Virus Detection and Identification: A General Process Overview

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Outline

- Question to address: Why is the virus identification process so lengthy?
- Virus Contaminations, General
- Virus Testing, Detection & Identification
- Conclusions

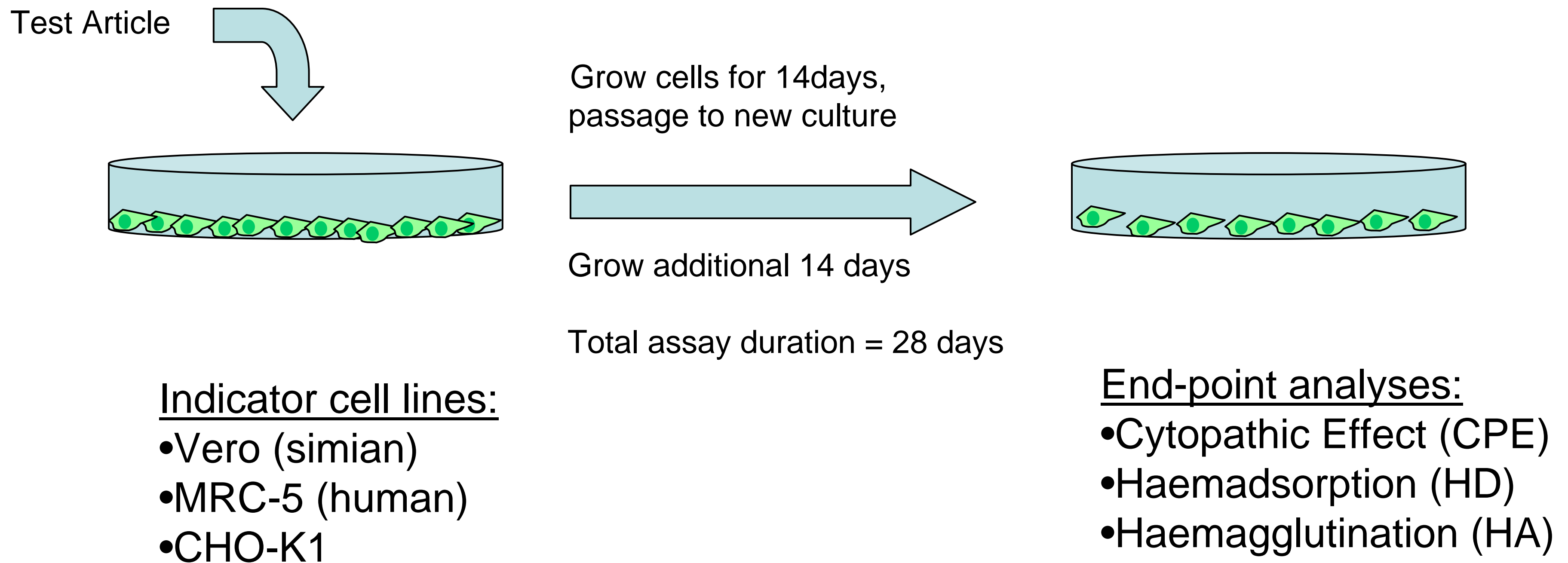
Introduction

- Viral contaminations are rare events in biotechnology industry
- In the last 30 years cases were reported of *viral & viral elements* presence in cell culture processes, final vaccine products, in transplant organs, or in cell based assays.

- Some *published* examples:

  - **MVM** (P. Nettleton, 1980: production BHK)
  - **MVM** (T. Hughes, 1991: production CHO)
  - **MVM** (R. Garnick, 1998: production reactor)
  - **BVDV** (E. Studar, 2002: viral RNA in some human vaccines)
  - **CV 2117** (A. Oehmig, 2003: first ever report of this agent)
  - **ALV/EAR** (A. Hussain, 2003: Avian retroviruses in YF vaccine)
  - **CVV** (R. Nims, 2008: multiple occurrences 2000, 2003, 2004)
  - **ERV** (D. Chen, 2008: serum used in the *in vitro* assay)
  - **CV2117** (Genzyme, 2008-2009: production reactor)
  - **PCV-1, 2** (J. Victoria, 2010: MPS technique applied on rotavirus vaccines)
  - **RD-114** (R. Yoshikawa, 2010: canine vaccines from CRFK cells)
  - **Kodoko, Arenavirus** (3 fatal organ transplants, G. Palacios, 2008, NEJM)

in vitro cell culture assay (ICH Q5, PTC 1993)
- The industry standard for adventitious virus testing



Indicator cell lines:
- Vero (simian)
- MRC-5 (human)
- CHO-K1

In vitro assay is a very valuable tool. It relies on the use of cell culture, enabling amplification & detection of *a live, biologically potent virus*. This represents the most important value of the method.

Virus testing process (cGMP environment)

- Two main phases of the virus testing process:
  - Phase 1: Virus detection and verification of detection
    - Using the current *in vitro* assay with 3 cell types
    - This typically leads to an “OOS” result
  - Phase 2: Virus identification (ID)
    - This is usually handled by way of “OOS investigation”

Phase 1: Virus detection

- The *presence* of a virus in the *in vitro* assay can be detected by visible cellular changes (CPE) or HA/HD using red blood cells from different animal species, or combination thereof
  - 28 days assay duration, or sooner if changes are noted
  - OOS result (‘unexpected’ result)

- Re-testing to confirm (verify) initial observations
  - The same (retention) and different sample(s) at the same laboratory
  - Re-testing at another lab
  - Re-testing using different detector cells
  - Electron microscopy may be indicated
  - *In vivo* assay is occasionally requested at this stage

- Goal of virus detection phase:
  - To generate *unambiguous* results for virus presence
  - This phase can be short, but also rather time consuming if data is ambiguous

Phase 2: Virus identification

- **ID Goal**: To generate *accurate* and *unambiguous* virus identification results

- **Virus Visualization & ID by Electron Microscopy (EM)**
  - *Negative stain* EM: helpful tool, but does not usually identify the virus
  - *Immuno-EM*, using specific anti-viral antibodies, achieves virus ID

- **Identification of Viral Antigens/Proteins:**
  - Immunofluorescent assay, using specific anti-viral antibodies
  - Immunoperoxidase test, using specific anti-viral antibodies
  - Antibody capture ELISA, using specific anti-viral antibodies for plate coating
  - Western Blot (WB) assay, using specific anti-viral antibodies
  - Inhibition of HA or HD, using specific anti-viral antibodies
  - Virus neutralization test, using specific anti-viral antibodies

  - Metaproteomic analysis: looking for viral proteome sequences

Phase 2: Virus identification, cont.

Identification of Viral Nucleic Acids (NA)

A. Conventional methods:

- NA amplification methods (PCR, SDA, 3SR, NASBA, LAMP)
- NA hybridization (FISH, Southern & Northern blot)

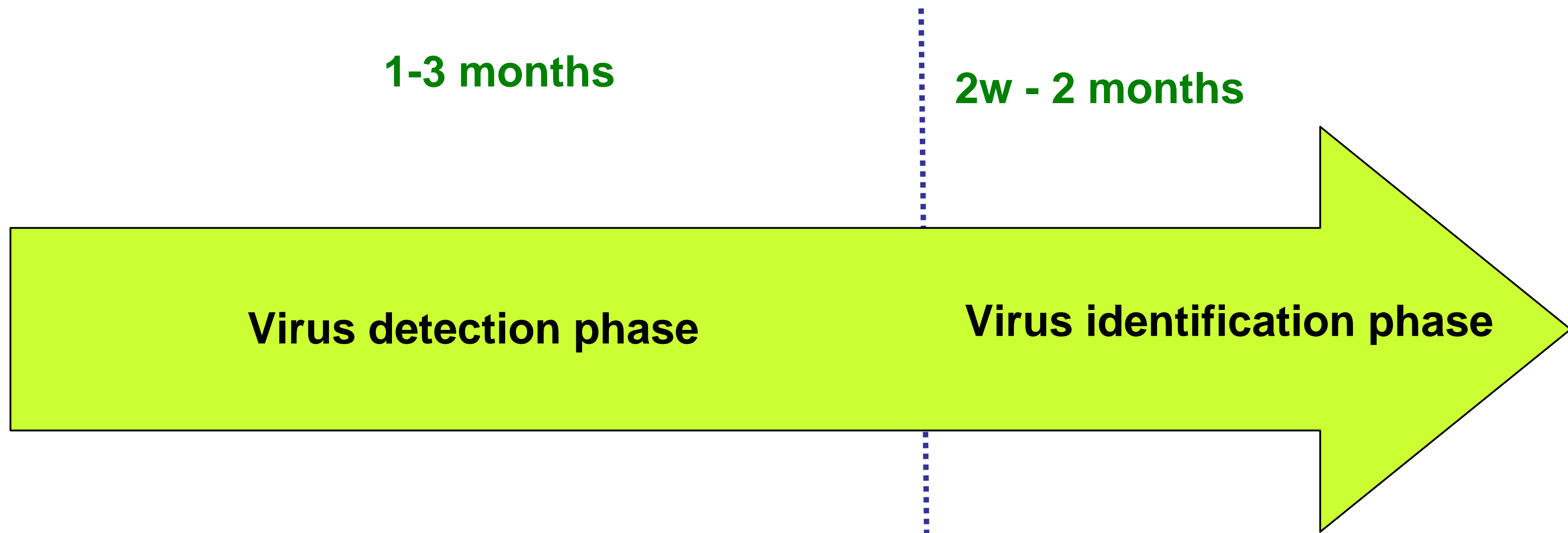
B. Novel techniques:

- Pan-viral microarrays (available in different formats)
- Metagenomic analysis (deep sequencing, MPS)

  – These methods provide fast turnaround, can test for many/all viruses simultaneously, can detect novel un-sequenced agents

  – For routine testing application, solid method understanding, amenability to validate, and established criteria for data handling & interpretation are critical

Virus identification can be a lengthy process



This applies to "cultivable" viruses – those that can infect the cells, replicate, and produce CPE and/or HA/HD

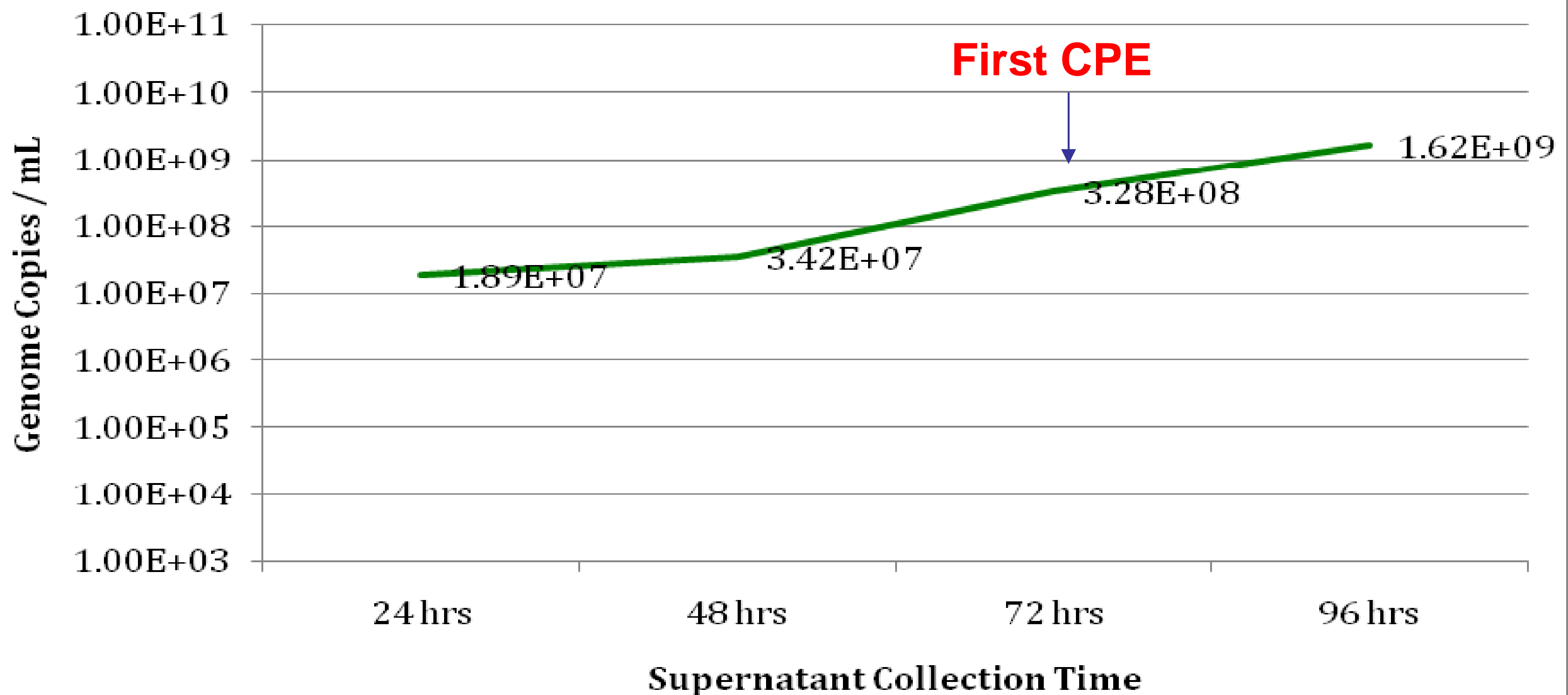
What contributes to the length of virus ID?

- **Assay itself**
  - Design & duration
- **Type of virus & virus strain**
  - Some known viruses are easier to detect & identify (less time involved)
  - Some known viruses are slow growing, produce weak CPE, and weak HA
  - Other viruses produce *neither* visible CPE nor clear HA (can be missed)
  - New or emerging viruses can be encountered – needed more testing time
- **Laboratory factors & GMP compliance factors**
  - Scheduling and turnaround time
  - Availability of ID assays
  - Availability of validated assays
  - Availability of specific reagents
  - Availability of technology platforms
  - Final result reporting and turnaround
- **Human factor**
  - Expertise & training
  - Human errors

Case: Calicivirus (Vesivirus) detection & ID at Genzyme

- “New” agent – only once observed previously in CHO
- Poorly published, not well documented
- No specific detection reagents commercially available
- Weakly cytophatic in cell culture, unless well adapted
- Produces weak but consistent HA with select animal RBC

VV2117: In Vitro virus kinetics in roller bottles, lab adapted virus



Vesivirus identification at Genzyme

- In spite of inconsistent results, *In Vitro* assay, coupled with additional cell culture investigation, helped to *detect* a virus.
- *Verification* of initial virus detection was also inconsistent
- Virus *identity* was achieved in-house by two orthogonal methods:
  - Viral proteome analysis: Nano-LC/MS/MS technology
  - Nucleic acid ID: Virus identity was confirmed by RT-PCR

Limitations of the current *In Vitro* assay format

- Three cell lines used
- Detector cells employed in the assay do not detect all viruses
- Current assay end points can be weak, subjective and hard to observe
- Many viruses produce neither CPE nor HD/HA – these will be missed
- Assay reagents can affect cell growth and interfere with virus
  - Media composition, serum supplement
  - Cells: seeding density, growth, health, age, cell density at inoculation
  - Variability of red blood cells
- Sample type, volume and handling can impact virus detection
- Inter-laboratory variations in assay details

Conclusions

- cGMP virus testing relies on the current 28-day cell based *In vitro* cell culture-based assay as the main testing protocol
- Some viruses can be detected more rapidly than others
- Some viruses may pass undetected because they produce neither CPE or HA/HD in the *in vitro* cell culture assay
- Virus identification can be a time consuming process that must rely on the application of the best scientific principles and in conformance with GLP/GMP standards in order to ensure unambiguous virus detection and accurate virus identification/confirmation

Conclusions

- Employment of well trained virologists can help shorten the "OOS investigation" time

- Future generation of methods for virus testing of biotech products should take advantage of novel virus detection technologies, used either as stand alone methods or in conjunction with *in vitro* cell culture as better assay end points.

Thank You

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