



Health Research Symposium 2021

Implementing Evidence-based Research in the Era of COVID-19 and other Global Health Challenges

Implementing Research Findings in Clinical Practice

23 November 2021

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COVID190107

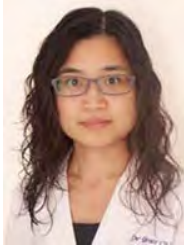
Comprehensive clinical, virological, microbiological, immunological and laboratory monitoring of patients hospitalized with Coronavirus Diseases (COVID-19)

COVID19F06

Early biomarkers in SARS-CoV-2 infection: correlation with short/medium/long-term clinical outcomes, and implications on acute patient management and long-term medical and health care



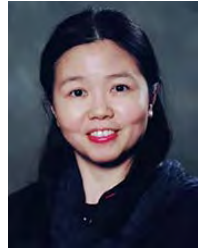
David Hui



Grace Lui



Albert Li



Renee Chan



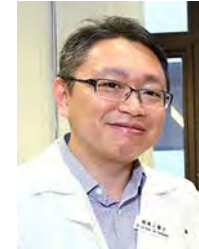
CK Wong



Lowell Ling



Martin Wong



Christopher Lai



Rita Ng



Public Health Laboratory Services Branch



Prince of Wales Hospital



United Christian Hospital





Performance of self-sampling options



Deep throat saliva (DTS)

深喉唾液

程序：



1. 預備以下急症室/門診所提供的物品：一個樣本瓶、兩個樣本袋、紙巾



2. 以肥皂及清水洗手或以酒精搓手液潔手



3. 檢查樣本瓶上的個人資料是否正確



4. 打開樣本袋及樣本瓶蓋



5. 於喉嚨發出「Kruuua」的聲音以清來自咽喉的唾液



6. 除下外科口罩，將唾液吐入樣本瓶內，避免唾液沾到樣本瓶外面。
(如量太少，重覆以上步驟)



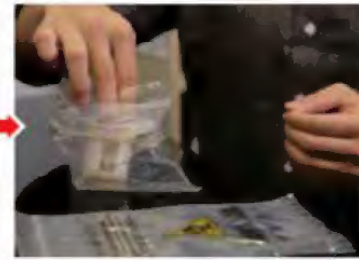
7. 戴上外科口罩



8. 蓋好及扭緊樣本瓶蓋，確保沒有滲漏



9. 用紙巾抹乾淨樣本瓶表面

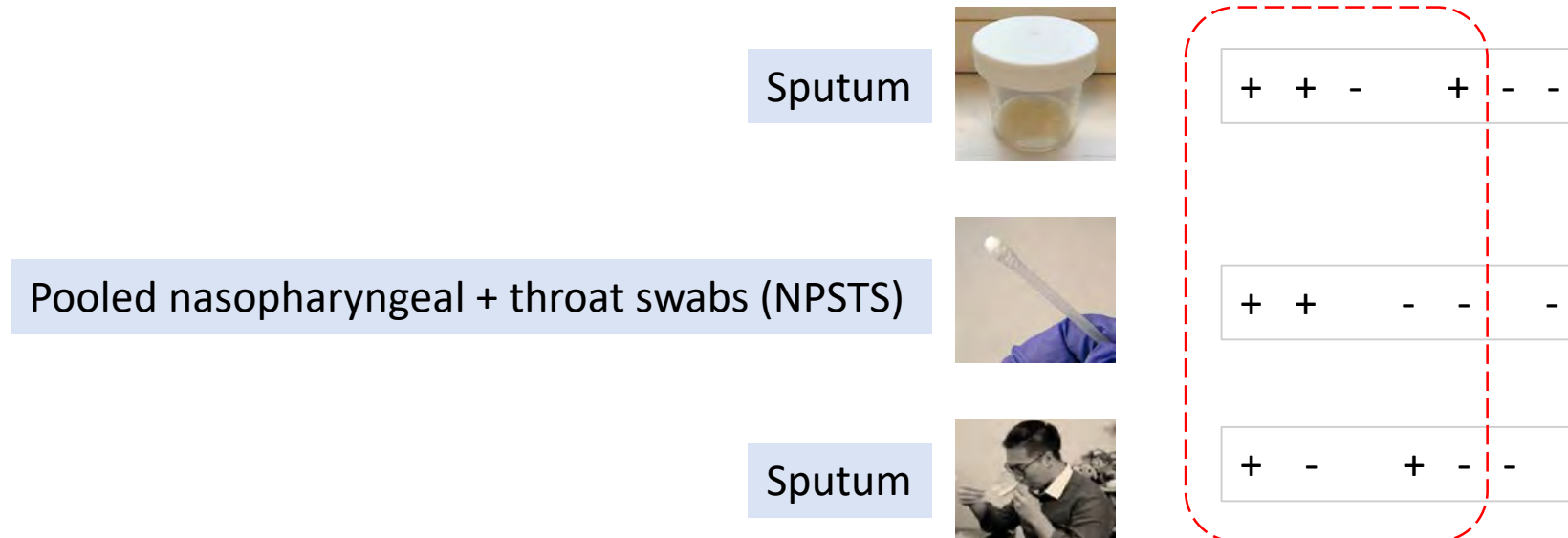


10. 將樣本瓶放入樣本袋內，確保瓶身直立沒有滲漏，並以肥皂及清水洗手或以酒精搓手液潔手

Deep throat saliva (DTS)

563 serial samples:
 150 deep throat saliva (DTS)
 309 pooled nasopharyngeal & throat swabs (NPSTS)
 104 sputum
 2 hospitals: PWH & UCH
 50 COVID confirmed patients

All specimens collected during virus shedding period were included



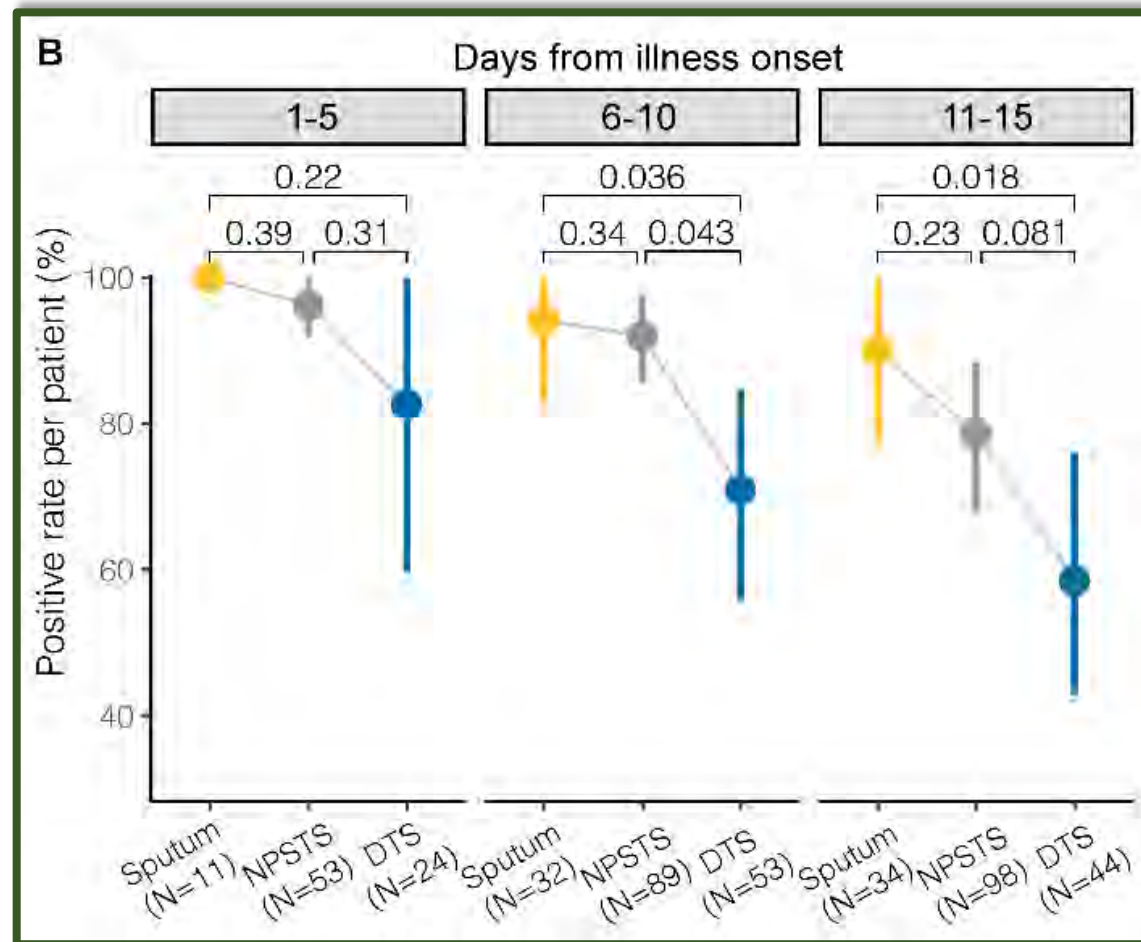
Deep throat saliva (DTS)

Overall

	Positive rate	
	Per specimen (N=563)	Per patient (mean) (N=50)
Deep throat saliva (DTS)	68.7%	72.3%
Pooled nasopharyngeal & throat swabs (NPSTS)	80.9%	82.6%
Sputum	89.4%	91.7%

Deep throat saliva (DTS)

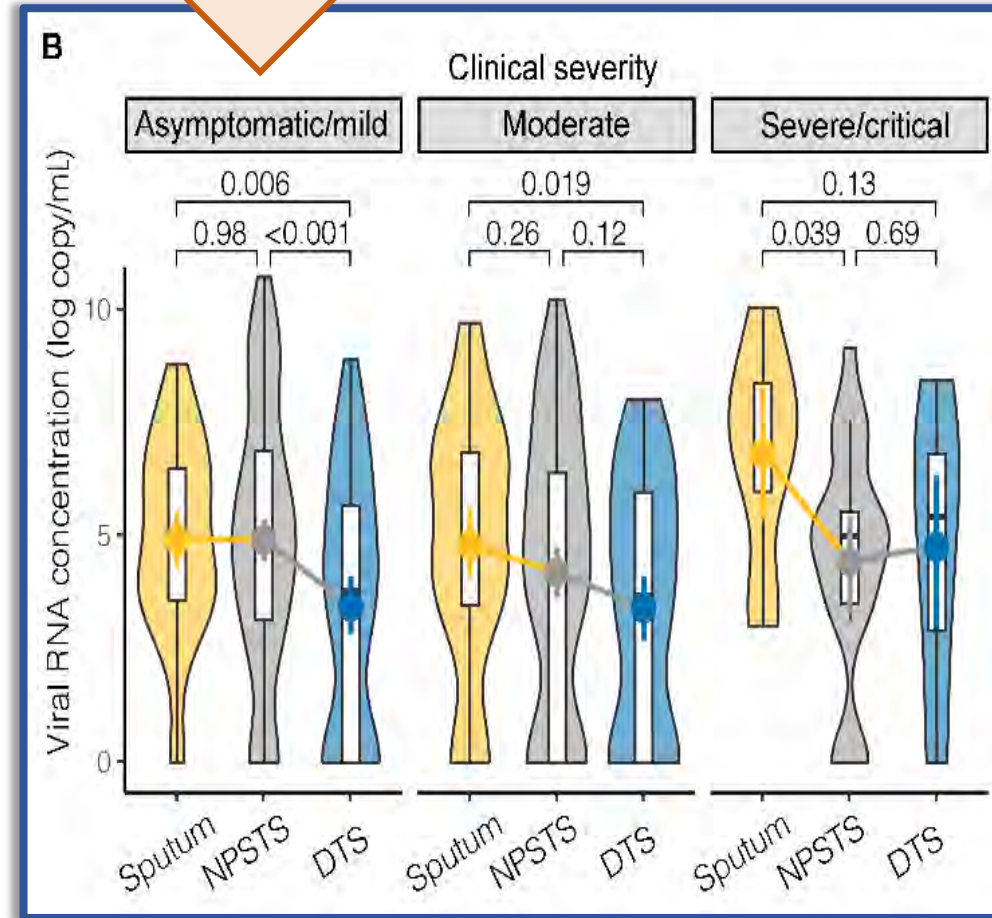
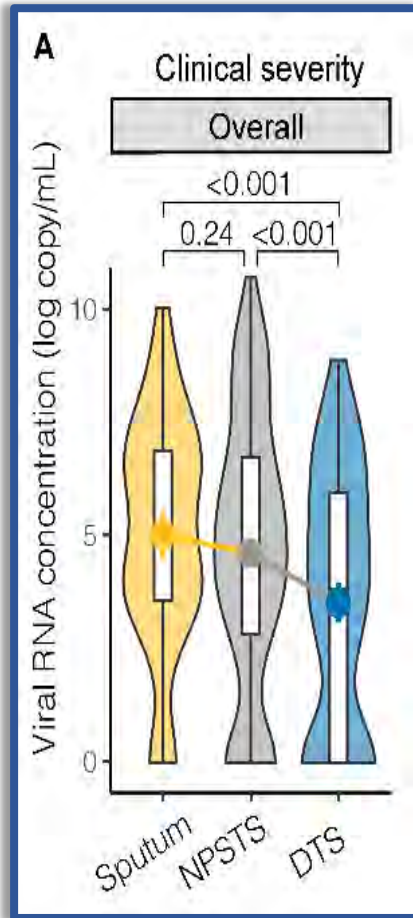
Time of collection



Deep throat saliva (DTS)

Severity

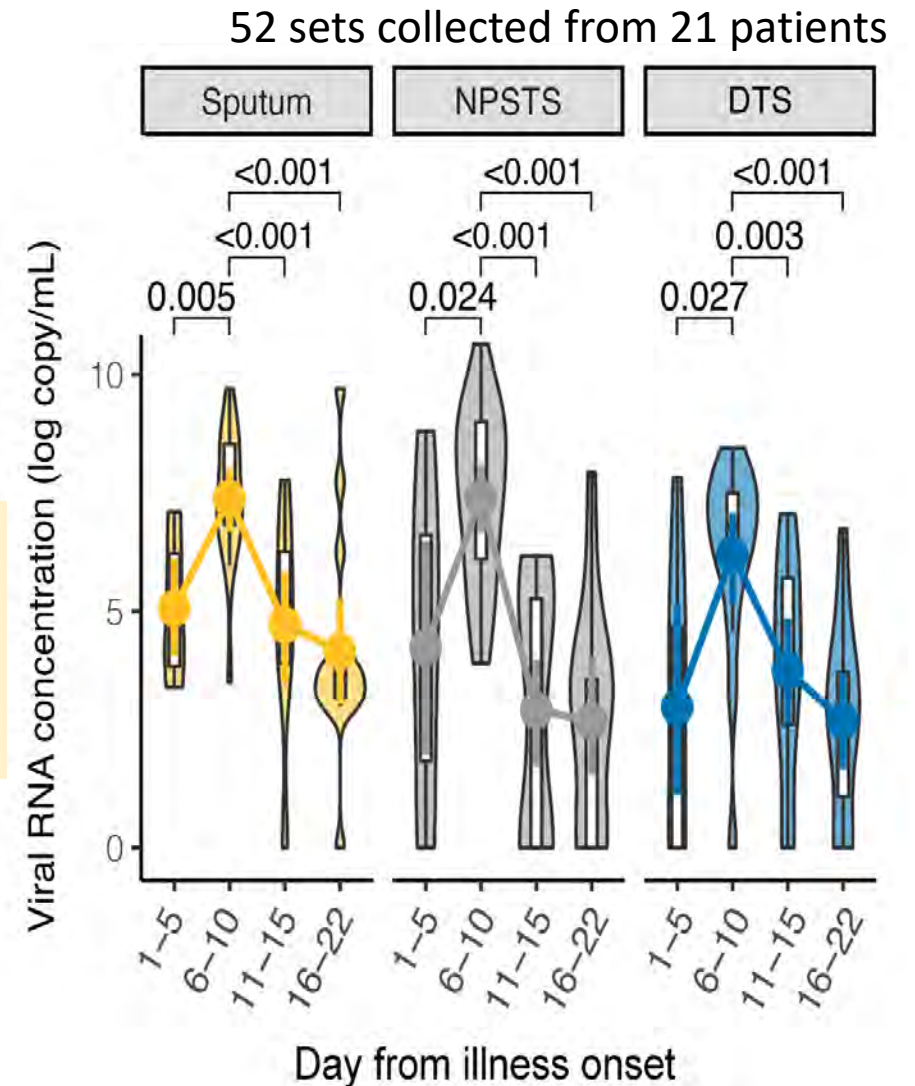
Dx yield ↓ asym / mild cases



Deep throat saliva (DTS)

Head-to-head comparison
Synchronized specimens
(collected on the same day)

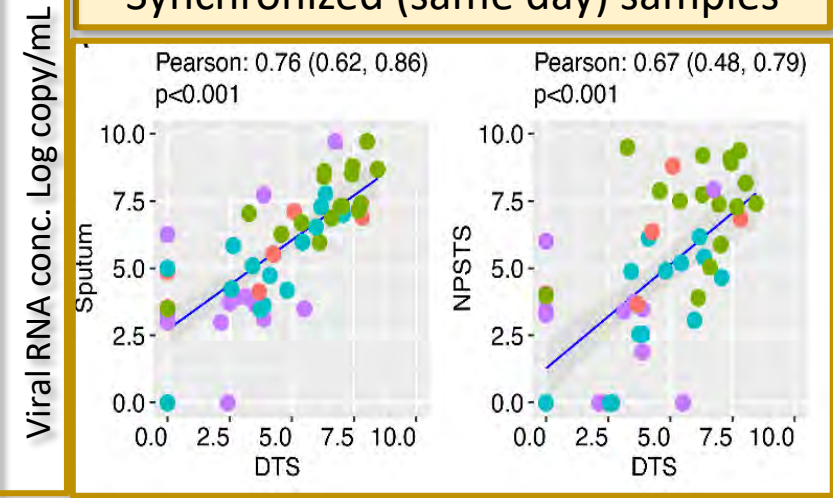
- ❑ Significantly **higher** virus conc. at **Day 6-10** for all specimen types
- ❑ **DTS** yields the **lowest** virus conc. for nearly **all** time frames



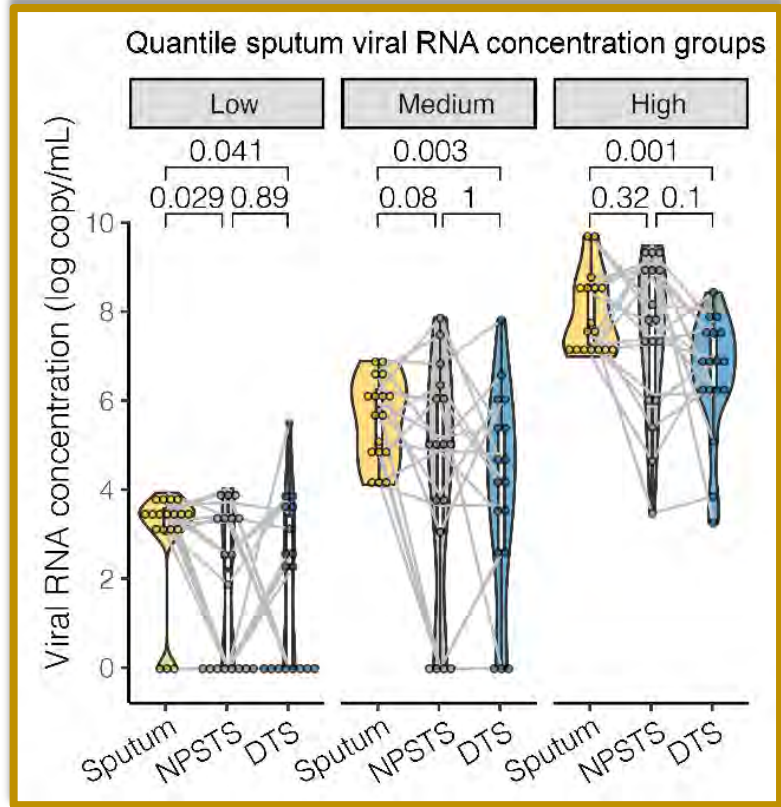
Deep throat saliva (DTS)

Correlation ∞ sputum

Synchronized (same day) samples



Days from illness onset: 1-5 (red), 6-10 (green), 11-15 (cyan), 16-22 (purple)



DTS False-Negative Rate

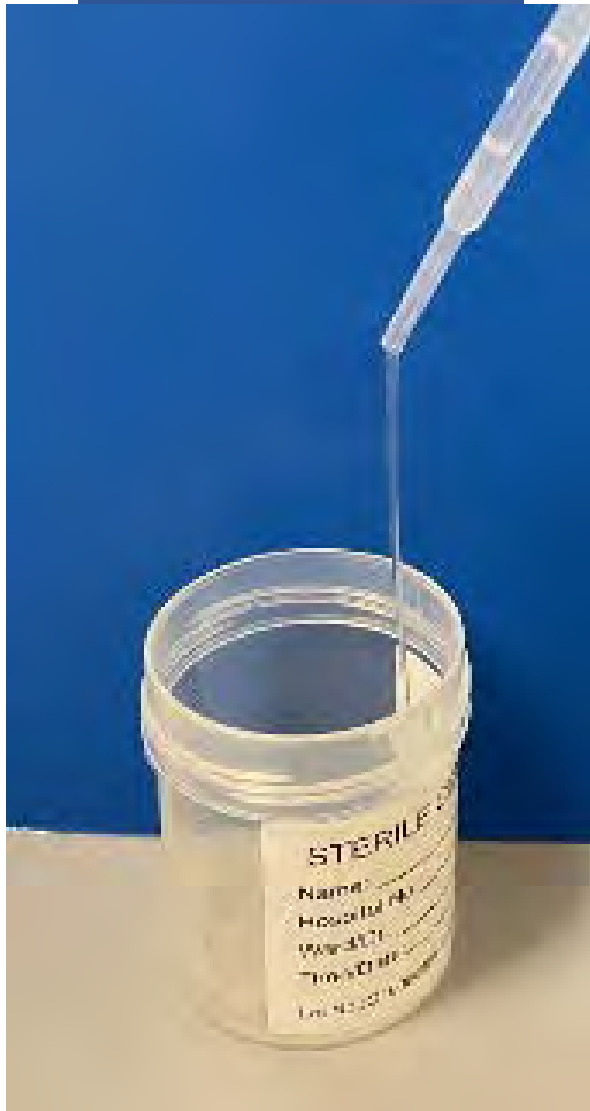
48 DTS collected 1st week

- ❑ Sputum producers: **8.3%**
- ❑ Non-sputum producers: **22.2%**

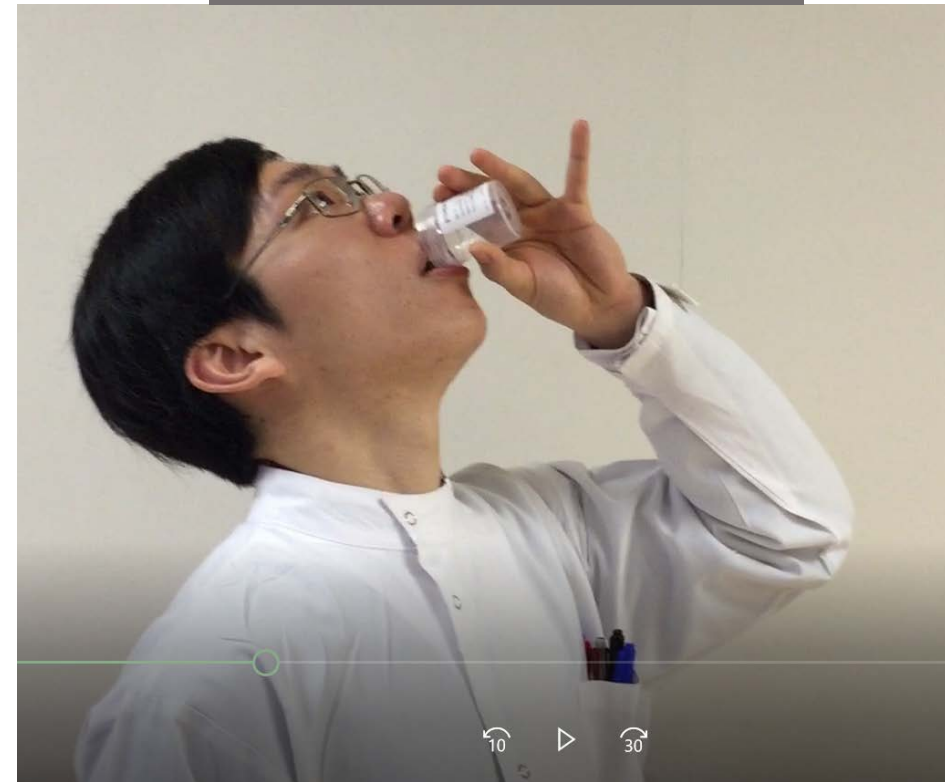
DST False-Negative rate:

- **2.6 x ↑** in patients without sputum
- 70% patients not produce sputum in 1st week

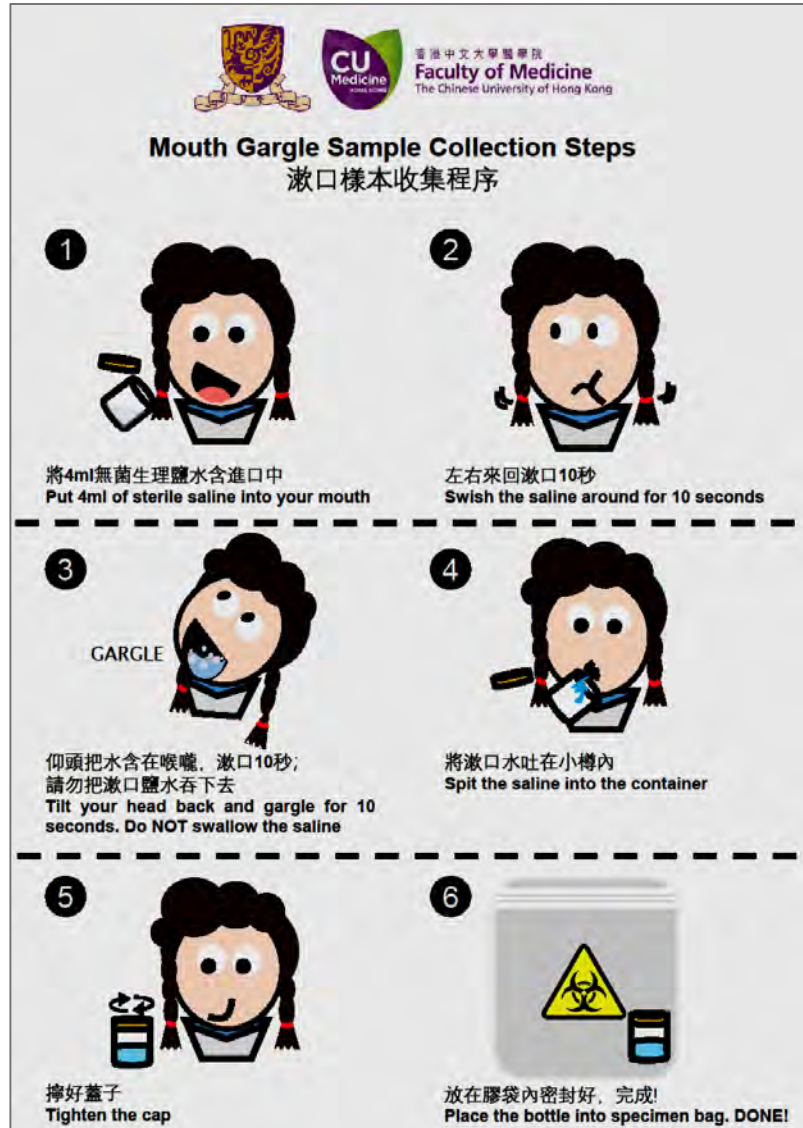
Deep throat saliva



Mouth gargle with saline



Self-collect specimen - mouth gargle with saline

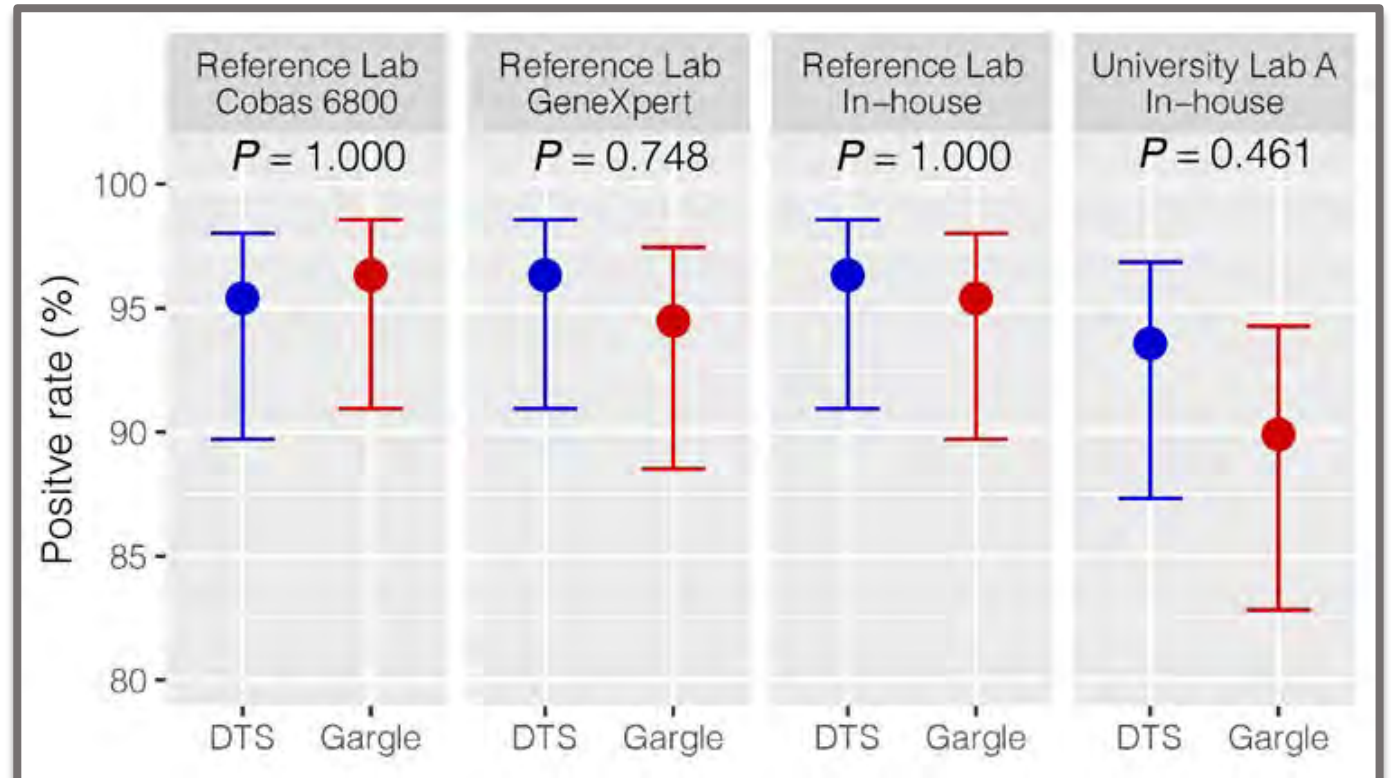


Gargle with saline vs. deep throat saliva (DTS)

PWH : 49 COVID patients

2 paedi (12 & 17 yr)

109 synchronized samples



Self-collect specimen – nasal epithelial lining fluid (NELF)



Self-collect specimen – nasal epithelial lining fluid (NELF)

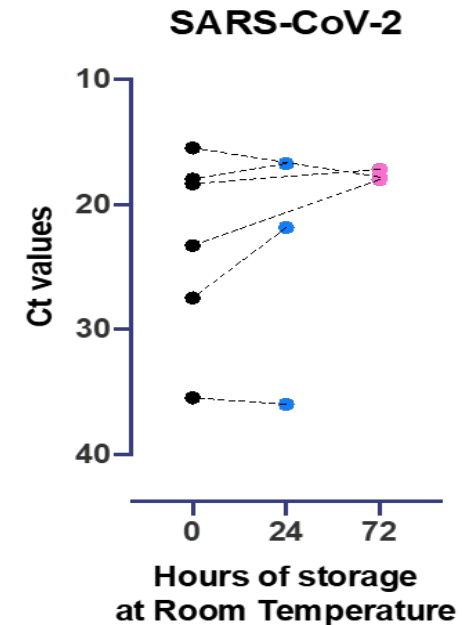
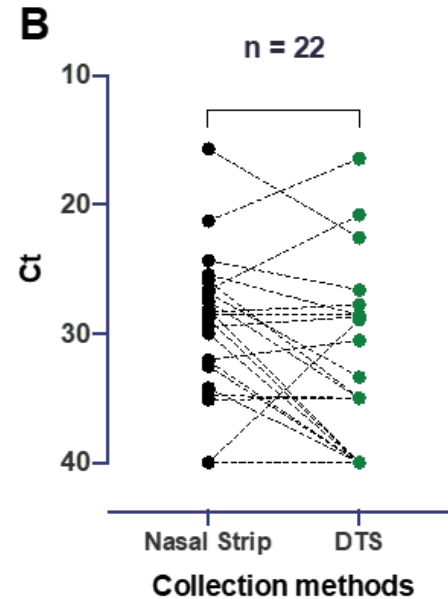
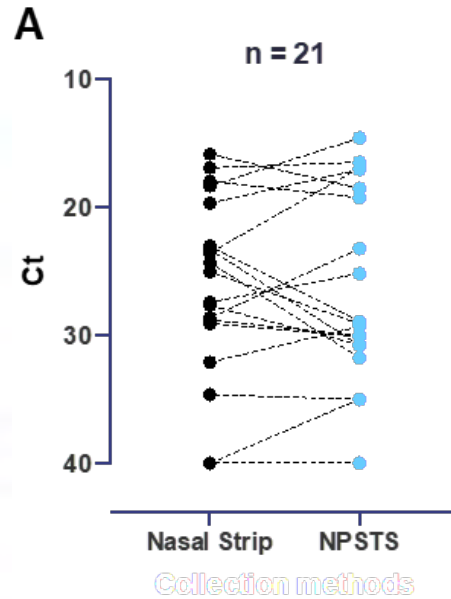
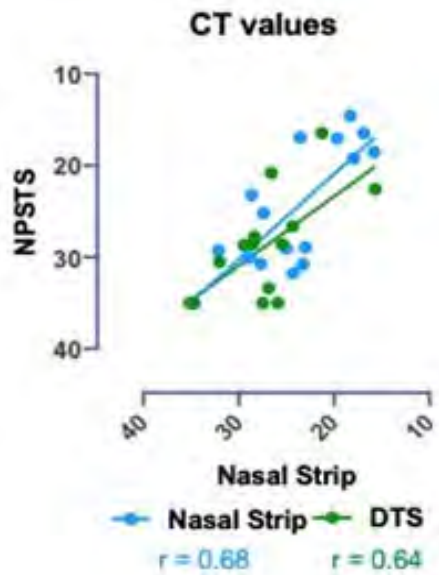
Sensitivity

94% c.f. Nasopharyngeal + throat swabs (NPSTS)
1.4x higher than saliva (DTS)

Stability

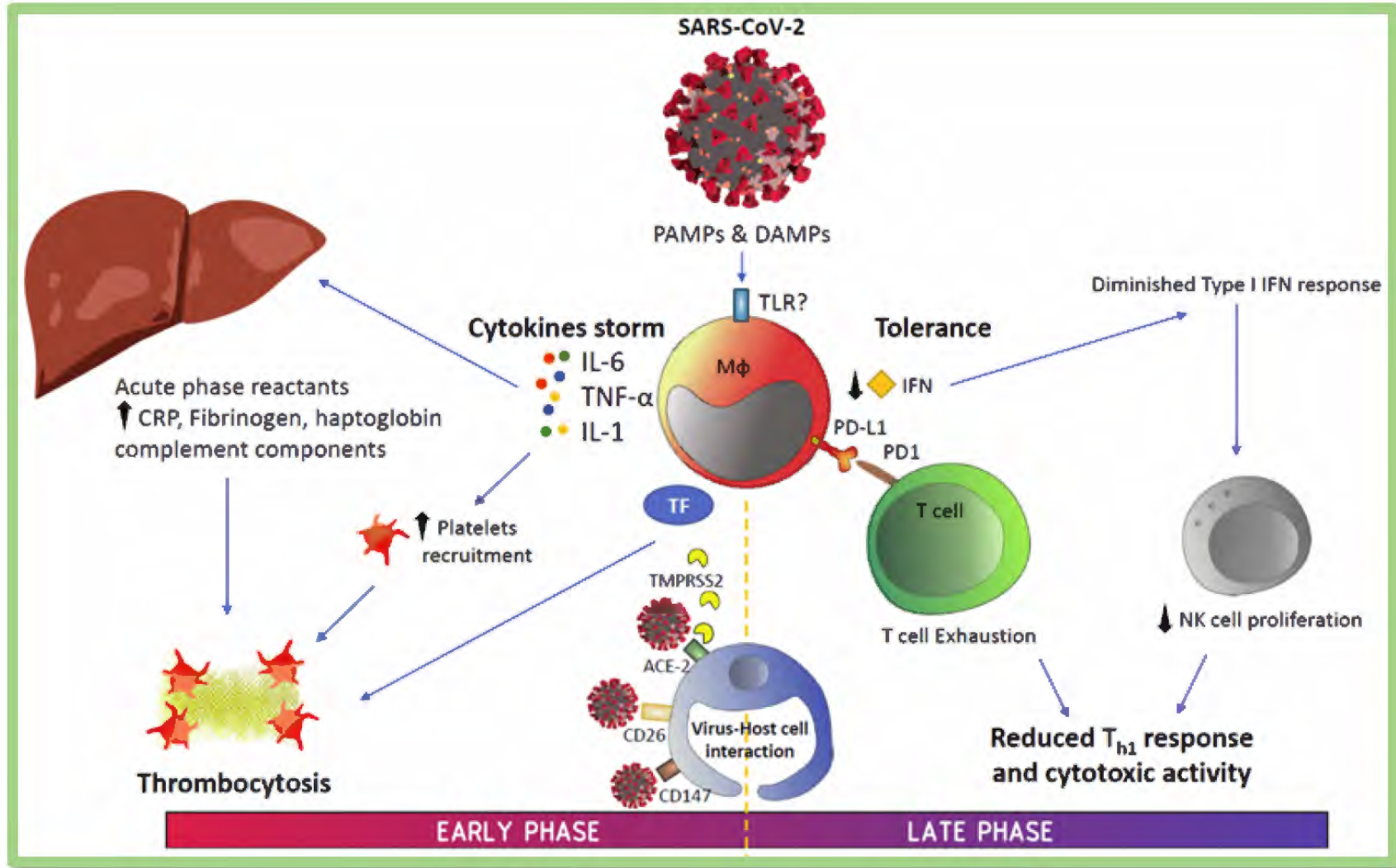
Over 3 days at room temp.

Pooled nasopharyngeal & throat swabs



NELF
A good option of self-collect specimen for older children

Early immune markers in COVID-19



Cytokine markers in COVID-19

40 patients (24-72 yr, 53% male, 18% smokers)

- 8 mild (no pneumonia)
- 15 moderate (pneumonia)
- 17 severe/critical (O₂ / ventilation)

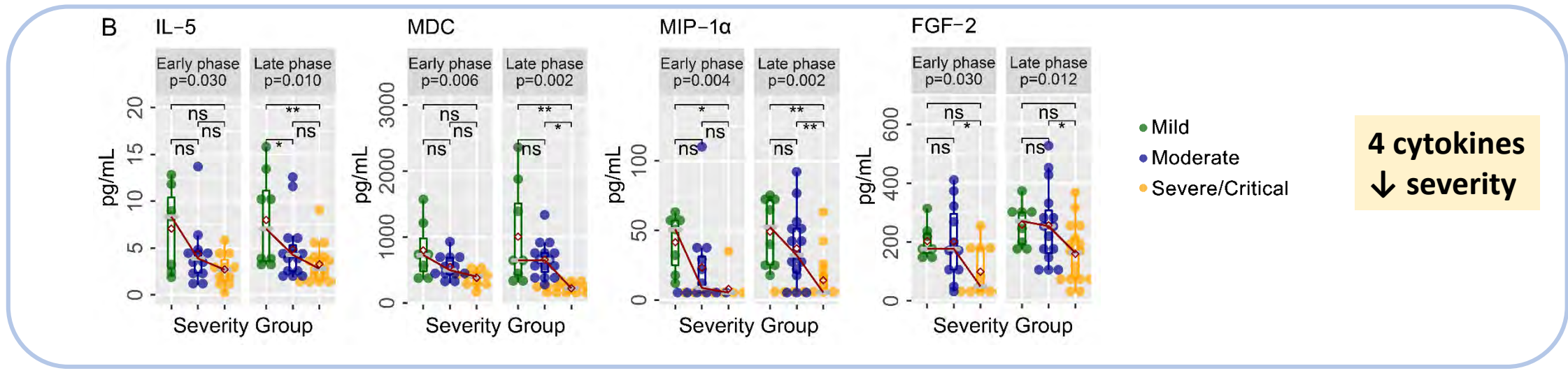
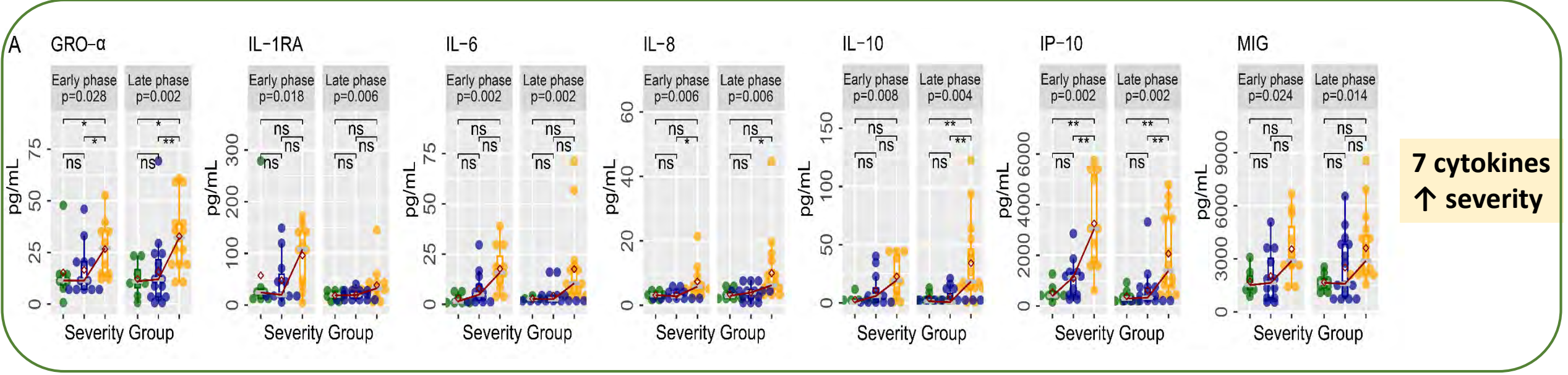
Early phase
 <7 days from illness onset

Late (critical) phase
 8-12 days from illness onset

40 cytokines

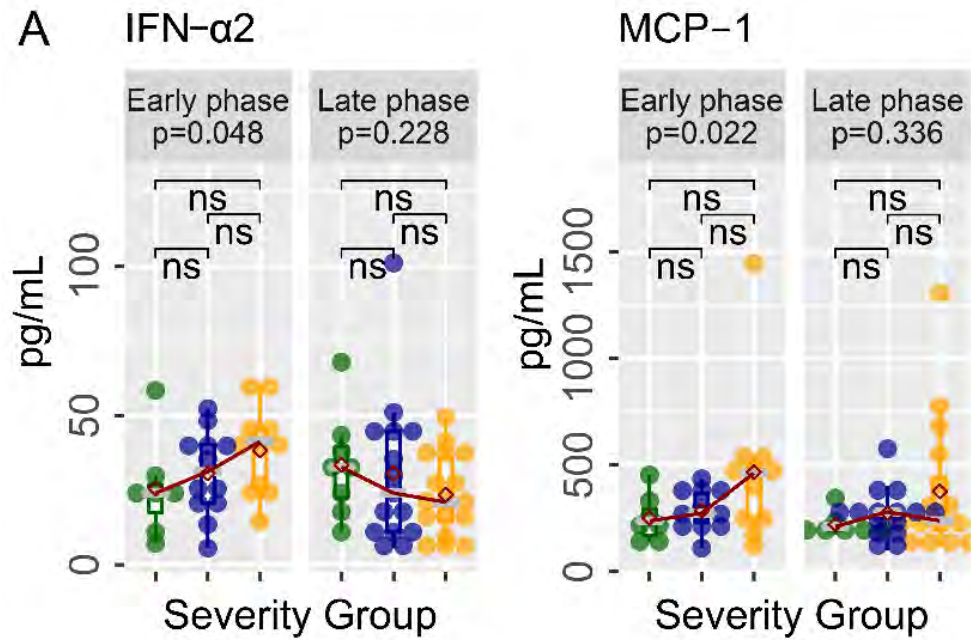
sCD40L, EGF, Eotaxin/CCL11, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO- α , IFN- α 2, IFN- γ , IL-1 α , IL-1 β , IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IL-18, IP-10, MCP-1, MCP-3, MDC (CCL22), MIG/CXCL9, MIP-1 α , MIP-1 β , TGF- α , TNF- α , TNF- β and VEGF.

11 cytokines consistently different in **both** early- and late-phase

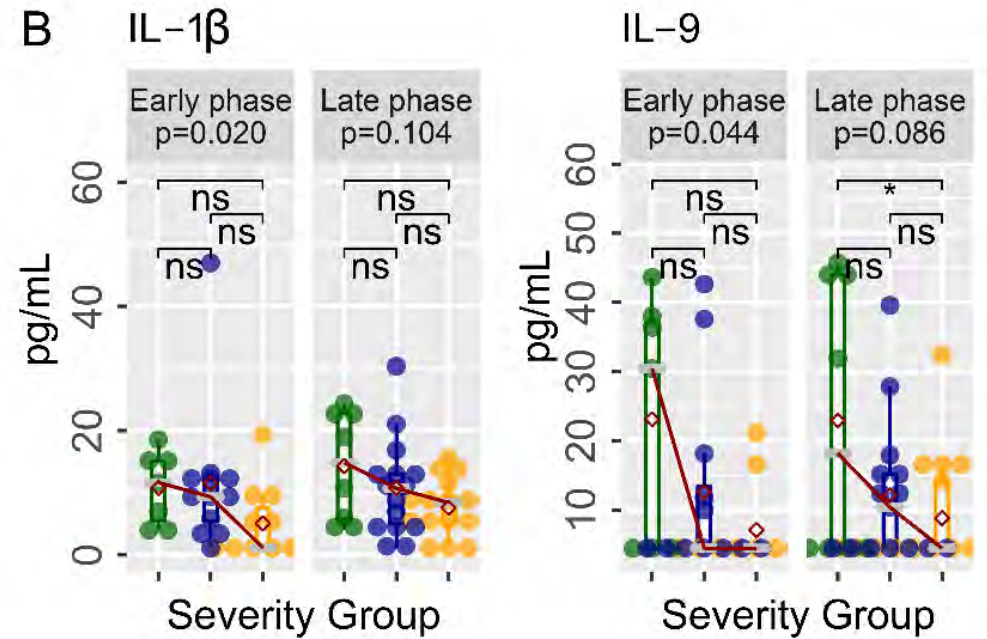


4 cytokines associate with severity **only** in early phase, **not** late-phase

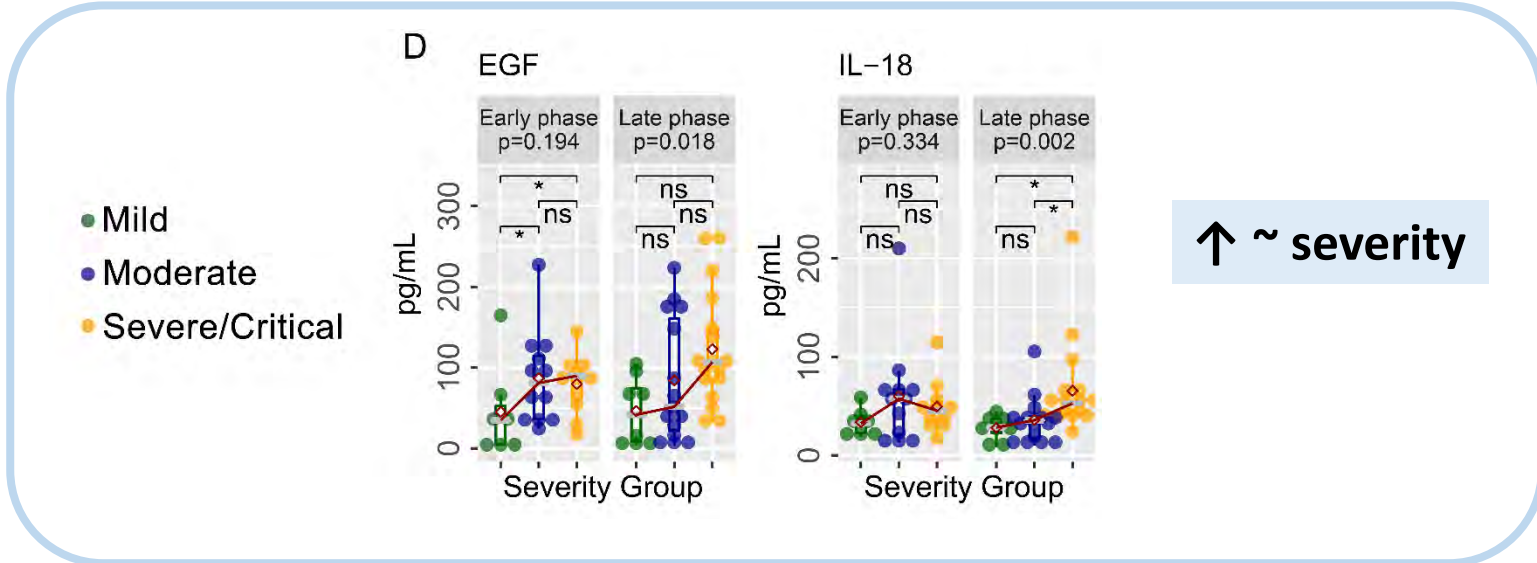
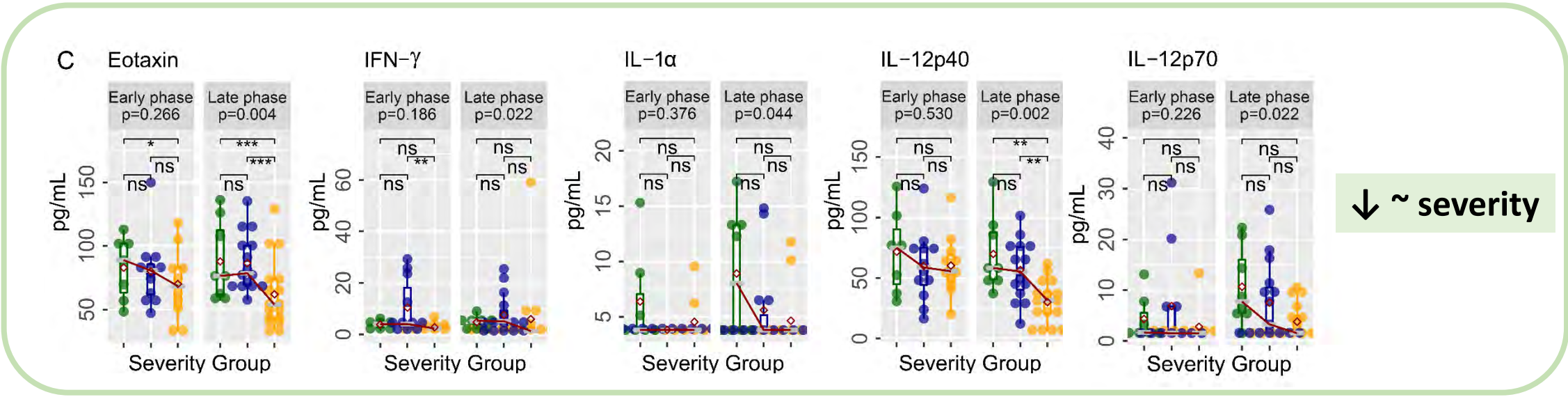
↑ ~ severity



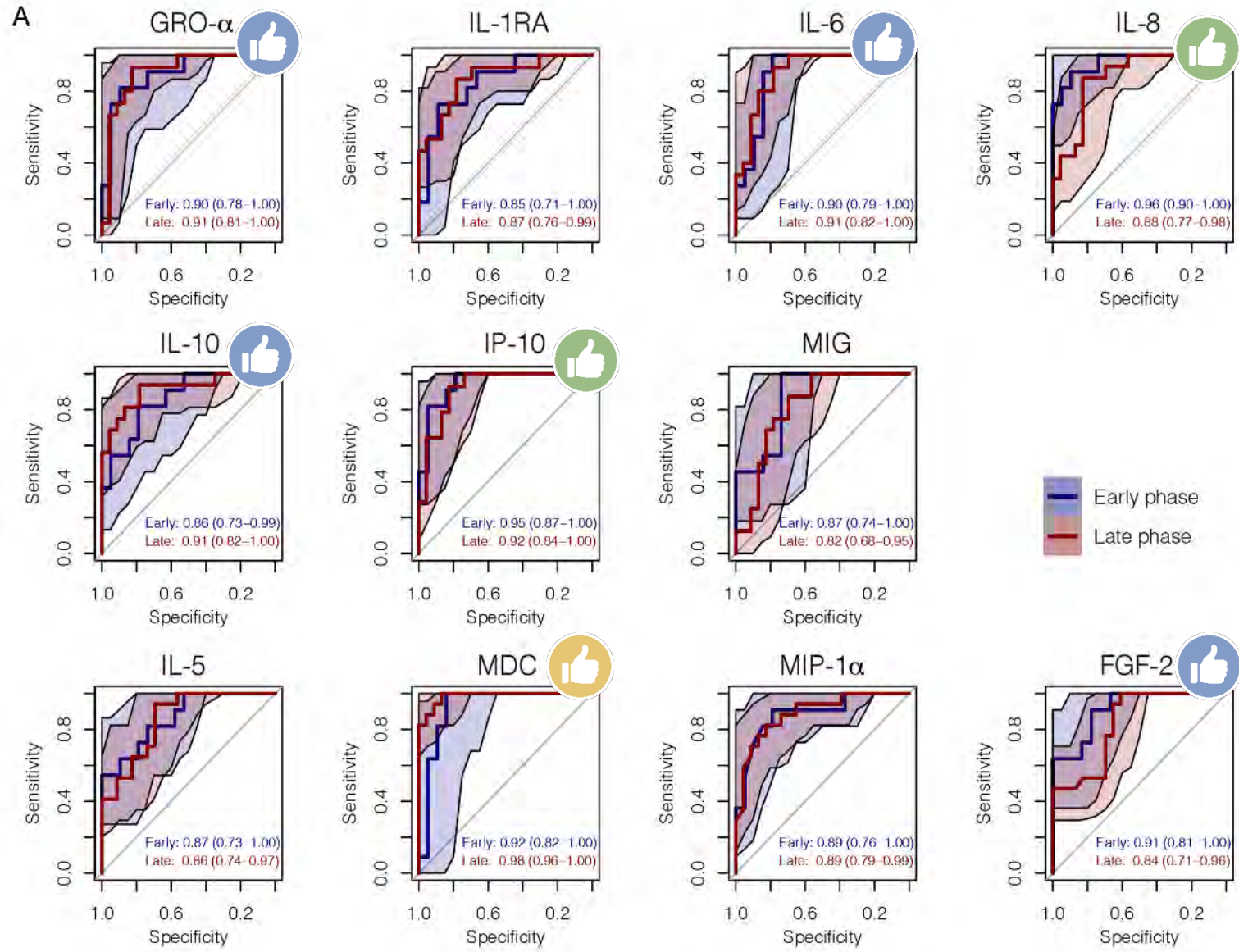
↓ ~ severity



7 cytokines associate with severity **only** in late-phase, **not** early-phase



Predict severe/critical infections



Early biomarker < 7 days

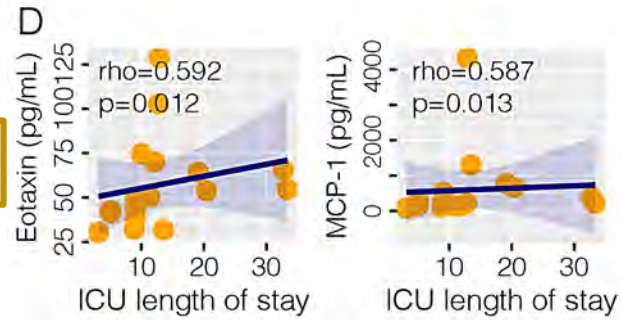
Late biomarker 8-12 days

Potential biomarker

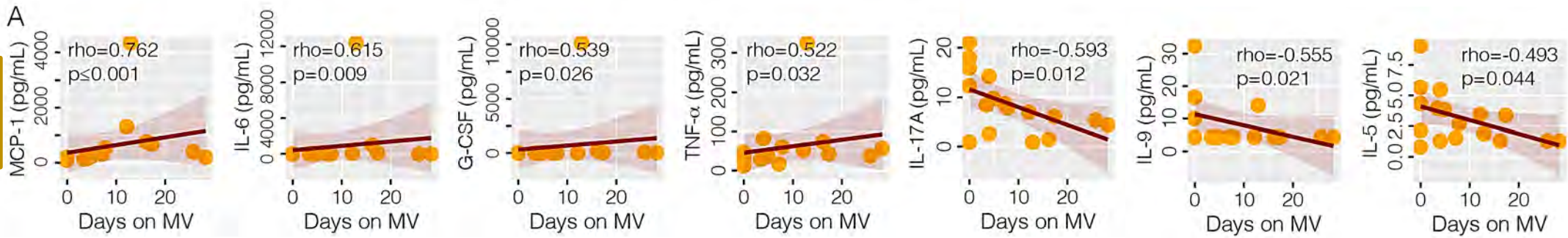
Age-adjusted

Critical patients – Late phase (cytokine at ICU admission)

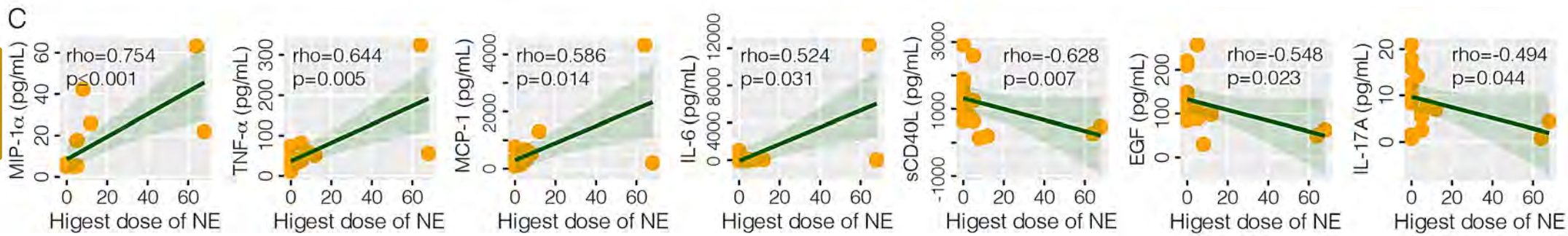
ICU stay



Ventilation duration



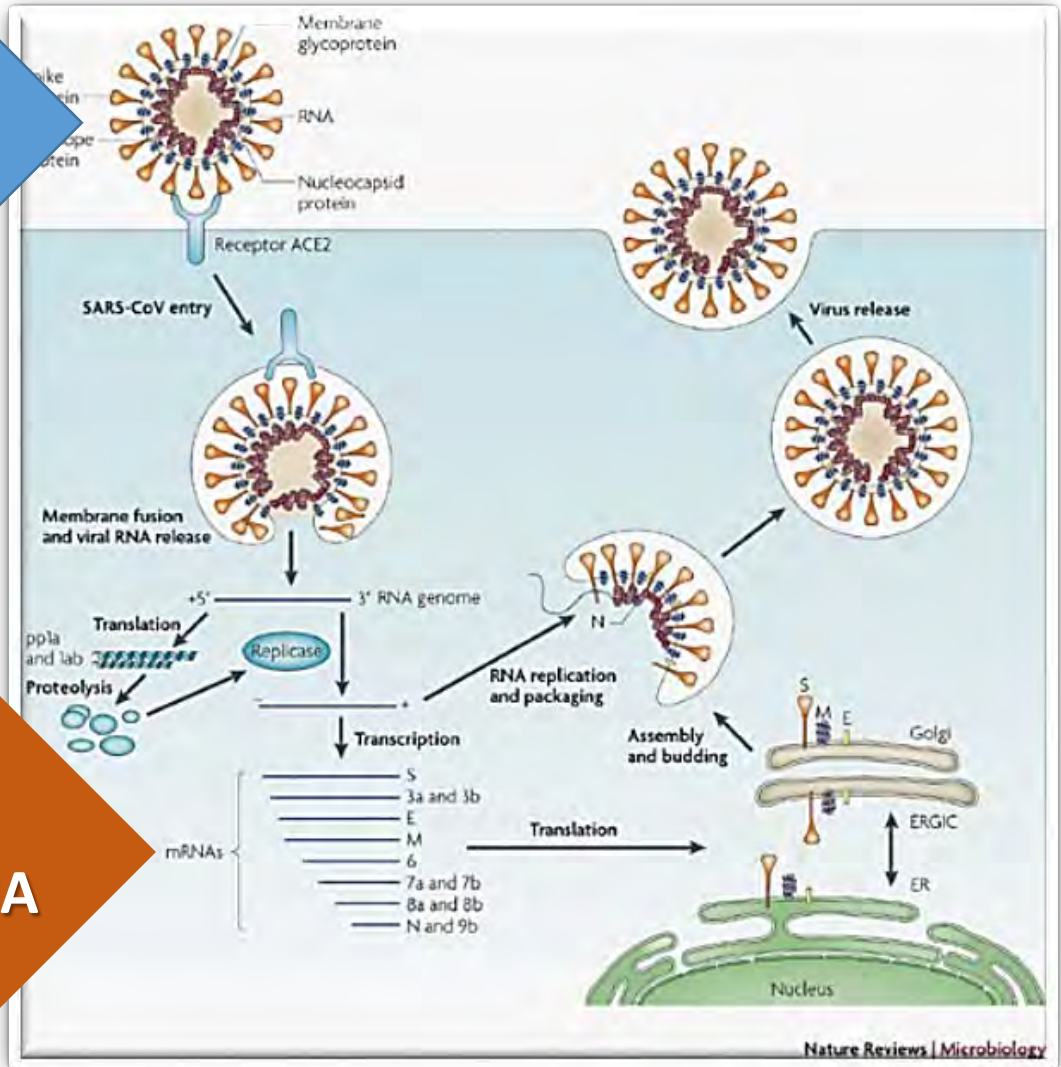
Highest dose Norepinephrine



Subgenomic RNA

Diagnostic PCR ⇒ Genomic RNA

Monitoring PCR ⇒ ???
Sub-genomic RNA



Subgenomic RNA profile

376 resp. samples from individual COVID-19 patients
 Ct values: 12.2 – 32.5
 Collection: median 6 (0-31) days from onset
 Asymptomatic : 7%
 Mild: 35%
 Moderate (pneumonia) : 37%
 Critical (O₂ /ventilation): 21%

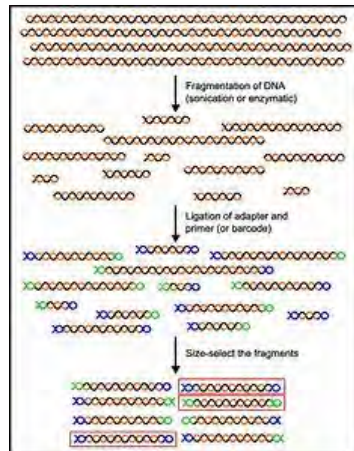
124 serial samples (2-47 days from onset)

- 45 upper resp.
- 37 lower resp.
- 42 stool

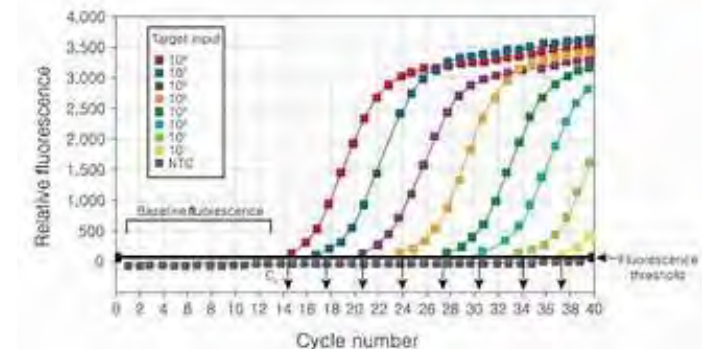
10 patients

- 4 mild
- 5 moderate
- 1 critical

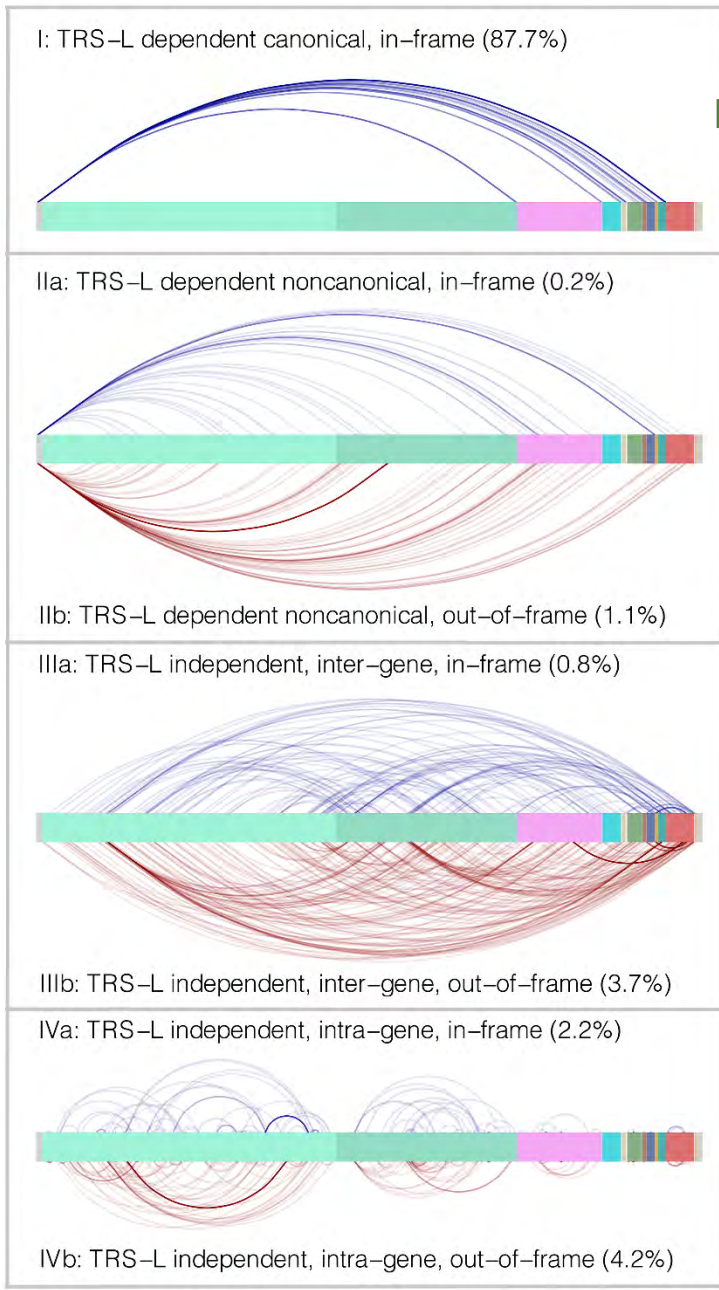
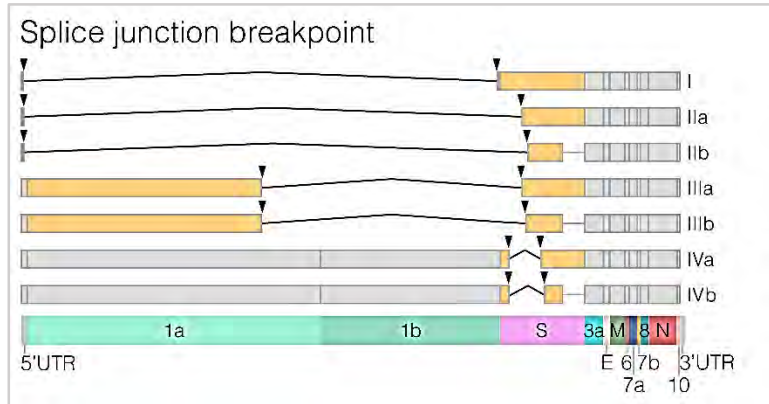
Catch all – Next Generation Sequencing



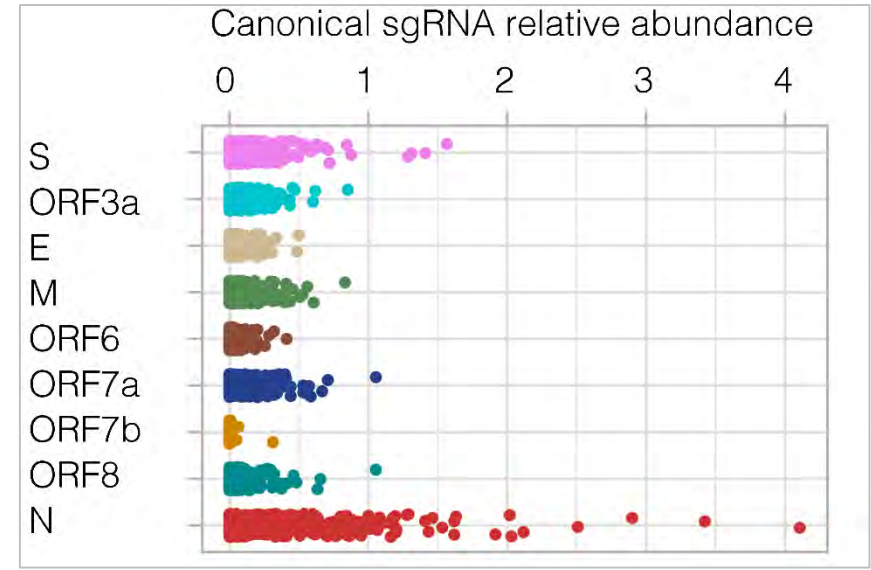
Real-time PCR targeting specific subgenomic RNA

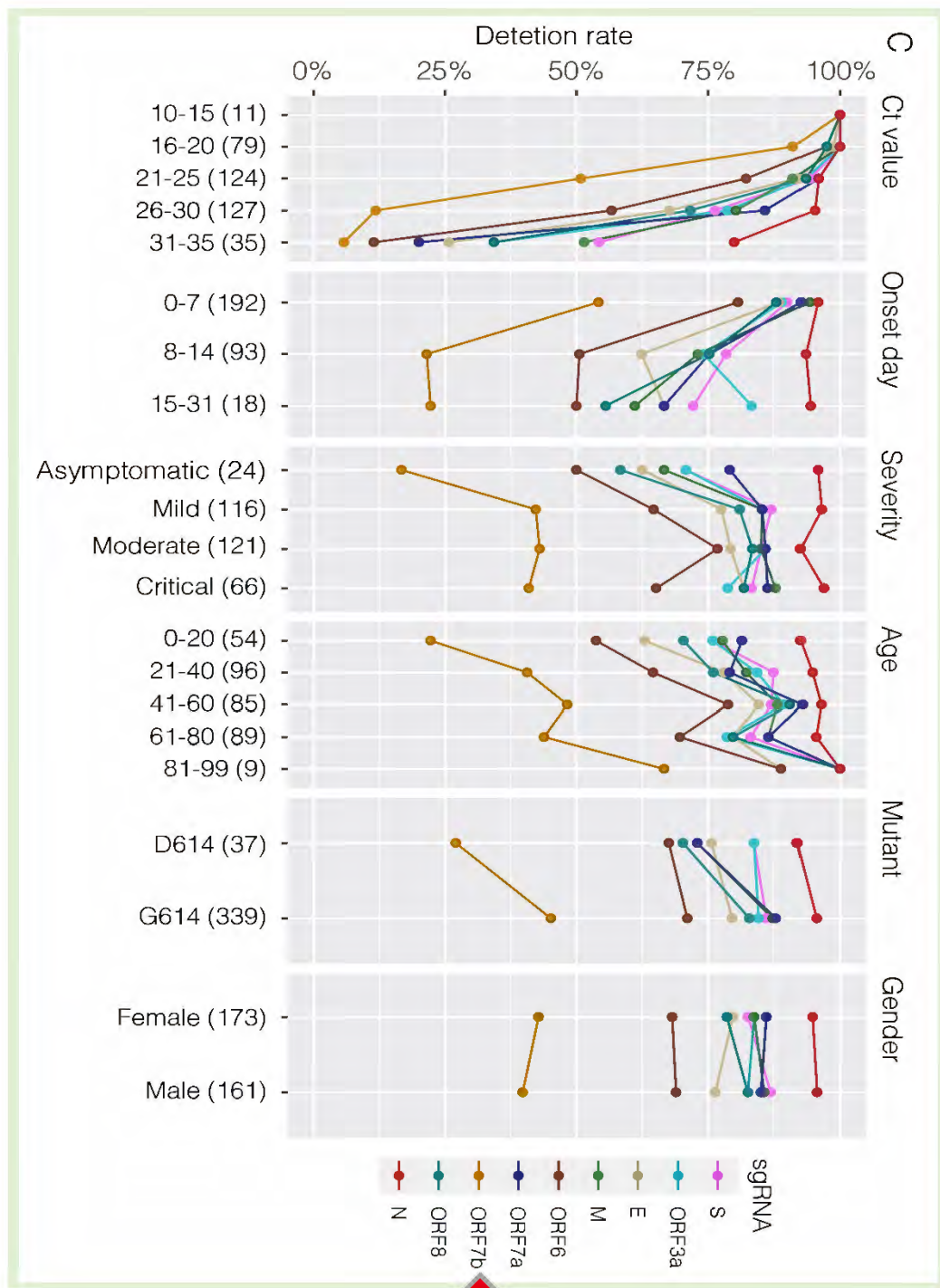
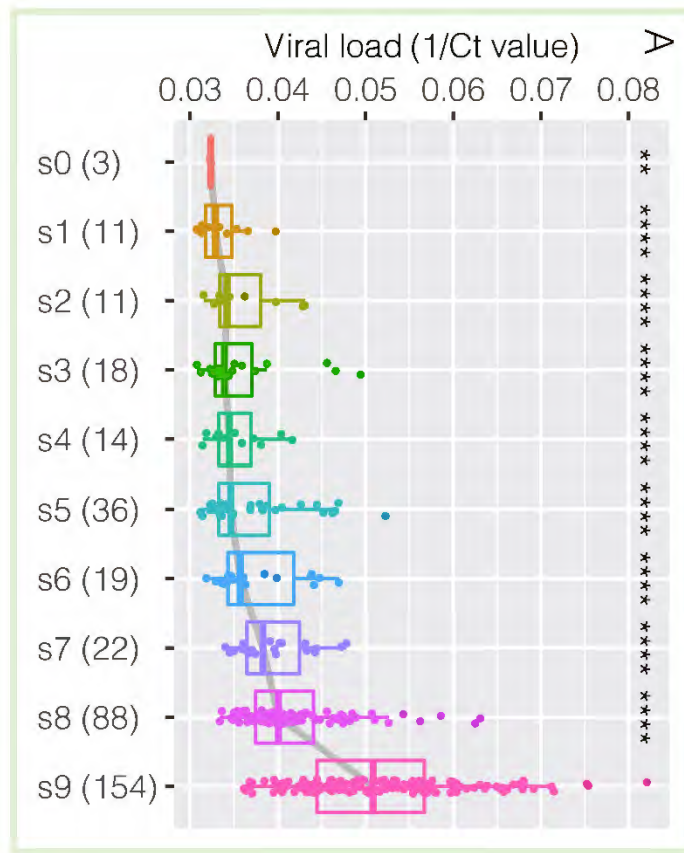
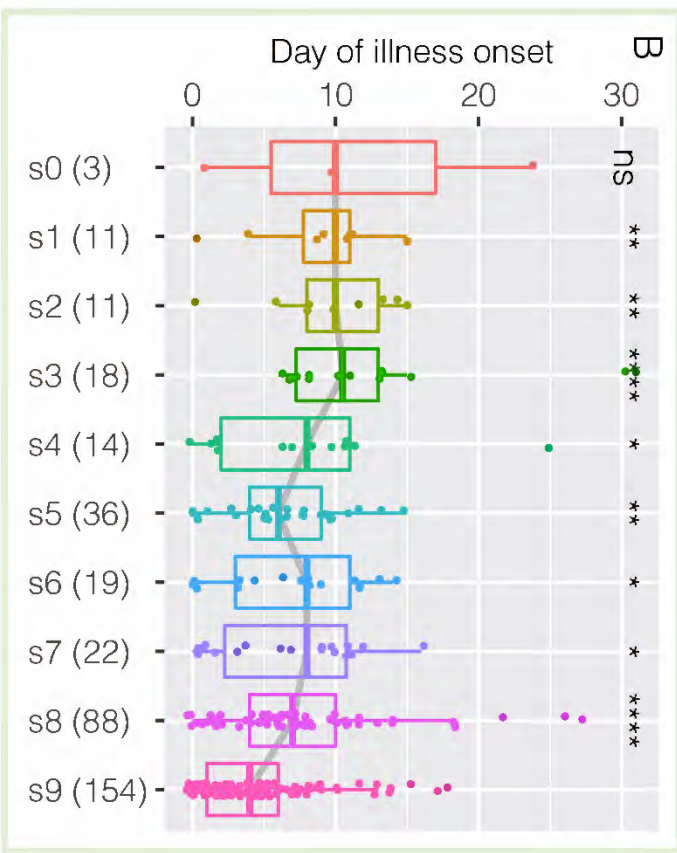


Subgenomic RNA profile

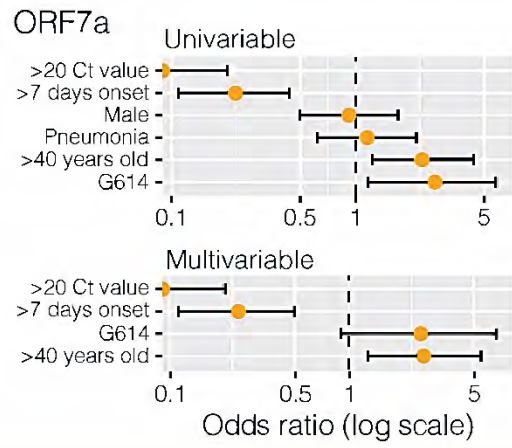
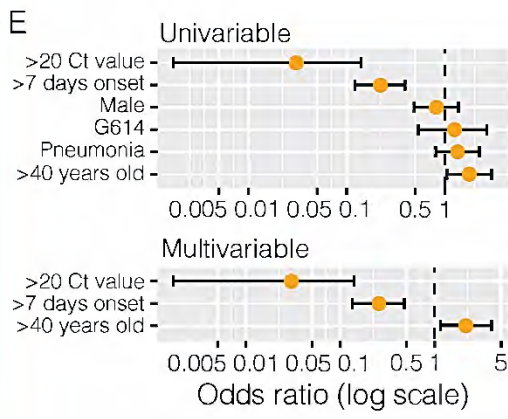
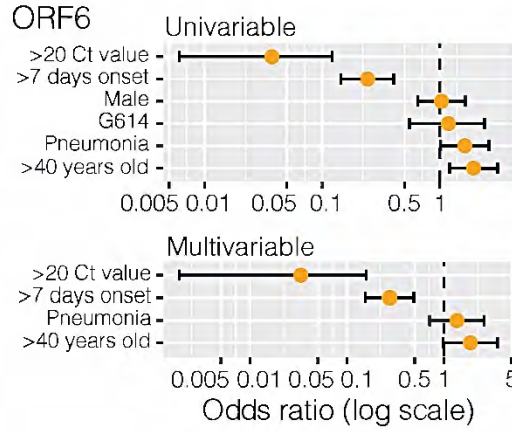
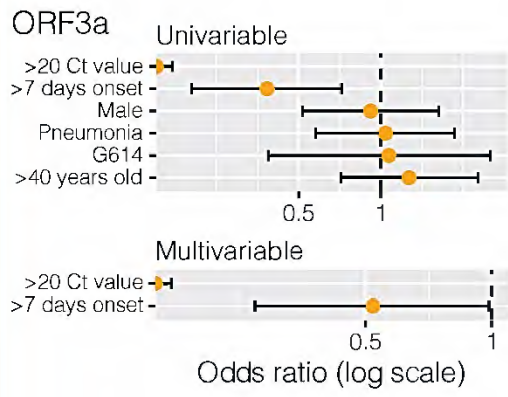
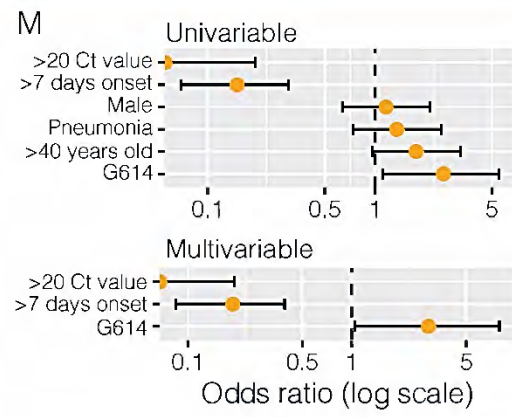
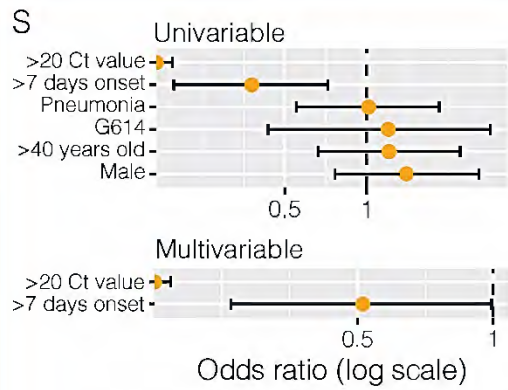


88%

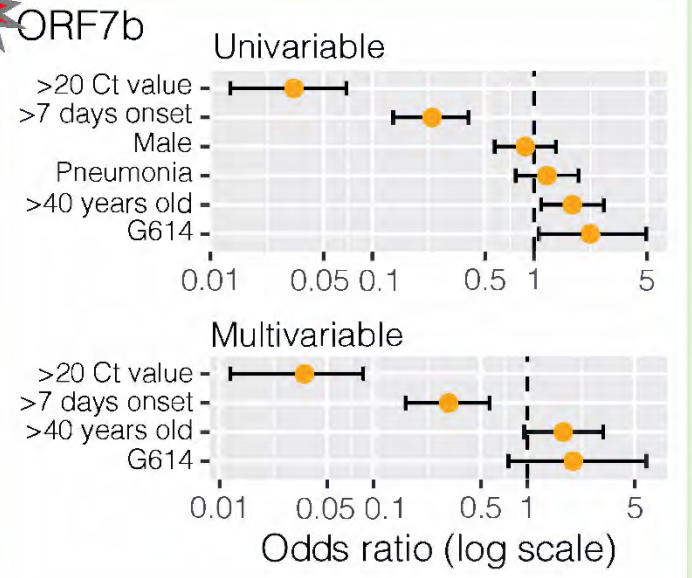
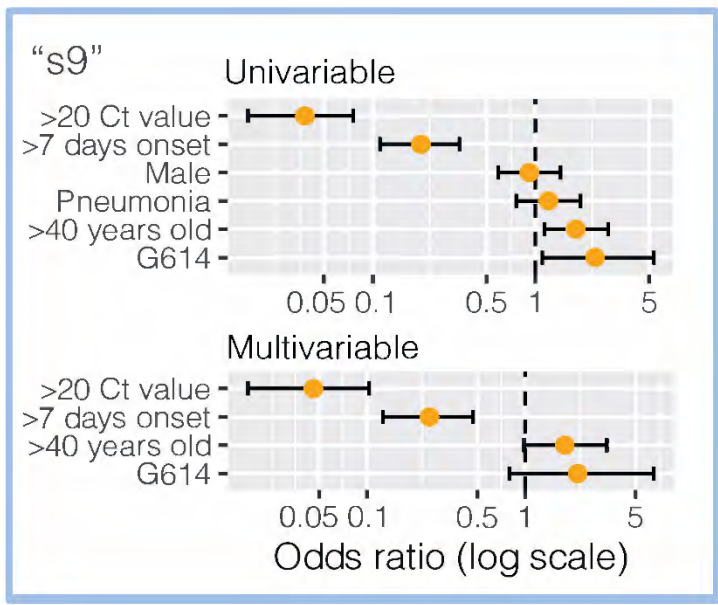




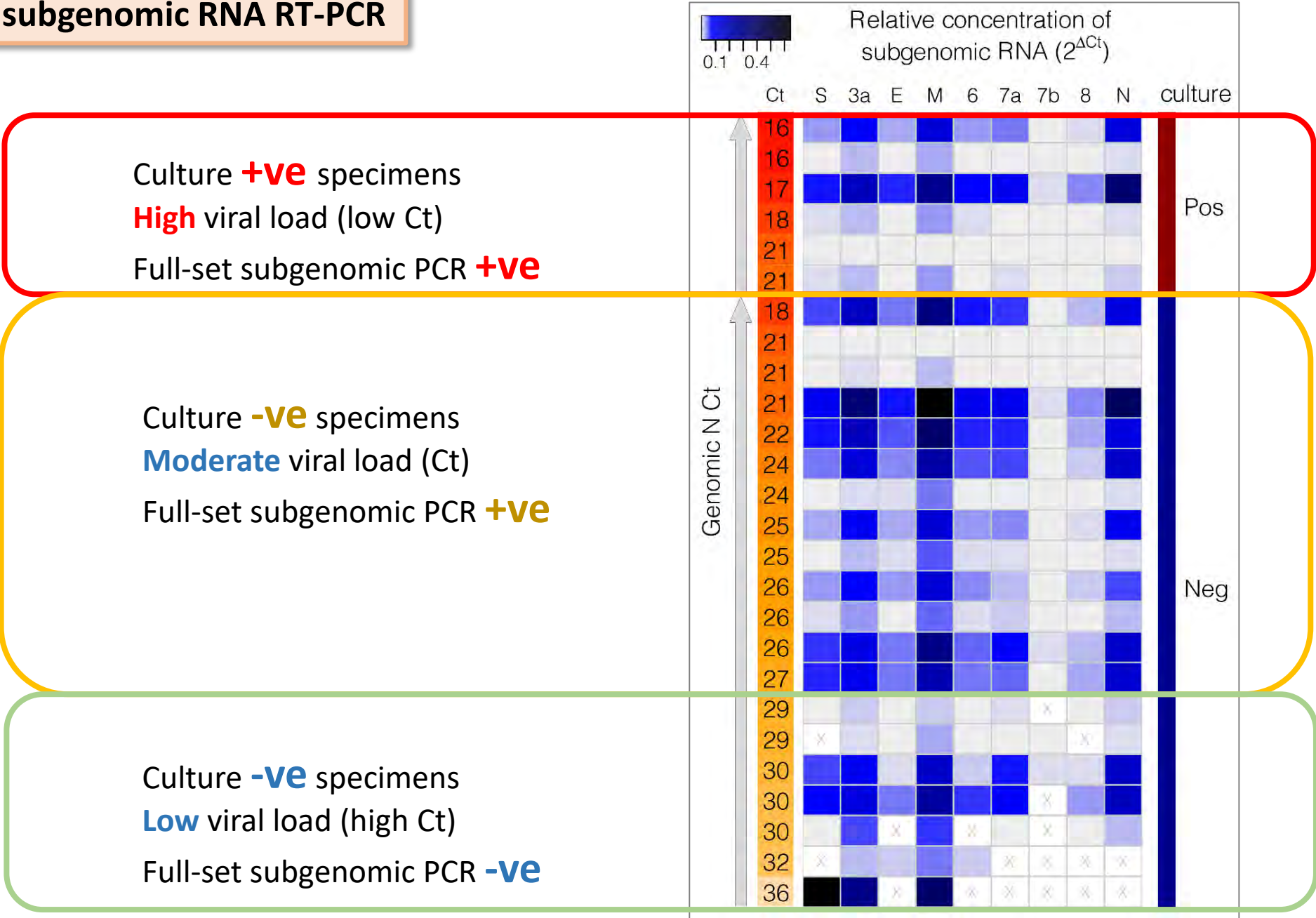
9 subgenomic RNAs vs. patient characteristics



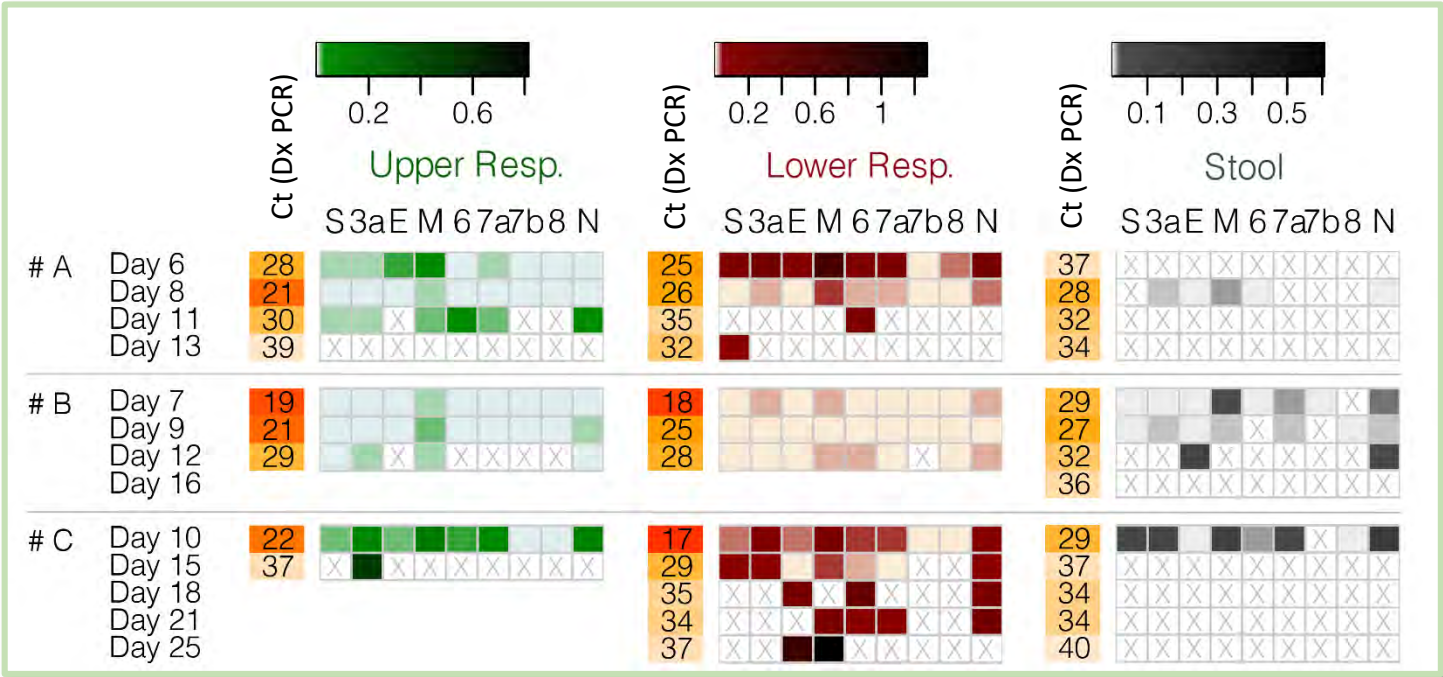
Full set of 9 subgenomic RNAs



Virus isolation vs. subgenomic RNA RT-PCR



Serial specimens tested POSITIVE by diagnostic (genomic) PCR

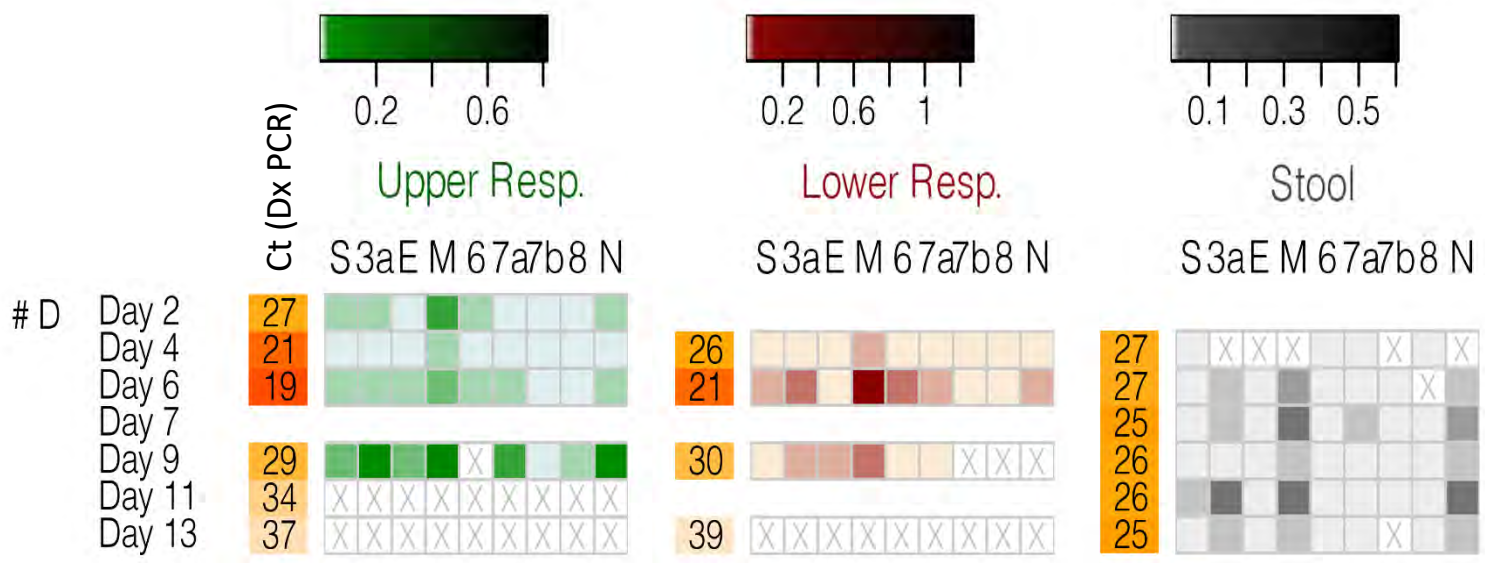


Upper & lower resp. & stool diagnostic PCR +ve for 2-3 weeks

Full-set sgRNA +ve **only** upto 10 day from onset

None of stool samples had full-set sgRNA

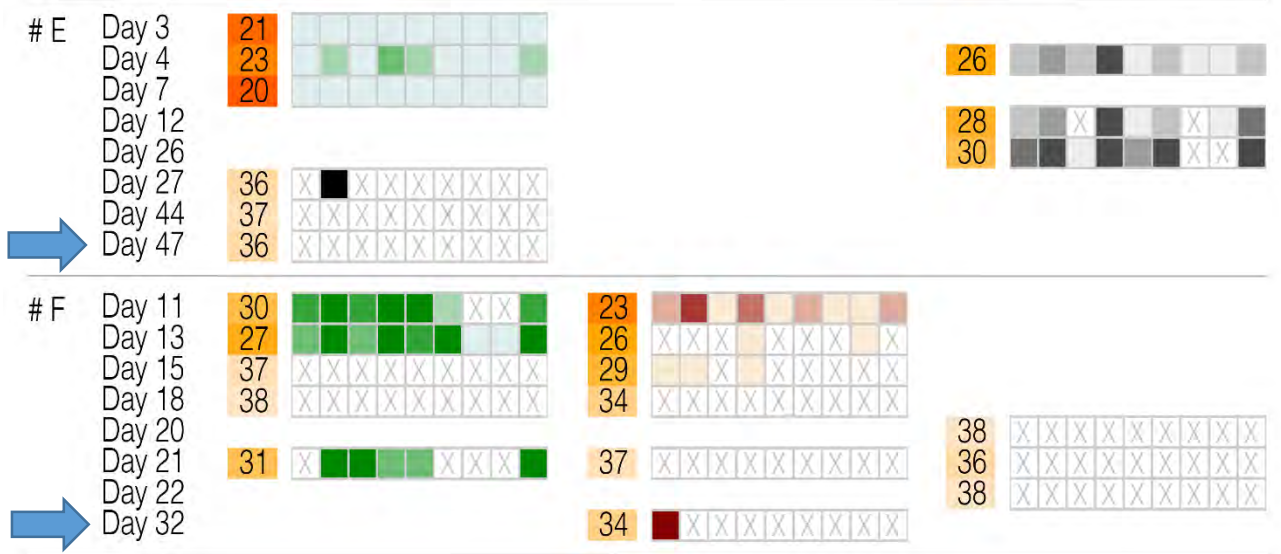
Serial specimens tested POSITIVE by diagnostic (genomic) PCR



Full-set subgenomic RNA PCR +ve

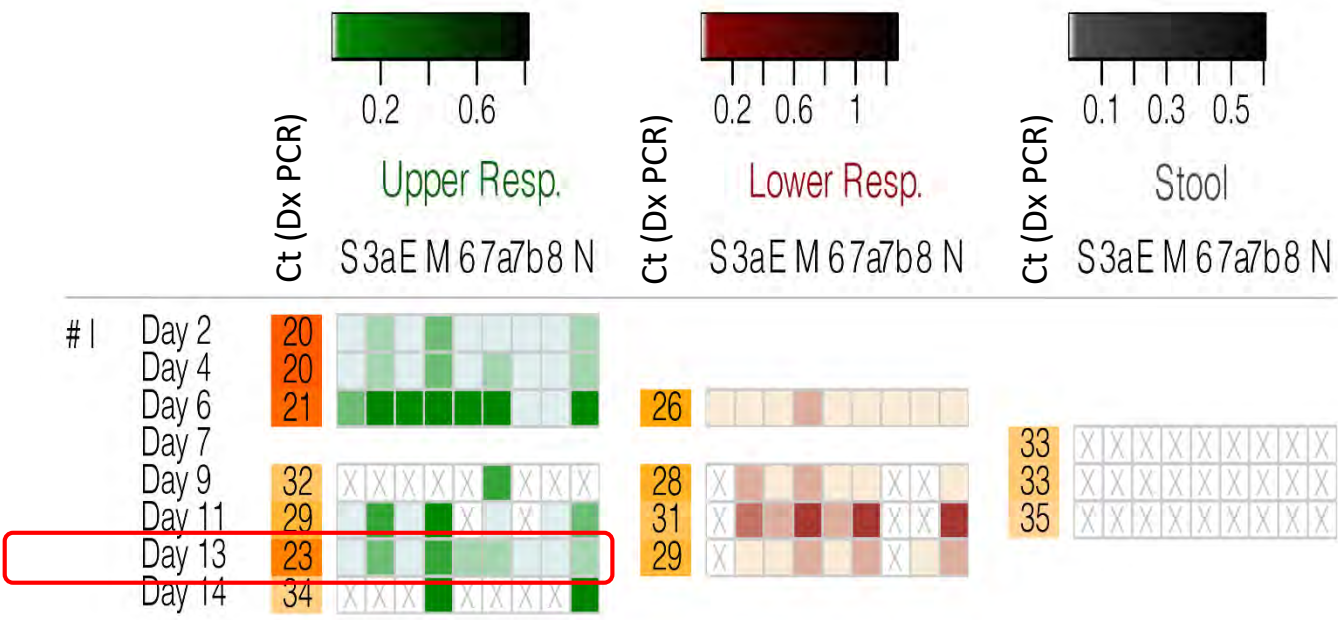
- Upper resp.
- Lower resp.
- Stool

Serial specimens tested POSITIVE by diagnostic (genomic) PCR



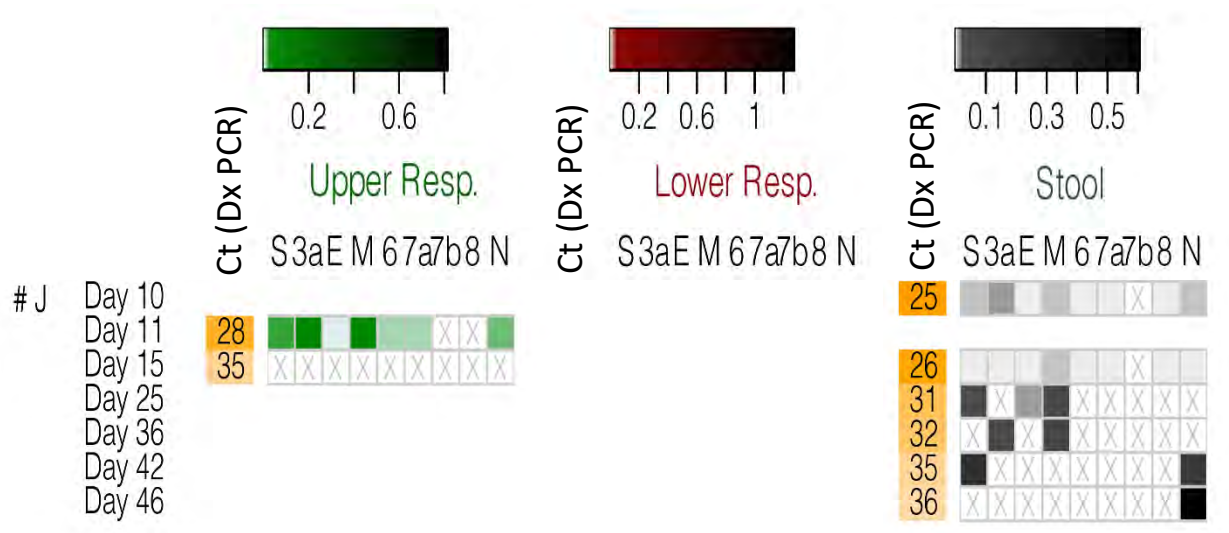
Prolonged diagnostic (genomic) PCR +ve
Full-set subgenomic RNA PCR only +ve in early samples

Serial specimens tested POSITIVE by diagnostic (genomic) PCR



Full-set subgenomic RNA PCR
 Became +ve **again**

Serial specimens tested POSITIVE by diagnostic (genomic) PCR



Prolonged diagnostic PCR +ve in stool
 Subgenomic RNA PCR **all -ve**

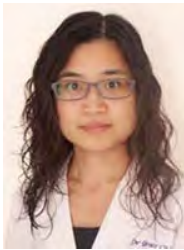
Acknowledgments



Food and Health Bureau
The Government of the Hong Kong Special Administrative Region



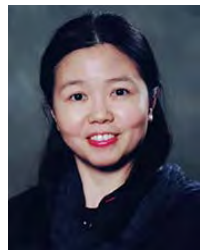
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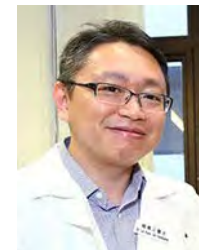
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Prince of Wales Hospital



United Christian Hospital



香港中文大學
The Chinese University of Hong Kong



香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Thank you

