

NeutNet:

Standardisation
of HIV Neutralisation Assays
to be used
in Vaccine Research and Clinical Trials



The BACKGROUND



WHO-UNAIDS MEETING ON "PROGRESS IN THE DEVELOPMENT AND STANDARDIZATION OF METHODS TO MEASURE ANTI-HIV-1 NEUTRALIZING ANTIBODIES IN HIV VACCINE RESEARCH AND CLINICAL TRIALS"

**DIBIT,
SAN RAFFAELE SCIENTIFIC INSTITUTE
MILAN, ITALY
7 TO 8 AUGUST 2003**

Africa

Ethiopia

Kenya

South Africa

Uganda

Asia

Thailand

Europe

France

Italy

Sweden

U.K.

South America

Brazil

USA

Considering HIV neutralisation, several **OBSTACLES** have to be dealt with:

One is virus **VARIABILITY**. The first question is, therefore, which virus strains to use ? Field isolates or molecularly cloned viruses ? Should the classification of genetic subtypes be considered ? Should receptor use of the virus be considered ?

The second difficulty is to choose the appropriate **TARGET CELL** for the neutralisation assay. Established cell lines or primary cells and, if primary cells, which cell type ?

The third problem is: how to run the **ASSAY** ? Should this be single cycle or multiple cycle ? What readout to use ? What would serve as a positive control reagent that would have a broad cross reactivity to many HIV-1 isolates ?

**THESE QUESTIONS ARE NOT AT ALL TRIVIAL,
THERE ARE AT LEAST TWO CONTRADICTORY
ANSWERS TO EACH.**

NeutNet : January 2005

Duke, Durham

NYU, New York

Monogram, San Francisco

HJF, Rockville

FCSR, Milan

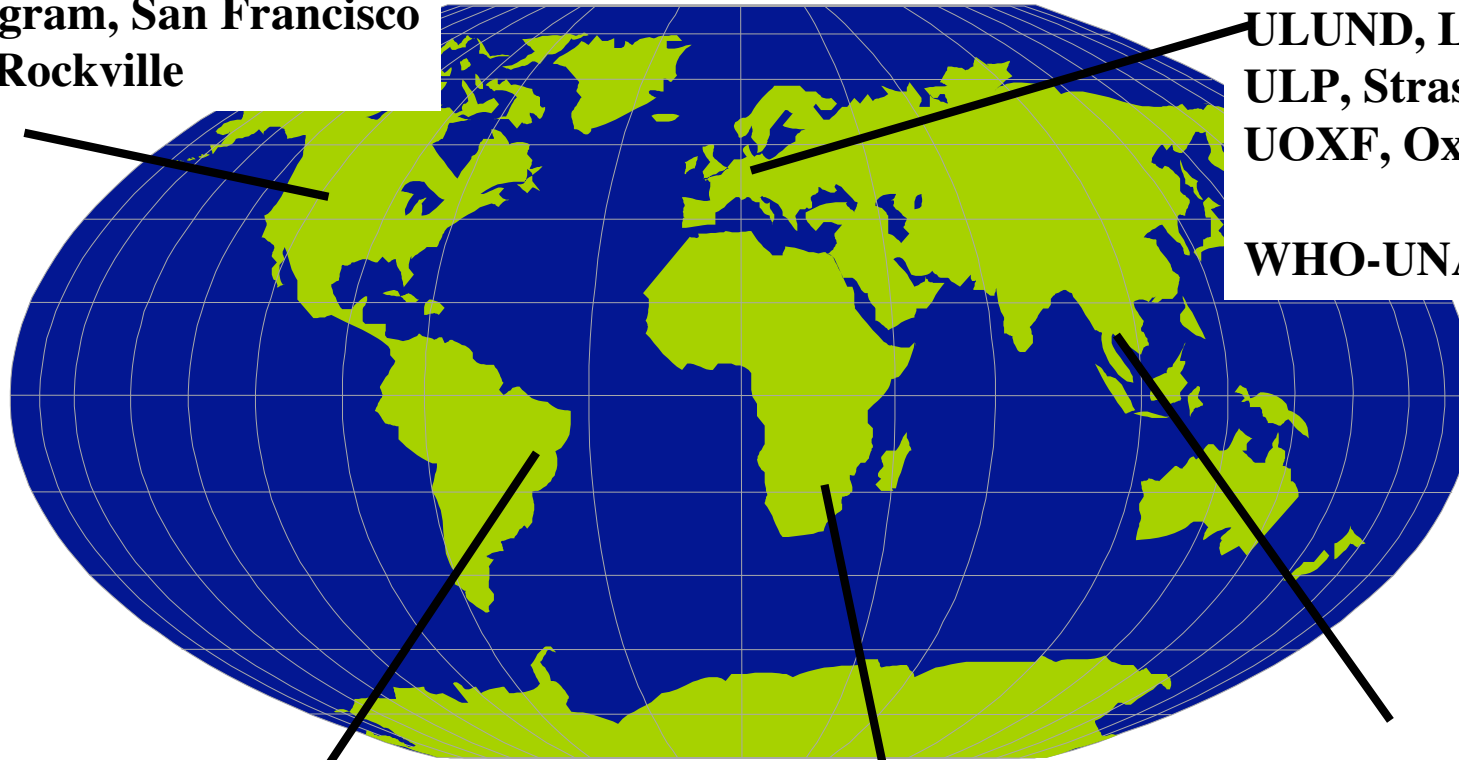
NIBSC, London

ULUND, Lund

ULP, Strasbourg

UOXF, Oxford

WHO-UNAIDS, Geneve



FIOCRUZ, Rio de Janeiro

NICD, Johannesburg

NHRBC, Bangkok

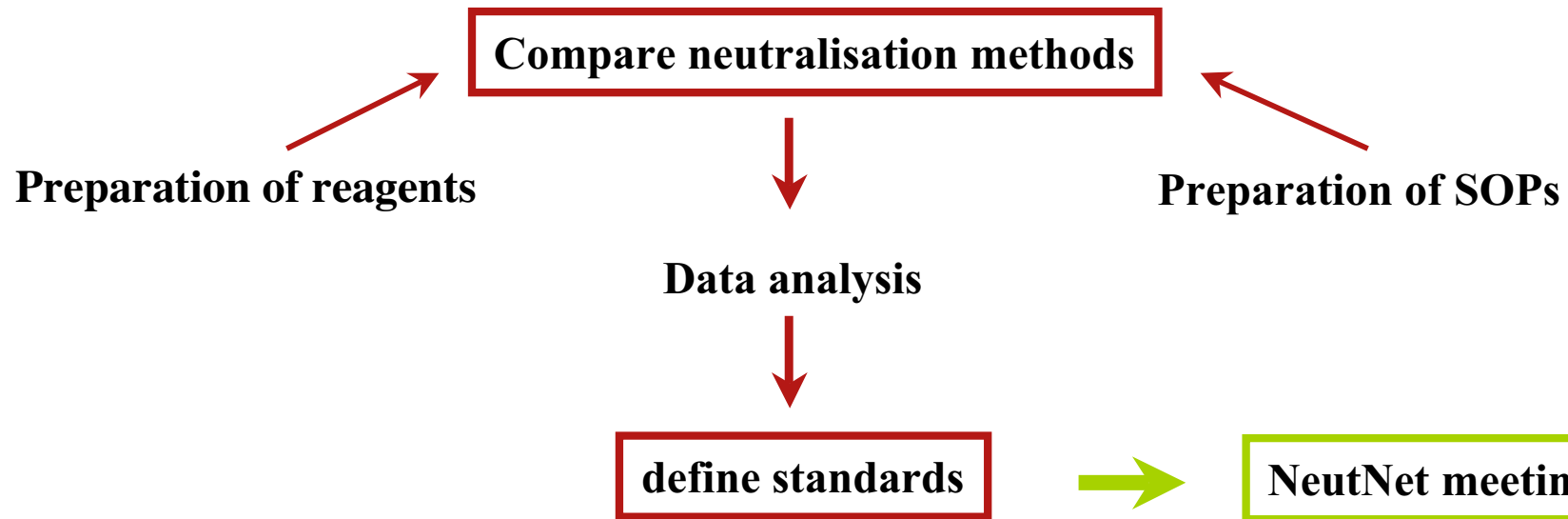
NeutNet: GOALS

Analytical studies for standardisation of HIV neutralisation assays

- **Organise an initial study to compare different neutralisation methods and define standards.**
- **Organise a subsequent study to compare polyclonal serologic reagents to define the best conditions to determine neutralising activity.**

NeutNet: GOALS

PHASE ONE:

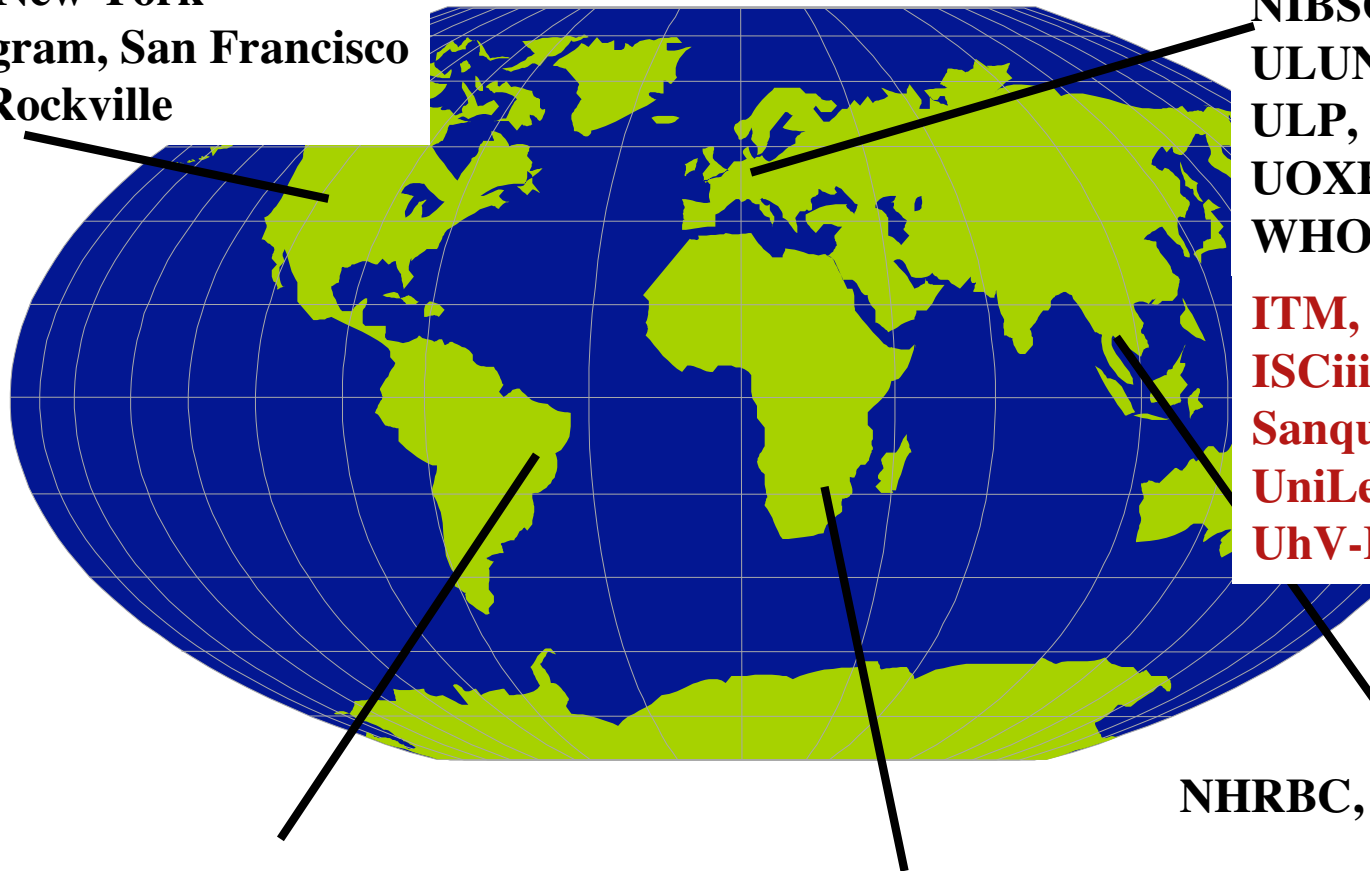


PHASE TWO:



NeutNet : September 2005

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PHASE 1

Compare different neutralisation methods

Study Variables

- **Target cells:**
 - PBMC, CD8-depleted and non-depleted**
 - MDM**
 - U87-CD4-CXCR4/CCR5**
 - GHOST-CXCR4/CCR5**
 - 3T3.T4. CXCR4/CCR5**
 - TMZ-bl**
- **Virus stocks/plasmids:**
 - Uncloned viruses produced in PBMC**
 - Pseudotyped viruses produced in 293T cells**
 - Recombinant viruses**
- **Single cycle and multiple-cycle infection**
- **Multiple read-outs:**
 - p24 ELISA**
 - p24 FACS**
 - Luciferase reporter gene expression**
 - Real time PCR**
 - Fluorescence microscopy**

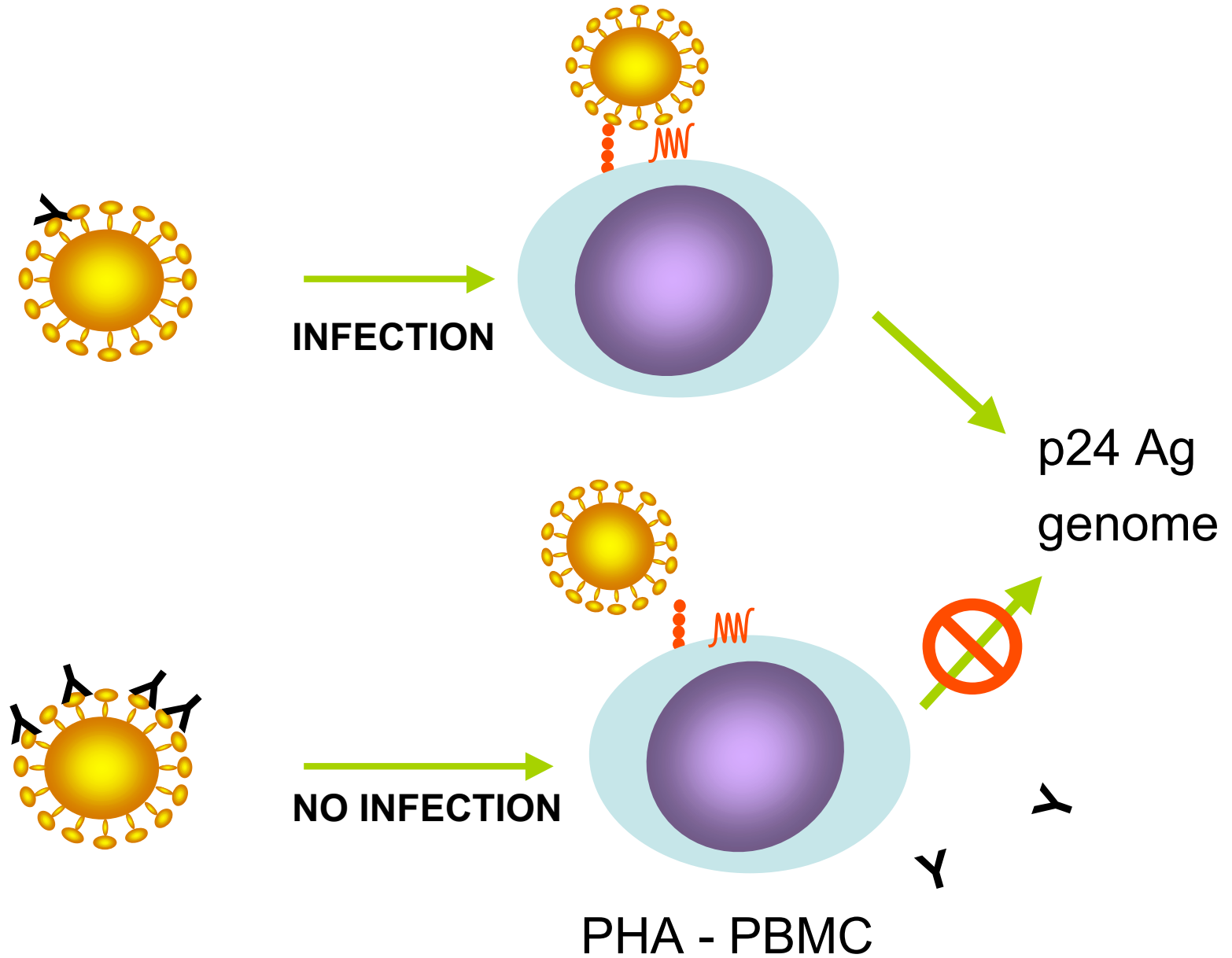
Assay	Virus type	Cell target	Infection	Read-out assay	Read-out day
<u>p24 reduction</u>	Isolate	PBMC	MR	ELISA	7
	Isolate	PBMC	MR	ELISA	7 and 10
	Isolate	PBMC	MR	ELISA	7 and 14
<u>IC p24</u>	High titer isolate	PBMC or MDM	SR	Flow cytometry	2
	Isolate	CD8 - PBMC	MR	Flow cytometry	3-7
<u>PCR</u>	Isolate	PBMC	MR	Real Time PCR	1-4
<u>Plaque reduction</u>	Isolate	U87 or Ghost	MR	Syncytia w. light microscopy	3
<u>Fusion</u>	Isolate	PM-1	SR*	β -Galactosidase	2 hours
<u>Pseudotyped virus</u>	Clones	TZM-bl	SR	Luciferase	2
	Clones	3T3.T4.	SR	Luciferase	2
	Clones	Ghost	SR	Luciferase	2
<u>Recombinant virus</u>	Isolate or clones	U87 CCR5/CXCR4	SR	Luciferase	3
	Isolate or clones	U87 or Ghost	SR or MR	Luciferase	SR: 24 or 48h MR: 5 d



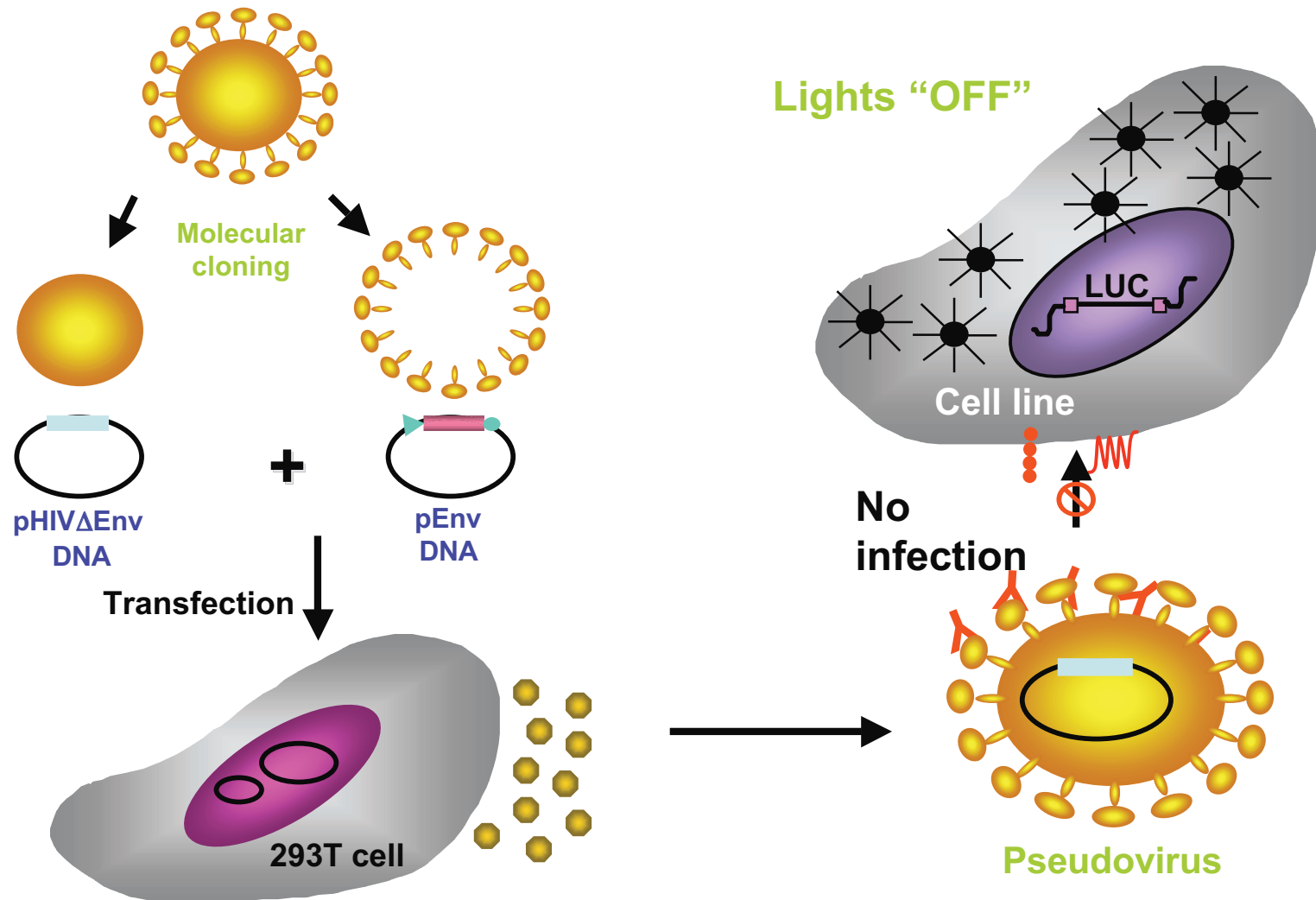
MR= multiple round, SR= single round; IC =intracellular;

*Limited to cell-surface envelope/receptor interaction

HIV ISOLATE NEUTRALIZATION ASSAY



HIV PSEUDOVIRUS NEUTRALIZATION ASSAY



PROPERTIES

PSEUDOVIRION

Cell line

Non infectious (BSL 2)

Quick (< 3 d)

Clonal (plasmid)

Less labour intensive

HTP possible

Cell Culture ISOLATE

PBMC / Cell lines

BSL 3

Time consuming

Quasispecies

Labour intensive

??

NeutNet Reference VIRUS Panel

- Clade (2A, 2B, 2C, 1D, 2CRF 01 + 3B US-panel)
- Phenotype (9 R5 and 3 X4)
- Vaccine Strains (SF162, MNp, CM244)
- Neutralization sensitivity
 - Sensitive, moderate, resistant

Subtype	Virus code	Virus phenotype	Neutralisation sensitivity
A	92RW009	R5	Sensitive
A	VI 191	R5	Sensitive
B	SF162*	R5	Sensitive
B	MN(P)*	X4	Sensitive
C	DU174	R5	Moderately Sensitive
C	92BR025	R5	Moderately Sensitive
D	92UG024	X4	Moderately Resistant
CRF01	CM244*	R5	Resistant
CRF01	<i>NP1525</i>	X4	Moderately Sensitive
B (US)	QH0692.42	R5	Moderately Sensitive
B (US)	<i>CAAN5325.A2</i>	R5	Moderately Resistant
B (US)	AC10.0.29	R5	Moderately Sensitive

* designates vaccine strains.

NeutNet Reference INHIBITOR Panel

- **Monoclonals (25ug/ml, 6 2-fold dilutions)**
 - **Individual: 447-D, 4E10**
 - **TriMab (2F5, 2G12, IgG1b12)**
- **Other reagents**
 - **sCD4 (10ug/ml, 6 2-fold dilutions)**
- **Sera / Serum pools (HIV+ and HIV-)**
 - **whole sera / purified Ig**

NeutNet

Standardisation of HIV Neutralisation Assays to be used in Vaccine Research and Clinical Trials

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