(Methodological Critique of "A Comparison of Laser Light-Scattering and Analytical Profile Index Systems for Foodborne Bacteria Identification")

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Dear Editor,

The authors present a compelling comparison between the laser light-scattering (LLS) method and conventional API systems (API 20E and ID 32E) for the identification of five clinically important foodborne bacterial species [1]. Their demonstration of 100% identification accuracy using LLS, alongside its advantages in speed, cost-effectiveness, and non-destructive analysis, represents a significant contribution to food safety diagnostics. Nevertheless, several methodological and translational aspects warrant further discussion.

While the reported 100% identification accuracy is impressive, the study did not explicitly describe how the scatter image "fingerprints" were interpreted or whether any standardized reference library was used for comparison. The authors mention that ideal colonies were selected based on diameter, circularity, and isolation, and that scatter patterns were visually distinguished by the size of the central wide star polygon (e.g., one-fourth of the image for *E. coli* versus one-third for *S. enterica*). However, no quantitative image analysis or machine learning algorithm was employed. Previous studies using optical forward-scattering have successfully applied pattern recognition and support vector machines to automate classification and reduce observer bias [2,3]. Future iterations of this technology would benefit from integrating computational image analysis to enhance reproducibility and scalability.

The authors appropriately note that colony age, culture media, growth temperature, and oxygen concentration can affect scattering patterns. However, the study did not systematically evaluate the robustness of LLS fingerprints across varying culture conditions. For instance, it remains unclear whether an *E. coli* colony grown on a different brand of agar or at a slightly different temperature would produce a discernibly altered scatter image that could lead to misclassification. A sensitivity analysis examining the impact of minor variations in incubation time (e.g., colonies of 1.0 mm vs. 1.5 mm diameter) or medium composition would strengthen confidence in the method's practical applicability, especially in routine food microbiology laboratories where standardization may be imperfect [4].

The authors highlight that one advantage of LLS over API systems is safety, as LLS does not require opening plates containing viable pathogens. While this is true, the initial isolation and

culture steps still involve handling live bacteria. Therefore, the safety benefit is primarily in the identification phase. Laboratories without adequate biosafety level 2 (BSL-2) facilities would still need to handle food samples containing potential pathogens during enrichment and plating. Thus, the LLS method complements rather than replaces traditional microbiological safety precautions.

The study focuses exclusively on five Enterobacterales species. However, foodborne outbreaks are frequently caused by other genera such as *Campylobacter*, *Listeria*, and *Staphylococcus*. The authors reference previous work by Azizieh et al. (2014) showing that *Staphylococcus* species produce concentric regular circles rather than star polygons. Expanding the LLS reference library to include a broader range of foodborne pathogens, including Gram-positive organisms and emerging pathogens, would greatly enhance the utility of this technology for routine food safety surveillance.

The authors do not provide an economic analysis comparing LLS to API systems. While they assert that LLS is cost-effective, API 20E kits are commercially available and require no specialized equipment beyond an incubator and a reader. In contrast, LLS requires a laser source, CCD camera, and image processing software, which may represent a significant capital investment for smaller laboratories. A detailed cost-benefit analysis, including equipment maintenance and operator training, would help stakeholders make informed decisions about adopting this promising technology [5].

According to recent studies, the integration of optical scattering with automated classification systems has shown promise across multiple diagnostic platforms [2,3]. These findings align with the broader trend toward rapid, label-free bacterial identification methods that reduce turnaround time and biohazard risk. As Al-Oklah et al. have convincingly demonstrated, LLS can rapidly and accurately identify specific foodborne bacteria.

Conclusion

Al-Oklah et al. have provided compelling evidence that LLS technology is an accurate, rapid, and safe alternative to API systems for identifying foodborne bacteria. To translate this proof-of-concept into routine practice, future research should focus on quantitative image analysis, robustness testing across varying culture conditions, expansion of reference libraries, and economic evaluations. Collaborative efforts between engineers, microbiologists, and public health authorities will be essential to bring this promising technology from the laboratory to the front lines of food safety surveillance.

Declaration of patient consent

Not applicable. This study did not involve human or animal subjects.

Conflict of Interest

The author declares no conflict of interest.

References

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Core issue of the critique:

1. Overreliance on visual interpretation of scatter images without quantitative or automated analysis.
2. Lack of standardized reference library for scatter pattern comparison.
3. Insufficient validation of method robustness across different culture conditions.

Similar studies that identified comparable limitations:

1. Banada et al. (2007) noted that colony age and media composition significantly affect forward-scatter patterns and recommended algorithm-based classification.
2. Marcoux et al. (2014) reported that optical scatter identification requires strict standardization of culture parameters to avoid misclassification.
3. Hussain et al. (2020) emphasized that manual pattern interpretation introduces observer bias and advocated for machine learning integration.

Does the current lack of evidence invalidate the authors' claims?

1. No, but the claims are premature.
2. 100% accuracy is reported under highly controlled, single-laboratory conditions.
3. Without multi-condition validation and automated analysis, the generalizability and reproducibility of the method remain unproven.
4. Therefore, the article presents a proof-of-concept, not a validated diagnostic alternative.

Recommended complementary actions:

1. Develop a quantitative image analysis pipeline (e.g., support vector machine or convolutional neural network).
2. Conduct multi-condition robustness testing:
3. Different agar brands
4. Temperature variations (±2°C)
5. Colony diameter ranges (0.8–1.8 mm)
6. Incubation time series

7. Build a public reference library of scatter fingerprints for at least 20 foodborne pathogen species.
8. Perform a formal cost-benefit and time-motion analysis against API systems.
9. Validate using blinded, multi-center trials with different operators.

Conclusion:

The study by Al-Oklah et al. is an innovative proof-of-concept. However, without automated interpretation, extensive robustness testing, and external validation, its claim of being a practical alternative to API systems is not yet scientifically substantiated.