ABBOTT RAPID DIAGNOSTICS

Abbott

# Molecular Diagnostics & Point of Care Testing

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Meeting Name
Location
day | month | year

# Disclaimers

- I am a salaried employee of  Abbott Rapid Diagnostics
- ARDx produces molecular testing devices for use at the POC
- All data in this presentation can be found in product labeling or peer reviewed literature
- This will be a balanced presentation describing the characteristics of various molecular POCT systems

# 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 symptoms
  - Cough
  - Fever
  - Chills
  - Difficulty breathing
- Influenza
  - Cough
  - Fever
  - Chills
  - Malaise

## Treatment is different

- Bacteria
  - Broad spectrum antibiotic
  - Narrow spectrum antibiotic
- Influenza
  - Antiviral
  - Treat symptoms only

## 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 and *C. difficile*

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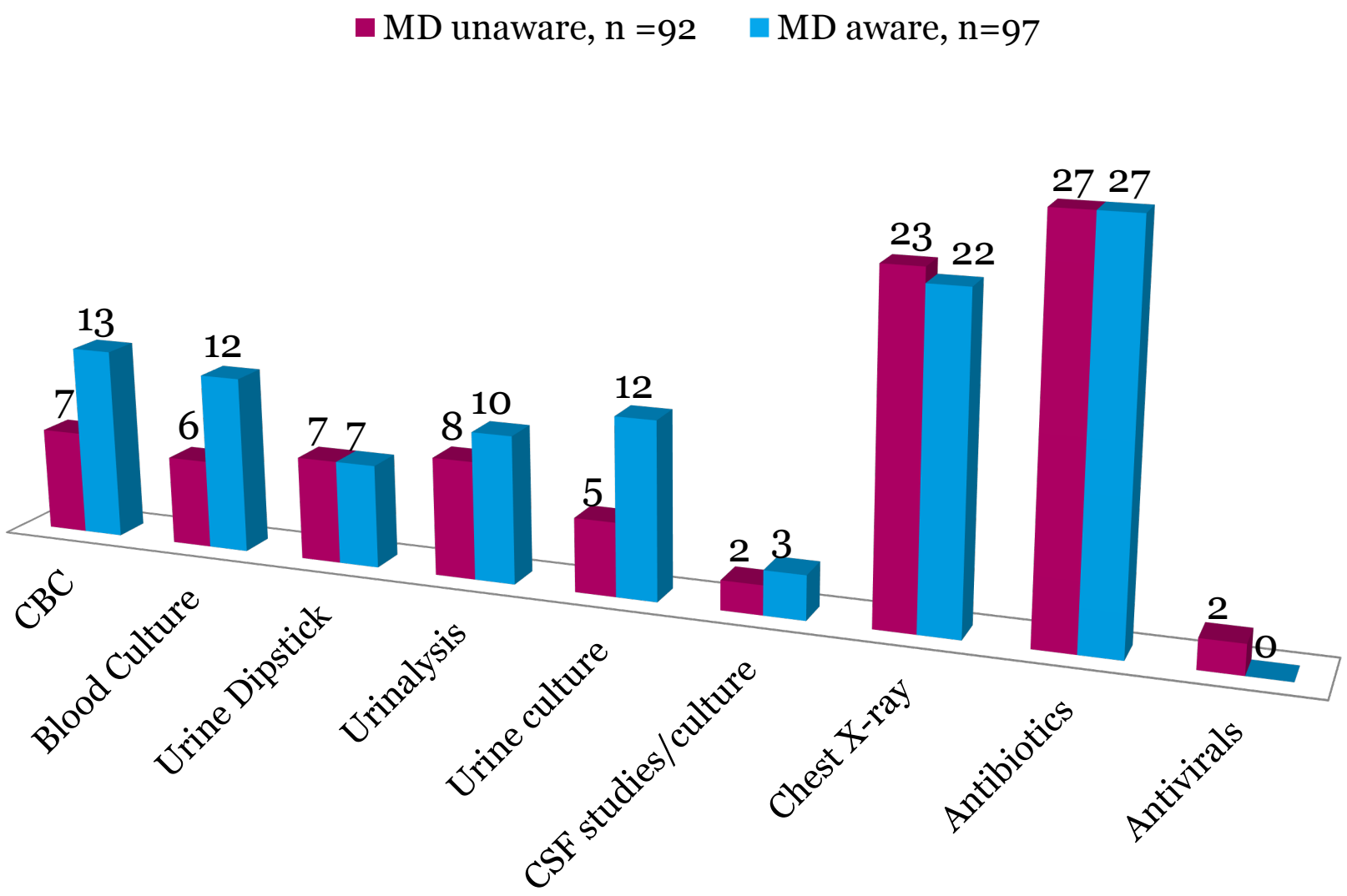
# Results – Flu Negative




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

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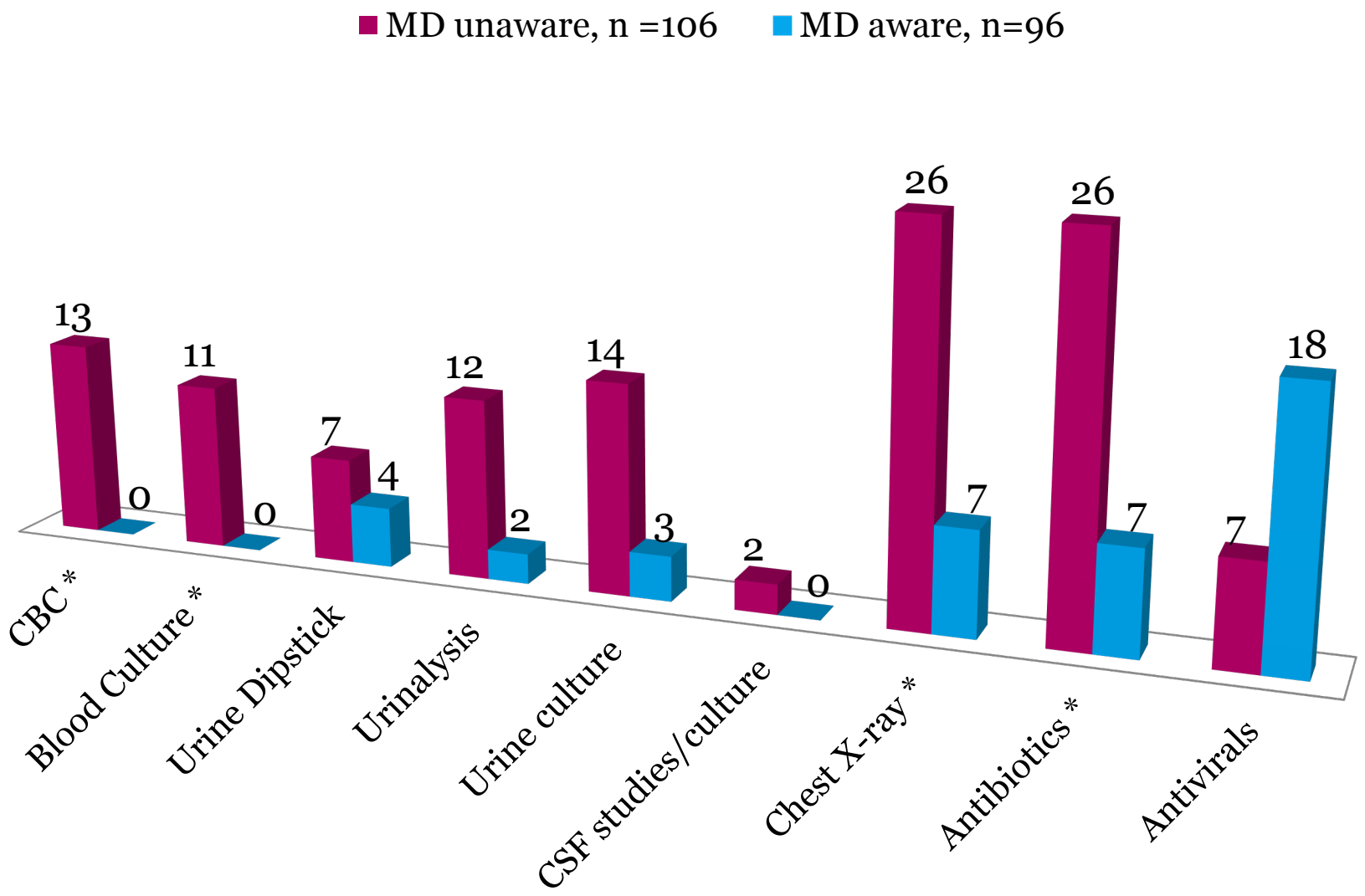
# Results – Flu Positive

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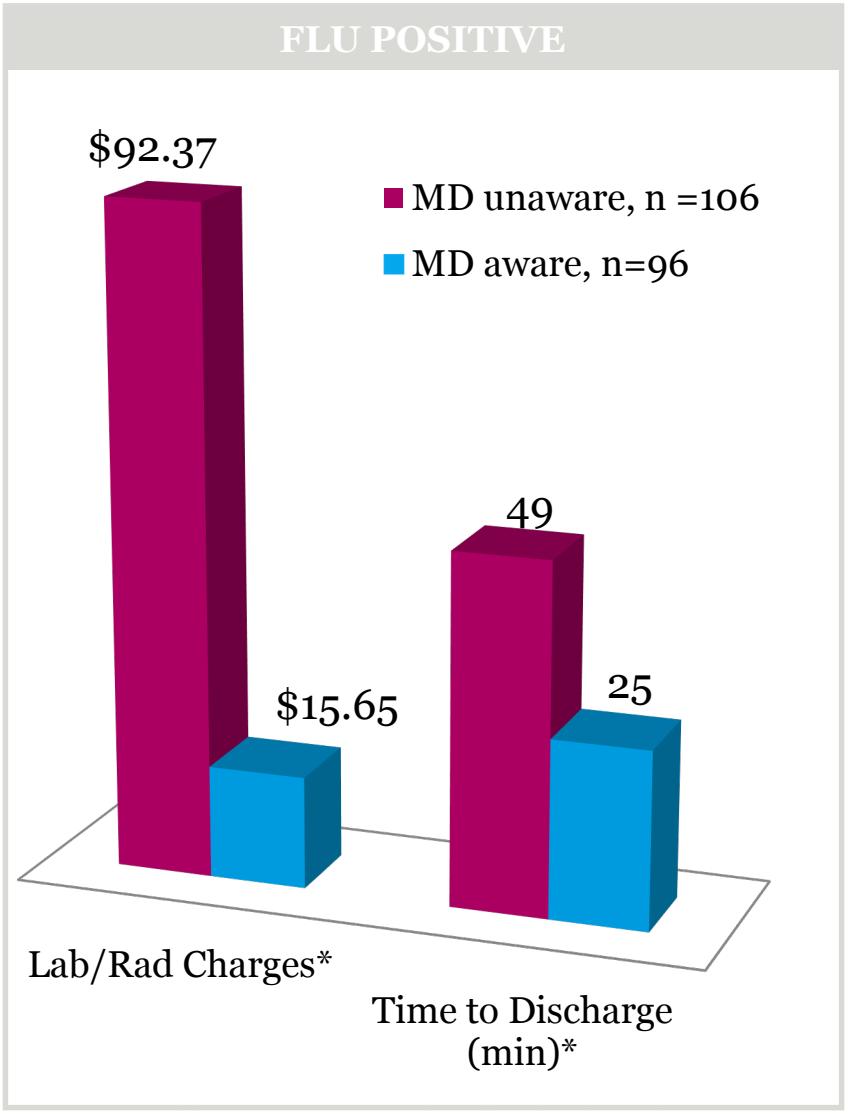
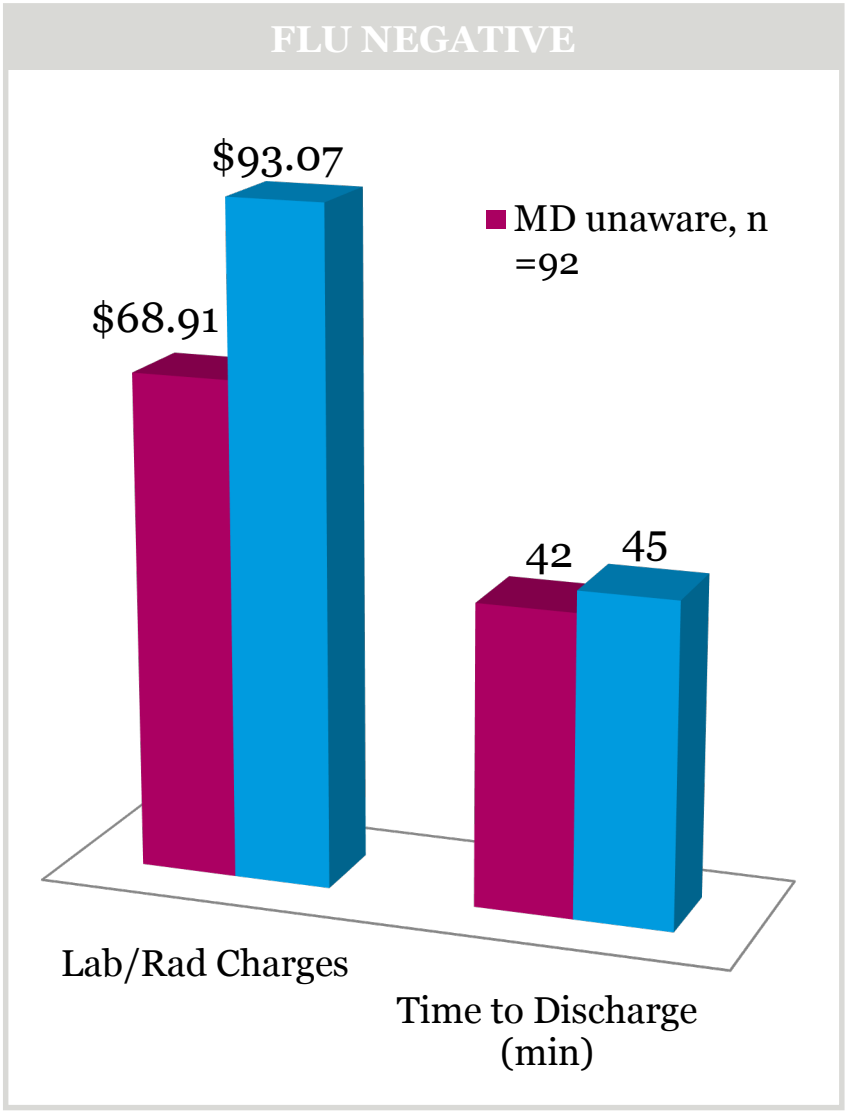
# Key Operational Metrics




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

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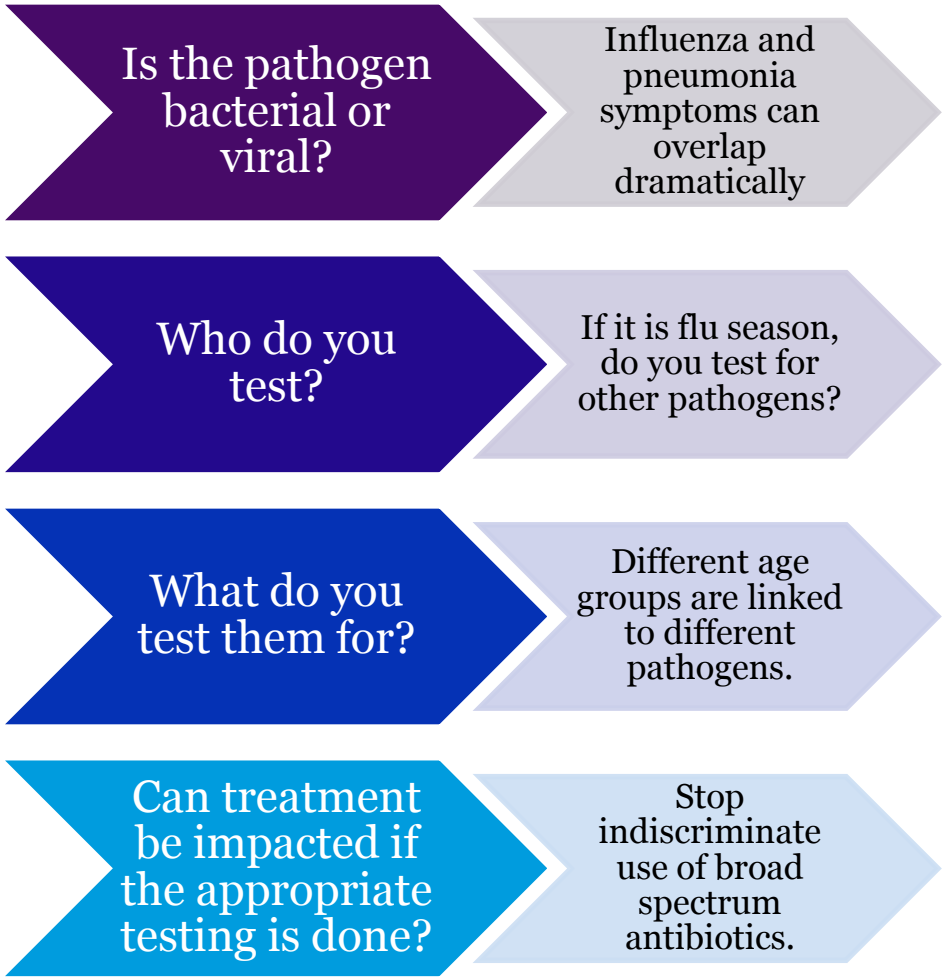
# Treating Respiratory Diseases in the Emergency Department

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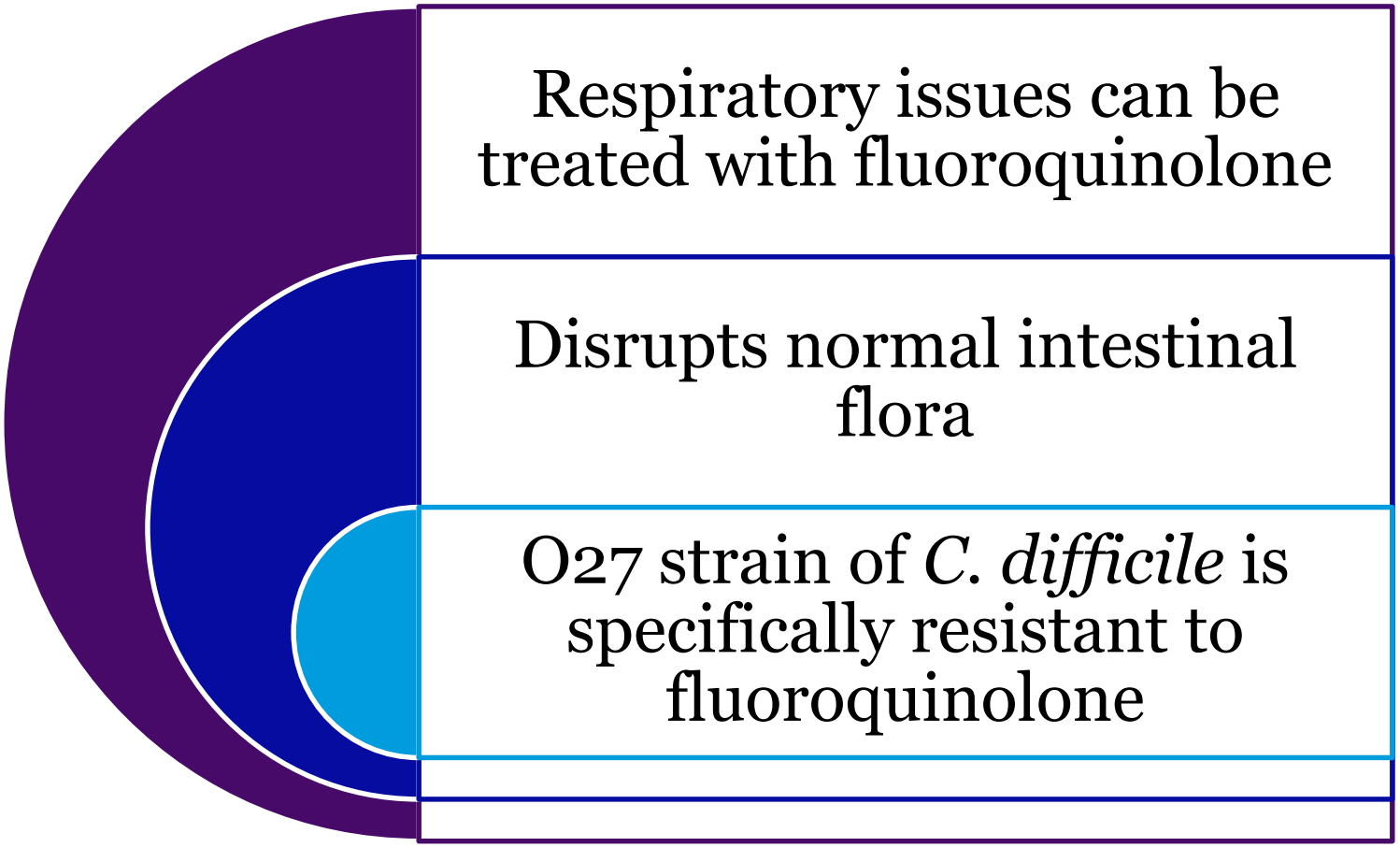
# Misuse of Antibiotics Can Lead to Other Medical Issues

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# Issues with Clinical Samples

Viral titer is highest in first 48 hours

Proper sample collection is necessary

Dilution in transport media

# Rapid 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.

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# Molecular Tests

## Pro

- For respiratory specimens, high performance
- Same day results

## Con

- Turn around time from lab may be extensive, especially if batching specimens
- Expensive
- May require experienced technicians, labs, dedicated equipment, etc.

# Pros and Cons of Molecular Testing

## Pros

Good for pathogens that you only have when you are sick

- Influenza

Good for living things which would have RNA/DNA

Good to see if active infection & can test where the infection is

- Not things like sepsis

## Cons

May only be a screen for bacteria/viruses that people may normally carry

- *Clostridium difficile*

Bad for non living things

- Protein, DOA

Bad for past infection

- Want test that detects antibody

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# 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,
  - **Alere™ q** - Competitive Reporter Amplification
  - Cepheid GeneXpert®
  - BD Affirm™ VPIII – direct probe
  - Biocartis Idylla™ – qPC
  - Enigma® MiniLab™ – qPCR - Flu A/B, RSV
  - Roche cobas® Liat – Lab in a tube
  - Spartan RX (PGx) – PCR

## Isothermal

- Rely on the ability to do the reaction at a single temperature
- Meridian ***illumi***gene® - LAMP (loop mediated isothermal amplification)
- Quidel Solana® – HDA (Helicase dependent amplification)
- **Alere™ i** – NEAR (Nicking enzyme amplification reaction)

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# 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

- realtime PCR

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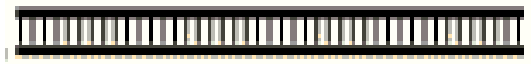
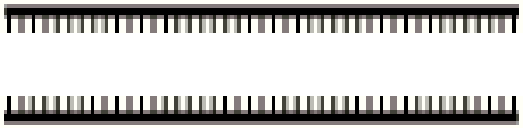
# PCR Cycle

**Double-stranded DNA**



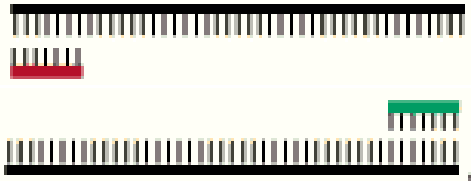
**95° Denaturation**

**Heating separates strands**

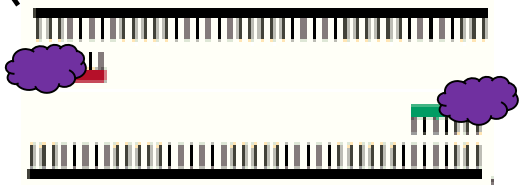


**72° Extension**

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



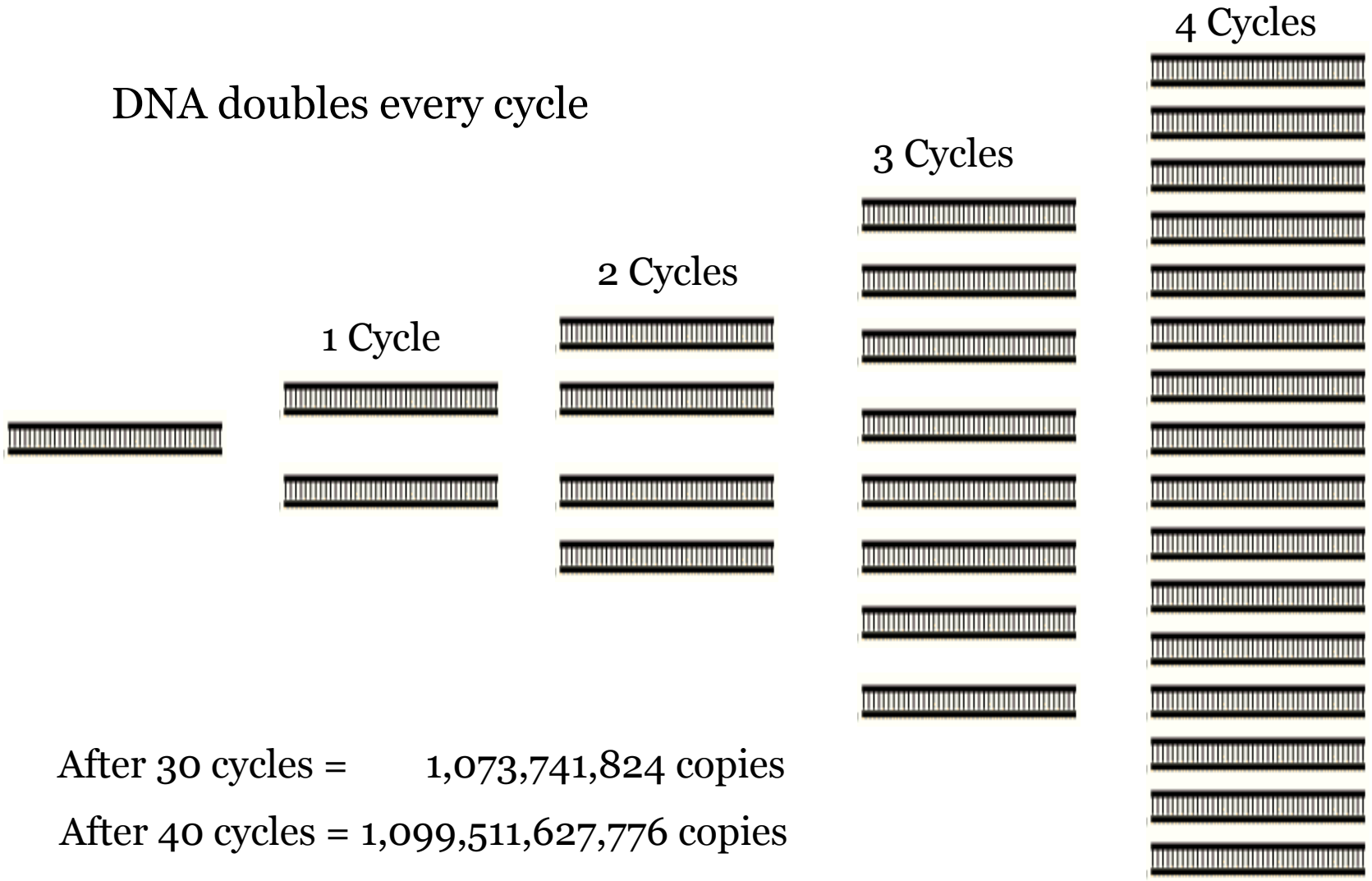
**57° Annealing**



**Taq Polymerase Binds at Primer Sites**

# PCR Amplification

DNA doubles every cycle




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

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

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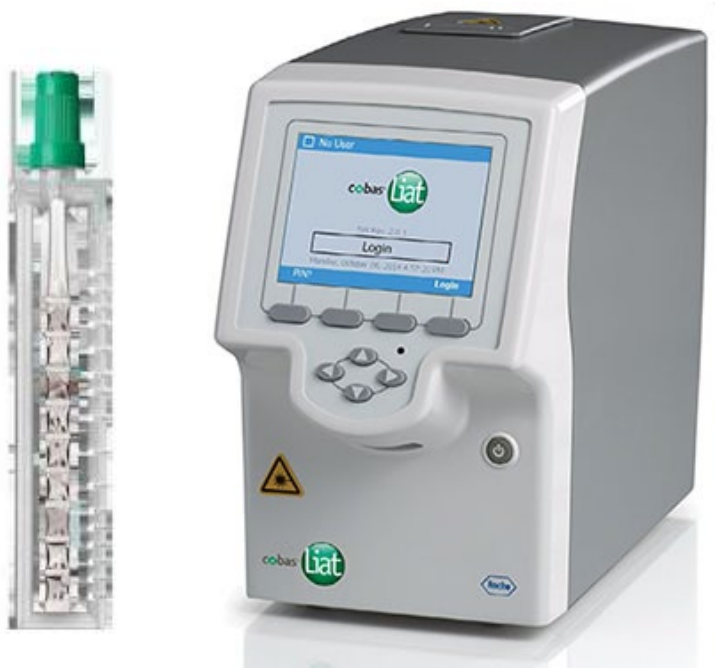
# Roche cobas® Liat - Lab In a Tube




20 minutes to results Flu

15 minutes to results Strep A

Footprint 4.5 x 9.5 x 7.5

Weight 8.3 lbs

## Flu A/B

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

## Strep A

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

## RSV

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

Data sourced from Roche Product Labeling
Permission granted by Roche Diagnostics

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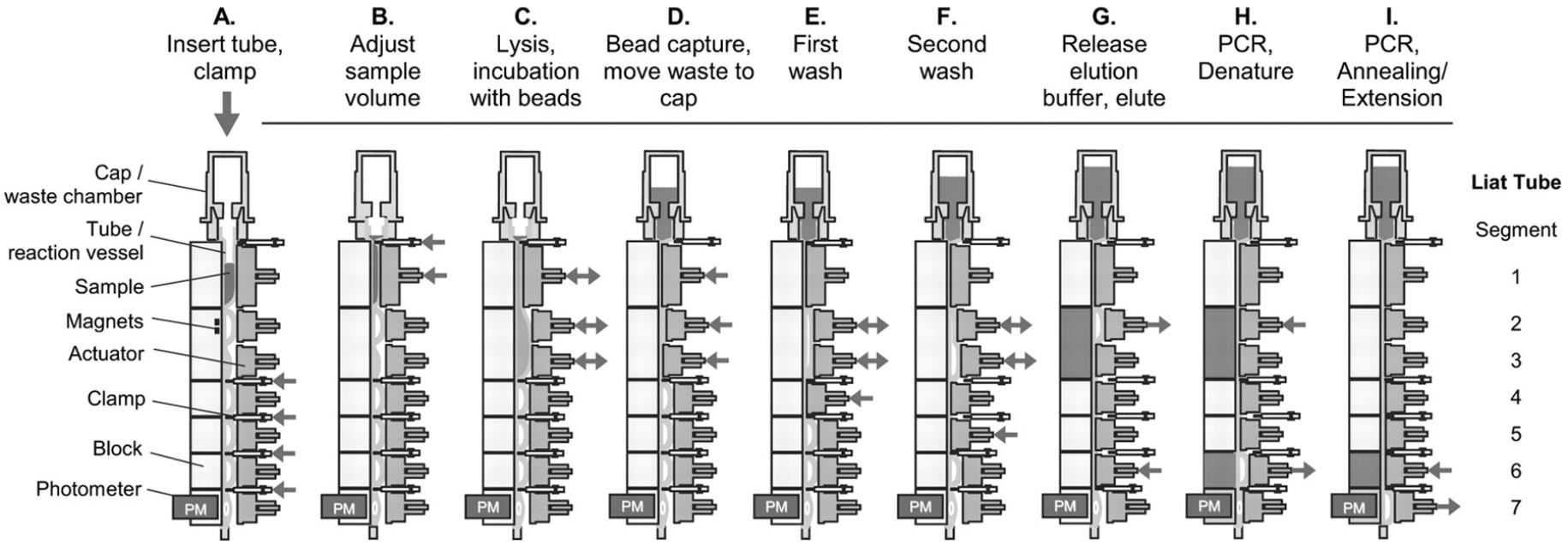
# Sample processing in the Liat Tube




Tanriverdi, Chen, Chen. A rapid and automated sample to result HIV load test for near patient application. J Infect Dis. 2010;201:S52-S58 by permission of Oxford University Press

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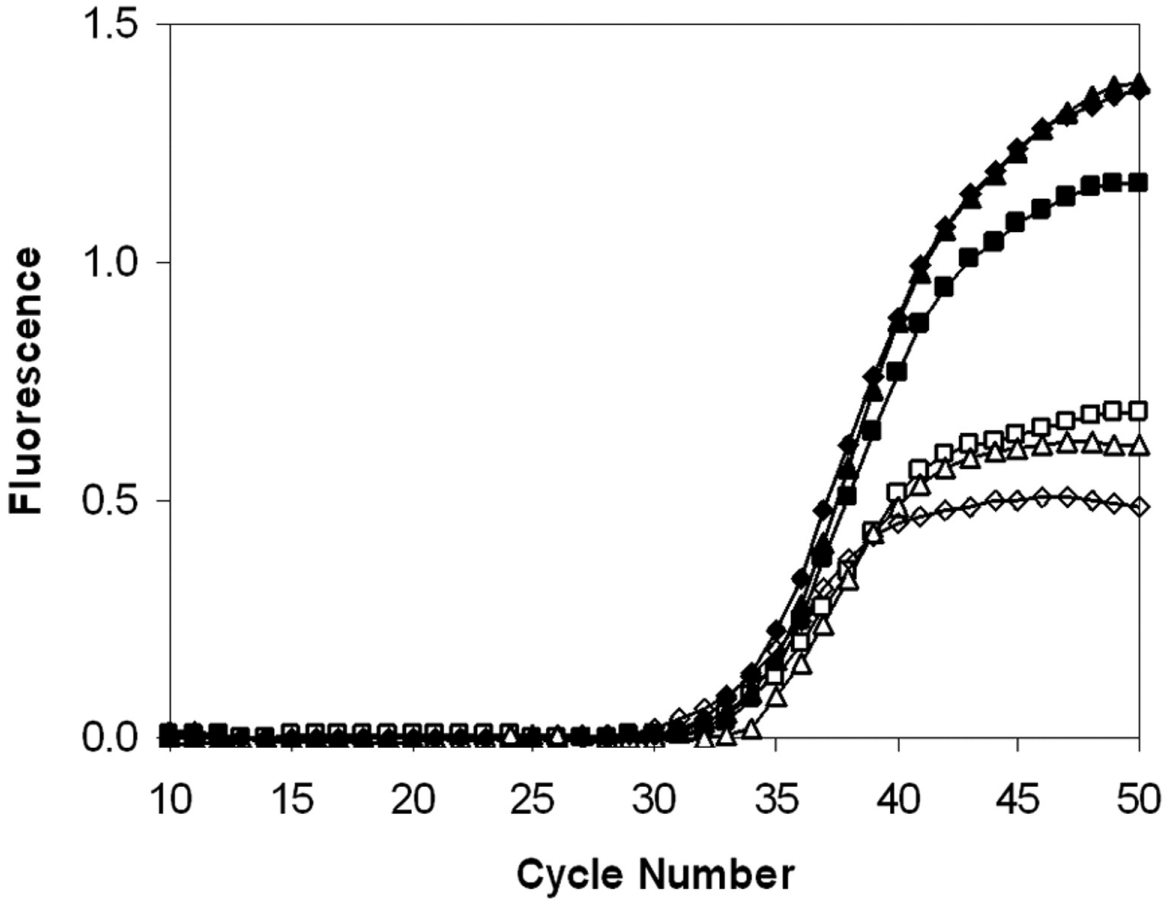
# Liat HIV Quant Assay amplification plot




Tanriverdi, Chen, Chen. A rapid and automated sample to result HIV load test for near patient application. J Infect Dis. 2010;201:S52-S58 by permission of Oxford University Press

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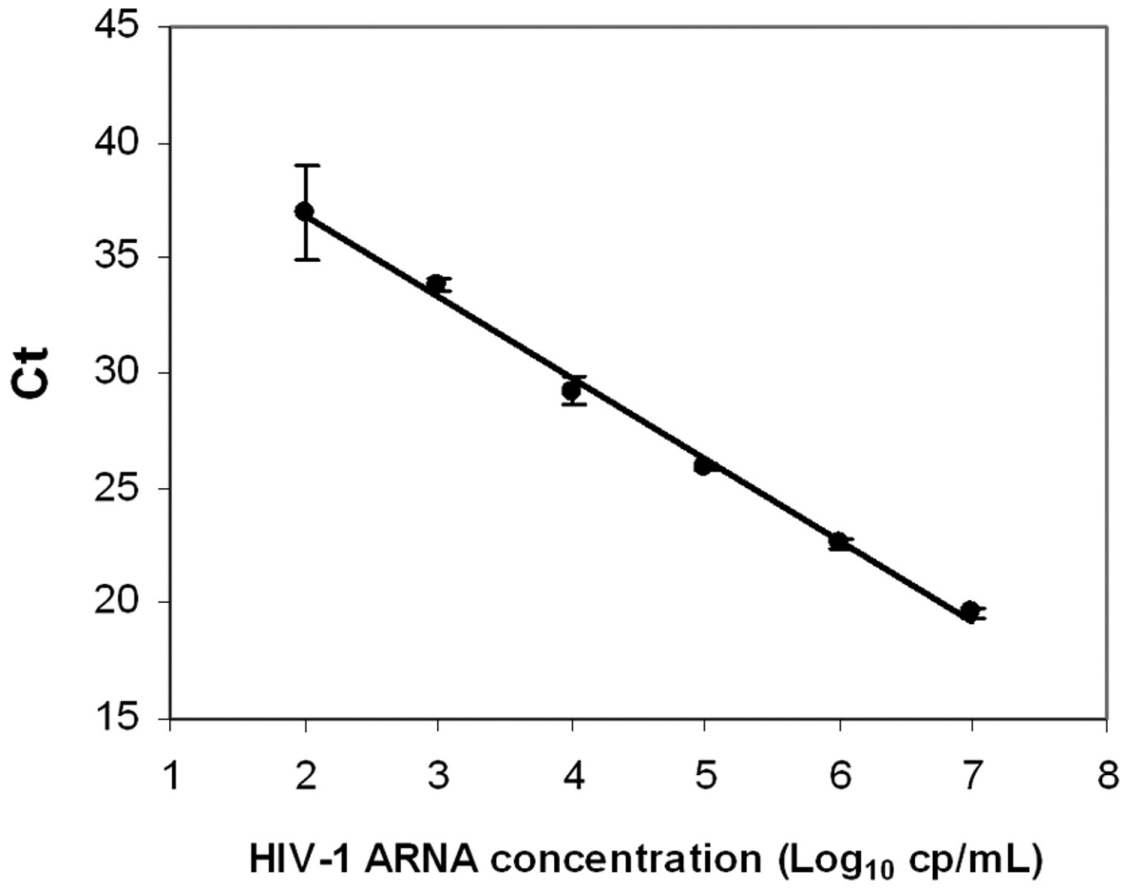
# Linearity of the Liat HIV Quant Assay

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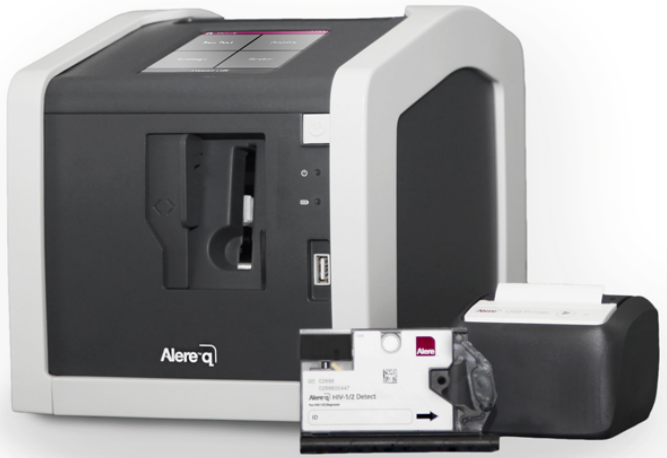
# The **Alere™ q**

- 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

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# The **Alere™ q** 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


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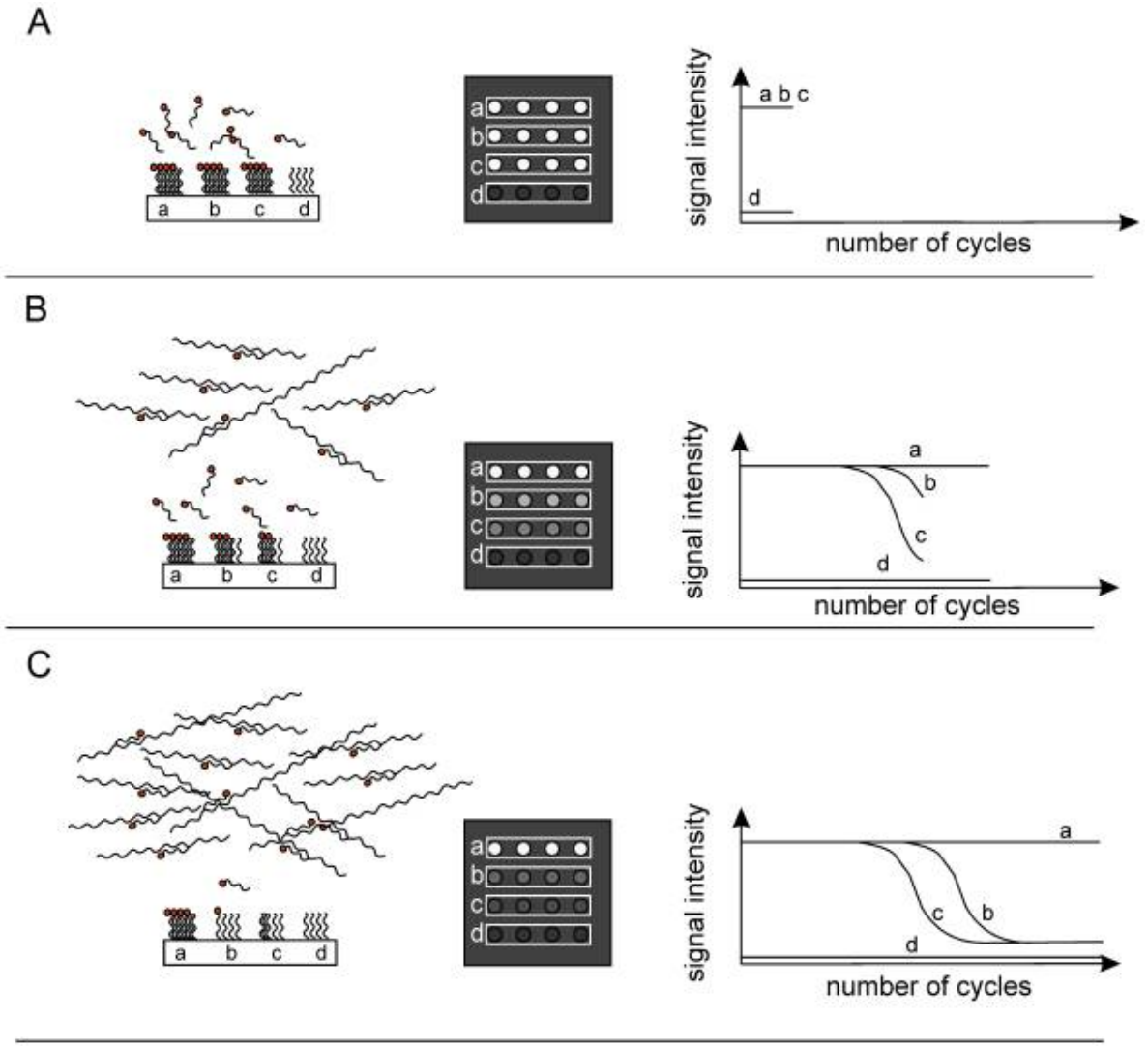
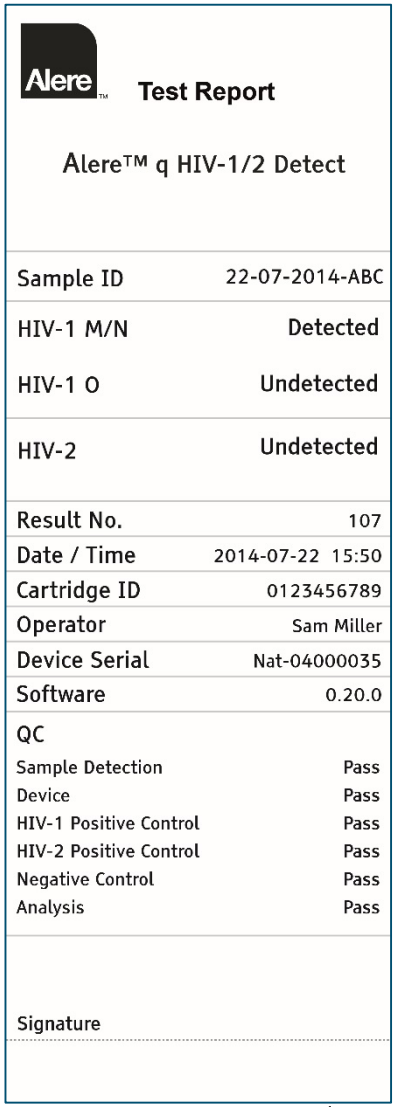
# Competitive Reporter Monitored Amplification




Permission granted from: Ullrich T et al (2012) Competitive Reporter Monitored Amplification (CMA) - Quantification of Molecular Targets by Real Time Monitoring of Competitive Reporter Hybridization. PLoS ONE 7(4): e35438. doi:10.1371/journal.pone.0035438

# Alere™ q HIV 1/2 Detect – Performing a Test



Alere™ Test Report

Alere™ q HIV-1/2 Detect

| | |
|---|---|
| Sample ID | 22-07-2014-ABC |
| HIV-1 M/N | Detected |
| HIV-1 O | Undetected |
| HIV-2 | Undetected |
| Result No. | 107 |
| Date / Time | 2014-07-22 15:50 |
| Cartridge ID | 0123456789 |
| Operator | Sam Miller |
| Device Serial | Nat-04000035 |
| Software | 0.20.0 |

QC

| | |
|---|---|
| Sample Detection | Pass |
| Device | Pass |
| HIV-1 Positive Control | Pass |
| HIV-2 Positive Control | Pass |
| Negative Control | Pass |
| Analysis | Pass |

Signature

## Test Results:

For HIV-1 (subtypes M/N and O) and HIV-2 a **qualitative** (detected/undetected) result is given.

## QC Parameters:

Sample Detection: control for sufficient sample volume
Device: multiple QC parameters for the functionality of **Alere™ q**
HIV-1 Positive Control: internal amplification control for HIV-1
HIV-2 Positive Control: internal amplification control for HIV-2
Negative Control: control for non-specific hybridization
Analysis: multiple QC parameters for the Analysis process, incl. positive hybridization control

**Failing of at least one of these controls renders the test invalid.**

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# Alere™ q HIV 1/2: Mozambique EID Study

- Blinded cross-sectional study of 827 HIV-exposed infants (1-18 months)
- **Alere™ q** HIV 1/2 performed by nurses at POC in 4 primary health care centres and 1 hospital ward
- Reference Method: Roche Diagnostics PCR at the reference laboratory

| AGE | Overall | HIV-pos* | %-pos |
|---|---|---|---|
| 1-2m | 500 | 19 | 3.8% |
| 2-3m | 124 | 6 | 4.8% |
| 3-6m | 111 | 12 | 10.8% |
| 6-9m | 58 | 14 | 24.1% |
| >9m | 34 | 14 | 41.1% |
| TOTAL | 827 | 65 | 7.9% |

*** HIV-positivity defined by the Roche technology**

| | | Conventional Results | |
|---|---|---|---|
| | | Positives | Negatives |
| POC NAT Results | Positives | 64 | 1 |
| | Negatives | 1 | 761 |

| Overall agreement | 95% C.I. | Positive percent agreement | 95% C.I. | Negative percent agreement | 95% C.I. |
|---|---|---|---|---|---|
| 99.8% | 99.1 - 100% | 98.5% | 95.5 - 100% | 99.9% | 99.3 - 100% |

| Cohen's Kappa | 95% C.I. | McNemar's Test | p-value |
|---|---|---|---|
| 0.981 | 0.960 - 1.000 | 0.500 | 0.480 |

Data sourced from Jani et al. „J Acquir Immune Defic Syndr Volume 67, Number 1, September 1, 2014

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# GeneXpert® - Cepheid


Not Yet Available

- **75 minutes to results**
  - 2 min hands on time
- **Broad molecular menu**
  - 11 FDA approved assays*
- **Footprint 3 x 4.2 x 9.1”**
- **2.2 lbs**
- **Battery powered**

Data sourced from Cepheid Product Labeling
Permission granted by Cepheid

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# 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

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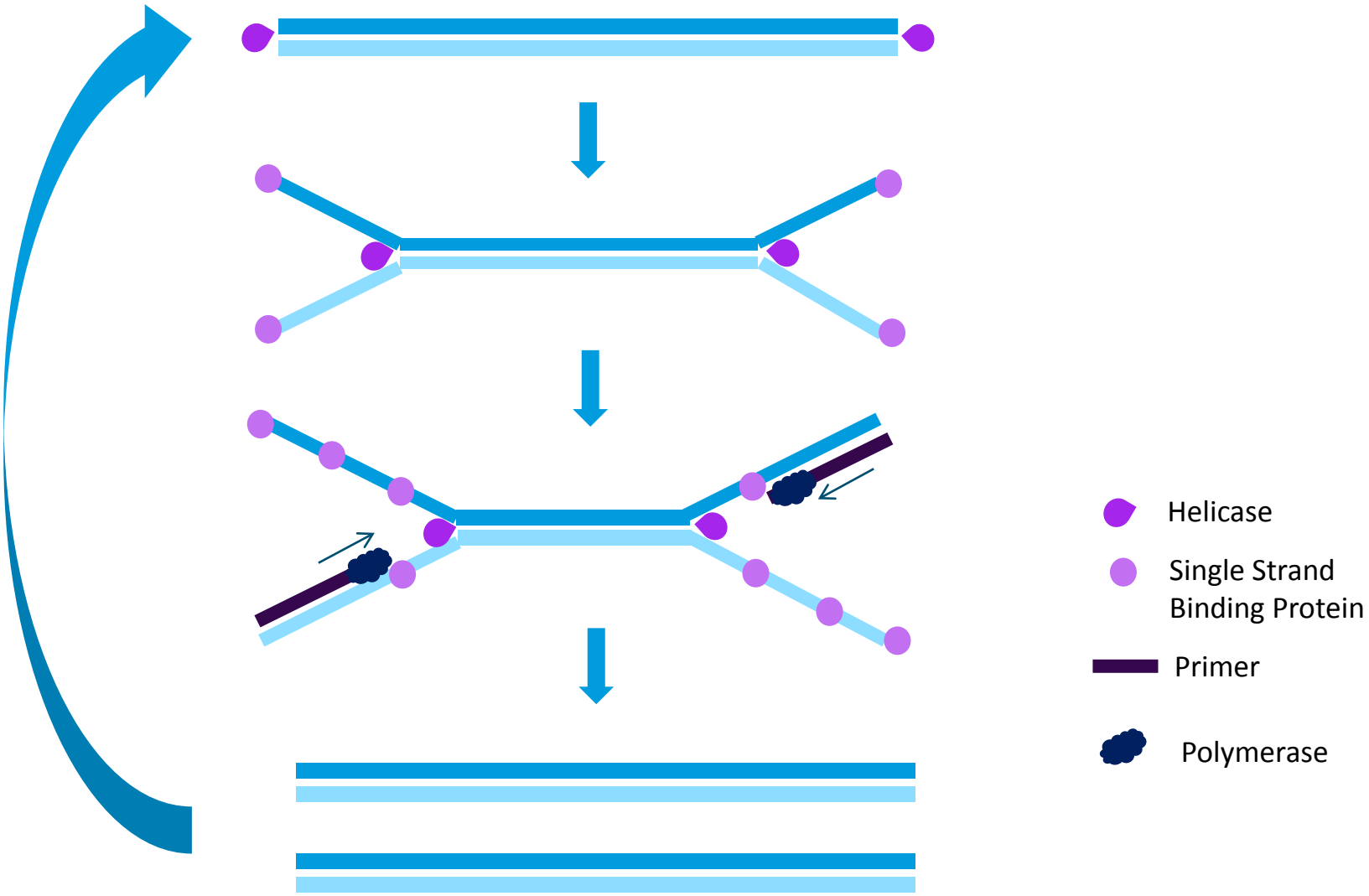
# Helicase Dependent Amplification Assays



# Solana® - Quidel


**35 minutes to results**

- Including heat pretreatment step

**Small footprint (9.4” x 9.4” x 5.9”)**

**8.8 lbs**

**Battery pack available**

**GAS only FDA approved test**

- Sensitivity – 98.2%
- Specificity – 97.2%
- LOD – 6.81 x $10^4$ CFU/mL

Data sourced from Quidel Product Labeling

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# 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.

Data sourced from Quidel Product Labeling

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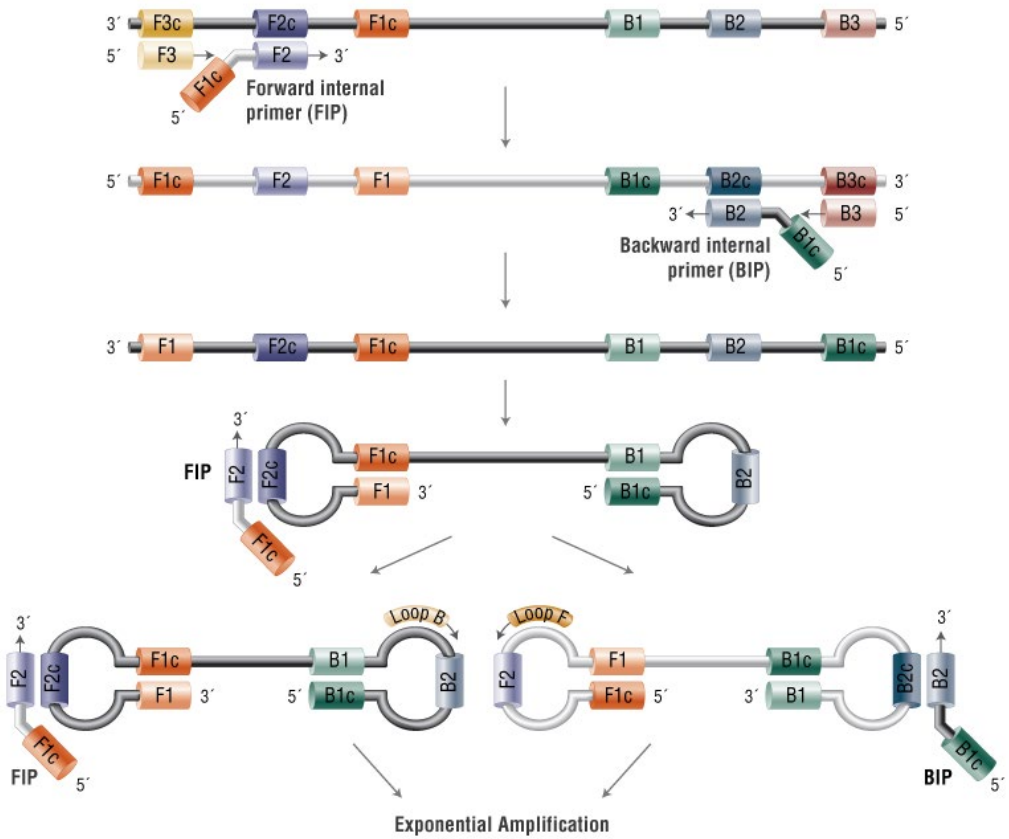
# Loop Mediated Amplification

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)




Reprinted from Trends in Parasitology, 31/8, Alhassan, Li, Poole, Carlow, Expanding the MDx toolbox for filarial diagnosis and surveillance, 391-400, (2015), with permission from Elsevier

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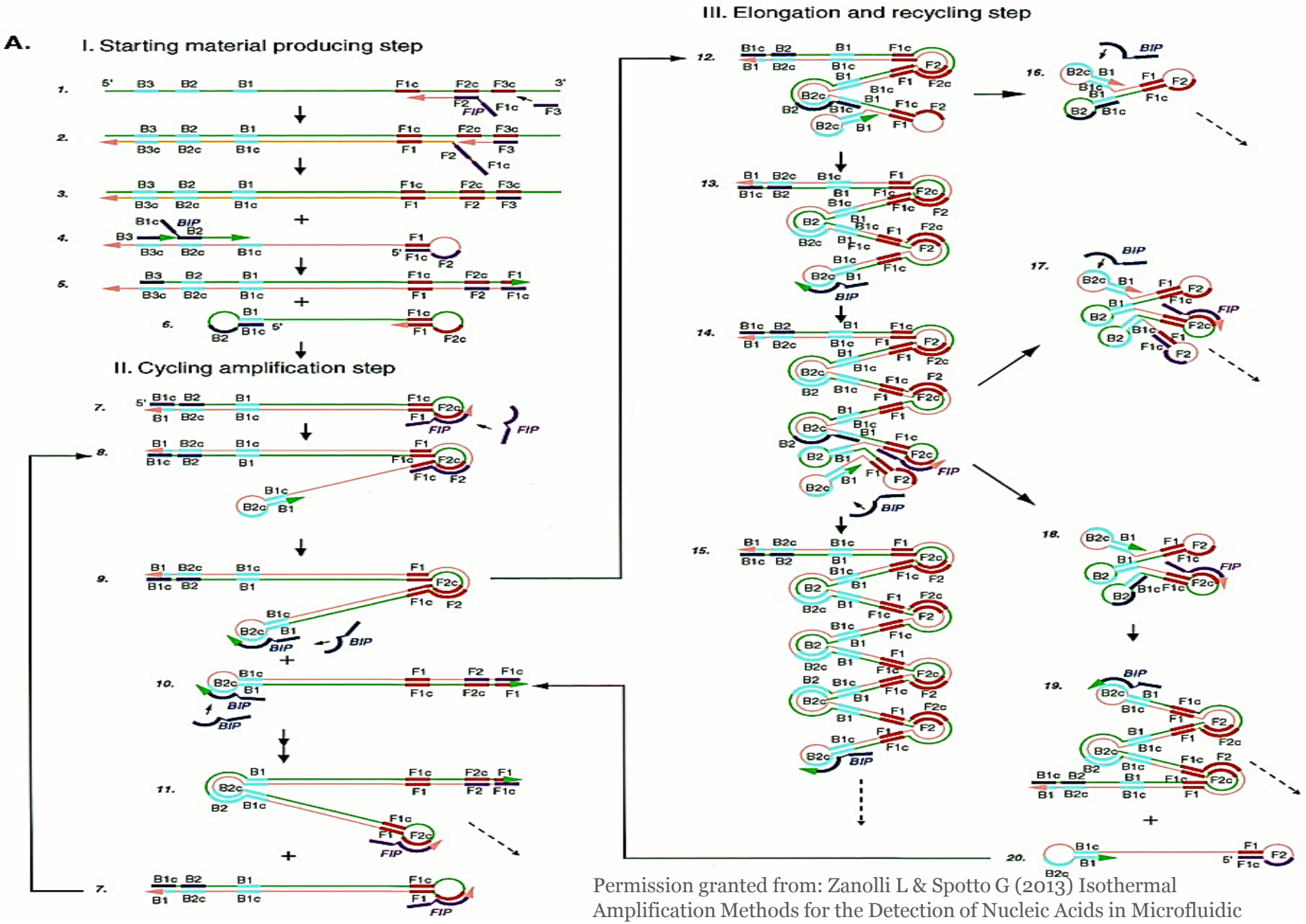


Permission granted from: Zanolli L & Spotto G (2013) Isothermal Amplification Methods for the Detection of Nucleic Acids in Microfluidic Devices, Biosensors 2013, 3, 18-43; doi:10.3390/bios3010018

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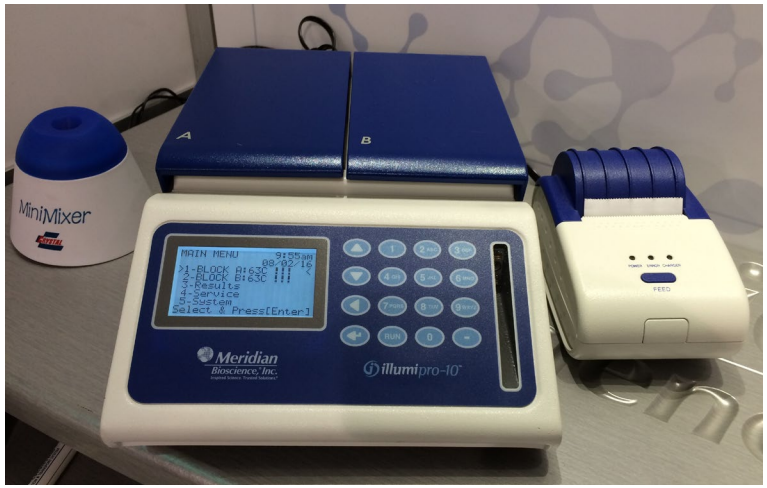
# LAMP final products are stem loop DNAs

**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**

# *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

6 FDA approved tests – C. difficile, GAS, GBS, HSV 1&2, Mycoplasma, Pertusis

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

Data sourced from Meridian Bioscience Product Labeling

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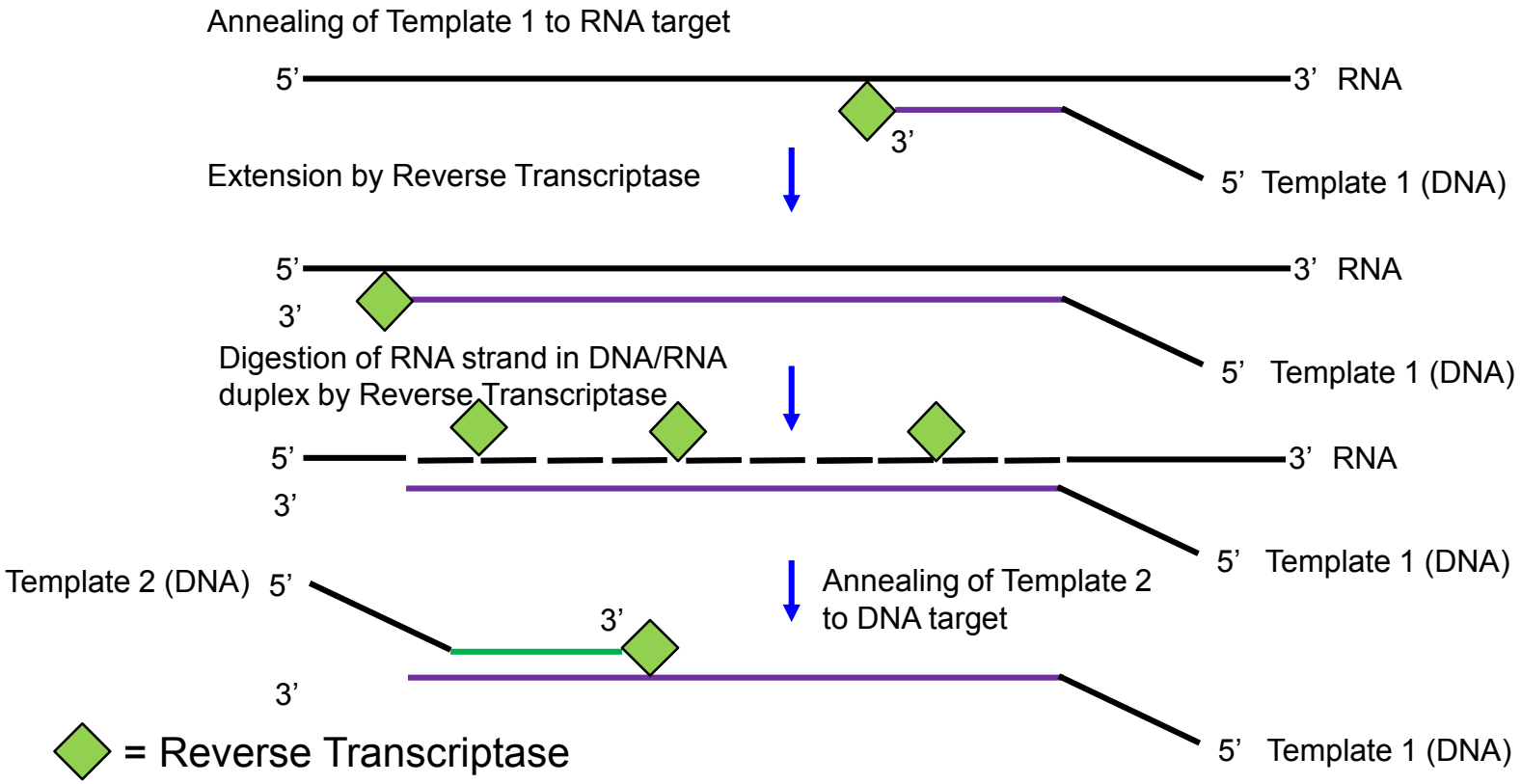
# NEAR Mechanism – Amplification from RNA

- **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

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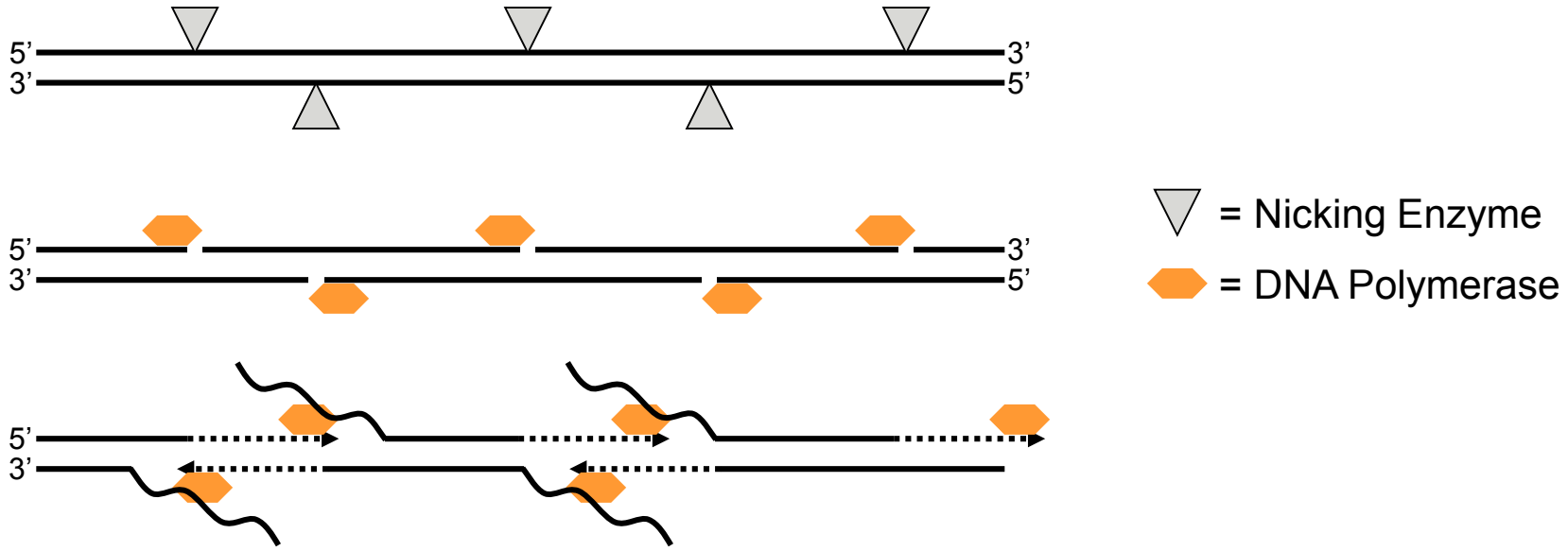
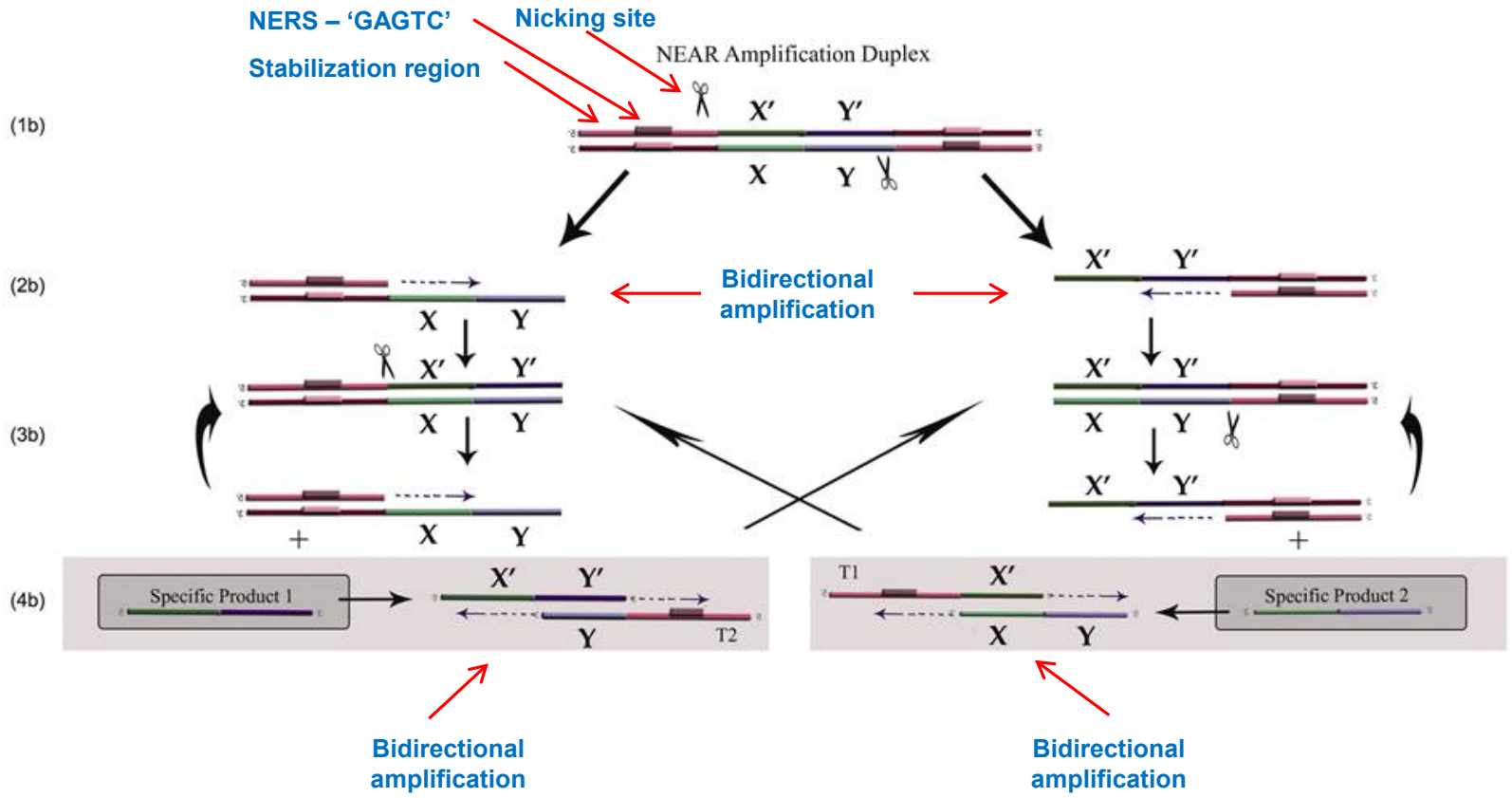
# NEAR Mechanism – Amplification from dsDNA

- **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



# Alere™ i 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

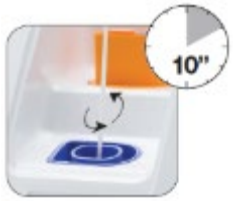
# Alere™ i System

## Extraction

**Acidic / Basic conditioning or enzymatic (Ply C)**

**Place test base & sample receiver in Alere™ i**

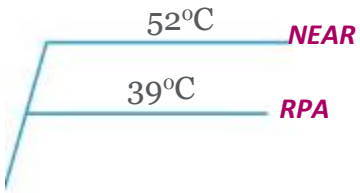
**Add swab to sample receiver**




**Transfer sample extract to Test Base**

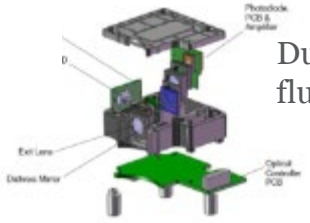


## Amplification




## Detection

Dual reaction tube



Dual channel fluorescence

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# Flu Clinical Trial Results

## Alere™ i Influenza A & B Performance vs. Culture

### Flu A

| | Culture + | Culture - |
|---|---|---|
| Alere™ i + | 92 | 66 |
| Alere™ i - | 2 | 411 |

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

### Flu B

| | Culture + | Culture - |
|---|---|---|
| Alere™ i + | 74 | 17 |
| Alere™ i - | 6 | 472 |

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

## Alere™ i Influenza A & B Performance vs. RT-PCR

### Flu A

| | RT-PCR + | RT-PCR - |
|---|---|---|
| Alere™ i + | 147 | 11 |
| Alere™ i - | 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 - |
|---|---|---|
| Alere™ i + | 123 | 7 |
| Alere™ i - | 2 | 500 |

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

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# Summary of POCT nucleic acid amplification methods

| | cobas® Liat | Alere™ q | GeneXpert® | Solana® | illumigene® | Alere™ i |
|---|---|---|---|---|---|---|
| Technology | PCR | PCR | PCR | HDA | LAMP | NEAR |
| DNA Amplification | Y | Y | Y | Y | Y | Y |
| RNA amplification | Y | Y | Y | Y | Y | Y |
| "Denaturing" agent | Heat | Heat | Heat | Helicase | Betaine | Restriction enzymes |
| Pretreatment Required | N | N | Y/N | Y | Y | N |
| # of enzymes | 1 | 1 | 1 | 2 | 1 | 2 |
| Temp (°C) | 95/72/57 | 95/72/57 | 95/72/57 | 64 | 60-65 | 52 |
| Time to Result (min) | <20 | 55 | 75 | 35 | <60 | <15 |
| Multiple Amplifications | Y | Y | Y | Y | N | N |

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Thank you

# Questions?

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