Instructions for MARIA® data processing

Raw Data Export into Microsoft Excel	Use Export function of the Luminex or Bio-Plex software. If data export is not an option, copy the following information for each allergen into a separate spreadsheet tab: • Standard and sample IDs • Median Fluorescent Intensities (MFI) and MFI-background for standards and samples (at all dilutions) • Standard deviation and %CV for duplicate standards • Observed (measured) concentration for standards and samples • Expected (actual) concentrations for standards • (Observed/Expected)x100 ratio for standards • Dilution factor for each sample Add one additional spreadsheet tab for data summary
Raw Data Formatting (a Macro is available to perform these tasks)	Standard Curve: a. Evaluate "(Obs/Exp)x100" results and mark all values between, and including, 85% and 115%. b. Mark all corresponding "MFI-background" values for the standards. c. The CV% for duplicate standards should be less than 15. If CVs is greater than 15% are present, pipets and pipetting technique should be checked. Sample data: d. Use conditional formatting (under Format tab in Excel) to mark all sample "MFI-background" values that do not fall within defined usable MFI range. e. Transfer markings to the corresponding sample "Observed Concentration" values. f. Repeat the formatting procedure for data in each allergen spreadsheet tab. From each allergen tab, copy the sample IDs, Observed Concentration, and sample dilution and paste into the Summary sheet.
Results Selection	• Use only values based on MFI within the usable MFI range of the standard curve, as defined above. • If two or more values fall within usable MFI range: 1. Use geometric mean of results, if results are within 30%CV. 2. If calculated concentration results increase significantly with increasing dilution, two different scenarios may apply and are resolved by checking the MFI development of the sample dilutions: a. A low MFI that does not decrease significantly with increasing dilution indicates a matrix effect. This leads to a mathematical amplification of a low background signal during calculation of allergen concentration x dilution. This matrix effect is addressed by selecting the result based on the lowest dilution in the assay, i.e. without the artificial amplification. The matrix effect is most likely encountered in certain Der f 1 and Bla g 2 samples. b. A high MFI that does not decrease significantly during the first two dilution steps indicates that the allergen concentration of the sample is high enough to saturate the assay. Select results only after the MFI decreases as expected with increasing dilution. This saturation effect is most likely encountered for samples very high in Fel d 1 and Can f 1.
Defining Lower Limit of Detection (LLOD)	Define the lowest usable point of the standard curve for each allergen and multiply the expected allergen concentration of this point by the lowest dilution factor used in the assay.