Delivering Your New Diagnostic Test into a Clinical Laboratory

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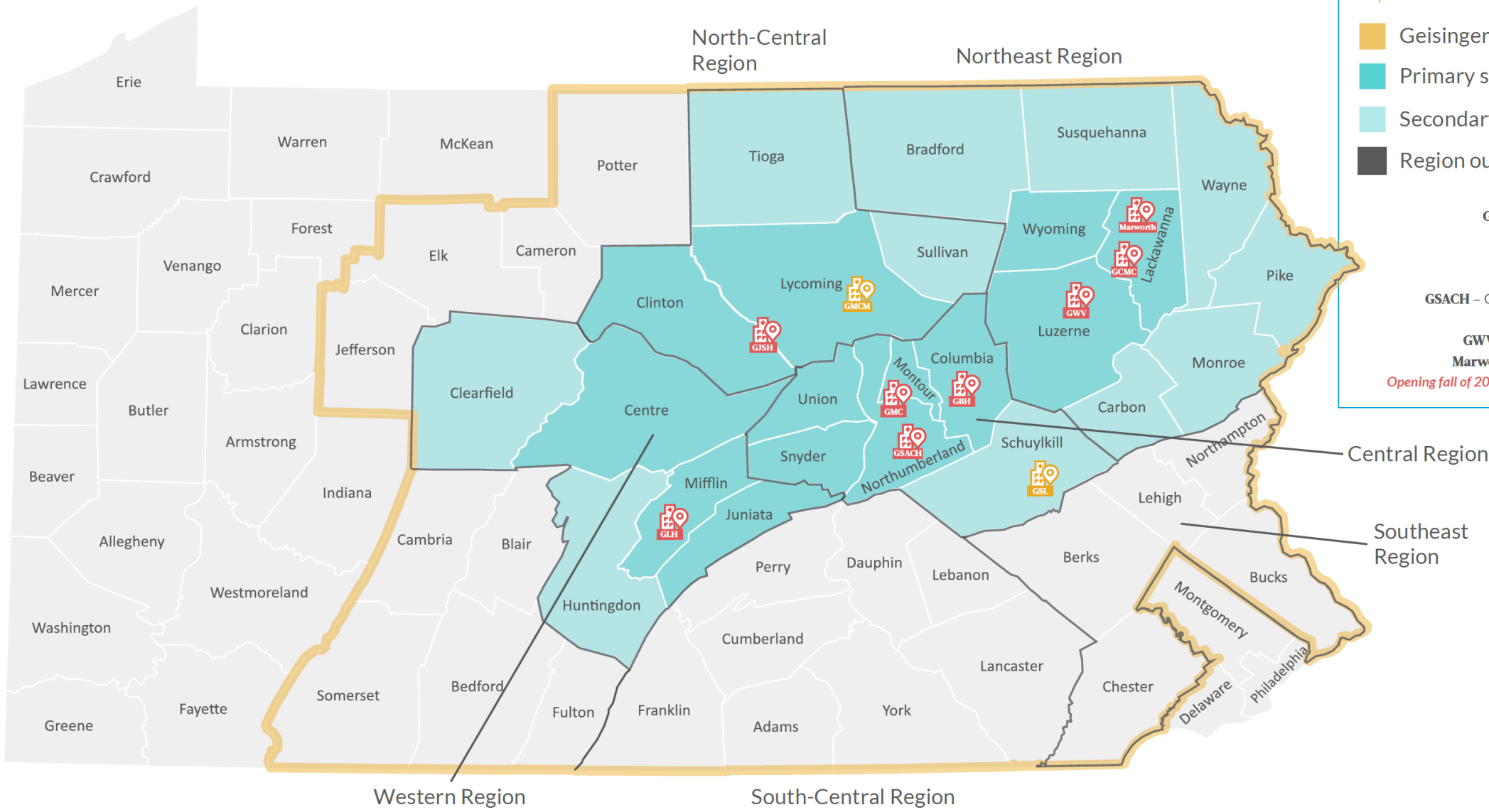
Geisinger Diagnostic Medicine Institute
Laboratory Medicine

125	CLIA certified sites
11	patient service centers
42	couriers on the road daily
1100	employees
45	pathologists and doctoral scientists
15 million	billable tests performed annually

Geisinger service area



Region and service area map key

- Geisinger inpatient facility
- Geisinger affiliated hospital
- Geisinger Health Plan service area
- Primary service area
- Secondary service area
- Region outline

GBH – Geisinger Bloomsburg Hospital
GCMC – Geisinger Community Medical Center
GJSH – Geisinger Jersey Shore Hospital
GLH – Geisinger Lewistown Hospital
GMC – Geisinger Medical Center
GSACH – Geisinger Shamokin Area Community Hospital
GSL – Geisinger St. Luke's
GWV – Geisinger Wyoming Valley Medical Center
Marworth – Geisinger Marworth Treatment Center
Opening fall of 2021 **GMCM** – Geisinger Medical Center Muncy

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Objectives

Traditional Laboratory Test Methods for SARS-CoV-2

- Describe the major Emergency Use Authorization (EUA) method categories used in U.S. clinical laboratories to detect SARS-CoV-2 in patient samples
- Review molecular diagnostic methods and common molecular viral targets used for testing patient samples for SARS-CoV-2
- Contrast SARS-CoV-2 patient testing to testing for the purpose of surveillance and epidemiology
- Discuss potential impact of SARS-CoV-2 on EUA test methods

Emergency Use Authorization (EUA) Methods for SARS-CoV-2

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Clinical Laboratory Testing Categories

Note: Definitions may vary slightly by organization

Clinical/Diagnostic Testing

Symptoms include:

Respiratory

Circulatory or clotting

Neurological

Systemic

Surveillance Testing

Health-care related: Checking for viral shedding: pre-procedure testing, pre-discharge from hospital, etc. (e.g., to long-term care)

Non-healthcare related: Schools, businesses, teams, pre-travel

Contact Testing

Post-Exposure testing

Contact tracing

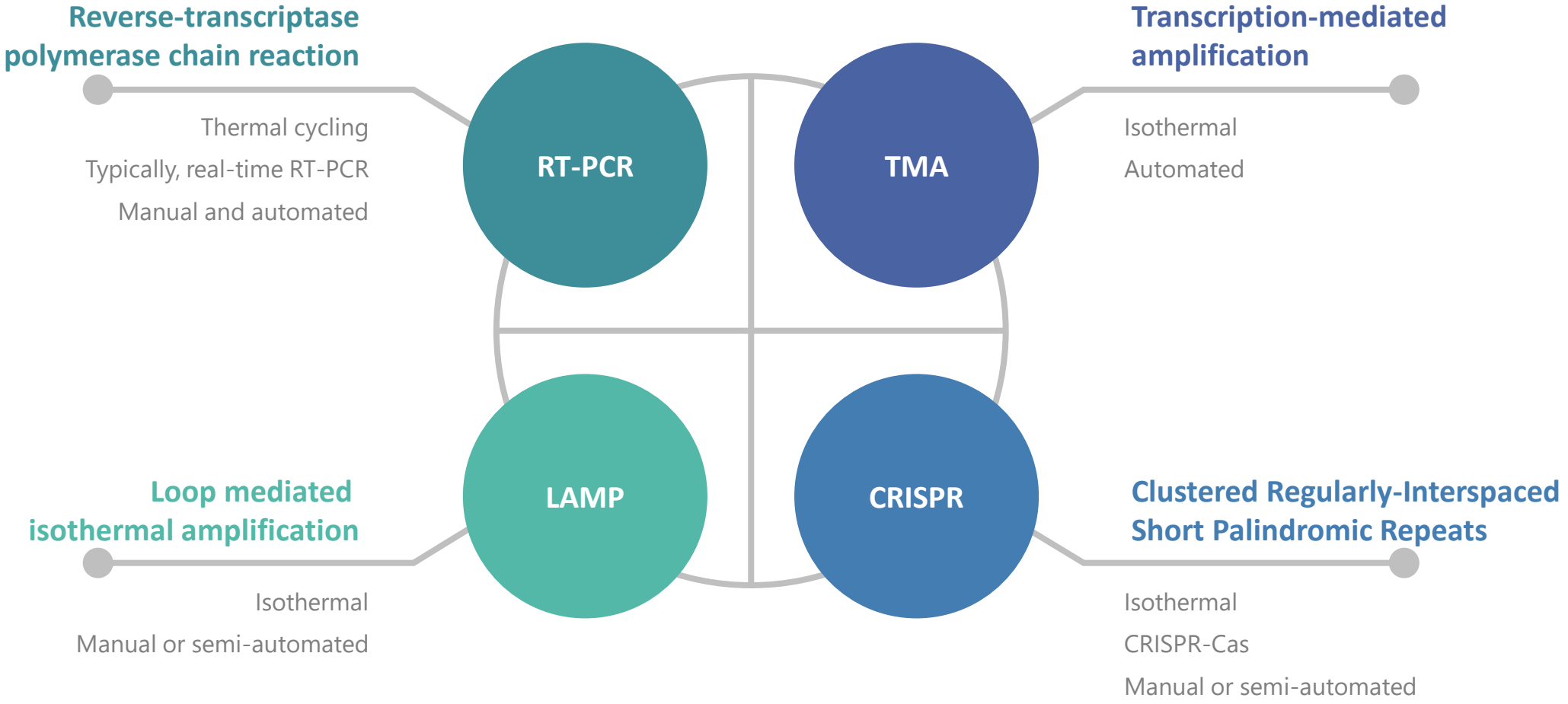
Epidemiologic Testing

Public health surveillance

Research

Diagnostic tests commonly used in high-complexity laboratories

EUAs tests for SARS-CoV-2



Reverse transcription polymerase chain reaction (RT-PCR)

Clinical diagnosis

Surveillance

Epidemiology

RT-PCR

Used to detect specific regions of viral RNA

Combines reverse transcription of RNA into DNA (cDNA) with polymerase chain reaction (PCR), for amplification of specific cDNA targets, exponentially doubling amplicon with each cycle

Real-time RT-PCR: amplification reaction is monitored with signal from bound fluorescence probes

Reverse transcription

1. RNA Strand, start codon AUG, ends with poly A tail

2. Oligo dT primer hybridizes to poly A tail

3. Primer extension by reverse transcriptase (adding dNTPs)

4. Full 1st strand completed: Synthetic strand elongatation

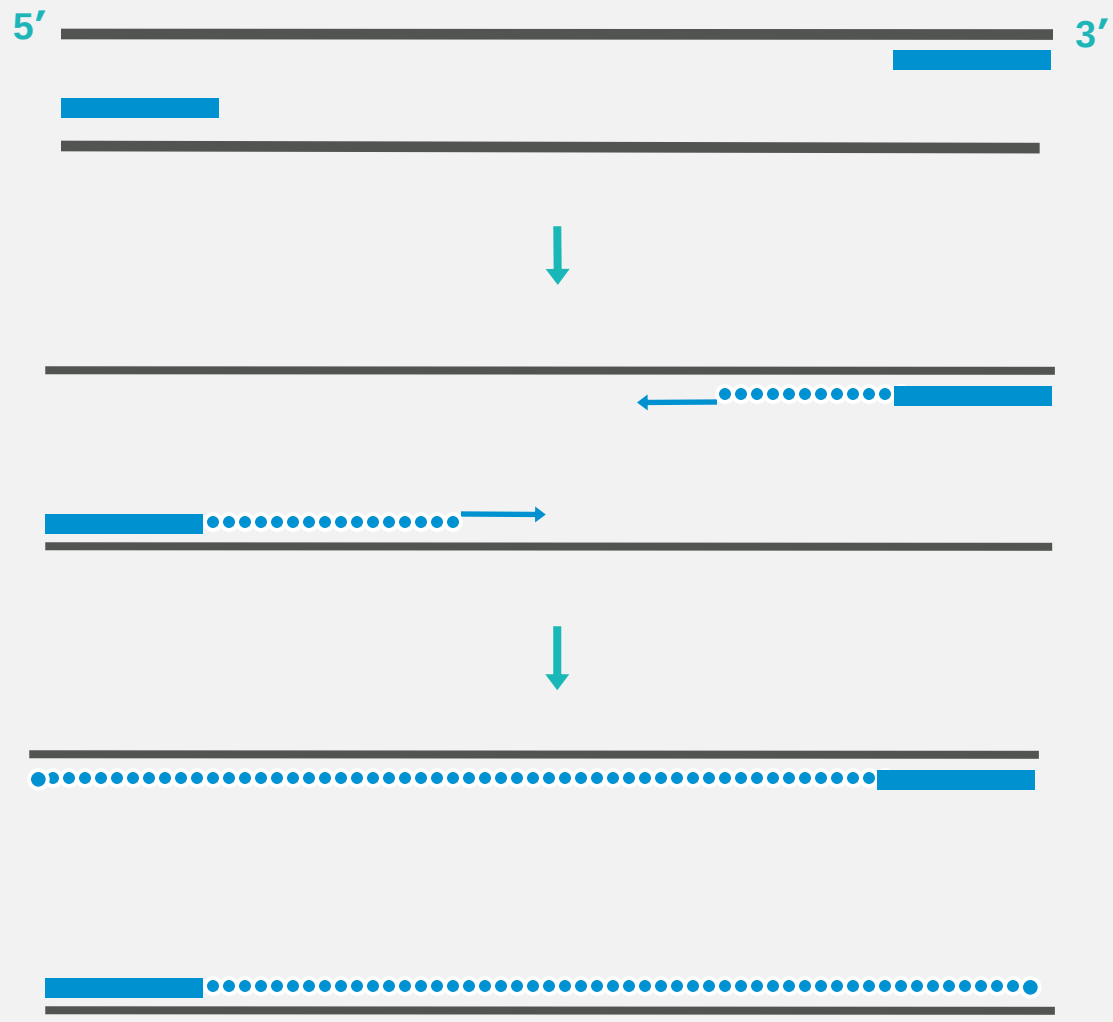
RNA Template

Primer

cDNA

Wolk, D.M. Mitchel S. Patel, R. 2001. Principles of Molecular Microbiology Testing Methods. Infect Dis Clin N America. 15(4) 1157-1204

PCR from cDNA



1. Denature DNA Strands @ 95°C

2. Primer anneals (hybridizes) to denatured cDNA @ 55-68°C

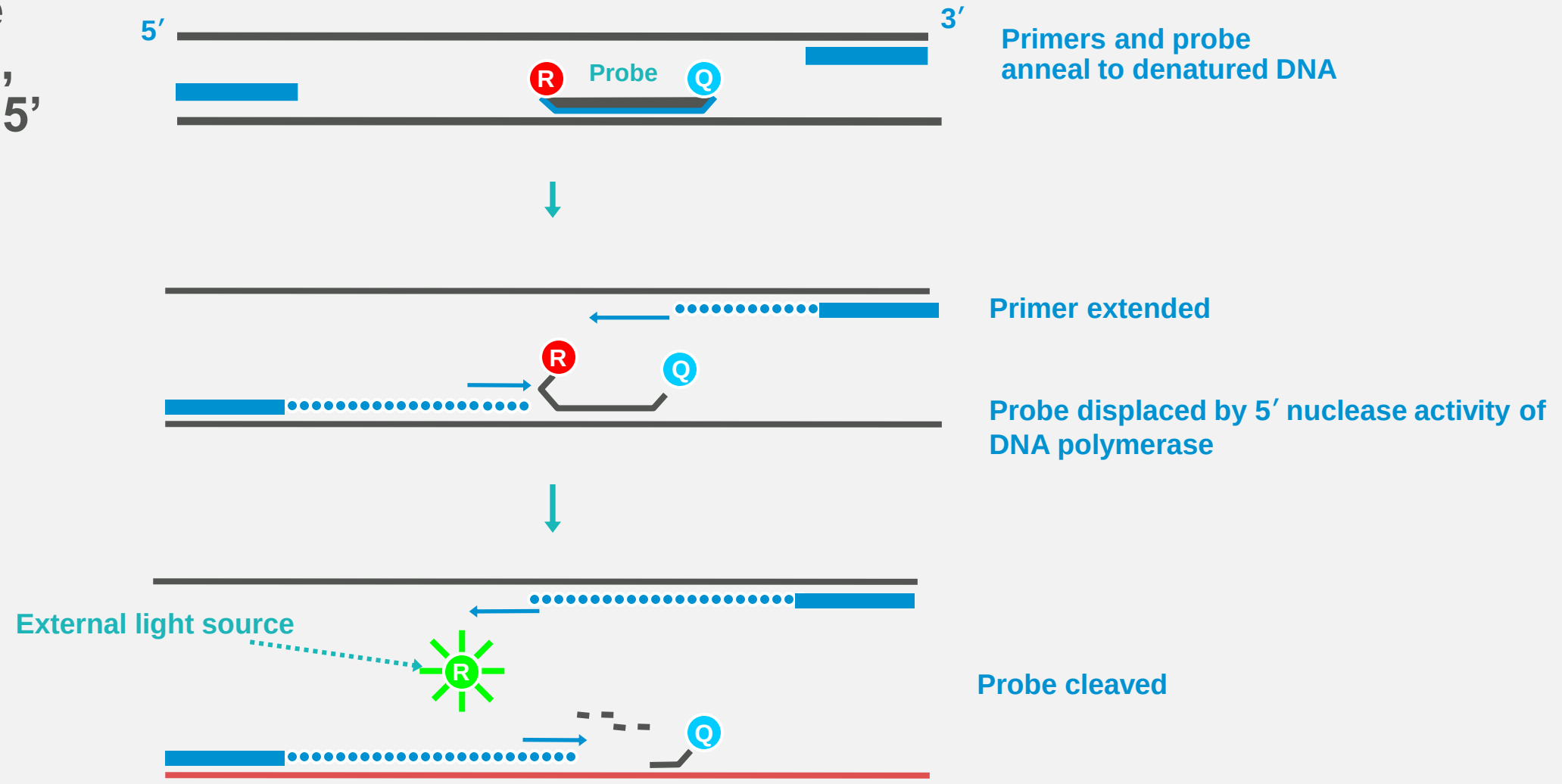
3. Primer extension by DNA polymerase @ 68-72°C

4. Full Cycle completed: Synthetic strand elongatation

Template **Primer** **Amplicon**

Wolk, D.M. Mitchel S. Patel, R. 2001. Principles of Molecular Microbiology Testing Methods. Infect Dis Clin N America. 15(4) 1157-1204

Real-time PCR: e.g., TaqMan® 5’ Nuclease Assay



R Reporter dyes FAM, TET, VIC, JOE

Q Quencher molecule TAMRA

R Fluorescent emission of reporter dye

•••• **Amplicon**

Wolk, D.M. Mitchel S. Patel, R. 2001. Principles of Molecular Microbiology Testing Methods. Infect Dis Clin N America. 15(4) 1157-1204

Transcription Mediated Amplification (TMA)

Clinical diagnosis

Surveillance

Epidemiology

TMA

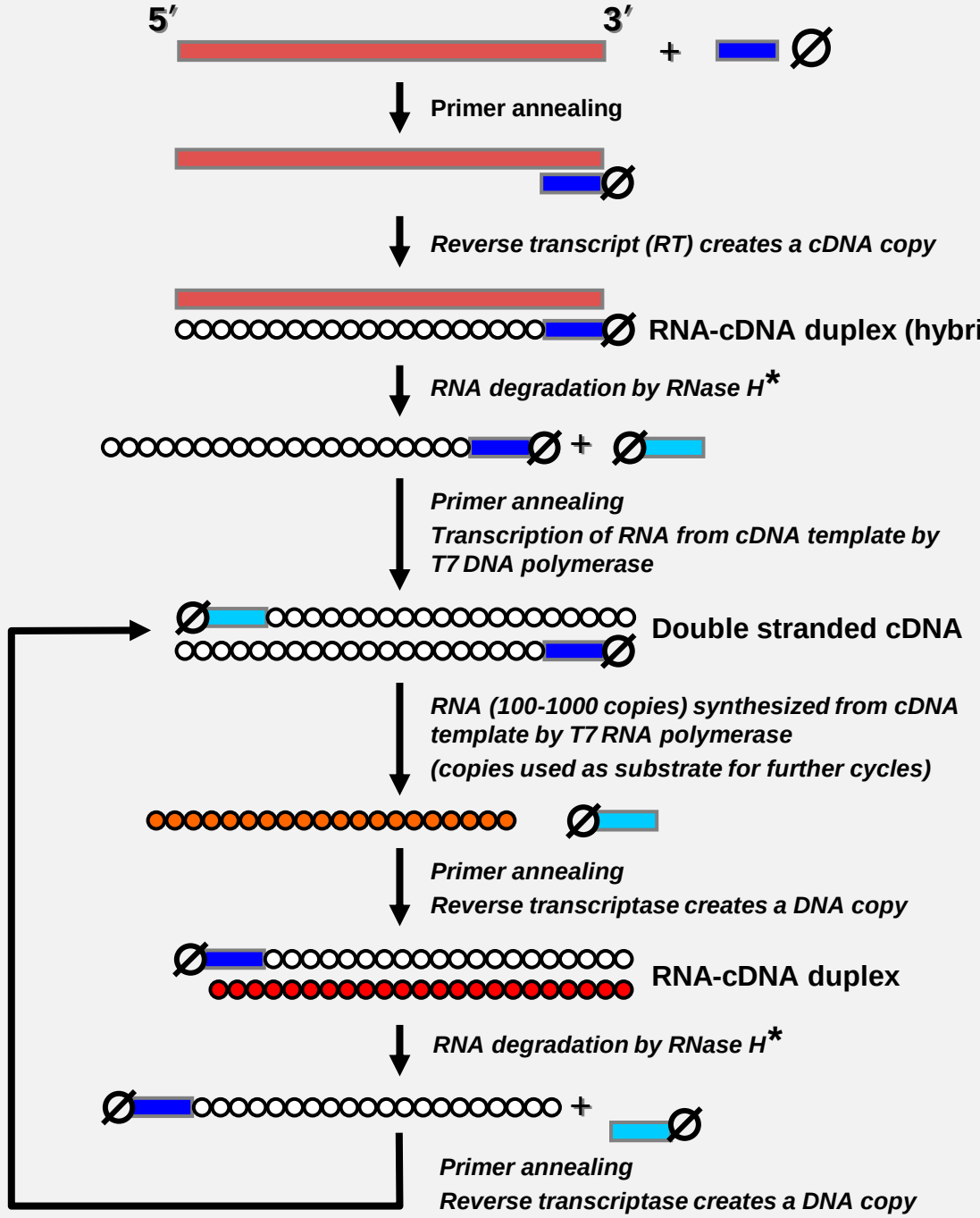
Isothermal method - no change to temperature

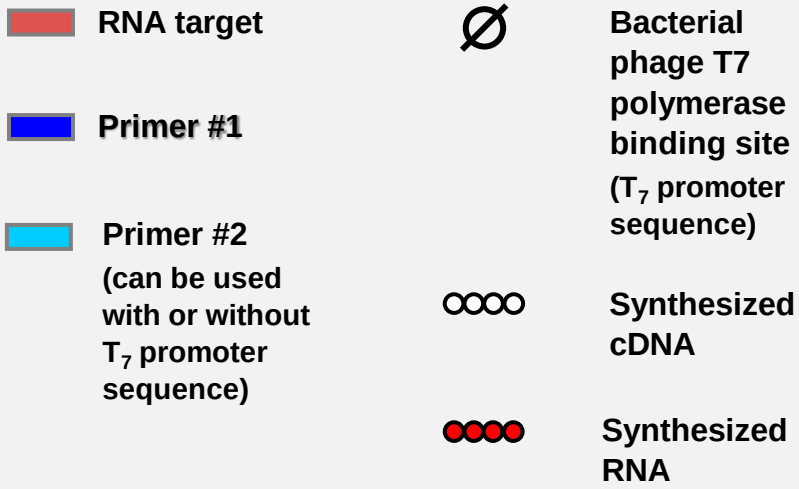
Single-tube nucleic acid amplification system utilizing two enzymes, RNA polymerase and reverse transcriptase

TMA produces RNA amplicon rather than DNA amplicon

TMA produces 100–1000 copies per cycle, resulting in a 10 billion fold increase of copies within about 15–30 minutes.

TMA





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RT-Loop-mediated isothermal amplification (RT-LAMP)

Clinical diagnosis

Surveillance

Epidemiology

RT-LAMP

cDNA amplification under isothermal conditions

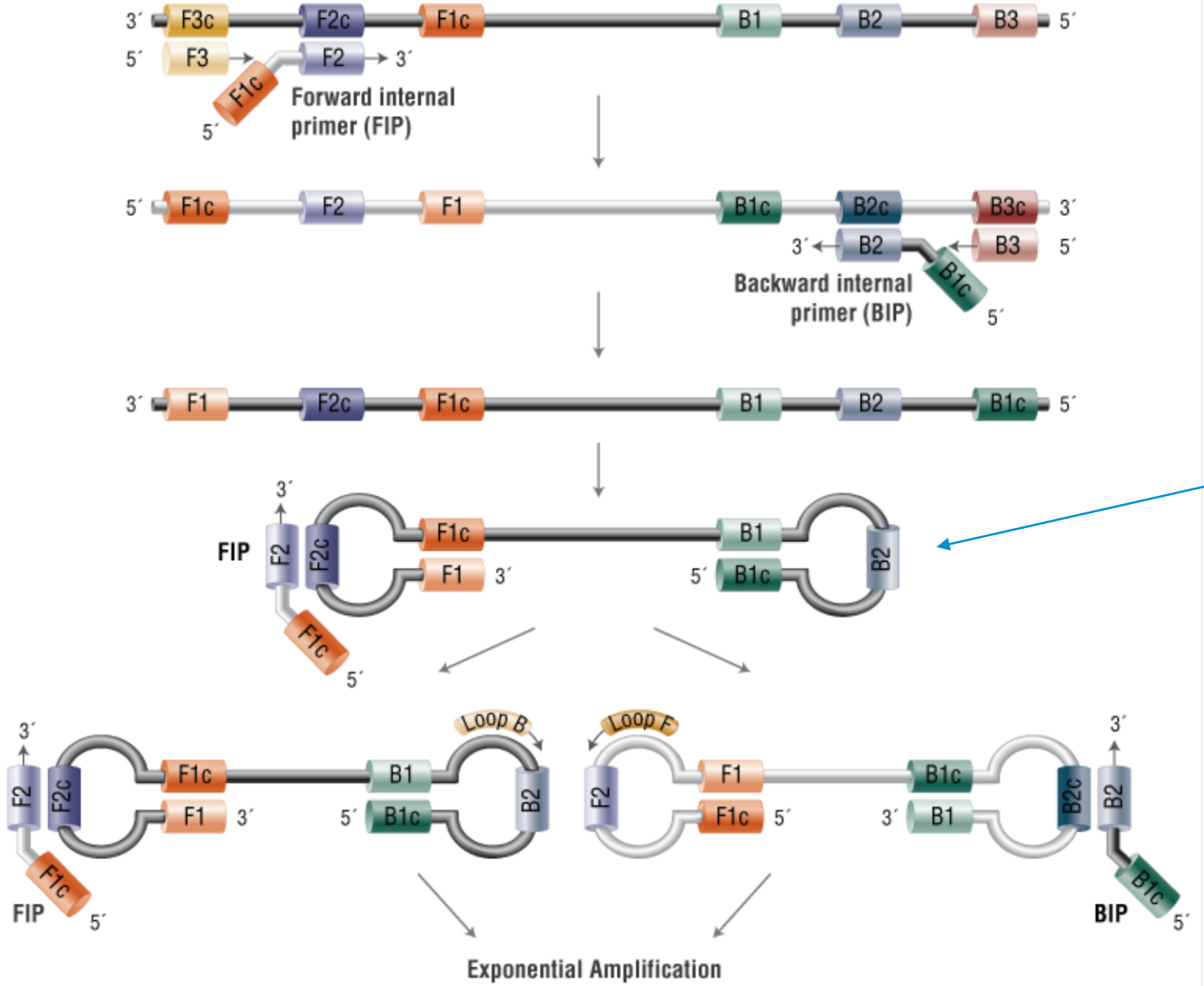
Uses 4-6 primers recognizing 6-8 distinct regions of target cDNA.

A strand-displacing DNA polymerase initiates synthesis and 2 of the primers form loop structures to facilitate subsequent rounds of amplification

Turbidity caused by magnesium pyrophosphate, a by-product of the amplification reaction, is produced in proportion to the amount of amplified products

The presence of turbidity indicates the presence of amplicon

LAMP



https://international.neb.com/applications/dna-amplification-pcr-and-qpcr/isothermal-amplification/loop-mediated-isothermal-amplification-lamp

Clustered **R**egularly-**I**nterspaced **S**hort **P**alindromic **R**epeats - CRISPR-associated genes (**CRISPR-Cas)**

Clinical diagnosis

Surveillance

Epidemiology

CRISPR-Cas

Viral RNA transcribed for cDNA amplification under isothermal conditions

Fast (~30 min)

Pathogen-specific crRNAs can be designed as long as unique genomic sequences have been identified

Multiplex applications allows multiple quenched fluorescent reporters to be used in the same reaction alongside multiple Cas enzymes

CRISPR-Cas

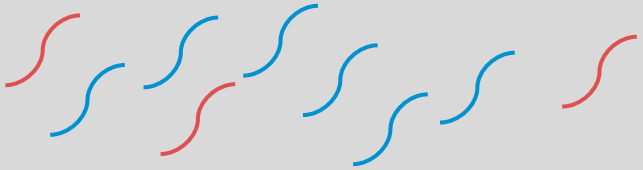
Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK)

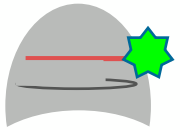
Viral RNA target undergoes reverse transcription to become cDNA for amplification

RT-LAMP and recombinase polymerase amplification (RPA)

Amplified cDNA

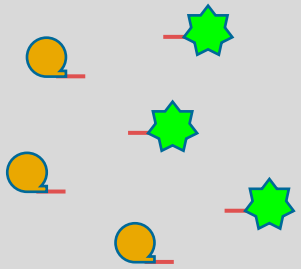
T7 Transcription





Cas13a

Cas13a recognizes CRISPR RNA (crRNA) target, then non-specific RNA-guided endonuclease activity cleaves the RNA probe and reporter dye is separated from quencher



Fluorescent reporter dye is detected

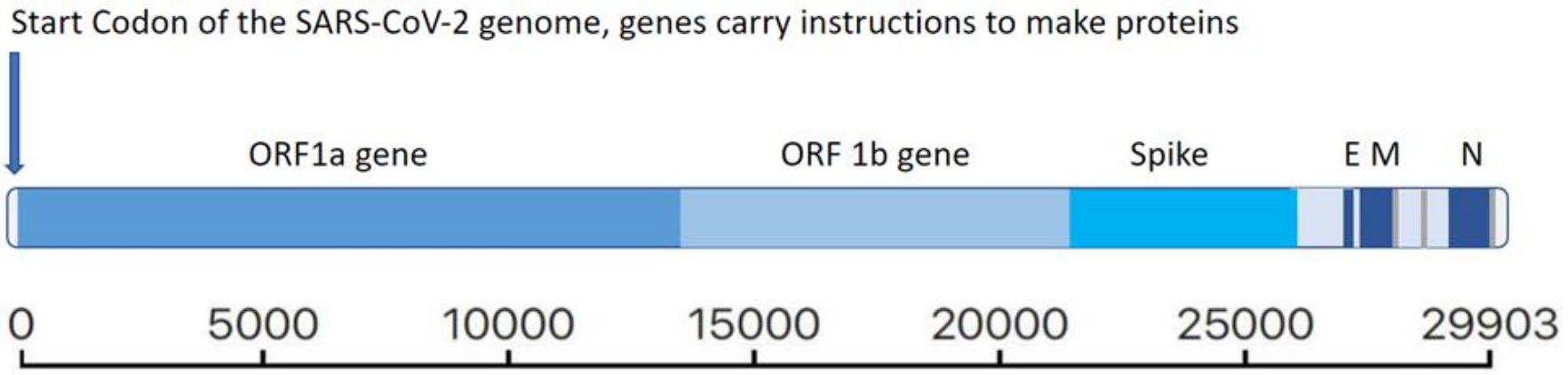
https://bitesizebio.com/47701/crispr-detect-viral-pathogens/

What is a SARS-CoV-2 variant and why worry about it in diagnsotics?

Routine testing implications

Gene in SARS-CoV-2

Figure 1: Important genes found in the SARS-CoV-2 viral genome include those that are transribed and translated by human cell machinery to create the ORF1a and 1b viral proteins, and the spike (S), envelope (E), matrix (M), and nucleocapsid (N) proteins.



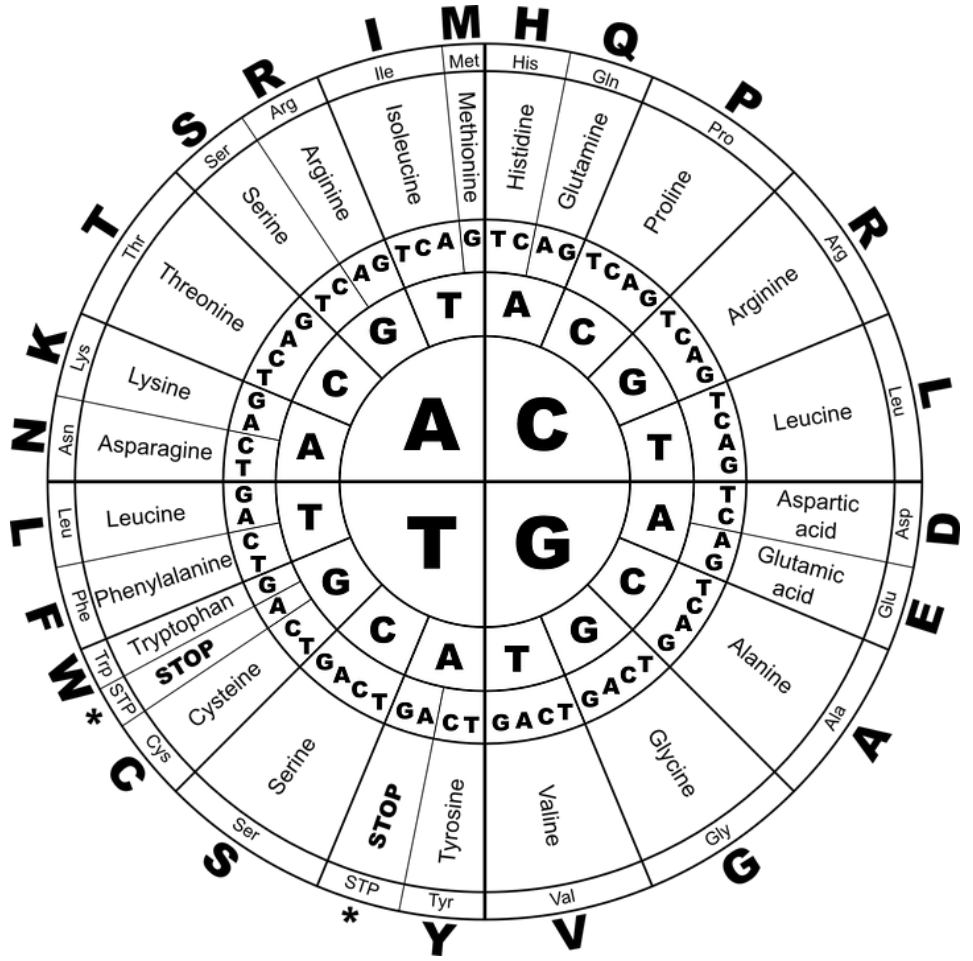
Genes
N1, N2, N3
N2, E
ORF, HKU-N
ORF1, E
ORF1a, E
ORF1ab
ORF1ab, S
RdRp, N
S, M

Genetic Mutations in Viruses

Mutation	Definition
Substitutions	≥ 1 nucleic acids are interchanged within the viral genome
Insertions	≥ 1nucleic acids are added from the viral genome
Deletions	≥ 1 nucleic acids are removed from the viral genome
Recombination	sections of viral genomes are exchanged
Mutation Rate	Speed that virus mutates

(RNA viruses mutate faster than DNA viruses)

Mutation Language



Vigilance for Strain Variation and Test Performance

Many thousands of SARS-CoV-2 variants

Subtypes categorized into larger groupings (e.g., lineages or clades)

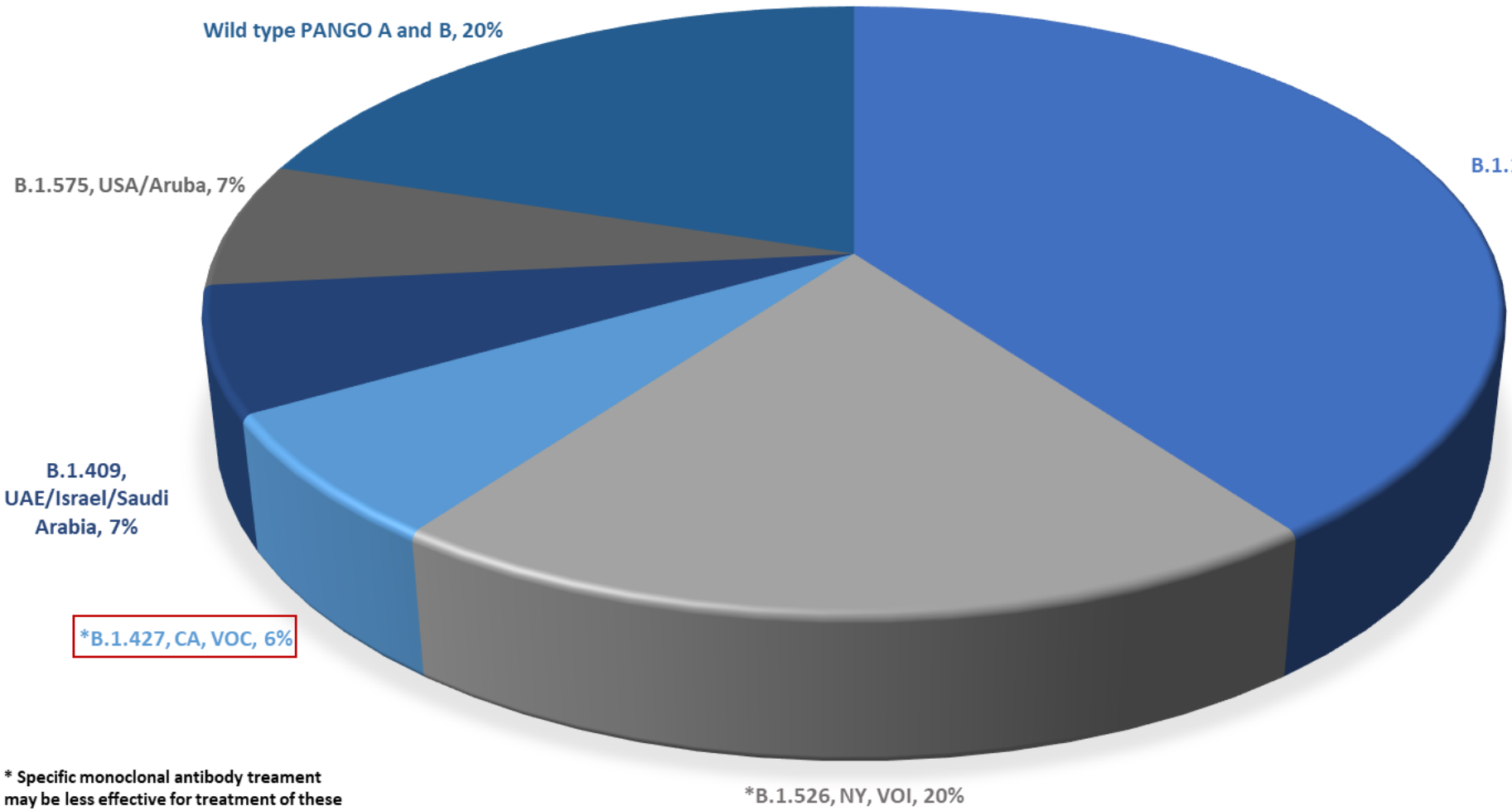
In silico

Assay designer monitors known mutations and assess whether or not the mutation will make a change in the binding of primers or probes

In vitro

Clinical samples of strain variants are tested with the assay to document accuracy

DISTRIBUTION OF SARS-COV-2 VARIANTS IN GEISINGER REGION
80% ARE MUTANTS; > 70% ARE CDC VARIANTS OF CONCERNS OR VARIANTS OF INTEREST



* Specific monoclonal antibody treament may be less effective for treatment of these SARS-CoV-2 variants

Courtesy of the Diagnostic Medicine Insitute

For SARS-COV-2, Nomenclature varies

Three main nomenclatures systems are proposed

Global Initiative on Sharing All Influenza Data

- Adapted for SARS-CoV-2
- Designates 8 global clades (S, O, L, V, G, GH, GR, and GV)

Nextstrain (real-time tracking)

- 11 major clades (19A, 19B, and 20A–20I) as of January 2021

Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLIN)

- Dynamic nomenclature that focuses on actively circulating virus lineages and those that spread to new locations
- 6 major lineages (A, B, B.1, B.1.1, B.1.177, B.1.1.7)

https://en.wikipedia.org/wiki/Variants_of_SARS-CoV-2#Nomenclature

Public health institutes can institute their own nomenclature system to track specific variants

E.g. MicrobeTrace (Centers for Disease Control and Prevention):

Users can map transmission networks based on

- person-to-person contacts
- pathogen-to-pathogen genetic distance
- person-to-place exposures

https://www.cdc.gov/genomics/events/microbetrace.htm

Variant of High Consequence (VOHC)

Clear evidence that prevention measures or medical countermeasures (MCMs), such as demonstrated failure of diagnostics, or have significantly reduced effectiveness relative to previously circulating variants

Requires notification to WHO under the International Health Regulations, reporting to CDC, an announcement of strategies to prevent or contain transmission, and recommendations to update treatments and vaccines

Currently there are no SARS-CoV-2 variants that rise to the level of high consequence

https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html#Consequence

Variants of Concern (VOC)

A variant for which there is evidence of an increase in transmissibility, more severe disease, significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures

https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html#Concern

Variant of Interest (VOI)

Specific genetic markers associated with changes

- Receptor binding
- Reduced neutralization by antibodies generated against previous infection or vaccination
- Reduced efficacy of treatments
- Potential diagnostic impact
- Predicted increase in transmissibility or disease severity

A VOI might require altering public health actions

- Enhanced sequence surveillance, laboratory characterization, or epidemiological investigations
- Assess ease of spread, severity of disease, efficacy of therapeutics, and vaccine protection

Current VOC in the U.S. are being closely monitored and characterized
CDC summary as of 5/25/2021, only 1 has testing impact

Strain	Transmission/Transmissibility	Increased severity	Impact on susceptibility to the combination of EUA monoclonal antibody (mAb) treatments	Impact on neutralization by convalescent and post-vaccination sera	Test impact
B.1.1.7	~50% increased transmission	Yes	No	Minimal	**S gene**

https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-viral-mutations-impact-covid-19-tests
https://www.medtechdive.com/news/fda-flags-covid-19-false-negative-risk-from-virus-variant/593120/
https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html#Concern

Enriching for the Likelihood of Finding Variants

Concepts to Consider

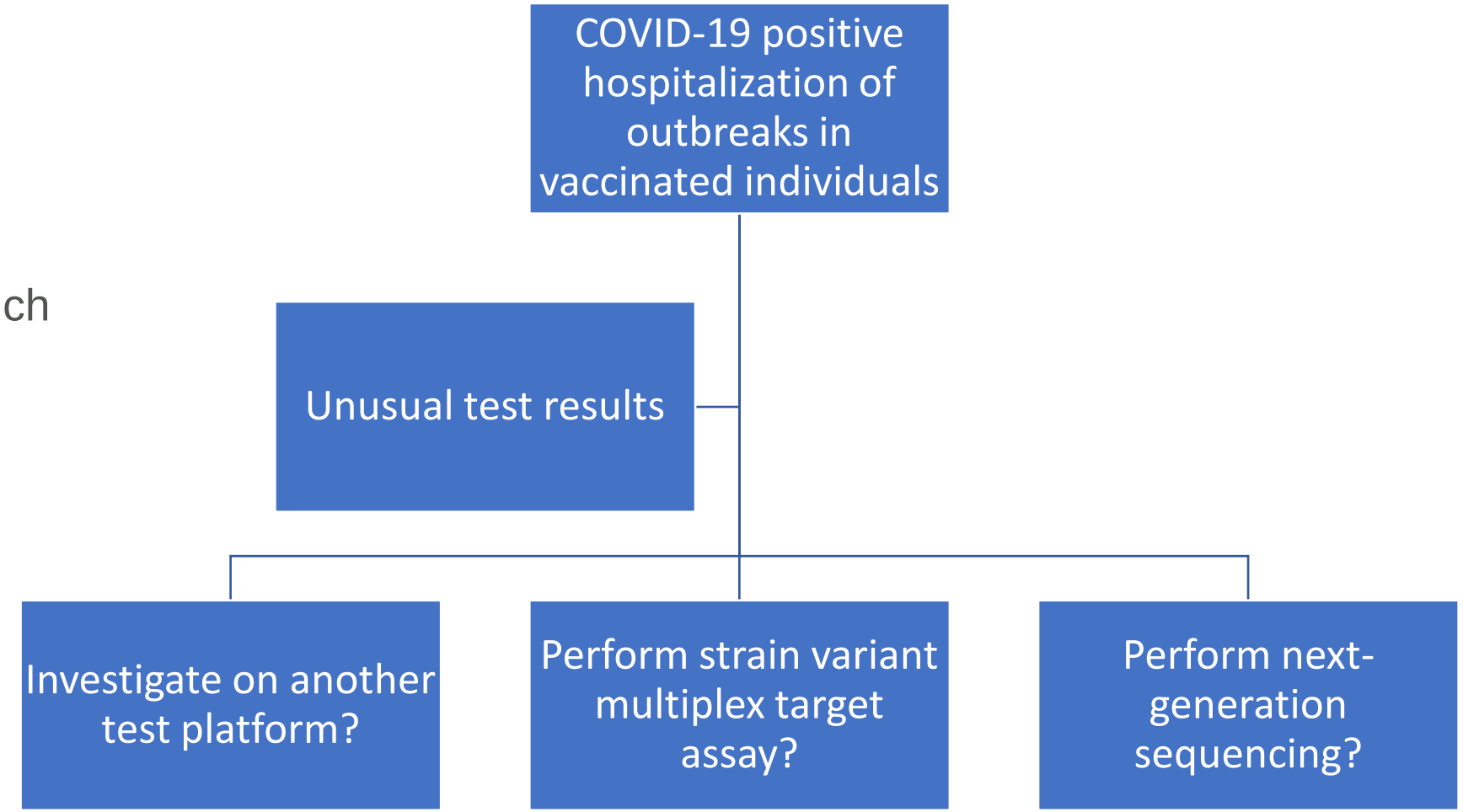
Cost

Expected turn-around time

Epidemiology/Outbreak needs

Which population to test to enrich the identification of variants

NGS or RT-PCR panels for variant detection



Banking or Testing of Clinical Sample

UTM, VTM, saline, RNAlater, or other RNA preservative

Use dedicated pipettes and supplies, RNAse-free zone

RNA processing precautions are required to avoid degradation

Extraction then freezing: Consider dilution to fill tube (dead space can cause water hydrolysis over time)

Thank you.

Stay positive, and test negative.

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