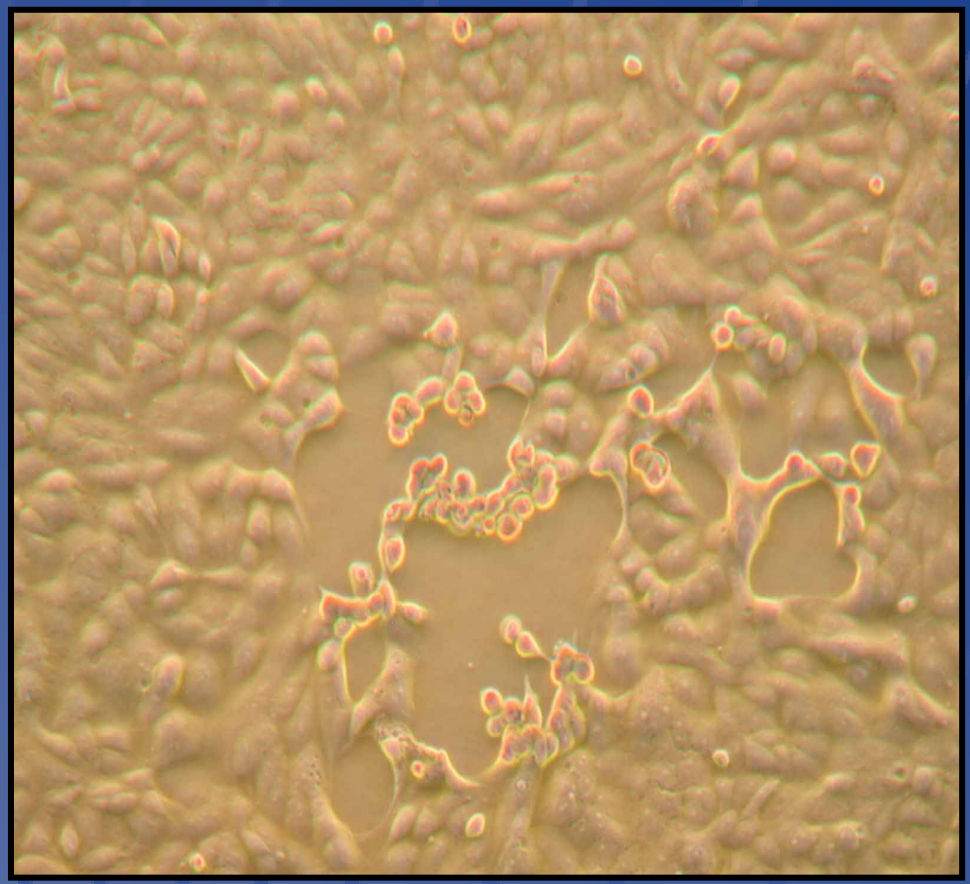
SARS Diagnostics

Preparedness

- Performance of current diagnostic tests
  - Real-time RT-PCR
  - Serology
- New diagnostic tools
- Optimal specimen types and timing
- Quality assessment
- Other respiratory pathogens – “rule-out testing”

SARS Diagnostics

Cell culture



P Rollin, Special Pathogens Branch

BSL-3 Activity

Restricted culture range

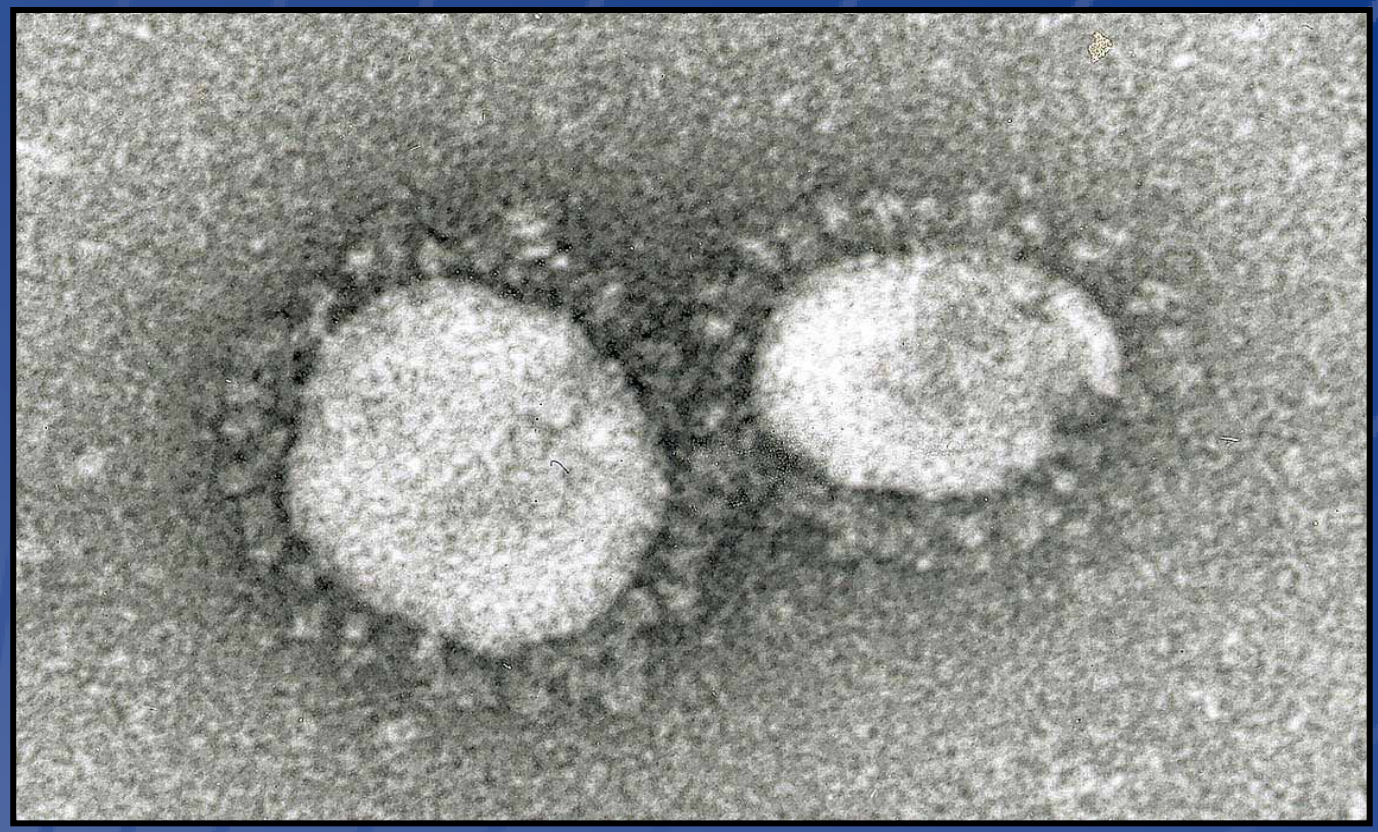
Vero E6 cells

CPE:

- **focal**
- **cell rounding**
- **retractile appearance**

SARS Diagnostics

Electron Microscopy



C Humphrey, Pathology Activity Program

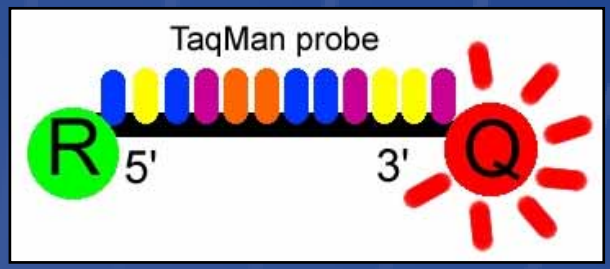
SARS Diagnostics

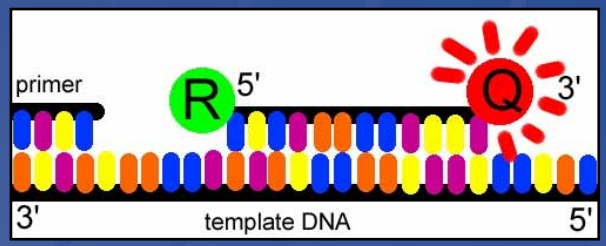
Real-time RT-PCR

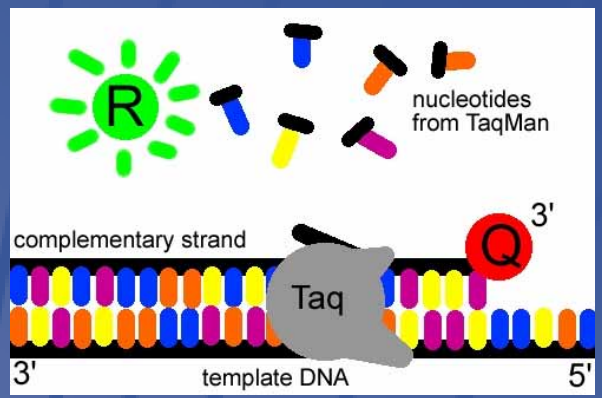
- Conventional vs Real-time RT-PCR (TaqMan™)
  - increased sensitivity (1-10 transcript copies)
  - increased speed/throughput
  - quantitative
  - reduced risk of amplicon contamination
- Multiple genetic targets
  - nucleocapsid and polymerase genes
  - amplification of 2 of the 3 targets required for a positive test

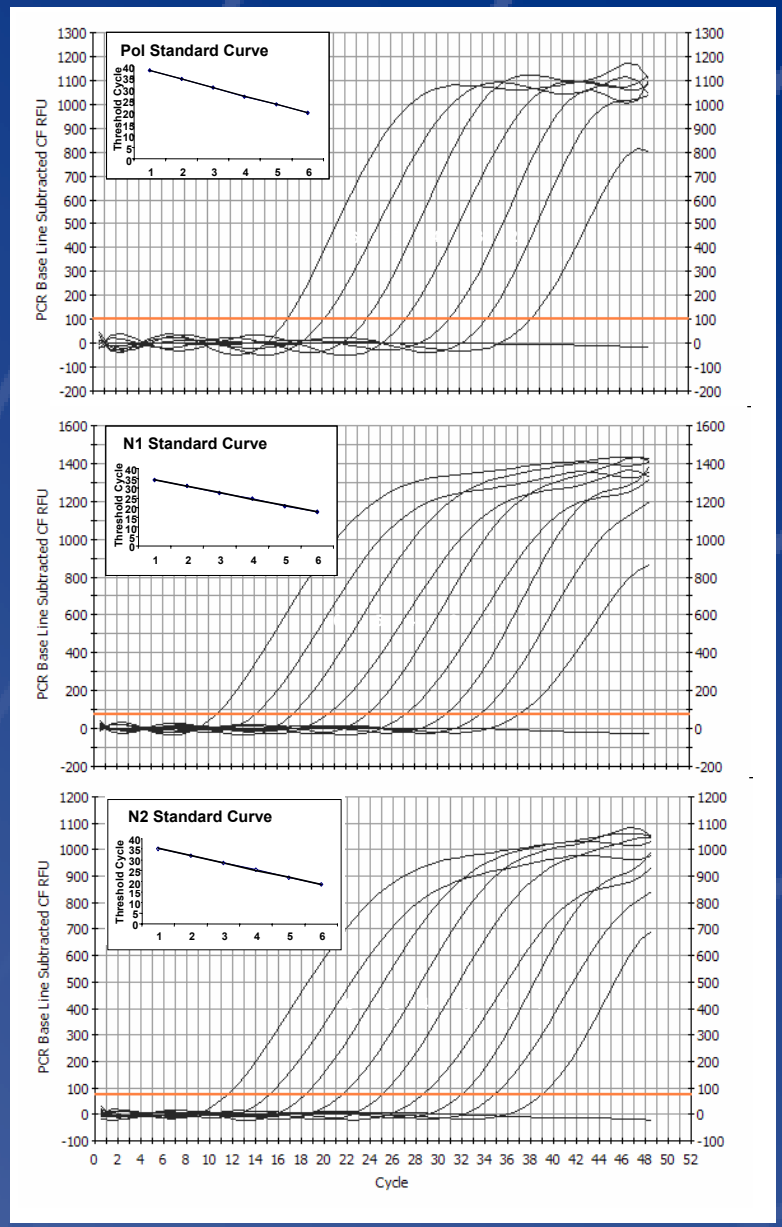
SARS Diagnostics

Real-time RT-PCR









SARS Diagnostics

RT-PCR – Interpretation of Test Results

- Potential for false negative results
  - low titer virus in respiratory secretions in first few days after onset of illness
- Potential for false positive results
  - contamination from previously amplified DNA
  - cross-contamination between specimens
- A positive test result should be considered provisional until confirmed by independent testing
- A negative test result does not rule out SARS and should not affect patient management decisions

SARS Diagnostics

RT-PCR – Interpretation of Test Results

- Confirmation of a positive SARS RT-PCR test (specimen)
  - repeat the RT-PCR from new aliquot of the original sample
  - if positive, have the sample tested in a second laboratory
- Positive SARS diagnostic test finding (patient)
  - at least 2 different clinical specimens
  - the same specimen type collected on 2 or more days

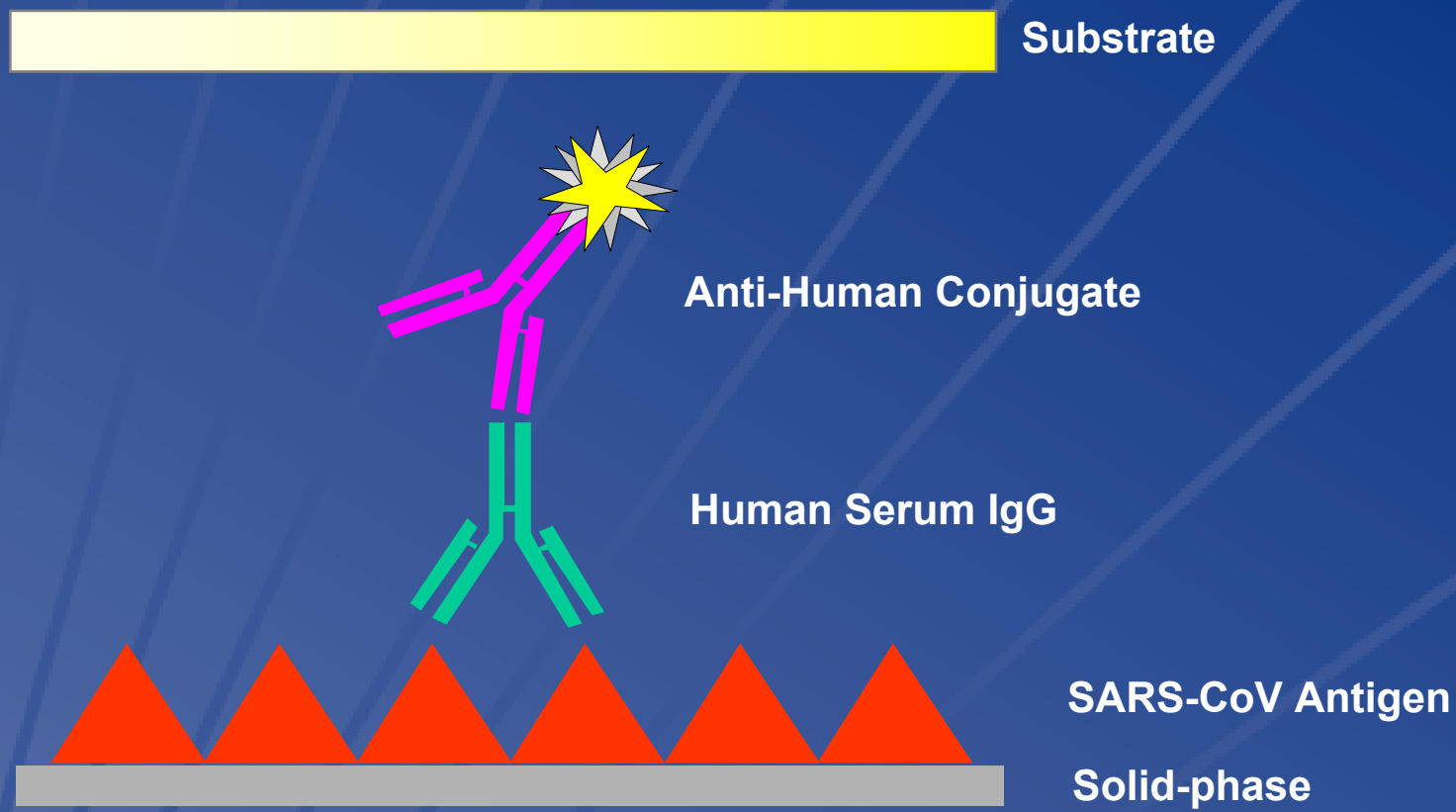
SARS Diagnostics

Serology – Current EIA

- Serology appears to be highly specific
  - no reactions with other documented CoV infections (OC43 and 229E)
  - no reactions with “normal” blood donors (U.S. and Hong Kong populations)
- Serology can be positive in as few as 8 to 10 days after onset of symptoms
- Serology cannot be considered negative until >28 days after onset of symptoms

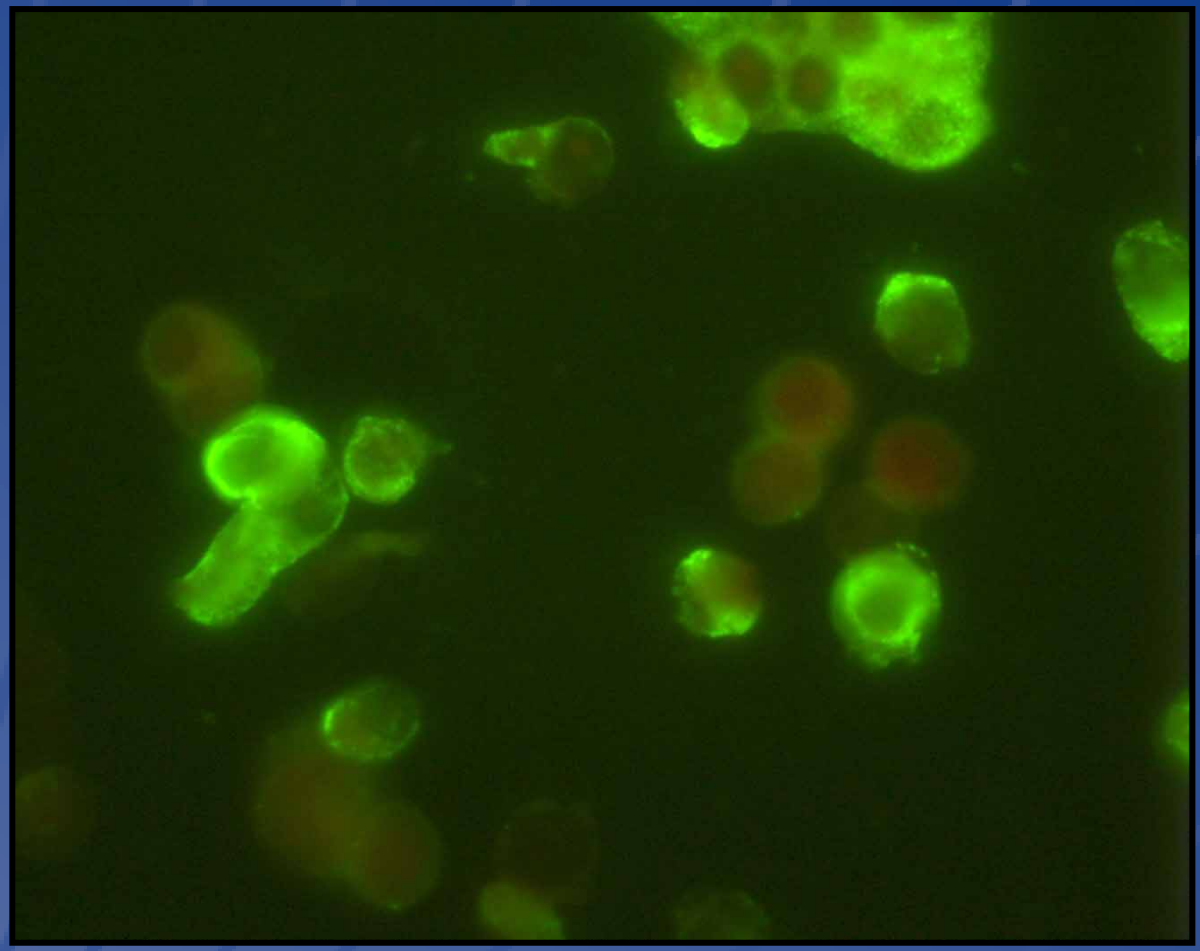
SARS Diagnostics

Antibody tests: Enzyme immunoassay



SARS Diagnostics

Antibody tests: Immunofluorescence Assay



SARS Diagnostics

Serology - New assays

- Native virus vs recombinant antigens
  - nucleocapsid, spike, and membrane proteins
  - safety, standardization, and sensitivity
  - need to rule out cross-reactions with other human coronaviruses
- IgM assays
  - IgM antibodies may be detectable earlier in the course of infection
  - Transient response
- Neutralization and other immunological markers

SARS Diagnostics

Specimen Selection and Timing

- Respiratory tract specimens
  - LRT > URT
  - Sputum > NP aspirates > NP/OP swabs
  - More sample
  - Multiple samples
- Others specimens
  - Blood plasma
  - Stool
- Timing of specimen collection

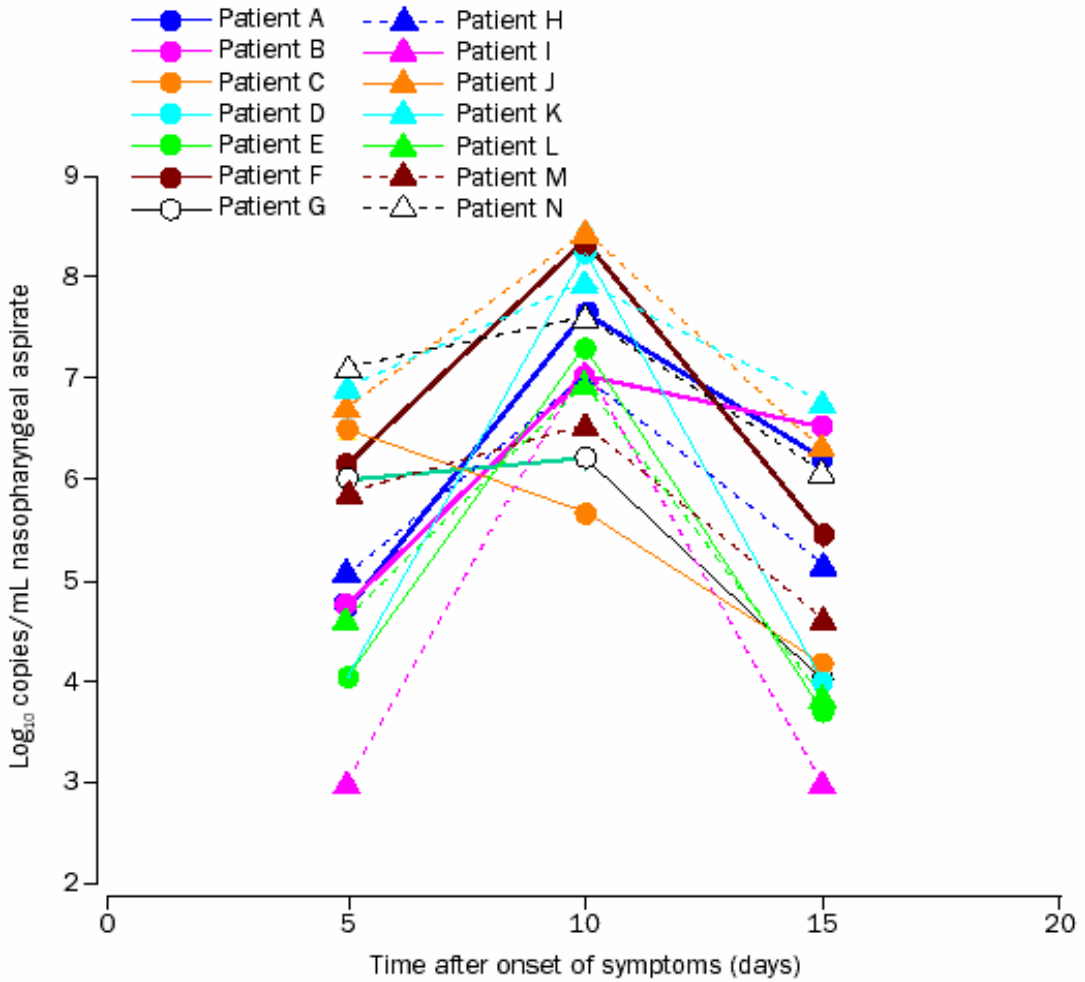
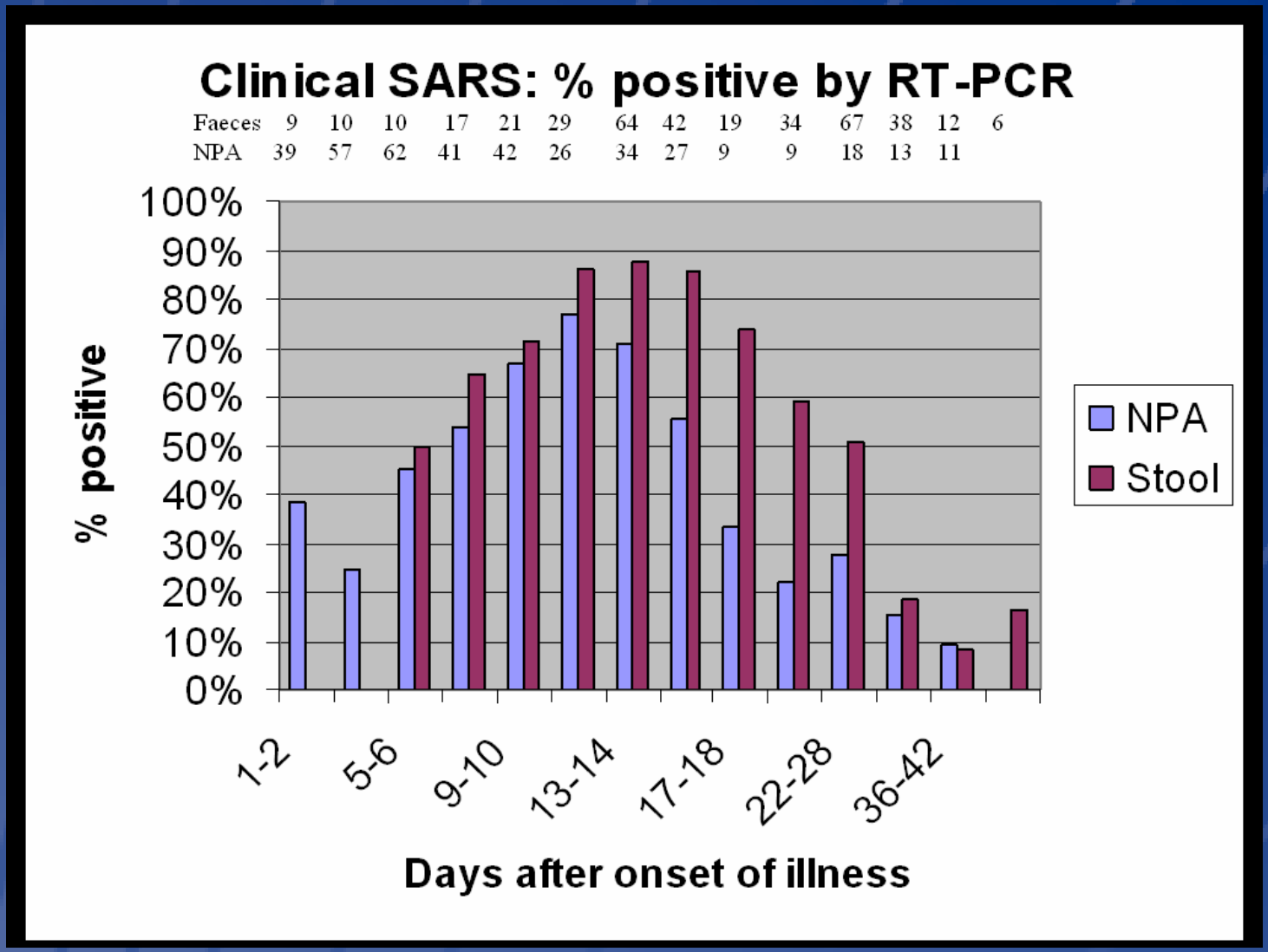


Figure 4: **Sequential quantitative RT-PCR for SARS-associated coronavirus in nasopharyngeal aspirates of 14 SARS patients**

Peiris et al: Lancet, May 24, 2003



Peiris: personal communication

SARS Diagnostics

Specimen Selection and Timing

Specimen	<1 week post symptom onset	1 - 3 weeks post symptom onset	>3 weeks post symptom onset
Serum (separator tube)	++	++	++
Blood plasma (EDTA)	++	+	-
Respiratory (BAL, sputum, nasal aspirate & wash, np/op swabs)	+	++	+
Stool	+	++	++

SARS Diagnostics

Quality Assessment

- QA CDC
  - Standardized test controls
  - Internal CDC confirmatory testing
  - External WHO quality assurance study
- QA LRN & APHL
  - Confirmatory testing
  - Proficiency testing

SARS Diagnostics

Other Respiratory Pathogens – "rule-out testing"

Other respiratory pathogens, U.S. SARS surveillance, Mach-July, 2003.

M. pneumoniae	C. pneumoniae	L. pneumophila	Influenza A or B	hMPV	hPIV 1,2, 3	RSV	Adeno	Picornavirus (rhinovirus)
22/200 (11%)	2/197 (1%)	0/196 (0%)	15/140 (11%)	9/150 (6%)	10/150 (7%)	1/150 (0.7%)	7/150 (5%)	18/61 (30%)

Schrag SJ et al. SARS surveillance in the United States during the Emergency Public Heath Response, March-July, 2003. EID (In press).

SARS Diagnostics

Other Respiratory Pathogens – "rule-out testing"

- CDC can provide guidance on test selection
  - What other tests are available?
  - What are their performance characteristics?
- CDC can provide guidance on testing
  - Clinical presentation
  - Demographics (e.g., age)
  - Seasonality (NREVSS)
- CDC can provide RT-PCR protocols for other respiratory pathogens

SARS Diagnostics

Key Messages

- SARS diagnostic assays are sensitive and specific, but may not provide definitive diagnosis early in the illness
- Changes in the quantity, type, and timing of specimens collected may improve detection of SARS-CoV infection
- Rapid and accurate diagnosis of other respiratory pathogens associated with SARS-like illness may help rule out SARS-CoV infection and calm public fears
- Interpretation of test results must take into consideration possibility of false positives and negatives; a clear strategy to minimize such possibilities and to confirm test results are essential