Mixture Models for Single-Cell Assays with Application to Vaccine Studies: Supplementary Materials

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Appendix A: Constrained beta-binomial model

We can define a model where we constrain the stimulated proportions under the alternative model such that $p_s > p_u$. In this case, the only changes required are for the alternative marginal likelihood L₁ defined in the main manuscript by (1). Due to the constraint, the normalizing constant of the prior under the alternative (model \mathcal{M}_1) is not given by $B(\alpha_u, \beta_u)B(\alpha_s, \beta_s)$ but requires computing

$$Z(\alpha_u, \beta_u, \alpha_s, \beta_s) = \int_0^1 p_u^{\alpha_u - 1} (1 - p_u)^{\beta_u - 1} \int_{p_u}^1 p_s^{\alpha_s - 1} (1 - p_s)^{\beta_s - 1} dp_s dp_u.$$

Using this expression, the constrained alternative marginal likelihood can be written as

$$L_1(\alpha_u, \beta_u, \alpha_s, \beta_s | \mathbf{y}_i) = \binom{N_{ui}}{n_{ui}} \binom{N_{si}}{n_{si}} \frac{Z(n_{ui} + \alpha_u, N_{ui} - n_{ui} + \beta_u, n_{si} + \alpha_s, N_{si} - n_{si} + \beta_s)}{Z(\alpha_u, \beta_u, \alpha_s, \beta_s)}$$

In general, there is no closed-form expression for $Z(\cdot)$, and a numerical approximation must be used. Let us denote by $\tilde{Z}(\alpha_u, \beta_u, \alpha_s, \beta_s)$ the approximation. A natural way to estimate \tilde{Z} is to use Monte Carlo integration. Indeed, we can write

$$\tilde{Z}(\alpha_u, \beta_u, \alpha_s, \beta_s) = B(\alpha_u, \beta_u)B(\alpha_s, \beta_s) \int_0^1 \frac{p_u^{\alpha_u - 1}(1 - p_u)^{\beta_u - 1}}{B(\alpha_u, \beta_u)} [1 - F_{\alpha_s, \beta_s}(p_u)]dp_u$$
(1)

where F_{α_s,β_s} is the cumulative distribution function of a beta random variable with parameters α_s and β_s . Using this identity, it can be seen that $\tilde{Z}(\alpha_u,\beta_u,\alpha_s,\beta_s)$ can be approximated by

$$\tilde{Z}(\alpha_u, \beta_u, \alpha_s, \beta_s) \approx B(\alpha_u, \beta_u)B(\alpha_s, \beta_s) \sum_{k=1}^{K} [1 - F_{\alpha_s, \beta_s}(X_k)]$$

where the X_k 's are *iid* beta distributed random variables with parameters α_s , β_s and K is the number of terms used in the Monte Carlo approximation. This approximation works relatively well with our EM implementation and does not significantly increase the computing time. Unfortunately, the number of terms (*i.e.* value of K) required for the approximation to be good might be large and computing such a normalizing constant at each iteration would significantly slow down our MCMC implementation. As it tuns out, a better

approximation can be obtained when α_s and β_s are integers. In this case, the cdf function in (1) can be calculated exactly using integration by parts, as follows,

$$F_{\alpha_s,\beta_s}(p_u) = \sum_{j=\beta_s}^{\beta_s + \alpha_s - 1} \frac{(\beta_s + \alpha_s - 1)!}{j!(\beta_s + \alpha_s - j)!} (1 - p_u)^j p_u^{\beta_s + \alpha_s - j}.$$

Then using this identity, we obtain

$$Z(\alpha_u, \beta_u, \alpha_s, \beta_s) = B(\alpha_s, \beta_s) \sum_{j=\beta_s}^{\beta_s + \alpha_s - 1} \frac{(\beta_s + \alpha_s - 1)!}{j!(\beta_s + \alpha_s - j)!} \int_0^1 (1 - p_u)^{\beta_u - 1 + j} p_u^{\alpha_u - 1 + \beta_s + \alpha_s - j} dp_u$$

$$= B(\alpha_s, \beta_s) \sum_{j=\beta_s}^{\beta_s + \alpha_s - 1} \frac{(\beta_s + \alpha_s - 1)!}{j!(\beta_s + \alpha_s - j)!} B(\beta_u + j) B(\alpha_u + \beta_s + \alpha_s - j).$$

Typically, in ICS data α_s is relatively small leading to relatively few terms in the sum. However, the use of this exact identity in our MCMC algorithm requires the use of discrete priors on α_s and β_s , which can be restrictive in terms of fit (e.g., if the true α_s is less than one) and can render mixing in the MCMC more difficult. In addition, even though the computation is exact and much faster for small values of α_s , which is typically the case with ICS data, it is still more demanding than the unconstrained model. In our case, we have decided to use the unconstrained model and simply fix the z_i to zero if the empirical proportion for the un-stimulated sample, p_u , is greater than that of the stimulation sample, p_s . Indeed, in the one-sided case, if $p_u > p_s$ the associated individual should be a non-responder and thus $z_i = 0$. In our experience, this computational shortcut performs just as well as the true one-sided implementation while being computationally much less demanding.

Appendix B: Computational details for the beta-binomial model

Marginal likelihood derivations

For a given subject i, the null marginal likelihood is obtained after integrating out the prior from the likelihood for model \mathcal{M}_0 , as follows,

$$\begin{split} \mathbf{L}_{0}(\alpha_{u},\beta_{u}|n_{si},n_{ui}) &= \int_{0}^{1} \Pr(n_{si},n_{ui}|p_{u})\pi(p_{u}|\alpha_{u},\beta_{u})\mathrm{d}p_{u} \\ &= \int_{0}^{1} \Pr(n_{s}|p_{u})\Pr(n_{u}|p_{u})\pi(p_{u}|\alpha_{u},\beta_{u})\mathrm{d}p_{u} \\ &= \int_{0}^{1} \binom{N_{s}}{n_{s}}p_{u}^{n_{s}}(1-p_{u})^{N_{s}-n_{s}}\binom{N_{u}}{n_{u}}p_{u}^{n_{u}}(1-p_{u})^{N_{u}-n_{u}}\frac{1}{\mathbf{B}(\alpha_{u},\beta_{u})}p_{u}^{\alpha_{u}-1}(1-p_{u})^{\beta_{u}-1}\mathrm{d}p_{u} \\ &= \binom{N_{s}}{n_{s}}\binom{N_{u}}{n_{u}}\frac{1}{\mathbf{B}(\alpha_{u},\beta_{u})}\int_{0}^{1}p_{u}^{n_{s}+n_{u}+\alpha_{u}-1}(1-p_{u})^{N_{s}+N_{u}-n_{s}-n_{u}+\beta_{u}-1}\mathrm{d}p_{u} \\ &= \binom{N_{s}}{n_{s}}\binom{N_{u}}{n_{u}}\frac{\mathbf{B}(n_{s}+n_{u}+\alpha_{u},N_{s}+N_{u}-n_{s}-n_{u}+\beta_{0})}{\mathbf{B}(\alpha_{u},\beta_{u})}. \end{split}$$

Similarly, the alternative marginal likelihood for a given subject is defined as,

$$\begin{split} \mathbf{L}_{1}(\alpha_{u},\beta_{u},\alpha_{s},\beta_{s}|n_{si},n_{ui}) &= \int_{0}^{1} \int_{0}^{1} \Pr(n_{si},n_{ui}|p_{u},p_{s})\pi(p_{u},p_{s}|\alpha_{u},\beta_{u},\alpha_{s},\beta_{s}) \mathrm{d}p_{s} \mathrm{d}p_{u} \\ &= \int_{0}^{1} \int_{0}^{1} \binom{N_{s}}{n_{s}} p_{s}^{n_{s}} (1-p_{s})^{N_{s}-n_{s}} \binom{N_{u}}{n_{u}} p_{u}^{n_{u}} (1-p_{u})^{N_{u}-n_{u}} \frac{1}{\mathbf{B}(\alpha_{u},\beta_{u})} p_{u}^{\alpha_{u}-1} (1-p_{u})^{\beta_{u}-1} \cdot \\ &= \frac{1}{\mathbf{B}(\alpha_{s},\beta_{s})} p_{s}^{\alpha_{s}-1} (1-p_{s})^{\beta_{s}-1} \mathrm{d}p_{s} \mathrm{d}p_{u} \\ &= \binom{N_{s}}{n_{s}} \binom{N_{u}}{n_{u}} \frac{1}{\mathbf{B}(\alpha_{u},\beta_{u})} \frac{1}{\mathbf{B}(\alpha_{s},\beta_{s})} \int_{0}^{1} p_{u}^{n_{u}+\alpha_{u}-1} (1-p_{u})^{N_{u}-n_{u}+\beta_{u}-1} \cdot \\ &\int_{0}^{1} p_{s}^{n_{s}+\alpha_{s}-1} (1-p_{s})^{N_{s}-n_{s}+\beta_{s}-1} \mathrm{d}p_{s} \mathrm{d}p_{u} \\ &= \binom{N_{s}}{n_{s}} \binom{N_{u}}{n_{u}} \frac{\mathbf{B}(n_{u}+\alpha_{u},N_{u}-n_{u}+\beta_{u}) \mathbf{B}(n_{s}+\alpha_{s},N_{s}-n_{s}+\beta_{s})}{\mathbf{B}(\alpha_{u},\beta_{u}) \mathbf{B}(\alpha_{s},\beta_{s})} . \end{split}$$

MCMC algorithm

In what follows, we use (x|y) to denote the conditional distribution of x given y. In particular, we use $(x|\cdots)$ to denote the distribution of x conditional on everything else in the model. Our MCMC algorithms cycles through the following steps:

- 1. Update each α_u , β_u , α_s and β_s by Metropolis-Hastings using a Gaussian symmetric proposal where the variance of the proposal is tuned for each parameter using the approach of Gelman *and others* (2004).
- 2. Update w by Gibbs sampling using the full conditional,

$$(w|\cdots) \sim \text{Beta}(\sum_{i} z_i, \sum_{i} (1-z_i)).$$

3. for each i, update z_i by Gibbs sampling using the following full conditional,

$$(z_i|\cdots) \sim \mathrm{B}(1,p_i).$$

where.

$$p_i = \frac{w \cdot L_1(\alpha_u, \beta_u, \alpha_s, \beta_s | n_{ui}, n_{si})}{w \cdot L_1(\alpha_u, \beta_u, \alpha_s, \beta_s | n_{ui}, n_{si}) + (1 - w) \cdot L_0(\alpha_u, \beta_u | n_{ui}, n_{si})}.$$

For each updated parameter, step 1 above involves the calculation of the following acceptance ratio, (e.g. α_u , below),

$$\frac{L(\alpha_u^{\text{new}}, \beta_u, \alpha_s, \beta_s | \cdots) \pi(\alpha_u^{\text{new}})}{L(\alpha_u, \beta_u, \alpha_s, \beta_s | \cdots) \pi(\alpha_u)}$$

where L is the complete marginal likelihood conditional on \mathbf{z} defined as,

$$L(\alpha_u, \beta_u, \alpha_s, \beta_s | \mathbf{n}_u, \mathbf{n}_s, \mathbf{z}) = \prod_i L_{z_i}(\alpha_u, \beta_u, \alpha_s, \beta_s | \mathbf{n}_u, \mathbf{n}_s)$$

and π is the prior distribution of α_u . The obvious changes in the above expression are made for the acceptance ratios of α_s , β_s , β_u . In our case each parameter has the same exponential prior with mean 1,000

Appendix C: Computational details for the Dirichlet-multinomial model

Marginal likelihood derivations

Because our Dirichlet-multinomial is a direct extension of the beta-binomial model, the marginal likelihoods

are obtained in the exact same fashion. For a given subject, the null marginal likelihood is defined as

$$\begin{split} \mathbf{L}_{0}(\boldsymbol{\alpha}_{u}|\mathbf{n}_{si},\mathbf{n}_{ui}) &= \int \cdots \int \mathrm{Pr}(\mathbf{n}_{si},\mathbf{n}_{ui}|\mathbf{p}_{u})\pi(\mathbf{p}_{u}|\boldsymbol{\alpha}_{u})\mathrm{d}\mathbf{p}_{u} \\ &= \int \cdots \int \frac{\mathbf{N}_{s}!}{\prod_{k} n_{sik}!} \prod_{k} p_{uk}^{n_{sik}} \frac{\mathbf{N}_{u}!}{\prod_{k} n_{uik}!} \prod_{k} p_{uk}^{n_{uik}} \frac{1}{\mathrm{B}(\boldsymbol{\alpha}_{u})} \prod_{k} p_{uk}^{\alpha_{uk}-1} \mathrm{d}\mathbf{p}_{u} \\ &= \frac{\mathbf{N}_{s}!}{\prod_{k} n_{sik}!} \frac{\mathbf{N}_{u}!}{\prod_{k} n_{uik}!} \frac{1}{\mathrm{B}(\boldsymbol{\alpha}_{u})} \int_{0}^{1} \prod_{k} p_{uk}^{n_{sik}+n_{uik}+\alpha_{uk}-1} \mathrm{d}\mathbf{p}_{u} \\ &= \frac{\mathbf{N}_{s}!}{\prod_{k} n_{sik}!} \frac{\mathbf{N}_{u}!}{\prod_{k} n_{uik}!} \frac{\mathrm{B}(\mathbf{n}_{si}+\mathbf{n}_{u}+\boldsymbol{\alpha}_{u})}{\mathrm{B}(\boldsymbol{\alpha}_{u})} \end{split}$$

Similarly, the alternative marginal likelihood for a given subject is:

$$\begin{split} \mathrm{L}_{1}(\boldsymbol{\alpha}_{u}, \boldsymbol{\alpha}_{s} | \mathbf{n}_{ui}, \mathbf{n}_{si}) = & \frac{\mathbf{N}_{s}!}{\prod_{k} n_{sik}!} \frac{\mathbf{N}_{u}!}{\prod_{k} n_{uik}!} \frac{1}{\mathrm{B}(\boldsymbol{\alpha}_{u}) \mathrm{B}(\boldsymbol{\alpha}_{s})} \int \cdots \int \prod_{k} p_{uk}^{n_{uik} + \alpha_{uk} - 1} \int \cdots \int \prod_{k} p_{sk}^{n_{sik} + \alpha_{sk} - 1} \mathrm{d}\mathbf{p}_{u} \mathrm{d}\mathbf{p}_{s} \\ = & \frac{\mathbf{N}_{s}!}{\prod_{k} n_{sik}!} \frac{\mathbf{N}_{u}!}{\prod_{k} n_{uik}!} \frac{\mathrm{B}(\mathbf{n}_{ui} + \boldsymbol{\alpha}_{u}) \mathrm{B}(\mathbf{n}_{si} + \boldsymbol{\alpha}_{s})}{\mathrm{B}(\boldsymbol{\alpha}_{u}) \mathrm{B}(\boldsymbol{\alpha}_{s})}. \end{split}$$

MCMC algorithm

The MCMC algorithm for the Dirichlet-multinomial model is analogous to the beta-binomial, above. The parameter vectors $\boldsymbol{\alpha}_s$, $\boldsymbol{\alpha}_u$ are updated component-wise:

- 1. Update each α_{sk} , α_{uk} using a Gaussian symmetric proposal distribution with the variance of each proposal tuned using the approach of Gelman *and others* (2004).
- 2. Update w by Gibbs sampling using the full conditional,

$$(w|\cdots) \sim \text{Beta}(\sum_{i} z_i, \sum_{i} (1 - z_i))$$

3. For each i, update z_i using the full conditional,

$$(z_i|\cdots) \sim \mathrm{B}(1,p_i)$$

where

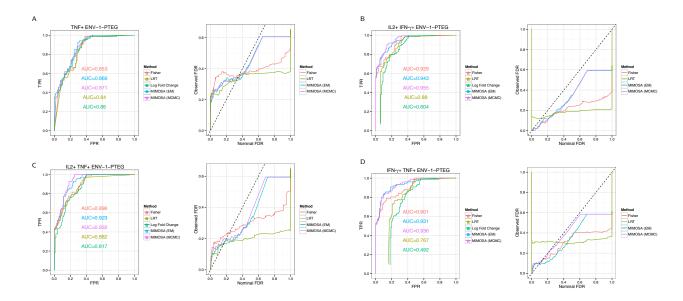
$$p_i = \frac{w \cdot L_1(\boldsymbol{\alpha}_u, \boldsymbol{\alpha}_s | \mathbf{n}_{si}, \mathbf{n}_{ui})}{w \cdot L_1(\boldsymbol{\alpha}_u, \boldsymbol{\alpha}_s | \mathbf{n}_{si}, \mathbf{n}_{ui}) + (1 - w) \cdot L_0(\boldsymbol{\alpha}_u | \mathbf{n}_{si}, \mathbf{n}_{ui})}$$

For each parameter component updated in step 1 above, compute the acceptance ratio (e.g. α_{uk} , below):

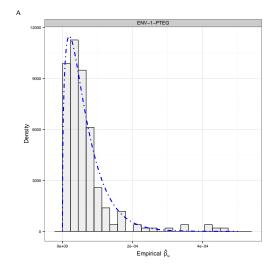
$$\frac{\mathrm{L}(\alpha_{uk}^{\mathrm{new}}, \boldsymbol{\alpha}_{u\{-k\}}, \boldsymbol{\alpha}_{s}| \cdots) \pi(\alpha_{uk}^{\mathrm{new}})}{\mathrm{L}(\boldsymbol{\alpha}_{u}, \boldsymbol{\alpha}_{s}| \cdots) \pi(\alpha_{uk})}$$

where π is the prior distribution of the parameter, and $\alpha_{u\{-k\}} = \{\alpha_{uj} : j \neq k\}$. Again, we have used the same exponential prior with mean 1,000 for each parameter. L is the complete marginal likelihood conditional on \mathbf{z} , defined as

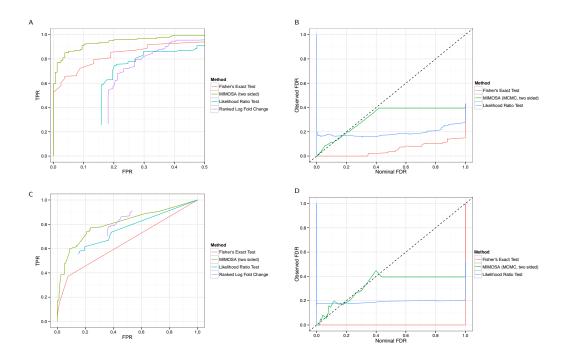
$$\mathrm{L}(oldsymbol{lpha}_u, oldsymbol{lpha}_s | \mathbf{n}_{si}, \mathbf{n}_{ui}, \mathbf{z}) = \prod_i \mathrm{L}_{z_i}(oldsymbol{lpha}_u, oldsymbol{lpha}_s | \mathbf{n}_{si}, \mathbf{n}_{ui}).$$



Supplementary Figure 1: Comparison of MIMOSA on other cytokines and cytokine combinations for ENV-1-PTEG stimulated CD4+ T-cells from the HVTN065 trial. This figure appears in color in the electronic version of this article.



Supplementary Figure 2: Histogram of the empirical proportions of unstimulated cells and overlaid posterior densities of the beta distribution with α_u and β_u estimated from the data for ENV-1-PTEG stimulated, IFN- γ +, CD4+ T-cells, demonstrating that the assumption of a common distribution for p_{iu} across subjects is reasonable.



Supplementary Figure 3: Unconstrained MIMOSA model fit to data from a model violating model assumptions and to two-sided data with small counts. Data was simulated from a model where proportions were sampled from a truncated normal distribution over [0,1] rather than a Beta distribution. A) The average ROC from 10 simulation with N=5,000 events. B) The average observed and nominal FDR from 10 simulations with N=5,000 events. C) Average ROC for N=1,000 events D) Average observed and nominal FDR for N=1,000 events.

References

Gelman, Andrew, Carlin, John B, Stern, Hal S and Rubin, Donald B. (2004). Bayesian Data Analysis. Chapman & Hall/CRC.