Diagnostics for Control of Hepatitis A What do we Need and Why?

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Overview

- Role of Diagnostics in Vaccine Preventable Diseases
- Hepatitis A Diagnostics
- Specialized Assays for Hepatitis A
  - Serologic
  - Molecular

Vaccine 20002: 3043–3045

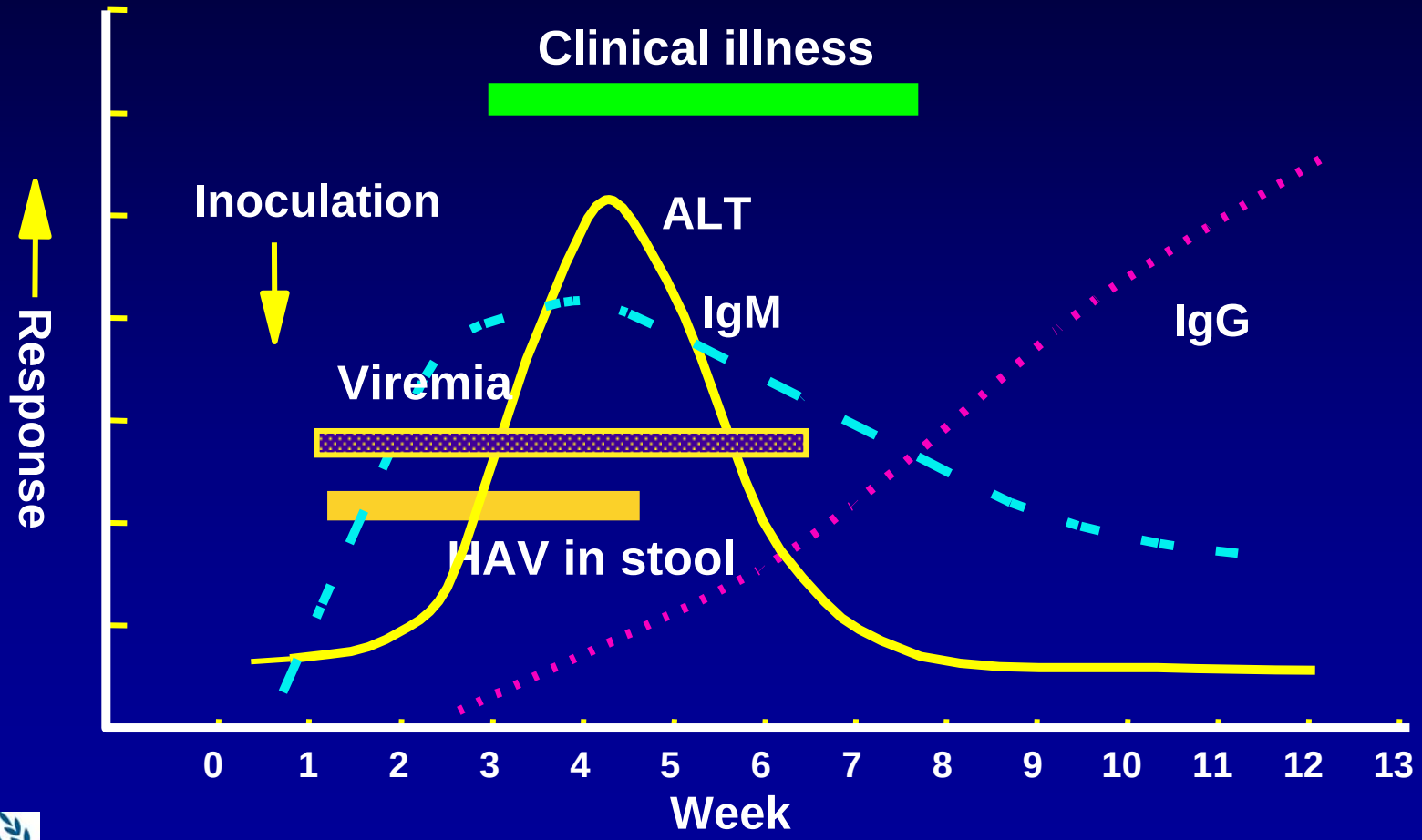
Some “Definitions”

- Diagnostics
  - widely used
  - commercially available
- Specialized Assays
  - Generally for research only
  - Not widely available
  - May be commercially available

Diagnostic Tests and Assays: Vaccine Preventable Diseases

- **Pre - Vaccine**
  - Diagnosis of acute disease (epidemiology / disease burden)
- **Vaccine Assessment**
  - Detection of infection
  - Assessment of vaccine response
- **Vaccine Introduction**
  - Effectiveness
  - Long-term effects

Events in Hepatitis A Virus Infection



Adapted from: J Med Virol 1988; 26:315-326; J Infect Dis 2000; 182:12-17

Diagnosis of Acute Disease

- Differential diagnosis of jaundice and acute febrile illness
- Clinical management
- Surveillance
  - Outbreak detection
  - Disease burden estimates
  - Post-introduction vaccine effectiveness
- Epidemiologic studies
- Clinical trials

IgM Anti-HAV

- An excellent diagnostic test among persons with symptoms suggestive of hepatitis
  - High sensitivity & specificity
  - High predictive values positive and negative
  - "Detuned" to improve specificity – only positive 4-6 months after symptom onset
- Transiently positive following vaccination (8-20%) – usually not a diagnostic problem

IgM Anti-HAV

- The *downside* = not widely used in countries where hepatitis A is endemic
  - differential diagnosis of acute hepatitis
  - (IgM anti-HAV & IgM anti-HBc)
  - non-icteric syndromes that could be hepatitis A (e.g., febrile illness in children)
  - Relatively high cost
  - No rapid test formats

Assessment of Hepatitis A Vaccination

- **Short-term Vaccine Response (total anti-HAV)**
  - Clinical trials
  - Epidemiologic studies
  - Problems:
    - Diagnostic test = lower levels of detection
    - Diagnostic test must be modified, not generally applicable to vaccinated persons
    - Measures antibody to structural proteins (vaccine and wild-type infection)

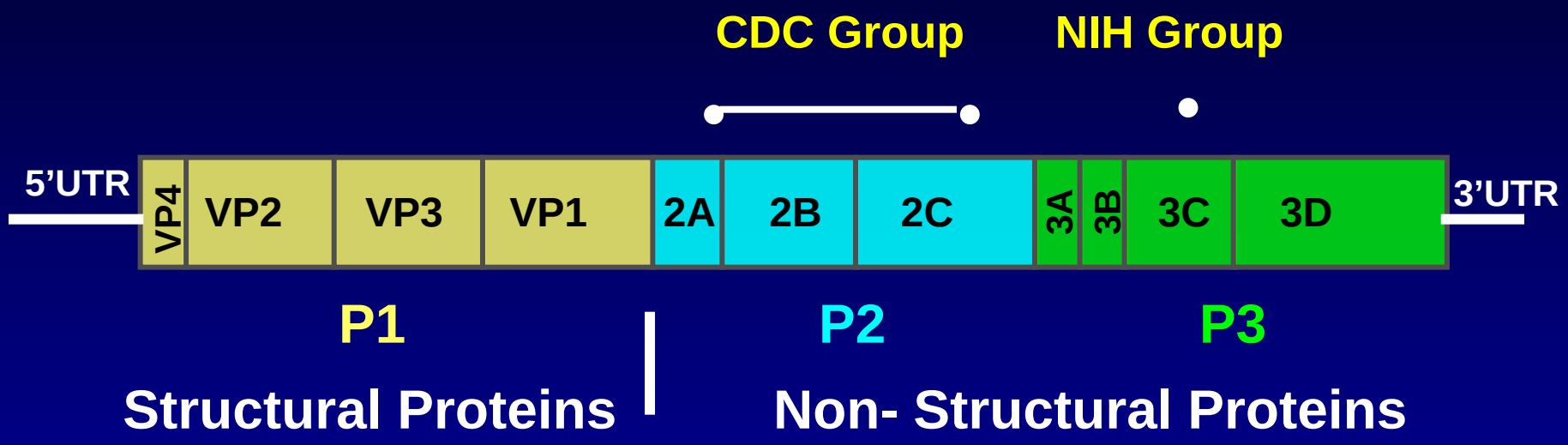
Assessment of Hepatitis A Vaccination

- **Long-term**
  - Antibody persistence (total anti-HAV)
  - Breakthrough infections
    - Clinically evident (IgM anti-HAV)
    - Inapparent - problematic
      - Virus detection – have to be lucky
      - Antibody to HAV non-structural proteins

Assessment of Hepatitis A Vaccination

- **Antibody to non-structural (replication) antigens of HAV**
  - Response to proteins produced during viral replication
  - Not present following vaccination with inactivated viral vaccines
  - Could identify subclinical infections in vaccinated population

Studies of Antibodies to HAV Non-Structural Proteins



CDC Group

Robertson BR, et al J Med Virol 176: 593 (1993)

NIH Group

Stewart DR, et al JID 176: 593 (1997)

Kabrane-Laziz Y, et al. Vaccine 19: 2878 (2001)

Antibodies to Non-Structural Proteins

- *Proof of concept:* antibodies can be detected
- *Limitations* = sensitivity
  - High viral replication = high rate of detection (>95%)
  - Low viral replication (e.g., attenuated vaccine) = low rate of detection (~25%)
  - Poor detection of persons with low levels of viral replication (small sample sizes)
  - Unknown – identification of persons with breakthrough infections following vaccination

Summary
Serologic Antibody Assays / Tests

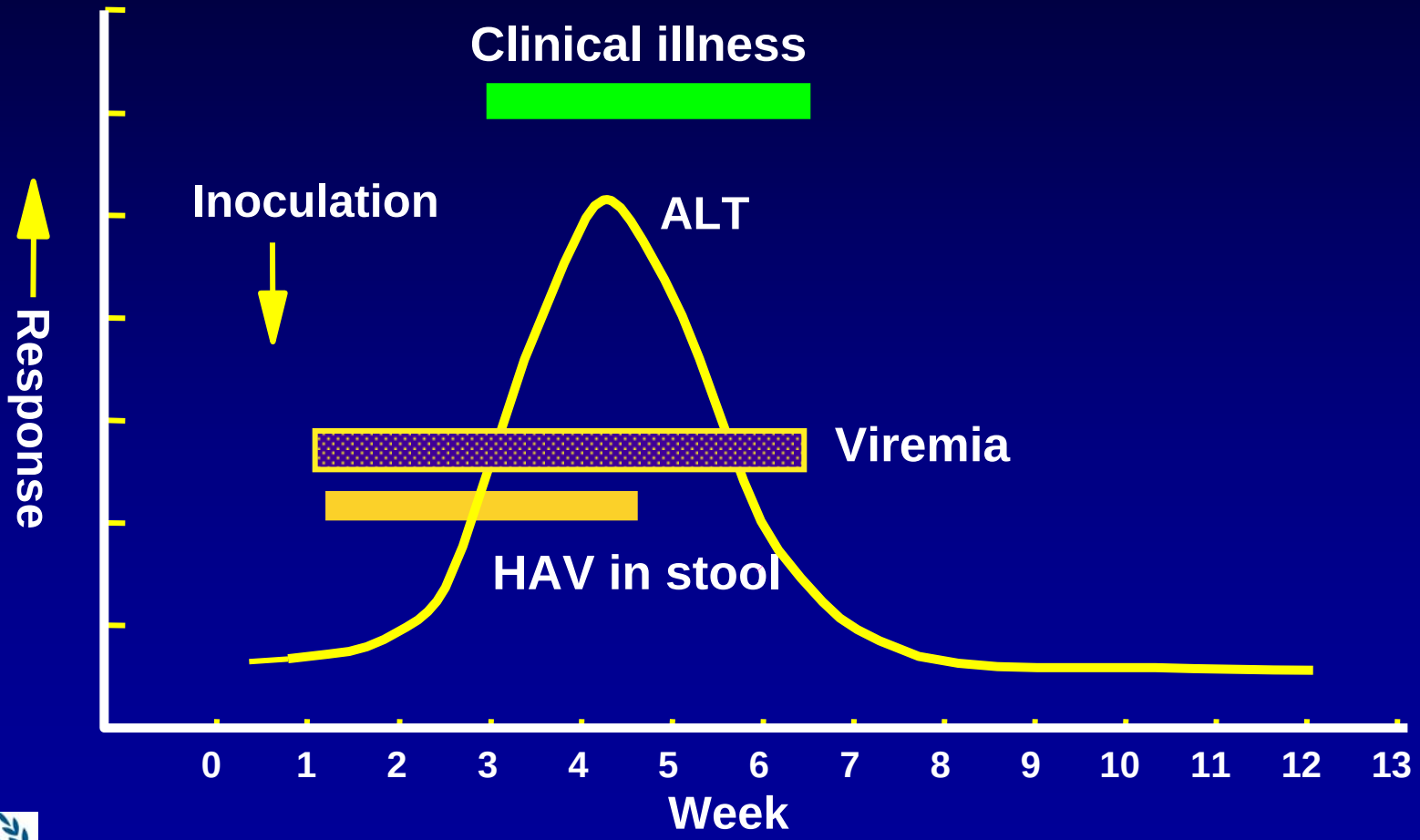
- Excellent diagnostic test – IgM anti-HAV
  - *More widespread use*
- Possible need for special assays
  - Total anti-HAV
    - ***more sensitive***
  - Antibody to non-structural proteins (anti-C3)
    - ***more sensitive***

Molecular Diagnostics

Uses

- **Virus Detection**
  - Humans during infection
  - Environmental samples
- **Molecular epidemiology**
  - Transmission patterns
  - Virus evolution

Events in Hepatitis A Virus Infection



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Detection of HAV RNA in Serum

Time from symptom onset to blood draw

Days	Positive (%)
<0	100
0 -13	93.4
14 -27	93.5
28 -41	63.3

Not affected by source of infection, gender, race, or age

Source: J Infect Dis 2000; 182:12-17 and CDC unpublished data

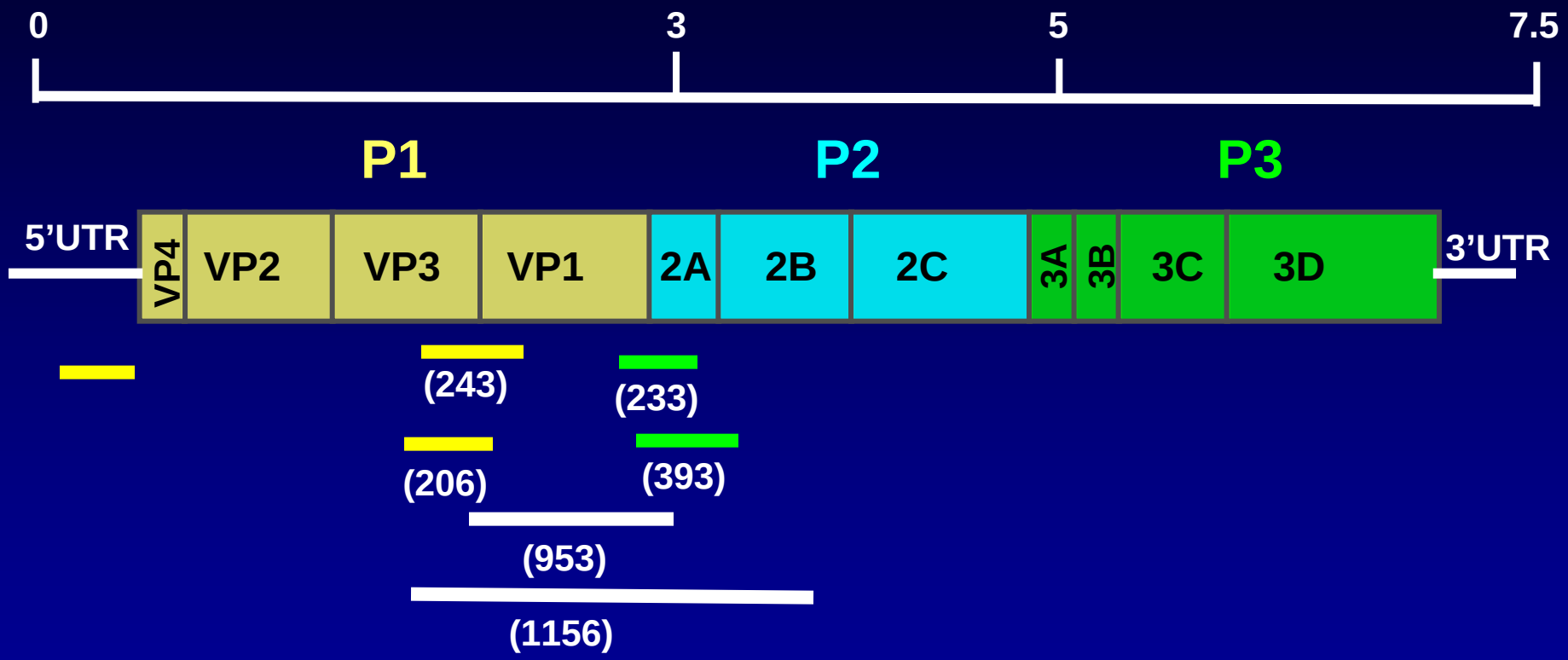
Virus Detection in Environmental Samples Challenges

- Material often NOT same material implicated in outbreak
- Foods – (e.g., berries, onions, shellfish)
  - **Special extraction methods to release virus from food surfaces / matrices and large biomass**
  - **Concentration of extracts from large volumes**
- Water and sewerage
  - Large volumes require concentration (e.g., membranes)
- Multiplex for other enterically transmitted agents (e.g., noro and caliciviruses)

Virus Detection in Environmental Samples

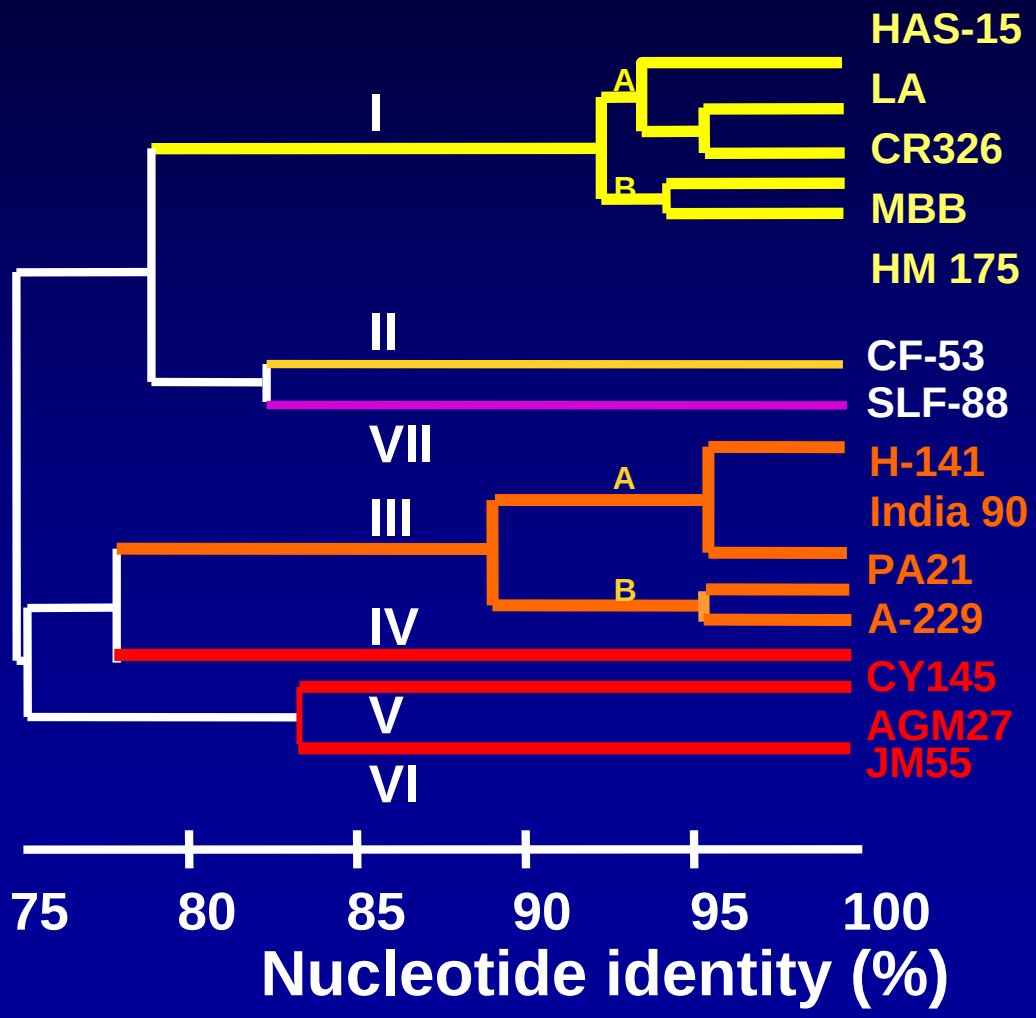
- **Nucleic acid amplification**
  - Inhibitors from food components, or elution and concentration methods
  - Detection of infectious virus
    - **Immuno-capture RT-PCR**
  - Amplification methods
    - **Dependent on throughput needs and lab capacity (e.g., real time, quantitative, RT-PCR)**

Regions Commonly Used to Amplify Hepatitis A Virus



From: Clinical Microbiology Reviews (2006) 19: 63

Genetic Relatedness of HAV



- Relatively low degree of nucleotide variation across genome regions
- 7 genotypes
  - 4 human
  - 3 simian
- Enough variation to determine relatedness of isolates using relatively short sequence fragments

Uses of Molecular Epidemiology

- **Sources of Virus Transmission**
  - Food / water / other environmental
  - Risk factors – MSM, IDU
  - Blood / Blood Products
- **Transmission Patterns within Populations**
- **Monitoring Vaccine Effectiveness**

Sources of Virus Transmission

- Food / water / other environmental
  - Simultaneous outbreaks in multiple locations
    - **Multiple food sources – e.g., berries, green onions, shellfish**
- Risk factors
  - Outbreaks in IDUs
  - Disease transmission patterns among MSM
- **Transmission Patterns after Vaccination**

Multistate Outbreak of Hepatitis A Associated with Frozen Strawberries, 1997

- Lessons Learned
  - Could identify small number of cases using markers of genetic relatedness
  - Required high-throughput molecular diagnostics
  - Required large data base of genetic sequences for general population
  - Required previously agreed upon sequenced regions for comparison

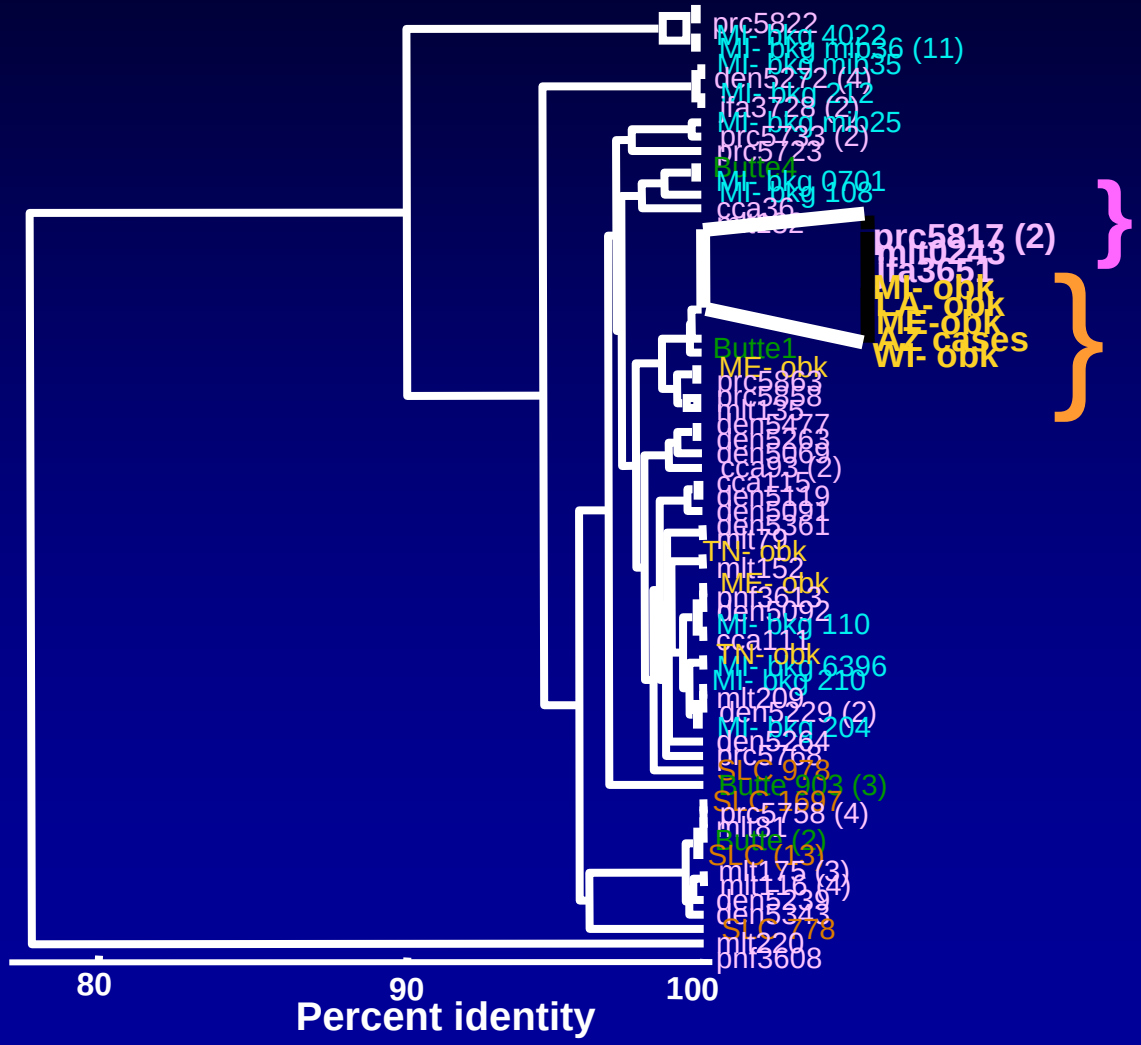
Hutin et al. NEJM 1999; 340:595-602

Relatedness of HAV from Cases who Ate Frozen Strawberries from Same Processor

State	# Cases	# Sera available	# with outbreak sequence
Michigan	198	118	118
Tennessee	2	1	0
Wisconsin	5	5	5
Louisiana*	4	2	2
Maine	29	10	8
Arizona	10	7	7
USA	-	98	4

*Commercial product

Multi-state Outbreak of Hepatitis A Associated with Frozen Strawberries, United States, 1997



Sentinel Counties
Tacoma, Portland, Birmingham

Michigan (118)
Louisiana (2)
Maine (8)
Arizona (7)
Wisconsin (5)

Hutin et al. NEJM 1999; 340:595-602

Summary

- **Have powerful tools for molecular diagnostics**
- Genetic markers (molecular epidemiology) has increased our knowledge of HAV transmission
- Must continue sharing information about strains
- We have tools to show elimination of HAV transmission in immunized populations

Vaccines don't Prevent Disease
<u>Vaccination</u> Prevents Disease

Dedication

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Betty Robertson