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rFC Validation - Simpler than you thought

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Title page

rFC validation – Simpler than you think!

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Some users have been hesitant to take on the validation of alternative methods. However, recombinant factor C (rFC) tests are so highly analogous to the horseshoe crab-sourced *Limulus* amebocyte lysate (LAL) tests that users should consider their advantages. They include sustainability, safeguarding the supply chain due to geographical diversity, accuracy, specificity, and reproducibility. Also noteworthy is that rFC is gaining acceptance in most of the world, with its own compendial chapter in the European pharmacopoeia (2.6.32)(1) and a new section written in the Chinese Pharmacopeia. Although given the delay of the USP, let us consider the "ease of validation" according to USP <1225> (2) as detailed in Q&A format below.

What needs to be done for rFC Method Validation? With an operationally qualified reader installed, generally a full validation can be achieved in as little as 2 days, with 6 rFC assays, incl. 2 operators and 3 reagent lots to determine all necessary validation parameters.

What is Bacterial Endotoxin Testing (BET) Validation? USP <85> (3) requires verification of bacterial endotoxin tests using LAL, but not validation. Verification is a check of the product's suitability in the test matrix, whereas validation requires the demonstrations of multiple parameters (accuracy etc., see below). As they are considered alternative methods to LAL tests in the USP, rFC assays require initial validation for the method using purified water. That is the Performance Qualification 1 (PQ1), a one-time per site test. Subsequently, the verification/suitability testing (PQ2) is the same as BET <85>, a test for interference factors (inhibition/enhancement, see Figure 1).

Why favor an rFC assay? rFC is produced from a natural genetic sequence from horseshoe crabs and produced in a tightly controlled biotechnological process. Thus, it is more standardized and reproducible across lots than the animal-extracted LAL (4),(5). Rising demand can be met sustainably by up-scaling rFC manufacturing without constraints such as the population of or access to horseshoe crabs. Moreover, some rFC reagents are stable at room temperature once combined and allow all-day use from a single preparation.

What about the authorities? The FDA and the EDQM have both strongly recognized these advantages and equivalency. They both published guidance that specifically mentioned rFC assays for BET (6),(7). The FDA's 2012 Q&A guidance document refers to USP chapter <1225> Validation of Compendial Procedures (2) (ICH Q2(R1)(8)) to demonstrate the desired validation parameters. Accordingly, rFC validation demonstrates 8 acceptable aspects: accuracy, precision, specificity, linearity, range, detection limit, quantitation limit and robustness.

Do we need to demonstrate all 8 aspects? No. Primary validation of rFC assays (4)(9) and scientific literature (10) have already been successfully used with FDA approved products. This type of validation compared rFC assays to kinetic-turbidimetric and -chromogenic LAL tests from different manufacturers and clearly demonstrated that rFC assays were equivalent or superior to the LAL tests for all of the mentioned quality attributes. Accordingly, rFC users may omit robustness testing and considerably tailor their efforts on the other aspects.

What does specificity mean for BET? According to USP (2), specificity refers to the ability to detect endotoxin in the presence of other substances ("exclusivity"). However, specificity may also be interpreted as the capability of detecting different varieties of endotoxin ("inclusivity"). The Pharmaceutical and Medical Device Agency of Japan (PMDA) had up to 5 laboratories compare three rFC and LAL assays, respectively, on 18 purified lipopolysaccharides, 5 crudely purified endotoxin samples from cultivated bacteria and 6 water samples with endogenous endotoxin ("naturally occurring endotoxin", NOE)(11),(12). A fundamental difference between rFC and LAL could not be shown and therefore demonstrated equivalent specificity. A metastudy on rFC vs. LAL comparisons confirmed these results (13).

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Is a side-by-side comparison to LAL required for validating an rFC assay? You do not need to use both methods in parallel. If an LAL test has not been established, there is not any transition to begin with. On the other hand, previous LAL users can simply refer to the PMDA studies (11)(12), a comparative review (13) and a Primary Validation (4) for this purpose, i.e. focus on rFC exclusively. Respective data from LAL testing needs to be compared, if it is available, only for demonstrating rFC Method Suitability on three lots of product, i.e. investigation of inhibition/enhancement (compendial Test for Interfering Factors).

With the aforementioned comparative studies and some Preparatory Testing, rFC can be quickly validated and working as intended in a given laboratory.

rFC manufacturers may support rFC users by supplying Primary Validation reports as well as ready-to-fill-out protocols and worksheets. Thus, Method Validation and Suitability testing becomes a straight-forward process (see Figure 1). Adding hardware and software Installation and Operational Qualification (IQ, OQ), Preparatory Testing and operator training – the same as required for LAL – rFC establishment can take just 5 days in the laboratory.

What comes next? rFC will become compendial in Europe on 1 January 2021 (1) and the Method Validation requirement will thus become obsolete. As respective chapter Ph.Eur. 2.6.32 became effective on 1 July 2020, rFC implementation can already start without Method Validation. Given the pharmacopeia harmonization pushed by the ICH, USP and JP/China will follow in time. Then again, should 6 assays keep you from adopting a more standardized, sustainable, specific and stable reagent right now? Do not let the fear of validation stop you from receiving the benefits of rFC.

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Conflict of Interest Declaration

Thomas Uhlig, Kevin L. Williams and Brendan Tindall are employees of bioMérieux, manufacturer of rFC assays. There is not any other financial interest.

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Figure Captions

Figure 1: Qualification process for BET, both LAL and rFC assays.

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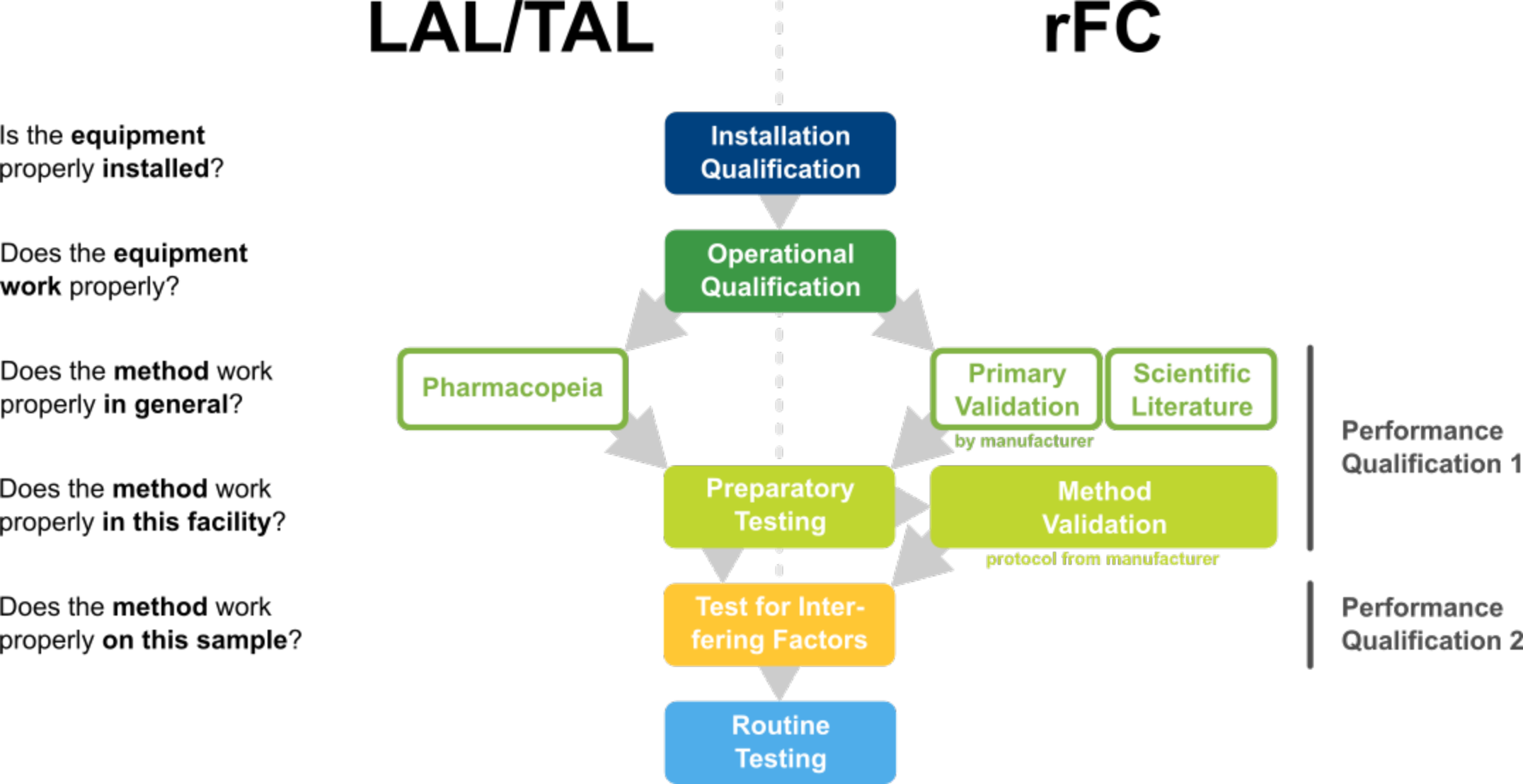
LAL/TAL
rFC
Is the **equipment** properly **installed**?
Does the **equipment work** properly?
Does the **method** work properly **in general**?
Does the **method** work properly **in this facility**?
Does the **method** work properly **on this sample**?
Installation Qualification
Operational Qualification
Pharmacopeia
Primary Validation
by manufacturer
Scientific Literature
Preparatory Testing
Method Validation
protocol from manufacturer
Test for Inter-fering Factors
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