December 2025

Understanding barriers to adoption of sustainable alternate methods for endotoxin testing

Regulatory pathway

PATH

Background

Our goal

To develop a roadmap to accelerate the adoption of alternate, animal-free test methods, such as the rFC assay, for vaccines and other biologics. This work focuses on low- and middle-income country manufacturers and regulators.

Session focus: Identifying and discussing the regulatory challenges

This regulatory session will focus on identifying the regulatory barriers that impede this transition to help inform the regulatory portion of the roadmap.

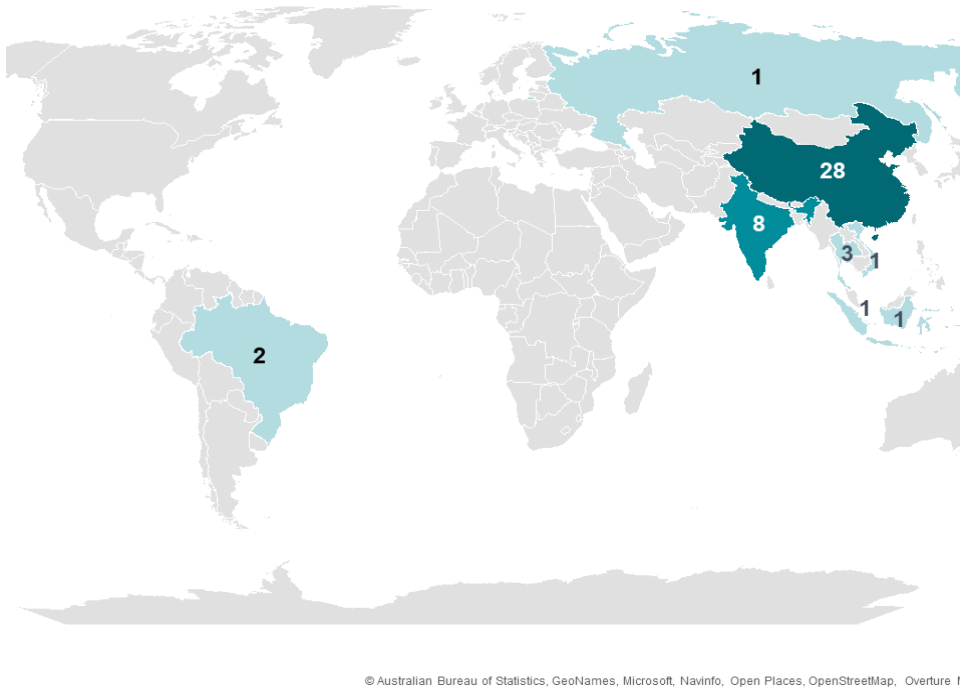
Photo: NPR/Ariane Mueller

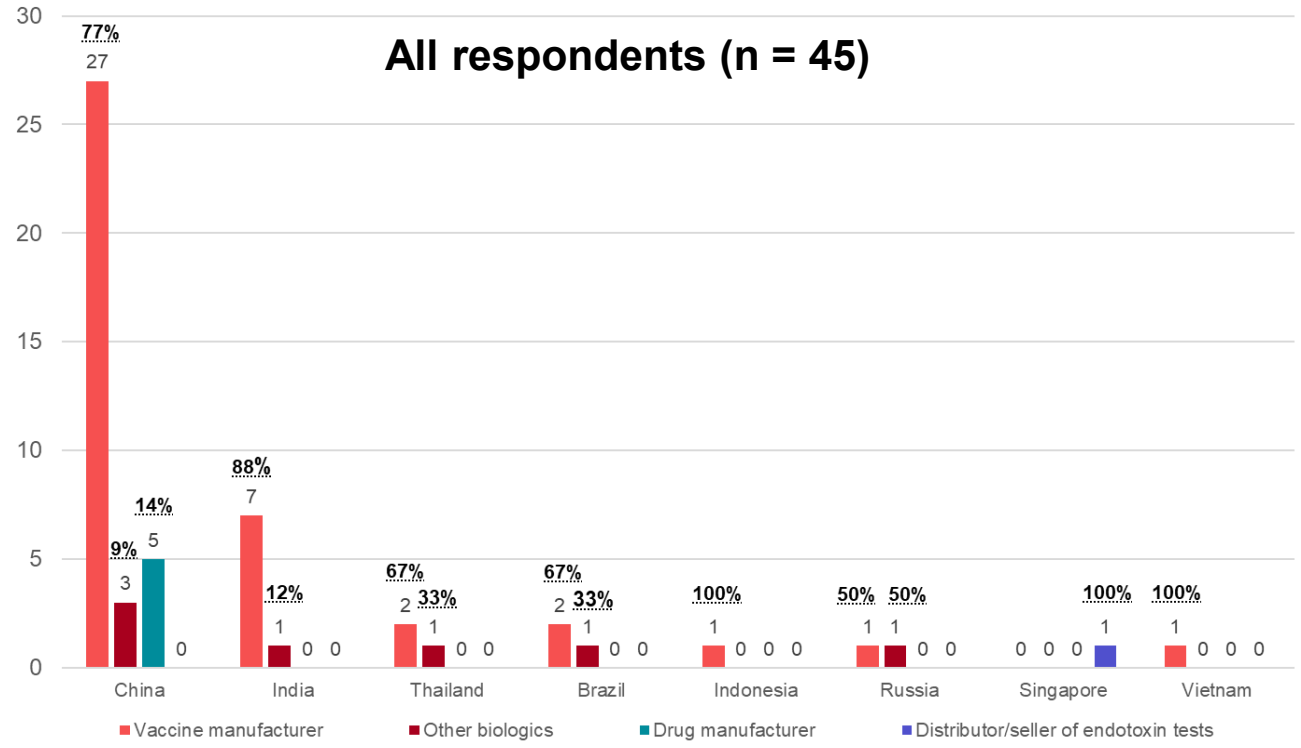
Marius M, Vacher F, Bonnevay T. Comparison of *Limulus* amoebocyte lysate and recombinant factor C assays for endotoxin detection in four human vaccines with complex matrices. *PDA Journal of Pharmaceutical Science and Technology*. 2020;74(4):394–407. https://doi.org/10.5731/pdajpst.2019.010389

Abbreviation: rFC, recombinant factor C.

Vaccine manufacturers represented the largest group of respondents across all countries

Where is your company located and which of the following best describes you as a stakeholder?





- Most respondents were from China and India, with vaccine manufacturers forming the largest group across all countries.
- The countries represented were China (28), India (8), Thailand (3), Brazil (2), Indonesia (1), Singapore (1), Vietnam (1), and Russia (1).

Regulatory approval is the most significant barrier to adoption of the rFC method

What challenges does your organization face in adopting the rFC method?

Primary challenges

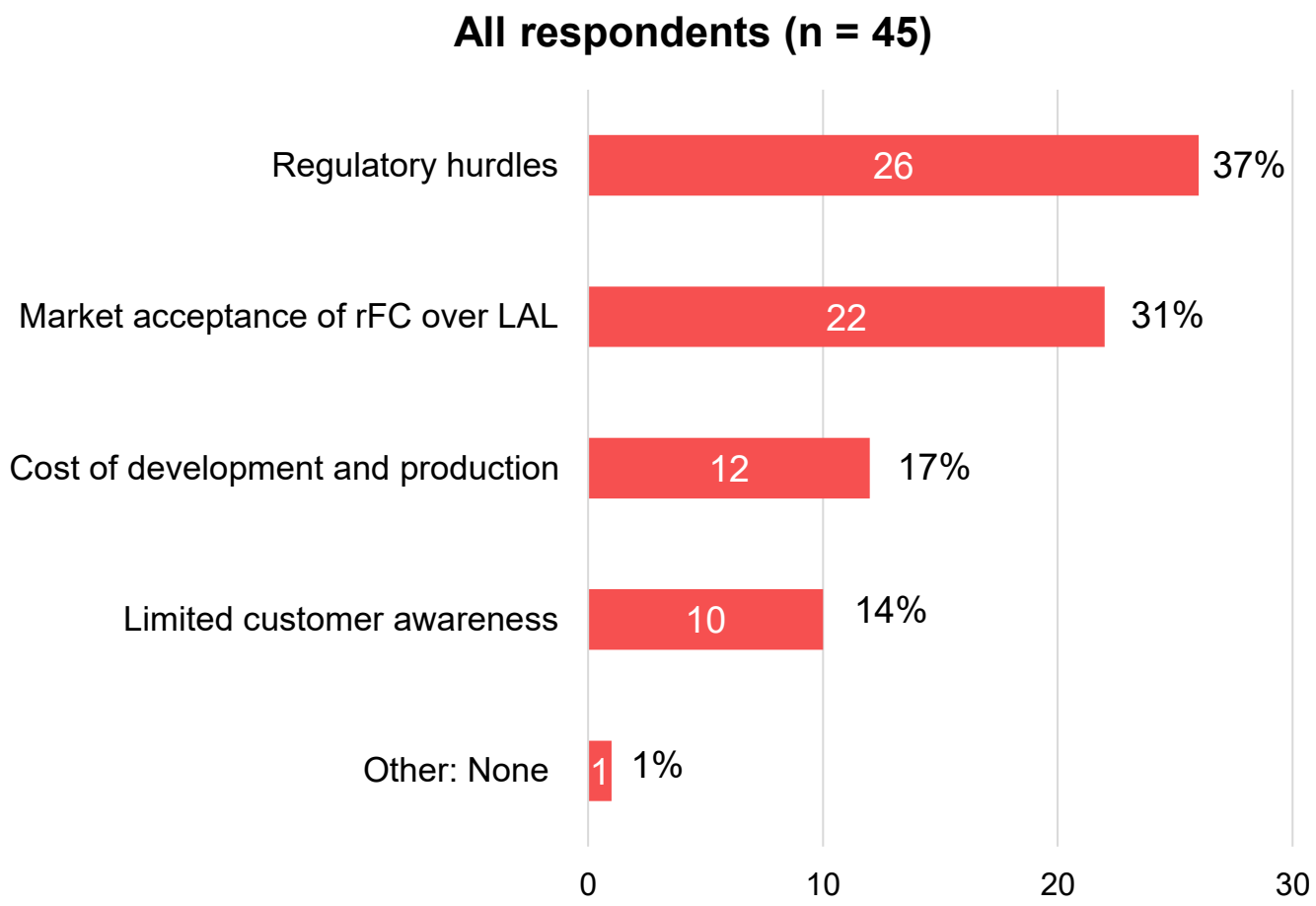
- Regulatory hurdles (37%) represent the most significant barrier.
- Achieving market acceptance of rFC methods over established LAL assays (31%) is a substantial hurdle.

Secondary challenges

- The financial investment required for rFC test development and production (17%) was noted as a considerable barrier.
- Limited customer awareness (14%) of the rFC test would hinder adoption.

No challenges

- One respondent reported no challenges.



Abbreviations: LAL, *Limulus* amebocyte lysate; rFC, recombinant Factor C.

Summary of online survey results

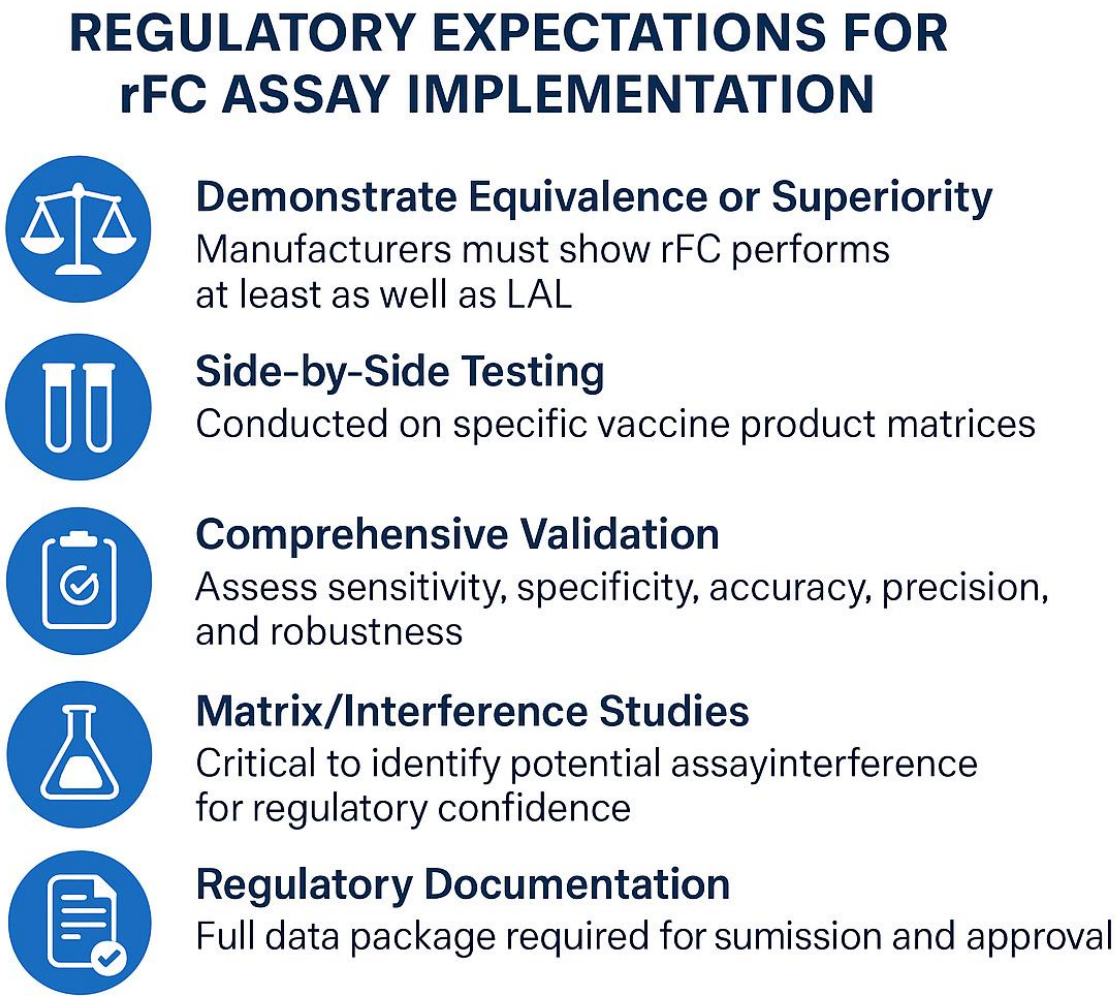
- There is growing interest in rFC adoption, particularly in lower-income countries, with many planning adoption in the next 3 to 5 years.
- The main barriers to rFC adoption reported by respondents include **regulatory hurdles,** market acceptance, and high initial costs.

Abbreviation: rFC, recombinant Factor C.

Regulatory acceptance and pharmacopeial landscape

Abbreviations: EP, European Pharmacopeia; JP, Japanese Pharmacopeia; LAL, *Limulus* amebocyte lysate; rFC, recombinant Factor C; USP, United States Pharmacopeia.

Validation and bridging study requirements



Abbreviations: LAL, *Limulus* amebocyte lysate; rFC, recombinant Factor C.

Regulatory submission and post-approval considerations

Bridging Data: Submitted via post-approval supplements/variations for licensed products

Product Coverage: Submissions needed for each impacted product, including multivalent vaccines; matrixing or bundling may be possible

Agency Timelines: Requirements and review times vary (FDA, EMA, WHO, etc.)

Pre-Submission Meetings: Consultations can clarify expectations and reduce risk

Approval Delays: Can impact product launch or supply schedules

Post-Approval Inspections: Verify adherence to new methods

Planning & Supply: Careful SKU and supply management essential for marketed products

Abbreviations: EMA, European Medicines Agency; FDA, United States Food and Drug Administration; SKU, stock-keeping unit; WHO, World Health Organization.

Regional and global harmonization challenges

Regulatory Variability

rFC acceptance differs across countnies and regions

Fragmented Landscape

Global regulatory differences complicate multi-market submissions

Multiple Validations

Separate validation efforts may be needed for each region

Supply Chain & Market Impact

Variability affects vaccine supply and launch strategies

Harmonization Efforts

WHO and international bodies working to align policies

Abbreviations: rFC, recombinant Factor C; WHO, World Health Organization.

Operational and Practical Implementation Barriers

SOP & QC Updates
Update procedures, quality systems, and train personnel

Equipment Investment
Capital needed for specialized tools (e.g. fluorescence readers)

Parallel Testing
Run LAL alongside rFC during transition for compliance

Supply Chain Management
Secure rFC reagents and consumables

Adoption Challenges
Possible disruptions and increased resource demands

Abbreviations: LAL, *Limulus* amebocyte lysate; QC, quality control; rFC, recombinant Factor C; SOP, standard operating procedure.

Regulatory risk and conservative approaches

Safety First: Vaccines given to healthy individuals; stringent testing for adventitious agents required

Assay Caution: Reluctance to accept changes without strong supporting data

Potential Delays: Heightened scrutiny, extra queries, or review delays

Risk Mitigation: Proactive measures to minimize regulatory risk

Engage Early: Continuous dialogue with regulators builds trust and eases acceptance

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Practical decision-making framework

- Assess product-specific needs and risk tolerance
- Plan validation and bridging studies carefully
- Address operational readiness: training, equipment, supply chain
- Engage regulators early to facilitate acceptance

Acknowledgments

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Group discussion

Group discussion: Shaping the roadmap

Group 1: rFC adoption landscape

Group 2: Regulatory engagement experience

Group 3: Bridging and validation capacity

Group 4: Inputs to roadmap

Group 5: Future engagement and collaboration

Abbreviation: rFC, recombinant Factor C.

Group 1: rFC adoption landscape

- What is the current state of rFC adoption in your organization or region?
- What factors drove or delayed adoption?
- Are there trends in adoption across types of organizations such as regulatory bodies or manufacturers?

Goal:

Capture the diversity of adoption status and the influencing factors, including technical, regulatory, and organizational elements.

Abbreviation: rFC, recombinant Factor C.

Group 2: Regulatory engagement experience

- Has your organization submitted rFC data to regulatory authorities?
- Which regulators have been most supportive or most cautious?
- What were the main questions or concerns raised during the review process?
- Was lack of harmonization between agencies an issue?

Goal:

Understand real-world experiences in engaging regulators and identify common regulatory barriers.

Abbreviation: rFC, recombinant Factor C.

Group 3: Bridging and validation capacity

- Has your team started or completed bridging studies for rFC?
- What were the main challenges (e.g., cost, technical barriers, or internal capacity)?
- Did you encounter issues like matrix interference or uncertainty with comparator methods?
- What kind of internal or external support was needed?

Goal:

Surface key validation and bridging challenges and what it would take to overcome them in different settings.

Abbreviation: rFC, recombinant Factor C.

Group 4: Inputs to roadmap

- What types of support would most help your organization adopt rFC?
- Are validated protocols or shared submissions more helpful than training or funding?
- What roles should regulators, donors, or technical partners play in enabling adoption?

Goal:

Identify priority support mechanisms and build consensus on what should be included in a practical, actionable roadmap.

Abbreviation: rFC, recombinant Factor C.

Group 5: Future engagement and collaboration

- Is your organization interested in contributing to a future roadmap or working group?
- What kinds of collaboration would be most helpful (e.g., peer exchange, template development, or joint case studies)?
- What would encourage deeper engagement from your side?

Goal:

Gauge the level of interest in future collaboration and identify mechanisms for sustained involvement.

Group topics

Group	Goal	Facilitator prompts
Group 1: rFC adoption landscape	Capture the diversity of adoption status and the influencing factors, including technical, regulatory, and organizational elements.	• What is the current state of rFC adoption in your organization or region? • What factors drove or delayed adoption? • Are there trends in adoption across types of organizations such as regulatory bodies or manufacturers?
Group 2: Regulatory engagement experience	Understand real-world experiences in engaging regulators and identify common regulatory barriers.	• Has your organization submitted rFC data to regulatory authorities? • Which regulators have been most supportive or most cautious? • What were the main questions or concerns raised during the review process? • Was lack of harmonization between agencies an issue?
Group 3: Bridging and validation capacity	Surface key validation and bridging challenges and what it would take to overcome them in different settings.	• Has your team started or completed bridging studies for rFC? • What were the main challenges (e.g., cost, technical barriers, or internal capacity)? • Did you encounter issues like matrix interference or uncertainty with comparator methods? • What kind of internal or external support was needed?
Group 4: Inputs to roadmap	Identify priority support mechanisms and build consensus on what should be included in a practical, actionable roadmap.	• What types of support would most help your organization adopt rFC? • Are validated protocols or shared submissions more helpful than training or funding? • What roles should regulators, donors, or technical partners play in enabling adoption?
Group 5: Future engagement and collaboration	Gauge the level of interest in future collaboration and identify mechanisms for sustained involvement.	• Is your organization interested in contributing to a future roadmap or working group? • What kinds of collaboration would be most helpful (e.g., peer exchange, template development, or joint case studies)? • What would encourage deeper engagement from your side?

Abbreviation: rFC, recombinant Factor C.

Short questionnaire

Before we close, we'd appreciate it if each of you could fill out a short questionnaire. It gives us your personal perspective and helps inform practical next steps for roadmap development.

Your input is valuable and greatly appreciated!