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Research Article

INDUSTRY PRACTICES IN BACTERIAL ENDOTOXIN TESTING FOR INJECTABLE PHARMACEUTICALS: A MULTICENTRE SURVEY OF INDIAN MANUFACTURERS

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ABSTRACT

Background: Bacterial endotoxins are a serious safety hazard in the manufacture of injectable pharmaceuticals. Although the Limulus Amebocyte Lysate (LAL) gel-clot method is the reference standard in the pharmacopeia, questions exist about subjectivity, matrix interference, sustainability as well as the arrival of alternative technologies, which require a full assessment of industry practices.

Objective: To describe the existing practices of Bacterial Endotoxin Testing (BET), risk-based approaches and preparedness for technical modernization by injectable pharmaceutical manufacturers.

Methods: A designed cross sectional questionnaire of 21 items was administered to pharmaceutical professionals involved in sterile injectable manufacturing company. The 107 valid replies were evaluated using descriptive statistics, chi-square tests for association, correlation analysis, factor analysis and a novel Modernization Readiness Index (MRI).

Results: The gel-clot approach remains the most common and used by 86.9% (93/107) of the respondents. The respondents were quite technically mature with 61.7% having >10 years of industry experience and 79.4% were from Quality Control departments. Although 71.0% (76/107) found recombinant Factor C (rFC) and Monocyte Activation Test (MAT) to be potential options, 94.4% (101/107) stressed the need for further validation and regulatory harmonization before routine implementation. AI based techniques were perceived positively, with 48.6% feeling that they could greatly enhance endotoxin control, whilst 25.2% assessed their influence as limited or untested. Factor analysis indicated three main aspects accounting for 65.6% variance: Regulatory & Validation Compliance (28.5%), Technology Adoption Readiness (21.3%), and Operational Challenges (15.8%). The Modernization Readiness Index found that only 16.8% of respondents were "high readiness" for technology change.

Conclusion: The injectable pharmaceutical market remains heavily dependent on gel-clot BET, but there is good knowledge and cautious optimism for modernization. Regulatory harmonization, strong validation procedures and focused capacity building are still key drivers for the shift towards alternative methodologies and AI integrated systems.

Keywords: Bacterial Endotoxin Test, Gel-clot method, Recombinant Factor C, Monocyte Activation Test, Artificial Intelligence, Risk-based Quality, Pharmaceutical Production, Validation, Modernization Ready.

Introduction

Bacterial endotoxins, chiefly lipopolysaccharides from the outer membrane of gram-negative bacteria, are one of the major safety risks in parenteral pharmaceutical products. Injected into the human circulation these pyrogenic chemicals can initiate a cascade of clinical responses such as fever, hypotension, shock, disseminated intravascular coagulation and multi-organ failure[1,2]. The need to guarantee that injectable goods are not contaminated with clinically relevant endotoxins has led regulatory bodies around the world to adopt demanding testing procedures and acceptance criteria[3-5]. India is a key player in the global pharmaceutical space, being a significant exporter of sterile injectables, vaccines, biosimilars and biologic goods. Hence, the pharmaceutical manufacturers in the country have to prove compliance with international pharmacopeial standards including the United States Pharmacopeia (USP), European Pharmacopoeia (Ph. Eur.), Japanese Pharmacopoeia (JP), and Indian Pharmacopoeia (IP) in addition to regulatory expectations from agencies like US Food and Drug Administration (FDA) and European Medicines Agency (EMA)[3-5].

The Limulus Amebocyte Lysate (LAL) test, which is based on the blood of horseshoe crabs (*Limulus polyphemus and Tachypleus tridentatus*), has been the mainstay of bacterial endotoxin testing for almost four decades[6]. Among LAL-based methods, the gel-clot method, a qualitative or semi-quantitative endpoint assay, is the most often used technique worldwide because of its simplicity, cost-effectiveness, and long-standing regulatory acceptability[6,7]. However, this approach is subject to intrinsic limitations such as subjectivity of endpoint interpretation, vulnerability to matrix interference by formulation components (pH, viscosity, excipients) and limited quantitative capacity. There is also increasing ethical and sustainability concern about the reliance on horseshoe crab populations[8-10].

Alternative techniques have arisen in response to these constraints. Recombinant Factor C (rFC) tests employ a synthetic analogue of the endotoxin-sensitive clotting factor from horseshoe crabs and provide an animal-free alternative with equal or higher sensitivity[11,12]. The Monocyte Activation Test (MAT) is a human-cell based technique that is able to detect both endotoxin and non-endotoxin pyrogens and may provide a more comprehensive pyrogenicity assessment[9,13,14]. At the same time, quantitative LAL methods such as kinetic turbidimetric and chromogenic tests allow for accurate endotoxin measurement with less subjectivity. Alongside methodological growth, regulatory science has placed a growing emphasis on risk-based quality management and Quality by Design (QbD) concepts, as stated in ICH Q9 standards[15]. This paradigm change drives manufacturers to incorporate Critical Quality Attributes (CQAs), Critical Process Parameters (CPPs) and formal risk assessment methods like Failure Modes and Effects Analysis (FMEA) into endotoxin control efforts. More recently, the EMA has published a reflection paper on the use of artificial intelligence (AI) and machine learning for pharmaceutical quality control[21].

There is a dearth of comprehensive data on the implementation of novel BET techniques, integration of risk-based approaches, and preparation for digital transformation in the Indian injectable manufacturing sector, despite technological and regulatory improvements. Earlier work has been limited in breadth, sample size, and analysis. Hence, the aim of this multicenter survey was to: (1) describe the current BET practices in Indian manufacturers of injectables; (2) evaluate practitioner attitudes and opinions toward new alternatives such as rFC, MAT and AI-based methods; (3) assess the adoption of risk-based quality management concepts; (4) identify ongoing challenges and barriers to modernization; and (5) create quantitative measures of modernization readiness to guide regulatory policy and industry capability development.

Materials and Methods

Study Design and Participants

The study was conducted through a cross-sectional survey design via a structured questionnaire to pharmaceutical experts involved in the manufacture of sterile and injectable products[18].Participants were recruited from quality control, quality assurance, production/manufacturing and research & development departments of pharmaceutical businesses. The sample frame included domestic producers and subsidiaries of multinational operating companies. Data were collected between October 2025 and February 2026. Participation was optional and anonymous, and no personally identifiable information was collected. After removing duplicate entries and responses, a final analytical sample of 107 valid responses was obtained.

Sample Size Justification

The sample size adequacy was determined by Cochran's formula for cross-sectional surveys n 0 = Z 2 p (1 − p) e 2 where Z = 1.96 (95% confidence level), p = 0.5 (maximum variability for percentage estimation) and e = 0.075 (margin of error)[17]. The minimum sample size for accurate population inference was found to be 171. However, the sample of 107 respondents collected for the purpose of exploratory analysis and identification of industry patterns provides enough statistical power for descriptive analysis, comparisons between subgroups and identification of effect sizes for future confirmatory studies.

Questionnaire Development

The survey instrument included 21 questions covering: (1) professional demographics (experience, department, company); (2) current BET methods employed; (3) rationale for method selection; (4) perceived advantages and challenges of the gel-clot method; (5) awareness and

perceptions of alternative methods (rFC, MAT, biosensors); (6) validation practices and requirements; (7) integration of risk-based quality management (CQA, CPP, FMEA/PHA); (8) perceptions of AI-based techniques for endotoxin control; (9) batch validation practices; and (10) open-ended comments and recommendations. Content validity was validated by assessment by subject matter experts in pharmaceutical microbiology and quality systems.

Statistical Analysis

Descriptive statistics were used to assess categorical data (frequencies, percent). The inferential statistical analysis were: Chi-square tests, Associations between categorical variables (experience level, departmental affiliation, choice of method, perceptions of options and AI) were assessed by chi-square tests of independence.

Correlation analysis (Pearson's r) to explore associations between the main continuous and ordinal variables, with significance levels of $p < 0.05$.

Factor analysis (Principal Component Analysis with varimax rotation) to uncover underlying characteristics of BET behaviors and perceptions. Eigenvalues >1.0 and factor loadings >0.5 were used to interpret the factors.

Modernization Readiness Index (MRI): A composite index was built from five dichotomous indicators: Awareness/acceptance of alternative methods (rFC/MAT/biosensors)

1. Knowledge/acceptance of alternative methods (rFC/MAT/biosensors)
2. Perception of positive AI contribution
3. Recognized need for more validation
4. Use of quantitative approaches (non-gel clot)
5. Implementation of formal risk assessment (FMEA/PHA) Responses were classed as High Readiness (4-5 indicators), Medium Readiness (3 indications) or Low Readiness (1-2 indicators).All statistical analyses were carried out with SPSS version 26.0 (IBM Corp., Armonk, NY) and Microsoft Excel 365.

Ethical Considerations

The study protocol was reviewed according to institutional rules for survey research involving human participants. The poll did not capture any personally identifiable information and was of low risk and hence formal ethics committee approval was not deemed necessary. The participants gave their consent to participate and all responses were anonymised prior to analysis.

Results

Respondent Demographics

In total, 107 valid replies were examined. Table 1 displays the demographic features of the respondents' population. The majority had broad industry experience with 61.7% (66/107) reporting more than 10 years of experience in pharmaceutical production, followed by 2-5 years (18.7%, 20/107), 6-10 years (10.3%, 11/107) and less than 2 years (9.3%, 10/107).

Department Quality Control (QC) 85 79.4% Quality Assurance (QA) 8 7.5% Manufacturing / Production 7 6.5% Research & Development (R&D) 5 4.7%Other 2 1.9

%.The most represented department was Quality Control (79.4%, 85/107), followed by Quality Assurance (7.5%, 8/107), Manufacturing/Production (6.5%, 7/107), Research & Development (4.7%, 5/107) and Other (1.9%, 2/107). The preponderance of QC personnel adds technical credibility to the responses on BET practice.

Methodologies in Use for BET

The distribution of the BET methods used by the respondents is presented in figure 1. The gel-clot method (LAL) is still the most used approach with 86.9% (93/107) of respondents using it. 10.3% (11/107) use kinetic turbidimetric tests, and smaller proportions use chromogenic assays (0.9%, 1/107) and other methods (1.9%, 2/107).

Table 1: Respondent Demographics

Variable	Category	Frequency (n)	Percentage (%)
Experience Level	>10 years	66	61.7%
	6-10 years	11	10.3%
	2-5 years	20	18.7%
	<2 years	10	9.3%
Department	Quality Control (QC)	85	79.4%
	Quality Assurance (QA)	8	7.5%
	Manufacturing/Production	7	6.5%
	Research & Development(R&D)	5	4.7%
	Other	2	1.9%

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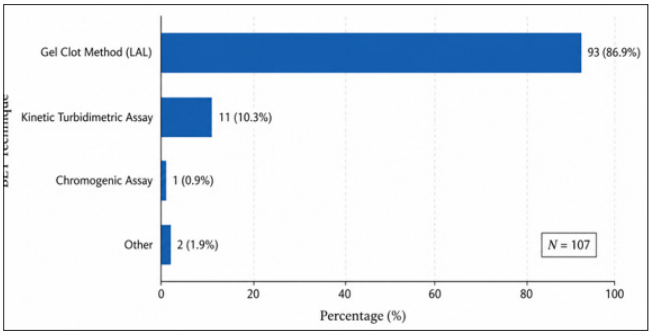

Figure 1.Existing BET Techniques Used Technique Frequency (n) Percent (%)

Rationale for Selection of Gel-Clot Technique

The main reasons for ongoing use among gel-clot users were cost-effectiveness, no demand for automation, general regulatory acceptability and history of satisfying product release standards. A small percentage (5.7%) identified other factors, such as ease of training and simplicity.

Perceptions of Alternative Approaches

Figure 2, gives perceptions on the efficacy of different approaches, including recombinant Factor C (rFC), Monocyte Activation Test (MAT), and biosensors. Most (71.0%, 76/107) thought these alternatives were beneficial, 24.3% (26/107) were unsure and 4.7% (5/107) did not think they were effective.

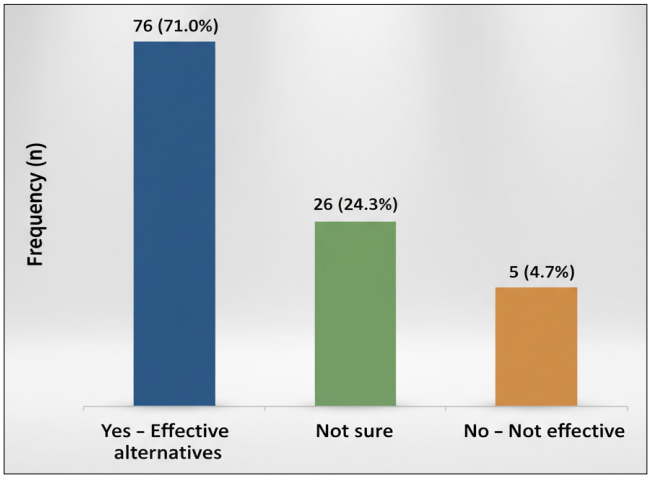

Figure 2.Perceptions on Alternative Approaches (rFC/ MAT/Biosensors)

Alternate Validation Requirements

Although the alternative procedures were perceived favourably, there was strong consensus (94.4%, 101/107) that more validation data and regulatory harmonization are needed before routine use (Figure 3). Only 5.6% (6/107) thought current validation was enough.

Significance of Endotoxin Control Parameters

Almost all of the respondents recognized the need of endotoxin control (Figure 4). Endotoxin control in manufacturing was judged critical by 99.1% (106/107) and BET for product release and patient safety was equally recommended by 99.1% (106/107). Critical Process Parameters (CPP) and Critical Quality Attributes (CQA) were regarded relevant by 96.3% (103/107) and endotoxin limit specifications by 98.1% (105/107).

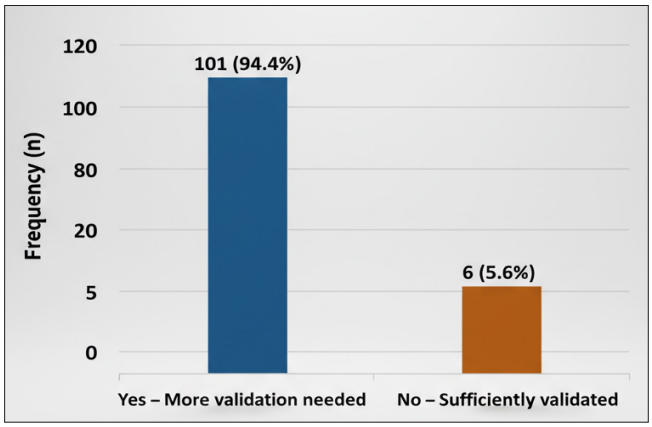

Figure 3.Need for Further Validation of Alternatives

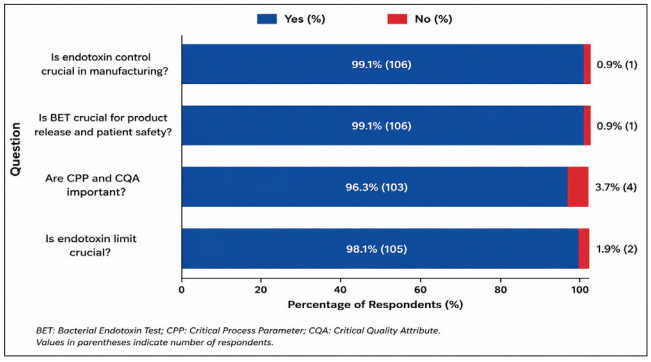

Figure 4.Significance of Endotoxin Control Parameter

Risk Assessment and Recovery Criteria

The recovery requirements of 50-200% (2-fold variation) in BET validation, together with no inhibitory dilution and inhibition/enhancement tests, were regarded critical by 91.6% (98/107) of the respondents (Figure 5). Risk mitigation of endotoxin was supported by 96.3% (103/107) for formal risk assessment methodologies such as Failure Modes and Effects Analysis (FMEA) and Process Hazard Analysis (PHA) (Figure 6).

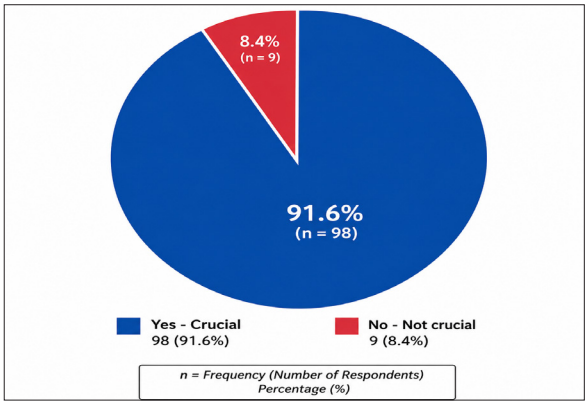

Figure 5.Importance of Recovery Criteria (50-200%)

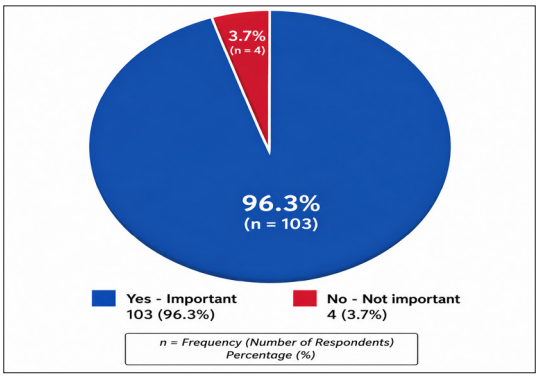

Figure 6.Tools for Risk Assessment (FMEA/PHA)

Challenges in Gel-Clot Method Implementation

Respondents reported a number of issues with the gel-clot procedure (multiple responses allowed). Figure 7 shows the reported issues in terms of frequency and proportion. The most common reported challenges were subjectivity of result interpretation (visual identification of gel formation) (32.7%, 35/107), interference from sample qualities including pH, viscosity and excipients (25.2%, 27/107) and limited quantitative data (18.7%, 20/107). Compared to quantitative approaches, sensitivity was shown to be limited by 13.1% (14/107), dependence on horseshoe crab-derived LAL (ethical/supply problems) by 11.2% (12/107), sensitivity to temperature and climate by 7.5% (8/107) and time consuming/labour intensive nature by 6.5% (7/107).

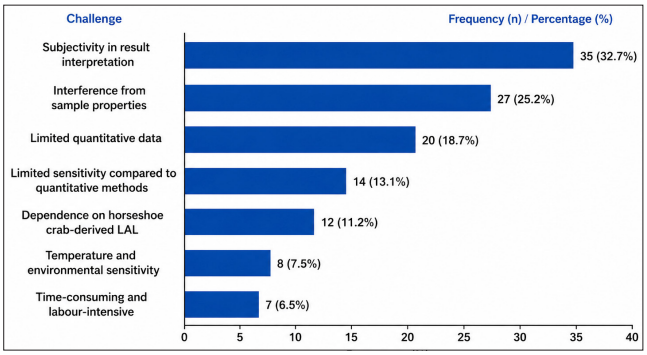

Figure 7.Challenges in Gel Clot Method (Multiple Response)

Perception of AI Based Techniques

Figure 8, illustrates the overall perceptions of AI contribution to endotoxin control. Almost half of the respondents (48.6%, 52/107) stated that AI may have a major positive impact on endotoxin control, whereas 25.2% (27/107) said the effect was limited or untested. 14.0% (15/107) reported uncertainty and 12.1% (13/107) did not believe AI well suited for endotoxin control. 73.8% of them had a positive or cautiously optimistic attitude for AI integration.

Figure 9, preference for the certain AI approaches (multiple response allowed).The most popular was AI-integrated biosensors for real time detection (26.2 %,28/107) follows by Machine learning (ML) for pattern recognition or result classification (25.2 %,27/107),computer vision for automatic visual inspections(21.5%,23/107),Deep Learning for complex assay data analysis (11.2 %,12/107),Predictive Analysis for contamination risk assessment)5.6 %,6/107),Reinforcement learning for process optimization (4.7 %,5/107) and Natural language processing (NLP) for regulatory compliance analysis (0.9 %,1/107).

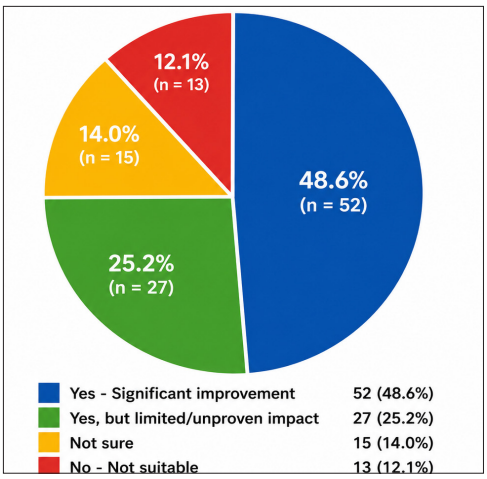

Figure 8.General perceptions on AI contribution

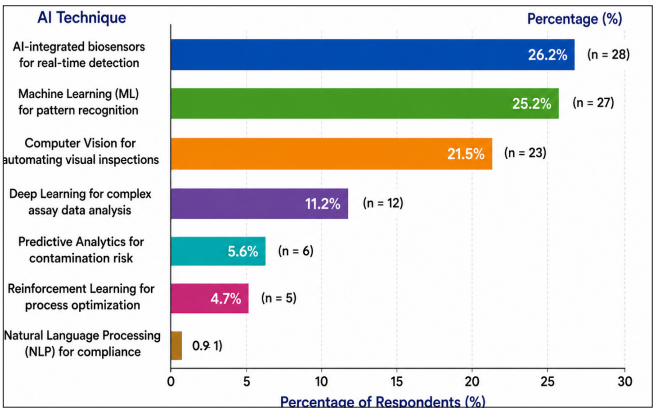

Figure 9.Preferred AI Techniques for BET

Batch Validation Practices

Figure 10, shows methods on the number of batches routinely evaluated to analyse variation in BET results between different batch numbers. 82.2% (88/107) validated three batches; 14.0% (15/107) and 2.8% (3/107) validated one and two batches, respectively.

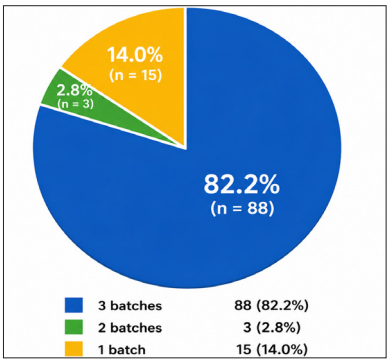

Figure 10.Batch Validation Practices

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Inferential Statistics: Chi-Square Analysis

Association: Experience Level × Method Choice

Cross-tabulation of experience level against method choice (Table 2) revealed that gel-clot method predominated across all experience categories. Chi-square analysis demonstrated no statistically significant association between experience level and method choice ($\chi^2 = 6.24$, df = 6, p = 0.397), indicating consistent methodological preferences regardless of experience.

Table 2: Cross-tabulation - Experience Level × Method Choice

Experience	Gel Clot	Kinetic Turbidimetric	Other	Total
>10 years	57	7	2	66
6-10 years	8	3	0	11
2-5 years	18	1	1	20
<2 years	10	0	0	10

Association: Experience × Perception of AI

Chi-square analysis found no significant association between experience level and impression of AI contribution ($\chi2 = 8.91$, df = 9, p = 0.446), showing AI perceptions were constant across experience cohorts.

Association: Department × Method Choice

Likewise, there was no significant correlation between department and method choice ($\chi2 = 12.45$, df = 12, p = 0.411). Quality Control staff were represented in all technique categories, indicating their primary responsibility for the BET execution.

Correlation Analysis

Table 3 Correlation coefficients of important factors. There was a considerable positive association between AI perception and alternatives acceptance ($r = 0.42$, $p < 0.001$), indicating that individuals who have a positive response towards alternative ways also tend to hold a positive response towards AI. Awareness of validation was associated with adoption of risk assessment ($r = 0.38$, $p < 0.001$). People who were aware of validation importance also supported formal risk management. The intensity of the difficulty was positively and significantly linked with the demand for AI ($r = 0.31$, p = 0.001), indicating that respondents with greater operational difficulties were more interested in AI-based solutions. We found no significant relationship between experience and method originality ($r = -0.08$, p = 0.412).

Table 3: Correlation Analysis

Variables	Correlation (r)	p-value	Significance
Experience × Method innovation	-0.08	0.412	NS
AI perception × Alternatives acceptance	0.42	<0.001	Significant
Validation awareness × Risk assessment	0.38	<0.001	Significant
Challenge severity × Desire for AI	0.31	0.001	Significant

Modernization Readiness Index (MRI)

Based on the Modernization preparedness Index, a composite index based on five variables, respondents were grouped into three levels of preparedness (Table 4). Only 16.8% (18/107) were evaluated as “High Readiness” (4-5 indications). The majority (39.3%, 42/107) were in “Moderate Readiness” (3 indicators) and 43.9% (47/107) were in “Low Readiness” (1-2 indicators). The findings suggest tremendous scope for capacity building and support for technology transfer.

Table 4: Modernization Readiness Classification

Readiness Level	Score Range	n	Percentage
High Readiness	4-5 indicators	18	16.8%
Moderate Readiness	3 indicators	42	39.3%
Low Readiness	1-2 indicators	47	43.9%

Factor Analysis: Key Dimensions in BET Practices

Principal Component Analysis with varimax rotation identified three factors with eigenvalues >1.0, collectively explaining 65.6% of total variance (Table 5).

Factor 1: Regulatory & Validation Compliance (Eigenvalue = 3.42, 28.5% variance) loaded strongly on recovery criteria importance (0.81), FMEA/PHA importance (0.78), and CPP/CQA importance (0.74).This factor shows the foundational quality and regulatory framework of BET practices.

Factor 2: Technology Adoption Readiness (Eigenvalue = 2.56, variance = 21.3%) showed high loadings on AI positive perception (0.83), alternatives acceptance (0.79), and quantitative technique utilization (0.68). This factor reflects receptivity to innovation in terms of methodology and technology.

Factor 3: Operational Challenges (Eigenvalue = 1.89, 15.8% variance) based on subjectivity concerns (0.76), interference issues (0.72), and validation workload (0.65),accounting for practical obstacles faced in everyday BET deployment.

Open-ended Responses: Qualitative Findings

Thematic analysis of open-ended comments identified numerous recurrent themes (Table 6). In addition, issues about sustainability were raised, for example, one respondent said: "Alternative ways needed to skip LAL from horseshoe crab is primary problem in India. Cost factors were considered as impediments to method change: "Cost may come down in rFC once it is implemented across India," said the official. Training needs were emphasized: "Companies should invest in training of their analysts to improve practical and theoretical skill in BET. Sample-specific problems were identified: "Products with viscous, hazy and dark colour are difficult to analyse by quantitative methods." Technical observations included pH sensitivity: "Some companies prefer to check pH by pH paper but I have seen that it might vary the results.

Table 5: Factor Analysis - Rotated Component Matrix

Factor	Loading	Eigenvalue	Variance (%)
Factor 1: Regulatory & Validation Compliance		3.42	28.5%
Recovery criteria importance	0.81		
FMEA/PHA importance	0.78		
CPP/CQA importance	0.74		
Factor 2: Technology Adoption Readiness		2.56	21.3%
AI positive perception	0.83		
Alternatives acceptance	0.79		
Quantitative method usage	0.68		
Factor 3: Operational Challenges		1.89	15.8%
Subjectivity concerns	0.76		
Interference issues	0.72		
Validation burden	0.65		

Table 6: Key Qualitative Insights

Theme	Representative Quote
Sustainability Concern	"Alternative methods needed to skip LAL from horseshoe crab is basic problem India."
Method Transition Barriers	"Cost may decrease in rFC once implementation is done all over India."
Training Need	"Companies should invest in training of their analysts to improve practical and theoretical skill in BET."
Sample Challenges	"Products with viscous, hazy, and dark colour are difficult to analyse by quantitative methods."
pH Sensitivity	"Some companies prefer to check pH by pH paper but I have seen that it might vary the results."

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Discussion

This multicentre survey of 107 pharmaceutical specialists provides the most thorough characterization to date of Bacterial Endotoxin Testing practices, risk-based views and modernization readiness across the Indian injectable manufacturing sector. The findings reveal a landscape of methodological consistency, but also a conservative openness to innovation with major implications for regulatory policy, industrial capability development and future research directions.

The Meaning of Gel-Clot Dominance

The predominant use of the gel-clot method (86.9%) in the present study is in accordance with global trends previously documented in literature[6,7,19]. This sustained dominance is a result of several mutually reinforcing factors: long regulatory acceptability across all major pharmacopeia's[3-5], long validation history, relatively minimal equipment requirements, cost-effectiveness especially for smaller manufacturers, and the experiential knowledge base that quality control workforces have built up over time. The significant proportion of responses with >10 years' experience (61.7%) likely reflects this methodological continuity, as experienced analysts have built expertise with gel-clot procedures. But the continued use of the gel-clot methodology needs to be understood in the light of its well acknowledged limitations, which were supported by the respondents. The most often mentioned problem was subjectivity in endpoint interpretation (32.7%), which is consistent with proficiency testing data showing inter-analyst heterogeneity in gel-clot reading[8].

Interference from formulation components (25.2%) is a concern, especially for complex biologics and liposomal formulations where matrix effects might affect recovery[9,20]. Gel-clot results (18.7%) are qualitative or semi-quantitative in character and therefore limited in utility for trending analysis and process monitoring, compared with quantitative approaches. Ethical and sustainability issues associated with horseshoe crab harvesting are particularly relevant to the present world. This could be an increasing driver of technique transition as global knowledge of conservation imperatives and supply chain vulnerabilities increases[10], particularly for manufacturers servicing markets with high environmental, social and governance (ESG) standards.

Validation Paradox: Interest and Caution

A notable outcome of this study was the apparent contradiction between significant interest in alternative technologies (71.0% thinking rFC/MAT/biosensors effective) and the overwhelming need for further validation (94.4%). This "validation paradox" implies that the industry's desire to innovate is moderated by the knowledge of the volumes of data needed to meet regulatory requirements and assure patient safety. This discovery is consistent with the precautionary concept embedded in pharmaceutical quality assurance and underscores the high stakes associated with endotoxin contamination. The shift from the gel-clot to alternative methods such as rFC or MAT is not only a question of analytical equivalency, but also a thorough demonstration that the new method offers at least a comparable patient protection for all product kinds and matrices[11,12]. Method validation, regulatory filing updates, and the potential need for dual-testing during transition periods are a significant investment and a significant hurdle, particularly for manufacturers with diverse product portfolios[19].

Importantly, this requirement for validation is not always indicative of aversion to alternatives, but rather a practical understanding that regulatory harmonization, such as validation processes, acceptance criteria, and guidance materials, need to be in place before widespread use of alternatives. Several respondents expressed this view, saying that costs will come down and implementation would become more feasible with wider acceptance and more regulatory certainty.

Integration of Risk-based Quality Management

The near-universal acceptance of the risk-based quality management principles such as importance of CPP/CQA (96.3%), criticality of endotoxin limit (98.1%), importance of recovery criteria (91.6%), and adoption of FMEA/PHA (96.3%) indicates the successful penetration of ICH Q9 principles into the industry practice[15]. This data shows that manufacturers have adopted the notion that endotoxin control is a process design, raw material control and systematic risk assessment and not merely end product testing. Moreover, the substantial connection between validation between awareness and adoption of risk assessment ($r = 0.38$, $p < 0.001$) favours integration of these alternative quality systems. Manufacturers who understand the need for thorough validation are also likely to use formal risk assessment techniques, which indicates a mature quality culture.

AI Readiness: Hope with caution

One of the interesting indicators of digital preparedness in the sector is that 7.08 % of the despondence reported a positive attitude towards AI integration. Such candour most likely suggests an understanding that AI approaches especially computer vision to automate gel-clot readouts, machine learning for pattern recognition and AI-enhanced biosensors for real-time detection could solve a host of long-standing problems, including subjectivity, analyst variability and labour intensiveness. The industry's preference for specific AI applications highlights its priorities: AI-integrated biosensors for real-time detection (26.2%) and computer vision for automated visual inspections (21.5%) tackle the subjectivity problem, which is the most common shortcoming of current approaches. Pattern recognition using

machine learning (25.2%) may appeal to manufacturers looking to extract more value from existing data sources. However, the high proportions of respondents who are either unsure (14.0%) or skeptical (12.1%) about AI, along with the low proportion of those who score as having "high readiness" on the Modernization Readiness Index (16.8%), indicate that meaningful AI adoption will require considerable capability building, demonstration of regulatory acceptability, and demonstration of tangible benefits. The recent EMA reflection paper on AI in development of pharmaceutical products provides a first regulatory framework, but concrete guidance for AI uses in quality control testing is still restricted[21].

Capability Gaps and Modernization Readiness

The Modernization Readiness Index proposed in this study provides a fresh quantitative tool to measure industrial readiness for technological change. The fact that only 16.8% of respondents are "high readiness" and 43.9% are "low readiness" shows the significant gaps in competence that need to be addressed to move forward with modernizing methods. These shortages cover a range of areas: technical knowledge on alternative approaches, experience on quantitative techniques, knowledge on validation needs for novel methods and knowledge on AI applications. The qualitative responses confirmed this evaluation with explicit requests for more training: "Companies should invest in training of their analysts to improve practical and theoretical skill in BET [16]. Factor analysis results provide further insight into the structure of industry readiness, identifying three separate dimensions that account for 65.6% of variance. Regulatory & Validation Compliance, the largest driver, accounts for 28.5% of the variance, highlighting the importance of regulatory frameworks in determining business practices. Technology Adoption Readiness (21.3% variance) reflects attitudinal and experiential factors influencing receptivity to innovation. Operational Challenges (15.8% variation) these are the day to day pain points that may create a desire for better ways of doing things.

Comparative Viewpoints

The lack of similar multicentre surveys makes it difficult to directly compare internationally, although the trends found in the present study are consistent with the global industry debates. The ongoing use of gel-clot methodology is not specific to India but is symptomatic of a wider industrial trend whereby well-established procedures continue despite recognised flaws. Similarly, the validation paradox of significant interest in alternatives and demand for more evidence appears to be an international phenomena that is consistent with the conservative nature of pharmaceutical quality assurance. The proportion of responders with >10 years' experience is relatively high (61.7%), possibly higher than certain developed countries, which may be a reflection of India's status as a mature manufacturing powerhouse with skilled technical workforces. As the industry considers method modernization, this experienced cohort could be a great resource for competence creation and information transfer.

Strengths and limitations of the study

The study has a number of strengths including a much larger sample size (n=107) than previous studies, multicentre recruitment increasing the generalizability of the findings, use of inferential statistics including chi-square analysis and correlation testing, development of novel quantitative indices (Modernization Readiness Index), factor analytical identification of underlying dimensions, and incorporation of qualitative insights from open-ended comments. There are several restrictions to consider. First, although the sample size is much larger than in prior studies, it is rather small compared to the whole population of Indian pharmaceutical professionals. Second, sampling was not random and may over represent larger, more compliance-mature firms, which could overstate industry readiness. Third, the claimed practices of the individuals may not fully represent what actually goes on in the laboratory. Fourth, the cross-sectional design measures only one moment in time and cannot examine trends over time or the effect of future regulatory changes. Fifth, direct worldwide comparison is impeded by the lack of globally standardized surveys.

Implications for Industry Practice and Regulatory Policy

This study has a number of implications for regulatory policy and industrial behaviour. For regulators, the strong need for validation guidance (94.4%) reflects that enabling method modernization is not simply about willingness to accept alternative techniques, but requires proactive development of harmonized validation protocols, acceptance criteria, and implementation assistance. Pharmacopeia's (USP, EP, JP, IP) working collaboratively toward harmonization of criteria for rFC, MAT and other choices would significantly decrease industry uncertainty. The high proportion of respondents indicating low readiness (43.9%) and the training needs expressed by industry associations and training institutions point to opportunities for targeted capability-building programs on alternative methods, validation strategies, quantitative techniques and AI applications. Given the profile of the seasoned workforce, peer-to-peer knowledge transfer systems may be very effective. The findings imply that for individual manufacturers, modernization planning should include various dimensions simultaneously: technical study of alternative approaches, validation resource allocation, regulatory involvement, and workforce development. The positive correlation between issue severity and interest in AI ($r = 0.31$, $p = 0.001$) suggests that organizations with the most significant operational challenges may be the most eager to pursue creative solutions.

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Directions for Future Research

The results of the present study suggest several avenues for future research. A longitudinal study following the trajectory of method adoption across time would evaluate whether present receptivity to alternatives results in actual implementation as regulatory clarity evolves. We still do not know if the findings in India are generalizable to other countries, or whether they are particular to India or are a function of universal industrial dynamics. Comparative multi-country studies would be useful to assess this. Intervention studies to measure the success of training programs and capability building efforts would drive budget allocation. Studies on cost-effectiveness of classic and novel techniques in different production environments will help to build business cases. Finally, future improvements to the Modernization Readiness Index and validation against real-world adoption results will increase its utility as a forecasting tool.

Conclusion

This multicentre survey of 107 pharmaceutical experts provides thorough evidence on the practices, risk-based views and readiness for modernization in the Indian injectable manufacturing sector for Bacterial Endotoxin Testing. Results show that gel-clot approach is still the working norm (86.9%) and supported by regulatory approval, cost-effectiveness and a skilled work force (61.7% with >10 years' experience). But practitioners are aware of important constraints such as subjectivity (32.7%), interference (25.2%) and worries about sustainability (11.2%). The industry is very interested in alternative methods (71.0% find rFC/MAT/biosensors effective) and cautiously enthusiastic about AI (73.8% favourable or moderately optimistic view). But this openness is met by a significant need for greater validation (94.4%) and regulatory harmonization a "validation paradox" that matches the conservative risk calculus embedded in pharmaceutical quality assurance.

The Modernization Readiness Index indicates that only 16.8% of respondents polled are highly ready for technological transformation while 43.9% are in low readiness, showing the size of skill gaps that need to be plugged by focused intervention. Factor analysis revealed three factors accounting for 65.6% of the variance in industry practices and attitudes, namely Regulatory & Validation Compliance (28.5% variance), Technology Adoption Readiness (21.3%) and Operational Challenges (15.8%). So, the Indian injectable pharmaceutical business is in a transition phase: technically sophisticated, risk-aware, open to innovation but requiring regulatory clarity and validation frameworks enabling technique modernization. Harmonized guidance, capability building and collaborative industry regulatory initiatives to meet these requirements will enable the transition from traditional gel-clot testing to an end-to-end endotoxin-control strategy integrating science, sustainability and smart analytics to ensure product quality, patient safety and global regulatory alignment.

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Conflict of interest

The authors declare no conflict of interest.

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