# Antibody Avidity and Antigen-Specific Antibody Profiling during HCV Infection

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**Assay Development and Diagnostic Reference Team**

**Division of Viral Hepatitis**

**CDC**

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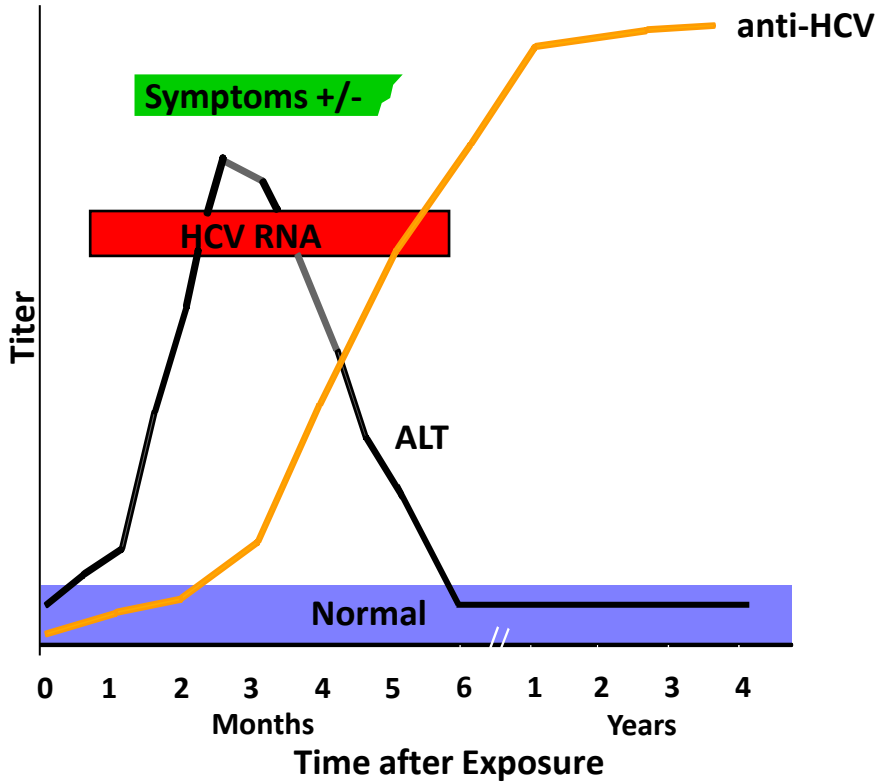
# Serologic Pattern of HCV Infection




**Acute**

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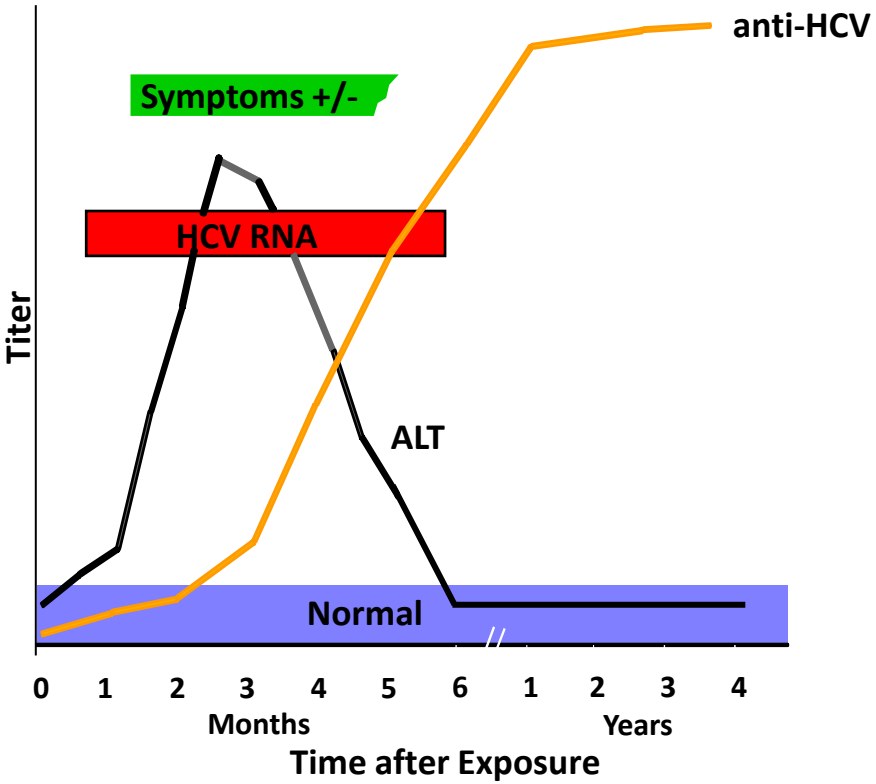
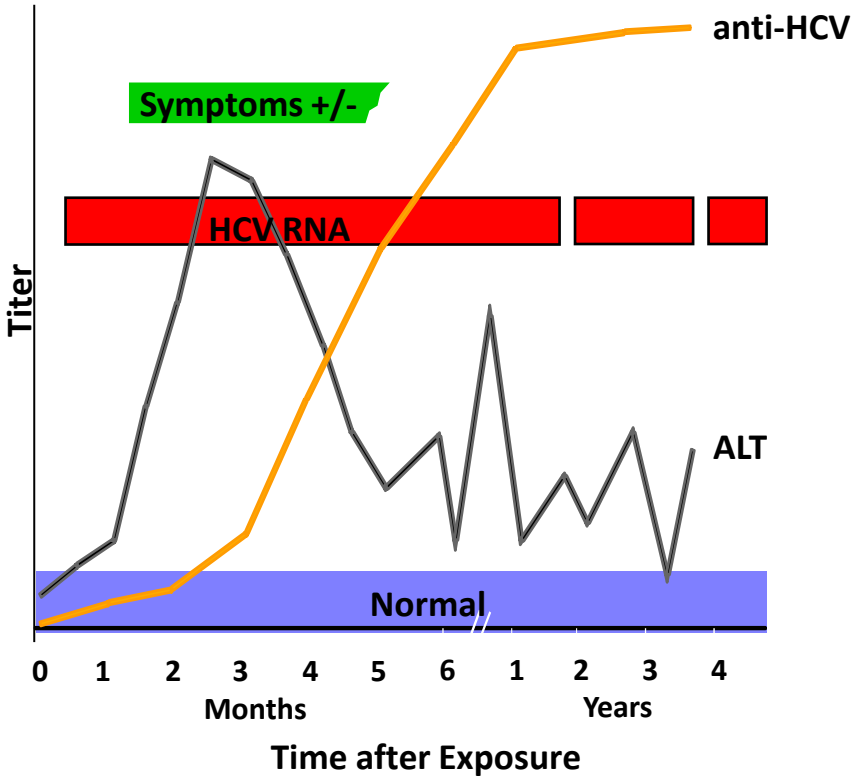
# Serologic Pattern of HCV Infection




**Acute**




**Chronic**

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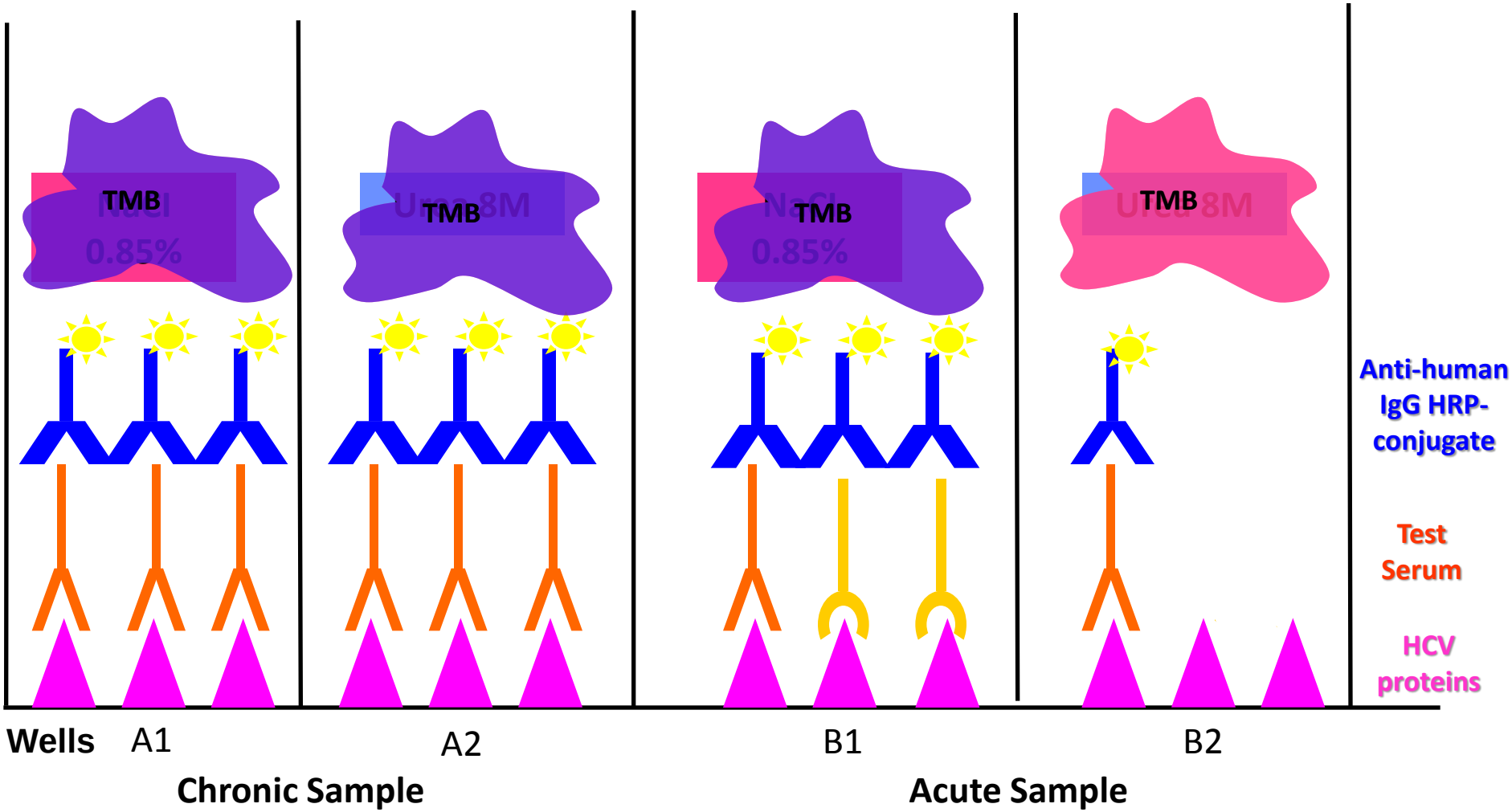
# Background

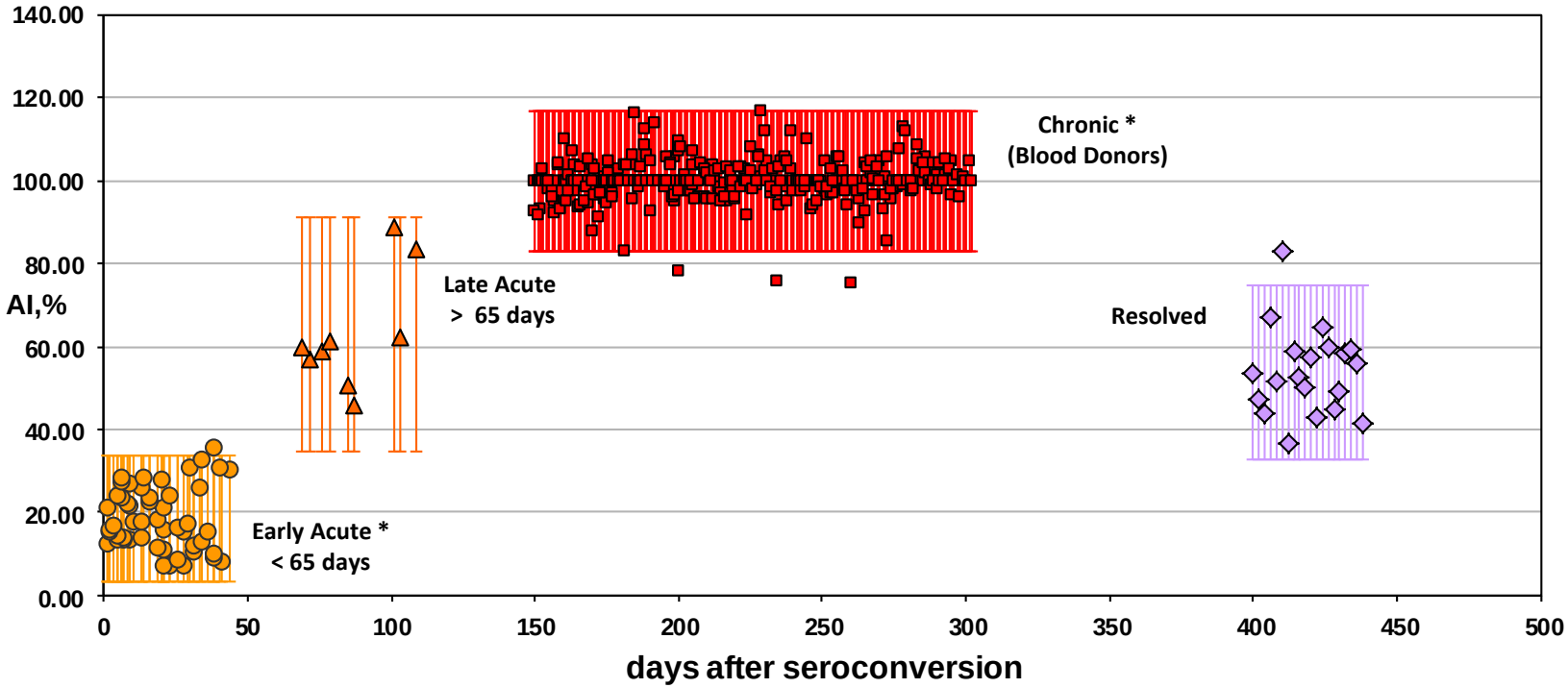
- The diagnosis of incident HCV infections has relied upon a combination of epidemiologic risk factor assessment and screening for ALT activity and HCV antibody.
- It is very difficult to identify seroconversion to anti-HCV outside of prospective studies.
- The early identification of patients with acute HCV infection, followed by treatment with IFN-α can lead to eradication of the virus in nearly all cases in contrast to a much lower rate (42 – 82%) if the treatment starts during chronic disease.

# Development of Anti-HCV IgG Avidity Assay

$$AI = OD_{8M\ Urea} / OD_{0.85\%\ NaCl} * 100\%$$



# AI Differences among 4 Study Groups




*Klimashevskaya et al., JCM, 2007, 45:3400–3403*

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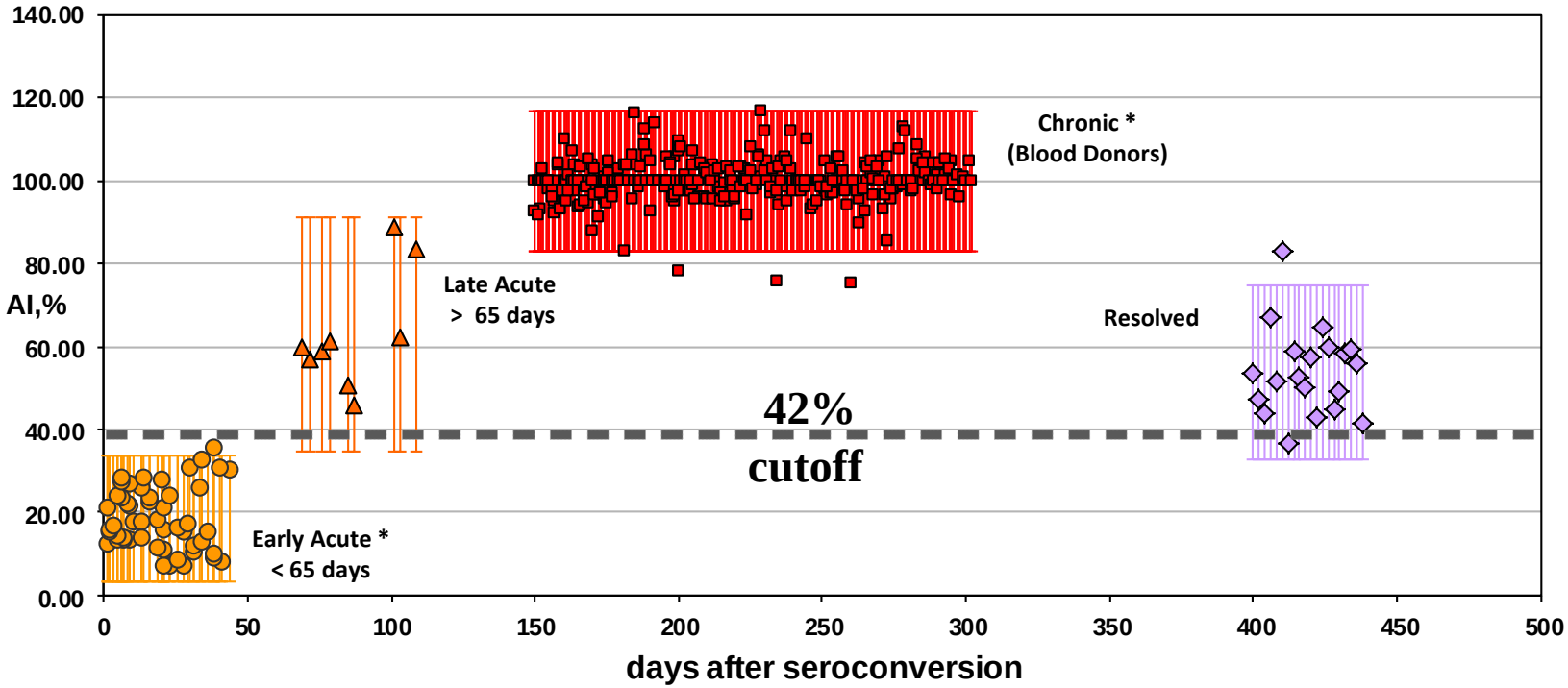


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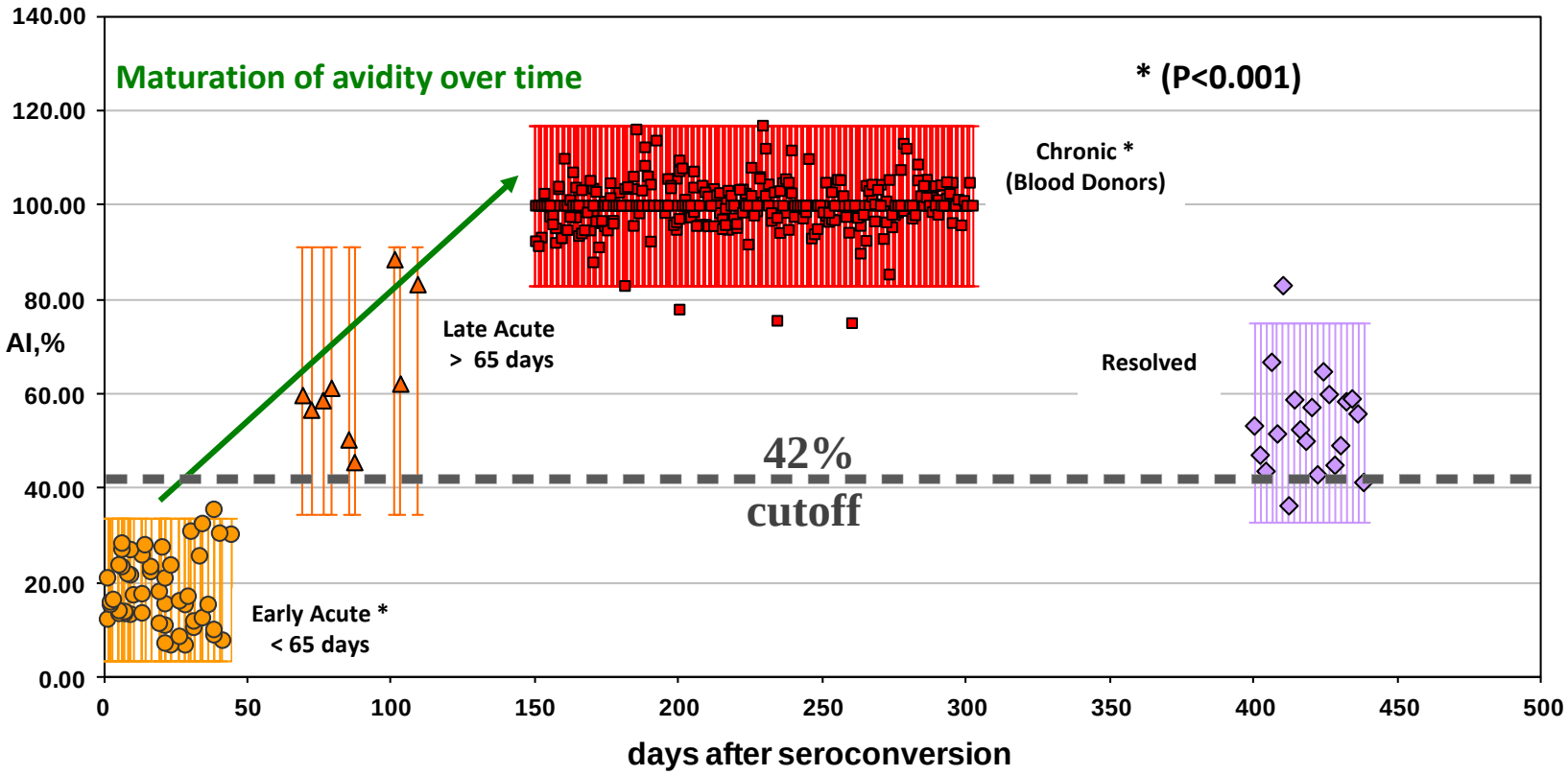


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# Development of high throughput assay for identification of Acute HCV Infection

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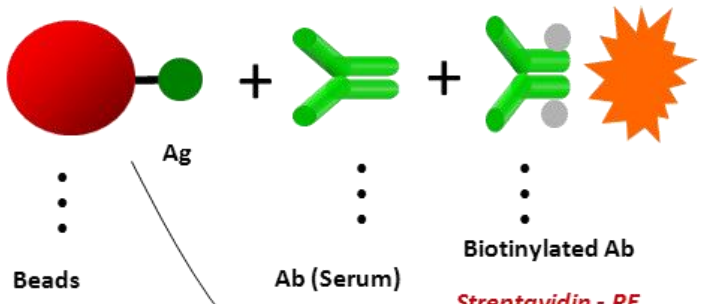
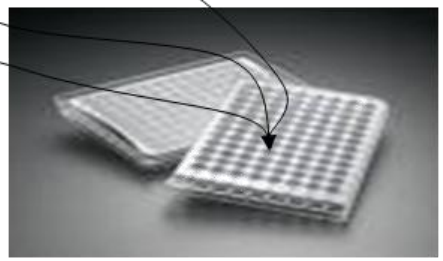
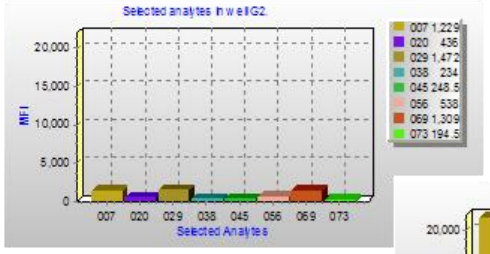
# HCV IgG Detection using LUMINEX System

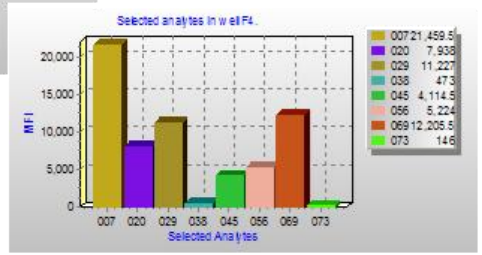
Antibody Capture Assay

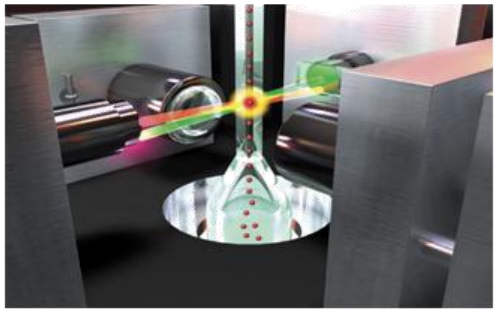


Data analyzes



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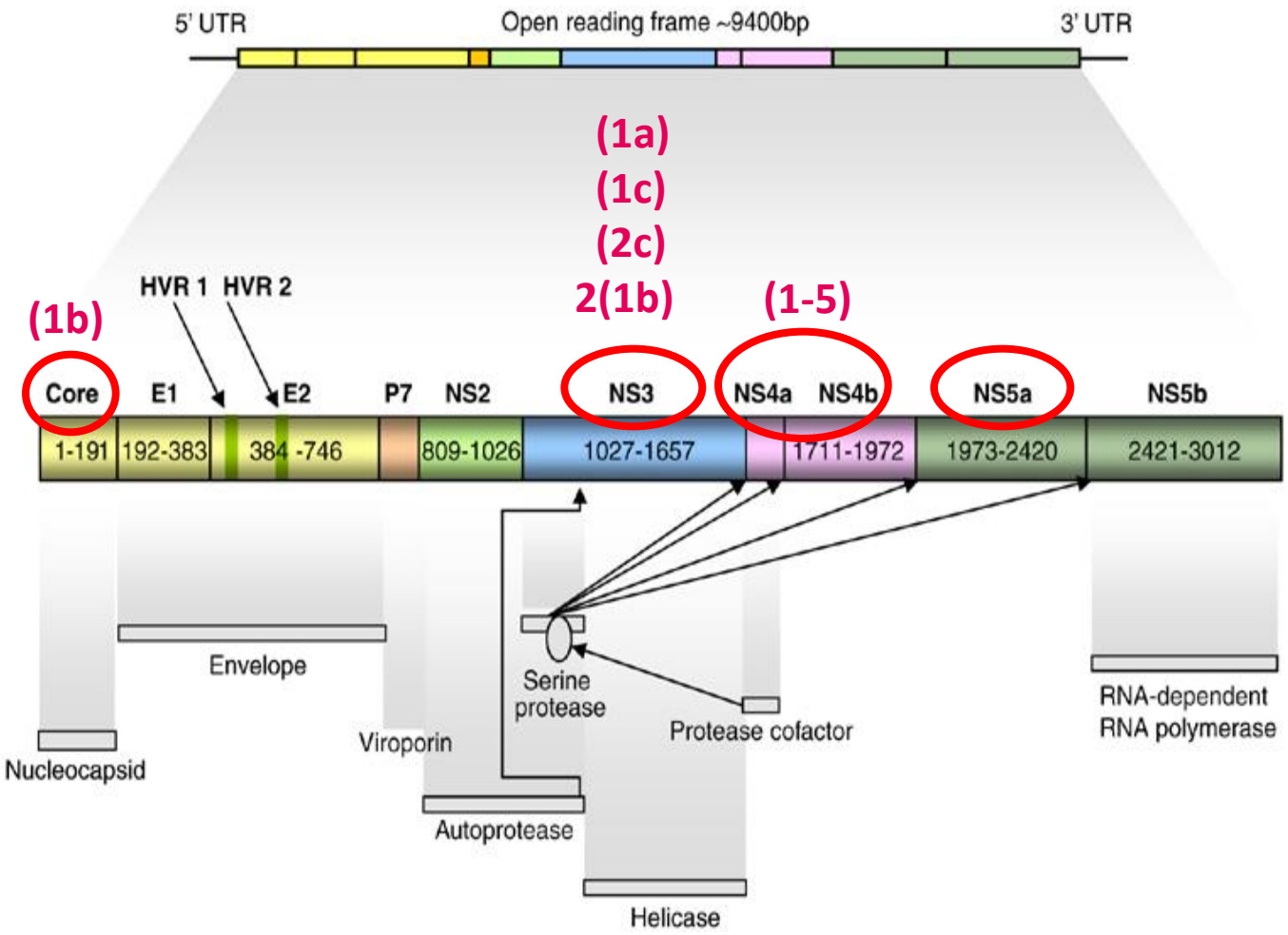
# Hepatitis C Virus

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# Evaluation Panel

- **Acute Infection:**

  Anti-HCV seroconversion panels

  – **Less than 62 days after seroconversion to anti-HCV (n=99)**

- **Chronic Infection:**

  Blood donors (BBI and American Red Cross)

  – **Anti-HCV and PCR positive**

  **(n=141)**

# Methods

- **Eight HCV recombinant antigens**
- **Cutoff value**
  - Mean MFI value of NHS samples + 2 SD
- **Signal/Cutoff = MFI/Cutoff**
- **Statistical evaluation**
  - Student T-test ($P < 0.001$)
- **Classifier** (MATLAB)
  - Multivariate Logistic Regression Model

**(MFI ) Mean Fluorescence Intensity**

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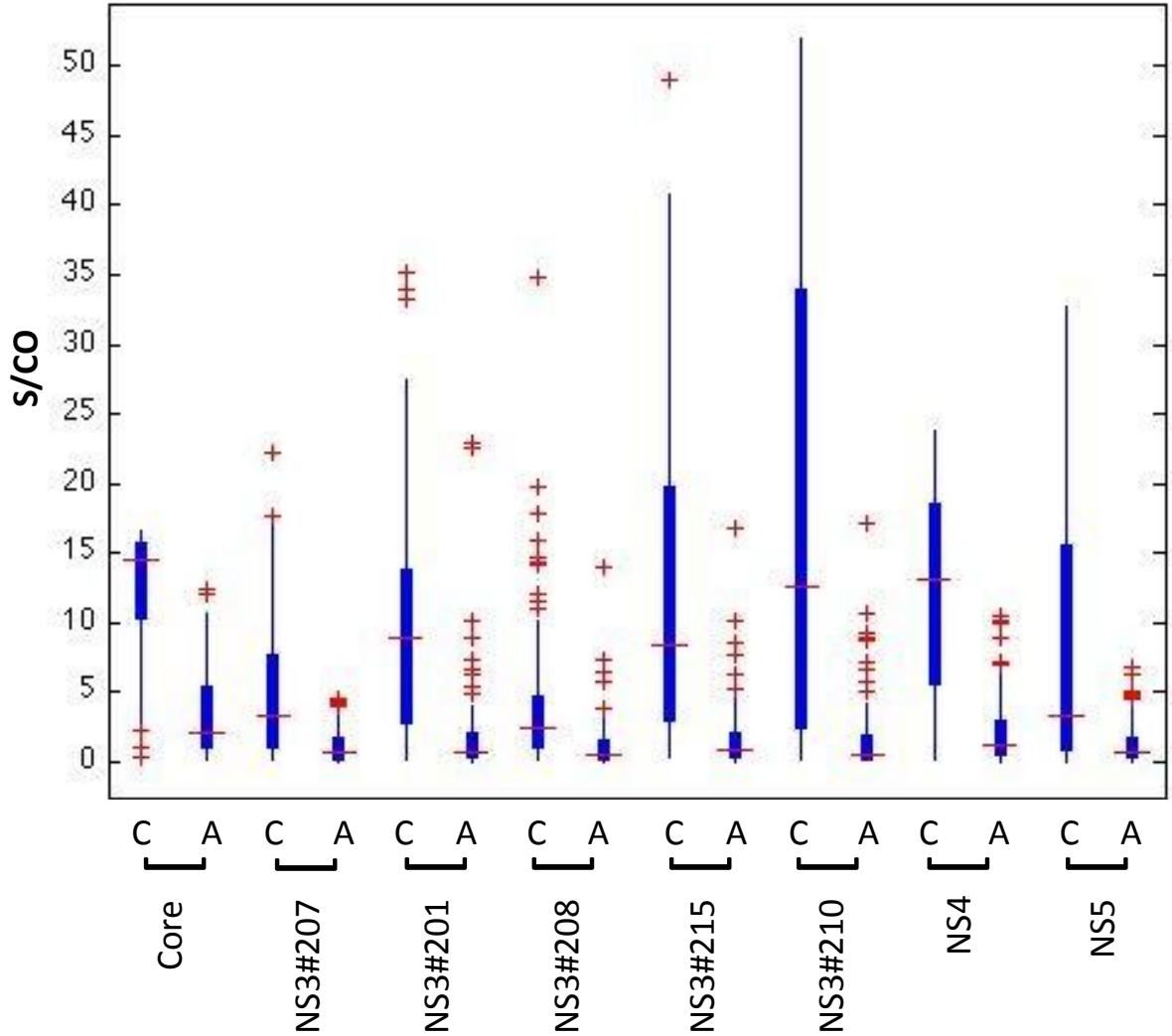
# Distribution of S/CO values per antigen in acute and chronic groups




C = Chronic
A = Acute

*Araujo et al., JCM, 2011, 49:54–57*

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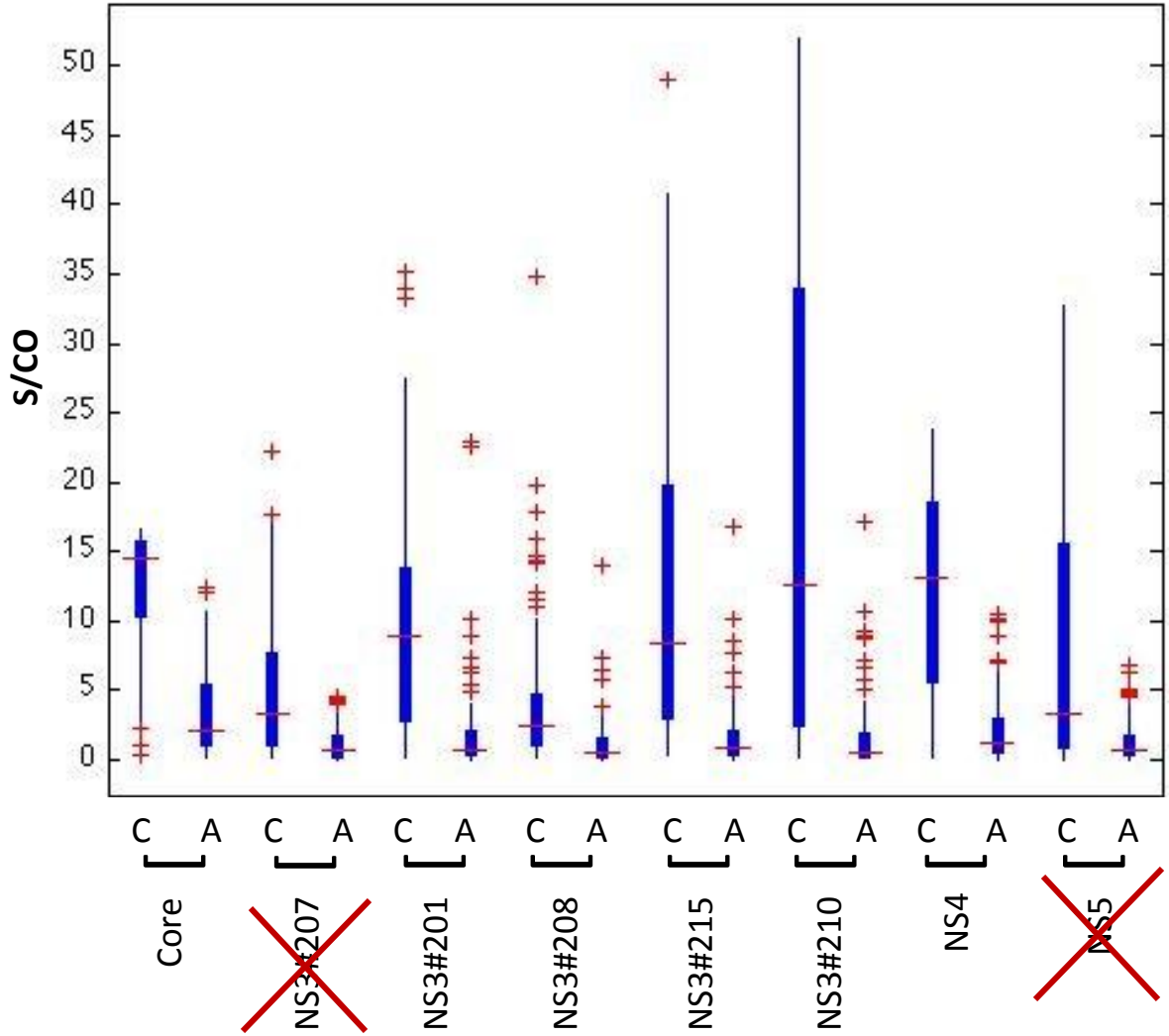
# Distribution of S/CO values per antigen in acute and chronic groups




C = Chronic
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# Serum Samples from HCVCU Cohort

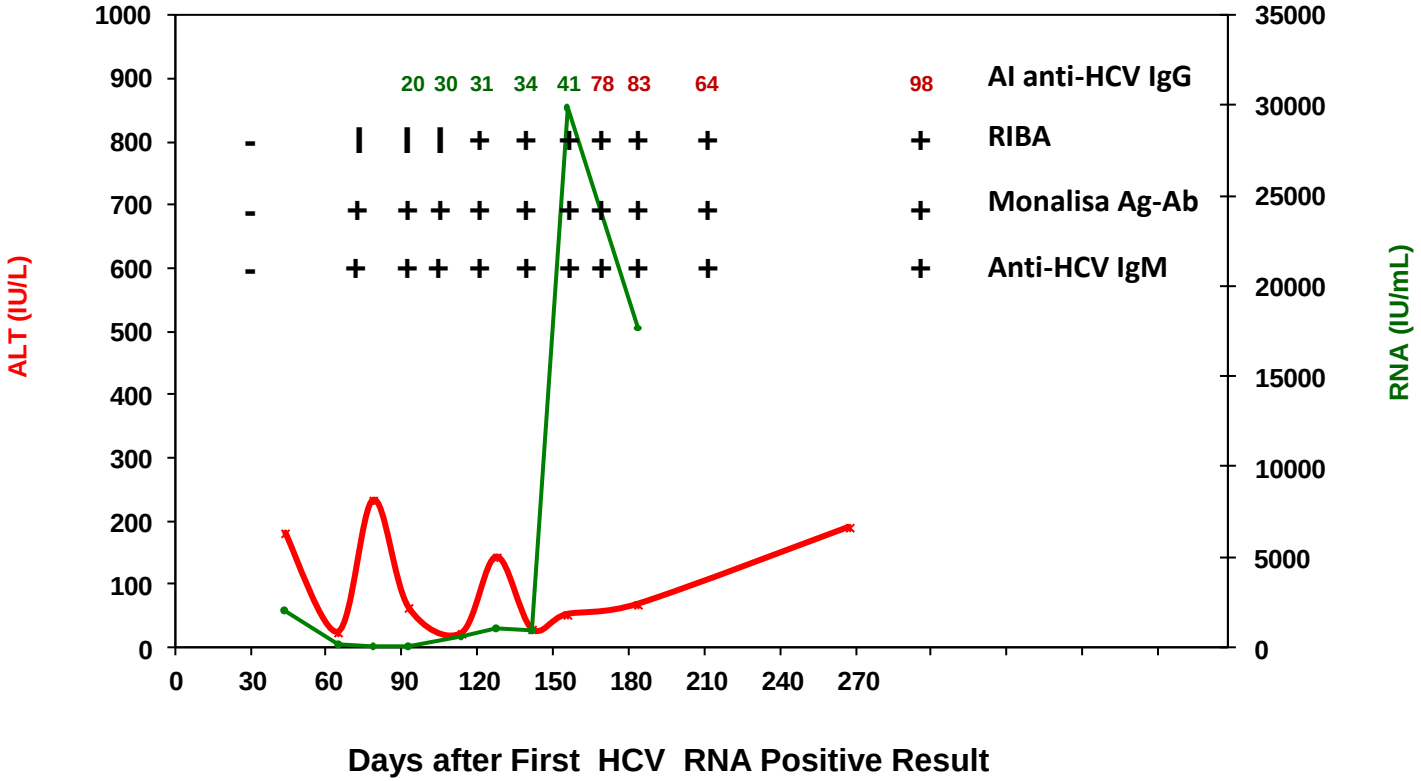
| Patient ID | Number of samples | Gender | Race | Year of Birth | Treatment | Clearance | Genotype | Reinfection | Description |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6 | F | LATINO | 1986 | Yes | Yes | 1 | | Prospective Infection (Cleared on Antiviral RX) |
| 2 | 11 | M | WHITE | 1980 | No | | 1 | | Prospective Infection |
| 3 | 7 | M | WHITE | 1980 | No | Yes | 1 | | Prospective Infection |
| 4 | 4 | F | WHITE | 1986 | No | | ? | | Prospective Infection |
| 5 | 3 | M | WHITE | 1979 | No | | 1 | | Prospective Infection |
| 7 | 14 | M | M | 1986 | No | | 1 | Yes | Prospective Infection and Reinfection |
| 8 | 2 | M | WHITE | 1985 | No | | 3 | | Prospective Infection |
| 9 | 15 | M | WHITE | 1986 | No | | 3 | Yes | Prospective Infection |
| 10 | 1 | M | LATINO | 1970 | Yes | | 3 | | Prospective Infection |
| 18 | 2 | F | WHITE | 1980 | No | | 1 | | Prospective Infection |
| 19 | 3 | F | BLACK | 1981 | No | | ? | | Prospective Infection w/Lag |
| 22 | 4 | M | WHITE | 1985 | No | | ? | | Prospective Infection w/Lag |
| 24 | 6 | F | M | 1976 | No | | ? | | Prospective Infection w/Lag |
| 28 | 12 | M | WHITE | 1983 | No | | 1a | | Window Peroid Infection |
| 29 | 2 | M | WHITE | 1985 | No | | 1 | | Window Peroid Infection |
| 32 | 1 | F | WHITE | 1981 | No | | 1 | | Window Peroid Infection |
| 34 | 1 | M | WHITE | 1983 | No | | 1 | | Window Peroid Infection |
| 39 | 1 | M | WHITE | 1985 | No | | 2 | | Window Peroid Infection |
| 45 | 8 | M | M | 1977 | No | | ? | | Window Peroid Infection |
| 46 | 3 | F | WHITE | 1977 | No | | 3 | | Apparent Seronegative Infection (HIV) |
| 51 | 3 | F | WHITE | 1985 | No | | ? | | Possible Window Period Infection |
| 53 | 4 | F | WHITE | 1980 | No | | ? | Yes | Probable Reinfection |
| 54 | 2 | M | LATINO | 1974 | No | | 3 | Yes | Probable Reinfection |
| 55 | 7 | F | WHITE | 1978 | No | | ? | Yes | Probable Reinfection |
| 56 | 13 | F | M | 1985 | No | | 1 | Yes | Probable Reinfection |
| 57 | 24 | M | LATINO | 1974 | No | | 1 | Yes | Probable Reinfection |
| 58 | 5 | F | WHITE | 1985 | No | | 1 | Yes | Probable Reinfection |
| 60 | 16 | M | WHITE | 1982 | No | | 3 | Yes | Probable Reinfection |
| 61 | 21 | F | WHITE | 1985 | No | | 1/3 | Yes | Reinfection |

# Sample Distribution during Period of Infection

| Days after 1st HCV RNA Positive Result | Samples (n) | Window Period | Prospective | Reinfection |
|---|---|---|---|---|
| **0 -180** | 78 | 8 | 28 | 42 |
| **180 – 365** | 38 | 7 | 7 | 24 |
| **> 365** | 51 | 9 | 12 | 30 |

# HCVCU Study

## Patient 2 - Prospective infection
## 25 y white male, untreated – HCV genotype 1

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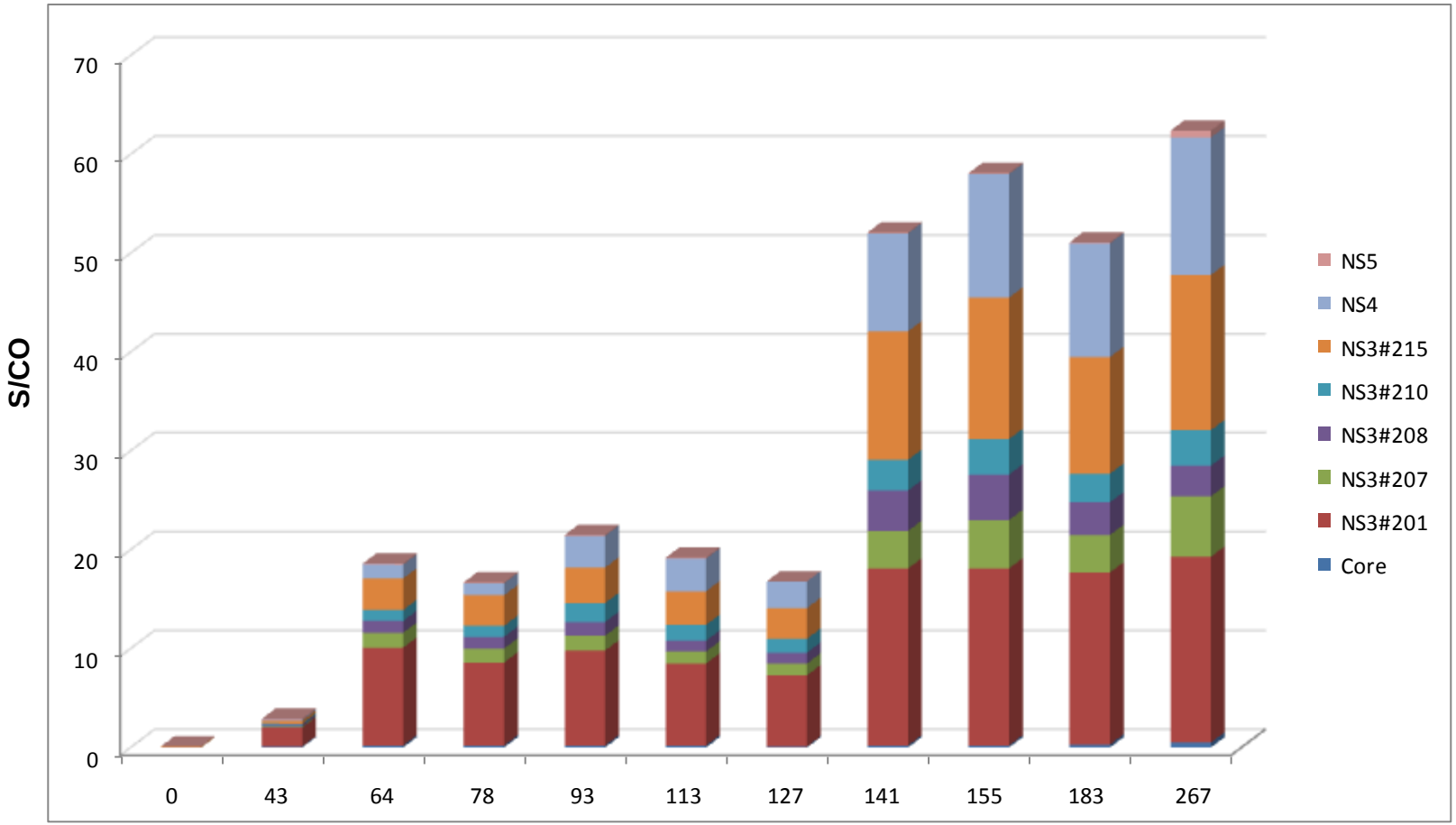
# HCVCU Study

## Anti-HCV IgG Luminex Assay

### Patient 2 - Prospective infection




Days after First HCV RNA Positive Result

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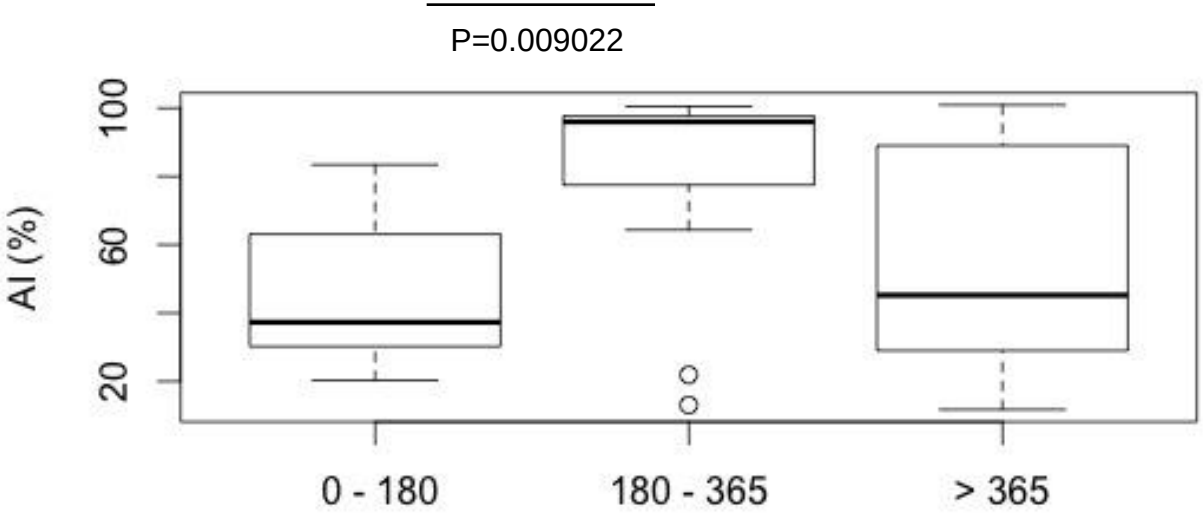
# Distribution of AI Results over Time

**Prospective Infection**




**Days After 1st HCV RNA Positive Result**

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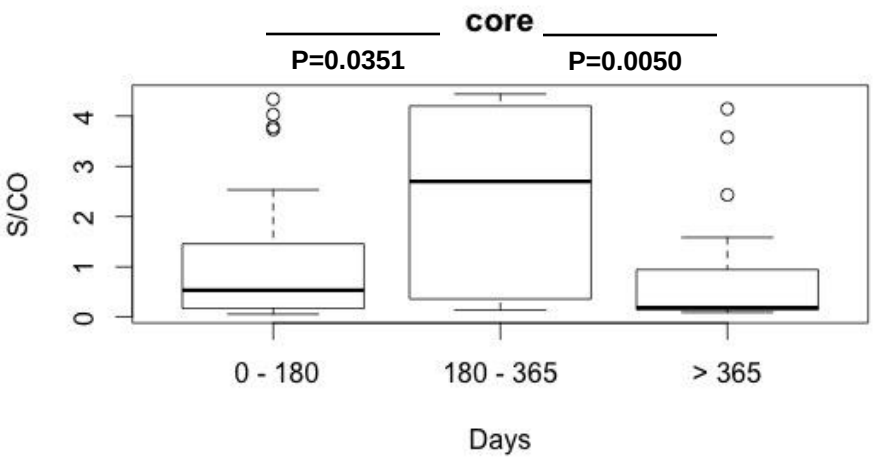
# Distribution of Antibody Reactivities to Structural and Non-structural HCV proteins



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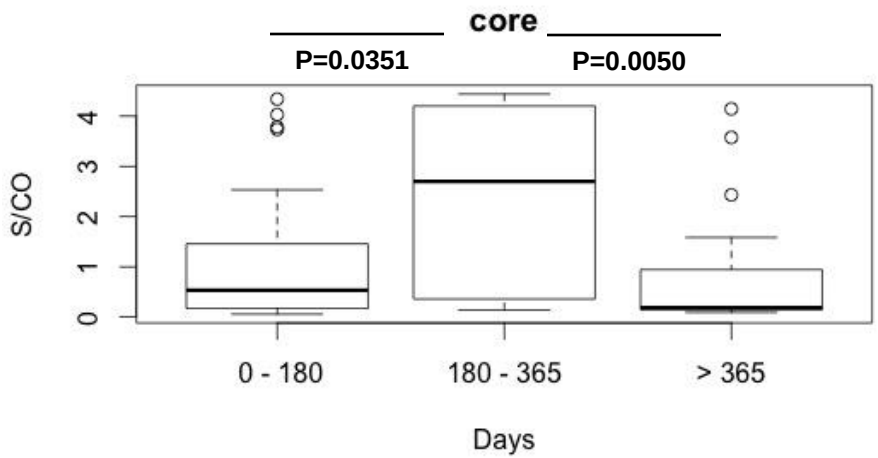


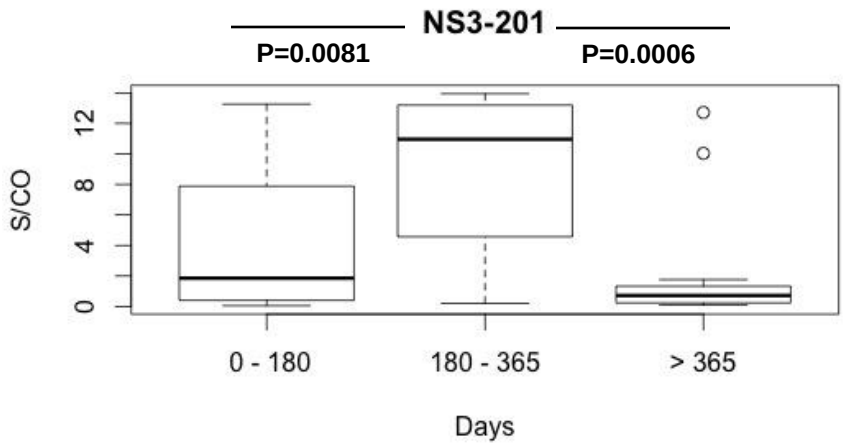


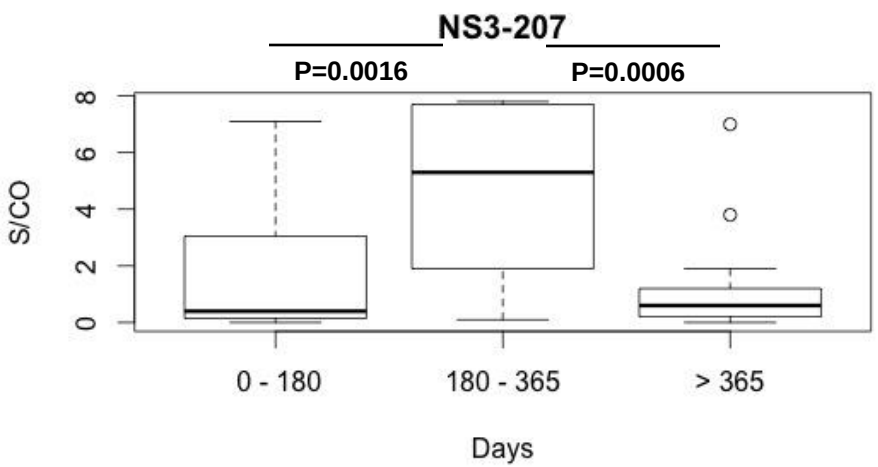


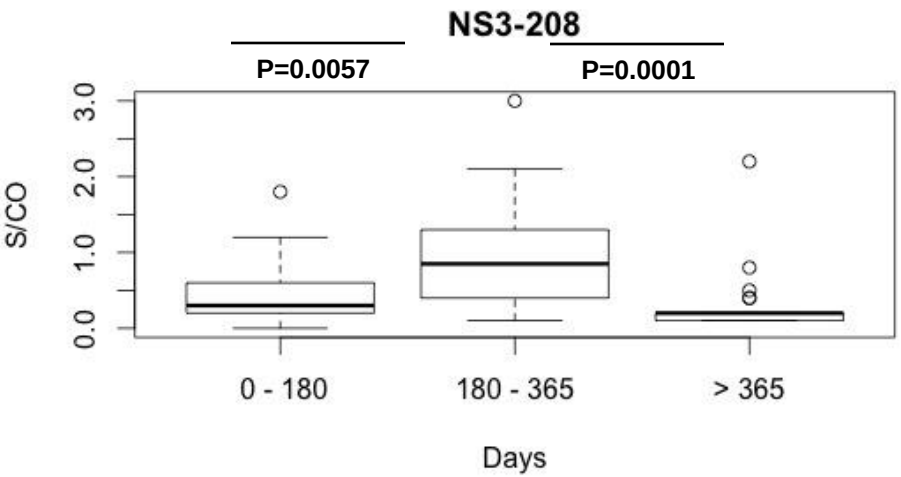

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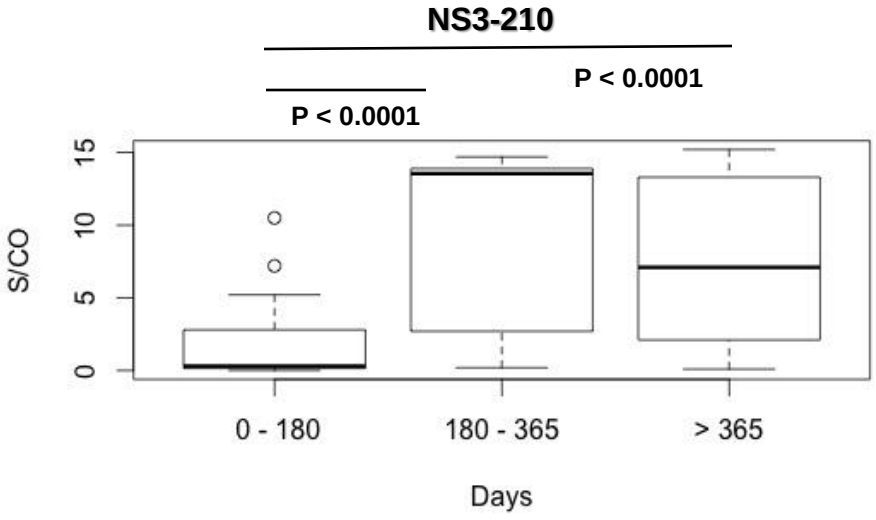


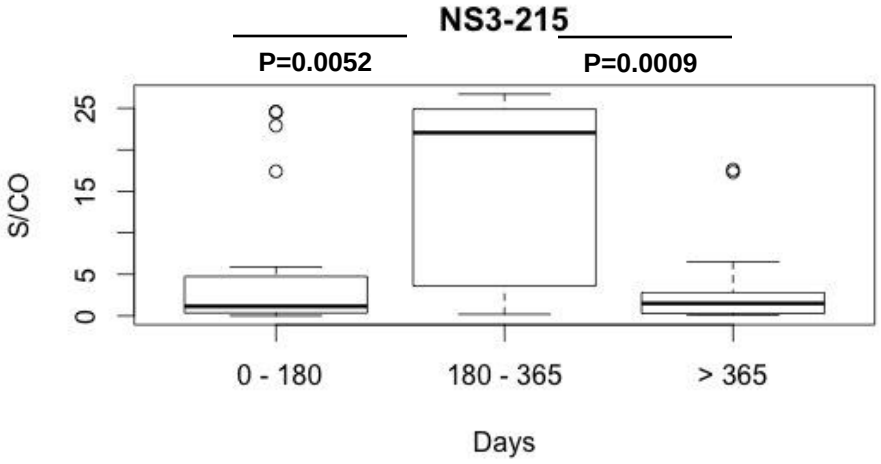


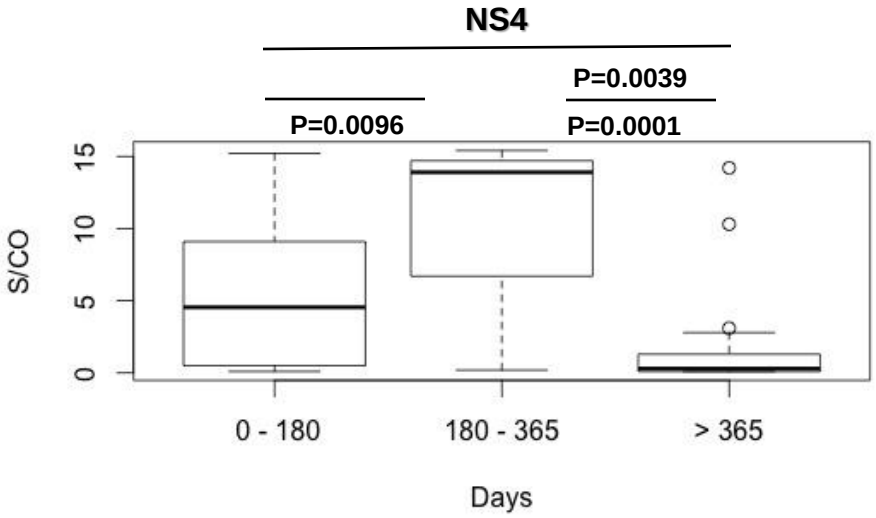


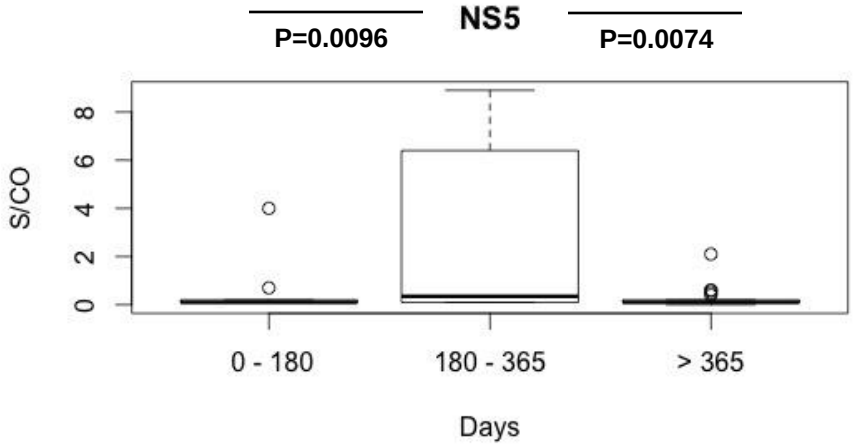

# Summary and Conclusion (I)

- There is a significant difference in the Avidity Index (AI) of anti-HCV IgG in samples collected during early acute and chronic phases of HCV infection.
- In the HCV Luminex assay the reactivity to the 6 HCV recombinant proteins distinguished acute and chronic infections with an accuracy of approximately 91% and 97% respectively.
- A multiplexed HCV recombinant-antigen microsphere immunoassay can be used to detect HCV infections and potentially distinguish acute from chronic HCV infections.

# Summary and Conclusion (II)

- Avidity Index assay identified 75% of the specimens collected during acute phase of HCV infection.
- Preliminary data on the Luminex assay, using all 8 HCV recombinant antigens show that S/CO values increase during early chronic phase of infection.
- The AI and the Luminex HCV Assays require further evaluation in clinical settings.

# Acknowledgement


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