Influence of Magnetic Beads on RealFast™ Assays

The influence of residual magnetic beads after DNA extraction from whole blood has already been demonstrated, and Fe^{3+} ions derived from magnetic microspheres may be present in the solution.[1] Depending on the coating of the beads, a potential interference with PCR might be the consequence. Commercial magnetic beads for nucleic acid separation can be coated with a multitude of matrices based on silica, porous glass, cellulose, agarose, polystyrene and silane.[2]

We investigated two types of magnetic beads, which are frequently used by customers of ViennaLab Diagnostics. Experiments included testing of three RealFast™ Assays (MTHFR 677C>T RealFast™ Assay, HLA-B27 RealFast™ Assay and MTHFR mpx RealFast™ Assay) with a dilution series from 0.001 to 3 µg/µl of magnetic beads in the reaction mix. Of note, semi-quantitative RealFast™ Assays were not part of this study.

In summary, the influence of magnetic beads on the RealFast™ Assay performance was minor within the concentration range of 0.001 to 0.1 µg/µl for both types of beads, but increased at higher concentrations. For safety, the lowest concentration observed for each of the magnetic beads tolerated by one of the RealFast™ Assays was used to define the overall **critical concentration** for all other assays and **was determined to be 1 µg/µl** (Figure 1).

To overcome potential problems with the interpretation of RealFast™ Assays under conditions, where a high concentration of residual magnetic beads is present, **extracted DNA should be diluted to 2 ng/µl** using PCR grade water. Subsequently, the **sample should be centrifuged for 5 min at 12,000 rpm** and the **supernatant should be used for PCR**. This results in sufficiently high amplification plateaus and clear genotyping calls (Figure 2). Cq values are expected to increase, but typically stay within the accepted range (≤ 37).

Due to the multitude of different types of magnetic beads and extraction protocols, care should be taken when extrapolating our results to all products available on the market. However, the investigated products from two manufacturers showed a relatively similar behaviour, which indicates that **at least certain types of magnetic beads can have an inhibiting effect on the performance of the RealFast™ Assays at high or excessive concentrations**.

References:

[1] Spanová A, Horák D, Soudková E, Rittich B. Magnetic hydrophilic methacrylate-based polymer microspheres designed for polymerase chain reactions applications. J Chromatogr B Analyt Technol Biomed Life Sci. 2004 Feb 5;800(1-2):27-32. doi: 10.1016/j.jchromb.2003.09.010. PMID: 14698232.

[2] Berensmeier S. Magnetic particles for the separation and purification of nucleic acids. Appl Microbiol Biotechnol. 2006 Dec;73(3):495-504. doi: 10.1007/s00253-006-0675-0. Epub 2006 Oct 25. PMID: 17063328; PMCID: PMC7080036.

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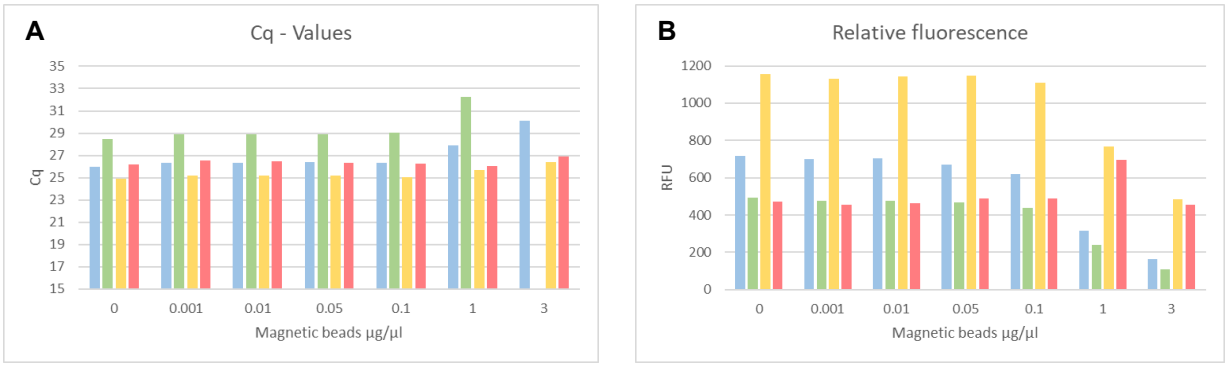

Figure 1: Dilution series of magnetic beads in a compound heterozygous DNA sample tested with the 7-165 MTHFR mpx RealFast™ Assay. The influence of magnetic beads on the assay performance is minor within the concentration range of 0.001 to 0.1 µg/µl. The critical concentration is reached at 1 µg/µl. **A**. Cq values (Cq) change significantly at a beads concentration of 1 µg/µl. At 3 µg/µl, the amplification curve in the HEX channel (green) does not cross the threshold, leading to a drop out of signal. **B**. Amplification plateaus measured in relative fluorescence units (RFU) start to decrease significantly at the critical concentration (1 µg/µl) in the FAM (blue), HEX (green) and ROX (yellow) channel. RFU values in the Cy5 (red) channel rise at 1 µg/µl to decrease again at 3 µg/µl.

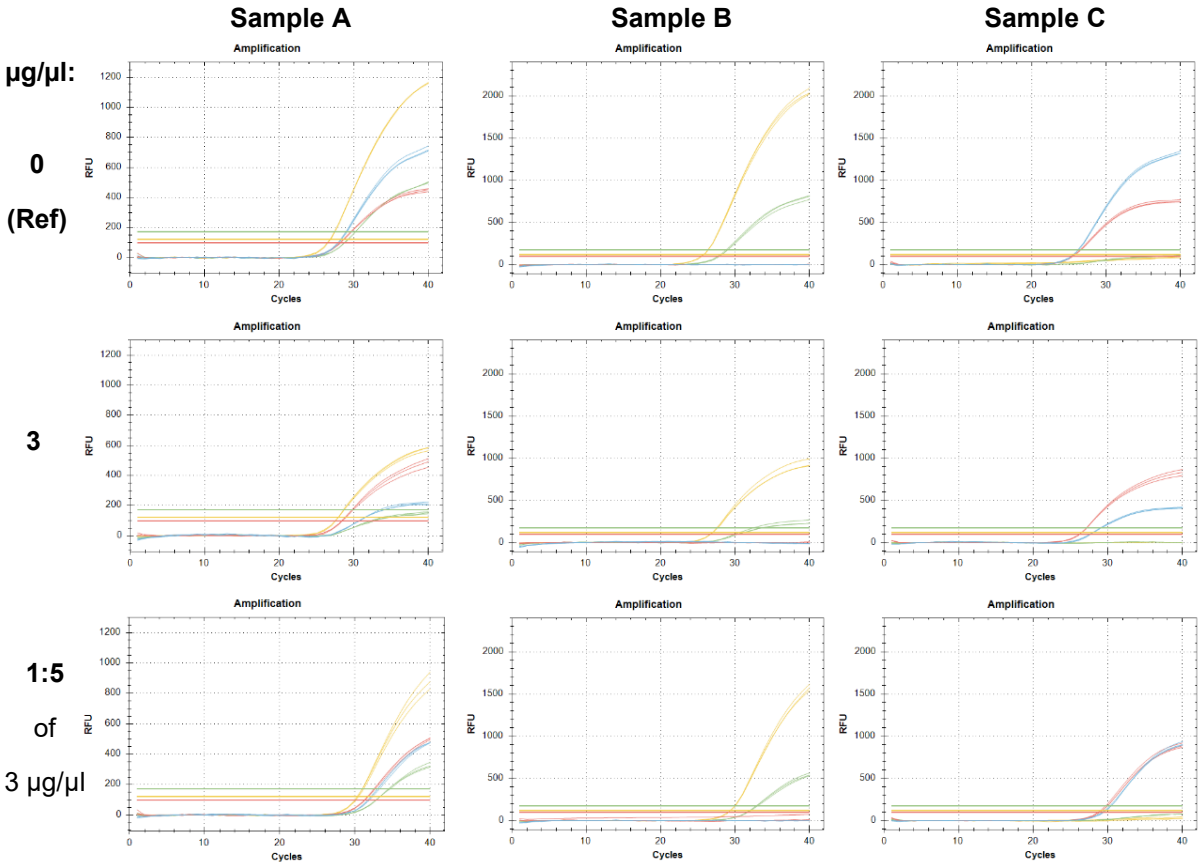

Figure 2: Effect of residual magnetic beads in extracted DNA on the performance of the 7-165 MTHFR mpx RealFast™ Assay. Shown are the amplification curves for three DNAs (5 ng/µl) in triplicates without magnetic beads in the reaction (*0; Ref*), with 3 µg/µl residual magnetic beads, as well as DNAs with 3 µg/µl of magnetic beads, which were subsequently diluted 1:5 with nuclease-free water (*1:5*). A final concentration of 3 µg/µl of beads leads to a significant decrease of amplification plateaus, hindering interpretation in case of the heterozygous *Sample A*. A dilution of these DNA samples caused an expected increase of Cq values, but also raised the amplification plateaus, thereby enabling the correct interpretation.