Real-Time Virus Quantification Using Direct Affinity-Based Detection

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Chief Technology Officer
Small Particle Detection
Why Total Particle Count Matters

Infectivity assays alone don’t provide enough information about viral preparations.

- Evidence that non-infective particles are biologically active
- Emergence of non-infective vaccines - VLPs, recombinant proteins, etc.
- Infective titers and total particle numbers are essential for viral characterization
- Previously not possible due to lack of rapid means of total particle quantification

<table>
<thead>
<tr>
<th>Virus</th>
<th>Particle-to-PFU Ratio (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>20-100</td>
</tr>
<tr>
<td>Alphavirus</td>
<td>1-2</td>
</tr>
<tr>
<td>Herpes Virus</td>
<td>50-200</td>
</tr>
<tr>
<td>Influenza</td>
<td>20-50</td>
</tr>
<tr>
<td>Papillomavirus</td>
<td>10,000</td>
</tr>
<tr>
<td>Polio</td>
<td>30-1,000</td>
</tr>
<tr>
<td>Polyomavirus</td>
<td>38-50</td>
</tr>
<tr>
<td>Simian Virus 40</td>
<td>100-200</td>
</tr>
<tr>
<td>Pox</td>
<td>1-100</td>
</tr>
<tr>
<td>Reovirus</td>
<td>10</td>
</tr>
</tbody>
</table>

Source: http://www.virology.ws/2011/01/21/are-all-virus-particles-infectious/
Traditional Methods of Virus Quantification

Virus Quantification

• Traditional virus quantification:
  • Time-consuming
  • Expensive
  • Technical
  • Variable
  • Subjective
  • 50-70 years old

• Emerging importance of non-infective particles

• New vaccine modalities are non-infective (e.g. VLPs)
## Virus Particle Counting Methods

### Emerging

<table>
<thead>
<tr>
<th>Technique</th>
<th>Precision</th>
<th>Time</th>
<th>Labor</th>
<th>Cost per Sample</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFF-MALS</td>
<td>High</td>
<td>Hours</td>
<td>Medium</td>
<td>Medium</td>
<td>Sensitive, label free</td>
<td>Limited virus data, technical</td>
</tr>
<tr>
<td>TRPS</td>
<td>Medium</td>
<td>Minutes</td>
<td>Low</td>
<td>Low</td>
<td>Multiple metrics like size and charge</td>
<td>Limited virus data, inability to detect empty particles</td>
</tr>
<tr>
<td>NTA</td>
<td>Medium</td>
<td>Hours</td>
<td>Medium</td>
<td>Low</td>
<td>Direct visual measure</td>
<td>Limited virus data, inability to detect empty particles</td>
</tr>
<tr>
<td>Virus Counter®</td>
<td>High</td>
<td>Minutes</td>
<td>Low</td>
<td>Low</td>
<td>Rapid correlation to infectivity, highly validated, biologically relevant</td>
<td>Some sample challenges, use only with intact virus particles</td>
</tr>
</tbody>
</table>

**FFF-MALS** = Field Flow Fractionation-Multi-Angle Light Scattering  
**TRPS** = Tunable Resistive Pulse Sensing  
**NTA** = Nanoparticle Tracking Analysis
Combo Dye – The Dyes

**Combo Dye**

**Nucleic Acid Stain**
- Affinity $\sim 1 \times 10^{-5}$ to $1 \times 10^{-8}$ M

![Chemical structure of a nucleic acid dye]

**Protein Stain**
- Affinity similar to nucleic acid dyes

![Chemical structure of a protein stain]

**Fluorogenic Dyes**
Hydrodynamic Focusing
Optical Sub-System

- Laser
- Excitation Optic
- Collection Optic
- Flow Cell
- Mirror
- Spatial Filter
- Dichroic Mirror
- Bandpass Filter
- Protein / ViroTag® 2 PMT
- Nucleic Acid / ViroTag® 1 PMT

Diagram showing the components of an optical sub-system.
Combo Dye Virus Staining

Fluorogenic Dyes
Additional Detail from Voltage Peak Heights and Widths
Determining total intact particle count is essential since there is growing evidence that the number of non-infectious viral particles is of significant biological importance and can impact both in vitro and in vivo studies.
Success of the Virus Counter / ComboDye


ComboDye – What We’ve Learned

**Competition**

Nucleic Acid Dye

Xanthophylls (egg yolk)

**Salts**

Salt [ ]’s > 60 mM may inhibit Nucleic Acid Dye Binding

**pH**

Affects on Emission Wavelength

**Detergent Micelles**

Activation via Insertion into Hydrophobic regions

**Added (stabilizing) Proteins**

Activate Protein Dye
Rapid Virus Cleanup System

• Originally developed for egg-grown influenza

• Sample cleanup removes interfering impurities for accurate measurement on the Virus Counter

• Demonstrated effective for other growth systems and viruses: Dengue, infectious bronchitis virus, Marek’s virus, norovirus and varicella

• Improves data quality for multiple analytical assays, including Virus Counter and plaque titer

• Both 6-sample and 96-well format kits available
Adding Value and Expanding Utility

**Combo Dye**

- Higher Affinity @1 x 10^{-9} to 1 x 10^{-12} M
  - ~10,000 x higher affinity than ComboDyes
- Highly specific
  - Well studied/published clones, minimal x-reactivity
- Broad Applicability
  - Enveloped Viruses
  - Non-Enveloped Viruses
  - Virus-Like Particles (VLPs)
  - Exosomes
- Limited Sample Challenges
  - High Salt OK
  - Detergents OK
  - Protein OK
- Scalable for simultaneous detection of two serotypes

**Antibodies**

- Great in “clean” systems
  - A. Birch et al. / BioProcessing Journal 13 (2) 32-39
  - C.A. Rossi et al. / Viruses 7 (2015) 857-872
- Limited to Enveloped Viruses
- Limited to Intact Viruses
- Some Sample Challenges
- Antibody-Based Detection
  - Higher Affinity @1 x 10^{-9} to 1 x 10^{-12} M
    - ~10,000 x higher affinity than ComboDyes
  - Highly specific
    - Well studied/published clones, minimal x-reactivity
  - Broad Applicability
    - Enveloped Viruses
    - Non-Enveloped Viruses
    - Virus-Like Particles (VLPs)
    - Exosomes
  - Limited Sample Challenges
    - High Salt OK
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    - Protein OK
  - Scalable for simultaneous detection of two serotypes
• Optimizing virus growth can result in higher yields and a more uniform baculovirus construct in less time

• Using the Virus Counter to quickly analyze:
  • Different growth conditions in real-time
  • Effects of temperature, pH, time and media composition on viral growth
  • Troubleshoot batches with abnormal behavior quickly and without delay
  • Spot constructs with poor or no growth before spending the time trying to use them to express protein
Reprogramming Viruses to Treat Disease

- Includes anticancer oncolytic viruses, viral vectors for gene therapy and viral immunotherapy
- A number of viruses have been used for Virotherapy: Adenovirus, adeno-associated virus, foamy virus, herpes simplex virus, lentivirus, measles, Newcastle virus, poliovirus, pox virus, reovirus, retrovirus, Seneca Valley virus, vaccinia

"Given the potential toxicity of the adenoviral particles themselves, CBER recommends that patient dosing be based on particle number."

Guidance for Human Somatic Cell Therapy & Gene Therapy, FDA Centers for Biologics Evaluation & Research
Initial Considerations, F/P and Concentration

No-Wash Systems

• **F/P = Fluorochrome to Protein (antibody) ratio**, the number of fluorochrome molecules on each antibody
  
  ✓ High enough to cover for the MESF* of the instrument
  ✓ Low enough to prevent false positives and competing background

• **Concentration**
  
  ✓ High enough to cover for the MESF of the instrument
  ✓ Low enough to prevent false positives and competing background
  ✓ Must cover dynamic range of instrument

*Mean Equivalent Soluble Fluorescence*
Anti-Baculovirus gp64 - ViroTag® BCVB (Baculovirus Budded)

Baculovirus – Budded, Expressing gp64

Immuno-Gold Staining of gp64
ViroTag® BCVB vs. ComboDye

Antibody based detection coming out consistently below ComboDye counts i.e., closer to plaque titer numbers.
ViroTag® BCVB Specificity Testing

Anti-gp64, ComboDye vs. Baculovirus and Lenti-virus

Lower Limit of Detection

Log Df

Ab Linearity

\[ y = -0.7681x + 7.2353 \]

\[ R^2 = 0.9968 \]

Ab

CD Expected

LentiVirus Negative Ctrl

Linear (Ab)

Budded virus (BV)
Cap structure
Major envelope glycoprotein gp64
Capsid
Capsid base

Lentivirus
Overcoming Protein Interference with ViroTag® BCVB

Medium Containing FBS

- Decreasing FBS
- Constant FBS
BacMam Particle Enumeration using ViroTag® BCVB

BacMam Gene Delivery System
Testimonial on First ViroTag® Affinity Reagent - Baculovirus

“I think that one major take-home here is that the assay worked so well the first time. That is a major advance and illustrates the robustness of the assay. The assay is simple and straightforward….ComboDye would not work under these conditions because of the high protein level from the serum.”

Chris Kemp, Ph.D.
President, kempbio
Anti-Adenovirus, Hexon Protein - ViroTag® ADVX (Adenovirus Cross (x) Reactive*)

Adenovirus – Hexon Protein, *all Serotypes

Transmission Electron Micrograph of Two Adenovirus Particles
ViroTag® ADVX – A Pan Anti-Adenovirus Reagent

Have also tested adenovirus serotypes 2, 3 and 6 with success (data not shown).
Baculovirus also tested negative (data not shown).
ViroTag® Workflow

Pipet 5 µL of ViroTag® Reagent

Mix and incubate protected from light for 30 minutes

Into sample vials containing 200 µL sample with virus within the dynamic range of the instrument

No Wash Assay!

Analyze
ViroTag® Software
Virus Counter® 3100 Reagent Kits

Virus Counter® 3100 Reagent Kit 200

- Sufficient reagents to process up to 200 samples
- Includes sample vials, combo dyes, controls and fluids
- Orderable with ComboDye or ViroTag®

Virus Counter® 3100 Autosampler Reagent Kit

- Sufficient reagents to process one 96-well plate
- Includes 96-well plate, combo dyes, controls and fluids

Virus Counter® 3100 Reagent Kit 200
Summary - ViroTag®

Direct Antibody-Based Virus Quantification in Minutes!

The ViroTag® detection system provides mission-critical data in real-time.

Features

• Hydrodynamically focused virus stream
• Automated background and threshold setting
• Highly specific and well vetted antibody clones
• All reagents, vials, fluids, controls in one kit
• High precision
• Low cost per sample
• Low assay time

Benefits

• Biologically relevant read-out
• Biologically specific read-out
• Future proofing for coming FDA and other regulatory requirements
Virus Counter® Customers

- **VACCINE MANUFACTURERS**
  - sanofi pasteur
  - MedImmune
  - Protein Sciences Corporation
  - Crucell
  - Novartis
  - redbiotec
  - GeoVax
  - Novavax

- **BIOPHARMACEUTICAL/BIOTECHNOLOGY/CRO/CMO**
  - AMGEN
  - IDT
  - biogen idec
  - Genentech
  - H3 Medigen

- **BIOPROCESS EQUIPMENT/REAGENT MFG.**
  - GE Healthcare
  - proteros
  - Kempbio

- **COLLEGE/UNIVERSITIES**
  - Sun Yat-sen University
  - New York Structural Biology Center
  - Oregon Health & Science University
  - University of Maryland
  - Texas Biomedical Research Institute

- **GOVERNMENT RESEARCH LABS & PRIVATE RESEARCH INSTITUTIONS**
  - FDA
  - National Institutes of Health
  - USAMRRIID
  - United States Army Medical Research Institute of Infectious Diseases
  - Lanzhou Institute of Biological Products Co., Ltd.
QUESTIONS?

See me outside the meeting room!