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
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

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

VP4 VP2 VP3 VP1 2A 2B 2C 3A 3B 3C 3D

5'UTR 3'UTR

P1 Structural Proteins  P2 Non- Structural Proteins  P3

CDC Group  

NIH Group  
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

Inoculation

Clinical illness

ALT

Viremia

HAV in stool

Detection of HAV RNA in Serum

Time from symptom onset to blood draw

<table>
<thead>
<tr>
<th>Days</th>
<th>Positive (%)</th>
</tr>
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<tbody>
<tr>
<td>&lt;0</td>
<td>100</td>
</tr>
<tr>
<td>0 -13</td>
<td>93.4</td>
</tr>
<tr>
<td>14 -27</td>
<td>93.5</td>
</tr>
<tr>
<td>28 -41</td>
<td>63.3</td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>State</th>
<th># Cases</th>
<th># Sera available</th>
<th># with outbreak sequence</th>
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<tbody>
<tr>
<td>Michigan</td>
<td>198</td>
<td>118</td>
<td>118</td>
</tr>
<tr>
<td>Tennessee</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Louisiana*</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Maine</td>
<td>29</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Arizona</td>
<td>10</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>USA</td>
<td>-</td>
<td>98</td>
<td>4</td>
</tr>
</tbody>
</table>

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

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
Vaccination Prevents Disease
Dedication

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