Use of NGS in adventitious virus screening of biological medicinal products: Regulatory requirements

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Disclaimer

- Koen Brusselmans is a Quality assessor for biological medicines at Sciensano (Institute of Public Health Belgium).
- performs assessments and provides advice for:
  - Belgian Medicines Agency (FAMHP)
  - European Medicines Agency (EMA).
- Koen Brusselmans is member of the Biologics Working Party (BWP) at the EMA.
- This presentation represents a personal view and may not necessarily reflect the official position of the FAMHP, EMA, BWP, CHMP, EDQM, WHO and/or any other regulatory authorities.

Introduction

- Several guidelines describe the test methods for virus screening:
  ICH Q5A (viral safety of biotech products), Ph.Eur.2.6.16 (viral vaccines)
  - *In vitro* tests: inoculation on different cell lines (with broad/different tropism to cover a wide range of viruses).
  - *In vivo* tests in (suckling) mice and embryonated eggs; antibody production tests in rodents.
  - Retrovirus: RT activity, electron microscopy, infectivity.
  - Specific virus tests (risk-based): PCR.

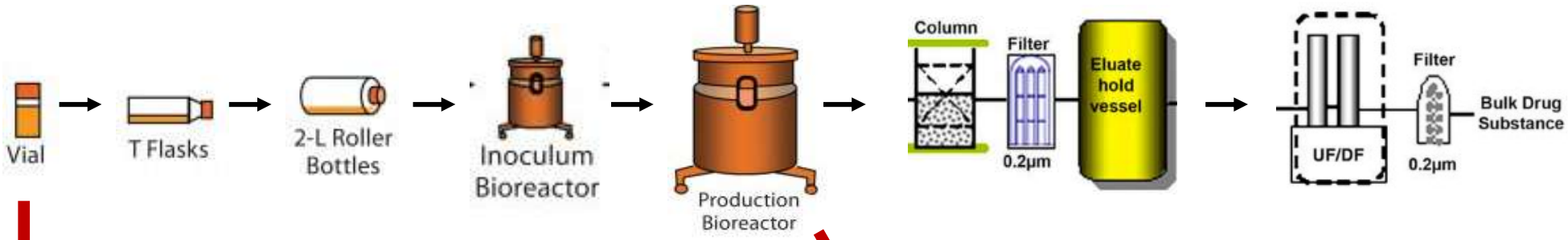
Use of NGS in virus screening

Several guidelines mention the use of new techniques, including NGS.

- Ph.Eur.2.6.16 (viral vaccines)
- Ph.Eur.5.2.14 (Substitution of *in vivo* methods by *in vitro* methods for the quality control of vaccines: Detection of viral extraneous agents by novel molecular methods)
- ICH Q5A (R1)
- Draft ICH Q5A(R2)

- NGS is acceptable to complement or replace existing virus screening methods.
- Current guidelines provide only limited information on NGS method validation.
- What is considered adequate and sufficient for NGS method validation ?
  - > **depends on the intended purpose**.

Use of NGS in virus screening



Upstream process

Production

purification/filling

Starting material

Crude harvest

During the last 5-10 years, NGS is frequently proposed :

1) As a supplementary test in addition to the classic test methods.
2) To replace the *in vivo* virus tests for qualification of starting materials (cell bank ,virus bank).
3) To replace *in vitro* virus tests (and specific PCR tests) for qualification of starting materials (cell bank ,virus bank).
4) To replace virus screening of the crude harvest (usually *in vitro* virus test).

Validation requirements for NGS

Case 1: NGS is used as a supplementary test for qualification of starting materials (cell bank/virus bank).

- NGS is only performed as supplementary test to gain additional information.
- As cell/virus bank has been sufficiently qualified according to applicable guidelines (using classic test methods), no specific requirements are expected for the NGS.

Validation requirements for NGS

Case 2: NGS is proposed to replace the *in vivo* virus testing for qualification of starting materials (cell bank/virus bank).

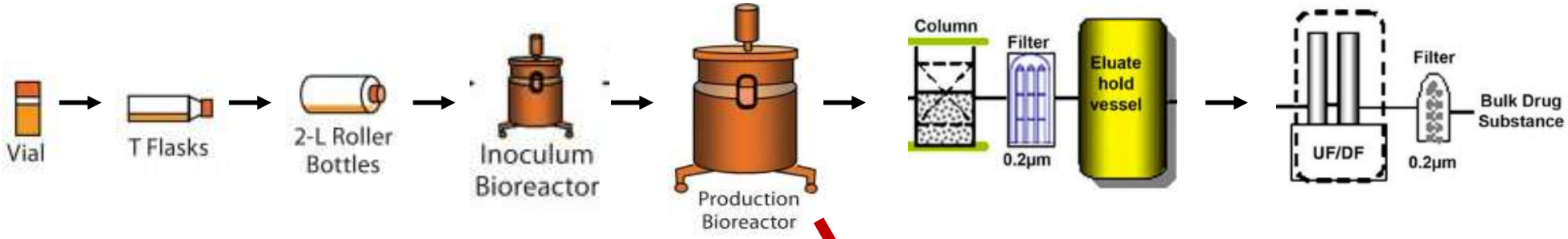
- *In vivo* testing > time consuming, costly, not animal-friendly, high variability. NGS is considered a suitable alternative.
  EU: it is recommended to replace *in vivo virus* testing by NGS.
- NGS needs to be validated.
  Validation should cover: Sample preparation, library preparation, sequencing and data analysis.
- Spiking experiments are needed with properly justified model viruses.
- Sensitivity of the NGS method should be evaluated. Comparison with classic *in vivo* screening methods is recommended. No head-to-head comparison.

Validation requirements for NGS

Case 3: NGS is proposed to replace the *in vitro* and *in vivo* virus testing (and specific NAT tests) for qualification of starting materials (cell bank/virus bank).

- NGS as only screening method > possible according to current guidelines.
- NGS validation may be more challenging.
  NGS should at least be equivalent to *in vitro* testing.
- Spiking experiments should include a broader virus panel.
- Recommended to compare NGS sensitivity with that of the *in vitro* test methods. Head-to-head comparison not strictly needed.
  Sensitivity should be sufficiently high for broad panel of model viruses.

Validation requirements for NGS



Upstream process | Production | purification/filling

Crude harvest

Case 4: NGS is proposed to replace the *in vitro* virus testing on the harvest.

- Similar to case 3 (NGS to replace *in vitro* testing on starting materials): also in this case, the NGS validation may be more challenging.
- NGS should at least be equivalent to *in vitro* testing.
  Spiking experiments should include a broader virus panel.
  Head-to-head comparison not strictly needed.
  Sensitivity should be sufficiently high for broad panel of model viruses.

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Sensitivity and breadth of detection of high-throughput sequencing for adventitious virus detection

Robert L. Charlebois[1], Sarmitha Sathiamoorthy[2], Carine Logvinoff[3], Lucy Gisonni-Lex[1], Laurent Mallet[3] and Siemon H. S. Ng[1]✉

High-throughput sequencing (HTS) is capable of broad virus detection encompassing both known and unknown adventitious viruses in a variety of sample matrices. We describe the development of a general-purpose HTS-based method for the detection of adventitious viruses. Performance was evaluated using 16 viruses equivalent to well-characterized National Institutes of Health (NIH) virus stocks and another six viruses of interest. A viral vaccine crude harvest and a cell substrate matrix were spiked with 22 viruses. Specificity was demonstrated for all 22 viruses at the species level. Our method was capable of detecting and identifying adventitious viruses spiked at 10^4 genome copies per milliliter in a viral vaccine crude harvest and 0.01 viral genome copies spiked per cell in a cell substrate matrix. Moreover, 9 of the 11 NIH model viruses with published in vivo data were detected by HTS with an equivalent or better sensitivity (in a viral vaccine crude harvest). Our general-purpose HTS method is unbiased and highly sensitive for the detection of adventitious viruses, and has a large breadth of detection, which may obviate the need to perform in vivo testing.

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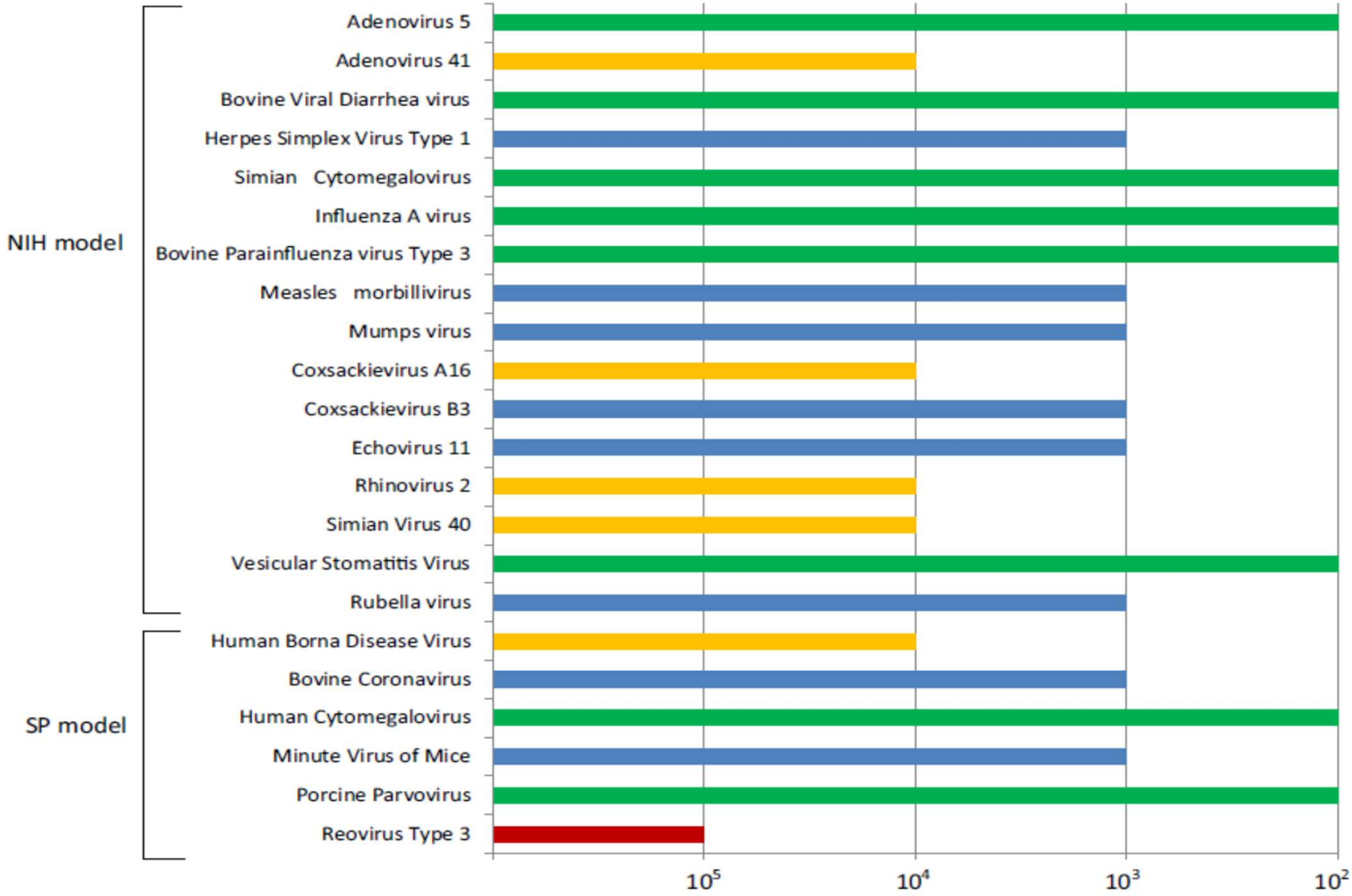
Study by Sanofi: evaluation of NGS method performance for virus screening



NGS data (Sanofi) for 22 model viruses: LOD, genome copies/ml in viral harvest.

Longer bar > better sensitivity. (*Charlebois et al*, 2020)

NGS study *Charlebois et al.*

- Overall, NGS showed good to very good sensitivity for 21 of the 22 viruses.
  Exception: Reovirus (lower sensitivity).

- Comparison of NGS data versus *in vivo* testing:
  NGS was shown to be superior (except for influenza virus and VSV, but also for these 2 viruses the NGS sensitivity was acceptable).

- Based on the published data, this NGS method would most likely be acceptable to replace the *in vivo* virus testing of starting materials (virus seed, cell bank): broad virus range, good sensitivity.

- This study is an excellent example for companies intending to use and validate an NGS method for screening of starting materials.

Conclusion

- NGS is strongly recommended as supplementary test to the classic tests as it combines a broad detection range and (in most cases) a high sensitivity.
- If NGS replaces classic methods, then the NGS method should be validated : Sample preparation, library preparation, sequencing and data analysis.
- NGS is considered a good alternative for *in vivo* virus testing.
- NGS validation is more challenging if intended to replace *in vitro* virus testing.
- Sanofi study (*Charlebois et al.*, 2020): very useful example for NGS validation.
- If NGS shows low sensitivity for particular viruses > include specific NAT test (or an appropriate *in vitro* test).
- If same validated NGS method is used for different matrix > no need to completely repeat NGS validation. Matrix-specific confirmatory validation with a limited but properly justified set of model viruses may be sufficient.
- If NGS generates positive results > infectivity assays needed for confirmation.