



## ROOT CAUSE ANALYSIS OF INCONSISTENT BACTERIAL CULTURE MEDIA QUALITY USING THE FISHBONE (ISHIKAWA) DIAGRAM: A CASE STUDY

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### ABSTRACT

Inconsistent bacterial culture media quality can compromise diagnostic accuracy and laboratory reliability in clinical microbiology. This study aimed to identify the root causes of bacterial culture media quality inconsistency using a Fishbone (Ishikawa) Diagram approach. A descriptive qualitative case study was conducted at the Microbiology and Biotechnology Laboratory, Faculty of Health Sciences, Universitas Anwar Medika, Indonesia, based on three laboratory quality complaints involving poor bacterial growth, contamination of prepared media, and inconsistent antimicrobial susceptibility test results. Data were collected through direct observation of media preparation processes, review of standard operating procedures, equipment calibration records, and structured discussions with laboratory personnel. Root cause analysis was performed using the 6M framework, including man, method, material, machine, measurement, and environment. The analysis revealed that method-related and measurement-related factors were the most dominant contributors, particularly non-standardized media preparation procedures, absence of routine growth promotion and sterility testing, and instability of sterilization and incubation equipment. Human-related and environmental factors acted as secondary contributors. These findings indicate that culture media quality inconsistency is primarily driven by systemic process weaknesses rather than isolated individual errors. Implementation of standardized procedures, strengthened quality control systems, and regular equipment monitoring is essential to improve laboratory performance and diagnostic reliability.

Keywords: Bacterial; Culture media; Fishbone analysis; Root cause

### INTRODUCTION

Bacterial culture media play a fundamental role in clinical microbiology laboratories by supporting the growth, isolation, and identification of pathogenic microorganisms. The quality and consistency of culture media directly influence diagnostic accuracy, antimicrobial susceptibility testing, and patient management outcomes (Araújo Neto et al., 2025). Variations in media composition, sterility, pH, moisture content, and nutrient stability may lead to false-negative results, contamination, or inconsistent microbial growth patterns, potentially

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compromising clinical decision-making (Garcia et al., 2018). In clinical laboratory practice, inconsistent media quality has been reported as a significant contributor to laboratory errors, particularly during pre-analytical and analytical phases (Allard et al., 2018). Improper preparation procedures, inadequate sterilization, equipment malfunction, and insufficient quality control measures may collectively affect media performance (ISO, 2017). Furthermore, human factors such as insufficient training, high workload, and procedural non-compliance also increase the risk of variability in laboratory outputs (Antonios et al., 2021a).

Root cause analysis (RCA) has been widely adopted in healthcare quality management as a systematic approach to identify underlying factors responsible for process failures and quality deviations (Martin-Delgado et al., 2020). Among RCA tools, the Fishbone (Ishikawa) Diagram is recognized as an effective method for categorizing contributing factors into structured domains such as manpower, methods, materials, machinery, measurement, and environment (Kumah et al., 2024). This approach enables laboratories to visualize complex cause-effect relationships and develop targeted corrective actions.

Previous studies have demonstrated the effectiveness of fishbone analysis in identifying systemic weaknesses in healthcare services, laboratory workflows, and clinical processes (Aminmansour et al., 2025) (Mushtaq et al., 2018). However, limited studies specifically focus on bacterial culture media quality inconsistency using complaint-based case analysis. Complaints reported by clinicians and laboratory users represent valuable indicators of quality gaps that are often not captured through routine internal audits (Antonios et al., 2021b). Furthermore, studies applying complaint-based root cause analysis specifically to bacterial culture media quality within microbiology laboratory quality assurance systems remain limited, particularly in the context of structured Fishbone (6M) analysis aligned with laboratory quality management standards such as ISO 15189. Accordingly, this study aimed to identify the root causes of inconsistent bacterial culture media quality using a Fishbone (Ishikawa) Diagram based on three laboratory quality complaints related to poor bacterial growth, media contamination, and inconsistent antimicrobial susceptibility testing results.

## **METHODS**

### **Study Design**

This study applied a descriptive qualitative case study design using a root cause analysis (RCA) framework supported by the Fishbone (Ishikawa) Diagram. The RCA approach was selected because it enables systematic identification of underlying process failures and contributing factors in healthcare and laboratory quality improvement settings (Martin-Delgado et al., 2020) (Liepelt et al., 2023). The fishbone diagram method was used to visualize cause-effect relationships and categorize potential root causes in a structured manner (Kumah et al., 2024). Ethical approval was obtained from the Institutional Review Board of Universitas Anwar Medika, Indonesia. This study used laboratory quality complaint data without involving patient identifiers, and all data were analyzed anonymously.



## Study Setting

The study was conducted at the Microbiology and Biotechnology Laboratory, Faculty of Health Sciences, Universitas Anwar Medika, Indonesia. This laboratory routinely performs bacterial culture, isolation, identification, and antimicrobial susceptibility testing for educational and research purposes. Culture media are prepared both from commercially available dehydrated media and through in-house preparation procedures following institutional standard operating protocols (Indrawati et al., 2023).

## Case Selection

Three laboratory quality complaints related to bacterial culture media inconsistency were purposively selected as study cases. The selected complaints included: (1) poor bacterial growth on prepared culture media, (2) contamination of culture plates during incubation, and (3) inconsistent antimicrobial susceptibility test results associated with Mueller-Hinton agar performance. Complaint-based case selection was applied because reported quality incidents and user feedback represent important indicators of latent system weaknesses that are often not detected through routine internal audits (Mukagendaneza et al., 2019).

## Data Collection

Data were collected using multiple sources to ensure triangulation and enhance the validity of findings. These sources included direct observation of culture media preparation procedures, review of laboratory standard operating procedures (SOPs), examination of equipment calibration and maintenance records, and semi-structured discussions with laboratory personnel responsible for media preparation and quality control activities. The triangulation approach was applied in accordance with laboratory quality management recommendations to reduce bias and improve data reliability (Roy & Das, 2019).

## Fishbone Analysis Procedure

The fishbone analysis was conducted using a structured multi-step process. First, each complaint was clearly defined to establish the primary quality deviation. Second, potential contributing factors were identified through guided brainstorming sessions involving laboratory staff. Third, identified causes were classified using the 6M framework, including manpower, methods, materials, machinery, measurement, and environment (Sancho et al., 2025) (Leal et al., 2021). This classification framework is widely applied in healthcare quality improvement studies to systematically organize root causes (Roy & Das, 2019). Fishbone diagrams were then constructed for each complaint to visualize causal relationships and identify dominant contributing factors.

## Data Analysis

Qualitative data were analyzed by grouping identified causes into thematic categories based on the 6M domains. Recurrent factors across the three complaints were compared and ranked according to frequency and operational impact on laboratory performance. This approach enabled identification of key root causes and supported the formulation of targeted corrective and preventive actions. Similar analytical strategies have been used in previous healthcare quality improvement and incident analysis studies (Mushtaq et al., 2018).

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## RESULT AND DISCUSSION

The fishbone analysis revealed that inconsistent bacterial culture media quality in the Microbiology and Biotechnology Laboratory of Universitas Anwar Medika was influenced by multiple interrelated technical and operational factors. Across the three analyzed complaints, method-related and measurement-related domains were identified as the most dominant contributors, followed by machine performance and human-related factors (Antonios et al., 2021a). These findings indicate that laboratory quality issues were primarily driven by systemic process weaknesses rather than isolated individual errors, which is consistent with previous reports on healthcare quality failures (Martin-Delgado et al., 2020).

The synthesis of root cause analysis identified three dominant contributing domains affecting bacterial culture media quality, namely method, measurement, and machine factors. Within the method domain, the primary root cause was the absence of standardized operating procedures (SOPs) for media preparation, which potentially resulted in procedural variability and inconsistent media performance. In the measurement domain, the lack of routine growth performance testing was identified as a critical factor limiting early detection of media quality deviations prior to laboratory use. Meanwhile, within the machine domain, temperature instability during media preparation and storage processes was recognized as a major contributing factor that may affect media sterility and bacterial growth consistency. These findings highlight that procedural standardization, systematic performance verification, and equipment temperature control represent key priority areas for improving bacterial culture media reliability in microbiology laboratory practice.

The first complaint (Figure 1) involving poor bacterial growth on culture media, the root cause analysis demonstrated that non-standardized media preparation procedures, prolonged cooling time prior to agar pouring, and inadequate storage conditions of dehydrated media significantly affected microbial growth performance. Equipment-related issues, particularly unstable autoclave sterilization temperatures, further compromised media quality (Beleza & Almeida, 2021). Additionally, the absence of routine growth promotion testing prevented early detection of suboptimal media performance. Similar observations have been reported in clinical microbiology laboratories, where deviations in preparation protocols and insufficient quality verification procedures were shown to directly impact culture reliability (Garcia et al., 2018).

The second complaint (Figure 2) related to contamination of prepared culture media highlighted the role of aseptic practice deficiencies and environmental control problems. Inadequate aseptic techniques during plate pouring, prolonged exposure of open plates, and lack of laminar airflow cabinet validation were identified as key contributing factors. Environmental conditions, including high humidity levels and insufficient workspace sanitation, further increased the risk of microbial contamination. These findings align with World Health Organization laboratory biosafety guidelines, which emphasize the importance of controlled environments and validated airflow systems to minimize contamination risks (WHO, 2016). Previous studies have also reported that contamination events are frequently associated with human procedural errors interacting with environmental factors (Mushtaq et al., 2018).



The third complaint (Figure 3) concerning inconsistent antimicrobial susceptibility test results revealed that variability in agar depth, non-standardized pH levels of Mueller-Hinton agar, and inconsistent incubation conditions were the primary contributing factors. Temperature fluctuations in incubators and the absence of routine media performance verification further exacerbated test result variability. These findings are consistent with international antimicrobial susceptibility testing standards, which highlight the critical role of media thickness, composition, and incubation stability in ensuring reproducible and accurate results (Bourbeau & Ledebauer, 2013)(Leber, 2016).

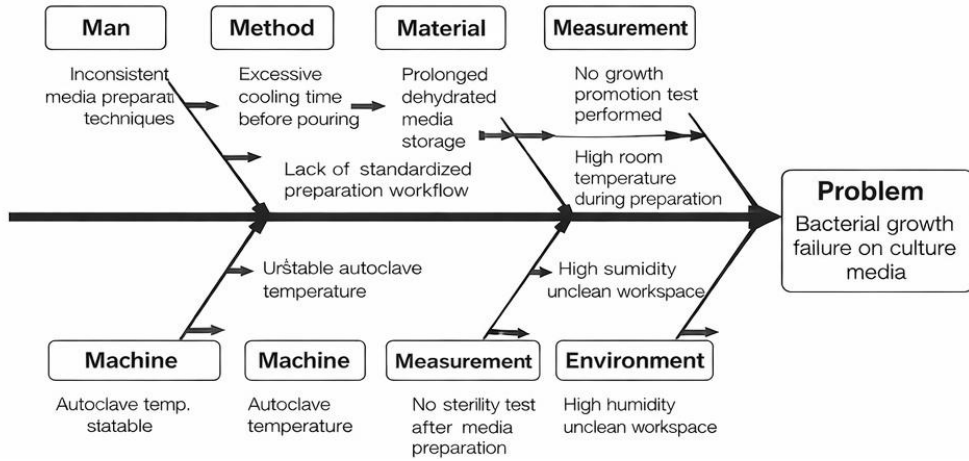
Comparative analysis across the three complaints demonstrated that procedural standardization gaps and insufficient quality control systems represented the most recurrent root causes. Method-related issues were present in all cases, while measurement-related deficiencies were identified in two out of three complaints. Equipment instability and human factor limitations were also frequently observed as secondary contributors. This pattern confirms previous findings that laboratory quality problems typically emerge from interactions between procedural weaknesses, monitoring system failures, and infrastructure limitations (Iregbu et al., 2019).

This study has several limitations. First, the analysis was based on three laboratory quality complaints from a single microbiology laboratory setting, which may limit the generalizability of the findings. Second, the root cause identification relied primarily on qualitative Fishbone-based root cause analysis without quantitative verification of each contributing factor. Third, the investigation focused only on selected dominant domains identified during the complaint-based evaluation, while other potential contributors within the laboratory quality management system may also influence bacterial culture media performance. Future studies involving larger datasets and multicenter laboratory settings are recommended to strengthen the robustness of the findings and support broader implementation of complaint-based root cause analysis in microbiology laboratory quality improvement programs.

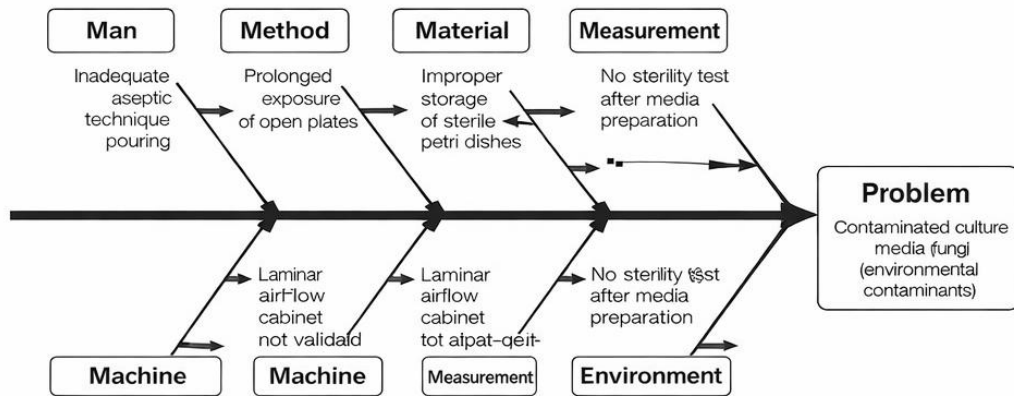
The fishbone-based root cause analysis effectively visualized the complex cause–effect relationships underlying bacterial culture media quality inconsistency and provided a structured framework for identifying dominant contributing factors. The results emphasize the importance of strengthening laboratory standard operating procedures, implementing routine quality verification systems, and improving equipment performance monitoring to enhance diagnostic reliability and laboratory service quality.

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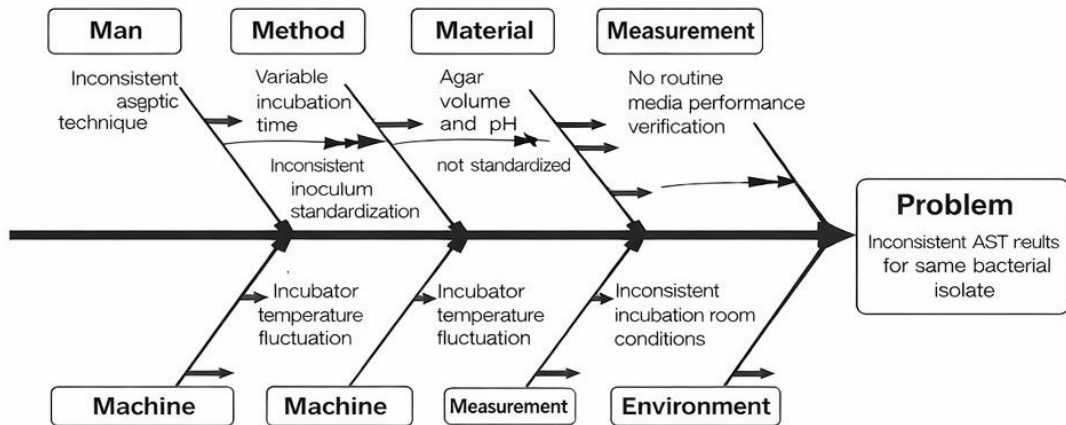
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**Figure 1.** Fishbone (Ishikawa) diagram illustrating root causes of poor bacterial growth on culture media in the Microbiology and Biotechnology Laboratory



**Figure 2.** Fishbone (Ishikawa) diagram showing contributing factors associated with contamination of prepared culture media.



**Figure 3** Fishbone (Ishikawa) diagram presenting root causes of inconsistent antimicrobial susceptibility test results related to culture media performance.



This study demonstrated that inconsistent bacterial culture media quality was predominantly influenced by procedural non-standardization, insufficient quality control mechanisms, and equipment performance variability. These findings reinforce the widely accepted view that laboratory errors are largely systemic rather than solely attributable to individual human mistakes (Srinivasaragavan et al., 2024). Across the three analyzed complaints, method-related and measurement-related factors emerged as the most recurrent root causes, highlighting weaknesses in standard operating procedures (SOPs) and internal quality assurance systems.

The identification of method-related issues as dominant contributors aligns with previous studies in clinical microbiology laboratories, which reported that minor deviations in media preparation protocols—such as cooling time, agar depth, and incubation conditions—can significantly affect bacterial growth and test reproducibility (Garcia et al., 2018). Similarly, a study by (Drancourt et al., 2016) emphasized that analytical-phase variability, including culture media preparation inconsistencies, remains a major source of diagnostic error despite advances in laboratory automation.

Measurement-related deficiencies, particularly the absence of routine growth promotion testing and sterility verification, were also prominent in this study. According to ISO 17025 and WHO laboratory quality management guidelines, verification of culture media performance is a critical requirement to ensure reliability and reproducibility of microbiological results (ISO, 2017; WHO, 2016). Failure to implement such controls increases the risk of false-negative cultures and undetected contamination, as observed in the first and second complaints. Comparable findings were reported by Westgard and Darcy (2019), who highlighted that inadequate quality indicators in microbiology laboratories often lead to delayed error detection.

Human-related factors, including inconsistent aseptic technique and limited competency reinforcement, contributed notably to contamination-related complaints. These findings are consistent with those of Mushtaq et al. (2018), who reported that human factors frequently interact with system weaknesses to exacerbate laboratory incidents. Moreover, a recent qualitative study by (Liepelt et al., 2023) found that staff workload and insufficient procedural reinforcement significantly increase the likelihood of biosafety and contamination events in laboratory settings.

Equipment-related issues, particularly autoclave and incubator temperature instability, were identified as secondary yet critical contributors. Previous research has shown that improper sterilization parameters and fluctuating incubation temperatures can compromise media integrity and antimicrobial susceptibility testing outcomes (Miller & Tang, 2009)(Shakir et al., 2023). These findings support the recommendation that preventive maintenance and continuous monitoring of laboratory equipment are essential components of quality assurance systems.

Environmental factors, such as high humidity and inadequate workspace sanitation, further contributed to contamination risks. WHO (2016) and CDC laboratory biosafety guidance emphasize that environmental control is an integral part of microbiology laboratory quality management. Studies conducted within the last decade have consistently shown that environmental monitoring and airflow validation are associated with reduced contamination rates (Wang et al., 2022).

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The use of the Fishbone (Ishikawa) Diagram in this study proved effective in visualizing complex cause–effect relationships and identifying dominant root causes across multiple complaints. Similar applications of fishbone-based root cause analysis in healthcare and laboratory settings have demonstrated its value in guiding targeted quality improvement interventions (Kumah et al., 2024)(Prada et al., 2019). Complaint-based analysis, as applied in this study, also aligns with evidence suggesting that user-reported incidents provide critical insights into latent system failures not captured by routine audits (Reader et al., 2014). This study corroborate existing evidence from the past decade indicating that strengthening procedural standardization, quality control systems, equipment maintenance, and staff competency development is essential for improving bacterial culture media quality and laboratory diagnostic reliability.

## CONCLUSION

This research demonstrates that inconsistent bacterial culture media quality is primarily driven by procedural non-standardization, insufficient quality control systems, and equipment performance variability. The Fishbone (Ishikawa) Diagram proved to be an effective root cause analysis tool for identifying interconnected laboratory process failures. Implementation of structured quality improvement strategies, including SOP standardization, routine media verification, equipment calibration, and staff training, is essential to enhance laboratory reliability and diagnostic accuracy. The findings provide practical guidance for microbiology laboratories aiming to strengthen internal quality assurance systems.

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