Evaluation of microbial qPCR workflows using engineered Saccharomyces cerevisiae.

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Abstract:

AIMS: We describe the development and interlaboratory study of modified Saccharomyces cerevisiae as a candidate material to evaluate a full detection workflow including DNA extraction and quantitative polymerase chain reaction (qPCR).

METHODS AND RESULTS:

S. cerevisiae NE095 was prepared by stable insertion of DNA sequence External RNA Control Consortium-00095 into S. cerevisiae. Participants were asked to detect the engineered strain by qPCR for 3 different concentrations: high (quantification cycle <37), intermediate (quantification cycle 37-43), and low (quantification cycle >43).

CONCLUSIONS:

The NE095 strain was successfully detected by all participants, with the high concentration indicating a potential target concentration for a reference material.

SIGNIFICANCE AND IMPACT OF THE STUDY:

The engineered yeast has potential to support measurement assurance for the analytical process of qPCR, encompassing the workflow from DNA extraction to qPCR. This material can also support process assessment for other DNA-based detection technologies.

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