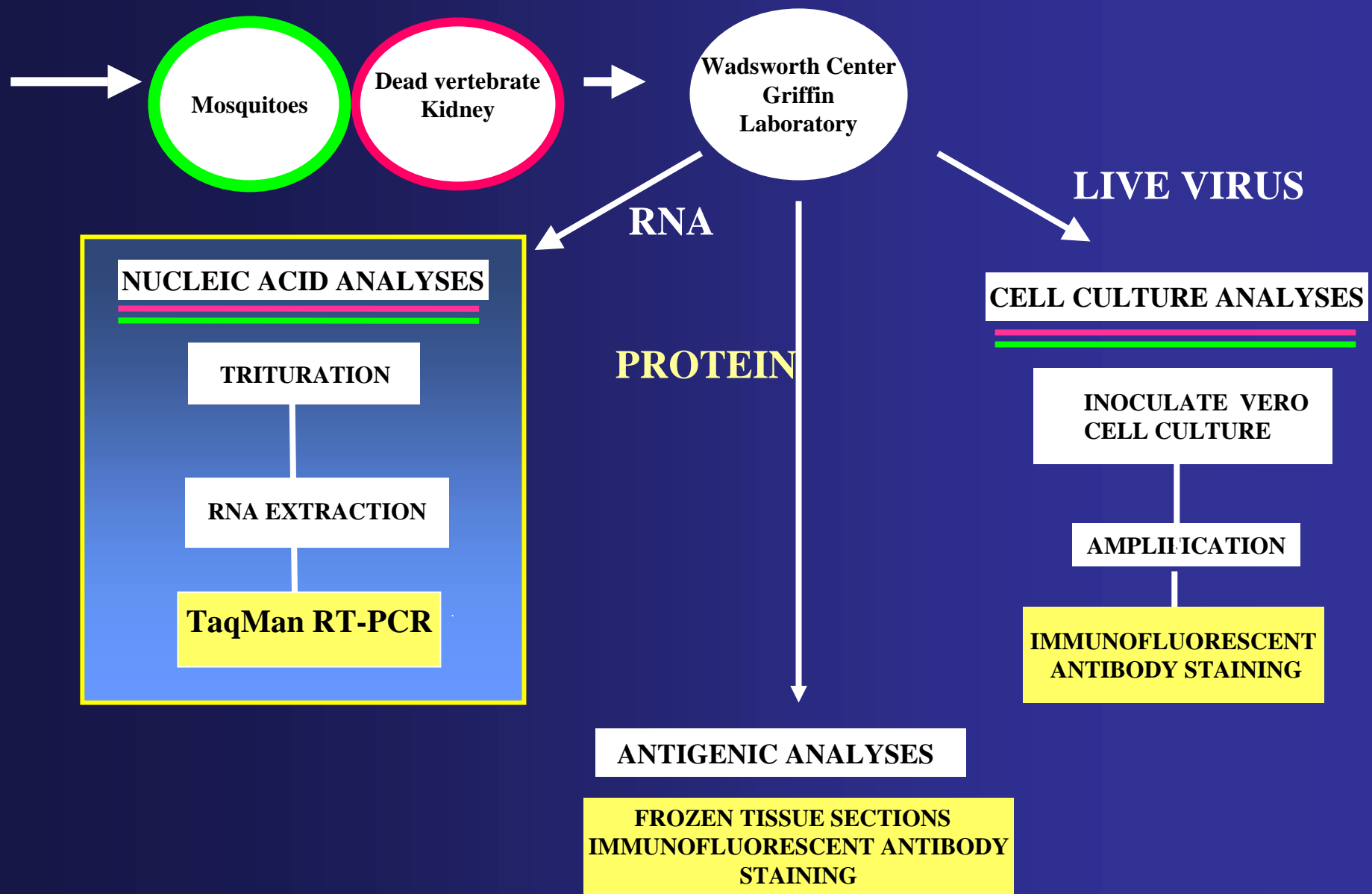


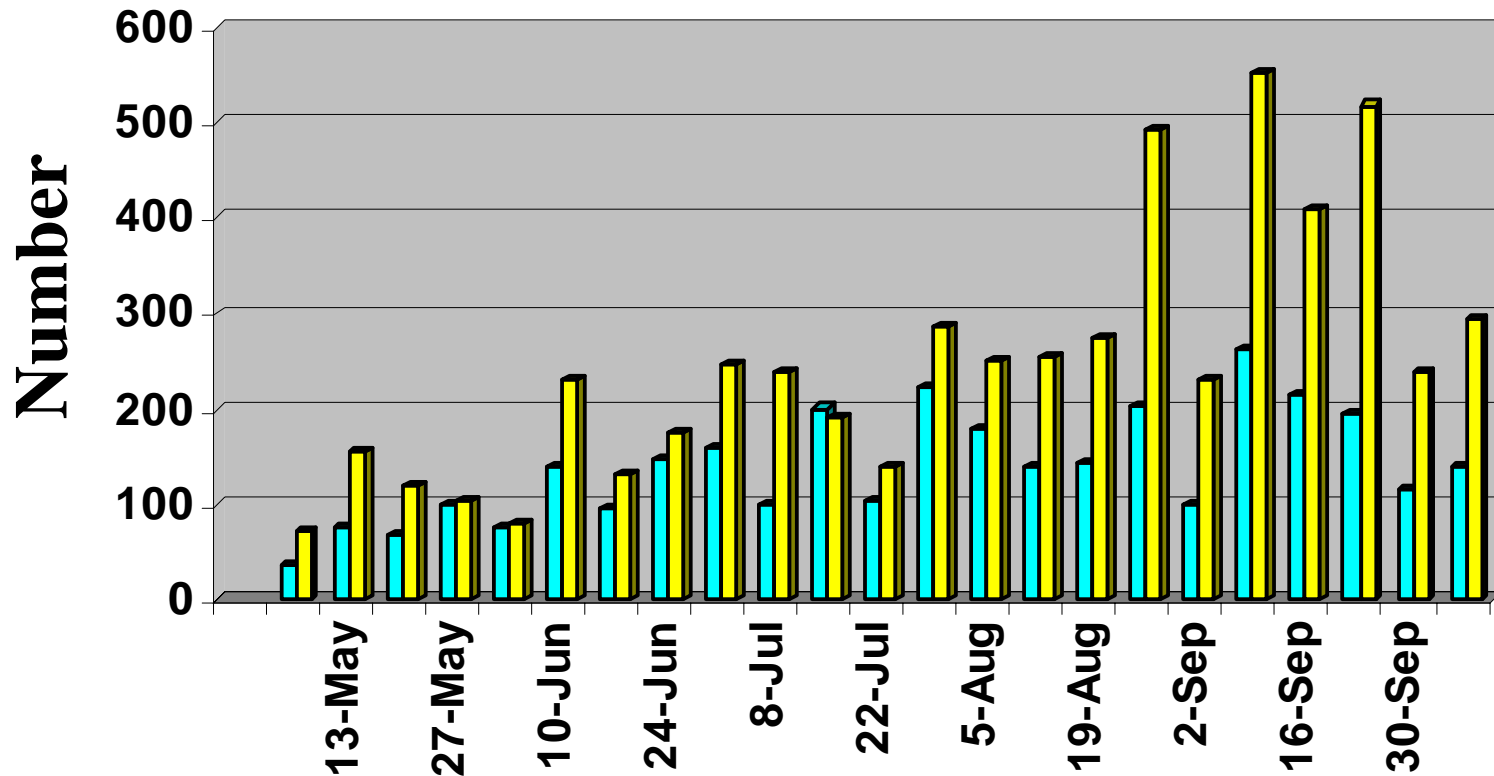
**HIGH THROUGHPUT
TESTING FOR WEST NILE
AND OTHER
ARBOVIRUSES:
A Case Study**

**The Arbovirus Laboratory
Wadsworth Center
New York State Dept of Health**

Flow chart for virologic testing of **vertebrate** and **mosquito** specimens



RT-PCR assays on avian tissue, 2001

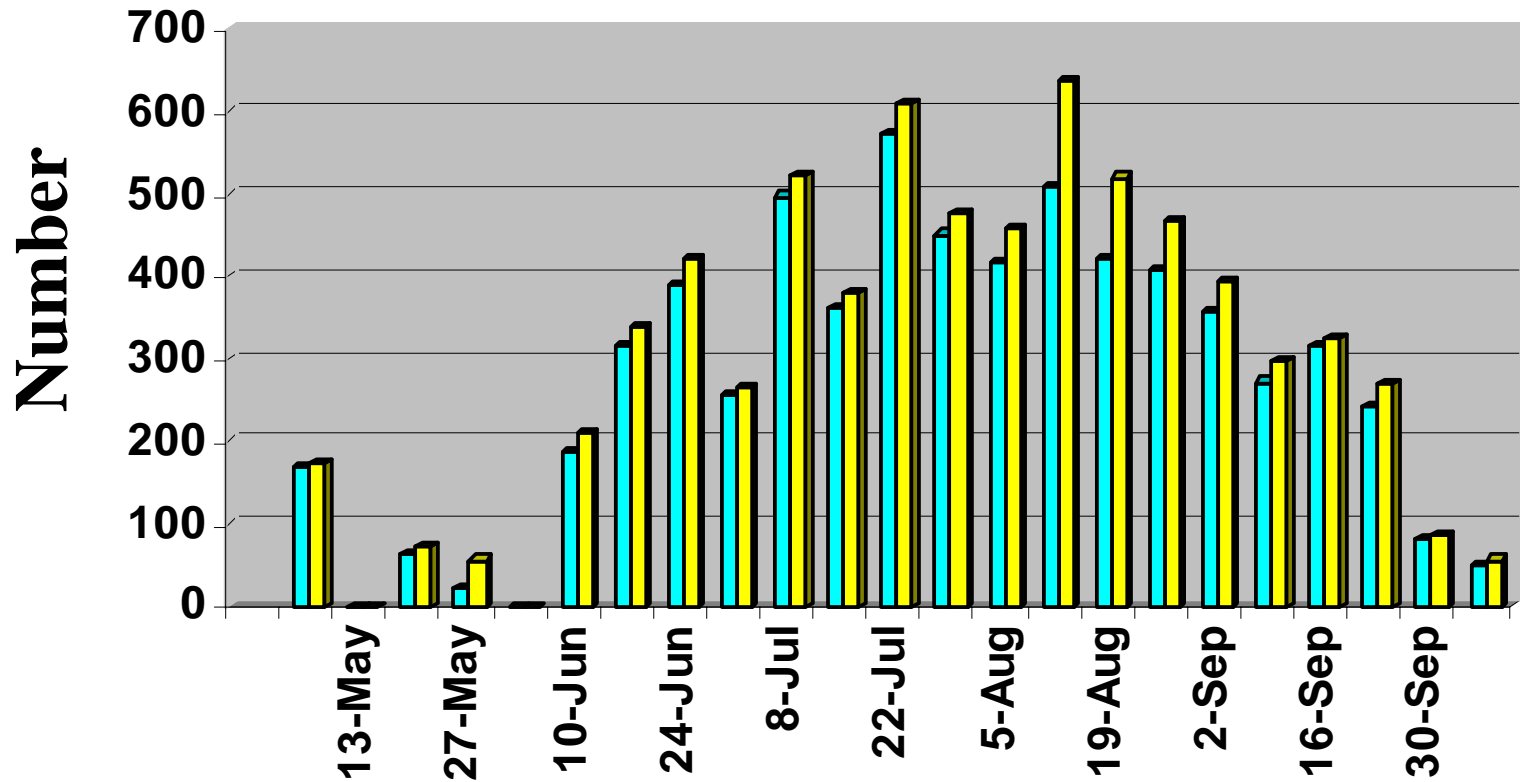


Week of year

Tests done

Birds tested

RT-PCR assays on mosquito pools, 2001



Week of year



Tests done



Mosq pools tested

TRITURATION: Qiagen Mixer Mill MM 300



Disrupts

2 x 96 samples (1.2 ml)

or

2 x 24 samples (2.0 ml)

in 2-4 min

High Throughput Testing

- **Automated Nucleic Acid Workstation**
 - Automates sample and reaction preparation for nucleic acid analysis
 - **increase in productivity**
 - **cost efficient**
 - **high quality of product**
 - **decreased cross-contamination**
 - **consistency and reproducibility**

ABI Prism 6700

- Class II biosafety cabinet enclosure (**HEPA filtered**)
- Sets up **dilutions** and **replicate** samples in up to four 96-well output trays for TaqMan analysis
- **Automatically seals** output trays with a full cover optical blanket and holds them at 4C
- Completely **compatible** with TaqMan sequence detection system without additional manipulation
 - Software synergy
- **One-step process** from RNA purification to assay plate
- **Disadvantage:** Chemistry works on cells only







Protocol | Deckspace | Instrument

Select Protocols

 Lysis/DNA Precipitation

6700 Standard Lysis

View

New

 RNA/DNA Archive

2002 RNA Purification

View

New

 cDNA Archive

6700 Standard cDNA

View

New

 Dilution Archive

1:10 Dilution

View

New

 Assay

Real Neat-1186-3136

View

New

Enter Archive Sample Names

Sample Name:

| Well | Pos. | Name |
|------|------|------------|
| B10 | 22 | 01004511-1 |
| B11 | 23 | 01004512-1 |
| B12 | 24 | 01004513-1 |
| C1 | 25 | 01004514-1 |
| C2 | 26 | 01004525-1 |
| C3 | 27 | 01004526-1 |
| C4 | 28 | 01004527-2 |
| C5 | 29 | 02000089-1 |
| C6 | 30 | 02000090-1 |
| C7 | 31 | 02000091-1 |
| C8 | 32 | 02000092-1 |
| C9 | 33 | B1 |
| C10 | 34 | 02000093-1 |
| C11 | 35 | 02000094-1 |
| C12 | 36 | 02000095-1 |
| D1 | 37 | 02000096-1 |
| D2 | 38 | 02000098-1 |
| D3 | 39 | 02000099-1 |
| D4 | 40 | 02000100-1 |
| D5 | 41 | 02000101-1 |
| D6 | 42 | 02000103-1 |
| D7 | 43 | 02000104-1 |

Select Input Plate Type

Input Plate Type: RNA Archive

Archive Plate Consumable ID:

Select Samples for Assay Protocol

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|----|----|----|----|----|----|----|----|----|----|----|----|
| A | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| B | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| C | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 |
| D | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 |
| E | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 |
| F | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 |
| G | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 |
| H | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 |





Preview Assay Protocol Output

Import Sample Names From File

File Edit View Setup Instrument Help

Protocol | Deckspace | Instrument

Turn Off Peltiers

 Start Pause**Instrument Status** Archive and Lysis Plates: 4 °C Assay Plates: 11 °C Master Mix, Standards,
Controls, Dilution Plates: 4 °C

Current Task: Peltier Cooling On

Status: Idle

Time Remaining: 0 Hr 0 Min 0 Sec

Run Log | Protocol Detail | Sample List | Consumable Details

2002-03-04, 01:07:43 PM - Assay Station temp: 11 °C. Dilution Station temp: 3 °C. Archive Station temp: 4 °C.
2002-03-04, 01:12:50 PM - Assay Station temp: 11 °C. Dilution Station temp: 4 °C. Archive Station temp: 4 °C.
2002-03-04, 01:25:22 PM - Begin sealing Output Plates
2002-03-04, 01:25:22 PM - Waiting for Sealer to come to correct temperature.
2002-03-04, 01:25:30 PM - Plate sealer is heated.
2002-03-04, 01:27:09 PM - Waiting for Sealer to come to correct temperature.
2002-03-04, 01:27:26 PM - Plate sealer is heated.
2002-03-04, 01:29:06 PM - Completed Sealing Output Plates.
2002-03-04, 01:29:06 PM - Completed placing Samples, Standards and Controls.
2002-03-04, 01:29:06 PM - Assay Protocol Complete
2002-03-04, 01:29:13 PM - Starting Peltiers cooling.
2002-03-04, 01:45:22 PM - ConductTransaction() failed in ThermalUpdateThread.run(): java.lang.Exception: Time out error. Robot not responding in ReceiveOnly()
2002-03-04, 01:45:28 PM - Peltiers failed to turn off while terminating Peltier thread: Time out error. Robot not responding in ReceiveOnly()



Print Tab

| DESCRIPTION | | ASSAY COST per sample* | PERSON HOURS (Hands on labor)** |
|---|------------------------------|---------------------------------------|--|
| Tissue sorting, excision, homogenization | Birds/Mammals | \$0.75 | 2 hr per 96-well plate |
| | Mosquitoes | \$0.50 | 3 hr per 96-well plate |
| Isolation of RNA | <u>RNeasy Method</u> | | |
| | Birds/Mammals | \$3.25 | 5 h per 96-well plate |
| | Mosquitoes | \$3.25 | 5 h per 96-well plate |
| | <u>ABI 6700 Robot</u> | | |
| | Birds/Mammals | \$2.00 | 1 h per 96-well plate |
| | Mosquitoes | \$2.75 | 1 h per 96-well plate |
| Real-time RT- PCR (TaqMan) | Manual setup | \$3.25 | 1 h per 96-well plate |
| | ABI 6700 Robot | \$3.85 | 30 m per 96-well plate |

* Specific supplies only (no equipment, personnel, general supplies)

**Robot run time: 85 m RNA extraction; 45 m TaqMan set-up

Comparison of TaqMan Ct values on RNA samples from naturally infected bird kidneys

| Crow kidney | ABI Prism 6700 | | RNeasy | | Ratio (6700:RNeasy) |
|-------------|---------------------|-----|---------------------|-----|---------------------|
| | Mean C _t | SD | Mean C _t | SD | |
| A | 14.97 | 0.8 | 16.56 | 0.7 | 0.90 |
| B | 17.85 | 0.6 | 18.45 | 0.6 | 0.97 |
| C | 14.59 | 0.6 | 16.93 | 0.5 | 0.86 |
| D | 14.71 | 0.7 | 16.78 | 0.6 | 0.88 |
| E | 17.57 | 0.4 | 21.95 | 1.3 | 0.80 |
| F | 30.13 | 1.5 | 29.54 | 5.5 | 1.02 |
| G | 40.00 | 0.0 | 40.00 | 0.0 | 1.00 |

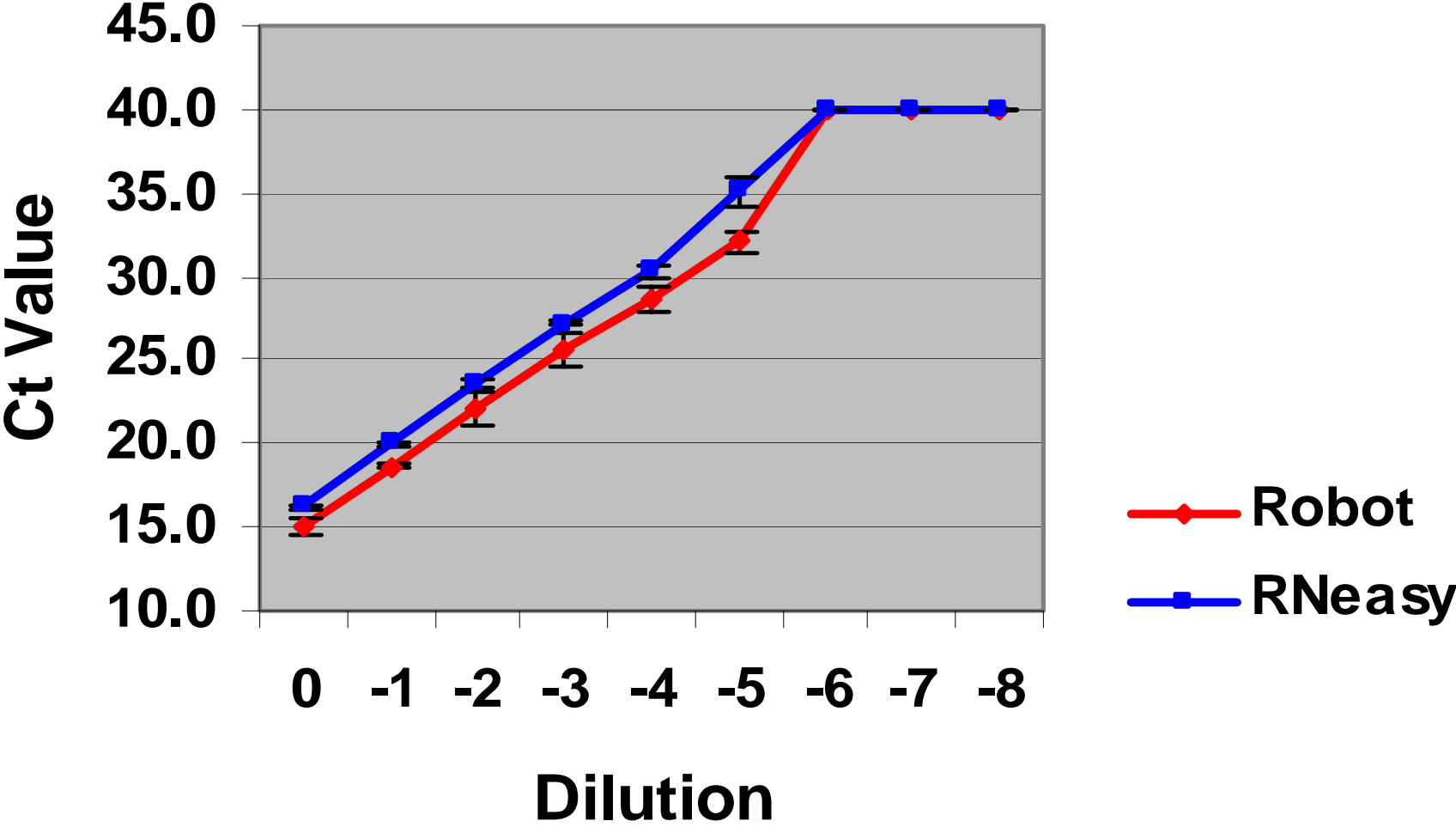
Reproducibility of robotic assays (**INTRA**-assay variability)

| Crow kidney | Ct Values | | | | | |
|----------------|-------------|-----------|-----------|-------------|-----------|-----------|
| | WNV NS1 | | | WNV env | | |
| | <i>Mean</i> | <i>SD</i> | <i>CV</i> | <i>Mean</i> | <i>SD</i> | <i>CV</i> |
| 227 | 16.7 | 0.51 | 3.0% | 15.8 | 0.39 | 2.5% |
| 228 | 16.2 | 0.35 | 2.2% | 15.4 | 0.22 | 1.5% |
| 229 | 15.9 | 0.19 | 1.2% | 15.3 | 0.11 | 0.7% |
| 230 | 15.8 | 0.22 | 1.4% | 15.2 | 0.13 | 0.8% |
| 231 | 16.9 | 0.31 | 1.8% | 16.0 | 0.32 | 2.0% |
| 232 | 17.1 | 0.17 | 1.0% | 16.1 | 0.03 | 0.2% |
| 233 | 16.5 | 0.90 | 5.5% | 15.7 | 0.62 | 3.9% |
| 234 | 15.8 | 0.25 | 1.6% | 15.8 | 0.25 | 1.6% |
| 235 | 16.4 | 0.11 | 0.7% | 16.4 | 0.11 | 0.7% |
| 236 | 16.1 | 0.05 | 0.3% | 16.1 | 0.05 | 0.3% |
| 302 | 16.4 | 0.20 | 1.2% | 16.4 | 0.20 | 1.2% |
| 303 | 16.9 | 0.42 | 2.5% | 16.9 | 0.42 | 2.5% |
| 304 | 16.6 | 0.53 | 3.2% | 16.6 | 0.53 | 3.2% |

Reproducibility of robotic assays (**INTER**-assay variability)

| Bird^a | Tissue Homogenate^b | <i>mean Ct^c</i> | <i>SD^d</i> | <i>%CV^e</i> |
|-------------------------|--------------------------------------|-----------------------------------|------------------------------|-------------------------------|
| 2445 | P126 | 16.1 | 0.94 | 5.9% |
| | P127 | 15.1 | 1.06 | 7.0% |
| | P128 | 16.6 | 0.77 | 4.6% |
| | P129 | 15.8 | 0.59 | 3.7% |
| | P130 | 15.4 | 1.10 | 7.1% |
| 473 | P152 | 18.1 | 0.39 | 2.2% |
| | P153 | 18.4 | 0.51 | 2.8% |
| | P154 | 17.8 | 0.38 | 2.1% |
| | P155 | 18.6 | 0.44 | 2.4% |
| | P156 | 18.3 | 1.51 | 8.2% |
| 3200 | P217 | 15.3 | 0.29 | 1.9% |
| | P218 | 15.7 | 0.30 | 1.9% |
| | P219 | 15.9 | 1.88 | 11.8% |
| | P220 | 15.6 | 1.27 | 8.1% |

Comparison of linearity of assays on avian tissue



Comparative assays on infected mosquito parts*

| Infected Mosquito Part | Ct Value | |
|------------------------|----------|--------|
| | Robot | RNeasy |
| 1 Leg | 40.0 | 32.4 |
| | 31.8 | 31.8 |
| 2 Legs | 29.9 | 30.6 |
| | 30.3 | 32.4 |
| Abdomen | 23.5 | 25.2 |
| Head | 26.7 | 24.7 |
| | 25.9 | 26.5 |
| Thorax | 22.5 | 23.0 |

* Added to pool of 50 uninfected mosquitoes

High Throughput Testing

Qiagen 9604 nucleic acid workstation



QIAGEN 9604 (Lanciotti data)

| | Manual Ext. | Robot | Robot |
|---------------|-------------|-------------|-------------|
| PFU in sample | Nov 8 2000 | nov 14 2000 | nov 13 2000 |
| 1000 | 24.01 | 23.46 | 23.04 |
| 100 | 27.06 | 26.4 | 25.51 |
| 10 | 30.21 | 31.47 | 29.65 |
| 1 | 34.28 | 33.66 | 32.13 |
| 0.1 | 36.18 | 37.69 | 38.35 |
| 0.1 | 36.63 | 37.1 | 36.1 |
| 0.01 | 45 | 39.34 | 40.84 |
| 0.01 | 45 | 45 | 45 |

Summary of High Throughput Techniques

- Submission of sample data to laboratory on Excel spreadsheets
- High capacity mixer mill
- Robotic workstation for RNA extraction and real time RT-PCR setup
- Real time RT-PCR

Arbovirus Laboratory staff

