Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Inter-Laboratory Reproducibility and Diagnostic Agreement of Antigen-Capture ELISAs for Bovine Viral Diarrhea Virus Detection in Ear-Notch Tissue: An Exploratory Method–Comparison Study

Maziar Kaveh Baghbadorani[1]

kaveh@ut.ac.ir

University of Tehran

Massoud Talebkhan Garoussi[1]

University of Tehran

Hossein Aminianfar[2]

University of Tehran

Research Article

Keywords: bovine viral diarrhea virus, BVDV, antigen-capture ELISA, ear notch, diagnostic agreement, inter-laboratory reproducibility, surveillance

Posted Date: November 25th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11108867/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

Reliable detection of BVDV-infected calves and identification of persistently infected animals are essential for effective control programs, yet routine ear-notch antigen-capture ELISA (Ag-ELISA) results may not be fully reproducible across laboratories. A prospective exploratory method-comparison study by testing 25 clinically selected Holstein calves aged 4–8 weeks was conducted to evaluate this hypothesis.

Materials and Methods

Each ear biopsy was divided into three aliquots and tested independently by three veterinary diagnostic laboratories; Laboratories 1 and 3 used the IDEXX® BVDV Ag/Serum Plus ELISA targeting Erns, while laboratory 2 utilized the ID Screen® BVD p80 Antigen Capture ELISA. Buffy-coat RT-qPCR served as an independent molecular reference.

Results

Twenty-three calves were RT-qPCR-positive and two were negative. Sensitivity relative to RT-qPCR was 78.3% (95% CI 56.3–92.5) in Laboratories 1 and 2 and 82.6% (61.2–95.0) in Laboratory 3. All laboratories classified both RT-qPCR-negative calves as negative; observed specificity was 100%, but the 95% CI was 15.8–100%. Pairwise categorical agreement ranged from 64% to 72%, Cohen's kappa from 0.066 to 0.274, and Gwet's AC1 from 0.415 to 0.545. Only 13/25 calves (52%) received the same final classification in all three laboratories. Despite use of the same IDEXX assay, Laboratories 1 and 3 showed poor quantitative concordance (Lin's CCC = 0.243) and wide Bland–Altman limits of agreement (− 3.704 to 2.717 OD units).

Conclusions

Aggregate diagnostic performance relative to RT-qPCR did not translate into consistent individual-animal classification or interchangeable quantitative results between laboratories. Because the sample was small, clinically enriched, and included only two RT-qPCR-negative calves, diagnostic-performance estimates should be considered exploratory. For longitudinal BVDV surveillance, analytical continuity, standardized sample handling, external quality assurance, and parallel comparison of representative specimens are advisable when laboratories or assay platforms change.

1. Introduction

Bovine viral diarrhea virus (BVDV) remains an important cause of reproductive and production losses in cattle worldwide. The magnitude of these losses varies among herds and production systems but may include impaired fertility, embryonic and fetal loss, calf mortality, reduced growth, and decreased milk production [1–4]. Persistently infected (PI) cattle are particularly important in BVDV epidemiology because they can continuously shed large quantities of virus and maintain transmission within and among herds. Consequently, most BVDV control and eradication programs rely on systematic identification and removal of PI animals together with appropriate biosecurity, surveillance, and, where applicable, vaccination strategies [5–10].

Since the earliest descriptions of bovine viral diarrhea in the 1940s and subsequent isolation of BVDV in the 1950s, understanding of the disease, its pathogenesis, epidemiology, and control has developed considerably. Over subsequent decades, advances in virology and diagnostic methodology enabled increasingly effective surveillance and control strategies, eventually contributing to successful regional and national eradication programs. These experiences have demonstrated that BVD elimination is achievable when surveillance is combined with systematic identification and removal of PI animals and sustained herd-level control measures [9, 11]. Progress in BVDV control has therefore been closely linked to improvements in diagnostic technologies capable of identifying infected animals rapidly and reliably.

Several diagnostic approaches are currently available for BVDV detection, including virus isolation, immunologically based methods such as immunofluorescence assay, immunohistochemistry, and antigen-capture enzyme-linked immunosorbent assay (Ag-ELISA), as well as molecular techniques including conventional RT-PCR and real-time RT-PCR (RT-qPCR) [12]. Among these methods, ear-notch capture Ag-ELISA in neonates is widely used for routine surveillance because tissue can be collected easily from young calves during identification or ear tagging, while testing is rapid, relatively inexpensive, and suitable for high-throughput screening. Controlled validation studies have demonstrated high diagnostic performance of ear-notch Ag-ELISA under defined conditions. Nevertheless, diagnostic accuracy under controlled conditions represents only one aspect of test utility. For surveillance and eradication programs, the consistency with which different diagnostic laboratories reproduce results from the same animal is also critically important.

Inter-laboratory reproducibility is especially relevant because herd-management decisions are ultimately made at the individual-animal level. Differences in tissue extraction, incubation conditions, washing procedures, reagent preparation, plate-reader calibration, operator technique, and interpretation of results close to manufacturer-defined decision thresholds may influence both categorical classification and quantitative assay outputs. Such variation becomes particularly important in longitudinal surveillance programs in which a herd may change laboratory, personnel, testing procedure, or assay platform over time. In these circumstances, apparent changes in the proportion of positive animals could potentially reflect variation in the diagnostic system rather than a true change in BVDV infection pressure. Maintaining analytical continuity is therefore important in eradication programs in which diagnostic results directly influence animal removal, herd classification, and subsequent control measures [13].

Previous proficiency and validation studies provide reassuring evidence regarding the performance of BVDV antigen-capture ELISAs under standardized conditions. However, standardized proficiency panels may not fully reproduce the biological heterogeneity and routine pre-analytical variability associated with clinical ear-tissue specimens. Previous investigations have also shown that qualitative Ag-ELISA classification may remain stable even when optical-density values vary with specimen preparation and processing conditions, whereas inter-laboratory proficiency trials using standardized specimens have generally demonstrated high qualitative reliability [14–16]. Thus, an important practical knowledge gap remains: comparatively little is known about individual-sample reproducibility when aliquots originating from the same clinical ear biopsy are processed independently through routine workflows in different diagnostic laboratories. Determining whether different test kits and laboratories produce comparable individual-animal classifications and quantitative signals under such conditions is directly relevant to surveillance quality assurance.

The present study arose from a practical diagnostic problem in a large closed commercial dairy herd in which routine newborn-calf testing and other epidemiological indicators were not fully consistent. We therefore conducted a prospective exploratory method-comparison study in which aliquots derived from the same ear biopsy were independently tested in three veterinary diagnostic laboratories. The primary objective was to determine categorical inter-laboratory agreement at the individual-calf level. A second primary objective was to assess quantitative reproducibility between Laboratories 1 and 3, which used the same commercial antigen-capture ELISA platform, thereby allowing laboratory-associated variability to be evaluated without the major confounding effect of a different commercial assay. Comparison of Ag-ELISA classifications with an independent buffy-coat RT-qPCR result was included as a secondary exploratory objective rather than as definitive validation of PI status.

We hypothesized that similar overall positivity rates could coexist with substantial disagreement at the individual-animal level and that meaningful quantitative variability could persist even among laboratories using the same commercial Ag-ELISA platform.

2. Materials and Methods

Study design, setting, and reporting framework

We conducted a prospective, cross-sectional method-comparison study in April 2025. Specimens were collected before the participating laboratories performed the Ag-ELISAs and the independent molecular reference test, and reporting was prepared with reference to STARD 2015 [17] and ARRIVE 2.0 [18], as applicable to this diagnostic field study. The study herd was a commercial Holstein dairy in Abyek County, Qazvin Province, Iran, with a total population of approximately 5,500 cattle, including 2,450 lactating cows, a mean parity of 2.29, and an average daily milk production of 43.5 kg/cow. No live replacement cattle had been introduced for more than 25 years; replacements were home reared and breeding was by artificial insemination. The herd had not used BVDV vaccination before or during the investigation.

Herd surveillance context and participant selection

The herd used routine newborn ear-tissue Ag-ELISA screening as part of a test-and-remove program. In accordance with the manufacturer's instructions and to improve diagnostic accuracy in identifying PI calves, those with an initial positive result were retested approximately three weeks later, before being classified as PI. Historical surveillance raised concern about ongoing BVDV circulation: 57/65 (89%) replacement heifers aged 9–12 months were antibody-positive in spot testing; the bulk-tank milk antibody ELISA percent positivity increased from 54 to 82; and the annual proportion of calves classified as PI by the routine program increased from 29/2448 (1.18%) in 2021 to 68/2465 (2.75%) in 2025. These herd-level observations provided the operational context for the method-comparison study; they were not used to define the infection status of the 25 enrolled calves.

We purposively enrolled 25 calves aged 4–8 weeks that had chronic, nonspecific findings compatible with BVDV infection, mainly persistent diarrhea, poor growth, and reduced weight gain. This approach deliberately enriched the sample for potentially positive or borderline specimens; the enrolled calves were therefore neither a consecutive series nor representative of herd prevalence. No formal a priori sample-size calculation was performed. The sample size was determined pragmatically by the number of clinically eligible calves available during the study period for this exploratory method-comparison study. Because this was a diagnostic method-comparison study in which every enrolled calf underwent all index and reference tests, random allocation to experimental groups was not applicable.

Specimen collection, aliquoting, transport, and masking

Ear tissue was collected aseptically from the distal pinna. Hair was trimmed when needed, gloves were changed between each sampling as appropriate, and the sampling scissors were rinsed, disinfected with 70% ethanol, and allowed to air-dry between collections. Each biopsy was then divided aseptically into three approximately equal pieces, one for each laboratory. The laboratories therefore received tissue from the same biopsy, although the fragments were not microscopically identical. Each aliquot was placed in a labelled leak-proof tube containing an equivalent volume of phosphate-buffered saline.

The three aliquots were dispatched on the same day at 2–8°C and were not frozen. They reached the laboratories within 2–6 h of collection, and antigen extraction was started within 8 h. From that point onward, each laboratory followed the extraction and assay timing specified for its kit. Unique study codes were used throughout. Personnel performing the Ag-ELISAs were unaware of the calves’ clinical status, the results from the other laboratories, and the RT-qPCR result.

Jugular blood was collected at the same visit into K2-EDTA tubes. Buffy-coat fractions were prepared and sent under refrigeration to an independent diagnostic laboratory for BVDV RT-qPCR. The molecular testing was performed independently of the three Ag-ELISA laboratories. The overall specimen allocation and testing workflow are summarized in Fig. 1.

Index tests: antigen-capture ELISAs

Laboratories 1 and 3 used the IDEXX BVDV Ag/Serum Plus ELISA (IDEXX Laboratories), which targets the BVDV Erns (E0) antigen. Ear tissue was soaked in the manufacturer-specified ear-notch buffer (150–250 µL) at 18–26°C for 12–72 h; overnight extraction was routinely used. Fifty microliters of tissue extract and 50 µL of detection solution were incubated for 2 h at 37 ± 3°C, followed by washing, HRP-conjugated monoclonal antibody incubation, TMB substrate development, stop solution, and optical-density reading at 450 nm with 650 nm as reference. Run validity required mean negative-control absorbance ≤ 0.250 and positive-minus-negative control absorbance ≥ 0.150. Corrected OD (S − N) ≤ 0.200 was negative, > 0.200 to ≤ 0.300 suspect, and > 0.300 positive, according to the manufacturer-defined criteria.

Laboratory 2 used the ID Screen® BVD p80 Antigen Capture ELISA (Innovative Diagnostics/IDvet, Grabels, France), targeting p80 (NS2-3). Tissue extracts were assayed according to the manufacturer's protocol, including 60 min incubation at 37°C, washing, HRP-conjugated detection antibody, substrate development, and OD reading at 450 nm. The run included two positive and two negative controls; mean positive-control OD was 1.3535, mean negative-control OD was 0.0175, and the positive-to-negative control ratio was 77.34, satisfying the stated validity requirements. Results were normalized as:

$S/P\% = [(OD_{sample} - OD_{NC}) / (OD_{PC} - OD_{NC})] \times 100.$

According to the manufacturer’s recommended interpretation criterion, samples with S/P < 35% were classified as negative and those with S/P ≥ 35% as positive. Using the plate-control values, this criterion corresponded to a plate-specific sample-well OD threshold of approximately 0.485. For exploratory quantitative analyses, corrected OD values were used as the continuous Laboratory 2 output. Because Laboratory 2 employed a different commercial ELISA platform targeting p80/NS2-3, these corrected OD values were not considered directly commutable with the corrected OD values generated by the Erns-based IDEXX assay used in Laboratories 1 and 3. One Laboratory 3 specimen initially fell in the IDEXX suspect interval. It was retested according to the manufacturer’s instructions and classified positive on retest. The final binary retest classification was used in the diagnostic-performance and inter-laboratory agreement analyses; the initial suspect result is reported transparently in the Results. Manufacturer-defined cut-offs were prespecified and were not optimized from the study data. The ELISA plate layouts for all three laboratories, together with the representative Laboratory 2 plate, are shown in Fig. 2.

Independent molecular reference classification

BVDV RNA was extracted from buffy coat using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) and tested with the virotype® BVDV RT-PCR Kit (QIAGEN Leipzig GmbH, Leipzig, Germany). The one-step real-time RT-PCR used a 25-µL reaction containing 5 µL extracted RNA, with reverse transcription at 50°C for 20 min, activation at 95°C for 15 min, followed by 40 cycles of 95°C for 30 s, 57°C for 45 s, and 68°C for 45 s. BVDV-specific amplification was detected in FAM and an internal control in HEX. Positive and negative controls were included in each run.

Samples with a valid FAM signal and Cq < 40 were classified as BVDV RNA-positive according to the interpretation used by the independent laboratory. A valid HEX internal-control signal without FAM amplification was classified as negative, whereas samples lacking both signals were predefined as inconclusive.

Outcomes and statistical analysis

The primary outcomes were (i) categorical inter-laboratory agreement at the individual-calf level and (ii) quantitative reproducibility for laboratories using the same commercial assay platform. Secondary exploratory outcomes described diagnostic performance relative to RT-qPCR and cross-platform quantitative associations.

For each laboratory, the final manufacturer-defined Ag-ELISA classification was cross-tabulated against RT-qPCR. We calculated sensitivity, specificity, PPV, NPV, overall accuracy, and LR−, with exact binomial 95% confidence intervals for proportions. No false-positive Ag-ELISA results occurred among the two RT-qPCR-negative calves. In such a small denominator, LR + and the diagnostic odds ratio become mathematically extreme and clinically unstable, so they were not emphasized. As a secondary exploratory analysis, ROC curves and areas under the curve (AUCs) were calculated from continuous assay outputs, with 95% confidence intervals estimated from DeLong variance [19]. Pairwise AUC comparisons used DeLong's test but were treated as supportive analyses because the molecular-reference classification was highly imbalanced, with only two RT-qPCR-negative calves; these comparisons are reported in Additional file 1: Table S1. Manufacturer-specified decision limits were retained throughout, and no cut-off was optimized using the study data.

Agreement among laboratories was described using overall, positive, and negative percent agreement together with Cohen's kappa and Gwet's AC1 for each pair. Fleiss' kappa was used for the three-laboratory comparison [20]. We also recorded complete three-way concordance and the number of calves with at least one discordant classification. No single coefficient was treated as definitive because chance-corrected statistics are affected by the balance of positive and negative results.

Pearson and Spearman coefficients were used to describe association between quantitative outputs, not agreement. Agreement itself was examined with Lin's CCC and Bland–Altman bias and 95% limits of agreement [21, 22]. The comparison between Laboratories 1 and 3 was the primary quantitative reproducibility analysis because both used the IDEXX assay and the same antigen target. Laboratory 2 used a different antigen target and commercial assay platform; its categorical interpretation was based on S/P%, whereas corrected OD values were used for exploratory continuous analyses. Although corrected OD values were available for all three laboratories, the Laboratory 2 measurements were not considered directly commutable with those from Laboratories 1 and 3 because the assays differed in platform and target antigen. Quantitative comparisons involving Laboratory 2 were therefore treated as assay-specific exploratory analyses and were not interpreted as evidence of calibration equivalence. These cross-platform associations, together with the exploratory all-laboratory ICC(2,1), are reported separately in Additional file 1: Table S2 [23]. Pairwise scatterplots were used to visualize quantitative

relationships, with the line of identity (y = x) indicating perfect numerical agreement where the scales were directly comparable. For the primary same-platform comparison between Laboratories 1 and 3, the scatterplot was presented together with the corresponding Bland–Altman plot to provide complementary visual assessments of association and agreement.

Passing–Bablok regression was used as a complementary method-comparison procedure to assess potential constant and proportional differences among laboratory measurements without requiring normally distributed measurement errors. The regression slope and intercept were estimated together with their 95% confidence intervals (CIs). Evidence of proportional difference was considered absent when the 95% CI of the slope included the line-of-identity value of 1, whereas evidence of constant difference was considered absent when the 95% CI of the intercept included 0. The linearity assumption underlying Passing–Bablok regression was evaluated using the modified CUSUM test described by Passing and Bablok [24]. The maximum absolute cumulative sum was standardized as:

$$H = \text{Max } |\text{CUSUM}| / \sqrt{(n_{neg} + 1)}$$

where nneg denotes the number of observations lying below the fitted regression line. H was compared with the Kolmogorov–Smirnov critical value of 1.36 at α = 0.05; H > 1.36 was considered evidence of significant deviation from linearity.

Because the number of paired specimens was limited (n = 25), Passing–Bablok estimates were interpreted together with Lin's concordance correlation coefficient and Bland–Altman analysis rather than as independent evidence of analytical equivalence.

All 25 calves had a final result from each Ag-ELISA and a valid RT-qPCR classification; there were no missing or inconclusive results. Tests were two-sided with $P < 0.05$. The original statistical analyses were performed in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Additional method-comparison analyses, including Passing–Bablok regression and the modified CUSUM assessment of linearity, were independently recalculated from the 25 paired Laboratory 1 and Laboratory 3 observations using Python 3.13.5 (Python Software Foundation), with NumPy 2.3.5 and SciPy 1.17.0. The Passing–Bablok and modified CUSUM procedures were implemented according to the approach described by Passing and Bablok [24]. Given the small, clinically enriched sample, interpretation focused on effect estimates, confidence intervals, and the pattern of agreement rather than on P values alone.

3. Results

Participant flow and reference classification

All 25 calves completed the three Ag-ELISA assessments and the buffy-coat RT-qPCR test. Twenty-three calves (92%) were RT-qPCR-positive and two (8%) were negative. No molecular result was inconclusive and no index-test result was missing. The high proportion of RT-qPCR-positive calves reflects the

purposive clinical selection used for this comparison and should not be read as an estimate of herd prevalence.

Secondary exploratory diagnostic performance relative to RT-qPCR

Laboratories 1 and 2 identified 18 of the 23 RT-qPCR-positive calves as Ag-ELISA-positive, corresponding to a sensitivity of 78.26% (95% CI 56.30–92.54%). Laboratory 3 identified 19 of 23, giving a sensitivity of 82.61% (61.22–95.05%). Both RT-qPCR-negative calves were classified as negative by all three laboratories. The resulting observed specificity was 100%, but the exact 95% CI was 15.81–100%, illustrating how little precision is available with only two reference-negative animals. PPV and likelihood-ratio estimates are affected by the same limitation (Table 1).

Table 1
Exploratory diagnostic performance of Ag-ELISA classifications relative to RT-qPCR.

Index test	TP / FP / TN / FN	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy % (95% CI)	LR−
Laboratory 1 (IDEXX Erns)	18 / 0 / 2 / 5	78.26 (56.30–92.54)	100 (15.81–100)	100 (81.47–100)	28.57 (3.67–70.96)	80.0 (59.30–93.17)	0.217
Laboratory 2 (ID Screen p80)	18 / 0 / 2 / 5	78.26 (56.30–92.54)	100 (15.81–100)	100 (81.47–100)	28.57 (3.67–70.96)	80.0 (59.30–93.17)	0.217
Laboratory 3 (IDEXX Erns)	19 / 0 / 2 / 4	82.61 (61.22–95.05)	100 (15.81–100)	100 (82.35–100)	33.33 (4.33–77.72)	84.0 (63.92–95.46)	0.174

PPV, positive predictive value; NPV, negative predictive value; LR−, negative likelihood ratio. Specificity was imprecisely estimated because only two RT-qPCR-negative calves were included. Predictive values should be interpreted cautiously because the study population was clinically enriched and was not representative of routine screening prevalence.

As a secondary exploratory analysis, continuous assay outputs showed high apparent discrimination relative to the RT-qPCR classification, with AUCs of 0.957 (95% CI 0.871–1.000), 0.935 (95% CI 0.789–1.000), and 0.913 (95% CI 0.781–1.000) for Laboratories 1, 2, and 3, respectively. These estimates should be interpreted cautiously because only two calves were RT-qPCR-negative, resulting in substantial class imbalance and limited precision for assessment of specificity and discrimination. Pairwise DeLong comparisons did not demonstrate statistically significant differences among laboratories and are reported in Additional file 1: Table S1.

Categorical inter-laboratory agreement

Laboratories 1 and 2 each gave 18 positives and 7 negatives final results. Laboratory 3 initially reported 18 positives, 6 negatives, and 1 suspect result; the suspect sample became positive when retested according to the manufacturer's instructions, giving 19 positives and 6 negatives final classifications. Despite these similar overall positivity rates, the same calves were not always classified alike by the three laboratories. Mean pairwise observed agreement across the three laboratory comparisons was 68.0%. As shown in Table 2, pairwise categorical agreement ranged from 64.0% to 72.0%, with consistently higher positive than negative agreement; however, the low Cohen's κ values and modest Gwet's AC1 coefficients demonstrate that similar overall positivity rates did not translate into reliable agreement at the individual-calf level. Only 13 of 25 calves (52.0%) had complete three-way agreement; the remaining 12 calves had at least one discordant result. Fleiss' κ was 0.182. Positive agreement exceeded negative agreement in every pair, a pattern consistent with greater classification instability among negative or lower-reactivity specimens.

Table 2
Pairwise categorical inter-laboratory agreement.

Laboratory pair	Observed agreement %	Positive agreement %	Negative agreement %	Cohen's κ	Gwet's AC1 (95% CI)
Laboratory 1 vs 2	68.0	77.8	42.9	0.206	0.464 (0.078–0.850)
Laboratory 1 vs 3	72.0	81.1	46.2	0.274	0.545 (0.187–0.902)
Laboratory 2 vs 3	64.0	75.7	30.8	0.066	0.415 (0.012–0.817)

Laboratory 3 uses its final retest classification. Agreement measures are presented together because the positive/negative distribution was imbalanced.

Descriptive statistics of assay-specific quantitative outputs

All three laboratories analyzed the same 25 ear-notch tissue samples, and descriptive distributions of their assay-specific continuous outputs are summarized in Table 3. For the directly comparable IDEXX measurements, Laboratory 1 had a mean corrected OD of 1.690 (median 1.834), whereas Laboratory 3 had a mean corrected OD of 2.184 (median 3.210) and greater dispersion (SD 1.506; IQR 3.109). Laboratory 2 had a mean corrected OD of 1.694 and a median of 1.997. Although corrected OD values were used for quantitative description in all three laboratories, the Laboratory 2 measurements should not be compared directly with those from Laboratories 1 and 3 because Laboratory 2 used a different commercial assay platform targeting p80/NS2-3, whereas Laboratories 1 and 3 used the Erns-based IDEXX platform. Across-specimen quantitative values showed substantial dispersion and were generally

negatively skewed, with relatively flat distributions. Given the small sample size and the observed distributional asymmetry, both Pearson and Spearman correlation coefficients were examined to characterize quantitative associations between laboratory measurements.

Table 3
Descriptive statistics of laboratory-specific quantitative ELISA outputs.

Laboratory	n	Mean ± SD	Median	Minimum	Maximum	IQR	Skewness	Kurtosis
Lab 1 (IDEXX corrected OD)	25	1.69 ± 1.16	1.83	0.008	3.563	2.264	-0.364	-1.035
Lab 2 (ID Screen corrected OD)	25	1.69 ± 1.09	2.00	0.017	3.000	2.018	-0.562	-1.117
Lab 3 (IDEXX corrected OD)	25	2.18 ± 1.51	3.21	0.008	3.480	3.109	-0.651	-1.624

Corrected OD values are reported for all three laboratories. Laboratories 1 and 3 used the same IDEXX Erns antigen-capture ELISA, whereas Laboratory 2 used the ID Screen p80/NS2-3 assay. Because Laboratory 2 differed in assay platform and target antigen, its corrected OD values should not be interpreted as directly commutable with those obtained from Laboratories 1 and 3.

Quantitative reproducibility and analytical comparability

Given the small sample size and the observed asymmetry of the quantitative distributions, both Pearson and Spearman coefficients were examined to describe associations between measurements. The primary quantitative reproducibility analysis focused on Laboratories 1 and 3 because both laboratories used the same IDEXX BVDV Ag/Serum Plus ELISA targeting the Erns antigen. The results of this same-platform comparison are summarized in Table 4. Pearson and Spearman coefficients were interpreted as measures of association, whereas Lin's concordance correlation coefficient (CCC) and Bland–Altman analysis were used to evaluate absolute agreement and quantitative reproducibility. Laboratories 1 and 3 showed a moderate Spearman rank association ($\rho = 0.419$, $P = 0.037$), whereas Pearson correlation was weak and not statistically significant ($r = 0.268$, $P = 0.195$). Despite some similarity in the ranking of specimens, absolute agreement was poor, with Lin's CCC of 0.243 (Table 4). The graphical comparison is presented in Fig. 3a, where individual corrected OD measurements showed substantial dispersion around the line of identity, indicating limited correspondence in absolute values between the two laboratories.

Table 4
Same-platform quantitative reproducibility between Laboratories 1 and 3.

Laboratory pair	Platform relation	Pearson r (P)	Spearman ρ (P)	Lin's CCC	Bland–Altman bias	95% limits of agreement
Laboratory 1 vs 3	Same IDEXX platform	0.268 (0.195)	0.419 (0.037)	0.243	−0.493	−3.704 to 2.717

Laboratories 1 and 3 used the IDEXX Erns antigen-capture ELISA. Pearson and Spearman coefficients describe association, whereas Lin's CCC and Bland–Altman statistics describe agreement.

Bland–Altman analysis further demonstrated limited quantitative agreement between Laboratories 1 and 3 (Fig. 3b). The mean difference (Laboratory 1 − Laboratory 3) was − 0.493 OD units (95% CI: −1.170 to 0.183), and the paired t-test did not indicate a statistically significant mean difference (P = 0.145). However, the 95% limits of agreement were broad, extending from − 3.704 to 2.717 OD units. Thus, the absence of a significant average bias did not imply satisfactory agreement, because substantial differences remained at the individual-specimen level. As a complementary method-comparison analysis, Passing–Bablok regression for Laboratories 1 and 3 yielded a slope of 1.053 (95% CI: 0.128–1.817) and an intercept of 0.581 (95% CI: −0.420 to 2.979). Although the confidence interval of the slope included 1 and that of the intercept included 0, the modified CUSUM test indicated significant deviation from linearity. The maximum absolute CUSUM was 6.000, corresponding to a standardized statistic of H = 1.664, which exceeded the 5% critical value of 1.36 (approximate P = 0.008). Accordingly, the Passing–Bablok coefficients were regarded as exploratory and were not interpreted as evidence of analytical equivalence or absence of systematic bias across the entire measurement range.

The exploratory ICC(2,1) calculated across all three laboratories was 0.135 (95% CI: −0.085 to 0.410), also indicating poor overall reproducibility. However, this estimate should be interpreted cautiously because Laboratory 2 used a different antigen target, normalization procedure, and numerical scale. Quantitative comparisons involving Laboratory 2 were therefore considered assay-specific exploratory analyses rather than evidence of calibration equivalence and are presented separately in Additional file 1: Table S2.

4. Discussion

The principal finding of this study was that similar overall positivity rates did not ensure that individual calves received the same classification across laboratories. Only 13 of 25 calves (52%) showed complete three-way categorical agreement, and pairwise observed agreement ranged from 64% to 72%. This discordance is operationally relevant because BVDV control decisions are made at the level of individual animals rather than laboratory-level positivity rates. The comparison between Laboratories 1 and 3 was particularly informative because both used the same commercial IDEXX Erns assay, yet quantitative concordance was limited. Diagnostic-performance analyses relative to buffy-coat RT-qPCR

were secondary and exploratory; with only two RT-qPCR-negative calves, specificity, ROC-based discrimination, predictive values, and related estimates were necessarily imprecise and should not be interpreted as formal validation of either Ag-ELISA platform.

The epidemiological context of the study herd is consistent with substantial BVDV circulation previously documented in Iran and the broader West Asian region. In Qazvin Province, where the present herd is located, a survey of 2205 cattle from 59 industrial dairy farms reported an overall BVDV seroprevalence of approximately 74.5%, with 76.2% seropositivity specifically in Abyek region, Qazvin, Iran [25]. Other Iranian investigations have reported considerable but heterogeneous levels of BVDV exposure across dairy populations, while persistent infection has also been documented, including an estimated PI prevalence of approximately 3.2% in industrial dairy herds around Mashhad, Iran [26, 27]. At the regional level, Scharnböck et al. [28] classified pooled animal-level PI prevalence as low (≤ 0.8%) in Europe, North America, and Australia, intermediate (> 0.8–1.6%) in East Asia, and high (> 1.6%) in West Asia, with a pooled estimate of approximately 2.6% for West Asia. The 89% seropositivity among replacement heifers and the 2.75% annual proportion of newborn calves classified as PI in the present herd are therefore epidemiologically compatible with substantial BVDV circulation in this regional setting. However, these values should not be directly equated with published prevalence estimates because populations, sampling strategies, diagnostic methods, and definitions of PI status differed among studies. Against this epidemiological background, the reliability of individual-animal classification becomes particularly important, providing the rationale for examining how the Ag-ELISA results obtained in the present study compare with previously reported diagnostic performance.

The apparent sensitivities observed in the present study (78.3–82.6% relative to buffy-coat RT-qPCR) were lower than those reported in several controlled evaluations of ear-notch antigen-capture ELISA. Kühne et al. [29], Cornish et al. [30], Hill et al. [31], and McDougall [32] reported very high sensitivity for detection of PI cattle, in some studies approaching 100%. These investigations generally included well-characterized PI animals and were conducted under validated testing conditions, whereas persistent infection was not established longitudinally in the present study. Their diagnostic-performance estimates are therefore not directly comparable with the apparent sensitivities calculated here. Nevertheless, high overall test performance does not exclude occasional failure of antigen detection under field conditions. False-negative Erns Ag-ELISA results have been documented in confirmed PI calves, including approximately 3.4% (8/233) in one investigation and approximately 2.7% of newborn PI calves in the Tyrol BVDV control program [33, 34].

Experimental evidence further indicates that BVDV detectability in young calves can be influenced by maternally derived antibodies, although the magnitude and duration of interference appear to depend on specimen type, diagnostic platform, and viral antigen target. Saucedo et al. [35] demonstrated transient interference of colostrum-derived antibodies with several BVDV diagnostic approaches early in life, whereas Fux and Wolf [34] reported different temporal effects on Erns- and NS3-based antigen detection in colostrum-fed PI calves, with more prolonged suppression of NS3 detection in skin biopsies. These findings are relevant because the calves in the present study were 4–8 weeks old and the evaluated

commercial assays targeted different viral antigens. Similarly, an Iranian investigation of 2173 aborted-fetus specimens reported BVDV detection rates of 15.96% by Erns Ag-ELISA and 18.49% by RT-PCR [36], further illustrating that antigen- and RNA-based methods may yield discordant classifications, although differences in specimen type, population, and clinical context preclude direct extrapolation to ear-notch PI screening.

The lower apparent sensitivity observed here should therefore be interpreted cautiously and may reflect several overlapping biological, analytical, and methodological factors. Potential contributors include genuine limitations of Ag-ELISA detection under field conditions, variation in antigen abundance or tissue distribution, age or antibody-related effects, specimen heterogeneity, assay-target characteristics, extraction and processing procedures, and characteristics of the molecular reference classification. The high exploratory AUC estimates should likewise be regarded as descriptive evidence of separation between molecular-reference categories within this clinically enriched sample rather than as precise estimates of diagnostic discrimination in a representative screening population.

Interpretation also requires distinguishing detection of BVDV at a single time point from confirmation of persistent infection. Cornish et al. [30] demonstrated that acutely infected calves may also be Ag-ELISA-positive, whereas Tavella et al. [37] reported that 435 out of 583 initially Erns Ag-ELISA-positive calves undergoing follow-up in the Bolzano control program were subsequently positive by RT-PCR. These observations emphasize that a single positive antigen or molecular result should not automatically be equated with confirmed PI status, particularly when the classification will inform irreversible animal-level management decisions.

Inter-laboratory proficiency studies provide an important context for the present findings. In a German BVDV eradication-program ring trial, Wernike and Beer [15] reported correct antigen-ELISA classification of a standardized panel of ear-notch and serum specimens. In a subsequent international trial involving 40 veterinary diagnostic laboratories, an Erns-based antigen ELISA also reliably identified BVDV-positive and pestivirus-negative proficiency specimens, whereas virus isolation was less consistently successful and RT-qPCR quantification was influenced by RNA-extraction method [16]. These results appear more reassuring than the agreement observed in the present study, but the difference is not necessarily contradictory. Proficiency materials are specifically prepared and characterized for inter-laboratory assessment, whereas the present study used aliquots of heterogeneous clinical ear tissue that subsequently underwent routine laboratory workflows. The contrast suggests that pre-analytical and analytical variability may become more apparent in field specimens even when an assay performs robustly with standardized materials.

The collective same-platform analyses indicated limited quantitative interchangeability between Laboratories 1 and 3 rather than a simple uniform calibration difference. Lin’s concordance correlation coefficient was 0.243, Pearson correlation was weak ($r = 0.268$, $P = 0.195$), and the scatterplot showed substantial dispersion around the line of identity. Bland–Altman analysis yielded a mean Laboratory 1 minus Laboratory 3 difference of − 0.493 OD units, but the absence of statistically significant mean bias

should not be interpreted as satisfactory agreement because the 95% limits of agreement were broad (−3.704 to 2.717 OD units). Individual specimens could therefore differ markedly despite the lack of a consistent average directional bias. This pattern is consistent with Quinet et al. [14], who showed that quantitative OD output from the IDEXX Ag/Serum Plus assay could vary with specimen-preparation and timing conditions while qualitative positivity was retained. Passing–Bablok regression also requires cautious interpretation: although the confidence intervals for the slope and intercept included 1 and 0, respectively, the modified CUSUM test indicated significant deviation from linearity. The regression coefficients therefore do not establish analytical equivalence or demonstrate that a single linear transformation adequately relates measurements between the two laboratories. Taken together, the CCC, scatterplot, Bland–Altman, and exploratory Passing–Bablok/CUSUM analyses indicate substantial specimen-level quantitative variability even when the same commercial Erns assay is used.

Several biological and analytical mechanisms may contribute to this same-platform variability. Although the three aliquots originated from the same ear biopsy, division of the biopsy could reduce but not eliminate within-tissue heterogeneity because BVDV antigen is associated with particular cellular compartments rather than being distributed uniformly throughout skin tissue [38, 39]. Following aliquoting, the laboratories also differed in routine aspects of extraction, reagent preparation, incubation, washing, reader calibration, operator technique, and local environmental conditions. Such variation may be particularly influential for specimens with lower antigen reactivity or values near a manufacturer-defined decision boundary, where relatively modest analytical changes can alter categorical classification. This interpretation is consistent with the protocol-dependent OD shifts described by Quinet et al. [14] and with the present observation that positive agreement exceeded negative agreement for each laboratory pair. Strongly reactive specimens may remain on the same side of the cut-off despite moderate analytical variation, whereas lower-reactivity specimens may be more susceptible to reclassification. Gwet's AC1 was consistently higher than Cohen's kappa because of the imbalance in positive and negative classifications; consequently, both coefficients are best interpreted together with observed, positive, and negative agreement rather than in isolation [20].

Quantitative results involving Laboratory 2 require a distinct interpretation because both the commercial assay platform and viral antigen target differed from those used in Laboratories 1 and 3. Laboratory 2 employed the ID Screen assay targeting the p80/NS2-3 nonstructural-antigen system, whereas Laboratories 1 and 3 used the IDEXX assay targeting Erns. Although corrected OD values were available for all three laboratories, Laboratory 2 values were not considered directly commutable with those from Laboratories 1 and 3 because they were generated by a different assay platform directed against a different antigen system. Experimental evidence from Fux and Wolf [34] indicates that Erns- and NS3-based antigen detection may respond differently to biological conditions such as maternally derived antibodies. These observations are mechanistically relevant to the p80/NS2-3 nonstructural-antigen platform used by Laboratory 2, but the NS3 assay evaluated by Fux and Wolf was not identical to the commercial assay used in the present study. Cross-platform quantitative comparisons involving Laboratory 2 were therefore retained only as exploratory assay-specific analyses and are reported separately in Additional file 1: Table S2 rather than being interpreted as evidence of calibration

agreement. Categorical comparison nevertheless remains informative because each laboratory applied the manufacturer-defined interpretation criteria for its own assay to answer the same operational question of positive versus negative classification. Because Laboratory 2 was the only laboratory using the p80/NS2-3 platform, laboratory, assay-platform, and antigen-target effects were inherently confounded, and the present design cannot determine the contribution of each factor to the observed differences [12, 34].

These findings have practical implications for BVDV surveillance and eradication programs because both inter-laboratory variability and diagnostic misclassification can influence animal- and herd-level decisions. The consequences of false-negative and false-positive classifications are not equivalent. Failure to identify a true PI animal may allow continuous viral shedding and transmission within the herd, with potential consequences for reproductive performance and the generation of additional PI offspring. Conversely, a false-positive classification acted upon without adequate confirmation may result in unnecessary removal of a non-PI calf and loss of productive or genetic value. In low-prevalence screening populations, even modest reductions in specificity may also substantially reduce positive predictive value, further emphasizing the importance of appropriate confirmation strategies.

At the surveillance level, changes in test positivity may represent genuine changes in BVDV transmission, but they may also arise from changes in laboratory, extraction procedure, assay platform, or interpretation process, particularly for lower-reactivity specimens near a decision threshold. Field experience supports ear-notch Ag-ELISA as a scalable tool for BVDV control while also emphasizing the importance of confirmatory algorithms and analytical continuity [14, 37]. Before changing laboratory or assay platform, parallel testing of a representative specimen set may provide a useful bridging assessment between systems. Standardized sampling and transport, controlled extraction conditions, equipment calibration, staff competency, internal quality control, and participation in external proficiency testing should accompany such transitions [13, 15, 16]. Together, these measures can reduce the risk that analytical variation is mistaken for a genuine epidemiological change in infection pressure.

Several limitations should be considered. The sample was small (n = 25), clinically enriched, and not representative of routine newborn screening. Only two calves were RT-qPCR-negative, resulting in substantial imprecision in specificity, ROC-based discrimination, predictive values, and related diagnostic-performance estimates. RT-qPCR and Ag-ELISA were performed on different specimens and measured different analytes, and a single positive RT-qPCR result did not establish persistent infection. Serial molecular testing and virus isolation were not performed to resolve PI status. Laboratory and assay effects also could not be separated because only one laboratory used the p80/NS2-3 platform, whose quantitative output was not directly commutable with the IDEXX corrected-OD scale. Finally, dividing a single biopsy into three pieces reduced shared pre-sampling variability but could not ensure microscopically identical tissue composition. These constraints limit diagnostic-accuracy and mechanistic inferences but do not negate the observed inter-laboratory disagreement among the specimens actually tested.

The study nevertheless has several features that strengthen its reproducibility findings. All laboratories tested aliquots derived from the same original biopsy, transport conditions were kept similar, and testing was performed independently. The Ag-ELISA laboratories were masked to one another's results and to the molecular reference classification, reducing the opportunity for interpretive influence. The study also addressed reproducibility at two complementary levels: whether laboratories assigned the same calf to the same diagnostic category and whether the two laboratories using the same commercial assay generated comparable quantitative signals. The Laboratory 1–Laboratory 3 comparison therefore provides a particularly informative quality-assurance assessment because it removes the major difference in commercial platform while retaining realistic between-laboratory workflow variation. Rather than challenging the established value of ear-notch Ag-ELISA, the findings complement previous validation and proficiency studies by showing that clinically relevant inter-laboratory variation may still occur with field specimens and support formal bridging, standardization, and proficiency assessment when laboratory systems are changed or surveillance results are compared over time.

Conclusions

In this exploratory comparison, ear-notch BVDV Ag-ELISA results were not fully reproducible across three diagnostic laboratories, even though all laboratories tested aliquots from the same original biopsy. Individual-calf classifications were not consistently reproduced, and the two laboratories using the same IDEXX assay showed poor quantitative agreement. Diagnostic-performance analyses relative to RT-qPCR were secondary and remain uncertain because the sample was small, clinically enriched, and included only two RT-qPCR-negative calves. For longitudinal surveillance, maintaining analytical continuity is desirable where feasible. When changes in laboratory or assay platform are necessary, parallel testing of representative specimens, standardized sample handling, external quality assurance, and appropriate confirmatory algorithms may reduce the risk of interpreting analytical variation as epidemiological change.

Abbreviations

BVDV
bovine viral diarrhea virus
Ag-ELISA
antigen-capture enzyme-linked immunosorbent assay
RT-qPCR
real-time reverse-transcription polymerase chain reaction
PI
persistently infected
OD
optical density
S/P
sample-to-positive ratio

AUC
area under the receiver operating characteristic curve
PPV
positive predictive value
NPV
negative predictive value
LR−
negative likelihood ratio
CCC
concordance correlation coefficient
ICC
intra-class correlation coefficient
CI
confidence interval
CUSUM
cumulative sum.

Declarations

Ethics approval and consent to participate

All animal welfare conditions and sampling procedures in this study were conducted in accordance with the ethical guidelines of the Faculty of Veterinary Medicine, University of Tehran, and received approval from its Biomedical Research Ethics Committee. Written informed consent for participation of the animals in the study and for collection of biological samples was obtained from the herd owner or an authorized farm representative before enrollment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

Not applicable.

Additional files

Additional file 1 (.docx). Supplementary diagnostic and cross-platform analyses. Contains Table S1 (pairwise DeLong comparisons of AUCs) and Table S2 (exploratory cross-platform quantitative associations and agreement involving Laboratory 2).

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contribution

MKB: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing—original draft, project administration. MTG: Conceptualization, methodology, supervision, validation, writing—review and editing. HA: Methodology, investigation, validation, resources, writing—review and editing. All authors read and approved the final manuscript.

Acknowledgement

The authors thank the farm management, Mr. Javad Torabi, and the calf-rearing staff for their valuable cooperation and assistance.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

1. Richter V, Lebl K, Baumgartner W, Obritzhauser W, Käsbohrer A, Pinior B. A systematic worldwide review of the direct monetary losses in cattle due to bovine viral diarrhoea virus infection. Vet J. 2017;220:80–7. https://doi.org/10.1016/j.tvjl.2017.01.005.
2. Richter V, Kattwinkel E, Firth CL, Marschik T, Dangelmaier M, Trauffler M, et al. Mapping the global prevalence of bovine viral diarrhoea virus infection and its associated mitigation programmes. Vet Rec. 2019;184:711. https://doi.org/10.1136/vr.105354.
3. Pinior B, Garcia S, Minviel JJ, Raboisson D. Epidemiological factors and mitigation measures influencing production losses in cattle due to bovine viral diarrhoea virus infection: a meta-analysis. Transbound Emerg Dis. 2019;66:2426–39. https://doi.org/10.1111/tbed.13300.

4. Rana EA, Gogoi-Tiwari J, Aleri J, Prodhan MA, Akter SH, Annandale H, et al. Molecular epidemiology and control strategies for BVDV: a global systematic review from 2000 to 2025. Vet Med Int. 2025;2025:6732453. https://doi.org/10.1155/vmi/6732453.
5. Lindberg A, Brownlie J, Gunn GJ, Houe H, Moennig V, Saatkamp HW, et al. The control of bovine viral diarrhoea virus in Europe: today and in the future. Rev Sci Tech. 2006;25:961–79. https://doi.org/10.20506/rst.25.3.1703.
6. Ståhl K, Alenius S. BVDV control and eradication in Europe—an update. Jpn J Vet Res. 2012;60(Suppl):S31–9.
7. Reichel MP, Lanyon SR, Hill FI. Perspectives on current challenges and opportunities for bovine viral diarrhoea virus eradication in Australia and New Zealand. Pathogens. 2018;7:14. https://doi.org/10.3390/pathogens7010014.
8. Moennig V, Becher P. Control of bovine viral diarrhea. Pathogens. 2018;7:29. https://doi.org/10.3390/pathogens7010029.
9. Moennig V, Yarnall MJ. The long journey to BVD eradication. Pathogens. 2021;10:1292. https://doi.org/10.3390/pathogens10101292.
10. Walz PH, Chamorro MF, Falkenberg SM, Passler T, van der Meer F, Woolums AR, et al. Bovine viral diarrhea virus: an updated American College of Veterinary Internal Medicine consensus statement with focus on virus biology, hosts, immunosuppression, and vaccination. J Vet Intern Med. 2020;34:1690–706. https://doi.org/10.1111/jvim.15816.
11. Goens SD. The evolution of bovine viral diarrhea: a review. Can Vet J. 2002;43:946–54.
12. Wang Y, Pang F. Diagnosis of bovine viral diarrhea virus: an overview of currently available methods. Front Microbiol. 2024;15:1370050. https://doi.org/10.3389/fmicb.2024.1370050.
13. Caldow GL, Carty H, Berezowski J. National BVDV eradication programme: assessing the utility of the spot test. Prev Vet Med. 2026;251:106828. https://doi.org/10.1016/j.prevetmed.2026.106828.
14. Quinet C, Czaplicki G, Dion E, Dal Pozzo F, Kurz A, Saegerman C. First results in the use of bovine ear notch tag for bovine viral diarrhoea virus detection and genetic analysis. PLoS ONE. 2016;11:e0164451. https://doi.org/10.1371/journal.pone.0164451.
15. Wernike K, Beer M. Diagnostics in the context of an eradication program: results of the German bovine viral diarrhea proficiency trial. Vet Microbiol. 2019;239:108452. https://doi.org/10.1016/j.vetmic.2019.108452.
16. Wernike K, Beer M. Comparison of bovine viral diarrhea virus detection methods: results of an international proficiency trial. Vet Microbiol. 2024;290:109985. https://doi.org/10.1016/j.vetmic.2024.109985.
17. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ. 2015;351:h5527. https://doi.org/10.1136/bmj.h5527.
18. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. BMC Vet Res. 2020;16:242.

https://doi.org/10.1186/s12917-020-02451-y.

19. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988;44:837–45. https://doi.org/10.2307/2531595.
20. Gwet KL. Computing inter-rater reliability and its variance in the presence of high agreement. Br J Math Stat Psychol. 2008;61:29–48. https://doi.org/10.1348/000711006X126600.
21. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1:307–10. https://doi.org/10.1016/S0140-6736(86)90837-8.
22. Lin LI. A concordance correlation coefficient to evaluate reproducibility. Biometrics. 1989;45:255–68. https://doi.org/10.2307/2532051.
23. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med. 2016;15:155–63. https://doi.org/10.1016/j.jcm.2016.02.012.
24. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. J Clin Chem Clin Biochem. 1983;21:709–20. https://doi.org/10.1515/cclm.1983.21.11.709.
25. Bahonar AR, Nekouie Jahromi OA, Omidvarian MJ, Najjar E, Shokri MR, Mirzaie K. Bovine viral diarrhea in Qazvin Province (Iran): a seroprevalence study. J Vet Res. 2011;66:319–23.
26. Talebkhan Garoussi M, Haghparast AR, Rafati MS. The prevalence of bovine viral diarrhea virus in persistently infected cows in industrial dairy herds in suburb of Mashhad-Iran. Iran J Vet Med. 2011;5:198–203. https://doi.org/10.22059/ijvm.2011.23574.
27. Javdani Shahedin G, Dorood D, Mousavi Nasab SD, Talebkhan Garoussi M. Epidemiology of bovine viral diarrhea virus (BVDV) infection in dairy cattle herds-Iran from 2000 to 2020. Iran J Virol. 2022;16:68–75.
28. Scharnböck B, Roch FF, Richter V, Funke C, Firth CL, Obritzhauser W, et al. A meta-analysis of bovine viral diarrhoea virus (BVDV) prevalences in the global cattle population. Sci Rep. 2018;8:14420. https://doi.org/10.1038/s41598-018-32831-2.
29. Kühne S, Schroeder C, Holmquist G, Wolf G, Horner S, Brem G, et al. Detection of bovine viral diarrhoea virus infected cattle—testing tissue samples derived from ear tagging using an Erns capture ELISA. J Vet Med B Infect Dis Vet Public Health. 2005;52:272–7. https://doi.org/10.1111/j.1439-0450.2005.00861.x.
30. Cornish TE, van Olphen AL, Cavender JL, Edwards JM, Jaeger PT, Vieyra LL, et al. Comparison of ear notch immunohistochemistry, ear notch antigen-capture ELISA, and buffy coat virus isolation for detection of calves persistently infected with bovine viral diarrhea virus. J Vet Diagn Invest. 2005;17:110–7. https://doi.org/10.1177/104063870501700203.
31. Hill FI, Reichel MP, McCoy RJ, Tisdall DJ. Evaluation of two commercial enzyme-linked immunosorbent assays for detection of bovine viral diarrhoea virus in serum and skin biopsies of cattle. N Z Vet J. 2007;55:45–8. https://doi.org/10.1080/00480169.2007.36734.

32. McDougall S. Effect of calf age on bovine viral diarrhea virus tests. J Vet Diagn Invest. 2021;33:528–37. https://doi.org/10.1177/1040638721998821.
33. Oettl J, Schöpf KC, Matt M, Dünser M, Auer G, Wolf G, et al. Fortschritte bei der Eradikation der Bovinen Virus Diarrhoe (BVD) mit Ohrgewebe- und Blutuntersuchungen im Bundesland Tirol. Wien Tierärztl Monatsschr. 2010;97:203–9.
34. Fux R, Wolf G. Transient elimination of circulating bovine viral diarrhoea virus by colostral antibodies in persistently infected calves: a pitfall for BVDV-eradication programs? Vet Microbiol. 2012;161:13–9. https://doi.org/10.1016/j.vetmic.2012.07.001.
35. Saucedo M, Chamorro MF, Passler T, Huber L, Kennedy S, Bayne JE, et al. Colostrum-derived immunity reduces detection of calves persistently infected with bovine viral diarrhea virus by common diagnostic tests. J Am Vet Med Assoc. 2026;264:202–10. https://doi.org/10.2460/javma.25.06.0397.
36. Safarpoor Dehkordi F. Prevalence study of bovine viral diarrhea virus by evaluation of antigen capture ELISA and RT-PCR assay in bovine, ovine, caprine, buffalo and camel aborted fetuses in Iran. AMB Express. 2011;1:32. https://doi.org/10.1186/2191-0855-1-32.
37. Tavella A, Zambotto P, Stifter E, Lombardo D, Rabini M, Robatscher E, et al. Retrospective evaluation of data over five years of the BVDV prevention program using ear notch samples from the autonomous province of Bolzano (Italy). Berl Munch Tierarztl Wochenschr. 2012;125:326–31. https://doi.org/10.2376/0005-9366-125-326.
38. Njaa BL, Clark EG, Janzen E, Ellis JA, Haines DM. Diagnosis of persistent bovine viral diarrhea virus infection by immunohistochemical staining of formalin-fixed skin biopsy specimens. J Vet Diagn Invest. 2000;12:393–9. https://doi.org/10.1177/104063870001200501.
39. Brodersen BW. Bovine viral diarrhea virus infections: manifestations of infection and recent advances in understanding pathogenesis and control. Vet Pathol. 2014;51:453–64. https://doi.org/10.1177/0300985813520250.

Figures

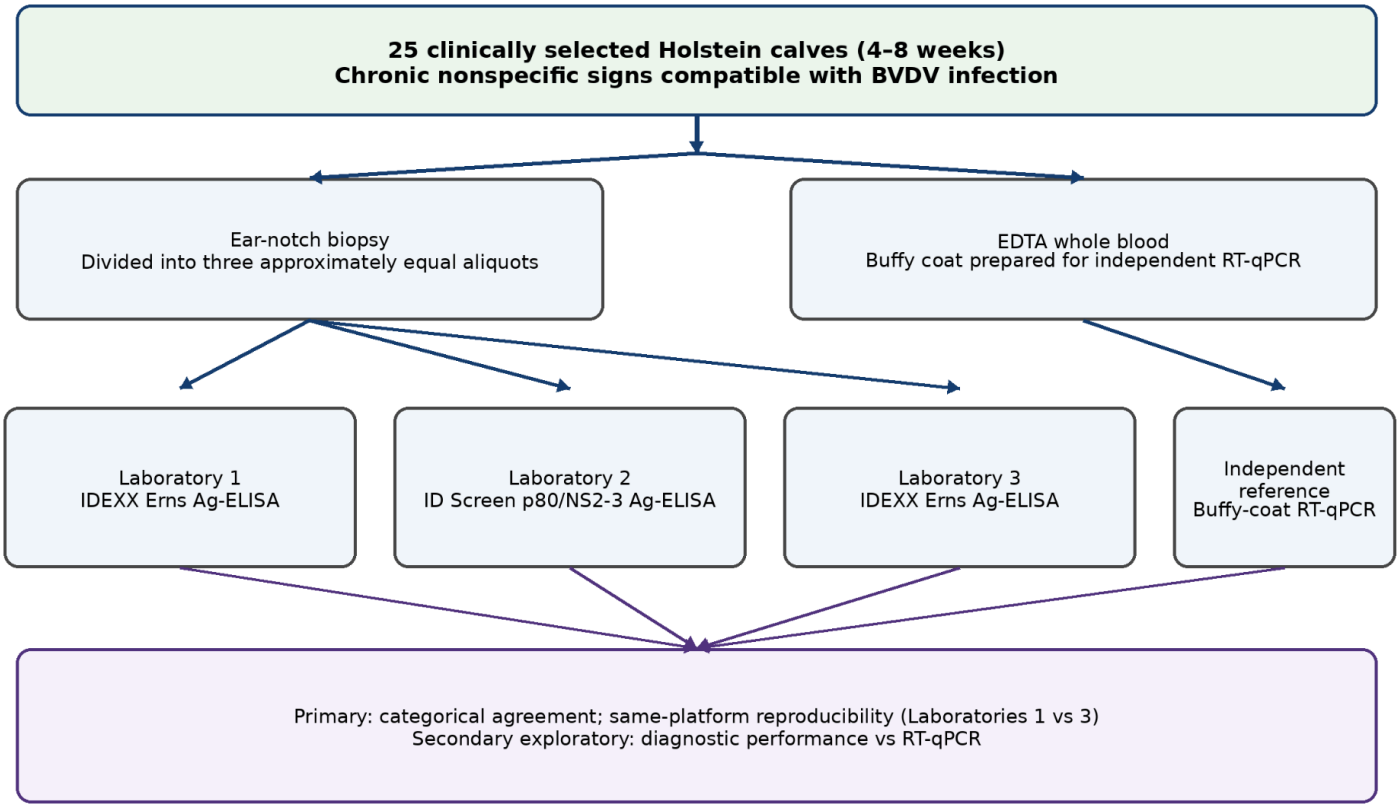


Figure 1

Study flow for inter-laboratory BVDV ear-notch testing. Each of the 25 clinically selected calves provided one ear-notch biopsy, which was divided into three aliquots for independent Ag-ELISA testing, and one EDTA-anticoagulated blood sample for independent buffy-coat RT-qPCR. Laboratories 1 and 3 used the IDEXX Erns assay; Laboratory 2 used the ID Screen p80/NS2-3 assay. Primary analyses assessed categorical agreement and same-platform quantitative reproducibility; diagnostic performance relative to RT-qPCR was secondary and exploratory.

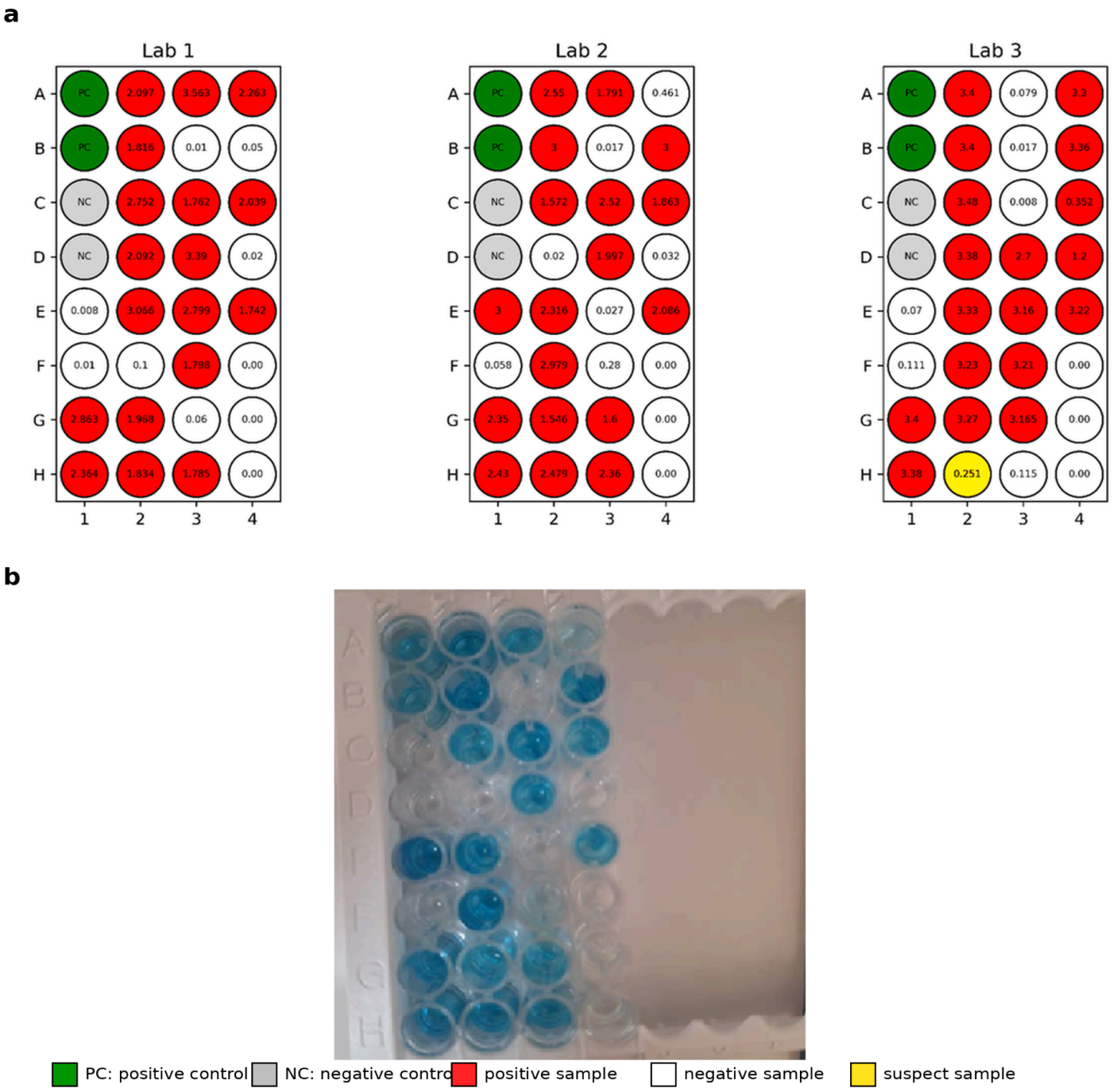


Figure 2

ELISA plate layouts and representative Laboratory 2 plate. (a) Schematic 32-well layouts for Laboratories 1, 2, and 3. Each well is labelled with its optical density (OD). Positive controls (PC) and negative controls (NC) are identified within the graphic. Samples were classified using laboratory-specific manufacturer-defined criteria: corrected OD >0.300 for Laboratories 1 and 3 and S/P ≥35% for Laboratory 2. For the Laboratory 2 plate shown, the S/P criterion corresponded to a sample-well OD threshold of approximately 0.485. Red indicates positive samples, white negative samples, and yellow the initially

suspect Laboratory 3 sample. (b) Laboratory 2 plate after testing. Wells F4, G4, and H4 were intentionally unused and are shown as 0.00.

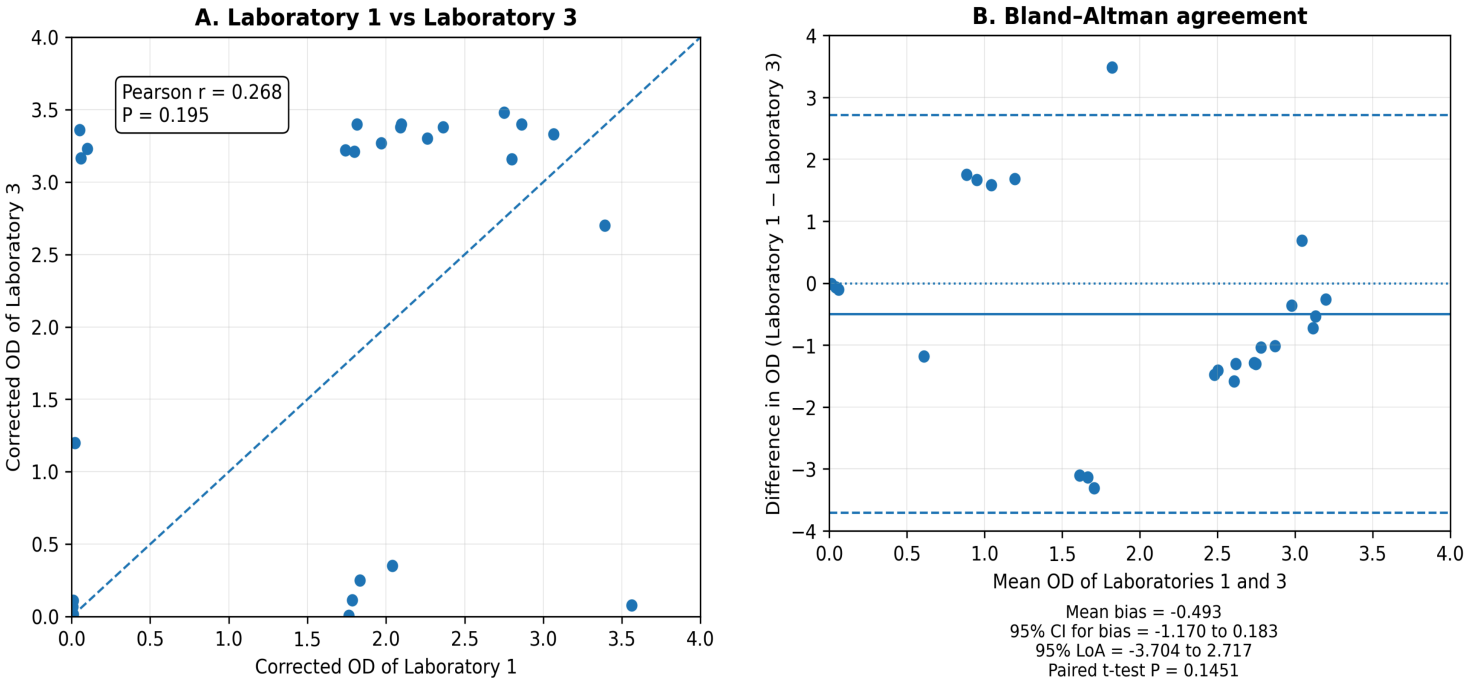


Figure 3

Same-platform quantitative agreement between Laboratories 1 and 3. (a) Scatterplot of corrected OD values with the line of identity (y = x). (b) Bland–Altman plot of the same paired measurements; the solid line indicates mean bias, the dotted line zero difference, and dashed lines the 95% limits of agreement. Pearson r = 0.268 (P = 0.195); mean bias = −0.493 OD units; 95% limits of agreement = −3.704 to 2.717.