Vaccination with Human Hookworm Vaccine “Necator americanus Aspartic Protease-1 M74” Generates Neutralizing Antibodies and a Potent Immune Response in BALB/c Mice

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Life Cycle of *Necator americanus* Human Hookworm

1. Eggs in feces
2. Rhabditiform larva hatches
3. Filariform larva penetrates skin
4. Filariform larva
5. Adults in small intestine

= Infective Stage
=d = Diagnostic Stage

[Link to CDC website: http://www.dpd.cdc.gov/dpdx]
Necator americanus Degradation of Host Hemoglobin and Vaccine Targets

Ingested Erythrocytes → Hemoglobin

NAb (Anti-Na-APR-1 IgG) → Na-APR-1 (Hemoglobinase)

Heme and Globin (Toxic)

NAb (Anti-Na-GST-1 IgG) → Na-GST-1 (Detoxification Enzyme)

Heme Na-GST-1 Complex
Method† (MOD=Fluorescence)

7-methoxycoumarin-4-acetyl-GKPILFFRLK(dinitrophenyl)-d-Arg-amide (fluorophor)

Cleavage site

7-methoxycoumarin-4-acetyl-GKPIFFRLK(dinitrophenyl)-d-Arg-amide (quencher)

Na-APR-1wt

7-methoxycoumarin-4-acetyl-GKPILFFRLK(dinitrophenyl)-d-Arg-amide (fluorophor)

Na-APR-1wt

Anti-Na-APR-1 M74 IgG

†Reaction Buffer - 50 mM Sodium Acetate (pH = 6.0).
Steps for Generation and Purification of Polyclonal Mouse IgG†

Protein G Sepharose column

Nanosep Filter

**Total IgG**
(Spectrophotometer)

†50 BALB/c mice vaccinated twice with 9.33µg Na-APR-1 M74 + 74.64µg Alhydrogel®.

Neutralizing Capacity of purified IgG with 250 ng of Na-APR-1wt and 1µM Cathepsin-D Substrate in 50mM Sodium Acetate, pH 6.0.

<table>
<thead>
<tr>
<th></th>
<th>IgG (µg)</th>
<th>Operators</th>
<th>% Inhibition*</th>
<th>Intra-plate %CV</th>
<th>Inter Operator %CV</th>
<th>All Runs %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 1</td>
<td>5</td>
<td>1</td>
<td>48.93</td>
<td>4.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>42.58</td>
<td>6.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Run 2</td>
<td></td>
<td>1</td>
<td>60.44</td>
<td>19.39</td>
<td></td>
<td>10.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>52.29</td>
<td>7.68</td>
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</table>

*RFU of the purified IgG compared to RFU of Negative IgG (commercial mouse IgG).
Note: All the components were incubated at 37°C for 30 Min. RFU=Relative fluorescence Units.
Neutralizing Capacity of 8, 4, 2 and 1 µg of IgG with 250ng of Na-APR-1wt

Note: All the components were incubated at 37°C for 30 Min.
RFU of Positive IgG compared to RFU of Negative IgG (commercial mouse IgG).
RFU=Relative fluorescence Units.
Potency Testing of \( Na\)-APR-1 M74 Clinical Drug Product$^+$
BALB/c mice immunized Subcutaneously with \( Na\)-APR-1/Alhydrogel\(^\circledR\) at time 1, 7 months post manufacture. (10 mice in 1 group)

<table>
<thead>
<tr>
<th>Na-APR-1 (µg)</th>
<th>Alhydrogel(^\circledR) (µg)</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>400</td>
<td>0.5</td>
</tr>
<tr>
<td>50.00</td>
<td>N/A</td>
<td>0.5</td>
</tr>
<tr>
<td>50.00</td>
<td>400.00</td>
<td>0.5</td>
</tr>
<tr>
<td>28.57</td>
<td>228.56</td>
<td>0.2857</td>
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<tr>
<td>16.33</td>
<td>130.64</td>
<td>0.1633</td>
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<tr>
<td>9.33</td>
<td>74.64</td>
<td>0.0933</td>
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<tr>
<td>5.33</td>
<td>42.64</td>
<td>0.0533</td>
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<tr>
<td>3.05</td>
<td>24.40</td>
<td>0.0305</td>
</tr>
<tr>
<td>1.74</td>
<td>13.92</td>
<td>0.0174</td>
</tr>
<tr>
<td>0.99</td>
<td>7.92</td>
<td>0.0099</td>
</tr>
</tbody>
</table>

Day → -1 0 28 42

B = Bleed
V = Vaccination
S = Sacrificed

$^+$0.1mg/mL \( Na\)-APR-1 (M74) and 0.8mg/mL Alhydrogel\(^\circledR\) in 150 mM NaCl/10mM imidazole/0.3% Empigen BB
Standard Calibration Curves (22 curves from 22 Anti Na-APR-1 M74 IgG ELISA Plates)

LOQ and RT

Standard Reference Serum

Day → -1 0 28 42

B V1 V2 S

B= Bleed
V= Vaccination (subcutaneously)
S= Sacrificed

9.33µg Na-APR-1 M74 + 74.64µg Alhydrogel®
### Responders on Study Day 28 Post-Prime Vaccination

<table>
<thead>
<tr>
<th>Fractional Doses (μg)</th>
<th>Responders</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Na-APR-1 M74</strong></td>
<td><strong>Alhydrogel®</strong></td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>400</td>
<td>0</td>
</tr>
<tr>
<td>50.00</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>50.00</td>
<td>400.00</td>
<td>9</td>
</tr>
<tr>
<td>50.00</td>
<td>228.56</td>
<td>10</td>
</tr>
<tr>
<td>28.57</td>
<td>130.64</td>
<td>6</td>
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<tr>
<td>16.33</td>
<td>74.64</td>
<td>3</td>
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<tr>
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<td>5.33</td>
<td>24.40</td>
<td>0</td>
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<tr>
<td>3.05</td>
<td>13.92</td>
<td>0</td>
</tr>
<tr>
<td>1.74</td>
<td>7.92</td>
<td>0</td>
</tr>
<tr>
<td>0.99</td>
<td>14.15</td>
<td>11.46</td>
</tr>
</tbody>
</table>

**ED50 (Effective Dose 50) (μg)**

**95% Fiducial Limits (μg)**

10.47 -- 18.93  
4.86 -- 27.42
ED50 on Study Day 28 Post-Prime Vaccination

- 1 Month
- 7 Month

Log (Dilution of Vaccine)

Probit

ED50 = Antilog(1.06) = 11.46 µg
ED50 = Antilog(1.15) = 14.15 µg

Na-APR-1(µg) 0.99 1.74 3.05 5.33 9.33 16.33 28.57 50
Alhydrogel® (µg) 7.96 13.93 24.37 42.65 74.64 130.61 228.57 400
Relative Potency (RP) of *Na-APR-1 M74 Vaccine*

![Graph showing relative potency over time with RP = 1.230 at 7 months post manufacture.](image-url)
Summary

- Five microgram of purified IgG from BALB/c mice vaccinated with 9.33 µg Na-APR-1 M74 and 76.64 µg Alhydrogel® neutralized 51.06% of the enzymatic activity of 250 ng of Na-APR-1wt

- An excellent dose response (% inhibition vs IgG) was observed

- The standard reference serum generated an excellent standard calibration curves (SCCs) as well as an excellent global standard calibration curve (GSCC) using an Anti-Na-APR-1 M74 IgG ELISA

- Na-APR-1 M74 vaccine generated a potent immune response in Balb/c as evident by the generation of ED50 at time 1 and 7 month post-manufacture

- Na-APR-1 M74 vaccine became 1.23 times more potent at time 7 month when compared to its potency at time 1 month post-manufacture
Acknowledgments

- This project is supported by the Sabin Vaccine Institute through funding from the Bill and Melinda Gates Foundation and the Dutch Government