

Severe Acute Respiratory Syndrome (SARS)

Laboratory Diagnostics

SARS Diagnostics

Should we be testing for SARS?

In the absence of documented SARS transmission, diagnostic testing for SARS-CoV should **NOT** be considered unless there is a high index of suspicion for SARS (e.g., a strongly suggestive clinical presentation and a travel history to a previously affected SARS area) and no alternative respiratory diagnosis.



SARS Diagnostics

Word on the Street

“[Labs are] not fully comfortable with the PCR assay for SARS because much about it is unknown.”

APHL: Public Health News

“State Public Health Laboratories Put to the Test with SARS”

SARS Diagnostics

Preparedness

- **Performance of current diagnostic tests**
- **New diagnostic tools**
- **Optimal specimen types and timing**
- **Quality control**
- **Other respiratory pathogens – “rule-out testing”**



SARS Diagnostics

Cell culture



BSL-3 Activity

Restricted culture range

Vero E6 cells

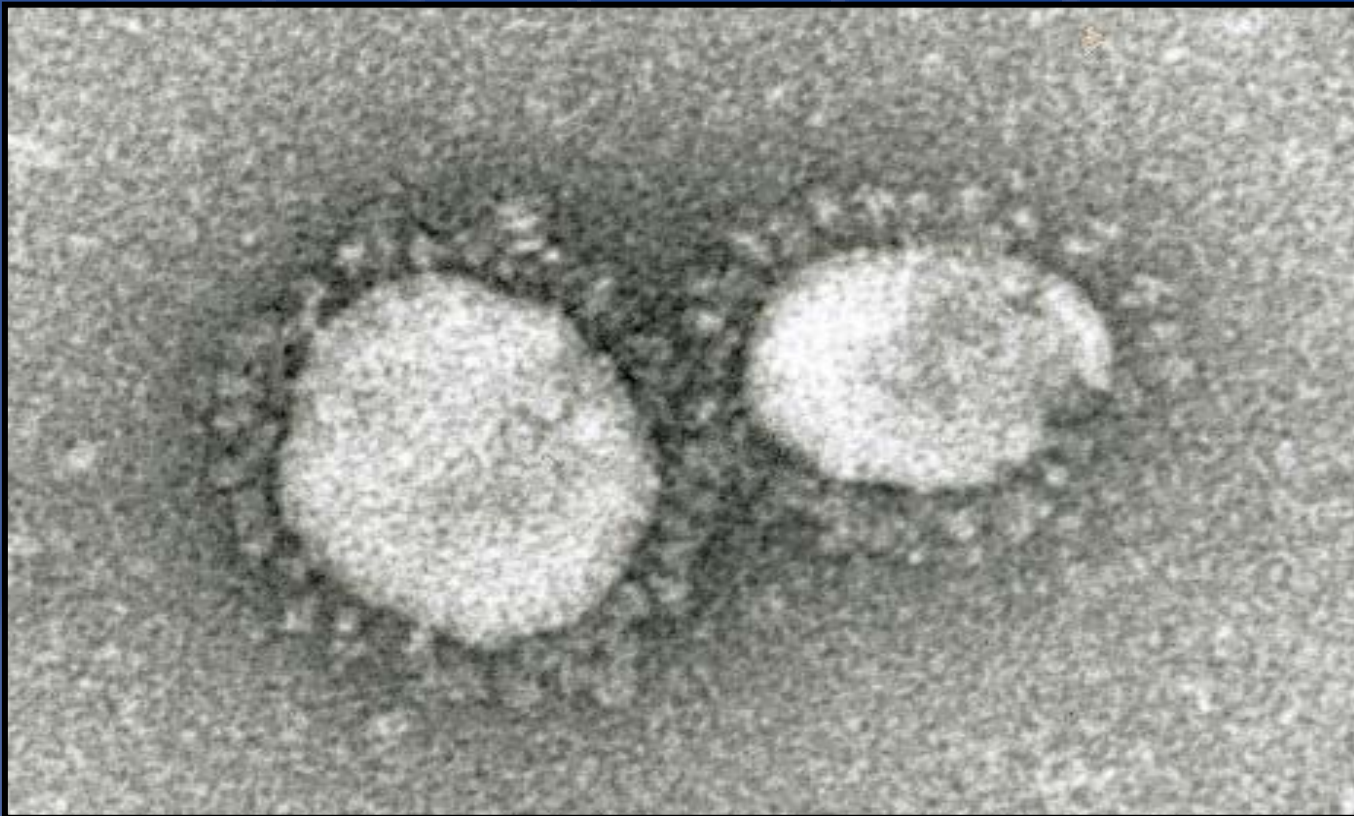
CPE:

- focal
- cell rounding
- refractile appearance

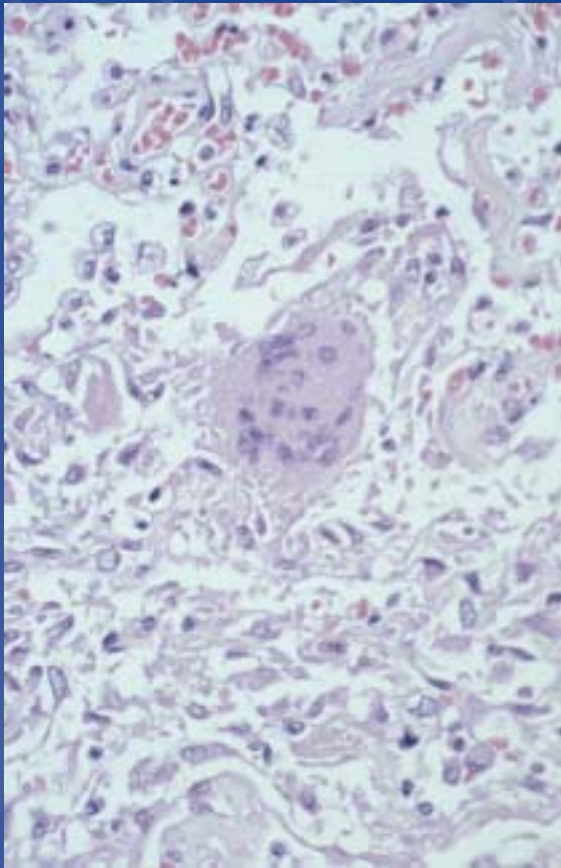
P Rollin, Special Pathogens Branch

***SARS* Diagnostics**

Electron Microscopy

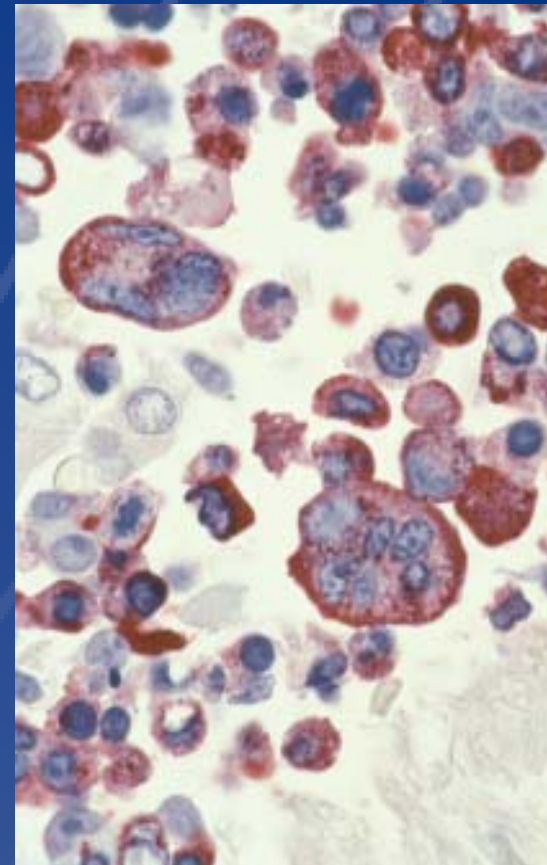


C Humphrey, Pathology Activity Program



**Syncytial giant cell
in lung of SARS patient**

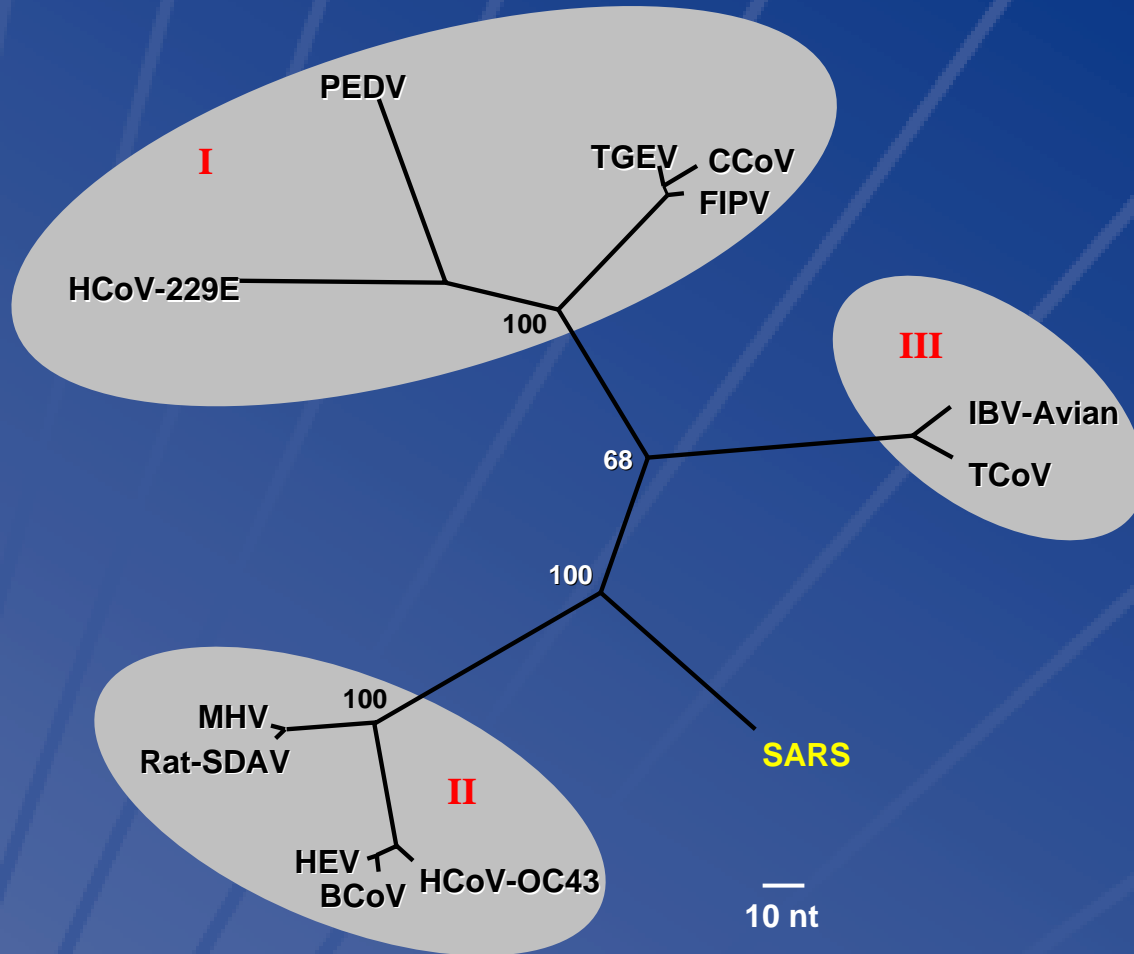
S Zaki, Pathology Activity Program



**IHC staining of syncytial cells
in coronavirus-infected Vero
cells (antigenic group I antisera)**

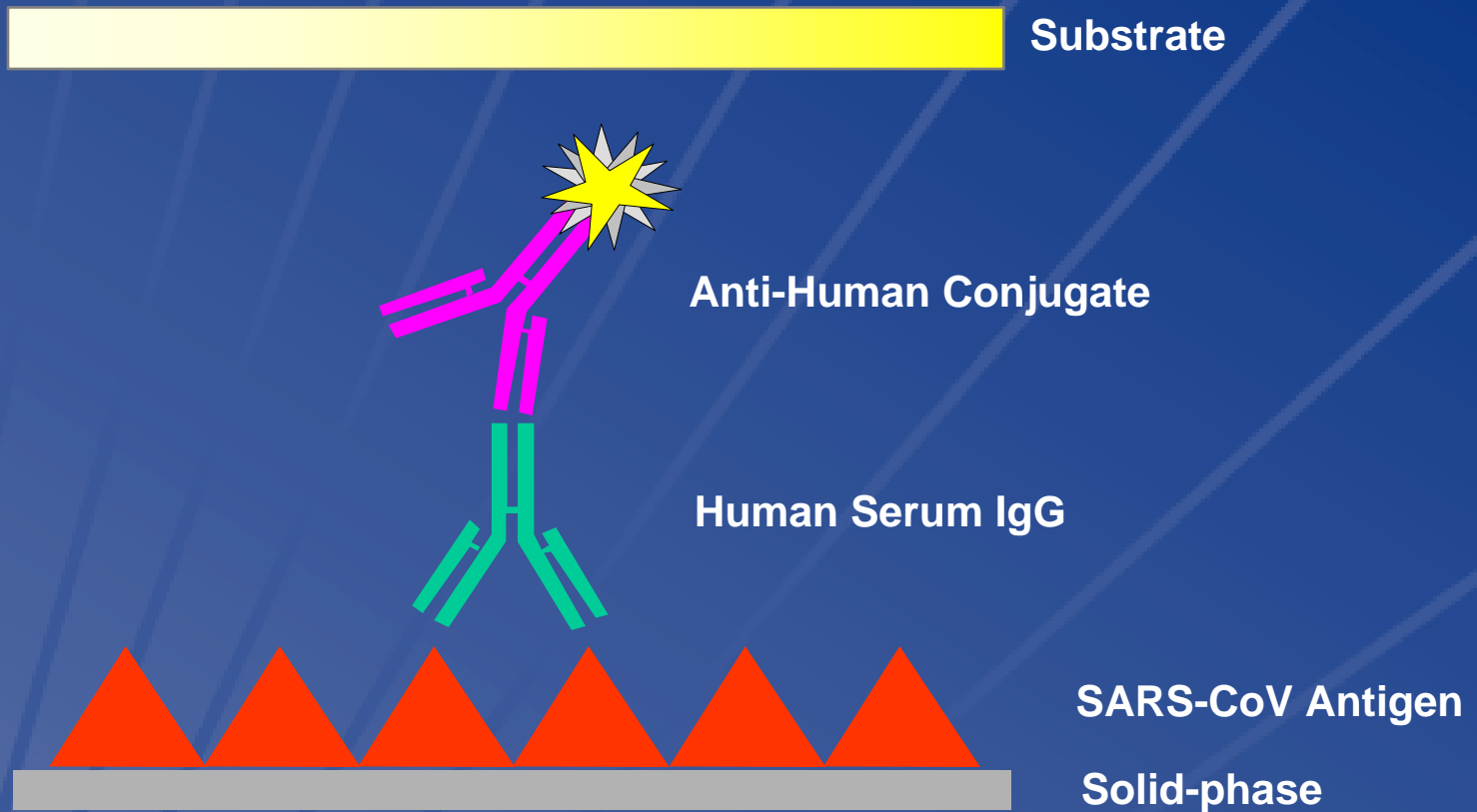
SARS Diagnostics

Phylogenetic Analysis



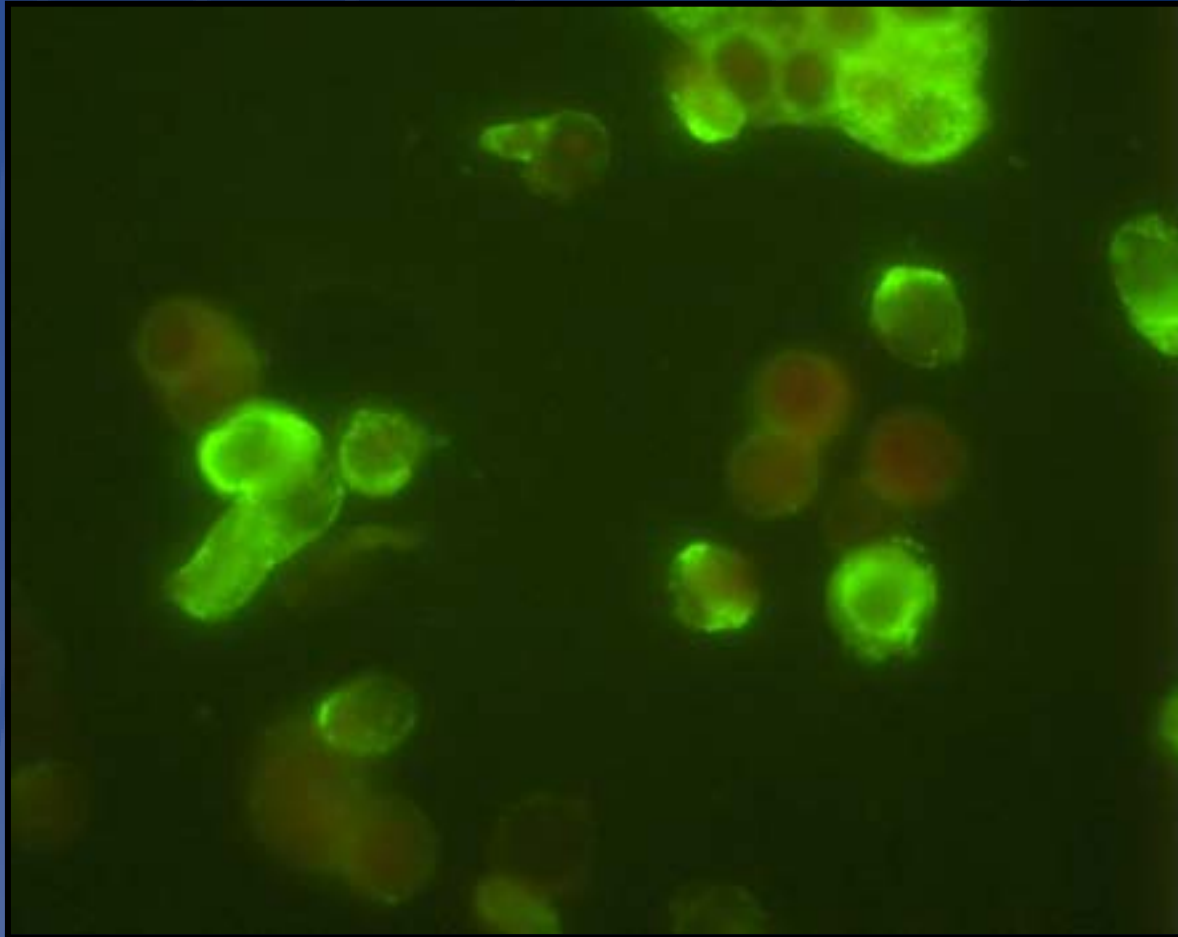
SARS Diagnostics

Antibody tests: Enzyme immunoassay



SARS Diagnostics

Antibody tests: Immunofluorescence Assay



SARS Diagnostics

RT-PCR – First Generation Tests



SARS Diagnostics

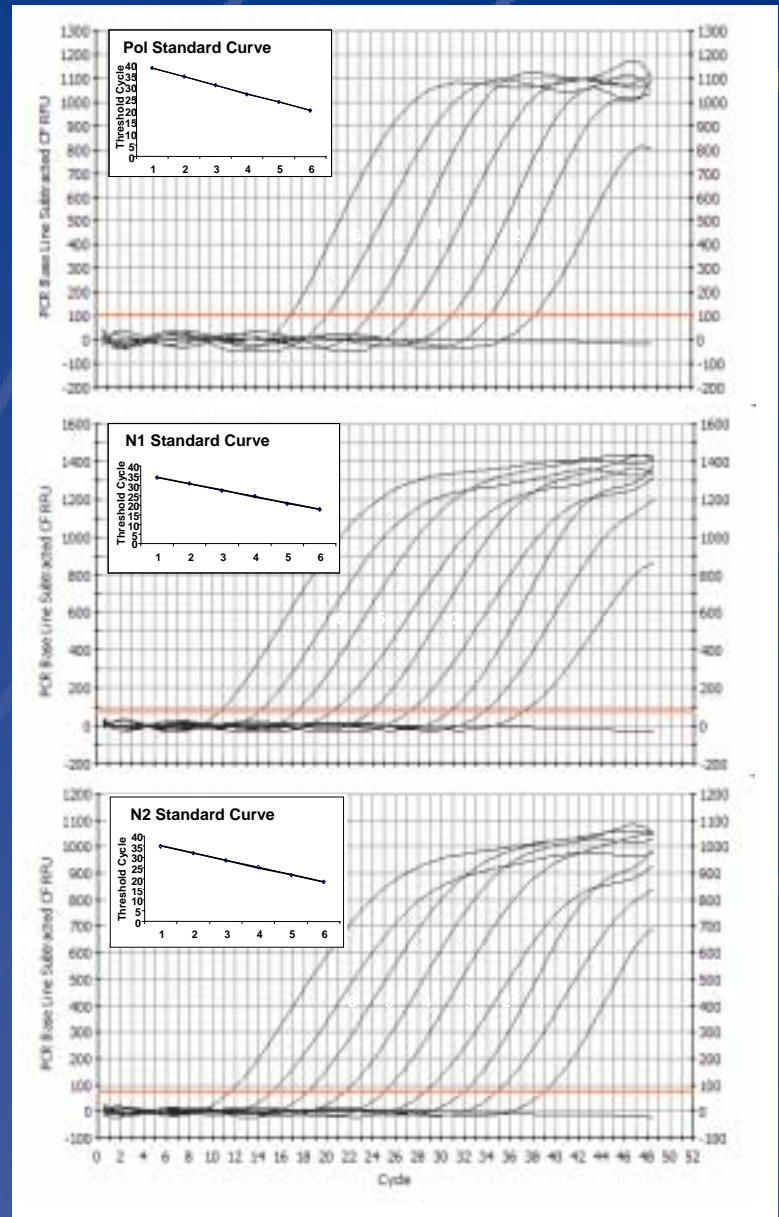
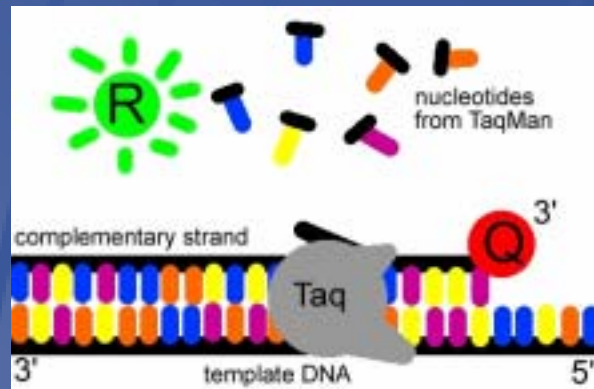
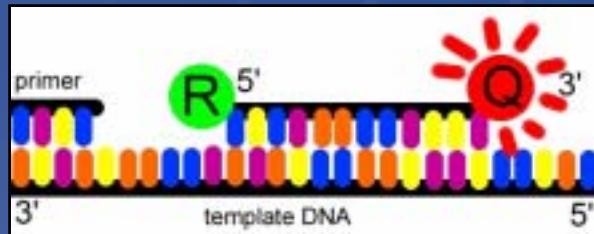
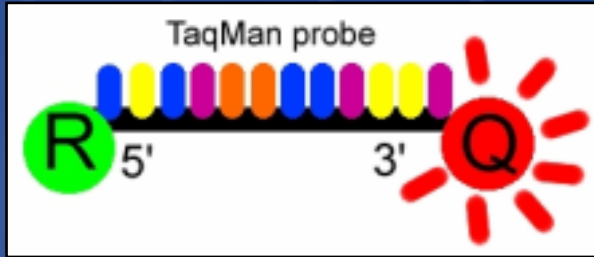
Real-time RT-PCR

- **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

Real-time RT-PCR

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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**
- **All test results must be interpreted in the context of the clinical and epidemiological findings**



SARS Diagnostics

RT-PCR – Interpretation of Test Results

- **Confirmation of a positive SARS RT-PCR test**
 - repeat the RT-PCR using the original sample
 - have the sample tested in a second laboratory
- **Positive SARS diagnostic test finding**
 - at least 2 different clinical specimens
 - the same specimen collected on 2 or more days

WHO recommendations: <http://who.int/csr/sars/labmethods/en/>



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

Serology - New assays

- **Native virus vs recombinant antigens**
 - **nucleocapsid, spike, and membrane proteins**
 - **safety, standardization, and sensitivity**
- **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 > aspirates > NP/OP washes > NP/OP swabs**
 - **more sample**
 - **multiple samples**
- **Others specimens**
 - **blood plasma**
 - **stool**
- **Timing of specimen collection**



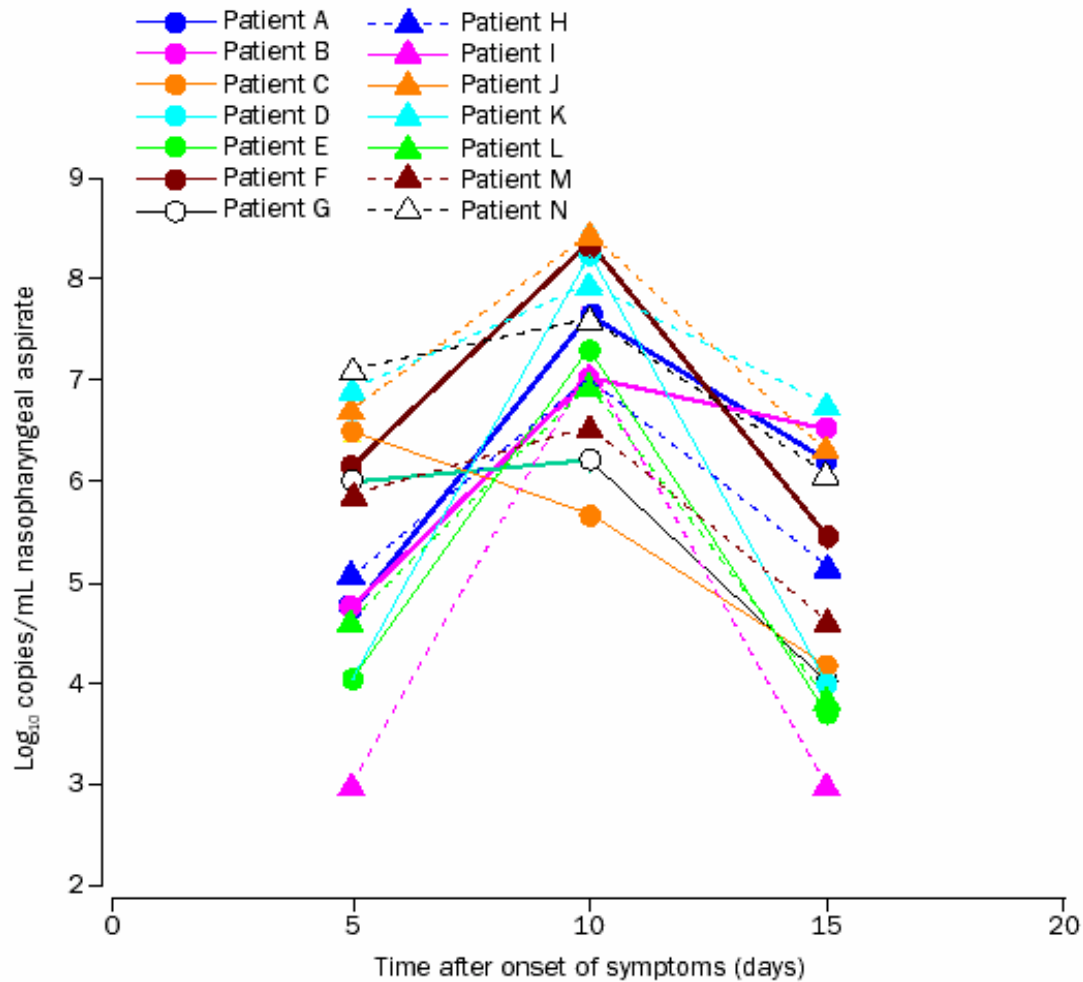
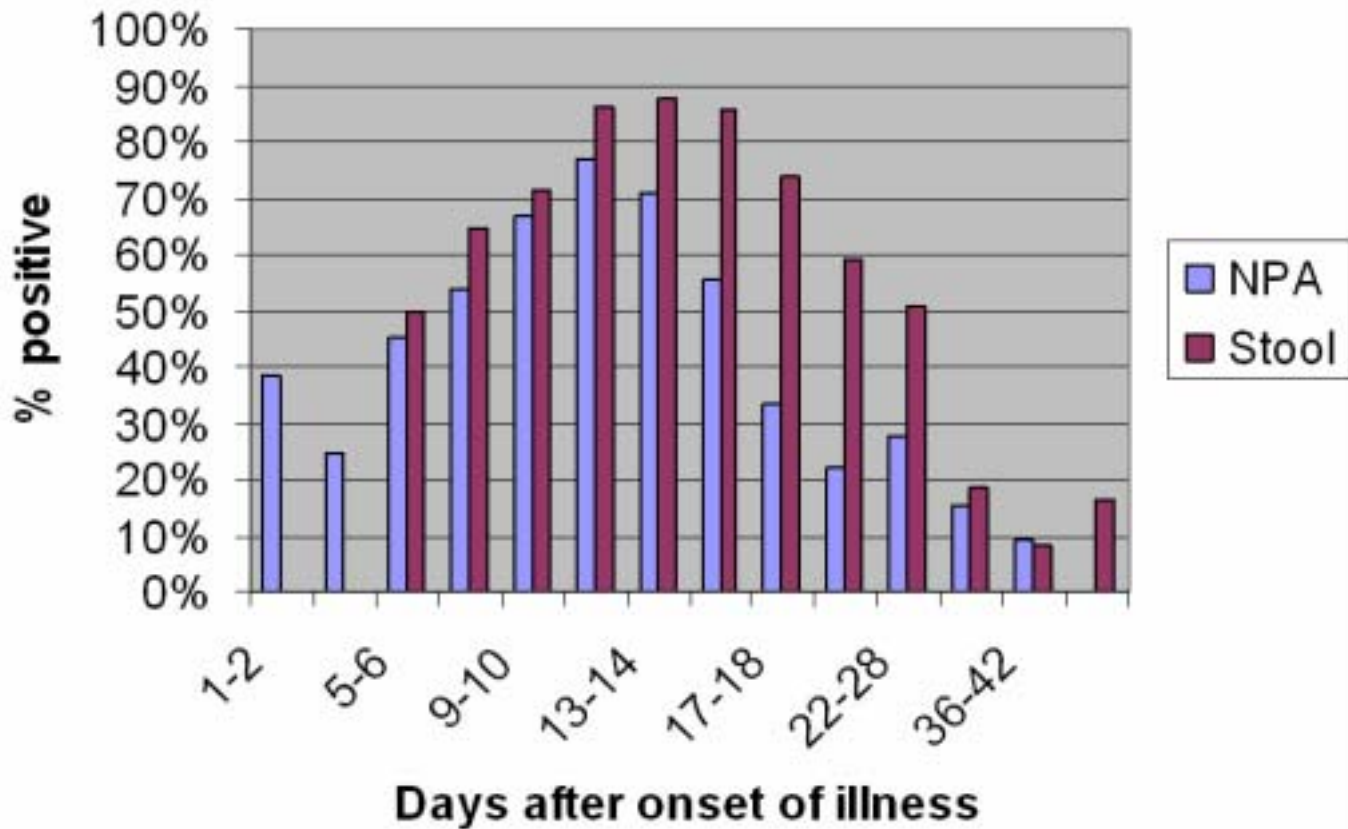


Figure 4: **Sequential quantitative RT-PCR for SARS-associated coronavirus in nasopharyngeal aspirates of 14 SARS patients**

Peiris et al: Lancet, May 24, 2003

Clinical SARS: % positive by RT-PCR

Faeces	9	10	10	17	21	29	64	42	19	34	67	38	12	6
NPA	39	57	62	41	42	26	34	27	9	9	18	13	11	



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 (EDTA)	++	+	-
Respiratory (sputum, nasal aspirate/wash, np/op swabs)	+	++	+
Stool	+	++	++

SARS Diagnostics

Quality Assessment

- **QA CDC**
 - **Standardized test controls**
 - **Internal confirmatory testing**
 - **External WHO quality assurance study**
- **QA LRN & APHL**
 - **Identical assays performed at CDC**
 - **Confirmatory testing**
 - **Proficiency testing**



SARS Diagnostics

Other Respiratory Pathogens – “Rule-out testing”

- **Why test for other respiratory pathogens?**
 - **may help rule out concerns about SARS**
 - **possibility of mixed infections**
- **When to test for other respiratory pathogens?**
- **What respiratory pathogens to test for?**
- **Which tests to use?**



SARS Diagnostics

Other Respiratory Pathogens – “Rule-out testing”

Other respiratory pathogens, U.S. SARS surveillance, March-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 Health Response, March-July, 2003. EID (In press).

SARS Diagnostics

Other Respiratory Pathogens – “Rule-out testing”

- **Provide guidance on test**
 - **What other tests are available?**
 - **What are their performance characteristics?**
- **Provide guidance on testing**
 - **Clinical presentation**
 - **Demographics (e.g., age)**
 - **Seasonality (NREVSS)**
- **Provide RT-PCR protocols**



SARS Diagnostics

Key Messages

- **Serologic and RT-PCR assays are sensitive and specific, but may not provide definitive diagnosis early in illness**
- **Changes in the type, timing and quantity of specimens collected may improve detection**
- **Interpretation of test results must take into consideration possibility of false positives and negatives**
- **Confirmation of positive results by a qualified second laboratory essential when SARS is infrequent**



SARS Diagnostics

Preparedness – Web Resources

- **Specimen packaging & shipping**

<http://www.cdc.gov/ncidod/sars/packingspecimens-sars.htm>

- **Specimen handling & processing**

<http://www.cdc.gov/ncidod/sars/sarslabguide.htm>

- **Specimen testing: RT-PCR**

<http://www.cdc.gov/ncidod/sars/lab/rtpcr/index.htm>

- **Specimen testing: Serology**

<http://www.cdc.gov/ncidod/sars/lab/eia/index.htm>

- **Emerging Infectious Diseases**

http://www.cdc.gov/ncidod/EID/sars_links.htm