Pediatric Clinical Microbiology: Influence of the season

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Objectives

- Describe the seasonality of respiratory viruses
- Discuss influence of disease prevalence on predictive values of the test
- Discuss important pathogens in children and recent trends in their prevalence
- Discuss latest updates on pathogens that affect children

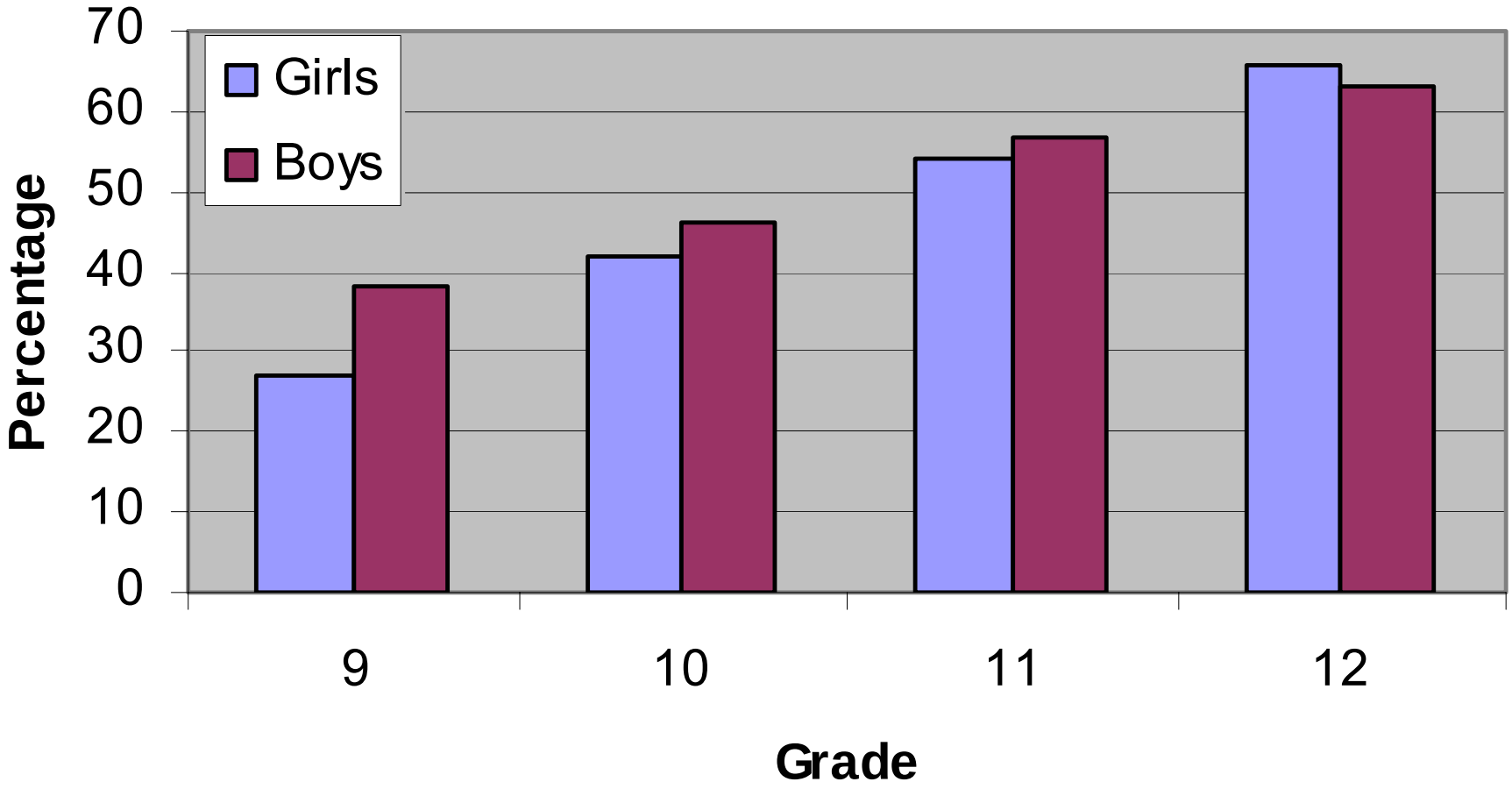
Influence of the Season

- Spring Season: Out door activity, accidental injuries, animal and human bites, Spring break- increase in STD!!!
- Summer: aseptic meningitis, Blood cultures, *S. aureus* infections, dermatophytes, diarrheal diseases
- Fall: Back to school- communicable diseases- Pertussis, Group A strep, diarrheal diseases
- Winter: Respiratory viruses, viral gastroenteritis, Group A strep, bacterial pneumonia, blood culture.

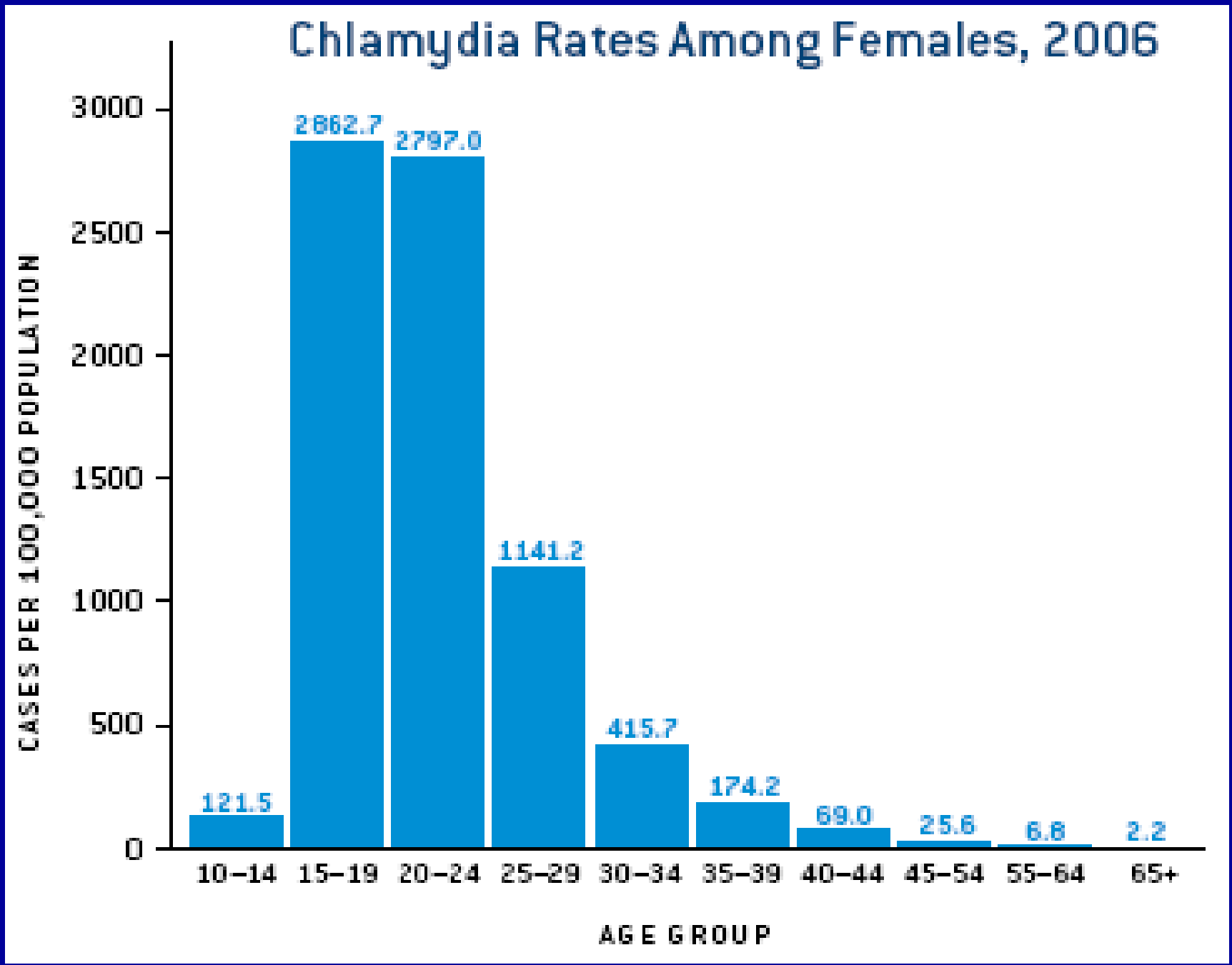
Infectious Diseases in Children

- Diseases that are influenced by human behavior- STD, injuries
- Diseases that occur yearly as epidemics- respiratory infection, rotavirus
- Diseases that are influenced by control measures (vaccination) - Pertussis, *S. pneumoniae*

Sexual Activity in High School Students



CDC: MMWR Vol 57/SS-4


Chlamydia Rates Among Females, 2006
CASES PER 100,000 POPULATION
3000
2500
2000
1500
1000
500
0
2862.7
2797.0
1141.2
415.7
174.2
121.5
69.0
25.6
6.8
2.2
10–14
15–19
20–24
25–29
30–34
35–39
40–44
45–54
55–64
65+
AGE GROUP

Spring activity CT/GC

- *Chlamydia trachomatis* and *Neisseria gonorrhea* are the most common STD in persons aged ≤25 years
- Sequelae include PID, ectopic pregnancy, and infertility.
- Asymptomatic infection is common. Annual screening of all sexually active women aged ≤25 years is recommended by CDC. Men and older women- screen on sexual risk assessment.
- NAAT is the gold standard for CT and GC testing. False negative due to competitive inhibition may occur when either pathogen is in high concentration.
- GC - Increase in ciprofloxacin resistance. CDC recommends cefixime or ceftriaxone as alternate drug regimen.

Sexual Abuse Management

- *N. gonorrhoeae* : Specimens from the pharynx and anus in both boys and girls, the vagina in girls, and the urethra in boys.
- *C. trachomatis*: anus in both boys and girls and from the vagina in girls. Pharyngeal specimens for *C. trachomatis* are not recommended
- CDC recommendation- Test for CT and GC by culture; GC requires confirmation by two different tests
- CT and GC by NAAT –Insufficient data to assess utility, can be used if confirmation by culture or alternate FDA-cleared nucleic acid amplification test that targets a different sequence from the initial test

Summer: Aseptic Meningitis

- Enteroviruses are the most common cause of aseptic meningitis in children during the summer months.
- Family *Picornaviridae*, about 4 species of Human enterovirus (A,B,C,D) and 68 serotypes are known; molecular identification 79-101.
- Febrile illness, aseptic meningitis, encephalitis, paralysis, myocarditis and neonatal enteroviral sepsis.
- Clinical symptoms mimic bacterial meningitis and septicemia
- Rapid diagnosis is important for patient management, restrict antibiotic usage, reduce length of hospitalization and cost savings

Enterovirus- Surveillance

- 10-15 million infections each year in US.
- Echoviruses 9, 11, 30, 6 and coxsackievirus B5 account for nearly half of the infections
- Parechovirus 1 (echovirus 22) accounted for nearly 2% cases, mainly respiratory. Severe disease with 11% mortality.
- Summer fall seasonality- June to October about 78% cases identified
- Children <1 yo account for about 44% of cases.
- Human Parechovirus (HPeV) 1, 2, recently 3, 4,5 and 6 closely related to enterovirus- reassigned to genus *Parechovirus*
- Wolthers *et al* CID 2008: HPeV in 5% of CSF during 2004 to 2007 (enterovirus 14%) –sepsis like illness and meningitis

CDC: MMWR Vol 55/No. SS-8

Enterovirus- Diagnosis

- Culture of CSF-poor sensitivity
- Reverse transcriptase PCR of CSF has highest sensitivity- Absence of CSF pleocytosis in children >2 months was highly predictive of negative RT-PCR result (Mulford *et al* 2004)
- Several realtime PCR reagents are available in the market as ASR
- Automated molecular platform “GeneXpert” provides opportunity for on demand testing.
- Rapid reporting:
  - Hamilton *et al* 2000, positive PCR 10% >2 days, culture 38% > 2 days in hospital
  - Robinson *et al* 2002 PCR result in <24 hrs Vs >24 hrs- reduced antibiotic use and $3000 difference in hospital charges.

Blood culture in children

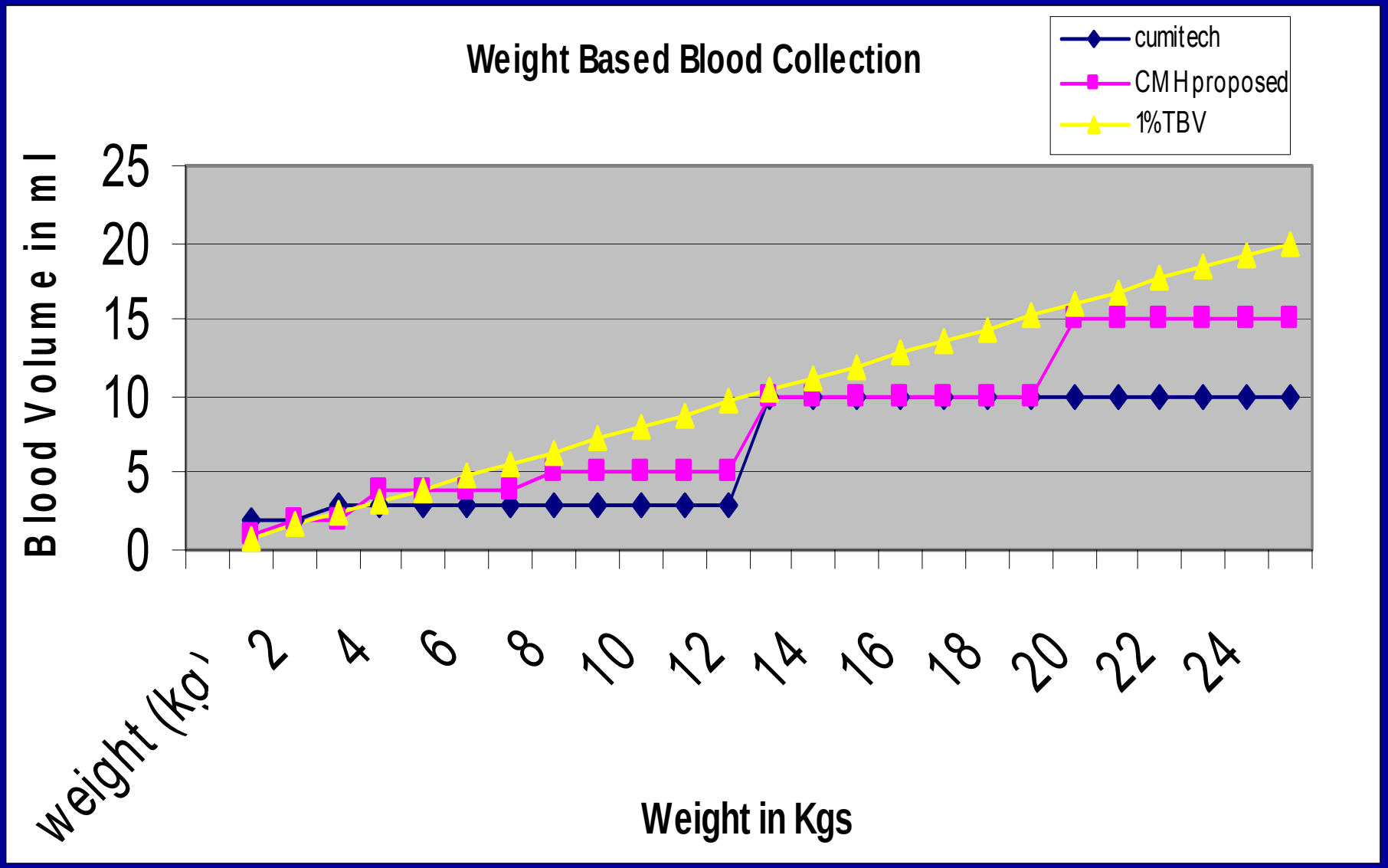
- Blood Culture Set- adequate volume of blood inoculated in to 1 or more vials
- Adult studies have shown that Blood to broth ratio should be a minimum 1:5 or greater dilution of blood.
- Cockerill *et al* CID 2004- Review of ~38K blood culture results. >3 blood cultures in a 24 hr period are required for sensitivity >95%. 1st (65%), 2nd (80%), 3 (96%).
- More than one culture is important both for increasing diagnostic yield and also for interpretation of positive result.
- Neonates-Usually only one culture obtained due to low blood volume and increased transfusion requirements.

Weight based guidelines

- Volume of blood obtained for each blood culture is the most important variable.
- Despite many organisms occurring in high concentrations, low density bacteremia is also recorded for most pathogens.
- Kellog JCM 2000-Quantitative blood cultures in children low level bacteremia (<10 CFU/ml) in 68% infants up to 2 months and 60% of children from birth to 15yrs. 23% episode <1CFU.
- Cockerill *et al* CID 2004- 20 ml Vs 10 (yield increased by 30%) 30ml Vs 10ml 47%, 40ml Vs 10ml- 58%.

Weight based volume collection

- Total Blood volume – premature- 90ml/kg, term newborn- 80ml /kg, Infants and preschoolers – 70ml /kg
- Cumitech collect up to 4-4.5% of total body weight
- St. Jude study- Weight based criteria increased yield from 12% to 23%;CHOP 2ml Vs 6ml recovery increased significantly
- Reller *et al* ASM Abstract– relative blood volume: >20K bottles studied. Volume of blood cultured, body weight and volume cultured per kg were significant in multivariate analysis. Odds of bacteremia increased 31% if blood volume was 0. 5ml to 1 ml Vs <0.5ml.


Weight Based Blood Collection
cumitech
CMH proposed
1%TBV
Blood Volume in ml
25
20
15
10
5
0
weight (kg)
2
4
6
8
10
12
14
16
18
20
22
24
Weight in Kgs

Patient weight in Kg	Blood volume to collect	Media type* and inoculation volume	
		Peds plus	Aerobic plus
1 to 3	1ml	0.5ml	0.5ml
3.1 to 6	2ml	1ml	1ml
6.1 to 9	4 ml	2ml	2ml
9.1 to 12	6ml	3ml	3ml
12.1 to 20	8ml	4ml	4ml
20.1 to 25	10ml	5ml	5ml
25.1 to 40	15ml	5ml	10ml
> 40	20ml	N/A	10ml X 2 bottles

CMH- Weight-based guideline

- Blood volume requirements distributed to nursing staff
- Education and training provided to each clinical section
- Each blood bottle was pre-weighed before delivery to clinical areas and weighed post inoculation
- One year data was compiled and analyzed to determine compliance and impact of weight-based blood volume collection on detection of BSI.
- Blood culture yield was significantly higher in the compliant group versus the non-compliant group irrespective of the location.

BSI in Neonates

- Stoll *et al* Peds 2002. Late onset sepsis: NICHD Neonatal Research Network, September 1, 1998 - August 31, 2000
  - Total 1313 BSI in 6950 patients (VLBW 401 to 1500 gms)
  - Gram-positive organisms 922 (70.2), Gram-negatives 231 (17.6) and Fungi 160 (12.2)
  - CoNS - 629 (47.9) 276 (44%) CONS were definite infections and 353 (56%) were possible infections.
- Rubin *et al* 2002 conducted a multicenter survey on practices of neonatologists in 35 hospitals
  - CoNS -54% of BSI, 83% of clinicians drew only one blood culture.
  - 60% prescribed a vancomycin regimen
  - 47-85% completed full course of antibiotics when only one blood culture was obtained Vs 22-47% when 1of 2 blood cultures grew CoNS.

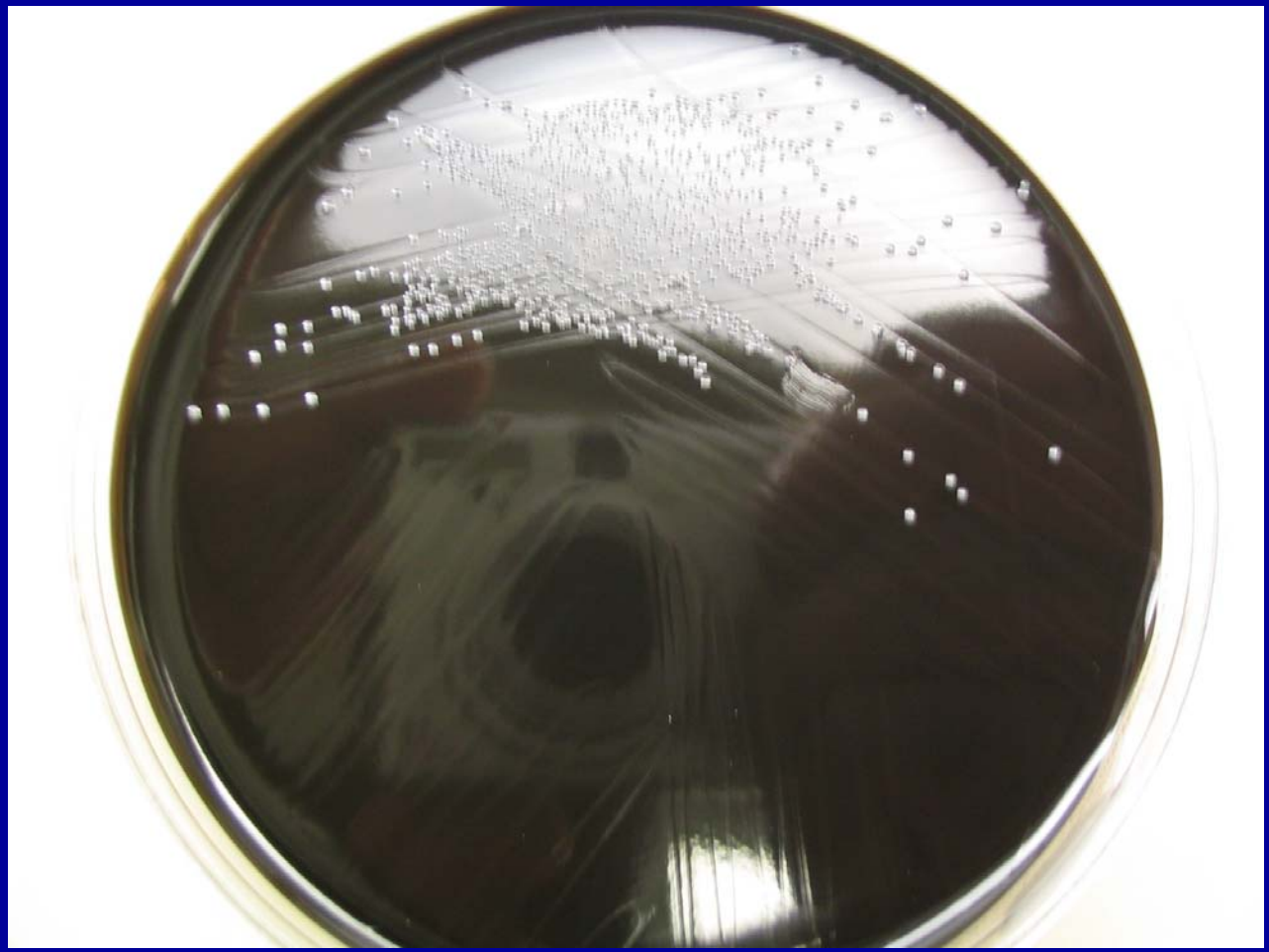
Fall Season: Pertussis

- Highly contagious respiratory infection caused by *Bordetella pertussis* less commonly by *B. parapertussis, B. holmesii* and *B. bronchiseptica*
- Outbreaks first described in 16th century, *Bordetella pertussis* isolated in 1906
- Estimated 48.5 million cases/yr; 285,000 deaths worldwide in 2001.
- Incubation period 5-10 days (up to 21 days), Insidious onset, Catarrhal stage 1-2 weeks, Paroxysmal Cough stage 1-6 weeks, Convalescence- weeks to months
- Incidence increase in summer and early fall months – Jul to Sep

Laboratory Diagnosis

- Specimen- nasopharyngeal swab, aspirates and washes.
- Culture- special transport and culture media required
- DFA- rapid, inexpensive, non-specific and insensitive
- PCR- Promoter for PT, upstream Porin, IS elements (IS *481* and IS*1001*) and Cya A. increase sensitivity by 2-4 fold over Cx
- Serology- 4 fold increase, cross reactivity of FHA, IgG and IgA to PT gives the best results

B. pertussis on Charcoal blood agar



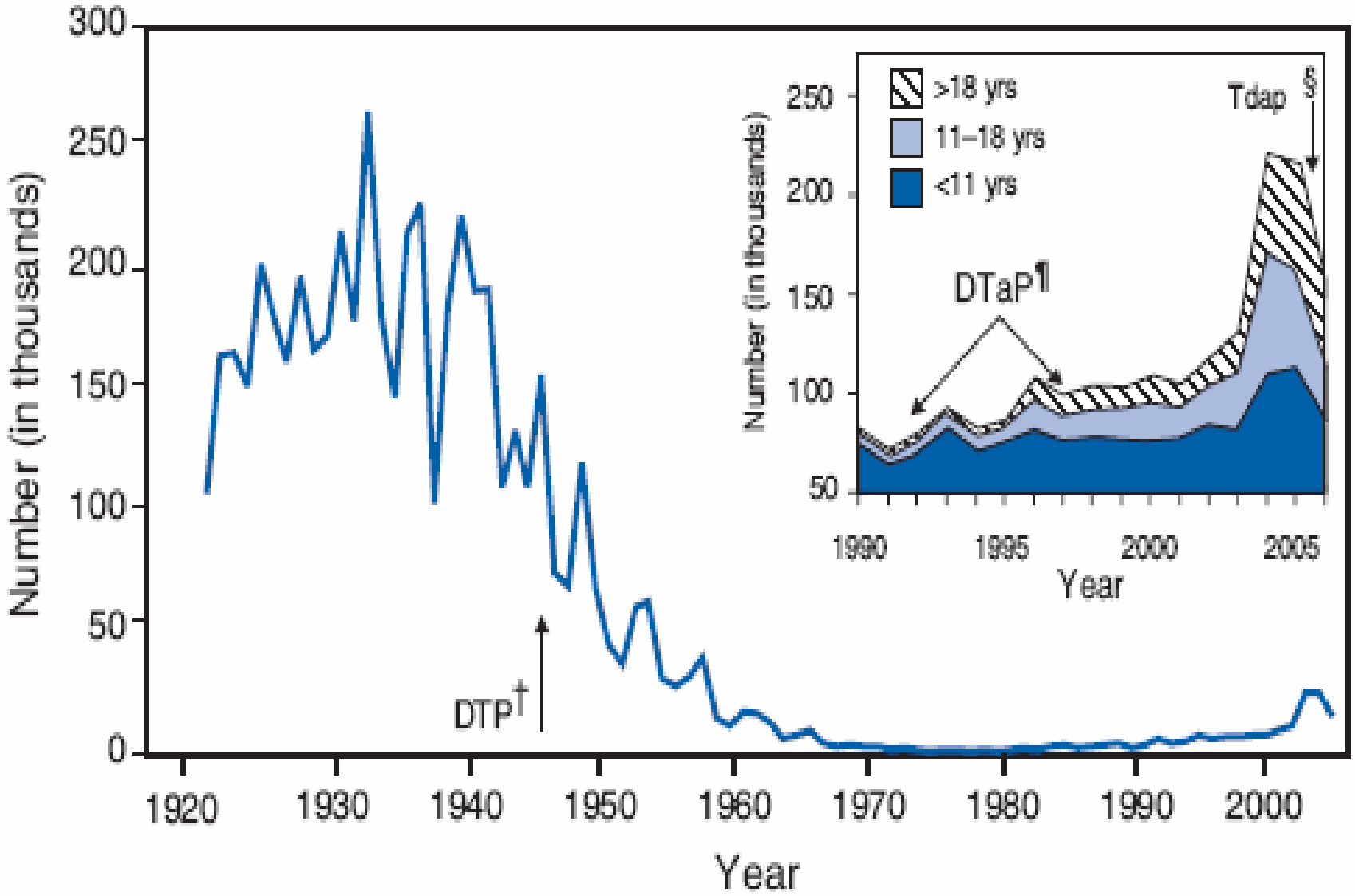
Small, gray, domed
Very shiny, mercury drops

Pertussis False Alarm

- MMWR Aug 24, 2007. Reported three outbreaks with equivocal laboratory findings and atypical clinical and epidemiological characteristics.
- April 2004 Tennessee-1459 cases, March 2006 134 cases in New Hampshire hospital, Sep 2006- 507 HCW.
- Tatti et al DMID 2008- describe dual-target PCR for detection of *B. pertussis* especially for outbreak investigation. IS481 and ptxS1 gene.
- 145 outbreak specimens- of which 127 Cx negative: 27 /123 were IS481 pos (35-40 Ct), ptxS1 gene neg.

Shift in Pertussis cases

	1933-39	1978-81	1997-2000
<1yr	7.5	53.5	29.4
1-4yr	41.1	26.5	11.1
5-9yr	46	8.2	9.8
10-14yrs	4	5.4	29.4
>15yr	0.9	6.5	20.4


Number (in thousands)
300
250
200
150
100
50
0
1920
1930
1940
1950
1960
1970
1980
1990
2000
Year
DTP†
Number (in thousands)
250
200
150
100
50
>18 yrs
11–18 yrs
<11 yrs
Tdap §
DTaP¶
1990
1995
2000
2005
Year

Pertussis-Why not eradicated

- Waning immunity of vaccine, Protection wanes 3-5 years after immunization
- Antibody levels fall to pre-infection levels 5 years after natural infection, Not measurable by 12 years
- Incomplete immunization of children
- Carrier status in adults and adolescents?
- Variable vaccine efficacy
- Under diagnosis of disease
- Lack of adolescent/booster vaccine- New vaccine available Tdap

Winter Season -Respiratory Viruses

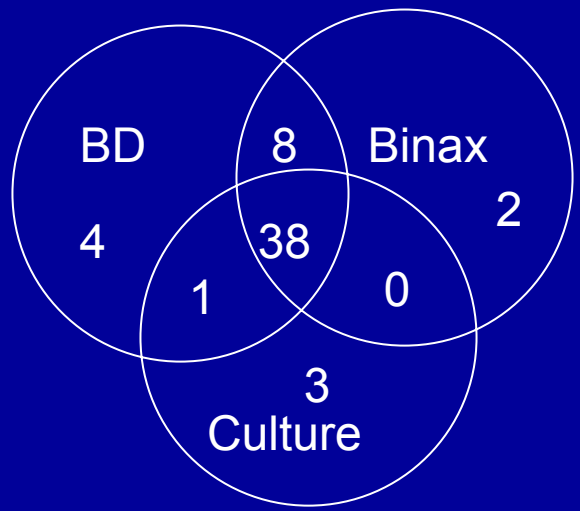
- Emergency Department Triage Algorithm
- Age 1wk to 2 months: Temp >38°C : Obtain nasopharyngeal aspirate for viral antigen test
- Age 2 months to 36 months: Temp >39°C
  - If URI symptoms present – Flu and RSV tested
  - If URI absent – Flu Test

Respiratory syncytial virus

- Enveloped RNA paramyxovirus
- Acute respiratory illness in all ages.
- In children cause of bronchiolitis and pneumonia.
- High risk- heart disease, pulmonary hyper tension, BPD, prematurity and immunodefeiceiny/ immunosuppression.
- Annual epidemics. Shedding 3-8 days. In infants 3 to 4 weeks.
- Treatment- supportive, maintenance of respiratory status and oxygen requirement. Ribavirin therapy.
- Prevention: RSV-IGIV and Palivizumab. Five doses once monthly during season given to premature infants and Chronic lung disease patients less than 24 months.


BD
RSV
C
T
BD
RSV
C
T
OCT 3 2005

	Sensitivity	Specificity	PPV	NPV
BD	91%	94%	94%	90%
BN	91%	100%	100%	90%.



R. Selvarangan *etal* IDSA 2005

RSV infection

Test results

1% RSV	*Positive*	*Negative*	
Positive	**TP = 19**	**FP = 80**	**PPV** TP / (TP + FP) 19/ 99 = **19%**
Negative	**FN = 1**	**TN = 1900**	**NPV** TN / (TN + FN) 1900 / 1901 **100%**
	Sensitivity TP / (TP + FN) 19 / 20 = 95%	**Specificity** TN / (FP + TN) 1900 / 1980 = **96%**	

Beginning and end of the season-
It is advisable to confirm all positives by culture due to low PPV

RSV infection

Test results	20 % RSV	*Positive*	*Negative*	
	Positive	**TP = 380**	**FP = 64**	**PPV** TP / (TP + FP) 380/ 444 = **86%**
	Negative	**FN = 20**	**TN = 1536**	**NPV** TN / (TN + FN) 1536 / 1556 **99%**
		Sensitivity TP / (TP + FN) 380 / 400 = **95%**	**Specificity** TN / (FP + TN) 1536 / 1600 = **96%**	

Influenza in children

- Member of the Orthomyxovirus, Influenza type A and B cause seasonal epidemics in humans.
- Children aged 0–4 years
  - 500/100,000 children for those with high-risk medical conditions
  - 100/100,000 children for those without high-risk medical conditions.
  - Highest among children aged 0–1 years and are comparable to rates reported among persons aged ≥ 65 years
- Complications: Otitis media, Bacterial Pneumonia, Encephalitis, encephalopathy, Myocarditis, Myositis

Influenza-Diagnostics

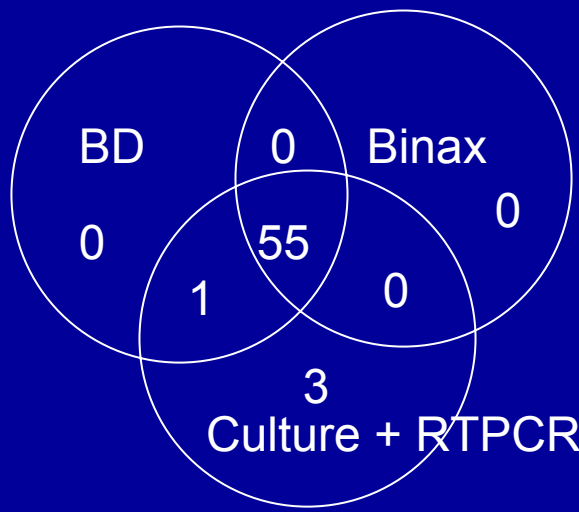
- DFA- Highly sensitive, Labor intensive, Subjective
- Cell culture- Tube and shell vials
- PCR- FDA approved- RVP-Luminex and Prodesse assays
- Rapid flow lateral immunochromatographic assays
- False-positive influenza test results are more likely to occur when disease prevalence is low; beginning and end of the influenza season.
- False-negative influenza test results are more likely to occur when disease prevalence is high; height of the influenza season.

Influenza


BinaxNOW
Influenza A & B
Test
FLU B
FLU A
CONTROL
37
(+)
(-)
SEP 15 2005
binax


BD
37
A
B
SEP 15 2005

	Sensitivity	Specificity	PPV	NPV
BD	95%	100%	100%	93%
BN	93%	100%	100%	91%.



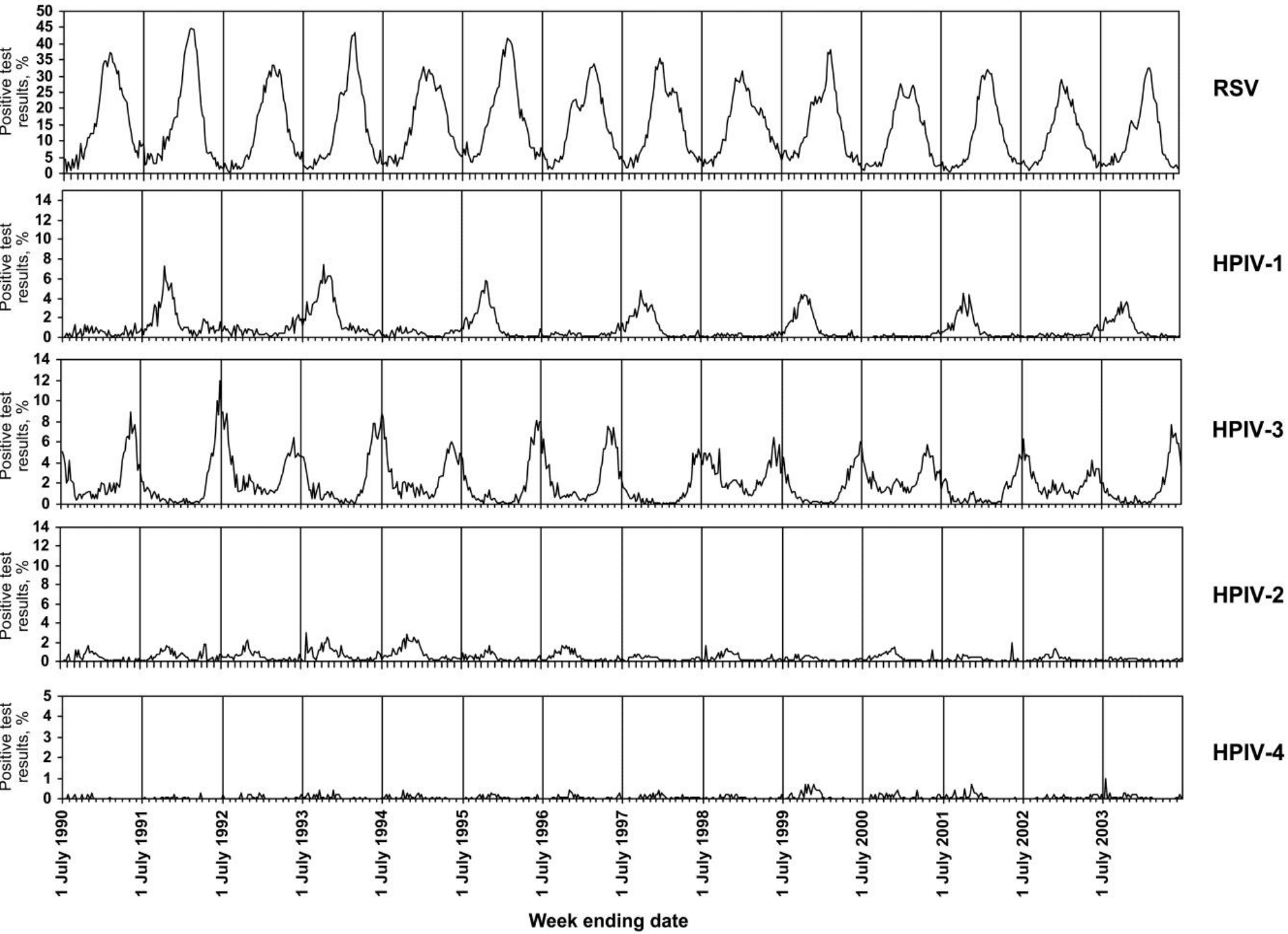
R. Selvarangan *et al* PASCV 2006

Influenza

- Vaccine: All children 6mo to 4yrs- Inactivated vaccine (TIV) and Live attenuated vaccine (LAIV) (healthy children 2-4 yrs and 5-49 yrs)
- Adamantanes (amantadine and rimantadine) not suitable for treatment or prophylaxis due to high resistance
- Neuraminidase inhibitors: Oseltamavir Rx and Prophylaxis >1yr old, Zanamavir Rx >7yr and Prophylaxis >5yr

Parainfluenza: seasonality

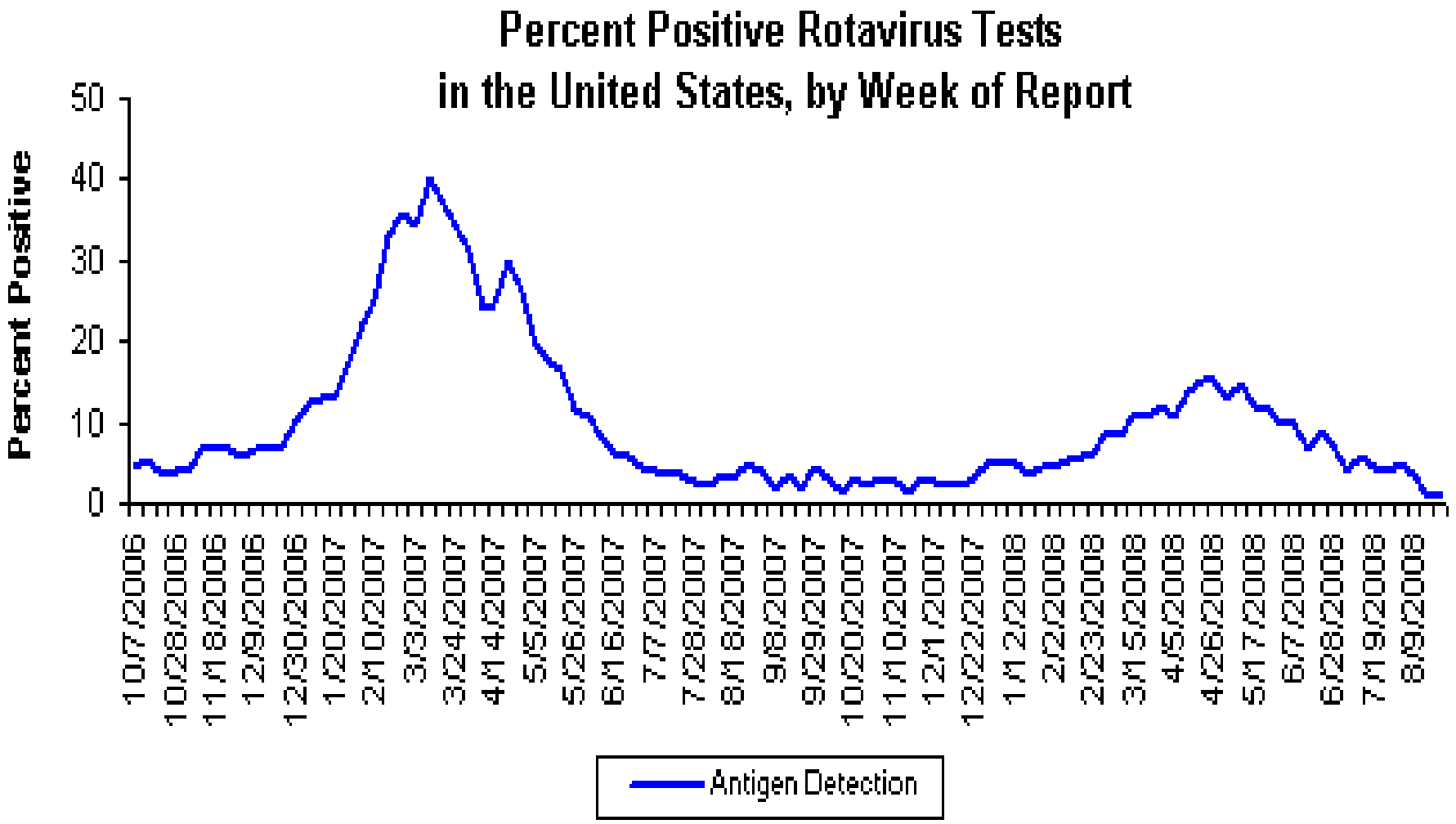
- Fry *et al* CID 2006:43
- Surveillance NREVS 1990-2004, 1.3 million HPIV tests (40.6K positive for HPV), about 69 labs/year
- Four HPIV serotypes are recognized – common cold, LRI- bronchiolitis and pneumonia. Second to RSV for hospitalizations in <5 yr old (2-17%)
- HPIV3- April/June, HPIV2 Oct/Nov- increased activity of HPIV1 occurred biennially during Sep/Dec on odd-numbered years.
- When HPIV1 was not circulating more HPIV3 was reported.
- HPIV3 (21K) >HPIV1(10K) >HPIV 2 (5K) >HPIV4 (1K)
- Represent about 3% respiratory virus per year.


Positive test results, %
RSV
HPIV-1
HPIV-3
HPIV-2
HPIV-4
1 July 1990
1 July 1991
1 July 1992
1 July 1993
1 July 1994
1 July 1995
1 July 1996
1 July 1997
1 July 1998
1 July 1999
1 July 2000
1 July 2001
1 July 2002
1 July 2003
Week ending date

Viral Gastroenteritis

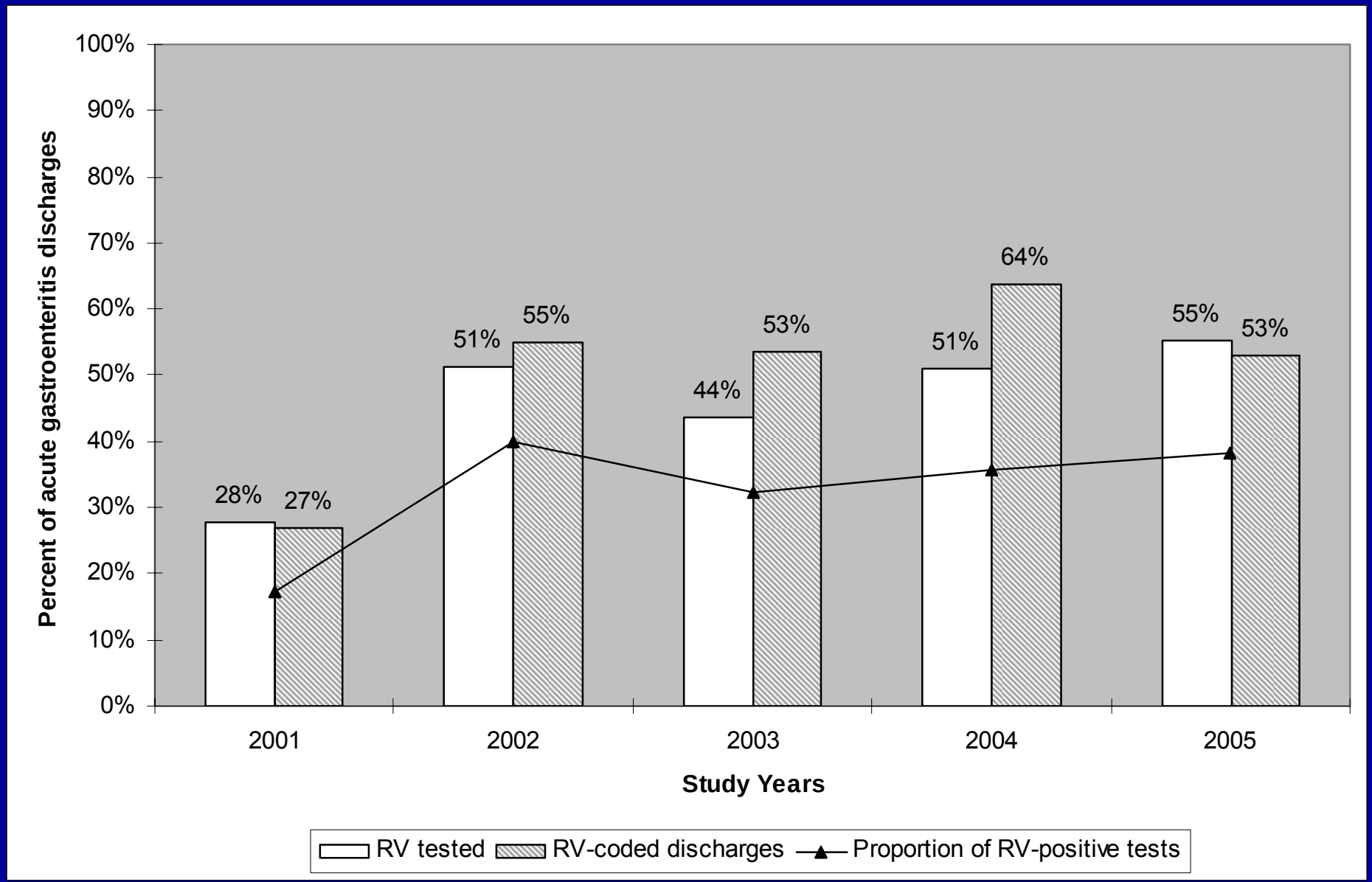
- Rotavirus is the most common viral pathogen causing gastroenteritis in children less than 5 years old.
- Estimated to cause 2.7 million cases and ~225K hospitalization
- Yearly winter epidemics occur
- EIA and lateral flow tests offer rapid testing capability with high sensitivity

Rotavirus



http://www.cdc.gov/ncidod/dvrd/revb/nrevss/

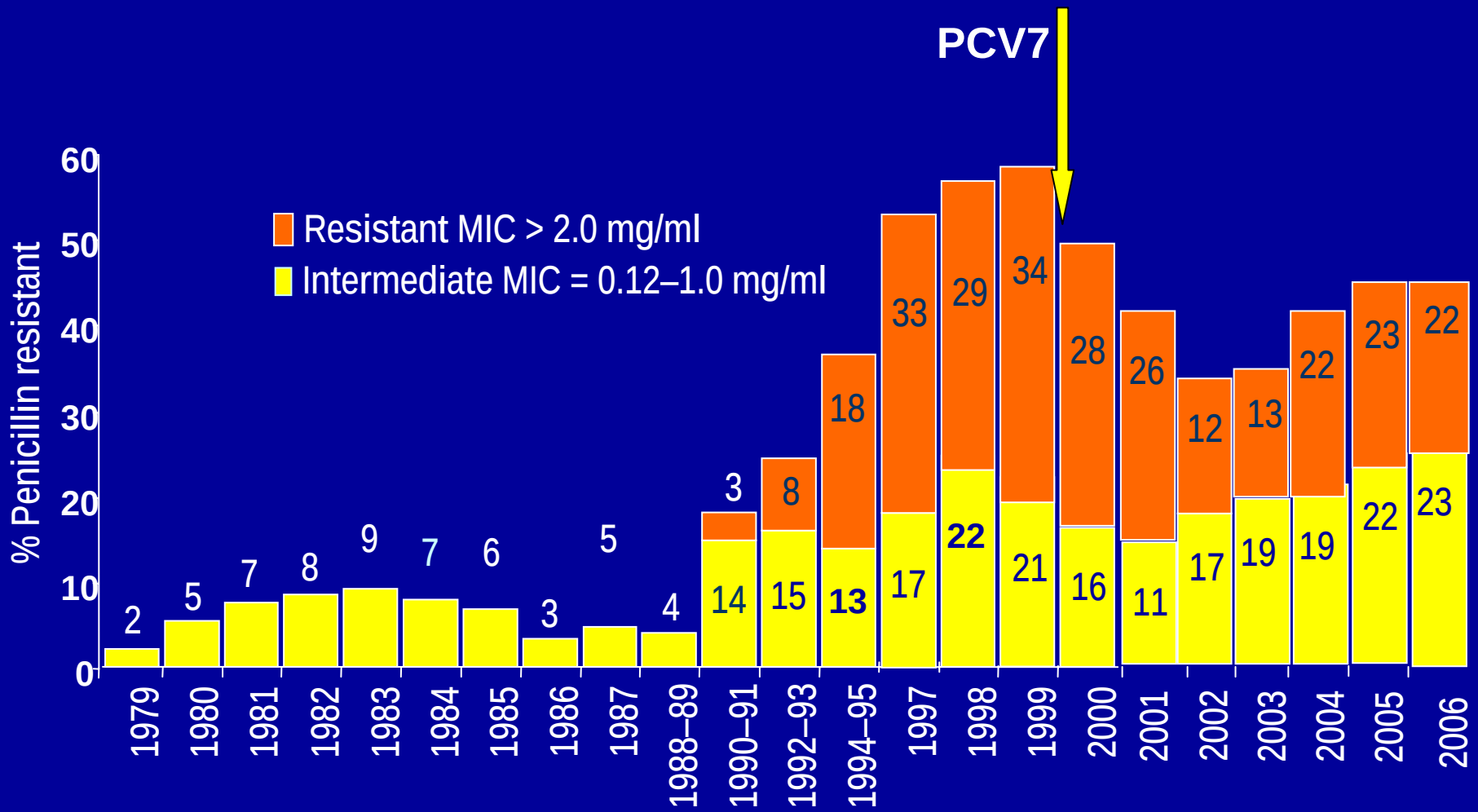
Rotavirus Testing, coding and incidence 2001 to 2005



Increase in Pneumococcus

- Leading cause of meningitis, sepsis, pneumonia and Otitis media in children
- Young children respond poorly to polysaccharide vaccine; new conjugate heptavalent vaccine is effective in young children (Feb 2000) – 4, 6B, 9V, 14, 18C, 19F, 23F.
- Vaccine initially effective but new serotypes emerge 28% in 2001, 66% in 2002, ~90% in recent years
- Emergence of drug-resistant pneumococci Serotype 19A
- New vaccine in the horizon 13 and 11 valent

Resistance pre vs post PCV-7 Use



1. Doern GV. *AJM* 1995; **99**(suppl 6B):3S–7S.
2. Jacobs MR *et al*. *AAC* 1999; **43**:1901–1908.
3. Harrison ICASS 2006 265:22-35.
4. Harrison, Woods et al. JAC 2008 (In Press)

Slide borrowed from Dr. Harrison, Prof. ID-CMH

Summary

- Both natural (Season) and man made (vaccination/control) changes impact prevalence of infectious disease
- Vaccination for common pediatric diseases have significantly reduced the incidence of infection
- Newer serotypes may emerge in the community under pressure from vaccination
- Periodic surveillance is essential to effectively control infection