CLINICAL MICROBIOLOGY SERVICE

VIRUS HUNTERS & RESPIRATORY TRACT DETECTIVES

Dr. Susan Whittier

VIRUSES
THE LOW DOWN

- Obligate intracellular organisms
- Require metabolically active cells for efficient replication
- There is no universal cell line
- Certain viruses cannot be cultured in traditional cell culture monolayers
  - Rotavirus
  - Metapneumovirus
  - Hepatitis

THE USUAL SUSPECTS

- DNA viruses
  - Adenovirus
  - CMV
  - HSV 1 & 2
  - Varicella Zoster
- RNA viruses
  - Enteroviruses
  - Influenza A & B
  - Parainfluenza 1-3
  - RSV
  - Rotavirus

THE MYTHS OF VIROLOGY

- TAT is too long
  - DFA & Culture
- Can't treat a virus
- Academic pursuit
- Testing is not standardized
- Minimal impact on patient care

PREDICTING THE PATHOGEN

- Age
  - Pediatrics
  - Adults
- Season
  - Enteroviruses: Summer/Fall
  - Influenza & RSV: Winter
  - Rotavirus: Winter/Spring
- Immune status
- Geography

- Skin
  - HSV, VZV, Entero
- Eye
  - Adeno, HSV, VZV, Entero
- CNS
  - Entero, HSV, CMV, VZV
- GI
  - Rota, Adeno, Entero
- Genital
  - HSV
VIRUSES - CLINICAL SYNDROME

- COMMON COLD
  - RHINO, CORONA, ENTERO, PARA 1-3, ADENO
- PHARYNGITIS
  - ENTERO, ADENO, EBV, HSV
- CROUP
  - PARA 1 & 2, RSV
- BRONCHIOLITIS
  - RSV, PARA 3, INFLU A & B
- PNEUMONIA
  - RSV, PARA 1-3, INFLU A & B, ADENO, CMV, VZV

PEDIATRIC CASE

OCTOBER, 2003: A 3 MTH OLD INFANT PRESENTED TO THE PEDS ED A “CROUP-LIKE” ILLNESS WITH LOW-GRADE FEVER. THE CHILD DID NOT HAVE A RECENT TRAVEL HISTORY.

MENU OF METHODOLOGIES

- RAPID ANTIGEN DETECTION
  - EIA
    - HSV, RSV, INFLUENZA A & B, ADENO 40/41, ROTAVIRUS
  - DFA
    - CMV, HSV, VZV, INFLU A & B PARAFLU 1-3, RSV, ADENO
- ROUTINE CULTURE
- SHELL VIAL CULTURE
- NUCLEIC ACID AMPLIFICATION

PATIENT RESULTS

- EIA
  - POSITIVE FOR FLU A
  - NEGATIVE FOR RSV
- DFA
  - POSITIVE FOR FLU A
  - NEGATIVE FOR RSV
- CULTURE
  - POSITIVE FLU A
  - SENT TO CDC & WHO FOR SUBTYPING

INFLUENZA TYPES A & B

<table>
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<tr>
<th>EIA</th>
<th>Flu A</th>
<th>SEN</th>
<th>71-95%</th>
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<tr>
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<td></td>
<td></td>
<td>91%</td>
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<tr>
<td>Flu B</td>
<td>SEN</td>
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<td>70-87%</td>
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<tr>
<td>SPEC</td>
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<td>98%</td>
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ROUTE CULTURE

- TURNAROUND TIMES
  - INCUBATE FOR UP TO 6 WEEKS
    - PRIMARILY FOR CMV
  - AVERAGE TAT
    - 5 TO 10 DAYS
  - HSV
    - 60% POSITIVE BY DAY 1
    - 90% POSITIVE BY DAY 2
    - ~100% POSITIVE BY DAY 5

FIRST CASE IN NYC-OCT
- TEXAS HAD LARGEST # CASES
- SCHOOL OUTBREAK IN HOUSTON 10/04
- STRAIN WAS H3N2
- ANTIGENICALLY SIMILAR TO VACCINE STRAIN
DIRECT ANTIGEN DETECTION
- IMMUNOFLUORESCENCE
- TARGETED TESTING VS BROAD
- RESTRICTED ACCEPTABLE SPECIMENS
- VARIABLE SENSITIVITY
- CULTURE BACK-UP NECESSARY
- REQUIRES $10^3 – 10^6$ VIRUSES/ML

THE ABCs OF INFLUENZA
- 114,000 HOSPITALIZATIONS & 20,000 DEATHS/YR IN U.S.
- TYPES B & C
  - ONLY HUMANS (C IS VERY RARE)
- INFLUENZA A
  - AQUATIC BIRDS ARE NATURAL HOSTS & SERVE AS RESERVOIRS
  - INFECTS HUMANS, OTHER MAMMALS (SWINE, ETC.), & BIRDS
  - PIGS PROPOSED AS “MIXING VESSELS” FOR GENETIC REASSORTMENT BETWEEN HUMAN & AVIAN FLU A

DFA
ADVANTAGES
- Relatively Rapid
  - 1-2 hrs
- Does not require cold specimen transport
- Amenable to small batch testing
- Assess specimen quality
- Highly specific

DFA
DISADVANTAGES
- Subjective read
- High complexity
- Skilled Personnel
- Fluorescent microscope
- Slide prep is time-consuming
- Lower Sensitivity
  - Requires adequate number of cells
- Longer turnaround time than EIA

INFLUENZA SUBTYPES
- INFLUENZA SUBTYPES BASED UPON SURFACE GLYCOPROTEINS
  - Hemagglutinin Activity (HA)
  - Neuraminidase Activity (NA)
- NA CLEAVES CELL MUCIN BARRIER & HA FUSES TO CELLS SIALIC ACID RESIDUES, ENABLING VIRAL ADSORPTION & PENETRATION
- 15 HA & 9 NA SUBTYPES
  - H1-H3 & N1-N2 CAUSE OF WIDESPREAD DISEASE IN HUMANS

INFLUENZA
- ANTIGENIC DRIFT
  - Mutations in HA & NA
  - Occurs during viral replication
- ANTIGENIC SHIFT
  - Only occurs with Influenza A
  - Trading of RNA segments between animal & human strains
- GENETIC REASSORTMENT BETWEEN SPECIES SPECIFIC VIRUSES HAS BEEN ASSOCIATED WITH PANDEMICS

DIRECT FLUORESCENT ANTIGEN

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<th>Flu</th>
<th>SENS</th>
<th>SPEC</th>
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<tbody>
<tr>
<td>A</td>
<td>84%</td>
<td>95%</td>
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<tr>
<td>B</td>
<td>83%</td>
<td>99%</td>
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ADEQUATE SPECIMEN FOR DFA
- > 200 CELLS/SLIDE
- > 20 CILIATED EPITHELIAL CELLS
ANTIGENIC DRIFT
GRADUAL ANTIGENIC CHANGE WITHOUT A CHANGE IN SUBTYPE

H3N2 H3N2 H3N2 H3N2
1968 1975 1993 2004
HONG KONG VICTORIA BEIJING FUJIAN

SMALL GENETIC CHANGES REQUIRE ANNUAL VACCINATION

FLU PANDEMICS 20TH CENTURY
1. "SPANISH FLU" (1918-1919)
   - H1N1 STRAIN
   - KILLED 50 - 100 MILLION WORLD WIDE 500,000 U.S.
   - VERY VIRULENT
   - CYTOKINE STORM IN 15- 45 YO
   - GENETIC MATERIAL FROM 1918 BEING ANALYZED
     - CLOSELY RELATED TO SWINE VIRUSES
     - PIG TO HUMAN TRANSMISSION
     - GENE MUTATIONS OF AVIAN VIRUS
       - NOT REASSORTMENT
2. "ASIAN FLU" (1957)
   - H2N2 STRAIN
   - KILLED 1 MILLION GLOBALLY, 70,000 U.S.
   - 3 OF THE 8 RNA SEGMENTS WERE RELATED TO AVIAN INFLUENZA VIRUSES (REASSORTMENT)

ANTIGENIC SHIFT
SUDDEN COMPLETE ANTIGENIC CHANGE DUE TO HA AND/OR NA SUBTYPE SUBSTITUTION

H1N1 H2N2 H3N2
1918 1957 1968
SPANISH ASIAN HONG KONG

FLU PANDEMICS 20TH CENTURY
- "HONG KONG FLU" (1968)
  - CAUSED BY H3N2 STRAIN
  - HA GENE SEGMENT – AVIAN ORIGIN
  - KILLED 40,000 U.S.
  - LOWER MORTALITY DUE TO HA-ONLY SHIFT, NOT NA
  - 2 DUCK-DERIVED GENES & 6 HUMAN

INTERSPECIES TRANSMISSION

FLU FROM CHICKENS TO HUMANS
1997 HONG KONG H5N1 INFLUENZA
18 CASES & 6 DEATHS
- INDEX CASE - 3-YR-OLD BOY
- PATIENT DIED OF EXTENSIVE INFLUENZA PNEUMONIA COMPLICATED BY REYE’S SYNDROME
- FIRST DOCUMENTED OUTBREAK OF AVIAN INFLUENZA A VIRUS IN HUMANS
- INCIDENT ESTABLISHED THAT AVIAN INFLUENZA VIRUSES CAN INFECT HUMANS WITHOUT PASSAGE THROUGH INTERMEDIATE HOSTS
- ALL GENE SEGMENTS WERE AVIAN, WHICH PROBABLY LIMITED ITS PANDEMIC POTENTIAL
1st CASE OF HUMAN TO HUMAN TRANSMISSION 2004
An 11-YR OLD GIRL IN THAILAND
• DIED OF PNEUMONIA SEPT 8 (H5N1)
• RESIDED WITH 32-YEAR AUNT (ALSO INF)
• BOTH HAD CONTACT WITH INF. CHICKENS
• GIRL'S MOTHER FROM BANGKOK PROVIDED BEDSIDE CARE FOR DAUGHTER UNTIL CHILD'S DEATH
• MOTHER FELL ILL & DIED (SEPT 20) UPON RETURN TO BANGKOK

H5:N1 TREATMENT
• AMANTADINE
• RIMANTIDINE
  TWO NEW NEURAMINIDASE INHIBITORS FOR TREATMENT OF UNCOMPPLICATED INFLUENZA A & B
• ZANAMIVIR
• OSELTAMIVIR
  ✓ EFFECTIVE AGAINST H5N1
  ✓ CURRENTLY BEING STOCKPILED IN PREPARATION FOR THE NEXT PANDEMIC
  ✓ 2/05, 14 YO VIETNAMESE GIRL
  ✓ 1ST CASE OF RESISTANCE
  ✓ ZANAMIVIR ADDED TO STOCKPILE

AVIAN INFLUENZA
• 1997 HONG KONG H5N1 P,H
• 1999 CHINA & HONG KONG H9N2 Pig,H
• 2003 CHINA & HONG KONG H5N1 P,H
• 2003 NETHERLANDS H7N7 P, H
• 2003 HONG KONG H9N2 Pig,H
• 2003 NEW YORK H7N2 P, H
• 2004 THAILAND & VIETNAM H5N1 P, H
• 2004 CANADA H7N3 P, H
• 2005 THAILAND H5N1 P, H
• 2005 ROMANIA, TURKEY H5N1 P
• 2005 COLOMBIA SA H5N1 P
• 2005 LONDON H5N1Parrot
• 2005 GERMANY H5N1Birds

P = POULTRY, H = HUMAN

HIGH ALERT
• RULE OUT INFLUENZA IS HIGH PRIORITY
• WHY? “FLU-LIKE” PRODROME
  ✓ INHALATIONAL ANTHRAX
  ✓ SARS
  ✓ H5 HONG KONG STRAIN !!!

PANDEMIC FLU WAITING IN THE WINGS
• POULTRY DISEASE
  ✓ 10 EUR-ASIAN COUNTRIES
• AFFECTS LARGE # OF ANIMAL SP.
• 118 HUMAN CASES, 61 DEATHS
• HIGHLY PROBABLE HUMAN TO HUMAN TRANSMISSION REPORTED
• RESISTANT TO OLDER CLASS OF ANTI-VIRALS

CASE HISTORY
• 74 YO FEMALE WITH SEVERE RESPIRATORY DISTRESS
  • 5 DAY PRIOR TO ADMISSION DEVELOPED COUGH & RHINITIS
  • 2 DAYS LATER BEGAN WHEEZING, DEVELOPED FEVER
  • BROUGHT TO ED WHEN LETHARGIC
  • ONE GRANDCHILD REPORTED TO BE COUGHING, AND HER SON HAD A “COLD”
  • PUT IN RESPIRATORY ISOLATION IN MICU PENDING MICRO RESULTS
WHAT SPECIMENS SHOULD BE SENT TO R/O VIRAL INFECTION?

- NASOPHARYNGEAL WASH OR SWAB
  - Sensitivity is optimal
- THROAT SWAB (VIRAL TRANSPORT MEDIUM)
  - Sensitivity is suboptimal
- TRACHEAL ASPIRATE
- SPECIMEN TRANSPORT
  - Hand deliver immediately to microbiology lab

RSV INFECTION

- ADULTS
  - Mild course
- ELDERLY & PEDIATRICS
  - Lower respiratory infections
- INFANTS & CHILDREN <2 YRS
  - First MTHS of life
    - 40% pneumonia
    - 90% bronchiolitis
  - By 2 yrs, nearly all have had RSV infection

EIA AG DETECTION ASSAYS

ADVANTAGES
- Rapid (15-40 min)
- Point of care testing
- Moderate or waived complexity
- Can do one-zies

DISADVANTAGES
- High volume labs
  - Cannot do >5 at once
- Unable to assess specimen quality
- Poor sensitivity

RSV FACTS

- RNA virus
- 2 antigenic subtypes A & B
- Spread through respiratory secretions by close contact with infected persons/object
- Cause repeated infections throughout life
- Virus unstable in environment
- Causes community outbreaks (day care) & nosocomial infections

LAB DX RSV

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<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td>EIA</td>
<td>52-98%</td>
<td>80-100%</td>
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<tr>
<td>DFA</td>
<td>75-97%</td>
<td>74-100%</td>
</tr>
<tr>
<td>SHELL VIAL</td>
<td>75-85%</td>
<td>100%</td>
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MOLECULAR TESTING RESPIRATORY VIRUSES

- Those we cannot grow easily
  - hMPV
- Those we don’t want to grow
  - SARS
  - Avian influenza
- Labile viruses
  - RSV
- Impact on clinical management
  - Influenza
  - Meningitis/encephalitis

Real time multi-plexed PCR
WHAT GOES AROUND COMES AROUND……

• HER 78 YO HUSBAND PRESENTS TO THE ED FEBRILE (103), TACHYPMIC WITH SHAKING CHILLS
• PUT IN RESPIRATORY ISOLATION IN MICU PENDING MICRO RESULTS
• CHEST RADIOGRAPH SHOWED INFILTRATE IN RIGHT LOBE

WHAT IS THE DIFFERENTIAL DX?

• VIRAL INFECTION?
  ✓ RSV EIA & DFA TESTS WERE NEGATIVE

• BACTERIAL INFECTION?
  ✓ X-RAY FINDINGS INDICATE LOBAR PNEUMONIA
  ✓ DISCRETE LOBE IN LUNG IS AFFECTED

SPECIMENS SENT TO R/O BACTERIAL INFECTION

• SPECIMEN COLLECTION
  ✓ SPUTUM
  ✓ BRONCHOSCOPIC ASPIRATES
• MICROBIOLOGY TESTS
  ✓ GRAM STAIN & CULTURE
  ✓ ANTIMICROBIC SUSCEPTIBILITY
  ✓ STREP PNEUMO URINE AG TEST
• DAY 1
  ✓ GRAM POSITIVE COCCI PAIRS & CHAINS
  ✓ URINE ANTIGEN TEST POSITIVE FOR S. PNEUMONIAE
• DAY 2
  ✓ BIOCHEMICAL TESTS
    STREPTOCOCCUS PNEUMONIAE

SUSPECT BACTERIAL PATHOGENS

• GRAM-POSITIVE BACTERIA
  ✓ S. pneumoniae - community acquired
  ✓ S. aureus - nosocomial
• GRAM-NEGATIVE BACTERIA
  ✓ Enterobacteriaceae - nosocomial
    • K. pneumoniae, E. coli, Serratia
  ✓ P. aeruginosa - nosocomial
  ✓ H. influenzae - community acquired
  ✓ Legionella sp. - community & nosocomial

S. PNEUMONIAE

<table>
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<tr>
<th></th>
<th>URINE AG</th>
<th>BLOOD CULTURE</th>
<th>SPUTUM CULTURE</th>
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<tbody>
<tr>
<td>SENS (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPEC (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT</td>
<td></td>
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PNEUMOCOCCUS URINE AG

• DETECTS C-POLYSACCHARIDE CELL WALL ANTIGEN COMMON TO ALL SEROTYPES
• NASOPHARYNEAL COLONIZATION
  ✓ 5-10% HEALTHY ADULTS
  ✓ 20-40% HEALTHY CHILDREN
• ADULTS: BEST CORRELATION
  ✓ DETECTS BACTEREMIC & NONBACTEREMIC PNEUMONIA
S. PNEUMONIAE

- Most common & important cause bacterial disease
- Occult bacteremia, meningitis, pneumonia – 17,000/yr; < 5 years
- Acute otitis media, acute bacterial sinusitis
- Peak age 6-12 months
- High risk groups (asplenia, HIV, day care, sickle cell anemia)

Déjà Vu All Over Again

- 1 week later
  - 53 y.o. male status post-renal transplant, develops a high fever and pneumonia-like picture
  - 48 y.o. female status post-renal transplant, develops a high fever and pneumonia-like picture
  - Sound familiar??

Déjà Vu All Over Again

- RF is a 46 y.o. male 7 days status post-renal transplant
- While still in the hospital
  - Fever to 102
  - Chest x ray c/w pneumonia
  - Blood cultures neg x 3
  - Sputum cultures x 3
    - GS = many polys/nos
    - Normal flora
  - AFB & fungal cultures negative to date
  - Condition worsening

Déjà Vu All Over Again

- One week later a BAL is obtained
  - GS = many polys/nos
  - Culture = GNR
    - Slow growing
    - Weakly catalase +
    - Weakly oxidase +
    - No growth on MacConkey
  - Legionella Micdaedi

Déjà Vu All Over Again

- Epidemiologic investigation begins in conjunction with NYC and NYS DOHs
- 12 additional patients had serologic evidence of infection
  - L. Micdaedi was isolated from several hot water sources
    - Showers & sinks in patient rooms
    - Heated water recirculation loop
  - PFGE confirmed clonality

Legionella

- Severe infection & death associated with
  - Cigarette smoking
  - Immunosuppression
  - COPD
  - Renal failure
  - Alcoholism
- Environmental sources
  - Faucets
  - Showerheads
  - Fountains
- May require a biofilm for colonization
  - Multiply within amoebae
## Legionella Identification

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<th>Test</th>
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<th>Spec</th>
<th>Species</th>
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<tr>
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<tr>
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<td>25-70</td>
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<tr>
<td>UR AG</td>
<td>70-90</td>
<td>&gt;99</td>
<td><em>L. pneum</em> type 1</td>
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