Testing the Maximum by the Mean in Quantitative Group Tests

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Abstract

Group testing, introduced by Dorfman in 1943, increases the efficiency of screening individuals for low prevalence diseases. A wider use of this kind of methodology is restricted by the loss of sensitivity inherent to the mixture of samples. Moreover, as this methodology attains greater cost reduction in the cases of lower prevalence (and, consequently, a higher optimal batch size), the phenomenon of rarefaction is crucial to understand that sensitivity reduction. Suppose, with no loss of generality, that an experimental individual test consists in determining if the amount of substance overpasses some prefixed threshold l. For a pooled sample of size n, the amount of substance of interest is represented by $(Y_1, \cdots, Y_n)$, with mean $\overline{Y}_n$ and maximum M_n. The goal is to know if any of the individual samples exceeds the threshold l, that is, $M_n > l$. It is shown that the dependence between $\overline{Y}_n$ and M_n has a crucial role in deciding the use of group testing since a higher dependence corresponds to more information about M_n given by the observed value of $\overline{Y}_n$.

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1 Introduction

The original idea of [1] was to use pooled samples on the screening of the defective members of a population (classification problem) in order to reduce the expected number of tests. In Dorfman's algorithm first stage, specimens are grouped for batched testing. If a pooled test is negative, all individuals in the pooled sample are declared negative. Otherwise, individual tests are performed. The optimal batch size minimizes the expected number of tests. Several extensions of this algorithm may be found in [2, 10, 11]. Alternative algorithms are presented in [5].

The seminal work of [9] deals with the problem of estimating the proportion of the defective members of a population (estimation problem).

The use of group testing schemes is usually restricted to qualitative analyses (presence or absence of the infection), without measuring any quantitative variable (antigens or antibodies or bacteria counts, or proportion of specific cells, or weight or volume of some chemical compound). If some continuous test outcome is available, it is usually transformed into a dichotomous outcome (cf. [13]). There are few works that deal with continuous outcomes (cf. [12,13]), but even those consider only the estimation problem. In this work, we present two possible methodologies that allow the application of Dorfman's algorithm to the classification problem when the test outcome is a continuous variable.

This work outline is as follows. Section 2 presents a discussion on the group testing procedure originally defined by Dorfman and the effect of the rarefaction phenomenon or the dilution effect (cf. [4]) in the sensitivity and the specificity of individual tests. In Sect. 3, two methodologies are proposed to conduct pooled sample tests with continuous outcomes. The importance of the correlation between the sample mean and the sample maximum is discussed when rarefaction may disturb the quality of group testing. The final remarks are presented in Sect. 4, where some suggestions for further investigation are given.

2 Dorfman's Procedures and Its Extensions

Let p denote the prevalence rate of the infection and the independent Bernoulli random variables X_i, with $i = 1, \cdots, N$, represent the presence $(X_i = 1)$ or absence $(X_i = 0)$ of the infection in the ith population individual. Furthermore, let $+$ and $-$ represent, respectively, the result of an individual test as positive and negative. The error of an individual test is usually assessed by two probabilities: the sensitivity and the specificity. The probability of getting a correct result on one individual test performed on a healthy individual is defined as the test specificity, that is, $\varphi_e = \mathbb{P}(-|\, X_i = 0)$. More important is the probability of detecting an infected individual, that is, the test sensitivity $\varphi_s = \mathbb{P}(+|\, X_i = 1)$. These definitions could be extended to pooled sample procedures (cf. [5,8]). Thus, using a sample of size n, the pooled sensitivity is defined by $\varphi_s^{[n]} = \mathbb{P}(+|\, \sum_{i=1}^{n} X_i > 0)$ and the pooled specificity by $\varphi_e^{[n]} = \mathbb{P}(-|\, \sum_{i=1}^{n} X_i = 0)$. The sensitivity $\varphi_s^{[n]}$ depends

on the number m of infected members as a result of the dilution of the fluid and its rarefaction. Therefore, using $\varphi_s^{[m,n]} = \mathbb{P}(+|\sum_{i=1}^n X_i = m)$ and applying Bayes theorem we obtain

$$\varphi_s^{[n]} = \frac{\sum_{j=1}^n \mathbb{P}\left(+, \sum_{i=1}^n X_i = j\right)}{\mathbb{P}\left(\sum_{i=1}^n X_i > 0\right)} = \sum_{j=1}^n \frac{\binom{n}{j} p^j (1-p)^{n-j}}{1-(1-p)^n} \varphi_s^{[j,n]} = \sum_{j=1}^n \lambda_j \varphi_s^{[j,n]}, \tag{1}$$

where $\sum_{j=1}^n \lambda_j = 1$.

In our problem both the healthy and the infected individual possess some substance of interest in the samples for testing. Suppose also that the amount of substance in a healthy individual follows some continuous distribution $Y \frown D_{\boldsymbol{\theta}_0}$ and that the amount of substance in an infected individual is $Y^* = \beta_0 + \beta_1 Y$ (or $Y^* \frown D_{\boldsymbol{\theta}_1}$ where $\boldsymbol{\theta}_0$ and $\boldsymbol{\theta}_1$ stand for distinct parameter vectors). We consider the cases where D is an exponential distribution, Gaussian distribution and Pareto distribution.

For an individual test, the hypothesis to be considered are

$$H_0 : X_i = 0 \text{ versus } H_1 : X_i = 1, \tag{2}$$

where the null hypothesis is equivalent to state that the amount of substance of the ith sample is described by $Y_i \frown D_{\boldsymbol{\theta}_0}$. A rule to decide if a sample is to be declared positive or negative is well defined for a known $D_{\boldsymbol{\theta}_0}$. For a significance level α, the null hypothesis is to be rejected if the amount of substance Y exceeds some fixed threshold $l = F_{D_{\boldsymbol{\theta}_0}}^{-1}(1-\alpha)$ where $F_{D_{\boldsymbol{\theta}_0}}^{-1}(1-\alpha)$ stands for the generalized inverse of the distribution function $D_{\boldsymbol{\theta}_0}$ at the point $1-\alpha$ (a similar reasoning is applied if the rule is to declare a positive sample when the amount of substance is lower than some threshold l). Hence, the test significance level coincides with $1-\varphi_e$ (i.e. $\varphi_e = 1-\alpha$). The power of the test is given by the probability that an infected sample is declared infected, that is, $1-\beta = 1-F_{D_{\boldsymbol{\theta}_1}}(l)$. Thus, the power of the test is equal to the sensitivity of the experimental test. Of course, if some further sources of error in the experimental test are considered, this correspondence between the two types of error of the hypothesis test and the two measures of the quality may not be valid. However, our goal is to show the association between these concepts.

As an example, consider $D_{\boldsymbol{\theta}_0} \equiv \mathrm{N}(\mu_0, \sigma_0)$ and $D_{\boldsymbol{\theta}_1} \equiv \mathrm{N}(\mu_1, \sigma_1)$ where $\mathrm{N}(\mu, \sigma)$ stands for a Gaussian distribution with mean μ and standard deviation σ. We will assume, with no loss in generality, $\mu_1 > \mu_0$ and let $\sigma = \sigma_1/\sigma_0$ and $\mu = \mu_1 - \mu_0 > 0$. Thus, in (2) we have $l = \mu_0 + \sigma_0 \Phi^{-1}(1-\alpha)$ and the power of the test is $1-\beta = 1-\Phi\left(\frac{l-\mu_1}{\sigma_1}\right) = 1-\Phi\left(\frac{\Phi^{-1}(1-\alpha)}{\sigma} - \frac{\mu}{\sigma_1}\right)$, where Φ denotes the cumulative distribution function of a standard Gaussian random variable. It is no surprise to verify that the test power increases with the difference of the mean values $\mu = \mu_1 - \mu_0$.

In Dorfman's methodology and its extensions to a number of stages greater than two, the decision whether a sample is or isn't infected depends firstly on the result of the pooled sample test. If the pooled sample test is considered to be positive further individual/grouped sample tests have to be conducted. The main problem here is to decide whether a pooled sample contains at least one infected individual since the substance of interest may be present both in healthy and infected individuals. This leads to the hypothesis test

$$H_0 : \sum_{i=1}^{n} X_i = 0 \; versus \; H_1 : \sum_{i=1}^{n} X_i > 0, \tag{3}$$

where the null hypothesis is equivalent to state that the amount of substance of interest is described by the $\sum_{i=1}^{n} Y_i$ of independent and identically distributed random variables to $Y \frown D_{\theta_0}$. As in the individual tests, it is necessary to establish a threshold (as a function of n) to decide if a pooled sample is or is not classified as a mixture of at least one infected individual. In Sect. 3 we propose two different methodologies in order to decide whether the null hypothesis should be rejected or maintained.

The process of getting a pooled sample is as follows. The same amount of sample is taken from n individuals and mixed (homogeneously). The new mixed sample is now tested. In a low prevalence case, a maximum of one infected sample in the pooled sample occurs with high probability. Hence, due to rarefaction the effect of this sample in the total amount of some substance in the pooled sample could be quite low. We raise this question in order to keep in mind that if the distributions D_{θ_0} and D_{θ_1} are not quite different the pooled sensitivity of the test could be seriously compromised. Some works incorporating rarefaction use some previous knowledge about this phenomenon (i.e. [12]). However, those works don't take advantage from this possibly known distributions. These pooled sample tests will be treated in detail in the next section.

3 The Pooled Sample Tests

When using pooled samples, the experimental test provides information on the batched sample as a whole although the experimenter wants to know if any of the individual samples exceeds the prefixed threshold l. The process of decision of the hypothesis test (3) isn't as obvious as the decision on individual testing represented by (2), because to classify a pooled sample it is previously needed to identify the samples in which $M_n = \max(Y_1, \cdots, Y_n)$ overpasses the threshold l using only the information of the sample mean (the only quantity observed). In this work, we discuss two different methodologies for deciding whether to reject or to maintain the null hypothesis of the pooled test (3).

Table 1 Simulation of correlation between mean $\overline{Y}_n$ and maximum M_n (1,000,000 replicates)

n	Standard Gaussian	Standard exponential	Pareto $\theta = 5$	Pareto $\theta = 3$	Pareto $\theta = 1$
2	0.8585	0.9483	0.9679	0.9801	0.9999450
3	0.7699	0.9034	0.9412	0.9646	0.9999819
5	0.6632	0.8413	0.8977	0.9378	0.9999714
50	0.3283	0.4919	0.6330	0.7607	0.9999983
100	0.2323	0.4062	0.5473	0.6889	0.9999786

3.1 T_1 Methodology: Using the Distribution of the Sample Mean

When mixing n healthy samples $Y_1, \cdots, Y_n$ and then extracting a portion $1/n$ of the total amount for batched testing, the amount of substance of interest is given by the random variable $C_{0,n}$ where $C_{m,n}$ is given by

$$C_{m,n} = \frac{\sum_{i=1}^{n-m} Y_i + \sum_{i=1}^{m} Y_i^*}{n}. \tag{4}$$

The random variable $C_{m,n}$ represents the amount of substance in a batched sample of size n with m infected individual samples. The null hypothesis of the hypothesis test (3) is rejected if $C_{0,n} > q_{1-\alpha}$ where $F_{C_{0,n}}(q_{1-\alpha}) = 1 - \alpha$ and $F_{C_{0,n}}$ stands for the distribution function of the random variable $C_{0,n}$.

If there is an infected individual in the pooled sample, the main problem is to know whether the observed value of the "mean" random variable $C_{m,n}$, with $m \geq 1$, is influenced by the presence of m infected samples.

A pooled sample that contains at least one defective individual should be screened as positive. Thus, a pooled sample is classified as defective if the sample maximum $M_n = \max(Y_1, \cdots, Y_n)$ overpasses the prefixed threshold l. However, the researcher uses only information about the mean to attain a decision that concerns only to the sample maximum. Hence, it is expected that the chance of deciding correctly increases with the dependence between the sample mean and sample maximum. For the three distributions mentioned above, the correlation between the sample mean and the sample maximum is computed for different sample sizes. All values presented in Table 1 were obtained by simulation (using software R) although we can get the same results analytically for the exponential distribution $\left[\rho_{M_n,\overline{Y}_n} = \sum_{i=1}^{n} i^{-1} \left(n \sum_{i=1}^{n} i^{-2}\right)^{-0.5}\right]$ and by numerical approximation for the Gaussian case (e.g. using $\rho_{M_n,\overline{Y}_n} = \left(\sqrt{n}\sigma_{M_n}\right)^{-1}$ with σ_{M_n} given in [7]). Thus, the simulation is an excellent resource to obtain good approximations of the theoretical value of the correlations.

The correlation decreases as n increases as expected. For the Pareto distribution, with shape parameter θ, the correlation is high even for n as high as 50. This is probably related to the heavy tails of this distribution. Otherwise, the correlation is quite moderate for the Gaussian distribution, and therefore, the power of test (3) is expected to be rather low. We simulate the Pareto(1) case to point out that the

sequence of correlations converges to 1 when θ decreases (although, for $\theta = 1$, the mean does not exist and therefore the simulation cannot be interpreted as an estimate of the theoretical value of the correlations). For the exponential and Gaussian distributions the correlations are independent of the parameters values.

3.2 $\mathbf{T_2}$ Methodology: Using a Simulation Method

Let α be the significance level of the hypothesis test (3). The aim is to reject the null hypothesis if at least one of the individual samples exceeds the threshold l. Therefore, under H_0,

$$\mathbb{P}(M_n \leq l) = \mathbb{P}(Y_1 \leq l, \cdots, Y_n \leq l) = F^n_{D_{\boldsymbol{\theta}_0}}(l) = 1-\alpha \Leftrightarrow l = F^{-1}_{D_{\boldsymbol{\theta}_0}}\left((1-\alpha)^{\frac{1}{n}}\right). \tag{5}$$

The computation of the generalized inverse distribution function $F^{-1}_{D_{\boldsymbol{\theta}_0}}$ is not generally straightforward but the use of simulation provides good approximations for the value of l (quantile $\sqrt[n]{1-\alpha}$ of distribution $D_{\boldsymbol{\theta}_0}$). Simulation is the core of this methodology. Let $\left(Y_{1j}, \cdots, Y_{nj}\right)_{j=1,\cdots,N}$ be N samples of size n generated by simulation that verify $Y_{ij} \frown D_{\boldsymbol{\theta}_0}$. Consider the N samples ordered by the sample maximum. Then, the k samples whose maximum is closest to l are chosen where k is an arbitrary number (in the simulations performed in Sect. 3.3 it was used $N = 10^5$ and k equals to 1 % of N). The mean sample of those k samples is computed and taken as the threshold l^* of decision for the pooled sample test, that is, if the mean sample exceeds l^* the pooled sample is declared infected.

3.3 Simulations Results

In this subsection, we compare the use of these two methodologies and their effects on sensitivity and specificity. Gaussian, exponential, and Pareto distribution are considered. The calculations are all done using simulation (via software R) although some calculus could be done analytically ($\overline{Y}_n \frown \mathrm{N}\left(\mu_0, \sigma_0/\sqrt{n}\right)$ if $Y \frown \mathrm{N}(\mu_0, \sigma_0)$ and $\overline{Y}_n \frown \mathrm{Gamma}\left(n, \frac{\lambda}{n}\right)$ if $Y \frown \mathrm{Exp}(\lambda)$).

Tables 2 and 3 present the specificity $\varphi_e^{[n]}$ and sensitivity $\varphi_s^{[n]}$ of a pooled sample test, applying methodologies $\mathbf{T_1}$ and $\mathbf{T_2}$ and assuming for each distribution that $D_{\boldsymbol{\theta}_1}$ is just a translation of $D_{\boldsymbol{\theta}_0}$. The translation is chosen in order to keep both sensitivity φ_s and specificity φ_e of individual tests equal to 0.95 (case 1) and to 0.995 (case 2). In all simulations we use the most efficient value for n in Dorfman's methodology [1].

The patterns observed in each methodology were already expectable according to Liu et al. [6]. In the $\mathbf{T_1}$ methodology, the loss of specificity of the test is low but it results in higher sensitivity loss than when using second methodology. Otherwise, the $\mathbf{T_2}$ methodology specificity loss is very close to the one using the $\mathbf{T_1}$

Table 2 Hypothesis tests simulation, Gaussian and exponential distribution (100,000 replicates)

		Case 1: $\varphi_s^{[1]} = \varphi_e^{[1]} = 0.95$				Case 2: $\varphi_s^{[1]} = \varphi_e^{[1]} = 0.995$			
		$\mathbf{T_1}$		$\mathbf{T_2}$		$\mathbf{T_1}$		$\mathbf{T_2}$	
p	$1-\alpha$	$\varphi_s^{[n]}$	$\varphi_e^{[n]}$	$\varphi_s^{[n]}$	$\varphi_e^{[n]}$	$\varphi_s^{[n]}$	$\varphi_e^{[n]}$	$\varphi_s^{[n]}$	$\varphi_e^{[n]}$
Gaussian distribution									
0.15	0.90	0.7746	0.8994	0.8573	0.8283	0.9623	0.9002	0.9830	0.8231
($n = 3$)	0.95	0.6627	0.9504	0.8012	0.8818	0.9229	0.9501	0.9728	0.8707
	0.99	0.4310	0.9900	0.6902	0.9408	0.7835	0.9901	0.9319	0.9427
0.05	0.90	0.6127	0.9003	0.7636	0.7971	0.8628	0.8992	0.9371	0.7960
($n = 5$)	0.95	0.4804	0.9501	0.7109	0.8417	0.7716	0.9500	0.9202	0.8320
	0.99	0.2534	0.9901	0.9490	0.9082	0.5431	0.9900	0.8445	0.9137
0.01	0.90	0.4066	0.8997	0.6600	0.7343	0.6261	0.9001	0.8277	0.7404
($n = 11$)	0.95	0.2759	0.9497	0.5917	0.7874	0.4880	0.9502	0.8122	0.7606
	0.99	0.1061	0.9899	0.5266	0.8338	0.2491	0.9900	0.7337	0.8347
Exponential distribution									
0.15	0.90	0.9423	0.8984	0.9923	0.8825	1.00	0.9019	1.00	0.8856
($n = 3$)	0.95	0.5496	0.9492	0.6673	0.9339	1.00	0.9554	1.00	0.9429
	0.99	0.1626	0.9894	0.2464	0.9792	0.6413	0.9895	0.9596	0.9820
0.05	0.90	0.8206	0.9005	0.9644	0.8698	1.00	0.8975	1.00	0.8667
($n = 5$)	0.95	0.4240	0.9486	0.6660	0.9198	1.00	0.9494	1.00	0.9229
	0.99	0.0792	0.9900	0.2195	0.9746	0.4019	0.9903	0.8979	0.9737
0.01	0.90	0.7668	0.9015	0.9907	0.8371	1.00	0.8957	1.00	0.8220
($n = 11$)	0.95	0.3514	0.9487	0.8710	0.8842	0.9521	0.9472	1.00	0.8896
	0.99	0.0256	0.9910	0.3292	0.9525	0.1896	0.9902	0.9367	0.9512

methodology and it performs better in what concerns to the sensitivity. We advise the use of the second methodology, since it has a better sensitivity behaviour.

4 Conclusion

The phenomenon of rarefaction can have a great effect in the quality of a pooled sample test. When the sample mean and the sample maximum correlation is high this effect is minimized and the use of batched samples is recommended.

When the correlation is low, the presence of an infected individual in the pooled sample has low effect on the amount of substance in the pooled sample. Therefore it is difficult to detect this infected sample. In this case, we have to be very careful when using pooled samples. Our recommendation is to use a pooled sample dimension lower than the optimal size obtained just by considering the relative cost of a specific methodology.

Further investigation may be conducted by considering a different null hypothesis in the test (3). As [3] points out, when the use of batched samples provides greatest savings (low prevalences), an infected pooled sample is almost certainly a pooled sample with just one infected sample ($\lambda_1 \approx 1$ for the efficient value for n in (1),

Table 3 Hypothesis tests simulation, Pareto distribution (100,000 replicates)

		Case 1: $\varphi_s^{[1]} = \varphi_e^{[1]} = 0.95$				Case 2: $\varphi_s^{[1]} = \varphi_e^{[1]} = 0.995$			
		$\mathbf{T_1}$		$\mathbf{T_2}$		$\mathbf{T_1}$		$\mathbf{T_2}$	
p	$1-\alpha$	$\varphi_s^{[n]}$	$\varphi_e^{[n]}$	$\varphi_s^{[n]}$	$\varphi_e^{[n]}$	$\varphi_s^{[n]}$	$\varphi_e^{[n]}$	$\varphi_s^{[n]}$	$\varphi_e^{[n]}$
Pareto(5)									
0.15	0.90	0.5813	0.9010	0.6300	0.8883	1.00	0.8995	1.00	0.8928
$n = 3$	0.95	0.3616	0.9473	0.3994	0.9408	1.00	0.9495	1.00	0.9422
	0.99	0.0646	0.9896	0.0802	0.9874	0.4166	0.9898	0.5075	0.9873
0.05	0.90	0.4189	0.9001	0.4725	0.8827	0.9924	0.8861	0.9987	0.8861
$n = 5$	0.95	0.2248	0.9507	0.2875	0.9353	0.8087	0.9514	0.9084	0.9362
	0.99	0.0406	0.9901	0.0583	0.9862	0.2526	0.9892	0.3350	0.9842
0.01	0.90	0.2585	0.9000	0.3555	0.8551	0.6771	0.9004	0.7738	0.8687
$n = 11$	0.95	0.1365	0.9504	0.2113	0.9207	0.4294	0.9497	0.5940	0.9168
	0.99	0.0263	0.9900	0.0554	0.9800	0.1118	0.9902	0.2054	0.9881
Pareto(3)									
0.15	0.90	0.5691	0.9019	0.5883	0.8978	1.00	0.8994	1.00	0.8905
($n = 3$)	0.95	0.2900	0.9518	0.3216	0.9479	1.00	0.9534	1.00	0.9494
	0.99	0.0287	0.9891	0.0325	0.9886	0.3409	0.9904	0.3840	0.9893
0.05	0.90	0.3669	0.9047	0.3996	0.8966	1.00	0.8984	1.00	0.8873
($n = 5$)	0.95	0.1836	0.9529	0.2189	0.9457	0.9359	0.9529	0.9793	0.9472
	0.99	0.0286	0.9901	0.0339	0.9887	0.1924	0.9903	0.2304	0.9886
0.01	0.90	0.2265	0.9032	0.2714	0.8803	0.7715	0.9002	0.8551	0.8768
($n = 11$)	0.95	0.1030	0.9490	0.1337	0.9382	0.4418	0.9492	0.5234	0.9355
	0.99	0.0183	0.9888	0.0220	0.9863	0.0772	0.9904	0.1089	0.9875

therefore the sensitivity $\varphi_s^{[1,n]}$ is crucial in $\varphi_s^{[n]}$ determination). This means that the study of the hypothesis test

$$H_0 : \sum_{i=1}^{n} X_i = 1 \; versus \; H_1 : \sum_{i=1}^{n} X_i = 0$$

is quite general and may be an alternative to follow up.

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