



# Bayesian Estimation of RGP90 ELISA Parameters for Diagnosis of Equine Infectious Anemia

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## Abstract

**Objective:** Equine Infectious Anemia (EIA) is caused by a retrovirus. The infected animal is the main source of the virus, and a laboratory diagnostic test is essential for the identification of infected horses when EIA cannot be definitively diagnosed clinically. EIA can be diagnosed based on serology, and serology methods often have limitations due to the uncertainty of sensitivity and specificity estimates. Our aim was to investigate the accuracy of these serological tests with a Bayesian model, as a gold standard for the identification of EIAV does not exist.

**Methods:** Validation studies for serological tests for EIA diagnosis are necessary. Using ROC curve analysis, we examined three possible cut-off values, 0.220, 0.228 and 0.232, for the rgp90 ELISA. In this study, we performed a Bayesian analysis of diagnostic data from an enzyme-linked immunosorbent assay (ELISA) of recombinant envelope glycoprotein gp90 and the classical agar gel immunodiffusion (AGID) test. For each scenario cut-off, we estimated the sensitivity and specificity of each test separately and of the two tests in combination.

**Results:** The upper limits of the posterior equally tailed 95% credible intervals for the Sensitivities (Se) and Specificities (Sp) of these two tests were as follows: AGID test alone, Se 85% and Sp 99%; ELISA alone, Se 99% and Sp 97%; and for the tests in combination, AGID test, Se 99% and Sp 100%; and ELISA, Se 99% and Sp 97%.

**Conclusion:** In this study, the Bayesian method was found to be a valuable tool for estimating the sensitivities and specificities of ELISA and AGID tests. In addition, the combination of those two tests was found to have better diagnostic accuracy than either test alone.

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**Keywords:** EIA; Sensitivity; Specificity; Bayesian model; ROC curve.



## Introduction

Equine Infectious Anemia (EIA) is caused by the lentivirus EIA virus (EIAV) of the *Retroviridae* family, which induces persistent infection in equids with recurrent cycles of viremia and fever episodes [1]. Blood from persistently infected horses is a potential the predominant source of EIAV transmission. EIA can be diagnosed using serology and molecular methods, and diagnosis is more difficult early in the course of infection because horses can be seronegative for up to 45 days post-infection [2].

According to the World Organization for Animal Health [3], Agar Gel Immune Diffusion (AGID) tests [2] and enzyme-linked immunosorbent assays (ELISAs) [4] are accurate, reliable tests for the detection of EIAV in horses, except in animals in the early stages of infection and in foals of infected dams.

The OIE has recommended that a positive test result by ELISA should be retested using the AGID test to confirm EIA diagnosis. Both of these tests in series are needed to confirm the diagnosis of EIA because the high sensitivity of ELISA can result in false positive results [5].

However, the serological test currently used in many countries for EIA control programs, is the AGID test [6]. In the last few years, the detection of EIA antibodies by ELISA has been described and used in some countries where this test is manufactured under variety of formats, competitive and non-competitive ELISAs [3], and validation studies have indicated agreement between the results of these ELISAs and the AGID assay.

Among the test methods available for the diagnosis of EIA, both ELISA and AGID tests are considered suitable for determining if a population is free of infection, the efficiency of eradication policies and if an individual animal is free of infection. However, neither of the two tests is the 'recommended method' for these purposes in the OIE Terrestrial Manual [3]; thus, further study of the accuracy of these methods is warranted.

The globalization of trade in animals has resulted in efforts to improve and control the analytical and diagnostic quality of all tests because methods often have limitations due to the uncertainty of sensitivity and specificity estimates [7, 8].

Diagnostic accuracy studies address how well a test identifies the condition of interest [9]. Therefore, statistical methods have been used to improve validation studies of diagnostic tests. Joseph et al. [10] showed that a Bayesian approach can be used to obtain interpretable posterior distributions for all unknown parameters relative to a given prior distribution in the absence of a gold standard. Bayesian methods can be applied in many areas of scientific research, and in the last few years, the application of Bayesian methods in the veterinary sciences, especially in the area of test validation, has increased [11-14].

Another statistical approach to improve validation studies is the receiver operating characteristic (ROC) curve. ROC plots can be used for the selection of an appropriate cut-off value for a test. In addition, ROC plots should be considered useful complements to estimates of sensitivity and specificity in test evaluation studies [15].

Our aim was to apply the statistical methods of the ROC curve and the Bayesian model to study the performance characteristics (sensitivity and specificity) of rgp90 ELISA [16], a diagnostic test from Brazil, in relation to the reference AGID [2] assay.

## Methodology

### Data under study

The data examined here included 1006 serological test results. The results were obtained from the Laboratório de Retrovíroses do Departamento de Medicina Veterinária Preventiva da Universidade Federal de Minas Gerais, Brazil. Equine serum samples were collected from the blood of EIAV naturally infected and uninfected equids from several areas of the State of Minas Gerais and examined by the classical method, AGID [2], and by rgp90 ELISA [16]. Blood samples for the diagnosis of equine infectious anemia were collected as part of the usual official scheme on farms, and animal welfare regulations were strictly respected. The collection protocol and study were approved by the Instituto Mineiro de Agropecuária and Escola de Veterinária da Universidade Federal de Minas Gerais.

### Statistical analysis

Data were entered into Stata 10.0 (Stata Corporation, College Station, Texas, USA) software, and ROC curve analysis was performed. The parameters ( $\alpha$ ,  $\beta$ ) were obtained by a computer program written in S-Plus. The Bayesian analysis was performed using WinBUGS software [17]. For Bayesian inference, Bayes Diagnostic Tests [18] were interactively performed using WinBUGS, which is a software package used to calculate marginal Bayesian posterior distributions via Gibbs sampling when data are available from one, two or three diagnostic tests.

### ROC curve analysis

An Excel database containing results from the indirect rgp90 ELISA and AGID test was sent from Laboratório de Retrovíroses do Departamento de Medicina Veterinária Preventiva da Universidade Federal de Minas Gerais, Brazil. The indirect rgp90 ELISA results were presented as optical densities (ODs). The AGID test results were reported as positive and negative. The Excel database was imported into Stata 10.0 software, and AGID test results were categorized as 0 (negative result) and 1 (positive result).

The rgp90 ELISA OD values ranged from 0.020 to 1.063 and were correlated with the results of the AGID (reference test) for the selection of the best rgp90 ELISA cut-off value. The command "roctab" [19] was used to perform nonparametric ROC analysis. The points on the nonparametric ROC curve were generated by using each outcome of the diagnostic test as a classification cutpoint and computing the corresponding sensitivity versus 1-specificity. The detail option was used to assess outputs in a table displaying the trade-off between sensitivity and specificity, the percentage of subjects correctly classified and the two likelihood ratios suggested by Choi [20], the likelihood ratio for a positive test result (LR+) and the likelihood ratio for a negative test result (LR-).

Thus, three cut-off values were selected, and the results cross-classified in a 2x2 table. The parameters used to select optical density cut-off values for the rgp90 ELISA for use in the Bayesian analysis were the maximum value of the sum of Se and Sp, a high level of correct classification of samples, a high LR+ and a lower LR- [21]. After the cut-off values were selected, the Bayesian analysis was performed.

## Estimation of Se and Sp using the bayesian model

### Priors

A Bayesian model similar to that used by Joseph et al. [10] was used to estimate the sensitivities and the specificities of the rgp90 ELISA and AGID tests, as a gold standard for the identification of EIAV does not exist.

Joseph et al. [10] stated that an important step in any Bayesian analysis is obtaining a prior distribution. A review of the literature or an expert opinion can be used to obtain beta prior distributions [22]. A beta distribution provides a flexible means of modeling uncertainty for parameters ranging from 0 to 1. The beta distribution is considered appropriate to model binomial probabilities, such as sensitivity and specificity [11,12].

The beta distribution has two shape parameters ( $\alpha$  and  $\beta$ ), and stochastic variability is often modeled using the equations  $\alpha = K + 1$  and  $\beta = n - K + 1$  [23]. In this study, the particular beta prior density for each test parameter was selected by matching the center of the range with the mean of the beta distribution,  $\alpha / (\alpha + \beta)$ , and by matching the standard deviation of the beta distribution [11]. After creating all equally tailed 95 percent probability intervals for the 2 x 2 table, the sensitivities and specificities for each selected cut-off value for rgp90 ELISA were determined. The ranges were used from Se = Sp= (0%-100%) for AGID test and Se = (94%-100%), (93%-98%), (90%-99%) and Sp = (95%-98%), (96%-97%), (96%-98%), respectively, for the three cut-offs, 0.220, 0.228 and 0.232. The sensitivities and specificities were simulated from the beta distributions with Se  $\sim$  beta ( $\alpha, \beta$ ) = (119.25, 3.89), (93.71, 4.11), and (93.30, 5.19) and from the beta distributions with Sp  $\sim$  beta ( $\alpha, \beta$ ) = (935.49, 35.23), (916.33, 35.23), and (919.23, 30.28), respectively, for the three cut-offs, 0.220, 0.228 and 0.232. The prevalence ( $\pi$ ) of the disease in the population was plotted with ( $\alpha=\beta=1$ ). The AGID test was performed with uniform beta distributions set to beta (1, 1) for all parameters.

### Model

In this study, the Bayesian model was applied to examine two diagnostic tests, neither of which were considered the gold standard. The likelihood function to produce update distribution for parameters of interest [24] was used to derive posterior distributions using Bayes theorem. The methods used here can be applied when the results of two diagnostic tests for the same disease are available on a randomly selected sample of subjects and when neither test is considered to be the gold standard. The Bayesian model was used to determine the marginal posterior densities that provide complete information about the sensitivities,  $S_1$  and  $S_2$ , specificities,  $C_1$  and  $C_2$ , and positive (PPV) and negative (NPV) predictive values of each test. Details are given in Joseph et al. [10].

After prior values for the parameters of prevalence, sensitivity and specificity were entered for each test, we entered the number of patients with each combination of test results (with the three different cut-offs). We used initial values generated by Win BUGS and ran only one Markov Chain (software packages are available at <http://www.medicine.mcgill.ca/joseph>).

For the analyses presented in this paper, the Gibbs sampler was run for 20,500 cycles; the first 500 cycles ("burn-in" period) were used to assess convergence, and the last 20,000 cycles were used for inference. The Gibbs sampler was used to obtain numerical approximations of exact posterior inference. Con-

vergence was assessed by considering plots of running means of the parameters of interest and was determined when these plots stabilized after a certain number of samples [24]. We used Gardner [11] and Enoe [24] as sources of information for the technical details of the Gibbs sampler.

The analyses were run using data from each diagnostic test alone and from the combination of the two tests. Each analysis was repeated from several different starting values, and convergence was assumed only if all runs provided very similar posterior distributions. Thus, summaries of the marginal posterior densities for the prevalence of EIA and the properties of the diagnostic tests ( $S_1, S_2, C_1, C_2, PPV_1, PPV_2, NPV_1, NPV_2$ ) in the absence of a gold standard diagnostic test were obtained.

## Results

### ROC analysis

The adjustments of all values for sensitivity versus 1-specificity are shown in Figure 1. The optimal density values between 0.220 and 0.232 were the points on the ROC curve with closest relative coordinates (0,1). The area under the ROC curve (AUC) indicated that the rgp90 ELISA was 98.2% accurate.

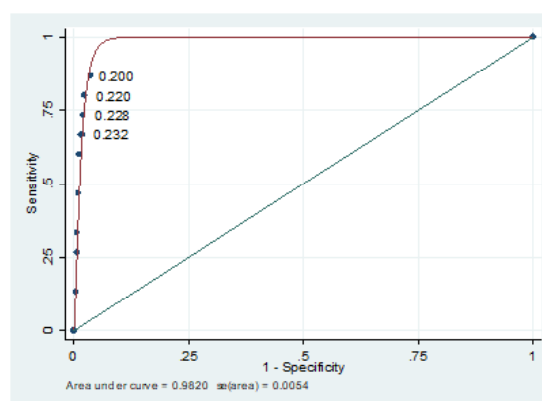


Figure 1: ROC Analysis.

### Trade-off

A total of 244 cutpoints and the discriminatory characteristics (Se, Sp, Correctly Classified, LR+, LR-) from 1006 rgp 90 ELISA ODs were obtained by ROC analysis (data not shown). Of these, 24 of the trade-off results, those ranging from 0.020 to 0.268 were summarized. Each cutpoint corresponds to a point on the nonparametric ROC curve. The last cutpoint (> 0.268) corresponds to the point at (0,0). The optimal density cut-off values for rgp90 ELISA were 0.220, 0.228 and 0.232 and for the 1006 samples, those cut-off values corresponded with sums of Se and Sp of 177.56, 171.22 and 164.67, respectively, with a high level of correct classification of samples, a high LR+ and a lower LR-. Variations from 97.27% to 97.49% were observed in the correctly classified results. Se ranged from 80.00% to 66.67%, and Sp values of 97.56% and 98.00% were found. The values of LR + ranged from 32.72 to 33.3 and the values of LR- ranged from 0.20 to 0.34. For OD cut-off values between 0.160 and 0.200, the sums of Se and Sp ranged from 181.23 to 182.89. For the OD cut-off values below 0.140, the sums of Se and Sp ranged from 100.00 to 168.39.

### Summary of 1006 serological tests

Table 1 summarizes the test results for the rgp90 ELISA and AGID test, with the application of three cut-off values. The results obtained for 1006 samples tested by rgp90 ELISA using

each cut-off value were as follows. For the 0.220 cut-off, 92 samples were concordantly positive and 878 were concordantly negative; the remaining 36 samples showed discordant results. For the 0.228 cut-off, 91 samples were concordantly positive and 881 were concordantly negative; the remaining 34 samples showed discordant results. For the 0.232 cut-off, 90 samples were concordantly positive and 882 were concordantly negative; the remaining 34 samples showed discordant results.

### Posterior credible intervals for parameters

The upper limits of the posterior equally tailed 95% credible intervals for the sensitivities (Se) and specificities (Sp) were as follows: AGID test alone, Se 85.1% and Sp 99.3%; ELISA alone, Se 99.1% and Sp 97.4%; and for the tests in combination, AGID test, Se 99.7% and Sp 100%; and ELISA, Se 99.2% and Sp 97.0%.

### Discussion

According to Florkowski [9], the diagnostic parameters of a test are not intrinsic properties of the test and are critically dependent upon the clinical context within which they are employed. In addition, different laboratories may have different test sensitivities and specificities depending of the available equipment and the level of expertise of the persons performing the test [10]. Therefore, it is essential that proper evaluations of tests are conducted. The performance characteristics of an assay must be known in order to determine the suitability of its potential application for a particular purpose [25]. In this study, we analyzed the rgp90 ELISA and AGID test with 1006 samples from naturally EIAV infected animals. An advanced statistical approach was used to evaluate these diagnostic tests. There is agreement in the literature [13, 26, 27] that Bayesian model provides important evidence of validity, which increases the credibility of this model.

In this study, the specificity of rgp90 ELISA alone had a 95 percent credible interval of 0.95-0.97 (specificities were similar for the three cut-offs, 0.220, 0.228 and 0.232), and the convergence point of the sensitivity of rgp90 ELISA was 0.93 to 0.90 and 0.89 (sensitivities were different for the three cut-offs, 0.220, 0.228 and 0.232). Using the cut-offs 0.220 and 0.228, the 95 percent posterior credible intervals for the sensitivity (0.94-0.99) and specificity (0.96-0.97) for both tests in combination were equivalent and that for the sensitivity (0.91-0.98) with the 0.232 cut-off was lower than that with the other two cutpoints. The upper limit of the posterior equally tailed 95% credible interval for the sensitivity of the AGID test alone was 0.85. In accordance with the results of Scicluna [28], the AGID test for the identification of EIAV in equids can generate some false negative results.

Since 1987, the some authors have studied the use of a combination of tests to improve the accuracy of the diagnosis of EIA [29,30,31]. A combination of tests uses the increased power of ELISA test on negative samples and the increased power of the AGID test for positive samples [32]. With this interpretation series, only animals that tested positive by both tests were considered positive for EIAV [21]. Issel [32] tested the utility of a combination, using ELISA test first, followed by AGID test and the use of immunoblot tests with the samples that were positive by ELISA test and that were negative by the AGID test.

In a study by Scicluna et al.[28], the series of examinations used with different serological tests was especially relevant, not only in the final phases of the eradication of the infection when the probability of recent infections is higher but also for the di-

agnosis of equids with constant weak-positive to null AGID test reactivities.

The statistical model applied in this study indicated that both tests are considered equally imperfect [26]. The AGID test remains the gold standard serology test for EIA because of its proven correlation with results in horse inoculation tests for EIA [32]. However, the results of this study indicate that AGID tests generate false negative results. Issel et al. [32] found EIAV genetic sequences in a number of persistently infected horses and mules whose serums were interpreted as negative/equivocal by the AGID test and as positive by more than one ELISA test and by immunoblot tests.

The traditional method for assessing a new test is to compare it to a gold standard. Because of practical difficulties, however, we must often use the accepted diagnostic method, which might be closer to a "bronze" standard. This can produce considerable difficulties in test evaluation [21]. Thus, researchers have begun to use statistical analyses to help estimate the test characteristics in the absence of a gold standard. Bayesian inference approaches have been applied to estimate test performance in the absence of a gold standard in many studies [10,13, 24].

Our study used a Bayesian model in the absence of gold standard to determine the performance characteristics of two assays. Traditionally, the tests were considered conditionally independent. However, for Gardner et al. [33], the two test outcomes for a given animal are likely to be correlated if both tests measure a similar biological phenomenon. In this study, the authors considered the two tests to be conditionally independent when the sensitivity or specificity of the second test did not depend on the results of the first test among infected or non-infected individuals. Rahman [27] evaluated the sensitivities and specificities of multiple indirect ELISA tests in the absence of gold standard. As none of the three tests examined were considered a gold standard, and the tests were not conditionally independent, constraints were necessarily imposed on a subset of the parameters.

Another statistical tool used in this study was the ROC curve. In the graph of sensitivity versus 1-specificity, the three operating points (0.220, 0.228 and 0.232) were the coordinates that were the closest on the ROC curve (0,1). Based on Gardner and Greiner [15], the ROC curve was used in this study to facilitate the analysis of the results of a test obtained by different studies.

The study of the trade-off between Se and Sp with cut-off values of 0.228 and 0.232 showed an increase of correctly classified samples of 97.49% and increases in specificity of 97.89% and 98.00%, respectively. Additionally, sensitivity decreased from 80.00% (cut-off 0.220) to 73.33% (cut-off 0.228) and 66.67% (cut-off 0.232). Despite the advantages of the cut-off of 0.232, 97.49% correct classification and 98.00% specificity, the loss of sensitivity with this cut-off value suggests an increase in the rate of false negative classification. Raising the cut-off increases Sp (less false positives) and decreases Se (more false negatives). Lowering the cut-off value has the opposite effect. Thus, which cut-off to use depends on the relative seriousness of a false negative versus a false positive test result [21].

Although the sum of Se and Sp was 181.23 and 182.89 more than those with the cut-offs 0.220, 0.228 and 0.232, the percentage of correctly classified samples decreased from 97.27%

(0.220) to 96.07% (0.200) and 83.00% (0.160). The percentage of correctly classified samples with cut-offs below 0.200 (0.200 to 0.160) was 13.07% more than with higher cut-off values (1.42%). In addition, the LR+ decreased from 32.72 (0.220) to 22.94 (0.200) and 5.32 (0.160) with the cut-offs 0.220, 0.228 and 0.232, respectively, suggesting a decrease in the ratio of the probability of a positive test among the truly positive subjects to the probability of a positive test among the truly negative subjects [20]. For example, the selection of a cut-off  $\geq 0.160$  indicates that all samples with OD 0.160 or greater are classified as positive. Consequently, all positive samples are correctly classified (sensitivity= 100%), but not all negative samples are classified correctly (specificity= 81.23%). Using this cut-off value, 83.00% of the 1006 samples were correctly classified. However, the LR+ with this cut-off value was low (5.32).

The 2 x 2 table shows that increasing the optical density cut-off amplifies the agreement of negative results for the AGID test and rgp90 ELISA from 878 to 881 or 882 (Table 1). The Bayesian analysis of the results of ELISA alone with the cut-off 0.228 showed a positive predictive value (PPV) of 0.755 and negative predictive value (NPV) of 0.995, which were both better than those with the other cut-offs (0.220 and 0.232).

The level of agreement between the two tests was high (97%, 972/1006) (Table 1). The apparent prevalence was 9.44%. However, in the Bayesian model, the medians of the prevalence of disease for the two tests were different (AGID  $\pi = 0.498$  and rgp90 ELISA ( $\pi = 0.095$ )). This result can be explained by the fact that the prevalence ( $\pi$ ) of the disease in the population was plotted with ( $\alpha = \beta = 1$ ) for the two tests. In others words, a uniform a priori was used [10].

Furthermore, the AGID test was performed with uniform beta distributions set to beta (1,1) for all parameters. This distribution was used to evaluate the upper limits of the posterior equally tailed 95% credible intervals for the parameters of the AGID test. The application of these methods results in 95% intervals that include the uncertainty inherent in the parameters of the test [18].

### Conclusions

In this study, the Bayesian method was a valuable tool for estimating the uncertainty of the sensitivities and specificities of diagnostic tests. In addition, the results from the combination of the two tests showed better diagnostic accuracy than either test alone.

The use of the ROC curve and the Bayesian model in sequence can enhance the assessment of the accuracy of the rgp90 ELISA.

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### References

1. Montelaro RC, Ball JM, Rushlow KE. Equine retroviruses. In *The Retroviridae* (2th edn). Edited by Jay A. Levy. New York: Plenum Press. 1993; 2: 257-343.
2. Coggins L, Norcross NL, Nusbaum SR. Diagnosis of equine infectious anemia by immunodiffusion test. *Am J Vet.* 1972; 33: 11-18.

3. OIE (World Organisation for Animal Health) Equine infectious anemia. In *Manual Diagnostic Test and vaccines for terrestrial animals*. Edited by OIE, Paris: OIE. 2017.
4. Suzuki T, Uedas S, Samejima T. Enzyme-linked immunosorbent assay for diagnosis of equine infectious anaemia. *Vet Microbiol.* 1982; 7: 307-316.
5. Marenzoni ML, Cuteri V, De Parri F, Danzetta ML, Yilmaz Z, et al. A pilot study on the epidemiological status of equine infectious anaemia, equine viral arteritis, glanders, and dourine in Turkey. *Turk J Vet Anim Sci.* 2013; 37: 76-80.
6. Paré J, Simard C. Comparison of commercial enzyme-linked immunosorbent assays and agar gel immunodiffusion tests for the serodiagnosis of equine infectious anemia. *Can J Vet Res.* 2004; 68: 254-258.
7. Caporale V, Nannini D, Ricci L. Quality assurance in veterinary diagnostic laboratories. *Rev Sci Tech OIE.* 1998; 17: 459-468.
8. Greiner M, Gardner IA. Epidemiologic issues in the validation of veterinary diagnostic tests. *Prev Vet Med.* 2000; 45: 3-22.
9. Florkowisk CM. Sensitivity, Specificity, Receiver-Operating Characteristic (ROC) Curves and Likelihood Ratios: Communicating the Performance of Diagnostic Tests. *Clin Biochem Rev.* 2008; 29: 83-87.
10. Joseph L, Gyorkos TW, Coupal L. Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. *Am J Epidemiol.* 1995; 141: 263-272.
11. Gardner IA. The utility of Bayes' theorem and Bayesian inference in veterinary clinical practice and research. *Aust Vet J.* 2002; 80: 758-761.
12. Brascum AJ, Gardner IA, Johnson WO. Bayesian modeling of animal-and herd-level prevalences. *Prev Vet Med.* 2004; 66: 101-112.
13. Page AL, Alberti KP, Mondonge V, Rauzier J, Quilici ML et al. Evaluation of a Rapid test for Diagnosis of Cholera in the Absence of a Gold Standard. *Plos ONE.* 2012; 7: 1-6.
14. Onisko A, Druzdzel MJ. Impact of precision of Bayesian network parameters on accuracy of medical diagnostic systems. *AIME.* 2013; 57: 197-206.
15. Gardner I, Greiner M. Receiver-operating characteristic curves and likelihood ratios: improvements over traditional methods for the evaluation and application of veterinary clinical pathology tests. *Vet Clin Pathol.* 2006; 35: 8-17.
16. Reis JKP, Diniz RS, Haddad JPA, Ferraz IBF, Carvalho AF, et al. Recombinant envelope protein (rgp90) ELISA for equine infectious anemia virus provides comparable results to the agar gel immunodiffusion. *J Virol Methods.* 2012; 180: 62-67.
17. Win BUGS Project.
18. Joseph L: BayesDiagnosticTests. Estimation of Disease Prevalence and the Parameters of one, two or three Diagnostic Tests.
19. Receiver operating characteristic (ROC) analysis.
20. Choi BCK. Slopes of a receiver operating characteristic curve and likelihood ratios for a diagnostic test. *Am J Epidemiol.* 1998; 148: 1127-1132.
21. Dohoo I, Martin W, Stryhn H. Screening diagnostic tests. In *Veterinary Epidemiologic Research* (2th edn). Charlottetown: VER. 2009.
22. Suess EA, Gardner IA, Johnson WO. Hierarchical Bayesian model for prevalence inferences and determination of a country's sta-

- tus for an animal pathogen. *Prev Vet Med.* 2002; 55: 155-171.
23. Vose D. *Quantitative Risk Analysis: a Guide to Monte Carlo Simulation Modelling.* Chichester: Wiley. 1996.
24. Enøe C, Georgiadis MP, Johnson WO. Estimation of sensitivity and specificity of diagnostics tests and disease prevalence when the true disease state is unknown. *Prev Vet Med.* 2000; 45: 61-81.
25. Schmitt B. Differential diagnostic test technology: Sensitivity and specificity, an OIE validation perspective. *Biologicals.* 2005; 33: 211-213.
26. Brascum AJ, Gardner IA, Johnson WO. Estimation of diagnostic-test sensitivity and specificity through Bayesian modeling. *Prev Vet Med.* 2005; 68: 145-163.
27. Rahman AKMA, Saegerman C, Berkvens D, Fretin D, Gani MO. Bayesian estimation of true prevalence, sensitivity and specificity of indirect ELISA, Rose Bengal Test and Slow Agglutination Test for the diagnosis of brucellosis in sheep and goats in Bangladesh. *Prev Vet Med.* 2012.
28. Scicluna MT, Issel CJ, Cook FR, Manna G, Cersini A. Is a diagnostic system based exclusively on agar gel immunodiffusion adequate for controlling the spread of equine infectious anaemia? *Vet Microbiol.* 2013; 165: 123-134.
29. Issel CJ, Cook RF. A review of techniques for the serologic diagnosis of equine infectious anemia. *J Vet Diagn.* 1993; 5: 137-141.
30. Issel CJ, Rwambo PM, Montelaro RC. Evolution of equine infectious anemia diagnostic tests: recognition of a need for detection of anti-EIAV glycoprotein antibodies. In *Equine Infectious Diseases V Proceeding of the Fifth International Conference: 7-10 October 1987, Lexington.* Edited by David G. Powell: University of Kentucky. 1987.
31. Mcconnico RS, Floyd CF, Issel CJ, Lehenbauer TW, Rodgers S. Evaluation of techniques for the serological diagnosis of equine infectious anaemia in a subclinically infected herd of reproductively active horses. In *Equine Infectious Diseases VIII Proceeding of the Eighth International Conference: 23-26 March, 1998, Dubai.* Edited by Ulrich: Rand W Publications. 1988.
32. Issel CJ, Scicluna MT, Cook SJ, Cook RF, Caprioli A. Challenges and proposed solutions for more accurate serological diagnosis of equine infectious anaemia. *Vet Rec.* 2013; 172: 210.
33. Gardner IA, Stryhn H, Lind P, Collins MT. Conditional dependence between tests affects the diagnosis and surveillance of animal diseases. *Prev Vet Med.* 2000; 45: 107-122.