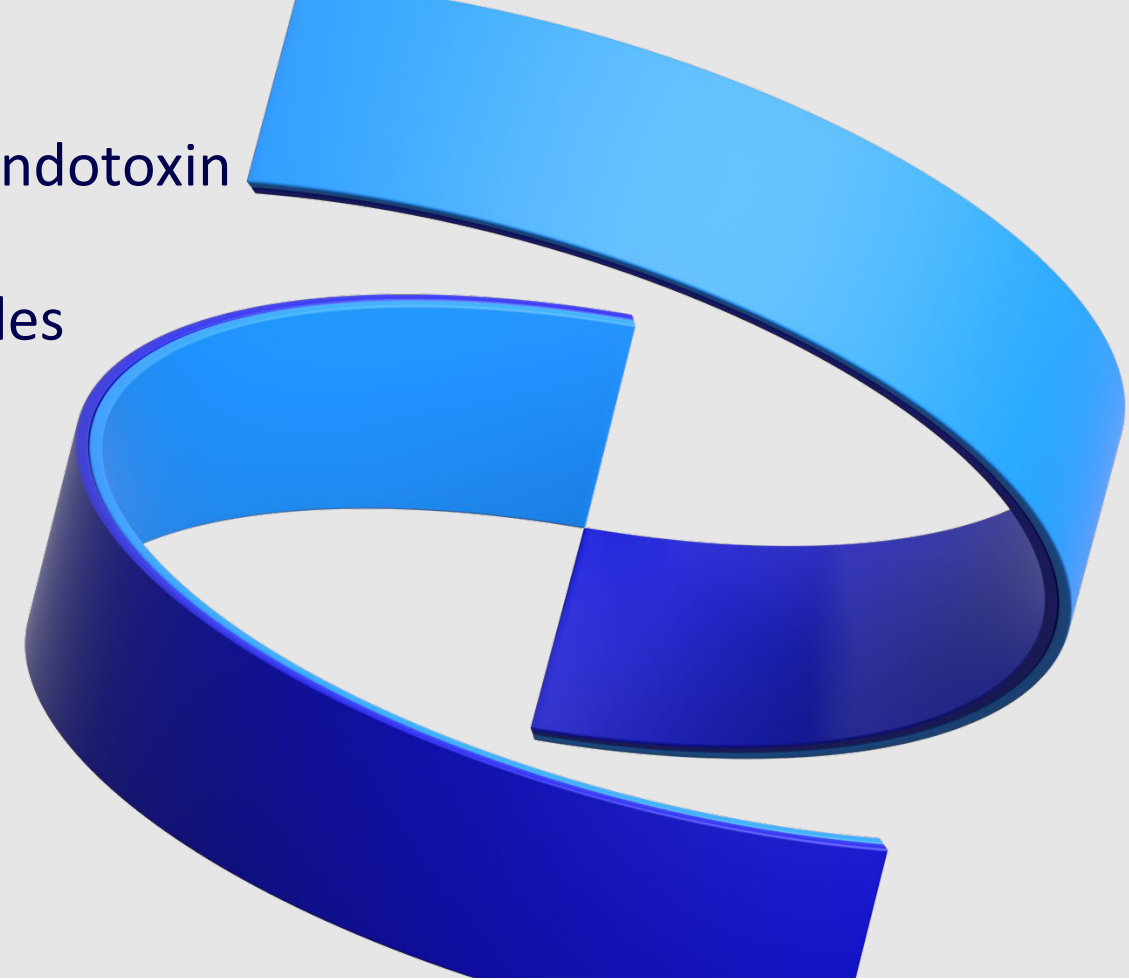
Pfizer Study Comparing Endotoxin Test Methods on Biopharmaceutical Samples

15 November 2021



Ned Mozier

ABSTRACT

A comparison of **two methods for endotoxin testing** was conducted on samples obtained one of our **biopharmaceutical production** facilities. One method includes naturally-sourced Limulus Amebocyte Lysate (**LAL**) – containing reagents in a multi-cartridge system (MCS) and the other recombinantly manufactured factor C (**rFC**). Samples included non-potable **city water** (CW), clean steam and Water for Injection (WFI) obtained over multiple days and tested at the same time, in the same lab, with the same reagents on fresh samples. Tests were conducted on 60 CW samples **with and without endotoxin-specific buffer (ESB)**. Both methods were found to have excellent and similar sensitivity and gave undetectable levels of endotoxin in clean steam and WFI samples; and **detectable endotoxin in all CW sample**. For these samples, the LAL and rFC gave similar results, well within the range of spike recovery allowed in routine percent product control (50-150%). The MCS LAL method showed more reduction of signal than rFC when ESB was added, suggesting **the presence of glucans** the CW samples. Overall these data demonstrate that **both methods are suitable for testing of incoming water samples to assure quality during biopharmaceutical manufacturing.**

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Submission Request

Deadline for Submission: September 20, 2021

USP plans to host a Virtual Open Forum to discuss alternatives to compendial reagents used in the Bacterial Endotoxins Test. USP posted a call for additional data on the comparability of alternative methods to <85> as well as a study proposal in June 2021. USP invites you to submit abstracts for a non-promotional, data-driven, 30-minute oral presentation on new (not yet published) comparability studies using alternative methods, specifically studies that demonstrate comparability using samples (e.g., pharmaceutical products, manufacturing materials, raw materials, manufacturing environment) that contain endotoxins from autochthonous microorganisms. This event for the Bacterial Endotoxins Test will be held virtually on November 15-16.

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From Merriam-Webster on line on 23 Sep 2021

autochthonous adjective

Save Word

au·toch·tho·nous | \ ȯ-ˈtäk-thə-nəs \

Definition of *autochthonous*

1 : INDIGENOUS, NATIVE
// an *autochthonous* people
// *autochthonous* plants

2 : formed or originating in the place where found
// *autochthonous* rock
// an *autochthonous* infection

Water is locally sourced and therefore "native" to our facility

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Topics

2013 Publication Comparing Research Samples by multiple LAL and Lonza rFC test kits

2019 Unpublished results Comparing Incoming Water Samples by one LAL and one rFC test method

Both studies conclude no consistent or important differences between rFC and LAL. The over-reporting by LAL due to non-specificity (for glucans) can be an issue for products with glucans, but generally over-reporting is not an issue for most projects.

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Pfizer Published Data

Comparison of Limulus amoebocyte lysate test methods for endotoxin measurement in protein solutions. Chen, L. and Mozier, N., J Pharm Biomed Anal, Vol 80, pp. 180-185, 2013

- Samples were collected over a long time because rarely do we have laboratory / research

Table 2
Glucan content in the protein samples.

Sample #	Sample description	Glucan (pg/ml)
1	mAb 1 Drug substance	Neg
2	mAb 2 in-process sample	Neg
3	mAb 3 in-process sample	112
6	Protein 1 culture harvest (yeast)	7600
7	mAb 4 clarified broth	20
8	mAb 5 in-process sample	>20,000
10	Protein 2 in lipid formulation	47
11	mAb 6 in-process sample	Neg
12	Protein 1 in-process sample	1500
13	Protein 2 in-process sample	Neg

The ten samples were measured for (1–3) beta-D-glucan content using the Glucatell assay kit from Associates of Cape Cod. The source and nature of these samples were also listed.

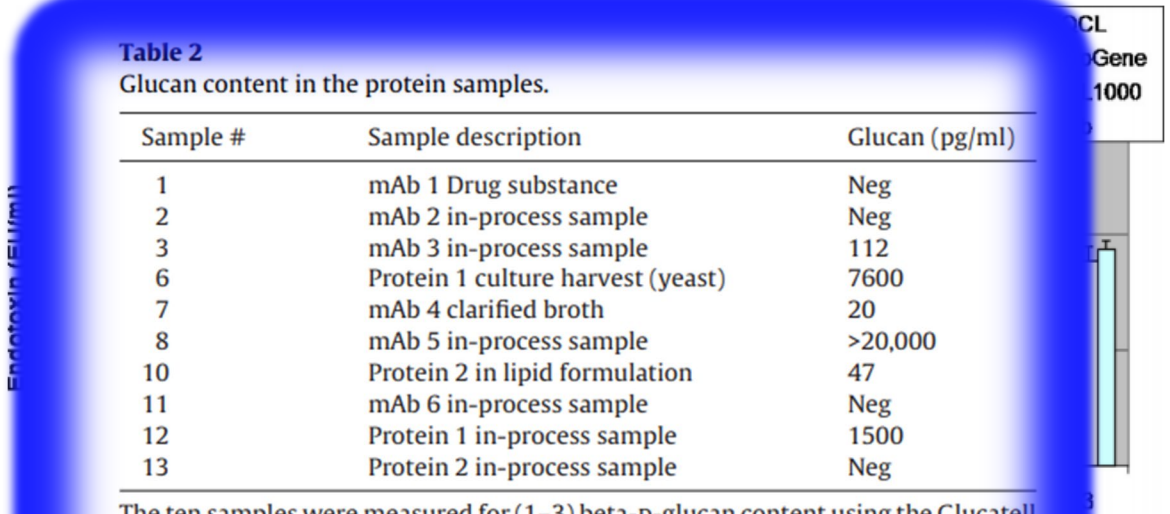

Fig. 3. Endotoxin potencies of t… …erent LAL endotoxin methods; kinetic turbidimetric method (Turb), kinet… …combinant Factor C method (PyroGene). Two sets of sample aliquots were used for each method. The average results for each method were graphed. The error bars are standard deviations from the duplicate assays. Endotoxin results were graphed in log-scale.

adequate to block all types of glucans

Purpose of Study Comparing Water Samples

- Evaluate suitability of an alternative endotoxin assay for samples relevant to recombinant production in one of our clinical manufacturing plants
- Compare a recombinant C method (Endozyme II GO, Biomerieux) to an LAL-based Multi-cartridge Endotoxin Detection System (MCS, Charles River Labs)
- Evaluate water samples from one of our clinical manufacturing facilities used to make recombinant biopharmaceutical products and vaccines

Potential Benefits

- Add another test option for endotoxin testing (supply chain continuity)
- Less reliance on horseshoe crabs if adequate similarity is demonstrated
- Less use of non-animal derived materials consistent with Pfizer RRR goals

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Study Design

Variable	Protocol to control for variable
Analyst	Tested by the same analyst using both assay kits / devices
Lab	The same laboratory used for all tests
Assay Kit	The same kit lot# used for each test
Sample stability	Fresh, unfrozen samples tested in both kits at the same time
Additional Evaluations	**Rationale**
Test with and without endotoxin specific buffer	To determine if glucans are present, understand if %PPC is affected
Test RSE	To eliminate any calibration bias during our application
Determine %PPC, precision	Assure suitability to USP<85>
Sample Considerations	Rationale
Incoming water before & after purification	Test over a period of weeks, head to head in both assays

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Control limits are <256 EU/mL for unprocessed water, <0.25 EU/mL for purified

WFI / clean steam (19 samples tested), spec <0.25 EU/mL

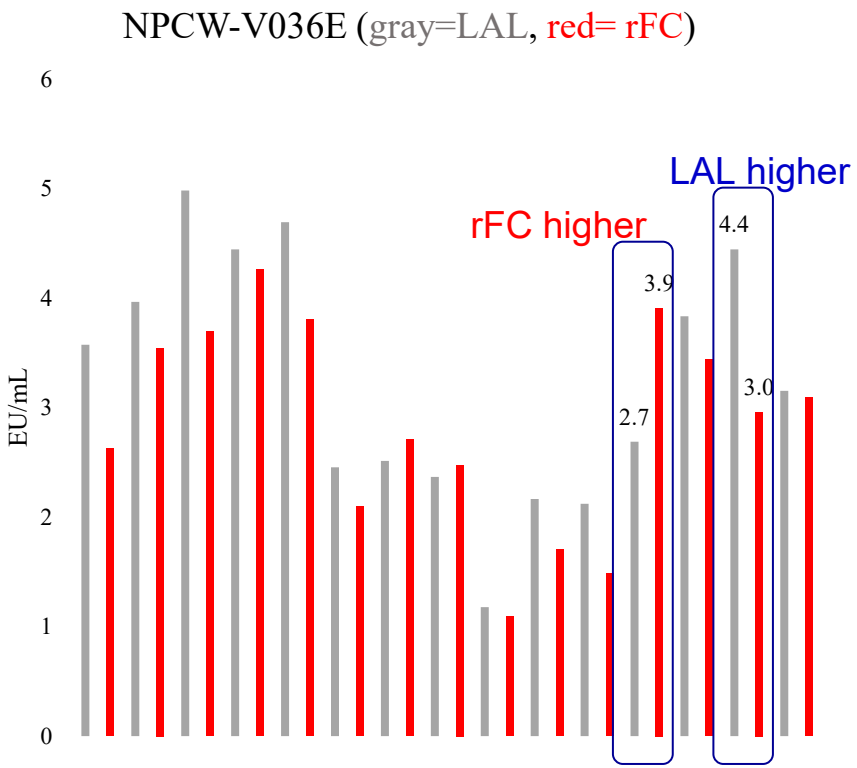
All samples in both assays were <0.05 EU/mL

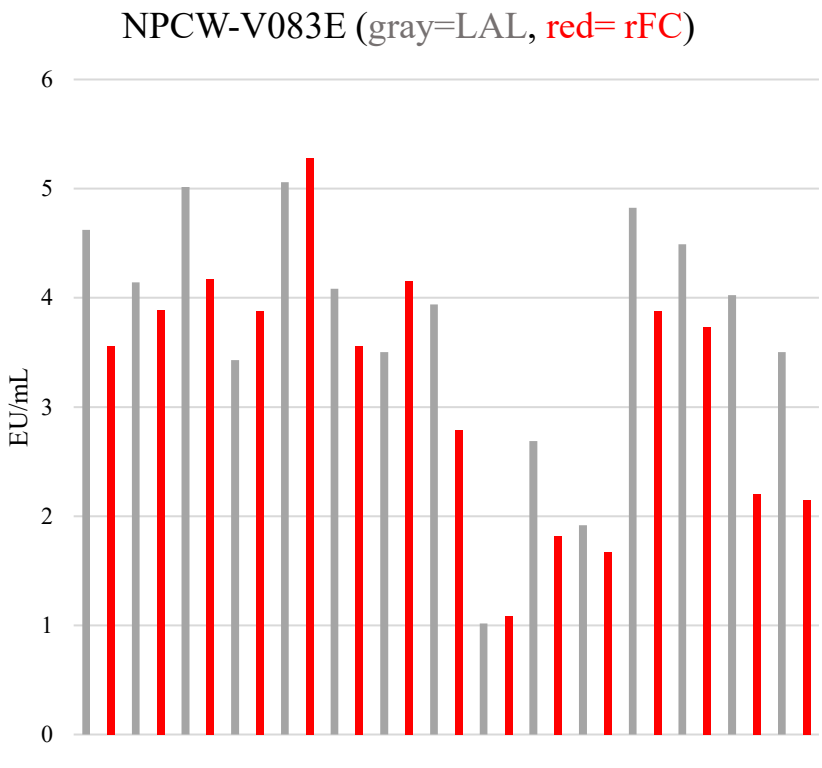
Learnings

- ESB added to samples reduced, on average, MCS by 25% and 15% for rFC (vs dilution in water)
  - ✓ Greater reduction by MCS suggests low level of beta glucans which can activate factor G in LAL
  - ✓ Small reduction in rFC in ESB vs WFI suggests nonspecific masking of endotoxin due to reagents
  - ✓ ESBs are only one type of glucan, not expected to block all types or quantitatively correlated with glucans
  - ✓ For this reason, ESB was included for all comparison testing (both MCS & rFC)

- Rates of %PPC failures need attention in a QC setting
- Calibration accuracy was evaluated by testing RSE multiple times in both assays
  - MCS was off by 27% (over-reporting)
  - rFC was off by 8% (under-reporting)
  - Comparisons were adjusted accordingly

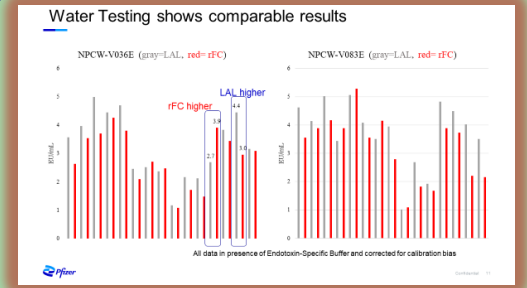
Water Testing shows comparable results





All data in presence of Endotoxin-Specific Buffer and corrected for calibration bias

Same Data, Different Scale to show vs. limit



nelmingly sensitive to our production needs

Limit is 256 EU/mL

Conclusions from our Water System testing

- Both methods are suitable for the incoming raw water used for biopharmaceutical productions
- LAL and rFC tests give very similar results (with ESB added)
  - All results within 50-200% of each other
  - Overall average shows LAL 18% higher than rFC
    - ✓ In 7 cases rFC gives higher results
    - ✓ In 23 cases MCS gives higher results
      - ❖ Likely due to presence of unblocked beta glucans
- We conclude the small average difference is irrelevant to our needs, is well within the differences between LAL kits, and likely due to non-specificity for due to incompletely blocked glucans that are present.

Overall Conclusions

- LAL and recombinant methods produce very similar results in a broad set of samples including those relevant to biopharmaceutical manufacturing
  - ✓ There is as much or more variation between LAL as between recombinant & LAL
- Recombinant reagents offer several advantages
  - ✓ Biotechnology processes are inherently more reliable for supply chains
  - ✓ Not reliant on sourcing from horseshoe crabs
    - ➢ Thus, better aligned with Pfizer's 3R's goals
- We recommend recombinant methods be included in compendia based on these data plus that widely published by others