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Quantifying the HIV Reservoir with Dilution Assays and Deep Viral Sequencing

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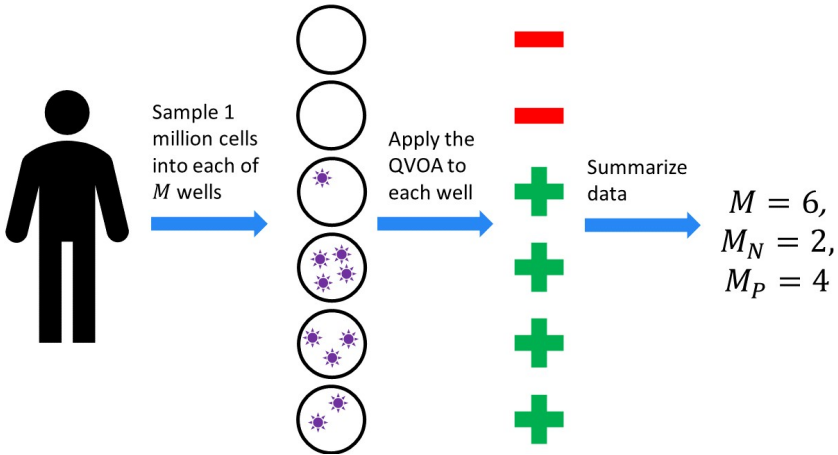
Motivation: Why Quantify the HIV Reservoir?

- For people living with HIV, antiretroviral therapies can help them achieve **viral suppression**
- Despite viral suppression, a **reservoir of latently infected cells**, which are undetectable by the immune system, remain
- The HIV reservoir is often measured in **infectious units per million**, or **IUPM**
- A barrier in HIV cure research is the need to **reliably quantify the IUPM** of the HIV reservoir

Quantitative Viral Outgrowth Assay

- One standard method for quantifying the HIV reservoir is a **serial limiting dilution (SLD) assay** called the **Quantitative Viral Outgrowth Assay**, or **QVOA**
- Cells are sampled from one individual into multiple wells
- The QVOA tests each well for the presence of **at least one HIV-infected cell**

Quantitative Viral Outgrowth Assay: Example



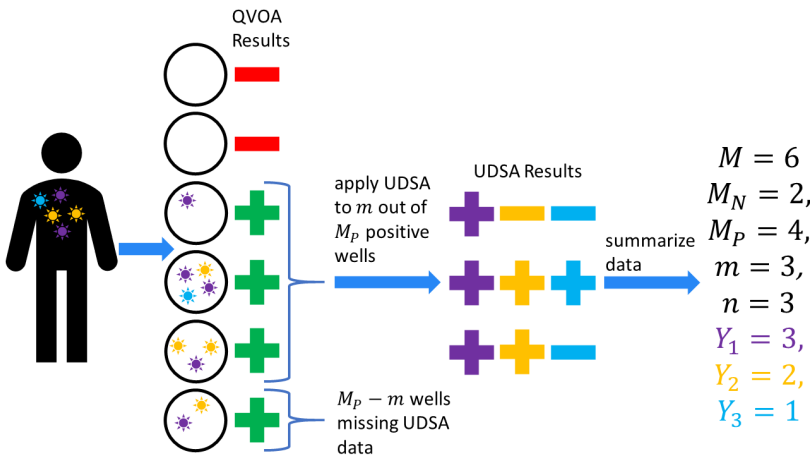
Quantitative Viral Outgrowth Assay: Inference

- latent cell counts $X_1, \ldots, X_M \sim \text{Poisson}(\Lambda)$
- observed indicators $W_j = 1(X_j > 0)$
- estimand: IUPM $\Lambda = \text{E}(X_j)$
- MLE for $\text{E}(W_j) = 1 - \exp(-\Lambda)$ is sample mean $\frac{M_P}{M}$
- MLE for Λ is then $\widehat{\Lambda} = -\log(1 - \frac{M_P}{M})$

Ultra Deep Sequencing Assay

- The **Ultra Deep Sequencing Assay**, or **UDSA**, is a more sophisticated assay used in addition to the QVOA
  - typically applied to wells that the QVOA has identified as HIV-positive
  - tests for the presence of **distinct viral lineages (DVLs)**

Ultra Deep Sequencing Assay: Example



Inference about the IUPM

- Let λ_i be the **DVL-specific IUPM** for DVL i and $\boldsymbol{\lambda} = (\lambda_1, \ldots, \lambda_n)^T$
- Let $\Lambda = \sum_{i=1}^{n} \lambda_i$ be the **IUPM** (over all DVLs of HIV)
- Our goal is to estimate Λ with $\widehat{\Lambda} = \sum_{i=1}^{n} \widehat{\lambda}_i$
- With no missing data, MLE for λ_i is $\widehat{\lambda}_i = -\log(1 - \frac{Y_i}{M})$
- Not so easy with missing data...

Inference about the IUPM

The **observed-data likelihood** is $L(\boldsymbol{\lambda}|M_N, \boldsymbol{Y}) =$

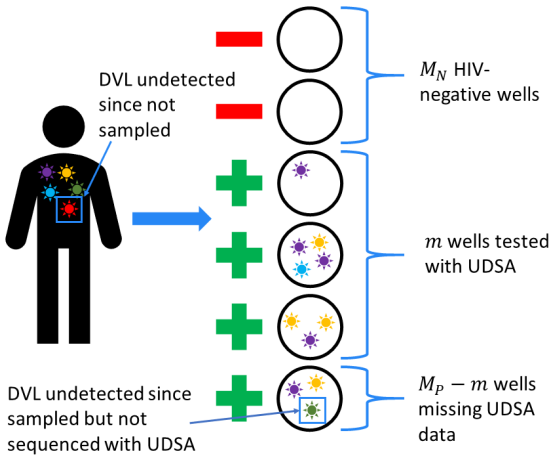
$$\underbrace{\left[\prod_{i=1}^{n}\{1-\exp(-\lambda_i)\}^{Y_i}\exp(-\lambda_i)^{M_N+m-Y_i}\right]}_{\text{complete data}}\underbrace{\{1-\exp(-\Lambda)\}^{M-(M_N+m)}}_{\text{missing data}}$$

- Maximized numerically to get the MLE $\widehat{\boldsymbol{\lambda}}$
- The **MLE for the IUPM** is $\widehat{\Lambda} = \sum_{i=1}^{n}\widehat{\lambda}_i$
- $\widehat{\boldsymbol{\lambda}}$ (and thus $\widehat{\Lambda}$) is consistent and asymptotically normal

Bias Correction for Small Samples

- The MLE $\widehat{\boldsymbol{\lambda}}$ is **upwardly biased** in small samples (i.e., small M)
- A **bias-corrected MLE** $\widehat{\boldsymbol{\lambda}}^*$ is adapted from Hashemi & Schneider PLoS ONE 2021
- $\widehat{\boldsymbol{\lambda}}^* = \widehat{\boldsymbol{\lambda}} - B(\widehat{\boldsymbol{\lambda}})$, where $B(\widehat{\boldsymbol{\lambda}})$ is an estimate of the bias of $\widehat{\boldsymbol{\lambda}}$
- This bias correction reduces the order of the bias from $\mathcal{O}(M^{-1})$ to $\mathcal{O}(M^{-2})$

Undetected Viral Lineages: Example



- Existing DVLs: $n' = 5$
- Detected DVLs: $n = 3$
- True IUPM: $\Lambda = \lambda_1 + \lambda_2 + \lambda_3 + \lambda_4 + \lambda_5$
- Estimated IUPM: $\widehat{\Lambda} = \widehat{\lambda}_1 + \widehat{\lambda}_2 + \widehat{\lambda}_3$

Undetected Viral Lineages: Problem

- Recall: $\widehat{\Lambda} = \widehat{\lambda}_1 + \cdots + \widehat{\lambda}_n$
- n is the number of **observed DVLs**. There may exist $n' > n$ DVLs in the source population, leaving $n' - n$ DVLs **undetected**
- Then $\Lambda = \lambda_1 + \cdots + \lambda_n + \lambda_{n+1} + \cdots + \lambda_{n'}$, which is greater than $\lambda_1 + \cdots + \lambda_n$
- Does this mean $\widehat{\Lambda}$ is a poor estimator of Λ?

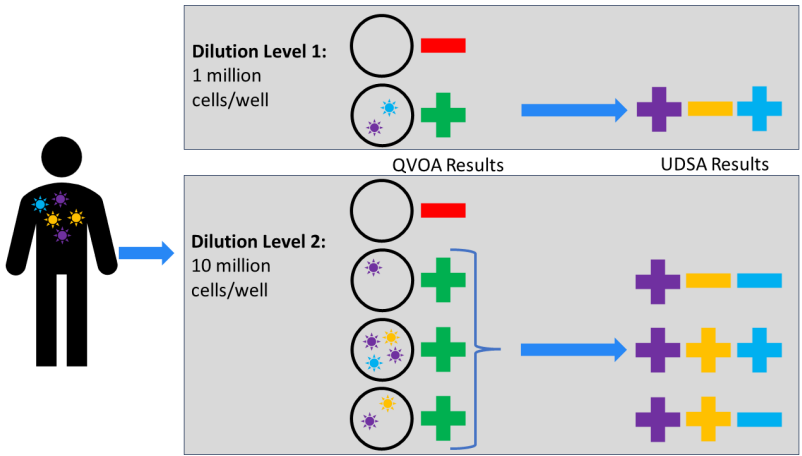
Undetected Viral Lineages: Solution

- Consider an **augmented likelihood** that accounts for possibly undetected DVLs

$$L'(\lambda'_1, \ldots, \lambda'_n, \lambda'_{n+1}, \ldots, \lambda'_{n'} | M_N, Y_1, \ldots, Y_n, Y_{n+1} = 0, \ldots, Y_{n'} = 0)$$

- The maximizer $\widehat{\boldsymbol{\lambda}}'$ of the augmented likelihood L' must satisfy $\widehat{\lambda}'_{n+1} = 0, \ldots, \widehat{\lambda}'_{n'} = 0$
- Thus, $\widehat{\Lambda} = \sum_{i=1}^{n} \widehat{\lambda}_i = \sum_{i=1}^{n'} \widehat{\lambda}'_i$
- The augmented likelihood L' and the original likelihood L **lead to the same MLE** $\widehat{\Lambda}$

Multiple Dilution Levels: Example



Extension to Multiple Dilution Levels

- Often, the QVOA and UDSA are applied to wells of **multiple dilution levels**
- Previous estimation methods can handle QVOA data from multiple dilutions *or* QVOA and UDSA data from one dilution level
- We proposed a more general estimator that can handle both
  - The bias-corrected MLE and the undetected DVL result extends to the multiple dilutions setting

Simulation Results

IUPM of $\Lambda = 1$, single dilution level, and 75% of HIV-positive wells tested with UDSA

		MLE			Bias-Corrected MLE		
n'	M	Bias	ESE	CP	Bias	ESE	CP
12	12	0.04	0.33	0.94	−0.02	0.31	0.96
12	24	0.03	0.22	0.95	−0.01	0.21	0.96
18	12	0.05	0.32	0.94	−0.01	0.31	0.96
18	24	0.03	0.23	0.95	0.00	0.22	0.96

ESE: empirical standard error of $\widehat{\Lambda}$,
CP: empirical coverage probability of 95% confidence interval

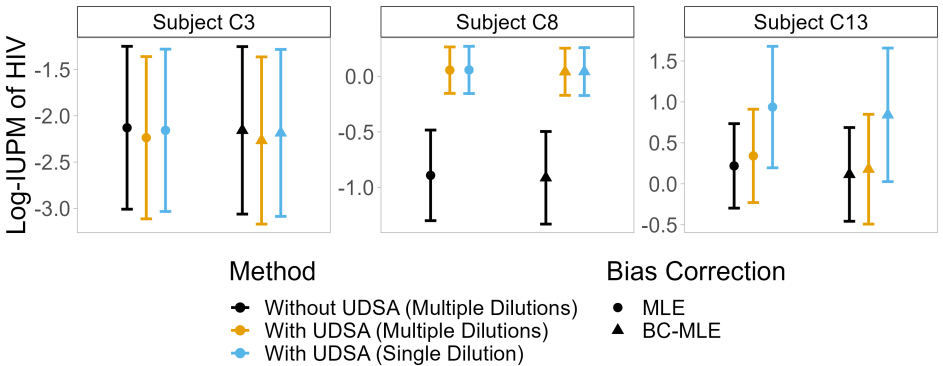
Application to HIV Data

QVOA and UDSA data on individuals living with HIV and taking antiretroviral treatment were obtained from the UNC HIV Cure Center

- For each individual, cells were sampled into various wells at **3–4 distinct dilution levels** and tested with the QVOA
- A subset of wells from **one dilution level was deep-sequenced**

Subject ID	DLVs (n)	Wells (M)	Positive Wells (M_P)	UDSA Wells (m)
C3	4	18, 6, 6	5, 0, 0	5, 0, 0
C8	26	36, 6, 6	22, 2, 1	22, 0, 0
C13	7	18, 6, 6, 6	16, 4, 3, 0	0, 4, 0, 0

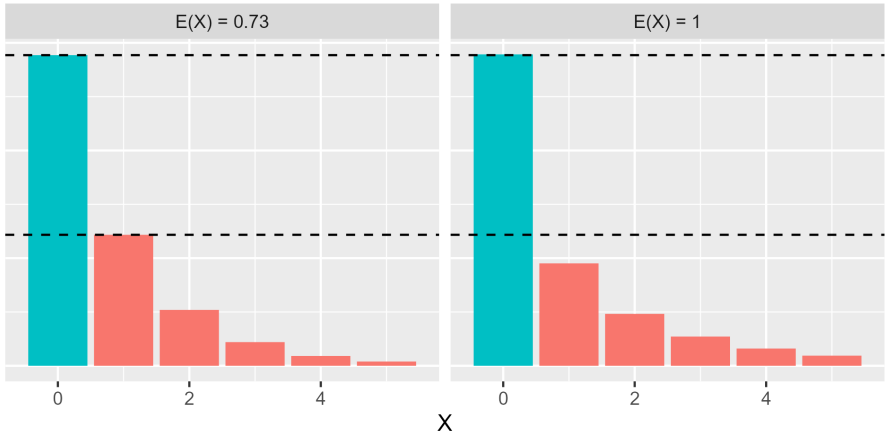
Application to HIV Data



Overdispersed Cell Counts: Problem

- Currently assuming latent cell counts are $X_i \sim \text{Poisson}(\lambda_i)$
- Allows inference on $\Lambda = \text{E}(X_i)$ based on observed $W_i = 1(X_i > 0)$
- Poisson assumption based on the approximation to $\text{Binomial}(n, p)$ with large n and small p.
- May be concern that Poisson assumptions doesn't hold
- **Overdispersion**: $\text{Var}(X_i) > \text{E}(X_i)$

Overdispersed Cell Counts: Example



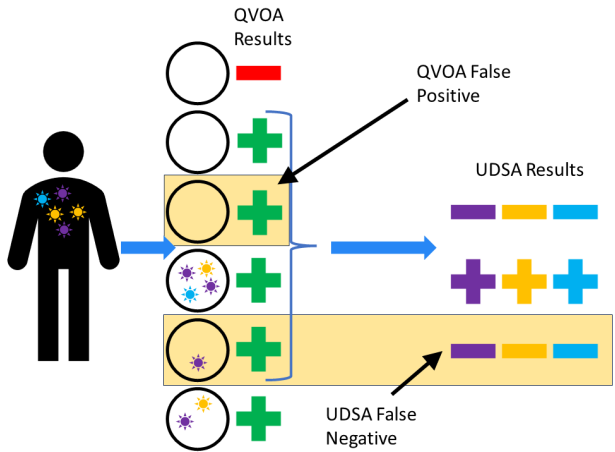
Overdispersed Cell Counts: Solution

- Model X_i as negative binomial with mean parameter λ_i and dispersion parameter $\gamma \in [0, \infty)$
- $\mathrm{P}(X_i > 0) = 1 - (\gamma\lambda_i + 1)^{-1/\gamma}$
- When $\gamma = 0$, reduces to Poisson case
- With multiple dilution level data, $\boldsymbol{\lambda}, \gamma$ are identifiable
- Can estimate $\boldsymbol{\lambda}, \gamma$
- Likelihood ratio test of overdispersion $H_0 : \gamma = 0$
  - null parameter on boundary of parameter space
  - asymptotic null distribution is $0.5\chi^2_0 + 0.5\chi^2_1$

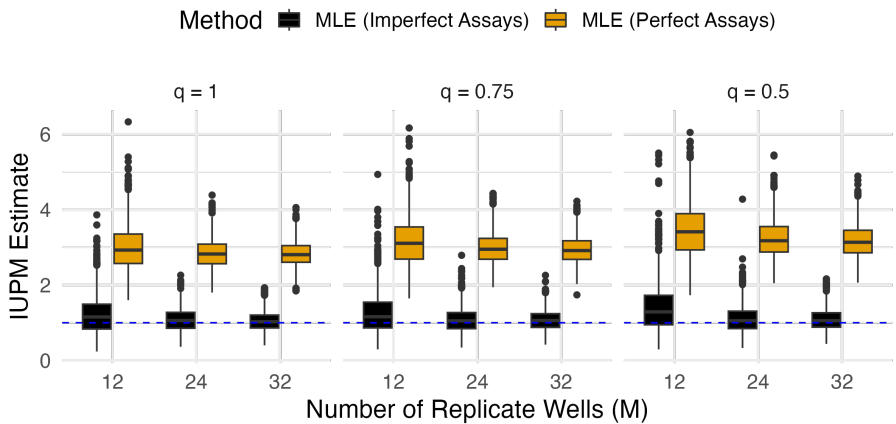
Imperfect Assays: Problem

- So far, we've assumed that the two assays (QVOA and UDSA) have perfect sensitivity and specificity
- **Sensitivity**: Probability of a positive result given the well is truly infected
- **Specificity**: Probability of a negative result given well is truly not infected
- Need to account for false positives and negatives in estimation of Λ

Imperfect Assays: Example



Imperfect Assays: Simulation Results



(single dilution level, 90% sensitivity and specificity of both assays)

Want to Learn More?

Paper:

https://pubmed.ncbi.nlm.nih.gov/38364812/

R package:

www.https://github.com/sarahlotspeich/SLDeepAssay

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Thank you! Any questions?

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