General Guidance on Single-Well Designs (Non-longitudinal or Longitudinal)

Single-well designs have one well assigned to each specimen. As such, technical error cannot be estimated so the utility of Maecker et al. (2020) cannot be applied. This document sketches a general and approximate approach for correction of nonspecific binding artifact under single-well designs, non-longitudinal or longitudinal.

The primary research hypothesis of interest can usually be formulated as a regression model. Let M_{ij} be the MFI value of the j^{th} cytokine on the i^{th} specimen. Consider a research design comparing two groups. Let $g_{ij} = 0$ for the first group and $g_{ij} = 1$ for the second group. We can then fit the following regression model:

$$M_{ij} = \beta_0 + \beta_1 g_{ij} + R_{ij},$$

where β_0 and β_1 are regression coefficients to be estimated and R_{ij} is residual (i.e., unexplained) error. When we fit this model, hypothesis testing regarding β_1 provides a test of the difference between groups. This test for the group difference can be corrected for nonspecific binding by including the logarithm of nonspecific binding MFI, n_{ij}, as a covariate:

$$M_{ij} = \beta_0 + \beta_1 g_{ij} + \beta_2 n_{ij} + R_{ij}.$$

Likewise, we can also correct this test for group differences for plate effects by including plate as a covariate (or set of covariates) in the model.

$$M_{ij} = \beta_0 + \beta_1 g_{ij} + \beta_2 n_{ij} + \beta_3 p_{2ij} + \beta_4 p_{3ij} + R_{ij}.$$

Here we are assuming three plates, with $p_{2ij} = 1$ for data from plate 2 and $p_{2ij} = 0$ otherwise and $p_{3ij} = 1$ for data from plate 3 and $p_{3ij} = 0$ otherwise. (Plate 1 has $p_{2ij} = 0$ and $p_{3ij} = 0$.) This modeling approach assumes plates are relatively evenly balanced with respect to the different levels of the factor(s) of interest (e.g., roughly equal quantities of the two groups on each plate). In R, the test for the group effect can be obtained from

```
summary(lm(m ~ g + n + p2 + p3, data = MyData))
```

This approach is completely general. For instance, suppose that instead of a group effect, the primary hypothesis concerns BMI b_{ij} with adjustment for the effects of age a_{ij} in an experiment conducted on four plates:

$$M_{ij} = \beta_0 + \beta_1 b_{ij} + \beta_2 n_{ij} + \beta_3 p_{2ij} + \beta_4 p_{3ij} + \beta_5 p_{4ij} + \beta_6 a_{ij} + R_{ij}$$

and in R

```
summary(lm(m ~ b + n + p2 + p3 + p4 + a, data = MyData))
```

A longitudinal study would have time d_{itj} as the primary variable of interest:

$$M_{itj} = \beta_0 + \beta_1 d_{itj} + \beta_2 n_{itj} + \beta_3 p_{2itj} + \beta_4 p_{3itj} + P_{ij} + R_{itj}$$

This example has three plates. Here t indexes the t^{th} time point and P_{ij} is the (random) effect of the i^{th} participant (e.g., Pinheiro and Bates 2000, p. 35). In R,

```
summary(lme(m ~ d + n + p2 + p3, random = ~1|P, data = MyData))
```

(e.g., Pinheiro and Bates 2000, p. 35). See "Example R Script for Analysis of Longitudinal, Single-Well Luminex® Data Sets" for more details on longitudinal modeling of single-well designs.

Conclusions:

Throughout all of the examples given here, we correct for nonspecific binding and plate effects by simply including these as covariates. Use of nonspecific binding as a covariate in this way will tend to under-correct for this artifact compared to the method of Maecker et al. (2020) that requires at least two wells per specimen.

References:

Maecker, H. T., Rosenberg-Hasson, Y., Kolstadt, K. D., Steen, V. D., & Chung, L. S. 2020. A novel utility to correct for plate/batch/lot and nonspecific binding artifacts in Luminex data. Journal of Immunology 204:3425-3433.

Pinheiro, J. C., & Bates, D. M. 2000. Mixed-effects models in S and S-PLUS. New York: Springer.