

# **Comprehensive characterization of cellular immune responses induced by MVA-HIV-1 in a Phase I randomized, controlled trial**

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# Initial Phase 1 Design

## First-in-man study of MVA-E (n=48)

- Dose escalation, route comparison
- Vaccinia naïve, healthy volunteers 18-49

Part A: Low dose (n=24)  $10^6$  ID v  $10^7$  IM pfu (US)

Part B: High dose (n=24)  $10^7$  ID v  $10^8$  IM pfu (US, Thailand)

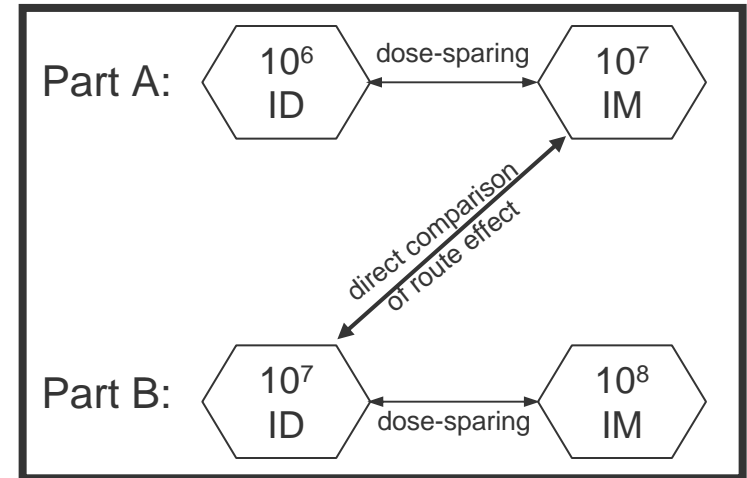
## Endpoints

### 1. Safety:

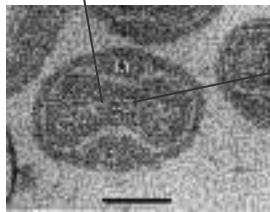
- No Serious AEs related to vaccination
- Expected local cutaneous symptoms in ID route

### 2. Immunogenicity:

- Chromium Release CTL
- IFN- $\gamma$  ELISPOT
- WB ICS IL-2/IFN- $\gamma$
- Multiparameter Flow Cytometry
- Antibody binding (ELISA) and HIV diagnostic testing (EIA, Western Blots, NAT)



# Recombinant MVA-HIV structure (WRAIR/NIH)



0.1 $\mu$

MVA-E is a live recombinant poxvirus vector

Engineered to express the following HIV-1 genes: env/gag/pol

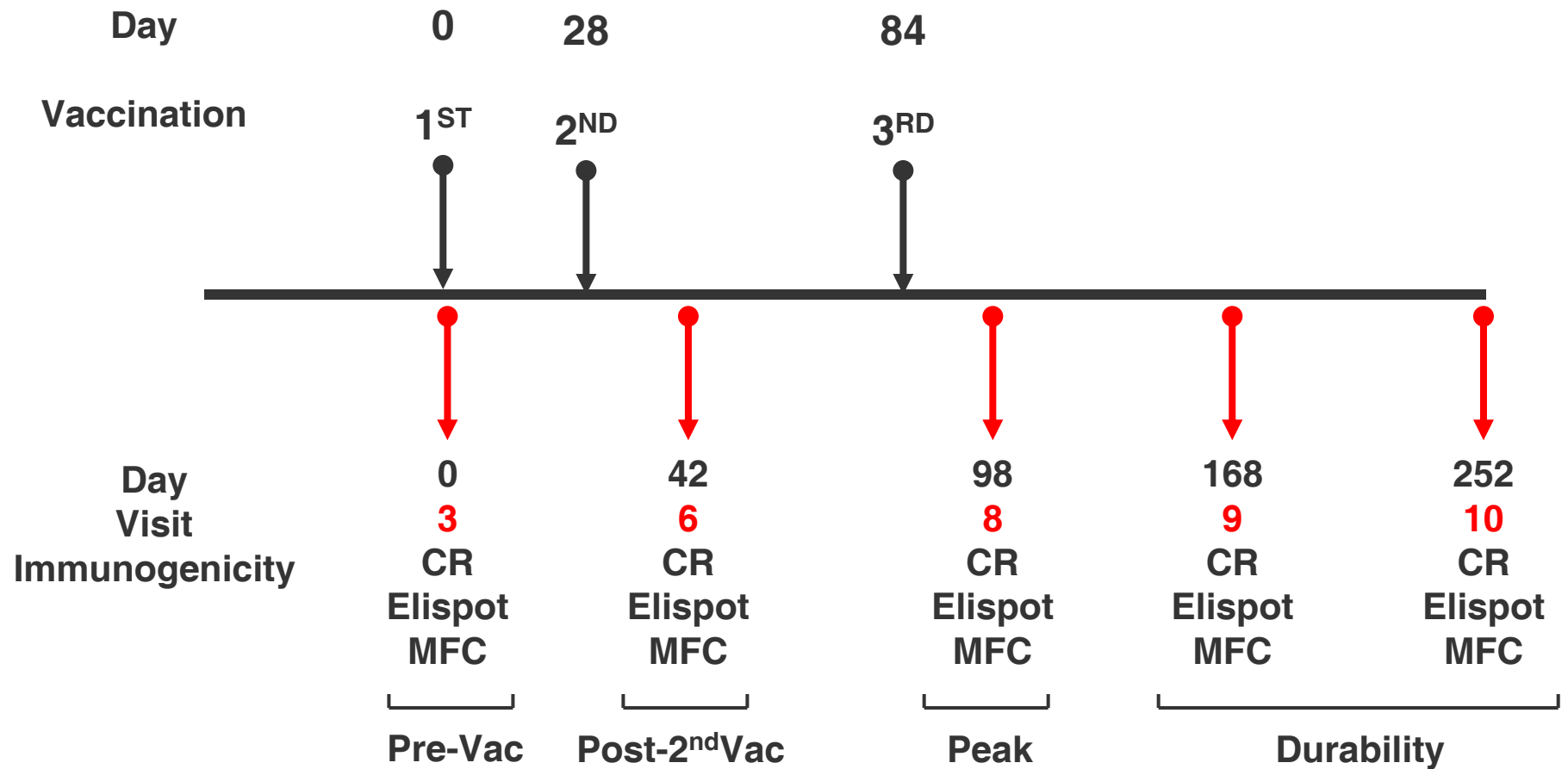
gp150 (Subtype CRF\_01AE, CM235) and gag/pol\* (Subtype A, CM240)

enveloped, DS DNA linear  
Replicates in cytoplasm  
Largest and most complex viruses  
Visible under light microscope  
Approx. 300 x 200 nm

Other Subtypes to match other areas:

- Kenya MVA-A
- Tanzania MVA-C
- Uganda MVA-D

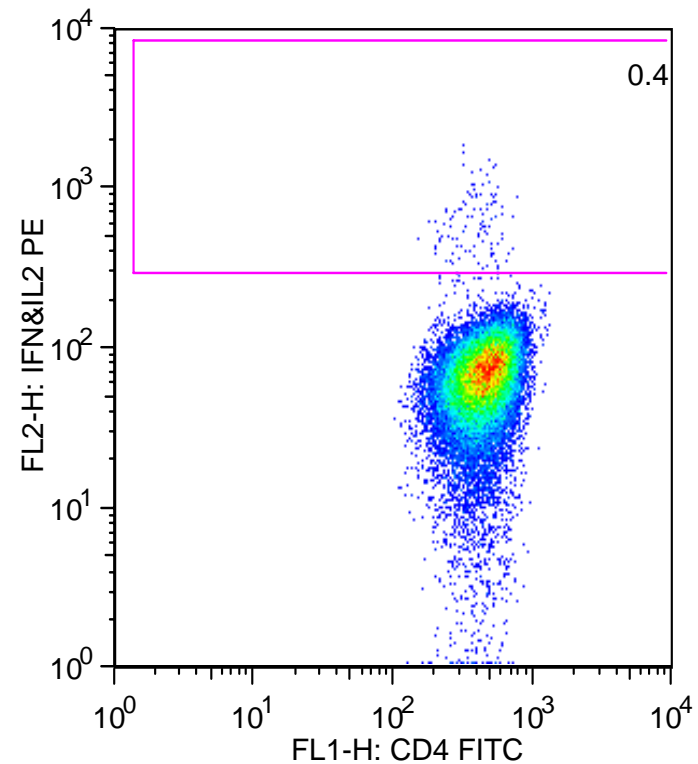
# RV158 Immunomonitoring Schema



# Whole Blood ICS IL-2/IFN- $\gamma$

High dose only

- Standard protocol at both sites
- Positive = 3x bkgrd and >0.05% (over background).
- Mainly CD4 + responses
- 50% RR both sites: env>gag
- Range for env responses:  
Thailand [0.06-0.4]  
Rockville [0.09-0.12]



CD4	Any env any time post vaccination	Any gag any time post vaccination
Rockville	4/12	1/12
Thailand	5/10	2/10

# CD8<sup>+</sup>-dep Chromium Release Cytolytic Activity (CTL)

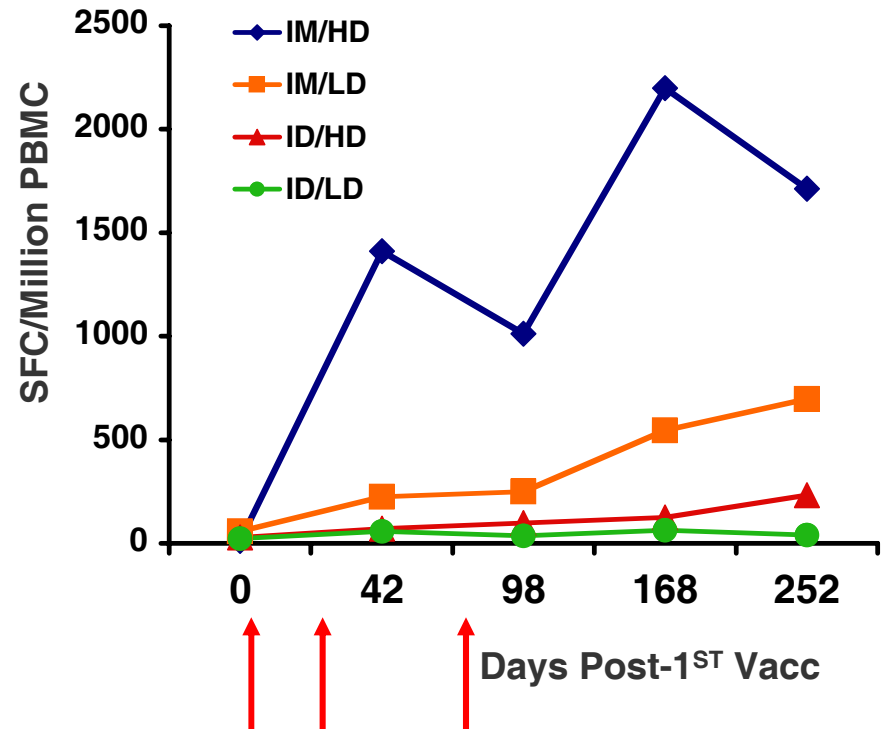
- <sup>51</sup>Cr release assay after 14d IVS with rMVA
- Cold-Target inhibition required due to MVA potent responses
- Positive response > 10% lysis at 1 E:T ratio
- CD8 or CD4 dependence required > 50% reduction of lytic activity with depletion

CD8 Dependent Cumulative Prevalence (by Route/Dose)						
Antigen	Pre-Vacc	IM/LD	ID/LD	IM/HD	ID/HD	Any Post-Vacc
	(38)	(13)	(13)	(6)	(6)	(38)
MVA	1	9	10	5	5	29
Env	0	5	6	4	3	18
Gag/Pol	0	2	0	2	1	5

- 76% RR to vector backbone
- 47% RR to HIV-insert genes
- CTL activity Env > Gag

# Anti-Vector (MVA) IFN- $\gamma$ Elispot Response

- IFN- $\gamma$  Elispots against MVA p581 backbone
- Some low-level background responses pre-vaccination
- Responses show good vaccine “take”
- 65% response rate to vector backbone
- Dose response observed against MVA
- Differences between routes also (IM>ID)
- 35% response rate to HIV-insert genes
- Env and gag responses more balanced



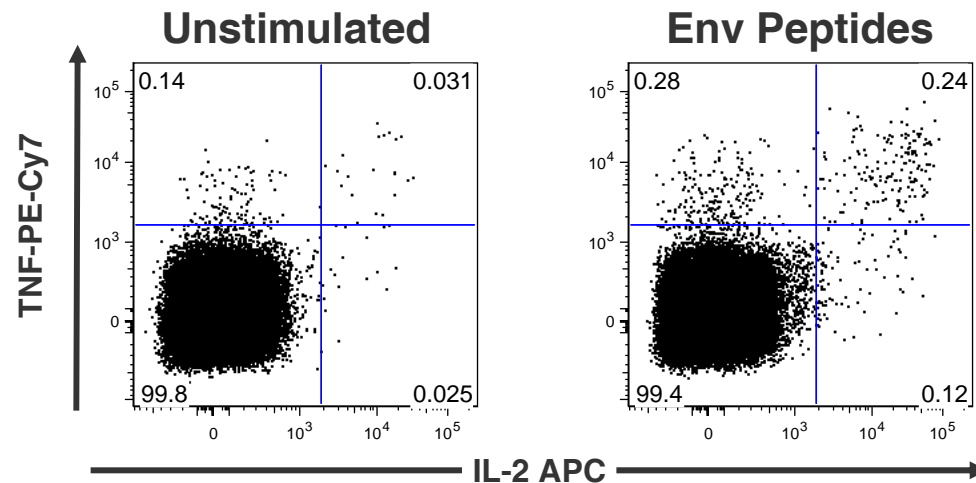
IFN- $\gamma$  Elispot Cumulative Prevalence (by Route/Dose)

Antigen	Pre-Vacc	IM/LD	ID/LD	IM/HD	ID/HD	Any Post-Vacc
	(37)	(12)	(13)	(6)	(6)	(37)
MVA	12	9	5	5	5	24
Env	0	4	2	5	2	13
Gag/Pol	0	4	2	2	1	9

# Polyfunctional Flow Cytometry Analysis of Low-Dose IM Route

- Polyfunctional ICS detected mainly IL-2/TNF- $\alpha$  responses against HIV-inserts
- $\alpha$ -Vector responses limited due to timing of protein transport blocking agents
- Responses indicate good vaccine “take”
- 50% response rate to vector backbone
- 60% response rate to HIV-insert genes
- Preliminary CD4+ predominant (need to analyze other arms)

CD4 Functional Responses					
Cumulative Prevalence Post-Vacc (N=10)					
Antigen	CD107a	MIP-1 $\beta$	IFN- $\gamma$	IL-2	TNF- $\alpha$
MVA	0	2	5	5	5
Env	0	0	2	6	4
Gag	0	1	2	3	3



# Conclusions



MVA-E alone was immunogenic and capable of self boosting after 3rd dose

## 1. Chromium release assays:

- qualitative but most sensitive and functional assay
- indicates good HIV specific CD8<sup>+</sup> lytic capacity (env>>gag)

## 2. Interferon- $\gamma$ ELISPOTs:

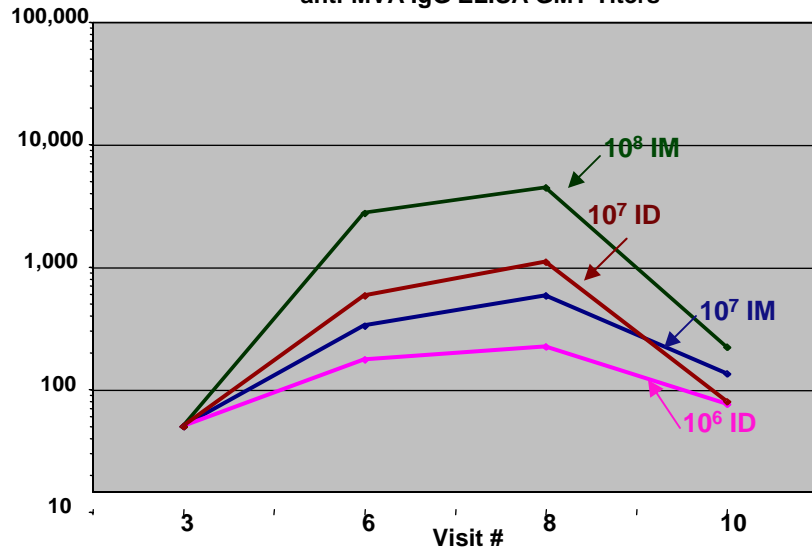
- less sensitive, limited single parameter information
- anti-vector dose response and route effects

## 3. Multiparameter Polyfunctional Cytometry (Preliminary):

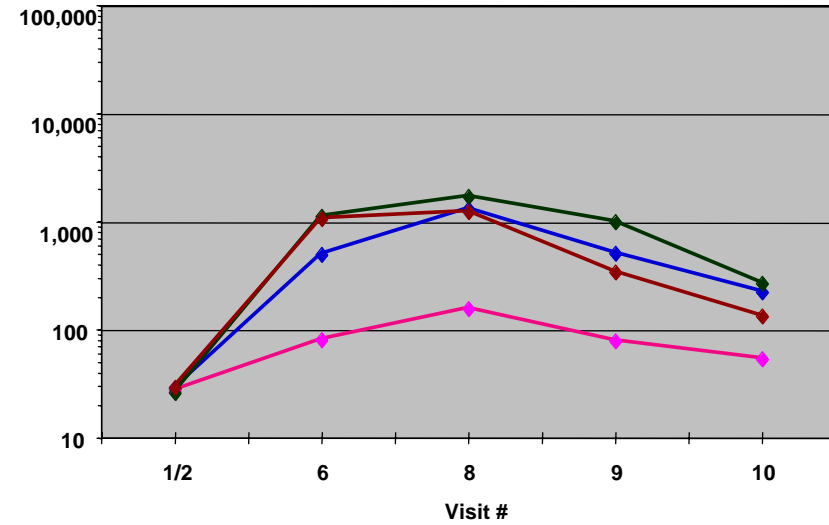
- mainly CD4 (env>gag) immune responses (same WB ICS)
- most responders double cytokine producers (IL-2/TNF- $\alpha$ )
- continued evolution of immune responses over time

# MVA-HIV-1 Antibody Data

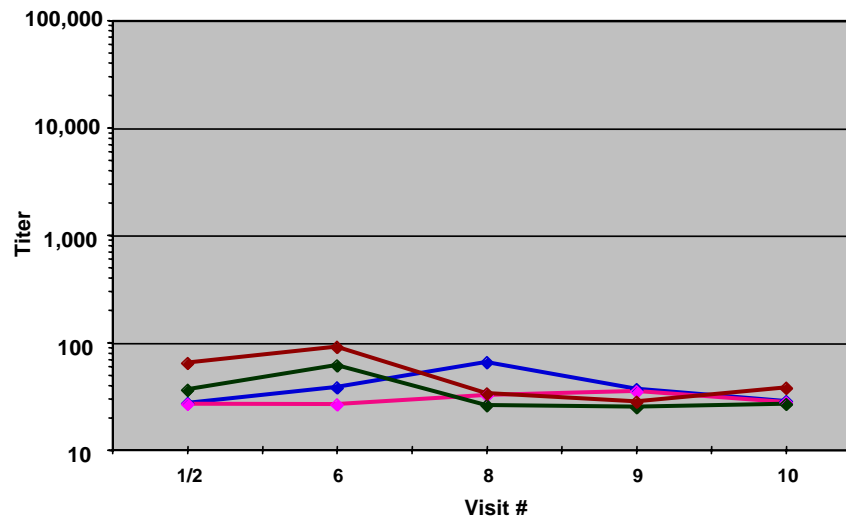
anti-MVA IgG ELISA GMT Titers



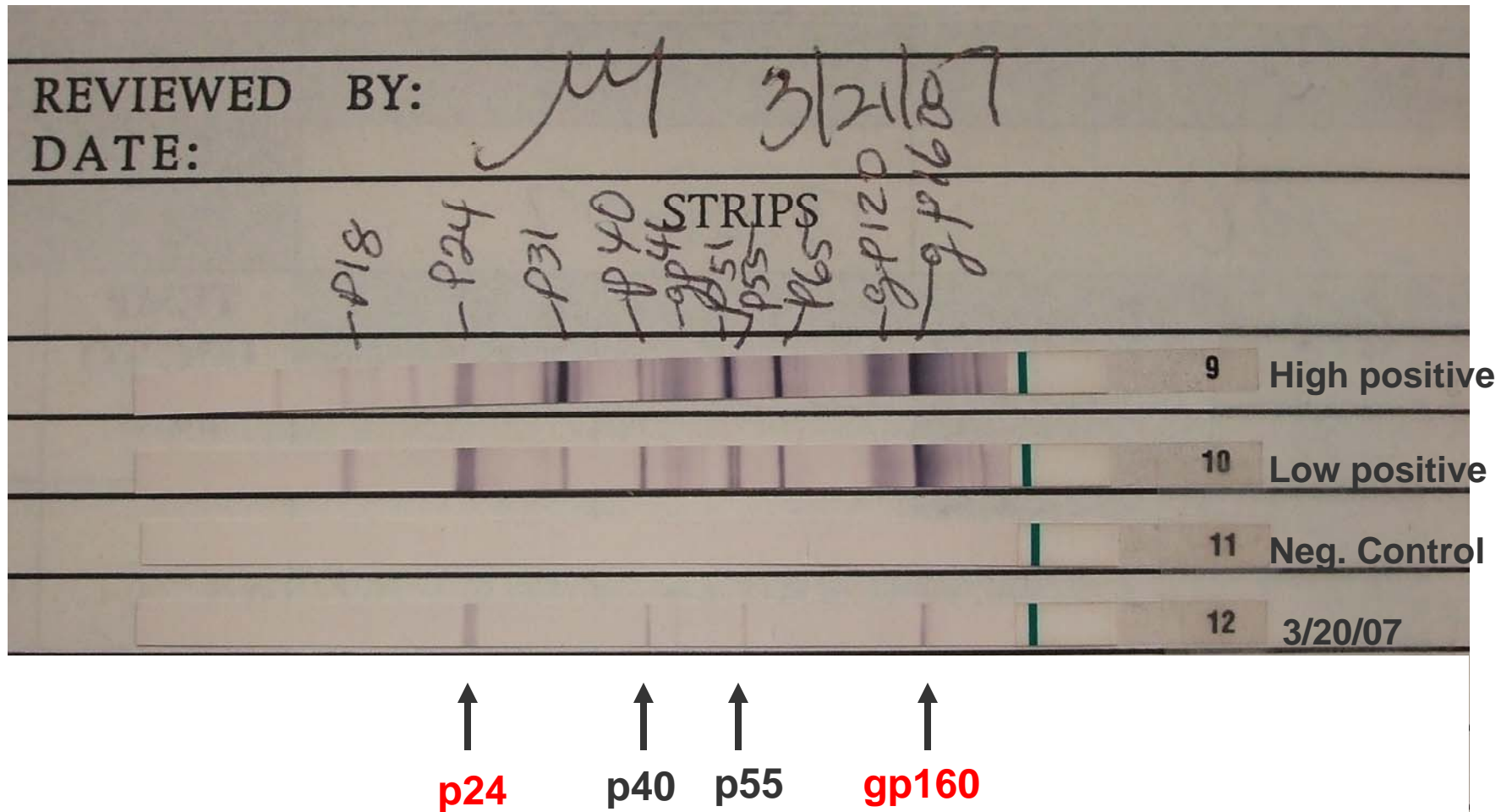
p24 (E. coli) IgG ELISA GMT Titers



gp140cm235 IgG ELISA GMT Titers



# High degree of Western blot reactivity (Env and gag)



# Acknowledgements

- All Clinical Trial participants
- Clinical Staff:
  - Rockville: V. Ngauy, M. Robb, MJ Humphries, K Duffy, Y Lewis, L. Zhu
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