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ESTABLISHMENT OF A REAL-TIME DETECTION METHOD FOR PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS USING A SURFACE-ENHANCED RAMAN SCATTERING SYSTEM

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major swine pathogen for which rapid, field-adaptable screening tools are needed. This study established complementary quantitative real-time polymerase chain reaction (qPCR) and surface-enhanced Raman scattering (SERS) workflows for PRRSV detection in swine specimens. Matrix-specific SERS preprocessing procedures were developed for serum, feces, and oral swabs, and a TaqMan-based qPCR standard operating procedure was used as the reference method. Serial PRRSV preparations from 8.152 to 2.152 $\log_{10}$ copies/mL were evaluated by applying 2 µL of each processed sample to a SERS chip and measuring the spectra with a portable 785-nm Raman system. Detectable PRRSV preparations consistently exhibited seven characteristic peaks at approximately 832, 1,032, 1,154, 1,276, 1,374, 1,502, and 1,604 cm^{-1}. The SERS detection limit was 3.152 $\log_{10}$ copies/mL (approximately 1.42×10^3 copies/mL), whereas the qPCR detection limit was 14.2 copies/mL. A total of 50 field specimens, including 20 blood-derived serum samples, 20 fecal samples, and 10 oral swabs, were tested. The qPCR identified 10 positive and 40 negative specimens. SERS correctly identified 9 of the 10 qPCR-positive specimens and all 40 qPCR-negative specimens. The single false-negative specimen contained 125.3 copies/mL, below the SERS analytical detection limit. Using qPCR as the reference, the preliminary sensitivity and specificity of SERS were 90% and 100%, respectively. These findings support SERS as a rapid screening platform for PRRSV, particularly when viral loads exceed the analytical detection limit; molecular confirmation remains advisable for negative specimens with suspected low viral loads.

Keywords: Porcine Reproductive and Respiratory Syndrome Virus; PRRSV; Surface-Enhanced Raman Scattering; SERS; Raman Spectroscopy; Quantitative Real-Time PCR; Rapid Screening; Swine

1 INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV), the causative agent of porcine reproductive and respiratory syndrome, is an RNA virus associated with reproductive failure and respiratory disease in pigs. In intensive swine production systems, rapid recognition of infected animals is important because clinical manifestations may be variable, mixed infections can complicate interpretation, and delayed diagnosis may facilitate transmission within and between herds. Laboratory confirmation is therefore essential for timely disease surveillance and herd-level management.

Nucleic-acid amplification methods, particularly quantitative real-time polymerase chain reaction (qPCR), provide sensitive and quantitative detection of PRRSV. Nevertheless, conventional molecular testing requires RNA extraction, reagent preparation, thermal cycling, and trained personnel. These requirements can limit immediate decision-making when rapid screening is needed at or near the farm. A complementary platform that uses a small sample volume and produces an immediately interpretable molecular fingerprint may therefore improve field-oriented surveillance.

Raman spectroscopy characterizes molecular vibrations through inelastic light scattering. Surface-enhanced Raman scattering (SERS) increases Raman signal intensity when analytes interact with nanostructured metallic surfaces, allowing complex biological materials to be examined with high sensitivity [5–8]. SERS has been applied to pathogen identification and to the characterization of virus particles, virus-infected cells, and virus-containing clinical matrices [9–22]. Previous studies have also explored SERS for rapid detection of animal and swine pathogens [1-4].

The present study established a PRRSV-specific rapid diagnostic workflow that combined a laboratory qPCR reference method with SERS sample preprocessing, Raman fingerprint database construction, and cloud-assisted spectral comparison. The objectives were to define the characteristic PRRSV Raman pattern and analytical detection limit, evaluate the reproducibility of the reference spectrum, determine whether PRRSV-associated signals could be recognized in serum, fecal, and oral-swab matrices, and estimate preliminary diagnostic sensitivity and specificity using qPCR as the reference method.

2 MATERIALS AND METHODS

2.1 Animals and housing conditions at the traditional pig farm

The study was conducted at a traditional pig farm operated by the Agricultural Technology Research Institute (ATRI). Pigs were housed in biosecure facilities maintained free of major swine diseases, and experiments involving zoonotic agents were not permitted. Personnel followed strict entry procedures and wore appropriate personal protective equipment, including dedicated clothing, footwear, gloves, masks, and caps or disposable coveralls. Routine health surveillance and vaccination requirements were implemented to reduce risks associated with zoonotic pathogens and animal allergens. Housing temperature and relative humidity were controlled, and a 12-h light/12-h dark photoperiod was maintained. Pigs had ad libitum access to a standard diet and fresh water and were allowed to acclimate for one week before data collection. All procedures were reviewed and approved by the ATRI Institutional Animal Care and Use Committee (IACUC No. 113085) and were conducted in accordance with institutional animal-care guidelines.

2.2 Study design and specimen set

The method-establishment study comprised two complementary components. First, a quantified PRRSV preparation was tested over a concentration range from 8.152 to 2.152 $\log_{10}$ copies/mL to establish the SERS reference fingerprint and analytical detection limit. Repeated PRRSV measurements were also examined to assess the consistency of characteristic peak positions. Second, 50 field specimens were evaluated, including 20 blood-derived serum samples, 20 fecal samples, and 10 oral swabs. Each specimen was classified by qPCR and independently assessed by SERS through comparison with the PRRSV reference spectrum. The qPCR result was used as the reference classification for the calculation of preliminary SERS sensitivity and specificity.

2.3 SERS sample preprocessing

2.3.1 Fecal specimens

A 0.015-g fecal aliquot was suspended in 20 μL of sterile normal saline and held at 4 °C for 30 min. The suspension was centrifuged at 2,000 rpm for 15 min at 4 °C, and the recovered supernatant was subsequently centrifuged at 5,000 rpm for 10 min at 4 °C. After removal of the supernatant, the pellet was washed with 500 μL of sterile Sodium chloride solution (< 0.15 M) and centrifuged at 5,000 rpm for 10 min at 4 °C. This sodium chloride wash was performed twice. The pellet was then washed once with 500 μL of sterile deionized water and centrifuged at 5,000 rpm for 10 min at 4 °C. The final pellet was resuspended in 10 μL of sterile deionized water for SERS measurement.

2.3.2 Serum specimens

Blood samples collected without anticoagulant were centrifuged at 3,000 rpm for 15 min at 4 °C to obtain serum. For SERS preprocessing, 150 μL of serum was centrifuged at 5,000 rpm for 10 min at 4 °C, and the supernatant was discarded. The pellet was washed with 500 μL of sterile Sodium chloride solution (< 0.15 M), followed by centrifugation at 5,000 rpm for 10 min at 4 °C; this wash was repeated twice. The pellet was then washed once with 500 μL of sterile deionized water and centrifuged at 5,000 rpm for 10 min at 4 °C. The final pellet was resuspended in 10 μL of sterile deionized water for SERS measurement.

2.3.3 Oral-swab specimens

Each oral swab was immersed in 1 mL of sterile normal saline and held at 4 °C for 30 min. The suspension was centrifuged at 2,000 rpm for 15 min at 4 °C, and the supernatant was transferred and centrifuged at 5,000 rpm for 10 min at 4 °C. After removal of the supernatant, the pellet was washed twice with 500 μL of sterile Sodium chloride solution (< 0.15 M), with centrifugation at 5,000 rpm for 10 min at 4 °C after each wash. The pellet was then washed once with 500 μL of sterile deionized water and centrifuged at 5,000 rpm for 10 min at 4 °C. The final pellet was resuspended in 10 μL of sterile deionized water for SERS measurement.

2.4 PRRSV qPCR reference procedure

A PRRSV nucleic-acid detection procedure was established to determine whether PRRSV-specific nucleic acid was present in the specimens and to quantify viral concentration. Samples underwent matrix-appropriate clarification or homogenization followed by automated RNA purification using a LabTurbo 48 nucleic-acid extraction system. The purified RNA was analyzed using a TaqMan-based quantitative real-time PCR assay on an ABI 7500 Fast Real-Time PCR System. Reaction mixtures containing the TaqMan reagents, RNA samples, and quantified standards were prepared in 96-well reaction plates under contamination-controlled conditions.

Amplification was performed in Fast mode for 40 cycles. Quantification was based on the standard curve, with the fluorescence threshold set to 0.2 and the baseline set to cycles 1–10. A run was accepted when the standard-curve slope was between −3.1 and −3.6 and the coefficient of determination (R^2) exceeded 0.99. The established qPCR standard-curve range was 1.42×10^8 to 1.42×10^1 copies/mL, and the analytical detection limit was 14.2 copies/mL.

Table 1: Operational settings and acceptance criteria for the PRRSV qPCR reference procedure.

Parameter	Established setting or criterion
Reference SOP	ATRI_PRRS_001
Nucleic-acid extraction platform	LabTurbo 48 automated extraction system
Amplification platform	ABI 7500 Fast Real-Time PCR System
Detection chemistry	TaqMan-based quantitative real-time PCR
Instrument mode	Fast mode
Number of amplification cycles	40
Fluorescence threshold	0.2
Baseline cycles	1–10
Acceptable standard-curve slope	−3.1 to −3.6
Acceptable coefficient of determination	$R^2 > 0.99$

2.5 PRRSV Raman fingerprint database and specimen classification

2.5.1 Establishment of the PRRSV Raman fingerprint database

Two microliters of each PRRSV preparation were applied to a SERS detection chip and subjected to Raman measurement. Raw spectra were exported and analyzed using SpectraView software to identify characteristic peak positions and compare spectral profiles across viral concentrations. The processed spectra were organized as standard reference profiles and used to establish a preliminary PRRSV Raman fingerprint database.

2.5.2 SERS classification of swine specimens

After matrix-specific preprocessing, 2 μL of each serum, fecal, or oral-swab specimen was applied to the SERS chip. The resulting spectrum was compared with the PRRSV reference pattern, using the 8.152 $\log_{10}$ copies/mL preparation as the principal comparison standard. A specimen was classified as SERS-positive when the characteristic PRRSV peak pattern aligned with the reference profile; specimens that did not reproduce the characteristic peak positions and overall pattern were classified as SERS-negative.

2.6 Raman spectrometer and cloud-assisted analysis

Measurements were performed using the RAPID Surface Raman Spectroscopy Rapid Detection Cloud System and Phan^2 SERS chips. Before each analytical run, the chip was evaluated using the substrate-validation function. Successful validation was defined by detection of the chip internal-standard signal at approximately 520 cm^{-1} together with the expected smoothly rising substrate profile at higher Raman shifts. After substrate confirmation, the measurement type was set to "unclassified," the prepared specimen was applied to the chip, and real-time measurement was initiated. Upon completion, the system automatically entered the cloud-comparison procedure, and the Raman spectra were downloaded for subsequent interpretation.

The portable Raman instrument used a 785-nm laser with power ≤ 100 mW, a spectral range of 200–1,800 cm^{-1}, and spectral resolution < 10 cm^{-1}. The instrument dimensions were approximately 46 × 35 × 20 cm, and the weight was < 10 kg. Raw and preprocessed spectra were reviewed using SpectraView.

2.7 Diagnostic performance analysis

qPCR classifications were used as the reference standard. A true-positive result was defined as a specimen positive by both qPCR and SERS, a false-negative result as qPCR-positive but SERS-negative, a false-positive result as qPCR-negative but SERS-positive, and a true-negative result as negative by both methods. Sensitivity was calculated as true positives divided by all qPCR-positive specimens, and specificity was calculated as true negatives divided by all qPCR-negative specimens.

3 RESULTS

3.1 Establishment of the PRRSV qPCR and SERS standard operating procedures

The PRRSV qPCR procedure (ATRI_PRRS_001) and matrix-specific SERS preprocessing procedure (ATRI_SERS_001) were successfully established. The qPCR standard curve covered 1.42×10^8 to 1.42×10^1 copies/mL and met the predefined slope and R^2 acceptance criteria. The analytical detection limit of qPCR was 14.2 copies/mL. In comparison, the SERS concentration series was evaluated from 8.152 to 2.152 $\log_{10}$ copies/mL, with reliable detection maintained to 3.152 $\log_{10}$ copies/mL.

Table 2: Analytical ranges and detection limits of the PRRSV qPCR and SERS workflows.

Method	Evaluated or quantifiable range	Analytical detection limit	Interpretation
qPCR	1.42×10^8 to 1.42×10^1 copies/mL	14.2 copies/mL	Sensitive quantitative reference method
SERS	8.152 to 2.152 $\log_{10}$ copies/mL evaluated; reproducible pattern from 8.152 to 3.152 $\log_{10}$ copies/mL	3.152 $\log_{10}$ copies/mL (approximately 1.42×10^3 copies/mL)	Rapid portable screening method

3.2 Substrate validation, PRRSV spectral fingerprint, and SERS detection limit

Before each SERS run, substrate validation confirmed the instrument response. The chip generated an internal-standard peak at approximately 520 cm^{-1} and the expected smoothly rising high-wavenumber profile, indicating acceptable chip and instrument performance. PRRSV preparations from 8.152 to 3.152 $\log_{10}$ copies/mL generated comparable preprocessed spectra with seven prominent characteristic peaks at approximately 832, 1,032, 1,154, 1,276, 1,374, 1,502, and 1,604 cm^{-1} (Figure 1). Although signal intensity varied with concentration, the characteristic peak positions remained identifiable.

The 2.152 $\log_{10}$ copies/mL preparation did not produce a reliable PRRSV fingerprint. Therefore, the SERS analytical detection limit was defined as 3.152 $\log_{10}$ copies/mL, equivalent to approximately 1.42×10^3 copies/mL. This limit was higher than the qPCR detection limit of 14.2 copies/mL, demonstrating lower analytical sensitivity. However, the SERS

system required only a small sample volume and provided rapid, portable measurement, supporting its use as a preliminary screening method.

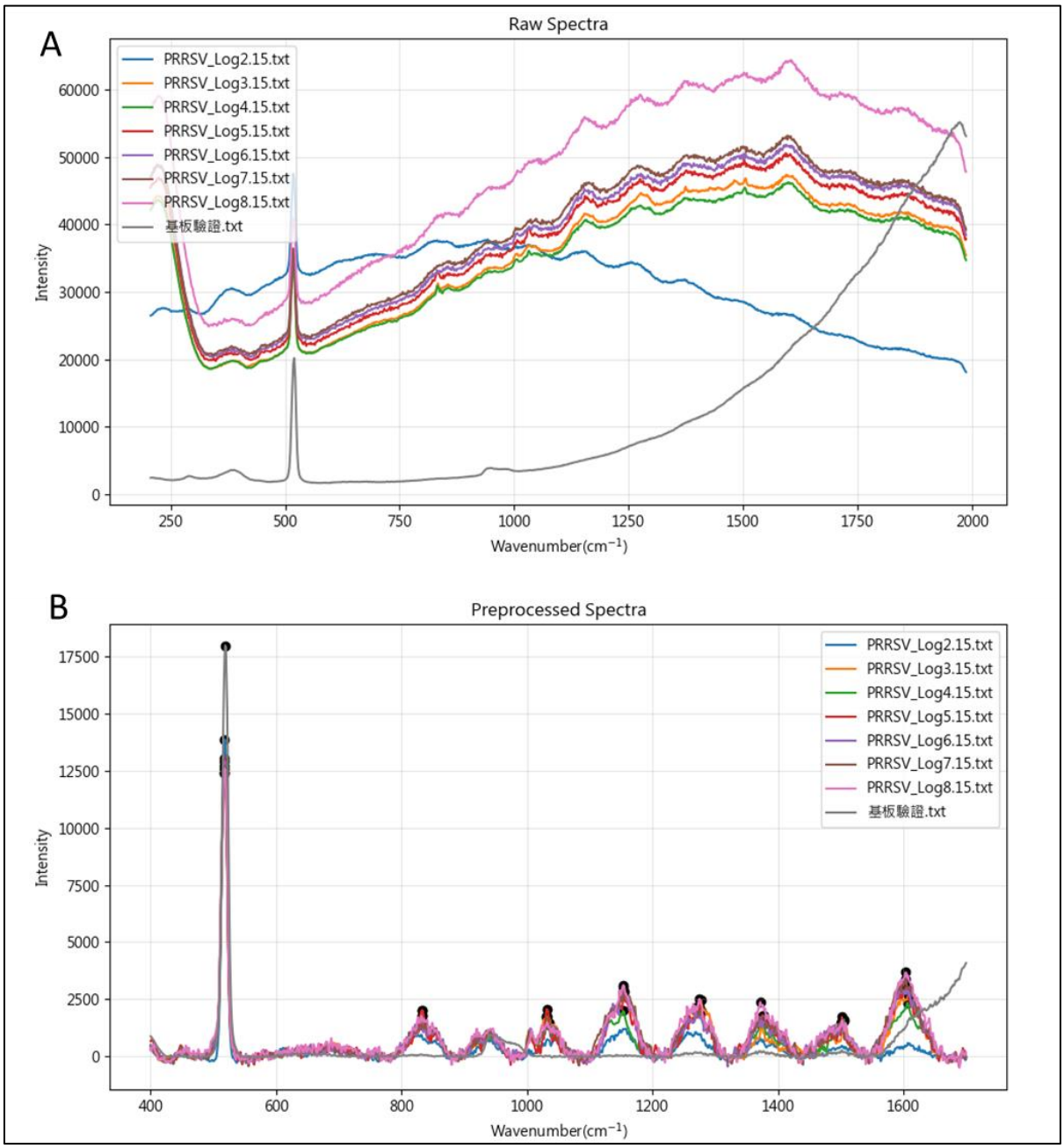

Figure 1: Concentration-dependent SERS detection of PRRSV. (A) Raw spectra of PRRSV preparations ranging from 8.152 to 2.152 $\log_{10}$ copies/mL and the substrate control. (B) Preprocessed spectra. Detectable preparations retained characteristic peaks at approximately 832, 1,032, 1,154, 1,276, 1,374, 1,502, and 1,604 cm^{-1}. The 2.152 $\log_{10}$ copies/mL preparation did not yield a reliable PRRSV pattern; the SERS detection limit was therefore 3.152 $\log_{10}$ copies/mL (approximately 1.42×10^3 copies/mL).

3.3 Consistency of the PRRSV reference spectrum

Repeated PRRSV reference measurements produced closely aligned raw and preprocessed spectral profiles (Figure 2). The principal PRRSV peak positions remained consistent despite differences in overall signal intensity. The narrow peak near 520 cm^{-1} was also observed in the SERS chip control and was treated as the substrate internal standard rather than a PRRSV-specific marker. These findings supported use of the multipeak PRRSV pattern as the reference fingerprint for subsequent specimen comparison.

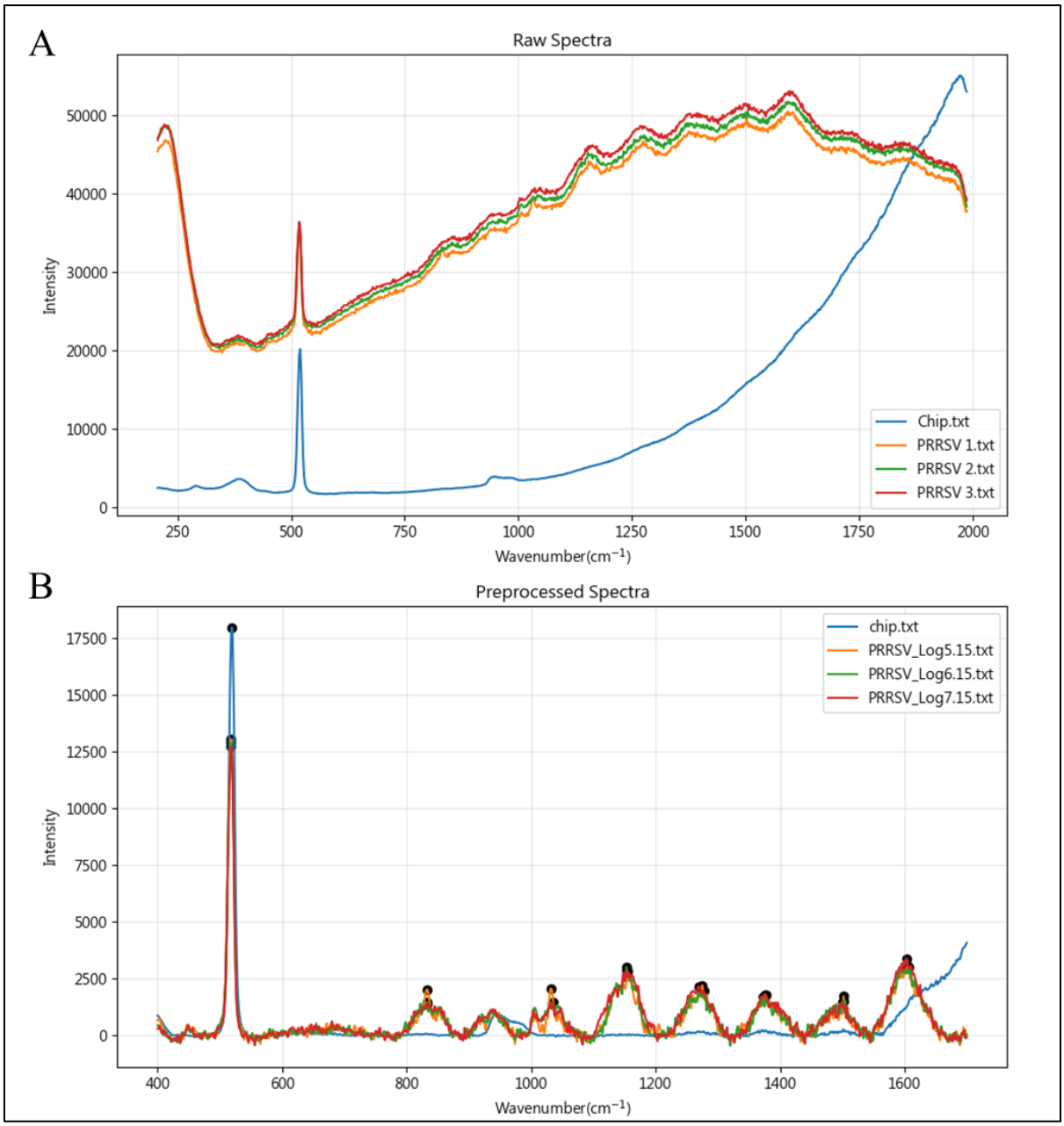

Figure 2: PRRSV reference Raman spectra. (A) Raw spectra from PRRSV measurements and the SERS chip control. (B) Preprocessed spectra. The principal PRRSV peak positions were reproducible, whereas the narrow band near 520 cm^{-1} was also present in the chip control and served as the substrate internal-standard signal.

3.4 SERS classification of PRRSV-positive and PRRSV-negative specimens

Using the 8.152 log_{10} copies/mL PRRSV preparation as the reference, the spectrum of a qPCR-positive blood-derived serum specimen reproduced the seven characteristic peaks at approximately 832, 1,032, 1,154, 1,276, 1,374, 1,502, and 1,604 cm^{-1} and was therefore classified as SERS-positive (Figure 3). Differences in relative intensity were observed, but the overall peak positions and fingerprint were consistent with the PRRSV reference.

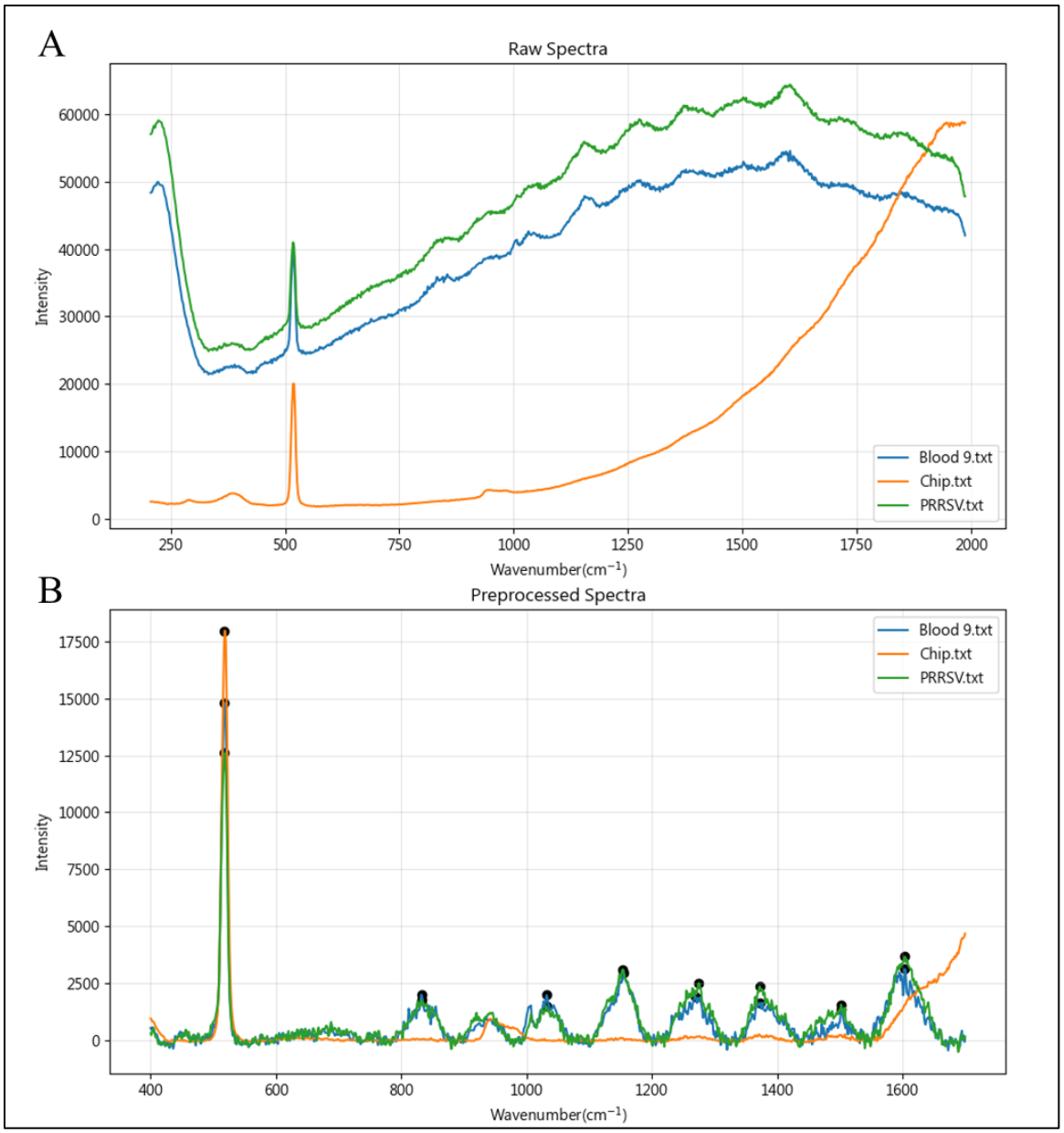

Figure 3: SERS profile of a PRRSV-positive blood-derived serum specimen. (A) Raw spectra of the positive specimen, PRRSV reference, and chip control. (B) Preprocessed spectra. The specimen reproduced the major PRRSV peak positions and was classified as SERS-positive.

In contrast, qPCR-negative blood-derived and fecal specimens did not reproduce the complete PRRSV characteristic pattern when compared with the same reference preparation (Figure 4). Although substrate- and matrix-associated signals were present, the major peak positions and relative-intensity profile did not align with the PRRSV reference, and the specimens were classified as SERS-negative. The same spectral-comparison procedure was applied to the oral-swab specimens included in the field validation set.

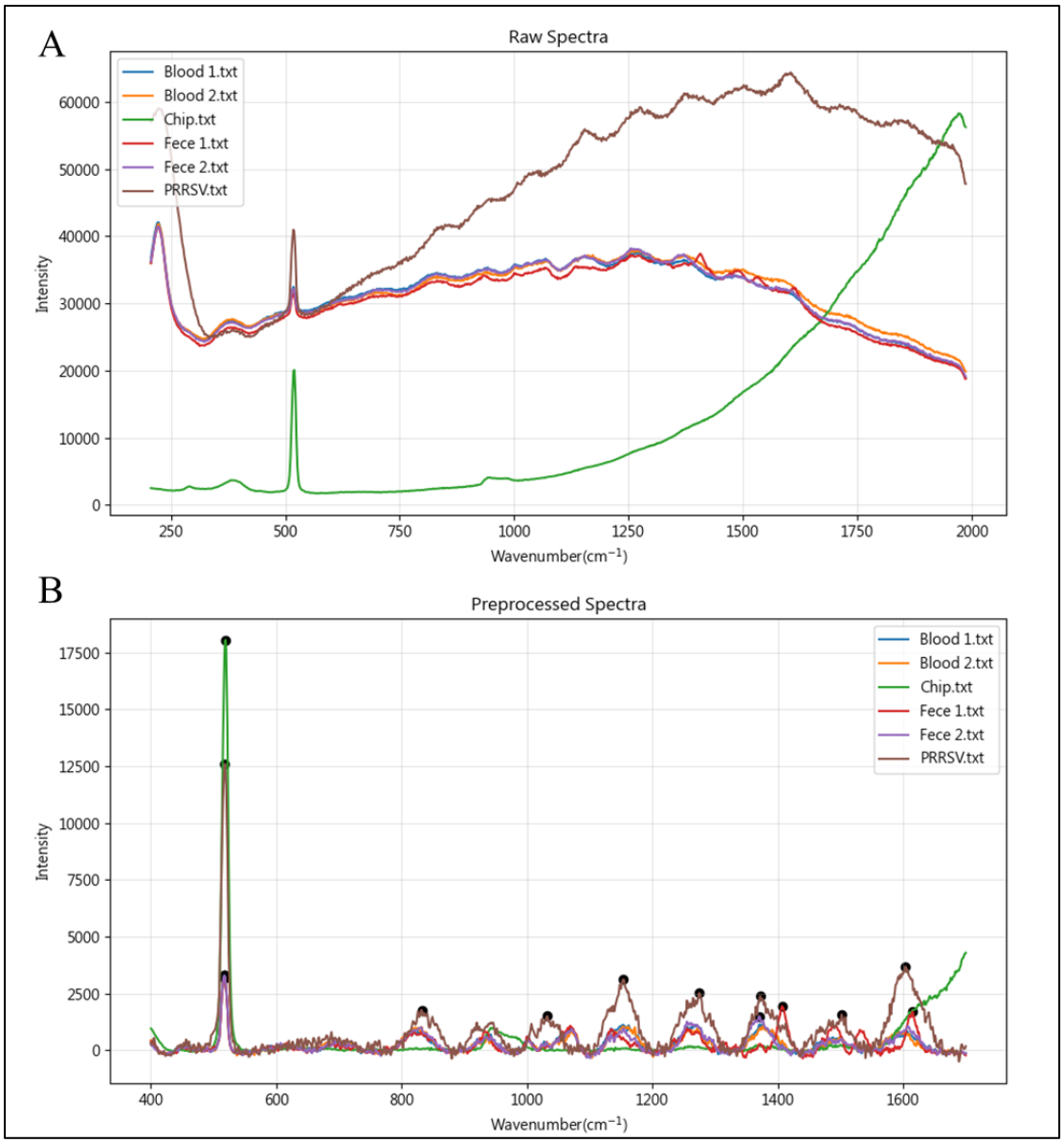

Figure 4: Comparison of the PRRSV reference spectrum with PRRSV-negative blood-derived and fecal specimens. (A) Raw spectra of two negative blood-derived specimens, two negative fecal specimens, the PRRSV reference, and the chip control. (B) Preprocessed spectra. The negative specimens did not reproduce the complete PRRSV reference fingerprint and were classified as SERS-negative.

3.5 Preliminary diagnostic performance in field specimens

Among the 50 field specimens, qPCR identified 10 PRRSV-positive and 40 PRRSV-negative specimens. SERS classified 9 of the 10 qPCR-positive specimens as positive and classified one as negative. The false-negative specimen contained 125.3 copies/mL by qPCR, which was below the established SERS detection limit of approximately 1.42×10^3 copies/mL. All 40 qPCR-negative specimens were also negative by SERS, and no false-positive SERS results were observed. Using qPCR as the reference, the preliminary SERS sensitivity was 90% and the specificity was 100% (Table 3).

Table 3: Cross-classification of 50 swine specimens by qPCR and SERS.

qPCR reference result	SERS positive	SERS negative	Total
Positive	9	1	10
Negative	0	40	40
Total	9	41	50

Sensitivity: 9/10 = 90%; specificity: 40/40 = 100%.

4 DISCUSSION

This study established an integrated PRRSV rapid-detection workflow comprising a qPCR reference procedure, matrix-specific SERS sample preprocessing, a preliminary PRRSV Raman fingerprint database, and cloud-assisted spectral classification. PRRSV generated a reproducible multipeak spectrum characterized by peaks near 832, 1,032, 1,154, 1,276, 1,374, 1,502, and 1,604 cm^{-1}. These peak positions were retained over a broad concentration range and in a qPCR-positive blood-derived specimen, supporting their use as a working PRRSV fingerprint under the present chip, instrument, and preprocessing conditions [1-7].

The SERS analytical detection limit was approximately 1.42×10^3 copies/mL, whereas the qPCR detection limit was 14.2 copies/mL. The approximately 100-fold difference in analytical sensitivity explains the single discordant field result: the qPCR-positive/SERS-negative specimen contained 125.3 copies/mL, a concentration below the SERS threshold. Consequently, a negative SERS result cannot exclude a low-level PRRSV infection. SERS is therefore best positioned as a rapid screening method, with qPCR confirmation recommended for specimens from clinically suspected animals or epidemiologically high-risk herds [8-15].

The field-specimen comparison yielded preliminary sensitivity and specificity estimates of 90% and 100%, respectively. The absence of false-positive results suggests that full-pattern comparison may distinguish the PRRSV fingerprint from background signals in serum, fecal, and oral-swab matrices. Nevertheless, the positive sample size was limited to 10 specimens, and the point estimates should not be interpreted as definitive diagnostic-performance parameters. Larger blinded studies are needed to determine confidence intervals, predictive values, matrix-specific performance, repeatability, reproducibility, and cross-reactivity with other common swine viruses.

The substrate peak at approximately 520 cm^{-1} was useful as an internal quality-control signal but was not specific to PRRSV. Similarly, biological matrices produced multiple Raman bands that may overlap individual viral peaks. Classification should therefore rely on the complete multipeak pattern rather than a single Raman shift. Future development should incorporate standardized baseline correction and normalization, predefined peak-position tolerances, and multivariate or machine-learning classifiers. These approaches may reduce operator-dependent interpretation and improve robustness across chips, instruments, and sample matrices [16-20].

A practical advantage of the SERS workflow is the use of only 2 μL of prepared sample together with a portable instrument and cloud-assisted comparison. The measurement stage is rapid after preprocessing, making the platform potentially useful for near-farm triage and targeted selection of specimens for molecular confirmation. However, the current centrifugation and washing procedures remain laboratory dependent. Simplified extraction cartridges, lower-speed concentration methods, and prospective on-site evaluations will be required before routine farm deployment [21-22].

Several limitations should be considered. The PRRSV strain or genotype, propagation and inactivation conditions, complete qPCR primer/probe and thermal-cycling details, and specimen-level metadata were not available in the supplied dataset. In addition, the sodium chloride wash concentration and the distinction between whole blood and serum terminology should be standardized. These details are required for full methodological reproducibility and should be completed before journal submission [1-4].

5 CONCLUSION

A PRRSV qPCR reference procedure and SERS-based rapid screening workflow were established for serum, fecal, and oral-swab specimens. PRRSV produced a reproducible seven-peak Raman fingerprint, and the SERS analytical detection limit was 3.152 $\log_{10}$ copies/mL (approximately 1.42×10^3 copies/mL). In 50 field specimens, SERS identified 9 of 10 qPCR-positive specimens and all 40 qPCR-negative specimens, corresponding to preliminary sensitivity of 90% and specificity of 100%. The only false-negative specimen had a viral concentration below the SERS detection limit. These findings support SERS as a rapid and portable PRRSV screening method, while qPCR remains necessary for sensitive confirmation, particularly in specimens with low viral loads.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC-113085 approved by the IACUC ethics committee.

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