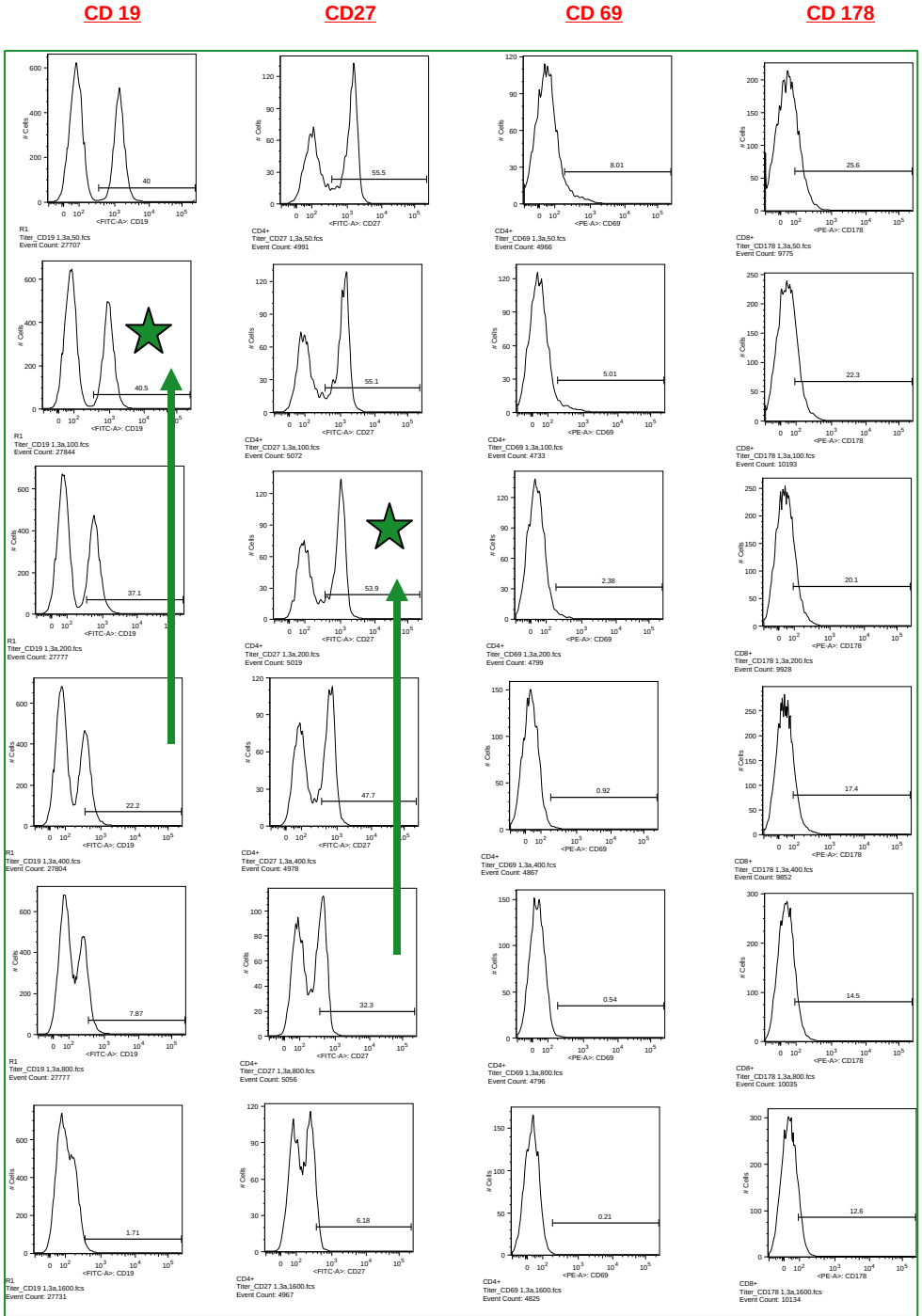

Antibody Titration for FACS Staining

•Histograms show % positive cells for each antigen-fluorophore over a range of doubling dilutions from the stock antibody.

•CD19-FITC shows a significant drop at the 400:1 dilution going from 37.1 to 22.2 % positivity whereas CD27-FITC exhibits a significant drop between the 400:1 and 800:1 dilutions.

•Being conservative one should move up two dilutions from the point of the significant drop in % positivity to establish the titration and hence working concentration for a given antibody. Therefore CD19 and CD27 would be put at 100:1 and 200:1 respectively.

•CD69-PE and CD178-PE would be examples of poor antibody titration as demonstrated by the lack of a clear peak of positivity and need to be reevaluated. Lack of positivity in these cases could be due to numerous reasons. Anything from having a bad antibody to needing an infected animal in order to see a particular rare marker could explain these results. Solutions may involve anything from a different visualization or gating strategy to a different staining approach such as an indirect method using biotin in order to amplify a weak signal. The myriad of possibilities is so application specific that it is beyond the scope of this generalized explanation of antibody titration methodology.

Working dilution for antibody.