December 2025

Understanding barriers to adoption of sustainable alternate methods for endotoxin testing

Cost analysis

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 countries' manufacturers and regulators.

Session focus: Identifying and discussing the costing barriers

This costing session will focus on identifying the core costing barriers that impede this transition based on the initial survey results. The outcome will help inform the costing portion of the roadmap.

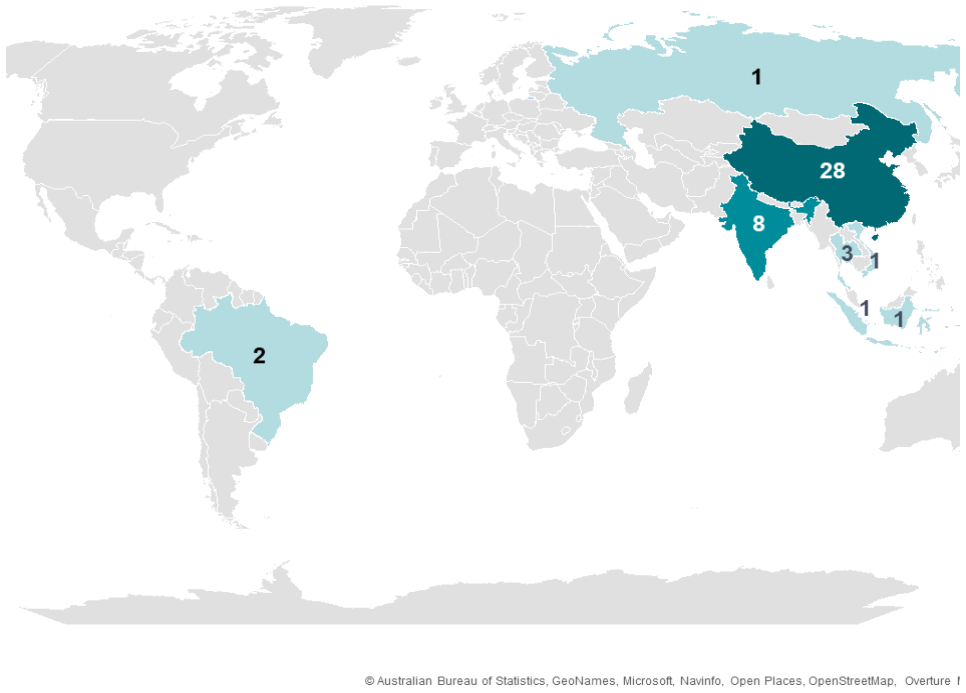
Photo: NPR/Ariane Mueller

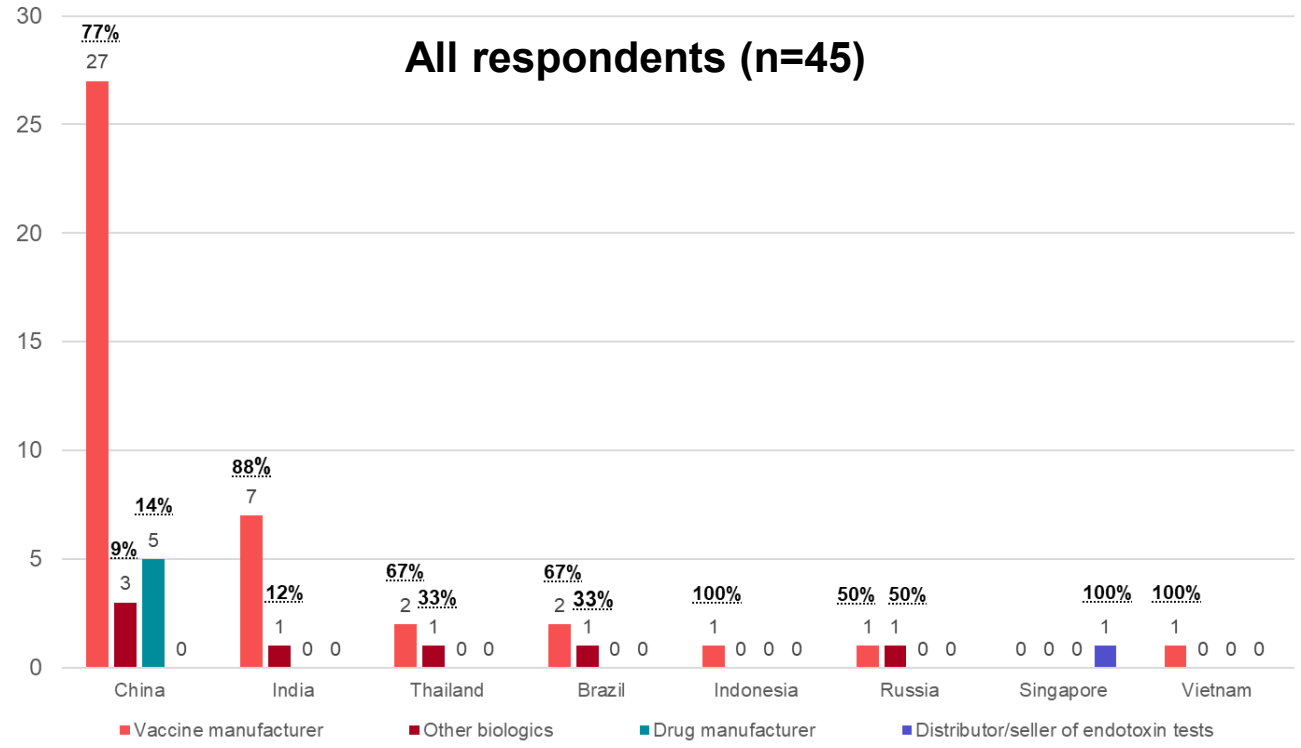
Marius M, Vacher F, Bonnevay T. Comparison of *Limulus* amoebocyte lysate and recombinant factor C assays for endotoxin detection in four human vaccines with complex matrices. *PDA Journal of Pharmaceutical Science and Technology*. 2020;74(4):394–407. https://doi.org/10.5731/pdajpst.2019.010389

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).

Cost of development and production of rFC tests poses significant barrier to recombinant method adoption

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

Primary challenges:

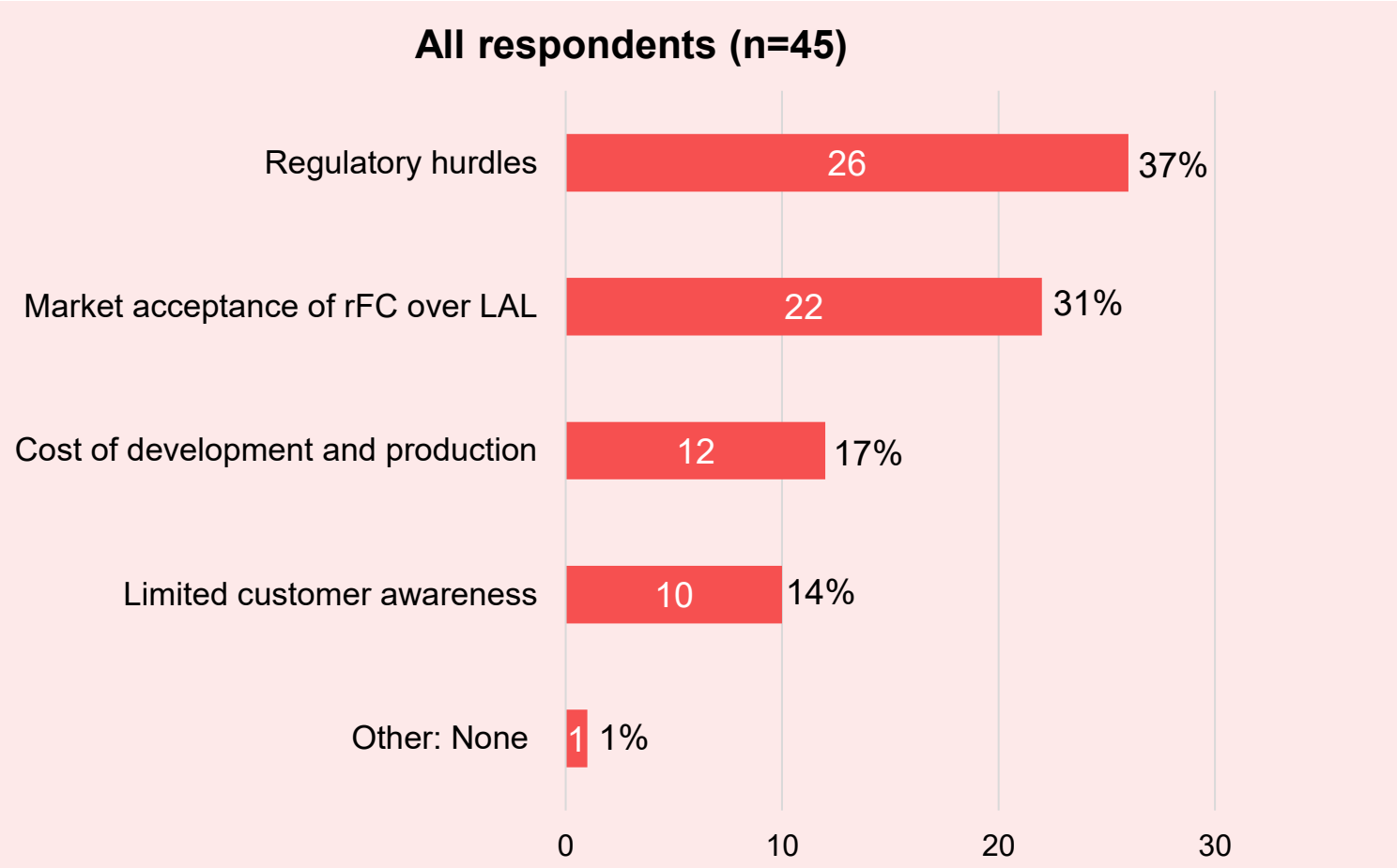
- Regulatory hurdles (37%) represented the most significant barrier to adoption.
- Achieving market acceptance of rFC methods over established LAL assays (31%) represented another significant hurdle.

Secondary challenges:

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

No challenges

- One respondent reported no challenges.



Abbreviations: LAL, limulus amebocyte lysate; rFC, recombinant factor C.

Significance of cost analysis

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The value of cost modeling

Clarifies the trade-offs by comparing true operational and one-time costs (training, equipment, regulatory bridging) between LAL and rFC.

Reveals volume-driven inflection points at which rFC becomes cost competitive and highlights long-term efficiency or compliance advantages.

Builds a defensible business case for stakeholders by quantifying financial impact, risk reduction, and strategic alignment with sustainability and supply chain goals.

Abbreviations: LAL, limulus amebocyte lysate; rFC, recombinant factor C.

Key assumptions in cost modeling

- **Volumes**: “Low,” “Mid,” and “High” represent increasing annual throughput; cost per batch/unit scales accordingly.
- **Costs**: One-time costs (training, equipment, regulatory bridging) are annualized; recurring costs include reagents, labor, and repeat testing.
- **Performance**: rFC is assumed to have lower repeat-test rates and more consistent performance than LAL.
- **Labor & reagents**: Labor rates hold constant; reagent use per test is consistent across volumes.
- **Regulatory**: Bridging requirements apply only to rFC and are treated as a single-year investment.
- **Scope**: Prices are in US dollars and exclude recombinant overhead, facility allocations, and external shocks (e.g., supply disruptions).

Abbreviations: LAL, limulus amebocyte lysate; rFC, recombinant factor C.

Key assumptions in cost modeling cont.

Production & testing parameters

- Product format:
  - Lyophilized, single-dose vial across all volumes
- Batch size & frequency:
  - Low volume: 10,000 doses, 10 batches/year
  - Mid volume: 50,000 doses, 50 batches/year
  - High volume: 100,000+ doses, 100+ batches/year
- Tests per batch:
  - 2 tests per batch (1 for release + 1 for stability)

Production volumes & testing setup			
Parameter	Low volume	Mid volume	High volume
Batch size	10,000 doses	50,000 doses	100,000+ doses
Batches per year	10	50	100+
Tests per batch	2 (release + stability)		
Total tests/year	20	100	200

8 Gavi, the Vaccine Alliance. Measles and measles-rubella vaccine support. Accessed November 18, 2025. https://www.gavi.org/types-support/vaccine-support/measles-and-measles-rubella

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Cost categories and assumptions

Recurring costs		
Cost component	LAL assumptions	rFC assumptions
Reagent cost/test	$13.00 (low) → $5.50 (high)	$8.44 (low) → $3.00 (high)
Reagent waste	~10% waste + 40%–50% QC markup	~1%–2% waste + 10%–15% QC markup
Labor/test	3 hrs. at $30/hr. = $90	2 hrs. at $30/hr. = $60
Repeat test rate	10.0%	1.5%

One-time/annualized costs			
Category	LAL annualized cost	rFC annualized cost	Assumptions
Training	$0	$1,061	$3,000 one-time, amortized over 3 years
Equipment depreciation	$803	$1,605	$5,000 (LAL) vs. $10,000 (rFC), over 7 years
Regulatory bridging	$0	$5,459	$25,000, amortized over 5 years

Abbreviations: LAL, limulus amebocyte lysate; QC, quality control; rFC, recombinant factor C.

Lonza. Kinetic-QCL® Kinetic Chromogenic LAL Assay, 192 Test Kit. Accessed November 11, 2025. https://bioscience.lonza.com/lonza_bs/US/en/Endotoxin-Detection/p/0000000000000182378/Kinetic-QCL-Kinetic-Chromogenic-LAL-Assay%2C-192-Test-Kit

Lonza. PyroGene® Recombinant Factor C Endpoint Fluorescent Assay. Accessed November 11, 2025. https://bioscience.lonza.com/lonza_bs/US/en/Endotoxin-Detection/p/0000000000000182383/PyroGene-Recombinant-Factor-C-Endpoint-Fluorescent-Assay

Cost model summary – LAL versus rFC, by volume

Cost component	Low volume	Mid volume	High volume
LAL one-time (annualized)			
Training	$0	$0	$0
Equipment depreciation	$803	$803	$803
Regulatory bridging	$0	$0	$0
Subtotal	**$803**	**$803**	**$803**
Recurring costs			
Reagents	$260	$750	$1,100
Labor	$900	$4,500	$9,000
Repeat tests/QA	$116	$525	$1,010
Subtotal	**$1,276**	**$5,775**	**$11,110**
Total annual cost	**$2,079**	**$6,578**	**$11,913**

Low volume	Mid volume	High volume
rFC one-time (annualized)		
$1,061	$1,061	$1,061
$1,605	$1,605	$1,605
$5,459	$5,459	$5,459
$8,125	**$8,125**	**$8,125**
Recurring costs		
$169	$450	$600
$600	$3,000	$6,000
$12	$52	$99
$780	**$3,502**	**$6,699**
$8,905	**$11,626**	**$14,824**

Abbreviations: LAL, limulus amebocyte lysate; QA, quality analysis; rFC, recombinant factor C.

Efficiency metrics

Metric	LAL	rFC
Cost per batch	$208 (low) → $119 (high)	$890 (low) → $148 (high)
Cost per unit (dose)	$0.0208 → $0.0012	$0.0890 → $0.0015

- LAL has lower cost at a small scale due to no bridging/training investment.
- rFC becomes more cost-efficient at higher volumes, despite higher upfront costs.
- Both methods scale well, with cost per dose dropping ~90% from low to high volume.
- At high volume, cost per dose is nearly equivalent for LAL and rFC.
- Both methods show significant cost reduction per dose as production scales.

Abbreviations: LAL, limulus amebocyte lysate; rFC, recombinant factor C.

Suggested roadmap for rFC adoption

SHORT-TERM (0–6 MONTHS)

Feasibility & Planning

- Review current LAL testing workflows for Gavi vaccines
- Identify high-volume products/sites for rFC piloting
- Conduct cost-benefit analysis based on diverse scale of production
- Engage regulatory authorities on bridging expectations
- Run small-scale rFC versus LAL comparability tests

MID-TERM (6–18 MONTHS)

Validation & Transition

- Perform validation and regulatory bridging studies
- Train QC staff on rFC protocols
- Procure and validate fluorescence plate readers
- Update quality management systems
- Submit supporting documentation for regulatory approval

LONG-TERM (18–36 MONTHS)

Scale-Up & Integration

- Expand rFC to additional vaccine products/sites
- Track improvements in cost, repeat rates, and throughput
- Assess alignment with sustainability and animal-free goals
- Gradually phase down LAL use

Use this roadmap as a **flexible guide** to build your own rFC transition plan for vaccines/Gavi-supported vaccines.

Abbreviations: LAL, limulus amebocyte lysate; QC, quality control; rFC, recombinant factor C.

Acknowledgments

PATH gratefully acknowledges the manufacturers and individuals who participated in our online survey and who have provided us with data. In addition, we acknowledge the leadership of Manjari Lal, who provided critical review and feedback on this presentation.

PATH also acknowledges Laura Viviani, who supported us throughout the session planning process. Her dedication and attention to detail were instrumental in making this session a success.

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Group discussion

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Group discussion: Shaping the roadmap

Group 1: Scale and production assumptions

Group 2: Reagent and labor costs

Group 3: Repeat testing and variability

Group 4: Transition costs and equipment needs

Group 5: Open reflections and roadmap priorities

Group 1: Scale and production assumptions

Discussion questions:

- What are typical batch sizes for measles or other Gavi-supported vaccines in your facility?
- Do the batch sizes used (10K, 50K, 100K+) match your operations?
- Are you working at smaller or larger scales than modeled?

Goal:

Confirm or refine the volume tiers used in the cost analysis for more realistic modeling.

Group 2: Reagent and labor costs

Discussion questions:

- We've shared estimated annual reagent costs for LAL and rFC across three production volumes:
  - How do these compare to your actual costs? Are yours higher, lower, or about the same?
  - If you don't know the exact cost, can you tell us who your reagent suppliers are?
- Are prices stable, or do they change often?
- Our model assumes 2–3 hours of hands-on labor per test. Does this reflect your team's experience with LAL and rFC?
- Have you observed any time savings, fewer repeats, or lower troubleshooting effort with rFC?

Goal:

Explore cost and labor variability across regions, methods, and facility types.

Abbreviations: LAL, limulus amebocyte lysate; rFC, recombinant factor C.

Group 3: Repeat testing and variability

Discussion questions:

- How often do LAL tests require repeats in your lab?
- What usually causes test repeats: variability, contamination, or other issues?
- Have you observed fewer repeats or more consistent results with rFC?

Goal:

Evaluate how assay consistency affects workload, cost, and decision timelines.

Abbreviations: LAL, limulus amebocyte lysate; rFC, recombinant factor C.

Group 4: Transition costs and equipment needs

Discussion questions:

- Are the equipment costs reasonable in your context ($5K LAL reader, $10K rFC reader)?
- Is $3,000 a realistic estimate for staff training?
- Does the $25,000 regulatory bridging cost seem high, low, or about right?
- Are there any hidden costs or overlooked issues in switching platforms?

Goal:

Capture perceptions of the true costs and risks of transition to inform support planning.

Abbreviations: LAL, limulus amebocyte lysate; rFC, recombinant factor C.

Group 5: Open reflections and roadmap priorities

Discussion questions:

- What is the most significant barrier to switching from LAL to rFC in your setting?
- Where could technical assistance or financial support make the greatest impact?
- What would help you create your own roadmap for adopting rFC?

Goal:

Gather practical input to shape a flexible, regionally informed roadmap.

Abbreviations: LAL, limulus amebocyte lysate; rFC, recombinant factor C.

Group topics

Group	Goal	Facilitator prompts
Group 1: Scale and production assumptions	Confirm or refine the volume tiers used in the cost analysis for more realistic modeling.	• What are typical batch sizes for measles or other Gavi-supported vaccines in your facility? • Do the batch sizes used (10K, 50K, 100K+) match your operations? • Are you working at smaller or larger scales than modeled?

Group 2: Reagent and labor costs	Explore cost and labor variability across regions, methods, and facility types.	• We've shared estimated annual reagent costs for LAL and rFC across three production volumes:
		  • How do these compare to your actual costs? Are yours higher, lower, or about the same?
		  • If you don't know the exact cost, can you tell us who your reagent suppliers are?
		• Are prices stable, or do they change often?
		• Our model assumes 2–3 hours of hands-on labor per test. Does this reflect your team's experience with LAL and rFC?
		• Have you observed any time savings, fewer repeats, or lower troubleshooting effort with rFC?

Group 3: Repeat testing and variability	Evaluate how assay consistency affects workload, cost, and decision timelines.	• How often do LAL tests require repeats in your lab? • What usually causes test repeats: variability, contamination, or other issues? • Have you observed fewer repeats or more consistent results with rFC?
Group 4: Transition costs and equipment needs	Capture perceptions of the true costs and risks of transition to inform support planning.	• Are the equipment costs reasonable in your context ($5K LAL reader, $10K rFC reader)? • Is $3,000 a realistic estimate for staff training? • Does the $25,000 regulatory bridging cost seem high, low, or about right? • Are there any hidden costs or overlooked issues in switching platforms?
Group 5: Open reflections and roadmap priorities	Gather practical input to shape a flexible, regionally informed roadmap.	• What is the most significant barrier to switching from LAL to rFC in your setting? • Where could technical assistance or financial support make the greatest impact? • What would help you create your own roadmap for adopting rFC?

Abbreviations: LAL, limulus amebocyte lysate; QC, quality control; rFC, recombinant factor C.

Short questionnaire

Before we close, we'd appreciate 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!

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