Cepheid GeneXpert® Technology Overview

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Cepheid is proud to be part of Danaher.

Agenda

1. GeneXpert® Module
2. GeneXpert® Cartridge & Reagents
3. Extraction
4. Real-Time PCR
5. Good Laboratory Practices

Cepheid
A better way.

GeneXpert® Module

GeneXpert® Systems

Accessibility

- On demand, random access

Automated

- Sample nucleic acid extraction, PCR amplification, and detection

Scalability

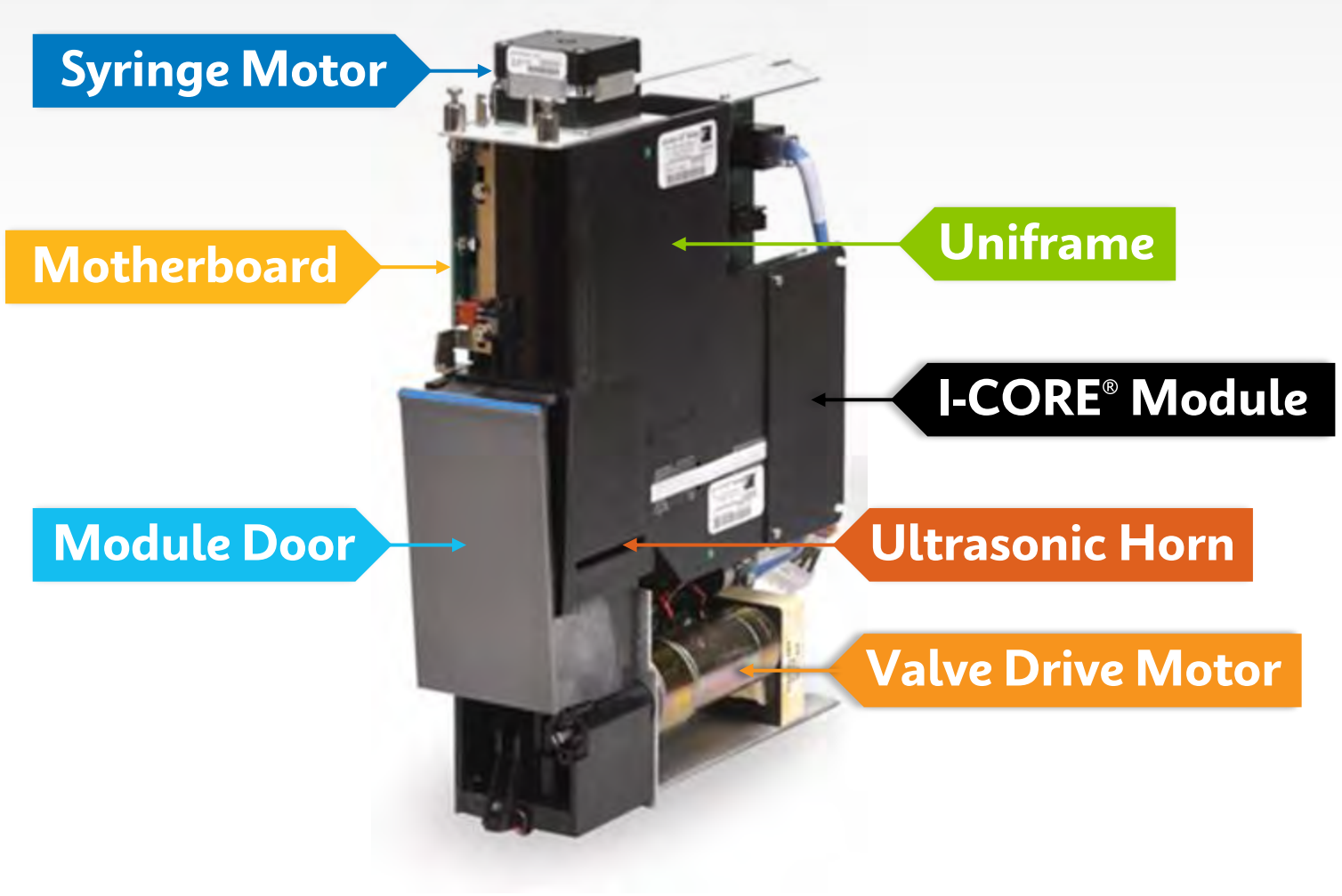
- Scalable across systems

Consistency

- Same module across all systems

- GeneXpert Technology (Module and Cartridge design) allows for ***the same quality*** of testing ***across all platforms***

GeneXpert® Module Mechanics



GeneXpert® Cartridge & Reagents

GeneXpert® Cartridge

Cartridge Body

- Reagents On Board that include Liquid Reagents and PCR beads
- Self-contained system

Valve Body

- Rubber stopper helps move the fluids and provides a barrier that protects the modules from contamination
- Filter to capture nucleic acids or cells

Cartridge Lid

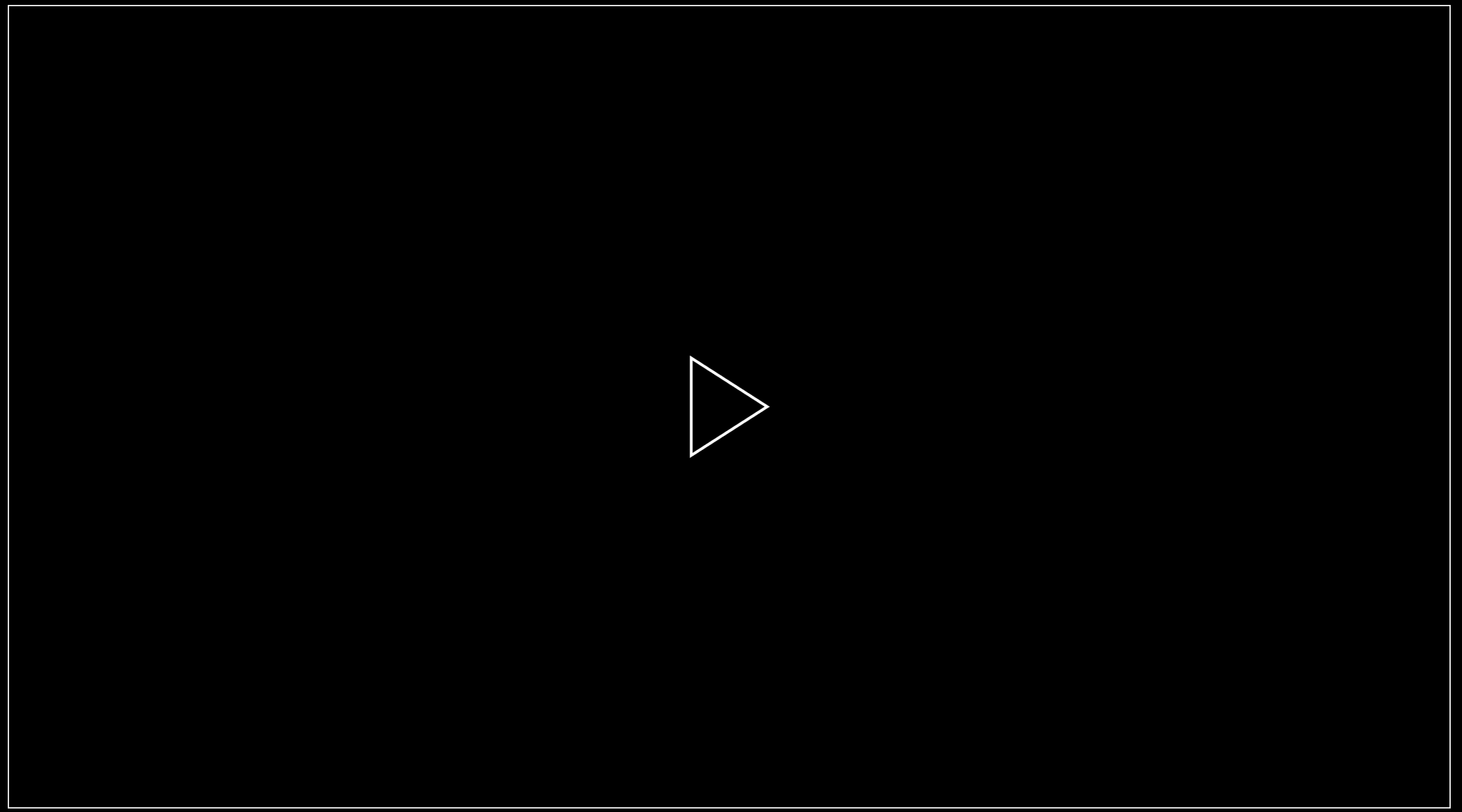
- Factory-sealed for stability
- Opening lid vents chambers
- Access to add sample

Reaction Tube

- Reaction chamber for RT-PCR
- Thin walls for fast thermal cycling

Cartridge Foot

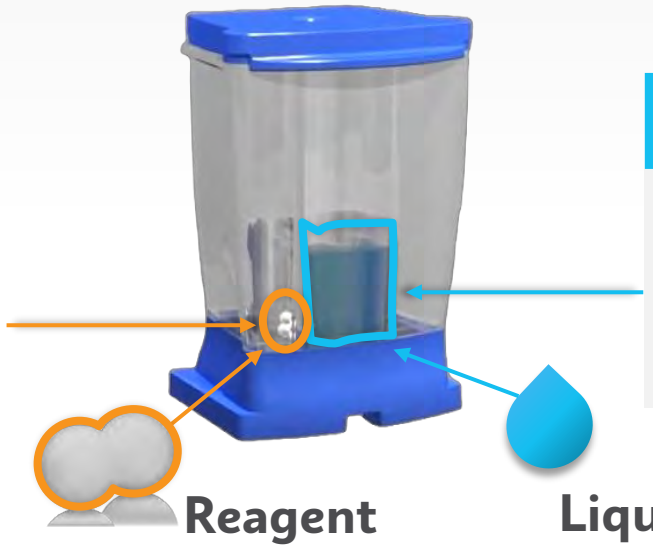
- Sits Cartridge on counter
- Snaps Cartridge assembly together



GeneXpert® Cartridge On-Board Reagents

Reagent PCR Beads

- **Enzyme (EZR) Beads**
  - Contains polymerase enzyme and other components needed for PCR to function
- **Target Specific Reagent (TSR) Beads**
  - Contains primers and probes for specific target sequences
- **Internal Control**
  - Sample Processing Control and/or Sample Adequacy Control



Reagent PCR Beads

Liquid Reagents

Not actual size: enlarged for demonstration

Liquid Reagents

- Contains reagents to treat patient samples, remove unwanted components, recover nucleic acids, and reconstitute the PCR beads

Extraction in GeneXpert® Cartridge

Filter in Valve Body

- Captures nucleic acid or cells

Extraction

- **Mechanical** - Ultrasonic horn and glass beads lyse hardy cells or spores
- **Chemical** - Lysis reagent buffers and filter to capture free nucleic acids

Cartridge Body

Extracted Nucleic Acid

- Resuspends reagent PCR beads
- Mixture moves to reaction tube for Real-Time PCR

Reagent Waste

- Waste is self-contained

Amplification and Detection in GeneXpert® Cartridge

Amplification

- Multiple PCR cycles amplify nucleic acids and generate fluorescent signal for targets that are present in the sample

Detection

- Detection of fluorescent probes
- Amplification curve with Ct value

Reaction Tube

Melt

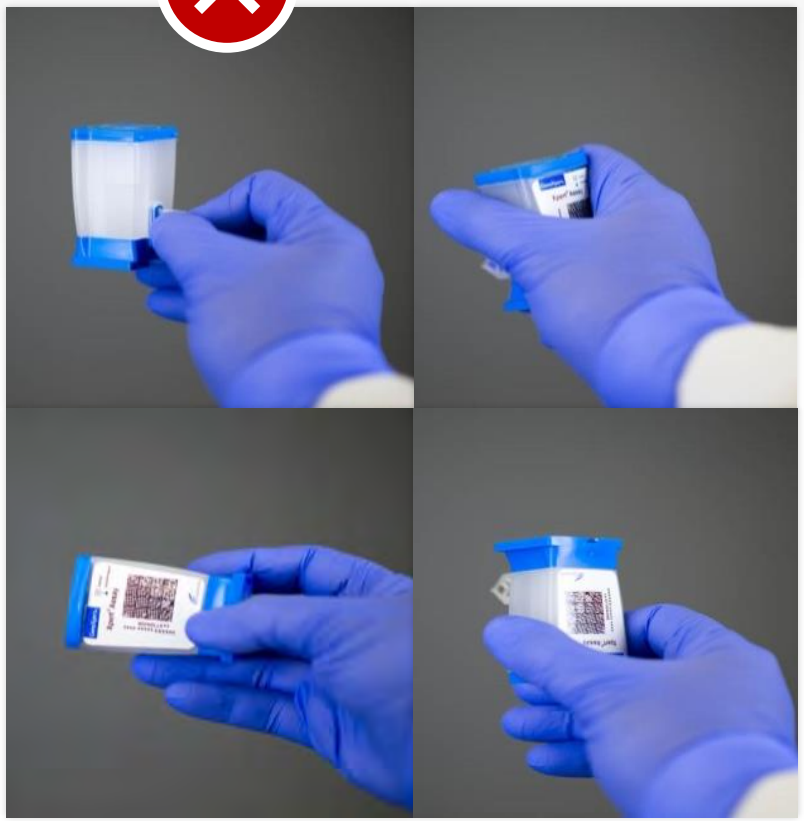
- Some assays employ post-PCR melt analysis to identify mutations or additional targets

Proper Cartridge Handling Techniques

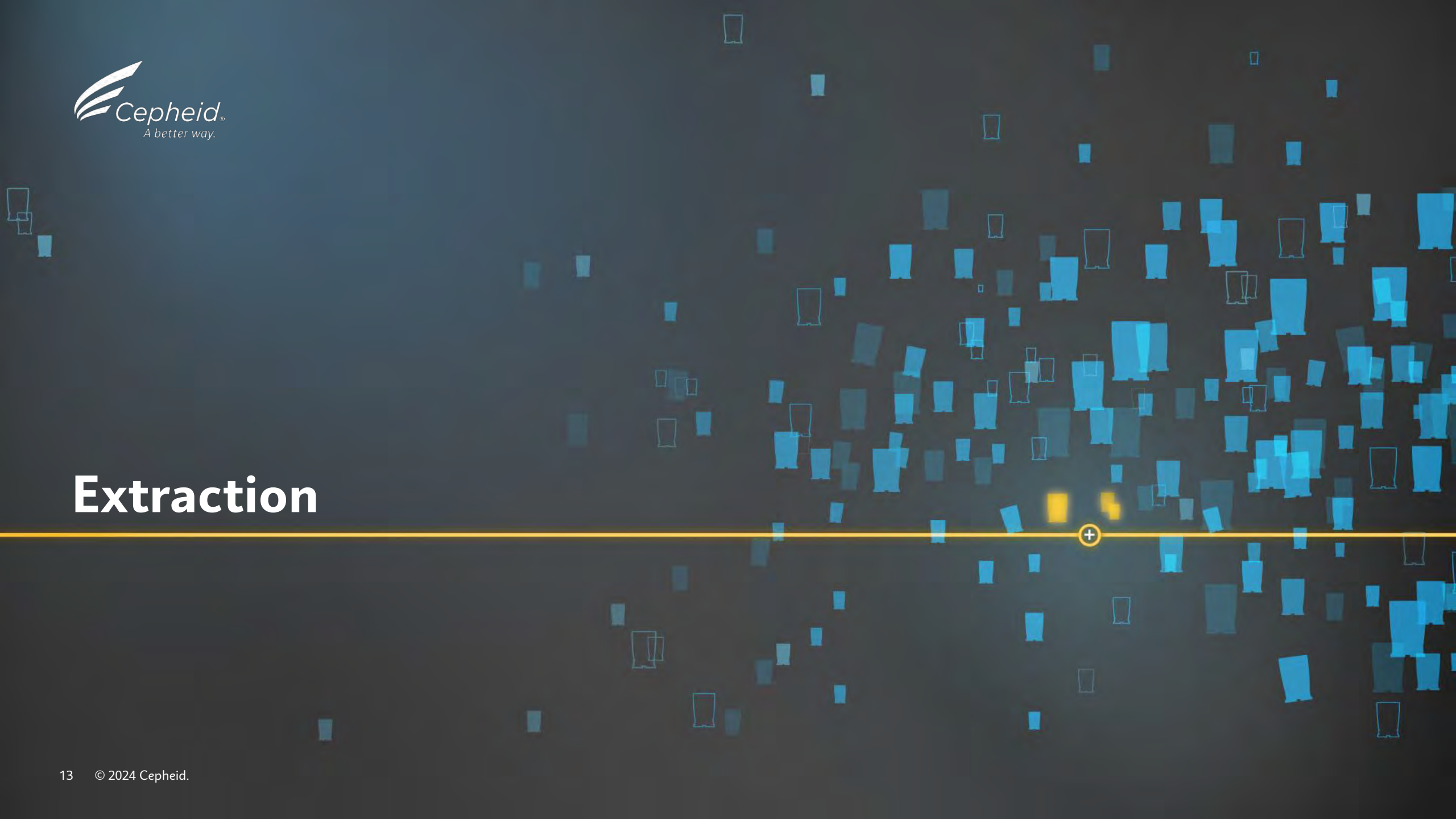
Correct

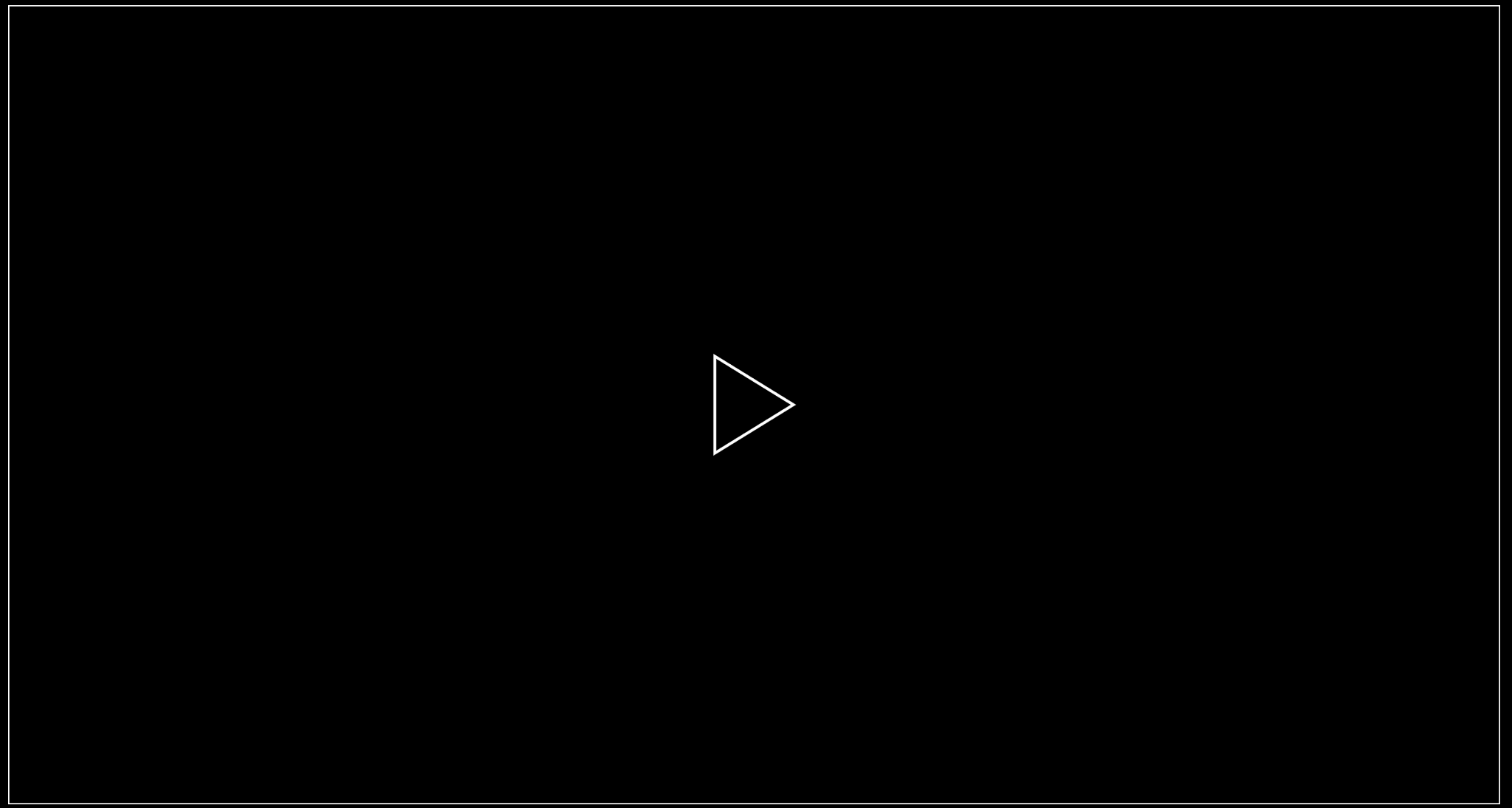
- ✓ Do not touch the reaction tube
- ✓ Keep the cartridge upright after seal has been broken
- ✓ Do not tilt when scanning the cartridge

Incorrect



Extraction





Extraction

- Once sample is loaded into the cartridge and a run is started, the GeneXpert® System automates all sample processing and nucleic acid extraction steps

- Sample processing steps and PCR reactions are customized for each assay using a pre-programmed Assay Definition File (ADF)

Extraction

- Cepheid cartridges incorporate different types of extraction pathways depending on the specimen type and target:

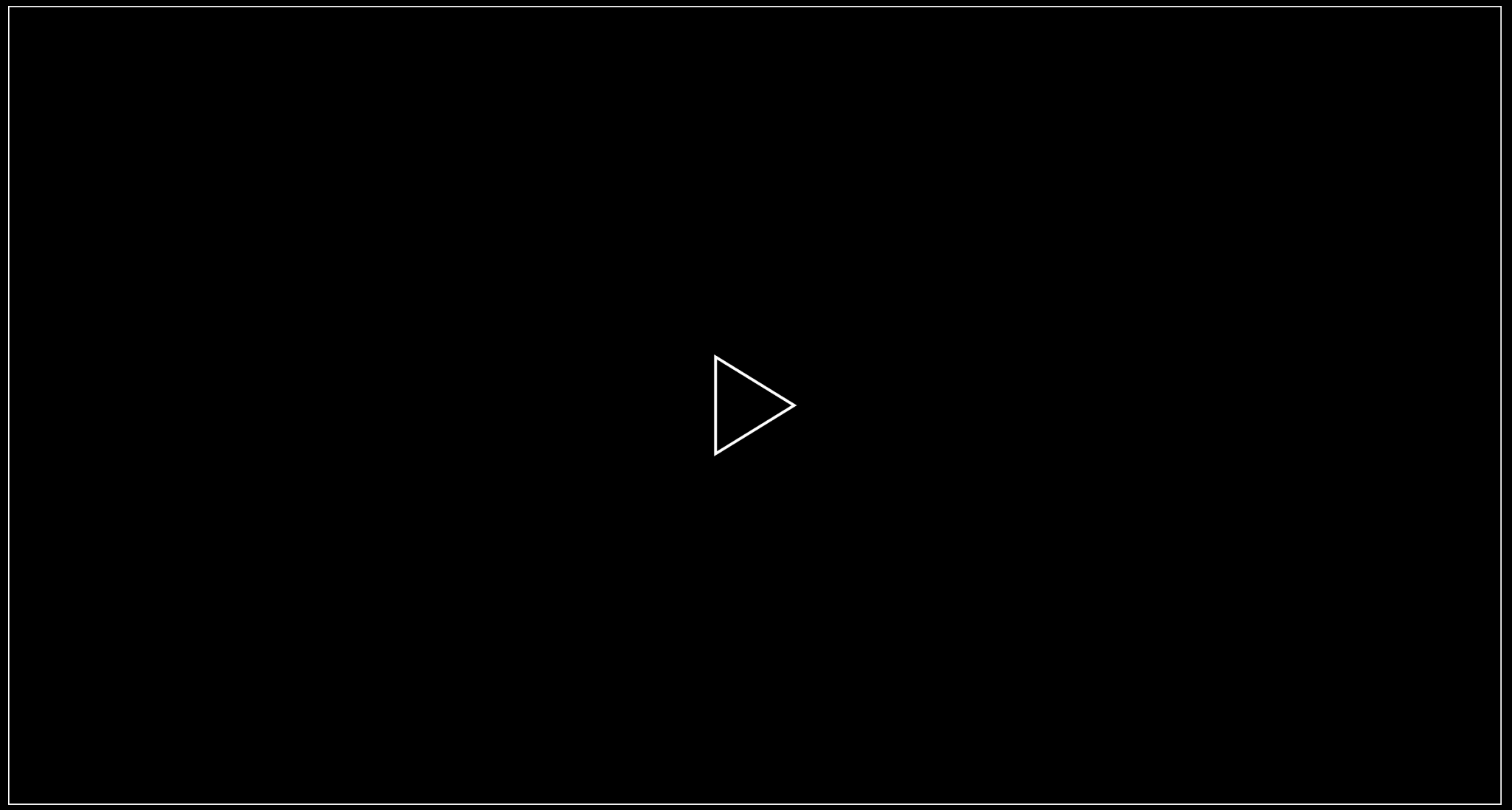
Mechanical Lysis

- **Hardier cells and spores** require **mechanical** lysis to extract
- Ultrasonic horn and glass beads lyse hardy cells or spores

Chemical Lysis

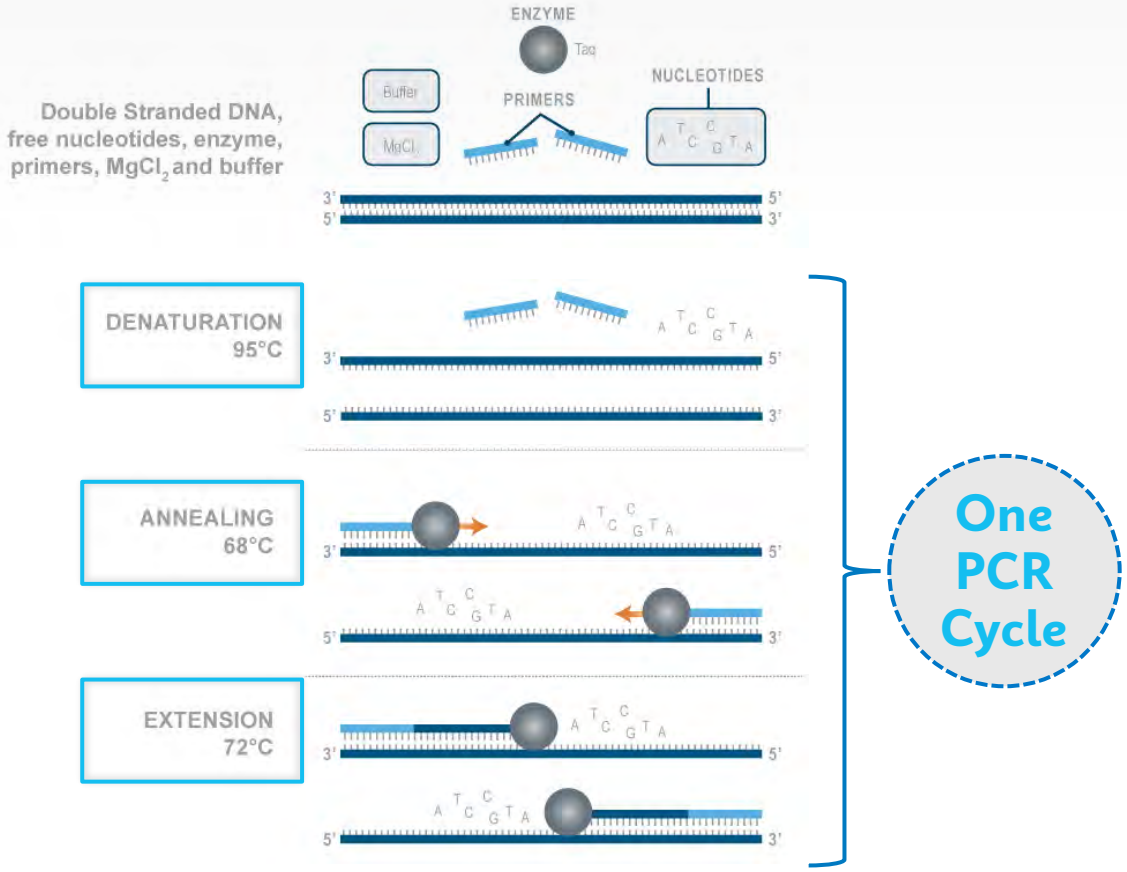
- **Viruses and weaker cells** can be **chemically** lysed, and their nucleic acids bound to a filter column
- Lysis reagent buffers and filter to capture free nucleic acids

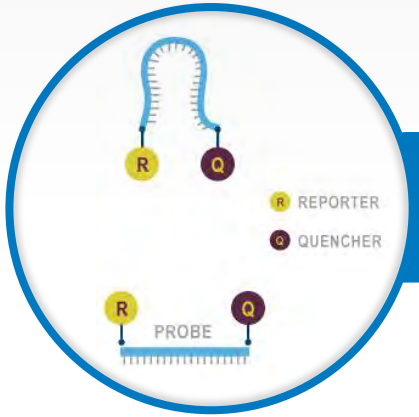
Real-Time PCR



Real Time PCR – Amplification & Detection

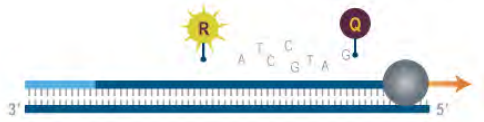
- PCR is a technique for amplifying DNA or cDNA to a detectable fluorescence signal.





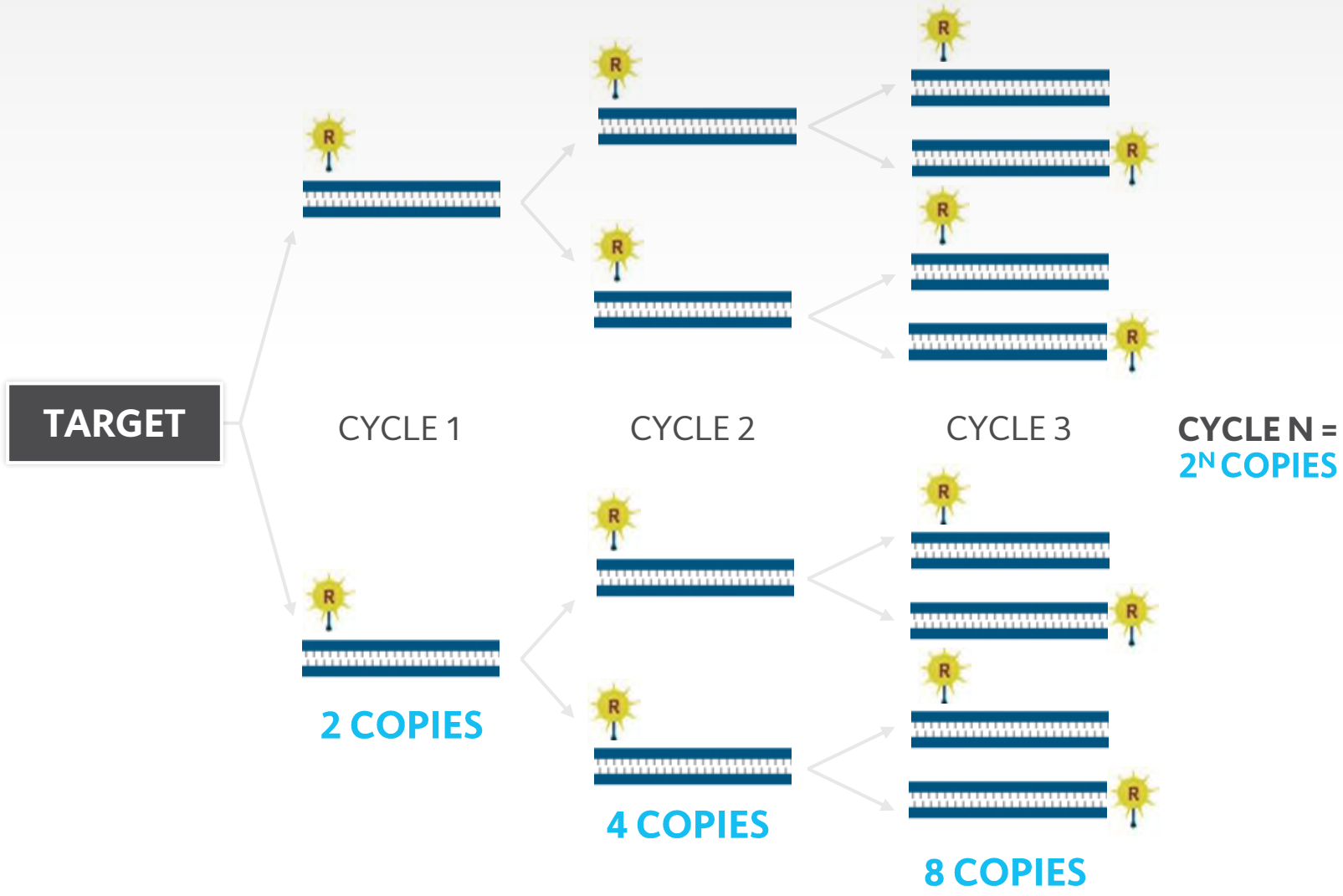
Different type of probes available depending on target and test design

1. Both Primer and Probe will bind to specific target sequences during the Annealing step



2. Reporter released during Extension step, providing a Fluorescence signal that is measurable

Exponential Increase in DNA Copies



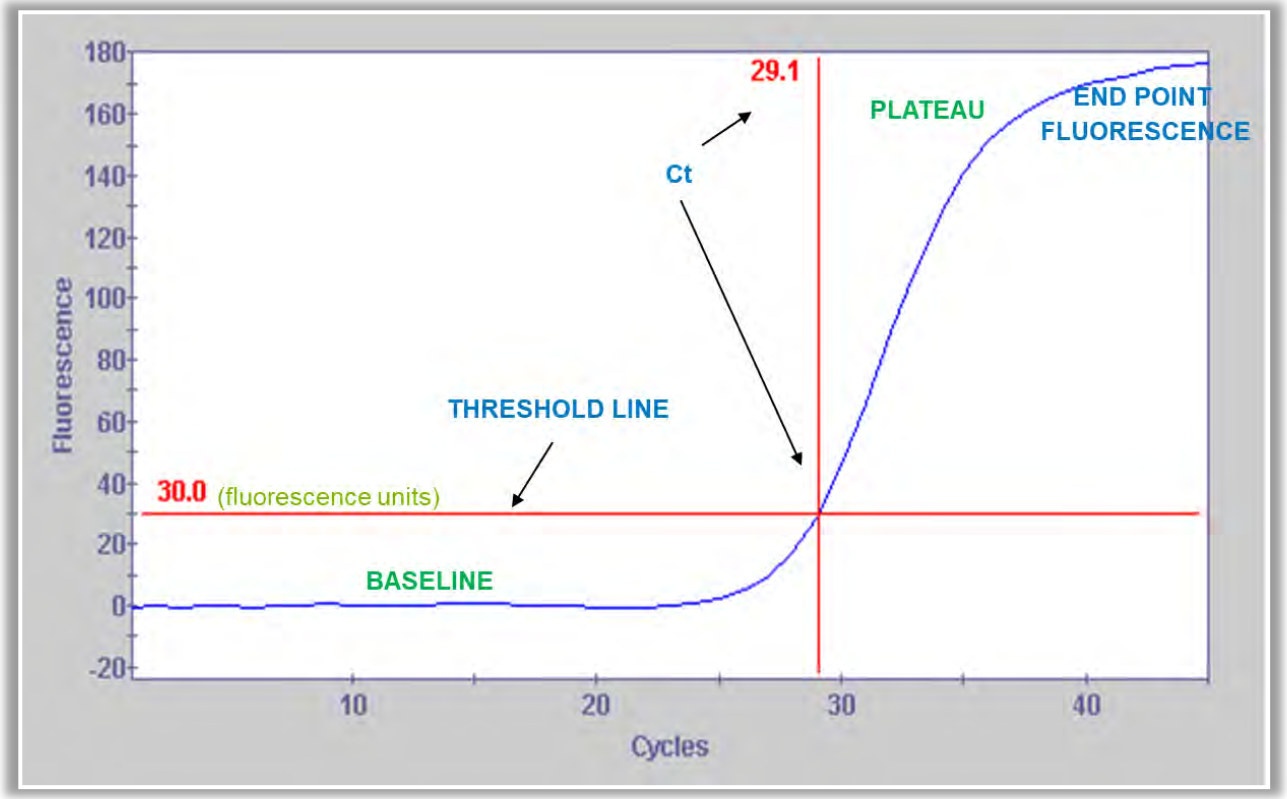
Fluorescence

- Each Reporter has a fluorescence that is **specific for each target** that is **measurable** in a channel
- The number of fluorescence signals are in **direct proportion** with the **number of copies being amplified**

Amplification Curve and Ct

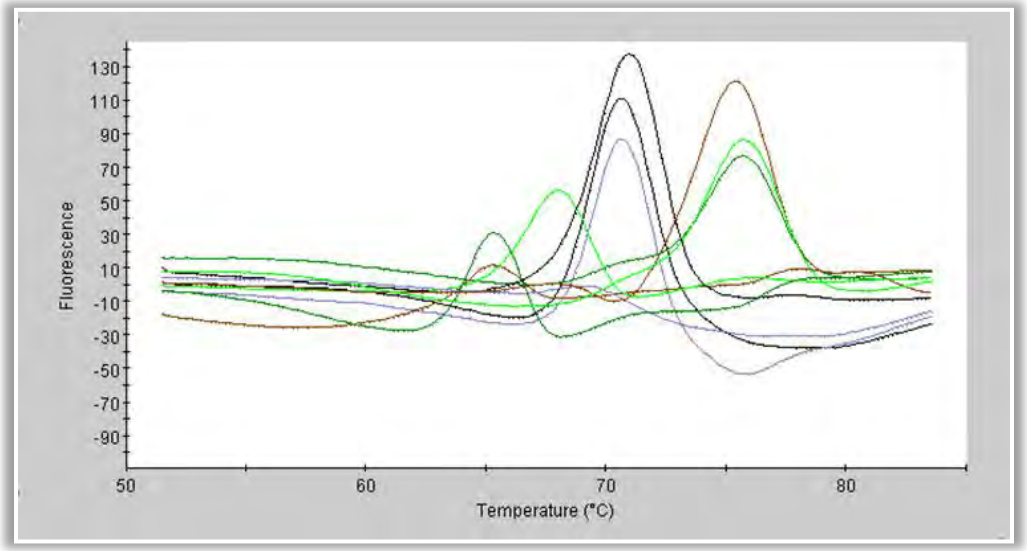
- The GeneXpert software continuously measures the amplification fluorescence per cycle.

The number of reaction cycles it takes to reach the threshold is called the “cycle threshold” (Ct) value.



Post-PCR Melt Analysis

- Melt profile analysis is a post-PCR stage in which the thermal denaturation of a melt probe + amplified DNA target provides a characteristic fluorescence vs temperature signature that can identify types of species or mutations such as single nucleotide polymorphism (e.g. Xpert® MTB/RIF tests) present in a sample.



Good Laboratory Practices

Good Laboratory Practice Review

Personal Protective Equipment (PPE)

- Wear clean lab coats, safety glasses, and gloves
- Change gloves between processing samples

Equipment

- Follow the manufacturer's requirements for instrument maintenance
- Setup samples away from the instrument
- Use filtered pipette tips when recommended

Lab Bench Area

- Clean work surfaces routinely with:
  - ✓ 1:10 dilution of household bleach*
  - ✓ 70% ethanol solution
- After cleaning, ensure work surfaces are dry and gloves are changed

*Final Active Chlorine concentration should be 0.5% regardless of the household bleach concentration

Specimens, Samples, and Kits Storage

- Store specimens and sample away from kit to prevent contamination

Waste Disposal Warnings and Precautions

- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents and require use of standard precautions.

- Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents.

- These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures.

- **Please Note:** Used cartridges may contain potentially infectious materials, as well as highly amplified PCR target(s).
- **Do not open or attempt to alter any part of the cartridge for disposal.**
- If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

Q&A

Thank You

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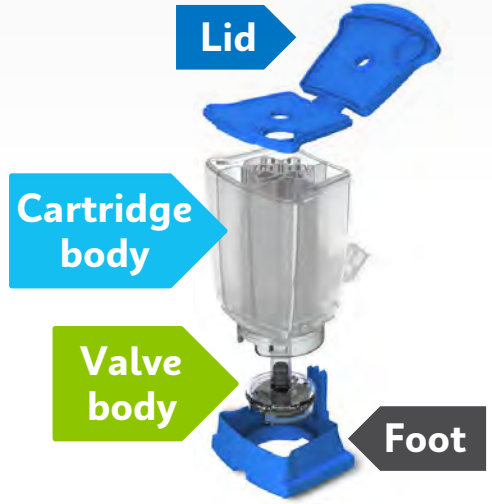
Check Your Knowledge

Question 1

- Which part of the cartridge moves liquid reagents and patient sample?

  (Select all that apply, then click the orange box to check your answer.)

- [ ] Valve body
- [ ] Lid
- [ ] Foot
- [ ] Cartridge body



Check Your Answer

Question 2

- To decrease the risk of cross-contamination, what should you be doing?
  (Select all that apply, then click the orange box to check your answer.)

- ☐ Cleaning surfaces with 1:10 dilution of bleach and 70% ethanol
- ☐ Change gloves after processing each sample
- ☐ Clean with Lysol wipes
- ☐ Save your samples next to your instrument

Check Your Answer

Question 3

- What does the test detect during PCR when a target is present?
  (Choose one best response to the question and click its blue square to check your answer.)

A	B	C	D
Florescence	Chemical Change	Heat	Air Pressure

Question 4

- Which of the following are true about the Cepheid® Module?
  (Select all that apply, then click the orange box to check your answer.)

- ☐ You must run the same test at the same time in each module
- ☐ The sample does not come into contact with the instrument
- ☐ Module function is universal between all Cepheid® instruments
- ☐ Should be replaced by someone at your institution if needed

Check Your Answer

Question 5

- Which parts of the cartridge can you remove before disposal?
  (Select all that apply, then click the orange box to check your answer.)

- [ ] Lid
- [ ] Reaction Tube
- [ ] Foot
- [ ] Do not alter or remove any part of the cartridge

Check Your Answer