Bluetongue laboratory diagnosis: a ring test to evaluate serological results using a competitive ELISA kit

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Summary

The occurrence of bluetongue (BT) in Italy prompted an increase in disease surveillance. Thus a competitive enzyme-linked immunosorbent assay (c-ELISA) to detect immunoglobulins to BT virus (BTV) was developed and distributed amongst 27 laboratories comprising the Italian veterinary diagnostic laboratories network to screen field sera. This ring test enabled comparison of the results and the evaluation of the reproducibility of the method. The c-ELISA developed by the National Reference Centre for Exotic Diseases (c-ELISA-IZSA&M) was compared also against a commercially available c-ELISA. In addition, results obtained by the Centre of Athens Veterinary Institutions are presented.

Keywords

Antibody detection – Bluetongue – Competitive enzyme-linked immunosorbent assay – Diagnosis – Ring test.

Introduction

Bluetongue (BT) disease occurred in Italy for the first time in 2000 and spread to a large area of the country (1). Surveillance plans and vaccination campaigns were implemented in regions affected and at risk. The serological surveillance plan stipulated that sentinel animals had to be tested fortnightly for early detection of virus circulation (2). Testing was performed using a competitive enzyme-linked immunosorbent assay (c-ELISA) kit produced by a private company. The National Reference Centre for Exotic Diseases developed a c-ELISA method referred to as the (c-ELISA-IZSA&M [Istituto Zooprofilattico Sperimentale, dell'Abruzzo e del Molise 'G. Caporale', Teramo]) which had high levels of sensitivity and specificity (6). This kit was distributed to all national laboratories involved in the BT serological plan. To verify laboratory performance, an inter-laboratory ring test was designed and implemented (3, 4, 6), whereby a panel of sera had to be tested simultaneously using the commercial kit and the c-ELISA-IZSA&M kit. The ring test enabled an evaluation to be made of laboratory performance in terms of accuracy of results and also provided an opportunity to evaluate the reproducibility of the c-ELISA-IZSA&M kit, adding new information to its validation (5). The c-ELISA-IZSA&M method was also supplied to the Laboratory of Virology of the Centre of Athens Veterinary Institutions in Greece. A panel of 162 sera was tested using both methods and the results compared using Cohen's K agreement index.

Materials and methods

Reference sera

A test panel comprised three sera: a strong-positive serum (from an infected animal), a negative serum (from an uninfected animal) and a weak-positive serum obtained by blending the positive and negative sera. Sera had been filtered through glass filters and 0.22 µm durapore® membranes. Sera (0.5 ml) were then distributed into vials and freeze-

dried. Homogeneity and stability of the product were then evaluated.

Inter-laboratory testing scheme

An identification number was assigned to all participating laboratories, each of which received 30 blind samples: 14 negatives, 10 weak-positives and 6 strong-positives. Each sample was identified using a unique code for each participant and was tested using both the c-ELISA-IZSA&M and a commercial kit. Results were entered on a standardised form and returned within 15 days of the despatch of samples.

Statistical evaluation of results

Results were analysed using a Bayesian approach (8). The beta distribution, based on the results from each laboratory, was calculated and used to express the probability of each laboratory to give a correct result and the uncertainty of this estimate, using the following formula:

$$Beta(\alpha_1,\alpha_2)=\frac{x^{\alpha_1-1}(1-x)^{\alpha_2-1}}{\int_0^1 t^{\alpha_1-1}(1-t)^{\alpha_2-1}dt}$$

Where

α_1 = correct results +1

α_2 = tested samples –correct results+1.

The number of samples to be tested was chosen taking into account the statistical significance of the results. For this reason, 30 samples were despatched since a laboratory giving 30 correct results out of 30 has a 95% probability of providing correct results at least for 90.7% of tested sera. The comparison between the commercial c-ELISA and the c-ELISA-IZSA&M was performed using the McNemar χ^2 test and Cohen's K agreement index (7).

Results

Figures 1 and 2 show the estimates of the percentage of correct results of participants using both kits. Using the c-ELISA-IZSA&M, 24 of 27 laboratories obtained 100% correct results, whereas two laboratories obtained 29/30 correct results and one laboratory 28/30 correct results. Using the c-ELISA commercial kit, the results obtained ranged from 20 to 30 correct results. Results obtained using both assay methods are reported in Table I. The test results compared with the true status of samples are shown in Tables II and III. Reproducibility distributions are shown in Figure 3.

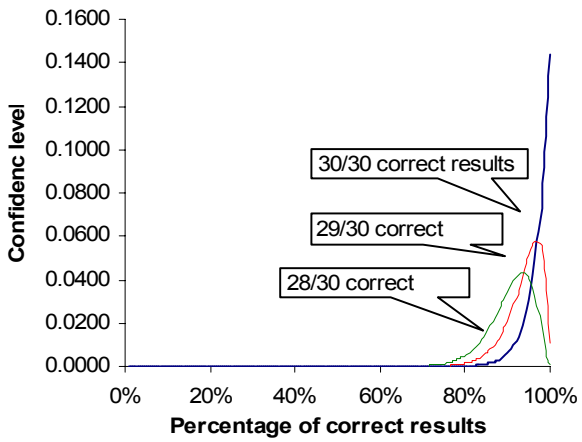

Figure 1
Distribution of correct results of participating laboratories using the c-ELISA-IZSA&M kit

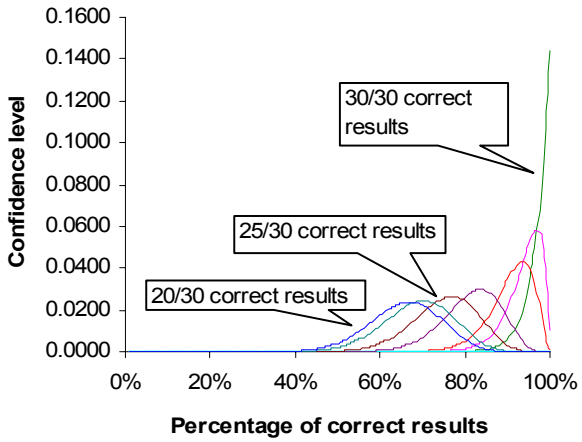

Figure 2
Distribution of correct results of participating laboratories using the c-ELISA commercial kit

Table I
Contingency table: c-ELISA commercial kit and c-ELISA-IZSA&M kit

		c-ELISA-IZSA&M Negative	Positive	Total
c-ELISA commercial kit	**Negative**	377	48	425
	Positive	2	382	384
Total		379	430	809

c-ELISA competitive enzyme-linked immunosorbent assay

IZAM Istituto Zooprofilattico Sperimentale, dell'Abruzzo e del Molise

χ^2 McNemar = 40.5 $p<0.0001$

Cohen's K agreement index = 0.88 $p<0.0001$

Table II
Contingency table: c-ELISA commercial kit and the true status of samples

		True status of samples Negative	Positive	Total
c-ELISA commercial kit	**Negative**	375	50	425
	Positive	2	382	384
Total		377	432	809

c-ELISA competitive enzyme-linked immunosorbent assay

Table III
Contingency table c-ELISA-IZSA&M kit and the true status of samples

		True status of samples Negative	Positive	Total
c-ELISA-IZSA&M kit	**Negative**	376	3	379
	Positive	1	429	430
Total		377	432	809

c-ELISA competitive enzyme-linked immunosorbent assay
IZAM Istituto Zooprofilattico Sperimentale, dell'Abruzzo e del Molise

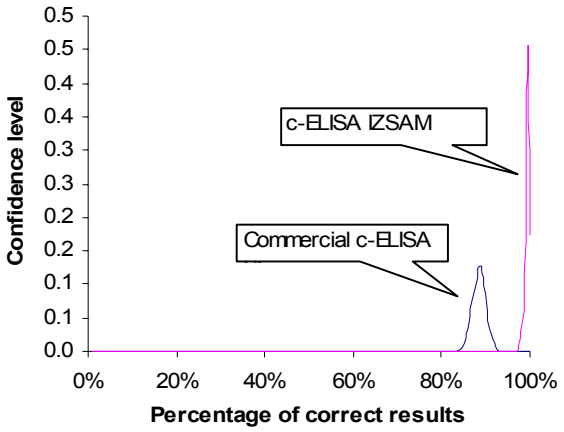

Figure 3
Reproducibility distributions of the c-ELISA-IZSA&M and the c-ELISA commercial kits

With regard to the results obtained using the c-ELISA-IZSA&M and the c-ELISA commercial kit by the Laboratory of Virology in Athens, the Cohen's K agreement index was = 0.891 (Table IV). This value (close to 1) gives almost perfect agreement between the two tests (9).

Table IV
Contingency table: c-ELISA commercial kit and the c-ELISA-IZSA&M kit
Laboratory of Virology, Centre of Athens Veterinary Institutions, Greece

		c-ELISA commercial kit Negative	Positive	Total
c-ELISA-IZSA&M kit	**Negative**	102	1	103
	Positive	7	52	59
Total		109	53	162

c-ELISA competitive enzyme-linked immunosorbent assay
IZAM Istituto Zooprofilattico Sperimentale, dell'Abruzzo e del Molise
Cohen's K agreement index = 0.891

Discussion and conclusion

As shown in Figure 1, the performance of each of the participants was satisfactory when using the c-ELISA-IZSA&M kit. Due to sample size, the uncertainty of the estimate of correct results will be reduced further when other proficiency tests, analysing at least 120-150 serum samples, are performed. Laboratories failing one test result have a 83-99% probability of obtaining a correct result with a 95% confidence level. Therefore, a more appropriate evaluation of these laboratories will be possible only after a greater number of samples have been screened. The opportunity of performing repetitions of the same panel of samples meant that the reproducibility distribution of the c-ELISA-IZSA&M could be evaluated, thereby completing verification of the characteristics of the method and the sensitivity and specificity values calculated previously (100% and 99.1%, respectively) (5). In a given serology laboratory, proficiency testing and evaluation of results are usually performed by analysis of a single serum sample. In this study, the results obtained using a panel of different serum samples were combined so as to obtain an overall evaluation of participating laboratories. As far as the comparison between the two methods is concerned, although Cohen's K agreement index shows almost perfect agreement between the two methods (K = 0.81), the McNemar test highlights a significant difference in the results (Table I).

The variability of results was higher when using the commercial c-ELISA kit as shown in Figure 2 and also the reproducibility of this test was less precise than that of the c-ELISA-IZSA&M kit (Fig. 3).

The results obtained by the Laboratory of Virology in Athens indicate that the c-ELISA-IZSA&M kit is sensitive. However, it is not possible to evaluate its specificity since the serum neutralisation test was not

performed and the true sanitary status of donor animals was unknown.

Acknowledgements

The authors wish to thank all those who participated in this study, namely: Giuseppe Addis (IZS Cagliari), Nicola Cavaliere (IZS Foggia), Francesco Casalinuovo (IZS Catanzaro), Rita Catanzariti (IZS Taranto), Giuseppina Ciccarese (IZS Lecce), Renato Ugo Condoleo (IZS Latina), Pasquale Cossu (IZS Nuoro), Luigi De Grossi (IZS Viterbo), Gian Mario De Mia (IZS Perugia), Dario Deni (IZS Arezzo) Annalisa Guercio (IZS Palermo), Maria Guidoni (IZS Grosseto), Riccardo Forletta (IZS Pisa), Giuseppe Lucifora (IZS Cosenza), Luisa Marati (IZS Portici), Loretta Masoero (IZS Torino), Stefano Nardelli (IZS Padova), Annalisa Oggiano (IZS Sassari), Antonio Parisi (IZS Putignano), Vincenzo Quaranta (IZS Potenza), Elena Rocchegiani (IZS Ancona), Angelo Ruiu (IZS Oristano), Giovanni Sala (IZS Brescia), Gianfranco Santagada (IZS Matera), Maria Teresa Scicluna (IZS Roma) and Elena Teneggi (IZS La Spezia).

Thanks are also extended to Massimo D'Ancona for his technical contribution and Attilio Pini, for his supervision of the preparation of the manuscript.

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