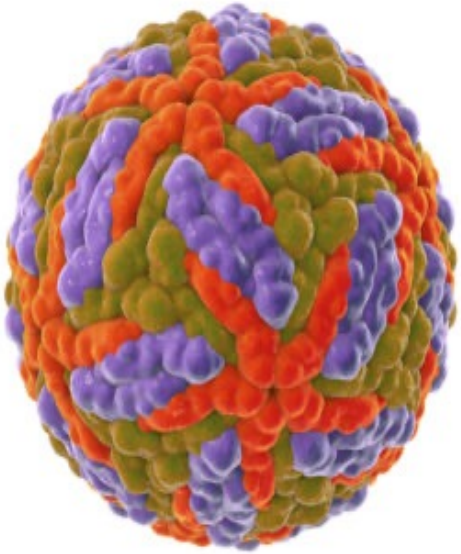
Monkey Neurovirulence testing of Yellow fever vaccine and nOPV and its refinement and replacement opportunities at BioE



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Neurovirulence Test MNVT : Current Status

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- Currently, MNVT remains an accepted in-vivo quality/safety /consistency for neurotropic live attenuated vaccines (classically YF 17D and poliovirus/OPV).

- It's widely used to confirm attenuation of seed/work/production lots before release.

- For nOPV2 (the new genetically stabilized OPV2 candidates), manufacturers used the WHO monkey neurovirulence procedure during development and manufacture to confirm attenuation of seed and production lots.
- For nOPV2 DS manufacturing, Each bulk should be tested for MNVT test .

Main problems with MNVT (Required Complete Replacement)

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- Ethical & practical: requires many non-human primates, with associated welfare and supply constraints, cost and biosafety requirements.
- Biological variability & limited predictiveness: the test has inter-laboratory variability and sometimes limited correlation to human adverse event rates (especially for many modern, rationally attenuated strains). That limits interpretability and regulator/manufacturer burden.
- Regulatory approvals and acceptance to 3Rs: WHO and expert bodies are actively working to reduce, refine, and replace primate tests where possible (recent WHO draft guidance and ECBS working groups)

Alternatives Opportunities available for nOPV

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1. **Transgenic mouse neurovirulence test** (tg-mouse NVT)Transgenic mice expressing the human poliovirus receptor were validated and accepted by WHO for OPV neurovirulence in collaborative studies; many regulatory frameworks **allow the tg-mouse NVT as an alternative to monkeys** for poliovirus. This is a mature, practicable replacement for OPV.
2. **Molecular approaches (MAPREC** / sequencing / genetic marker assays)For poliovirus, MAPREC (molecular amplification and probe restriction) and targeted assays that quantify reversion markers are established for OPV ;
3. **More recently NGS/deep sequencing to** track minor neurovirulence-associated variants has become a powerful tool to complement or replace in vivo steps for well-characterized strains for nOPV.

Alternatives Opportunities available for Yellow fever vaccine

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1. **Mouse Neurovirulence / LD 50 as alternative to Neurotropism test in MNVT :** Data is more comparable with MNVT as it refinement to use lower species i.e Monkey to mouse. This is a mature, practicable replacement for YFV Primate testing.

2. **Viscerotropism and Immunogenicity** can also be performed on rodents and non- rodents.

3. **NGS/deep sequencing: Most sensitive tool to replace MNVT and Mouse Neurovirulence test.** The key challenge is identification of molecular determinants of attenuation and comparison in MNVT which is regulatory expectation. There are **24 potential attenuation sites for YFV**

4. **Collaborative studies** are required with regulatory agencies like WHO,MHRA and local regulatory authorities will facilitate immediate implementation .

(Neurovirulence of live attenuated yellow fever 17D vaccine virus in Mice)

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Results			
Group No.	Batch No.	Concentrations/0.03mL	Result: LD50 (IU/0.03mL)
G1	WHO 17D Yellow Fever Vaccine virus reference batch NIBSC code: 168-73	300, 100, 33.33, 11.11, 3.70, 1.23, 0.41, 0.14, 0.05	7.36
G2	Live-attenuated Yellow Fever (17D) Master Virus (17D 213- 77 strain) Batch No.: MVSB/YFV/001		>300
G3	Live-attenuated Yellow Fever (17D) Working Virus (213-77 strain) Batch No.: WVSB/YFV/001		>300
G4	Final lot of yellow fever vaccine (Live, Attenuated) (Freeze dried) (01 dose) Batch No.: PCT/YFV/1D/001A/24		144.22
G5	Yellow fever vaccine (Live) –Competitor egg based Vaccine		11.11
G6	Placebo for Yellow Fever Vaccine (Live, Attenuated) (Freeze dried) Batch No.: PCT/YFV/PL/001A/24		0

NGS for YFV Potential Attenuation sites

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S.No	Gene	Position (nt)	Nucleotide Ref in 17D	Amino acid Ref in 17D
1.	*M*	854	T	Phe
2.	*E*	1127	A	Arg
3.	*E*	1482	T	Val
4.	*E*	1491	T	Ile
5.	*E*	1572	C	Thr
6.	*E*	1870	A	Ile
7.	*E*	1887	T	Phe
8.	*E*	2112	G	Arg
9.	*E*	2193	T	Val
10.	*NS1*	3371	G	Val
11.	*NS2A*	3860	G	Val
12.	*NS2A*	4007	G	Ala
13.	*NS2A*	4022	G	Ala
14.	*NS2A*	4056	T	Phe
15.	*NS2B*	4505	C	Leu
16.	*NS3*	6023	A	Asn
17.	*NS4A*	6876	C	Ala
18.	*NS4B*	7171	G	Met
19.	*NS5*	10142	A	Lys
20.	*NS5*	10338	T	Leu
21.	*(3´ NCR)*	10367	C	-
22.	*(3´ NCR)*	10418	C	-
23.	*(3´ NCR)*	10800	A	-
24.	*(3´ NCR)*	10847	C	-

Summary :

	YFV	OPV
Master virus seed	Yes	Yes
Working virus seed	Yes	Yes
Drug substance	No	Yes
What is tested	1. Viscerotropism test 2. Immunogenicity test 3. Neurotropism test	1 Neurovirulence
Reference virus	Yes WHO reference virus, 168-73, Attenuated strain	Sabin OPV virus, Attenuated strain
Containment requirements	BSL 2	GAP IV
Determinants of attenuation	Not known 24 potential sites	Known One primary attenuation site
Use of HTS as alternative	Sufficient data a not available	Recommended by WHO
Other lower mammals as alternative	Opportunity available need validation 1. Viscerotropism test: not available 2. Immunogenicity in Rabbits/ Rats/ Hamsters 2. Neurotropism test: LD 50 in mouse (Potency test used earlier) as alternative	TG Mouse available

The monument commemorates the sacrifice of the mice in genetic research used to understand biological and physiological mechanisms for developing new drugs and curing of diseases

Thank You

References

- Konz JO et al., *Evaluation and validation of next-generation sequencing to support lot release for a novel type 2 oral poliovirus vaccine* (Vaccine X; 2021) — NGS validation and spike-in approach used for nOPV2 lot release
- Wahid R. et al., *Evaluating stability of attenuated Sabin and two novel type-2 OPV candidates* (2022) — mTgmNVT correlation with genetic data. Nature
- WHO neurovirulence and transgenic-mouse NVT SOPs / TRS guidance (WHO polio vaccine recommendations).
- WHO TRS 1045 Annex 2 (OPV & nOPV) and WHO TRS 978 Annex 05 (YFV)