



Medicines & Healthcare products
Regulatory Agency

An MHRA perspective on bioassays

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CASSS Bioassays, 16-17th April 2018, Washington DC



What products do we licence? MHRA

Biological Products:

- Products extracted from tissues / blood, e.g. Urokinase;
- Heparins;
- Polyclonal Abs;
- Plasma-derived FVIII;
- Non-genetically engineered vaccines, e.g. flu vaccines;



National / DCP / Mutual
Recognition procedures

Biotechnology Products:

- *E Coli* / CHO derived proteins;
- Advanced Therapy Medicinal Products;
 - Monoclonal antibodies;
 - Rec FVIII;
- Recombinant vaccines, e.g. Gardasil;



Centralised EU licence
mandatory



Content



- General expectations for bioassays
- Potency and effector functions for biosimilars
- New *Ph. Eur.* monographs for etanercept and infliximab
- A regulator's perspective on some novel methods and approaches to potency assays



How is potency defined and where is it reported in dossier?

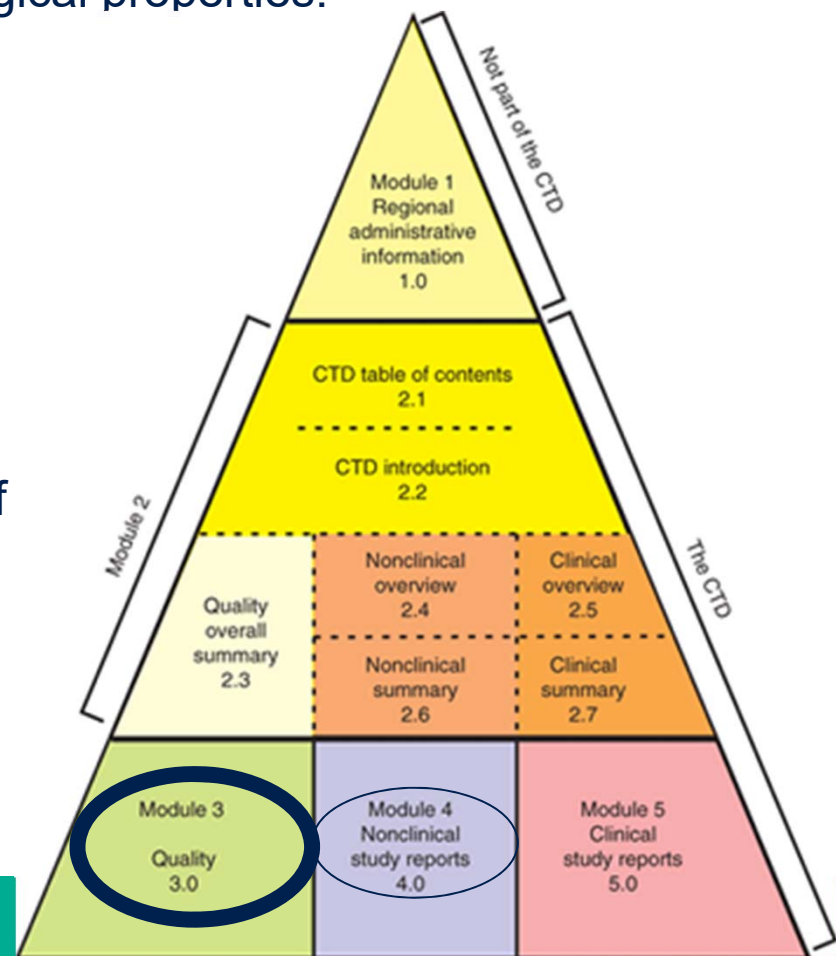
ICH Q6B guidance

Potency = 'The measure of the biological activity using a suitably quantitative biological assay, based on the attribute of the product which is linked to the relevant biological properties.'

CTD:

Information on bioassays should be reported in module 3 section S.3 Characterization and section S.4 Specification.

Bioassays used in the comparability exercise of biosimilars are expected in the regional information section of module 3 (3.2.R).



Bioassay requirements



- **Reflective of MOA**
- **Sensitive to relevant physico-chemical changes**
- **Stability indicating (detection of degradation)**
- **Usable as release assay**
- **Bioassays evolve during lifecycle of product:**



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

14 September 2017
EMA/CHMP/BWP/534898/2008 rev. 1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on the requirements for quality documentation
concerning biological investigational medicinal products in
clinical trials

- **Binding assay may be acceptable to support early clinical phase while a clinically more relevant assay is required by the pivotal clinical trial**
- **Bioassay qualification is sufficient during Phase I and II but extensive validation is required for Phase III and MAA**



Biosimilars



**Guideline
EMA/CHMP/BWP/247713/2012
(quality of biosimilars):**

Comparative evaluation of

- **Biological activity**
- **Physicochemical properties**
- **Purity and impurities**



Together, these demonstrate that quality of the biosimilar product is similar to the reference medicinal product (RMP)



Functional assays for biosimilars



- Multiple MOAs can only be assessed using well-designed assays
- Both Fab and Fc-mediated functions should be evaluated
- Relevance of effector functions is sometimes different for different indications
→ Example adalimumab biosimilars:
 - The target molecule TNF- α in Crohn's disease is membrane-bound
 - Thus, extrapolation to Crohn's disease requires convincing evidence related to these other potential mechanisms, e.g. the binding and effector functions of adalimumab biosimilars in the context of membrane-bound TNF- α .



Biosimilars



- Some changes (chemical or physical) may not necessarily have an impact on potency and effector functions
- However, even minor differences in glycosylation may have substantial effects (e.g., impact of absence of fucose residue on ADCC) → correlation between glycosylation and biological activity not trivial

➤ **What happens if changes in RMP are observed over time (years)?**

- Small changes: part of quality profile
- Clear shift in a particular quality attribute (QA), e.g. glycans
 - Ranges identified before and after the observed shift in quality profile are representative of RMP
 - QA values outside or between the range(s) should be appropriately justified (potential impact)



Considerations



- **Setting of acceptance criteria to be derived from the data pool obtained from the reference product**
- **Representative profile of reference product on the market**
 - As many batches as possible, over the longest period of time (multiple different batches/ages)
 - If only a few batches analysed, narrower QTPP?
- **Biosimilar comparability exercise**
 - Range of quality attributes based on RMP data
 - Arguments for mean $\pm$ nSD or tolerance intervals not usually accepted
- **Statistical approaches for calculation of comparability ranges to be justified**

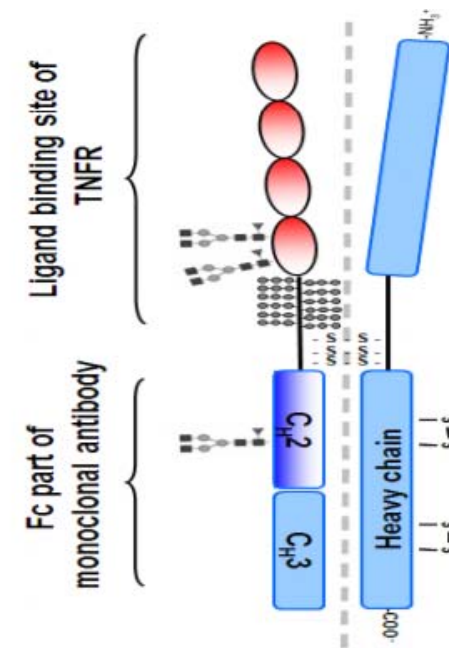


Etanercept biosimilars: *Erelzi* (source: EPAR)



Bioassays comparing Enbrel/EU, Enbrel/US and Erelzi were conducted to investigate:

- Binding and functional neutralization of TNF α and LT α in reporter gene assay
- Binding to TNF α using SPR
- Binding to human Fc receptors (Fc γ RI, Fc γ RII, Fc γ RIII and neonatal Fc receptor (FcRn)) using SPR
- Binding to C1q using ELISA
- TNF α neutralization in a cell based assay using the ability of etanercept to inhibit TNF α -mediated apoptosis.
- The ability of etanercept to initiate ADCC- and CDC-mediated depletion of transmembrane TNF α -expressing target cells using suitable cell-based assays.
- Binding to tmTNF α on stably tmTNF-transfected human cell lines.
- Caspase induction in tmTNF-transfected cell lines



**Schematic representation of etanercept
(from Erelzi EPAR)**



Etanercept biosimilars: *Erelzi* (source: EPAR)



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Table 4: Binding assays and in-vitro bioassays

	Test	Method / cell line	Key findings
Binding assays	TNF- α binding assay	Surface plasmon resonance assay	Comparable potency
	Fc γ RIIIa (F158 and V158) binding assay	Surface plasmon resonance assay	Comparable K_D
	Fc γ RIIIb binding assay	Surface plasmon resonance assay	Comparable K_D
	Fc γ RIIa binding assay	Surface plasmon resonance assay	Comparable K_D
	Fc γ RIIb binding assay	Surface plasmon resonance assay	Comparable K_D
	Fc γ RIa binding assay	Surface plasmon resonance assay	Comparable K_D



Etanercept biosimilars: *Erelzi* (source: EPAR)



Table 4: Binding assays and in-vitro bioassays

	Test	Method / cell line	Key findings
In-vitro bioassays	FcRn binding assay	Surface plasmon resonance assay	Comparable K_D
	C1q binding	C1q binding ELISA	Comparable binding
	TNF- α neutralization reporter gene assay	Luciferase reporter gene assay	Comparable potency
	TNF- β neutralization reporter gene assay	Luciferase reporter gene assay	Comparable potency
	Apoptosis inhibition assay	Cell based apoptosis assay	Comparable inhibition
	ADCC assay	Cell based ADCC assay, ADCC surrogate assay	ADCC activity of Erelzi lower than ADCC activity of Enbrel ¹⁾
	CDC assay	Cell based CDC assay	Slightly outside the range for CDC activity

¹⁾ It has been established in previous comparability assessments that Erelzi shows a lower activity in assays quantifying ADCC activity than Enbrel. This difference is consistent with differences in N-glycosylation and is considered to have no adverse impact on patient safety and efficacy, as ADCC not considered a mode of action in indications for which EU-authorized Enbrel is currently licensed.



Conclusions



- ***Erelzi* binding assays and in vitro bioassays showed high similarity; however:**
- **Quantitative differences in N-glycans were observed in the head-to-head analyses for non-fucosylated and alpha-galactosylated N-glycans, as well as for high mannose structures.**
- **Lower ADCC activity was observed for Erelzi compared to Enbrel. In order to exclude a relevant contribution of ADCC, the Applicant submitted appropriate data to justify the claim that neither Erelzi nor Enbrel were able to induce ADCC activity under more physiological conditions.**
- **CDC is also not expected to be involved in the MOA for etanercept. Therefore, the small difference detected between Enbrel and Erelzi is considered clinically irrelevant.**

- **The MA for Erelzi was granted in 2017**

Date of issue of marketing authorisation valid throughout the European Union	23/06/2017
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Etanercept biosimilars: *Benepali* (source: EPAR)



-Measurement of binding to TNF- α and LT α 3 (TNF- β) by the FRET assay shows that the ranges for the binding activity of Benepali and EU Enbrel relative to the bioassay standard are similar.

-The TNF- α neutralisation assay (reporter gene) demonstrates that the potency of Benepali is within the similarity range defined for the similarity exercise.

-A wide range of Fc related biological assays were performed. Minor differences were observed in some assays between Benepali and Enbrel.



Etanercept biosimilars: *Benepali* (source: EPAR)



- The afucosylated glycan content in Benepali is higher than observed for EU Enbrel.
- However, ADCC is not considered to be a MOA of etanercept so these glycan differences are not considered clinically meaningful. The results from ADCC analysis demonstrate similar low ADCC activity between Benepali and Enbrel.
- The neutral galactosylated glycan content in Benepali seems to be more variable than observed for EU Enbrel. However, CDC is not known to be a MOA of etanercept, and the results from analysis demonstrate similar CDC activity between Benepali and Enbrel.

-The MA for Benepali was granted in 2016

Date of issue of <u>marketing</u> <u>authorisation valid</u> throughout the European Union	14/01/2016
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Biosimilars



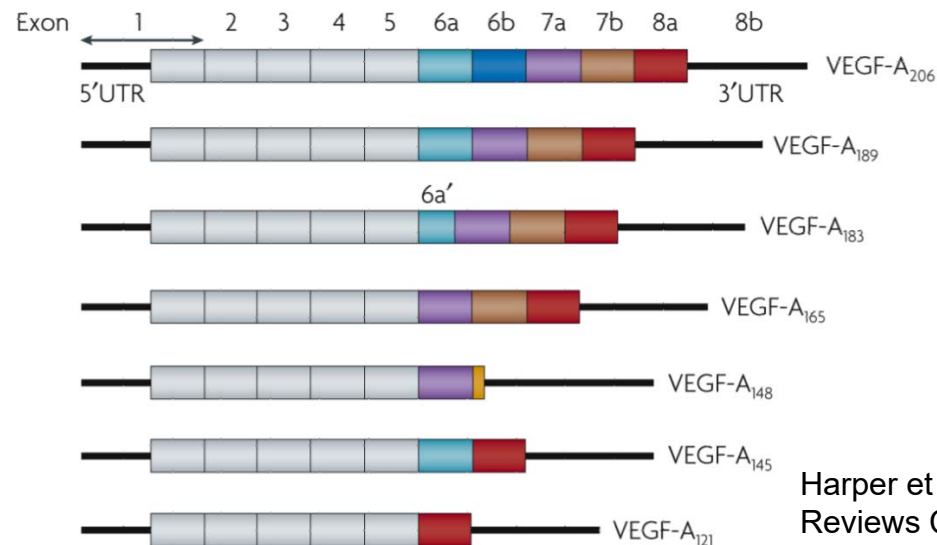
- **Differences identified?**
 - Impact on biological activity
 - use range of assays where possible
 - Support arguments with data/orthogonal analysis
 - Consider potential impact on safety and efficacy
- **Only those differences known/proven to have no impact on clinical efficacy and/or safety should be acceptable without additional justification.**
- **The in-vivo net contribution of different modes of action described for one mAb is sometimes incompletely understood and may also be different in different indications.**



Bevacizumab biosimilars: Need for ADCC testing?



- ADCC is generally not considered to be an important MOA for bevacizumab, which acts by binding soluble VEGF. Binding of VEGF leads to diminished angiogenesis in various cancers. Since ADCC will only occur when cells are targeted, this effector function would not be relevant.
- Yet, VEGF comprises different isoforms : whereas VEGF 121, 145 and 165 isoforms are soluble, VEGF 189 and 206 isoforms are mainly cell-associated.



Harper et al, Nature Reviews Cancer, 2008



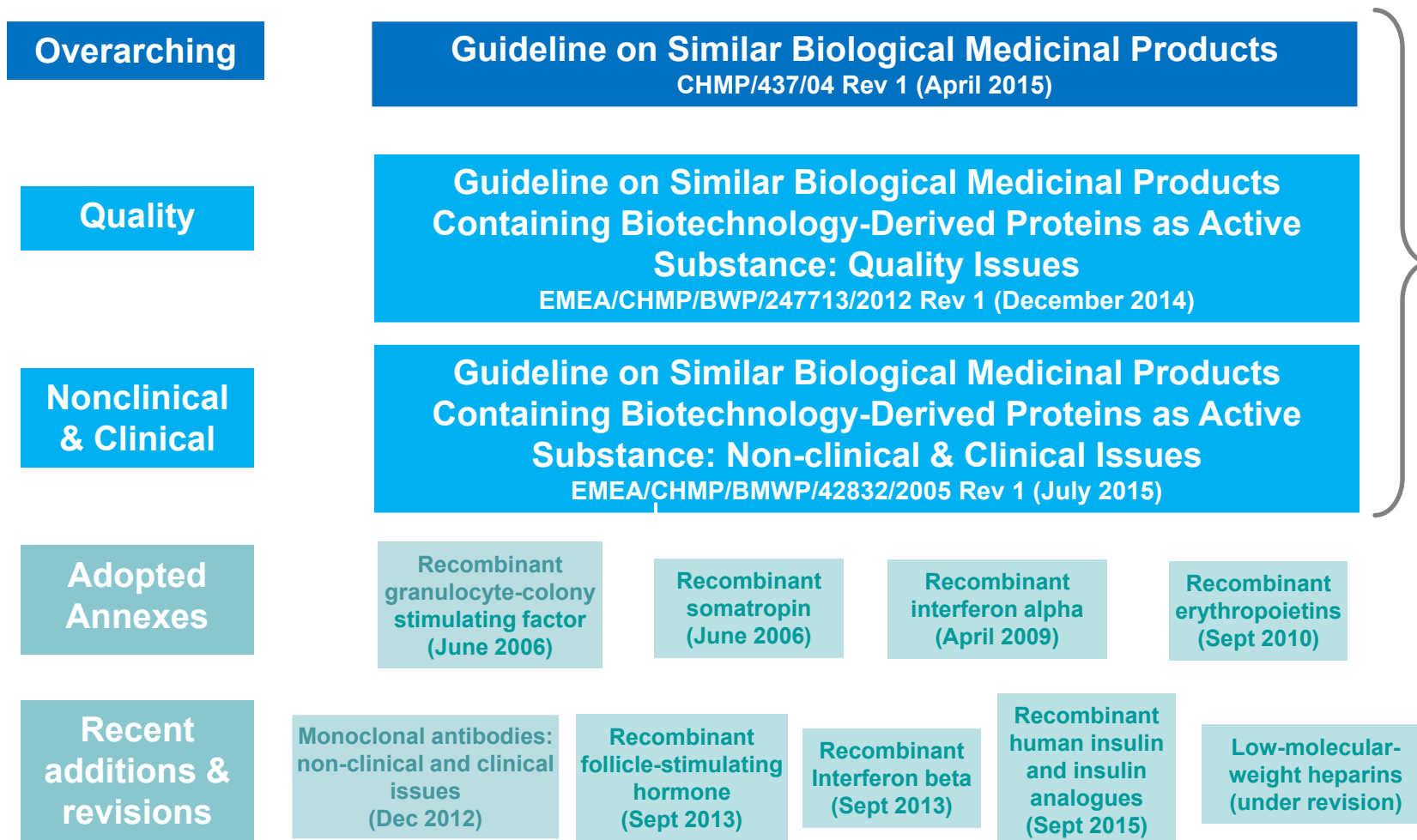
Bevacizumab biosimilars: Need for ADCC testing?



- While VEGF 121 and 165 are the predominant isoforms in tumorigenesis, it has been observed that VEGF 189 is expressed in lung and colon cancers.
- Thus, bevacizumab may also bind to cell-associated VEGF 189 and is thereby theoretically capable of mediating Fc-related effector functions.
- Conclusion: ADCC/CDC should be analysed during comparability assessment or the absence of relevant effects should be demonstrated utilizing a suitable assay format.
- The clinical relevance of the functional aspect is always an important point. In case there is doubt about the clinical relevance, comparative data on a specific MOA should be included.



Guidelines for EU Similar Biological Medicinal Products



New *Ph. Eur.* monograph: Etanercept



- **Monograph for etanercept (2895) was recently published in *Ph Eur* Supplement 9.5**
- **describes apoptosis assay for etanercept and will become effective on 1 July 2018**

- o ***'Potency. The potency of etanercept is determined by comparison of dilutions of the test preparation with the dilutions of etanercept BRP using a suitable cell-based assay based on the inhibitory action of etanercept on the biological activity of TNF- α and a suitable readout for assessing this inhibitory effect.***

The following procedure has been found suitable.

- o ***Carry out an apoptosis-based assay based on the ability of etanercept to inhibit TNF- α induced apoptosis in histiocytic lymphoma cell line U937 (ATCC No. CRL-1593.2) via caspase activation. The U937 cells are incubated with varying dilutions of test and reference preparations of etanercept in the presence of TNF- α . They are then incubated with Caspase-Glo 3/7 reagent.... '***

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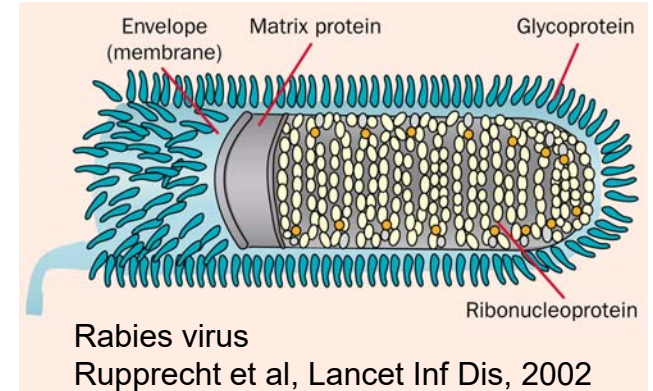
New *Ph. Eur.* monograph: Infliximab



- The monograph for infliximab (2928) has been published in *PharmEuropa* and was adopted in Nov.
- The monograph describes the cell-based inhibition assay for infliximab
- It will be part of Ph Eur Supplement 9.6 and will become effective in January 2019



Novel approaches to bioassays – the pseudotype virus neutralization assay



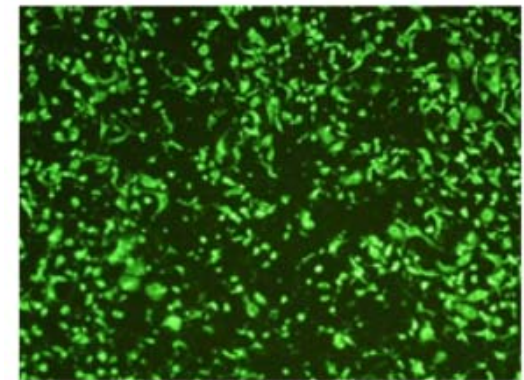
- **Testing the potency of new Mabs and vaccines against viruses including Ebola and Rabies has challenges:**
 - Need for handling live virus in high-security labs
 - The majority of endemic countries have resource-limited laboratories and performing any neutralization assays under high containment presents several financial and logistical challenges.
- **To address these issues, the pseudotype neutralization assay (PNA) has been developed → Pseudotypes are viruses that carry the core of one virus and the envelope of another.**
- **Viral pseudotypes have been constructed using lentiviral backbones and an analytical method has been established that utilizes these replication-incompetent viruses to accurately measure neutralizing antibody titres.**



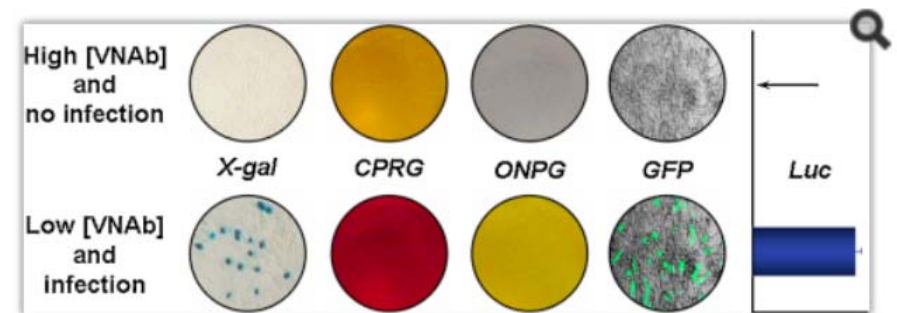
Novel approaches to bioassays – the pseudotype virus neutralization assay



- Pseudotyped rabies virus has rabies G protein on surface
- Rabies pseudotypes were demonstrated to be stable during freeze–thaw cycles and storage at room temperature
- PNA has been compared with routinely used FAVN assay (live virus) = *Ph. Eur.* assay, currently used by WHO/OIE reference laboratories to evaluate potency of rabies immune globulins & vaccines
- Collaborative study has been evaluating PNA performance and shown promising results
- PNA might be incorporated in relevant guidance/monographs in the future



Current *Ph. Eur.* assay using live rabies virus: Fluorescent Antibody Virus Neutralization (FAVN) assay



Repertoire of reporter genes that can be carried within pseudotypes (Wright et al, Vaccine, 2009)





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New MHRA HQ in London's Canary Wharf from June 2018

Thanks for listening

