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Protocols

Evaluation of dried blood spot testing for serological monitoring of epizootic and zoonotic pathogens in domestic pigs

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ABSTRACT

Dried blood spots (DBS) constitute a stable, cost-efficient sampling matrix that can be collected in a minimally invasive manner. Although widely adopted in human medicine, their use in veterinary diagnostics remains limited. This study aimed to establish and validate DBS elution protocols for use in commercial ELISAs to detect antibodies against Hepatitis E virus (HEV), African swine fever virus (ASFV) and Aujeszky's disease virus (ADV) in domestic pigs. DBS were prepared from EDTA blood, dried serum spots (DSS) from serum. Additional DBS samples were prepared after spiking blood from healthy pigs with antibodies. Various parameters were evaluated to establish the final elution protocols, i.e. number of disks, type and volume of elution buffer, incubation time of the disks in elution buffer, and volume of eluate used for detection. Once the final protocols were in place, for each pathogen 52 DBS were tested in three independent runs. The diagnostic performance was evaluated by comparing the ELISA results of DBS eluates with the corresponding serum or plasma samples. For HEV, only one out of 52 DBS samples qualitatively did not match the plasma result in any of the three runs. For ASFV, all 52 DBS samples matched the qualitative results of the corresponding liquid samples. For ADV, two samples yielded false negative results in all three runs. The results suggest that DBS represent a practical and reliable alternative to liquid blood samples for antibody detection in pigs. Further validation with field samples and large-scale testing is needed.

1. Introduction

Dried blood spots (DBS) have been used as sample material since the early 20th century and are widely recognized in human medicine (Guthrie and Susi, 1963; Grüner et al., 2015). Initially used for neonatal screening of metabolic disorders, their application has since expanded to a broad range of fields, including infectious and metabolic disease diagnostics, therapeutic drug monitoring, toxicology, anti-doping control and epidemiological studies (Lim, 2018; Luginbühl and Gaugler, 2020; Samsonova et al., 2022). The range of analyses in which DBS have been successfully employed include PCR, Western blotting, hemagglutination assay, ELISA, and other types of immunoassays (Demirev, 2013; Gaugler et al., 2021; Virtudazo et al., 2024). DBS offer several advantages over liquid blood samples: (1) Sampling is minimally invasive, only requiring a short puncture of a superficial vein with a lancet. (2) A sample volume of just 10 – 30 μL is sufficient to prepare one spot. (3) DBS are classified as non-infectious, enabling their transport and handling under less stringent biosafety conditions (World Health Organization, 2024). (4) The high stability of DBS eliminates the need for a cold chain. (5) The compact format of the DBS cards allows for space-saving storage. (6) DBS samples can be prepared directly on site, even by non-specialist personnel (McDade et al., 2007; Santos et al., 2018; Samsonova et al., 2022). These features make DBS a promising tool also for veterinary medicine, yet they are not as commonly used as in human medicine (Saushkin et al., 2016; Wood et al., 2021; Lechmann et al., 2022; Samsonova et al., 2022).

Several studies have shown that blood collection in pigs is associated with a high level of stress, in some cases even with mortality, particularly when performed via the cranial vena cava or the jugular vein, as it usually requires restraint of the animal (Stephens and Rader, 1983; Fiderer et al., 2024; Papatsiros et al., 2024). The use of a maxillary sling is a common tool for restraining domestic pigs. It often results in strong vocalizations, resistance, and visible distress in the animal (Fiderer et al., 2024). Blood collection from the vena cava under restraint with a

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Table 1
Overview of materials used in the present study. ADV = Aujeszky's disease virus; ANSES = Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail; ASFV = African swine fever virus; DBS = dried blood spot; DSS = dried serum spot; HEV = Hepatitis E virus.

Virus	Sample Material	Origin	Number	Serostatus	Use
HEV	EDTA blood	Slaughterhouse Zurich	33	Positive	Unspiked DBS
	EDTA blood	Slaughterhouse Zurich	24	Negative	Unspiked DBS
	Serum	Institute of Virology, University of Zurich	15	Positive	DSS
	Serum	Institute of Virology, University of Zurich	7	Negative	DSS
	Serum	Institute of Virology, University of Zurich	2	Doubtful	DSS
ASFV	EDTA blood	Slaughterhouse Zurich	42	Negative	Unspiked DBS
	EDTA blood	ANSES, Ploufragan, France	5	Positive	Unspiked DBS
	Serum	Institute of Virology & Immunology, Mittelhäusern	10	Positive	Spiked DBS
ADV	EDTA blood	Slaughterhouse Zurich	32	Negative	Unspiked DBS
	Serum	ANSES, Ploufragan, France	25	Positive	Spiked DBS

Table 2
Overview of the different conditions used for manual DBS and DSS elution for HEV, ASFV, and ADV.

Virus	Number of disks	Elution buffer	Volume of elution buffer (µL)	Volume of eluate per well (µL)	Incubation time
HEV	5 DSS	Buffer B	500	50	18 h
	5 DSS	Buffer B	500	100	18 h
	5 DSS	Buffer B	500	50	1 h
	2 DBS	Buffer A	200	50	1 h
	2 DBS	Buffer B	200	50	1 h
	2 DBS	Buffer A	150	50	1 h
	3 DBS	Buffer A	200	50	1 h
	3 DBS	Buffer A	180	50	1 h
ASFV	2 DBS	Buffer B	200	50	18 h
	2 DBS	Buffer B	200	50	1 h
	2 DBS	Buffer A	200	50	1 h
ADV	1 DBS	Buffer A	150	50	1 h
	2 DBS	Buffer A	200	50	1 h

maxillary sling has been associated with acute increases in stress markers like cortisol and adrenocorticotropic hormone (ACTH), and prolonged stress responses compared to less invasive procedures like ear tagging (Merlot et al., 2011). In contrast, blood sampling from superficial ear or tail veins were shown to have no impact on plasma glucose, lactate, cortisol, or ACTH levels, and therefore could prove a viable and humane method for blood sampling in pigs (Merlot et al., 2011; Samsonova et al., 2022).

The diseases evaluated in the present study include African swine fever (ASF), Aujeszky's disease (AD), and Hepatitis E. ASF is a highly contagious and lethal viral disease in domestic pigs and Eurasian wild boar caused by *Asfivirus haemorrhagiae* (ICTV, 2025), also referred to as African swine fever virus (ASFV). It is characterized by hemorrhagic fever and causes significant economic losses in the pork industry (Alonso et al., 2018; Li et al., 2022; Velazquez-Salinas, 2025). In Europe the virus has been spreading since the early 2000s. Although some countries remain free of ASF, wild boar populations in neighboring affected regions pose a risk for disease introduction (Galli et al., 2022; Savioli et al., 2022; Vargas-Amado et al., 2022). Early detection of ASF is crucial to minimizing its impact, making effective surveillance and diagnostics essential for rapid response and disease control.

AD is a neurological, respiratory, and reproductive disorder caused by the herpesvirus *Varicellovirus suidalpha 1* (ICTV, 2025), also referred to as Aujeszky's disease virus (ADV) (Roizmann et al., 1992; Pomeranz et al., 2005; Mettenleiter, 2020). Several European countries have successfully eradicated AD from domestic pig populations. National surveillance programs are widely implemented to maintain the disease-free status.

Hepatitis E is an emerging zoonotic disease caused by *Paslahepevirus balayani* (ICTV, 2025), also known as Hepatitis E virus (HEV), which can cause both acute and chronic hepatitis in humans (Syed et al., 2018). HEV is primarily transmitted through contaminated water or undercooked meat from infected animals, with pigs being the main reservoir (Meng et al., 1997; Hoofnagle et al., 2012; Aslan and Balaban, 2020). Seroprevalence studies from several European countries demonstrate that HEV is widespread in domestic pig populations, with reported antibody prevalence rates ranging from 20 % to over 90 % (Burri et al., 2014; Salines et al., 2017; Lienhard et al., 2021).

The aim of this study was to establish and validate protocols for DBS elution to be used in commercial ELISAs for the detection of antibodies against these epizootic and zoonotic disease agents in domestic pigs. DBS were prepared from untreated as well as antibody-spiked EDTA blood samples from domestic pigs. Different elution conditions were evaluated which varied in number of eluted disks, type and volume of elution buffer, incubation time of the disks in the buffer, and volume of eluate used for detection. The eluates were tested in commercial ELISA formats according to the manufacturers' instructions. The performance of ELISA testing using DBS eluates was evaluated by comparing the results to those obtained from plasma or serum samples.

2. Materials and methods

2.1. Ethics statement

Ethical approval was not sought as the present study relied solely on stored samples originally collected for diagnostic purposes, or on freshly obtained samples collected by official veterinarians at the Zurich slaughterhouse during routine slaughter procedures.

2.2. Sample collection

For HEV, EDTA whole blood samples (EDTA K3E, 10 mL,Sarstedt AG, Nümbrecht, Germany) were collected from clinically healthy pigs during exsanguination at the Slaughterhouse Zurich. They were used to prepare unspiked DBS. Stored porcine serum samples (Institute of Virology Zurich) (Table 1) were used to prepare unspiked dried serum spots (DSS). For ASFV and ADV, seronegative EDTA whole blood samples from the Slaughterhouse Zurich were used directly for preparation of unspiked DBS, while after centrifugation the corresponding blood cell fractions were used for preparation of spiked DBS. For ASFV, additional seropositive EDTA whole blood samples obtained from the Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES, Ploufragan-Plouzané-Niort, France) were used for preparation of unspiked DBS, and seropositive serum samples from the Institute of Virology and Immunology (IVI, Mittelhäusern, Switzerland) for spiked DBS. For ADV, seropositive serum samples from the Institute of Virology and Immunology (IVI, Mittelhäusern, Switzerland) were used for spiked DBS (Table 1).

2.2.1. Preparation of DBS/DSS

For preparation of DBS/DSS, 20 µL of EDTA blood or serum were pipetted onto each of the four marked circles on a TFN filter paper card (AutoCollec format (85 × 53 mm), STERA Scientific AG, Switzerland). From each EDTA blood and serum sample, several DBS and DSS cards were prepared. The spotted cards were placed in racks to dry for at least four hours. Once dried, the DBS and DSS cards were stored in plastic

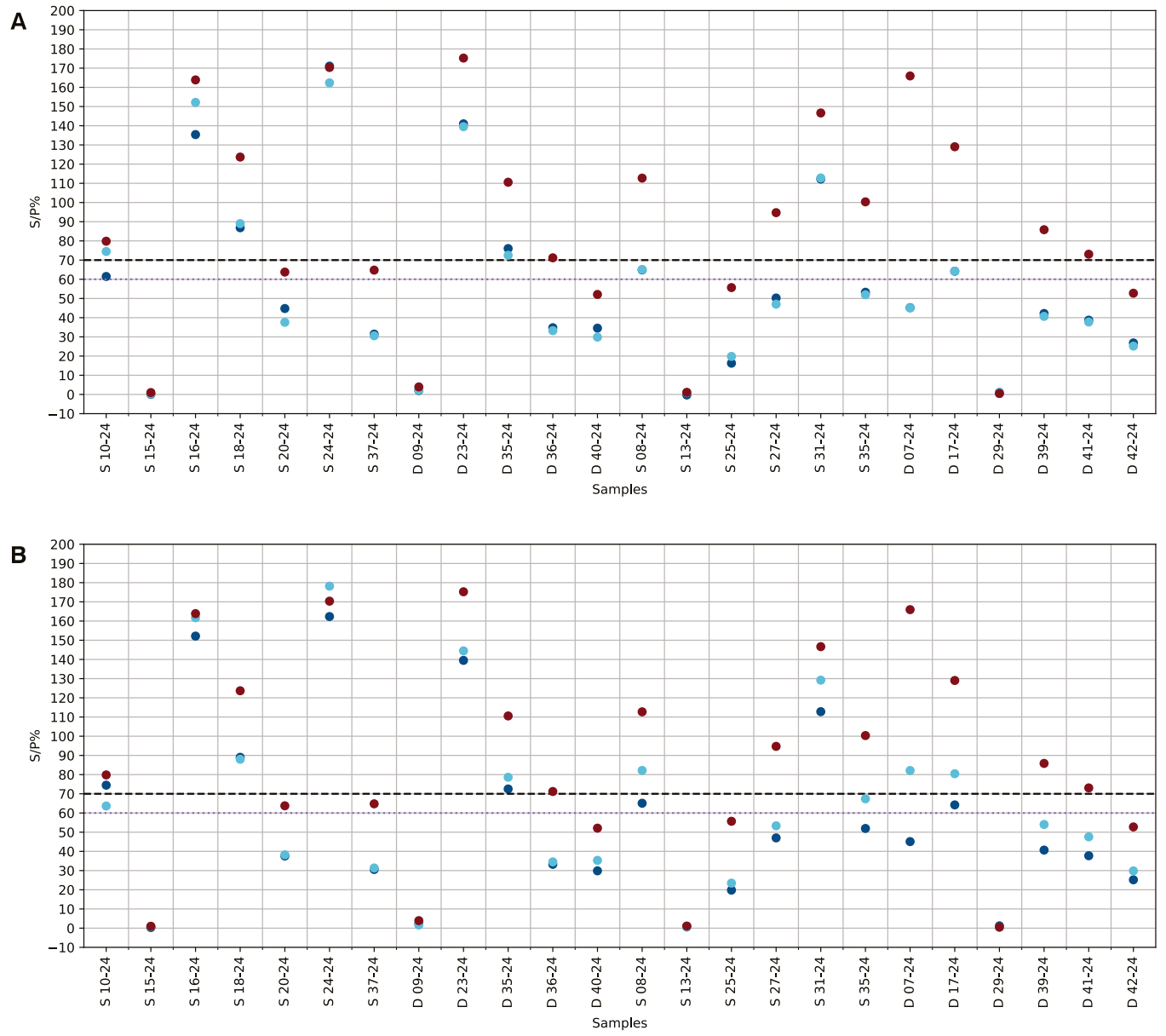

Fig. 1. HEV ELISA results of 24 serum (red dots) and DSS samples. DSS eluates were tested using two different volumes of eluate per ELISA well (A): 100 µL (dark blue dots) and 50 µL (light blue dots). For both conditions, five disks were eluted in 500 µL of Buffer B and incubated for one hour. DSS eluates were further tested using two different incubation times (B): 1 h (dark blue dots) and 18 h (light blue dots). Both conditions used five disks and 500 µL of Buffer B and 50 µL DBS eluate per ELISA well. The positive cutoff is indicated by a dashed black line, the negative cutoff by a dotted purple line. Samples with S/P% values between 60 % and 70 % are considered doubtful.

bags (Zipper-top poly bag, size 3 in. x 5 in., Minigrip, Thailand) containing a desiccant (R5 Pro, Indicating Silica Gel, RS Components SAS, Beauvais Cedex, France) and kept at −20°C until further use.

Following DBS preparation, the remaining EDTA blood was centrifuged at 868 × g for 10 min (Heraeus Megafuge 40 R, Thermo Fisher Scientific). The plasma was decanted into 3 mL tubes, stored short-term (approximately two weeks) at 6°C until the ELISA was completed, and subsequently frozen at –20°C.

2.2.2. *Preparation of antibody-spiked DBS samples*

For pathogens for which no (ADV) or only few seropositive field samples (ASFV) were available, antibody-spiked DBS were prepared. For this purpose, EDTA blood samples collected from healthy pigs at the slaughterhouse were utilized (Table 1.). Switzerland is considered officially free of both pathogens, suggesting that the likelihood of the slaughterhouse samples being seronegative was very high. After plasma separation by centrifugation, the cellular fraction of the blood was spiked with antibody-positive sera (Table 1.). The sera used originated from pigs that were naturally infected, vaccinated, or experimentally infected with the respective pathogens. To approximate a pig's physiological hematocrit of about 40 %, one part of blood cells was mixed with 1.5 parts of serum. DBS were then prepared as described above.

2.3. *DBS and DSS elution*

DBS and DSS were punched out on a cutting mat using a 6 mm diameter puncher (UniCore Punch Kit, 6.0 mm, Qiagen, Hilden, Germany). Based on measurements from approximately 100 DBS and DSS samples, the mean spot diameters were 7.7 mm and 10.5 mm, corresponding to approximately 12 µL whole blood and 6.5 µL plasma per punched Ø6 mm disk, respectively. The puncher was positioned vertically in the center of the blood spot, pressed down, and rotated around

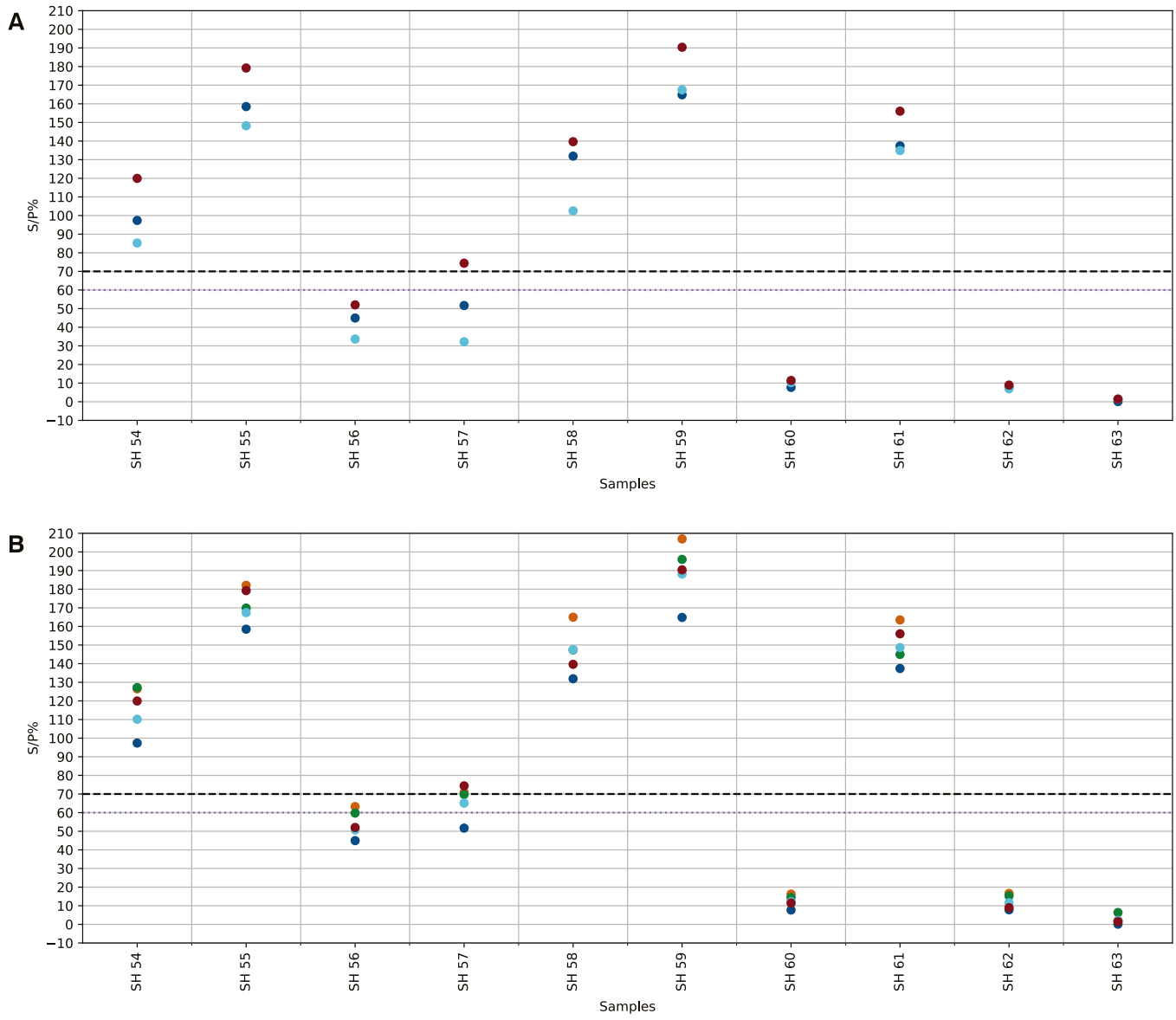

Fig. 2. HEV ELISA results of ten serum (red dots) and DBS samples. DBS eluates were tested using two different elution buffers (A): Buffer A (dark blue dots) and Buffer B (light blue dots). For both conditions two disks and 200 µL of elution buffer were incubated for one hour and 50 µL DBS eluate used per ELISA well. DBS eluates were further tested using four different conditions (B) (Table 2): Two disks eluted in 200 µL (dark blue dots), two disks eluted in 150 µL (light blue dots), three disks eluted in 200 µL (green dots), and three disks eluted in 180 µL buffer (orange dots). All conditions used Buffer A, a one-hour incubation and used 50 µL DBS eluate per ELISA well. The positive cutoff is indicated by a dashed black line, and the negative cutoff by a dotted purple line. Samples with S/P% values between 60 % and 70 % are considered doubtful.

its length-axis in both directions with small movements. The disks were then transferred into 1.5 mL safe-lock tubes. To prevent carryover, the puncher and cutting mat were wiped with PBS after processing each card. After adding elution buffer to the disks, the tubes were incubated on a thermoshaker (Eppendorf ThermoMixer C, Eppendorf, Switzerland) at 21°C and 800 rpm.

Two proprietary buffers, A and B (STERA Scientific AG, Basel, Switzerland), were used for elution. For each pathogen different elution conditions were tested (Table 2). The conditions varied in number of eluted disks, type and volume of elution buffer, incubation time of the disks in the buffer, and volume of eluate added to the wells of the ELISA plate.

2.3.1. *Hepatitis E virus*

HEV was the first pathogen used to establish a preliminary elution protocol, with both DBS and DSS tested. In the initial approach, DSS elution for HEV ELISA (ID Screen Hepatitis E Indirect Multi-species, Innovative Diagnostics, Grabels, France) was evaluated using five disks in 500 µL Buffer B (as an approximation to the 1:20 serum dilution used in the ELISA), two different incubation times (1 h and 18 h) and volumes of eluate used in the ELISA (50 µL, 100 µL) (Table 2). As the different conditions showed no substantial differences, the most practical ones, i.e. one hour incubation and 50 µL eluate per well, were selected for further trials using DBS. These were tested with elution Buffers A and B, each using 200 µL buffer with two disks. Subsequent experiments were performed with Buffer A using different disk numbers and buffer volumes (Table 2).

2.3.2. *African Swine Fever virus*

For ASFV, different conditions (Table 2) were tested using the ID Screen African Swine Fever Indirect ELISA (Innovative Diagnostics, Grabels, France). Incubation times of 18 h and one hour were evaluated,

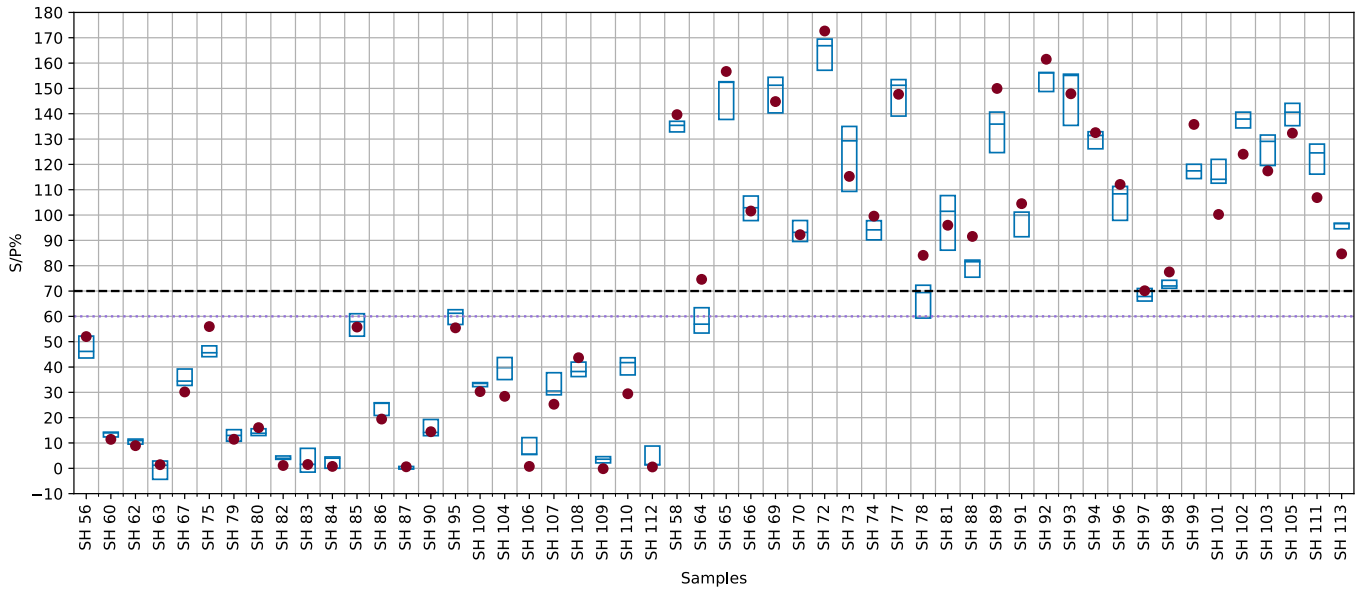

Fig. 3. HEV ELISA results of 52 plasma and DBS samples. Plasma samples (red dots) were tested in single approach within a single run. DBS were tested in three independent runs (boxes) after elution of two disks in 150 µL Buffer A, a one-hour incubation, and 50 µL of DBS eluate added per ELISA well. Each horizontal line of the box represents one ELISA run. The positive cutoff is indicated by a dashed black line, and the negative cutoff by a dotted purple line. Samples with S/P% values between 60 % and 70 % are considered doubtful.

using two disks and 200 µL of Buffer B. Elution was further evaluated with Buffers A and B, each using 200 µL buffer with two disks, one hour incubation and 50 µL eluate for the ELISA (Table 2).

2.3.3. *Aujeszky's disease virus*

For ADV, two conditions were evaluated using the IDEXX PRV/ADV gB Ab Test ELISA (IDEXX Laboratories, Inc., Westbrook, USA) (Table 2).

2.4. *Antibody ELISA*

DBS and DSS eluates, serum and plasma samples were tested in commercial antibody ELISAs. For serum and plasma samples, the assays were carried out according to the manufacturer's instructions. For DBS and DSS, the assay was started by dispensing eluate into the wells of the ELISA plate, which was followed by the first incubation step as per manufacturer's instructions.

For ADV and ASFV, serum, plasma, DBS, and DSS samples were always tested in duplicate. For the HEV ELISA, which is a bi-well test, serum, plasma, DBS, and DSS samples were tested in single approach. Using the final DBS protocol, only DBS samples were tested in three independent runs for each pathogen: each run in duplicate for ADV and ASFV, and in single-well approach for HEV.

The optical density (OD) of the samples was measured using an ELISA reader (Infinite F50, Tecan Group Ltd., Männedorf, Switzerland). The OD values of the samples were used in calculations provided by the manufacturer, where sample ODs were divided by either the negative or the positive control OD. This yielded S/N values for ADV and S/P% values for ASFV and HEV, which were then interpreted based on the respective cutoff values.

3. Results

3.1. *Hepatitis E virus (HEV)*

3.1.1. *Volume of eluate per well*

Twenty-four DSS samples were tested in the ID Screen Hepatitis E Indirect Multi-species ELISA (Innovative Diagnostics) using two different volumes of eluate per well (100 µL vs. 50 µL). For both conditions, five disks were eluted with 500 µL of Buffer B and incubated for one hour. All but one sample (S10–24) yielded the same qualitative result across both volumes (Fig. 1). Thirteen DSS results qualitatively matched the corresponding serum using 100 µL, and 14 using 50 µL eluate per well. Since the results did not differ significantly from one another, the 50 µL condition requiring less buffer and fewer sample disks was chosen for subsequent experiments.

3.1.2. *Incubation time*

Next, two incubation times – 1 h and 18 h - were compared using five disks with 500 µL of Buffer B and 50 µL of DSS eluate per ELISA well. Of the 24 DSS samples, 16 qualitatively matched the corresponding serum after 18-hour incubation, and 14 with one-hour incubation (Fig. 1). In over 70 % of the samples, the difference between the two incubation conditions was less than 10 %. Therefore, the one-hour incubation was selected for further optimization of the assay conditions, given its shorter processing time.

3.1.3. *Elution buffer*

DSS were then replaced by DBS because the concordance of the ELISA results from DSS and serum was unsatisfactory, and ultimately the spot should be obtained directly from the animal without having to centrifuge or otherwise treat the blood beforehand. For practical reasons, it was also decided to try the elution with fewer disks and buffer volumes, while keeping the dilution factor within a similar range. Ten DBS samples were tested using two disks and 200 µL of Buffers A and B (STERA Scientific AG). For both buffers, nine out of ten DBS samples qualitatively matched the corresponding plasma. Five tested negative and five positive, with one sample (SH 57) testing false negative with both buffers (Fig. 2). In seven out of ten cases Buffer A yielded lower deviations to the plasma compared to Buffer B. Therefore, the following elutions were done using Buffer A.

3.1.4. *Number of disks and buffer volume*

For further optimization of the DBS elution protocol using Buffer A, a one-hour incubation, and 50 µL of DBS eluate per ELISA well, ten DBS samples were tested with four different conditions, in which two or three disks were eluted with 150 µL, 180 µL or 200 µL of buffer (Table 2). Except for two samples (SH 56 and SH 57) all samples yielded the same qualitative results as the corresponding plasma sample (Fig. 2). Sample

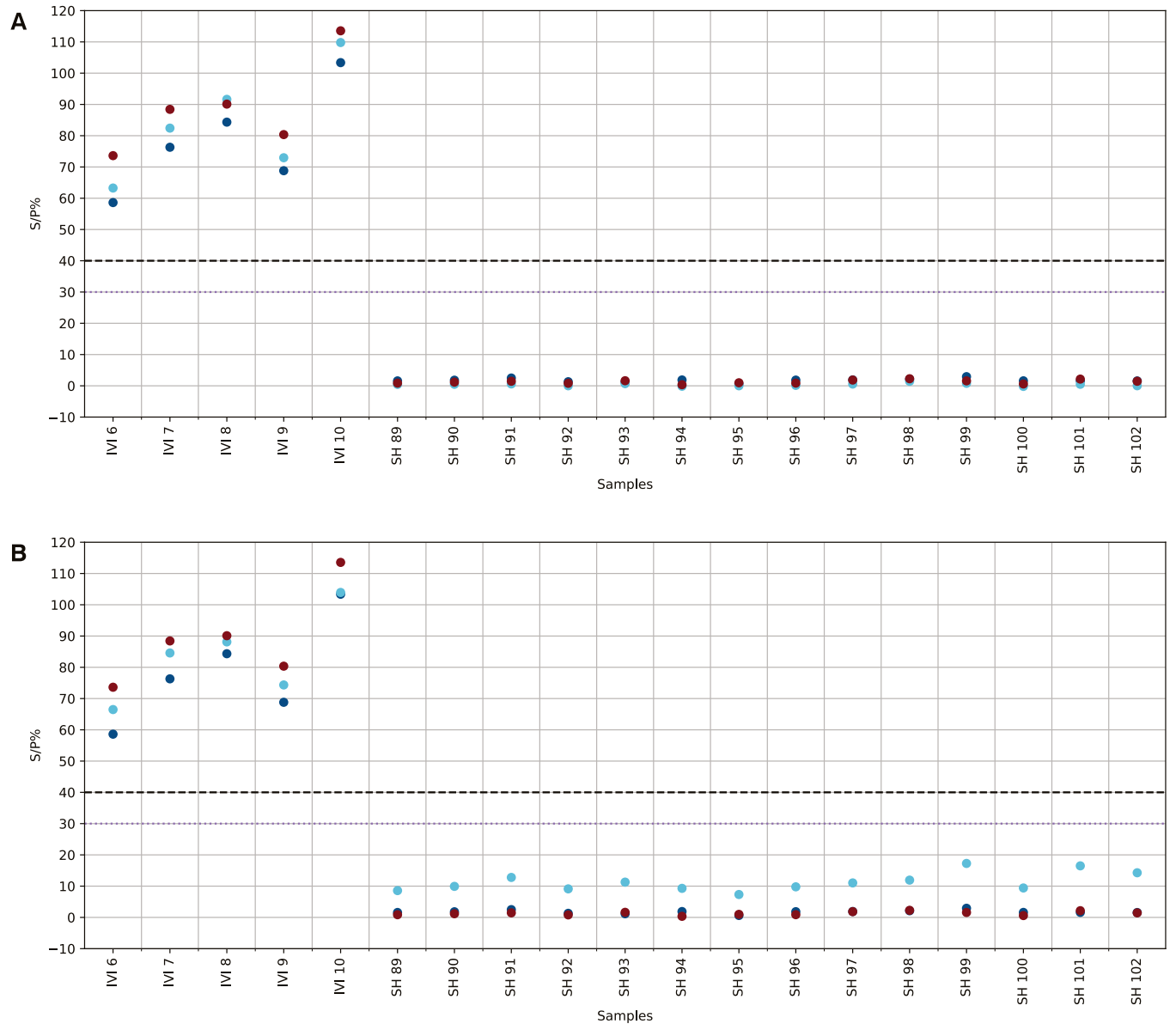

Fig. 4. ASFV ELISA results of 19 serum or plasma (red dots) and DBS samples. DBS eluates were tested using two different incubation times (A): 1 h (dark blue dots) and 18 h (light blue dots). For both conditions two disks were eluted in 200 µL of Buffer B and 50 µL DBS eluate used per ELISA well (Table 2). DBS eluates were further tested using two different elution buffers (B): Buffer A (light blue dots) and Buffer B (dark blue dots). For both conditions two disks and 200 µL of elution buffer were incubated for one-hour and 50 µL DBS eluate used per ELISA well (Table 2). The positive cutoff is indicated by a dashed black line, the negative cutoff by a dotted purple line. Samples with S/P% values between 30 % and 40 % are considered doubtful.

SH 56, which tested negative in the serum, yielded a doubtful result using three disks and 180 µL buffer. In contrast, SH 57, which tested positive in the serum, tested negative using two disks and 200 µL buffer, and doubtful with two disks and 150 µL as well as three disks and 200 µL buffer.

3.1.5. Optimized DBS protocol for three separate ELISA runs

Based on the results described above, the DBS elution protocol with two disks in 150 µL Buffer A, one hour incubation, and 50 µL of DBS eluate per ELISA well was used for testing 52 DBS samples in three independent ELISA runs.

Of 52 DBS samples, 47 qualitatively matched the corresponding plasma in all three runs (Fig. 3). Of the remaining five samples (SH 85, 95, 64, 78, 97), only one (SH 64) did not match the plasma result in any of the runs. The other four samples matched the plasma result in at least one run. The average deviation in S/P% values between DBS and plasma samples across all runs was 7.11 %. The variation between runs, calculated as the mean deviation across the three measurements, was 3.22 %.

3.2. African Swine Fever virus (ASFV)

3.2.1. Incubation time

Nineteen DBS samples (14 unspiked, 5 spiked) were tested in the ID Screen African Swine Fever Indirect ELISA (Innovative Diagnostics) using two disks with 200 µL of Buffer B and 50 µL of DBS eluate per ELISA well. Two incubation times were compared – 1 h and 18 h. All 19 DBS samples qualitatively matched their corresponding serum or plasma with both incubation times (Fig. 4). In over 73 % of the samples, the difference between the two conditions was less than 1.3 %. Therefore, all subsequent elutions were carried out with a one-hour incubation.

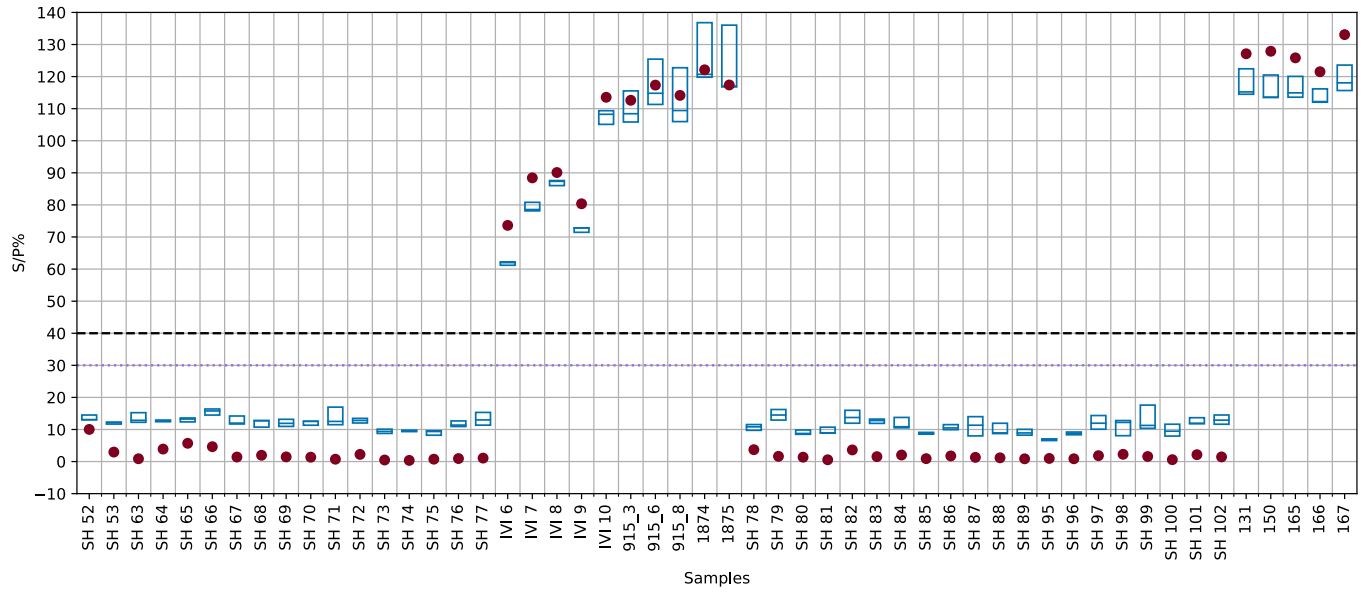

Fig. 5. ASFV ELISA results of 52 plasma or serum and DBS samples. Plasma and serum samples (red dots) were tested in duplicate in a single run. DBS were tested in three independent runs (boxes) after elution of two disks in 200 µL Buffer A for one hour and 50 µL of DBS eluate added per ELISA well. Each horizontal line of the box represents one ELISA run. The positive cutoff is indicated by a dashed black line, and the negative cutoff by a dotted purple line. Samples with S/P% values between 30 % and 40 % are considered doubtful.

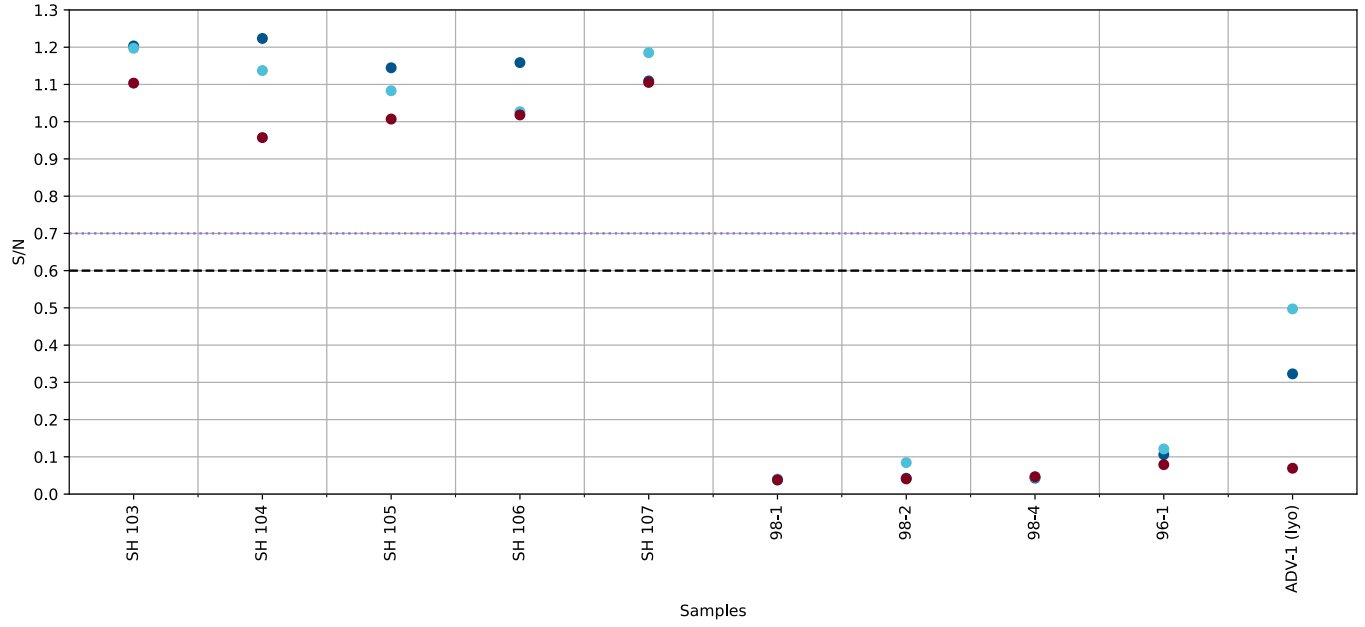

Fig. 6. ADV ELISA results of ten plasma or serum (red dots) and DBS samples. DBS eluates were tested using two different conditions: one disk eluted in 150 µL (dark blue dots) and two disks eluted in 200 µL buffer (light blue dots). Both used Buffer A, a one-hour incubation and 50 µL DBS eluate per ELISA well (Table 2). The positive cutoff is indicated by a dashed black line, and the negative cutoff by a dotted purple line. Samples with S/N values between 0.6 and 0.7 are considered doubtful.

3.2.2. Elution buffer

The same 19 DBS samples were tested using two disks and 200 µL of either Buffer A or Buffer B. For both buffers, all 19 DBS samples qualitatively matched the corresponding serum or plasma (Fig. 4). The deviation of S/P% values compared to the corresponding serum or plasma ranged from 1.98 % to 15.68 % for Buffer A and from 0.04 % to 15.01 % for Buffer B. Buffer A showed lower deviation in positive, Buffer B in seronegative samples. Given its lower deviation in positive samples, Buffer A was selected for the evaluation of the optimized DBS protocol in three separate runs.

3.2.3. Optimized DBS protocol for three separate ELISA runs

52 DBS samples (42 unspiked, 10 spiked) were evaluated in three independent ELISA runs using the DBS elution protocol with two disks in 200 µL Buffer A, one hour incubation, and 50 µL of DBS eluate per ELISA well. All 52 DBS samples qualitatively matched the corresponding serum or plasma: 37 tested negative and 15 positive (Fig. 5). The average deviation in S/P% values between DBS and serum or plasma samples across all runs was 9.15 %. The variation between runs, calculated as the mean deviation across the three measurements, was 1.62 %.

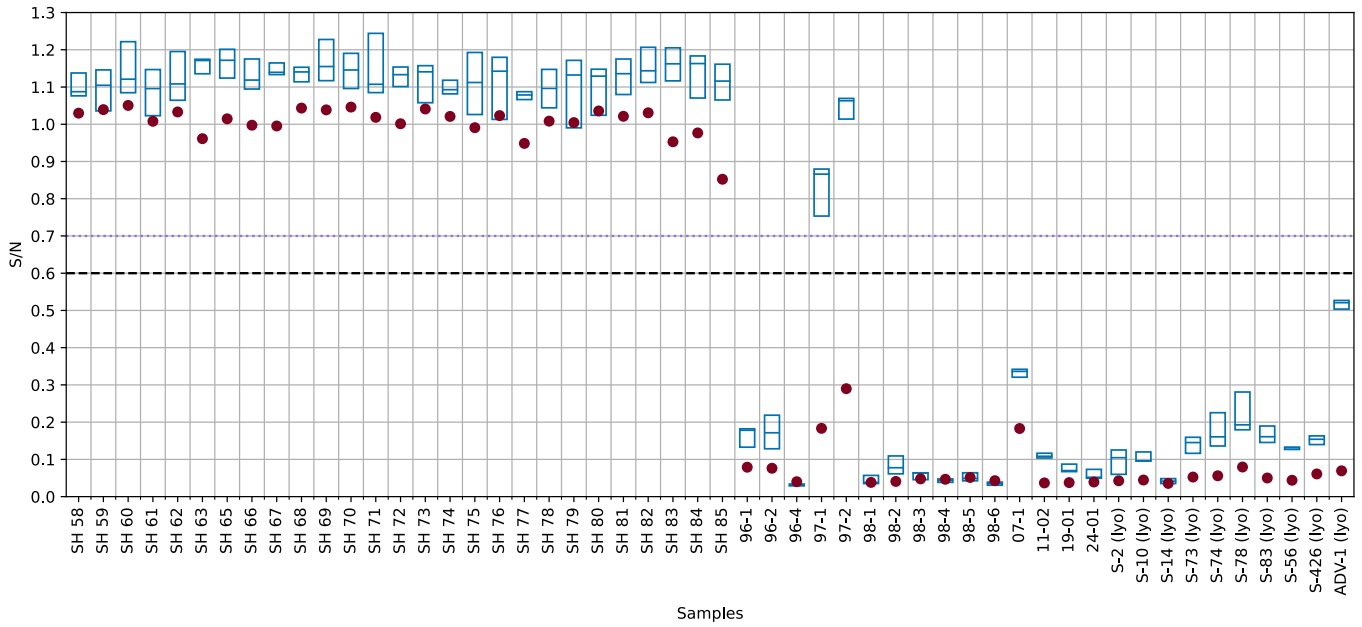

Fig. 7. ADV ELISA results of 52 plasma or serum and DBS samples. Plasma and serum samples (red dots) were tested in duplicate in a single run. DBS were tested in three independent runs (boxes) after elution of one disk in 150 μL Buffer A for one hour and 50 μL of DBS eluate added per ELISA well. Each horizontal line of the box represents one ELISA run. The positive cutoff is indicated by a dashed black line, and the negative cutoff by a dotted purple line. Samples with S/N values between 0.6 and 0.7 are considered doubtful. lyo = lyophilized serum used for spiking.

3.3. Aujeszky's disease virus (ADV)

3.3.1. Number of disks and buffer volume

Ten DBS samples were tested in the PRV/ADV gB Ab Test ELISA (IDEXX) using two different conditions, i.e. one disk eluted in 150 μL and two disks eluted in 200 μL Buffer A. For both conditions samples were incubated for one hour, and 50 μL of DBS eluate were used for the ELISA. All DBS samples yielded the same qualitative results as the corresponding serum or plasma sample (Fig. 6). The difference between the two conditions ranged from 0.0021 to 0.1744.

3.3.2. Optimized DBS protocol for three separate ELISA runs

Fifty-two DBS samples (27 unspiked, 25 spiked) were evaluated in three independent ELISA runs using the DBS elution protocol with one disk in 150 μL Buffer A, one hour incubation, and 50 μL of DBS eluate per ELISA well.

Fifty DBS samples qualitatively matched their corresponding serum or plasma in all three runs, with 29 testing negative and 23 positive (Fig. 7). Two samples yielded false-negative results in all three runs. The average deviation in S/N values between DBS and serum or plasma samples across all runs was 0.1228. The variation between runs, calculated as the mean deviation across the three measurements, was 0.0262.

4. Discussion

The objective of this study was to establish practical protocols for manual elution of DBS from domestic pig blood to be used for antibody testing in commercial ELISAs.

Three pathogens – ASFV, ADV and HEV – were selected for an initial evaluation of DBS testing in domestic pigs: (1) although HEV has only limited clinical relevance for pigs, it was an ideal candidate for evaluating the DBS method, as in contrast to ASFV and ADV access to seropositive and seronegative field samples in Swiss pigs was warranted, eliminating the need for spiking (Burri et al., 2014; Lienhard et al., 2021). (2) Given that in ASFV infected pigs the viral load is highest in blood, spleen and lymph nodes, DBS offer a safe alternative over liquid blood or tissue swab samples for collection, transport, and testing (Penrith et al., 2024). Whereas PCR is the standard method for early detection of acute ASFV infections, indirect methods such as ELISA are used to identify past exposure and provide a rapid and high-throughput option for large-scale screening in areas with endemic infection (Penrith et al., 2024; Zhang et al., 2025). (3) Since seropositivity in herpesviruses is associated with actual virus infection, a large proportion of laboratory diagnostic investigations (abortion investigations, national surveillance programs) is based on serology (Moynagh, 1997; Müller et al., 2011) using antibody ELISA (Müller et al., 2003).

The number of samples examined in the present study, and the conditions evaluated varied from pathogen to pathogen and depended on the sample material available. The use of only 50 μL instead of 100 μL eluate did not appear to have a significant impact on the outcome of the HEV ELISA (Fig. 1). Also, based on the HEV and ASFV results, an 18-hour incubation period did not appear to be significantly superior to a 1-hour incubation period (Fig. 1 and Fig. 4). For ASFV, a manufacturer-validated method for testing DBS eluates in the ELISA (Innovative Diagnostics) is available, in which two disks are to be eluted in 200 μL dilution buffer (contained in the ELISA kit) during 16 – 20 h at room temperature. Also in this protocol, 50 μL of the eluate are used for the ELISA. Across the various conditions (i.e. number of disks and volume of buffer) tested for HEV and ADV antibody detection, none proved to clearly and consistently perform better than the others (Fig. 2 and Fig. 6). For the final DBS elution protocols, the conditions that were most resource- and time-efficient were therefore selected. In general, these resulted in satisfactory accuracy and repeatability in the three ELISA runs for all three pathogens (Fig. 3, Fig. 5 and Fig. 7). When out of 156 measurements per pathogen doubtful results were rated as positive, diagnostic sensitivity and specificity for HEV, ASFV and ADV were 96.4 % and 95.8 %, 100 % and 100 %, and 96.2 % and 100 %, respectively. In the ADV ELISA, DBS samples spiked with lyophilized sera tendentially showed higher deviation from their corresponding serum values compared to DBS prepared from fresh serum, suggesting that matrix differences may influence S/N values. Two samples yielded false negative results in all three ADV runs (Fig. 7). Interestingly, according to the laboratory from which the samples were obtained, these were the only two samples that had not been heat-treated (30 min at 56°C). Ultimately, the reason for the discrepancy in the two samples remains

unclear.

Studies comparing the performance of antibody ELISA using DBS and serum/plasma from domestic pigs are scarce or do not exist. Randriamparany et al. (2016) evaluated DBS for ASFV antibody detection by Ingezim PPA Compac ELISA (Ingenasa, Spain), reporting a sensitivity of 93.8 % and specificity of 100.0 % when eluting a 40 mm^2 filter paper section in 100 µL of buffer (Ingenasa) for two hours at room temperature. For HEV and ADV, according to the authors' knowledge, there are no published studies from domestic pigs. On the human side, it was demonstrated that HEV antibodies can be reliably detected from DBS, with a specificity of 97.0 % and an overall sensitivity of 81.0 %, which is in a similar range as observed in the present study (Øverbø et al., 2022). Charrier et al. (2018) used DBS collected from domestic pigs in commercial ELISAs for large-scale field surveillance. Unfortunately, the procedure for DBS elution is not described and diagnostic sensitivity and specificity are not specified. Yet, their study provides evidence for the practical feasibility of DBS-based testing for ADV and HEV under field conditions.

The results from this study strongly suggest that DBS can serve as a viable and highly cost-effective alternative to liquid blood for antibody detection using ELISA, especially for large-scale or field-based surveillance. The results further support the hypothesis that DBS, with a thoroughly optimized protocol, can perform comparably or equally to serum or plasma samples in terms of diagnostic sensitivity and specificity in ELISA-based antibody detection.

Two key limitations should be acknowledged which need to be addressed in future studies. First, a part of the DBS was prepared from antibody-spiked blood. The protocols established in the present study require further testing on untreated samples directly collected from the animals. Ideally, these tests are accompanied by a comparison with a gold standard test. Second, the limited sample size ($n = 52$ per pathogen) restricts generalizability of the results. Large-scale studies are needed to confirm the reliability and robustness of the DBS elution protocols evaluated here.

CRediT authorship contribution statement

Ranja Steinhauer: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Eric Kübler:** Writing – review & editing, Validation, Resources, Methodology, Funding acquisition, Conceptualization. **Stefan Gaugler:** Writing – review & editing, Validation, Resources, Methodology, Funding acquisition, Conceptualization. **Cornel Fraefel:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Julia Lechmann:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

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Declaration of Competing Interest

Eric Kübler is an affiliate of STERA Scientific AG which aims to commercialize the findings described in this manuscript. All other authors declare no conflict of interest relevant to this work.

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Data availability

Data will be made available on request.

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