BioAgilytix

Proof-of-Concept for a Qualitative qPCR Assay for RCL Monitoring

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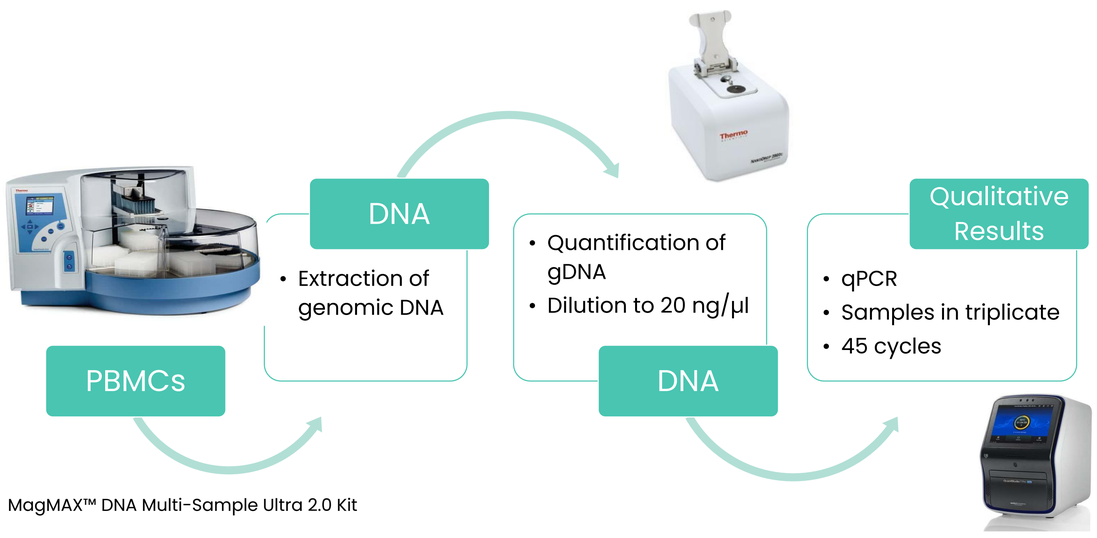
PURPOSE

qPCR assays for RCL monitoring are a rapid method to detect and usually quantify lentiviral genes, such as the envelope gene vesicular stomatitis virus G glycoprotein (VSV-G). Since FDA (Food and Drug Administration) guidance requires that all RCL positive results should be pursued by direct culture assay to obtain and characterize the infectious viral isolate[1], we concluded, that a quantitative assay is not necessarily needed to detect RCL. Therefore, we developed an alternative method for a highly sensitive, cost-efficient qualitative qPCR assay to assess the presence of the VSV-G sequence for the purpose of RCL monitoring. Our approach is based on recommendations for qualitative anti-drug-antibody assays, where a low positive control with an error rate of 1% monitors the assay at the sensitivity level.[2]

ASSAY WORKFLOW



MagMAX™ DNA Multi-Sample Ultra 2.0 Kit

Pictures from Thermo Fisher Scientific website

OBJECTIVE

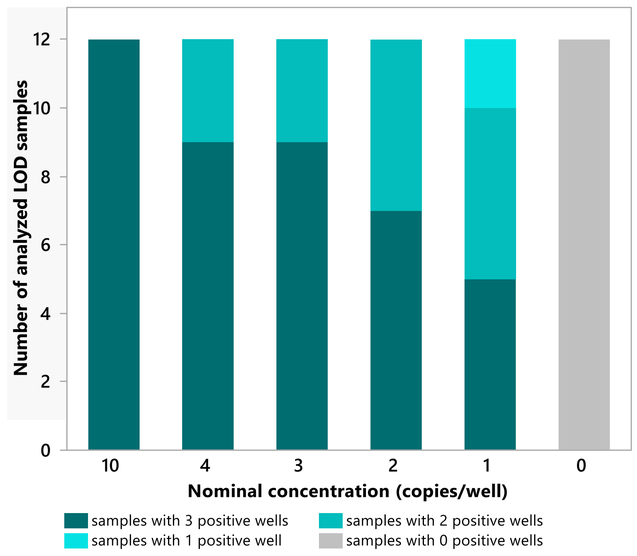
To perform the Limit of Detection (LOD) experiment and determine the LOD99 in order to set the low positive control (LPC) concentration. Then characterize the LPC by performing inter-assay precision and selectivity.

CONCLUSION

We developed a highly specific, selective, and sensitive assay with a robust performance in samples spiked at the LOD level. The negative scoring of the LPC in 1 of 30 cases demonstrates the applicability of the chosen approach for monitoring the assay at the sensitivity limit. With this qualitative method, we can reduce costs and increase sample throughput whilst maintaining reliability of the results.

RESULTS

Figure 1: Running LOD experiment.
12 samples per tier were analyzed in technical triplicates. Spiking 10 copies/well showed positive signals for VSV-G in all wells, while lower spike concentrations resulted in less positive wells in same cases. Due to high specificity of the VSV-G primers and probes and no background signals, two positive wells were assessed as overall positive result.



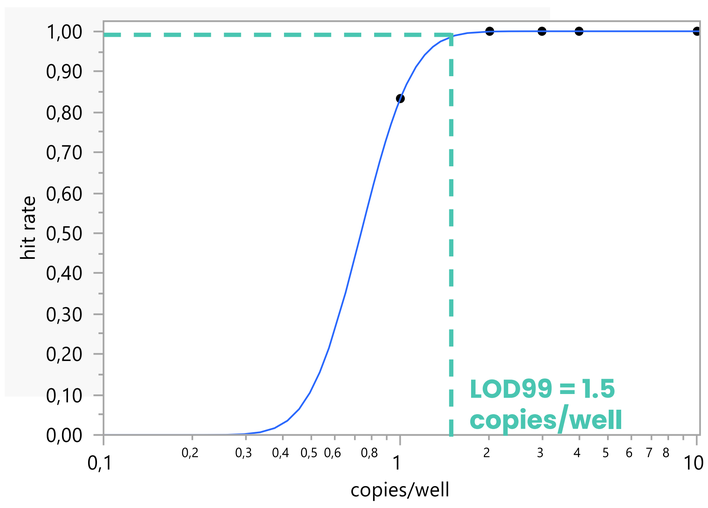
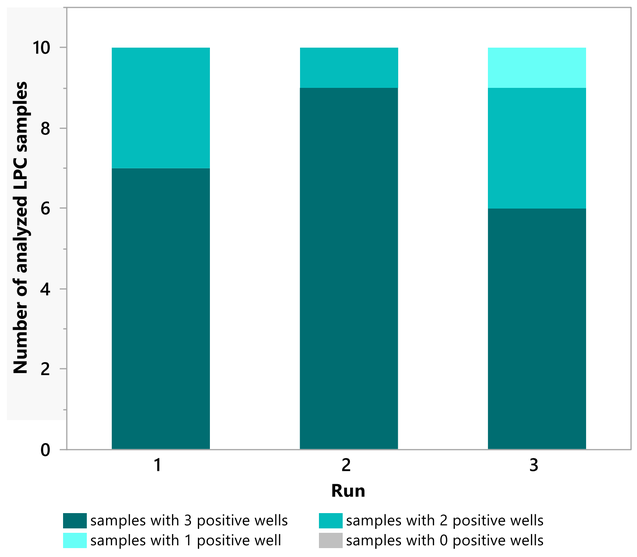


Figure 2: Determination of LOD99 to set LPC concentration.
Hit rates were determined based on the results from the LOD experiment and applied to Probit Analysis for determination of LOD99 (1.5 copies/well). LOD99 was chosen to setup a low positive control (LPC) with 1% failure rate. LPC concentration was set at 2 copies/well.

Figure 3: Testing LPC in inter-assay approach.
VSV-G was spiked in a gDNA donor pool with a final concentration of 2 copies/well. 10 samples per run were analyzed in technical triplicates. Acceptance criterion for an overall positive result was detection of VSV-G in 2 out of 3 wells for each sample. 29 samples were tested positive, while 1 sample was tested negative resulting in a failure rate of 3.3%.



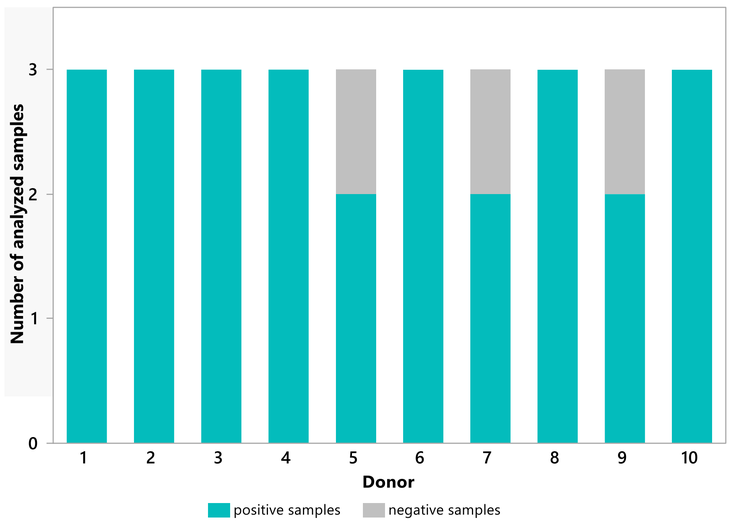


Figure 4: Testing LPC in selectivity approach.
VSV-G was spiked in gDNA from 10 individual donors at LPC concentration. Each donor was analyzed in three independent runs.
Detection of VSV-G in 2 out of 3 wells was assessed as overall positive result. 90% of all samples were tested positive.

REFERENCES

[1] FDA. Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up. Guidance for Industry. Jan 2020.
[2] Myler H, et al. Anti-drug Antibody Validation Testing and Reporting Harmonization. AAPS J (2022) 24:4. https://doi.org/10.1208/s12248-021-00649-y