

Diagnosis of Marek's disease by real time PCR from samples collected on FTA[®] cards

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Introduction

- Load of viral DNA measured by real time PCR
 - **Diagnosis of MD:** tumors, blood (challenge virus)
 - **Monitoring of vaccines:** blood, feather pulp (vaccine virus)
- DNA extracted from fresh or frozen biological samples
- Use of FTA[®] cards for collection and transportation of biological samples
- Quantification of viral DNA is critical in the diagnosis of MD

The Goal

- To determine if samples collected in FTA[®] cards can be used to quantify the load of MDV DNA (wild type and vaccines) by real time PCR
 - Blood
 - Feather pulp
 - Tumors

Materials and Methods

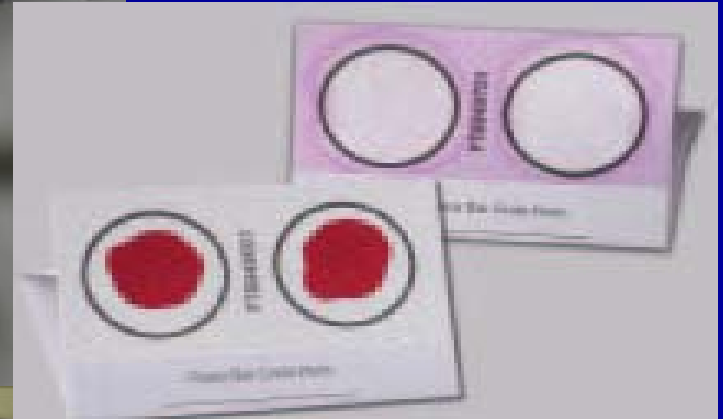
- Samples were collected from three different challenge studies at different times after infection
- Collection of samples: **Original and in FTA[®] cards**

Blood	Feather Pulp	Tumors
56	36	14

Material and Methods

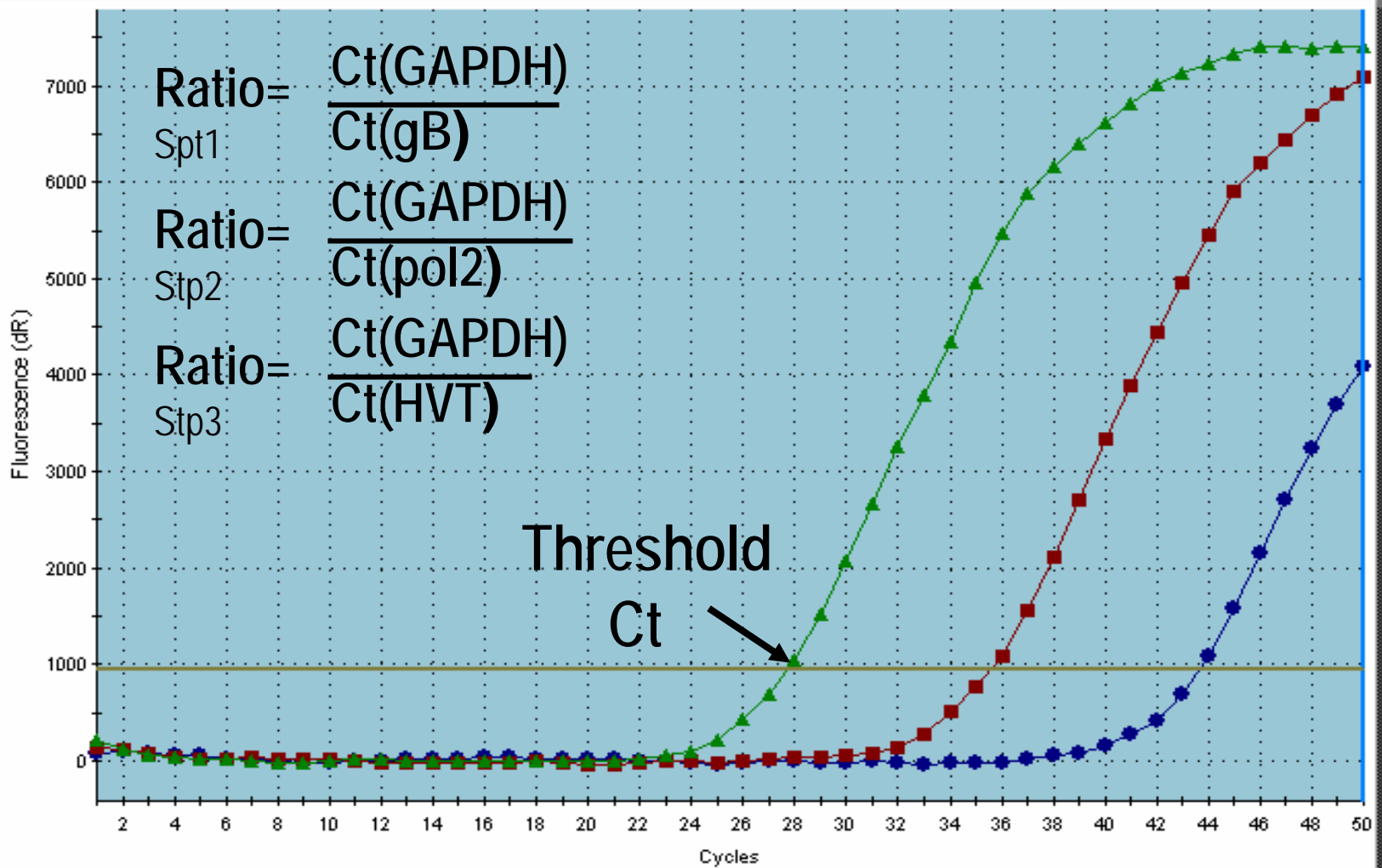
- **Real time PCR:**
 - SYBR® green (GAPDH)
 - Taq Man Probe (gB, pol2, HVT)
- **Primers:**
 - MDV **serotype 1** (gB)
 - MDV **serotype 2** (pol2)
 - MDV **serotype 3** HVT gene (spanning internal repeat regions)
 - Chicken genome** (GAPDH)
- **Statistical analysis:** Correlation test of Pearson

Material and Methods



Real time PCR

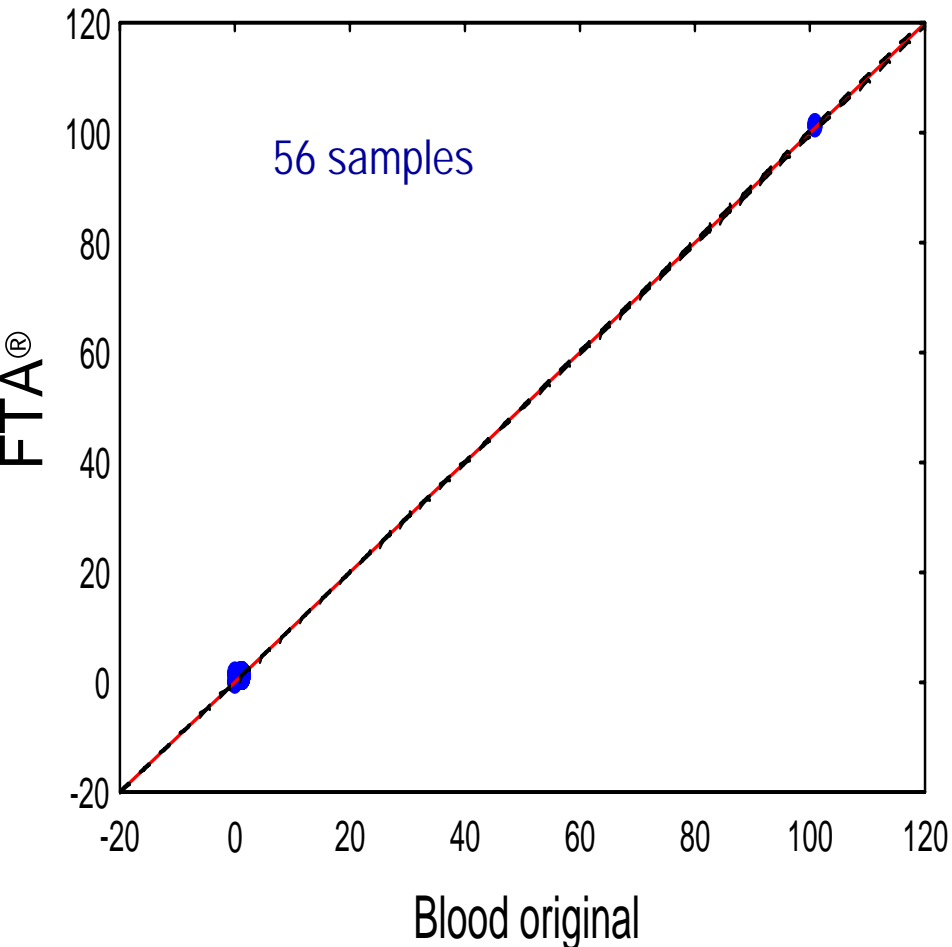
Amplification Plots



Use of FTA cards to measure viral DNA load (serotype 1) in blood and feather pulp

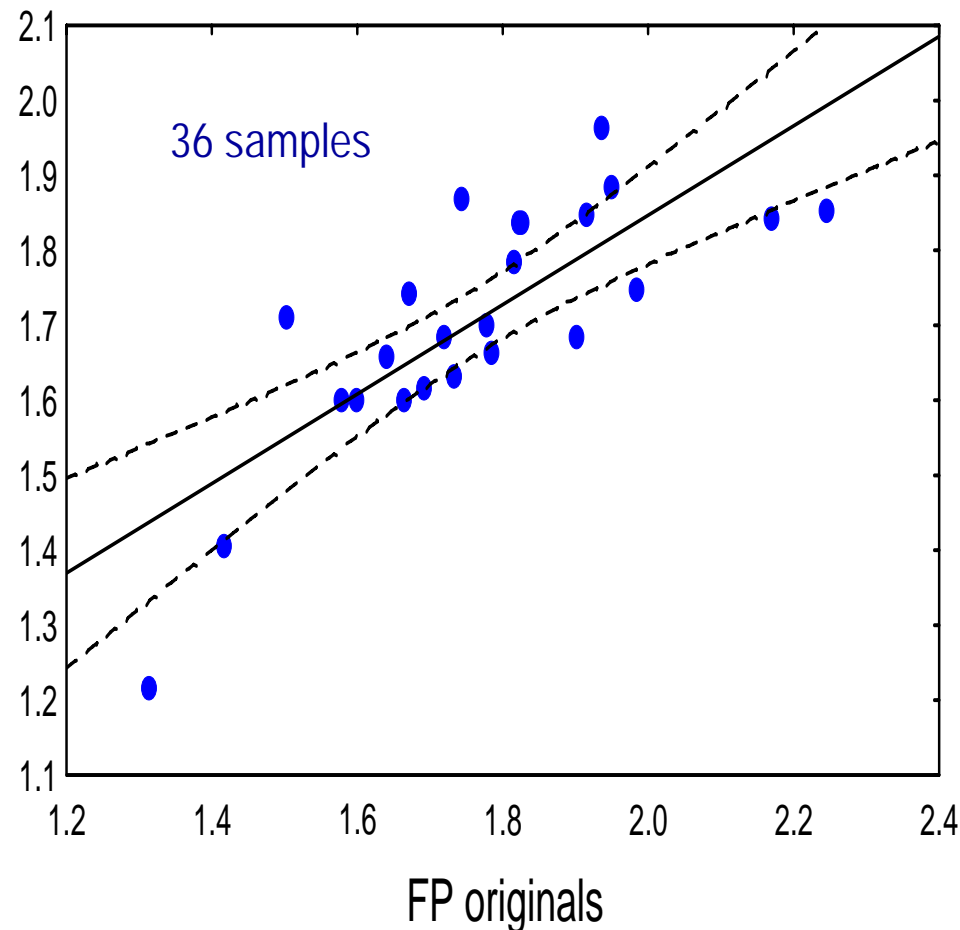
$$\text{GAPDH/gB FTA} = .01209 + .99970 * \text{GAPDH/gB orgnl.}$$

Correlation: $r = .99987$ $p < 0.05000$



$$\text{GAPDH/gB FTA} = .65381 + .59658 * \text{FP GAPDH/gB org.}$$

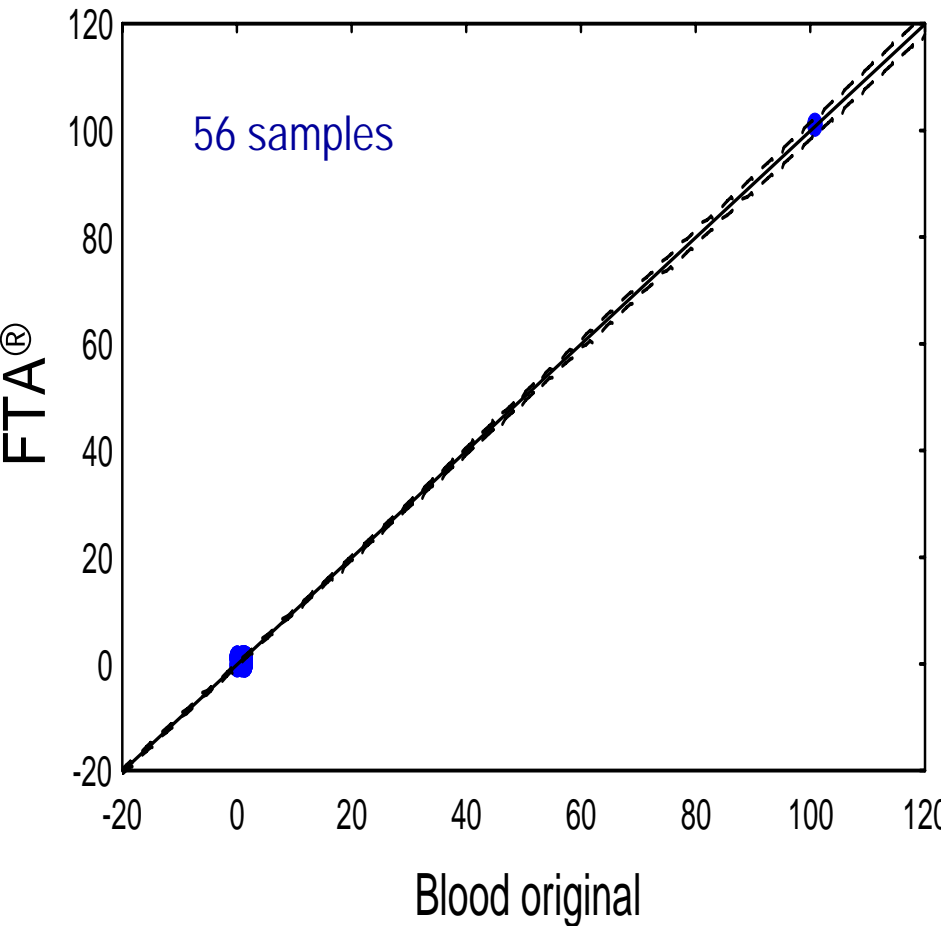
Correlation: $r = .78271$ $p < 0.05000$



Use of FTA cards to measure viral DNA load (serotype 2) in blood and feather pulp

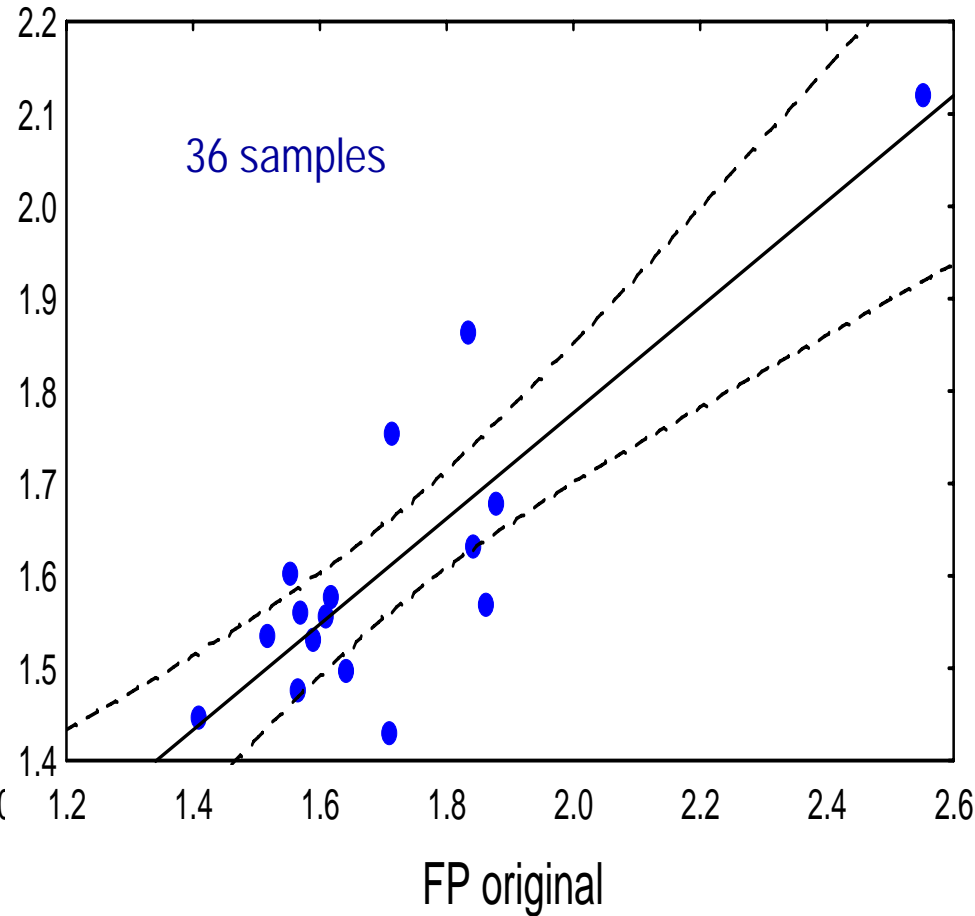
$$\text{GAPDH/SB1 FTA} = -.1456 + 1.0007 * \text{GAPDH/SB1 org.}$$

Correlation: $r = .99919$ $P < 0.05000$



$$\text{GAPDH/SB1 FTA} = .63216 + .57215 * \text{GAPDH/SB1 org.}$$

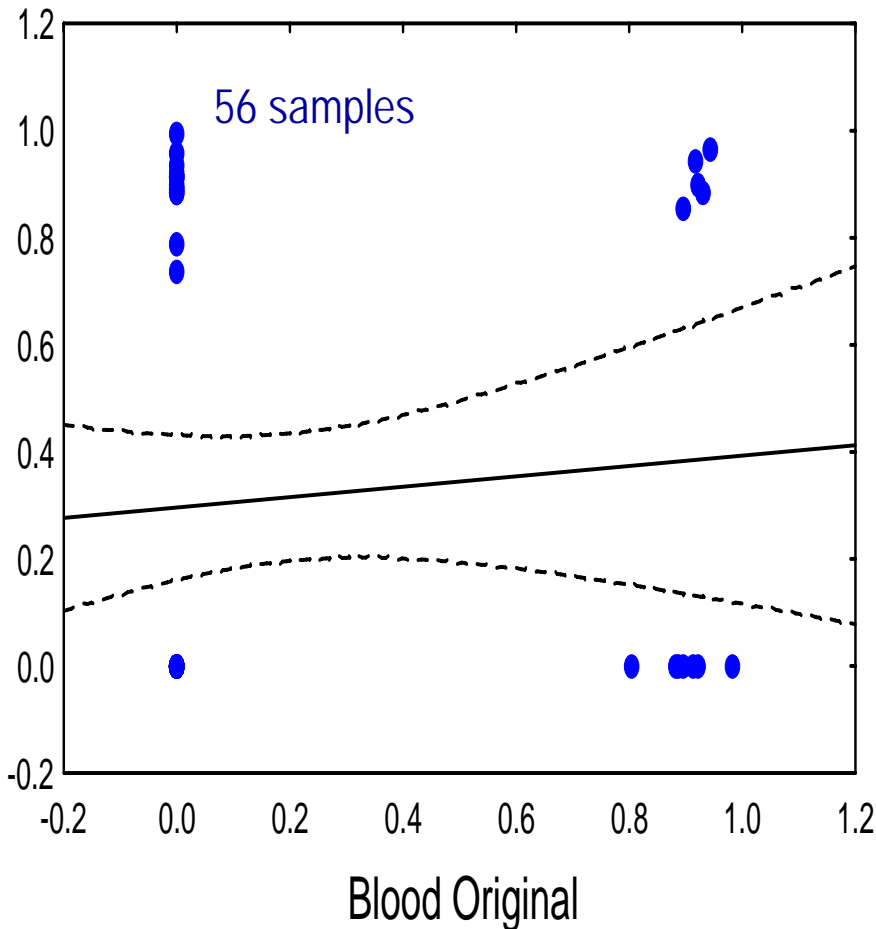
Correlation: $r = .85409$ $p < 0.05000$



Use of FTA cards to measure viral DNA load (serotype 3) in blood and feather pulp

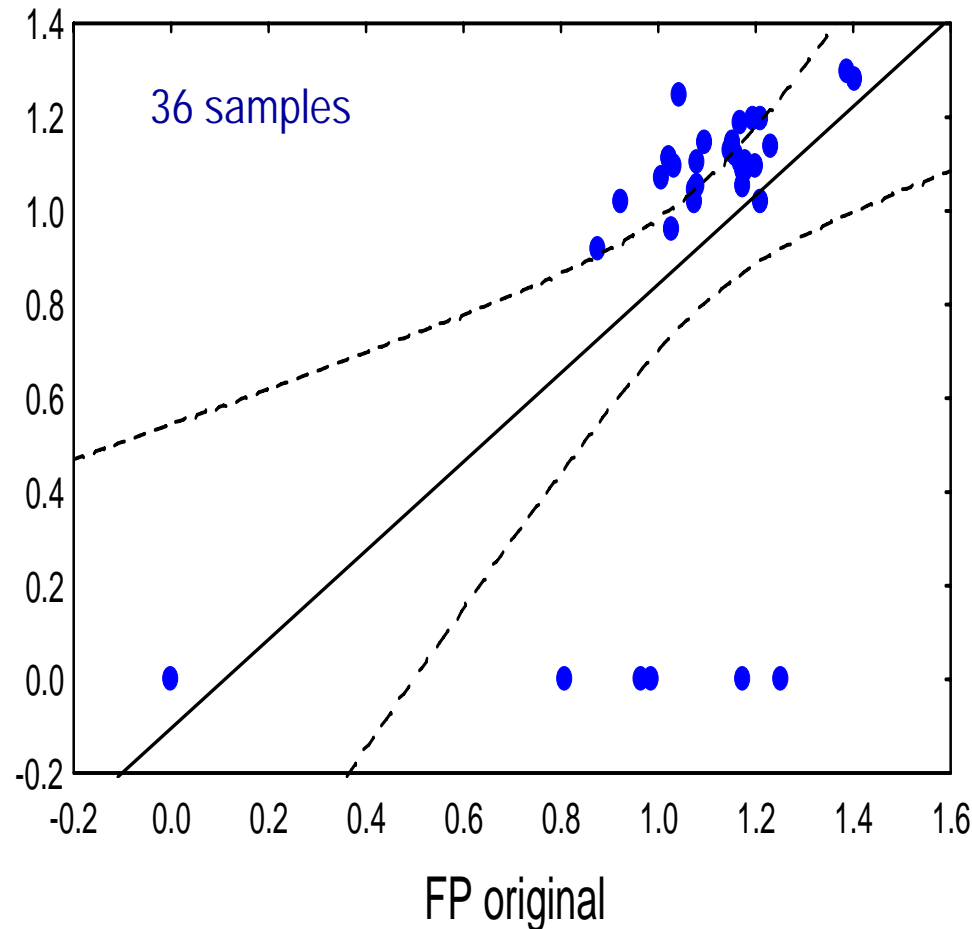
$$\text{GPDH/HVT FTA} = .29615 + .09697 * \text{GAPDH/HVT org.}$$

Correlation: $r = .08528$



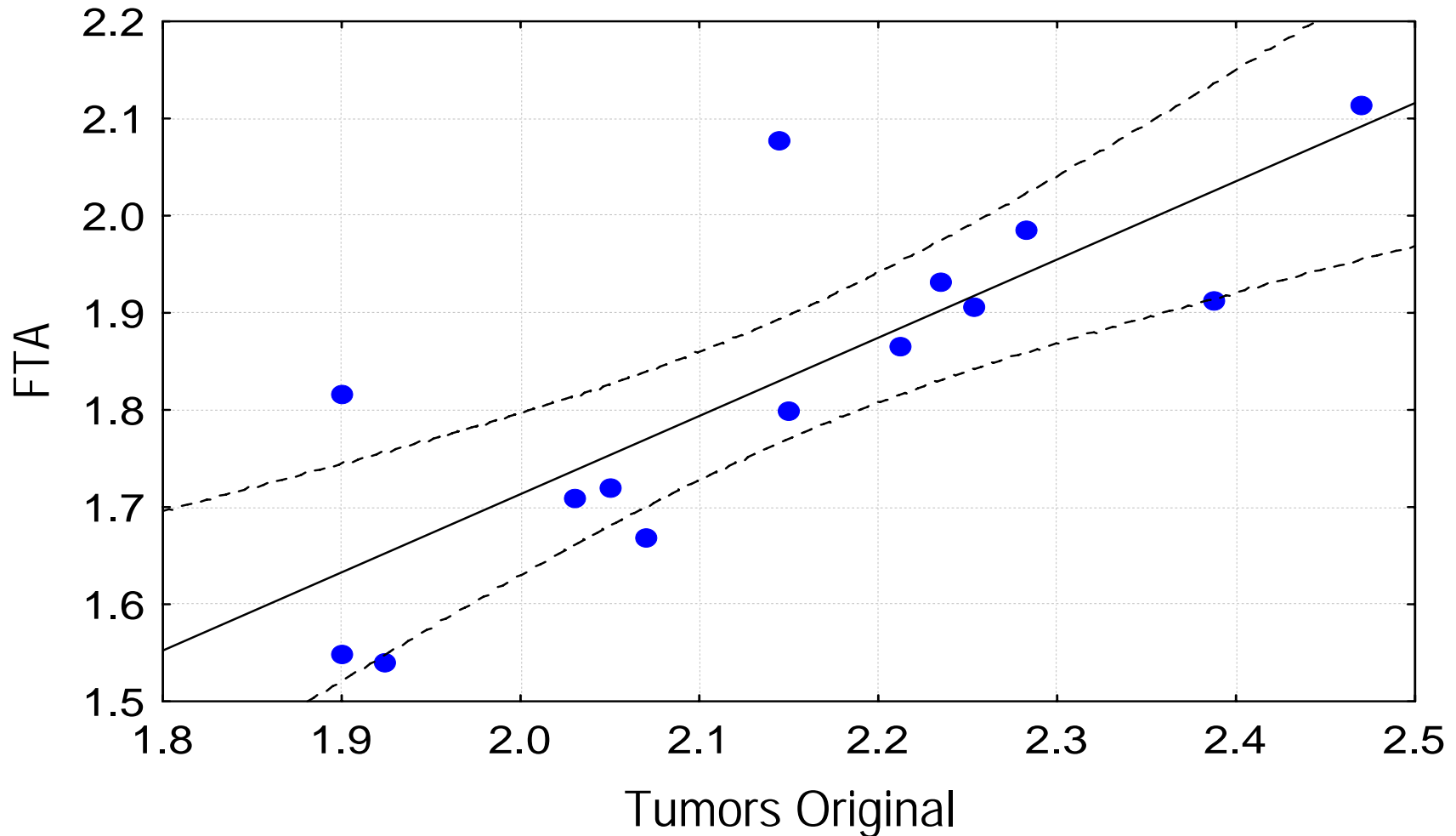
$$\text{GAPDH/HVT FTA} = -.1043 + .94784 * \text{GAPDH/HVT org}$$

Correlation: $r = .49598$ $p < 0.05000$



Use of FTA cards to measure viral DNA load (serotype 1) in tumors

GPDH/gB FTA = .10263 + .80534 * GPDH/gB org
Correlation: $r = .80333$ $p < 0.05000$



Conclusion

- **Correlation** between the load of MDV DNA in original samples and samples taken in FTA[®] cards is **statistically significant** for all three serotypes. Exception HVT in blood

Practical applications

- Imprints of tumors taken in FTA[®] cards can be used for the diagnosis of MD
- Samples of blood taken in FTA[®] cards can be used for early diagnosis of MD (wild type virus) and for monitoring serotype 2 MD vaccines
- Samples of feather pulp taken in FTA[®] cards can be used for monitoring serotypes 1, 2, and 3

Acknowledgments

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Thank you!!