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Bayesian analysis and classification of two Enzyme-Linked Immunosorbent Assay (ELISA) tests without a gold standard

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Abstract

Reconciling two quantitative ELISA tests for an antibody to an RNA virus, in a situation without a gold standard and where false negatives may occur, is the motivation for this work. False negatives occur when access of the antibody to the binding site is blocked. Based on the mechanism of the assay, a mixture of four bivariate normal distributions is proposed with the mixture probabilities depending on a two-stage latent variable model including the prevalence of the antibody in the population and the probabilities of blocking on each test. There is prior information on the prevalence of the antibody, and also on the probability of false negatives, and so a Bayesian analysis is used. The dependence between the two tests is modeled to be consistent with the biological mechanism. Bayesian decision theory is utilized for classification. The proposed method is applied to the motivating data set to classify the data into two groups: those with and those without the antibody. Simulation studies describe the properties of the estimation and the classification. Sensitivity to the choice of the prior distribution is also addressed by simulation. The same model with two levels of latent variables is applicable in other testing procedures such as quantitative polymerase chain reaction tests where false negatives occur when there is a mutation in the primer sequence.

Keywords

Bayesian analysis; Bayesian decision theory; diagnostic testing; mixture model

1. Introduction

In the absence of a diagnostic test with positive predictive value (PPV) and negative predictive value (NPV) extremely close to one (a gold standard), combining multiple imperfect tests to obtain a classification may be necessary [1]. Reconciling the results of two imperfect quantitative Enzyme-Linked Immunosorbent Assay (ELISA) tests is addressed

here. Based on the biological mechanism underlying the tests, a parametric model is proposed with two levels of latent variables. Prior information on the prevalence of the antibodies, and the probability of false negatives on each test is easily incorporated through the prior information. When the antibodies are present and neither test result is a false negative, the dependence between the two results is incorporated through a positive correlation.

An Enzyme Linked Immunosorbent Assay, or ELISA, is a test for the presence of a specific binding site on a specific antibody; a Polymerase Chain Reaction, or PCR, is a test for the presence of a specific subsequence of the genetic material of a virus or retrovirus (DNA or RNA respectively). In a sandwich ELISA, as described in Appendix A, if the binding site of the antibody of interest (Ab #2 in Figure A.1) on the E2 protein is physically blocked by MAb #1, then the result of the assay will be consistent with no Ab #2 being present and therefore be a false negative. It is reasonable to assume that if a false negative occurs then the quantitative result is not only consistent with no antibody being present but also, conditional on the false negative occurring, the false negative result is independent of the quantitative result of the other test. If neither test is a false negative then the two quantitative results are closely related as they both quantify Ab #2. Similar arguments result for real time PCR testing where two tests look for different subsequences of genetic coding on the virus: false negatives occurring when there is a mutation in a target subsequence.

Statistical methods for diagnostic testing with no gold standard were addressed by Hui and Walter [2]. They considered the case where the false positive (negative) rate of both the new test and the reference test is unknown. By applying two tests simultaneously to individuals from two populations with different prevalence of disease, and further assuming conditional independence, the sensitivity and specificity of both tests, together with the true prevalence in two populations, could be estimated by maximum likelihood (ML). The Hui-Walter model has been extensively discussed and extended since it was proposed. Vacek [3] discussed the impact of the conditional independence on the estimates of the error rates in the model, and Walter and Irwig [4] provided a thorough discussion of the method in different settings. Joseph et al. [5] developed Bayesian methods for the evaluation and implementation of the conditional independent tests. Hui and Zhou [6] summarized many available methods for qualitative diagnostic test evaluation, with special focus on estimating sensitivity and specificity without assuming the conditional independence. The Hui-Walter model and its extensions have also been applied to research in animal health as discussed by Enøe et al. [7]. The conditional dependence is accommodated via either an ML approach or a Bayesian approach, for example, by Qu et al. [8], Yang and Becker [9], Dendukuri and Joseph [10], Black and Craig [11]. These methods are applicable only to binary tests, and hence cannot be directly utilized for quantitative tests.

For a quantitative assay, the sensitivity and specificity are computed based on a certain classification rule with a specific threshold value. The two accuracy indices are dependent on the choice of the classification rule. Therefore, when the true disease status is unknown and there is no gold standard or no imperfect binary reference test, as in our motivating problem, it is necessary to establish a classification. Nielsen et al. [12] proposed estimation of the sensitivity and specificity pointwise over the whole range of cutoff values by the ML

method of the Hui-Walter model. However, the estimated receiver operating characteristic (ROC) curve obtained by connecting all the estimated (sensitivity, 1-specificity) values is not necessarily monotonic. Henkelman et al. [13] used a latent mixture model of two multivariate normal distributions to estimate the ROC curve for ordinal-scale tests, and Choi et al. [14] adopted the same parametric model and used the Bayesian method to estimate the ROC curve for continuous-scale tests. Both estimated curves are guaranteed to be monotone increasing. To relax the normality assumption, Hall and Zhou [15] proposed a nonparametric estimator for the ROC curves of continuous tests based on the conditional independence assumption. Zhou et al. [16] applied this estimator to estimate the ROC curves for ordinal tests in the absence of a gold standard. If there is an imperfect binary test, the ROC curve of the a continuous test can be estimated by comparing to the binary test using a Bayesian approach [17]. This approach assures the monotonicity of the ROC curve without any assumptions regarding to the distributions of the test results.

All of the aforementioned methods primarily focus on estimating the ROC curve to evaluate the tests. There is little guidance available on how to obtain a gold standard, or at least an imperfect classification by combining multiple quantitative tests. The main objective of this paper is to develop a model and method for combining multiple quantitative tests and deriving a classification rule. Section 2 proposes the two-level latent variable model and Section 3 derives the decision rule to classify samples. Simulation studies are provided in Section 4 to assess the Bayesian classification rule and Section 5 presents the results of the analysis of samples from 100 subjects each tested by two methods along with a prior distribution based on previous data. Section 6 is a discussion of the model's strengths and limitations. It explains how the model is also applicable to data from multiple quantitative (real time) PCRs. Appendix A gives a biological motivation for the presence of false negatives but not false positives and the background of samples in the example. Appendix B provides details of a sensitivity analysis for the motivating data set. Appendix C is an additional simulation study where the model is not well fit and the biological assumptions are violated.

2. Statistical Model

The model is motivated by the example of Section 5 and Appendix A.

2.1. Notation, Assumptions and Model

For the k^{th} sample, $k = 1, 2, \dots, n$, and the i^{th} test, $i = 1, 2$, let Y_{ik} be the observable result. Corresponding X_k, X_{ik} , $i = 1, 2, k = 1, 2, \dots, n$ are binary latent variables as below:

$$X_k = \begin{cases} 1 & \text{if the target antibodies are present in the blood sample } k. \\ 0 & \text{if the target antibodies are absent in the blood sample } k. \end{cases}$$

$$X_{ik} = \begin{cases} 1 & \text{if } X_k=1 \text{ and the binding site for test } i \text{ on sample } k \text{ is accessible.} \\ 0 & \text{if } X_k=1 \text{ and the binding site for test } i \text{ on sample } k \text{ is blocked.} \end{cases}$$

$$X_{ik}=0, \text{ if } X_k=0.$$

Assume that if antibodies are present ($X_k = 1$) and both tests have accessible binding sites ($X_{1k} = X_{2k} = 1$), then Y_{1k} and Y_{2k} are positively correlated. If antibodies are present but at

least one binding site is inaccessible, then Y_{1k} and Y_{2k} are independent. Similarly, if there are no antibodies present, $X_k = 0$, then Y_{1k} and Y_{2k} are independent and have the identical distribution as when antibodies are present but both binding sites are inaccessible ($X_k = 1$ and $X_{1k} = X_{2k} = 0$). The joint distribution of Y_{1k} and Y_{2k} conditioning on any combination of X_{1k} and X_{2k} is assumed to be bivariate normal. Hence Y_{1k} and Y_{2k} are jointly distributed as a mixture of four bivariate normal distributions conditioning on X_{1k} and X_{2k} , $k = 1, \dots, n$. The four distributions are defined:

$$\begin{aligned} \left(\begin{pmatrix} Y_{1k} \\ Y_{2k} \end{pmatrix} \middle| X_{1k}=X_{2k}=1, X_k=1 \right) &\sim N \left(\begin{pmatrix} \mu_{1P} \\ \mu_{2P} \end{pmatrix}, \begin{pmatrix} \sigma_{1P}^2 & \rho\sigma_{1P}\sigma_{2P} \\ \rho\sigma_{1P}\sigma_{2P} & \sigma_{2P}^2 \end{pmatrix} \right), \\ \left(\begin{pmatrix} Y_{1k} \\ Y_{2k} \end{pmatrix} \middle| X_{1k}=X_{2k}=0 \right) &\sim N \left(\begin{pmatrix} \mu_{1N} \\ \mu_{2N} \end{pmatrix}, \begin{pmatrix} \sigma_{1N}^2 & 0 \\ 0 & \sigma_{2N}^2 \end{pmatrix} \right), \\ \left(\begin{pmatrix} Y_{1k} \\ Y_{2k} \end{pmatrix} \middle| X_{1k}=1, X_{2k}=0, X_k=1 \right) &\sim N \left(\begin{pmatrix} \mu_{1P} \\ \mu_{2N} \end{pmatrix}, \begin{pmatrix} \sigma_{1P}^2 & 0 \\ 0 & \sigma_{2N}^2 \end{pmatrix} \right), \\ \left(\begin{pmatrix} Y_{1k} \\ Y_{2k} \end{pmatrix} \middle| X_{1k}=0, X_{2k}=1, X_k=1 \right) &\sim N \left(\begin{pmatrix} \mu_{1N} \\ \mu_{2P} \end{pmatrix}, \begin{pmatrix} \sigma_{1N}^2 & 0 \\ 0 & \sigma_{2P}^2 \end{pmatrix} \right), \end{aligned}$$

where the means μ_{1N} and μ_{2N} denote the means of Y_{1k} and Y_{2k} when either antibodies are absent (true negatives) or antibodies are present but binding site 1 or 2 respectively is inaccessible (false negatives). The means μ_{1P} and μ_{2P} denote the mean responses when antibodies are present and can bind. Based on the biological mechanism, the high test result values should correspond to higher chances of being “positive”. Hence we set a constraint that $\mu_{1P} > \mu_{1N}$ and $\mu_{2P} > \mu_{2N}$. To guarantee that the constraint holds, define new parameters $\beta_i = \log(\mu_{iP} - \mu_{iN})$ ($i = 1, 2$). Parameters $\sigma_{1N}^2, \sigma_{2N}^2, \sigma_{1P}^2$ and σ_{2P}^2 are variances, constrained to be positive. The positive correlation between Y_{1k} and Y_{2k} , if both binding site are accessible, is denoted by ρ with $0 < \rho < 1$.

Denote the prevalence of E2 antibodies $\phi = \Pr(X_k = 1)$, and denote the probability of the binding site being accessible in test i ($i = 1, 2$) if E2 antibodies are present as $\phi_i = \Pr(X_{ik} = 1 | X_k = 1)$. Then assuming latent variables X_{1k} and X_{2k} are independent conditional on $X_k = 1$, the mixture proportions are:

$$\begin{aligned} \Pr(X_{1k}=X_{2k}=1, X_k=1) &= \phi_1\phi_2\phi, \\ \Pr(X_{1k}=X_{2k}=0) &= (1-\phi_1)(1-\phi_2)\phi + (1-\phi), \\ \Pr(X_{1k}=1, X_{2k}=0, X_k=1) &= \phi_1(1-\phi_2)\phi, \\ \Pr(X_{1k}=0, X_{2k}=1, X_k=1) &= (1-\phi_1)\phi_2\phi. \end{aligned}$$

The unknown parameters are denoted as

$\underline{\psi} = (\phi, \phi_1, \phi_2, \mu_{1N}, \mu_{2N}, \beta_1, \beta_2, \sigma_{1N}^2, \sigma_{2N}^2, \sigma_{1P}^2, \sigma_{2P}^2, \rho)^T$. The values ϕ, ϕ_1, ϕ_2 , are probabilities and are between 0 and 1, as is the correlation ρ .

2.2. Parameter Estimation

2.2.1. Maximum Likelihood (ML) Estimation—The parameters $\underline{\psi}$ can be estimated by ML. The estimates (MLE) can be found using numerical optimization and an iterative approach as follows:

1. Choose a starting value for $\underline{\psi}_1(\phi, \phi_1, \phi_2)^T$
2. Maximize the log-likelihood as a function of $\underline{\psi}_2 = (\mu_{1N}, \mu_{2N}, \beta_1, \beta_2, \sigma_{1N}^2, \sigma_{2N}^2, \sigma_{1P}^2, \sigma_{2P}^2, \rho)^T$ for that fixed $\underline{\psi}_1$.
3. Denote the results as $\underline{\psi}_2 \hat{=} \underline{\psi}_1$ and then maximize the log-likelihood as a function of $\underline{\psi}_1$ for fixed $\underline{\psi}_2 = \underline{\psi}_2 \hat{=} \underline{\psi}_1$.
4. Denote the results as $\underline{\psi}_1 \hat{=}$ and use that as a starting value to repeat the steps above until the estimates converge.

Note that without the constraint that $\mu_{iP} = \mu_{iN}$ for $i = 1, 2$, the likelihood may be multimodal. There is a lack of identifiability without the constraint: the constraint requires high values of either test to be “positive” and low values to be “negative”. See Section 6 for more discussion.

2.2.2. Bayesian Estimation—In the motivating data set, there is some prior information available and this is used in constructing the prior distribution in Section 5. This prior distribution incorporates the constraint that $\mu_{iP} = \mu_{iN}$ for $i = 1, 2$. Because of the complexity of the model, it is impossible to obtain the marginal posterior distribution for parameters analytically. The Markov chain Monte-Carlo (MCMC) method is utilized to simulate samples from the marginal posterior distribution of each parameter. We use the software WinBUGS [18] to implement the MCMC method and use the R [19] package R2WinBUGS [20] to call WinBUGS. Similar results were obtained from a self contained R program. Code is available in a technical report [21].

3. Statistical Decision Rule

The classification decision is chosen after observing the values of the random variables Y_1 and Y_2 and computing the posterior distribution, denoted $p(\phi|data)$. The observed quantitative test results Y_1 and Y_2 provide information about the parameters ϕ . For a new sample with test results (Z_1, Z_2) , let the loss of classifying this sample as negative if it is in fact positive be L_1 and the loss of classifying this sample as positive if it is negative be L_2 . In addition, if the correct positive or negative classification is made, the loss is zero.

The posterior probability of E2 antibodies being present for (Z_1, Z_2) , $Pr(X = 1|Z_1, Z_2, data)$, abbreviated to PPP , is:

$$PPP = Pr(X=1|Z_1, Z_2, data) = \int Pr(X=1|Z_1, Z_2, \underline{\psi}) p(\underline{\psi}|data) d\underline{\psi} \\ = \int \frac{f(Z_1, Z_2|X=1, \underline{\psi}) Pr(X=1|\underline{\psi})}{f(Z_1, Z_2|X=1) Pr(X=1|\underline{\psi}) + f(Z_1, Z_2|X=0, \underline{\psi}) Pr(X=0|\underline{\psi})} p(\underline{\psi}|data) d\underline{\psi}. \quad (1)$$

Under Bayesian decision theory [22], the risk under the negative classification is $PPP \times L_1$, and the risk under the positive classification is $(1 - PPP) \times L_2$. The optimal Bayes decision for (Z_1, Z_2) based on the observed data is therefore the one that has the smaller risk. Hence, (Z_1, Z_2) is classified as positive if $(1 - PPP) \times L_2 < PPP \times L_1$, which is equivalent to $PPP > C$, where $C = 1/(1 + L_1/L_2)$. The value $C = 0.5$ corresponds to $L_1 = L_2$ and represents a symmetric loss of misclassification. In many applications $L_1 \neq L_2$ and any value of C between 0 and 1 can be obtained by choosing different values. For example, false negatives in disease screening may lead to no treatment and subsequently worse consequences of the disease: in this case it may be appropriate to choose $L_1 > L_2$. Alternatively if the treatment subsequent to a false positive result is toxic it may be appropriate to choose $L_2 > L_1$.

4. Simulation Studies

A simulation study, in a 4×3 factorial structure, was designed to assess the accuracy of the Bayesian classification rule developed above. The data are assumed to arise from either the mixture of four bivariate normal distributions or a similar mixture of four bivariate t distributions with 4 degrees of freedom. Skewed versions of the distributions are also used: the bivariate skew normal and skew t distributions with shape parameter -3 (right-skewed) [23]. The values of the parameters in the model are specified as follows

$$\begin{cases} \varphi=0.48; \varphi_1=0.9; \varphi_2=0.8; \\ \mu_{1N}=0.15; \mu_{2N}=0.24; \mu_{1P}=1; \mu_{2P}=0.9; \\ \sigma_{1N}^2=0.004; \sigma_{2N}^2=0.02; \sigma_{1P}^2=0.57; \sigma_{2P}^2=0.40; \\ \rho=0.54, \end{cases}$$

which are, approximately, the posterior means of the data set in Section 5. The posterior distribution is calculated using the mixture of bivariate normal distributions and prior distribution A, justified in Section 5. The classification under $C = 0.5, 0.7$ and 0.9 is implemented, respectively. Because the simulated data are generated with a known classification of each sample (gold standard), a linear discriminant analysis is also carried out; this assumes the model is a mixture of two bivariate normal distributions. The empirical measures of the diagnostic accuracy are computed based on 500 simulated data sets for each of the three Bayesian classification rules and the linear discriminant classifier (LDC). Computations were done in R [19] and WinBUGS [18]. The coda package [24] was used to calculate highest posterior density intervals and R2WinBUGS to call WinBUGS from R [20].

All the analyses converge and results are summarized in Table 1 and Table 2. Results in Table 1, indicate that even though the LDC uses more information, it assumes an incorrect distribution and it generally performs worse than the Bayesian classification method. See Su and Liu [25] for conditions under which the LDC is optimal. Among the three Bayesian classification rules with different cutoff values C , for any kind of data, a higher cutoff value C leads to a lower sensitivity and a higher specificity (the higher C is, the fewer samples are classified as positive). At any fixed C , the sensitivity for the t data is slightly higher than that for the normal data, while the specificity for the normal data is much greater than for the t

data. This is reasonable considering that the t distribution has fatter tails, hence the true negative group has more overlap with the true positives. The PPV and NPV have similar comparisons as the specificity and sensitivity, implying that the mis-specified model tends to overestimate the PPP for the t data, and hence more samples are classified as positive. Adding the skewness to the data does not affect the performance of the classification much but Table 2 indicates that the coverage probabilities of the 95% highest posterior density intervals for some parameters is very low in many cases. Note that the parameter estimation is biased under the mis-specified model, especially for the location and scale parameters when the true underlying marginal distribution is a mixture of skew normal or t , according to Table 2.

5. Illustrative Example

In the motivating example, a total of 100 blood specimens obtained from HIV infected subjects were tested for GBV-C E2 antibody with each of two tests: called the μ Plate Anti-HGenv ($i = 1$) and M5 ($i = 2$) assays. The two assays are variations on the sandwich ELISA and the differences between them are explained in more detail in Appendix A. False negatives occur in both tests when the binding site of the human antibody Ab #2 to the E2 protein is blocked by MAb #1. The additional material introduced in the μ Plate Anti-HGenv through the lysate may add additional noise that causes blocking.

Neither test is perfect and no commercial and validated test is available for the antibody, which means that there is no gold standard. Prior information is that false negatives due to blocking may occur approximately 10% of the time but there is uncertainty about this value. The parameters ϕ_1 and ϕ_2 are therefore assumed to have a $Beta(18, 2)$ distribution independently: the prior mean is therefore 0.90, the prior mode 0.94, and the prior standard deviation is 0.065.

For GBV-C, the prevalence of the target antibodies, ϕ , varies between populations but the average is thought to be about 50% in HIV-infected populations based on previous studies [26–31]. Note however that all these studies used imperfect tests. Based on this information, the prior distribution for ϕ is chosen as a $Beta(5, 5)$ which is symmetric on (0, 1) and centered at 0.5. For the correlation ρ between two quantitative results (true positives), a uniform distribution on (0, 1) was chosen as putting probability one on a positive correlation but otherwise being non-informative. The means, μ_{1N} and μ_{2N} , of the two tests when either no antibodies are present or a false negative occurs are assumed to have a normal distribution $N(0, 100)$. To incorporate the constraint that higher values of either test correspond to a greater chance of antibodies being present, recall that $\beta_i = \log(\mu_{iP} - \mu_{iN})$ and any prior distribution on β_i incorporates the constraint that $\mu_{iP} > \mu_{iN}$. Parameters β_1 and β_2 are assumed to be independent in the prior distribution and each has a $N(0, 10^4)$. The prior distribution for each of the inverse variances $\sigma_{1N}^{-2}, \sigma_{2N}^{-2}, \sigma_{1P}^{-2}, \sigma_{2P}^{-2}$ is assumed to be $\Gamma(0.01, 0.01)$ ($\Gamma(\alpha, \beta)$ is the Gamma distribution with the shape parameter α and rate parameter β) independently, representing a lack of specific information. This primary prior distribution is denoted as Prior A, and is as below:

$$\begin{aligned}
\varphi &\sim \text{Beta}(5, 5) \\
\varphi_1, \varphi_2 &\sim \text{Beta}(18, 2) \\
\mu_{1N}, \mu_{2N} &\sim N(0, 100) \\
\beta_1, \beta_2 &\sim N(0, 10000) \\
\sigma_{1N}^{-2}, \sigma_{2N}^{-2}, \sigma_{1P}^{-2}, \sigma_{2P}^{-2} &\sim \Gamma(0.01, 0.01). \\
\rho &\sim U(0, 1),
\end{aligned}$$

with all of the above assumed to be independent. Recall that $\beta_i = \log(\mu_{iP} - \mu_{iN})$ and so $\mu_{iP} > \mu_{iN}$ for $i = 1, 2$.

The observed data are plotted on the right panel in Figure 1 and the *PPP* for each of the 100 blood samples are shown as a histogram in the left panel. Because the classification was to be used in an analysis comparing antibody positive subjects to antibody negative subjects, a value $C = 0.5$ was used for classification: positively classified samples are in green, and negatively classified samples are in red. The samples with low results on both tests are classified as E2 antibody negative, and samples with high results on at least one test are classified as positive. This is consistent with the biological mechanism.

Details of the posterior distribution are in Table 3. Note that for ϕ_2 the prior and posterior standard deviation are almost identical, although the corresponding means (0.9 in the prior, and 0.842 in the posterior) and modes (0.944 in the prior, and 0.853 in the posterior) are different.

Appendix B provides a sensitivity analysis using six additional prior distributions (priors B, C, D, E, F and G). The classification with $C = 0.5$ leads to almost identical classifications under each prior distribution: the classifications differ in at most 3 samples for priors B, C, D and E, 7 samples for F and 8 samples for G. Note that F and G are the least informative prior distributions. See Figure B.1 for the classification and Figure B.2 for the posterior distributions. The figures indicate that marginal posterior distributions for some parameters (notably ϕ , ϕ_1 , ϕ_2) are sensitive to the choice of prior distribution. This is understandable as these parameters correspond to the latent variable structure. However, the classification is fairly robust.

6. Discussion

A two-level latent variable model is proposed for reconciling two tests in the absence of a gold standard. The model is consistent with a common biological mechanism given in Appendix A. If the data are from the assumed bivariate normal mixture distribution, or from a similar bivariate t mixture distribution, with or without skewness, the classification has a robust discriminating capability in the cases examined by simulations in Section 4.

The model assumes that conditioning on the antibodies being present ($X_k = 1$) and both binding sites being accessible ($X_{1k} = X_{2k} = 1$), the measurements are positively correlated. This is reasonable as they both measure the concentration of the antibody in the sample. If either the antibody is absent ($X_k = 0$), or it is present but in one or both of the tests the binding site is blocked, then the responses are independent. This conditional independence

assumption can be criticized, but in this case seems biologically very plausible. The two tests are carried out separately on different plates, so if the antibody is present in the sample, the blocking of the binding site for one test is independent from the blocking for the other test. Therefore, the results from the two tests are independent from each other unless both binding sites are accessible, in which case both quantitative results reflect the concentration of the antibody of interest. Conditional independence is reasonable in the other cases when one or both tests are false negatives.

In the biological mechanism, high values of a test result should correspond to “positive” classifications and low values to “negative”, and so the constraint $\mu_{iP} \geq \mu_{iN}$ for $i = 1, 2$ is implemented. Without the constraint, there is an identifiability question. Plots of the profile log-likelihood of μ_{iN} and μ_{iP} ($i = 1, 2$) indicate very well the issue in the parameter estimation for the example data set. The profile likelihoods for $i = 1, 2$ have a ridge, symmetric around the axis $\mu_{iP} = \mu_{iN}$ where the values of μ_{iP} and μ_{iN} can be interchanged without changing the likelihood much for each $i = 1, 2$. Omitting the constraint may lead to a classification that is inconsistent with the biological mechanism. The sensitivity analysis was repeated without the constraint, and if a starting value is chosen that does not satisfy the constraint, the analysis sometimes converges to a local mode at which the constraint does not hold.

The classification can also be achieved in the ML approach, although the likelihood maximization should be done carefully. Since from the ML aspect, the parameters are fixed but unknown, the PPP for each sample is estimated by the $\Pr(X = 1 | Z_1, Z_2, \hat{\psi})$ in the integrand of (1), where $\hat{\psi}$ are the MLE of ψ . Figure B.3 illustrates the histogram of PPP estimated by the ML approach and the corresponding classification. The classification is exactly the same as the classification under prior G. However, there are twelve parameters to be estimated and the sample size of the motivating data is just 100, which is relatively small to make asymptotic inferences. The Bayesian approach is preferred here to obtain more stable estimation because there does exist some prior information on parameters such as the prevalence and the probability of false negatives. Additionally, the Bayesian approach will lead to interpretable interval estimates that do not rely on asymptotic arguments.

The model is developed based on combining two ELISA tests for antibodies, but it can be extended easily to an arbitrary number of tests, or modified to accommodate different kinds of testing problems. For example, in a real time polymerase chain reaction (PCR) test, part of a virus genome is amplified and quantified. If a mutation occurs in that part of the genome, the primer does not detect the virus, and a false negative results. In RNA viruses especially, errors in transcription result frequently, and mutations (and hence false negatives) result.

There remain some limitations to this method. For example the sensitivity analysis of our example (Appendix B) shows that the shapes of the marginal posterior distributions are sensitive to the choice of the prior distribution, although the classification does not change much. In many situations classification is more important than parameter estimation.

The primary prior distribution (prior A) was informative on the prevalence (ϕ), and on the two probabilities of a true positive response (ϕ_1, ϕ_2). The prior distributions on these parameters were specified in discussion of the statistical authors (JZ and KC) with the physician-scientist author (JTS) from whose lab the data were generated and who developed one of the tests. Other more structured methods for the specification of a prior distribution, such as in Christensen [32], may be superior. The primary goal of the ELISA test results was classification because immunological responses to therapy were to be compared between two groups of subjects: comparing subjects infected with HIV alone to subjects infected with both HIV and GBV-C. The robustness of the classification to the prior distribution is reassuring.

The parametric assumption is another limitation to this method. In the model, the results from the two tests are assumed to be a mixture of multivariate normal distributions. In practice, this may not be warranted, and is hard to verify. Under a mis-specified model, it is not surprising for estimates of the parameters to be biased. For simulated data similar to our motivating example with some separation of the four components of the mixture model and the data corresponding to the simulations in Section 5, the classification appears to be quite robust. When there is a lack of separation in the four populations, the classification appears to have a low sensitivity in the examples examined and in addition, computational problem arises (Appendix C). Computation of the PPP for each sample however, may help in a classification system that includes three categories (positive/negative/indeterminate). Note, however, that a good ELISA test or PCR test should have some separation in the marginal distributions.

In addition, for a different population, a different prior distribution will be needed and the parameters may be different. For example, because HIV and GBV-C share the same modes of infection, the prevalence of the E2 antibody in an HIV-infected population is high, about 50%, whereas the prevalence in the general population of blood donors is much lower, about 5%. The prior distribution on the prevalence should be different for the two populations. Caution should also be used in using prior information from one population to extrapolate to a different population in constructing the prior. It is possible that the underlying level of what is being tested for (concentration of antibody in an ELISA, or concentration of virus in a PCR) is different in different populations and therefore the parameters of the mixture components involving true positive measurements will differ. It may be reasonable to assume that the true negative responses on a test are similar across populations and perhaps also the probability of a false negative. Different prior distributions for different populations are easily incorporated. Different populations may share the same model structure, but with different parameter values (perhaps, for example, with a hierarchical structure between populations).

To summarize, this method provides a reasonable method for combining the results of quantitative tests when there is no gold standard and when false negatives may occur fairly frequently, independently on each test, and the probability of a false negative does not depend on the underlying value of the quantitative variable. It provides a systematic way of combining the results so that sufficiently high values of any one test lead to a positive classification.

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Appendices

A. Details of the Motivating Example

GBV-C is a human RNA virus, not currently known to definitely cause any disease although a recent observational study suggested a potential link between GBV-C and non-Hodgkins lymphoma [33]. There is also evidence that people with HIV disease who are co-infected with GBV-C have prolonged survival [34]. In addition, one study found an association between GBV-C and response to an HIV therapy [35]. The mechanism for these mechanisms is under investigation [36–38]. At this time, there is no commercial and validated test available for GBV-C antibodies. When people with active infection (viremia) with GBV-C clear infection, antibodies develop that are directed against the viral envelope glycoprotein 2 (E2). Several Enzyme Linked Immunosorbent Assays (ELISAs) have been designed to detect the presence of E2 antibodies in human serum samples. ELISAs can be designed in several ways, but all GBV-C assays reported to date use E2 Monoclonal antibodies (MAb) which bind to the E2 protein at a specific site.

One test was developed by Roche Laboratories and is denoted the μ Plate Anti-HGenv test [39]. It is a variation of a “sandwich capture assay”. It uses full-length recombinant E2 protein in a Chinese Hamster Ovary (CHO) cell lysate (this contains other cellular material in addition to E2 protein). This lysate is treated with a specific murine monoclonal antibody (MAb #1) which binds to the E2 protein. MAb #1 is biotinylated and binds to the E2 in the lysate but supposedly not to the other cellular materials present. After MAb #1 is mixed with the E2, it is added to wells on a microtitre plate together with the human sample. The wells are coated with streptavidin which binds to the biotin on the MAb #1 (which has E2 protein attached). If there are GBV-C E2 antibodies in the human sample, these human antibodies (denoted as Ab #2) in Figure A.1 will bind to the E2 protein. When the plate is subsequently washed, the E2 protein-biotinylated MAb complex remains on the plate. In some samples however, the human antibodies will not bind because they are directed against the same region on E2 recognized by MAb #1 and their access is therefore blocked; blocking may also occur because of the additional cellular material in the lysate. This blocking is the

mechanism for false negatives. Anti-human IgG antibodies conjugated to an enzyme are then added to the wells, which attach to Ab #2. A colorimetric substrate for the enzyme is added afterward to allow determination of the concentration of enzyme present in the well, reflecting the amount of human anti-E2 antibody. Control wells to which no human serum is added are present on each plate to measure nonspecific material that may stick to Ab #2 and give rise to background fluorescence. The ultraviolet absorbance of color in the wells is measured and compared to the fluorescence of the control wells.

A second test (denoted M5), was developed in the Stapleton laboratory [40], and is a more common variation on the sandwich capture assay. The end result of the test is the same, as in Figure A.1, but the procedure to get there differs from the μ Plate Anti-HGenv ELISA. A murine MAb #1 specific for E2 protein is attached to microtiter plate wells. This MAb was provided by Dr. Alfred Engel, Roche Diagnostics, Penzburg, Germany. The MAb used may be the same as the MAb used in the μ Plate Anti-HGenv test; however, this information is proprietary. This antibody is not biotinylated. Semi-purified recombinant E2 protein for which the C-terminal membrane spanning domain is not included is added to wells. The plate is then washed and human serum samples applied. Human antibodies against E2 (Ab #2) will bind to the E2 protein, unless they have the same specificity as the murine capture MAb #1. Anti-Human IgG conjugated to an enzyme is added, and the colorimetric substrate to measure human IgG uses the same methods as the μ Plate Anti-HGenv assay. The result of both the μ Plate Anti-HGenv and the M5 test is quantitative; however, due to differences in the capture antibody, recombinant E2 protein, the quantitative results cannot be directly compared.

Figure A.1.
Diagram of sandwich capture ELISA test.

B. Summary of analyses and sensitivity to the prior distribution

Six additional prior distributions, priors B, C, D, E, F and G shown in Table B.1, are also used to analyze the example data. The marginal distributions are assumed to be mutually independent, as they are in prior A. Table B.2 lists the summary statistics of the posterior distributions under the six prior distributions. Figure B.1 illustrates the classification using $C = 0.5$. The histograms from the 6 additional prior distributions are not very different from each other and not very different from the histogram from prior A, especially around the threshold of $C = 0.5$. Figure B.2 shows the marginal posterior densities under each of the seven prior distributions. From these figures, the marginal posterior distributions are sensitive to the choice of the prior distributions. However the classifications under priors B, C, D and E differ from the classification under prior A for only 1, 3, 2 and 1 samples respectively, and the classifications under F and G differ for 7 and 8 samples respectively. The convergence of each chain is examined by Geweke's diagnostic [41], Heidelberger and Welch's diagnostic [42] and Raftery and Lewis's diagnostic [43]. All the seven chains converge.

Table B.1

Six alternative sets of prior distributions for the mixture model.

Parameter	Prior B	Prior C	Prior D	Prior E	Prior F	Prior G
ϕ	$Beta(5, 5)$	$Beta(5, 5)$	$Beta(5, 5)$	$Beta(1, 1)$	$Beta(5, 5)$	$Beta(1, 1)$
ϕ_1, ϕ_2	$Beta(2, \frac{2}{9})$	$Beta(1, 1)$	$(0.9)^*$	$Beta(18, 2)$	$Beta(18, 2)$	$Beta(1, 1)$
μ_{1N}, μ_{2N}	$N(0, 100)$	$N(0, 100)$	$N(0, 100)$	$N(0, 100)$	$N(0, 100)$	$N(0, 100)$
β_1, β_2	$N(0, 10^4)$	$N(0, 10^4)$	$N(0, 10^4)$	$N(0, 10^4)$	$N(0, 10^4)$	$N(0, 10^4)$
$\sigma_{1N}^{-2}, \sigma_{2N}^{-2}, \sigma_{1P}^{-2}, \sigma_{2P}^{-2}$	$\Gamma(0.01, 0.01)$	$\Gamma(0.01, 0.01)$	$\Gamma(0.01, 0.01)$	$\Gamma(0.01, 0.01)$	$Unif(0, 100)^{\dagger}$	$Unif(0, 100)^{\dagger}$
ρ	$Beta(1, 1)$	$Beta(1, 1)$	$Beta(1, 1)$	$Beta(1, 1)$	$Beta(1, 1)$	$Beta(1, 1)$

* (0.9) denotes a triangular distribution with the mode at 0.9.

 $\dagger$ The prior distribution is on σ instead of σ^{-2} .**Table B.2**

Summary statistics of posterior distributions under six sets of prior distributions for sensitivity analysis

Parameter	Prior B			Prior C			Prior D		
	Mean	SE	Median	Mean	SE	Median	Mean	SE	Median
ϕ	0.479	0.063	0.479	0.500	0.065	0.499	0.498	0.065	0.497
ϕ_1	0.921	0.063	0.932	0.871	0.069	0.880	0.864	0.063	0.873
ϕ_2	0.792	0.106	0.794	0.721	0.099	0.725	0.727	0.096	0.728
μ_{1N}	0.156	0.014	0.155	0.153	0.014	0.152	0.155	0.015	0.153
μ_{2N}	0.238	0.019	0.238	0.239	0.019	0.239	0.238	0.019	0.238
μ_{1P}	1.007	0.134	1.003	1.007	0.140	1.004	1.013	0.137	1.007
μ_{2P}	0.927	0.125	0.926	0.936	0.129	0.934	0.944	0.121	0.941
σ_{1N}^2	0.004	0.002	0.004	0.004	0.002	0.003	0.004	0.002	0.004
σ_{2N}^2	0.018	0.004	0.018	0.018	0.004	0.018	0.018	0.004	0.018
σ_{1P}^2	0.568	0.131	0.547	0.560	0.126	0.544	0.561	0.129	0.544
σ_{2P}^2	0.412	0.103	0.397	0.406	0.101	0.392	0.407	0.105	0.392
ρ	0.542	0.129	0.557	0.550	0.130	0.563	0.554	0.129	0.569

Parameter	Prior E			Prior F			Prior G		
	Mean	SE	Median	Mean	SE	Median	Mean	SE	Median
ϕ	0.472	0.065	0.470	0.538	0.056	0.539	0.472	0.065	0.470
ϕ_1	0.908	0.045	0.914	0.912	0.041	0.917	0.908	0.045	0.914
ϕ_2	0.844	0.067	0.848	0.814	0.071	0.818	0.844	0.067	0.848
μ_{1N}	0.157	0.015	0.157	0.139	0.008	0.138	0.157	0.015	0.157

Parameter	Prior E			Prior F			Prior G		
	Mean	SE	Median	Mean	SE	Median	Mean	SE	Median
μ_{2N}	0.237	0.019	0.237	0.234	0.019	0.234	0.237	0.019	0.237
μ_{1P}	1.031	0.135	1.028	0.925	0.113	0.925	1.031	0.135	1.028
μ_{2P}	0.917	0.123	0.914	0.842	0.110	0.839	0.917	0.123	0.914
σ_{1N}^2	0.004	0.002	0.004	0.001	0.001	0.001	0.004	0.002	0.004
σ_{2N}^2	0.018	0.004	0.018	0.017	0.004	0.017	0.018	0.004	0.018
σ_{1P}^2	0.563	0.127	0.545	0.566	0.119	0.551	0.563	0.127	0.545
σ_{2P}^2	0.418	0.102	0.402	0.425	0.099	0.411	0.418	0.102	0.402
ρ	0.554	0.126	0.567	0.614	0.109	0.626	0.554	0.126	0.567

C. Additional Simulation Study With Little Separation

Another simulation study was also performed to assess the performance of the Bayesian classifier on data without much separation in four populations. The parameters in the mixture of four bivariate normal distributions are configured as below with

$\sigma_{1N}^2, \sigma_{2N}^2, \sigma_{1P}^2, \sigma_{2P}^2$ considerably greater than in Section 4 to give additional overlap of the mixture components.

$$\begin{cases} \varphi=0.5; \varphi_1=0.9; \varphi_2=0.8; \\ \mu_{1N}=1; \mu_{2N}=0.81; \mu_{1P}=3.71; \mu_{2P}=1.51; \\ \sigma_{1N}^2=0.68; \sigma_{2N}^2=0.18; \sigma_{1P}^2=3.97; \sigma_{2P}^2=1.62; \\ \rho=0.56. \end{cases}$$

Figure C.1 illustrates the ROC curves of the two tests based on the true parameters, implying an inadequate discriminating power, similar in both tests. Computational problems arise in WinBUGS and Table C.1 and Table C.2 list the summary of the simulation study based only on 347 out of 500 MCMC simulations that were successful. The Bayesian classifications do not show an appealing performance in this scenario, although the rule with $C = 0.5$ still slightly outperforms the LDC. Moreover, the posterior estimates of the means and variances in the mixture distribution are substantially biased.

Figure C.2 demonstrates one simulated data set with the Bayesian classification using $C = 0.5$. There are a lot of false positives and false negatives based on the Bayesian rule. The sensitivity is only 0.38, compared to the sensitivity of 0.62 by the linear discriminant classifier using the true status in the simulated data. In reality, however, the linear discriminant classifier is no longer available in absence of a gold standard.

Figure B.1.

Plots of the classifications using six prior distributions B, C, D, E, F and G. The classification cutoff value $C = 0.5$ is used. Red represents the negative classification and green represents the positive classification.

Figure B.2.

Marginal posterior densities of parameters for prior A (solid), prior B (dashed), prior C (dotted), prior D (dotdash), prior E (longdash), prior F (twodash) and prior G (yellow solid).

Figure B.3.

Histograms of posterior probability of E2 antibodies being present and the classifications of the 100 blood samples with $C = 0.5$ estimated by the ML approach. Red represents the negative classification and green represents the positive classification.

Table C.1

Empirical sensitivity (Sen), specificity (Spe), positive predictive value (PPV) and negative predictive value (NPV) of the Bayesian classification rules and the linear discriminant classifier based on 347 out of 500 simulated data sets with little separation. MCSE is the Monte-Carlo standard error of the estimate.

	$C = 0.5$	$C = 0.7$	$C = 0.9$	LDC
Sen (MCSE)	0.590(0.238)	0.073(0.136)	0.009(0.061)	0.506(0.303)
Spe (MCSE)	0.486(0.220)	0.940(0.157)	0.980(0.134)	0.517(0.311)
PPV (MCSE)	0.552(0.087)	0.715(0.251)	0.768(0.342)	0.519(0.302)
NPV (MCSE)	0.569(0.092)	0.511(0.061)	0.502(0.053)	0.514(0.299)

Table C.2

Empirical properties of posterior estimates based on 347 out of 500 simulated data sets with little separation. RMSE is the root mean square error of the estimate, Width is the median width of the 95% highest posterior density interval and CP is the coverage probability of the 95% highest posterior density interval.

Parameter	Mean	SE	RMSE	Width	CP
$\phi = 0.5$	0.507	0.150	0.061	0.573	0.997
$\phi_1 = 0.9$	0.900	0.065	0.006	0.225	1.000
$\phi_2 = 0.8$	0.900	0.065	0.100	0.225	0.968
$\mu_{1N} = 1$	2.405	0.128	0.445	0.456	0.089
$\mu_{2N} = 0.81$	1.187	0.109	0.393	0.414	0.084
$\mu_{1P} = 3.97$	$> 10_6$	$> 10_6$	$> 10_6$	0.457	0.006
$\mu_{1P} = 1.51$	$> 10_6$	$> 10_6$	$> 10_6$	0.418	0.182
$\sigma_{1N}^2 = 0.68$	1.201	0.457	0.591	1.598	0.882
$\sigma_{2N}^2 = 0.18$	3.763	88.712	44.889	1.930	0.216
$\sigma_{1P}^2 = 3.97$	1.037	0.399	2.942	1.388	0.000
$\sigma_{2P}^2 = 1.62$	2.515	47.458	24.728	1.726	0.847
$\rho = 0.56$	0.495	0.185	0.124	0.688	0.980

Figure C.1.
ROC curves of the two tests in the illustrative example without a clear separation.

Figure C.2.

Bayesian classification ($C = 0.5$) in one simulated data set without a clear separation. Red represents the negative classification and green represents the positive classification. 6 of 50 true negatives are mis-classified as positive and 31 of 50 true positives are mis-classified as negative.

Figure 1.

Histograms of posterior probability of E2 antibodies being present and the classifications of the 100 blood samples with $C = 0.5$. Red represents the negative classification and green represents the positive classification.

Table 1

Empirical sensitivity (Sen), specificity (Spe), positive predictive value (PPV) and negative predictive value (NPV) of the Bayesian classification rule and the linear discriminant classifier based on 500 simulated data sets. MCSE is the Monte-Carlo standard error of the estimate.

	Normal	t_4	Skew-Normal	Skew- t_4
$C = 0.5$				
Sen (MCSE)	0.899(0.051)	0.931(0.039)	0.893(0.048)	0.964(0.025)
Spe (MCSE)	0.991(0.014)	0.948(0.034)	0.974(0.025)	0.945(0.037)
PPV (MCSE)	0.989(0.016)	0.944(0.036)	0.971(0.027)	0.944(0.036)
NPV (MCSE)	0.914(0.042)	0.937(0.035)	0.909(0.040)	0.965(0.024)
$C = 0.7$				
Sen (MCSE)	0.886(0.054)	0.922(0.043)	0.875(0.052)	0.961(0.026)
Spe (MCSE)	0.996(0.009)	0.962(0.029)	0.988(0.018)	0.956(0.032)
PPV (MCSE)	0.996(0.010)	0.958(0.032)	0.986(0.020)	0.955(0.032)
NPV (MCSE)	0.904(0.044)	0.931(0.038)	0.896(0.043)	0.963(0.025)
$C = 0.9$				
Sen (MCSE)	0.868(0.059)	0.909(0.045)	0.849(0.058)	0.956(0.028)
Spe (MCSE)	0.999(0.004)	0.977(0.022)	0.996(0.010)	0.970(0.028)
PPV (MCSE)	0.999(0.004)	0.973(0.026)	0.995(0.011)	0.968(0.028)
NPV (MCSE)	0.891(0.047)	0.921(0.040)	0.879(0.046)	0.959(0.026)
Linear Discriminant Classifier				
Sen (MCSE)	0.688(0.062)	0.725(0.063)	0.668(0.068)	0.614(0.152)
Spe (MCSE)	0.999(0.005)	0.999(0.007)	0.984(0.066)	0.848(0.289)
PPV (MCSE)	0.999(0.005)	0.998(0.009)	0.986(0.049)	0.893(0.164)
NPV (MCSE)	0.775(0.032)	0.799(0.035)	0.765(0.043)	0.669(0.174)

Table 2

Empirical properties of posterior estimates based on 500 simulated data sets. RMSE is the root mean square error of the estimate, Width is the median width of the 95% highest posterior density interval and CP is the coverage probability of the 95% highest posterior density interval.

Parameter	Normal						t_4						Skew Normal						Skew t_4					
	Mean	SE	RMSE	Width	CP	Mean	SE	RMSE	Width	CP	Mean	SE	RMSE	Width	CP	Mean	SE	RMSE	Width	CP				
$\phi=0.48$	0.476	0.053	0.049	0.205	0.972	0.503	0.053	0.053	0.205	0.950	0.488	0.056	0.050	0.217	0.978	0.512	0.051	0.056	0.197	0.932				
$\phi_1=0.9$	0.904	0.047	0.032	0.170	0.996	0.900	0.046	0.031	0.171	0.990	0.898	0.053	0.030	0.191	1.000	0.906	0.042	0.032	0.156	0.978				
$\phi_2=0.8$	0.864	0.061	0.075	0.230	0.820	0.876	0.055	0.086	0.209	0.690	0.882	0.063	0.088	0.225	0.782	0.882	0.051	0.089	0.191	0.610				
$\mu_{1N}=0.15$	0.150	0.010	0.009	0.038	0.962	0.151	0.008	0.008	0.033	0.966	0.117	0.008	0.034	0.033	0.030	0.190	0.009	0.041	0.036	0.008				
$\mu_{2N}=0.24$	0.242	0.021	0.021	0.080	0.942	0.242	0.018	0.017	0.069	0.956	0.171	0.018	0.072	0.070	0.066	0.326	0.020	0.088	0.078	0.012				
$\mu_1 \rho=1$	0.982	0.128	0.130	0.491	0.942	0.924	0.123	0.170	0.461	0.884	0.397	0.106	0.624	0.371	0.000	1.525	0.143	0.543	0.530	0.020				
$\mu_{2P}=0.9$	0.857	0.120	0.138	0.460	0.930	0.784	0.112	0.184	0.417	0.816	0.349	0.085	0.567	0.319	0.000	1.295	0.128	0.409	0.482	0.080				
$\sigma_{1N}^2=0.004$	0.005	0.001	0.001	0.004	0.950	0.003	0.001	0.001	0.003	0.780	0.003	0.001	0.001	0.003	0.822	0.004	0.001	0.001	0.004	0.952				
$\sigma_{2N}^2=0.02$	0.021	0.005	0.005	0.018	0.950	0.014	0.004	0.007	0.013	0.554	0.014	0.004	0.007	0.013	0.560	0.018	0.005	0.005	0.017	0.864				
$\sigma_{1P}^2=0.57$	0.595	0.139	0.136	0.504	0.942	0.614	0.140	0.360	0.466	0.726	0.368	0.092	0.225	0.317	0.400	0.911	0.207	1.257	0.638	0.642				
$\sigma_{2P}^2=0.4$	0.425	0.107	0.104	0.385	0.950	0.437	0.105	0.221	0.348	0.788	0.242	0.064	0.171	0.225	0.360	0.595	0.140	0.372	0.454	0.708				
$\rho=0.54$	0.470	0.135	0.141	0.515	0.912	0.500	0.123	0.168	0.468	0.798	0.210	0.122	0.344	0.425	0.222	0.327	0.125	0.274	0.468	0.508				

Table 3

Summary statistics of the posterior distributions.

Parameter	Mean	SE	Median
ϕ	0.478	0.063	0.477
ϕ_1	0.907	0.045	0.913
ϕ_2	0.842	0.066	0.846
μ_{1N}	0.157	0.015	0.156
μ_{2N}	0.237	0.019	0.237
μ_{1P}	1.029	0.137	1.029
μ_{2P}	0.916	0.125	0.913
σ_{1N}^2	0.004	0.002	0.004
σ_{2N}^2	0.018	0.004	0.017
σ_{1P}^2	0.564	0.128	0.546
σ_{2P}^2	0.417	0.104	0.402
ρ	0.555	0.126	0.569