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Matrix Pooling: An Accurate and Cost Effective Testing Algorithm for Detection of Acute HIV Infection

Bethany L. Hedt* Marcello Pagano†

*Harvard School of Public Health, bhedt@hsph.harvard.edu

†Harvard University, pagano@hsph.harvard.edu

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Abstract

Individuals newly infected with HIV have an increased viral load than at other phases of HIV infection, which is associated with a higher rate of transmission. Combined with ignorance about infection status, individuals in the acute phase not only contribute to the growing epidemic but are believed by some to be the driving force behind new infections. Unfortunately, due to the high costs of testing, individuals are not routinely tested for acute HIV infection. The matrix pooling algorithm we present is more economical than individual testing whilst improving accuracy — reducing the number of false positive and false negative test results. Although matrix pooling may require more tests compared to other pooling algorithms, the significant increase in accuracy and rapidity with which results are obtained makes this method more desirable when identifying acute HIV infections, even in resource poor settings.

1 Introduction

At the end of 2007, UNAIDS estimated that 33.2 million people were infected with the Human Immunodeficiency Virus (HIV). Of these, two and a half million were estimated to have occurred in 2007, highlighting the continuing presence of HIV as a major public health crisis [1]. Because the first few weeks of infection are marked with high viral loads, which is linked to increased infectiousness, and because newly infected individuals often are unaware of their infection status, these individuals in the early stage of infection play a critical role in HIV transmissions [2]. While it is valuable to identify these individuals for treatment and care, it is more importantly an opportunity to provide behavior change education to prevent further infections [[3],[4],[5],[6]]. Additionally, we can use information on recent infections to estimate HIV incidence, which informs program managers on current trends and the effectiveness of prevention efforts [7].

Several studies have investigated the impact of expanding HIV testing to include testing for new (acute) infections using Nucleic Acid Amplification Tests (NAAT). In a routine HIV testing environment in North Carolina, the addition of acute testing identified 23 newly infected individuals (out of more than 100,000 people tested). As a result, clinicians were able to provide emergency intervention for 48 sex partners and one fetus [8]. Other studies have found that additional NAAT testing on individuals in high risk populations who test negative with HIV antibody tests increases the number of identified infections by 4 to 10 % [[8],[9],[10],[11], [12]].

Despite the clear clinical benefit of identifying individuals in the early phase of infection, one hindrance to universal testing is the high costs associated with these tests. One solution for reducing costs is to use pooling methods. Dorfman first suggested pooling in 1943 with the primary goal of reducing the overall costs of testing for

syphilis [13]. While the Dorfman method reduces the expected number of tests, the sensitivity of the testing process is also reduced, resulting in an unappealing increase in false negative test results. In response, Litvak, Tu and Pagano [14] present an algorithm, T_2^+, that improves the accuracy of the pooled testing process by retesting all of the pools that initially test negative, increasing the overall sensitivity. Such confirmatory testing does on average require more tests than the standard Dorfman procedure; however, there remains a significant reduction in the number of tests as compared to individual testing at low prevalences. Since the introduction of T_2^+, the concept of retesting negative results to improve accuracy has been applied to other testing algorithms [15].

We propose a matrix pooling algorithm to test for new HIV infections that, at low prevalence settings, requires fewer tests per sample and yet increases overall accuracy when compared to individual testing. Additionally, this proposed method offers some benefits over other pooling algorithms with retesting. First, the matrix pooling algorithm reduces the amount of time needed to conduct the testing, in part because the two tests are performed concurrently, instead of sequentially. Additionally, each sample is tested in two unique pools, which is advantageous if there is a blocker or synergistic effect in the samples. The retesting aspect of the matrix pooling algorithm can lead to more tests compared to other pooling methods, but the increase in costs results in more individuals' disease status correctly identified.

For the purposes of this paper, we focus on individuals who test negative on HIV antibody tests, and assume these individuals are either infected or uninfected with HIV. Because of the very high sensitivity and specificity of the antibody tests, nearly all antibody test negative individuals are truly disease free or are in the acute phase of infection. Further, the prevalence of acute HIV infections in this group is low, the ideal

setting for pooling. A person newly infected with HIV will test antigen positive with probability S_e, the sensitivity of the test, and an uninfected person will test negative with probability S_p, the specificity of the test. We assume that the sensitivity and specificity of the test are preserved under the pooling process. Finally, we assume that all of the tests are independent.

In Section 2 of the paper, we outline current testing methods relevant to acute HIV, followed by a description of the matrix pooling algorithm in Section 3. In section 4, we discuss the timeliness of all algorithms to deliver positive results. Finally, in Section 5, we compare our proposed method to other testing methods, showing that in many settings our method has an improved accuracy and timeliness of results, though in some cases at the cost of increasing the expected number of tests.

2 Methods of Testing Relevant for Acute HIV

In the past, individual testing has been the standard method for identifying acute HIV. The drawback is that individual testing for new infections is very expensive. However, acute HIV infection is the ideal candidate for pooled testing because of the low prevalence of HIV infections in antibody test negative individuals (due to the short period in the acute phase of infection when antibodies are not detectable) and the high cost of the tests. In this section, we describe the sensitivity, specificity and expected number of tests for two additional testing methods — the modified Dorfman testing algorithm and T_2^+— because of their current use in testing for new infections of HIV or because of their role in the evolution of matrix pooling.

Quinn et al examine a method to test for acute HIV infection similar to Dorfman's pooling procedure with the addition of intermediate pools [16]. They combine n

samples into one large pool, and like the Dorfman method, if the pool tests negative, all of the samples are declared uninfected. If the pool tests positive, then the samples are divided into m subpools of size k, with $k = n/m$. The subpools are then tested, and all samples in subpools that test negative are declared uninfected. The samples in subpools that test positive are then tested individually. This method has been used in the identification of individuals in the acute stage of HIV [[9],[17],[8],[10],[18],[11],[12]], and henceforth, we refer to this method as the *modified Dorfman pooling algorithm.*

Finally, we present the T_2^+ algorithm here. Not only is this algorithm used for general HIV testing, but we utilize this procedure as a substep of matrix pooling. With T_2^+, the first step begins with testing a pool of n samples. If the pool tests negative, then the entire pool is retested. If the pool tests negative a second time, then the entire pool is declared uninfected. If the pool tests positive either at the first test or negative at the first test and positive at the retest, then the pool is divided into two subpools. These subpools are equal size if the original pool size, n, is even, and sizes $(n-1)/2$ and $(n+1)/2$ if n is odd. Henceforth, this division is referred to as the *halving step.* The subpools are then each treated like the original pool, and this testing procedure continues until each sample is determined to be positive or negative for infection [14].

Let ψ_i, ϕ_i, and $\mathcal{E}(N)_i$, for ($i = I, MD$, or T_2^+) denote the sensitivity, specificity and expected number of tests for the different testing algorithms. The sensitivity and specificity of individual testing is equal to the test properties ($\psi_I = S_e$ and $\phi_I = S_p$) and the expected number of tests is one ($\mathcal{E}(N)_I = 1$). The sensitivity of the modified Dorfman method is only a function of the test sensitivity ($\psi_{MD} = S_e^3$). For a fixed prevalence, the specificity and the expected number of tests ($\phi_{MD(n,m|p)}$ and $\mathcal{E}_{MD(n,m|p)}$) are functions of the total number of samples tested and the number of intermediate pools. These quantities are derived in Appendix A. Finally, the sensitiv-

ity, specificity and expected number of tests of T_2^+ ($\psi_{T_2^+(n)}$, $\phi_{T_2^+(n|p)}$, and $\mathcal{E}(N)_{T_2^+(n|p)}$) are derived by Litvak et al and for a fixed prevalence are a function of the original pool size, n ([14],[19]). These values for T_2^+ are summarized in Appendix A.

3 Matrix Pooling

Phatarfod and Sudbury (1994) and Xie et al (2001) describe a pooling algorithm that arranges nm samples in an $n \times m$ rectangular array and starts by testing the pools formed by each row and column [[20],[21]]. Both papers discuss the properties of this algorithm under the assumption of perfect sensitivity and specificity. We propose a pooling algorithm called matrix pooling (MT_2^+), a square array testing method that accommodates imperfect tests and is an extension of these algorithms and T_2^+. With MT_2^+, n^2 samples are randomly placed in an $n \times n$ matrix in order to form two sets of pools: the n pooled rows, $r_1, \ldots, r_n$, and the n pooled columns, $c_1, \ldots, c_n$. For simplicity, we restrict ourselves to a square testing array, but with obvious modifications, the method can easily be extended to rectangular arrays that are not square.

We give a brief description of the MT_2^+ testing algorithm and its properties below, with detailed derivations of sensitivity, specificity and expected number of tests provided separately [22]. At the first step of the matrix pooling, we test all the row pools and all the column pools. We declare the samples at the intersection of negative row and negative column tests disease free. Samples at the intersection of positive row and positive column tests are tested individually, with confirmatory retesting of negative results. Samples at the intersection of discordant row and column pool tests are submitted to T_2^+ with the other samples which have discordant results in

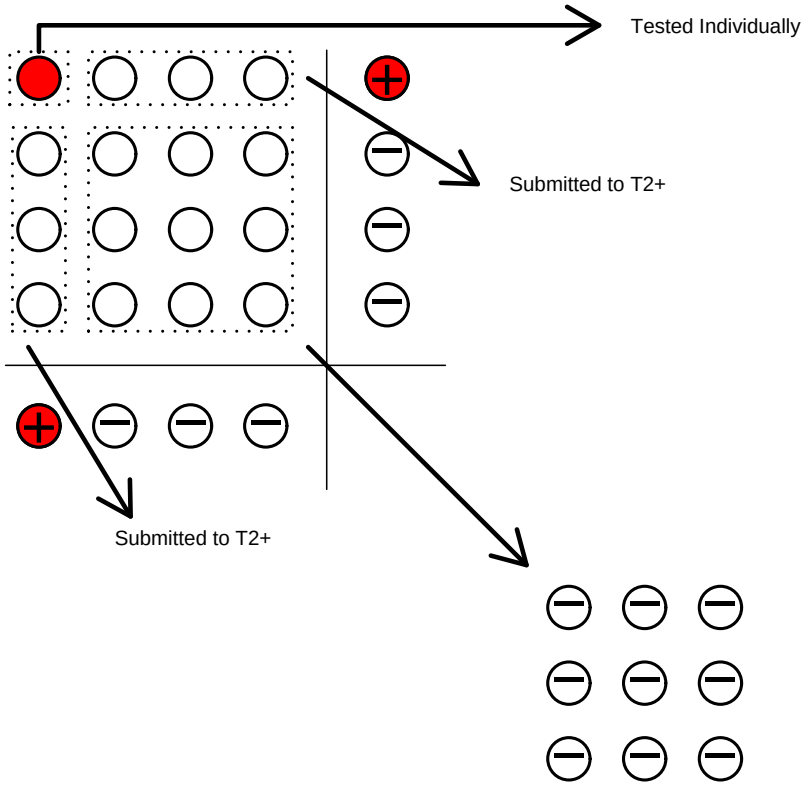

Figure 1: Matrix pooling testing algorithm with perfect tests.
The filled circles indicate an infected sample. The positive and negative symbols indicate row and column pools that test positive and negative, respectively.

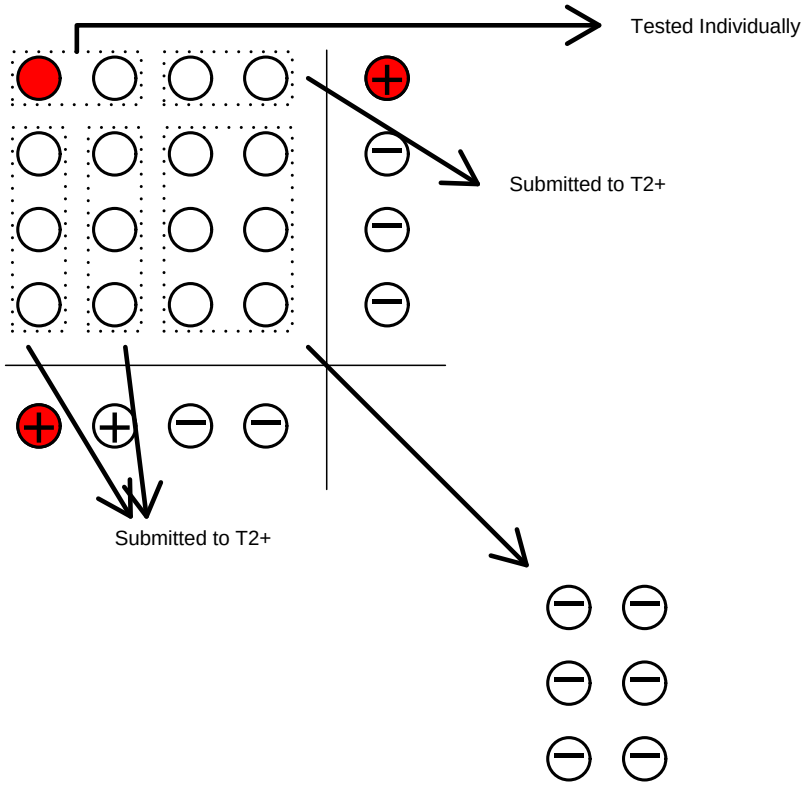

Figure 2: Matrix pooling testing algorithm with imperfect tests.
The filled circles indicate an infected sample. The positive and negative symbols indicate row and column pools that test positive and negative, respectively.

the same row, when the row pool has tested positive, and with the other samples which have discordant results in the same column, when the column pool has tested positive (Figures 1 and 2). There are three exceptions to the algorithm steps above. First, if all $2n$ row and column pools test positive, then each row pool is submitted to T_2^+ with a halving step. Second, if r row pools test positive and no column pools test positive, then each of the r row pools are submitted to T_2^+ with a halving step. Finally, if c column pools test positive and no row pools test positive, then each of the c row pools are submitted to T_2^+ with a halving step.

Suppose that one of the n^2 samples is from an infected individual, and that without loss of generality, this sample resides in the (n, n) cell. The sensitivity of MT_2^+, $\psi_{MT_2^+(n|p)}$, is the probability that this sample will be identified as positive at the end of MT_2^+, with pool size n and disease prevalence p, so that

$$\begin{aligned}\psi_{MT_2^+(n|p)} &= 2S_e(1-S_e)\left\{\sum_{r=0}^{n-1} pos_n^{(1)}(r,0|p)\left(\frac{a}{n}\psi_{T_2^+(a)}+\frac{b}{n}\psi_{T_2^+(b)}\right)+\right. \\ &\qquad \left.\sum_{r=0}^{n-1}\sum_{c=1}^{n-1} pos_n^{(1)}(r,c|p)\psi_{T_2^+(n-c)}\right\}+ \\ &\qquad S_e^2\left[pos_n^{(1)}(n-1,n-1|p)\left(\frac{a}{n}\psi_{T_2^+(a)}+\frac{b}{n}\psi_{T_2^+(b)}\right)+\right. \\ &\qquad \left.\left\{\sum_{r=0}^{n-1}\sum_{c=0}^{n-1} pos_n^{(1)}(r,c|p)-pos_n^{(1)}(n-1,n-1|p)\right\}(2S_e-S_e^2)\right].\end{aligned} \tag{1}$$

Here $pos_n^{(1)}(r,c|p)$ is the probability that r of the first $n-1$ row pools and c of the first $n-1$ column pools test positive and p is the prevalence of disease.

The specificity of matrix pooling, $\phi_{MT_2^+(n|p)}$, is the probability that an uninfected sample is identified as disease free at the end of the testing algorithm, and is dependent on both the matrix size and prevalence of disease. Without loss of generality, we fix the (n, n) cell to be a sample from an uninfected individual. Then, the specificity of matrix pooling, $\phi_{MT_2^+(n|p)}$, is

$$\begin{aligned}\phi_{MT_2^+(n|p)} &= 1 - \Bigg(2P(r_n^+)P(c_n^-) \Bigg[\sum_{c=1}^{n-1} \sum_{r=0}^{n-1} pos_n^{(1)}(r, c|p) \left(1 - \phi_{T_2^+(n-c|p_B^*)}\right) + \\ &\qquad \sum_{r=0}^{n-1} pos_n^{(1)}(r, 0|p) \left\{ \frac{a}{n} \left(1 - \phi_{T_2^+(a|p_B^*)}\right) + \frac{b}{n} \left(1 - \phi_{T_2^+(b|p_B^*)}\right) \right\} \Bigg] + \\ &\quad P(r_n^+)P(c_n^+) \Bigg[pos_n^{(1)}(n-1, n-1|p) \left\{ \frac{a}{n} \left(1 - \phi_{T_2^+(a|p_A^*)}\right) + \frac{b}{n} \left(1 - \phi_{T_2^+(b|p_A^*)}\right) \right\} + \\ &\qquad (1 - S_p^2) \left\{ \sum_{r=0}^{n-1} \sum_{c=0}^{n-1} pos_n^{(1)}(r, c|p) - pos_n^{(1)}(n-1, n-1|p) \right\} \Bigg] \Bigg), \end{aligned} \tag{2}$$

where, given that the (n, n) sample is uninfected, we have the following quantities: r_n^+ and c_n^+, the probability that the n^{th} row or n^{th} column test positive; r_n^- and c_n^-, the probability that the n^{th} row or n^{th} column test negative; p_A^*, the prevalence of infected samples in the n^{th} row with a positive column pool test, given that the n^{th} row tests positive; and p_B^*, the prevalence of infected samples in the n^{th} row with a negative column pool test, given that the n^{th} row tests positive.

Finally, let $E(N)_{MT_2^+(n|p)}$ denote the expected number of tests for matrix pooling with

an $n \times n$ testing matrix and disease prevalence, p. Then,

$$\begin{aligned}
\mathcal{E}(N)_{MT_2^+(n|p)} &= 2n\,[pos_n(0,0|p)+ \\
&\quad \sum_{r=1}^{n} r\left\{\mathcal{E}(N)_{T_2^+(a|p_B)} + \mathcal{E}(N)_{T_2^+(b|p_B)}\right\} pos_n(r,0|p) + \\
&\quad \sum_{c=1}^{n} c\left\{\mathcal{E}(N)_{T_2^+(a|p_B)} + \mathcal{E}(N)_{T_2^+(b|p_B)}\right\} pos_n(0,c|p) + \\
&\quad n\left\{\mathcal{E}(N)_{T_2^+(a|p_A)} + \mathcal{E}(N)_{T_2^+(b|p_A)}\right\} pos_n(n,n|p) + \\
&\quad \sum_{r=1}^{n}\sum_{c=1}^{n}(1-\delta_{rc,n^2})\left\{rc\mathcal{E}(N)_{T_2^+(1|p_A)} + r\mathcal{E}(N)_{T_2^+(n-c|p_B)}+ \right. \\
&\quad \left. c\mathcal{E}(N)_{T_2^+(n-r|p_B)}\right\} pos_n(r,c|p)\Big],
\end{aligned} \tag{3}$$

with $\delta_{rc,n^2} = 0$ if $rc = n^2$, and where p_A is the prevalence of infected samples at the intersection of positive row pool and column pool tests; p_B is the prevalence of infected samples at the intersection of discordant row and column pool tests; and $pos_n(r,c|p)$ denoting the probability that r out of n row pools and c out of n column pools test positive.

4 Number of Stages Required to Test for Acute HIV infection

The greatest value of testing for acute HIV infection is the potential of averting new infections. Therefore, it is necessary to quickly identify an individual in the acute stage of HIV infection in order to maximize the impact of intervention. Here, we consider minimizing the number of stages required to identify an infected individual as acutely infected, where the time required for each stage is the time needed to test and process the results. Note that this is a separate measure from the sensitivity, or

the probability correctly identifying an infected individual. Here, we only consider those correctly identified as infected, and determining how many stages of testing were required for this identification.

Let $\mathcal{S}_i$ denote the number of stages required to classify an infected individual as infected. Individual testing requires the fewest stages, as it only uses one test at one stage to classify an infected individual as such, $\mathcal{S}_I = 1$. With the modified Dorfman algorithm, an infected sample must test positive at three stages — first in the master pool, which is divided into subpools if it tests positive; second in the subpool; and then individually if the subpool tests positive. Thus, the modified Dorfman method requires three stages to correctly identify an infected individual, $\mathcal{S}_{MD} = 3$.

For T_2^+, the number of stages required to correctly identify an infected sample is a function of the original pool size and test sensitivity. For each n, $n = 2^{k-1}$, then any infected sample must be subdivided into $k-1$ additional subpools beyond the master pool to be determined positive, requiring a minimum of k and maximum $2k$ stages to be determined positive at the end of T_2^+. For $2^{k-1} \leq n < 2^k$, an infected sample requires $k-1$ subpools (k levels total) with probability $(2^k - n)/n$ and k subpools ($k+1$ levels total) with probability $1 - (2^k - n)/n$. Therefore, for any pool size n, the minimum number of stages to determine an infected sample as positive is k and the maximum number is $2(k+1)$.

The expected number of stages to identify an infected sample as positive for T_2^+ is

$$\begin{aligned} \mathcal{S}_{T_2^+(n)} &= \frac{1}{\psi_{T_2^+(n)}} \sum_{s=k}^{2(k+1)} s\, P(S = s \bigcap T^+ | D^+) \\ &= \frac{1}{\psi_{T_2^+(n)}} \mathcal{S}^+_{T_2^+(n)}, \end{aligned}$$

where $\mathcal{S}^+_{T_2^+(n)}$ is the expected number of stages from the joint distribution for the number of stages and the probability that the sample is identified as infected at the end of T_2^+. Therefore, we standardize this expected value by the probability that an infected sample tests positive, or the sensitivity of T_2^+, to obtain the final quantity, $\mathcal{S}_{T_2^+(n)}$. For the joint expected value, we have

$$\begin{aligned}\mathcal{S}^+_{T_2^+(n)} &= \frac{2^k-n}{n}\sum_{i=k}^{2k} i\binom{k}{i-k} S_e^k\,(1-S_e)^{i-k} \\ &\quad + \left(1-\frac{2^k-n}{n}\right)\sum_{j=k+1}^{2(k+1)} j\binom{k+1}{j-(k+1)} S_e^{(k+1)}\,(1-S_e)^{j-(k+1)}.\end{aligned}$$

The number of stages to correctly identify an infected individual with matrix pooling is a function of both the initial matrix size, the prevalence of disease, and test sensitivity and specificity. As with T_2^+, we first look at the expected number of stages with joint distribution of the number of stages and testing positive, conditional on the sample being infected, $\mathcal{S}^+_{MT_2^+(n|p)}$. We then standardize this quantity by the sensitivity of matrix pooling for the pool size and prevalence, so that $\mathcal{S}_{MT_2^+(n|p)} = \mathcal{S}^+_{MT_2^+(n|p)}/\psi_{MT_2^+(n|p)}$.

At the first round of testing, each sample is tested twice, in a row and column pool. However, this testing occurs simultaneously, so that the round of testing is considered one stage, and the probability that the sample is not identified as negative at this stage is $2S_e - S_e^2$. If the infected sample is at the intersection of a positive row and column pool (with probability S_e^2), and not all rows and columns test positive, then the sample is tested individually, with a confirmation for negative test. This results in an added expected number of additional stages of $3S_e - 2S_e^2$. If the sample is at the intersection of discordant row and column tests (with probability $(1-S_e)^2$) or if all

of the rows and columns test positive, then the sample is submitted to T_2^+ following the steps outlined in section 3. Therefore, the expected number of stages to correctly identify an infected sample is

$$\begin{aligned}\mathcal{S}^+_{MT_2^+(n|p)} &= (2S_e - S_e^2) + 2S_e(1-S_e)\left\{\sum_{r=0}^{n-1} pos_n^{(1)}(r,0|p)\left(\frac{a}{n}\mathcal{S}_{T_2^+(a)} + \frac{b}{n}\mathcal{S}_{T_2^+(b)}\right) + \right. \\ &\left. \sum_{r=0}^{n-1}\sum_{c=1}^{n-1} pos_n^{(1)}(r,c|p)\mathcal{S}_{T_2^+(n-c)}\right\} + \\ &S_e^2\left[pos_n^{(1)}(n-1,n-1|p)\left(\frac{a}{n}\mathcal{S}_{T_2^+(a)} + \frac{b}{n}\mathcal{S}_{T_2^+(b)}\right) + \right. \\ &\left.\left\{\sum_{r=0}^{n-1}\sum_{c=0}^{n-1} pos_n^{(1)}(r,c|p) - pos_n^{(1)}(n-1,n-1|p)\right\}(3S_e - 2S_e^2)\right],\end{aligned}$$

where $a = \lceil n/2 \rceil$ and $b = \lfloor n/2 \rfloor$.

5 Comparison of Matrix Pooling to Individual Testing, Modified Dorfman, and T_2^+

In this section, we first compare the accuracy, as measured by the false positive and false negative predictive values, and the expected number of tests per result of MT_2^+ to individual testing, the modified Dorfman pooling algorithm and T_2^+. The false negative predictive value (FNPV) is the probability that a sample identified as negative at the end of a testing algorithm is truly infected, and for testing method i, is written as

$$FNPV_i = \frac{(1-\psi_i)p}{(1-\psi_i)p + \phi_i(1-p)}.$$

Minimizing the FNPV in the case of acute HIV infection is imperative in order maximize the opportunities for risky behavior intervention during this highly infectious stage. In the case of screening blood, identifying an acutely infected individual prevents an infected unit of blood from entering the blood supply.

The false positive predictive value (FPPV) is the probability that a sample is determined to be positive is truly uninfected, and is expressed as

$$FPPV_i = \frac{(1-\phi_i)(1-p)}{(1-\phi_i)(1-p)+\psi_i p}.$$

Generally, minimizing this value is of second priority to minimizing the FNPV, arguing that eventually the individual falsely identified as positive will be determined to be uninfected. However, in the case of HIV, where infection can be highly stigmatized, a false positive result can lead to extreme emotional and, in some cases, physical harm. Therefore, it is important to consider minimizing this quantity as well.

Finally, a reduction in the expected number of tests per result should translate into decreased costs in the testing procedure. A reduction in cost can increase the feasibility of screening for acute infections, even in resource constrained environments.

Tables 1–3 show the analytic results for the FPPV, FNPV, and expected number of tests per result for three prevalence levels: 0.03%, 0.3% and 3.0%. These prevalences of acute infection in HIV antibody negative individuals approximate those observed in the general testing population in North Carolina, in the STD clinic population in San Francisco, and in the STD/dermatology clinic population in Malawi, respectively ([8], [10], [12]). We restrict our discussion to these levels of acute infection because they represent an extreme range of incidence rates — from a rate of new infections in a general testing population in a low prevalence setting to the rate of new infections

in a high risk population in a high prevalence setting. Our tables include two test accuracies, $S_e = S_p = 0.95$ and $S_e = S_p = 0.99$, reflecting the high accuracy in the testing methods for acute HIV.

We consider two scenarios of the modified Dorfman pooling algorithm (one with a master pool size of 90 and nine subpools, one with 50 samples in the master pool and five subpools), reflecting the pooling procedure used in the North Carolina and Malawi studies referenced to above. We present results for four matrix sizes — 4×4, 8×8, 12×12, and 16×16. Any matrix size larger than 16×16 requires more than 200 samples, which in many setting would take too long to collect. Finally, we present T_2^+ for pool sizes $n = 4, 8, 12$, and 16 for easy comparison to matrix pooling, and for $n = 50$ and 90 for comparison to the modified Dorfman method. This paper presents the analytic results in Tables 1 - 3, but each has been confirmed with simulations.

For the prevalence levels and combinations of sensitivity and specificity shown, matrix pooling out performs individual testing in all three areas: false positive predictive values, false negative predictive values and expected number of tests per result. Compared to individual testing, matrix pooling reduces the false positive predictive value by 20–80%, when test sensitivity and specificity is equal to 0.95, and 67–99% when the test sensitivity and specificity is equal to 0.99. The false negative predictive value is 8 – 10 times higher when testing samples individually, if the sensitivity and specificity is 0.95, and as much as fifty times higher if the sensitivity and specificity is 0.99. Even with this sizable increase of accuracy, matrix pooling requires between 30 – 85% fewer tests per result compared to individual testing.

Matrix pooling does not greatly impact the false positive predictive values compared to the modified Dorfman method at the lowest prevalence level when the sensitivity and specificity are 0.95. At the remaining prevalence levels and sensitivity/specificity

Table 1: Comparing matrix pooling to other testing algorithms, p = 3/10,000

prevalence = 3/10,000	FPPV		FNPV ($\times 10^{-6}$)		Expected Number of Tests per Result	
Individual Testing	0.9943	(0.9711)	15.79	(3.03)	1	(1)
MD(90,9)	0.5366	(0.0903)	42.81	(8.91)	0.0247	(0.0179)
MD(50,5)	0.5132	(0.0860)	42.81	(8.91)	0.0320	(0.0255)
$T_2^+(90)$	0.1287	(0.0257)	5.64	(0.23)	0.0331	(0.0287)
$T_2^+(50)$	0.1286	(0.0255)	5.01	(0.20)	0.0537	(0.0464)
$T_2^+(16)$	0.1311	(0.0203)	3.73	(0.15)	0.1549	(0.1329)
$T_2^+(12)$	0.2134	(0.0267)	3.49	(0.14)	0.2049	(0.1757)
$T_2^+(8)$	0.2978	(0.0208)	2.99	(0.12)	0.3049	(0.2615)
$T_2^+(4)$	0.7634	(0.0448)	2.25	(0.09)	0.6026	(0.5196)
$MT_2^+(16)$	0.4957	(0.0140)	1.75	(0.06)	0.1539	(0.1316)
$MT_2^+(12)$	0.5015	(0.0122)	1.71	(0.06)	0.2045	(0.1750)
$MT_2^+(8)$	0.5295	(0.0105)	1.66	(0.06)	0.3067	(0.2617)
$MT_2^+(4)$	0.7860	(0.0348)	1.58	(0.06)	0.6123	(0.5220)

The false positive and false negative predictive values and expected number of tests per result, for a sensitivity and specificity of 95% and sensitivity and specificity of 99% (in parentheses).

Table 2: Comparing matrix pooling to other testing algorithms, p = 3/1,000

prevalence = 3/1,000	FPPV		FNPV ($\times 10^{-5}$)		Expected Number of Tests per Result	
Individual Testing	0.9459	(0.7705)	15.83	(3.04)	1	(1)
MD(90,9)	0.4053	(0.0856)	42.97	(8.94)	0.0750	(0.0653)
MD(50,5)	0.3698	(0.0806)	42.96	(8.94)	0.0702	(0.0627)
$T_2^+(90)$	0.1281	(0.0256)	5.66	(0.23)	0.0861	(0.0781)
$T_2^+(50)$	0.1274	(0.0254)	5.02	(0.20)	0.0999	(0.0896)
$T_2^+(16)$	0.1100	(0.0202)	3.74	(0.15)	0.1865	(0.1627)
$T_2^+(12)$	0.1390	(0.0264)	3.50	(0.14)	0.2331	(0.2028)
$T_2^+(8)$	0.1303	(0.0203)	3.00	(0.12)	0.3275	(0.2832)
$T_2^+(4)$	0.2977	(0.0227)	2.25	(0.09)	0.6161	(0.5331)
$MT_2^+(16)$	0.2167	(0.0188)	1.76	(0.06)	0.1778	(0.1498)
$MT_2^+(12)$	0.1852	(0.0122)	1.73	(0.06)	0.2248	(0.1912)
$MT_2^+(8)$	0.1624	(0.0067)	1.67	(0.06)	0.3239	(0.2765)
$MT_2^+(4)$	0.3026	(0.0074)	1.58	(0.06)	0.6270	(0.5359)

The false positive and false negative predictive values and expected number of tests per result, for a sensitivity and specificity of 95% and sensitivity and specificity of 99% (in parentheses).

Table 3: Comparing matrix pooling to other testing algorithms, p = 3/100

prevalence = 3/100	FPPV		FNPV ($\times 10^{-4}$)		Expected Number of Tests per Result	
Individual Testing	0.6299	(0.2462)	16.25	(3.12)	1	1
MD(90,9)	0.3185	(0.0740)	44.47	(9.20)	0.3675	(0.3655)
MD(50,5)	0.3080	(0.0720)	44.43	(9.20)	0.3500	(0.3511)
$T_2^+(90)$	0.1232	(0.0248)	5.83	(0.23)	0.4777	(0.4772)
$T_2^+(50)$	0.1225	(0.0246)	5.18	(0.21)	0.4702	(0.4388)
$T_2^+(16)$	0.1042	(0.0197)	3.86	(0.16)	0.4709	(0.4524)
$T_2^+(12)$	0.1260	(0.0255)	3.61	(0.14)	0.4926	(0.4524)
$T_2^+(8)$	0.1056	(0.0197)	3.09	(0.12)	0.5392	(0.4874)
$T_2^+(4)$	0.1229	(0.0199)	2.32	(0.09)	0.7456	(0.6632)
$MT_2^+(16)$	0.3210	(0.0812)	1.81	(0.06)	0.5731	(0.5240)
$MT_2^+(12)$	0.2381	(0.0515)	1.78	(0.06)	0.5274	(0.4696)
$MT_2^+(8)$	0.1504	(0.0252)	1.74	(0.06)	0.5425	(0.4754)
$MT_2^+(4)$	0.1087	(0.0084)	1.64	(0.06)	0.7801	(0.6827)

The false positive and false negative predictive values and expected number of tests per result, for a sensitivity and specificity of 95% and sensitivity and specificity of 99% (in parentheses).

combinations, with few exceptions, matrix pooling improves the false positive predictive values as much as 66% over modified Dorfman (99% when sensitivity and specificity are 0.99). More importantly, the false negative predictive value of the modified Dorfman method is approximately 25 fold larger when the sensitivity and specificity are 0.95 compared to matrix pooling (nearly 150 times larger when sensitivity and specificity are 0.99). However, this great increase in accuracy comes at a cost. Matrix pooling does require two to ten times more tests per result compared to the modified Dorfman method.

The comparison of matrix pooling to T_2^+ is much more complicated. The FPPV is consistently the same or lower with T_2^+ compared to MT_2^+, as much as a sixth the rate. The FNPV of T_2^+ is marginally larger than MT_2^+, by an order of 1.3 – 3.6 times larger. However, the FNPV of T_2^+ is still considerably lower than individual testing and the modified Dorfman algorithm. When the T_2^+ pool size is the same as matrix pooling row or column pool sizes then both algorithms require approximately the same number of tests per result. However, larger T_2^+ pool sizes results in savings similar to what we observe with large pool sizes and the modified Dorfman algorithm, especially for lower prevalences.

The true benefit of MT_2^+ over T_2^+ is in the timeliness of correctly identifying an infected individual, allowing more time for interventions to avert new infections. Figure 3 shows the average number of stages required for identifying an infected individual as positive for T_2^+ and MT_2^+ ($\mathcal{S}_{T_2^+(n)}$ and $\mathcal{S}_{MT_2^+(n|p)}$). On average, matrix pooling requires between 2–3 stages to correctly identify an infected individual, for all matrix sizes and test sensitivity between 0.90–1.00. Note that we only present the graph for $p = 3/10,000$ and $S_p = 0.99$, but the results remain the same for the other prevalence levels and specificities presented in this paper. Even at the smallest pool size, $n = 4$,

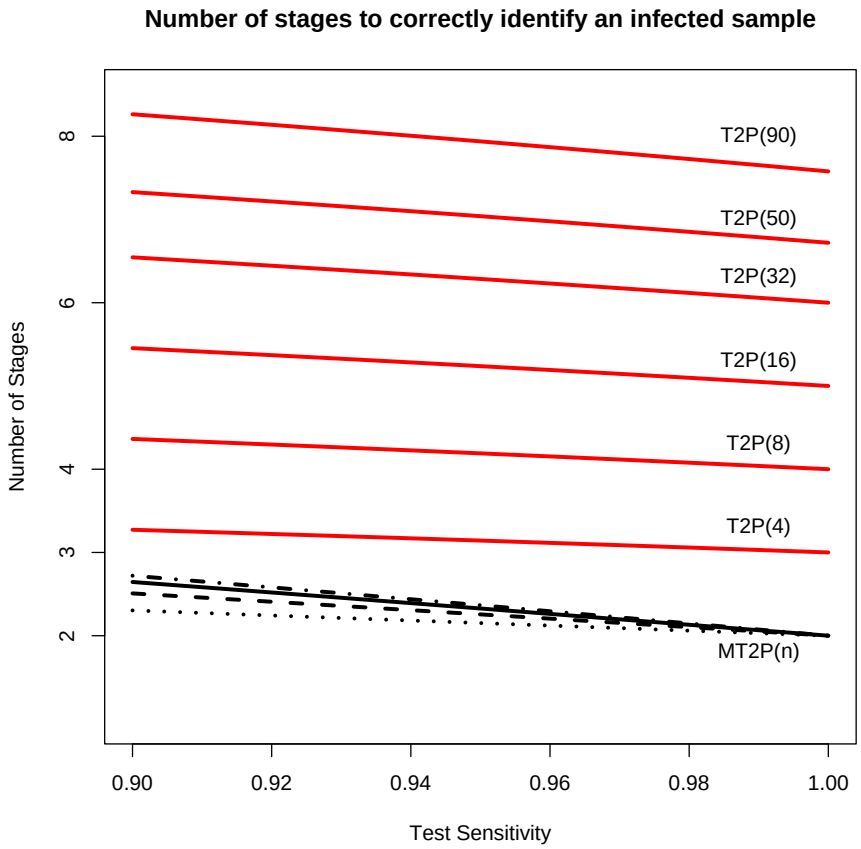

Figure 3: The number of stages required to correctly identify an infected sample. The red lines display results for T_2^+; the black lines for MT_2^+ (n=4, dotted; n=8, dashed; n=12, solid; n=16, dot-dashed). $S_p = 0.99, p = 3/10,000$.

T_2^+ requires at least three stages. This number increases so that at the largest pool size, $n = 90$, T_2^+ requires, on average, approximately eight stages, potentially losing the value of testing for acute infection for the purpose of intervention.

These results beg the question, how do we select a testing strategy for acute HIV in practice? For now, we will focus on selecting a testing algorithm based only on two parameters - the expected number of tests per result and the number of people in the acute phase of infection correctly identified by the testing algorithm. We no longer discuss T_2^+, because of the increased amount of time required to obtain the positive

results for an infected individual at the pool sizes where there is a clear benefit of T_2^+ over matrix pooling in terms of reducing the expected number of tests per sample.

For each prevalence level, we present the one modified Dorfman algorithm and one matrix pooling strategy that minimizes the expected number of tests per result, since the false negative predictive values within a testing method are approximately constant for a fixed prevalence, sensitivity and specificity. Suppose we anticipate testing 100,000 individuals. Tables 4 and 5 show the number of infected people correctly and incorrectly identified by each algorithm. These tables also present the number of tests required per infected sample identified and the additional number of tests per additional infected sample identified with matrix pooling compared to the modified Dorfman.

As noted before, the modified Dorfman is a cheaper method per case identified. However, by employing the more accurate matrix pooling algorithm, we can identify more acute cases, potentially averting more infections. For the sensitivity and specificity of 0.95, this requires on average 3,151 tests per additional case identified in the lowest prevalence setting, and 43.2 tests per additional case identified in the highest prevalence setting. Assuming a NAAT costs between \$30 – \$80, this results in approximately \$95,000 – \$252,000 per additional case identified in low prevalence settings and \$1,300 – \$3,500 in high prevalence settings. At a higher test sensitivity and specificity (0.99), it is more expensive to identify an additional case — as much as \$383,250 - \$1,022,000 in low prevalence areas and \$4,020 – \$10,720 in high prevalence areas. It is difficult to place a numeric value on identifying a new HIV infection. Factors such as the estimated number of infections averted per case identified, both in primary and secondary infections, influence this value. One must also consider the monies

Table 4: Number of tests per additional acute case identified, $S_e = S_p = 0.95$

	p=3/10,000		p=3/1,000		p=3/100	
	MD(90,9)	$MT_2^+(16)$	MD(50,5)	$MT_2^+(16)$	MD(90,9)	$MT_2^+(12)$
Number of Infected Samples Correctly Identified	25.7	29.8	257.2	298.2	2,572.1	2,982.9
Number of Infected Samples Identified as Uninfected	4.3	0.2	42.8	1.8	427.9	17.1
Number of Tests	2,470	15,390	7,020	17,780	35,000	52,740
Number of Tests per Infected Sample Identified	96.1	516.4	27.3	59.6	13.6	17.7
Number of Tests per Additional Infected Sample Identified		3,151.2		262.4		43.2

Table 5: Cost per additional acute case identified, $S_e = S_p = 0.99$

	p=3/10,000		p=3/1,000		p=3/100	
	MD(90,9)	$MT_2^+(16)$	MD(50,5)	$MT_2^+(16)$	MD(90,9)	$MT_2^+(12)$
Number of Infected Samples Correctly Identified	29.1	> 29.99	291.1	299.9	2,910.9	2,999.4
Number of Infected Samples Identified as Uninfected	0.9	< 0.01	8.9	0.1	89.1	0.6
Number of Tests	1,790	13,160	6,270	14,980	35,110	46,960
Number of Tests per Infected Sample Identified	61.5	438.8	21.5	49.9	12.1	15.7
Number of Tests per Additional Infected Sample Identified		12,775.3		989.8		133.9

saved in terms of treatment and care for the infections averted. Finally, identifying and intervening with acute HIV infections has huge potential impact for curbing the epidemic, emphasizing the value of prioritizing accuracy over cost. Ultimately, all of these factors must be considered on a case-by-case basis when determining the most appropriate algorithm for a particular scenario.

6 Conclusion

There are multiple tests available to identify an individual as HIV infected, either through antibody or antigen detection. The choice of test, or combination of tests, depends on the goals of testing as well as the availability of resources. Antibody detecting tests are most commonly used to identify individuals who are HIV infected for the purpose of clinical intervention because of the low cost. In many contexts, especially low prevalence situations, very few additional cases would be identified with antigen detection through NAAT. However, recent infection is marked with high viral load and can indicate recent risky behavior. This biologically supports evidence that individuals with acute HIV infection are drivers of the epidemic [2]. Therefore, identifying these individuals provides an excellent opportunity for interventions to prevent further infections.

Changing the way that a test is administered by pooling samples reduces the expected number of tests, and therefore makes detection of acute HIV affordable. If the single goal is to save money, then the modified Dorfman testing algorithm is the superior method compared to individual testing and matrix pooling. However, because of the extreme risks associated with missing an individual who is truly in the early phases of HIV infection, and negative consequences of falsely informing a person that

he/she is HIV positive, it is also important to factor in the accuracy of the test. In almost all cases presented here, the matrix pooling method performs better than the modified Dorfman algorithm and individual testing with respect to the false positive predictive value and matrix pooling has far better false negative predictive values at the plausible prevalences, sensitivities, and specificities.

The FPPV and FNPV of T_2^+ are very similar to that of matrix pooling, sometimes offering a slight advantage over MT_2^+ and at times performing slightly worse. This is not surprising since matrix pooling is an extension of the T_2^+ testing algorithm. However, MT_2^+ offers the clear benefit of identifying an infected individual as positive more quickly, which again may lead to faster, more effective interventions. Also, matrix pooling has the advantage of testing each sample in at least two unique pools. This property is beneficial if the chemical compositions of different samples can react, risking one sample blocking or amplifying the effect of another.

The matrix pooling testing algorithm is more complicated to implement when compared to individual testing and the modified Dorfman method. However, automating the procedure will allow testing for acute HIV infections using MT_2^+ with minimal human error introduced. Another possible limitation is that we derive our theory under the strong assumption that the sensitivity and specificity of an individual test is not compromised by the formation of pools. This is a common assumption when testing for acute HIV, and a testable assumption if one wants to use matrix pooling for other diagnostic settings. Also, if the sensitivity is slightly reduced by pooling, it is possible to adjust our overall algorithm estimates of sensitivity accordingly and appropriately compare to the other proposed methods.

Because of the decrease in costs, pooling techniques are currently being used to test for acute HIV infections. However, the accuracy of the testing is compromised by

the process. The matrix pooling method introduced here provides a more affordable alternative to testing as compared to individual testing, whilst reducing the false positive and false negative predictive values. Given these desirable testing properties, adopting the matrix pooling method is recommended, especially since it can make testing for acute HIV infection possible in many settings where it would otherwise not be feasible.

Appendix A: Properties of the Pooling Methods

A.1 Modified Dorfman

In this section, we present the derivations of the sensitivity, specificity and expected number of tests for the modified Dorfman method. In order for an infected sample to be declared positive via the modified Dorfman pooling method [13], it must test positive three times, first in the master pool, then in the subpool, then individually. Therefore, the sensitivity of the modified Dorfman method is $\psi_{MD} = S_e^3$.

To determine the specificity of the modified Dorfman algorithm, fix one sample in the pool to be uninfected. Let D_1^+ be the event that the master pool tests positive and D_1^- be the event that the master pool tests negative, given one sample is negative. Then

$$\begin{aligned} P(D_1^-) &= S_p(1-p)^{n-1} + (1-S_e)\left\{1-(1-p)^{n-1}\right\} \qquad \text{(A.1)} \\ P(D_1^+) &= 1 - P(D_1^-). \end{aligned}$$

Let D_2^+ be the event that the subpool tests positive and D_2^- be the event that the subpool tests negative, given one sample is uninfected and the master pool tests positive. Then, similarly

$$\begin{aligned} P(D_2^-) &= S_p(1-p^*)^{k-1} + (1-S_e)\left\{1-(1-p^*)^{k-1}\right\} \qquad \text{(A.2)} \\ P(D_2^+) &= 1 - P(D_2^-), \end{aligned}$$

where now p^* is the prevalence in the subpool, excluding the one sampled fixed to be

uninfected, given that the master pool tests positive. Thus,

$$\begin{aligned} p^* &= P(\text{sample } i \text{ is positive} | D_1^+ \text{ and one sample is negative}) \\ &= \frac{S_e p}{S_e p + \left[(1-p)^{n-2}(1-S_p) + S_e\{1-(1-p)^{n-2}\}\right](1-p)}. \end{aligned} \tag{A.3}$$

The specificity in the modified Dorfman method is the probability that the sample fixed to be uninfected is identified as disease negative by either the master pool testing negative, the subpool testing negative in the event that master pool tests positive, or the individual sample testing negative in the event that the master pool and subsequently the subpool tests positive. Using the above notation, the specificity of the modified Dorfman method is,

$$\phi_{MD(n,m|p)} = P(D_1^-) + P(D_1^+)\left\{P(D_2^-) + P(D_2^+)S_p\right\}. \tag{A.4}$$

To determine the expected number of tests for this method, let G_1^+ be the event that the master pool tests positive. Then

$$P(G_1^+) = (1-S_p)(1-p)^n + S_e\left\{1-(1-p)^n\right\}. \tag{A.5}$$

Let G_2^+ be the event that the subpool tests positive. Then,

$$P(G_2^+) = S_p(1-p')^k + (1-S_e)\left\{1-(1-p')^k\right\}, \tag{A.6}$$

where p' is the prevalence of infected samples in the subpool, given the master pool tests positive and thus

$$p' = \frac{S_e p}{S_e p + [(1-p)^{n-1}(1-S_p) + S_e\{1-(1-p)^{n-1}\}](1-p)}. \tag{A.7}$$

The required number of tests using the modified Dorfman testing algorithm is one, if the master pool tests negative, $1 + m + jk$ if the master pool tests positive and j of the m subpools test positive. Therefore, using the previous notation, the expected number of tests for the modified Dorfman method is

$$\mathcal{E}(N)_{MD(n,m|p)} \quad = \quad 1 + P(G_1^+)\left\{m + P(G_2^+)(mk)\right\} \tag{A.8}$$

A.2 $\mathbf{T}_2^+$

Here we summarize the sensitivity, specificity and expected number tests as derived elsewhere.

The sensitivity of T_2^+ is

$$\psi_{T_2^+(n)} = (2S_e - S_e^2)\left[\frac{a}{n}\psi_{T_2^+(a)} + \frac{b}{n}\psi_{T_2^+(b)}\right]$$

with $a = \lceil n/2 \rceil$ and $b = \lfloor n/2 \rfloor$. To start the iteration, we have $\psi_{T_2^+(1)} = (2S_e - S_e^2)$.

For specificity, we first define the probability of identifying an uninfected sample as positive, conditional on the number of infected samples in the remaining $n-1$ samples in the pool, y. Here,

$$
\begin{aligned}
FP(n|Y=0) &= \left[\frac{a}{n}FP(a|Y=0)+\frac{b}{n}FP(b|Y=0)\right](1-S_p^2) \\
FP(n|Y=c) &= \left[\frac{a}{n}\sum_{i=\lambda_a}^{\nu_a}\frac{\binom{a-1}{i}\binom{b}{c-i}}{\binom{n-1}{c}}FP(a|Y=i)+\frac{b}{n}\sum_{j=\lambda_b}^{\nu_b}\frac{\binom{b-1}{j}\binom{a}{c-j}}{\binom{n-1}{c}}FP(a|Y=j)\right](2S_e-S_e^2)
\end{aligned}
$$

where a and b are defined above and $\lambda_a = max(0, c-(m-a))$, $\nu_a = min(a-1, c)$, $\lambda_b = max(0, c-(m-b))$, and $\nu_b = min(b-1, c)$. The iteration starts with $FP(1|y = 0) = 1 - S_p^2$. Now, we have the false positive rate, conditional on p, as

$$
\begin{aligned}
FP(n|p) &= \sum_{i=0}^{n-1} FP(n|Y=i)P(Y=i), \text{ and} \\
\phi_{T_2^+(n|p)} &= 1 - FN(n|p) \qquad \text{(A.9)}
\end{aligned}
$$

where $P(Y = c)$ is based on the binomial distribution of $n-1$ samples and a prevalence p.

Finally, we first express the expected number of tests for T_2^+ conditioned on the number of positive samples in the pool, so that

$$
\begin{aligned}
\mathcal{E}(N)_{T_2^+(n|Y=0)} &= 1 + S_p + (1-S_p^2)\left[\mathcal{E}(N)_{T_2^+(a|Y=0)} + \mathcal{E}(N)_{T_2^+(b|Y=0)}\right] \\
\mathcal{E}(N)_{T_2^+(n|Y=c)} &= 2 - S_e + (2S_e - S_e^2)\left[\sum_{i=\lambda}^{\nu}\frac{\binom{a}{i}\binom{b}{c-i}}{\binom{n}{c}}[\mathcal{E}(N)_{T_2^+(a|Y=i)} \right. \\
&\qquad \left. + \mathcal{E}(N)_{T_2^+(b|Y=c-i)}]\right](2S_e - S_e^2),
\end{aligned}
$$

where $a = \lceil n/2 \rceil$, $b = \lfloor n/2 \rfloor$, $\lambda = max(0, c-b)$, and $\nu = min(a, c)$. The iteration

starts with $\mathcal{E}(N)_{T_2^+(1|Y=0)} = 1+S_p$ and $\mathcal{E}(N)_{T_2^+(1|Y=1)} = 2-S_e$. It follows immediately that the expected number of tests, conditional on the prevalence of disease, p, is

$$\mathcal{E}(N)_{T_2^+(n|p)} = \sum_{i=0}^{n} \mathcal{E}(N)_{T_2^+(n|Y=i)} P(Y=i).$$

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