Detection of SARS CoV 2 in respiratory specimens

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Methods of detection

- Nucleic acid amplification
  - Polymerase chain reaction (PCR) is the most commonly used
  - Gold standard for sensitivity and specificity
- Antigen detection
  - 80% sensitivity and 95% specificity compared with PCR
- Viral culture
  - Not used for diagnosis*
  - Much less sensitive than PCR
  - May indicate infectiousness

Biological factors that affect the diagnostic characteristics of the test

- Likelihood of detecting the virus when present in the specimen
  - Amount of virus
  - Type of specimen
  - Quality of specimen
  - Time after infection
  - Severity of infection
- Likelihood of false positive results
  - Cross reactivity
  - Contamination
  - Nonspecific binding of the primer or antibody

Relationship of the test result with the amount of virus in respiratory specimens

- ALL the diagnostic tests currently approved by the FDA are QUALITATIVE
- Limit of detection = the minimum amount of target necessary to detect the presence of the virus in the specimen
  - PCR: 180 to 300,000 RNA copies/ml
  - Antigen detection: equivalent to $6.6*10^6$ RNA copies/ml (BEI strain)
  - Viral culture: equivalent to $2*10^5$ RNA copies (BEI strain)

Factors that affect infectivity of respiratory specimens

- Amount of virus
- Time after initiation of infection
  - Symptomatic individuals without immune defects: median duration of viral shedding = 4 days s after initiation of symptoms; max duration = 8-9 days
  - Epidemiologic studies indicate that transmission starts ~2 days before initiation of symptoms and peaks during the first 4 days after initiation of symptoms

Can PCR predict risk of transmission?

YES and NO

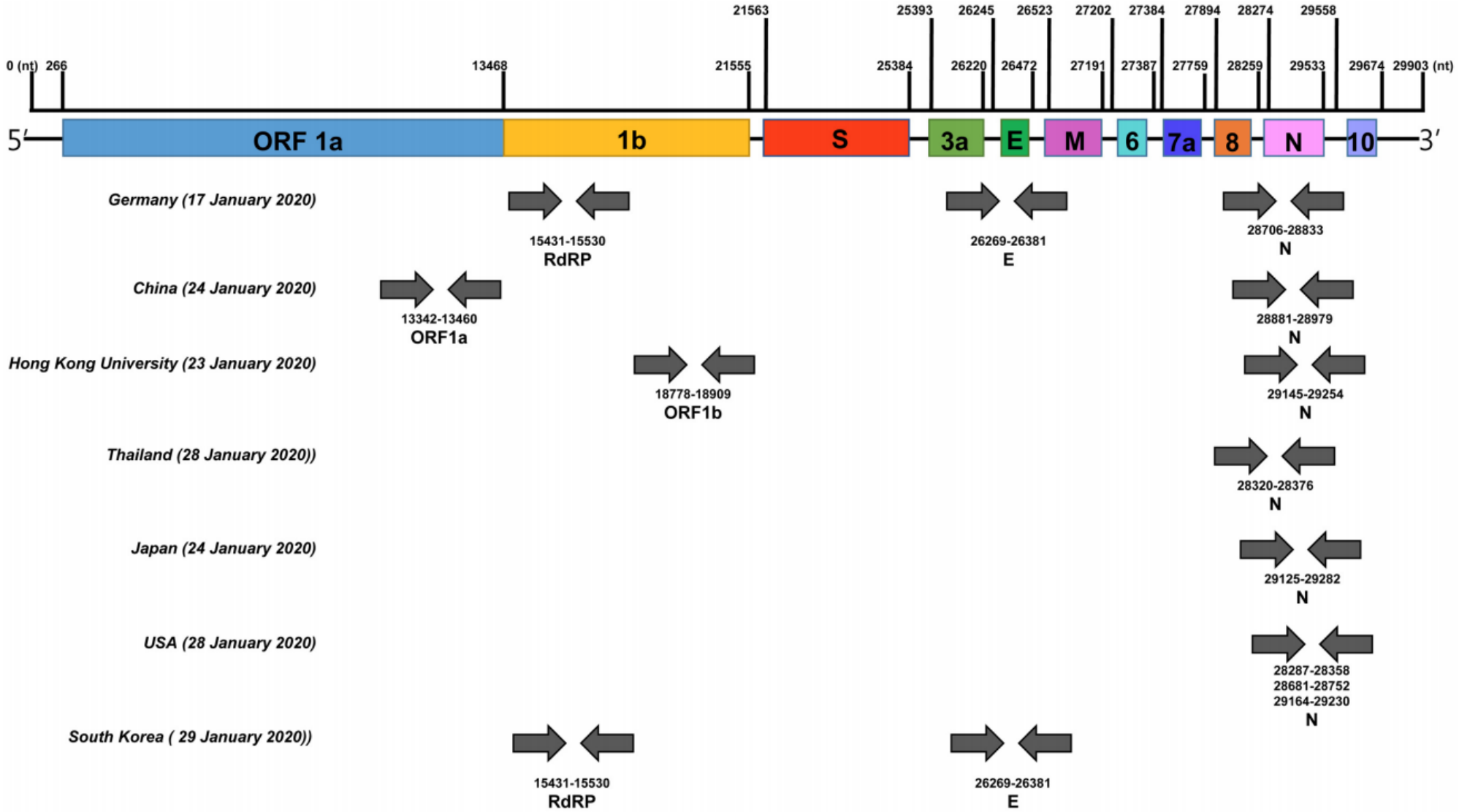
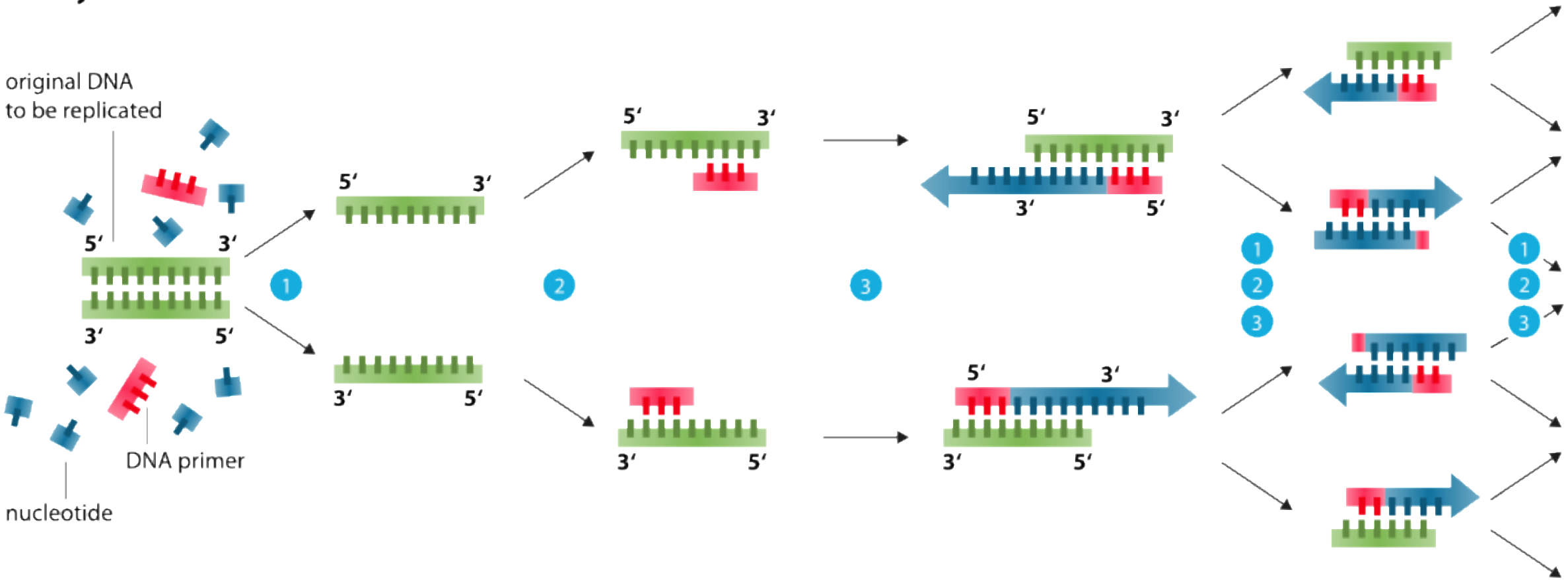


Fig. 5. A representative diagram showing currently available diagnostic primer sets on SARS-CoV-2 genome.
Numbers represent genome positions according to SARS-CoV-2 isolate Wuhan-Hu-1 (GenBank: MN908947.3). Each primer set for the diagnosis was indicated by grey arrows.

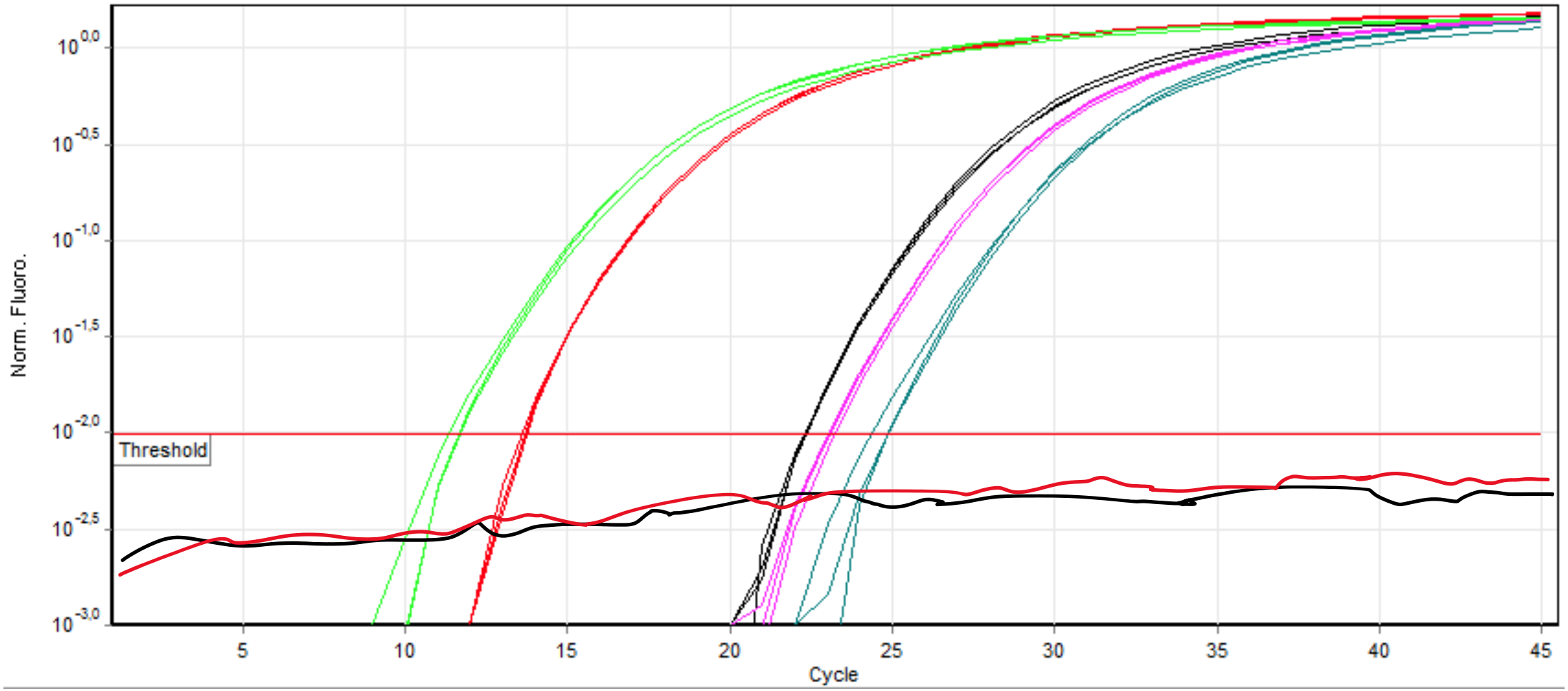
Ahn *et al.* doi: 10.4014/jmb.2003.03011

Polymerase chain reaction - PCR



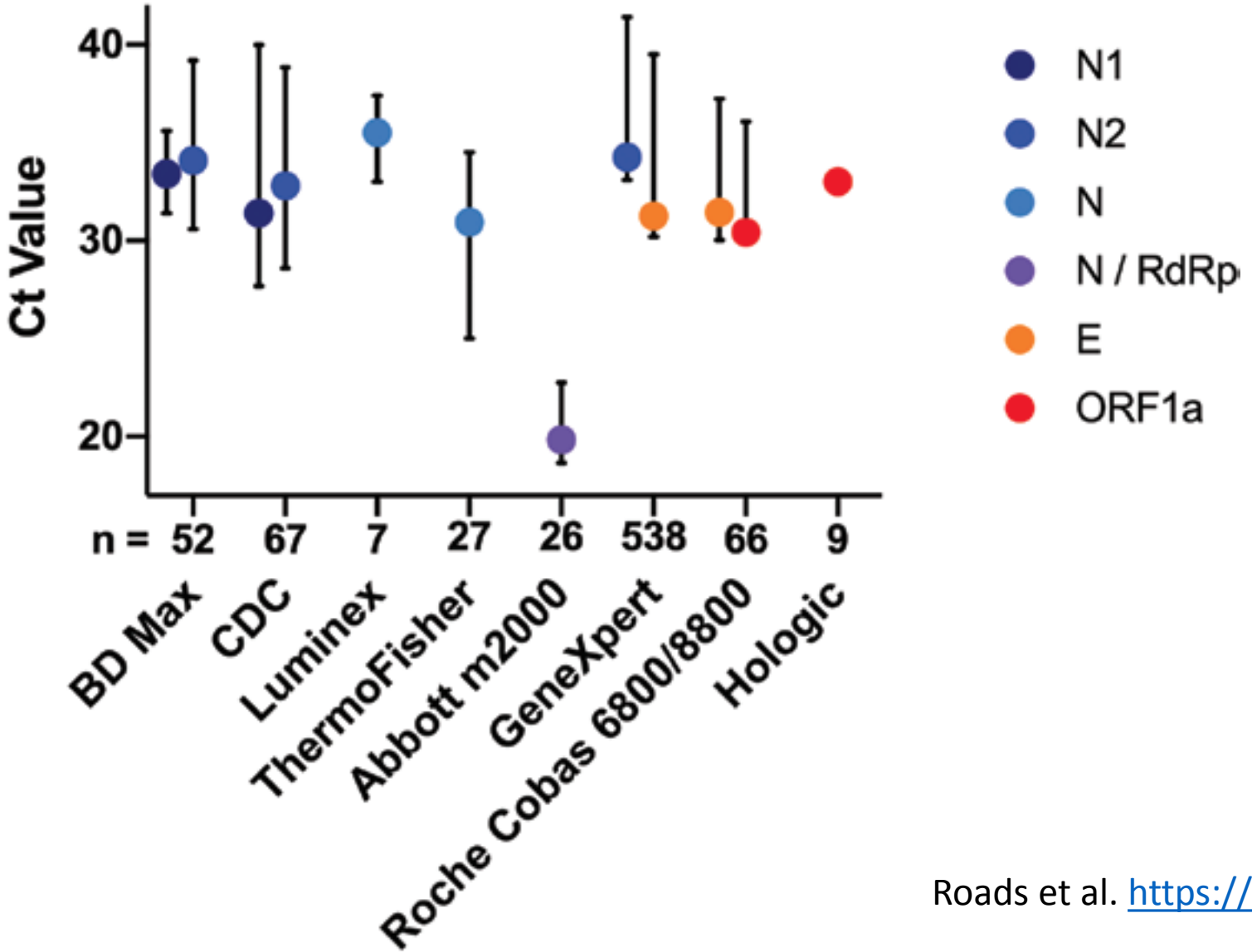
https://commons.wikimedia.org/wiki/File:Polymerase_chain_reaction.svg

Crossing-threshold (CT)



https://upload.wikimedia.org/wikipedia/commons/4/4e/Qpcr-cycling.png

CT variability across FDA-approved PCR methods



Roads et al. https://doi.org/10.1093/cid/ciaa1199

Studies correlating CT with culture

Study	N of samples Cx+/PCR+	No growth based on CT	No growth based on log(10) RNA copies/ml
Bullard	26/90	>24	5.98 ±0.18
Huang	44/112	>31.5	6.62±0.16
La Soola	129/611	>35.2	
Singanayagam	133/324	>35	
Gniazdowski	47/132	>23	
Basile	56/234	>32	
Ladhani	31/87	>35	
Young	21/100	>30	

Jefferson et al. https://doi.org/10.1101/2020.08.04.20167932

Summary

- PCR is currently the preferred test for diagnosis
- Time after initiation of symptoms is currently the most reproducible criterion for risk of transmission

Questions?