AgPath-ID™ BVDV: High Throughput Viral RNA Isolation and Detection of Bovine Viral Diarrhea Virus

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Bovine Viral Diarrhea Virus

- **12.3 kb, ss+RNA, enveloped virus in genus *Pestivirus* of the *Flaviviridae* family.**
- **GI disease, reproductive failure, respiratory illness.**
- **Major economic losses.**

  from Reproductive dysfunction.

- **Maintained in population through Persistently Infected (PI) cattle.**

  PI cattle shed virus throughout their lives.

MagMAX™ nucleic acid isolation technology

- **High throughput magnetic bead-based RNA isolation**

MagMAX™-96 Viral RNA Isolation
For viral RNA/DNA isolation from swabs, serum, and plasma

MagMAX™-96 Blood RNA Isolation
For viral and total RNA isolation from whole blood

MagMAX™-96 Total RNA Isolation
For viral and total RNA isolation from tissue or cell culture

AgPath-ID™ BVDV Kit

- One-step qRT-PCR assay
- Detects both Type I and Type II BVDV
- XenoRNA-01: Isolation and qRT-PCR control
- Optimized multiplex reaction targeting BVDV and XenoRNA-01 (control RNA)

MagMAX™ and AgPath-ID™ Advantage

- **Fast.**

  ~2 hrs for RNA isolation and detection.

- **Sensitive.**

  Detects < 100 copies of BVDV.

- **Diverse sample inputs.**

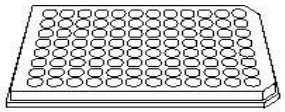
  Whole Blood, plasma, serum, raw milk, ear notch supernatant.

- **Robust.**

  No false positives in vaccinated cattle.

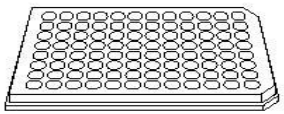
- **Easy to automate.**

High Throughput BVDV Detection Using Whole Blood



Blood/milk Samples

1. Add Lysis/Binding Solution
2. Add Bead Resuspension Mix
3. Shake for 5 min
4. Capture and wash beads twice
5. Treat with DNase
6. Add Lysis/Binding Solution and shake for 2 min
7. Capture and wash beads two more times
8. Elute total RNA



Sample RNA

- **Small sample volume required (50μl)**
- **Low elution volume streamlines the process**

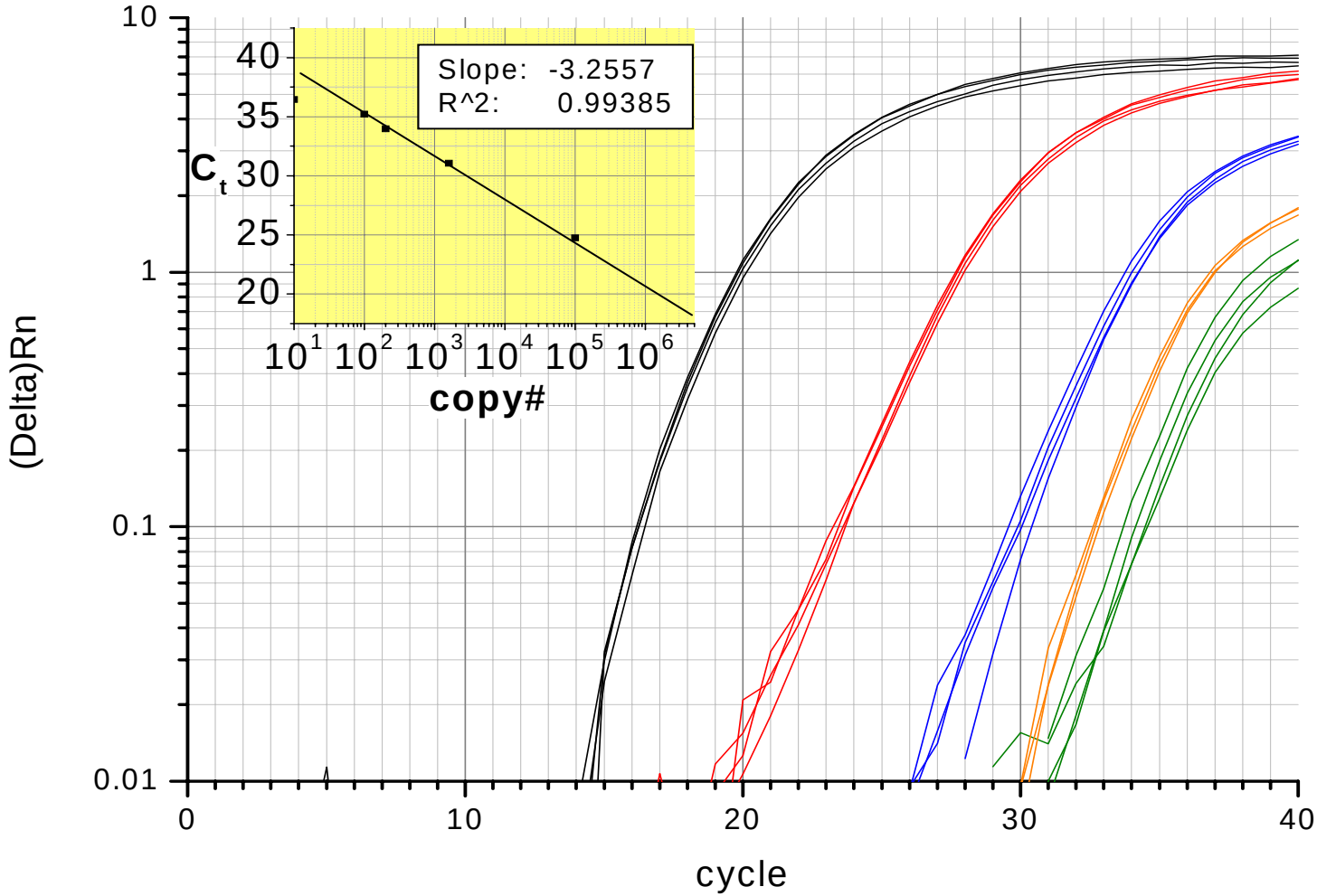
AgPath-ID™ BVDV Kit

- **25X Primer Probe mix**
  - BVDV (FAM/BHQ1)
  - XenoRNA-01 (CAL Fluor Orange 560/BHQ1)
- **40X Enzyme Mix**
- **2X qRT-PCR Buffer**
- **50X ROX Reference Dye**
- **Nuclease-free H20**
- **XenoRNA-01 Control RNA**
- **BVDV Transcript**

Experimental Overview

- **Examine detection sensitivity of AgPath-ID™**
- **Validate and confirm use of AgPath-ID™ in various samples matrixes**
- **Demonstrate no false positives in vaccinated cattle**
- **Examine consistency and efficiency of RNA Isolation**

AgPath-ID™ BVDV Kit Detection Sensitivity



Linear detection of BVDV RNA from 25M to 40 copies

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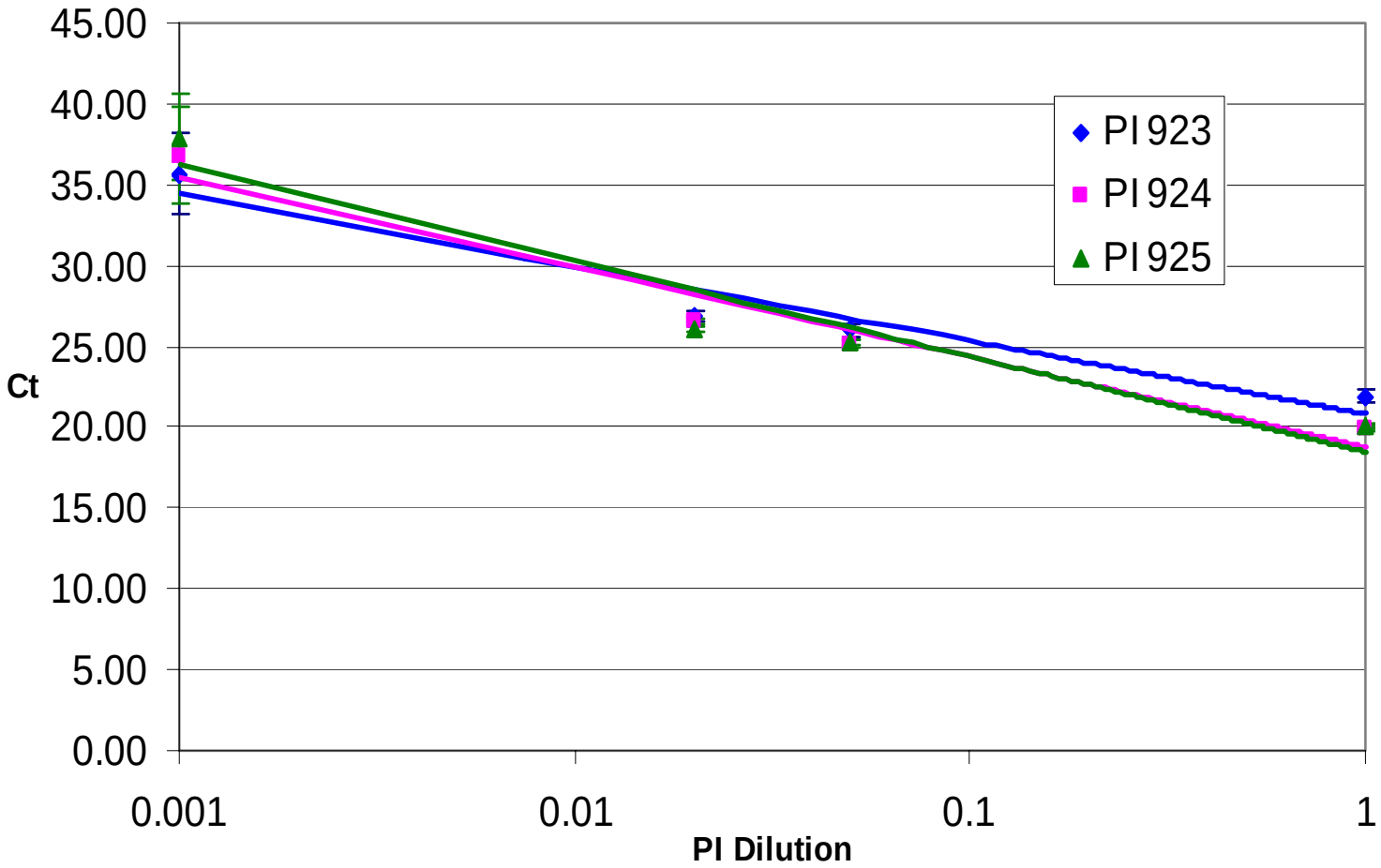
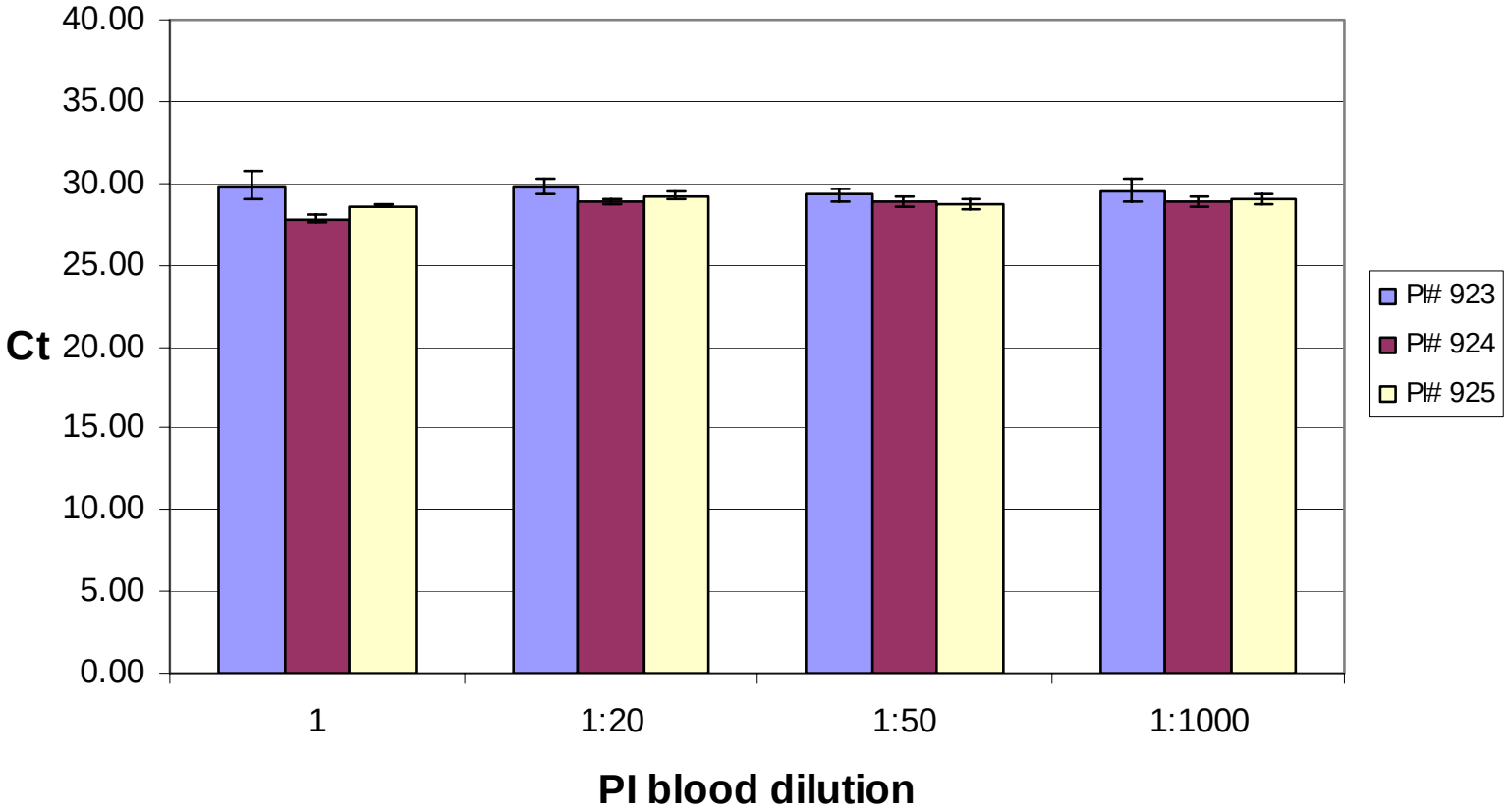
THE RNA COMPANY®

AgPath-ID™ BVDV Detection Sensitivity using Pooled Whole Blood Samples



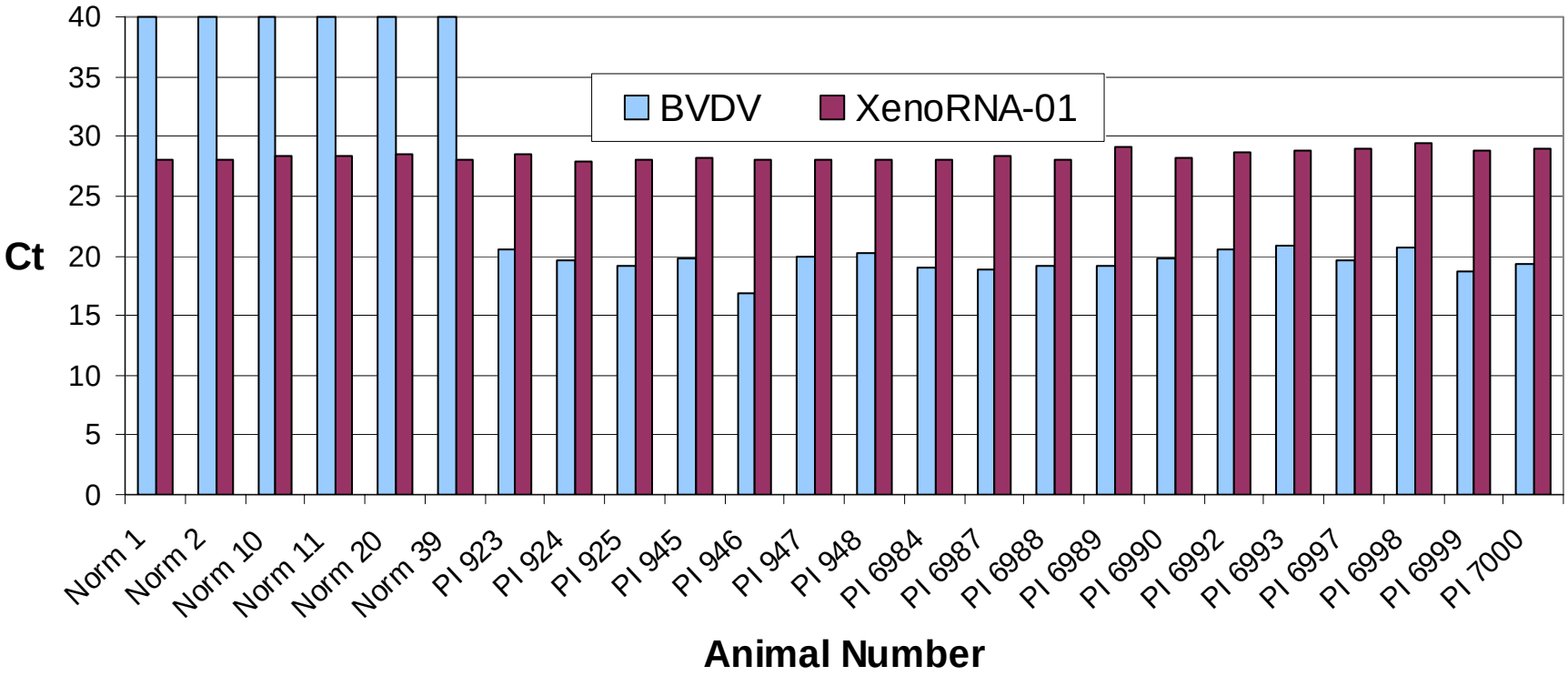
BVDV RNA can be detected in pools of 1000 samples.

No inhibition observed in pooled blood samples



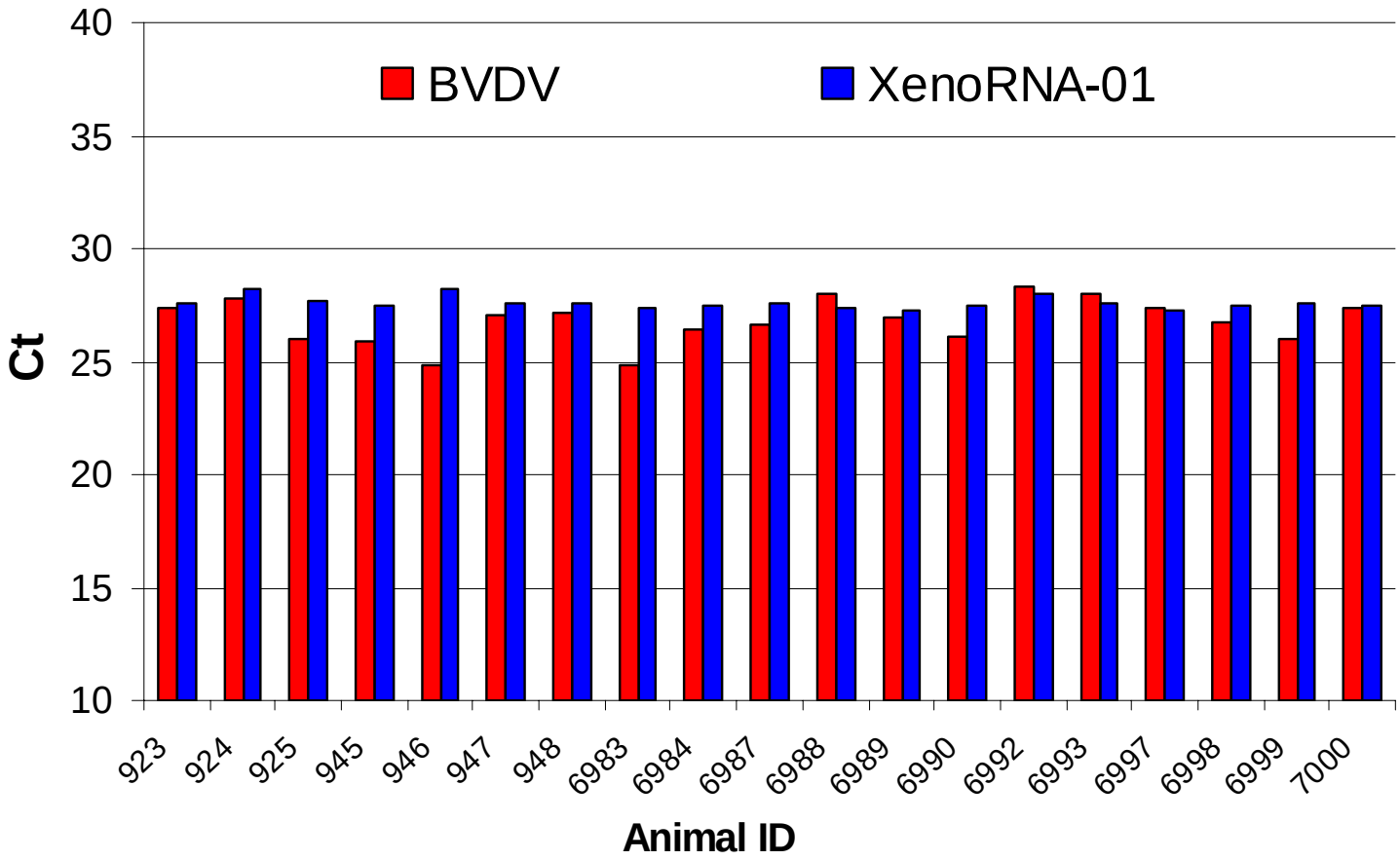
Consistent detection of XenoRNA-01 in pooled blood samples (Avg Ct=29.02+/-0.55)

BVDV Detection using Whole Blood



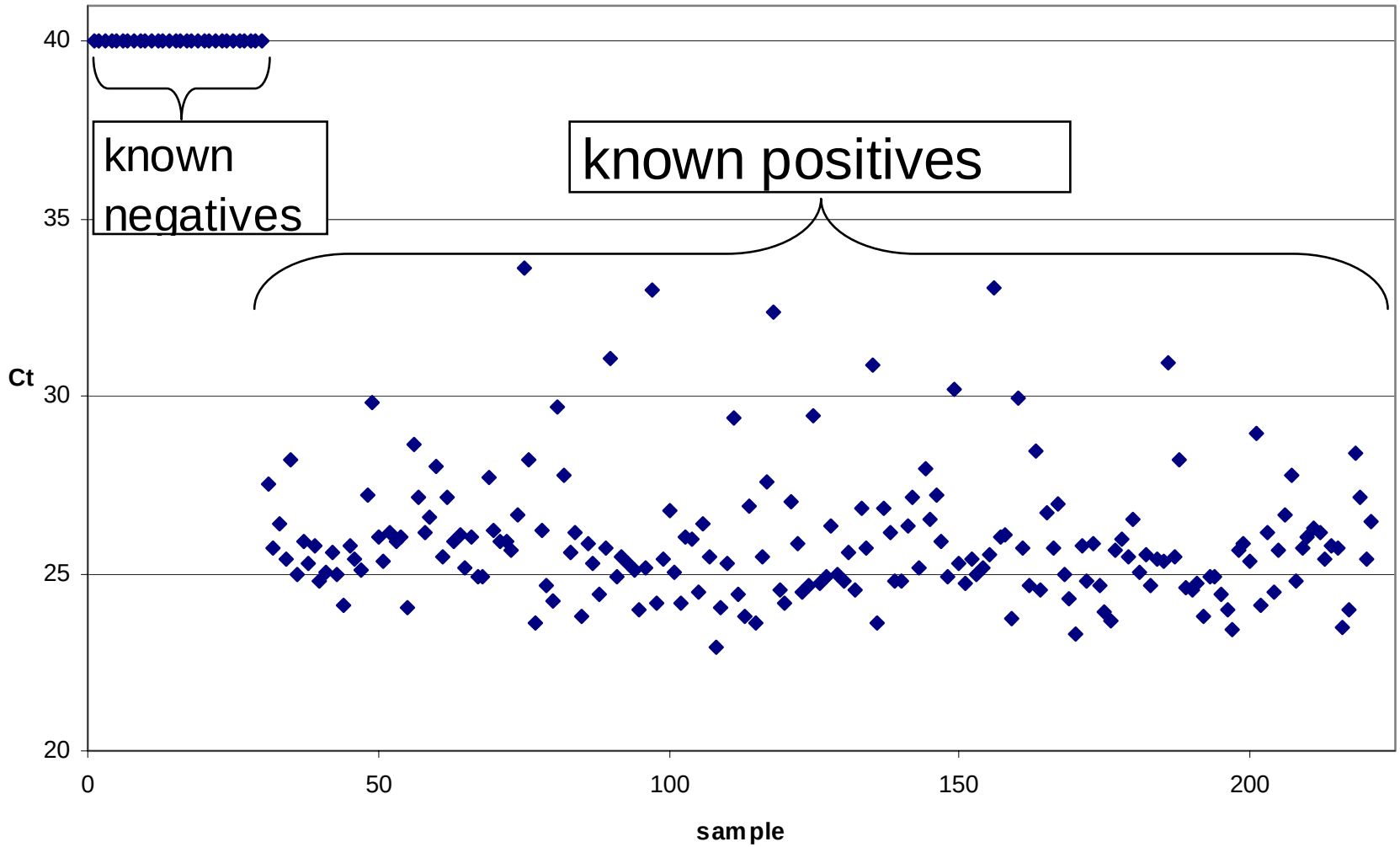
BVDV was successfully detected in all 18 positive samples with no false positive or negative.

BVDV Detection using Ear Notch Supernatant



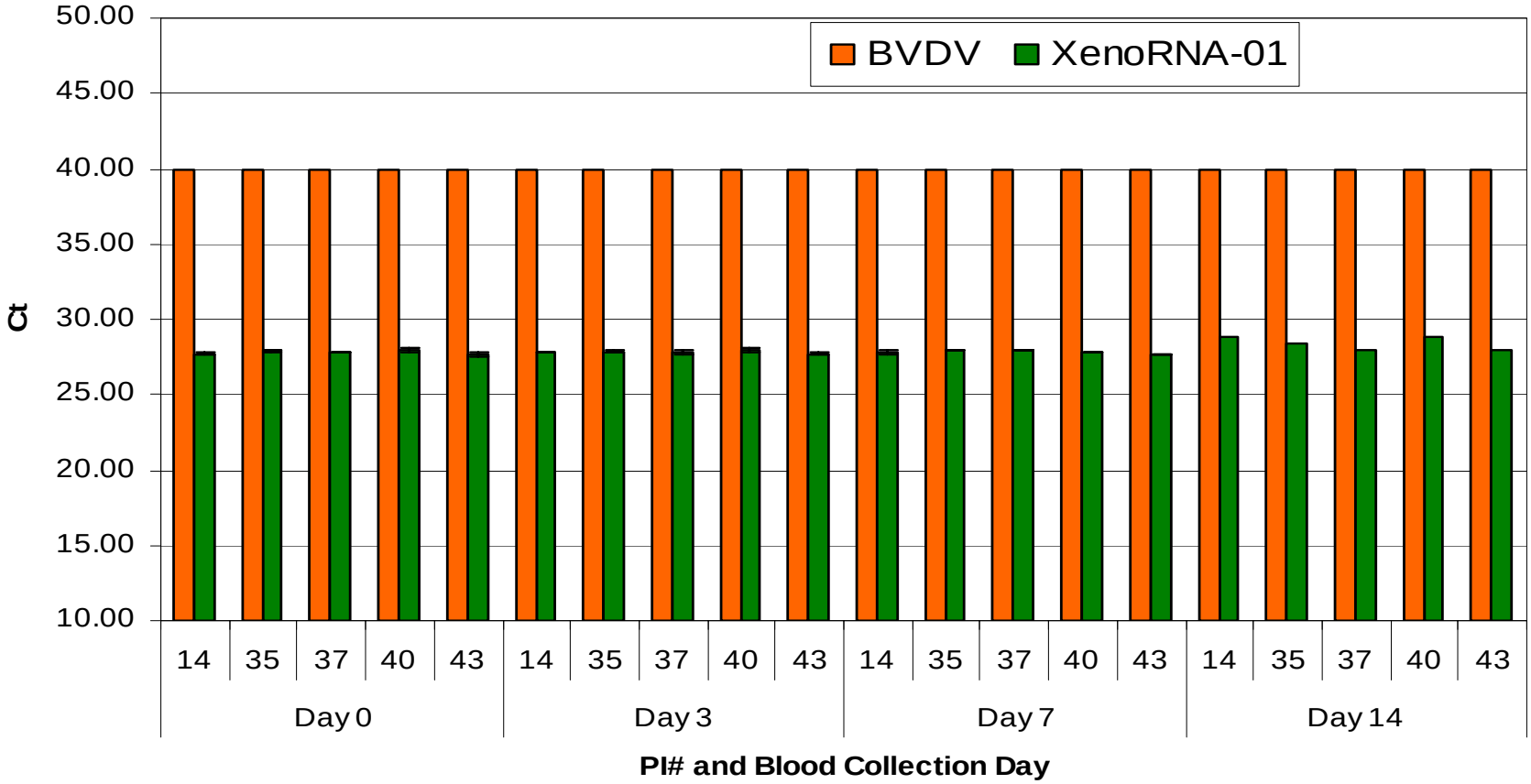
BVDV was detected in all 19 positive samples. Control XenoRNA-01 was consistently detected in all samples (Av. Ct 27.59+/- 0.27).

HT BVDV Detection using Ear Notch Supernatant



Ct of 40 = undetectable

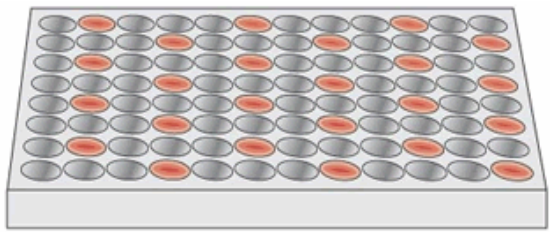
AgPath-ID™ BVDV Kit does not detect BVDV vaccine



5 Non-infected cows were vaccinated with Bovi-Shield® GOLD™ FP 5 L5 Bovine Rhiotracheitis-Virus Diarrhea-Parainfluenza$_3$-Respiratory Syncytial Virus Vaccine on day 0. **All vaccinated cattle tested negative for BVDV at all four time points.**

Ct of 40= Undetectable

HT BVDV RNA Isolation with Zero Cross-Contamination



+ sample (24)

- sample (72)

BVDV	1	2	3	4	5	6	7	8	9	10	11	12
A	***	22.92	***	***	***	22.74	***	***	***	22.91	***	***
B	***	***	***	22.91	***	***	***	23.1	***	***	***	22.77
C	***	23.13	***	***	***	23.17	***	***	***	23.03	***	***
D	***	***	***	23.08	***	***	***	22.69	***	***	***	22.61
E	***	23.18	***	***	***	23.32	***	***	***	22.64	***	***
F	***	***	***	23.26	***	***	***	23.04	***	***	***	22.77
G	***	23.41	***	***	***	23.68	***	***	***	23.37	***	***
H	***	***	***	23.64	***	***	***	23.41	***	***	***	23.11

*** Not Detected; Avg Ct: 23.08; STDEV: 0.30

Efficient and consistent BVDV detection in all positive wells.

Summary

- **AgPath-ID™ BVDV Kit allows rapid and accurate detection of BVDV from diverse sample matrixes**
- **Detection sensitivity < 100 copies of BVDV RNA or 1000-fold dilution of PI whole blood**
- **High sensitivity allows pooling of samples.**
- **No false positives from BVDV vaccine**
- **XenoRNA-01 monitors RNA isolation and qRT-PCR efficiency**

Acknowledgements

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