Medicines & Healthcare products Regulatory Agency

Setting the scene: Reference standards in the transition from *in vivo* to *in vitro* methods

Biological Reference Materials Webinar Series

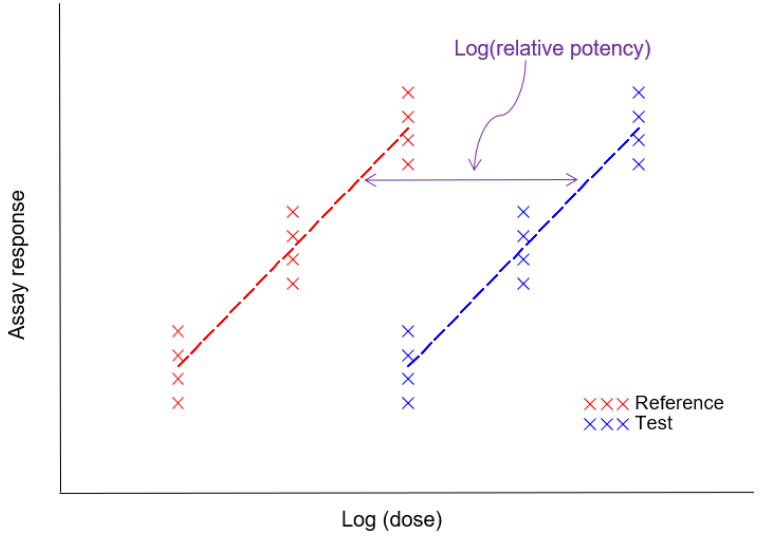


Paul Stickings, MHRA, UK

7 October 2026

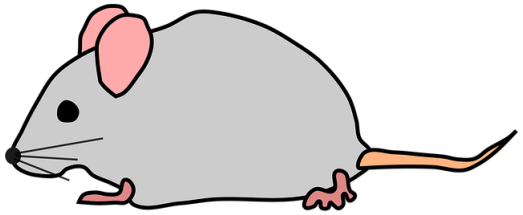
General principles

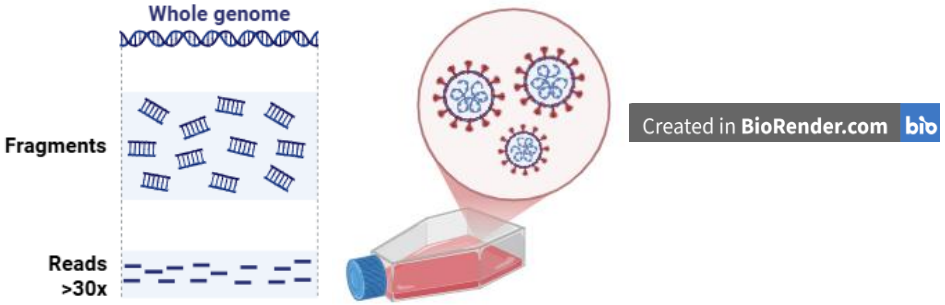
- Many Critical Quality Attributes (CQA) are measured using **comparative** assays where a reference standard is essential
- For some assays, the reference standard may be calibrated against an (external) higher order standard that defines the measurand
- In other cases, where no higher order standard exists, the reference standard will typically be a batch of the material being tested (e.g. a clinical batch or batch representative thereof)
- **Not all assays are comparative** and therefore may not require a reference standard (for the purposes of deriving the sample result)



Reference standards in the transition from..

in vivo to *in vitro*





In the transition from *in vivo* to *in vitro:*

- there may be no change regarding the need for / and the choice of reference standard

Or

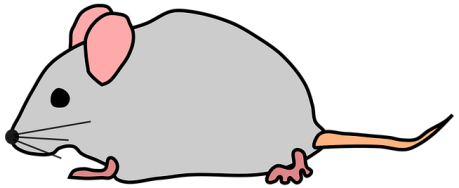
- the reference standard selected for the *in vitro* assay may be different (or perhaps used differently) to the one used for *in vivo* assay

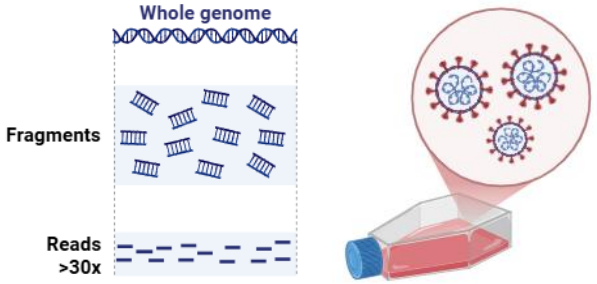
Or

- there may be a need to use a reference standard for the *in vitro* assay where previously one was not needed for the *in vivo* assay (and vice versa)

Example 1: TBEV vaccine potency

in vivo to *in vitro*





Reference standard = Reference standard

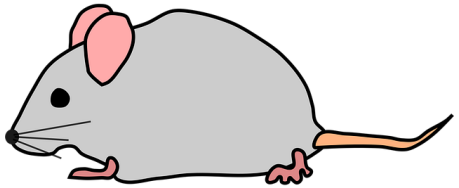
in vivo potency assay in mice
Potency of each batch expressed relative to in-house reference that is representative of the product

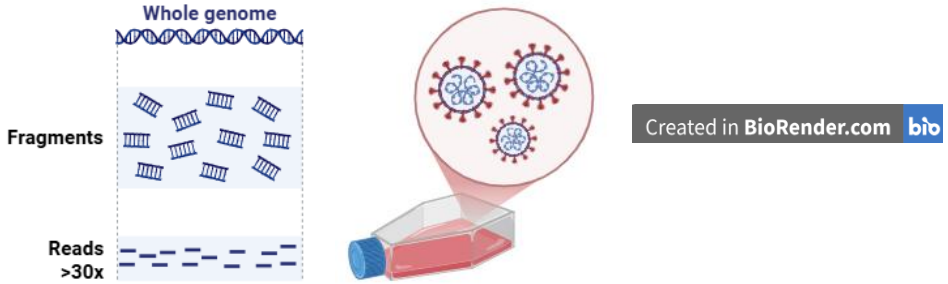
In vitro alternative is an immunoassay
Potency of each batch expressed relative to in-house reference that is representative of the product

The reference standard is **qualitatively similar** to the test sample so its suitability for use is not impacted by the transition from *in vivo* to *in vitro*

Example 2: Tetanus vaccine potency

in vivo to *in vitro*





Reference standard

~

Reference standard

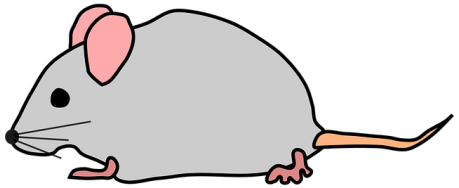
In vivo assay potency is expressed relative to a reference vaccine, calibrated in International Units (sometimes WHO or Pharmacopoeia standard is used directly, sometimes an in-house reference batch calibrated against it)

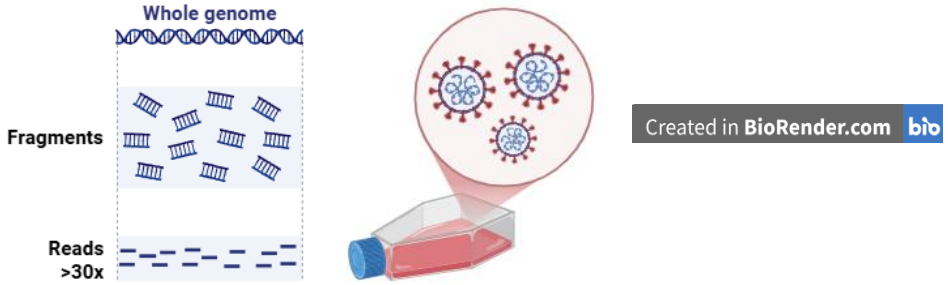
In vitro assay is an immunoassay (e.g. ELISA)
WHO / Pharmacopoeia standard is qualitatively different to the test sample and may not be the optimal choice for the transition to the *in vitro* assay
In vitro assay can be multiplexed where use of an in-house (combination) reference standard is likely to be needed

As with the TBEV example, where an in-house (product specific) reference standard is used for the *in vivo* assay, it will likely be equally suitable for use in the *in vitro* assay **but will be used differently** (no IU for antigenicity)

Example 3: Pyrogenicity

in vivo to *in vitro*





~~Reference standard~~

+

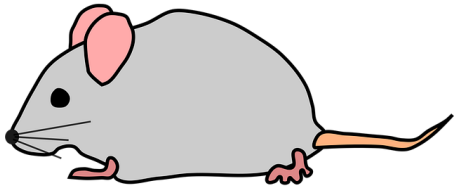
Reference standard

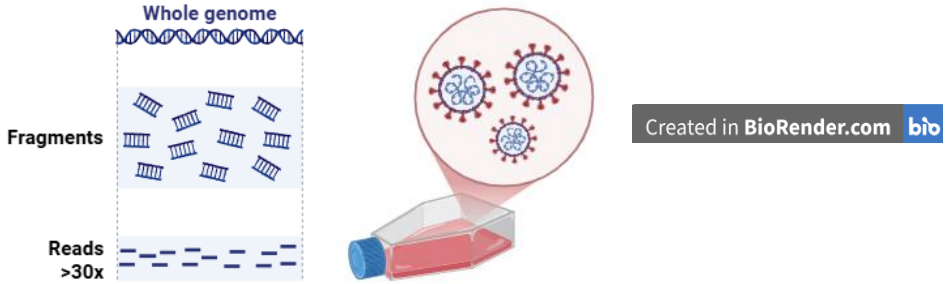
In vivo assay monitors temperature changes in rabbits
Does not require a reference standard

Whether the *in vitro* alternative is to measure/monitor **endotoxin** (e.g. LAL) or **non-endotoxin pyrogens** (e.g. monocyte activation test), a suitable reference standard will be needed

Example 4: Specific toxicity tetanus

in vivo to *in vitro*





Reference standard

Reference standard

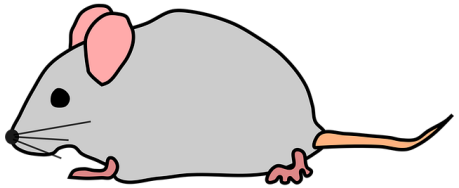
In vivo assay based on monitoring animals for clinical signs of tetanus intoxication – not a comparative method, no reference standard is needed

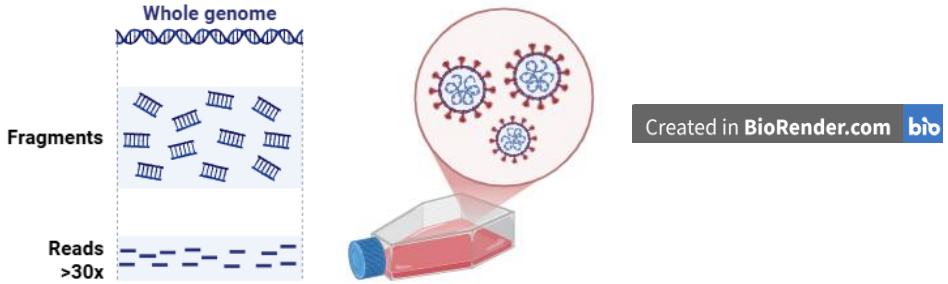
In vitro assays (whether BINACLE type assay or cell-based assay) are also not comparative methods and no reference standard is needed

BUT – the *in vitro* alternatives will need appropriate **control materials** (such as positive control toxin) to monitor sensitivity / consistency of the assay over time

Example 5: OPV neurovirulence

in vivo to *in vitro*





Reference standard

~~Reference standard~~

MNVT and TgmNVT both require an OPV reference standard for the interpretation of the results obtained with the test vaccine

A transition to high throughput sequencing will remove the need for a reference standard

As with other *in vitro* approaches, suitable **control materials** will be needed to ensure validity of molecular methods and to monitor consistency of the assay (this could include use of current *in vivo* OPV reference standard **repurposed and used in a different way** for some *in vitro* approaches or the use of synthetic or plasma derived nucleic acids)

Concluding remarks

- Where *in vitro* assays are being developed as an alternative to current *in vivo* models, the need for suitable reference and/or assay control materials must be considered at the earliest stage
  - The **need for a reference standard**, **what that standard looks like** and **how it is used** ultimately depends on **what is being measured** and **how it is being measured**

- Where reference standards are needed, there is no distinction between *in vitro* and *in vivo* in terms of the requirements for the chosen reference standard to be **well characterised**, **appropriately qualified** and **fit for its intended purpose**

- Wide availability of suitable, well characterised assay control materials can facilitate the transition from *in vivo* to *in vitro* methods and help to maximise impact (in terms of reducing the use of *in vivo* models)

Ongoing efforts at the MHRA

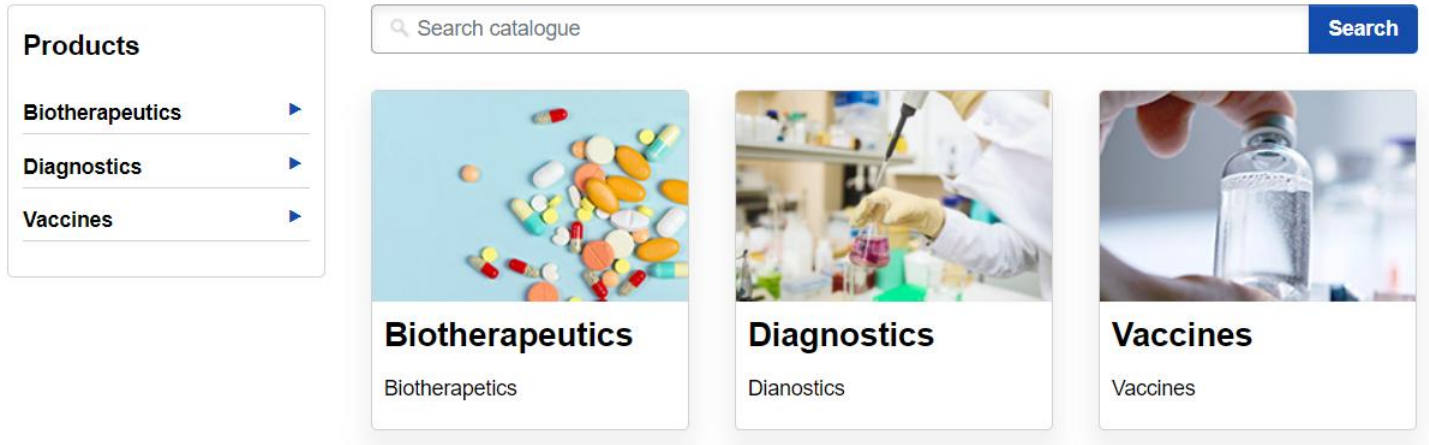
- We have a number of **ongoing projects** to develop new materials that will help support the transition from *in vivo* to *in vitro* methods, including:

- Development of **non-endotoxin pyrogen controls** (TLR agonists) to support development, validation and performance monitoring of monocyte activation test (in collaboration with PEI and EDQM)

- Development of a **stable, lyophilised tetanus toxin** preparation to support development, validation and performance monitoring of *in vitro* alternatives

- Development of **molecular controls for high throughput sequencing technologies** that are used to monitor genetic consistency of oral polio vaccines

Get in touch





Product catalogue (WHO and other standards): www.nibsc.org

Queries related to accounts / ordering: standards@nibsc.org

Express interest in collaborative study participation: paul.stickings@mhra.gov.uk

Copyright information

© Crown copyright 2026

Open Government Licence

Produced by the Medicines and Healthcare products Regulatory Agency (MHRA).

You may re-use this information (excluding logos) free of charge in any format or medium, under the terms of the Open Government Licence. To view this licence, visit http://www.nationalarchives.gov.uk/doc/open-government-licence or email: psi@nationalarchives.gsi.gov.uk

Where we have identified any third-party copyright material you will need to obtain permission from the copyright holders concerned.

The names, images and logos identifying the MHRA are proprietary marks. All logos belonging to the MHRA are registered trademarks and cannot be used without the agency's explicit permission.