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# Comparative evaluation of competitive and indirect ELISA kits for detection of bluetongue virus antibodies in ovine sera from an endemic region

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## Abstract

*Bluetongue virus* (BTV) remains an important pathogen of small ruminants in endemic regions, necessitating reliable serological tools for surveillance and diagnosis. This study evaluated the diagnostic performance of a newly developed indirect ELISA kit (LT-Biotech), based on a plant-derived recombinant VP7 antigen, in comparison with a commercial competitive ELISA (ID Screen® Bluetongue Competition, ID-Vet). A total of 56 ovine serum samples collected in a blue tongue-endemic region of the Middle East were tested in parallel using both assays.

Using an optimized cut-off value of OD = 0.5, the indirect ELISA demonstrated a diagnostic sensitivity of 90% and a specificity of 94% relative to the competitive ELISA. The positive and negative predictive values were 97% and 79%, respectively. The likelihood ratios (LR+ = 14.4; LR− = 0.11) indicated strong discriminative power, and the overall agreement between the two assays reached 91%, with a Cohen's kappa coefficient of 0.79, reflecting substantial concordance.

These findings show that the plant-derived VP7 indirect ELISA provides a balanced diagnostic profile and performs comparably to the commercial competitive ELISA. The assay represents a promising tool for routine serological screening and epidemiological monitoring of bluetongue in small ruminant.

**Keywords:** *Bluetongue virus*; Indirect ELISA; Plant-Derived VP7 Antigen

## 1. Introduction

*Bluetongue virus* (BTV) is a vector-borne *Orbivirus* transmitted primarily by *Culicoides* midges and responsible for significant morbidity and mortality in sheep and goats. Cattle, although typically asymptomatic, play a critical epidemiological role as reservoir hosts, maintaining viral circulation in endemic regions. Middle East and North Africa regions are recognized as an area with continuous BTV activity, where ruminant frequently develop antibodies following repeated exposure [1, 2, 3, 4].

The VP7 is the major group-specific core protein of *Bluetongue virus* and forms the outer layer of the viral core together with VP3. It is highly conserved across all known BTV serotypes and is abundantly expressed during infection. Because

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VP7 contains stable, serogroup-specific epitopes that are not affected by serotype variation, it represents an optimal antigen for ELISA-based detection of BTV antibodies [5, 6].

Serological assays are essential for BTV surveillance, trade certification, and outbreak investigation [7]. The most widely used diagnostic format is competitive ELISA (c-ELISA), which detects antibodies capable of blocking monoclonal antibody binding to the conserved VP7 antigen epitope. Indirect ELISA (i-ELISA) formats, in contrast, detect total binding antibodies and may offer improved sensitivity, particularly for low-titer or early immune responses [8].

Plant-based expression systems provide a promising platform for producing recombinant viral antigens, including BTV VP7 protein [9, 10, 11, 12]. The objective of this study was to compare the diagnostic sensitivity and specificity of a newly developed indirect ELISA (LT-Biotech), based on a plant-derived recombinant VP7 antigen (Lot 06.02.26), with those of a commercial competitive ELISA (ID Screen® Bluetongue Competition, ID-Vet) using a cohort of 56 sheep sera collected from a farm located in a bluetongue-endemic region of the Middle East.

## 2. Materials and Methods

### 2.1. Sample collection

A total of 56 ovine serum samples were collected from a farm located in a bluetongue-endemic region of the Middle East. All samples were stored at -20 °C until analysis. Sera were tested in parallel using two ELISA kits.

### 2.2. Indirect ELISA

The newly developed indirect ELISA (LT-Biotech) was based on a plant-derived recombinant VP7 antigen. Serum samples were tested according to the manufacturer's protocol. Optical density (OD) values were measured at 450 nm, and samples were classified as positive or negative using an optimized cut-off value of OD = 0.5, established on the internal validation data.

### 2.3. Competitive ELISA

All sera were also tested using a commercial competitive ELISA kit (ID Screen® Bluetongue Competition, ID-Vet), following the manufacturer's instructions. Results were interpreted according to the kit's recommended S/N% ratio, discrimination threshold was 40%.

### 2.4. Diagnostic comparison and statistical analysis

The diagnostic performance of the indirect ELISA was evaluated relative to the competitive ELISA, which served as the reference method. Agreement statistics to calculate sensitivity, specificity, accuracy, predictive values, likelihood ratios, and Cohen's kappa coefficient were computed using standard formulas [13].

## 3. Results

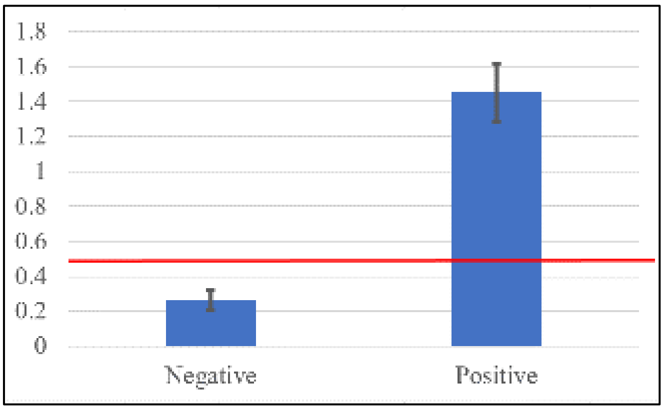
All 56 ovine serum samples were tested in parallel using the newly developed indirect ELISA (LT-Biotech) and the commercial competitive ELISA (ID-Vet). The competitive ELISA classified 40 samples as positive and 16 as negative, whereas the indirect ELISA, using the optimized cut-off value of OD = 0.5, identified 37 samples as positive and 19 as negative (Fig.1, Table 1).

A direct comparison of the two assays demonstrated high overall concordance. The indirect ELISA correctly identified 36 of 40 competitive ELISA positive samples (true positives) and 15 of 16 competitive ELISA negative samples (true negatives). Four samples were negative in the indirect ELISA but positive in the competitive ELISA (false negatives), and one sample was positive in the indirect ELISA but negative in the competitive ELISA (false positive).

Based on the 2×2 contingency table (TP = 36, FN = 4, FP = 1, TN = 15), the indirect ELISA demonstrated a diagnostic sensitivity of 90%, specificity of 94%, and an overall accuracy of 91%. The positive and negative predictive values were 97% and 79%, respectively, while the false-positive and false-negative rates were 6% and 10% (Table 2). The likelihood ratios (LR+ = 14.4; LR− = 0.11) indicated strong discriminative power at 95% CI.

Agreement analysis showed that the two assays classified 51 of 56 samples identically, corresponding to an observed agreement of 91%. Cohen's kappa coefficient was 0.79, indicating substantial agreement between the two ELISA formats.

Overall, these results demonstrate that the plant-derived VP7 indirect ELISA performs comparably to the commercial competitive ELISA and provides a balanced diagnostic profile suitable for routine serological testing in bluetongue-endemic regions.




X-axis: *Serum sample group* (Negative, Positive), Y-axis: $OD_{450}$ *value*

**Figure 1** OD 450 values obtained by indirect ELISA for Negative (n = 19) and Positive (n = 37) serum samples, separated using a cut-off value of 0.5 (red line). Error bars represent 95% confidence intervals ($p < 0.001$)

**Table 1** Summary of diagnostic performance of two ELISA kits

| | **i-ELISA Positive** | **i-ELISA Negative** | **Total** |
|---|---|---|---|
| c-ELISA Positive | 36 (TP*) | 4 (FN) | 40 |
| c-ELISA Negative | 1 (FP) | 15 (TN) | 16 |
| Total | 37 | 19 | 56 |

TP – True Positive, FP – False Positive, FN – False Negative, TN – True Negative.

**Table 2** Diagnostic and agreement statistics for the i-ELISA compared with the c-ELISA (n = 56)

| **Parameter** | **Value** | **95 % CI** | **Interpretation** |
|---|---|---|---|
| Sensitivity | 0.90 | 0.86-0.97 | High ability to detect true positives |
| Specificity | 0.94 | 0.70-1.00 | Strong discrimination of true negatives |
| Accuracy (Efficiency) | 0.91 | 0.80-0.97 | Overall correct classification |
| PPV (Positive Predictive Value) | 0.97 | 0.86-1.00 | Positive results are highly reliable |
| NPV (Negative Predictive Value) | 0.79 | 0.54-0.94 | Most negative results are true negatives |
| Positive Likelihood Ratio | 14.40 | 2.25-86.33 | Strong evidence for infection when test positive |
| Negative Likelihood Ratio | 0.11 | 0.00-0.27 | Strong evidence against infection when test negative |
| False positive rate | 0.06 | 0.00–0.30 | Proportion of negatives incorrectly classified as positive |
| False negative rate | 0.1 | 0.00–0.24 | Proportion of positives incorrectly classified as negative |
| Cohen’s kappa | 0.79 | 0.68-0.84 | Substantial agreement between assays |

## 4. Discussion

This study assessed the diagnostic performance of a newly developed indirect ELISA based on a plant-derived recombinant VP7 antigen by comparing it directly with a commercial competitive ELISA using the same set of 56 ovine serum samples from a bluetongue-endemic region. The head-to-head evaluation under identical testing conditions allowed a clear assessment of inter-assay agreement and relative diagnostic accuracy.

The indirect ELISA demonstrated strong diagnostic performance, correctly identifying most of competitive ELISA positive and negative samples. Its sensitivity (90%) and specificity (94%) indicate reliable detection of BTV-specific antibodies, while the high positive predictive value (97%) confirms that positive results are highly trustworthy. Although a small proportion of low-reactive samples were missing (false-negative rate 10%), the negative predictive value (79%) remained acceptable for field diagnostics. The likelihood ratios (LR+ = 14.4; LR− = 0.11) further support the assay's ability to distinguish between seropositive and seronegative animals.

Agreement analysis showed substantial concordance between the two ELISA formats, with 91% observed agreement and a Cohen's kappa of 0.79. These findings highlight the robustness of the plant-derived VP7 antigen and demonstrate that the optimized cut-off (OD = 0.5) provides a balanced diagnostic profile suitable for routine serological surveillance.

From an operational perspective, the indirect ELISA offers advantages for large-scale screening, including procedural simplicity, broad antibody isotype detection, and compatibility with high-throughput workflows. While the competitive ELISA remains a valuable confirmatory tool, the strong agreement observed in this study indicates that the indirect ELISA can serve as an effective primary diagnostic method. The successful application of plant-derived VP7 antigen also supports its potential for scalable and cost-efficient diagnostic production.

In addition, based on the diagnostic performance observed in this study, we plan to develop a rapid point-of-care (POC) test using a lateral flow immunoassay (LFIA) format incorporating plant-derived VP7 antigen. The assay will be designed as a double-antigen sandwich format, which enables the capture of multiple antibody subclasses, including IgM and IgA, in addition to IgG. This approach is expected to enhance early detection of *Bluetongue virus* exposure and provide a practical, field-deployable tool for rapid screening in endemic regions.

## 5. Conclusion

This study demonstrates that the plant-derived VP7 indirect ELISA provides high diagnostic accuracy and substantial agreement with a commercial competitive ELISA when applied to ovine serum samples from a bluetongue-endemic region. With a sensitivity of 90%, specificity of 94%, and a kappa value of 0.79, the assay reliably detects BTV-specific antibodies and is well suited for routine diagnostic use.

The optimized cut-off (OD = 0.5) ensures balanced performance, minimizing false-positive results while maintaining strong sensitivity. Combined with its simplicity and compatibility with high-throughput testing, the indirect ELISA represents a valuable tool for large-scale serological surveillance. Further validation using well-characterized reference sera from non-endemic regions will support formal receiver operating characteristic (ROC) analysis and refinement of the diagnostic threshold, facilitating broader implementation across diverse epidemiological settings.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that there is no conflict of interest.

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