Verification of Commercial AGID Kits for Equine Infectious Anemia Antibody Detection

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Background

The Agar gel immunodiffusion (AGID) test is the gold standard for the serological detection of Equine Infectious Anemia (EIA). However, the discontinuation of the previous kit requires a switch to new commercial alternatives. These alternatives must be verified to ensure that diagnostic standards are maintained for consistent and accurate disease surveillance.

Objectives

This study aimed to verify and compare the diagnostic performance of two commercial AGID test kits to determine their suitability for routine laboratory diagnosis.

Methods

The required sample size was determined in accordance with the WOAH Terrestrial Manual, Chapter 1.1.6 (Principles and methods of validation of diagnostic assays for infectious diseases). Based on the anticipated diagnostic performance of the two kits, a diagnostic sensitivity (DSe) of 97% and a diagnostic specificity (DSp) of 99%, at a 99% confidence level and 5% allowable error, the corresponding sampling table specified a minimum of 77 known positive and 26 known negative samples (103 in total). Accordingly, the verification was performed using a panel of 118 sera, comprising 81 known positive samples (including international reference materials from the National Veterinary Services Laboratories [NVSL], USA) and 37 known negative samples, thereby exceeding the minimum requirement.

Methods (cont.)

DSe and DSp for each kit were derived from a 2 × 2 contingency table against the known serum status, and inter-kit agreement was quantified using Cohen's kappa statistic, interpreted according to Landis and Koch (1977). Analytical specificity (ASp) was assessed for cross-reactivity against reference sera containing antibodies to African Horse Sickness (AHS), Equine Influenza (EI), Foot-and-Mouth Disease (FMD), Avian Influenza (H5, H7, H9), Newcastle Disease (ND), and Rabies. Repeatability was determined from 20 replicates of a known positive serum tested at undiluted, 1:4, and 1:8 dilutions. The predefined acceptance criteria were DSe ≥ 90%, DSp = 100%, a fully negative ASp, and kappa > 0.80.

Verified test: Agar gel immunodiffusion		Known positive	Known negative
	Positive	True positive (TP)	False positive (FP)
	Negative	False negative (FN)	True negative (TN)
	Total	TP+FN	FP+TN

Test kit	TP	FN	FP	TN	DSe (%)	DSp (%)
A	75	6	0	37	92.59	100
B	78	3	0	37	96.30	100

Results

The results indicated that kit A achieved a DSe of 92.59%, while kit B demonstrated a higher DSe of 96.30%. Both kits exhibited a DSp of 100% and an ASp of 100%, showing no cross-reactivity with the other tested pathogens. Additionally, repeatability testing for both commercial kits showed 100% agreement across all replicates and sample concentrations.

Conclusion

The verification study confirmed that both commercial AGID test kits exceed the established performance criteria (DSe ≥ 90% and DSp = 100%). These findings support the implementation of these kits as reliable replacements for discontinued diagnostic tools, ensuring the continued accuracy and continuity of EIA surveillance programs.

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