National Genetics Institute

Pooled HCV NAT Using the UltraQual™ HCV RT-PCR Assay

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National Genetics Institute

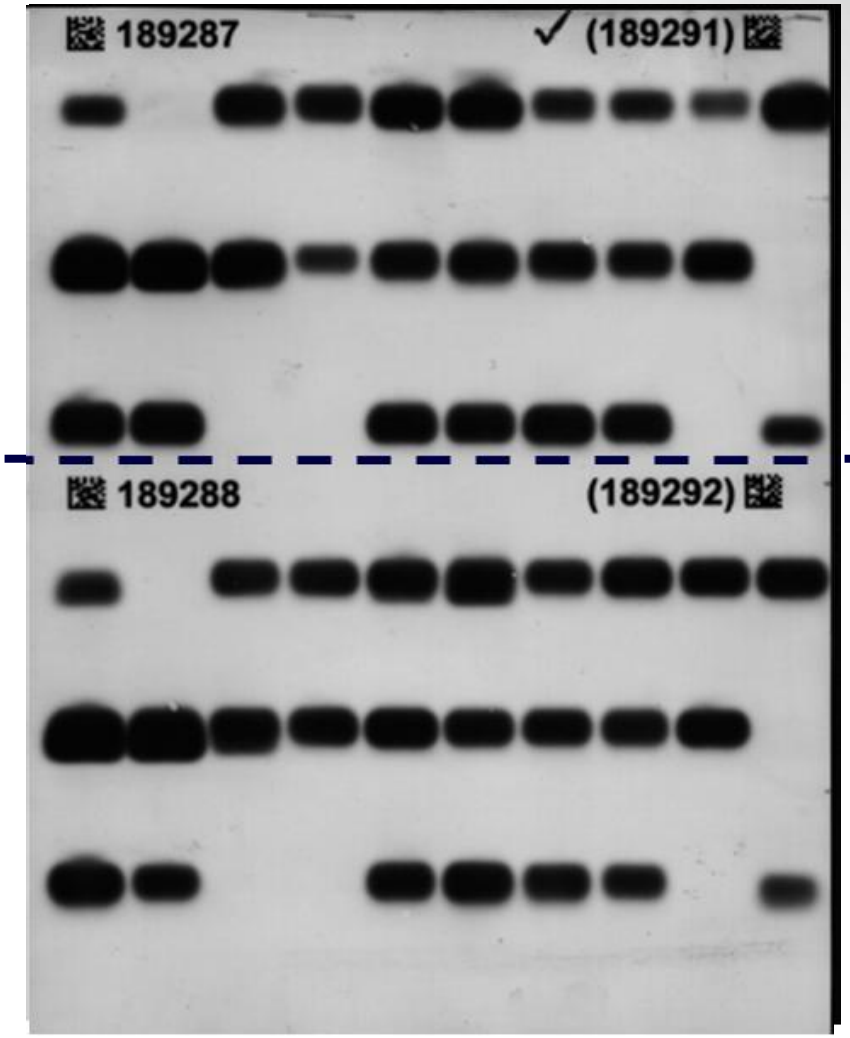
OUTLINE

- Assay Overview
- Pooling
- Clinical Study Data
- Prospective Data

ASSAY OVERVIEW

Primer Set 1

Primer Set 2



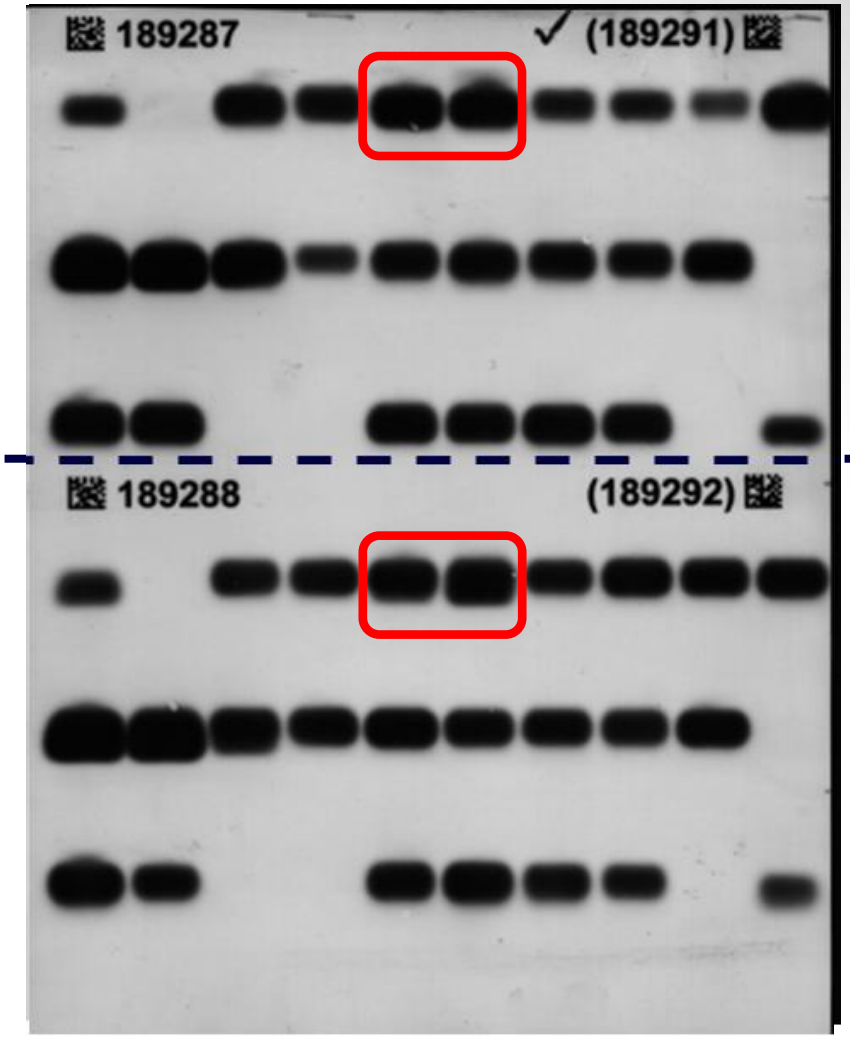
Re-Hybridized with Internal Control Probe

UltraQual™ HCV RT-PCR Raw Data

ASSAY OVERVIEW

Primer Set 1

Primer Set 2



One POSITIVE Master Pool Highlighted

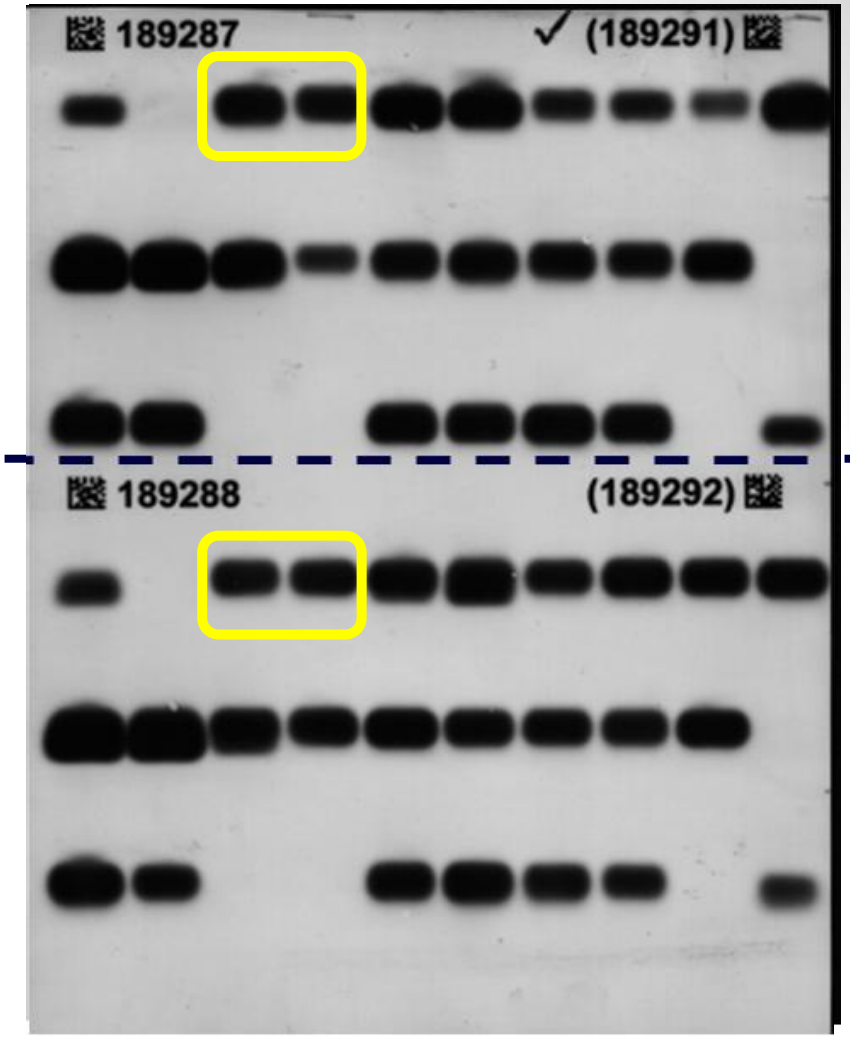
Re-Hybridized with Internal Control Probe

UltraQual™ HCV RT-PCR Raw Data

ASSAY OVERVIEW

Primer Set 1

Primer Set 2



One NEGATIVE Master Pool Highlighted

Re-Hybridized with Internal Control Probe

UltraQual™ HCV RT-PCR Raw Data

ASSAY OVERVIEW

NGI's UltraQual™ HCV RT-PCR Assay

Input Volume	Amplification Reactions	50% Detection Cutoff (IU/mL)	95% Detection Cutoff (IU/mL)	Assay Commonly Used For
1 mL	4	1.24	[redacted]	pools of 65 - 512
1 mL	2	2.44	10.4	pools of 64 or less
100 µL[1]	2	24.4	104	individual samples

[1] 100 µL assay sensitivities calculated by multiplying the 1 mL assay sensitivities by 10.

3,532 IU/mL for an individual sample in a pool of 512

Used for resolution of positive master pools

Validated for Detection of HCV Genotypes 1 - 6

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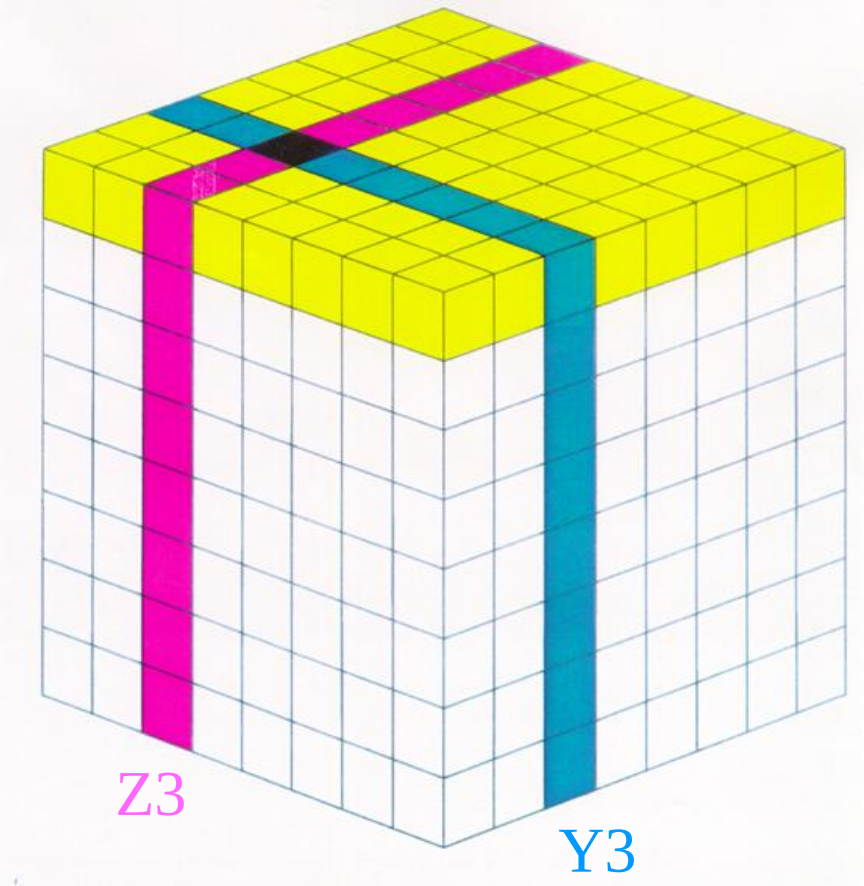
ROBOTIC POOLING

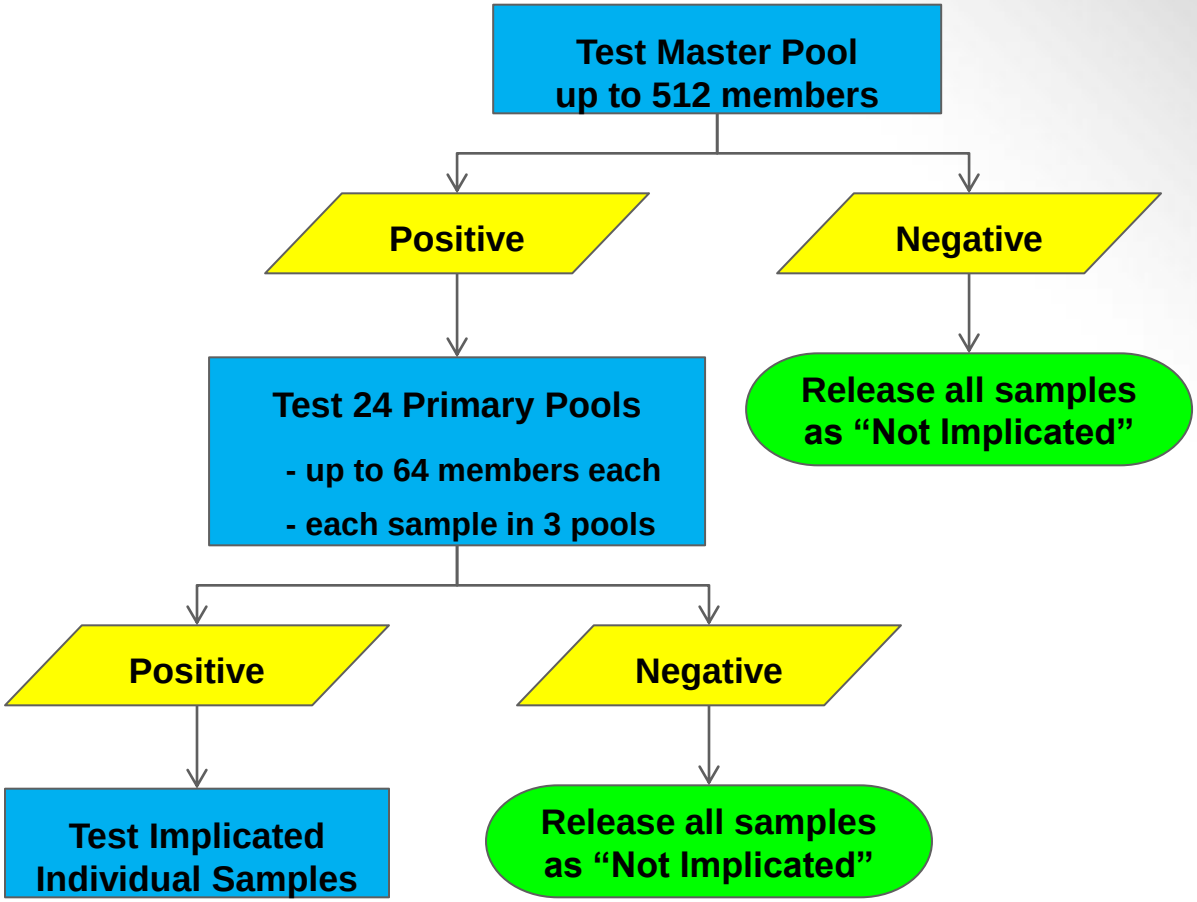
POOLING OVERVIEW

“Three-Dimensional” Pooling
8 x 8 x 8 Matrix



ngi_POOLING.flv

Basic Resolution Scheme

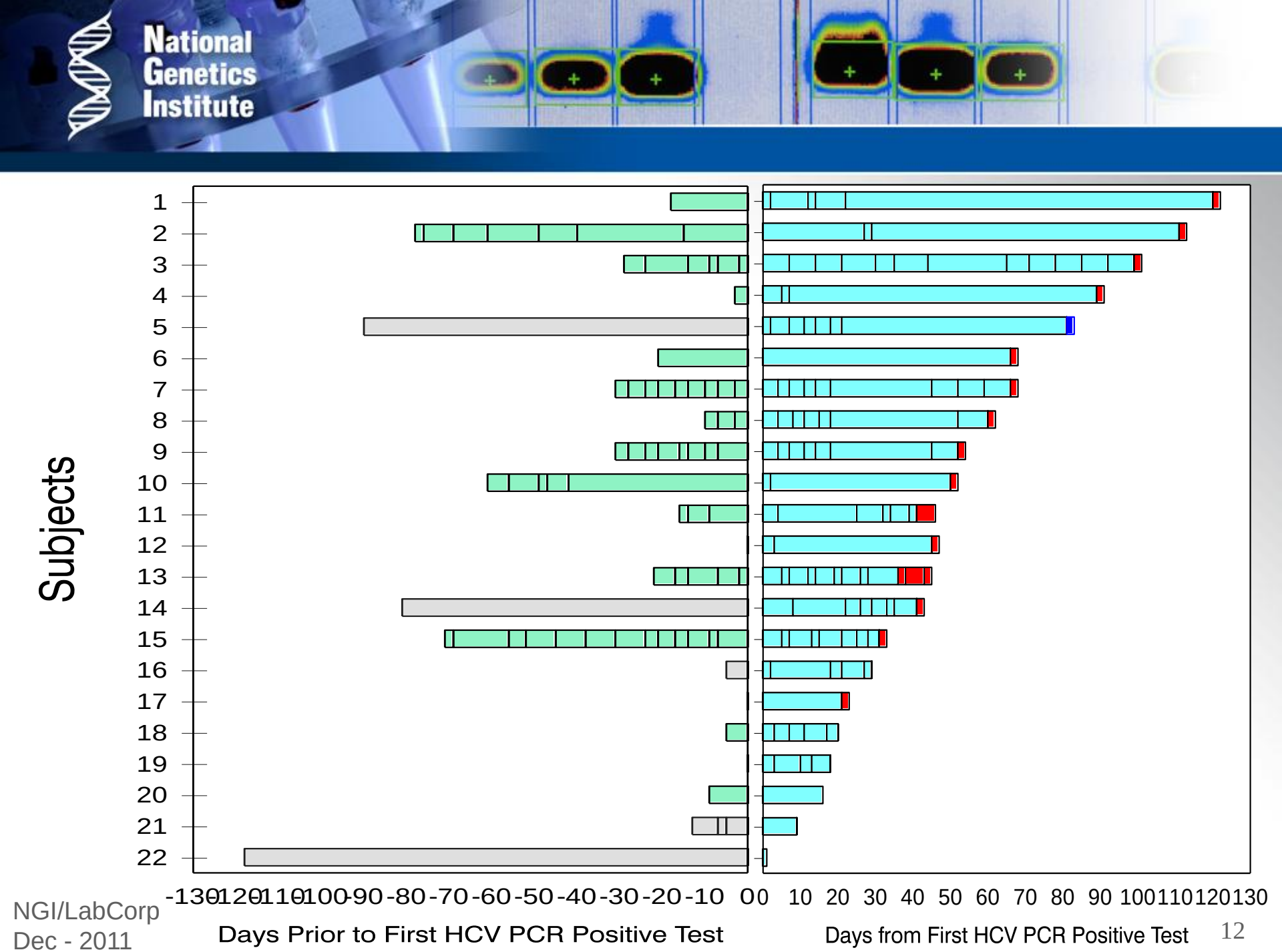


CLINICAL STUDY RESULTS

- 342,729 Source Plasma units screened
- 85 found HCV NAT + / anti-HCV EIA -
  (1 per 4,032 units or 0.025%)
- These window-period donations came from 22/48,000 donors
  (1 per 2,182 donors or 0.046%)

CLINICAL STUDY RESULTS

- Mean time to seroconversion 57+/- 14 days
- Range 22 to 120 days(Window Period)
- Average viral load of 1.36×10^{7} copies/mL
- Range (7.5×10^{3} - 5.6×10^{7} copies/mL)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Subjects
1
2
3
4
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14
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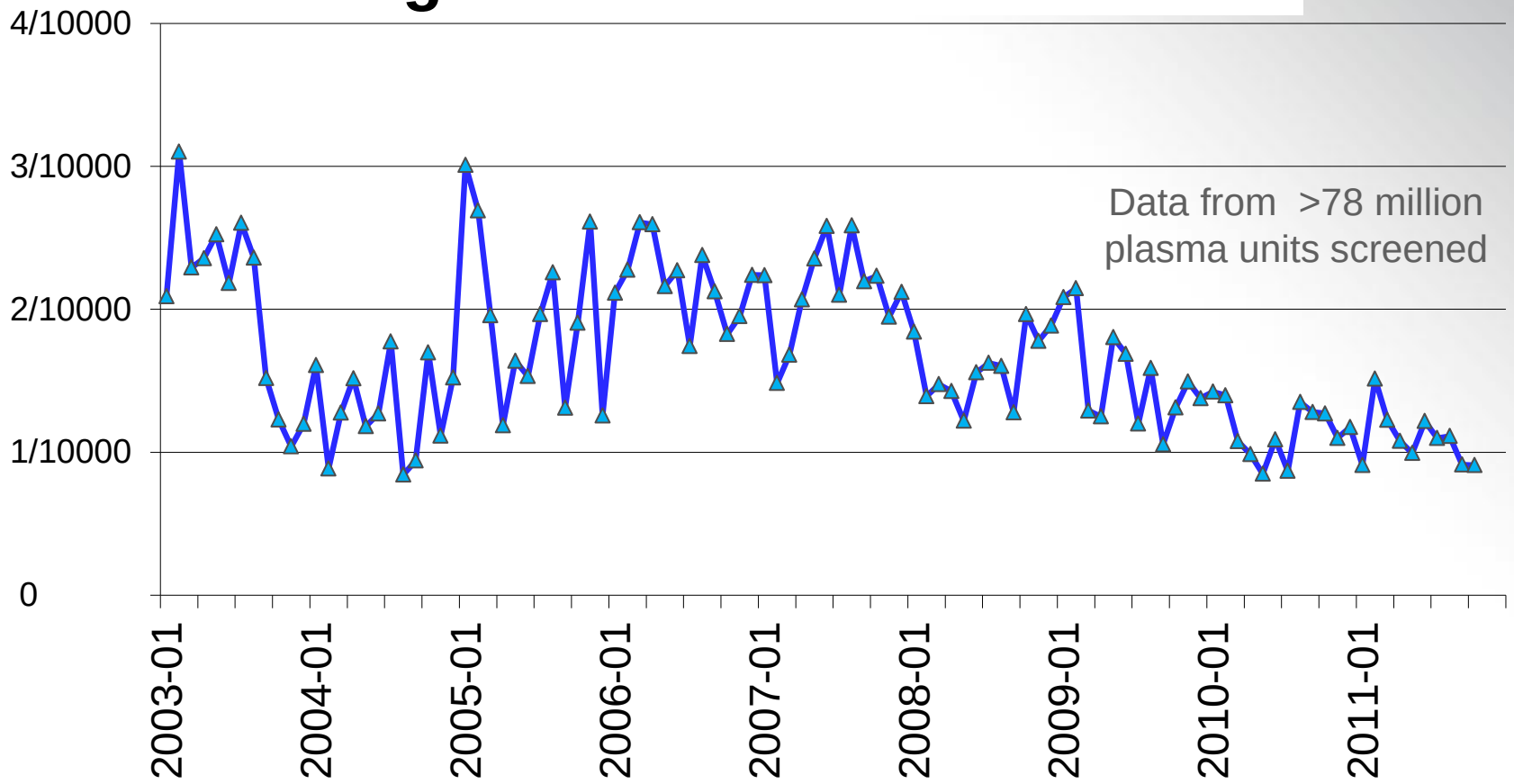
16
17
18
19
20
21
22
-130 -120 -110 -100 -90 -80 -70 -60 -50 -40 -30 -20 -10 0
0 10 20 30 40 50 60 70 80 90 100 110 120 130
Days Prior to First HCV PCR Positive Test
Days from First HCV PCR Positive Test

PROSPECTIVE DATA

HCV NAT Positivity in anti-HCV Negative Source Plasma Units