CLINICAL MICROBIOLOGY SERVICE

VIRUS HUNTERS
&
RESPIRATORY TRACT DETECTIVES

DR. SUSAN WHITTIER

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 COMMERCIALLY AVAILABLE

VIRUSES THE LOW DOWN

- OBLIGATE INTRACELLULAR ORGANISMS
- REQUIRE METABOLICALLY ACTIVE CELLS FOR EFFICENT 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 VIROLGY

- 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

- FIRST CASE IN NYC-OCT
  - ✓COLUMBIA PRESBY CHONY (2002, 2003)
- TEXAS HAD LARGEST # CASES
  - ✓SCHOOL OUTBREAK IN HOUSTON 10/04
  - ✓STRAIN WAS H3N2
    - ✓ANTIGENICALLY SIMILAR TO VACCINE STRAIN

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

INFLUENZA TYPES A & B EIA

Flu A	SEN	71-95%
	SPEC	91%
Flu B	SEN	70-87%
	SPEC	98%

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

DIRECT FLUORESCENT ANTIGEN

Flu A	SENS	84%
	SPEC	95%
Flu B	SENS	83%
	SPEC	99%

ADEQUATE SPECIMEN FOR DFA
- ✓ > 200 CELLS/SLIDE
- ✓ 20 CILIATED EPITHELIAL CELLS

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

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]

ANTIGENIC SHIFT

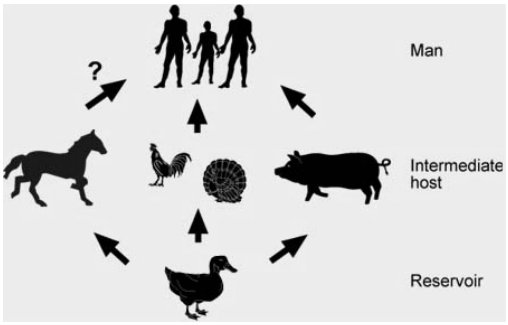
SUDDEN COMPLETE ANTIGENIC CHANGE DUE TO HA AND/OR NA SUBTYPE SUBSTITUTION

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

RECENT CASES RESISTANT

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

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

POSITIVE FOR RSV

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

LAB DX RSV

TEST	SENSITIVITY	SPECIFICITY
EIA	52-98%	80-100%
DFA	75-97%	74-100%
SHELL VIAL	75-85%	100%

RSV EIA +

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

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 INFILTRATE IN RIGHT LOBE

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*

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

S. PNEUMONIAE

	URINE AG	BLOOD CULTURE	SPUTUM CULTURE
SENS (%)			
SPEC (%)			
TAT			

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

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

- **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**

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***

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

TEST	SENS	SPEC	SPECIES
CULT	70%	100%	ALL
DFA	25-70	>95	*L. PNEUMOPHILA*
UR AG	70-90	>99	*L. PNEU* TYPE 1