened the way to an in vitro replacement [8]. The lysate test, universally called LAL after *Limulus* amebocyte lysate, became an official pharmacopoeial method, and the Food and Drug Administration (FDA) recognised it as a substitute for the rabbit pyrogen test for many products [9, 10]. It is sensitive, inexpensive per sample and fast. It is also a product of a living animal harvested from wild populations, and it responds to more than endotoxin. Both facts underlie the interest in a recombinant reagent that mimics only the endotoxin-sensing step of the cascade [11, 12, 13].

Terminology deserves a note, because the literature mixes terms. Pyrogen refers to any fever-inducing substance and includes endotoxin as well as non-endotoxin pyrogens such as peptidoglycan, lipoproteins and some viral or fungal components. The BET detects endotoxin and not other pyrogens, a limitation shared by LAL and recombinant reagents alike. Pharmacopoeial limits are expressed in endotoxin units (EU) calibrated against an international standard; one EU corresponds to roughly 0.1 ng of the reference lipopolysaccharide, although the mass varies with the preparation [4]. The limit for a product follows from the threshold pyrogenic dose, 5 EU per kilogram of body weight per hour for most parenteral products and a much lower figure, 0.2 EU per kilogram, for intrathecal use [10, 14]. Product-specific limits are then worked out from the maximum

dose.

## 2. ENDOTOXIN AND THE LAL CASCADE

Lipopolysaccharide has three regions: the lipid A anchor, a core oligosaccharide and the O-antigen polysaccharide chain. Lipid A carries the toxic activity, and its acylation pattern determines how strongly it stimulates human cells; hexa-acylated forms are potent, while under-acylated forms from some bacteria are much weaker [1]. Free and aggregated forms behave differently. In water, endotoxin forms micelles and vesicles, and it binds to proteins, lipids, cations and surfaces, which is why its measured activity depends on the matrix [1, 15]. Contamination arises from raw materials, water systems, excipients, filters, tubing and the cells used to make biologics. Gorbet and Sefton summarised how endotoxin on biomaterials and devices can drive inflammation even at low levels [5]. For a pharmacist the practical point is that endotoxin is a product-quality attribute, controlled by design of the process and not only by testing at the end.

The clinical side of endotoxin detection offers a useful parallel. Hurley reviewed methods for detecting endotoxaemia in patients and noted that circulating endotoxin is hard to measure in plasma, because it binds lipoproteins and other plasma components and because plasma inhibits the lysate reaction, so samples need pretreatment before testing [16]. The same masking principle reappears later in drug products. Beutler and Rietschel traced the story of endotoxin from early observations of fever after exposure to bacterial products to the identification of the receptor that senses lipid A [3]. For pharmaceutical purposes the important inheritance of that research is the idea of a threshold: below a certain dose per kilogram, healthy people do not respond, and testing against a limit is therefore meaningful [4, 10].

In the horseshoe crab the same recognition system protects against invasion. Factor C, a serine protease zymogen in the amebocytes, binds lipopolysaccharide and activates itself [17]. Activated Factor C converts Factor B, which in turn activates the proclotting enzyme; the clotting enzyme then cleaves

coagulogen to coagulin, which gels [11, 18]. A separate branch is triggered by (1→3)-β-D-glucans through Factor G and converges on the same proclotting enzyme [19]. The pharmacopoeial test exploits this cascade. In the gel-clot format, formation of a firm gel indicates endotoxin at or above the labelled sensitivity. In the turbidimetric format the rise in turbidity as coagulin forms is followed over time, and the chromogenic format uses a synthetic peptide substrate that releases a coloured product when cleaved by the clotting enzyme [4, 10].

Running the test well takes more than following a recipe. Reagents must be qualified, the standard curve must meet linearity criteria, and each product must pass an interference check: a known amount of endotoxin is added to the product at a non-inhibitory dilution, and its recovery must fall within 50–200% of the added quantity [10, 14]. The maximum valid dilution, calculated from the endotoxin limit and the lysate sensitivity, sets how far a sample can be diluted to overcome interference. Common interfering factors include extreme pH, chelating buffers such as citrate and phosphate that bind the divalent cations the cascade needs, surfactants, and proteins or antibodies in biologicals that bind or denature the enzymes [4, 14]. Dilution, pH adjustment and added magnesium are the standard remedies. These details matter in any comparison with recombinant reagents, because a new method faces exactly the same product-specific validation.

Two persistent weaknesses of lysate reagents are glucan sensitivity and lot-to-lot variability. Because Factor G responds to glucans, material contaminated with cellulose-derived or fungal glucan can give a false positive for endotoxin; Roslansky and Novitsky showed that glucans from filters and membranes could activate the lysate [19]. Reagent makers add glucan-blocking buffers to suppress the effect, though these may not remove it completely. Lysate is a biological extract pooled from animals in different physiological states, so potency, enzyme content and background reaction can differ between lots, and laboratories need to qualify each new lot [4, 20]. Novitsky also described

how the animals are collected and bled, with lysate production a long process that cannot be scaled quickly in a shortage [20]. Those practical problems, rather than any failure of sensitivity, are what motivate the search for alternatives.

Alternative sources of lysate exist within the horseshoe crab family. Three Asian species, the tri-spine horseshoe crab (*Tachypleus tridentatus*), the Indo-Pacific species (*Tachypleus gigas*) and the mangrove species (*Carcinoscorpius rotundicauda*), have long supplied reagents in Japan, China and Southeast Asia, and Factor C from the Singapore mangrove species was the first to be cloned [12, 21]. Reagents differ in cascade composition, glucan sensitivity and handling, but all remain dependent on wild harvest. Lysates from different species are used within the same pharmacopoeial framework, and each laboratory must validate the one it buys [4, 20].

### 3. RECOMBINANT FACTOR C ASSAY

Recombinant Factor C (rFC) takes only the first step of the cascade. Ding and colleagues cloned the gene for Factor C from the mangrove horseshoe crab, expressed it in insect cells, and showed that the product was activated by lipopolysaccharide [11, 21]. In the assay, rFC is mixed with the sample and a fluorogenic substrate. Endotoxin binding activates the enzyme, which cleaves the substrate and releases a fluorophore; fluorescence is read at the start and end of an incubation, and the increase is compared with a standard curve [12, 13]. Because there is no Factor B, proclotting enzyme or coagulogen, there is no clotting step, and with no Factor G there is no response to glucans. Most commercial kits use an endpoint rather than a kinetic read. This design is simple, and it is the main reason rFC is described as more specific for endotoxin than lysate.

Mizumura and co-workers took a different recombinant route and produced all three cascade enzymes in recombinant form, so that the reagent follows the kinetic pattern of natural lysate but without animal blood [22]. Such three-factor recombinant cascade reagents were meant to bring the behaviour of the recombinant test closer to that of lysate,

which could ease comparability arguments with regulators. The rFC design has the practical advantages of a defined composition and controllable manufacturing, with lot-to-lot consistency expected in principle since the protein is made in culture and not harvested from animals [12, 23]. Maloney and colleagues, describing a synthetic alternative to horseshoe crab blood for endotoxin detection, stressed these points together with sustainability [23]. Whether the change lowers the cost per test depends on volume and supplier.

How well does rFC perform in practice? Loverock and colleagues described an early procedure for recombinant Factor C and showed linear standard curves over a wide range, with sensitivity in the same region as sensitive lysate methods [13]. Bolden and Smith applied the reagent to a range of pharmaceutical products and reported spike recoveries within the usual pharmacopoeial window for most of them, alongside data on repeatability [24]. The reagent has also been compared with lysate in a long-running proficiency scheme, which allows the same blind samples to be tested by both kinds of method in many laboratories [25]. Those comparisons usually find that results from the two assay types agree closely for pure endotoxin standards and for most products, with occasional differences for particular matrices. This is the evidence base on which regulators and manufacturers mainly rely.

Interference deserves separate attention, since it is where a new reagent can win or lose acceptance. The rFC reaction is enzymatic like the lysate cascade, so chelators, high salt, extreme pH and proteases can still interfere, though the spectrum of interference differs. Fluorescence reading adds its own sources: coloured or fluorescent samples, quenching and turbidity can distort the signal, and analysts rely on sample blanks and spike recoveries to catch them [12, 24]. Because the cascade is shorter there are fewer enzymes to inhibit, but also fewer amplification steps, so the assay may be more sensitive to partial inhibition of its single enzyme. Reports differ on whether rFC tolerates particular formulations better or worse than lysate [24, 25]. Each product therefore requires its own

validation, just as with lysate.

Calibration is worth a short paragraph, because the numbers reported by different reagents are meaningful only against a common reference. Results are expressed in EU relative to a reference or control standard endotoxin, a purified lipopolysaccharide from *Escherichia coli* whose potency has been assigned against the international standard [4, 10]. Lysate and rFC reagents are both calibrated this way, so on a standard they should agree. The match is less certain for native endotoxins from other bacteria. Factor C binds lipid A, and lipid A structures differ between species in acylation and phosphorylation, so the response of a reagent to a given mass of native endotoxin may differ from its response to the standard [1, 4]. This is a reminder that the EU is an assay-defined unit, not a direct measure of mass or of toxicity.

A note on formats helps readers who are used to lysate. Commercial rFC kits are supplied for microplate use, with reagents mixed in the wells, incubated at a controlled temperature and read in a fluorescence plate reader, so the workflow resembles that of the chromogenic lysate assay [12, 24]. A standard curve of endotoxin, negative controls, and positive product controls are run on each plate. Because the read is at defined time points, incubation temperature and timing are more critical than with a kinetic read that tracks onset time. Plate readers need regular qualification, and analysts should check for fluorescent compounds in formulations before the first run. None of this is demanding, but it shows that moving to rFC changes equipment and data handling as well as the reagent.

#### 4. COMPARATIVE PERFORMANCE

Specificity is the most discussed difference. The glucan insensitivity of rFC avoids false positives from glucan-containing materials, including cellulose-based filters, some excipients and certain plant- or yeast-derived components [12, 19]. Silkworm larvae plasma tests were developed partly to detect peptidoglycan and glucan, which activate cascades that lack Factor C [26]. In practice, a lysate analyst can add glucan-blocking buffer, or compare signals with and without it, to see

whether a result arises from glucan, while an rFC user avoids the problem at source [12, 19]. There is a small reverse consideration. A reagent that is blind to glucan will also not warn the analyst of glucan contamination, which is not a pyrogen threat in the same sense but can signal a process or materials problem worth investigating.

Low endotoxin recovery (LER) has become the most debated phenomenon in biologics testing. In some formulations containing a chelator such as citrate or phosphate together with a non-ionic surfactant such as polysorbate, endotoxin spiked into the product loses detectable activity in lysate assays over time [15, 27]. Mechanistic work suggests that endotoxin becomes sequestered in mixed micelles and complexes with surfactant and chelator, so that it is not accessible to the assay reagents. Schwarz and colleagues showed that such masked endotoxin retained biological activity in cell-based assays, which means the loss of signal is not harmless [28]. Comparisons in the literature suggest the phenomenon is a property of the sample matrix and not of one reagent, though the extent may differ between methods [15, 27]. The usual response is hold-time studies with native endotoxin and, where needed, orthogonal assays.

Findings from the comparative literature are collected in table 1, which lists the assay attributes most often compared and notes where each reagent type holds an advantage. The entries are grouped by type of attribute in fig. 1: analytical performance, specificity, practical operation, and supply and regulation. Analytical performance entries mostly show equivalence, which is itself the main message of the comparison [13, 24, 25]. Specificity and supply favour rFC, while the long history and the established position in compendia favour lysate [23, 29]. No row shows a clear advantage of one reagent for all products, which is why method choice is made product by product. The table synthesises reports from different laboratories using different kits and should be read as a summary of tendencies, not as a head-to-head trial.

The statistics used to compare methods influence the conclusions drawn. Because

spike recovery is accepted anywhere between 50% and 200%, two methods can both pass and still give results that differ appreciably for a given sample [10, 14]. A correlation coefficient alone is therefore a poor judge of agreement, since two methods can correlate well and still differ systematically. Paired comparisons with differences plotted against means, equivalence testing with a pre-set margin, and agreement of pass-fail decisions near the limit are more informative. The proficiency scheme data cited earlier lend themselves to this kind of analysis because many laboratories tested the same blinded samples [25]. Regulatory text for the recombinant chapter expects demonstration of comparability along these lines, not simply a statement that results look similar [29].

On the bench the two approaches feel different. Lysate gel-clot tests need no instrument beyond a heating block, which makes them attractive in small laboratories, whereas kinetic assays need an incubating reader and software. rFC runs on a fluorescence microplate reader and, being an endpoint reading, is simpler in data handling although more dependent on the timing of incubation [12, 24]. Staff trained in lysate methods usually find the principle familiar, and most validation concepts carry over, including standard curve, spike recovery and negative control. Data integrity requirements apply to both. Laboratories running very large numbers of samples, for example water systems and in-process samples, may value the speed and plate format of rFC; those with a few lots a year may find that gel-clot suffices [4, 10].

**Table 1: Comparison of lysate and recombinant factor C endotoxin assays in published studies**

| Attribute                  | Reported comparison                                                                | Ref.     |
|----------------------------|------------------------------------------------------------------------------------|----------|
| Sensitivity and range      | Both reagent types report working ranges at similar low endotoxin concentrations   | [4, 13]  |
| Spike recovery in products | Most pharmaceutical products recover within the 50–200% window with either reagent | [24, 25] |
| Proficiency testing        | Results from rFC and lysate agree closely for most blinded samples                 | [25]     |

| Attribute              | Reported comparison                                                                                       | Ref.         |
|------------------------|-----------------------------------------------------------------------------------------------------------|--------------|
| Repeatability          | Acceptable with both; data across different kits remain limited                                           | [13, 24]     |
| Glucan response        | Lysate responds through Factor G unless blocked; rFC does not respond                                     | [12, 19]     |
| Low endotoxin recovery | Seen in chelator-surfactant formulations; appears matrix-driven for both reagent types                    | [15, 27, 28] |
| Non-endotoxin pyrogens | Neither detects them; the monocyte activation test is needed                                              | [30, 31]     |
| Lot variability        | Lysate varies as an animal extract; rFC is made in culture and expected to be more consistent             | [4, 20]      |
| Format and equipment   | Lysate: gel-clot, turbidimetric or chromogenic; rFC: fluorescence microplate                              | [10, 12]     |
| Animal supply          | Lysate depends on wild horseshoe crab harvest; rFC does not                                               | [23, 32, 33] |
| Compendial status      | Lysate described in main chapters; rFC has its own European chapter and FDA allows validated alternatives | [9, 14, 29]  |

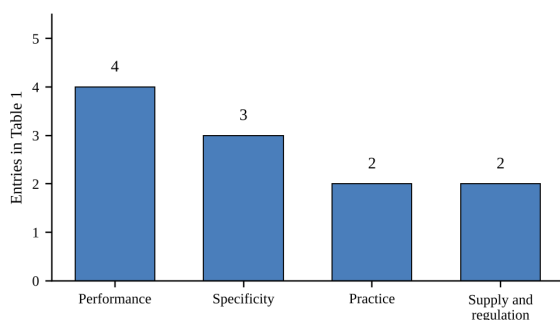


**Fig. 1: Number of entries in table 1 by type of attribute**

Neither reagent type detects non-endotoxin pyrogens. The monocyte activation test (MAT), which uses human monocytic cells or whole blood and measures cytokine release, was validated internationally and responds to endotoxin and to several non-endotoxin pyrogens [30, 31]. For products at risk of contamination with such material, MAT is a complement to endotoxin testing. It has limits of its own: donor variability, cost and slower turnaround. The rabbit pyrogen test, now largely replaced, still has a role in some cases [8, 30]. The choice between tests depends on

the product risk assessment, with MAT reserved for those where non-endotoxin contamination is plausible.

## 5. SUPPLY, REGULATORY STATUS AND PRACTICE

The sustainability argument is central to the debate. Limulus blood is collected from animals caught along the east coast of the United States, bled by a standard procedure and returned to the water. Mortality after bleeding and release has been estimated in several studies, with figures varying with handling and bleeding volume, and sublethal effects on behaviour and reproduction have been suggested [32, 33]. A regional assessment of the American horseshoe crab concluded that populations differ, with some regions showing declines driven by harvest for bait as well as biomedical use [32]. The Asian species face their own pressures. Biomedical use is a small fraction of total harvest but is concentrated and potentially lethal for the animals, so ecological arguments are used to justify moving to recombinant reagents, while the industry argues that bleeding is well managed [20, 23]. Both sides agree that more data would help.

The ecological context is wider than the crabs alone. Horseshoe crab eggs have been described as an important food for migrating shorebirds along parts of the east coast of North America, so that pressure on spawning populations has been argued to reach other species too [32]. Counting only the animals bled for lysate therefore understates the debate, because harvest for bait and the loss of spawning habitat act on the same populations. Industry-linked bodies point to bleeding protocols and return of animals, while conservation groups ask for independent monitoring [20, 33]. For a pharmacist responsible for purchasing, these arguments are not abstract. Environmental policy of the organisation, supplier audit and supply security all bear on the choice of reagent.

Regulatory acceptance has moved in stages. The FDA guidance accepts alternative endotoxin methods if validated as equivalent to the compendial test, which formally opened the door to recombinant reagents [9].

The United States and European pharmacopoeias describe the compendial test in terms of lysate [10, 14], and Europe has since added a separate general chapter that sets out an rFC test with validation and routine requirements [29]. Franco and colleagues discussed the pharmacopoeial position on endotoxins and the way harmonised chapters have developed [34]. Validation of a recombinant method against lysate generally requires showing comparable results on product-specific samples, using the same interfering-factor study, and evidence that the method suits the intended use. Manufacturers should check the current wording of their own marketing authorisation and compendial commitments before switching reagents.

A switch from lysate to rFC is a change-control project, not a purchase. Typical steps are a gap assessment against pharmacopoeial text; side-by-side testing of representative lots and spiked samples; confirmation that retests give the same pass–fail decisions; training and updating of procedures; and regulatory notification where the release method is part of the registered dossier [9, 29]. Tamura and co-workers described the long evolution of lysate technology and pointed out that newer methods inherit its validation logic [35]. In many reports, side-by-side data are accompanied by an assessment of the risk to patients if the new method were to read lower than the old one. A sound plan sets a decision rule in advance, such as equivalence of recoveries and of pass–fail outcomes for all tested lots, so that discrepancies are judged against criteria instead of preferences.

Testing is only one half of endotoxin control. Depyrogenation of glassware and equipment by dry heat at high temperature, commonly 250 °C for a set time, removes endotoxin that steam sterilisation leaves behind [36]. For protein products made in Gram-negative hosts, removal steps rely on ion-exchange, affinity or phase-separation methods, and reviews of these techniques note that they work best when the target protein and endotoxin differ in charge or hydrophobicity [37, 38]. Water for injection (WFI), excipients and filters are also routine sources,

and testing of incoming materials gives early warning. A reagent with fewer interferences makes in-process monitoring easier, because process fluids from chromatography often contain buffers and detergents that complicate lysate assays [4, 37]. Keeping the process clean is cheaper than finding endotoxin at release.

Routine water monitoring is one of the highest-volume uses of the BET, and it may be where a switch to rFC is easiest to justify. WFI has a pharmacopoeial endotoxin limit of 0.25 EU/mL, and samples are simple in composition, with few interfering substances [10, 14]. Hundreds of samples a month can be run on microplates, and any change in reagent can be bridged by testing parallel samples from the same loop. For such matrices the evidence of agreement between reagent types is strongest, so the validation burden is lightest. Laboratories can build confidence on this application before extending rFC to finished biologics, where interference and LER need more careful study.

## 6. LIMITATIONS AND FUTURE DIRECTIONS

The literature has several weaknesses. Many comparative studies come from reagent manufacturers or from laboratories with close links to them, and independent multi-site studies are few. Sample sets are usually limited to products the laboratory already makes, so complex biologics such as monoclonal antibodies, vaccines with adjuvants, cell and gene therapy products and lipid nanoparticles are less well represented [24, 25]. Most reports use spiked endotoxin, which is a convenient test of recovery but not the same as native endotoxin in process samples, as the LER literature demonstrates [15, 28]. Comparisons usually involve a single rFC kit against a single lysate, so differences between kits within each type are hidden. Finally, the question of whether equivalent analytical results imply equivalent patient safety is addressed only by argument, since no clinical outcome data exist for either reagent.

Publication bias is another concern. Validation work that goes smoothly gets written up, whereas products that failed

interference testing, required unusual dilutions or showed LER often remain in internal reports. The published picture is therefore likely to be more favourable than what laboratories experience with difficult formulations [24, 25]. Reviews of the field, including that by Tamura and colleagues, rely largely on published sources and share the same limitation [35]. Industry consortia and pharmacopoeial committees could improve matters by publishing anonymised collections of product-level validation outcomes with both reagent types. Even a modest shared database would let readers see how often each reagent needed special handling and for which classes of product.

Several research needs follow. Independent interlaboratory studies with native endotoxin from several bacterial species would show whether the two reagent types rank the potency of different lipopolysaccharide structures in the same way. This matters because lipid A structure varies between species, and potency in human cells does not always match reagent response [1, 2]. Studies of LER should compare lysate, rFC and cell-based readouts in the same samples over time, so that masked endotoxin can be characterised properly [27, 28]. Work on how to combine endotoxin tests with the MAT in a testing strategy would help products at risk of non-endotoxin pyrogens [30, 31]. More public data on reagent robustness, lot variation and kit-to-kit comparability would also let laboratories judge the risk of switching [25, 35].

For laboratories deciding now, a few practical conclusions seem justified. First, rFC is a credible, pharmacopoeially recognised reagent whose analytical performance in the published comparisons is close to that of lysate [13, 24, 25, 29]. Second, validation must remain product specific, and no reagent can be exempted from interference testing [10, 14]. Third, a laboratory with persistent glucan problems or concerns about supply has the strongest reasons to move [12, 23]. Fourth, a laboratory that is satisfied with its lysate method, with stable lots and no glucan issues, may reasonably wait for more data, since nothing in the literature suggests that lysate has become unsafe. Fifth, whichever

reagent is used, interpretation of results for biologics should consider LER and non-endotoxin pyrogens, and should not rest on a single test [27, 30].

The comparison also fits a wider pattern in pyrogen testing. Replacement of the rabbit test by in vitro methods followed the logic of replacement, reduction and refinement of animal use, and the monocyte-based tests continued it by using human cells in place of animals [30, 31]. Moving from lysate to a recombinant reagent applies the same logic to a blood-derived reagent. Laboratories sometimes underrate the cultural side of such a change: staff who have worked for years with lysate may distrust a new reagent, and a clear explanation of the biochemistry and of the validation data helps. A shared understanding of why the method is changing is part of good quality management and makes the validation exercise easier to complete.

## 7. CONCLUSION

Endotoxin testing with lysate and with recombinant Factor C measures the same hazard by closely related enzymatic routes. The published comparisons indicate that analytical performance is similar for standards and for most products, that rFC avoids glucan interference, and that it removes the dependence on horseshoe crab blood (table 1) [12, 23, 24, 25]. Lysate keeps the advantages of long experience, wide compendial coverage and a range of formats [10, 14]. Neither reagent addresses non-endotoxin pyrogens, and neither is free of matrix interference or low endotoxin recovery [15, 27, 30]. These shared limits matter as much as the differences, because they mean that careful product-specific validation is needed whichever reagent a laboratory chooses.

Because evidence from independent multi-site studies is still limited, the choice is best made product by product, using spike recovery, native endotoxin where possible and a clear decision rule agreed in advance [9, 28, 29]. Laboratories with supply concerns or glucan problems have the strongest reason to adopt rFC, while others can proceed more slowly. Over the next few years, accumulated

validation data should show whether the early agreement between the methods holds across the newer classes of biological medicine [34, 35]. Until then, a measured and well-documented approach is the safest course.

## 8. ABBREVIATIONS

BET-Bacterial endotoxins test, EU-Endotoxin unit, FDA-Food and Drug Administration, LAL-Limulus amoebocyte lysate, LER-Low endotoxin recovery, MAT-Monocyte activation test, rFC-Recombinant factor C, WFI-Water for injection

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All the authors have contributed equally.

## 11. CONFLICT OF INTERESTS

Declared none

## 12. REFERENCES

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