

Quantitative Detection of Antibodies to Aleutian Disease Virus in Dried Blood Spots as an Estimation of Hypergammaglobulinemia in Mink

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Abstract

Infections with Aleutian disease virus (ADV) cause a progressive hypergammaglobulinemia, immune complex formation and plasma cell infiltrations in internal organs which induce a multi-systemic disease with high mortality. Serum ADV antibodies have traditionally been diagnosed with counter immunoelectrophoresis (CIEP) as a gold standard for qualitative assessment separating infected from non-infected animals, but less laborious ELISA-methods have been confirmed to be equally sensitive. A way to simplify the diagnostics further could be to demonstrate antibodies in dried blood spot samples (DBS). However, quantitative analysis of ADV antibodies in DBS and its correlation to the degree of hypergammaglobulinemia have not been scientifically published. The aim of this paper was to describe the adaptation and validation of the VP2 ELISA to ADV antibody detection in DBS and compare the estimated antibody levels in DBS to CIEP results, the estimated antibody levels and albumin: gamma globulin (A:γG) ratio.

The VP2 ELISA worked technically well when transferred from serum to DBS with mean intra-assay and mean inter-assay coefficients of variation within $\pm 20\%$. The DBS VP2 ELISA had a sensitivity of 97.3% and specificity of 93.2% compared to CIEP. Further, we found a correlation coefficient between the level of antibodies in DBS and the A:γG ratio of -0.81. The correlation between the A:γG ratio and the OD₄₅₀ value was superior in DBS compared to serum samples from the same mink with the most pronounced difference at low A:γG ratios.

Our results confirmed that the VP2 ELISA could detect ADV antibodies in DBS with a high sensitivity and specificity when employing CIEP as gold standard. The antibody estimated with DBS VP2 ELISA were well correlated to the antibody titer and A:γG ratios in serum, and the DBS VP2 ELISA could be an applicable and preferable tool for estimating AD progression in mink.

Keywords: Aleutian disease virus; Antibody titer; ELISA; Enzyme-linked immunosorbent assay; VP2; Counter immunoelectrophoresis; Dried blood spots; Serum proteins; Hypergammaglobulinemia

Abbreviations

AD: Aleutian Disease; ADV: Aleutian Disease Virus; CIEP: Counter immunoelectrophoresis; ELISA: Enzyme-Linked Immunosorbent Assay; DBS: Dried Blood Spot samples; VP2: Virus capsid Protein 2; A:γG ratio: Albumin-Gammaglobulin ratio; MALDI-TOF: Matrix-Assisted- Laser Desorption/Ionization-Time of Flight; RT: Room Temperature; CV: Coefficient of Variation; OD: Optical Density; SD: Standard Deviation; ROC: Receiver Operating Characteristic

Introduction

Infections with Aleutian disease virus (ADV) causes a multi-systemic disease in mink and other mustelids with symptoms such as weight loss, polyuria, polydipsia, anemia, melena, poor reproductive performance and death [1]. Infected animals typically experience a progressive hypergammaglobulinemia that causes immune complex formations and plasma cell infiltrations in internal organs [2,3]. Aleutian disease (AD) is an important disease with a negative impact on animal health and economy throughout the mink producing countries of the world. Unfortunately, neither vaccines nor infections

with low pathogenic strains of the virus efficiently protect animals from future infections. Instead, mink breeders practice different forms of disease surveillance and methods to control the disease. [4-6].

During disease surveillance, serological analysis of ADV antibodies in individual mink is performed. Traditionally, blood has been sampled in capillary tubes for analysis of antibodies but with the introduction of ELISA for ADV antibody analysis, only a small amount of blood is required for analysis. Therefore, dried blood spot samples (DBS) have been evaluated as an alternative sampling method in order to simplify the sampling procedure, facilitate automation of the analyzing procedure and improve the animal welfare by using a less invasive sampling method [7-10]. Analyzing elutes of DBS with VP2 ELISA has shown a high agreement with results obtained with counter immunoelectrophoresis (CIEP) and thereby concluded sufficient for separating ADV infected from non-infected animals [8]. However, quantitative analysis of ADV antibodies in DBS has not yet been scientifically evaluated.

The need for quantitative assessment of ADV antibodies in mink has grown the past decade as a prerequisite for a new method of ADV control [11] due to the lack of success of eradicating the disease by stamping out which has been attempted since the 1970's [12,13]. The theory behind is that the morbidity and mortality from AD depend, not only on the virus strain, but also on host factors [14-17]. In a

clinical setting with widespread infection with ADV strains of low pathogenicity, individual mink within the herd will develop fatal disease at different pace and more AD tolerant mink will not express clinical symptoms during their lifetime even though they are infected with ADV. Therefore, focus has shifted from diagnosing AD to evaluation of the AD progression in the mink [11].

One way of estimating AD progression is by estimation of the degree of hypergammaglobulinemia, which is a typical characteristic of chronic progressive AD [11,18,19]. However, conventional electrophoresis for serum proteins is laborious, expensive and not suitable for a large number of animals, and indirect tests, such as the iodine agglutination test, are not sufficiently sensitive [11,20,21]. Consequently, a new definition of hypergammaglobulinemia in mink was recently suggested based on the estimation of the serum albumin: gamma globulin ratio (A:γG) with the use of MALDI-TOF [11]. Unfortunately, due to the large size of the serum proteins, not all MALDI-TOF instruments can be used for this purpose and there is a need for a simpler method to separate animals based on their disease progression, and indirectly, their ADV infection tolerance.

Several previous studies have shown correlations between the level of ADV antibodies, histological lesions and hypergammaglobulinemia [22-24]. However, these studies have either focused on the estimation of ADV antibodies in plasma with CIEP or in serum with VP2 ELISA. The aim of this paper was to describe the adaptation process and the validation of the VP2 ELISA, previously validated for analysis of serum [8,25], to ADV antibody detection in DBS and to present the results from the comparisons between the estimated antibody levels in DBS and the qualitative assessment of infection status by the gold standard (CIEP), the quantitatively estimated antibody levels and A:γG ratio in serum.

Materials and Methods

Collection and preparation of samples

Plasma and serum samples: Blood was collected by toe nail clips into capillary tubes without additive (serum) and with heparin as additive (plasma), and stored at +2-8°C until transport to the laboratory. The capillary tubes were centrifuged at 850G and stored at -20°C until analysis. Serum or plasma was harvested through breakage of the tubes.

Dried blood spot samples: Capillary blood was collected by toe nail clips on to filter papers (Munktell TFN, VWR International AB, Stockholm, Sweden) and air-dried at room temperature (RT). The filter papers were placed separately inside envelopes, transported to the laboratory and stored at RT until analysis. The day before the analysis, the DBS were punched manually to a size of 25 mm² which was equivalent to approximately 5 μL serum based on the average mink hematocrit value [26]. Each punched filter paper piece was placed in a separate well of a flat bottomed 96-well plate containing 200 μL elution buffer (PBS/T, SVA) over night at RT and shaken gently prior to removal of the eluates. The eluate in each well then corresponded to a serum dilution of 1:40 (5 μL diluted in 200 μL).

Detection of antibodies to ADV in serum by ELISA

Serum antibodies to ADV were analyzed with a previously described indirect ELISA system based on a VP2 antigen [25]. In brief, immunoplates (VWR International AB, Stockholm, Sweden) were coated overnight with the antigen diluted 1:1500 in coating buffer. The

serum samples were diluted 1:200 in dilution buffer. The plates were incubated for 60 min at RT followed by washing and 60 min incubation with conjugate solution at RT. After washing, substrate was added to each well and the plates were incubated for 15 min at RT before the reaction was stopped. The optical density was measured at 450 nm in a Sunrise ELISA microplate reader (Tecan Nordic AB, Mölndal, Sweden). The mean OD of the blank wells was subtracted from each result. Reference sera (negative and positive) were included on each plate: The positive reference serum was stepwise diluted to a concentration corresponding to OD₄₅₀=1.0 and used as the positive reference sera in the VP2 ELISA during the evaluation process. Pooled serum from animals from an ADV negative herd was diluted 1:200 and used as negative control.

Detection of antibodies to ADV in DBS by ELISA

DBS from 1008 mink from routine analysis of an 84 sampling series of mink from an ADV negative herd were eluted and the eluates further diluted 1:5 to correspond to serum diluted 1:200 (see above). The eluates were then analyzed with respect to presence of antibodies to ADV (defined as OD₄₅₀) in the VP2 antigen based indirect ELISA system according to the previously evaluated protocol as briefly described above [25]. On each micro titer plate, positive reference serum and negative controls were employed. All mink with OD₄₅₀ values exceeding the mean OD₄₅₀+2 SD of all the 1008 samples were re-evaluated with either CIEP (in plasma) or PCR (in spleen and mesenteric lymph nodes) in order to confirm that they were truly ADV negative.

Repeatability of the DBS VP2 ELISA

The degree of repeatability of the DBS VP2 ELISA was evaluated through intra-assay as well as inter-assay variability. The intra-assay variability was estimated by comparing the OD₄₅₀ values for 11 randomly selected samples from two ADV positive herds tested in six replicates in a single assay. This was repeated during two consecutive days. The coefficient of variation (CV) for each sample each day was calculated by dividing the standard deviation of the six replicates for each sample with the mean of the replicates. The overall intra-assay variability was computed as the mean CV for all the 11 samples.

The inter-assay variability was evaluated by comparing the mean value from the two duplicate wells for each sample analyzed during three consecutive days. The CV for each sample was computed as the standard deviation of the test results from the three assays divided by the mean test result for the assays. The overall inter-assay variability was again computed as the mean CV for all the samples [27].

Evaluation of DBS compared to the gold standard (CIEP)

Plasma samples and DBS from routine analysis of 300 mink in an ADV low prevalence herd were compared. The plasma samples were analyzed at Copenhagen Diagnostics, Glostrup, Denmark for detection of antibodies to ADV with CIEP. The DBS were eluted and diluted 1:20 before analysis with the same VP2 ELISA as above. On each micro titer plate, positive reference serum and negative controls were employed and the optical densities on each plate were corrected to a value of OD₄₅₀=1.0 for the positive reference control serum.

The OD₄₅₀ values for the CIEP negative and positive mink were compared with a paired t-test assuming unequal variances. To find the optimal cut-off value and evaluate the ability of the DBS ELISA to discriminate between CIEP negative and CIEP positive samples,

receiver operating characteristic (ROC) analysis was used. Sensitivity and specificity for the DBS ELISA compared to CIEP were then calculated using the selected cut-off.

Correlation between the OD₄₅₀ values (DBS and serum) and the level of hypergammaglobulinemia

DBS and serum samples 180 mink were collected from two infected herds with a known high prevalence of seropositive animals in order to evaluate the correlation between the OD₄₅₀ values for DBS and serum samples. The serum samples were diluted 1:200 and the DBS eluates were further diluted 1:20 (corresponding to a serum dilution of 1:800) before analysis with the indirect VP2 ELISA described above. On each micro titer plate, positive reference serum and negative controls were included and the optical densities of each plate was corrected to a value of OD₄₅₀=1.0 for the positive reference control serum. The Spearman rank correlation between the OD₄₅₀ values for DBS and serum samples was calculated for the paired samples. The linear association between serum and DBS as well as herd as a possible interacting factor was investigated using a linear regression model.

The concentration of serum proteins was determined by serum electrophoresis at the Department of Clinical Sciences at the Swedish University of Agricultural Sciences, Uppsala. The OD₄₅₀ values for DBS and serum samples versus the A:γG ratio in serum were graphically presented using the lowess command, and the Spearman rank correlations between DBS and A:γG ratio as well as between the serum samples and A:γG ratio were calculated. The linear association between serum/DBS and A:γG ratio was investigated using linear regression models. Prior to the analysis, OD₄₅₀ values for DBS were transformed using the natural logarithm (Ln) to improve its normality.

Further, using the disease definition suggested by Cepica et al. (2012), A:γG ratio was stratified into three categories (<5, 5-8, and >8). The mean OD₄₅₀ values for DBS and serum samples for each category were statistically assessed using a paired t-test assuming unequal variances. The prediction power of DBS was assessed by ROC analysis and the sensitivity as well as the specificity for the optimal cut-off was calculated [28].

Statistical analysis

All statistical analyses and graphical presentations were made in Stata (StataCorp, College Station, Texas 77845, USA).

Ethical approval and informed consent

The study was approved by the Swedish Ethical Committee in Gothenburg (Dnr 117-2012) prior to initiation. All farms participated voluntarily in the project.

Results

Detection of antibodies to ADV in DBS and determination of the optimal DBS dilution

The mean OD₄₅₀ value in the DBS eluates from 1008 ADV-free mink diluted 1:5 was 0.15 ± 0.067 with values ranging from 0.034 to 1.04. In total, 28 mink had OD₄₅₀ values exceeding 0.28 (mean+2SD). Of these 28 mink, four mink with OD₄₅₀ values above 0.42 (1.5 times the mean OD₄₅₀ value) were euthanized and tested negative to ADV by

PCR (spleen and mesenteric lymph nodes) and the remaining 24 were confirmed ADV negative by CIEP.

Seventy of the original 1008 samples were selected so that the selection of sera encompassed the four mink with the highest OD₄₅₀ values, three other false seropositive mink and in total 63 seronegative mink (nine negative sera from each of the seven sampling series as the false positive sera).

These 70 eluates were then diluted stepwise 1:5, 1:10, 1:15 and 1:20 (to correspond to serum concentrations of 1:200, 1:400, 1:600 and 1:800) and analyzed in duplicate in order to determine the optimal dilution of the DBS. The mean OD₄₅₀ values of the titrations are presented in Figure 1 showing that dilution 1:20 reduced the mean OD₄₅₀ values and more pronounced for mink with original OD₄₅₀ values exceeding 0.28. As a consequence, an eluate dilution of 1:20 (corresponding to a serum dilution 1:800) was concluded to be the dilution of choice. After determination of the optimal dilution, 555 DBS eluates from routine analysis of another two ADV negative herds were diluted 1:20 and analyzed to further validate the optimal DBS dilution.

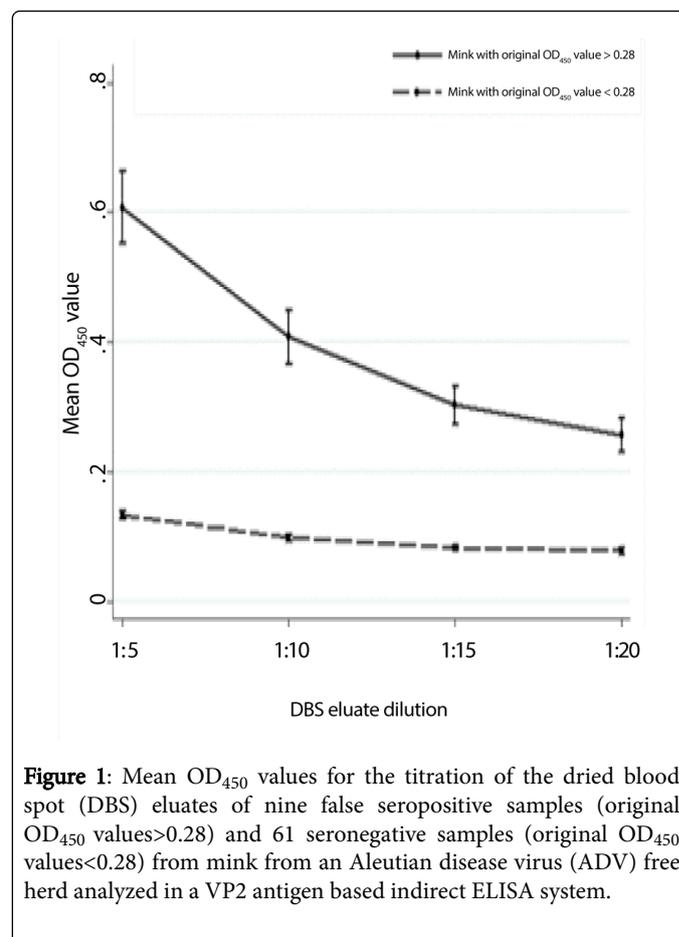


Figure 1: Mean OD₄₅₀ values for the titration of the dried blood spot (DBS) eluates of nine false seropositive samples (original OD₄₅₀ values > 0.28) and 61 seronegative samples (original OD₄₅₀ values < 0.28) from mink from an Aleutian disease virus (ADV) free herd analyzed in a VP2 antigen based indirect ELISA system.

Repeatability of the DBS VP2 ELISA

The mean OD₄₅₀ values of the 11 randomly selected samples ranged from 0.25 to 1.92, and thereby included mink with low as well as high antibody levels. The mean intra-assay CV was 5.2% (range: 3.7-8.9%) and 5.8% (range: 2.8-8.7%) for the two consecutive days, respectively, and the mean inter-assay was 12.0% (range: 1.2%-26.1%) (Table 1).

Inter-assay variability N=3 per sample			
Sample no	Mean OD ₄₅₀	SD	Inter-Assay CV (%)
1	0.25	0.04	16.1
2	0.26	0.04	16.5
3	0.30	0.00	1.2
4	0.49	0.04	8.6
5	1.10	0.25	22.5
6	1.10	0.07	6.3
7	1.14	0.06	5.6
8	1.22	0.10	8.1
9	1.25	0.15	12.1
10	1.46	0.13	8.6
11	1.92	0.50	26.1
Mean			12.0

Table 1: The mean optical density (OD₄₅₀), standard deviation (SD), and inter-assay coefficient of variation (CV) for 11 randomly selected Aleutian disease virus positive samples analyzed in a VP2 antigen based indirect ELISA system.

Evaluation of DBS compared to the gold standard (CIEP)

For the 300 mink from an ADV low prevalence herd analyzed with CIEP, 37 mink were seropositive (12%) and 263 were seronegative (88%). Table 2 shows a significantly higher mean OD₄₅₀ value in the DBS ELISA for CIEP positive mink than for CIEP negative mink (p<0.001). The area under the ROC curve was 0.98 (Figure 2) and the optimal cut-off in the low prevalence herd was OD₄₅₀=0.15 according to the ROC analysis. However, this cut-off should be compared to the calculated cut-off of OD₄₅₀=0.30 from the two ADV free herds.

The mean OD₄₅₀ of the 555 DBS eluates from routine analysis of two ADV negative herds analyzed with the dilution of 1:20 was 0.16 ± 0.07 (0.21 ± 0.06 and 0.11 ± 0.04, respectively). Therefore, the resulting numbers of ADV positive and negative samples in each assay as well as the sensitivity and specificity for both cut-offs are shown in Table 3.

	(n)	Mean OD ₄₅₀ values ± SD	95% CI
CIEP negative	263	0.10 ± 0.07	0.09-0.10
CIEP positive	37	0.64 ± 0.29	0.54-0.73

Table 2: Mean OD₄₅₀ values and 95% confidence interval (CI) for Aleutian disease virus (ADV) positive and ADV negative mink as determined by counter immunoelectrophoresis (CIEP).

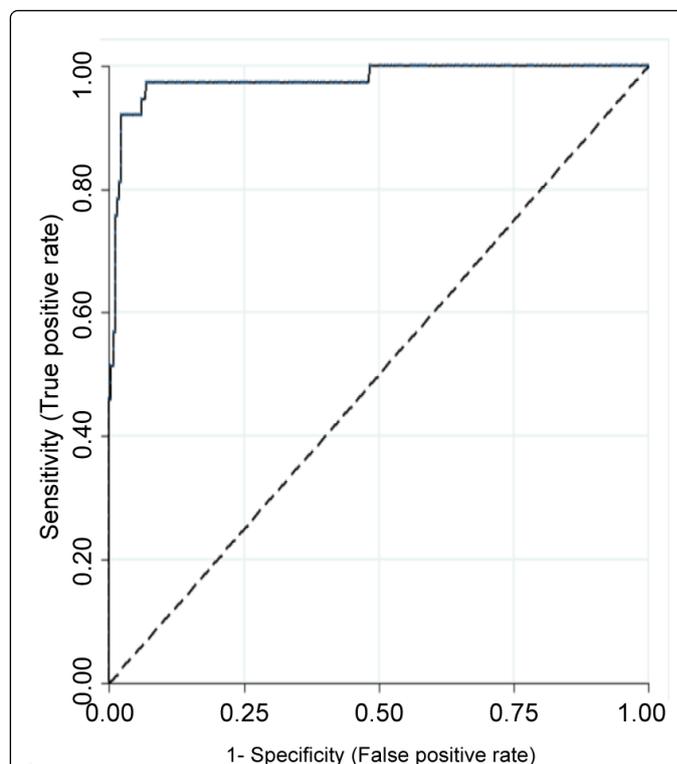


Figure 2: Receiver operating characteristics (ROC) curve for the evaluation of the dried blood spots samples (DBS) VP2 antigen based indirect ELISA system compared to the gold standard (counter immunoelectrophoresis-CIEP) for 300 mink from a low Aleutian disease virus prevalence herd (Area under curve=0.98).

	ELISA result	CIEP positive mink	CIEP negative mink	Total
0.15	Seropositive	36	18	54
	Seronegative	1	245	246
		37	263	300
	Sensitivity	97.3%		
	Specificity		93.2%	
0.30	Seropositive	33	6	39
	Seronegative	4	257	261
		37	263	300
	Sensitivity	89.2%		
	Specificity		97.7%	

Table 3: The number of Aleutian disease positive and negative mink, sensitivity and specificity of a dried blood spot samples (DBS) VP2 antigen based indirect ELISA system with cut-offs at 0.15 and 0.30, respectively, when using counter immunoelectrophoresis (CIEP) as the gold standard.

Correlation between OD₄₅₀ values for DBS and serum samples

DBS and serum from 180 mink from two ADV infected herds were analyzed. The OD₄₅₀ values for each mink in the two different analyses are presented in Figure 3 showing an exponential association between the DBS and serum OD₄₅₀ values. The mean OD₄₅₀ values in DBS and serum were 1.02 (range: 0.17-2.83) and 0.91 (range: 0.08-1.92), respectively, and the calculated Spearman rank correlation between the OD₄₅₀ values from the two analyses was 0.90.

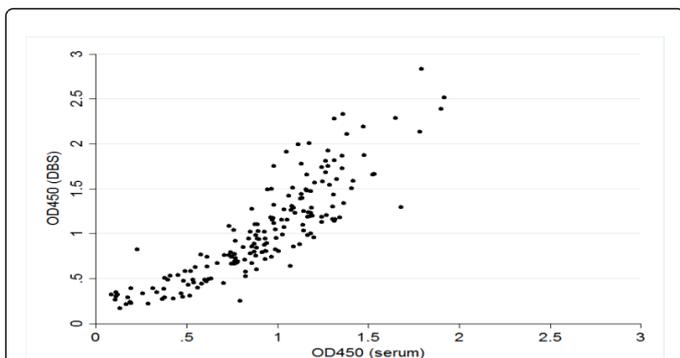


Figure 3: Individual OD₄₅₀ values obtained in serum and dried blood spot samples (DBS) from 180 mink from two Aleutian disease virus infected herds analyzed using a VP2 antigen based indirect ELISA system.

Correlation between the OD₄₅₀ values (DBS and serum) and the level of hypergammaglobulinemia

The Spearman correlation between the OD₄₅₀ and the A:γG ratio was -0.81 for DBS and -0.68 for serum. The results from the linear regression showed that both serum and DBS were significantly related to the A:γG ratio (p<0.001) with a R² value of 0.66 for ln OD₄₅₀ (DBS) and 0.47 for OD₄₅₀ (serum). Herd was not significantly associated with ln OD₄₅₀ (DBS) (p=97.7) nor OD₄₅₀ (serum) (p=27.9).

The t-test revealed that there was no significant difference between DBS and serum for A:γG ratios greater than five but for A:γG ratios less than five there was a significant difference with DBS showing a steeper slope than serum samples (Table 4 and Figure 4)

A:γG	(n)	Mean OD ₄₅₀ (DBS) ± SD	Mean OD ₄₅₀ (serum) ± SD	p-values
<5	97	1.35 ± 0.05	1.11 ± 0.03	<0.001
5-8	61	0.73 ± 0.04	0.75 ± 0.05	0.47
>8	22	0.42 ± 0.05	0.48 ± 0.07	0.15

Table 4: Mean OD₄₅₀ values in dried blood spot samples (DBS) and serum ± standard deviation (SD) for diseased, ambiguous and healthy mink (as determined by serum electrophoresis albumin: gamma globulin ratios (A:γG)) as well as p-value for the t-test for the difference between the compared means.

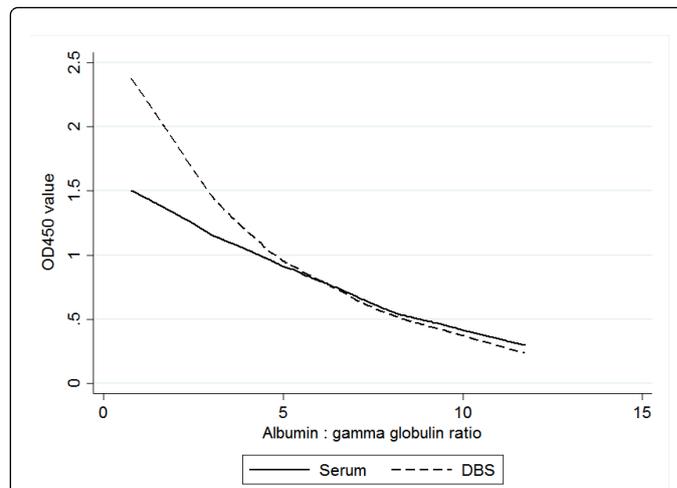


Figure 4: The mean Aleutian disease virus VP2 ELISA OD₄₅₀ values in dried blood spot samples (DBS) and serum for different serum electrophoresis albumin: gamma globulin ratios (A:γG).

Using ROC analysis to find the optimal OD₄₅₀ for the DBS ELISA to predict diseased animals, as defined as an A:γG less than 5, resulted in an area under the ROC curve of 0.89 (Figure 5) and an optimal cut-off of OD₄₅₀=0.825. The sensitivity and specificity of the DBS ELISA in predicting animals as diseased or non-diseased are shown in Table 5.

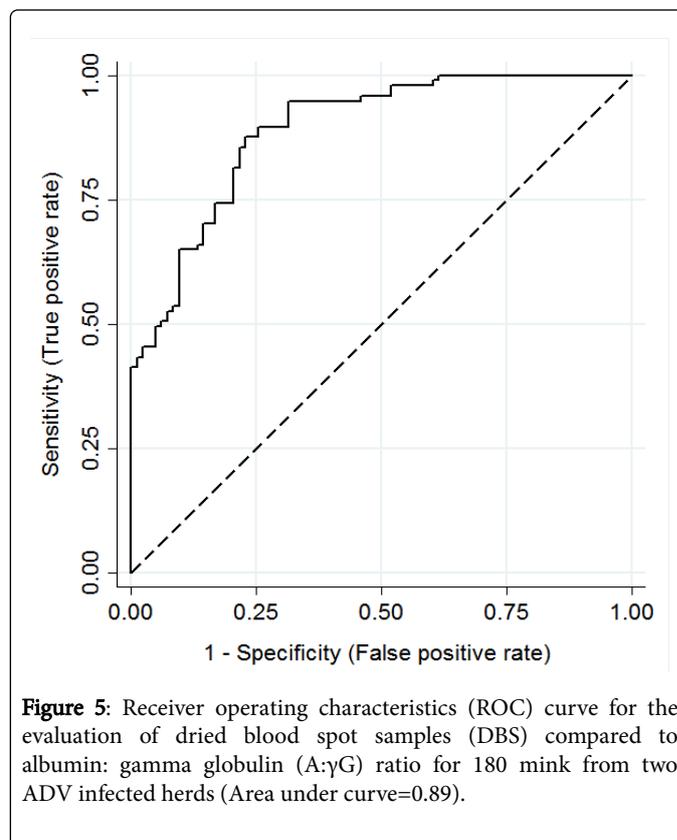


Figure 5: Receiver operating characteristics (ROC) curve for the evaluation of dried blood spot samples (DBS) compared to albumin: gamma globulin (A:γG) ratio for 180 mink from two ADV infected herds (Area under curve=0.89).

Categorization based on the OD ₄₅₀ - value	Categorization based on A:γG ratio		Total
	Diseased (A:γG ≤ 5)	Non-diseased (A:γG ≥ 5)	
Seropositive	85	20	105
Seronegative	12	63	75
Total	97	83	180
Sensitivity	87.6%		
Specificity		76.0%	

Table 5: Comparison of the number of mink, as well as the sensitivity and specificity, of mink categorized as having hypergammaglobulinemia (seropositive and seronegative, respectively) in the Aleutian disease virus VP2 ELISA (with a cut-off of 0.825) and the categorization based on the albumin: gamma globulin (A:γG) ratio (diseased and non-diseased, respectively).

Discussion

The VP2 ELISA used in the present study worked technically well when transferred to DBS and our results confirmed that antibodies to ADV can be detected in dried whole blood eluted from filter paper with good intra- and inter-assay validity. Both the mean intra-assay and the mean inter-assay coefficients of variation were within the acceptable marginal of ± 20% [27]. One of the tested samples had an inter-assay variability of 26.1%. This was still judged as acceptable since the OD₄₅₀ value of that sample was 1.92 which was outside the optimal reading area in the ELISA and the sample therefore ought to be diluted further in order to get a more accurate result. However, dilution to obtain a more accurate result is not necessary seen from a clinical perspective as it does not make any practical difference whether the mink has an OD₄₅₀ value of 1.5 or 2.5. Although the ELISA worked well the variability might be reduced if the possible non-homogenous antigen coating due to debris in the antigen solution prior to dilution could be prevented. According to the manufacturer, the antigen has been further purified since this study was carried out so the variability might be improved now. Another factor that could have influenced the results could be the extraction of the DBS. Only one elution buffer (PBS/T) was used and other elution buffers may alter the sensitivity, specificity as well as agreement between serum and DBS. Consequently, if other elution buffers should be used they need to be evaluated for their capacity to elute antibody, their non-specific reactivity and other undesired effects as well as optimal elution times.

When using CIEP as gold standard, the DBS VP2 ELISA had a high sensitivity and specificity, as previously also shown by others [8,24]. The sensitivity of the VP2 ELISA was lower when performed on DBS than what previously has been demonstrated in serum, but still very high (93.2% versus 99.7% for the serum VP2 ELISA [25]). One reason for this can be the cut-off chosen.

The cut-off chosen in the present study was the cut-off for maximizing the sensitivity and specificity together. When developing an ELISA, there will always be a trade-off between sensitivity and specificity. A lower cut-off value will increase the sensitivity but decrease the specificity with higher risk of false positive samples. For instance, using a cut-off at 0.10 instead of 0.15 would lead to a sensitivity of 97.3% and a specificity of 72.2% (results not shown). On

the other hand, increasing the cut-off to 0.30 (representing the mean+2 SD for the two negative herds together) would increase the specificity to 97.7% but decrease the sensitivity to 89.2%.

In the end, the choice of cut-off depends on the aim with the test. For screening purposes, it is beneficial to maximize sensitivity and detect infected animals as early as possible. If necessary, it is possible then to re-test the positive animals with another test with higher specificity. On the other hand, for selection of animals based on disease progression, it is less important and the correlation to hypergammaglobulinemia more relevant. Despite that the majority of the IgG comprise ADV-specific antibodies, it has been argued that quantitative ELISA analysis for antibodies cannot replace a direct test for hypergammaglobulinemia as the exact concentrations of antibodies and gamma globulins depend on many other factors such as the host, the virus strain and the total pathogen load [11,14-17,23]. Indeed, the ADV antibody levels have been shown to reach a plateau while the gamma globulins continue to increase due to production of anti-DNA antibodies, other antiviral antibodies other than to VP2, as well as polyclonal autoimmune antibodies [15,29,30]. However, we found a moderate to high correlation between the level of antibodies and the degree of hypergammaglobulinemia, as well as fairly high R² values for the regression models, which is in line with the results found by Farid et al. [24].

The correlation between the A:γG ratio and the OD₄₅₀-value, as well as the R² value, were generally superior in DBS compared to serum samples from the same mink. So although the quantitative comparison of OD₄₅₀ for paired samples of serum and DBS revealed a good agreement, the DBS VP2 ELISA was preferable over serum VP2 ELISA as a quantitative tool for predicting disease progression, as defined as the degree of hypergammaglobulinemia. Our results show that, in the absence of any available direct diagnostic method for defining the A:γG ratio, the DBS VP2 ELISA can be a fully applicable and preferable diagnostic tool for estimating AD progression of individual mink.

Conclusion

Our results confirmed that the VP2 antigen based ELISA could be optimized to detect antibodies to ADV in dried whole blood eluted from filter paper with a high sensitivity compared to CIEP as gold standard. The antibody titers estimated with DBS VP2 ELISA were well correlated to the antibody titers and ratio of A:γG in serum. The correlation between the A:γG ratio and the OD₄₅₀ value, as well as the R² value, were generally superior in DBS compared to serum samples from the same mink. The use of DBS VP2 ELISA could be concluded to be a fully applicable and preferable diagnostic tool for diagnosing AD progression in mink.

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