Molecular Diagnostics & Point of Care Testing

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molecular (nucleic acid) technology

detects and/or quantifies specific DNA/RNA sequences in a specimen

Uses

Identification of etiologic agents without need of culture.	Characterization of pathogen subspecies variation.	Determination of viral load	Direct detection of genes or gene mutations that confer drug resistance	Identify changes in a gene/ chromosome that may cause/affect the chance of developing a specific disease or disorder, e.g., cancer, genetic testing

Advantages of Rapid Testing for Infectious Diseases

Faster directed therapy to reduce:

- **antibiotic resistance**
- **hospital length-of-stay**

Less adverse consequences

Teachable moment

Reduced length-of-stay in Emergency Department

Timely application of **appropriate infection control** procedures

What are the issues of respiratory disease?

The symptoms of respiratory diseases are vague

- Pneumonia
  - Cough
  - Fever or Chills
  - Difficulty breathing
- Influenza
  - Cough
  - Fever or Chills
  - Malaise
- SARS CoV-2
  - Cough
  - Fever or Chills
  - Difficulty breathing
  - Muscle/Body aches
  - Loss of taste/smell

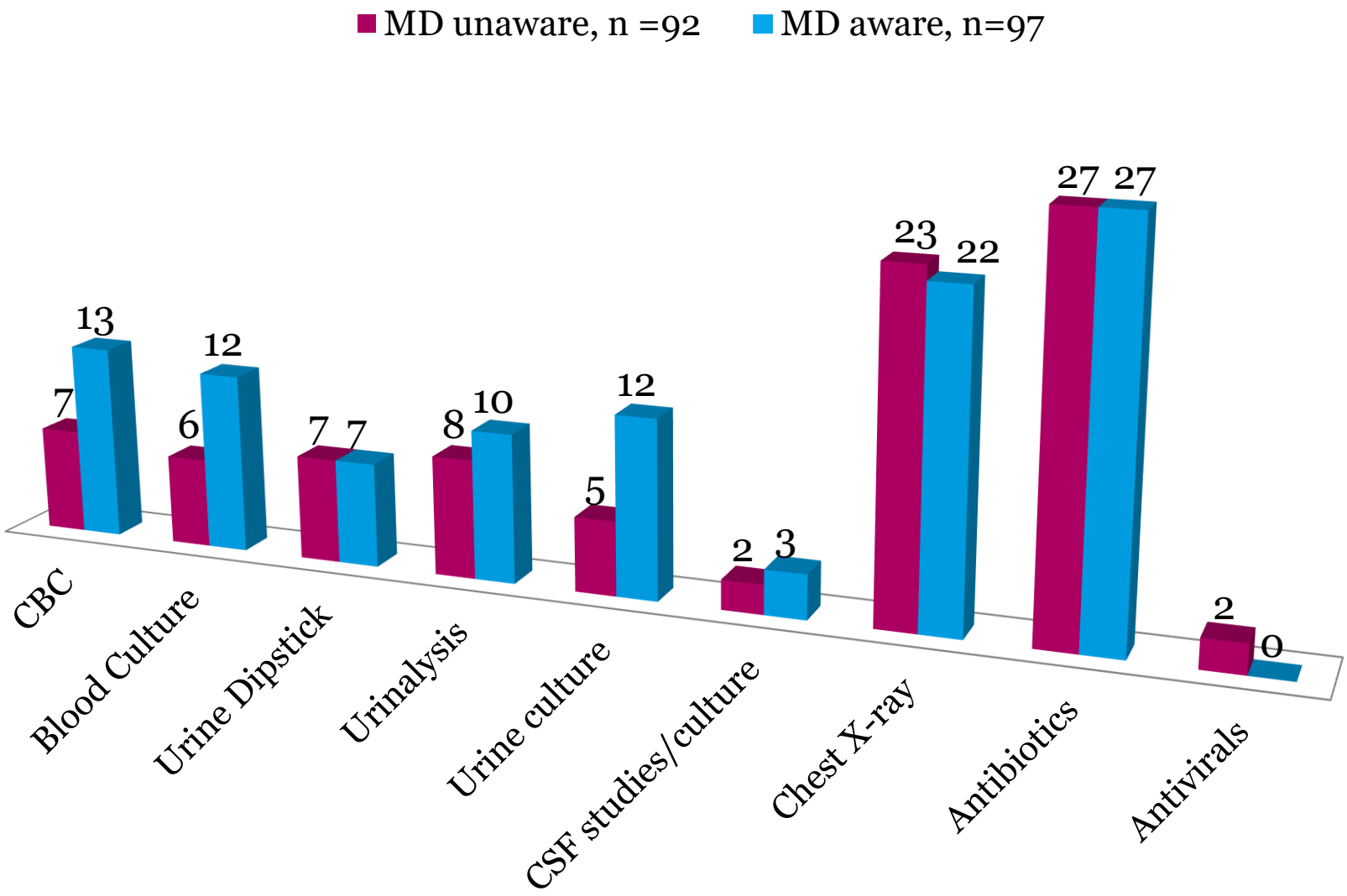
Treatment is different

- Bacteria
  - Broad spectrum antibiotic
  - Narrow spectrum antibiotic
- Influenza
  - Antiviral
  - Treat symptoms only
- SARS CoV-2
  - Monoclonal
  - Antiviral/Corticosteroid
  - Treat symptoms

Complications of mistreatment

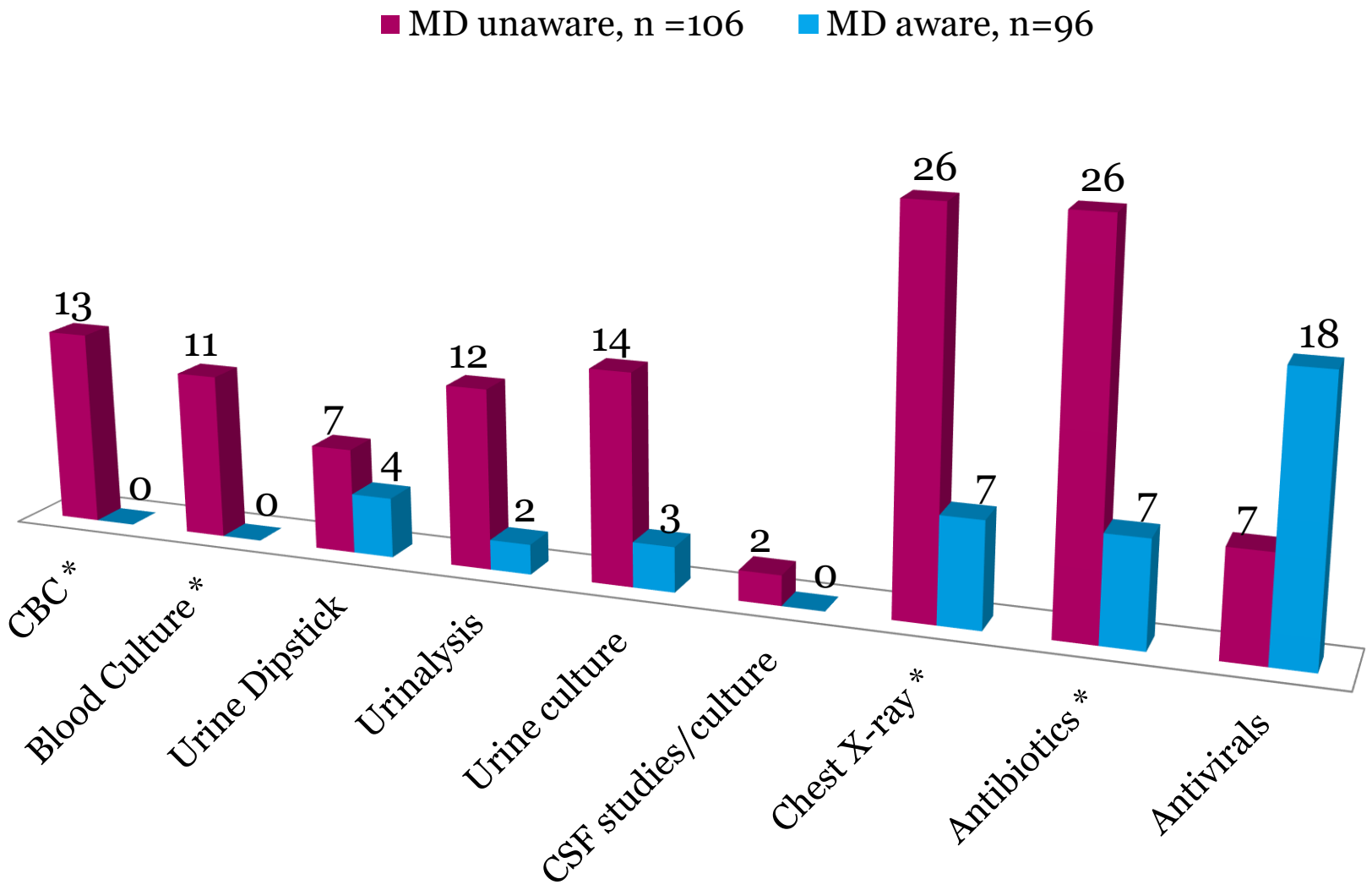
- Mistreatment of bacterial etiology
  - May increase morbidity/ mortality
  - May have longer hospital stay
  - May get *C. difficile*
- Mistreatment of influenza
  - May have increased resistance & *C. difficile*
- Mistreatment of SARCoV-2
  - May increase morbidity/ mortality
  - May enhance infection rate

Results – Flu Negative



Bonner, *et al,* Pediatrics (2003) 112:363-367

Results – Flu Positive



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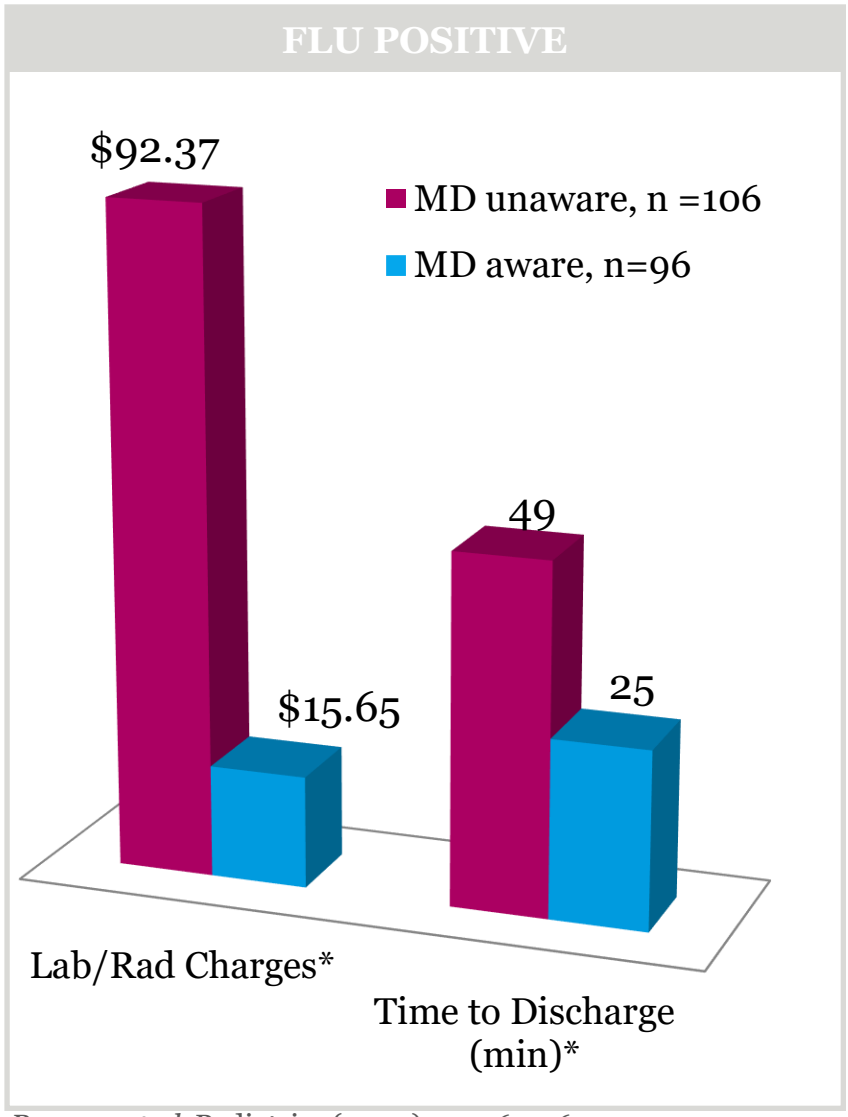
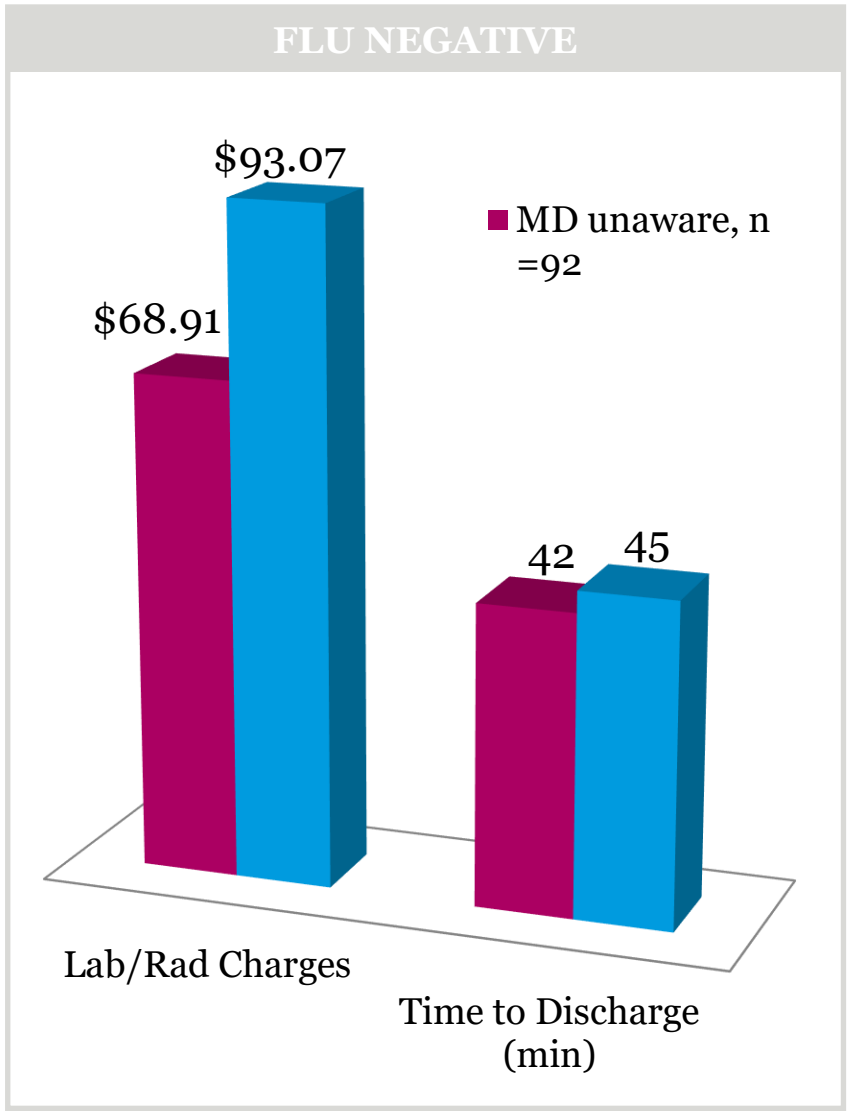
Key Operational Metrics





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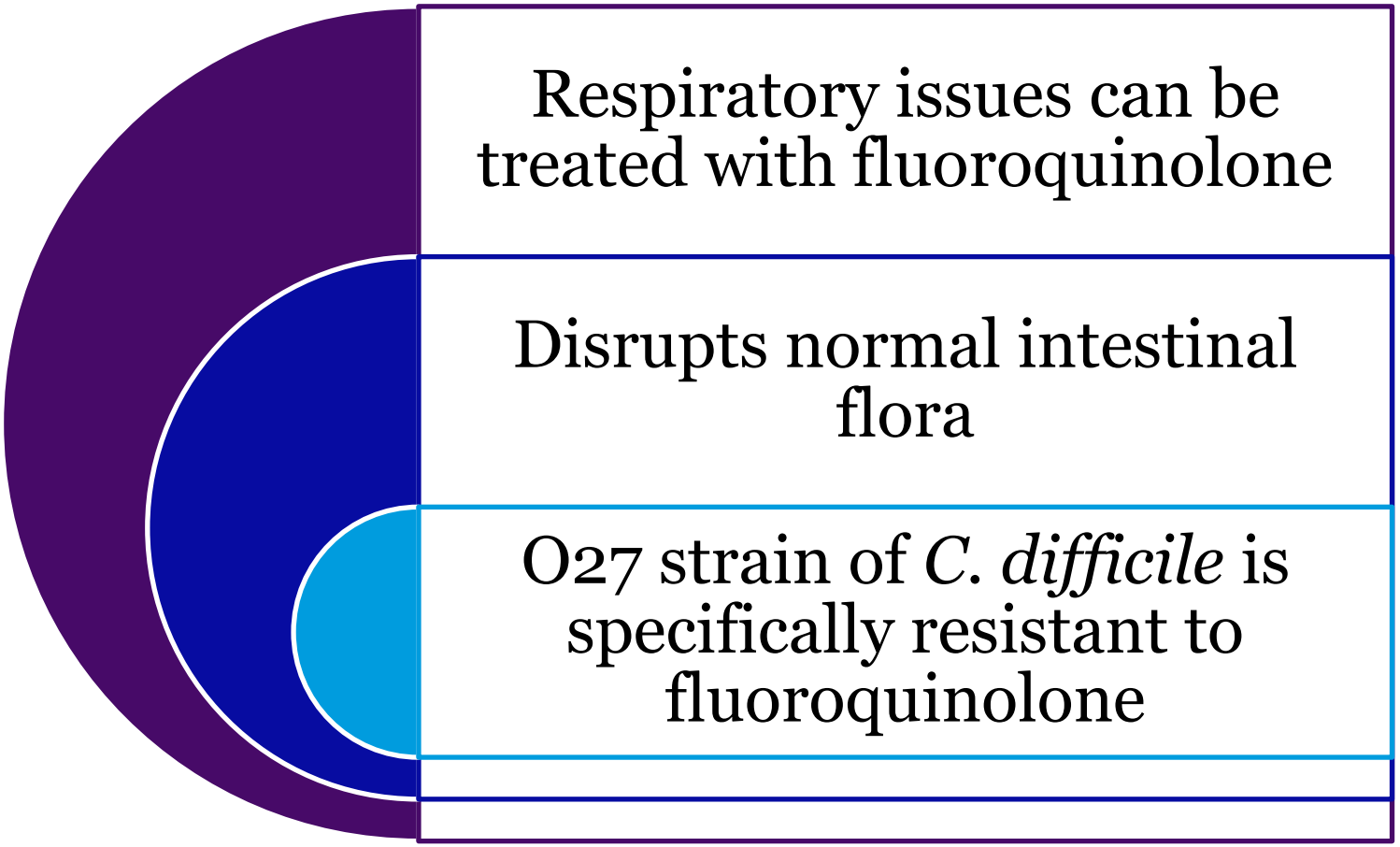
Treating Respiratory Diseases in the Emergency Department

Is the pathogen bacterial or viral?	Influenza and pneumonia symptoms can overlap dramatically
Who do you test?	If it is flu season, do you test for other pathogens?
What do you test them for?	Different age groups are linked to different pathogens.
Can treatment be impacted if the appropriate testing is done?	Stop indiscriminate use of broad spectrum antibiotics.

Misuse of Antibiotics Can Lead to Other Medical Issues



Issues with Clinical Samples

Viral titer is highest in first 48 hours

Proper sample collection is necessary

Dilution in transport media

Rapid Antigen Tests

Pro

- Tests take minimal time
- Some tests are so simple that they are CLIA-waived
- Can be used to triage patients
- Positive results can be used to rule out other issues like pneumonia so don't give unnecessary chest x-ray, antibiotics, etc.

Con

- Performance is not as good as culture, PCR, or DFA
- Often used as a screening test, usually with negatives requiring additional confirmation.

Molecular Tests

Pro

- For respiratory specimens, high performance
- Same day results
- Good for active infections

Con

- Turn around time from lab may be extensive, especially if batching specimens
- Expensive
- May require experienced technicians, labs, dedicated equipment, etc.
- May only be a screen for bacteria/viruses that people may normally carry
- Bad for past infection

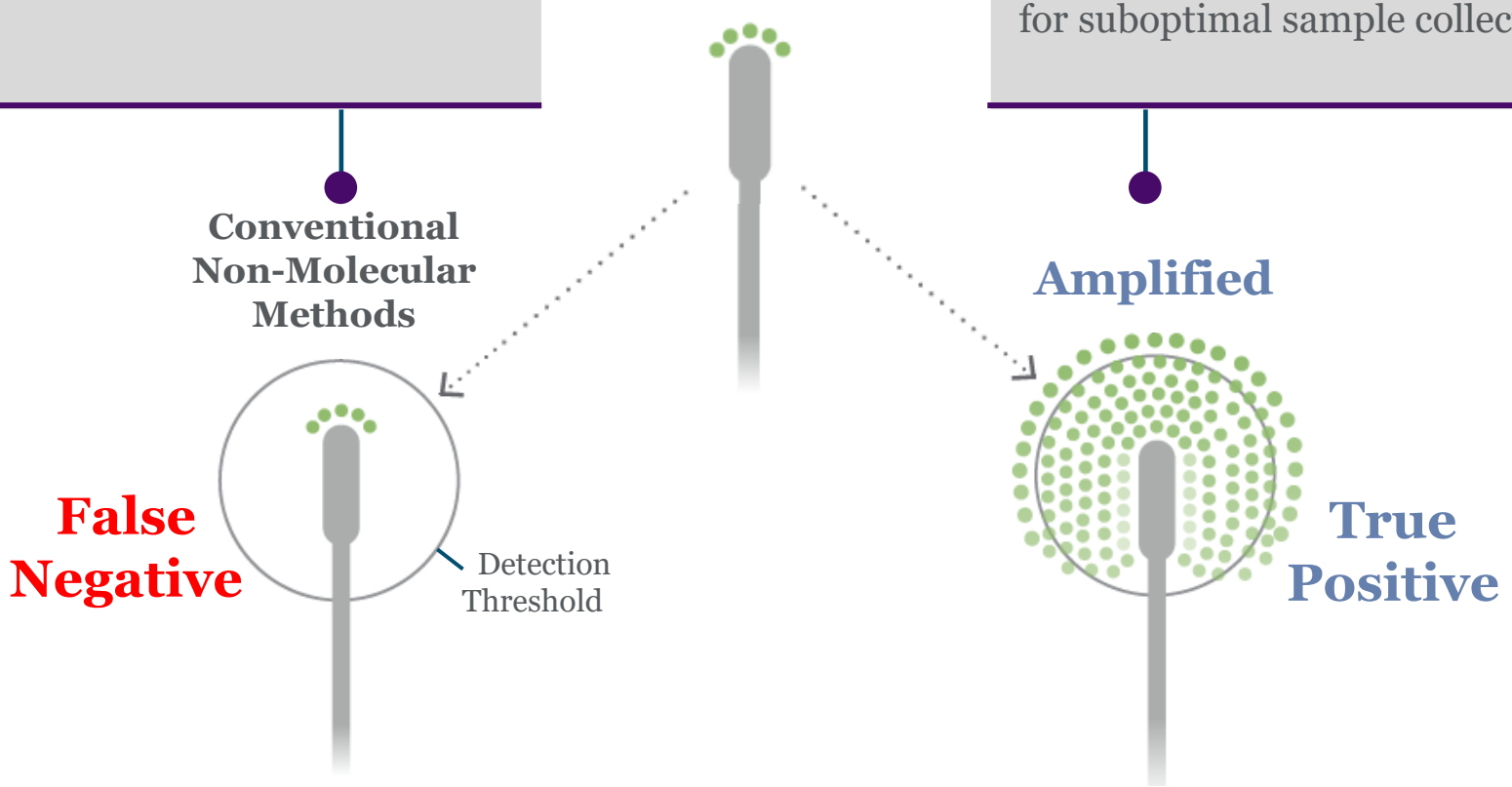
Why molecular?
The power of sample amplification

Conventional **non-molecular** methods can have suboptimal limits of detection.

Samples with low viral or bacterial load could result in a false negative.

With **molecular**, even a few hundred infectious particles can be amplified billions of times!

Amplification increases likelihood of detection, and may compensate for suboptimal sample collection.



IDSA Influenza Clinical Guidelines 2018

What tests should be used to diagnose Influenza?

10) Clinicians should use rapid molecular assays (ie. nucleic acid amplification tests) over rapid influenza diagnostic tests (RIDTs) in outpatients to improve detection of influenza virus infection (A-II) .

11) Clinicians should use reverse-transcription polymerase chain reaction (RT-PCR) or other molecular assays over other influenza tests in hospitalized patients to improve detections of influenza virus (A-II).

15) Clinicians should not use RIDTs in hospitalized patients except when more sensitive molecular assays are not available (A-II), and follow-up testing with RT-PCR or other molecular assays should be performed to confirm negative RIDT results (A-II)

Molecular Tests on the Market

PCR – Polymerase Chain Reaction

- Rely on the ability to amplify due to temperature cycling
- Many traditional molecular companies, e.g,
  - Abbott m-PIMA - Competitive Reporter Amplification
  - BioFire Diagnostics – qPCR
  - Cepheid GeneXpert
  - Roche cobas Liat – Lab in a tube
  - Mesa - Accula – OscAR PCR

Isothermal

- Rely on the ability to do the reaction at a single temperature
- Meridian Alethia - LAMP (loop mediated isothermal amplification)
- Quidel Solana – HDA (Helicase dependent amplification)
- Abbott ID NOW – NEAR (Nicking enzyme amplification reaction)

Introducing the Players in PCR

Patient sample containing DNA (or RNA)

- May or may not have target gene

Primers

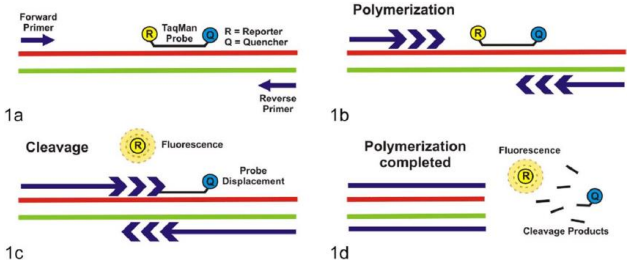
- short bits of manufactured DNA that recognize the target gene

Nucleotides

- building blocks of DNA

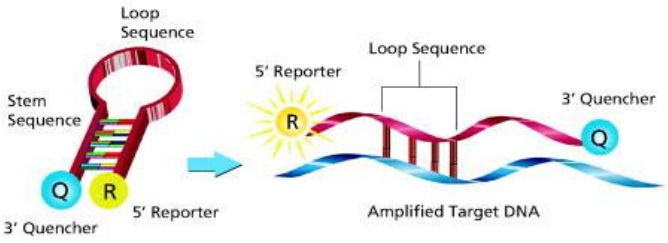
Taq Polymerase

- Enzyme that replicates DNA in a PCR reaction



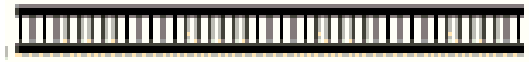
Fluorescent dye for reporting results

- TaqMan Probes
- Molecular Beacons
- Scorpions
- SYBR green



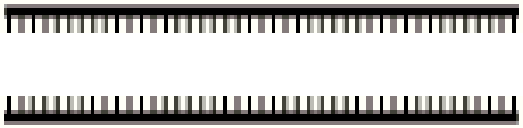
PCR Cycle

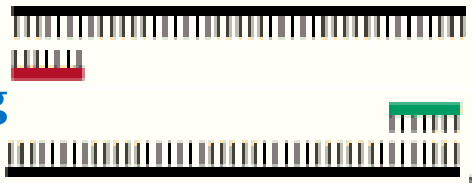
Double-stranded DNA



95° Denaturation

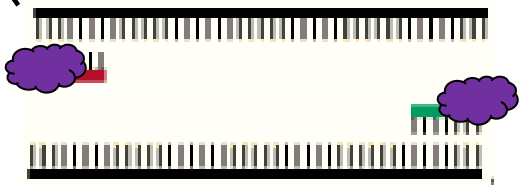
Heating separates strands





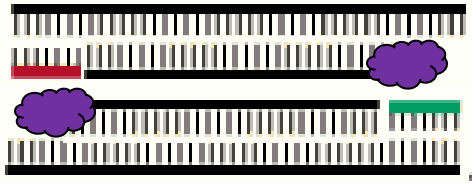
Primers Bind

57° Annealing



Taq Polymerase Binds at Primer Sites

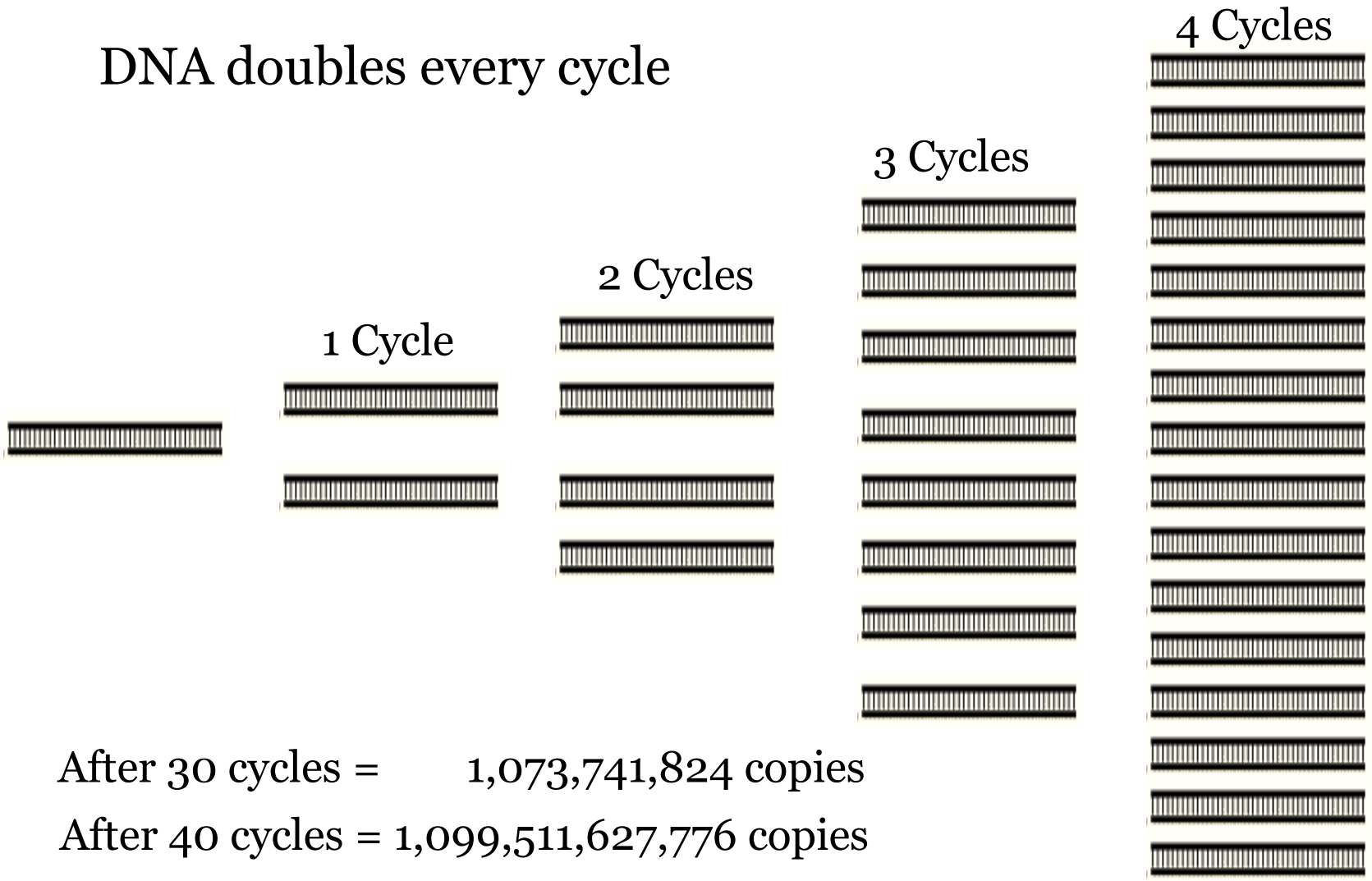
72° Extension



Taq Polymerase reads existing DNA strand to create a new matching one

PCR Amplification

DNA doubles every cycle



After 30 cycles = 1,073,741,824 copies

After 40 cycles = 1,099,511,627,776 copies

Roche Cobas Liat – "Lab In a Tube"

20 minutes to results Flu/RSV

15 minutes to results Strep A

Footprint 4.5 x 9.5 x 7.5

Weight 8.3 lbs

Flu A/B – Waived -Nasopharyngeal

- Sensitivity 100%/100%
- Specificity 96.8%/ 94.1%
- LOD 10^{-2} – 10^{-1} / 10^{-3} – 10^{-1} $TCID_{50}$/mL

Strep A - Waived

- Sensitivity 98.3%
- Specificity 94.2%
- LOD 5-20 CFU/mL

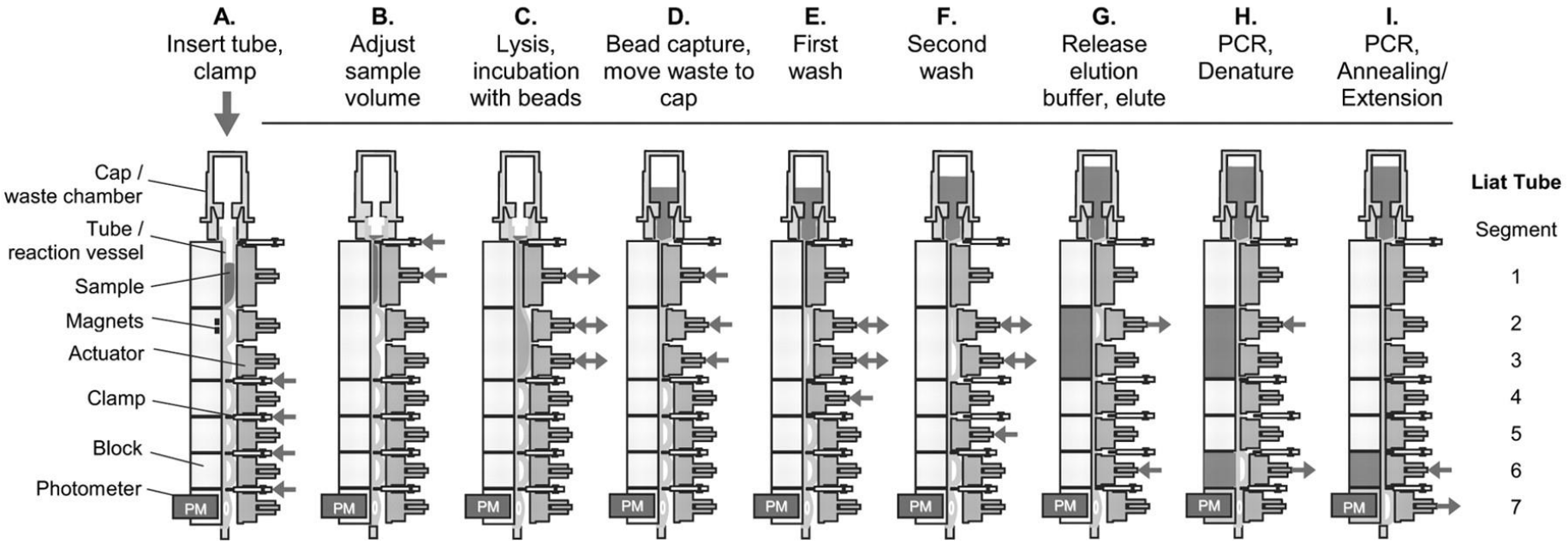
RSV - Waived

- Sensitivity 97.0%
- Specificity 98.7%
- LOD 4 CFU/mL

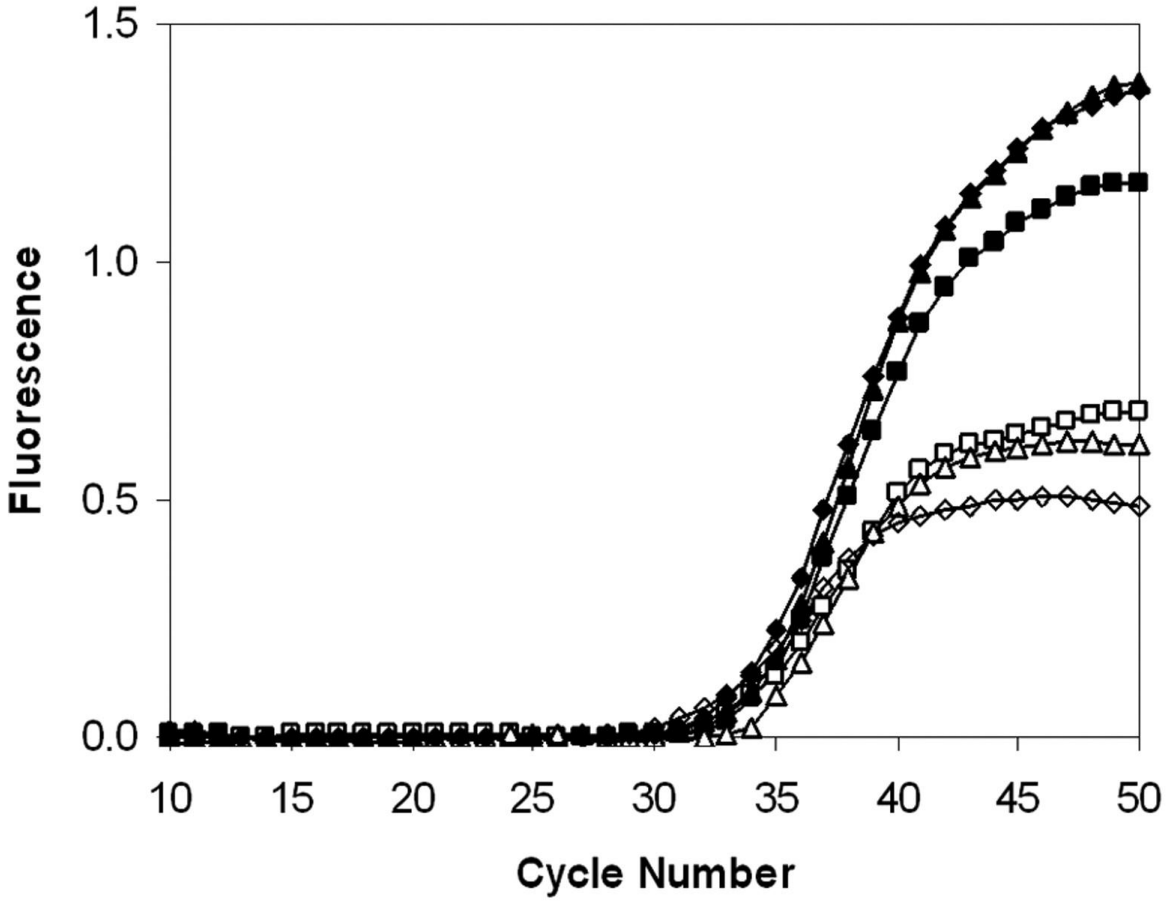
SARS-CoV 2/Flu A/B –NP, Naso- Waived

- PPA - 96.4%/100%/100%
- NPA - 98.7%/99.6%/99.7%
- LOD - 10^{-3} – 10^{-1} /10^{-2} – 10^{-1}/ 10^{-3} – 10^{-1} $TCID_{50}$/mL

Sample processing in the Liat Tube

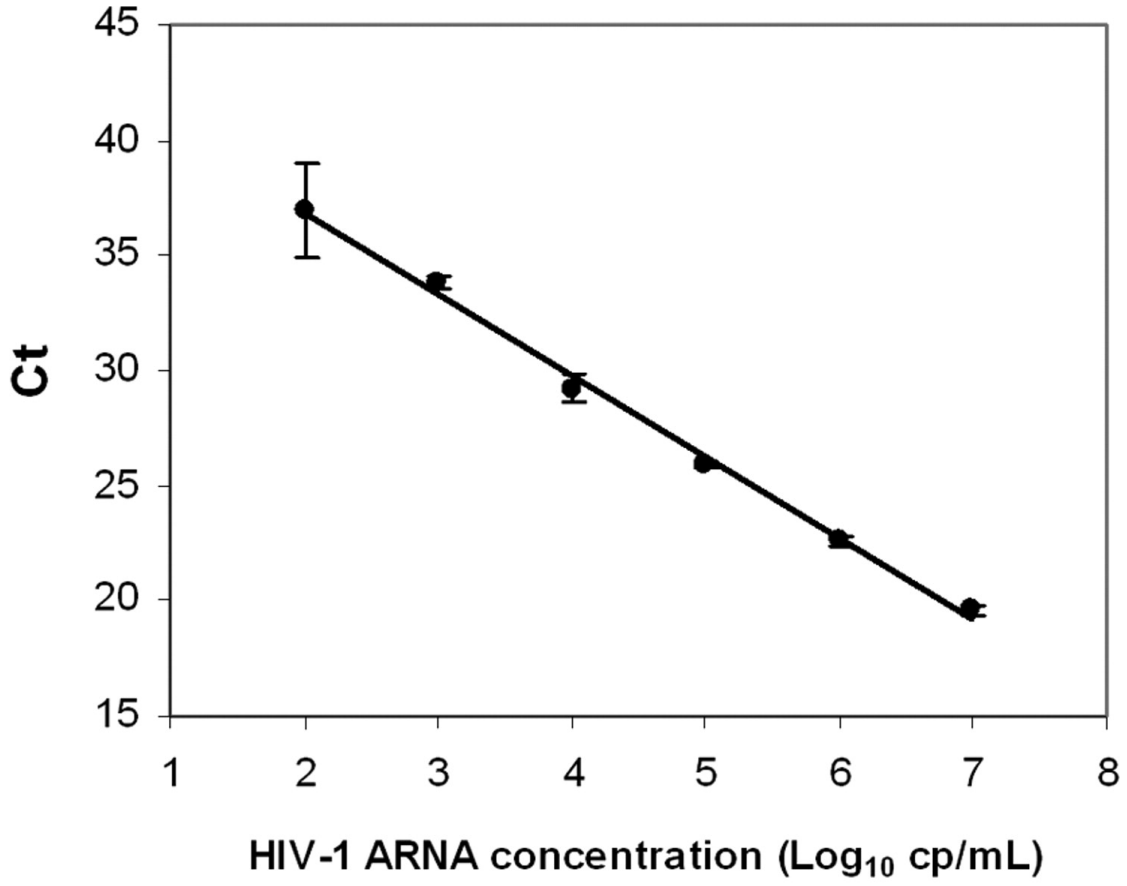


Liat HIV Quant Assay amplification plot



Tanriverdi, Chen, Chen. J Infect Dis. 2010;201:S52-S58

Linearity of the Liat HIV Quant Assay



Tanriverdi, Chen, Chen. J Infect Dis. 2010;201:S52-S58

GeneXpert - Cepheid

75 minutes to results *

- 2 min hands on time

Broad molecular menu

- 11 FDA approved assays

Footprint 3.7 x 12 x 11.7”

8.8 lbs

Battery powered

- WAIVED - Xpert Xpress Flu/RSV (N & NP), Strep A & SARS-CoV-2 EUA– 18-30 min

Flu A/B – Nasopharyngeal

- Sensitivity 100%/100%
- Specificity 96.8%/ 94.1%
- LOD 10^{-2} – 10^{-1} / 10^{-3} – 10^{-1} $TCID_{50}$/mL

RSV

- Sensitivity 97.0%
- Specificity 98.7%
- LOD 4 CFU/mL

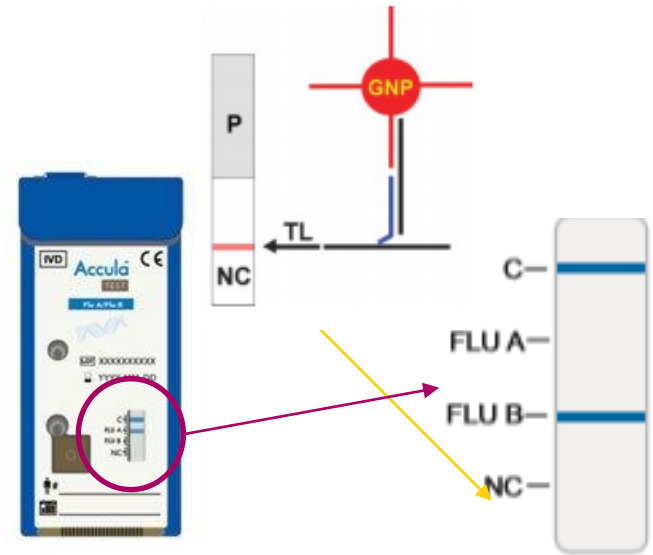
Strep A

- Sensitivity 98.3%
- Specificity 94.2%
- LOD 5-20 CFU/mL

SARS-CoV 2/Flu A/B – NP,OP,MT,AN

- PPA -92.8% (88.4 – 99.6)
- NPA -95.6% (85.2%-98.8%)
- LOD – $5x10^{-3}$ –$2x^{-2}$ –PFU/mL

Accula- Mesa Biotech



Visual Read

30 minutes to results

< 2 min hands on time

Footprint 5.7 x 3.9 x 3.8

Weight <1.4 lbs

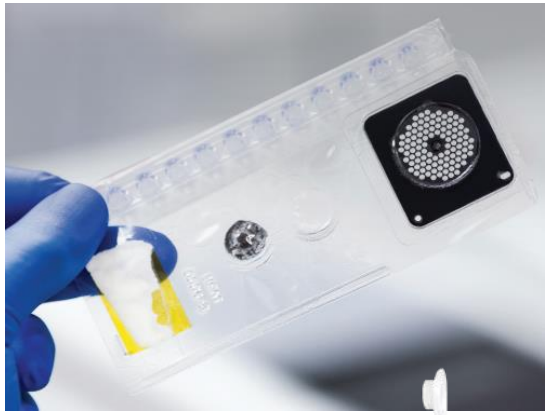
Strep A, RSV, SARS-CoV-2(MT, AN) – Waived

- SARS LoD – 150 copies/mL
  PPA 95.8% (78.9-99.9%)
  NPA 100% (86.8-100%)

Flu A/B – Waived – Naso & NP

- Sensitivity 97% / 94%
- Specificity 94% / 991%

BioFire Diagnostics



45-60 minutes to results

Footprint 25.4 x 39.3 x 16.5cm

Weight 40 lbs

Respiratory Panels – 19 tests

Flu A/B – Waived – Naso & NP

- Sensitivity 97% / 94%
- Specificity 94% / 991%

SARS CoV-2 – Waived (EUA) – NP, OP, MT, AN

- LoD 330GE/mL
- PPA 99.7% (88.2-99.6%)
- NPA 100% (95.8-100%)

Visby COVID-19 Point of Care Test

30 minutes to results

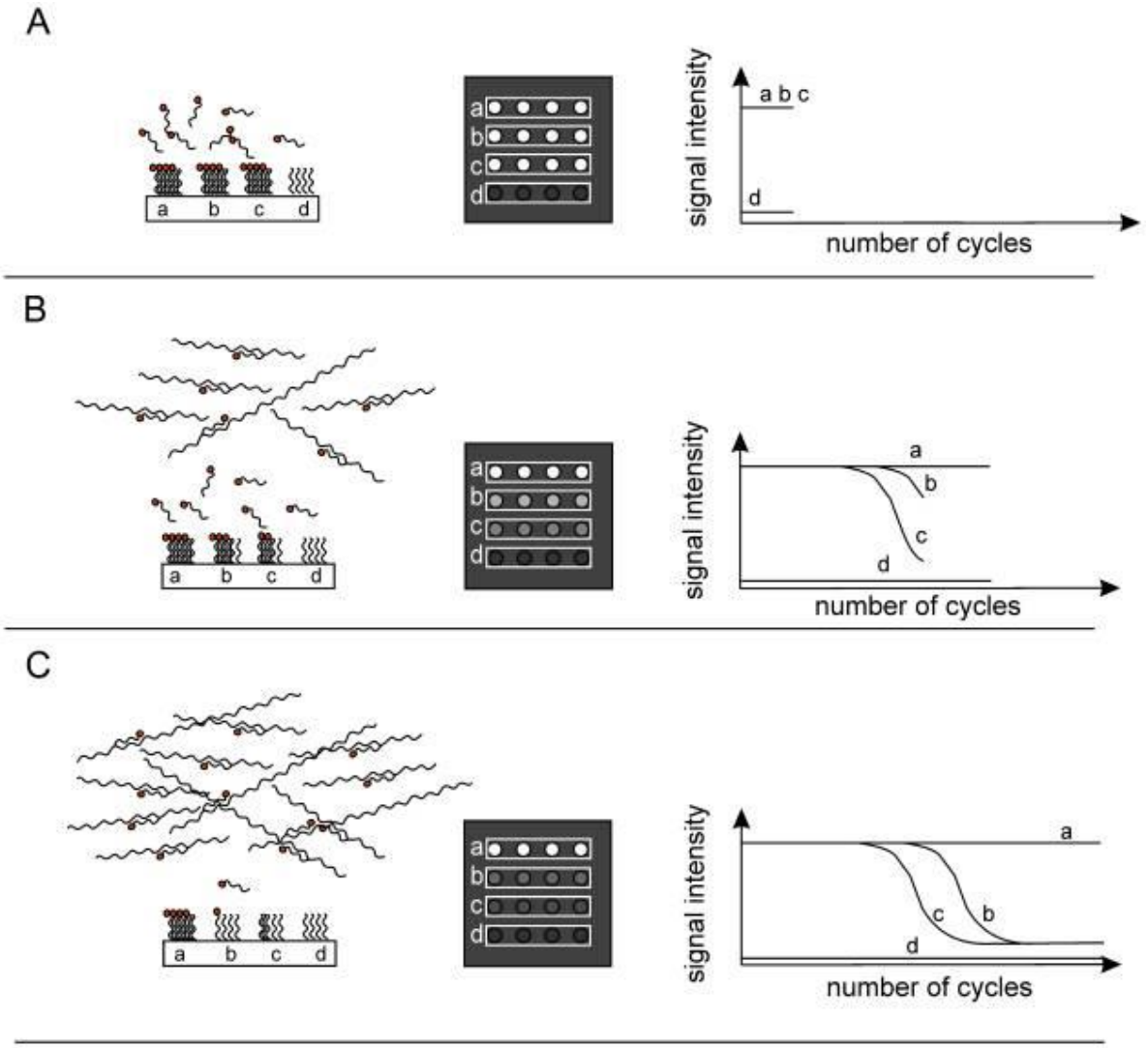
Nasopharyngeal, Mid-turbinate or Anterior Nasal Specimen Collection Swab

LoD – 435 genomic copies/swab

SARS CoV-2 –NP, AN, MT - Waived (EUA)

- PPA 100.0% (95% CI: 89.0%-100.0%)
- NPA 95.3% (95% CI: 87.1%-98.4%)

Competitive Reporter Monitored Amplification



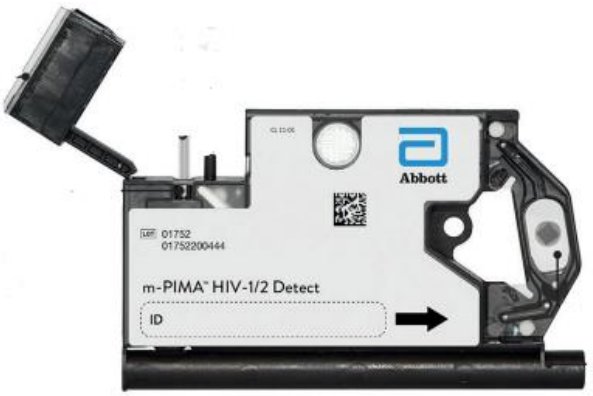
m-PIMA

- Portable bench-top real time (rt) Reverse Transcriptase (RT) PCR system for processing and analysis of Alere q HIV-1/2 test cartridges
- 50 minutes to results
- 7.8 kg (3.5 lbs)
- In-built battery to seamlessly bridge power outages

Not Available in US

The **m-PIMA** HIV-1/2 Detect Cartridge

- Qualitative measurements of HIV-1 (subtypes M/N and O) and HIV-2
- Low sample volume - only 25 µl of capillary/EDTA venous whole blood or plasma
- All reagents and controls enclosed in the test cartridge
- No manual sample processing
- Fully automated capture and enrichment of the specific RNA target, reverse transcription and real time PCR
- High speed target amplification and real time multiplex detection based on CMA (**C**ompetitive reporter **M**onitored **A**mplification) assay format



Isothermal Molecular Technologies

cHDA : Circular Helicase-dependent amplification
HDA : Helicase-dependent amplification
IMDA : Isothermal multiple displacement amplification
LAMP : Loop-mediated isothermal amplification
MPRCA : Multiply-primed rolling circle amplification
NASBA : Nucleic acid sequence based amplification
NEAR: Nicking enzyme amplification reaction
RAM : Ramification amplification method
RCA : Rolling circle amplification
RPA : Recombinase polymerase amplification
SDA : Strand displacement amplification
SMART : Signal mediated amplification of RNA technology
SPIA : Single primer isothermal amplification
TMA : Transcription mediated amplification

Isothermal Molecular Technologies

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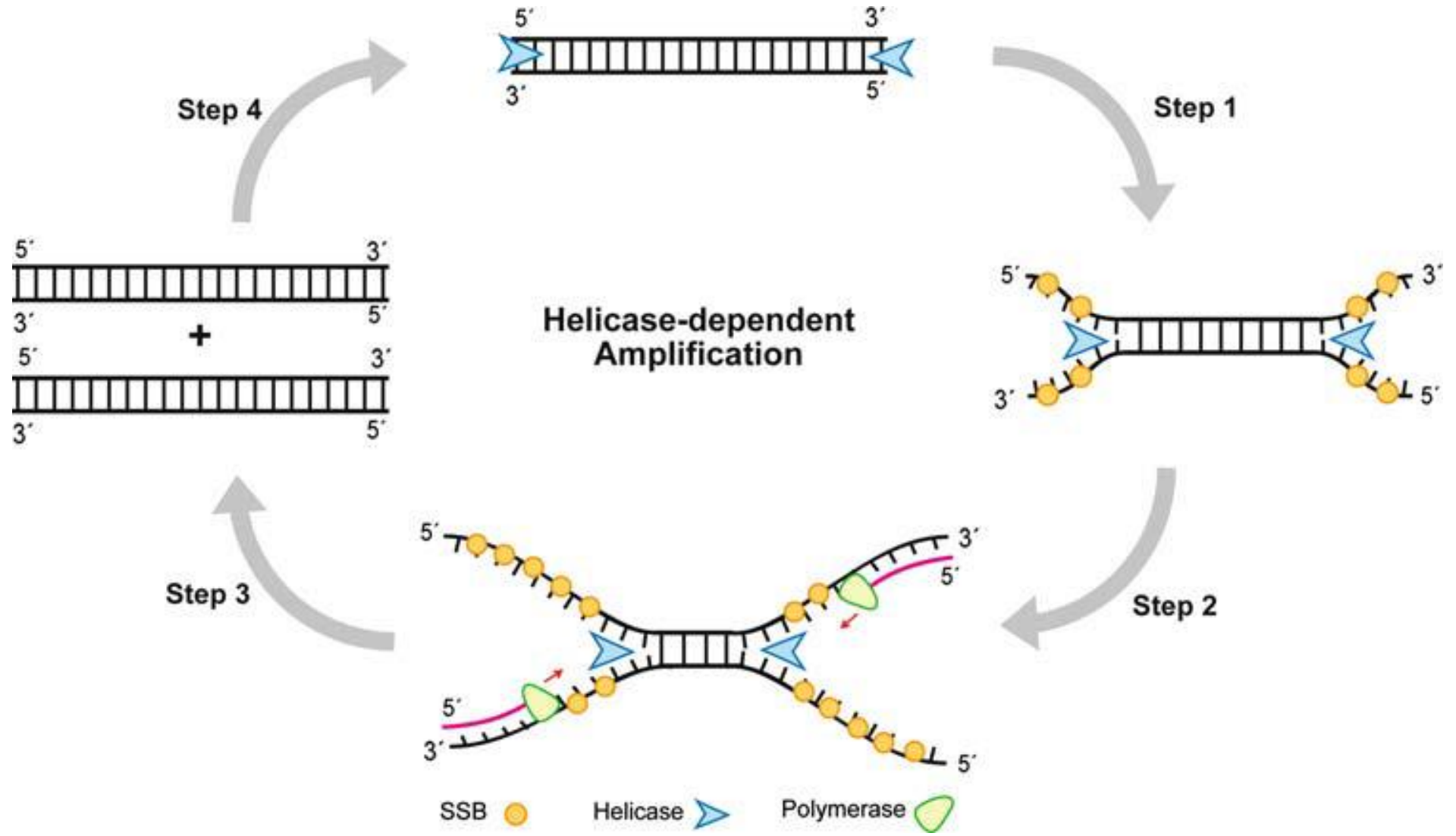
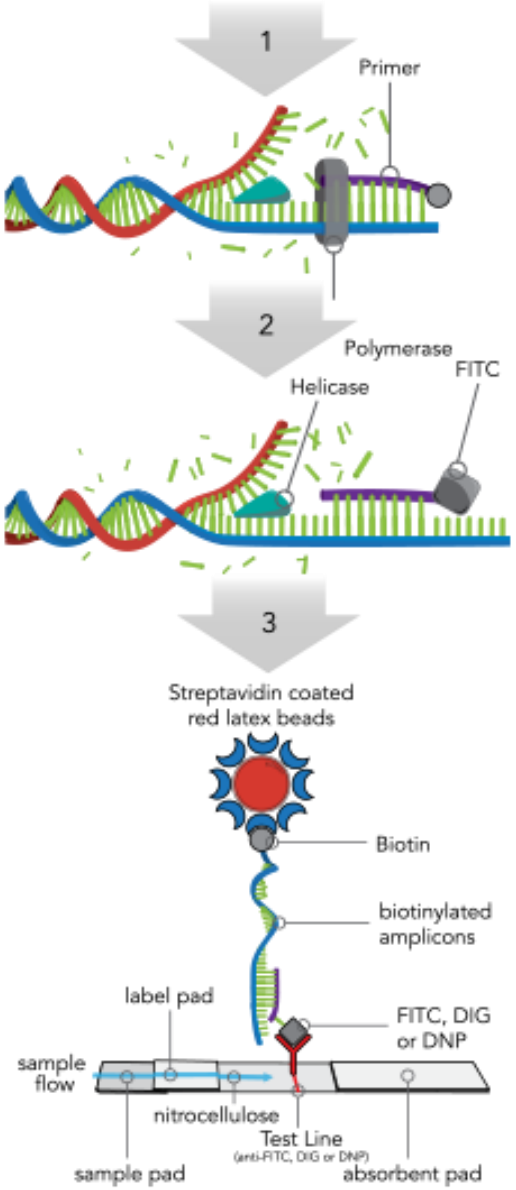

Helicase-dependent Amplification
Step 1
Step 2
Step 3
Step 4
5′
3′
SSB
Helicase
Polymerase

Helicase Dependent Amplification Assays



Solana - Quidel

35 minutes to results

- Including heat pretreatment step

< 2 minutes hands on time

Small footprint (9.4” x 9.4” x 5.9”)

8.8 lbs

Flu A/B (N & NP), C. difficile, GAS, GBS, HSV 1&2, HMPV, Pertussis, RSV, VZV, Trichomonas, SARS-CoV-2 (N & NP)

- GAS Sensitivity – 98.0%
- GAS Specificity – 97.7%
- GAS LOD – 400-430 CFU/mL
- SARS PPA – 97.8% (92.3-99.4%)
- SARS NPA – 99.5% (97.2-99.9%)
- SARS LOD – 1.16 x 10^4 cp/mL

Solana - Quidel

Step 1
Specific primers bind to target sequences that have been separated by the helicase.

Step 2
Specific DNA probes labeled with a quencher on one end and a fluorophore on the other end bind to the single-stranded biotinylated amplicons.

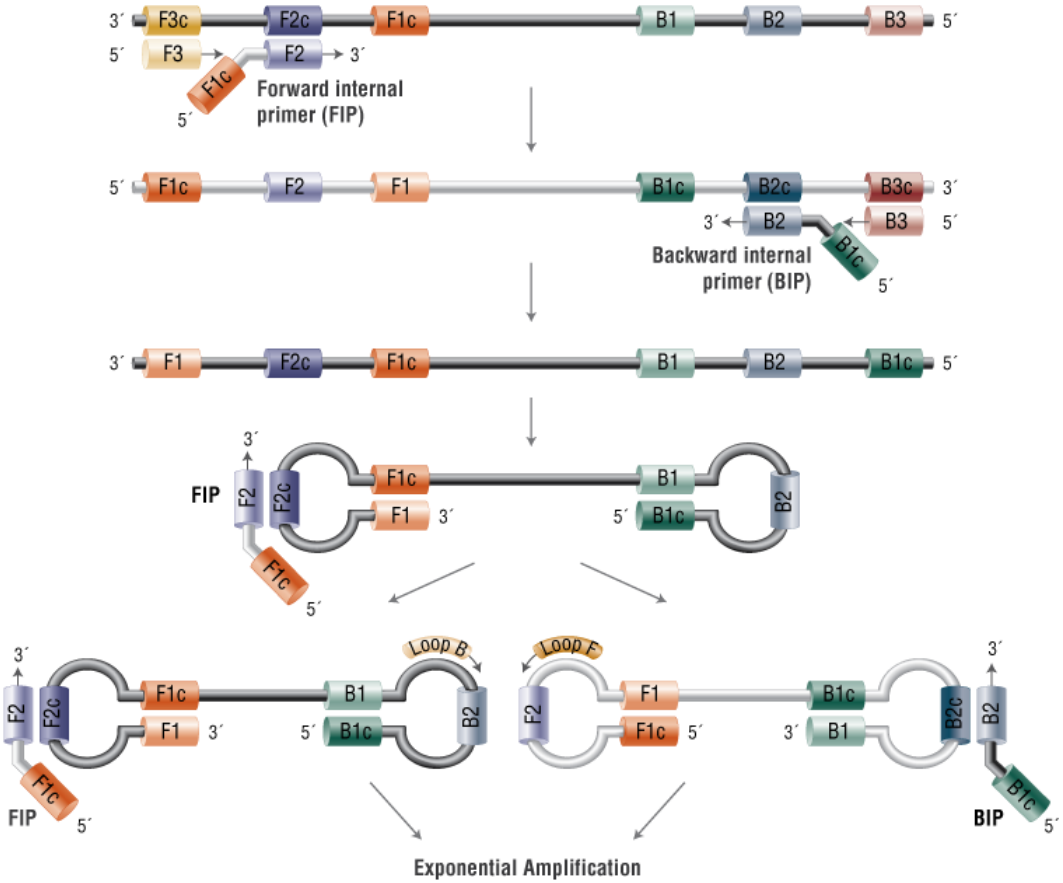
Step 3
Upon annealing to the amplicons, the fluorescence probes are cleaved and the fluorescence signal increases due to physical separation of fluorophore from quencher.

Loop Mediated Amplification

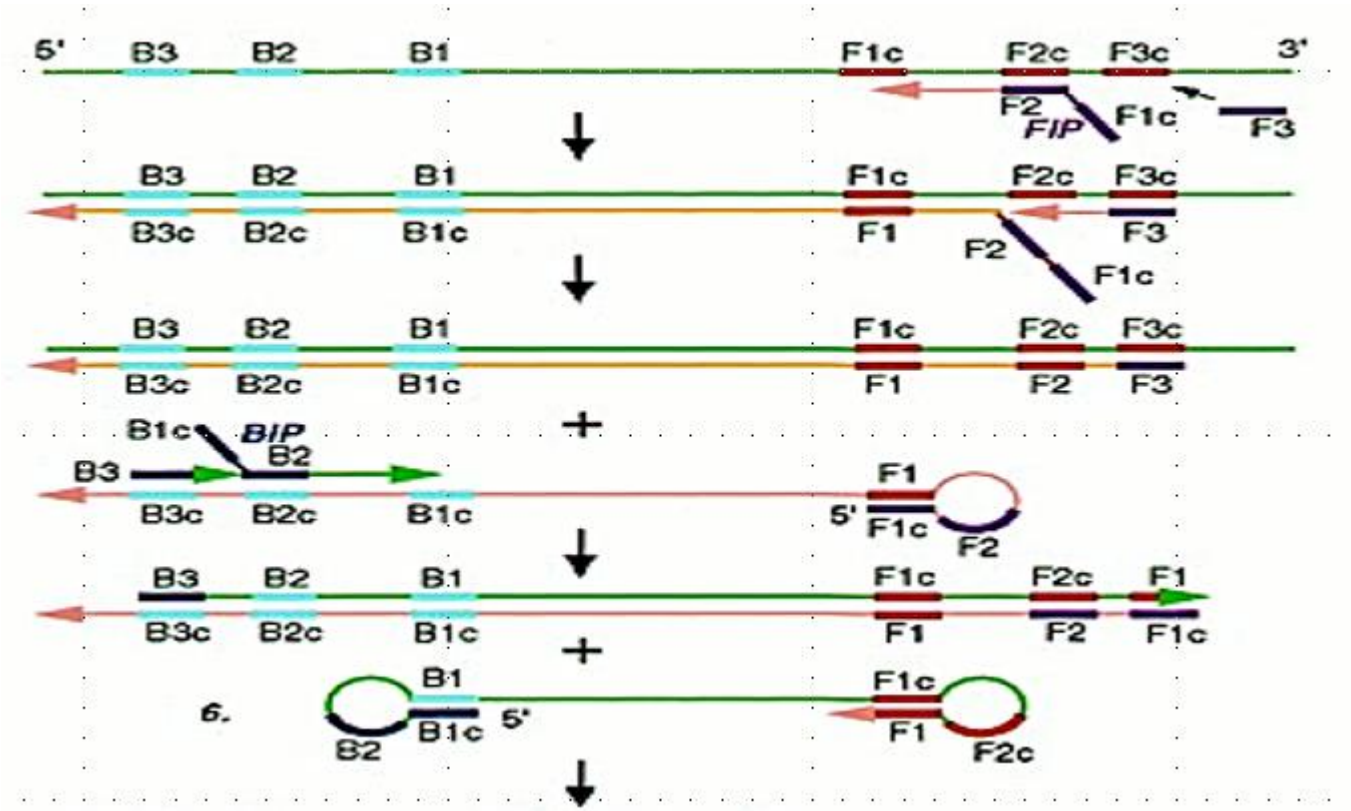
Use of 4–6 different primers to recognize 6-8 distinct regions

Outer primers are known as F3 and B3

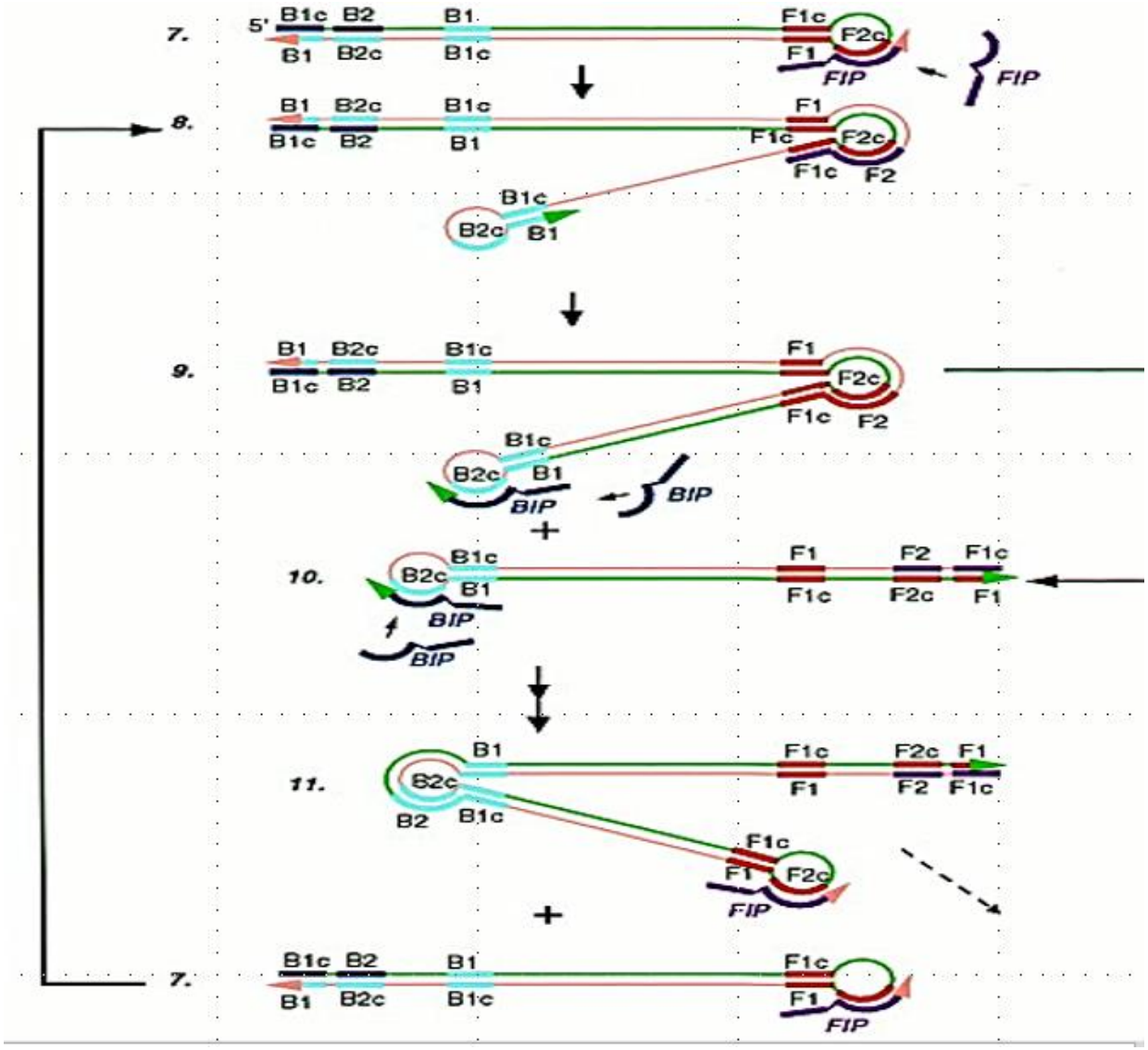
Inner primers are forward inner primer (FIB) and backward inner primer (BIP)



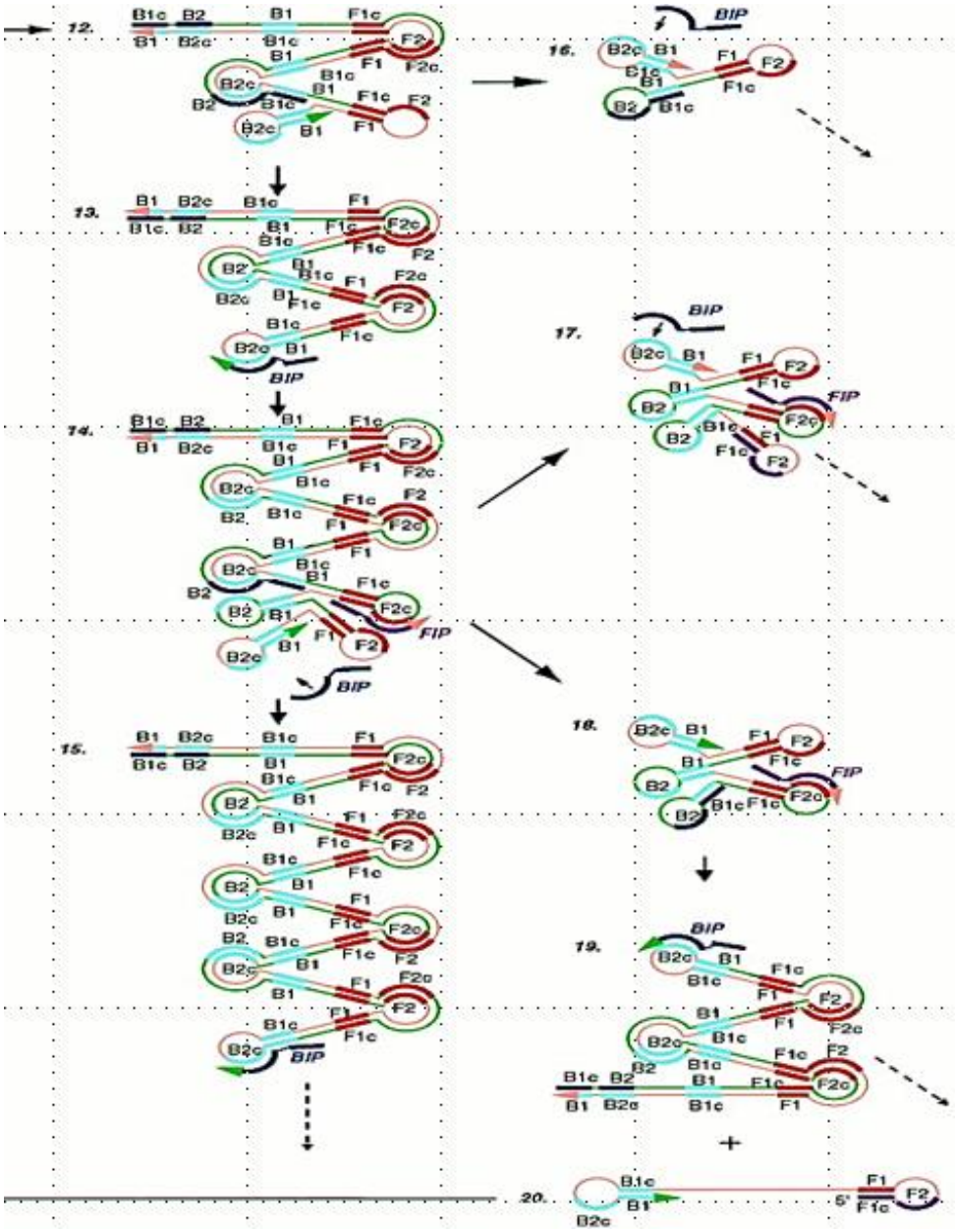
LAMP – Starting Material Production Step



LAMP – Cycle Amplification Step



LAMP – Elongation & Recycling Step



Advantages of LAMP

- Isothermal conditions and no sophisticated equipment required
- Both amplification and detection can be completed in single step
- High efficiency
- No need for DNA purification
- Visual detection

LAMP final products are stem loop DNAs

The final products are stem loop DNAs with several inverted repeats of the target and cauliflower-like structures with multiple loops due to hybridization between alternately inverted repeats in the same strand

Positive LAMP reactions can be visualized with the naked eye

Alethia (formerly ***illumi***gene) – Meridian Bioscience

< 60 minutes to results

- Including heat pretreatment step

< 2 minutes hands on time

Small footprint (8.3” x 11.5” x 3.7”)

6.5 lbs

Room temp storage

7 FDA approved tests – C. difficile, GAS, CMV,GBS, HSV 1&2, Mycoplasma, Pertussis

- GAS Sensitivity – 98.0%
- GAS Specificity – 97.7%
- GAS LOD – 400-430 CFU/mL

Lamp Based COVID-19 POC Diagnostics with EUAs

Cue – 20 minutes,NS, 99% sens, 98% spec, LOD-20 virions

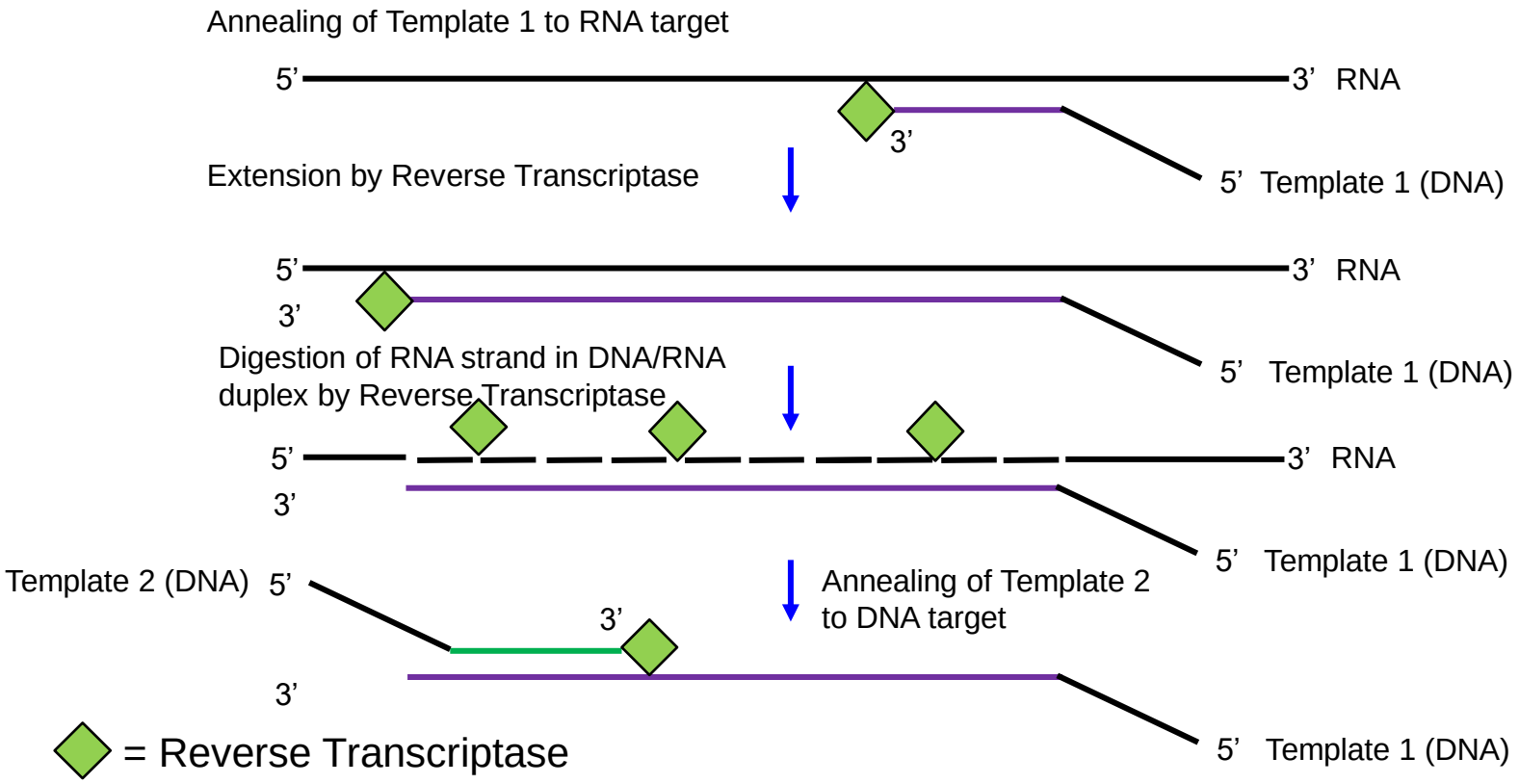
WAIVED

Lucira – 30 minutes, 94% PPA, 98% NPA, LOD-900 cp/mL home use

WAIVED

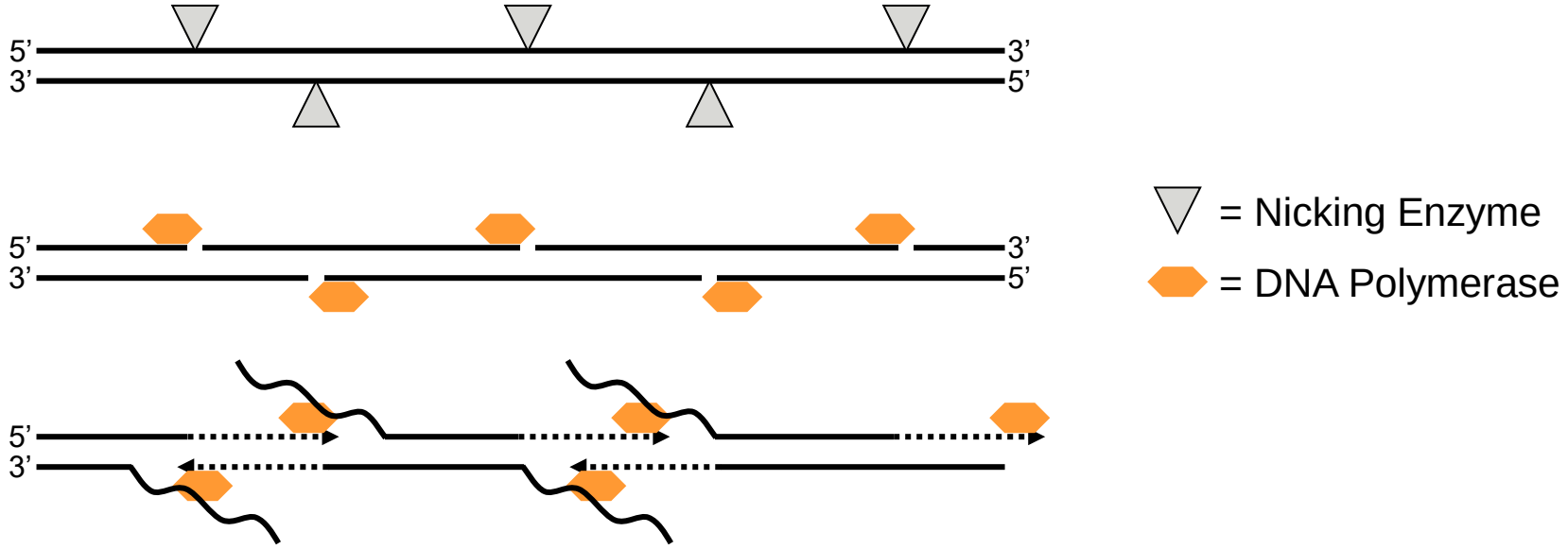
NEAR Mechanism – Amplification from RNA

- **NEAR amplifies target sequence directly from single stranded RNA**
  - No heat denaturation required
  - Reverse transcriptase, DNA polymerase & Nicking endonuclease
  - Converts single stranded RNA to single stranded DNA

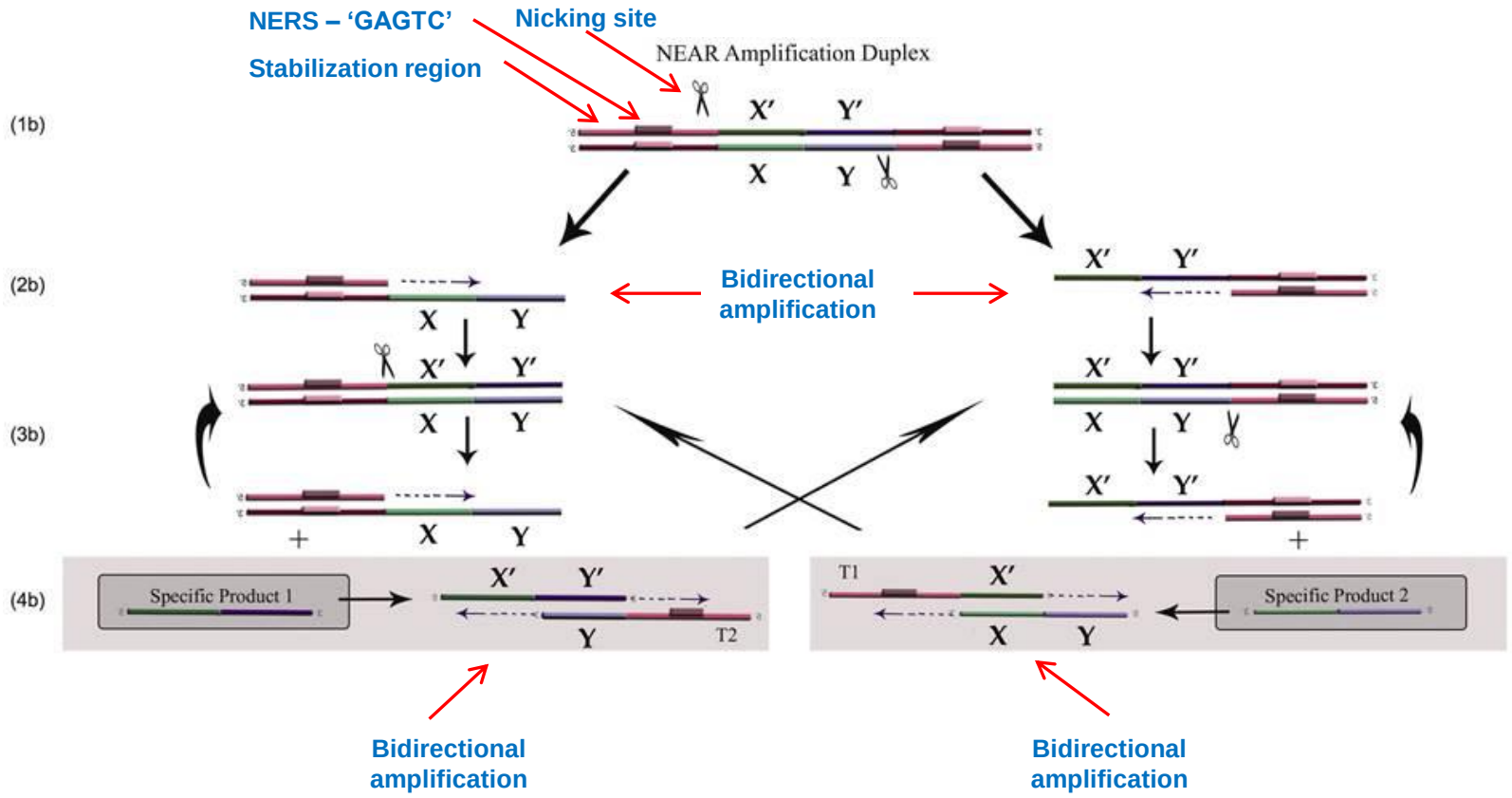


NEAR Mechanism – Amplification from dsDNA

- **Assay amplifies target sequence directly from double-stranded genomic DNA**
  - No heat denaturation required
  - Nicking Enzyme, DNA Polymerase
  - Creates single-strand copy of genome



NEAR Amplification Duplex – Bidirectional Amplification



ID NOW System

- < 15 minutes to results
- < 2 minutes hands on time
- Small footprint (8.15” W x 5.71” H x 7.64” D)
- 1.4 lbs. / 3 kg
- 3 approved tests – Flu A/B, GAS, RSV, SARS CoV-2 (EUA)
- Flu A/B – Nasal, Nasopharyngeal
- SARS CoV2 – Nasal, NP, Throat

WAIVED

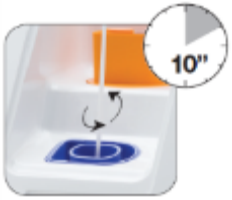
ID NOW System

Conditioning

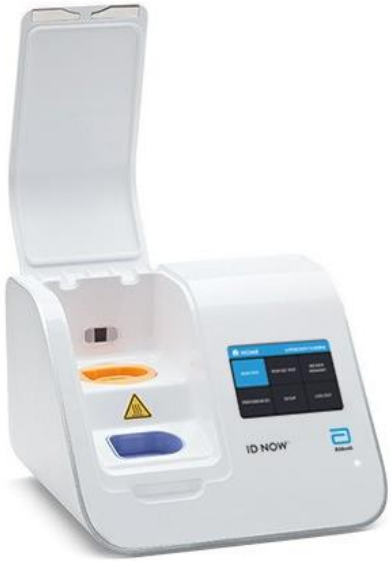
Acidic / Basic conditioning or enzymatic (Ply C)

Place test base & sample receiver in ID NOW™

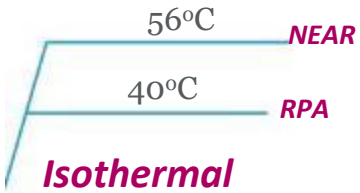
Add swab/VTM aliquot to sample receiver



Transfer conditioned sample to Test Base

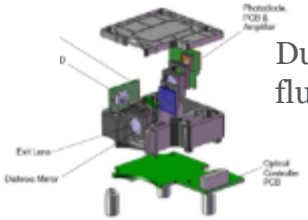


Amplification



Detection

Dual reaction tube



Dual channel fluorescence

Flu Clinical Trial Results

ID NOW Influenza A & B Performance vs. Culture

Flu A

		Culture +	Culture -
ID NOW	+	*92*	*66*
ID NOW	-	*2*	*411*

Sensitivity = 97.9% (92.6-99.4)
Specificity = 86.2% (82.8-89.0)

Flu B

		Culture +	Culture -
ID NOW	+	*74*	*17*
ID NOW	-	*6*	*472*

Sensitivity = 92.5% (84.6-96.5)
Specificity = 96.5% (94.5-97.8)

ID NOW Influenza A & B Performance vs. RT-PCR

Flu A

		RT-PCR +	RT-PCR -
ID NOW	+	*147*	*11*
ID NOW	-	*8*	*464*

Positive Percent Agreement = 94.8% (90.1-97.4)
Negative Percent Agreement = 97.7% (95.9-98.7)

Flu B

		RT-PCR +	RT-PCR -
ID NOW	+	*123*	*7*
ID NOW	-	*2*	*500*

Positive Percent Agreement = 98.4% (94.4-99.6)
Negative Percent Agreement = 99.4% (98.3-99.8)

SARS CoV-2 Test Agreement with the Expected Results by Sample Concentration Target Concentration

	# Concordant/ # Tested	% Agreement (95% CL)
2 x LOD	*20/20*	*100% (83.9 – 100%)*
5 x LOD	*10/10*	*100% (72.3 – 100%)*
NEGATIVE	30/30	*100% (72.3 – 100%)*

LoD
125 GE/mL

Post-Authorization Studies

Clinic

	PCR +	PCR -
ID NOW +	*21*	*0*
ID NOW -	*2*	*951*

PPA = 91.3%
NPA = 100%

Multi Site Urgent Care

	PCR +	PCR -
ID NOW +	*51*	*2*
ID NOW -	*2*	*375*

PPA = 96.2%
NPA = 99.5%

In-Patient

	PCR +	PCR -
ID NOW +	*75*	*24*
ID NOW -	*19*	*400*

PPA = 79.8%
NPA = 94.3%

Summary of POCT nucleic acid amplification methods

	Liat	m-PIMA	GeneXpert	Accula	BioFire	Visby
Technology	PCR	PCR	PCR	PCR	PCR	PCR
Waived	Y	N	Y/N	Y	Y	Y(EUA)
DNA Amplification	Y	Y	Y	Y	Y	Y
RNA amplification	Y	Y	Y	Y	Y	Y
"Denaturing" agent	Heat	Heat	Heat	Heat	Heat	Heat
Pretreatment Required	N	N	Y/N	N	Y	N
# of enzymes	1	1	1	1	1	1
Temp (^{o}C)	95/72 /57	95/72 /57	95/72 /57	95/72 /57	95/72 /57	95/72 /57
Time to Result (min)	<20	55	75	30	<60	30
Multiple Amplifications	Y	Y	Y	Y	Y	N

Summary of POCT nucleic acid amplification methods

	Solana	Alethia	Cue	Lucira	ID NOW
Technology	HDA	LAMP	LAMP	LAMP	NEAR
Waived	N	N	Y(EUA)	Y(EUA)	Y
DNA Amplification	Y	Y	Y	Y	Y
RNA amplification	Y	Y	Y	Y	Y
"Denaturing" agent	Helicase	Betaine	Betaine	Betaine	Restriction enzymes
Pretreatment Required	Y	Y	N	N	Y
# of enzymes	2	1	1	1	2
Temp (^{o}C)	64	60-65	60-65	60-65	52
Time to Result (min)	35	<60	20	30	<15
Multiple Amplifications	Y	N	N	N	Y

Specific Benefits of POC Molecular Testing in a Resource Limited Setting

Drastically improved TAT – no need to send to ref lab for confirmation

- Get results in real-time to act upon them

Same testing Standard of Care that would be found at tertiary care center

- Clinical confidence in test method/result for diagnosis

Clinic can perform testing that previously could only be done by specialized staff

Test accuracy and healthcare efficiency/lab stewardship

Offering new technology for community and outreach

QUESTIONS

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