**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**
  - ✔️ **ROTA VIRUS**
  - ✔️ **METAPNEUMOVIRUS**
  - ✔️ **HEPATITIS**
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

BUSTING THE MYTHS

• TAT CAN RANGE FROM MINUTES TO <72 HRS
  ✓ DIRECT AG
  ✓ MOLECULAR ASSAYS
• MANY ANTI-VIRAL AGENTS AVAILABLE
• CLINICALLY RELEVANT INFORMATION
• QUALITY REAGENTS & CELL LINES COMMERCIALY AVAILABLE
**THE USUAL SUSPECTS**

**DNA VIRUSES**
- ✓ Adenovirus
- ✓ CMV
- ✓ HSV 1 & 2
- ✓ Varicella Zoster

**RNA VIRUSES**
- ✓ Enteroviruses
- ✓ Influenza A & B
- ✓ Parainfluenza 1-3
- ✓ RSV
- ✓ Rotavirus

**PREDICTING THE PATHOGEN**

**AGE**
- ✓ Pediatrics
- ✓ Adults

**SEASON**
- ✓ Enteroviruses: Summer/Fall
- ✓ Influenza & RSV: Winter
- ✓ Rotavirus: Winter/Spring

**SKIN**
- ✓ HSV, VZV, Entero

**EYE**
- ✓ Adeno, HSV, VZV, Entero

**CNS**
- ✓ Entero, HSV, CMV, VZV

**GI**
- ✓ Rota, Adeno, Entero

**GENITAL**
- ✓ HSV

**IMMUNE STATUS**

**GEOGRAPHY**
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

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
ROUTINE 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

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.
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
  - ✓ FIRST CASE IN NYC-OCT
  - ✓ TEXAS HAD LARGEST # CASES
    - ✓ SCHOOL OUTBREAK IN HOUSTON 10/04
    - ✓ STRAIN WAS H3N2
    - ✓ ANTIGENICALLY SIMILAR TO VACCINE STRAIN

### INFLUENZA TYPES A & B

**EIA**

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<tr>
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<th>SEN</th>
<th>71-95%</th>
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<tbody>
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<td>SPEC</td>
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<tr>
<td>Flu B</td>
<td>SEN</td>
<td>70-87%</td>
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<tr>
<td></td>
<td>SPEC</td>
<td>98%</td>
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DIRECT ANTIGEN DETECTION

- IMMUNOFLUORESCENCE
- TARGETED TESTING VS BROAD
- RESTRICTED ACCEPTABLE SPECIMENS
- VARIABLE SENSITIVITY
- CULTURE BACK-UP NECESSARY
- REQUIRES $10^3$ – $10^6$ VIRUSES/ML

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
DIRECT FLUORESCENT ANTIGEN

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<tr>
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</tr>
<tr>
<td>Flu B</td>
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ADEQUATE SPECIMEN FOR DFA
✓ > 200 CELLS/SLIDE
✓ 20 CILIATED EPITHELIAL CELLS

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
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
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

ANTIGENIC SHIFT

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

H1N1  H2N2  H3N2
1918  1957  1968
SPANISH  ASIAN  HONG KONG
11

INTERSPECIES TRANSMISSION

FLU PANDEMICS 20TH CENTURY

1. "SPANISH FLU" (1918-1919)
   - H1NI 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]
**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

**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

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 H5N1 Parrot
• 2005 GERMANY H5N1 Birds

P = POULTRY, H = HUMAN
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

H5:N1 TREATMENT

- AMANTADINE
- RIMANTIDINE
  - TWO NEW NEURAMINIDASE INHIBITORS FOR TREATMENT OF UNCOMPLICATED 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
HIGH ALERT

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

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

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/OBJECTS
• CAUSE REPEATED INFECTIONS THROUGHOUT LIFE
• VIRUS UNSTABLE IN ENVIRONMENT
• CAUSES COMMUNITY OUTBREAKS (DAY CARE) & NOSOCOMIAL INFECTIONS

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
LAB DX RSV

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<tr>
<th>TEST</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
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<tbody>
<tr>
<td>EIA</td>
<td>52-98%</td>
<td>80-100%</td>
</tr>
<tr>
<td>DFA</td>
<td>75-97%</td>
<td>74-100%</td>
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<tr>
<td>SHELL VIAL</td>
<td>75-85%</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), TACHYPNIC WITH SHAKING CHILLS

• PUT IN RESPIRATORY ISOLATION IN MICU PENDING MICRO RESULTS

• CHEST RADIOGRAPH SHOWED INFLTRATE 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
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

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
## S. PNEUMONIAE

<table>
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<th>URINE AG</th>
<th>BLOOD CULTURE</th>
<th>SPUTUM CULTURE</th>
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<tr>
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<tr>
<td>SPEC (%)</td>
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<tr>
<td>TAT</td>
<td>20-30 min</td>
<td>24-48 hr</td>
<td>24-48 hr</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**

- 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 MICDADEI

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

- **EPIDEMIOLOGIC INVESTIGATION BEGINS IN CONJUNCTION WITH NYC AND NYS DOHs**
- **12 ADDITIONAL PATIENTS HAD SEROLOGIC EVIDENCE OF INFECTION**
- **L. MICDADEI 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>
<th>Sens</th>
<th>Spec</th>
<th>Species</th>
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<td>Ur Ag</td>
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