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Letter to the Editor

Critical Gaps in Analytical Validation of Nanozyme-Based Assays for Clinical Application

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Letter to the Editor

The rapid growth of nanozyme-based analytical platforms has created a lot of interest in their ability to detect pharmaceutical residues, environmental toxins, and clinically important small molecules. These systems have several advantages, including good catalytic stability, adjustable surface properties, and relatively low-cost production [1]. However, there still seems to be a noticeable gap between the promising experimental results and the level of analytical validation needed to use them in clinical laboratories on a regular basis. We write to highlight the need for further emphasis on systematic validation across the full spectrum of Analytical Validation criteria before nanozyme-based assays are considered “ready-to-use” diagnostic tools in the context of evidence-based laboratory medicine.

Accuracy, which is the most important part of any clinical assay, still does not seem to be proven well enough. Most nanozyme studies rely on spiked samples or simplified matrices rather than real patient specimens. In the absence of comparisons with certified reference methods or traceable standards, the reported recoveries may not fully reflect true analytical accuracy. The catalytic activity of nanozymes can be highly influenced by factors such as pH, ionic strength, and matrix composition [2]. As a result, accuracy established under idealized experimental conditions may not be extrapolated to complex biological fluids such as serum, urine, or whole blood.

Similarly, precision including both repeatability and intermediate precision, has not yet been sufficiently characterized. Many studies report single measurements or very limited repetitions, often without considering key sources of variability such as day-to-day, batch-to-batch, or operator-to-operator differences. It is also important to recognize that the catalytic activity of nanozymes may change over time due to factors such as surface oxidation, aggregation, or ligand desorption [3]. Without robust precision studies, the reproducibility of these assays under routine clinical workflow conditions [4] remains uncertain.

Claims of very high sensitivity and low limits of detection are often derived from optical measurements performed in purified or simplified solutions rather than clinically significant biological samples. However, endogenous chromogens, proteins, and varied metabolites present in actual biological matrices can affect signal accuracy or cause interference. Furthermore, limits of quantification, which are essential for interpreting results in a clinical context, are not adequately defined in most studies. For this reason, sensitivity data obtained under ideal conditions cannot be considered clinically valid without proper matrix-compatible validation [1, 2].

Specificity and selectivity are also significant limitations. Nanozymes often possess broad catalytic activity, leading to the oxidation of multiple substrates or interactions with structurally similar molecules [2, 3]. However, interference studies, an important component of analytical validation, are generally either absent or only superficially addressed. Potential cross-reactivity with commonly used substances such as pharmaceuticals, dietary compounds, hemoglobin, lipids, and environmental contaminants is rarely systematically evaluated. Without systematic interference testing, claims of specificity should be interpreted with caution.

In nanozyme-based assays, the reportable range and linearity are frequently not well characterized. Many studies present limited and narrow calibration curves without showing linearity across clinically relevant concentration intervals. In addition, factors such as non-linear catalytic behavior, substrate depletion, and nanozyme saturation further complicate quantitative assessment [1, 3]. However, clinical laboratory standards require clear definitions of measurement ranges, supported by regression analysis and analytically validated, rather than calibration approaches consisting of only a few points.

The robustness of the test, that is, its ability to maintain its performance against small but deliberate changes in conditions, has not been sufficiently demonstrated. Nanozyme performance is highly sensitive to many factors such as synthesis conditions,

particle size distribution, surface functionalization, and storage conditions [1, 2]. Therefore, even small alterations in temperature, buffer composition, or reaction time may lead to significant changes in catalytic response. Unless robustness studies are conducted that systematically evaluate such variables, the integration of these tests into routine laboratory practices seems difficult.

Another concerning is the limited use of method comparison studies with established gold standard techniques such as mass spectrometry (e.g. LC–MS/MS, GC–MS), or high-performance immunoassays. In analytical validation, demonstrating equivalence or superiority through statistically robust comparison with reference methods is essential [1, 5]. However, many nanozyme-based studies lack such external benchmarking, instead rely on internal calibration strategies.

Other critical validation components including carryover assessment, sample and reagent stability, quality control integration, and reference interval determination, are largely omitted. Clinical laboratories cannot adopt methods that lack defined quality control materials, have not demonstrated stability under routine storage conditions, or do not provide population-based interpretive frameworks [4].

Finally, the pre-analytical and post-analytical dimensions of validation are rarely addressed. Nanozyme assays often require manual preparation steps, unstable reagents, or non-standard detection platforms incompatible with automated analyzers. In the absence of workflow integration studies, claims regarding clinical applicability remain largely aspirational.

In summary, nanozyme-driven technologies represent an exciting and rapidly developing area of analytical chemistry. However, their current level of validation remains insufficient when assessed against the requirements of routine clinical laboratory use. Premature claims of diagnostic readiness may overstate their current capabilities. This may undemine scientific reliability and foster unrealistic clinical expectations. Therefore, we encourage the research community to adopt comprehensive analytical validation frameworks compliant with ISO 15189, CLSI guidelines, and the principles of evidence-based laboratory medicine before nanozyme-based assays are positioned as viable clinical tools.

Sincerely,

Declarations

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