

Real Time PCR for Detection of WNV from Feather Pulp Extracts



Muhammad Morshed, PhD, SCCM
Head, Zoonotic & Emerging Pathogens
Laboratory Services, BCCDC
Clinical Associate Professor
Pathology & Laboratory Medicine, UBC

Introduction

- **Emerging Infection Diseases** Vol. 10, No. 5
May 2004 by CDC, USGS, and NWHC
- Feather > Virus Isolation > RT-PCR
- Quick, Efficient, and Safer Sampling Method
- Possible non-lethal Sampling Method
- Quite Sensitive (better than Cloacal Swab, Kidney and Sleep Pools)
- 7 Days of Incubation
- Safety Concern in Viral Amplification

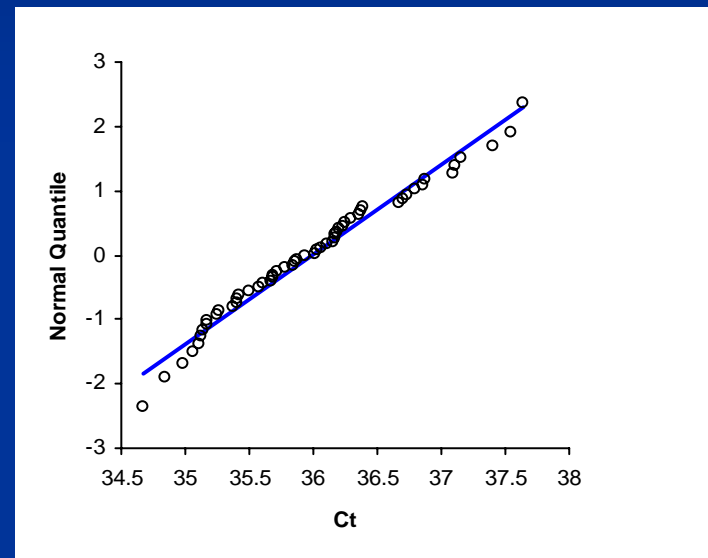
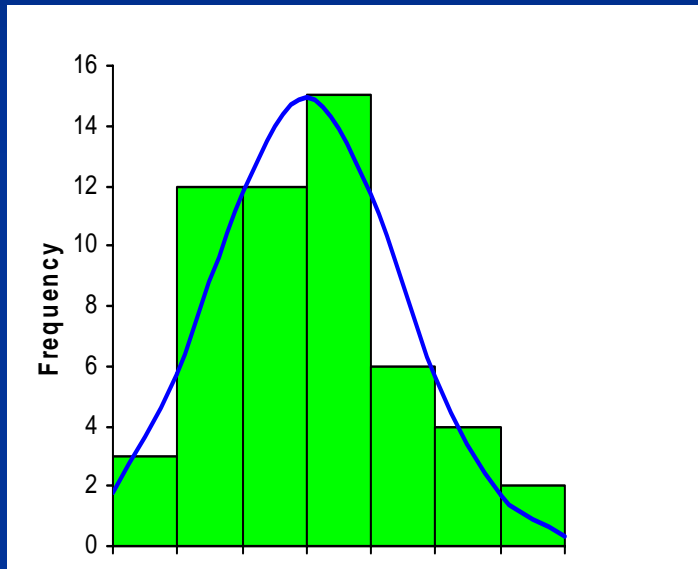
BCCDC Approach

- Direct Realtime RT-PCR from Feather Pulp Extraction (Short TA time, Less Costs, Safer)
- Internal Positive Control (QA)
- Method might be applied to other viral diseases carried by birds (i.e. Avian Flu)
- Sequencing and genotyping possible from positive samples

Methods

- **Samples:** Northern Goshawks, Cooper's Hawks, and Crow
- **Extraction:** QIAGEN Bead Beater and Viral RNA kit
- **Amplification:** Taqman RT-PCR (Multiplex in NS-5 & 3'NCR)
- **IPC:** Armored RNA (NS-5) Rf: J. Clin. Micro. 42(2):841-43; 2004
- **Analysis:** Taqman and 'Analyze-It' statistic software

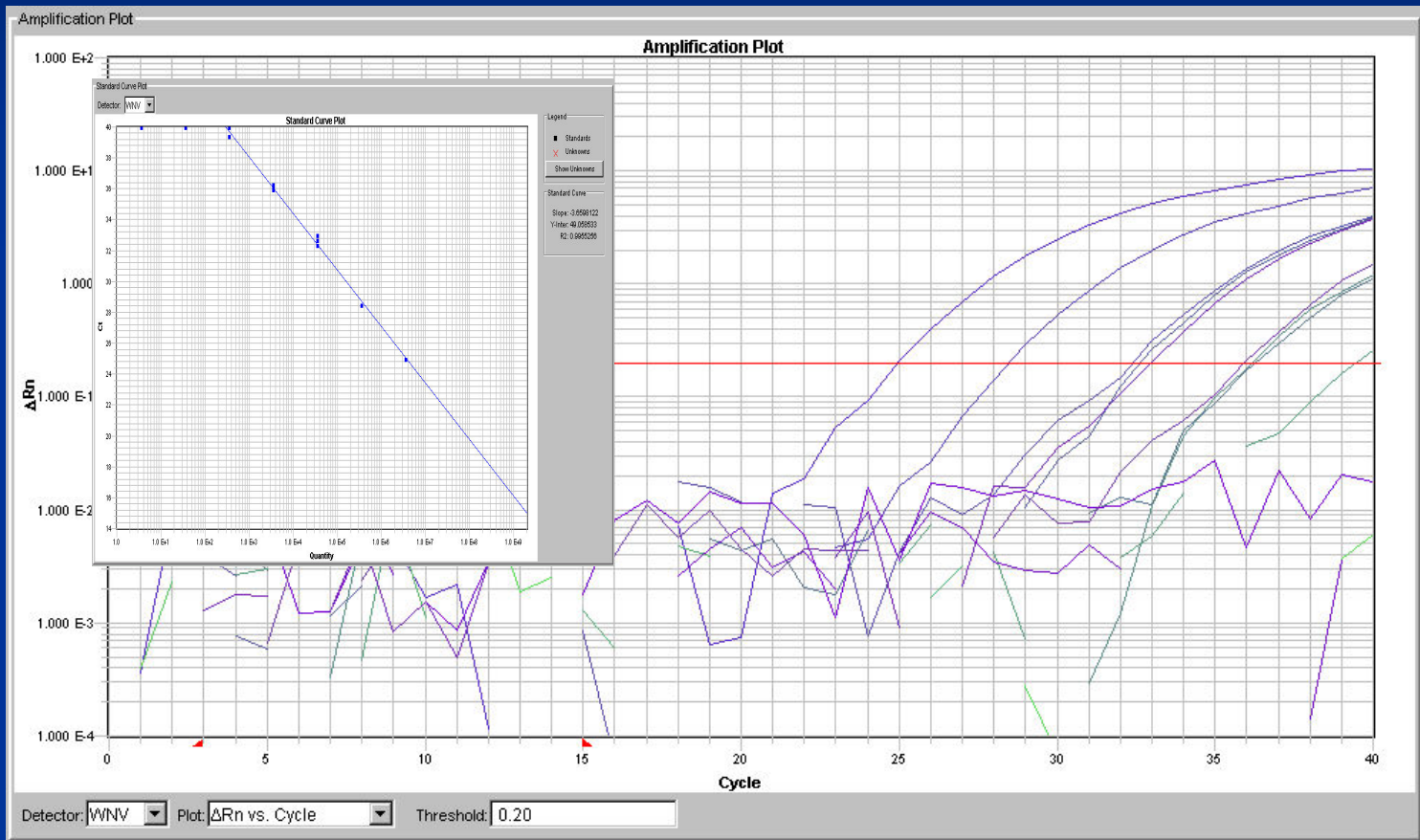
Data Distribution



The feather pulp data falls within two SD of the control, which indicates this data is statistically valid.

Recovery Rate: 95.6 % based on absolute quantification

Detection Limit



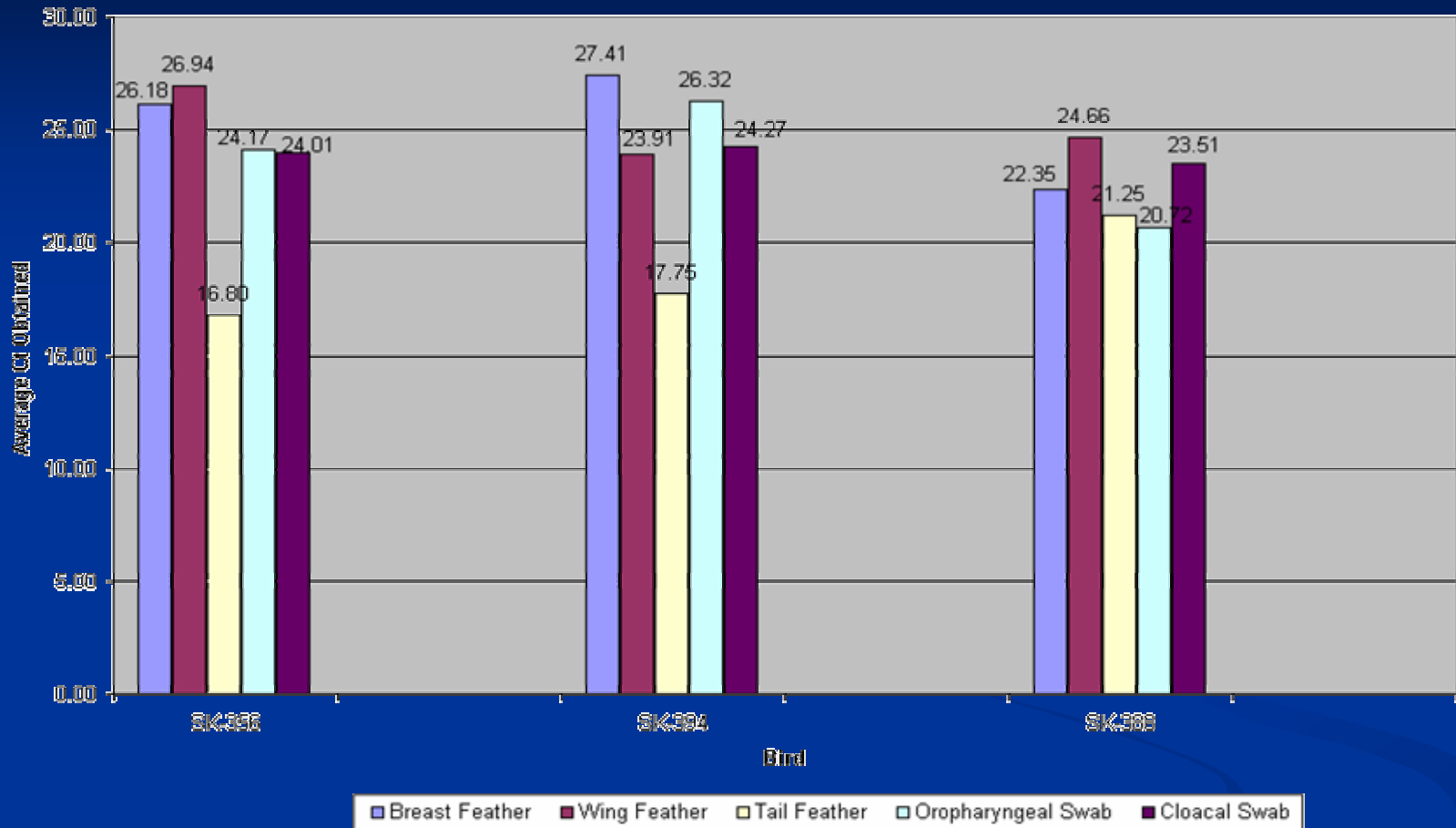
End Point, 1 - 10 copies/ μl , is determined based on Ct 40

Positive Range



Ct results for feathers from positive birds is ranged from 16 to 27, which is much higher than cut-off Ct 40

Sample Type Comparison



Comparison among breast feather, wing feather, tail feather, oropharyngeal swab and cloacal swab. Tail feather samples gave the most sensitive results.

Conclusions

- Good recovery rate and low end point suggest we can skip the cell culture step and shorten the TA time from 7 days with less safety concern.
- With limited positive samples, tail feather produced the best results in our hand. It could be the first choice for future sampling.
- This method has an advantage over VecTest, because the positive samples can be further sequenced and genotyped.
- We might be able to apply this method for other bird carried disease, such as Avian Flu.

Acknowledgements

- **Canadian Cooperative Wildlife Health Centre, SK**

F.A. Leighton and P. Zimmer

- **Ministry of Water, Land and Air Protection, BC**

H. Schwantje and E. McLaren

- **Ministry of Sustainable Resources, BC**

A. Stewart

- **University of Victoria, BC**

B. Harrower

- **BC Centre for Disease Control**

D. Jorgensen and M.K. Lee



Portrait of Elizabeth I with a feather fan, c.1585