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PP-036 [Bee Health]

Field-deployable molecular diagnosis of Sacbrood virus using a rapid point-of-care assay

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Introduction

- Sacbrood virus (SBV) is a significant pathogen affecting honeybee colonies, leading to larval mortality, colony weakening, and substantial economic losses in apiculture. In South Korea, SBV has caused severe outbreaks, particularly in *Apis cerana*, and remains a risk for *Apis mellifera*. While laboratory-based RT-qPCR offers high diagnostic accuracy, its reliance on centralized facilities causes delays due to sample transportation and long processing times, limiting its utility for timely disease control.
- To overcome these constraints, we developed the XQ SBV Dx Kit, a portable molecular assay for point-of-care (POC) SBV detection. This compact system integrates sample preparation, amplification, and real-time detection, enabling results within approximately 90 minutes significantly faster than conventional laboratory workflows. The primary aim was to evaluate the diagnostic accuracy, sensitivity, specificity, and field applicability of this assay, with a particular focus on its ability to detect low viral loads for early intervention and mitigation of SBV spread.

Materials & Methods

- **System Components**
  - ➢ XQ Station (Integrated nucleic acid extraction, amplification, and detection)
  - ➢ XQ SBV Dx Kit (Dedicated diagnostic kit for SBV)

- **Workflow**
  - ➢ Sample homogenization
  - ➢ Load 50 uL sample + 10 uL magnetic bead
  - ➢ Insert Kit into XQ Station
  - ➢ Total time : 90 min (45 cycle) / Positive Ct cut off : < 35
- **Comparison**: Laboratory RT-qPCR vs POC RT-qPCR using identical sample sets.
- **Performance metrics**: Diagnostic sensitivity, specificity, agreement rate, and limit of detection (LOD).

Results

SBV Titers (copies/reaction)	RT-qPCR test (Laboratory)		POC RT-qPCR test (XQ SBV Dx Kit)	
	% positive (out of 24)	Mean Ct value	% positive (out of 24)	Mean Ct value
10^0	0%	-	0%	-
10^1	58.3% (14/24)	37.71	83.3% (20/24)	34.11
10^2	95.8% (23/24)	34.55	95.8% (23/24)	30.82
10^3	100% (24/24)	30.85	100% (24/24)	26.62
10^4	100% (24/24)	27.57	100% (24/24)	23.11

Table 1. Comparison of the limit of detection (LOD) between laboratory RT-qPCR and point-of-care (POC) RT-qPCR for SBV. Each concentration was tested in 24 replicates. Both assays achieved an LOD of 10^2 copies per reaction, with 95.8% detection.

Pathogen	Ct value	PCR Protocol	RT-qPCR test (Laboratory)		POC RT-qPCR test (XQ SBV Dx Kit)	
			% positive (out of 3)	Interpretation	% positive (out of 3)	Interpretation
A. apis (chalkbrood)	28.45	qPCR	0 (0/3)	Not Detected	0 (0/3)	Not Detected
A. flavus (stonebrood)	23.54	qPCR	0 (0/3)	Not Detected	0 (0/3)	Not Detected
M. plutonius (EFB)	23.99	qPCR	0 (0/3)	Not Detected	0 (0/3)	Not Detected
P. larvae (AFB)	24.60	qPCR	0 (0/3)	Not Detected	0 (0/3)	Not Detected
V. ceranae (nosemosis)	24.35	qPCR	0 (0/3)	Not Detected	0 (0/3)	Not Detected
ABPV	23.95	RT-qPCR	0 (0/3)	Not Detected	0 (0/3)	Not Detected
BQCV	23.86	RT-qPCR	0 (0/3)	Not Detected	0 (0/3)	Not Detected
CBPV	24.00	RT-qPCR	0 (0/3)	Not Detected	0 (0/3)	Not Detected
DWV	25.75	RT-qPCR	0 (0/3)	Not Detected	0 (0/3)	Not Detected
IAPV	25.17	RT-qPCR	0 (0/3)	Not Detected	0 (0/3)	Not Detected
KBV	24.03	RT-qPCR	0 (0/3)	Not Detected	0 (0/3)	Not Detected

Table 2. Analytical specificity of the POC RT-qPCR assay. No cross-reactivity was observed with 11 major honeybee pathogens (6 viruses, 2 bacteria, and 3 fungi). All non-SBV pathogens tested negative, confirming high analytical specificity.

		POC RT-qPCR (XQ SBV DX Kit)	
		Detected	Not Detected
RT-qPCR (Laboratory)	Detected	63	1
	Not Detected	3	97
	Total	66	98
Positive percent agreement (PPA)		95.5%	
Negative percent agreement (NPA)		99.0%	
Overall agreement (OA)		97.6%	

Table 3. Clinical evaluation of the POC RT-qPCR assay. A total of 164 field samples (64 SBV-positive, 100 SBV-negative) were tested against laboratory RT-qPCR. The assay showed high clinical performance (Sensitivity 98.4%, Specificity 97.0%, PPA 95.5%, NPA 99.0%, OA 97.6%), confirming its reliability for field application.

Conclusion

- **The XQ SBV Dx Kit offers rapid, accurate, and field-deployable SBV detection.**
- **Detects low viral loads, enabling early intervention before clinical symptoms spread.**
- **Reduces dependency on centralized laboratories, improving response time and supporting sustainable beekeeping.**

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Sacbrood virus (SBV) is a significant pathogen affecting honeybee colonies, leading to larval mortality and economic losses in apiculture. While laboratory-based molecular diagnostic techniques provide accurate detection, their application in field settings is hindered by logistical challenges, including sample transportation delays and long processing times. These limitations highlight the need for a rapid, field-deployable diagnostic tool that enables on-site virus detection and immediate intervention. To address this, we developed the Q-POC SBV Kit, a portable molecular assay designed for point-of-care (POC) SBV detection. Unlike conventional RT-qPCR methods requiring centralized laboratory testing, the Q-POC SBV Kit delivers results within hours rather than days, eliminating the time lag caused by sample transportation. The system integrates sample processing, amplification, and real-time detection into a single compact platform, making it highly accessible for use directly in apiaries. Performance evaluations confirmed the high diagnostic accuracy, sensitivity, and specificity of the Q-POC SBV Kit, comparable to laboratory-based RT-qPCR. Its ability to detect low viral loads makes it particularly effective for early disease identification, supporting proactive management strategies to mitigate SBV spread. The system's efficiency, portability, and ease of use allow beekeepers and field researchers to conduct rapid diagnostics without requiring specialized technical expertise. By reducing reliance on centralized laboratory testing and enabling real-time on-site diagnosis, the Q-POC SBV Kit represents a major advancement in honeybee health monitoring. Its rapid detection capabilities empower beekeepers to implement timely control measures, ultimately improving colony health and enhancing the sustainability of apicultural practices.