Development of a Serologic Assay for Differentiating Acute from Chronic Hepatitis C Virus infection

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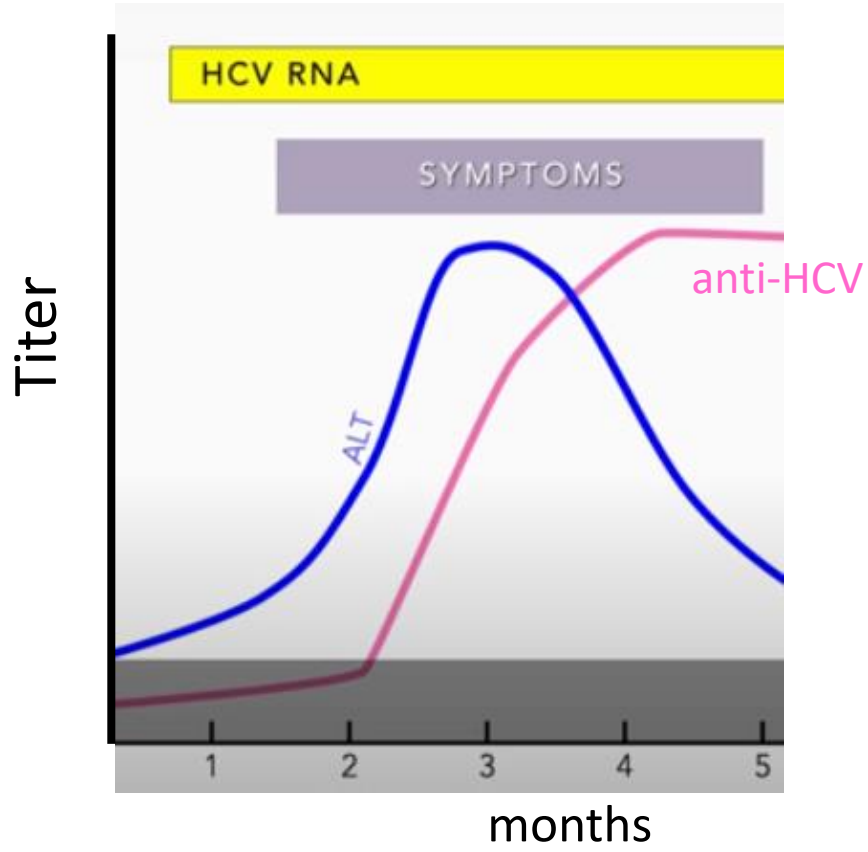
Hepatitis C virus

- Bloodborne hepatotropic virus
- Worldwide there are
  - ~ 1.5 million new infections annually
  - ~ 60 million chronic infections (lasting longer than 6 months)

WHO, 2021

Acute HCV infection (“recent”)



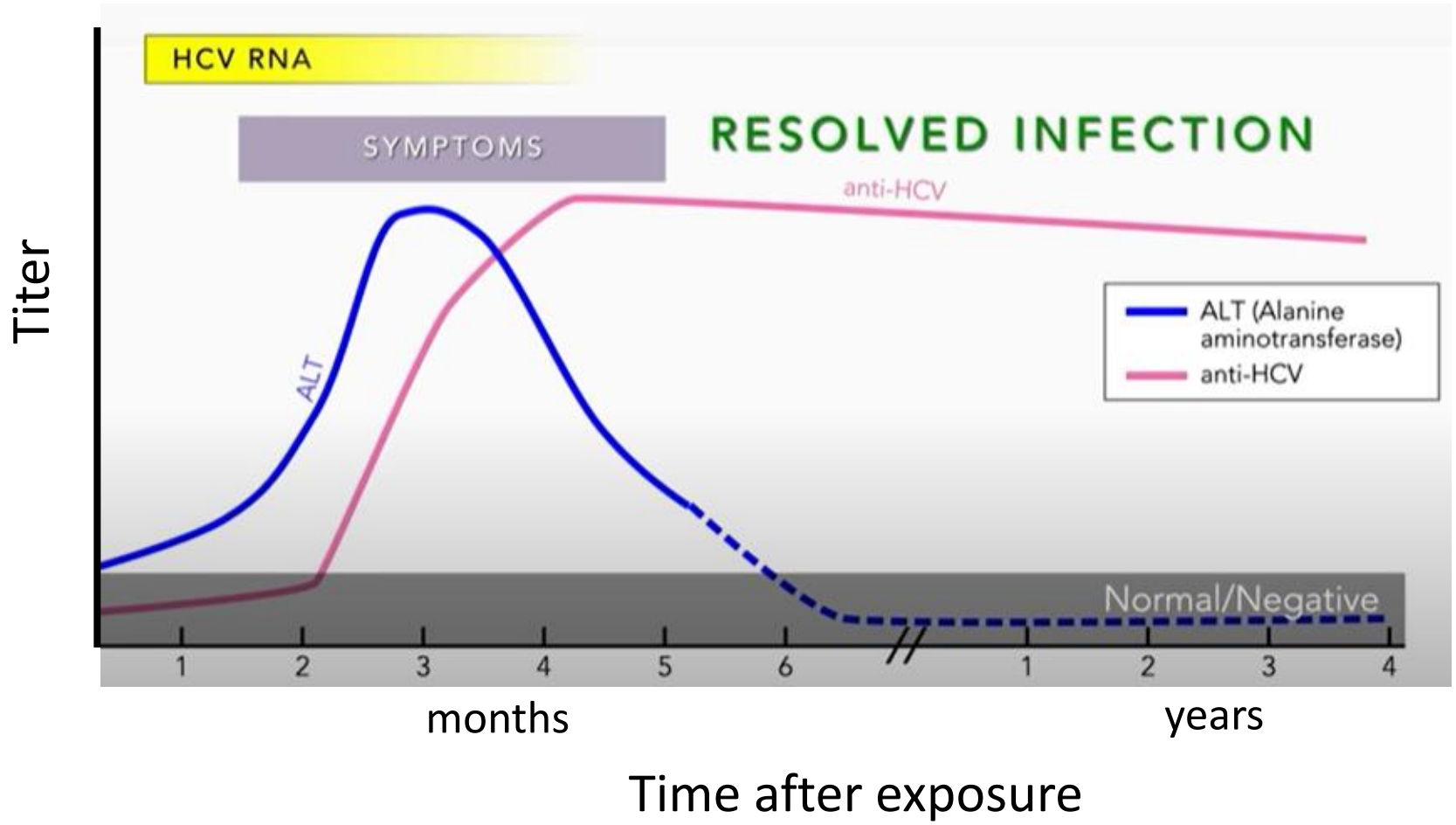
Only 15-30% of acute HCV infections are symptomatic.

Time after exposure

modified from cdc.gov/hepatitis

Resolved HCV infection

Less than half of acute infections resolve.



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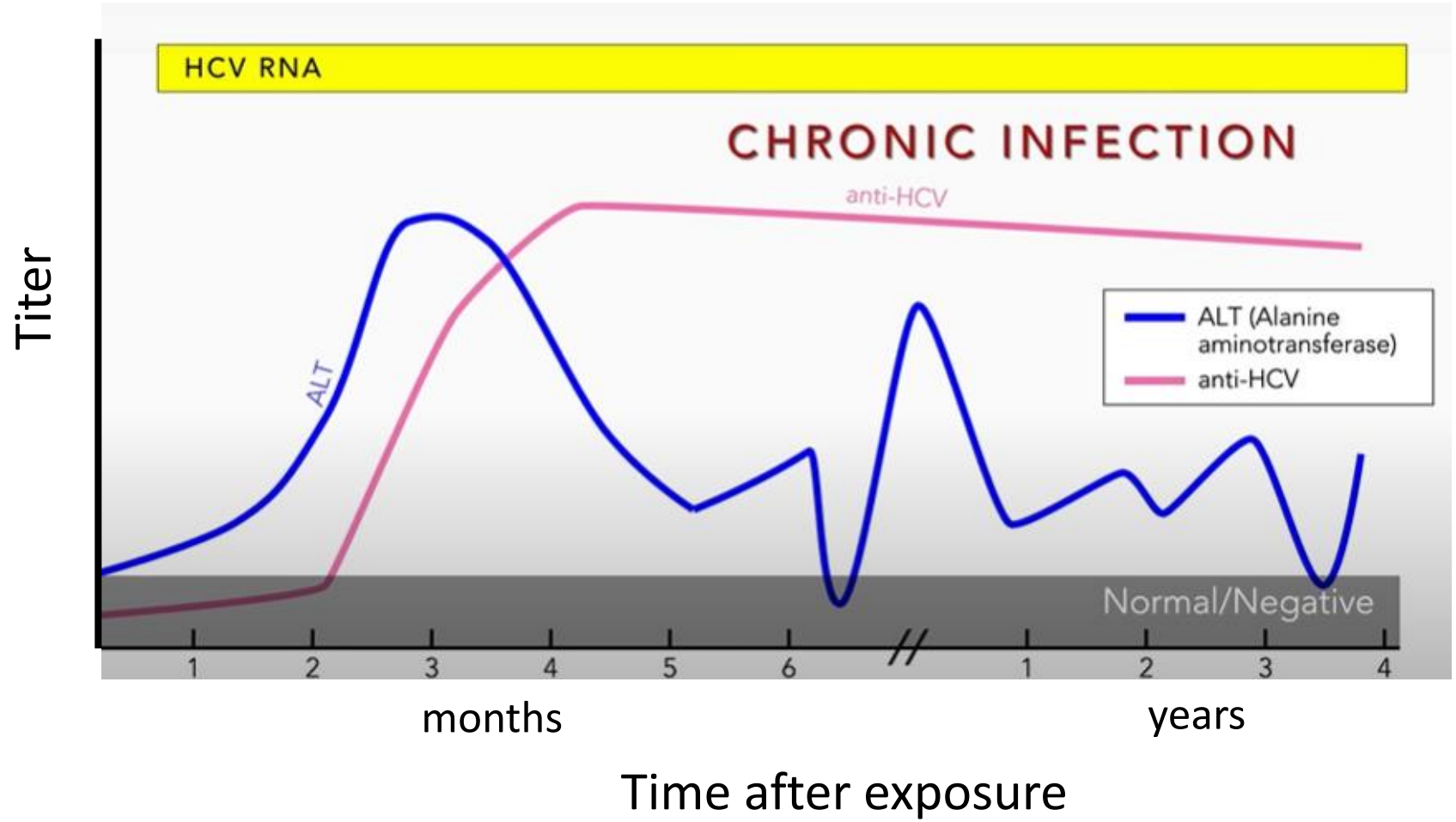
Chronic HCV infection ("long term")

Between 50-80% of acute infections become chronic.



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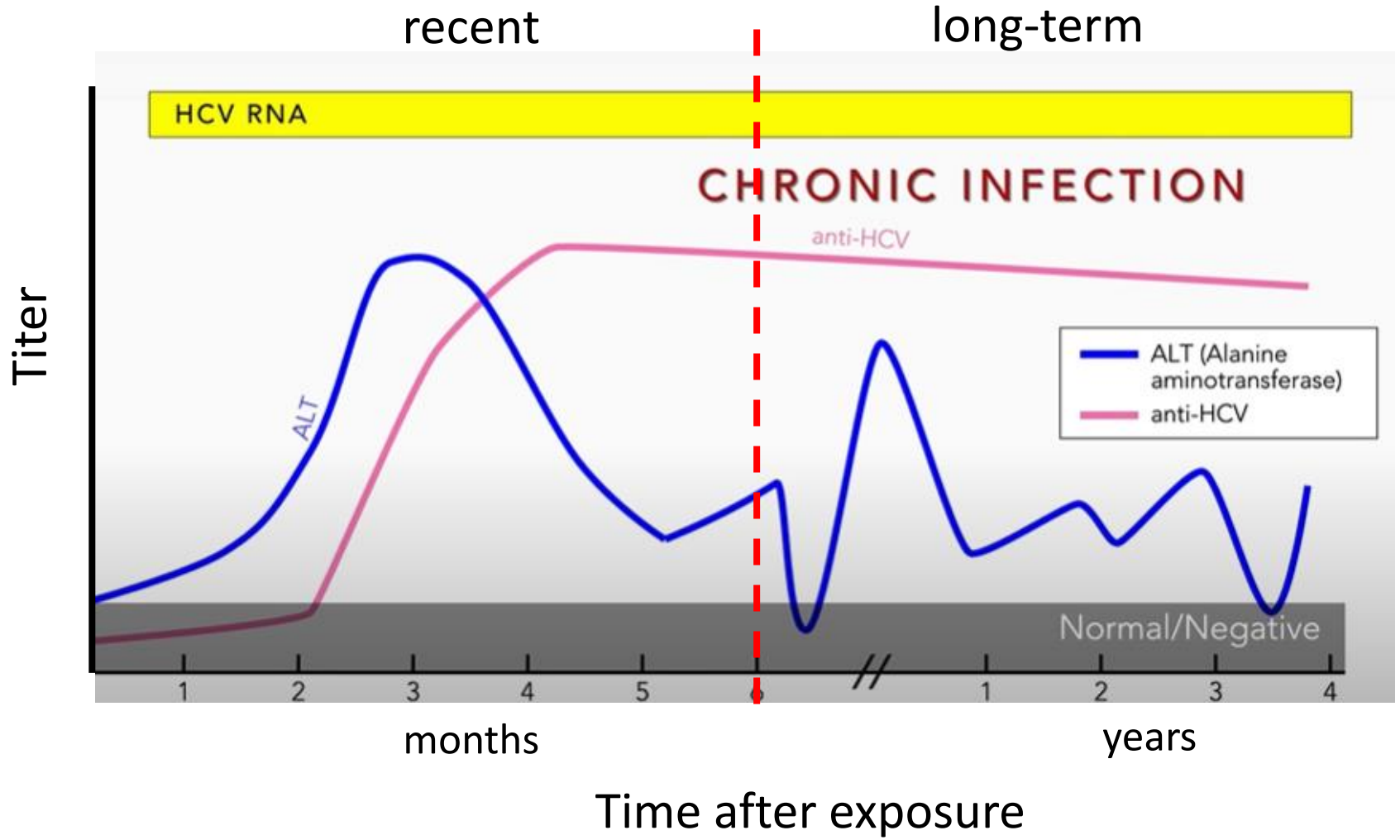
There is no routine method for differentiating recent from long term HCV infections



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Identifying acute or recent infections

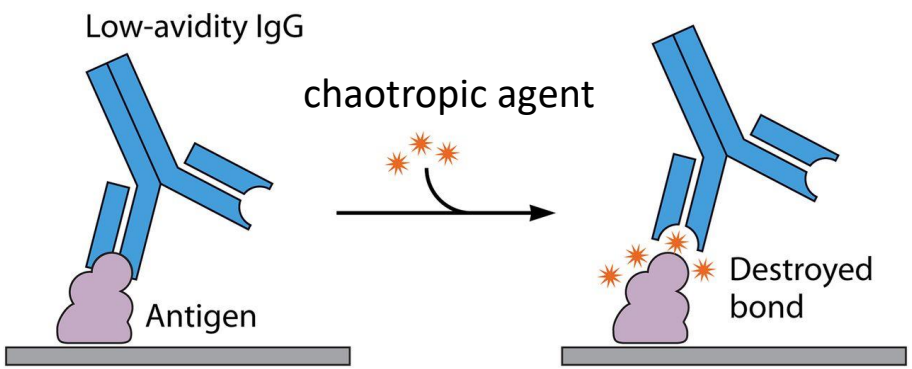
- Uses of incidence measurements
  - Monitor progress towards elimination (90% reduction – 2030 WHO goal)
  - Population surveillance
  - Evaluate effectiveness of infection interventions

- Challenges
  - Acute infections are typically asymptomatic.
  - Chronic infections can have symptomatic periods.
  - IgM is not a reliable marker during HCV infections
  - Documentation of seroconversion is rare.

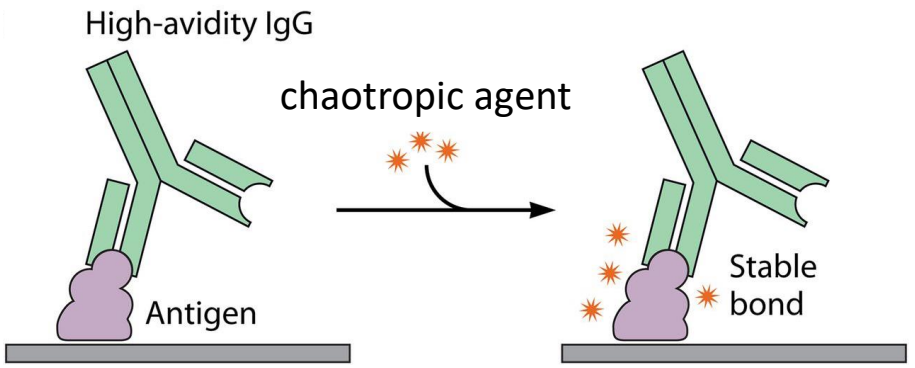
Antibody avidity: differentiating recent from long-term HCV infection

- Antibody avidity = strength of binding.
- Affinity maturation leads to higher antibody avidity over time.
- Avidity can be measured by an immunoassay including a chaotropic agent.

Recent infection



Long-term infection



Teimouri, A. *J. Clin. Micro*. 2020.

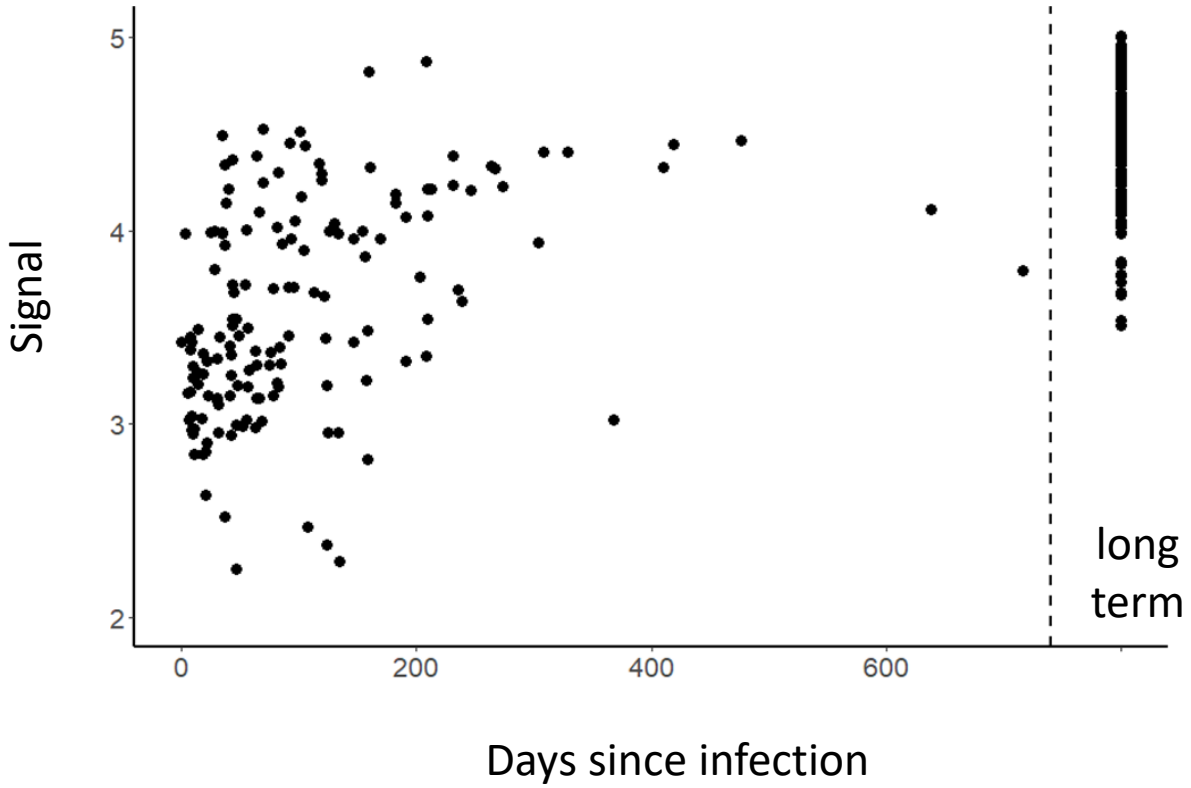
Parameters that define a recency test

- False recency rate (FRR)
  - 1-Specificity of recent result.

- Mean duration of recent infection (MDRI)
  - Used rather than sensitivity.
  - Timing of antibody avidity increase differs among individuals.

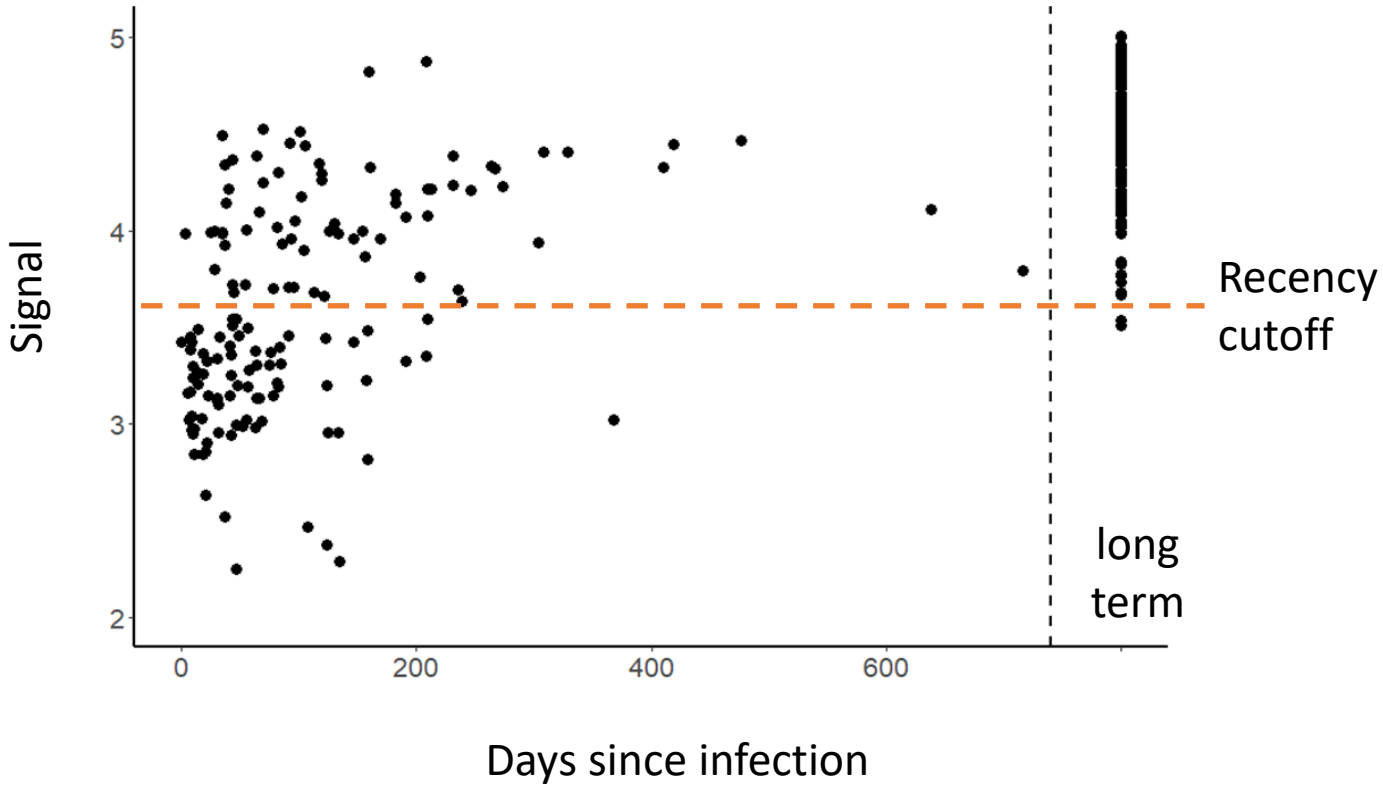
Parameters that define a recency test: FRR and MDRI

- Two sample groups



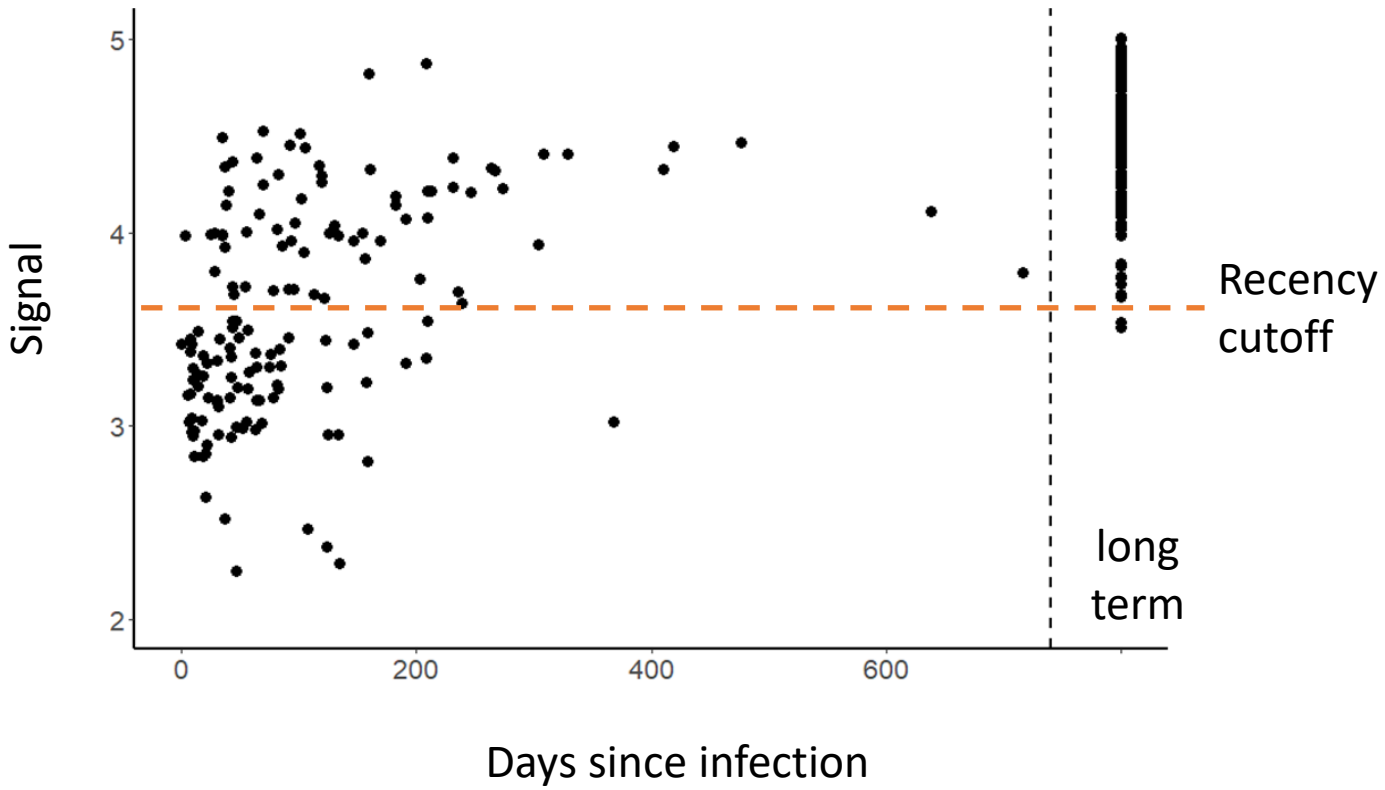
Parameters that define a recency test: FRR and MDRI

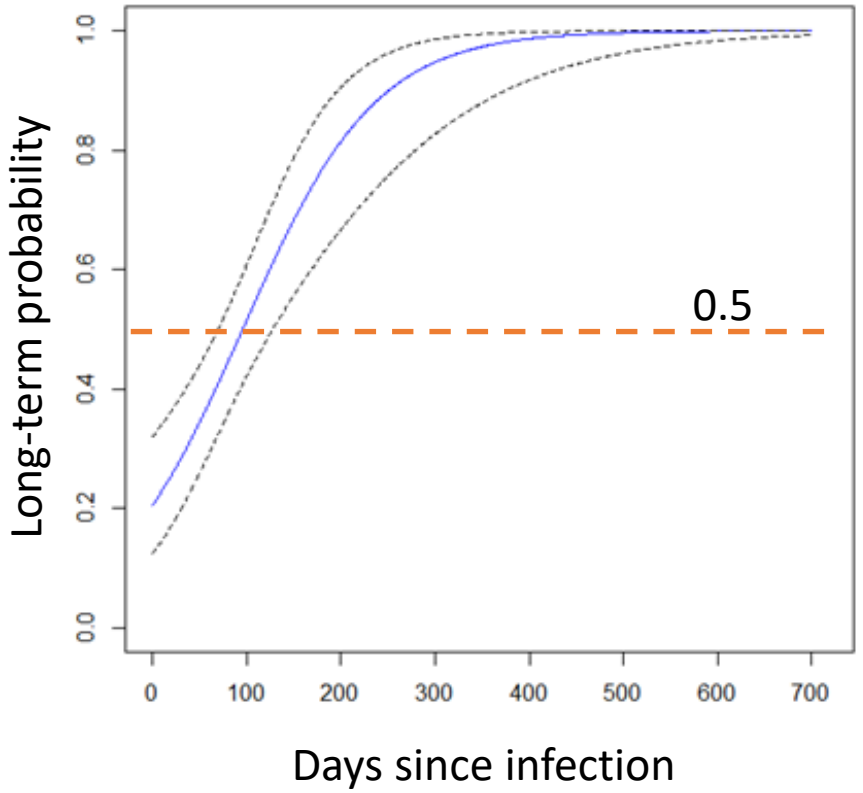
- False Receny Rate (FRR) : “specificity”



Parameters that define a recency test: FRR and MDRI

- Mean duration of recent infection (MDRI)





HCV antibody avidity-based recency tests using serum/plasma samples

Since estimated seroconversion

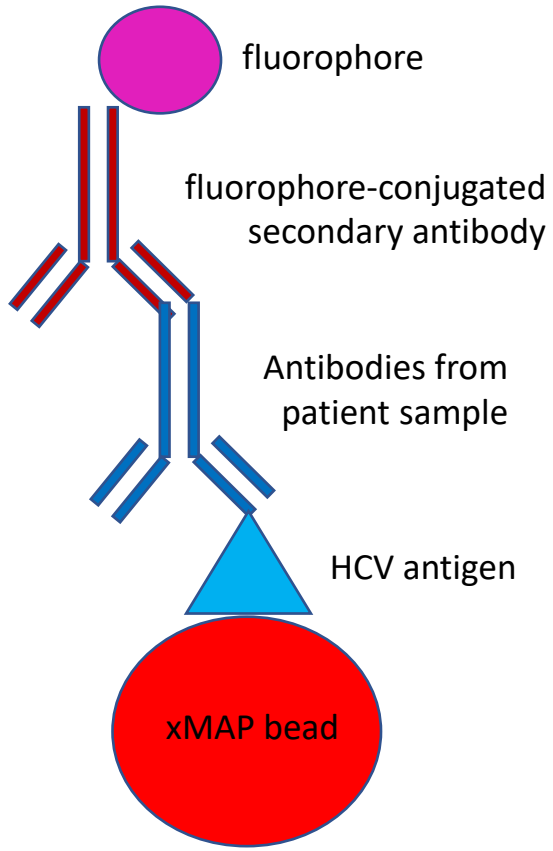
Study	FRR	MDRI
Boon, D. *J. Hepatology* . 2020.	0.4%	113 days (84-146)
Patel, E. *J. Inf. Dis.* 2016.	0.7% (0.2-1.8)	147 days (125-195)
Shepherd, SJ. *J. Med. Vir.* 2018.	4.0% (2.0-10.0)	116 days (98-135)

These studies use modified commercial anti-HCV immunoassays to make avidity measurements.

The assays detect antibodies targeting C, NS3, NS4, and NS5 viral proteins.
(optimized for early antibody detection in infected patients)

Examining how different anti-HCV antibodies affect recency measurements

- Indirect immunoassays using xMAP bead-based assay.
- Multiplex detection of antibodies targeting
  - C
  - NS3
  - NS4
  - NS5
  - HCV fusion protein
- Urea as chaotropic agent to measure antibody avidity.



Samples used to evaluate assay performance

- For FRR determination
  - 152 donors (long-term, but unknown time since last anti-HCV negative)
    - 1 sample per donor
    - anti-HCV and HCV RNA positive (no documented seroconversion)

- For MDRI determination
  - human- estimated seroconversion
    - 85 donors
    - 133 samples , 0-397 days (for single donors, samples are at least 60 days apart)

  - chimpanzee- experimentally infected
    - 20 animals
    - 180 samples , 0-730 days (~60 days between timepoints)

Anti-HCV antibody selection affects MDRI length

		human	chimpanzee
Antibodies targeting	**FRR**	**MDRI (since estimated seroconversion)**	**MDRI (since infection)**
C+NS3+NS4+NS5	0.7% (0.0-3.6)	58 (50-79)	97 (51-126)
C+NS4+NS5	0.7% (0.0-3.6)	116 (82-180)	151 (105-191)
Fusion HCV antigen	0.7% (0.0-3.6)	193 (137-384)	163 (131-194)

Summary

- Avidity-based HCV recency assays can be performed using xMAP bead-based immunoassay technology.
  - May perform similarly to modified commercial anti-HCV immunoassays.

- The MDRI can be tuned by the selection of anti-HCV antibodies being measured.
  - Inclusion of antibodies targeting NS3 shortens the MDRI.

- This assay could be used with a low probability of misclassifying long-term infections as recent.
  - Tool for estimating HCV incidence in population-based studies.