

User's Guide on Default Settings for VSpecimen, VPipette, and Fold

- Refer to Maecker et al. (2020) *Journal of Immunology* 204: 3425-3433.
- In Maecker et al. (2020), "Assumption 1" in the subsection on "Theory for estimating technical variance" describes the relationship between VSpecimen, VPipette, and Fold.
- Possible technical replicates per specimen = $(V_{\text{Specimen}} \times \text{Fold}) / V_{\text{Pipette}}$.
- Note that VSpecimen is the specimen volume that is actually diluted.
- Typical settings for each of the three parameters are as follows:
 - 1) Fold dilution is included in the specimen ID: DX3, DX10, DX100, DX40K
 - 2) VSpecimen: 5 μL to 100 μL

Dilution	VSpecimen
$\times 3$	50 to 75 μL
$\times 100$	5 to 10 μL
$\times 400$	5 to 10 μL
$\times 40,000$	5 to 20 μL

3) VPipette: 25 μL

- Important: Different kits should be run separately through the utility. Even if default settings are the same for different kits, each kit has unique NC MFI data that are an essential requirement.