**Research Article**

Statistics in Medicine

Received XXXX

(www.interscience.wiley.com) DOI: 10.1002/sim.0000

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

**Jingyang Zhang[1]*, Kathryn Chaloner[1,2], James H. McLinden[3] and Jack T. Stapleton[3]**

**This work is motivated by a problem in 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. 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 model including the prevalence of the antibody in the population and the probabilities of blocking on each test. Because there is prior information on the prevalence of the antibody, and also on the probability of false negatives, a Bayesian analysis is used. The dependence between the two tests is also 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. Copyright © 2010 John Wiley & Sons, Ltd.**

**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

[1]*Department of Biostatistics,* [2]*Department of Statistics and Actuarial Science,* [3]*Department of Internal Medicine, University of Iowa, Iowa City, IA*
* *Correspondence to: Jingyang Zhang, Department of Biostatistics, Iowa City, IA 52242, USA. Email: jingyang-zhang@uiowa.edu*


Copyright © 2010 John Wiley & Sons, Ltd.

incorporated through the prior information. When the antibodies are present and no false negatives occur 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 an ELISA, if the binding site of the antibody of interest (Ab #2 in Figure A.1) on E2 protein is physically blocked by MAb #1 as explained in Appendix A, 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 the tests look for two different subsequences of genetic coding on the virus: false negatives occurring when there is a mutation in a target subsequence and mutations occur independently in the two target sequences.

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 a 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 mixture of multivariate normal latent model 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 release 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 with multiple quantitative tests. The main objective of this paper is to develop a model and method for combining multiple continuous tests and deriving a classification rule. Section 2 proposes the two-level latent model and Section 3 derives the decision rule to classify

 Copyright © 2010 John Wiley & Sons, Ltd. 

samples. The results of the analysis of samples from 100 subjects each tested by two methods are presented in Section 4 along with a prior distribution based on previous data. A series of simulation studies is provided in Section 5. 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.

## 2. Statistical Model

### 2.1. Notation, Assumptions and Model

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

$$
\begin{aligned}
X_k &= \begin{cases} 1 & \text{if the GBV-C E2 antibodies are present in the blood sample } k. \\ 0 & \text{if the GBV-C E2 antibodies are absent in the blood sample } k. \end{cases} \\
X_{ik} &= \begin{cases} 1 & \text{if } X_k = 1 \text{ and the binding site for test } i \text{ on sample } k \text{ is accessible.} \\ 0 & \text{if } X_k = 1 \text{ and the binding site for test } i \text{ on sample } k \text{ is blocked.} \end{cases} \\
X_{ik} &= 0, \text{ if } X_k = 0.
\end{aligned}
$$

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 $X_{2k}$, $k = 1, \ldots, n$. 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 $\mu_{1N}$ and $\mu_{2N}$ 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 $\mu_{1P}$ and $\mu_{2P}$ denote the mean responses when antibodies are present and can bind. Based on the biological mechanisms, the high test result values should correspond to higher chance of being "positive". Hence we set a constraint that $\mu_{1P} \geq \mu_{1N}$ and $\mu_{2P} \geq \mu_{2N}$. To guarantee that the constraint holds, define new parameters $\beta_i = \log(\mu_{iP} - \mu_{iN})$ ($i = 1, 2$). Parameters $\sigma_{1N}^2$, $\sigma_{2N}^2$, $\sigma_{1P}^2$


Copyright © 2010 John Wiley & Sons, Ltd.

and $\sigma^2_{2P}$ are variances, constrained to be positive. The positive correlation between $Y_{1k}$ and $Y_{2k}$, if both binding site are accessible, is denoted by $\rho$ 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 $\underline{\psi} = (\phi, \phi_1, \phi_2, \mu_{1N}, \mu_{2N}, \beta_1, \beta_2, \sigma^2_{1N}, \sigma^2_{2N}, \sigma^2_{1P}, \sigma^2_{2P}, \rho)^T$. The values $\phi$, $\phi_1$, $\phi_2$ are probabilities and are between 0 and 1, as is the correlation $\rho$.

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

Note that without the constraint that $\mu_{iP} \geq \mu_{iN}$ 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 4. This prior distribution incorporates the constraint that $\mu_{iP} \geq \mu_{iN}$ 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 package `R2WinBUGS` [19] to call WinBUGS. Similar results were obtained from a self contained R [19] program. Code is available in Appendix C.

## 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 $p(\underline{\psi}|data)$. The observed quantitative test results $Y_1$ and $Y_2$ provide information about the parameters $\underline{\psi}$. 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 $L_2$, as illustrated in Table 1.

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:

 Copyright © 2010 John Wiley & Sons, Ltd. 

**Table 1.** Loss function in the decision function.

| | Classification Positive | Classification Negative |
|---|---|---|
| Antibodies are present | 0 | $L_1$ |
| Antibodies are absent | $L_2$ | 0 |

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

Under Bayesian decision theory [21], the risk under the negative classification is $PPP \cdot L_1$, and the risk under the positive classification is $(1 - PPP) \cdot L_2$. 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 $(1 - PPP) \cdot L_2 < PPP \cdot L_1$, 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 positive result is toxic it may be appropriate to choose $L_2 > L_1$.

## 4. Illustrative Example

In the motivating example, a total of 100 blood specimens obtained from HIV infected subjects were tested with each of the two tests: called the $\mu$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 $\mu$Plate Anti-HGenv through the lysate may add additional noise that causes blocking. Neither test is perfect and false negatives are thought to occur approximately 10% of the time. Moreover, no commercial and validated test is available for the antibody, which means that there is no gold standard in the data.

In this example, the prevalence of the target antibodies varies between populations but the average is thought to be about 50% in HIV-infected populations based on previous studies [22, 23, 24, 25, 26, 27], and the chance of blocking (false negatives) for each test is thought to be around 10%. Note however that these studies used imperfect tests. Based on this information, the prior distribution (prior A) is chosen as follows:

$$\begin{aligned}
\phi &\sim Beta(5, 5) \\
\phi_1, \phi_2 &\sim Beta(18, 2) \\
\mu_{1N}, \mu_{2N} &\sim N(0, 100) \\
\beta_1, \beta_2 &\sim N(0, 10000) \\
\sigma_{1N}^{-2}, \sigma_{2N}^{-2}, \sigma_{1P}^{-2}, \sigma_{2P}^{-2} &\sim \Gamma(0.01, 0.01). \\
\rho &\sim U(0, 1),
\end{aligned}$$

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


Copyright © 2010 John Wiley & Sons, Ltd.

**Table 2.** Summary statistics of the posterior distributions.

| Parameter | Mean | SD | Median |
|---|---|---|---|
| $\phi$ | 0.478 | 0.063 | 0.477 |
| $\phi_1$ | 0.907 | 0.045 | 0.913 |
| $\phi_2$ | 0.842 | 0.066 | 0.846 |
| $\mu_{1N}$ | 0.157 | 0.015 | 0.156 |
| $\mu_{2N}$ | 0.237 | 0.019 | 0.237 |
| $\mu_{1P}$ | 1.029 | 0.137 | 1.029 |
| $\mu_{2P}$ | 0.916 | 0.125 | 0.913 |
| $\sigma^2_{1N}$ | 0.004 | 0.002 | 0.004 |
| $\sigma^2_{2N}$ | 0.018 | 0.004 | 0.017 |
| $\sigma^2_{1P}$ | 0.564 | 0.128 | 0.546 |
| $\sigma^2_{2P}$ | 0.417 | 0.104 | 0.402 |
| $\rho$ | 0.555 | 0.126 | 0.569 |

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.

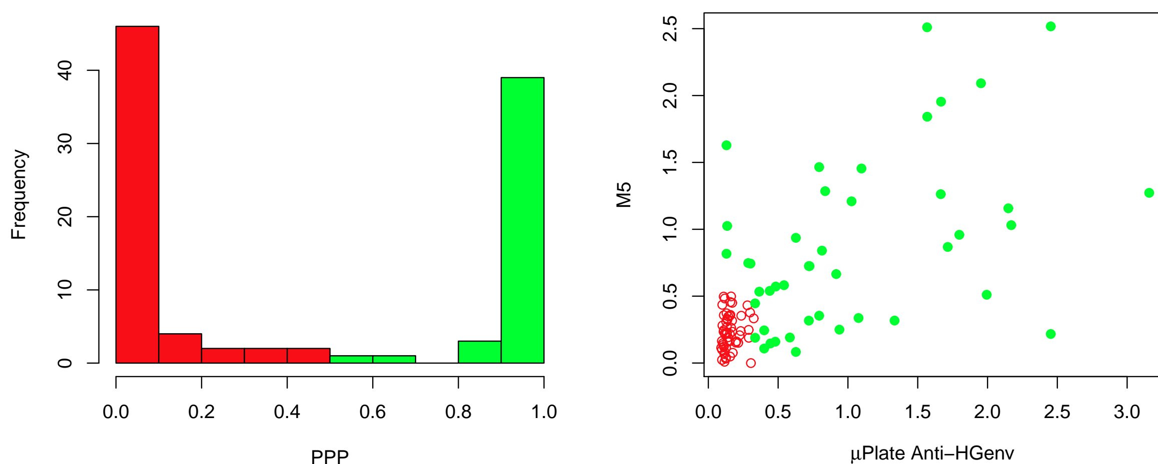


**Figure 1.** Histograms of posterior probability of E2 antibodies being present and the classifications of the 100 blood samples with $C = 0.5$. Red represents the negative classification and green represents the positive classification.

Details of the posterior distribution are in Table 2. Appendix B also gives 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 are sensitive to the choice of prior distribution, although the classification is not very sensitive.


Copyright © 2010 John Wiley & Sons, Ltd.

## 5. 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 have shape parameter $-3$ (right-skewed) [28]. The values of the parameters in the model are chosen to be close to the posterior means from the motivating example in Table 2. The posterior distribution is calculated using the mixture of bivariate normal distributions. The classification under $C = 0.5$, $0.7$ and $0.9$ is implemented. Because the simulated data is 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.

All the analyses converge and results are summarized in Table 3 and Table 4. Results in Table 3, indicate that even though the linear discriminant classifier uses more information, it assumes an incorrect distribution and it generally performs worse than the Bayesian classification method. 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 4 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 4.

## 6. Discussion

In this paper, a two-level latent model is proposed, which is consistent with the biological machenism. 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.

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 E2 antibody in the sample. If either the antibody is absent ($X_k = 0$), or it is present but in one 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 and 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". The constraint $\mu_{iP} \geq \mu_{iN}$ for $i = 1, 2$ is implemented by defining $\beta_i = \log(\mu_{iP} - \mu_{iN})$ $(i = 1, 2)$. Without the constraint, there is an identifiability question. Plots of profile log-likelihood of $\mu_{iN}$ and $\mu_{iP}$ $(i = 1, 2)$ 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

 Copyright © 2010 John Wiley & Sons, Ltd. 

**Table 3.** 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) |

around the axis $\mu_{iP} = \mu_{iN}$ where the values of $\mu_{iP}$ and $\mu_{iN}$ 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 also repeated without the constraint, and if 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. Since from the ML aspect, the parameters are fixed but unknown, the PPP for each sample is estimated by the $\Pr(X = 1|Z_1, Z_2, \hat{\underline{\psi}})$ in the integrand of (1), where $\hat{\underline{\psi}}$ are the MLE of $\underline{\psi}$. 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. In the model, 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.

The model is developed based on two ELISA tests for the E2 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 overall inferences and the classification do not change much .

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 with clear separation of the four components of the mixture model, the classification appears to be quite robust. When there is a lack of separation in the four populations, the classification is much harder and appears to have a low sensitivity in the examples

 Copyright © 2010 John Wiley & Sons, Ltd. 

**Table 4.** Empirical properties of posterior estimates based on 500 simulated data sets. MCSE is the Monte-Carlo standard error of the estimate and CP is the coverage probability of the 95% highest posterior density interval.

| | Normal | | | $t_4$ | | |
|---|---|---|---|---|---|---|
| Parameter | Mean (MCSE) | SD (MCSE) | CP | Mean (MCSE) | SD (MCSE) | CP |
| $\phi$ =0.480 | 0.476(0.049) | 0.053(0.002) | 0.972 | 0.503(0.048) | 0.053(0.002) | 0.950 |
| $\phi_1$ =0.900 | 0.904(0.032) | 0.047(0.008) | 0.996 | 0.900(0.031) | 0.046(0.007) | 0.990 |
| $\phi_2$ =0.800 | 0.864(0.040) | 0.061(0.009) | 0.820 | 0.876(0.040) | 0.055(0.009) | 0.690 |
| $\mu_{1N}$ =0.150 | 0.150(0.009) | 0.010(0.001) | 0.962 | 0.151(0.008) | 0.008(0.001) | 0.966 |
| $\mu_{2N}$ =0.240 | 0.242(0.021) | 0.021(0.003) | 0.942 | 0.242(0.017) | 0.018(0.003) | 0.956 |
| $\mu_{1P}$ =1.000 | 0.982(0.129) | 0.128(0.018) | 0.942 | 0.924(0.152) | 0.123(0.029) | 0.884 |
| $\mu_{2P}$ =0.900 | 0.857(0.131) | 0.120(0.021) | 0.930 | 0.784(0.142) | 0.112(0.030) | 0.816 |
| $\sigma^2_{1N}$ =0.004 | 0.005(0.001) | 0.001(0.000) | 0.950 | 0.003(0.001) | 0.001(0.000) | 0.780 |
| $\sigma^2_{2N}$ =0.020 | 0.021(0.005) | 0.005(0.001) | 0.950 | 0.014(0.004) | 0.004(0.001) | 0.554 |
| $\sigma^2_{1P}$ =0.570 | 0.595(0.133) | 0.139(0.034) | 0.942 | 0.614(0.358) | 0.140(0.088) | 0.726 |
| $\sigma^2_{2P}$ =0.400 | 0.425(0.101) | 0.107(0.028) | 0.950 | 0.437(0.218) | 0.105(0.058) | 0.788 |
| $\rho = 0.54$ | 0.470(0.122) | 0.135(0.019) | 0.912 | 0.500(0.163) | 0.123(0.027) | 0.798 |
| | Skew Normal | | | Skew $t_4$ | | |
| Parameter | Mean (MCSE) | SD (MCSE) | CP | Mean (MCSE) | SD (MCSE) | CP |
| $\phi$ =0.480 | 0.488(0.049) | 0.056(0.003) | 0.978 | 0.512(0.046) | 0.051(0.001) | 0.932 |
| $\phi_1$ =0.900 | 0.898(0.030) | 0.053(0.009) | 1.000 | 0.906(0.032) | 0.042(0.006) | 0.978 |
| $\phi_2$ =0.800 | 0.882(0.033) | 0.063(0.010) | 0.782 | 0.882(0.035) | 0.051(0.007) | 0.610 |
| $\mu_{1N}$ =0.150 | 0.117(0.008) | 0.008(0.001) | 0.030 | 0.190(0.009) | 0.009(0.001) | 0.008 |
| $\mu_{2N}$ =0.240 | 0.171(0.019) | 0.018(0.003) | 0.066 | 0.326(0.021) | 0.020(0.003) | 0.012 |
| $\mu_{1P}$ =1.000 | 0.397(0.161) | 0.106(0.032) | 0.000 | 1.525(0.139) | 0.143(0.046) | 0.020 |
| $\mu_{2P}$ =0.900 | 0.349(0.133) | 0.085(0.031) | 0.000 | 1.295(0.108) | 0.128(0.026) | 0.080 |
| $\sigma^2_{1N}$ =0.004 | 0.003(0.001) | 0.001(0.000) | 0.822 | 0.004(0.001) | 0.001(0.000) | 0.952 |
| $\sigma^2_{2N}$ =0.020 | 0.014(0.003) | 0.004(0.001) | 0.560 | 0.018(0.005) | 0.005(0.001) | 0.864 |
| $\sigma^2_{1P}$ =0.570 | 0.368(0.097) | 0.092(0.029) | 0.400 | 0.911(1.211) | 0.207(0.308) | 0.642 |
| $\sigma^2_{2P}$ =0.400 | 0.242(0.065) | 0.064(0.020) | 0.360 | 0.595(0.317) | 0.140(0.068) | 0.708 |
| $\rho = 0.54$ | 0.210(0.098) | 0.122(0.027) | 0.222 | 0.327(0.172) | 0.125(0.029) | 0.508 |

examined. Computation of the PPP for each sample however, may help in a classification system that includes three categories (positive/negative/indeterminate). In our example data there was some separation in the marginal distributions that led to a robust classification. A good ELISA test or PCR test, should have 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 infected 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.


Copyright © 2010 John Wiley & Sons, Ltd.

## Acknowledgement

This work was supported by grants from the National Institutes of Health: T32 GM077973 to Kathryn Chaloner and R01 AI058740 to Jack Stapleton. We are grateful to Dr. Alfred Engel, Roche Labboratories, Germany, for kindly providing antibodies. We are also grateful to the two referees for their helpful and insightful suggestions.

## References

1. Pepe MS. *The Statistical Evaluation of Medical Tests for Classification and Prediction*. Oxford University Press:New York, 2003.
2. Hui S, Walter S. Estimating the error rates of diagnostic tests. *Biometrics* March 1980; **36**(1):167–171.
3. Vacek P. The effect of conditional dependence on the evaluation of diagnostic tests. *Biometrics* 1985; **41**:959–968.
4. Walter S, Irwig L. Estimation of test error rates, disease prevalence and relative risk from estimation of test error rates, disease prevalence and relative risk from misclassified data: a review. *Journal of Clinical Epidemology* 1988; **41**:923–937, doi:10.1016/0895-4356(88)90110-2.
5. Joseph L, Gyorkos T, Coupal L. Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. *American Journal of Epidemiology* 1995; **141**(3):263–272.
6. Hui S, Zhou X. Evaluation of diagnostic tests without gold standards. *Statistical Methods in Medical Research* 1998; **7**:354–370, doi:10.1177/096228029800700404.
7. Enøe C, Georgiadis M, Johnson W. Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. *Preventive Veterinary Medicine* 2000; **45**:61–81, doi:10.1016/S0167-5877(00)00117-3.
8. Qu Y, Tan M, Kutner M. Random effects models for evaluating accuracy of diagnostic tests. *Biometrics* 1996; **52**:797–810.
9. Yang I, Becker M. Latent variable modeling of diagnostic accuracy. *Biometrics* 1997; **53**:948–958.
10. Dendukuri N, Joseph L. Baeysian approach to modeling the conditional dependence between multiple diagnostic tests. *Biometrics* 2001; **57**:158–167, doi:10.1111/j.0006-341X.2001.00158.x.
11. Black M, Craig B. Estimating disease prevalence in the absence of a gold standard. *Statistics in Medicine* 2002; **21**:2653–2669, doi:10.1002/sim.1178.
12. Nielsen S, Gronbaek C, Agger J, Houe H. Maximum-likelihood estimation of sensitivity and specificity of ELISAs and faecal culture for diagnosis of paratuberculosis. *Preventive Veterinary Medicine* 2002; **53**:191–204, doi:10.1016/S0167-5877(01)00280-X.
13. Henkelman R, Kay I, Bronskill M. Receiver operating characteristics (ROC) analysis without truth. *Medical Decision Making* 1990; **10**:24–29, doi:10.1177/0272989X9001000105.
14. Choi Y, Collins M, Gardner I. Bayesian inferences for receiver opterating characteristic curves in the absence of a gold standard. *Journal of Agricultural Biological and Environmental Statistics* 2006; **11**(19), doi:10.1198/108571106X110883.
15. Hall P, Zhou XH. Nonparametric estimation of component distributions in a multivariate mixture. *The Annals of Statistics* 2003; **31**:201–224, doi:10.1214/aos/1046294462.
16. Zhou X, Castelluccio P, Zhou C. Nonparametric estimation of ROC curves in the absence of a gold standard. *Biometrics* 2005; **61**:600–9, doi:10.1111/j.1541-0420.2005.00324.x.
17. Wang C, Turnbull B, Grohn Y, Nielsen S. Nonparametric estimation of ROC curves based on Bayesian models when the true disease state is unknown. *Journal of Agricultural, Biological, and Environmental Statistics* 2007; **12**:128–146, doi:10.1198/108571107X178095.
18. Lunn D, Thomas H, Best N, Spiegelhalter D. Winbugs – a bayesian modelling framework: concepts, structure, and extensibility. *Statistics and Computing* 2000; **10**:325–337.
19. R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria 2010. ISBN 3-900051-07-0.
20. Zhang J, Chaloner K, McLinden J, Stapleton J. Bayesian analysis and classification of two enzyme-linked immunosorbent assay (ELISA) tests without a gold standard. *Technical Report*, Department of Biostatistics, University of Iowa 2011.
21. DeGroot MH. *Optimal Statistical Decisions*. Wiley Classics Library, 2004.
22. Clevenbergh P, Durant J, Halfon P, Tran A, Manos T, Rahelinirina V, Yang G, Benzaken S, Ouzan D, Rampal P, *et al.*. High prevalence of GB virus C/hepatitis G virus infection in different risk groups of HIV-infected patients. *Clinical Microbiology and Infection* 1998; **4**:644–647, doi:10.1111/j.1469-0691.1998.tb00347.x.
23. Heringlake S, Ockenga J, Tillmann HL, Trautwein C, Meissner D, Stoll M, Hunt J, Jou C, Solomon N, Schmidt RE, *et al.*. GB Virus C/Hepatitis G Virus Infection: A Favorable Prognostic Factor in Human Immunodeficiency Virus-Infected Patients. *The Journal of Infectious Diseases* 1998; **177**(6):1723–1726, doi:10.1086/517431.
24. Bourlet T, Guglielminotti C, Evrard M, Berthelot P, Grattard F, Frésard A, Lucht FR, Pozzetto B. Prevalence of GBV-C/hepatitis G virus RNA and E2 antibody among subjects infected with human immunodeficiency virus type 1 after parenteral or sexual exposure. *Journal of Medical Virology* 1999; **58**:373–377, doi:10.1002/(SICI)1096-9071(199908)58:4⟨373::AID-JMV9⟩3.0.CO;2-1.

 Copyright © 2010 John Wiley & Sons, Ltd. 

25. Rey D, Vidinic-Moularde J, Meyer P, Schmitt C, Fritsch S, Lang JM, Stoll-Keller F. High Prevalence of GB Virus C/Hepatitis G Virus RNA and Antibodies in Patients Infected with Human Immunodeficiency Virus Type 1. *European Journal of Clinical Microbiology and Infectious Diseases* 2000; **19**:721–724, doi:10.1007/s100960000352.
26. Tillmann HL, Heiken H, Knapik-Botor A, Heringlake S, Ockenga J, Wilber JC, Goergen B, Detmer J, McMorrow M, Stoll M, *et al.*. Infection with gb virus c and reduced mortality among hiv-infected patients. *New England Journal of Medicine* 2001; **345**(10):715–724, doi:10.1056/NEJMoa010398.
27. Smith S, Donio M, Singh M, Fallon J, Jitendranath L, Chkrebtii N, Slim J, Finkel D, Perez G. Prevalence of GB virus type C in urban Americans infected with human immunodeficiency virus type 1. *Retrovirology* 2005; **2**:38, doi:10.1186/1742-4690-2-38.
28. Azzalini A. A class of distributions which includes the normal ones. *Scandinavian Journal of Statistics* 1985; **12**:171–178.
29. Krajden M, Yu A, Braybrook H, Lai A, Mak A, Chow R, Cook D, Tellier R, Petric M, Gascoyne R, *et al.*. GBV-C/hepatitis G virus infection and non-Hodgkin lymphoma: a case control study. *International Journal of Cancer* 2010; **126**, doi:10.1002/ijc.25035.
30. Zhang W, Chaloner K, Tillmann H, Williams C, Stapleton J. Effect of early and late GB virus C viraemia on survival of HIV-infected individuals: a meta-analysis. *HIV Medicine* 2006; **7**:173–180, doi:10.1111/j.1468-1293.2006.00366.x.
31. Stapleton J, Chaloner K, Zhang J, Klinzman D, Souza I, Xiang J, Landay F A, J P, R, Mitsuyasu R. GB virus C viremia is associated with reduced CD4 expansion following interleukin 2 therapy in HIV-infected people receiving HAART. *AIDS* 2009; **23**:605–610, doi:10.1097/QAD.0b013e32831f1b00.
32. Xiang J, McLinden J, Rydze R, Chang Q, Kaufman T, Klinzman D, Stapleton J. Viruses within the Flaviviridae decrease CD4 expression and inhibit HIV replication in human CD4+ cells. *Journal of Immunology* 2009; **183**:7860–7869, doi:10.4049/jimmunol.0902276.
33. Mohr E, Stapleton J. GB virus type C interactions with HIV: the role of envelope glycoproteins. *Journal of Viral Hepatitis* 2009; **16**:757–768, doi:10.1111/j.1365-2893.2009.01194.x.
34. Mohr E, Xiang J, McLinden J, Kaufman T, Chang Q, Montefiori D, Klinzman D, Stapleton J. GB virus type C envelope protein E2 elicits antibodies that react with a cellular antigen on HIV-1 particles and neutralize diverse HIV-1 isolates. *Journal of Immunology* 2010; **185**:4496–4505, doi:10.4049/jimmunol.1001980.
35. Tacke M, Kiyosawa K, Stark K, Schlueter V, Ofenloch-Haehnle B, Hess G, Engel AM. Detection of antibodies to a putative hepatitis G virus envelope protein. *Lancet* 1997; **349**:318–320, doi:10.1016/S0140-6736(96)06461-6.
36. McLinden J, Kaufman T, Xiang J, Chang Q, Klinzman D, Engel A, Hess G, Schmidt U, Houghton M, Stapleton J. Characterization of an immunodominant antigenic site on GB virus C glycoprotein E2 that is involved in cell binding. *Journal of Virology* 2006; **80**:2131–12 140, doi:10.1128/JVI.01206-06.
37. Geweke J. Evaluating the accuracy of sampling-based approaches to calculating posterior moments. *Bayesian Statistics*, vol. 4. Clarendon Press: Oxford, 1992.
38. Heidelberger P, Welch P. Simulation run length control in the presence of an initial transient. *Operationg Research* 1993; **31**:1109–1144.
39. Raftery A, Lewis S. One long run with diagnostics: Implementation strategies for Markov chain Monte Carlo. *Statistical Science* 1992; **7**:493–497.



Copyright © 2010 John Wiley & Sons, Ltd.

# 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 [29]. There is also evidence that people with HIV disease who are co-infected with GBV-C have prolonged survival [30]. In addition,one study found an association between GBV-C and response to an HIV therapy [31]. The mechanism for these mechanisms is under investigation [32, 33, 34]. 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 $\mu$Plate Anti-HGenv test [35]. 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 subseqently 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 [36], 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 $\mu$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 $\mu$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, again 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 $\mu$Plate Anti-HGenv assay. The result of both the $\mu$Plate Anti-HGenv and the M5 test is quantitative; however, due to differences in the capture antibody, recombinant E2 protein, the quantitative results can not be directly compared.


Copyright © 2010 John Wiley & Sons, Ltd.

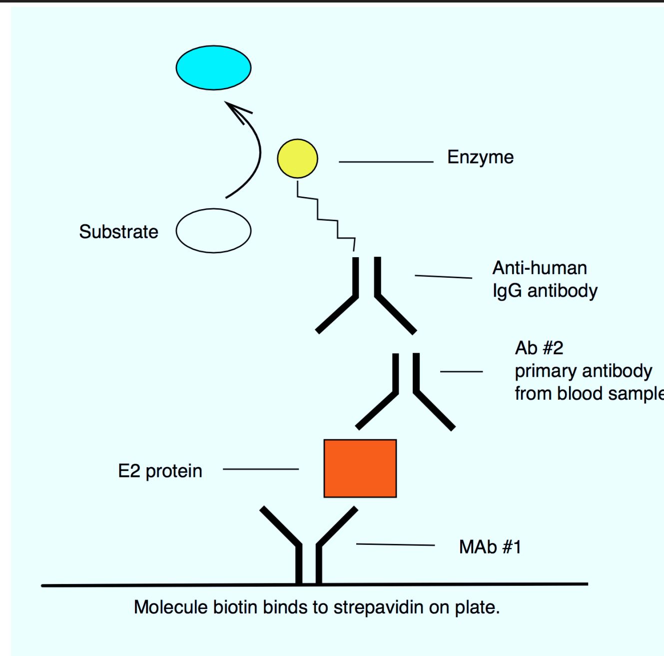


**Figure A.1.** Diagram of sandwich capture ELISA test.

## 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 are not very different from each other and from that with 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 [37], Heidelberger and Welch's diagnostic [38] and Raftery and Lewis's diagnostic [39]. 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 |
|---|---|---|---|---|---|---|
| $\phi$ | $Beta(5,5)$ | $Beta(5,5)$ | $Beta(5,5)$ | $Beta(1,1)$ | $Beta(5,5)$ | $Beta(1,1)$ |
| $\phi_1, \phi_2$ | $Beta(2, \frac{2}{9})$ | $Beta(1,1)$ | $\Delta(0.9)^*$ | $Beta(18,2)$ | $Beta(18,2)$ | $Beta(1,1)$ |
| $\mu_{1N}, \mu_{2N}, \mu_{1P}, \mu_{2P}$ | $N(0,100)$ | $N(0,100)$ | $N(0,100)$ | $N(0,100)$ | $N(0,100)$ | $N(0,100)$ |
| $\sigma_{1N}^{-2}, \sigma_{2N}^{-2}, \sigma_{1P}^{-2}, \sigma_{2P}^{-2}$ | $\Gamma(0.01,0.01)$ | $\Gamma(0.01,0.01)$ | $\Gamma(0.01,0.01)$ | $\Gamma(0.01,0.01)$ | $Unif(0,100)^\dagger$ | $Unif(0,100)^\dagger$ |
| $\rho$ | $Beta(1,1)$ | $Beta(1,1)$ | $Beta(1,1)$ | $Beta(1,1)$ | $Beta(1,1)$ | $Beta(1,1)$ |

$^*$ $\Delta(0.9)$ denotes a triangular distribution with the mode at $0.9$.
$^\dagger$ The prior distribution is on $\sigma$ instead of $\sigma^{-2}$.

 Copyright © 2010 John Wiley & Sons, Ltd. 

**Table B.2.** Summary statistics of posterior distributions under six sets of prior distributions for sensitivity analysis

| | Prior B | | | Prior C | | | Prior D | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameter | Mean | SD | Median | Mean | SD | Median | Mean | SD | Median |
| $\phi$ | 0.479 | 0.063 | 0.479 | 0.500 | 0.065 | 0.499 | 0.498 | 0.065 | 0.497 |
| $\phi_1$ | 0.921 | 0.063 | 0.932 | 0.871 | 0.069 | 0.880 | 0.864 | 0.063 | 0.873 |
| $\phi_2$ | 0.792 | 0.106 | 0.794 | 0.721 | 0.099 | 0.725 | 0.727 | 0.096 | 0.728 |
| $\mu_{1N}$ | 0.156 | 0.014 | 0.155 | 0.153 | 0.014 | 0.152 | 0.155 | 0.015 | 0.153 |
| $\mu_{2N}$ | 0.238 | 0.019 | 0.238 | 0.239 | 0.019 | 0.239 | 0.238 | 0.019 | 0.238 |
| $\mu_{1P}$ | 1.007 | 0.134 | 1.003 | 1.007 | 0.140 | 1.004 | 1.013 | 0.137 | 1.007 |
| $\mu_{2P}$ | 0.927 | 0.125 | 0.926 | 0.936 | 0.129 | 0.934 | 0.944 | 0.121 | 0.941 |
| $\sigma^2_{1N}$ | 0.004 | 0.002 | 0.004 | 0.004 | 0.002 | 0.003 | 0.004 | 0.002 | 0.004 |
| $\sigma^2_{2N}$ | 0.018 | 0.004 | 0.018 | 0.018 | 0.004 | 0.018 | 0.018 | 0.004 | 0.018 |
| $\sigma^2_{1P}$ | 0.568 | 0.131 | 0.547 | 0.560 | 0.126 | 0.544 | 0.561 | 0.129 | 0.544 |
| $\sigma^2_{2P}$ | 0.412 | 0.103 | 0.397 | 0.406 | 0.101 | 0.392 | 0.407 | 0.105 | 0.392 |
| $\rho$ | 0.542 | 0.129 | 0.557 | 0.550 | 0.130 | 0.563 | 0.554 | 0.129 | 0.569 |
| | Prior E | | | Prior F | | | Prior G | | |
| Parameter | Mean | SD | Median | Mean | SD | Median | Mean | SD | Median |
| $\phi$ | 0.472 | 0.065 | 0.470 | 0.538 | 0.056 | 0.539 | 0.472 | 0.065 | 0.470 |
| $\phi_1$ | 0.908 | 0.045 | 0.914 | 0.912 | 0.041 | 0.917 | 0.908 | 0.045 | 0.914 |
| $\phi_2$ | 0.844 | 0.067 | 0.848 | 0.814 | 0.071 | 0.818 | 0.844 | 0.067 | 0.848 |
| $\mu_{1N}$ | 0.157 | 0.015 | 0.157 | 0.139 | 0.008 | 0.138 | 0.157 | 0.015 | 0.157 |
| $\mu_{2N}$ | 0.237 | 0.019 | 0.237 | 0.234 | 0.019 | 0.234 | 0.237 | 0.019 | 0.237 |
| $\mu_{1P}$ | 1.031 | 0.135 | 1.028 | 0.925 | 0.113 | 0.925 | 1.031 | 0.135 | 1.028 |
| $\mu_{2P}$ | 0.917 | 0.123 | 0.914 | 0.842 | 0.110 | 0.839 | 0.917 | 0.123 | 0.914 |
| $\sigma^2_{1N}$ | 0.004 | 0.002 | 0.004 | 0.001 | 0.001 | 0.001 | 0.004 | 0.002 | 0.004 |
| $\sigma^2_{2N}$ | 0.018 | 0.004 | 0.018 | 0.017 | 0.004 | 0.017 | 0.018 | 0.004 | 0.018 |
| $\sigma^2_{1P}$ | 0.563 | 0.127 | 0.545 | 0.566 | 0.119 | 0.551 | 0.563 | 0.127 | 0.545 |
| $\sigma^2_{2P}$ | 0.418 | 0.102 | 0.402 | 0.425 | 0.099 | 0.411 | 0.418 | 0.102 | 0.402 |
| $\rho$ | 0.554 | 0.126 | 0.567 | 0.614 | 0.109 | 0.626 | 0.554 | 0.126 | 0.567 |

## C. WinBUGS and R code

```
# =========================================================================
#      WinBUGS function for the Bayesian analysis of the example data
# =========================================================================

# Notations:
#
# phi: the prevalence of the E2 antibody
# phi1 and phi2: the accessible probability of the binding site for each test
# mu1N, mu2N, mu1P, mu2P: the means of the normal marginal distributions
# mud1=log(mu1P - mu1N); mud2=log(mu2P - mu2N)
# sigma2_1N, sigma2_2N, sigma2_1P, sigma2_2P:
#  the variances of the normal marginal distributions
# tau1N, tau2N, tau1P, tau2P:
#  the precisions of the normal marginal distributions
# rho: the positive correlation of the two tests results
#  when the antibody is present and both test bind
# N: the sample size
# y: the data matrix (N*2)
```


Copyright © 2010 John Wiley & Sons, Ltd.

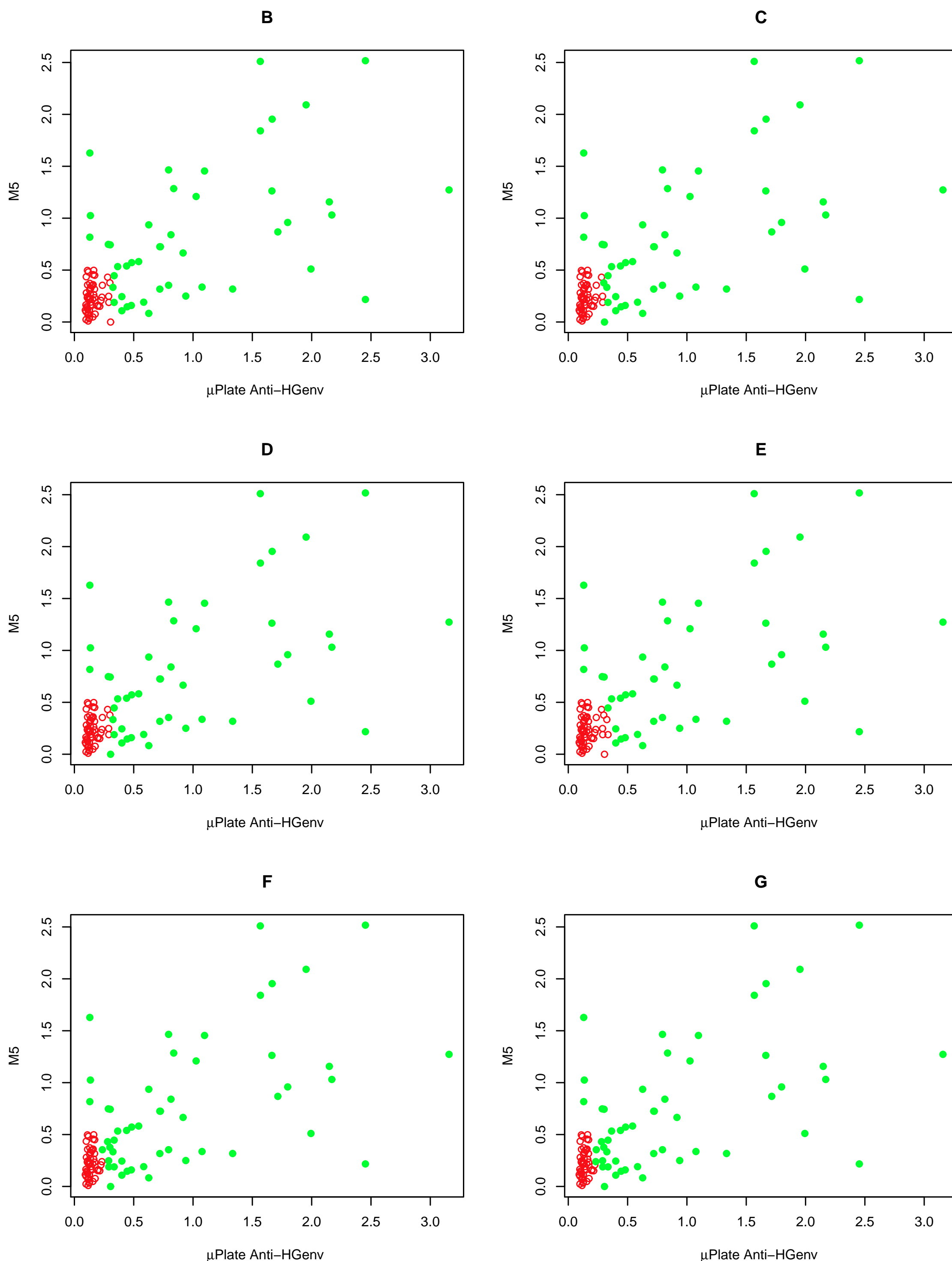


**Figure B.1.** Plots of the classifications using six prior distributions B, C, D, E, F and G. The classification cutoff value $C = 0.5$ is used. Red represents the negative classification and green represents the positive classification.

```
# C: vector of indicators for the 4 mixture elements
# p: vector of 4 mixture probabilities
#
# Seven prior distributions are all listed.
```

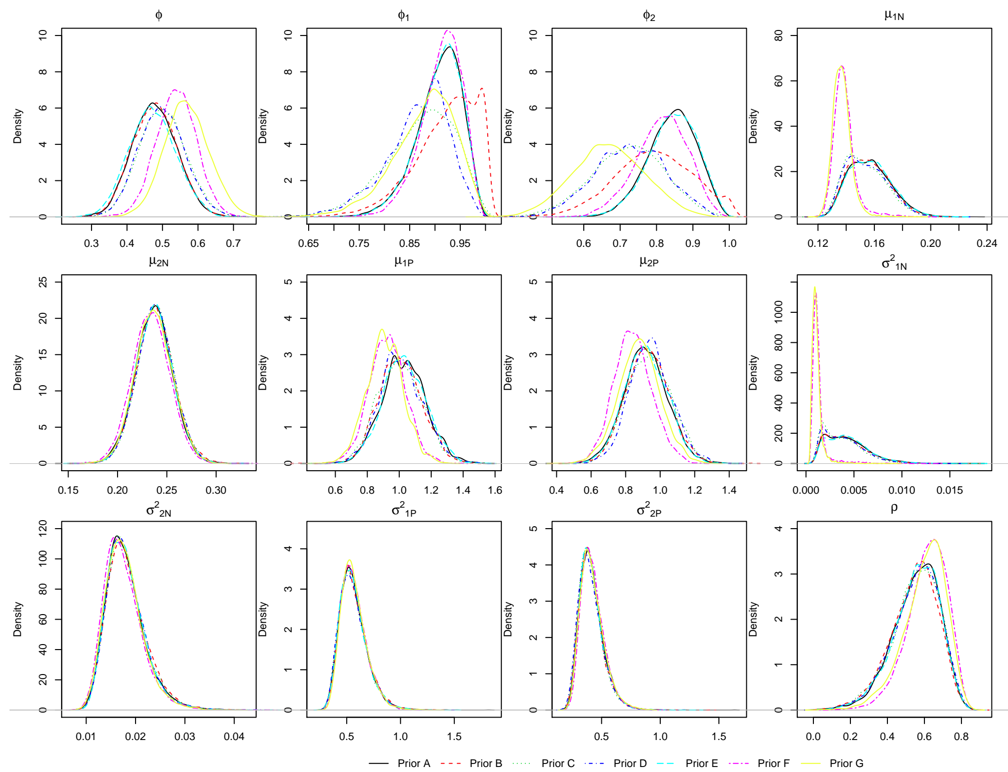


**Figure B.2.** Marginal posterior densities of parameters for prior A (solid), prior B (dashed), prior C (dotted), prior D (dotdash), prior E (longdash), prior F (twodash) and priof G (yello solid).

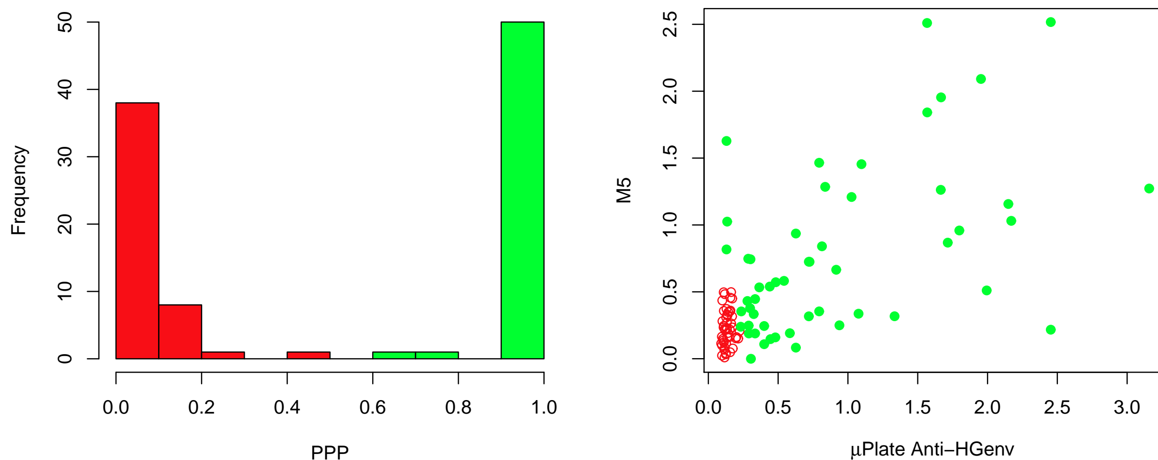


**Figure B.3.** Histograms of posterior probability of E2 antibodies being present and the classifications of the 100 blood samples with $C = 0.5$ estimated by the ML approach. Red represents the negative classification and green represents the positive classification.

```
# Comment out the unnecessary priors when use.
# The model is saved as ''model.txt''

model
{
# prior A
```


Copyright © 2010 John Wiley & Sons, Ltd.

*Prepared using* simauth.cls

```
phi ~ dbeta(5,5)
phi1 ~ dbeta(18, 2)
phi2 ~ dbeta(18, 2)

mu1N ~ dnorm(0, 1.0E-2)
mu2N ~ dnorm(0, 1.0E-2)
mud1 ~ dnorm(0, 1.0E-4)
mud2 ~ dnorm(0, 1.0E-4)
mu1P <- mu1N + exp(mud1)
mu2P <- mu2N + exp(mud2)

tau1N ~ dgamma(0.01, 0.01)
tau2N ~ dgamma(0.01, 0.01)
tau1P ~ dgamma(0.01, 0.01)
tau2P ~ dgamma(0.01, 0.01)
sigma2_1N <- 1/tau1N
sigma2_1P <- 1/tau1P
sigma2_2N <- 1/tau2N
sigma2_2P <- 1/tau2P

rho ~ dunif(0,1)

# prior B
phi ~ dbeta(5,5)
phi1 ~ dbeta(2, b)
phi2 ~ dbeta(2, b)
b <- 2/9

mu1N ~ dnorm(0, 1.0E-2)
mu2N ~ dnorm(0, 1.0E-2)
mud1 ~ dnorm(0, 1.0E-4)
mud2 ~ dnorm(0, 1.0E-4)
mu1P <- mu1N + exp(mud1)
mu2P <- mu2N + exp(mud2)

tau1N ~ dgamma(0.01, 0.01)
tau2N ~ dgamma(0.01, 0.01)
tau1P ~ dgamma(0.01, 0.01)
tau2P ~ dgamma(0.01, 0.01)
sigma2_1N <- 1/tau1N
sigma2_1P <- 1/tau1P
sigma2_2N <- 1/tau2N
sigma2_2P <- 1/tau2P

rho ~ dunif(0,1)
```

 Copyright © 2010 John Wiley & Sons, Ltd. 

```
# prior C
phi ~ dbeta(5,5)
phi1 ~ dbeta(1, 1)
phi2 ~ dbeta(1, 1)

mu1N ~ dnorm(0, 1.0E-2)
mu2N ~ dnorm(0, 1.0E-2)
mud1 ~ dnorm(0, 1.0E-4)
mud2 ~ dnorm(0, 1.0E-4)
mu1P <- mu1N + exp(mud1)
mu2P <- mu2N + exp(mud2)

tau1N ~ dgamma(0.01, 0.01)
tau2N ~ dgamma(0.01, 0.01)
tau1P ~ dgamma(0.01, 0.01)
tau2P ~ dgamma(0.01, 0.01)
sigma2_1N <- 1/tau1N
sigma2_1P <- 1/tau1P
sigma2_2N <- 1/tau2N
sigma2_2P <- 1/tau2P

rho ~ dunif(0,1)

# prior D
phi ~ dbeta(5,5)

Const <- 10000
b <- 20/9

zero1 <- 0
phi1 ~ dflat()
theta1 <- -log(b*phi1*step(phi1)*step(0.9-phi1)+
              (20-20*phi1)*step(phi1-0.9)*step(1-phi1)) + Const
zero1 ~ dpois(theta1)

zero2 <- 0
phi2 ~ dflat()
theta2 <- -log(b*phi2*step(phi2)*step(0.9-phi2)+
              (20-20*phi2)*step(phi2-0.9)*step(1-phi2)) + Const
zero2 ~ dpois(theta2)

mu1N ~ dnorm(0, 1.0E-2)
mu2N ~ dnorm(0, 1.0E-2)
mud1 ~ dnorm(0, 1.0E-4)
```


Copyright © 2010 John Wiley & Sons, Ltd.

```
mud2 ~ dnorm(0, 1.0E-4)
mu1P <- mu1N + exp(mud1)
mu2P <- mu2N + exp(mud2)

tau1N ~ dgamma(0.01, 0.01)
tau2N ~ dgamma(0.01, 0.01)
tau1P ~ dgamma(0.01, 0.01)
tau2P ~ dgamma(0.01, 0.01)
sigma2_1N <- 1/tau1N
sigma2_1P <- 1/tau1P
sigma2_2N <- 1/tau2N
sigma2_2P <- 1/tau2P

rho ~ dunif(0,1)

# prior E
phi ~ dbeta(1,1)
phi1 ~ dbeta(18, 2)
phi2 ~ dbeta(18, 2)

mu1N ~ dnorm(0, 1.0E-2)
mu2N ~ dnorm(0, 1.0E-2)
mud1 ~ dnorm(0, 1.0E-4)
mud2 ~ dnorm(0, 1.0E-4)
mu1P <- mu1N + exp(mud1)
mu2P <- mu2N + exp(mud2)

tau1N ~ dgamma(0.01, 0.01)
tau2N ~ dgamma(0.01, 0.01)
tau1P ~ dgamma(0.01, 0.01)
tau2P ~ dgamma(0.01, 0.01)
sigma2_1N <- 1/tau1N
sigma2_1P <- 1/tau1P
sigma2_2N <- 1/tau2N
sigma2_2P <- 1/tau2P

rho ~ dunif(0,1)

# prior F
phi ~ dbeta(5,5)
phi1 ~ dbeta(18, 2)
phi2 ~ dbeta(18, 2)

mu1N ~ dnorm(0, 1.0E-2)
mu2N ~ dnorm(0, 1.0E-2)
```

 Copyright © 2010 John Wiley & Sons, Ltd. 

```
mud1 ~ dnorm(0, 1.0E-4)
mud2 ~ dnorm(0, 1.0E-4)
mu1P <- mu1N + exp(mud1)
mu2P <- mu2N + exp(mud2)

sigma1N ~ dunif(0, 100)
sigma2N ~ dunif(0, 100)
sigma1P ~ dunif(0, 100)
sigma2P ~ dunif(0, 100)
sigma2_1N <- pow(sigma1N,2)
sigma2_1P <- pow(sigma1P,2)
sigma2_2N <- pow(sigma2N,2)
sigma2_2P <- pow(sigma2P,2)

tau1N <- 1/sigma2_1N
tau2N <- 1/sigma2_2N
tau1P <- 1/sigma2_1P
tau2P <- 1/sigma2_2P

rho ~ dunif(0,1)

# prior G
phi ~ dbeta(1,1)
phi1 ~ dbeta(1, 1)
phi2 ~ dbeta(1, 1)

mu1N ~ dnorm(0, 1.0E-2)
mu2N ~ dnorm(0, 1.0E-2)
mud1 ~ dnorm(0, 1.0E-4)
mud2 ~ dnorm(0, 1.0E-4)
mu1P <- mu1N + exp(mud1)
mu2P <- mu2N + exp(mud2)

sigma1N ~ dunif(0, 100)
sigma2N ~ dunif(0, 100)
sigma1P ~ dunif(0, 100)
sigma2P ~ dunif(0, 100)
sigma2_1N <- pow(sigma1N,2)
sigma2_1P <- pow(sigma1P,2)
sigma2_2N <- pow(sigma2N,2)
sigma2_2P <- pow(sigma2P,2)

tau1N <- 1/sigma2_1N
tau2N <- 1/sigma2_2N
tau1P <- 1/sigma2_1P
```

 Copyright © 2010 John Wiley & Sons, Ltd. 

```
tau2P <- 1/sigma2_2P

rho ~ dunif(0,1)

# likelihood of the ith data
for ( i in 1:N)
{
  y[i, 1:2 ] ~ dmnorm(mu[ C[i], 1:2 ], T[ C[i], 1:2 , 1:2 ] )
  C[i] ~ dcat(p[ 1:4])
}

p[1] <- phi * phi1 * phi2
p[2] <- phi * phi1 * (1 - phi2)
p[3] <- phi * (1 - phi1) * phi2
p[4] <- phi * (1 - phi1) * (1 - phi2) + 1-phi

mu[1, 1 ] <- mu1P
mu[1, 2 ] <- mu2P
mu[2, 1 ] <- mu1P
mu[2, 2 ] <- mu2N
mu[3, 1 ] <- mu1N
mu[3, 2 ] <- mu2P
mu[4, 1 ] <- mu1N
mu[4, 2 ] <- mu2N

sigma1[1, 1] <- 1/tau1P
sigma1[1, 2] <- rho * pow(tau1P * tau2P, -0.5)
sigma1[2, 1] <- rho * pow(tau1P * tau2P, -0.5)
sigma1[2, 2] <- 1/tau2P
T[1, 1:2, 1:2 ] <- inverse(sigma1[ , ])

sigma2[1, 1] <- 1/tau1P
sigma2[1, 2] <- 0
sigma2[2, 1] <- 0
sigma2[2, 2] <- 1/tau2N
T[2, 1:2, 1:2 ] <- inverse(sigma2[ , ])

sigma3[1, 1] <- 1/tau1N
sigma3[1, 2] <- 0
sigma3[2, 1] <- 0
sigma3[2, 2] <- 1/tau2P
T[3, 1:2, 1:2 ] <- inverse(sigma3[ , ])

sigma4[1, 1] <- 1/tau1N
sigma4[1, 2] <- 0
```

 Copyright © 2010 John Wiley & Sons, Ltd. 

```
sigma4[2, 1] <- 0
sigma4[2, 2] <- 1/tau2N
T[4, 1:2, 1:2 ] <- inverse(sigma4[ , ])
}

# ========================================================================
#      Calling WinBUGS in R
# ========================================================================

library(R2WinBUGS)

# N = sample size
# Y: N*2 data matrix
data <- list(
                N = 100,
                y = dput(Y, control="showAttributes")
              )

inits <- function()
{
  list( C = c(2, 3, 1, 4, 4, 2, 4, 2, 1, 2, 2, 2, 1, 3, 1, 4, 3, 3, 2, 2,
2, 4, 3, 2, 4, 2, 3, 2, 2, 3, 1, 3, 2, 1, 3, 3, 1, 3, 2, 2, 4,
3, 2, 3, 1, 1, 2, 1, 4, 4, 3, 4, 4, 3, 3, 2, 3, 1, 2, 3, 3, 1,
2, 2, 1, 3, 3, 1, 4, 3, 4, 4, 4, 4, 3, 3, 2, 1, 2, 2, 2, 1, 2,
2, 4, 2, 1, 1, 4, 2, 4, 1, 1, 1, 3, 4, 4, 3, 2, 1),
     phi = 0.5,phi1 = 0.5,phi2 = 0.5,mu1N = 0,mu2N = 0,mud1 = 0,
       mud2 = 0,tau1N = 1,tau2N = 1,tau1P = 1,tau2P = 1,rho = 0.5)
}

sim <- bugs(data, inits,
            model.file = "model.txt",
            n.iter = 15000, n.chains=1, n.thin=1, n.burnin=5000, digits=5,
            parameters.to.save = c("phi", "phi1", "phi2", "mu1N", "mu2N",
            "mu1P", "mu2P", "sigma2_1N", "sigma2_2N", "sigma2_1P", "sigma2_2P",
            "rho","mud1","mud2"),
            bugs.directory = "C:/Program Files/WinBUGS14/"
            )

# ========================================================================
#     MCMC in R
# ========================================================================

library(mvtnorm)
# log likelihood
loglik <- function(y,para)
```

 Copyright © 2010 John Wiley & Sons, Ltd. 

```
{
  res <- 0

  phi <- exp(para[1])/(1+exp(para[1]))
  phi1 <- exp(para[2])/(1+exp(para[2]))
  phi2 <- exp(para[3])/(1+exp(para[3]))
  mu1N <- para[4]
  mu2N <- para[5]
  b1 <- para[6]
  b2 <- para[7]
  tau1N <- exp(para[8])
  tau2N <- exp(para[9])
  tau1P <- exp(para[10])
  tau2P <- exp(para[11])
  rho <- exp(para[12])/(1+exp(para[12]))

  p1 <- phi * phi1 * phi2
  p2 <- phi * phi1 * ( 1 - phi2 )
  p3 <- phi * ( 1 - phi1 ) * phi2
  p4 <- phi * ( 1 - phi1 ) * ( 1 - phi2 ) + ( 1 - phi )

  mu1P <- mu1N + exp(b1)
  mu2P <- mu2N + exp(b2)

  var1N <- 1/tau1N
  var2N <- 1/tau2N
  var1P <- 1/tau1P
  var2P <- 1/tau2P

  meanNN <- c(mu1N, mu2N)
  covNN <- diag(c(var1N, var2N))

  meanPN <- c(mu1P, mu2N)
  covPN <- diag(c(var1P, var2N))

  meanNP <- c(mu1N, mu2P)
  covNP <- diag(c(var1N, var2P))

  meanPP <- c(mu1P, mu2P)
  covPP <- matrix(c(var1P, (rho*sqrt(var1P)*sqrt(var2P)), (rho*sqrt(var1P)*sqrt(var2P)),

  f1 <- dmvnorm(y, mean = meanPP, sigma = covPP)
  f2 <- dmvnorm(y, mean = meanPN, sigma = covPN)
  f3 <- dmvnorm(y, mean = meanNP, sigma = covNP)
  f4 <- dmvnorm(y, mean = meanNN, sigma = covNN)
```


Copyright © 2010 John Wiley & Sons, Ltd.

Statistics
in Medicine

```
  res <- log(p1 * f1 + p2 * f2 + p3 * f3 + p4 * f4)

  return(sum(res))
}

# log full posterior
logf <- function(y,para)
{
  phi <- exp(para[1])/(1+exp(para[1]))
  phi1 <- exp(para[2])/(1+exp(para[2]))
  phi2 <- exp(para[3])/(1+exp(para[3]))
  mu1N <- para[4]
  mu2N <- para[5]
  b1 <- para[6]
  b2 <- para[7]
  tau1N <- exp(para[8])
  tau2N <- exp(para[9])
  tau1P <- exp(para[10])
  tau2P <- exp(para[11])
  rho <- exp(para[12])/(1+exp(para[12]))

  full.loglik <- loglik(y,para) + dbeta(phi, 5, 5, log=T) +
                 log(exp(para[1])/(1+exp(para[1]))^2) + dbeta(phi1, 18, 2, log=T) +
                 log(exp(para[2])/(1+exp(para[2]))^2) + dbeta(phi2, 18, 2, log=T) +
                 log(exp(para[3])/(1+exp(para[3]))^2) +
                 dnorm(mu1N, 0, 10, log=T) + dnorm(mu2N, 0, 10, log=T) +
                 dnorm(b1, 0, 100, log=T) + dnorm( b2, 0, 100, log=T) +
                 dgamma(tau1N, 0.01, 0.01, log=T) + abs(para[8]) +
                 dgamma(tau2N, 0.01, 0.01, log=T) + abs(para[9]) +
                 dgamma(tau1P, 0.01, 0.01, log=T) + abs(para[10]) +
                 dgamma(tau2P, 0.01, 0.01, log=T) + abs(para[11]) +
                 dunif(rho, 0, 1, log=T) + log(exp(para[12])/(1+exp(para[12]))^2)
  return(full.loglik)
}

# M-H within Gibbs: proposal distribution is N(0,d)

# initial values: from the WinBUGS posterior means
winbugs <- read.table("winbug_summary.txt",header=T)
postmean <- winbugs[,1]
init <- rep(0,12)
library(boot)
init[1:3] <- logit(postmean[1:3])
init[4:5] <- postmean[4:5]
```

 Copyright © 2010 John Wiley & Sons, Ltd. 
*Prepared using* simauth.cls

```
init[6:7] <- postmean[13:14]
init[8:11] <- (-1) * log(postmean[8:11])
init[12] <- logit(postmean[12])
# sd of proposal distribution: from the result of WinBUGS
postsd <- winbugs[,2]
std <- rep(1,12)
std[1:3] <- postsd[1:3]/(postmean[1:3]*(1-postmean[1:3]))
std[4:5] <- postsd[4:5]
std[6:7] <- postsd[13:14]
std[8:11] <- postsd[8:11]/postmean[8:11]
std[12] <- postsd[12]/(postmean[12]*(1-postmean[12]))

mar.post <- function(dat=y, para=init, N, d=std, K=1)
{
  L <- length(para)
  v <- matrix(0, nrow=N, ncol=L)
  AcceptRate <- matrix(0,nrow=N, ncol=L)
  for (n in 1:N)
  {
    cat("n=",n)
    for (l in 1:L)
    {
      for (k in 1:K)
      {
        new.para.l <- rnorm(1, mean=para[l],sd=d[l])
        new.para <- para
        new.para[l] <- new.para.l
        logDensRatio <- logf(dat, new.para) - logf(dat, para)
        if (is.finite(logDensRatio) && log(runif(1)) < logDensRatio)
        {
          para[l] <- new.para.l
          AcceptRate[n,l] <- 1
        }
      }
    }
    cat(" para=",para,"\n")
    v[n,] <- para
  }
  return(list(v,AcceptRate))
}

sim <- mar.post(N=15000)
v <- sim[1]
A <- sim[2]
```

 Copyright © 2010 John Wiley & Sons, Ltd. 

```
# ========================================================================
#     function to calculate posterior positive prob. (PPP)
# ========================================================================

prob.1 <- function(y,para)
# para is the parameters saved from the ``bugs()'' function
  {
    phi <- para[1]
    phi1 <- para[2]
    phi2 <- para[3]
    mu1N <- para[4]
    mu2N <- para[5]
    mu1P <- para[6]
    mu2P <- para[7]
    var1N <- para[8]
    var2N <- para[9]
    var1P <- para[10]
    var2P <- para[11]
    rho <- para[12]

    p1 <- phi1 * phi2
    p2 <- phi1 * ( 1 - phi2 )
    p3 <- ( 1 - phi1 ) * phi2
    p4 <- ( 1 - phi1 ) * ( 1 - phi2 )

    meanNN <- c(mu1N, mu2N)
    covNN <- diag(c(var1N, var2N))

    meanPN <- c(mu1P, mu2N)
    covPN <- diag(c(var1P, var2N))

    meanNP <- c(mu1N, mu2P)
    covNP <- diag(c(var1N, var2P))

    meanPP <- c(mu1P, mu2P)
    covPP <- matrix(c(var1P, (rho*sqrt(var1P)*sqrt(var2P)),
     (rho*sqrt(var1P)*sqrt(var2P)), var2P),nrow=2)

    f1 <- dmvnorm(y, mean = meanPP, sigma = covPP)
    f2 <- dmvnorm(y, mean = meanPN, sigma = covPN)
    f3 <- dmvnorm(y, mean = meanNP, sigma = covNP)
    f4 <- dmvnorm(y, mean = meanNN, sigma = covNN)

    res <- (phi * (f1 * p1 + f2 * p2 + f3 * p3 + f4 * p4)) /
     (phi * (f1 * p1 + f2 * p2 + f3 * p3 + f4 * p4) + f4 * (1 - phi))
```

 Copyright © 2010 John Wiley & Sons, Ltd. 

```
  return (res)
}
```


Copyright © 2010 John Wiley & Sons, Ltd.