# Antibody Dependent Cellular Cytotxic activity: Past and Future

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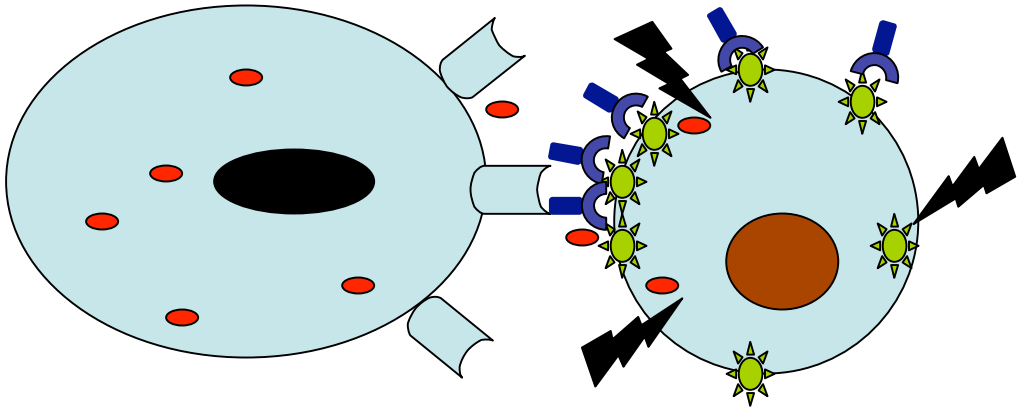
# Mechanism of Antibody Dependent Cellular Cytotoxicity (ADCC)

**ADCC Effector Cells**

**(NK, monocytes/macrophages, FcR-bearing cells))**

HIV-1-infected CD4 T cells



=HIV-1 gp120, gp41

= ADCC mediating Ab IgG1,
ADCC mediating Ab IgA

= Fc_RIIIa-receptor,
Fc_R receptor

# ADCC
# in Non-Primates Animal Models

- May be relevant in the protection against sexually transmitted pathogens such as *Chlamydia trachomatis* in mice.[2]
- ADCC activity has been shown to confer protection against cells infected with other envelope viruses which indicates that ADCC may contribute to protective efficacy.[1]
- Protects complement-deficient mice against lethal challenge of HSV. [1]
- ADCC may play a role in mice immunity to and the clearing of certain types of blood stage malaria. [3,4,5]


[1] Balachndran, N., S. Bacchetti, and W.E. Rawls. 1982. Infect. Immun.37:1132
[2]Moore, T., GA Ananaba, et al. 2002. Immunology 105:213
[3]Weidanz, W. P., J. Melancon-Kaplan, and L. A. Cavacini. 1990. *Immunol. Lett.* 25:87.
[4]Meding, S. J., and J. Langhorne. 1991. *Eur. J. Immunol*. 21:1433.
[5]H. Bouharoun-Tayoun *et al.*, 1995. *J. Exp. Med.* 182:409

# ADCC
# Humans and non-human primates

- Abs with anti-HIV ADCC activity appear early in acute infection, often preceding neutralizing Ab response.[6]
- ADCC has been associated with higher CD4+ T cell counts in subtype B HIV-infected humans.[7]
- Potent ADCC activity against viral envelope-coated targets have been observed in the sera of HIV-1 long-term non-progressors[8] as well as some cohorts of healthy children born to HIV-infected mothers. [9]
- ADCC activity has also been associated with slower disease progression in SIV-infected rhesus macaques. [10]
- Vaccine elicited ADCC activity is correlated with a significant reduction in plasma viremia during the acute phase of infection in Rhesus macaques challenged with SIVmac251. [11]

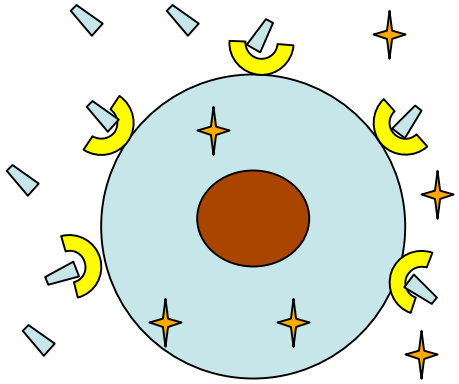
[6] Swayer,L., et al. 1990. *AIDS Res. Hum, Retrovir. 6:341*
[7]. Ahmad, R., et al 2001. J. Clin. Immunol. 21:227
[8]. Alsmadi, O., et al. 1997. *J. Virol.* 71:925
[9]. Ljunggren, K., et al. 1990. *J. Infect. Dis.* 161:198
[10]. Banks, ND et al 2002. *AIDS Res. Hum. Retrovir. 18:1197*
[11]. V. Raul Gomez-Roman, et al. 2005. *J. Immunol* 174:2185

# Antibody Dependent Cell Cytotoxicity Assay

1. 90’ NKR+gp120+$^{51}$Cr

**ADCC Target Cells**

**ADCC NK Effector Cells**



gp120-coated NKR cells

= gp120 = CD4 = HIV-1 = ADCC mediating Ab = Fc-receptor = $^{51}$Chromium

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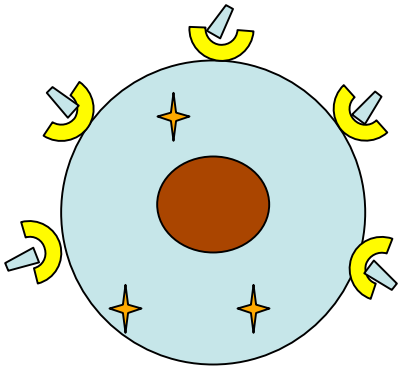

gp120-coated NKR cells

1. 90’ NKR+gp120+$^{51}$Cr
2. Wash x3

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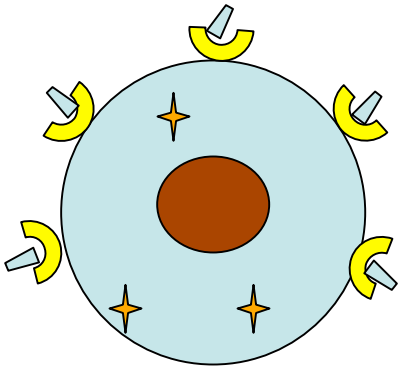
**ADCC NK Effector Cells**



gp120-coated NKR cells

1. 90’ NKR+gp120+$^{51}$Cr
2. Wash x3
3. Isolate PBMC from HIV$^{neg}$ donor

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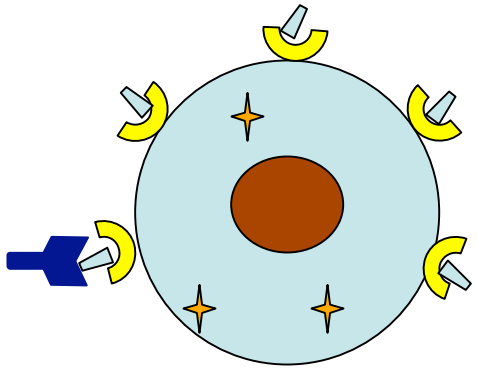

gp120-coated NKR cells

1. 90’ NKR+gp120+$^{51}$Cr
2. Wash x3
3. Isolate PBMC from HIV$^{neg}$ donor
4. Make serum dilution (1:20-1:2500)
5. Plate serum dilution in triplicate

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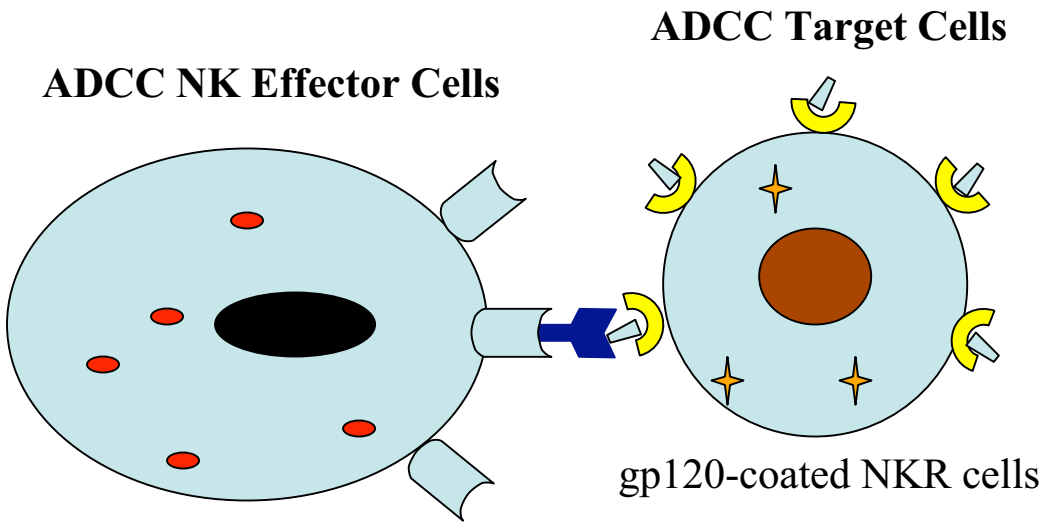
# Antibody Dependent Cell Cytotoxicity Assay

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2. Wash x3
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4. Make serum dilution (1:20-1:2500)
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6. Plate target cells
7. Plate Effector cells E:T=33:1

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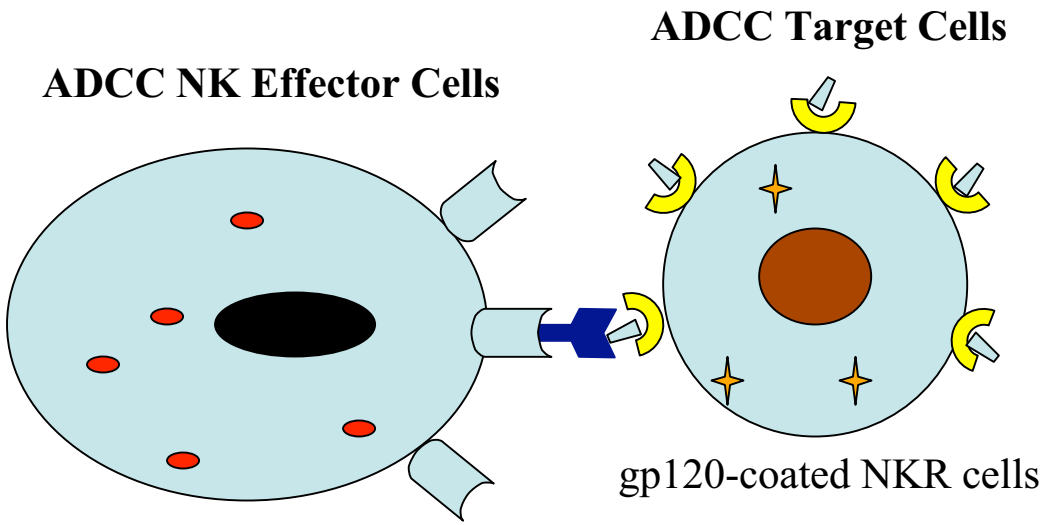


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3. Isolate PBMC from HIV$^{neg}$ donor
4. Make serum dilution (1:20-1:2500)
5. Plate serum dilution in triplicate
6. Plate target cells
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8. 6 hours incubation

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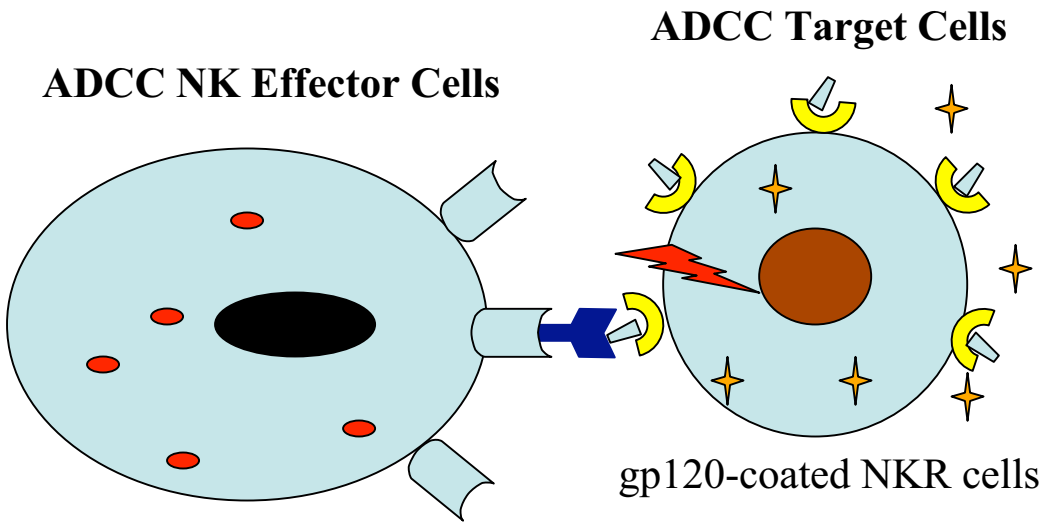


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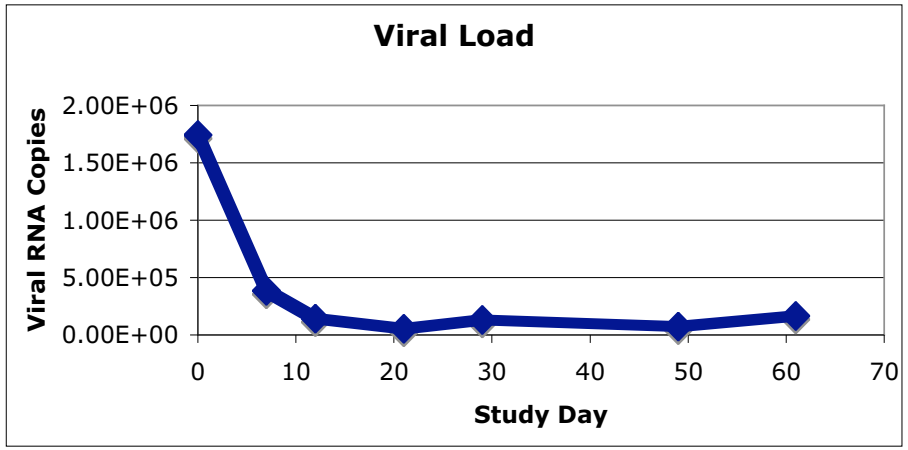
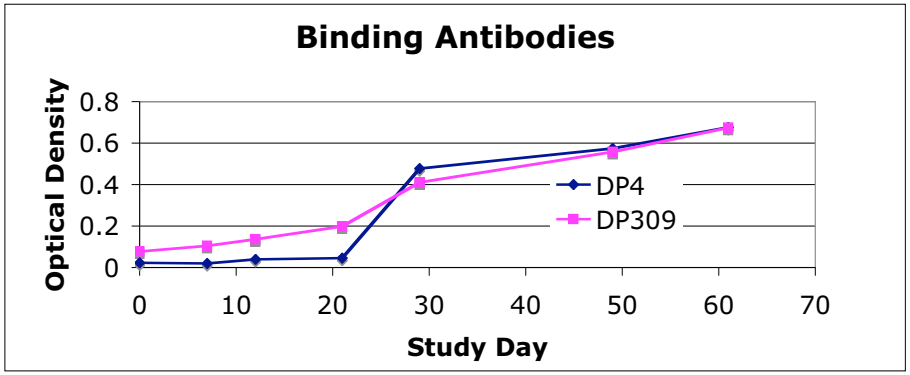
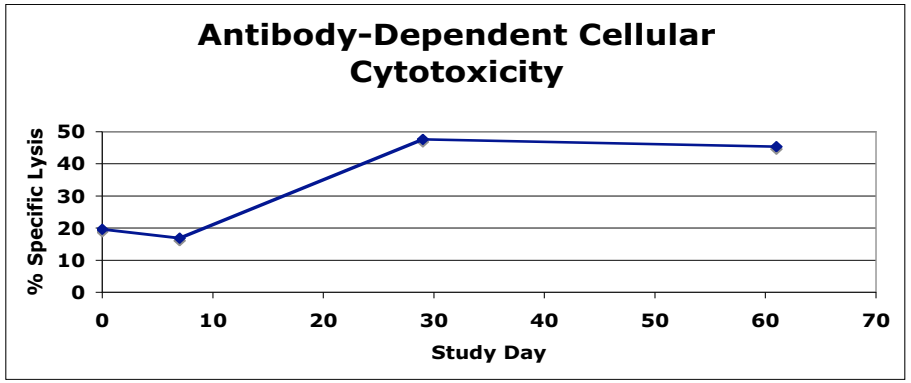
6. Plate target cells
7. Plate Effector cells E:T=33:1
8. 6 hours incubation
9. Calculate % SL as usual
10. Positive if %SL w/ serum >15%SL w/o serum

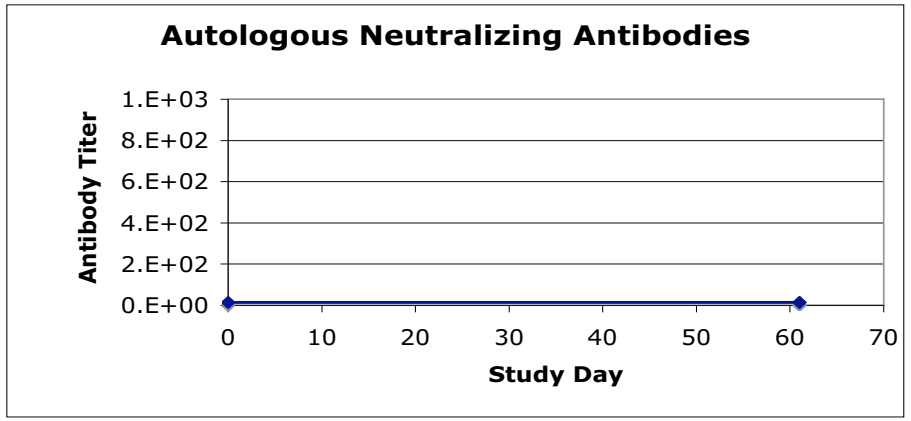
= gp120 = CD4 = HIV-1 = ADCC mediating Ab = Fc-receptor = $^{51}$Chromium

# Kinetic of ADCC-mediating Ab appearance in Subtype B-infected subjects: SC02 data representative of 20 subjects




G. Tomaras & G. Ferrari, unpublished data.

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# Limitations of current assay

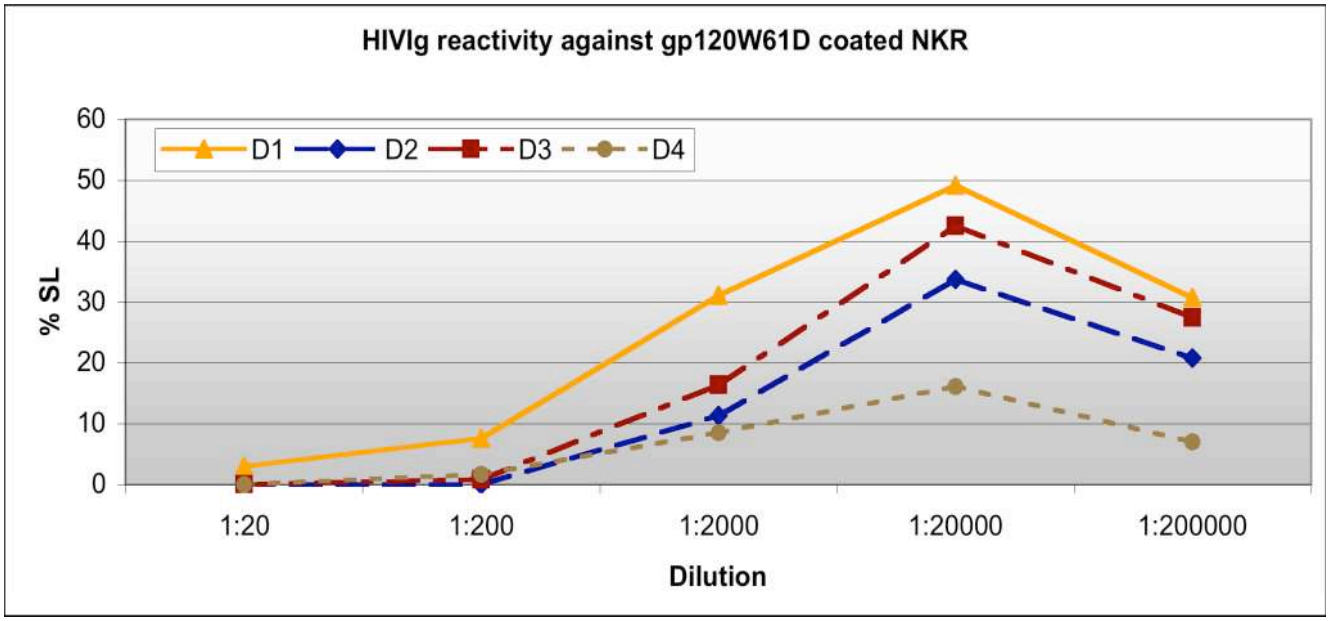
- **The use of fresh blood to run each assay makes it difficult to standardize the experiments**

# Influence of NK Cell Counts on detection of ADCC reactivities

A

| Sample | 3+/4+ | 3+/8+ | 3-/16+56+ |
|---|---|---|---|
| D1 | 24.55 | 26.07 | 39.16 |
| D2 | 44.74 | 28.18 | 17.82 |
| D3 | 53.25 | 20.98 | 17.16 |
| D4 | 50.59 | 28.41 | 5.48 |

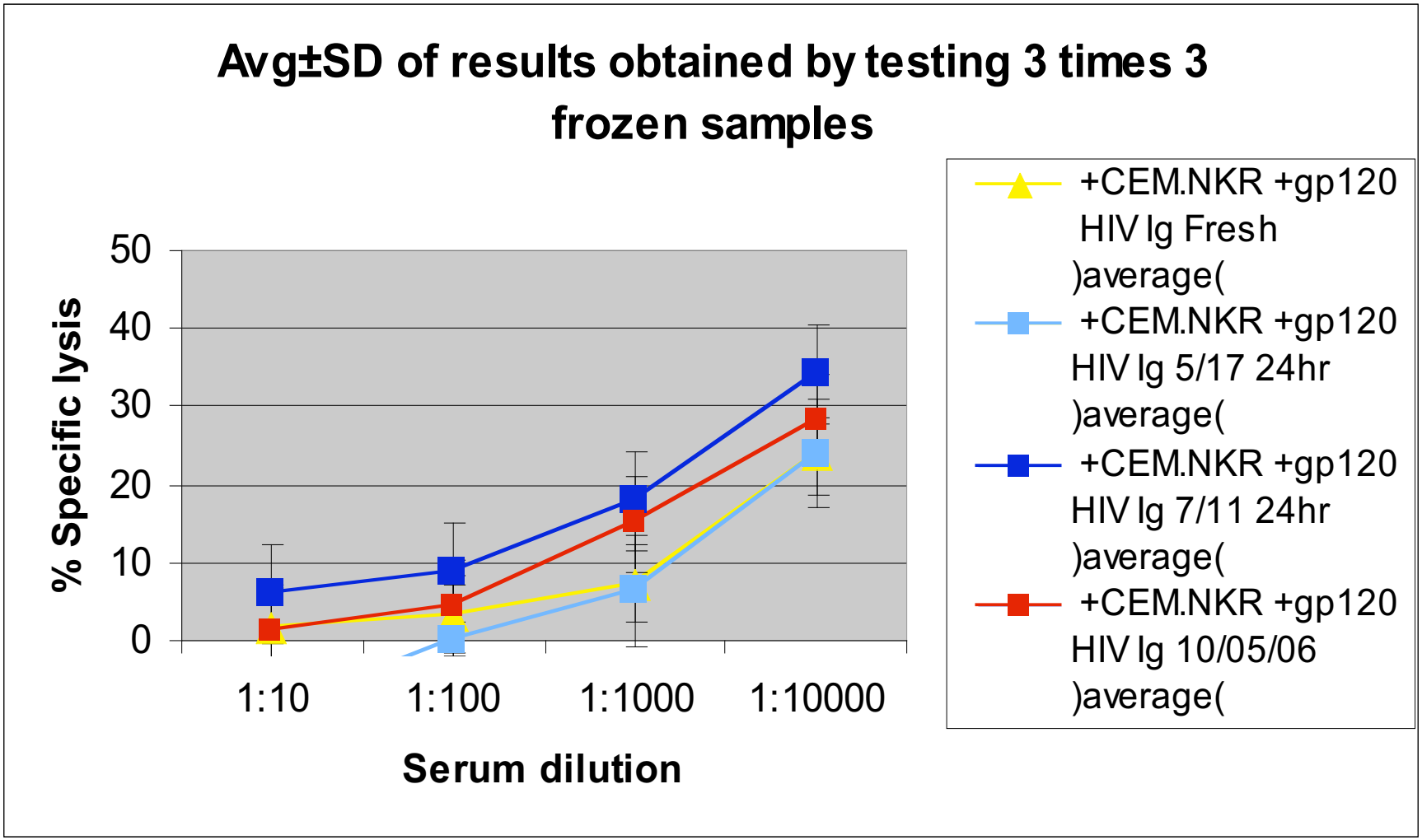
B




E:T ratio = 33:1 and 6 hrs incubation

# ADCC Effector Activity

## Fresh vs Frozen after o/n rest (24hr)

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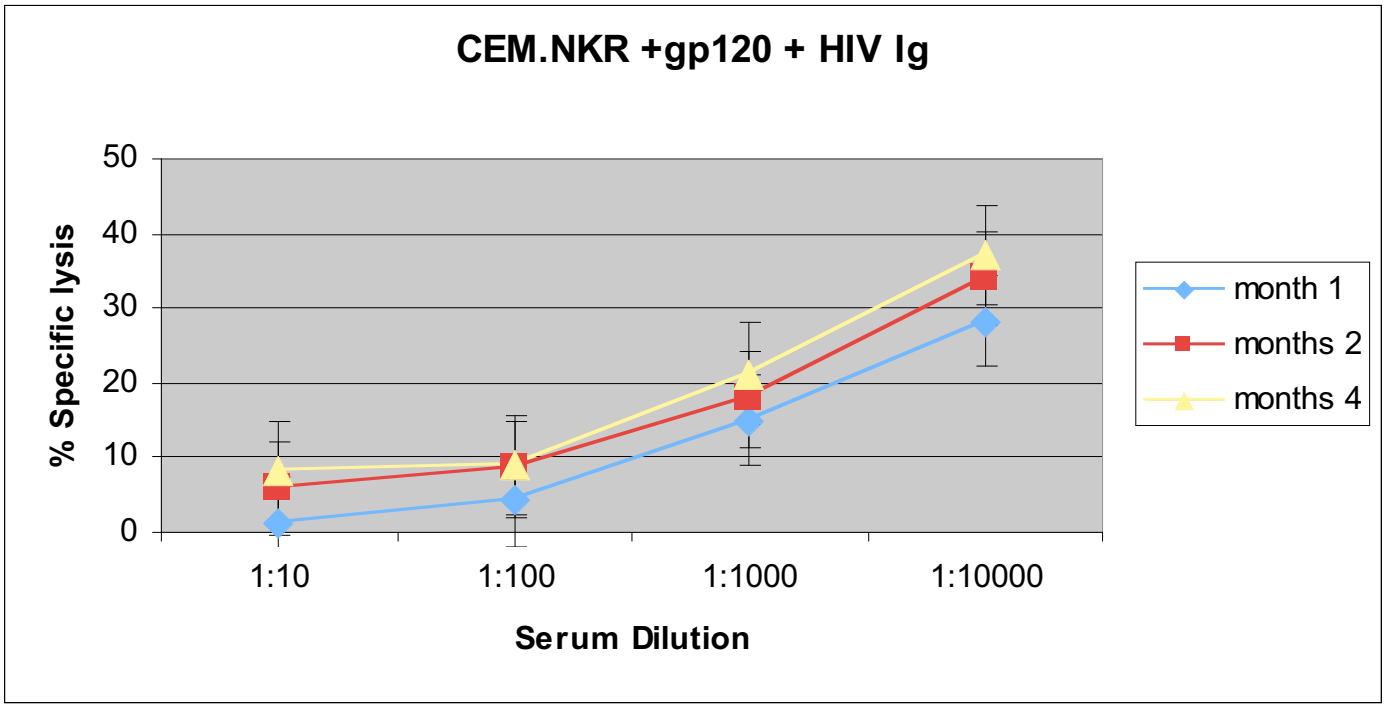
# Does detection decrease with Frozen samples?

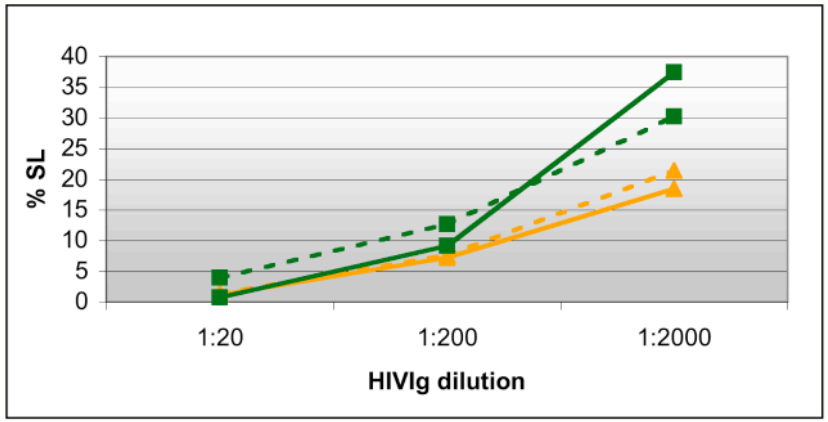


# Limitations of current assay

- The use of fresh blood to run each assay makes it difficult to standardize the experiments
- **Artificial target (gp120 coated CD4+ Tcells)**
- **Dependency on available gp120 products**
- **Each recombinant gp120 saturates CD4 molecules on target cells at different concentrations**

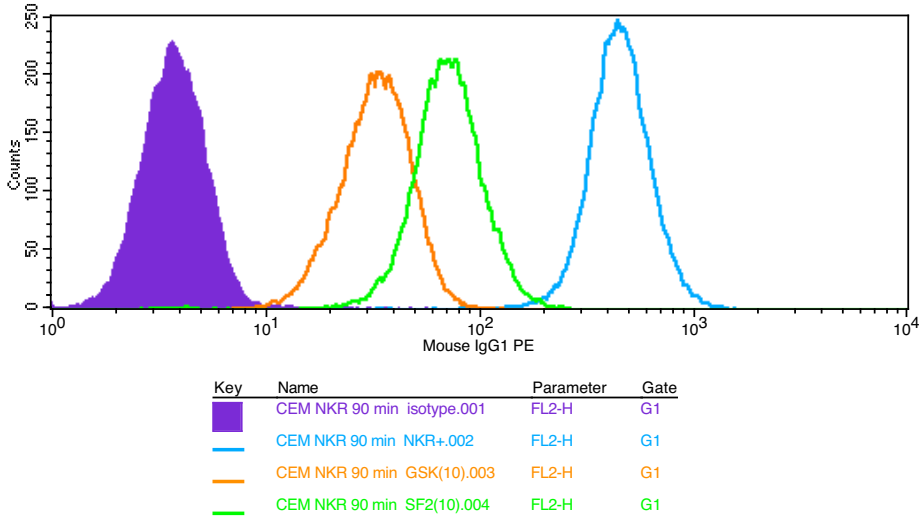
# Effects of optimal and suboptimal CD4 saturation on ADCC

A

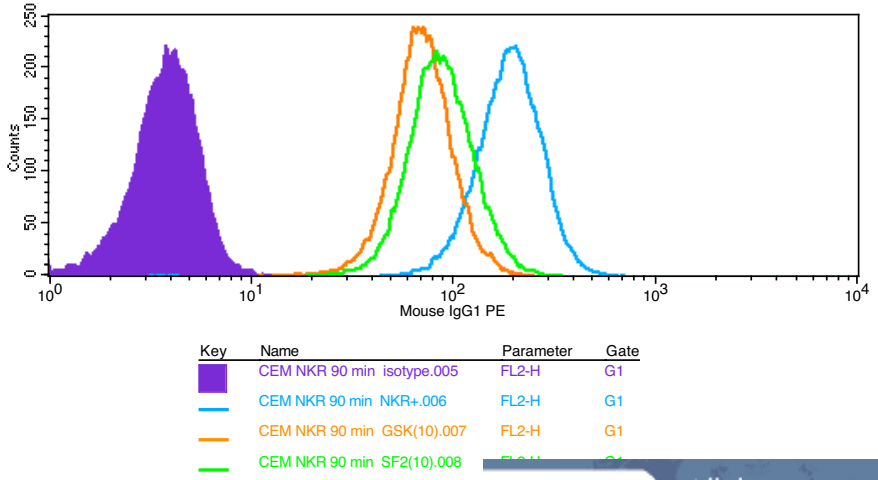



Dotted and solid lines represent %SL obtained with optimal and suboptimal saturation, respectively, of $gp120_{W61D}$ and $gp120_{SF2}$. Effector obtained from Donor 1.

B: Optimal saturation




C: Suboptimal saturation

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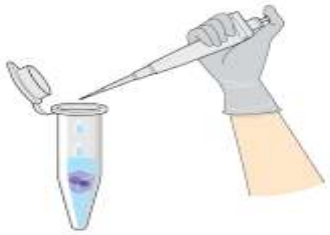
- **Envelope reagents not linked to those used to detect Nab**
- **Coating with gp120 does not show gp41 activity**

# Envelope Expressing Strategies

**DNA Plasmid containing gp160**

**Transfection Reagent**



**Incubate 45 minutes at RT**

**Add DNA:Transfection complex to CEM.NK$^{r}$ cells**

**Incubate for 24-48hrs**

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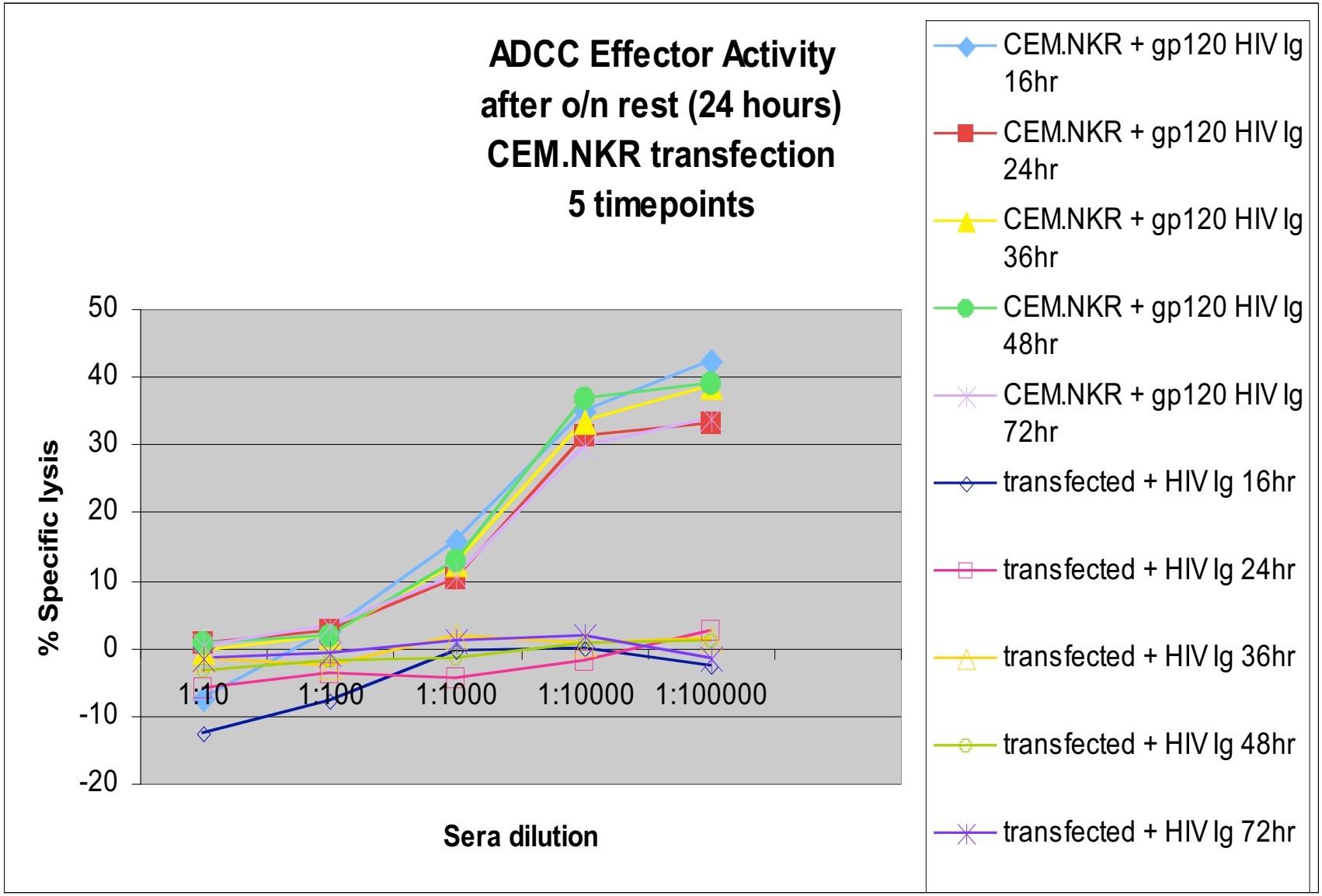

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- Envelope reagents not linked to those used to detect Nab
- **Coating with gp120 does not show gp41 activity**
- **Limitation of radioactive usage**
- **Health risks associated with working with radioactive materials**

# Acknowledgement


- Kina Armstrong
- Christopher Dibble

- David Montefiori
- Mira Biliska

- Georgia Tomaras
- Kent Weinhold