Group Testing with Blockers and Synergism

Minge Xie, Kay Tatsuoka, Jerome Sacks and S. Stanley Young

ABSTRACT

Discovery and development of a new drug can cost hundreds of millions of dollars. Pharmaceutical companies have routinely used group testing methodology (Dorfman 1943) as one of the efficient High Throughput Screening techniques to search for “lead” compounds among collections of hundreds of thousands of chemical compounds. The lead compounds can be modified to produce new and effective molecules, which eventually may lead to new drugs. This paper develops models and estimation procedures to obtain quantitative information from data in such applications. It investigates group testing procedures and studies cost efficiency when the standard assumption adopted by Dorfman, that tested items act independently of one another, is violated. The investigation is focused on, but not limited to, the square array pooling method (Phatarfod and Sudbury 1994), and the methodologies developed are illustrated through simulations and a drug discovery data set from the Glaxo Wellcome Inc.

Key Words: Cost efficiency; Drug discovery; EM algorithm; Estimation; Screening.

1. INTRODUCTION

The Dorfman (1943) group testing procedure, which tests samples in groups of individuals, can be a cost-effective alternative to screening each individual. In general, it deals with binary type (positive or negative) responses, and the method is especially effective in

\[\text{Reference:} \]
situations when the positive rate (the proportion of positives in a population, also known as prevalence in medical applications) is small but the number of individuals to be tested is large. Many applications use the group testing method to identify positive individuals, but some also use the group testing method to estimate the prevalence of positives in the population studied (cf. Sobel and Elashoff 1975; Gastwirth and Hammick 1989; Chen and Swallow 1990; and Hardwick, Page and Stout 1998). There is a large literature on Dorfman group testing method and its variants, both in statistics and in biomedical and industrial applications. Much of the statistical focus has been on the optimal group size and improved re-testing schemes (see, e.g., some recent publications, Yao and Hwang 1990; Hughes-Oliver and Swallow 1994; Phatarfod and Sudbury 1994; and Brookmeyer 1999). These re-testing schemes either improve efficiency of the screening process or have better statistical properties for estimation or inference. Sometimes the re-testing scheme is tailored for special applications. For instance, Litvak, Tu, and Pagano (1994) and Gastwirth and Johnson (1994) used different re-testing schemes to propose cost-effective quality control procedures under the context of HIV infection and drug use.

In this paper we consider the application of the group testing methodology in drug discovery. Pharmaceutical companies have amassed collections of hundreds of thousands of chemical compounds. In an early stage of drug discovery, the large collections of chemical compounds are screened to find highly active compounds, called “lead” compounds. The lead compounds are modified to produce new and effective molecules, which eventually may lead to new drugs. Since discovery and development of a new drug can cost hundreds of millions of dollars, it is critical to reduce the cost by developing economical ways to find new drugs. The group testing methodology, as one of the efficient High Throughput Screening techniques, is often used in the early screening stage of drug discovery.

One standard assumption adopted by Dorfman (1943) and many others is that a group with a positive response has at least one positive and a group with a negative response has no positives. In drug discovery, however, there are complications: (1) a small percentage of positive groups contain no single potent compounds, as a pair or some set of inactive compounds, when placed together, can result in a positive response; (2) some tested negative groups will contain a potent compound, because certain compounds will block the detection of a potent compound when placed in the same pool as the potent compound. Langfeldt,
Hughes-Oliver, Ghosh, and Young (1997) recognized the second complication, and they called those compounds that block the detection of potent compounds \textit{blockers}. Similar phenomena has been noted in blood testing applications by Phatarfod and Sudbury (1994).

With blockers in the pools, the false negative rate, defined as the probability of misidentifying a positive as a negative, is not zero. Phatarfod and Sudbury (1994) and Langfeldt et al. (1997) suggested a square array pooling method as a way to reduce the false negative rate. Phatarfod and Sudbury (1994) compared the cost efficiencies of a couple of squared array pooling methods with the standard Dorfman group testing method and later extended their discussions to the case of blockers. Langfeldt et al. (1997) calculated optimal pooling sizes of several group testing methods based on a proposed cost function and a set of hypothesized cost values. Both papers assumed the positive and blocker rates were known. In this paper, we study models, pooling strategies, and estimation problems in the presence of blockers (the second complication), and later extend the discussion to cases when additionally there exist synergistic or additive effects (the first complication). Most of our discussions are focused on, but not limited to, the square array pooling method. In addition, we also discuss cost-efficiency and provide a method to find optimal group sizes. The results have potential applications in other areas such as blood screening and HIV testing.

Throughout the paper we adopt two assumptions. First, the behavior of blockers and positive responses will not depend on the size of the pool. Our justification of this assumption is based on an internal technical report from Glaxo Wellcome. It is concluded that, when the pool size was less than or equal to 20, the dilution effect on group testing samples of chemical compounds is very slight for a wide range of assay methods. Second, in order to simplify the discussion, the assay method is assumed to be perfectly accurate, i.e., a positive sample (group or individual) will always be tested positive and a negative sample (group or individual) will always be tested negative. The methodologies developed in this paper can be directly extended to the cases when the assay method is not perfectly accurate. We adopt this assumption simply to avoid further complications in our notations and discussions.

The rest of the paper is arranged as follows. Section 2 discusses models and pooling schemes in the presence of blockers. The Dorfman pooling method (one-way) is extended to square array (two-way) and multiple-way pooling methods. Section 3 develops an estimation procedure for samples from a square array pooling method. Section 4 proposes a flexible
new scheme to select individuals for re-testing. Section 5 extends the model and method
developed in the previous sections to deal with synergism and additive effects in pools.
Section 6 provides discussion on cost efficiency and suggests a method to produce optimal
choices of pool size. Section 7 applies our methods to a drug discovery data set from Glaxo
Wellcome. Finally, Section 8 provides some further comments.

2. GROUP TESTING SCHEMES WITH BLOCKERS

The presence of the blockers in a group testing procedure has been noted in several
applications. In blood testing experiments, Phatarfod and Sudbury (1994) discussed the
possibility that, when samples are pooled, one blood sample can neutralize a positive sample.
For example, a sample from an individual with a very high titer for anti-HB$_2$Ag (the antibody
to Hepatitis B surface antigen) could mask a sample with low levels of HB$_2$Ag. Gastwirth and
Johnson (1994) stated that, due to the possible “slight loss of sensitivity”, i.e., slightly higher
chance to falsely declare a positive individual as a negative, the group testing methodology
had not been adopted in the United States to screen blood donors. The presence of blockers
may be one cause of this loss of sensitivity in group testing. Langfeldt et al. (1997) pointed
out the presence of “blocking compounds” in drug discovery applications. The “blocking
compounds” can “mask the effect of active compounds”, as certain chemical compounds
can bind with potent compounds and cause non-potent responses. Since the chance for an
individual to be both a positive and a blocker is extremely small, Phatarfod and Sudbury
(1994) and Langfeldt et al. (1997) assumed that an individual cannot be both a positive
and a blocker. So, each individual can be either a positive, or a blocker, or neither of them.

Suppose the individuals considered in the group testing are randomly selected from a
population. Let $W$ and $V$ be the indicator variables for an individual being a positive or a
blocker, respectively. $1 - W - V$ is the indicator variable for an individual being neither of
them. Since each individual falls into one of the three categories, instead of the standard
binomial model assumption, we model each individual by a trinomial distribution,

$$(W, V, 1 - W - V) \sim \text{Multinomial}(1; p, f, 1 - p - f),$$

where $p$ is the positive rate and $f$ is the blocker rate in the population. This trinomial model
assumption is implicit in Phatarfod and Sudbury (1994) and Langfeldt et al. (1997).
Suppose there are \( nk \) individuals randomly selected from a population and pooled into \( n \) groups of size \( k \). Then, a group response is positive if and only if it contains at least one positive individual and no blockers,

\[
X^{(l)} = \mathbf{1}_{\left( \sum_{i=1}^{k} W_{i}^{(l)} > 0, \sum_{i=1}^{k} V_{i}^{(l)} = 0 \right)}, \quad l = 1, 2, \cdots, n,
\]

where \( X^{(l)} \) is the test response of the \( l \)th group and \((W_{i}^{(l)}, V_{i}^{(l)})\) are the indicator variables for the \( i \)th individual in the \( l \)th group. The function \( \mathbf{1}_{C} \) in \( (2) \) is the indicator function that equals 1 (positive) if set \( C \) is true and equals 0 (negative) if \( C \) is false. Since without any extra effort \( V \) cannot be identified, we assume that \( V \)’s are not observed; the available observations are the group responses \( X \)’s and perhaps some of the \( W \)’s that are specified by a particular group testing scheme.

If \( f = 0 \), i.e., there are no blockers in the pools, \( (2) \) becomes the more standard binomial model assumption adopted by Dorfman (1943) and many others,

\[
X^{(l)} = \mathbf{1}_{\left( \sum_{i=1}^{k} W_{i}^{(l)} > 0 \right)}, \quad l = 1, 2, \cdots, n.
\]

Under the standard Dorfman scheme, the available observations include the group responses \( \mathbf{x} = (\mathbf{x}^{(1)}, \ldots, \mathbf{x}^{(n)}) \) and some individual responses, i.e., the decodes of the \( r = \sum_{i=1}^{n} \mathbf{x}^{(l)} \) positive groups, \( \mathbf{w}_{\text{obs}} = (w_{1}^{(1)}, \ldots, w_{k}^{(1)}, \ldots, w_{1}^{(r)}, \ldots, w_{k}^{(r)}) \), where, without loss of generality, we assumed that the first \( r \) groups are positive. When there are no blockers, the positive rate can be estimated by the number of identified positive individuals divided by \( nk \). Using only the group sample responses \( \mathbf{x} \), Gastwirth and Hammick (1989) obtained the maximum likelihood estimate of the positive rate \( \hat{p} \); their approach also covered situations when the assay method is not perfectly accurate.

With the presence of blockers, we cannot estimate the parameters \( p \) and \( f \) by using only the group responses \( \mathbf{x} \), since any pairs of \((p, f)\) with the same value of \((1-f)^k - (1-p-f)^k\) have the same likelihood and \( p \) and \( f \) are not identifiable. Under the standard Dorfman testing scheme, we have observations \((\mathbf{x}, \mathbf{w}_{\text{obs}})\). The likelihood function is

\[
L(p, f | \mathbf{x}, \mathbf{w}_{\text{obs}}) = \{1 - (1-f)^k + (1-p-f)^k\}^{n-r} (1-p-f)^{kr} \left( \frac{p}{1-p-f} \right)^{\sum_{i=1}^{r} \sum_{i=1}^{k} w_{i}^{(l)}}.
\]

By directly maximizing this function, we can obtain the estimates of \( p \) and \( f \).

In laboratories, individual samples are often placed in square or rectangular trays. Phatarfod and Sudbury (1994) proposed a square array (say, size \( k \times k \)) pooling method, in which
individual samples in each of the $k$ rows as well as each of the $k$ columns are pooled together, and the resulting $2k$ groups of mixed samples are tested. Suppose we have a total of $nk^2$ individuals placed in $n$ $k \times k$ square arrays. In the $l$th square array, $l = 1, \ldots, n$, the responses of the $i$th row-wise and the $j$th column-wise pooled groups are determined, respectively, by

$$X_i^{(l)} = 1_{\{\sum_{j=1}^{k} W_{ij}^{(l)} > 0, \sum_{j=1}^{k} V_{ij}^{(l)} = 0\}} \text{ and } Y_j^{(l)} = 1_{\{\sum_{i=1}^{n} W_{ij}^{(l)} > 0, \sum_{i=1}^{n} V_{ij}^{(l)} = 0\}},$$

where $(W_{ij}^{(l)}, V_{ij}^{(l)})$ are the indicator variables for the $(i, j)$ individual in the $l$th square array.

Phatarfod and Sudbury (1994) proposed a basic scheme (referred to as the AND scheme by Langfeldt et al., 1997) that only select individuals at the intersection of a positive row and a positive column for re-testing. They showed that, in several situations, the square array pooling method with the AND re-testing scheme requires less number of tests per individual than the standard Dorfman testing procedure does. However, Langfeldt et al. (1997) noted that, when there exist blockers, the square array pooling method with the AND re-testing scheme increases the false negative rate. Instead, they proposed a re-testing scheme (referred to as the OR scheme), in which individuals in either a positive column or a positive row are selected for re-testing. The OR scheme can reduce the false negative rate, but at the cost of selecting more individuals for re-testing.

The two-way square array pooling method can be extended to a three-way (cubic array) or even a multiple-way pooling method, in which each individuals are pooled three or multiple times. Results later in Section 6 suggest that the multiple-way pooling method can reduce the false negative rate, but often with the cost of requiring a larger number of tests per individual. To simplify our discussion, in the rest of this paper except in Section 6, we will focus only on the two-way square array pooling methods.

3. ESTIMATION PROCEDURE

In this section, we develop an EM algorithm (Dempster, Laird, and Rubin 1977) to estimate $p$ and $f$, using the observations from a square array pooling method. The observations can be either $(x, y)$ or $(x, y, w_{obs})$, where $x = \{(x_1^{(l)}, \ldots, x_k^{(l)})|l = 1, \ldots, n\}$ and $y = \{(y_1^{(l)}, \ldots, y_k^{(l)})|l = 1, \ldots, n\}$ are the row-wise and the column-wise group responses and $w_{obs}$ are the individual responses observed under a particular re-testing scheme. It can be shown that the parameters $(p, f)$ are identifiable when only observing the group responses $(x, y)$.
In the $l$th array, the row-wise group responses $X_1^{(l)}, \ldots, X_k^{(l)}$ are independent, as are the column-wise group responses $Y_1^{(l)}, \ldots, Y_k^{(l)}$, but any combination of $X^{(l)}$'s and $Y^{(l)}$'s are correlated. The joint distribution of $(x, y)$ or $(x, y, w_{\text{obs}})$ cannot be expressed explicitly. Direct maximization of the likelihood function is problematic. However, we know that the likelihood function of the individual responses $W$’s and $V$’s is simply

$$L(p, f | w, v) = \prod_{i=1}^{n} \prod_{j=1}^{k} P(W_{ij}^{(l)} = w_{ij}^{(l)}, V_{ij}^{(l)} = v_{ij}^{(l)}) = \prod_{i=1}^{n} \prod_{j=1}^{k} p^{w_{ij}^{(l)}} (1 - p - f)^{-w_{ij}^{(l)} - v_{ij}^{(l)}}. \quad (5)$$

By treating the latent individual responses $(w, v)$ as the full observations, we propose an EM algorithm to estimate $p$ and $f$, i.e., iterating between the following E and M steps:

**E-step:** Given the current estimates $(p_{\text{old}}, f_{\text{old}})$ and the observations in the $l$th array, $(x^{(l)}, y^{(l)}, w_{\text{obs}}^{(l)})$, calculate the conditional expectations of $W_{ij}^{(l)}$ and $V_{ij}^{(l)}$,

$$c_{i,j,l} = E\{W_{ij}^{(l)} | x^{(l)}, y^{(l)}, w_{\text{obs}}^{(l)}, (p_{\text{old}}, f_{\text{old}})\}, \quad d_{i,j,l} = E\{V_{ij}^{(l)} | x^{(l)}, y^{(l)}, w_{\text{obs}}^{(l)}, (p_{\text{old}}, f_{\text{old}})\}.$$  

**M-step:** Update the estimates $(p_{\text{new}}, f_{\text{new}})$ by averaging $c_{i,j,l}$ and $d_{i,j,l}$,

$$p_{\text{new}} = \frac{1}{nk^2} \sum_{i=1}^{n} \sum_{j=1}^{k} c_{i,j,l}, \quad f_{\text{new}} = \frac{1}{nk^2} \sum_{i=1}^{n} \sum_{j=1}^{k} d_{i,j,l}.$$  

To update the expectations $c_{i,j,l}$ and $d_{i,j,l}$ in the E-step, if $W_{ij}^{(l)} = w_{ij}^{(l)}$ is observed, then $c_{i,j,l} = w_{ij}^{(l)}$; if further $w_{ij}^{(l)} = 1$, then $d_{i,j,l} = 0$. However, if $W_{ij}^{(l)}$ is unobserved, $(W_{ij}^{(l)}, V_{ij}^{(l)})$ is conditionally correlated to all other (unobserved) individuals in the same square array, given $(x^{(l)}, y^{(l)})$ or $(x^{(l)}, y^{(l)}, w_{\text{obs}}^{(l)})$. The form of the joint conditional distribution $f(w^{(l)}, v^{(l)} | x^{(l)}, y^{(l)}, w_{\text{obs}}^{(l)}, (p_{\text{old}}, f_{\text{old}}))$ is complicated. The expectations in the E-step do not have explicit forms in general. Since any pair of $(W_{ij}^{(l)}, V_{ij}^{(l)})$ can take on at most three values, the fully-conditional distributions $f(w_{ij}^{(l)}, v_{ij}^{(l)} | x^{(l)}, y^{(l)}, w_{\text{obs}}^{(l)}, (p_{\text{old}}, f_{\text{old}}), \{w_{i'j'}^{(l)}, v_{i'j'}^{(l)} : (i', j') \neq (i, j)\}, (p, f))$ are explicit; they are provided in Appendix A. Based on this information, we use the simulation based Gibbs sampling technique to evaluate the expectations in the E-step:

- Simulate each $(w_{ij}^{(l)*}, v_{ij}^{(l)*})$, $1 \leq i, j \leq k$, in turn from the fully conditional distributions $f(w_{ij}^{(l)}, v_{ij}^{(l)} | x^{(l)}, y^{(l)}, w_{\text{obs}}^{(l)}, \{w_{i'j'}^{(l)}, v_{i'j'}^{(l)} : (i', j') \neq (i, j)\}, (p_{\text{old}}, f_{\text{old}}))$. Cycle the process $S$ times. The simulated samples form a Monte Carlo Markov Chain.

- From Theorem A in Appendix B, this Monte Carlo Markov Chain is convergent. When $S$ is large, $(w^{(l)*}, v^{(l)*}) = \{(w_{ij}^{(l)*}, v_{ij}^{(l)*})$, $1 \leq i, j \leq k\}$ can be viewed as samples from the joint conditional distribution $f(w^{(l)}, v^{(l)} | x^{(l)}, y^{(l)}, w_{\text{obs}}^{(l)}, (p_{\text{old}}, f_{\text{old}}))$.
Repeat the entire simulation \( T \) times. We obtain a set of \( T \) simulated samples \((w^*, v^*) = \{(w^{(l)*}, v^{(l)*})\}, l = 1, \ldots, n\).

The \( c_{i,j,l} \) and \( d_{i,j,l} \) in the E-step are evaluated by \( \sum w^{(l)*}_{ij}/T \) and \( \sum v^{(l)*}_{ij}/T \) respectively, where the summations are over the set of \( T \) Gibbs samples.

Based on the missing information principle and Louis' method (see, e.g., Tanner 1993, sec. 4.4.2, 4.4.3), we have the observed information matrix

\[-\frac{\partial^2 l(\eta|x, y, w_{\text{obs}})}{\partial \eta^2} = -\mathbb{E}_\eta \left\{ \frac{\partial^2 l(\eta|x, y, w)}{\partial \eta^2} \bigg| x, y, w_{\text{obs}} \right\} - \text{var}_\eta \left\{ \frac{\partial}{\partial \eta} l(\eta|x, y, w) \bigg| x, y, w_{\text{obs}} \right\},\]

where \( \eta = (\eta_1, \eta_2)^T = (\log\{p/(1-p)\}, \log\{f/(1-f)\})^T \). \( l(\eta|x, y, w_{\text{obs}}) \) and \( l(\eta|w, v) \) are the log-likelihood functions of \((x, y, w_{\text{obs}})\) and \((w, v)\), respectively. With the Gibbs samples available in the algorithm, this observed information matrix can be estimated by

\[H_n = -\frac{1}{T} \sum \frac{\partial^2 l(\eta|x^*, v^*)}{\partial \eta^2} - \left[ \frac{1}{T} \sum \left\{ \frac{\partial}{\partial \eta} l(\eta|x^*, v^*) \right\}^2 - \left\{ \frac{1}{T} \sum \frac{\partial}{\partial \eta} l(\eta|x^*, v^*) \right\} \right]^2,\]

where the summations are over the set of \( T \) Gibbs samples \((w^*, v^*)\) obtained in the final round of the EM iterations. The information matrix for \((p, f)\) can be obtained by transformation, and its inverse matrix is used to estimate the variance covariance matrix of \((\hat{p}, \hat{f})\). In order to obtain the (Wald-type) confidence intervals of \( p \) and \( f \), we first find the confidence intervals for \( \eta_1 \) and \( \eta_2 \), then transform them back to the scale of \( p \) and \( f \). This ensures the confidence intervals will be within \((0, 1)\).

Table 1 lists results of a simulation study on 12 simulated data sets. The 12 data sets were generated from 6 sets of 8000 Multinomial\((1; p, f, 1 - p - f)\) random samples, corresponding to \((p, f)\) values: (.015, .01), (.015, .001), (.01, .01), (.01, .001), (.005, .01) and (.005, .001), respectively. Each of the 6 sets of 8000 samples was randomly arranged onto \( k \times k \) square arrays for two different \( k \) values, \( k = 10 \) and \( k = 20 \). Then the row-wise and column-wise pooled group responses were obtained according to the pooling assumption (2). The 12 simulated data sets used in the study are these 12 sets of group responses. The first four columns of Table 1 list the population \((p, f)\) values and the sample proportions of the positives and the blockers in the simulated data sets. Due to the random sampling, these proportions are slightly different from the population \((p, f)\) values. We applied the EM algorithm to the 12 simulated data sets. The last four columns of Table 1 list the parameter
estimates and the associated 95% confidence intervals (in parentheses). It can be seen that, the EM algorithm provides accurate estimates of the sample proportions. Because, under our setting, a blocker does not affect the testing respond unless it is placed in a row (or column) containing a positive individual, far less information is available on blockers than on positives. The confidence intervals of $f$’s are wider than those of $p$’s. In fact, in the cases with $f = .001$ (total about 8 blockers), the information on $f$ is so little that its confidence intervals is essentially the whole parameter space $(0, 1)$ in several occasions. Also, as pointed out by the associate editor, the normality approximation of the maximal likelihood estimator $\hat{f}$ may not be very accurate in these cases. This also leads to wide confidence intervals.

The EM algorithm also provides the conditional probabilities of each individual being a positive or a blocker, conditional on all available observations. They are the $c_{i,j,l}$ and $d_{i,j,l}$ in the E-step of the final round of the EM iterations. These values can be used to make predictions of the positive individuals from the available observations. For example, let us take a look at the first simulated data set with $(p, f) = (.015,.01)$ and $k = 10$. In the 7th square array, the $(7,5)$ element is a positive, the $(10,7)$ element is a blocker and the rest are negatives. Only using the group responses, the algorithm predicts that the $(7,5)$ individual has a 98.84% chance to be positive, the other individuals in the 7th row or the 5th column have around a .30% chance to be positive, and the remaining individuals in the array have less than .05% chances. The probabilities of each individual being a blocker are also provided by the algorithm. However, it is harder to predict the blockers, since, as mentioned in the previous paragraph, the data contain less information on the blockers.

4. THRESHOLD TESTING SCHEME

As mentioned in Section 3, the proposed EM algorithm can also provide the probabilities of each individual being a positive or being a blocker. In an intermediate stage of a group testing procedure (for example, right after obtaining the observations of the group responses), this information can be used to select individuals for re-testing. For instance, one method is to test all individuals with (conditional) probability of being positive above a threshold value. We call this re-testing scheme the “threshold” method. For a given threshold value $h$, the selected individuals are those in set $A_h = \{(i,j,l) | \hat{w}_{ij}^{(l)} \geq h\}$, where $\hat{w}_{ij}^{(l)} = \hat{P}\{W_{ij}^{(l)} = 1 | x, y, w_{obs}\}$ is the probability of the $(i,j)$th individual in the $l$th array being a positive, available from the EM algorithm. Depending on the threshold value, the
number of individuals selected can vary. The smaller $h$ is, the more individuals will be selected for testing; Setting $h = 0$ would end up testing all individuals. The threshold scheme provides ample choices for screeners to select individuals for re-testing. From our experience, there always exist threshold values such that the corresponding threshold methods are the same or very close to the AND or the OR method. In this sense, both the AND and the OR method can be viewed as special threshold methods.

Before re-testing, the number of positives to be found in a threshold method can be estimated by $\sum \hat{w}_{ij}^{(l)}$, where the summation is over the individuals in $A_k$. The number of positives to be found in an AND or an OR or some other re-testing schemes can be also estimated in the same way, except that the summation is over the set of individuals selected for re-testing instead. This information can assist screeners in choosing from different re-testing schemes or threshold values before even testing any individuals. One way to determine the threshold $h$, for instance, can be illustrated through the simulation study in Section 3 in the case with $(p, f) = (.015,.01)$ and $k = 10$. Figure 1 indicates how one might choose the threshold. In Figure 1, the number of individuals selected for re-testing and the estimated number of positives to be found are plotted against the possible threshold values in the top and bottom figures, respectively. The dotted line in the bottom figure corresponds to the actual number of positives found after testing the selected individuals. Figure 1 indicates, for example, that a choice of the threshold $h = .003$ dramatically reduces the number of tests from 8000 with very little decrease in the number of positives found. For $h = .003$, we select 1767 individuals for re-testing (total $1767 + 80 \times 20 = 3367$ tests in the entire testing procedure). Corresponding to the solid lines in the bottom figure, we obtain an estimated 128.78 out of a total of 130.32 estimated positives. This is not far from the actual number from the dotted lines: we found 127 out of 128 positives in the simulated data.

Under the 12 simulation settings described in Section 3, we compare the standard Dorfman, the AND, the OR, and the threshold methods. The numbers compared are the false negative rate and the (expected) number of tests required per individual. When we use the threshold method, there are many choices of threshold $h$ values, leading to different results including some results the same or similar to the AND and the OR methods. To simplify our discussion and for our convenience, we pick $h = .003$. This $h$ value was not selected to be optimal in any sense. Depending on the setting and the need, we expect there will be
some other \( h \) values that will be more suitable in practice. In Table 2, the numbers listed under the Dorfman, the AND and the OR methods are obtained based on the theoretical formulas (7) and (8) provided later in Section 6. It is difficult to obtain a formula to evaluate the corresponding values under the threshold method. The values listed under the threshold method are the averages from 5 repeated simulations. The false negative rate under the OR method is always much smaller than those under the Dorfman or the AND methods, but with a price of requiring a larger number of tests per individual. For pool size of 10, the number of tests required under the Dorfman method is comparable or slightly less than those under the AND method; for the pool size of 20, the AND method requires the least number of tests, but also has the highest false negative rates. The threshold method with a fixed \( h = .003 \) is close to the OR method. The threshold method provides an alternative to the AND or OR re-testing scheme.

The threshold method is a two-step group testing procedure, the same as the Dorfman method, where in the second step one selects and tests a small number of individuals. In theory, one can develop a multiple-step threshold method by re-grouping the selected individuals in the late steps and testing the newly formed groups. However, in practice, the extra recording and back tracking would be logistically difficult. For instance, it leaves room for serious back tracking mistakes; note that under the square array pooling the groups formed at various steps will not necessarily be nested. One sequential multiple-step threshold method, that may be appropriate, is as follow. Start by testing the group responses; feed these testing results into the EM algorithm; individually test a few individuals that are predicted to be positive with high probability; feed their testing results back into the EM algorithm. Based on the new predictions, select and re-test a few more individuals, and feed their testing results back into the algorithm again, and so on. At each step we can update how many positives have been identified and estimate the number of the positives in the entire samples. A stopping rule could be constructed by controlling the ratio of the number of positives identified over the (estimated) total number of positives, or the process can stop when enough positive compounds are found.

5. EXTENSION TO CASES WITH SYNERGISM OR ADDITIVE EFFECTS

As discussed in the Introduction, a pool consisting entirely of negative individuals can
sometimes test positive, due to synergism or additive effects of two or more individuals. We extend the methods developed in the previous sections to deal with this type of complication. Although we may also be interested in identifying pairs or sets of negative compounds that act synergistically, at the early stage of the drug discovery, such situations are typically considered a source of contamination. This is because very little information is available on the chemical compounds at the early stage and it can be very expensive and time consuming to track down individuals acting synergistically.

The synergism or additive effects do not affect the testing response of a group unless the group contains neither a positive nor a blocker. We introduce a Bernoulli random variable $Z$ to model the possible synergism or additive effects in a group with neither a positive nor a blocker. Now, we extend the model assumption (2) to

$$X^{(l)} = 1_{\left(\sum_{i=1}^{k} W_{i}^{(l)} > 0, \sum_{i=1}^{k} V_{i}^{(l)} = 0\right)} + Z^{(l)} 1_{\left(\sum_{i=1}^{k} W_{i}^{(l)} = \sum_{i=1}^{k} V_{i}^{(l)} = 0\right)}, \quad l = 1, 2, \ldots, n,$$

where $Z^{(l)}$ is a Bernoulli random variable taking value 1 with probability $q$ and value 0 with probability $1 - q$. Comparing (6) with (2), we can see that they are exactly the same except in the case when a group contains neither positives nor blockers (i.e., $\sum_{i=1}^{k} W_{i}^{(l)} = \sum_{i=1}^{k} V_{i}^{(l)} = 0$). When a group does not contain any positives or blockers, (6) suggests that the group response can still be positive with a chance $P(Z = 1) = q$.

For a given type of assay, the parameter $q$ should be a function of pool size $k$, $q = q(k)$. For instance, $q(k)$ can perhaps be an increasing function of $k$, since the number of potential combinations increases with pool size. In estimation problems, the pool sizes are typically fixed. We have only an extra parameter $q$.

In a square array pooling case, by extending (4), we have a formula similar to (6). Let $z$ be the vector of all $Z$'s in the pools. The likelihood function of $(w, v, z)$ is

$$L(p, f, q|w, v, z) = \prod_{i=1}^{n} \left\{ \prod_{i,j} p^{w_{ij}}^{(l)} f^{v_{ij}}^{(l)} (1 - p - f)^{1 - w_{ij}^{(l)} - v_{ij}^{(l)}} \prod_{i} q^{z_{i}^{(l)}}^{(1 - q)^{1 - z_{i}^{(l)}}} \prod_{j} q^{z_{j}^{(l)}}^{(1 - q)^{1 - z_{j}^{(l)}}} \right\},$$

where $z_{i}^{(l)}$ and $z_{j}^{(l)}$ are the realizations of the random assignments $Z_{i}^{(l)}$ and $Z_{j}^{(l)}$ at the ith row-wise and jth column-wise pools, respectively. By treating $(w, v, z)$ as the full observations, we can extend the EM algorithm in Section 3 to the current setting. In the E-step, in addition to computing $c_{i,j,l}$ and $d_{i,j,l}$, we compute the conditional expectations, $e_{i,l} = E\{Z_{i}^{(l)}|x^{(l)}, y^{(l)}, w_{obs}, (p_{old}, f_{old}, q_{old})\}$ and $e_{j,l} = E\{Z_{j}^{(l)}|x^{(l)}, y^{(l)}, w_{obs}, (p_{old}, f_{old}, q_{old})\}$.
In the M-step, in addition to $p$ and $f$, we also update the extra parameter $q$, $q_{\text{new}} = \sum_{i=1}^{n} \{ \sum_{i=1}^{k} e_{i..l} + \sum_{j=1}^{k} e_{..j..l} \}/(2nk)$. Further technical details are provided in Appendix C.

The threshold re-testing scheme proposed in Section 4 can also be extended. Notice that, at the intermediate stage with only observations of the group responses, we cannot distinguish positive pools due to synergism or additive effects from positive pools containing positive individuals, and the parameters $(p, f, q)$ are not identifiable. We may adopt a working assumption that $q = 0$ (often we consider cases with small $q$ values). We can use the EM algorithm developed in Section 3 to obtain the estimates of $(p, f)$, say $(\hat{p}, \hat{f})$, and to compute the chance of each individual being a positive. So, the threshold re-testing scheme can apply. We should be aware, however, that by setting $q = 0$, both the estimates and predictions will be overestimated, since the positive pools due to synergistic effects are treated as if they contain positive individuals. After obtaining some individual observations, we can use the EM algorithm proposed in this section to obtain more accurate estimates of $p$ and $f$.

6. COST EFFICIENCY EVALUATION

When there are blockers in the samples, the false negative rate will be positive. In practice, we may be satisfied with a screening method with a small rate of false negatives, considering that most screening methods tolerate some errors. For instance, even in cases with serious social impact such as the HIV testing, ELISA, a commercial test kit used in initial screening, only achieves a level of about 99.5% accuracy (Gastwirth and Johnson, 1994). We may accept a screening method in which the chance of misclassifying a positive as a negative is less than a pre-specified small tolerance level, say $p_{c}$. Since in our setting the individuals identified as positives are among those selected individuals who have been tested individually, the false positive rate, defined as the probability of a negative to be declared as positive, is always zero (or only depends on the accuracy of the assay method if the assay method is not perfectly accurate). The price of reducing the false negative rate is the increase of the number of tests required and not the increase of the false positive rate.

Let $d$ be the number of times each individual has been pooled in a multiple-way pooling method, for example, $d = 2$ in a square array pooling method, and $d = 3$ in a cubic pooling method. Under an AND scheme or an OR scheme, the false negative rates are,

$$r^{\text{AND}} = 1 - (1 - f)^{d(k-1)} \quad \text{and} \quad r^{\text{OR}} = \{(1 - f)^{(k-1)}\}^d,$$

(7)
respectively. For a given set of \((f, k, d)\), we have \(r^{A\text{ND}} \geq r^{O\text{R}}\), i.e., the OR scheme has a smaller false negative rate, and the rates are the same only in the case when \(d = 1\). Both false negative rates are increasing functions of the blocker rate \(f\). Multiple-way pooling method will reduce the false negative rate under an OR scheme, but will increase the false negative rate under an AND scheme.

We measure the cost efficiency of a screening method by the expected number of tests per individual it requires. From direct computation, they are,

\[
ET^{A\text{ND}}(k) = \frac{d}{k} + p(1 - f)^{d(k-1)} + (1 - p - f) \sum_{s=0}^{d} \{q(k)(1-p-f)\}^{s}(1-f)^{k-1}-(1-p-f)^{k-1}d-s,
\]

\[
ET^{O\text{R}}(k) = \frac{d}{k} + p[1 - \{1-(1-f)^{k-1}\}]^{d} + (1-p-f)[1 - \{1-(1-f)^{k-1} + (1-q(k))(1-p-f)^{k-1}\}]d,
\]

for an AND and an OR methods, respectively. In the formulas, the notation \(q(k)\) is used to remind us that \(q\) can possibly be a function of the pool size \(k\). For a fixed set of values \((p, f, q, k, d)\), \(ET^{A\text{ND}} \leq ET^{O\text{R}}\), i.e., the AND scheme is more efficient than the OR scheme. Both \(ET^{A\text{ND}}(k)\) and \(ET^{O\text{R}}(k)\) are increasing functions of \(q\). The higher the \(q\) is, the more tests per individual are required. Too high a value of \(q\) will make a group testing method inefficient. If there is no synergism or additive effect in the pools, e.g., \(q(k) = 0\), the above formulas become,

\[
ET^{A\text{ND}}(k) = \frac{d}{k} + p(1 - f)^{d(k-1)} + (1 - p - f)\{(1 - f)^{k-1} - (1 - p - f)^{k-1}\}d,
\]

\[
ET^{O\text{R}}(k) = \frac{d}{k} + p[1 - \{1-(1-f)^{k-1}\}]^{d} + (1-p-f)[1 - \{1-(1-f)^{k-1} + (1-p-f)^{k-1}\}]d. \tag{8}
\]

To comply with the tolerance constraint discussed at the beginning of this section, we set, for a pre-specified tolerance level \(p_{T}\),

\[
r^{A\text{ND}} \leq p_{T} \quad \text{or} \quad r^{O\text{R}} \leq p_{T}.
\]

This leads to an upper bound for the pool size \(k\): \(k \leq k^{A\text{ND}} = 1 + \{\log(1-p_{T})^{1/d}\}/\{\log(1-f)\}\) for an AND method and \(k \leq k^{O\text{R}} = 1 + \{\log(1-p_{T}^{1/d})\}/\{\log(1-f)\}\) for an OR method. To obtain a meaningful upper bound, for a very small value \(p_{T}\), the blocker rate \(f\) should be small as well. Under the OR scheme, increasing \(d\) will increase the upper bound of the pool size. One referee pointed out that there might be connections between this and the multiple grouping methods discussed in Redman and King (1965) and Hammick and
Gastwirth (1994). If the parameters \((p, f)\) and the form of \(q(k)\) are known, for a given \(d\), we obtain the optimal pool sizes by solving minimization problems,

\[
k_{opt}^{AND} = \arg\min_{k: k \leq 1 + \frac{\log(1 - p_f)}{\log(1 - f)}} ET^{AND}(k) \quad \text{or} \quad k_{opt}^{OR} = \arg\min_{k: k \leq 1 + \frac{\log(1 - p_f)}{\log(1 - f)}} ET^{OR}(k).
\]

Table 3 lists the optimal pool sizes and the corresponding average numbers of tests required per individual for several combinations of \((p, f, d)\), assuming \(q(k) = 0\). The tolerance level is \(p_T = 10^{-4}\). If the blocker rate \(f\) is larger than \(10^{-4}\), a standard Dorfman’s pooling method \((d = 1)\) cannot achieve the required precision. To reduce the false negative rate, we consider 2 and 3-way pooling with the OR scheme. It can be seen that with \(d = 2\) and \(d = 3\), the group testing method can still be cost effective for samples with a moderate blocker rate. Since the AND method \((d = 2, 3)\) cannot achieve the required precision \(p_T = 10^{-4}\) for the listed \(f\) values, it is not included in Table 3.

When the tolerance level is moderately small, say, for example, \(p_T = 10^{-2}\), both the AND and OR schemes can satisfy the constraints for some values of blocker rate. Table 4 lists the optimal pooling sizes and the number of tests per individual, assuming \(q(k) = 0\). To consume space, we only list results for \(d = 1\) and 2. The lower half of Table 4 compares the AND and OR methods when \(d = 2\). Because of the tolerance constraint, the optimal pooling size for an AND scheme and for an OR scheme can be different. Sometimes, an OR scheme can be more efficient than an AND scheme! See the cases indicated by asterisks in Table 4.

7. APPLICATION TO DRUG DISCOVERY DATA

In an experiment conducted by Glaxo Wellcome, 8000 chemical compounds were put into liquid stores and stored in 100 plates with an array of \(8 \times 10\) wells. These 100 plates were arranged in a \(10 \times 10\) grid, and 10 empty plates (the same type) were placed at the end of the rows and columns. An \(8 \times 10\) gang transfer robot then transferred the \(8 \times 10\) chemicals in each of the plates in a row into the corresponding \(8 \times 10\) wells in the empty plate in the same row; the same was done for the columns. This gives rise to 20 plates or 1600 wells with 10 mixed compounds per well. The mixed compounds in the 1600 wells were then assayed (type of assay not disclosed). The compounds in the same (physical) plate were not mixed; the mixed compound samples were from the wells at the same position in the plates across the same rows or the same columns. Netherless, the 1600 samples of mixed compounds can
essentially be considered as if they were from the row and column pools of 80 (virtual) square arrays of size $10 \times 10$.

The reported assay response values were in fact continuous potency measurements. But these values were dichotomized and only the dichotomized responses are used in our analysis. The dichotomization is a common practice in drug discovery. It is based on the following practical considerations. First, the empirical distribution of the continuous assay values are often asymmetric, and no standard distribution assumption of the assay values is agreed upon. Second, these continuous measurements are often not very accurate. Third, in the late stages of the high throughput screening process, more accurate (different type) assay measurements will often be available and the measurements at the early screening stage will not be used. Fourth, and most importantly, it is felt necessary in drug discovery to simplify the analysis to speed up the screening process. Also, several pilot experiments performed on the same or similar assays support (empirically) the dichotomization approach. In these pilot experiments, both pooled group samples and each individual compound sample were tested. The results suggested that the potency values of groups were highly correlated to the maximum potency values of the individual chemical compounds of the same group.

One immediate question is how to dichotomize the continuous assay measurements. Often, a sample with an assay value higher than a given significant value, say $t_d$, is treated as potent. One approach used in Glaxo Wellcome to determine the $t_d$ value is to take $t_d = c + ks$, where the $c$ is the potency value corresponding to the highest mode of the individual assay values obtained in the pilot experiments (often in the log-scale) and $s$ is the sample standard error. The $k$ is often a value between 1 to 4, depending on the assay and the follow up costs in the following stages of the drug discovery process. The higher the costs, the larger the $k$ is, leading to less lead compounds studied. For the data set considered in the paper, our collaborators at Glaxo Wellcome suggested the use of the 90% quantile of the group response values, which is very close to the $t_d$ value they determined based on the aforementioned approach. In the statistical literature, dichotomization of continuous assay values has been discussed by Redman and King (1965, p. 869). In their paper, they suggested using a specified significance level $\alpha$, the assay values above the $100(1 - \alpha)$% percentile of the assay responses are considered as potent.

The data set used in our analysis consists the dichotomized group assay responses from
an 80 10 × 10 virtual square arrays. Upon examination of the data set, we found that a few square arrays have no positive group responses at one side but some positive group responses on the other side, which implies there are blockers. This is consistent with the findings in the pilot experiments. The pilot experiments also suggested that a small percent of the positive group responses are due to synergism or additive effects. However we cannot detect such an effect from the data available to us, since, as mentioned in Section 4, there is not enough information to estimate $q$ together with $p$ and $f$ when individual observations are not available. We adopt in our analysis a working assumption that $q = 0$.

From the pilot experiments, the positive rate and the blocker rate were about 1% to 2%. The group size was chosen to be 10 in the experiment, mainly due to an ad hoc application of Dorfman group testing result: the optimal size in a population with 100p% positives is close to $1/\sqrt{p}$ (Feller 1968, p. 239). In fact, based on our results in Section 6 (Table 4 with $p_T = .01$, $p = f = .01$), if an OR scheme is employed, we would have suggested the optimal size of 11. It turns out that the pool size used in the experiment is close to optimal.

We used (1) and (2) to model this group pooling data set, and applied the estimation procedure in Section 3 to estimate $p$ and $f$. The starting parameter values for the EM algorithm were moment-based estimates. After the algorithm converged, the estimates of the positive rate and blocker rate were $\hat{p} = .0150$ and $\hat{f} = .0299$, respectively. The 95% confidence intervals of $p$ and $f$ were (.0147, .0153) and (.0276, .0326). The result suggests that there are about 120 highly active compounds among the 8000 samples. It should be stressed that, since we did not model the synergism or additive effects, the $\hat{p}$ and $\hat{f}$ are, in fact, $\bar{p}$ and $\bar{f}$ in the notation of Section 5.

As discussed in Section 3, we can also predict highly active compounds (results not shown in the paper). This information can assist screeners choosing among different re-testing schemes. For instance, if the AND scheme is employed, it ends up selecting 216 individuals; we expect to find 66.04 highly active compounds out of the predicted 120 total positives in the 8000 compounds. If the OR scheme is employed, it ends up selecting 1384 individuals for re-testing testing and we expect to find 113.45 highly potent compounds. If a threshold scheme is suggested, Figure 2 is helpful. If, for example, we recommend testing the compounds predicted to have .01 or higher chance to be positive, then we end up testing 1186 individual compounds and expect to find 111.68 highly active compounds. This is
about 27% of the total 8000 tested if each compound is tested individually.

One ad hoc method that has been adopted in practice is to treat the compounds at the intersection of a positive row and a positive column as active compounds, and move to further phases of drug discovery without even testing the selected individual compounds. This ad hoc method is exactly the AND method, except that the step of re-testing the selected individuals is omitted. Based on the results from the EM algorithm output, it is quite clear that if the ad hoc AND method is employed, we could miss an estimated $1 - (1 - .0299)^{2 \times 9} = 42\%$ of the positives. Also, among the 120 lead compounds selected by the ad hoc AND method, there are estimated as many as $120 - 66.04 \approx 54$ non-potent chemicals.

8. DISCUSSION

This paper develops models and methods for pooled samples in situations when blockers and synergistic effects exist. The estimation algorithm not only provides parameter estimates, but also assigns probabilities of activity and blocker for each individual. A new testing scheme, that provides options to select different number of individuals for re-testing, is proposed. There is a trade off between the number of total tests required and the number of potential lead compounds found. If the compound collection is somewhat redundant, missing a few lead compounds may have little loss, as other compounds in the same class will be found. However in situations when the medical need is high, we may not want to miss any compounds with strong drug potential. A big advantage of our method is that we provide a way to quantify information at any stage of a group testing process. This information can be used to guide the screening process.

This paper also gives a method to obtain the optimal pooling size when the parameters are given. The cost efficiency computation suggests that the false negative rate can be reduced by a square array or a multiple-way pooling method (with appropriate re-testing scheme). If we want to minimize the chance of missing compounds with strong drug potential, a square array or a multiple-way pooling method appears to be a good choice. The optimization method can be used to determine the optimal pooling size in the design of experiments.

The ad hoc AND method discussed in the last paragraph of Section 7 can be viewed as a variation of the special case in Redman and King (1965) with $g = 2$: each compound is randomly assigned to and evaluated in two groups, and the individual potency values
are estimated by the average response of the two groups; if this average is bigger than or equal to the \((1 - \alpha)100\%\) percentile of the potency values, then the individual is taken to be active (See, Redman and King 1965 for details). Here one takes an average of the two group potency values and then dichotomizes the response; but in Section 7 we dichotomize the two group potency values first and then check whether both of them are active. Although these two approaches do not always lead to the same results, they are close; both methods identify individuals at the intersection of a highly potent row and a highly potent column as potential positives. One great advantage to dichotomizing the group potency values first is that it is much easier to relax the standard pooling assumption and extend the models.

In our paper, the blocker is defined intrinsically to block positive individuals in the same pool. Evidence for existence of blockers comes from the pilot experiments where both the pools and all individual compounds were tested and from the group testing results where row or column positives do not match. Although identifying the blockers themselves can be very important, experiments have not yet been performed to identify the blockers. There have been discussions and plans of further research in this direction.

**APPENDIX A: CONDITIONAL DISTRIBUTION OF \((W_{ij}^{(l)}, V_{ij}^{(l)})\) GIVEN \(x^{(l)}, y^{(l)}\) AND \((w_{ij'}^{(l)}, v_{ij'}^{(l)}), (i, j) \neq (i', j')\)**

Without loss of generality, we assume \(i = 1, j = 1\) and suppress the well index \(l\). The conditional distribution of \((W_{11}, V_{11})\) given the marginal observations and the others depends only on \(X_1, Y_1, W_R = \sum_{i=2}^{k} W_{i1}, W_C = \sum_{j=2}^{k} W_{1j}, V_R = \sum_{i=2}^{k} V_{i1},\) and \(V_C = \sum_{j=2}^{k} V_{j1}\).

Case 1. Event \(A^{++} = \{X_1 = 1, Y_1 = 1\}\) is true.

(i) If \(B_{11} = \{W_R or W_C = 0, V_R = V_C = 0\}\) is true, then \(P\{(W_{11}, V_{11}) = (1, 0) | A^{++} \cap B_{11}\} = 1\).

(ii) If \(B_{12} = \{W_R \geq 1, W_C \geq 1, V_R = V_C = 0\}\) is true, then \(P\{(W_{11}, V_{11}) = (1, 0) | A^{++} \cap B_{12}\} = p/(1 - f)\) and \(P\{(W_{11}, V_{11}) = (0, 0) | A^{++} \cap B_{11}\} = 1 - p/(1 - f)\).

Case 2. Event \(A^{+-} = \{X_1 = 1, Y_1 = 0\}\) is true. (Use the same formulas when \(A^{-+} = \{X_1 = 0, Y_1 = 1\}\) is true, but the column and row indices should be switched.)

(i) If \(B_{21} = \{W_R = 0\}\) is true, then \(P\{(W_{11}, V_{11}) = (1, 0) | A^{+-} \cap B_{21}\} = 1\).

(ii) If \(B_{22} = \{W_R \geq 1, V_C = 0\}\) is true, then \(P\{(W_{11}, V_{11}) = (0, 0) | A^{+-} \cap B_{22}\} = 1\).
(iii) If \( B_{23} = \{ W_R \geq 1, V_C \geq 1 \} \) is true, then we have \( P\{(W_{11}, V_{11}) = (1, 0)\mid A^++ \cap B_{23}\} = p/(1 - f) \) and \( P\{(W_{11}, V_{11}) = (0, 0)\mid A^++ \cap B_{23}\} = 1 - p/(1 - f) \).

Case 3. Event \( A^- = \{ X_1 = 0, Y_1 = 0 \} \) is true.

(i) If \( B_{31} = \{ V_R = V_C = 0 \} \) is true, then \( P\{(W_{11}, V_{11}) = (1, 0)\mid A^- \cap B_{31}\} = p \), \( P\{(W_{11}, V_{11}) = (0, 0)\mid A^- \cap B_{31}\} = f \) and \( P\{(W_{11}, V_{11}) = (0, 1)\mid A^- \cap B_{31}\} = 1 - p - f \); 
(ii) Event \( B_{32} = \{ W_R \geq 1, V_R = 0 \} \) (or \( W_C \geq 1, V_C = 0 \)) is true, then we have \( P\{(W_{11}, V_{11}) = (0, 1)\mid A^- \cap B_{32}\} = 1 \); 
(iii) Event \( B_{33} = \{ V_R \geq 1, W_C = V_C = 0 \} \) (or \( W_R = V_R = 0, V_C \geq 1 \)) is true, then \( P\{(W_{11}, V_{11}) = (1, 0)\mid A^- \cap B_{33}\} = p/(1 - f) \), \( P\{(W_{11}, V_{11}) = (0, 0)\mid A^- \cap B_{33}\} = 1 - p/(1 - f) \).

APPENDIX B: CONVERGENCE THEOREM OF THE GIBBS SAMPLES

Without loss of generality, we assume \( n = 1 \).

**Theorem A** The Monte Carlo Markov Chain formed from the Gibbs samples in Section 3 is ergodic. More specifically, denote \( \pi \) as the probability measure with the density function \( f(w, v|x, y, w_{obs}) \) on the measurable space \( ((1, 0), (0, 1), (0, 0))^k, \mathcal{B} \), \( \mathcal{B} \) is the Borel set. Let \( P(u, C) \) be the transition probability of the Markov Chain. We have

\[
\sup_{C \in \mathcal{B}} |P_B^S(u, C) - \pi(C)| \rightarrow s \rightarrow \infty 0, \text{for all } u = \{(w_{ij}, v_{ij}), i, j = 1, \ldots k\} \in K_{x, y, w_{obs}},
\]

where \( K_{x, y, w_{obs}} \subset \{(1, 0), (0, 1), (0, 0)\}^k \) and it is the collection of all possible state arrangements that match with the given observations \( x, y, w_{obs} \).

**Outline of the Proof.** Follow Section 2.2 of Tierney (1994), we can write

\[
P(u, C) = \int_{C} \prod_{r=1}^{k} \prod_{s=1}^{k} f(w_{rs}^*, v_{rs}^*) \{(\tilde{w}_{ij}, \tilde{v}_{ij}), (i, j) \neq (r, s)\}, x, y, w_{obs} dB_{rs} dB_{rs}^*
\]

where \( (\tilde{w}_{ij}, \tilde{v}_{ij}) = (w_{ij}^*, v_{ij}^*) \) if \( i < r \) or if \( i = r \) and \( j < s \); and \( (\tilde{w}_{ij}, \tilde{v}_{ij}) = (w_{ij}, v_{ij}) \), if \( i > r \) or if \( i = r \) and \( j > s \). From direct calculation, we have \( \int P(u, C)\pi(du) = \pi(u) \). So, \( \pi \) is an invariant probability measure. According to the standard result (e.g., Theorem 1 of Tierney, 1994), we only need to verify that this Markov Chain is \( \pi \)-irreducible and aperiodic. To do so, we only need to verify that, for each cell in the square array, all possible values (subject to match with the observations \( x, y, w_{obs} \)) can be achieved with a positive probability after a Gibbs sampling cycle, no matter what the initial value is. Detail verification involves discussion on different cases once at a time and it is omitted.
APPENDIX C: TECHNICAL DETAILS IN SECTION 5

I. Formula for $e_{i,l}^{(m)}$ ($e_{i,l}^{(m)}$) can be obtained similarly:

We suppress the EM cycle index $m$ and the pool index $l$. From direct computations,

\[
E(Z_i|x, y, w, v) = \frac{P(Z_i = 1, X_i = x_i | \sum_{j=1}^{k} W_{ij} = \sum_{j=1}^{k} w_{ij}, \sum_{j=1}^{k} V_{ij} = \sum_{j=1}^{k} v_{ij})}{P(X_i = x_i | \sum_{j=1}^{k} W_{ij} = \sum_{j=1}^{k} w_{ij}, \sum_{j=1}^{k} V_{ij} = \sum_{j=1}^{k} v_{ij})}
= \{q1(\sum_{j=1}^{k} w_{ij} > 0)I(x_i=0) + q1(\sum_{j=1}^{k} w_{ij} > 0, \sum_{j=1}^{k} v_{ij} = 0) + 1(\sum_{j=1}^{k} w_{ij} = \sum_{j=1}^{k} v_{ij} = 0)\}1(x_i=1).
\]

Thus, $e_{i,l} = E(Z_i|x = x, Y = y, W_{obs} = w_{obs}) = qP1(x_i=0) + (qP2 + P3)1(x_i=1)$,

where $P_1 = P(\sum_{j=1}^{k} V_{ij} > 0|x, y, w_{obs})$, $P_2 = P(\sum_{j=1}^{k} W_{ij} > 0, \sum_{j=1}^{k} V_{ij} = 0|x, y, w_{obs})$,

and $P_3 = P(\sum_{j=1}^{k} W_{ij} = \sum_{j=1}^{k} V_{ij} = 0|x, y, w_{obs})$. $P_1$, $P_2$ and $P_3$ can be estimated by

$(1/T) \sum 1(\sum_{j=1}^{k} w_{ij} > 0, \sum_{j=1}^{k} v_{ij} = 0)$, $(1/T) \sum 1(\sum_{j=1}^{k} w_{ij} = \sum_{j=1}^{k} v_{ij} = 0)$, and $(1/T) \sum 1(\sum_{j=1}^{k} v_{ij} > 0)$ respectively, where the $w^*$'s and $v^*$'s are the $T$ sets of Gibbs samples used in Section 3.

II. Gibbs Formula in Section 5,

There are two modifications in the Gibbs formula in Appendix A.

1) In the case 1 (i), write event $B_{1,1a} = \{W_R = W_C = 0, V_R = V_C = 0\}$ and event $B_{1,1b} = \{W_R = 0, V_R = V_C = 0\}$ or $B_{1,1b} = \{W_R = 0, V_R = V_C = 0\}$. We have

$P\{(W_{11}, V_{11}) = (1, 0)|A^{++} and B_{1,1a}\} = 1 - q^2$, $P\{(W_{11}, V_{11}) = (0, 0)|A^{++} and B_{1,1a}\} = q^2$;

$P\{(W_{11}, V_{11}) = (1, 0)|A^{++} and B_{1,1b}\} = 1 - q$, $P\{(W_{11}, V_{11}) = (0, 0)|A^{++} and B_{1,1b}\} = q$.

2) In the case 2 (i), denote event $B_{2,1a} = \{W_R = 0, V_C = 0\}$ and $B_{2,1a} = \{W_R = 0, V_C = 0\}$.

We have $P\{(W_{11}, V_{11}) = (0, 0)|A^{-+} and B_{2,1a}\} = 1$, $P\{(W_{11}, V_{11}) = (1, 0)|A^{-+} and B_{2,1b}\} = 1 - q$ and $P\{(W_{11}, V_{11}) = (0, 0)|A^{+-} and B_{2,1b}\} = q$.

REFERENCES


Table 1. Parameter Estimates of Six Simulated Data Sets of 8000 Compounds
(Arranged in Square Arrays of Size 10 × 10 and 20 × 20)

<table>
<thead>
<tr>
<th>Population Values</th>
<th>Sample Proportions</th>
<th>Parameter Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$</td>
<td>$f$</td>
<td>$p$</td>
</tr>
<tr>
<td>.015</td>
<td>.01</td>
<td>.016000</td>
</tr>
<tr>
<td>(.0159, .0165)</td>
<td>(.0083, .0131)</td>
<td>(.0150, .0161)</td>
</tr>
<tr>
<td>.015</td>
<td>.001</td>
<td>.012875</td>
</tr>
<tr>
<td>(.0127, .0132)</td>
<td>(.0, .7642)</td>
<td>(.0129, .0136)</td>
</tr>
<tr>
<td>.010</td>
<td>.01</td>
<td>.008875</td>
</tr>
<tr>
<td>(.0085, .0090)</td>
<td>(.0018, .0102)</td>
<td>(.0085, .0095)</td>
</tr>
<tr>
<td>.010</td>
<td>.001</td>
<td>.009375</td>
</tr>
<tr>
<td>(.0093, .0098)</td>
<td>(.0, .9998)</td>
<td>(.0092, .0097)</td>
</tr>
<tr>
<td>.005</td>
<td>.01</td>
<td>.006500</td>
</tr>
<tr>
<td>(.0061, .0066)</td>
<td>(.0007, .0270)</td>
<td>(.0065, .0072)</td>
</tr>
<tr>
<td>.005</td>
<td>.001</td>
<td>.004125</td>
</tr>
<tr>
<td>(.0039, .0044)</td>
<td>(0, .9999)</td>
<td>(.0038, .0043)</td>
</tr>
</tbody>
</table>

Note: The numbers in the parentheses are the corresponding 95% confidence intervals.

Table 2. Comparison of Dorfman, OR, AND and Threshold Methods
(Six Simulated Data Sets in Pool Size of 10 and 20)

<table>
<thead>
<tr>
<th>Pool Size 10</th>
<th>Pool Size 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$</td>
<td>$f$</td>
</tr>
<tr>
<td>.015</td>
<td>.01</td>
</tr>
<tr>
<td>.015</td>
<td>.001</td>
</tr>
<tr>
<td>.010</td>
<td>.01</td>
</tr>
<tr>
<td>.010</td>
<td>.001</td>
</tr>
<tr>
<td>.005</td>
<td>.01</td>
</tr>
<tr>
<td>.005</td>
<td>.001</td>
</tr>
<tr>
<td>.005</td>
<td>.001</td>
</tr>
</tbody>
</table>

Note: For each pair of $(p, f)$, the numbers at the first row are the false negative rates, the numbers at the second row (italic) are the expected number of tests required per individual. Under the threshold method, the threshold value $h = .003$ and the numbers in the table are obtained by taking averages of 5 repeated simulations.
Table 3. Optimal Group Sizes and Expected Number of Tests per Individual

\[ (p_T = 10^{-4}, \text{OR method}) \]

<table>
<thead>
<tr>
<th>( f \backslash p )</th>
<th>.0001</th>
<th>.0005</th>
<th>.001</th>
<th>.005</th>
<th>.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d = 1 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.0001</td>
<td>(2, .5002)</td>
<td>(2, .5010)</td>
<td>(2, .5020)</td>
<td>(2, .5100)</td>
<td>(2, .5199)</td>
</tr>
<tr>
<td>.0005</td>
<td>(101, .0395)</td>
<td>(46, .0878)</td>
<td>(33, .1234)</td>
<td>(15, .2684)</td>
<td>(11, .3719)</td>
</tr>
<tr>
<td>.005</td>
<td>(11, .1839)</td>
<td>(11, .1922)</td>
<td>(11, .2024)</td>
<td>(11, .2808)</td>
<td>(11, .3704)</td>
</tr>
<tr>
<td>.01</td>
<td>(3, .6672)</td>
<td>(3, .6916)</td>
<td>(3, .6912)</td>
<td>(3, .7153)</td>
<td></td>
</tr>
<tr>
<td>( d = 2 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.0001</td>
<td>(103, .0591)</td>
<td>(46, .1307)</td>
<td>(33, .1831)</td>
<td>(16, .3931)</td>
<td>(12, .5392)</td>
</tr>
<tr>
<td>.0005</td>
<td>(96, .0582)</td>
<td>(47, .1295)</td>
<td>(34, .1819)</td>
<td>(16, .3920)</td>
<td>(12, .5381)</td>
</tr>
<tr>
<td>.001</td>
<td>(48, .0760)</td>
<td>(48, .1280)</td>
<td>(34, .1805)</td>
<td>(16, .3906)</td>
<td>(12, .5367)</td>
</tr>
<tr>
<td>.005</td>
<td>(10, .3027)</td>
<td>(10, .3133)</td>
<td>(10, .3265)</td>
<td>(10, .4256)</td>
<td>(10, .5356)</td>
</tr>
<tr>
<td>.01</td>
<td>(5, .6013)</td>
<td>(5, .6062)</td>
<td>(5, .6125)</td>
<td>(5, .6608)</td>
<td>(5, .7181)</td>
</tr>
</tbody>
</table>

Note: The numbers in the parentheses are the optimal group sizes and the expected number of tests per individual.

Table 4. Optimal Group Sizes and Expected Number of Tests per Individual

\[ (p_T = 10^{-2}, \text{AND and OR schemes}) \]

<table>
<thead>
<tr>
<th>( f \backslash p )</th>
<th>.0001</th>
<th>.0005</th>
<th>.001</th>
<th>.005</th>
<th>.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d = 1 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.0001</td>
<td>(101, .0199)</td>
<td>(45, .0444)</td>
<td>(32, .0627)</td>
<td>(15, .1390)</td>
<td>(11, .1955)</td>
</tr>
<tr>
<td>.0005</td>
<td>(21, .0497)</td>
<td>(21, .0580)</td>
<td>(21, .0682)</td>
<td>(15, .1386)</td>
<td>(11, .1950)</td>
</tr>
<tr>
<td>.001</td>
<td>(11, .0920)</td>
<td>(11, .0963)</td>
<td>(11, .1017)</td>
<td>(11, .1440)</td>
<td>(11, .1945)</td>
</tr>
<tr>
<td>.005</td>
<td>(3, .3336)</td>
<td>(3, .3348)</td>
<td>(3, .3363)</td>
<td>(3, .3481)</td>
<td>(3, .3627)</td>
</tr>
<tr>
<td>.01</td>
<td>(2, .5002)</td>
<td>(2, .5010)</td>
<td>(2, .5020)</td>
<td>(2, .5099)</td>
<td>(2, .5197)</td>
</tr>
<tr>
<td>( d = 2 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.0001 AND</td>
<td>(51, .0393)</td>
<td>(51, .0403)</td>
<td>(51, .0426)</td>
<td>(38, .0859)</td>
<td>(25, .1352)</td>
</tr>
<tr>
<td>OR</td>
<td>(102, .0395)</td>
<td>(46, .0878)</td>
<td>(33, .1234)</td>
<td>(15, .1264)</td>
<td>(11, .3719)</td>
</tr>
<tr>
<td>.0005 AND</td>
<td>(11, .1819)</td>
<td>(11, .1823)</td>
<td>(11, .1829)</td>
<td>(11, .1891)</td>
<td>(11, .2007)</td>
</tr>
<tr>
<td>OR</td>
<td>(107, .0387)</td>
<td>(47, .0870)</td>
<td>(33, .1226)</td>
<td>(15, .2377)</td>
<td>(11, .3712)</td>
</tr>
<tr>
<td>.001 AND</td>
<td>(6, .3334)</td>
<td>(6, .3338)</td>
<td>(6, .3343)</td>
<td>(6, .3389)</td>
<td>(6, .3456)</td>
</tr>
<tr>
<td>OR</td>
<td>(106, .0377)</td>
<td>(48, .0860)</td>
<td>(34, .1216)</td>
<td>(15, .2669)</td>
<td>(11, .3704)</td>
</tr>
<tr>
<td>.005 AND</td>
<td>(2, 1.0000)</td>
<td>(2, 1.0000)</td>
<td>(2, 1.0000)</td>
<td>(2, 1.0000)</td>
<td>(2, 1.0000)</td>
</tr>
<tr>
<td>OR</td>
<td>(22, .0948)</td>
<td>(22, .1101)</td>
<td>(22, .1289)</td>
<td>(17, .2591)</td>
<td>(12, .3629)</td>
</tr>
<tr>
<td>.01 AND</td>
<td>(1, 1.0000)</td>
<td>(1, 1.0000)</td>
<td>(1, 1.0000)</td>
<td>(1, 1.0000)</td>
<td>(1, 1.0000)</td>
</tr>
<tr>
<td>OR</td>
<td>(11, .1837)</td>
<td>(11, .1913)</td>
<td>(11, .2007)</td>
<td>(11, .2828)</td>
<td>(11, .3554)</td>
</tr>
</tbody>
</table>

Note: The numbers in the parentheses are the optimal group sizes and the number of tests per individual. The cases indicated by asterisks are the cases when the (optimal) OR method is more efficient than the (optimal) AND method.
Figure 1: Threshold method (simulation example): for a given threshold value, the top figure provides the number of individuals to be selected for testing, and the bottom figure provides the estimated number of positive individuals to be found before testing (solid line) and the actual number of positives that will be found after testing (dotted line).

Figure 2: Threshold method (drug discovery data): for a selected threshold value, the top figure provides the number of individuals to be selected for testing, and the bottom figure provides the estimated number of positive individuals to be found.