
Microbiologics®

Quality Control in the Clinical and Molecular Laboratory - it's easier than you think!

An overview of Microbiologics' Quality Control Standards

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Microbiologics®

AGENDA

- LYFO DISK™ and KWIK-STIK™
  - Product formats
  - Primary customer use
  - Common complaints
- KWIK-STIK™ Plus
- Lab Elite™ CRM
- QC Sets and Panels
- QC Slides
- Parasite Suspensions
- Microbiologics Molecular Products

LYFO DISK™, KWIK-STIK™, KWIK-STIK™ Plus, and Lab Elite™ CRM Specifications

KWIK-STIK™

Features

- Self-contained unit with everything needed for hydration
  - Swab and ampoule of fluid
- Available in over 900 strains
- Qualitative
- Storage 2-8ºC
- Minimum Shelf-life of 8 months
- Accredited Reference Material under ISO 17034
- 3 passages or less from the reference culture

Packaging

- Orange plastic unit with a swab and ampoule of hydration fluid and a lyophilized pellet
- Available as a pack of 2 or 6
- Product codes = P, K, and U

PRODUCT CODES

- Product codes are the alphabetical identifier at the end of the catalog number. Example; 0335**P**
  - P = KWIK-STIK™ 2 Pack
  - K = KWIK-STIK™ 6 Pack
  - U = Single KWIK-STIK™

LYFO DISK™

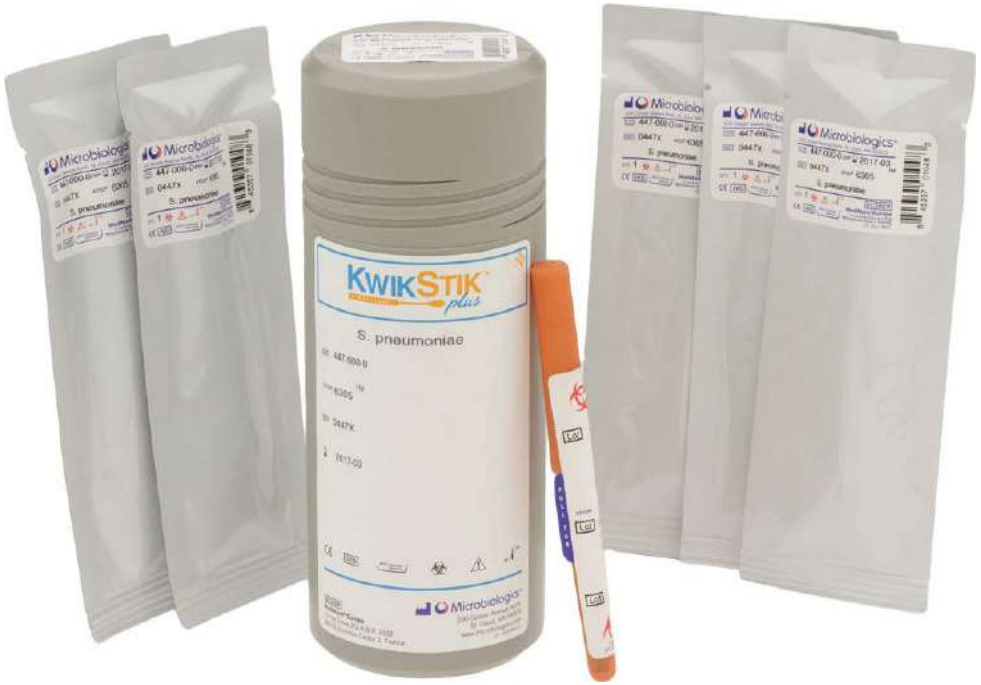
Features

- A vial of a single microorganism population
- Available in over 900 strains
- Qualitative
- Storage 2-8ºC
- Minimum Shelf-life of 8 months
- Accredited Reference Material under ISO 17034
- 3 passages or less from the reference culture

Packaging

- A glass vial with 6 lyophilized pellets and a dessicant
- Product code = L

KWIK-STIK™ Plus



Features

- Self-contained unit with everything needed for hydration
- Qualitative
- Storage 2-8ºC
- Minimum Shelf-life of 8 months
- Accredited Reference Material under ISO 17034
- 2 passages from the reference culture

Packaging

- Orange plastic unit with a swab and ampoule of hydration fluid and a lyophilized pellet
- Shipped as Five KWIK-STIK™ Plus units per canister
- Product codes = X

Lab Elite™ CRM

Features

- Self-contained unit with everything needed for hydration
- Qualitative
- Storage 2-8ºC
- Minimum Shelf-life of 8 months
- **Certified** Reference Material under ISO 17034
- 1 passage from the reference culture

Packaging

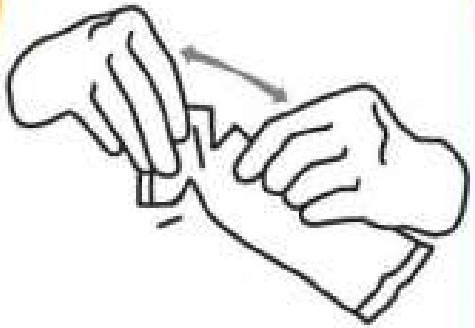
- Orange plastic unit with a swab and ampoule of hydration fluid and a lyophilized pellet
- Shipped as One Lab Elite™ CRM unit per canister
- Product codes = -CRM

HOW TO USE

HOW TO USE KWIK-STIK™, KWIK-STIK™ Plus, and Lab Elite™ CRM

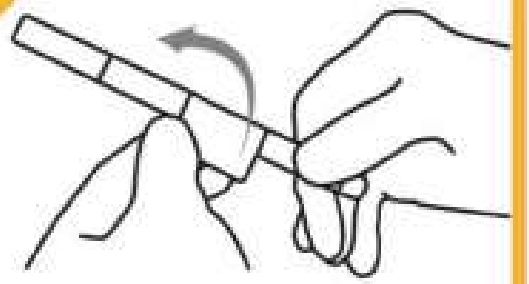
Microbiologics

1



Allow the unopened KWIK-STIK pouch to equilibrate to room temperature. Tear open pouch at notch and remove the KWIK-STIK unit.

2

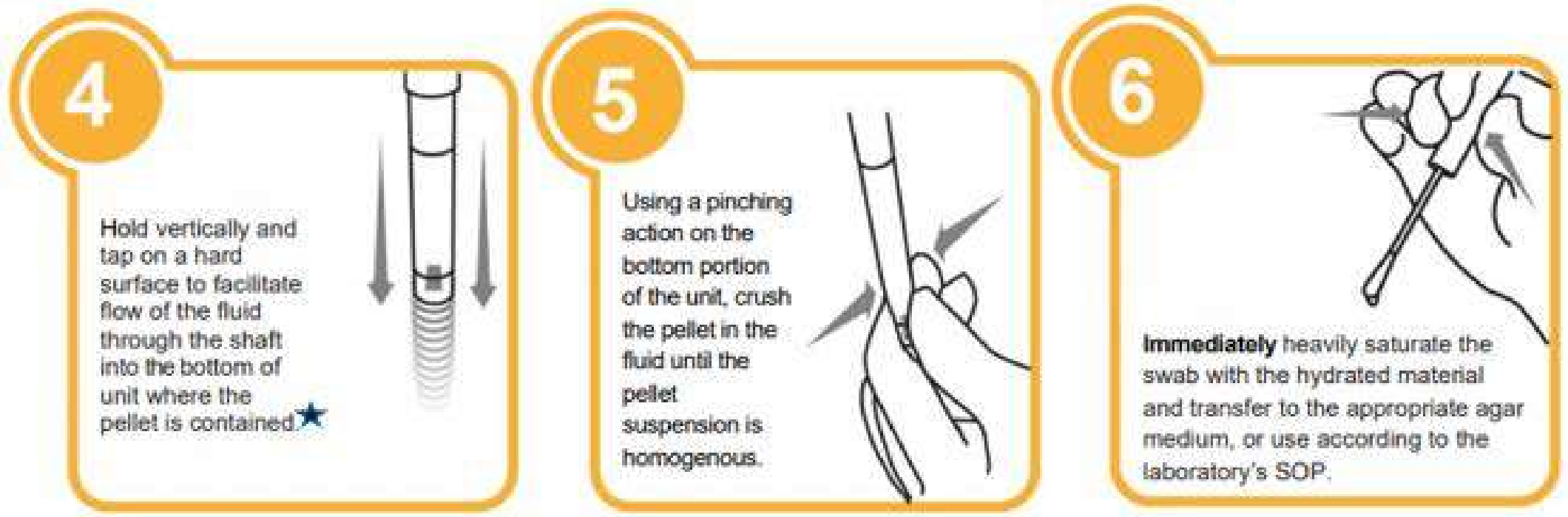


Tear off pull-tab portion on the label and attach it to the primary culture plate or QC record. Do not disassemble the device during

3

Over the edge of the work bench or counter, crack the ampoule at the top of the KWIK-STK (just below the fluid meniscus of the ampoule) found in the cap to release the hydrating fluid.

HOW TO USE KWIK-STIK™, KWIK-STIK™ Plus, and Lab Elite™ CRM



When the swab is removed from the KWIK-STIK™ unit, grey material must be present, and the swab appear wet.

HOW TO USE KWIK-STIK™, KWIK-STIK™ Plus, and Lab Elite™ CRM

Microbiologics

7

Inoculate the primary culture plate(s) by gently rolling the swab over one-third of the plate.

8

Using a sterile loop, streak to facilitate colony isolation.

9

Using proper biohazard disposal, discard the KWIK-STIK

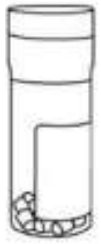
10

Immediately incubate the inverted inoculated primary culture plate(s) at temperature and conditions appropriate to the microorganism.

Culture method can be found on the product's page at microbiologics.com

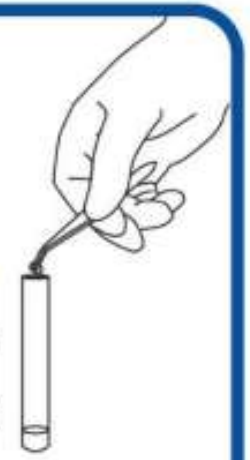
LYFO DISK™ How to Use

1. Remove the unopened LYFO DISK vial from 2°C to 8°C storage and allow to equilibrate to room temperature.



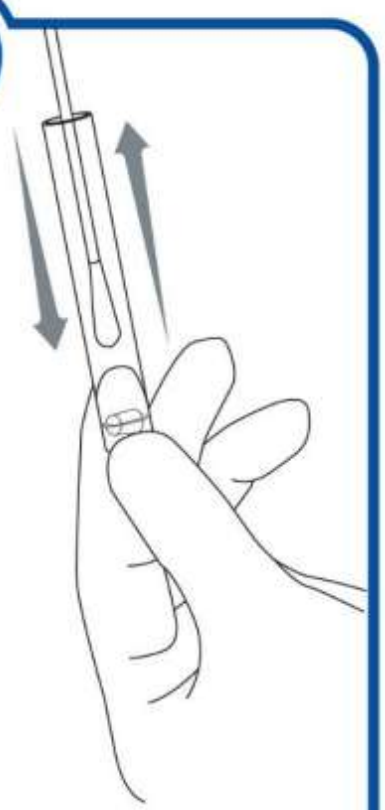
2. Aseptically remove 1 pellet with sterile forceps from the vial. Do not remove desiccant.

3. Place the pellet in 0.5 ml of sterile fluid (water, saline, TSB, or BHIB). Immediately stopper and recap vial and return to 2°C to 8°C storage.



LYFO DISK™ How to Use

4



Crush the pellet with a sterile swab until the suspension is homogenous.

Immediately heavily saturate the same swab with the hydrated material and transfer to agar medium.

5

Inoculate the primary culture plates(s) by gently rolling the swab over one-third of the plate.

6

Using a sterile loop, streak to facilitate colony isolation.

LYFO DISK™ How to Use

7

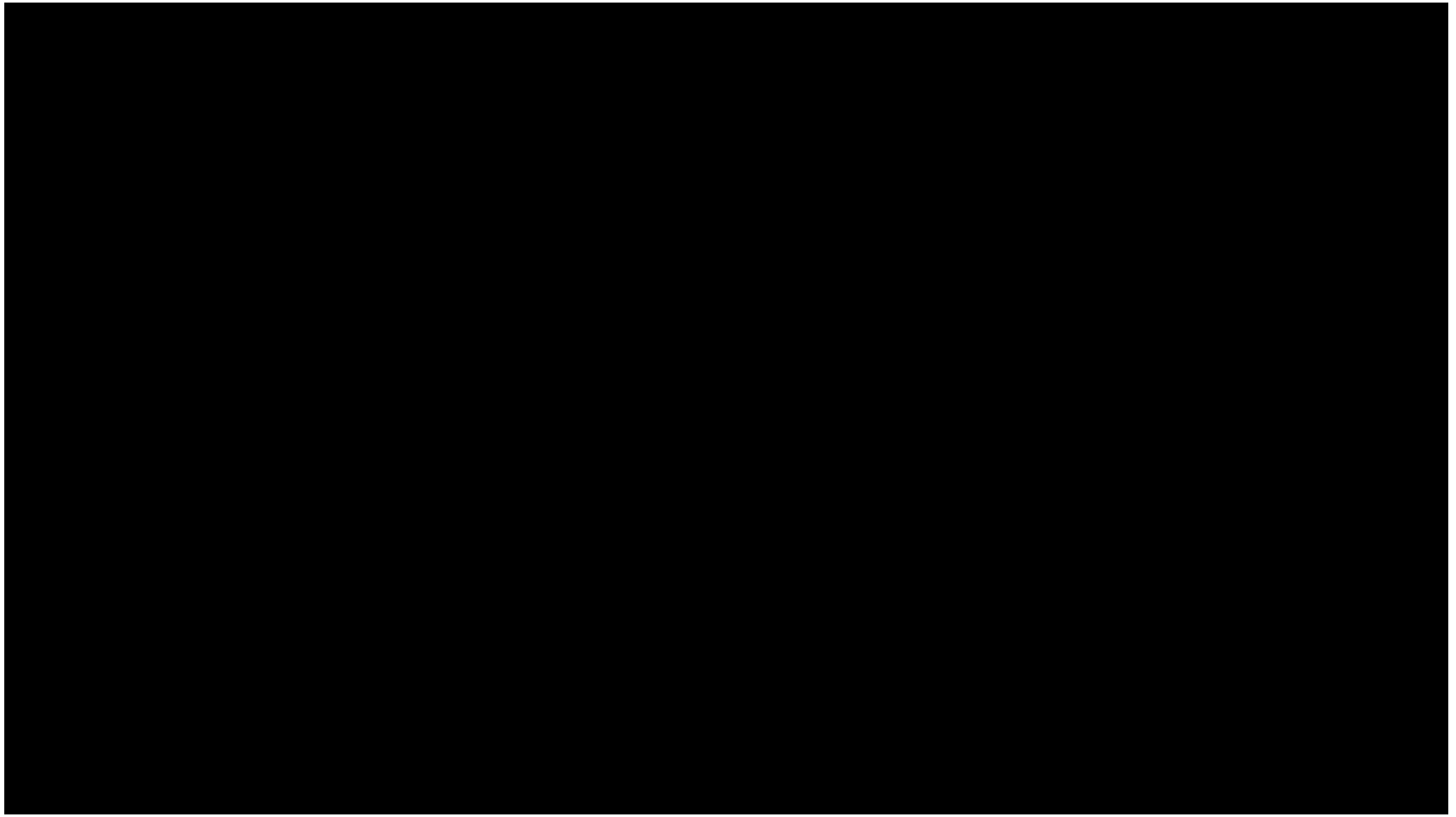
Using proper biohazard disposal discard the remaining hydrated material.

8

Immediately incubate the inverted inoculated primary culture plate(s) at temperature and conditions appropriate to the microorganism.

Culture method can be found on the product's page at microbiologics.com

HOW TO USE



PRIMARY CULTURE MEDIA

Microbiologics has validated the best primary media and conditions for strains produced

Validated growth conditions for primary growth are located:

- Culture Methods on website
- Recommended Growth Requirements TIB.081

Use non-selective agar/broth for primary growth

DO NOT use selective media

PRODUCT WARRANTY

KWIK-STIK™, LYFO DISK™, KWIK-STIK™ Plus, and Lab Elite™ CRM

- Will recover when using the media validated and described in TIB.081 – Recommended Culture methods
- Look a certain way on the agar plate or under the microscope
- Have specific biochemical reactions
- Be resistant or susceptible to certain antibiotic

The warranty is voided if the resuscitated culture is frozen

MICROORGANISM MAINTENANCE

SHORT-TERM PRESERVATION METHODS

Preservation Method	Pros	Cons
Sub-culturing/ Refrigeration	+ Low tech	- Highest risk of mutation - Risk of contamination - May exceed 5 passages
Sub-zero Freezing (-20°C)	+ Viability can be maintained for 1-2 yrs.	- Cellular damage due to ice crystals and electrolyte fluctuations - Risk of contamination - Freezer cost and maintenance

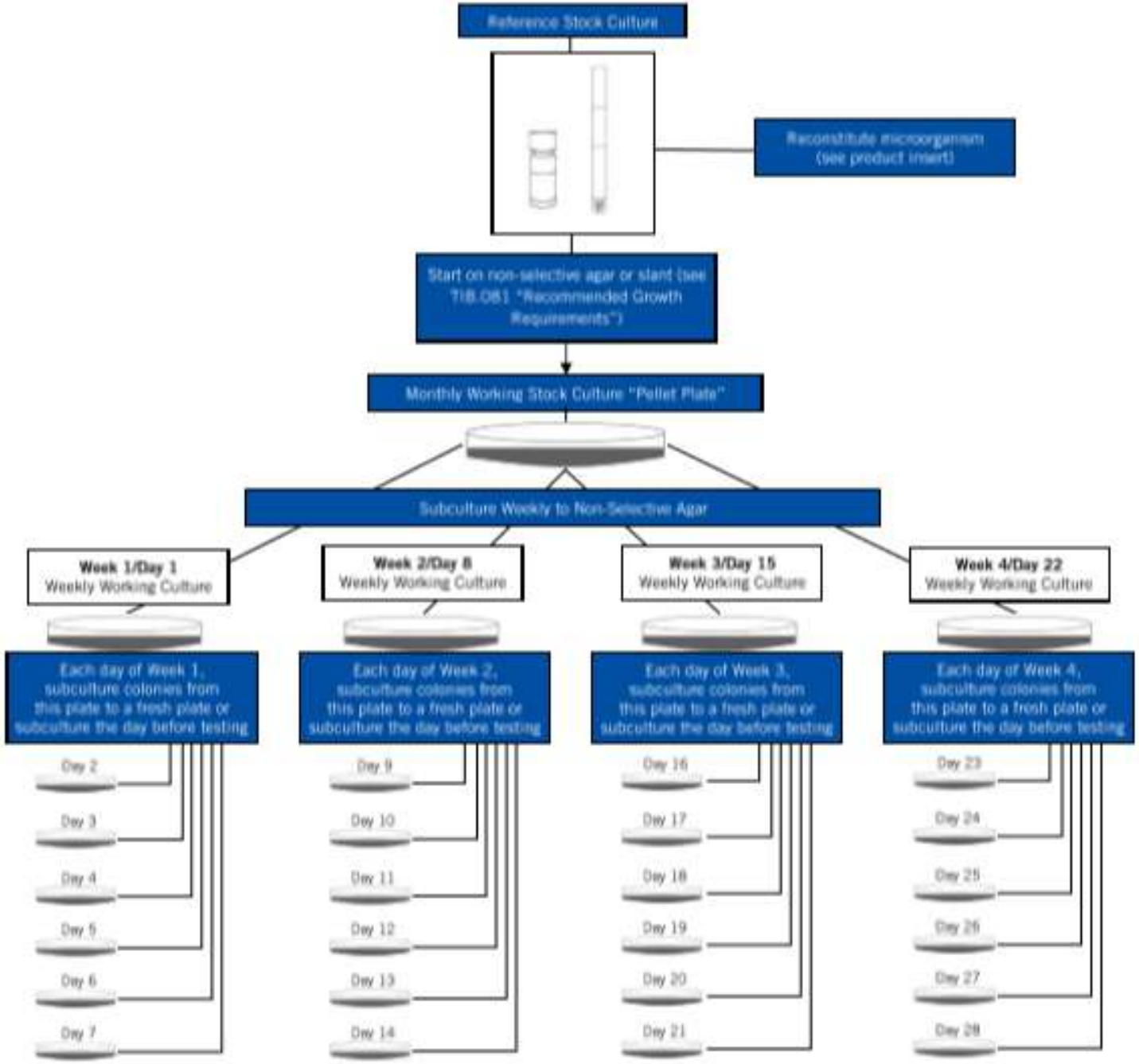
SHORT-TERM MAINTENANCE

Storage of Reconstituted Microorganisms

- Most quality control microorganisms can be maintained on nonselective agar plates or slants for up to four weeks at room temperature or in the refrigerator.[1, 4]
- Fastidious microorganisms have shorter survival periods than aerobic bacteria. They will need to be subcultured every few days. For example, *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* need to be subcultured every third day.
- Microbiologics has found the following storage conditions to be favorable for maintenance:

Category of Microorganism	Storage Conditions
Aerobic Bacteria	Store at 2-8°C. A few species of Bacillus remain viable for a longer period when stored at room temperature.
CO_2 Dependent Species	Store at room temperature in a candle jar or in a container with a CO2 packet.
Yeast and Fungi	Store at room temperature.
Anaerobes	Store in anaerobic conditions at room temperature.
Campylobacter	Store on chocolate agar at 35°C in microaerophilic conditions.

- Microorganisms stored at 4°C should not be used for certain tests. Consult manufacturer's instructions.
- If the resuscitated culture is frozen, Microbiologics cannot guarantee the stated characteristics of the product.


Reference Stock Culture
Reconstitute microorganism (see product insert)
Start on non-selective agar or slant (see TIB.081 "Recommended Growth Requirements")
Monthly Working Stock Culture "Pellet Plate"
Subculture Weekly to Non-Selective Agar
Week 1/Day 1 Weekly Working Culture
Week 2/Day 8 Weekly Working Culture
Week 3/Day 15 Weekly Working Culture
Week 4/Day 22 Weekly Working Culture
Each day of Week 1, subculture colonies from this plate to a fresh plate or subculture the day before testing
Each day of Week 2, subculture colonies from this plate to a fresh plate or subculture the day before testing
Each day of Week 3, subculture colonies from this plate to a fresh plate or subculture the day before testing
Each day of Week 4, subculture colonies from this plate to a fresh plate or subculture the day before testing
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14
Day 16
Day 17
Day 18
Day 19
Day 20
Day 21
Day 23
Day 24
Day 25
Day 26
Day 27
Day 28

LONG-TERM PRESERVATION METHODS

Preservation Method	Pros	Cons
Ultra-low Freezing **Cryogenic Freezing**	+ Reduces probability of mutation + Longer survival rate	- Labor intensive - Costly - Requires closely monitor temperature - Vulnerable to power outages and failures
Lyophilization	+ Reduced risk of intracellular ice crystallization; halts all enzymatic and non-enzymatic reactions + Easy storage	- Requires specialized equipment - Labor intensive - Lyophilization expertise required

LONG-TERM MAINTENANCE

We do not provide recommendations for cryopreservation of the resuscitated culture

- Not recommended by ATCC either
- Product performance no longer warranted

Custom Preservation

- Microbiologics offers a Fee based service to preserved customer's in-house strains
- Most popular format is enumerated but can be made into a KWIK-STIK™

GROWTH REQUIREMENTS

Microbiologics

Selection of Growth Requirements

1. Primary growth on a nonselective agar medium is preferred. Primary growth in a fluid medium should only occur in special instances or when recommended. Because of the manipulations required during hydration, it is difficult to obtain purity of a lyophilized strain in a fluid medium. A contaminant may completely overgrow and obscure the presence of the lyophilized strain.

2. The following information lists which method should be used to grow the various microorganism species. Descriptions of methods follow the microorganism list.

Common Uses

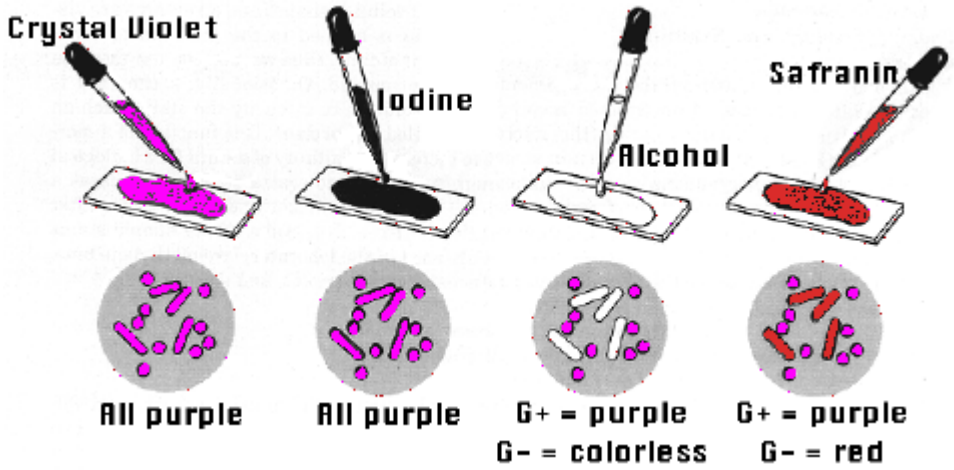
Quality Control of Basic Laboratory Tests:

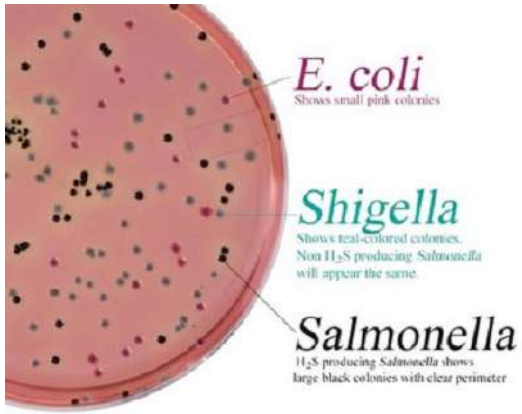
- Rapid Identification Systems (e.g. Vitek 2 or Bruker BioTyper)
- New lot QC of media and daily/weekly QC of media
  - Selective and differential features
  - Growth or no growth
- Susceptibility systems, disks and antibiotics
- Fermentation and utilization of sugars
- Oxidase and Catalase

IDENTIFICATION OF CULTURES

There are several ways to identify microorganisms:

- **Microscopy**
  - Gram Positive/Negative
  - Shape
  - Motility
- **Macroscopic**
  - Use of Selective and Differential Medias
  - Color
  - Shape
- **Phenotypic**
  - Ability to ferment certain sugars
  - Oxidase





Gram Stain

To remember the differences in the cell wall of gram positive & negative organisms - think of a boring, *long powerpoint presentation.*
Long ppt will be your mnemonic guide =D

Lipopolysaccharide
Outer membrane
Negative
Gram?

Positive
Peptidoglycan (thick)
Teichoic acid

Identification Using Instrumentation

Vitek – Growth based

- Each test is measures a specific characteristic
  - Ex. Enzyme hydrolysis, Growth in the presence of inhibitory substances, Fermentation of carbohydrates

Bruker Biotyper or Vitek MS – MALDI-TOF

- Matrix-Assisted Laser desorption/ionization Time Of Flight
- Based on the unique cellular protein profile of each microorganism
- Peaks are created based on the weight of the protein (mass/charge) and its abundance (intensity

Vitek

- **Each card is cycled through and read every 15 minutes**
- **It collects data on which wells have reacted and which have not and puts it into an algorithim to match to known biochemical patterns (the Bionumber)**
- **Once it has enough information it will stop reading the card and eject it**
- **Each card takes time to process:**
  - Identification: between 8-18 hours
  - Susceptibility: 16-24 hours for bacteria, 24-48 for yeast

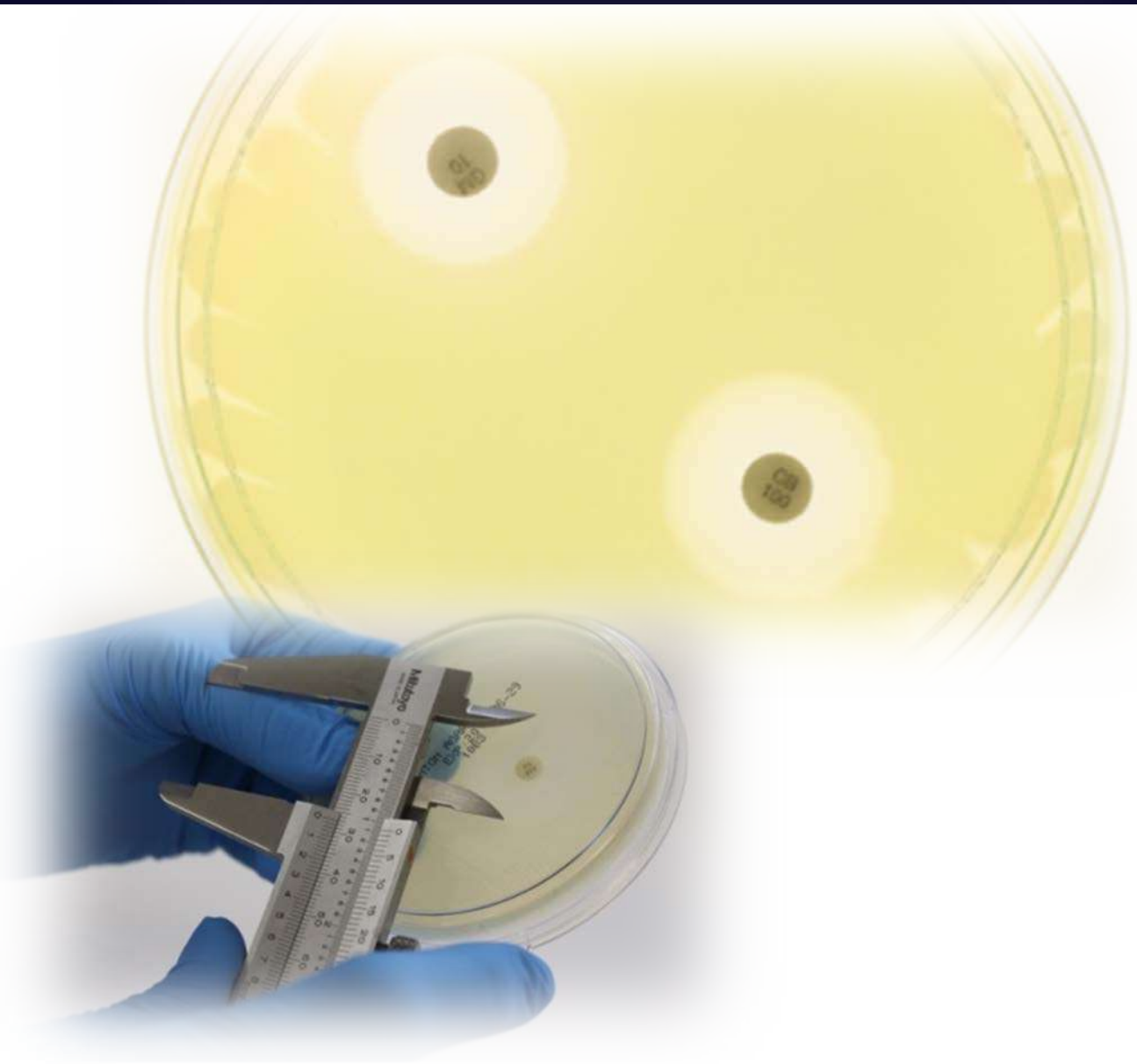
Vitek

Different cards for different types of organism groups

QC Example:

- KWIK-STIK™ 2 Pack *E. coli* ATCC 25922 Catalog Number 0335P
- Used to QC the Vitek 2 GN card
- Customer will grow the strain, make a suspension and run it like a patient sample
- The GN card is acceptable if specific reactions are met

Susceptibility



Used to determine how to treat a patient

Two common methods:

1. Disk Diffusion
2. Broth Microdilution

Can be performed Manually and using Instrumentation

QC Sets and Panels – KWIK-STIK™ 2 Pack

- **Vitek AST Panels**
- **Microscan Walkaway Panels**
- **BD Phoenix**
- **Sensititre**

All require live growth from a fresh (18-24hour) old culture. See instrument instructions for use for growth conditions

Susceptibility: Live vs. Molecular

Live Culture Methods

- Timely – can take 5 to 7 days to receive results
- Require expertise to perform
- Can be automated using instrumentation
- Provides true susceptibility data on the patient isolate

Molecular Methods

- Rapid – can indicate if resistance genes present in <3 hours on average
- Automation and improvements do require expertise to interpret results
- Only shows that a resistance gene is present – cannot confirm if it is active

Moral of the story: Live culture is still needed to know true susceptibility data and treatment options! Must be used in parallel with Molecular methods

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Moral of the story: Live culture is still needed to know true susceptibility data and treatment options! Must be used in parallel with Molecular methods

COMMON COMPLAINTS

LOW OR NO RECOVERY

What: Customer is not able to observe growth or as much growth as expected with a KWIK-STIK™

How it happens:

- Incorrect growth conditions used (eg. Media, temperature, atmosphere, time)
- Improper hydration of the pellet
- Incorrect storage conditions
- Exposure to extreme temperatures for extended periods of time

WRONG IDENTIFICATION

What: Customer obtains an ID that they do not expect or does not match what is listed on the product label

How it happens:

- Incorrect growth conditions used (eg. Media, temperature, atmosphere, time)
- Improper maintenance of the strain
- Using the wrong panel to identify the strain
- Limitations of the technology used
  - Example, difficult to differentiate strains of *Bacillus cereus* group using 16s rRNA sequencing with the MicroSeq due to close relatedness, need to use biochemical means to confirm ID

SUSCEPTIBILITY

What: Customer is obtaining results that are more or less resistant than expected

How it happens:

- Incorrect growth conditions used (eg. Media, temperature, atmosphere, time)
- Incorrect storage conditions
- Improper maintenance
  - Storage at -20ºC or less
  - Maintained on agar for more than 2-weeks
- Discs or panels not performing as expected

CONTAMINATION

What: Customer observes growth of a colony that is not listed on the product label.

- Eg. When inspecting the morphology, they should only see only colony type but two are observed. One is the listed E.coli but the other colony is S.aureus

How it happens:

- Culture media contaminated prior to inoculation
- Streaking to close to the edge of the plate
- Failure to use aseptic technique
- Using control material that is already contaminated

Parasite Suspensions

Parasite Suspensions

Features

- A 1ml vial of formalin fixed parasites at a particular developmental stage in a fecal suspension
- Qualitative
- Storage 15-35ºC
- Minimum Shelf-life of 8 months

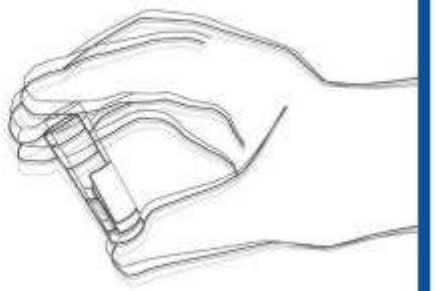
Packaging

- 1ml vial containing a suspension of parasites fixed in formalin in fecal material
- Product code = FPXX

How to Use

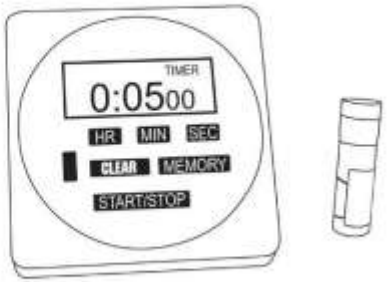
1

Thoroughly mix the suspension by vigorously shaking or vortexing.

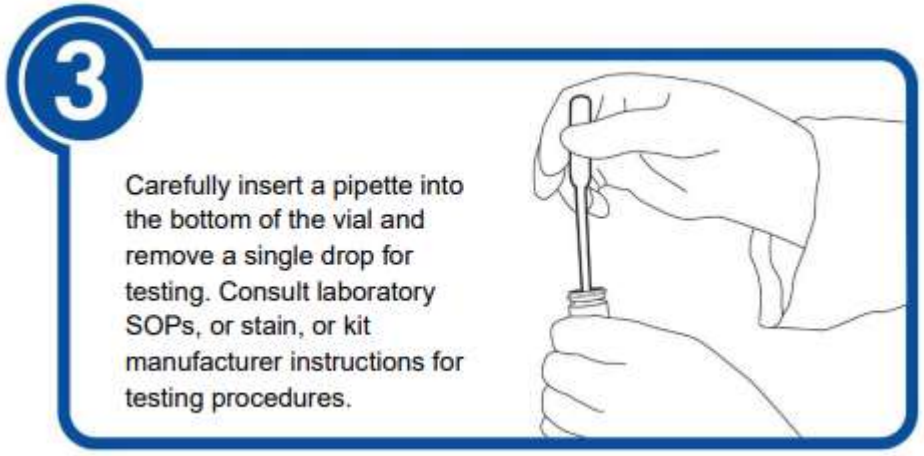


2

Allow the suspension to settle for 5-10 minutes before use in any testing procedure.



How to Use



- For O&P, place a drop of iodine on the slide and view under a 40x magnification – adjust a needed
- Due to the formalin, it is not recommended that this product be used in molecular applications such as qPCR
- Other parasites may be present than the one listed on the label – consult the Certificate of Analysis for further information

QC Microbiology Slides

QC Microbiology Slides

Features

- Box of 10 slides containing a specified fixed material
- Designed with common staining methods in mind
- Qualitative
- Storage 2-25ºC
- Minimum Shelf-life of 3 months

Packaging

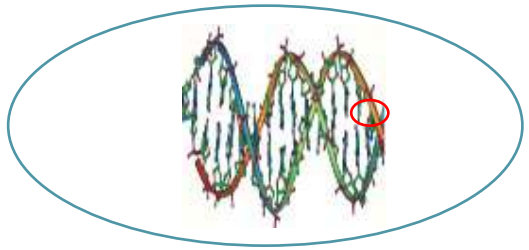
- A white cardboard box containing 10 slides
- Product code = SLXX-10

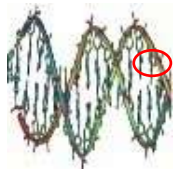
MICROBIOLOGICS MOLECULAR PRODUCTS

HELIX ELITE™ MOLECULAR STANDARDS

HELIX | ELITE™
MOLECULAR STANDARDS

- Inactivated (Extraction, Amplification, and Detection)
- Genomic DNA (Amplification and Detection)
- Synthetic (Amplification and Detection)

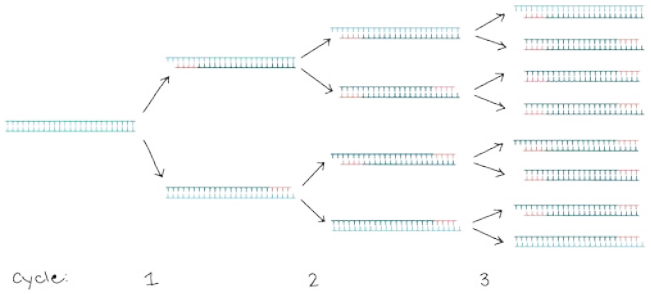




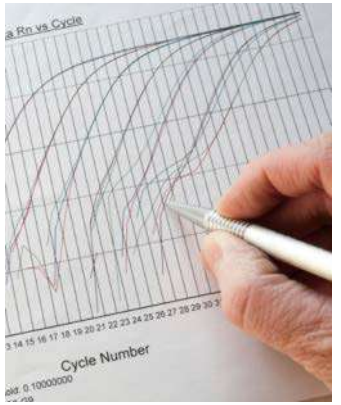
MEETING MOLECULAR CONTROL REQUIREMENTS

Extraction

Amplification



Detection



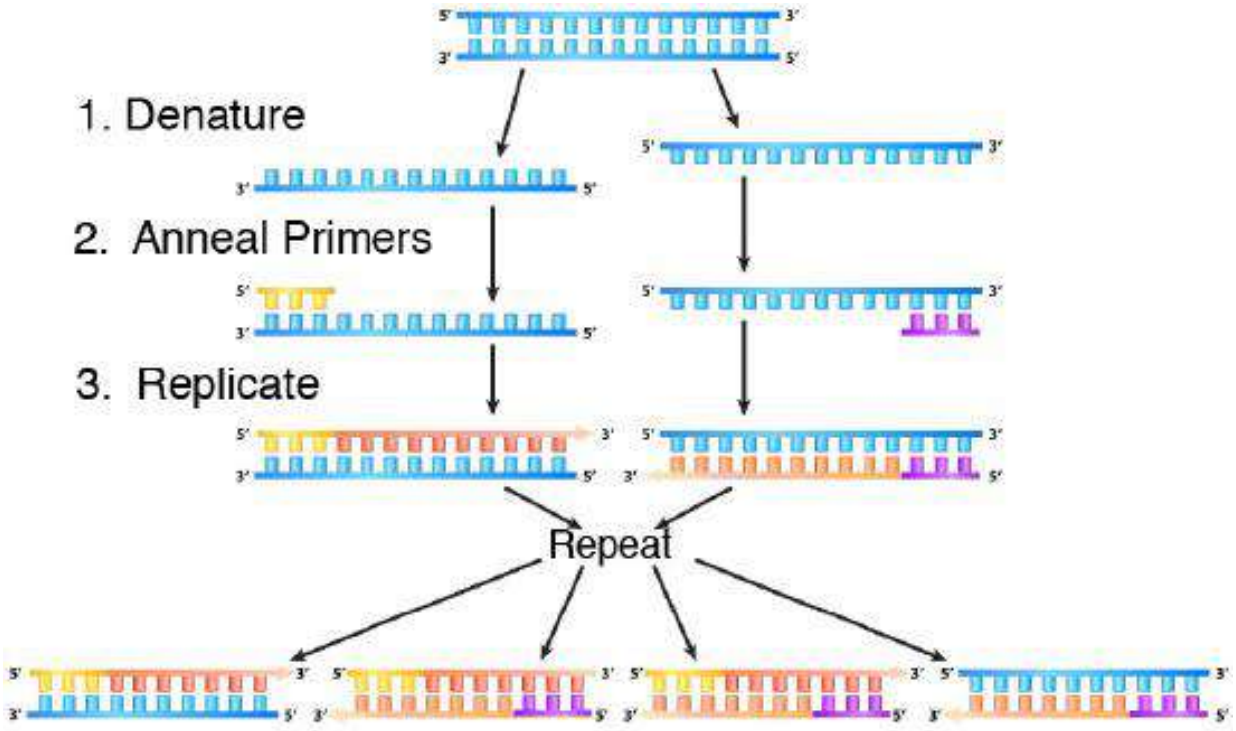
- There are multiple stages involved with any diagnostic test
- Each stage must be controlled; however, different formats may be required at each individual stage

STEP 1: EXTRACTION

Nucleic acids (DNA/RNA) are extracted from specimens either manually or using an automated method.

Control Format Required:
It is best to use whole bacteria or virus because it will mimic the patient sample.

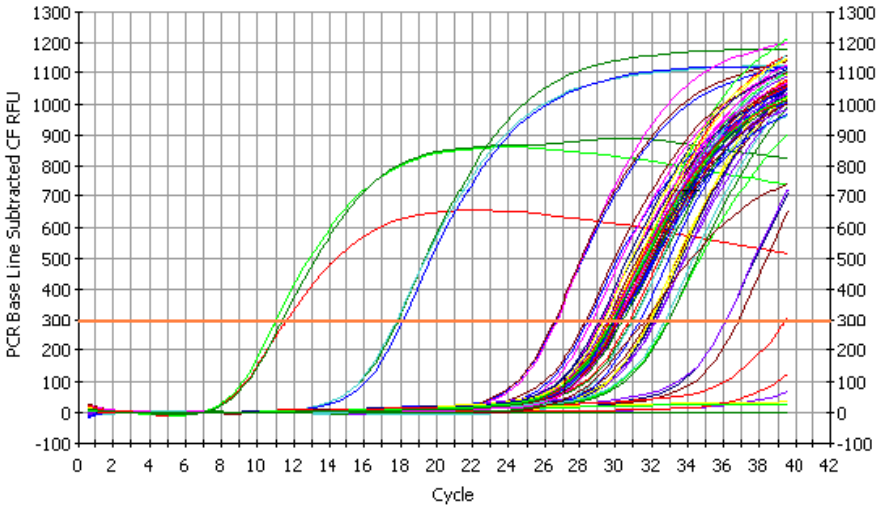
STEP 2: AMPLIFICATION

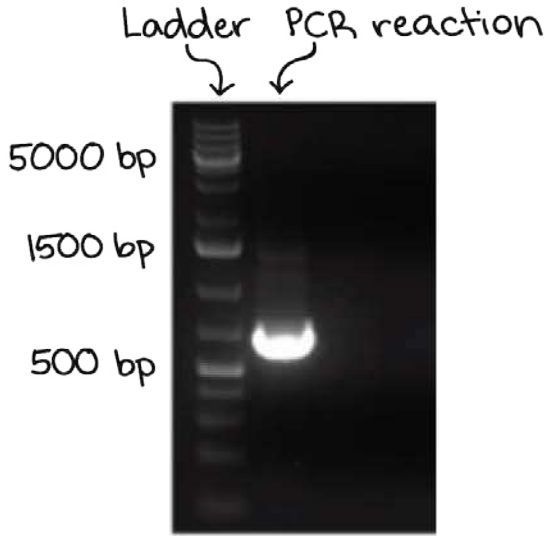


Production of multiple copies of a sequence of DNA for quantification or strain typing in a polymerase chain reaction
(PCR)

Control Format Required:
Positive controls could be purified nucleic acid,
such as a synthetic standard,
or a whole genomic extract

STEP 3: DETECTION





Visualization of the PCR material

- Is the correct band present on the agarose gel?
- How many cycles did it take to detect the target?
- Was there amplification?

APPLICATIONS

HELIX|ELITE™
MOLECULAR STANDARDS

- QC of commercial molecular testing systems
- Verification and Validation of Laboratory Developed Tests (LDTs)
- Sensitivity
- Specificity
- Assay Optimization
- Training
- Proficiency
- Lot-to-Lot Testing


GENOMIC DNA

Helix Elite™ GENOMIC EXTRACT

Format:

- Extracted DNA and purified from a low passage reference strain
- 2-25ºC Storage before hydration
  - At or below -20ºC after hydrated
- Genome copy number determined by qPCR
- Approx. 10^6 copies of genomic DNA per vial

Packaging

- Red plastic box
- 1 vial of genomic extract
- 1 vial of Helix Elite™ Molecular Standard water
- Instructions for Use

This product is being discontinued

USES

- Verification/Validation and control of Mycoplasma detection in cell cultures and media
- Drugs made using cell cultures tend to be for those already immunocompromised
  - Biologics such as Humira and Enbrel (RA treatment)
  - Monoclonal Antibodies for treatment of cancer and MS

SYNTHETIC CONTROLS

SYNTHETIC HELIX ELITE™ MOLECULAR STANDARDS

Format:

- Synthetic RNA or DNA designed as a consensus sequence of diagnostically relevant genetic target
- 2-8ºC Storage until rehydrated
  - At or below -20ºC after rehydration
- Multi-use vial

Packaging

- 1 (one) vial of dried synthetic DNA or RNA (approximately 100 reactions) 1
- 1(one) vial of molecular standard water for rehydration

HOW TO USE

1 Rehydration

Open the foil pouch and then centrifuge the synthetic **Helix Elite™ Molecular Standard** tube before opening the tube to avoid loss of the dried material.

2

Add 55 µl **Helix Elite™** molecular standard water to the **Helix Elite™ Molecular Standard** tube.

3

Incubate the **Helix Elite™ Molecular Standard** tube at 2°C-8°C for 15 minutes to allow for complete rehydration.

HOW TO USE

4

Mix the hydrated **Helix Elite™ Molecular Standard** by gently pipetting up and down several times.

Do not vortex as this may damage the nucleic acids.

5

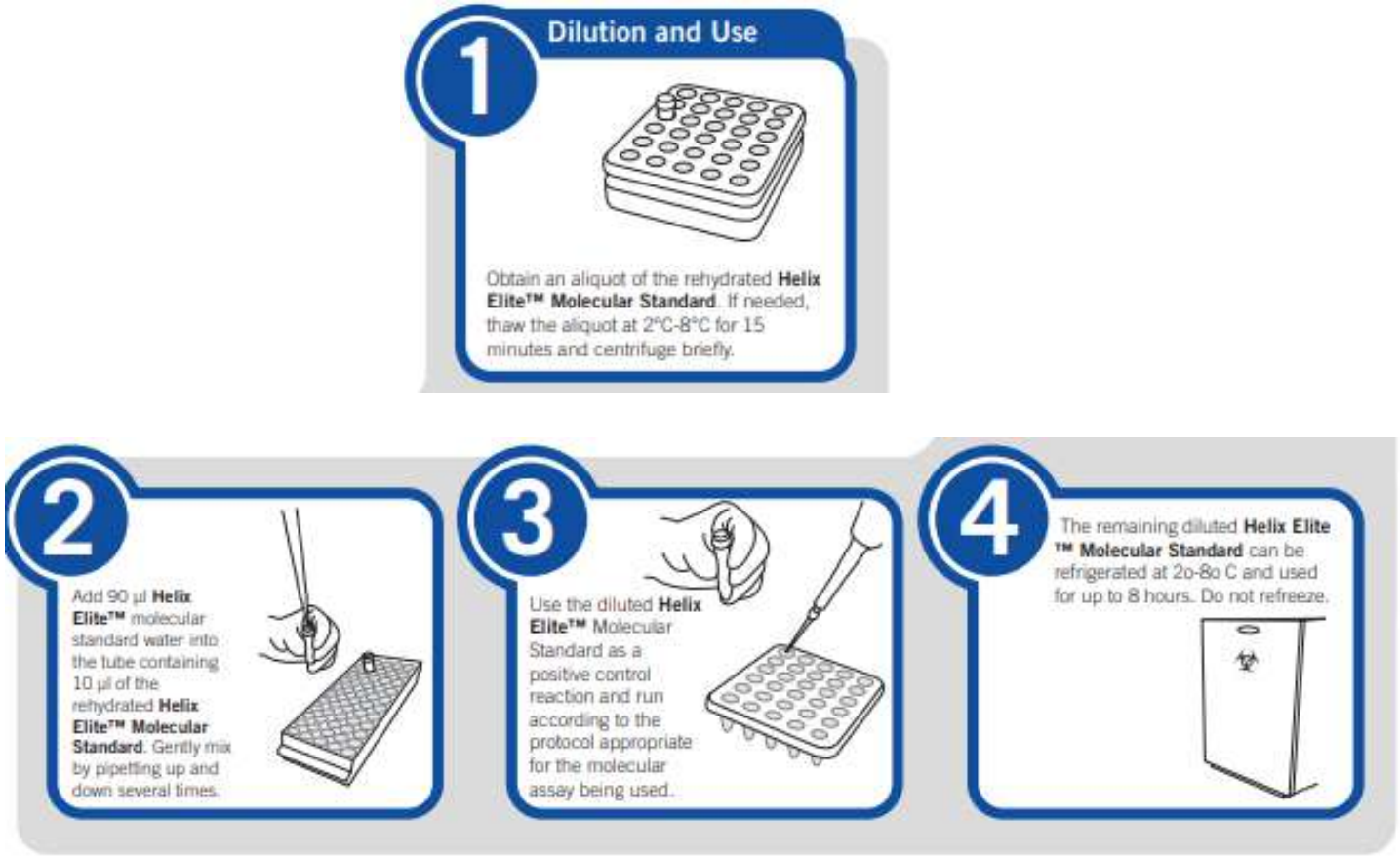
Briefly centrifuge to ensure all liquid is in the bottom of the tube.

6

Aliquot 10 µl of the rehydrated synthetic **Helix Elite™ Molecular Standard** into 5 new, labeled microcentrifuge tubes.

Store aliquots at or below -20°C. These tubes are concentrated stock tubes that must be diluted further for use in molecular assays.

HOW TO USE


1
Dilution and Use
Obtain an aliquot of the rehydrated **Helix Elite™ Molecular Standard**. If needed, thaw the aliquot at 2°C-8°C for 15 minutes and centrifuge briefly.
2
Add 90 µl **Helix Elite™** molecular standard water into the tube containing 10 µl of the rehydrated **Helix Elite™ Molecular Standard**. Gently mix by pipetting up and down several times.
3
Use the diluted **Helix Elite™** Molecular Standard as a positive control reaction and run according to the protocol appropriate for the molecular assay being used.
4
The remaining diluted **Helix Elite ™ Molecular Standard** can be refrigerated at 2o-8o C and used for up to 8 hours. Do not refreeze.

WHY SYNTHETIC HELIX ELITE™?

Manual Methods	Microbiologics Solutions
Time consuming (from 3 hours to 1 day)	Ready-to-use upon arrival
Cross contamination risk	DNA already extracted which helps to avoid cross contamination
Requires skilled technicians to perform extraction	Easy-to-use so minimal training is required
Live organism (health risk)	Non-viable (safe)

COMMON ISSUES

What: Assay is not detecting the desired microorganism/target

Why:

#1 – the primers and probes of the assay are designed to detect a different gene that what is contained in the synthetic sequence

#2 – over dilution of the synthetic material

#3 – contamination during processing

INACTIVATED CONTROLS

INACTIVATED HELIX ELITE™ MOLECULAR STANDARDS

Format:

- lyophilized pellet containing fully intact, inactivated organism(s)
  - Medium to high titer
  - Unassayed/qualitative
- IVD and CE Marked
- Quick dissolve
- Storage at 2 - 25ºC

Configuration:

- 5 individually packaged pellets
- Instructions for Use
- Certificate of Analysis

WHY A FULL PROCESS CONTROL?

Full process controls, from extraction through detection, are important because they are:

- Used as a surrogate for real samples to test efficiency of the extraction process
- Used as “Spike-In Controls” to determine the effect of the matrix on the overall recovery
- Processed at the same time and in the same manner as the patient sample to show test effectiveness

HELIX ELITE™ QC SETS AND PANELS

Format:

- Inactivated or Synthetic Pellets
- Inactivated or Synthetic Swabs
- Instrument Specific (Assayed)
- Syndrome Specific (Unassayed)
- Storage 2 – 25ºC
- Pre-Pooled if applicable
- Quick dissolve
- Designed to be hydrated to work like a patient sample

Configuration:

- Instructions for Use
- Certificate of Analysis

Microbiologics®
QC SETS AND PANELS

MOST POPULAR MOLECULAR QC SETS & PANELS

- **BD MAX™**
- **BioFire FilmArray®**
- **Cepheid GeneXpert®**

PROCESS SUMMARY

Products	Application	Extraction	Amplification	Detection
Inactivated	Process Control Verification/ Validation Testing	Yes	Yes	Yes
Genomic	Positive Control Verification/ Validation Testing	No	Yes	Yes
Synthetic	Positive Control Assay Development	No	Yes	Yes

Instrument Specific Controls

BD MAX™ CONTROL PRODUCTS: INACTIVATED PELLETS

- **Full process controls**
- **Pre-pooled when applicable to mimic processing of patient sample**
- **Ready-to-use**
- **Room temperature storage**
- **In vitro diagnostic (IVD)**

BD MAX™ ASSAYS AND MICROBIOLOGICS CONTROLS

BD	Microbiologics			
Assay	Cat # for Verification/Validation	Verification/Validation Product	Cat # for Ongoing QC	Ongoing QC Product
BD MAX™ VAGINAL PANEL	8208	Vaginal Verification Panel (Inactivated Pellet)	8209	Vaginal Control Panel (Inactivated Pellet)
BD MAX™ CT/GC/TV	8193	BD MAX™ CT/GC/TV 20-Day QC Panel	8228	CT/GC/TV Control Panel (Inactivated Pellet)
BD MAX™ GBS	8173	Strep B Organism Set (Live Culture)	8232	Group B Streptococcus (GBS) Control Panel (Inactivated Swab)
BD MAX™ ENTERIC BACTERIAL PANEL	8179	Enteric Bacterial Organism Set (Live Culture)	8231	Comprehensive Enteric Bacterial Control Panel
BD MAX™ EXTENDED ENTERIC BACTERIAL PANEL	8191	Extended Enteric Bacterial Verification Panel (Inactivated Pellet)	8231	Comprehensive Enteric Bacterial Control Panel
BD MAX™ ENTERIC PARASITE PANEL	8202	BD MAX™ Enteric Parasite 20-Day QC Panel	8204	BD MAX™ Enteric Parasite Control Panel
BD MAX™ ENTERIC VIRAL PANEL	8210	Enteric Viral Verification Panel	8211	Enteric Viral Control Panel
BD MAX™ CDIFF	8243	Cdiff Verification Panel (Live Culture)	8227	C. difficile Control Panel (Inactivated Swab)
BD MAX™ MRSA XT	8178	SA Organism Set (Live Culture)	HE0053NS, HE0054NS, HE0055NS	Methicillin-Resistant Staphylococcus aureus (MRSA) Inactivated Swab, Methicillin-Susceptible Staphylococcus aureus (MSSA) Inactivated Swab, Methicillin-Susceptible Staphylococcus epidermidis (MSSE) Inactivated Swab
BD MAX™ STAPHSR	8178	SA Organism Set (Live Culture)	HE0053NS, HE0054NS, HE0055NS	Methicillin-Resistant Staphylococcus aureus (MRSA) Inactivated Swab, Methicillin-Susceptible Staphylococcus aureus (MSSA) Inactivated Swab, Methicillin-Susceptible Staphylococcus epidermidis (MSSE) Inactivated Swab

HOW IT USE

1. **Transfer pellet or swab to sample buffer tube**
    i. If a swab, lift a couple mm from the bottom and snap the swab at top of the tube
2. **Vortex, invert, vortex**
3. **Process according to BD insert**

BioFire® CONTROL PRODUCTS

- **Full process control**
- **Pre-pooled pellets to mimic the processing of a patient sample**
- **Ready-to-use**
- **Room temperature storage**
- **In vitro diagnostic (IVD)**

BioFire's Panels and Microbiologics Controls

BioFire	Microbiologics			
Assay	**Cat # for Verification/ Validation**	**Verification/Validation Product**	**Cat # for Ongoing QC**	**Ongoing QC Product**
The BioFire® FilmArray® Respiratory Panel (RP)	N/A	N/A	8217	Respiratory (21 Targets) Control Panel
The BioFire® FilmArray® Respiratory Panel 2 (RP2)	N/A	N/A	8217	Respiratory (21 Targets) Control Panel
The BioFire® FilmArray® Respiratory Panel 2.1 (RP2.1)	N/A	N/A	8247	Respiratory (22 Targets) Control Panel
The BioFire® FilmArray® Blood Culture Identification Panel	8201	Blood Culture Identification (BCID) Verification Panel (Inactivated)	8215	Blood Culture Identification (BCID) Control Panel (Inactivated)
The BioFire® FilmArray® Gastrointestinal Panel	N/A	N/A	8236	Gastrointestinal (22 Targets) Control Panel
The BioFire® FilmArray® Meningitis/Encephalitis Panel	N/A	N/A	Coming in 2021	Meningitis/Encephalitis (14 Pathogens) Control Panel
The BioFire® FilmArray® Pneumonia Panel	N/A	N/A	Coming in 2021	Pneumonia (33 Targets) Control Panel

HOW TO USE

1. **Transfer the pellet to the specified minimum hydration volume**
2. **Vortex to dissolve material.**
3. **Start testing based on BioFire's Verification Protocol.**
4. **Store hydrated materials at 2°C-8°C for up to 72 hours for use throughout the remainder of the verification testing**

HOW TO USE

1. **Hydrate pellet with the minimum hydration volume**
    i. E.g. RP panel, hydrated one pellet in 300µl
2. **Vortex to mix**
3. **Process according to BioFire's insert**
    i. E.g. RP panel, add full 300µl suspension to sample vial

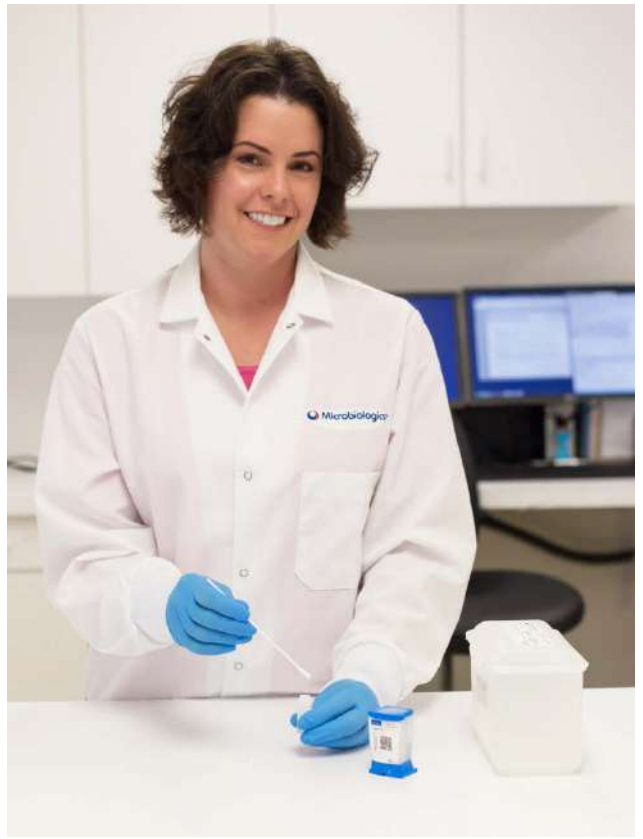
Cepheid GeneXpert PRODUCTS

Format:

- Inactivated swab format
- Positive and negative controls included in each kit
- Ready-to-use
- Room temperature storage
- In vitro diagnostic (IVD)

Configuration

- 6 swabs of each pool per kit
- Instructions for Use
- Certificate of Analysis



CEPHEID ASSAYS AND MICROBIOLOGICS CONTROLS

Cepheid	Microbiologics	
Assay	Cat # for Ongoing QC	Ongoing QC Product
Xpert® C. difficile	8200	Cepheid Xpert® C. difficile Control Panel
Xpert® C. difficile/Epi	8200	Cepheid Xpert® C. difficile Control Panel
Xpert® Carba-R	8187	Carbapenem-resistant Enterobacteriaceae (CRE) Control Panel (Inactivated Swab)
Xpert® CT/NG	8188	Cepheid Xpert® CT/NG Control Panel
Xpert® EV	8190	Enterovirus (EV) Control Panel (Inactivated Swab)
Xpert® Flu/RSV XC	8199	Cepheid Xpert® Respiratory Control Panel
Xpert® GBS	8194	Cepheid Xpert® GBS LB Control Panel
Xpert® GBS LB	8194	Cepheid Xpert® GBS LB Control Panel
Xpert® MRSA NxG	8195	Cepheid Xpert® MRSA/MRSA NxG Control Panel
Xpert® MRSA/SA BC	8196	Cepheid Xpert® SA Nasal Complete Control Panel
Xpert® MRSA/SA SSTI	8196	Cepheid Xpert® SA Nasal Complete Control Panel

CEPHEID ASSAYS AND MICROBIOLOGICS CONTROLS

Cepheid	Microbiologics	
Assay	**Cat # for Ongoing QC**	**Ongoing QC Product**
Xpert® MTB/RIF	8238, 8206	Rifampicin-Resistant Mycobacterium tuberculosis Positive Control Panel (Inactivated Pellet) and Rifampicin-Resistant Mycobacterium tuberculosis Negative Control (Inactivated Pellet)
Xpert® Norovirus	8205	Norovirus Control Panel (Inactivated Swab)
Xpert® SA Nasal Complete	8196	Cepheid Xpert® SA Nasal Complete Control Panel
Xpert® TV	8189	Trichomonas vaginalis (TV) Control Panel (Inactivated Swab)
Xpert® vanA	8203	Vancomycin Resistant Enterococcus (VRE) Control Panel (Inactivated Swab)
Xpert® Xpress Flu	8199	Cepheid Xpert® Respiratory Control Panel
Xpert® Xpress Flu/RSV	8199	Cepheid Xpert® Respiratory Control Panel
Xpert® Xpress Strep A	8219	Group A Streptococcus (GAS) Control Panel
Xpert® Xpress SARS-CoV-2/Flu/RSV	8246	Flu/RSV/SARS-CoV-2 Control Panel
Xpert® Xpress SARS-CoV-2	HE0066NS	Inactivated SARS-CoV-2 Whole Virus (Swab)

HOW TO USE

Instructions for Sample Preparation Reagent

1. Break Swab in Vial or Transport Medium Vial (check Xpert instructions)
2. Vortex
3. Transfer to cartridge and test per assay instructions!

Instructions for Direct Inoculation with Dry or Pre-Wet Swab

1. Break swab in cartridge
2. Test per assay instructions!

Syndromic Testing

SYNDROMIC TESTING GROUPS

- Blood Culture ID (BCID)
- Health Care Associated Infections (HAIs)
- Gastrointestinal (GI)
- Respiratory
- Women’s Health and STIs
- Emerging Diseases and Other

BLOOD CULTURE (BCID)

Microorganisms that cause sepsis (blood infection)

- Gram positive (S.aureus, Strepococcus pyogenes)
- Gram negative (E.coli, Psueodmonas, K.pneumoniae)
- Fungal (Candida albicans, Candida krusei)

BioFire FilmArray and Luminex Verigene Leaders in this testing

- All panels unassayed at this time

HEALTH CARE ASSOCIATED INFECTIONS (HAIs)

Microorganisms that cause disease commonly acquired at medical facilities or during long hospital stays

- MRSA : Methicillin Resistant Staphylococcus aureus
- C.diff: Clostridium difficile
- VRE: Vancomycin resistant Enterococcus
- CRE: Carbapenam Resistant Enterobacteriaciae

Cepheid a leader in this

Three assayed Cepheid panels (MRSA and C.diff)

GASTROINTESTINAL (GI)

Microorganisms that commonly cause gastrointestinal infections

- E.coli
- Salmonella
- Rotavirus
- Norovirus

Biofire, BD MAX and Cehied major players

Microorganism that cause respiratory infections

- Group A Strep (GAS) – Strep throat
- Influenza Virus
- Respiratory Syncytial Virus (RSV)
- Bordetella (Whooping cough)
- Adenovirus (common cold, bronchitis, pneumonia)

BioFire and Cepheid leaders

WOMEN’S HEALTH AND STI’s

Microorganisms associated with common infections to women and sexually transmitted infections (STIs)

- Chlamydia
- Neisseria gonorrhoeae (GC)
- Human Papillomavirus (HPV)

Cepheid and Hologic leaders

EMERGING DISEASES

Microorganisms that cause newly discovered, or diseases of currently concern

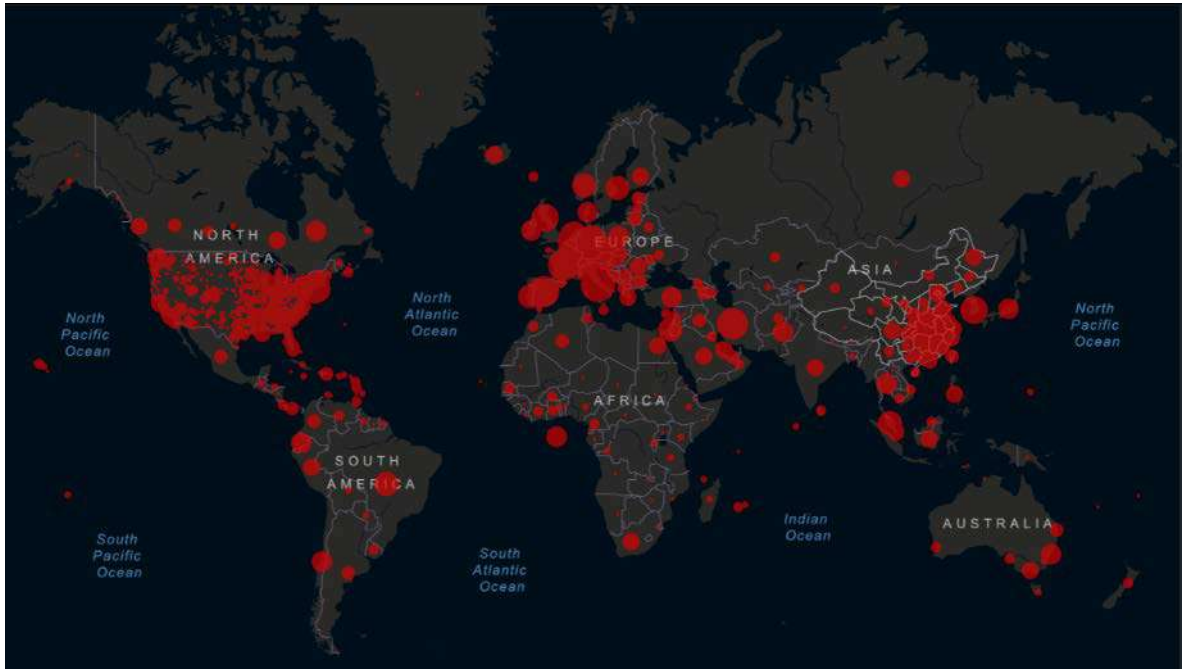
- Parasites : Cyclospora
- Cytomegalovirus (CMV)
- Treponema pallidum (Syphillis)

These are inactivated or synthetic Standards

SARS-CoV-2 Controls

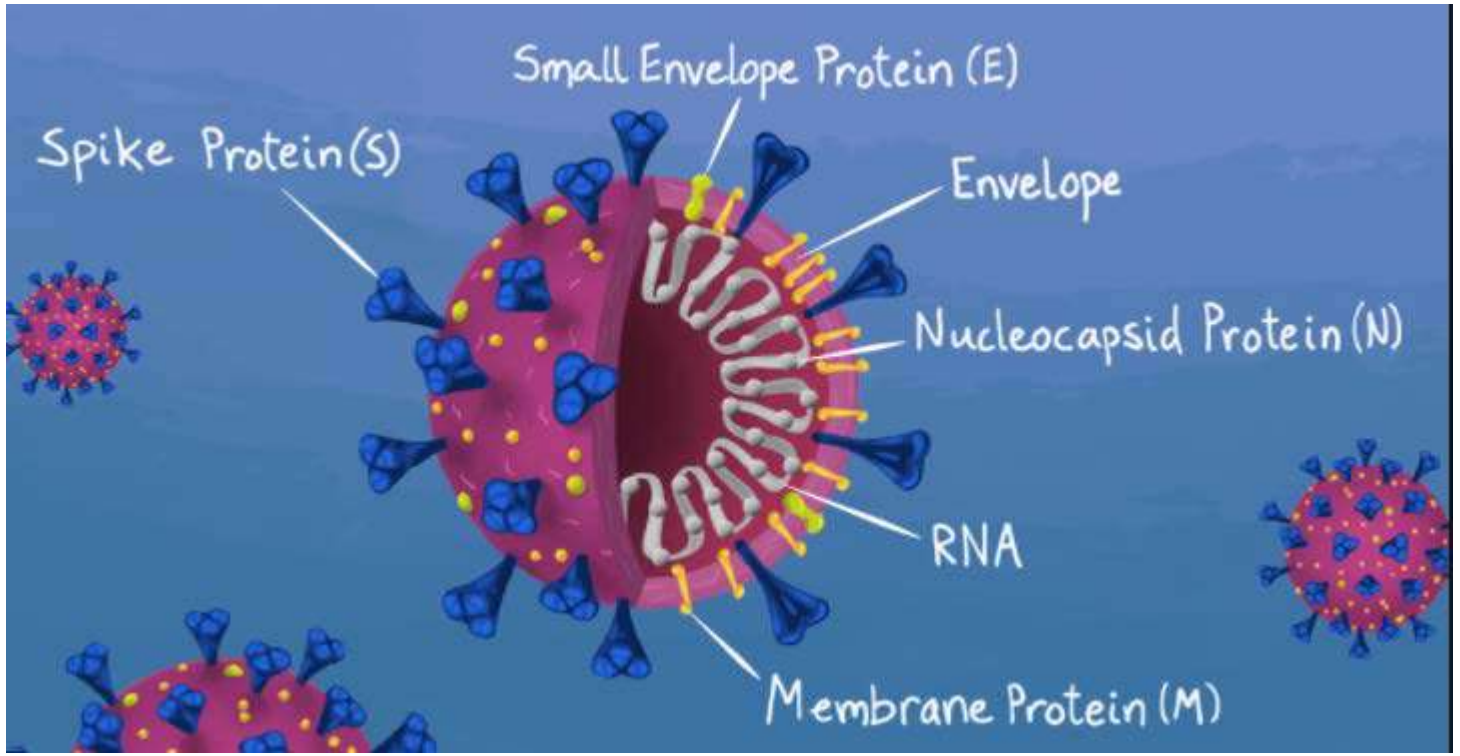
ABOUT SARS-COV-2

- 2019-2020 Coronavirus pandemic
- Caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
- The outbreak was first identified in Wuhan, Hubei, China, in December 2019
- As per Oct. 27th , 43.6M cases and 1.16M death in 189 countries



https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6

SARS-CoV-2 Virus Structure



Source: addgene blog

- Spike Protein – Facilitates entry into the host cell
- Membrane Protein – Promotes virus assembly
- Nucleocapsid Protein – Formation of nucleocapsid in RNA genome
- Envelope Protein – Involved with assembly, budding, and envelope formation
- RdRp – RNA dependent RNA polymerase – Located within the ORF1ab segment.

SARS-CoV-2 GENOME

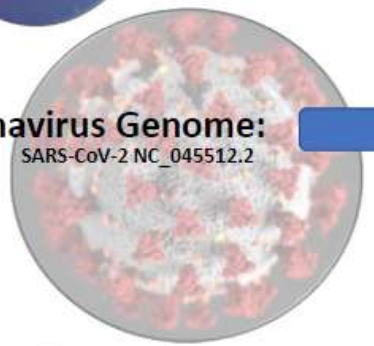
Naked RNA

Large (>1000 nt) RNA pieces of the coronavirus genome to be used as a positive control for assay development or testing for COVID-19

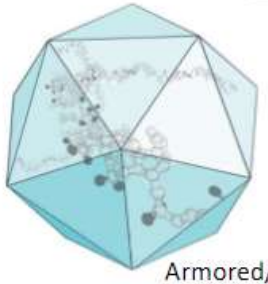
- This 3.3K nt RNA contains the second half of the RdRp gene and a small piece of the first half so as to include the Pasteur primer IP2.
- It does not contain the sequence shaded in light blue below.
- This RNA also contains the entire E gene.

- This 3.8K nt RNA contains the entire S gene and will work with any primers targeting this gene.

- This 1044 nt RNA contains almost the entire N gene
- It lacks the last 253 nt of N.
- It is compatible with all the CDC designed targets (N1, N2 and N3).
- It is compatible with Thailand N Gene targets.



Coronavirus Genome:
SARS-CoV-2 NC_045512.2

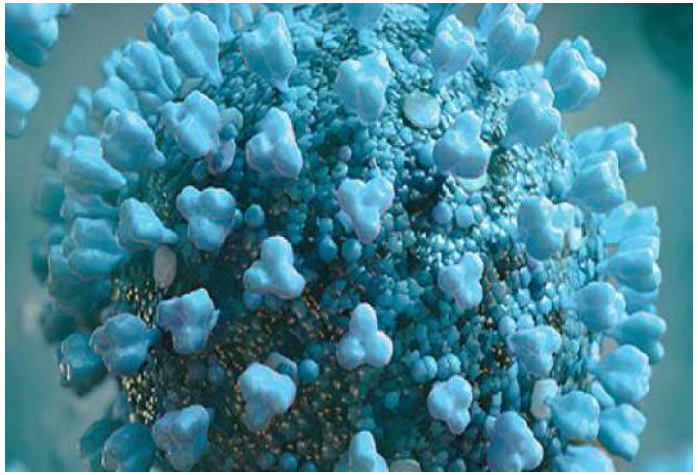


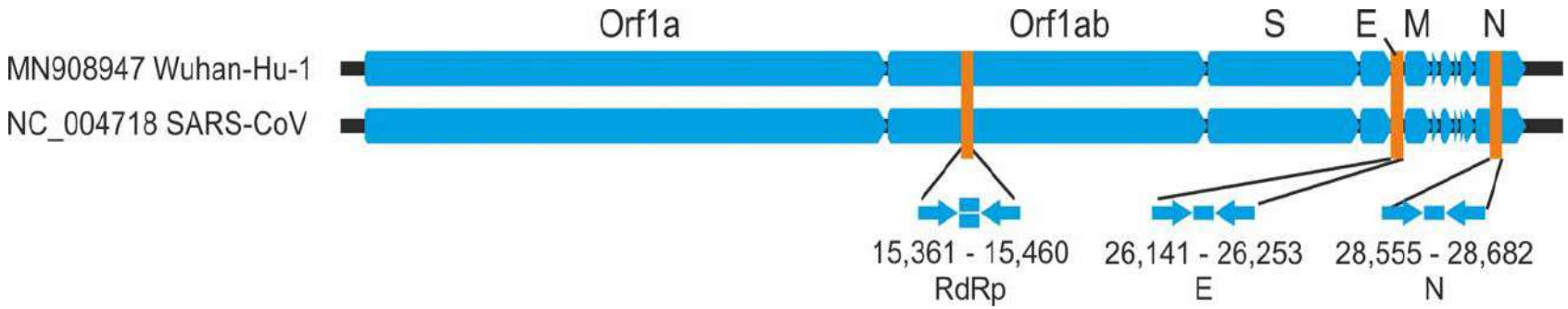
Small (<100 nt) RNA pieces of the coronavirus genome stitched together and protected by a proteinaceous coat to be used as a positive extraction control for assay development or testing for COVID-19

Armored/Protected/Shielded RNA

- This 1200 nt RNA contains very short regions specific to all the selected WHO identified primer binding sites (red squares on Coronavirus Genome).
- This RNA is contained within n Rnase-resistant proteinaceous coat so as to act as an extraction control.
- This is designed to work only with WHO identified primer sites as listed above—other sites within the coronavirus genome targeted by independent labs will not work with this product.

SARS-CoV-2 International Protocols





Berlin Protocol:
First line screening assay: E gene assay
Confirmatory assay: RdRp gene assay
Additional confirmatory assay: N gene assay

Pasteur Protocol:
First line screening assay: E gene assay
Confirmatory assay: RdRp gene assay
Additional confirmatory assay: N gene assay

China CDC:
Confirmatory assay: RdRp gene assay
Additional confirmatory assay: N gene assay

Hong Kong:
Confirmatory assay: Orf1b
Additional confirmatory assay: N gene assay

US CDC and Japan:
Confirmatory assay: N gene assay

https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance

MICROBIOLOGICS SARS-COV-2 PRODUCT OFFERING

Table 1. IVD SARS-CoV-2 Product Portfolio

Helix Elite Product Name	SARS-CoV-2 Synthetic RNA (N Gene Targets)	SARS-CoV-2 Synthetic RNA (N/E/RdRp/S Gene Targets)	SARS-CoV-2 Process Control (Pellet)	SARS-CoV-2 Process Control (Swab)	Inactivated SARS-CoV-2 Whole Virus (Pellet)	Inactivated SARS-CoV-2 Whole Virus (Swab)
Helix Elite Catalog Number	**HE0060S**	**HE0061S**	**HE0062S**	**HE0063S**	**HE0065N**	**HE0066NS**
Gene Targets	* N1, N2 & N3	* N/E/RdRp/S	* Orf1ab/RdRP/S/E/ ORF8/M/N	* Orf1ab/RdRP/S/E/ ORF8/M/N	Full genome	Full genome
Format	Dried Synthetic RNA	Dried Synthetic RNA	Synthetic RNA encapsulated in a phage protein envelope Lyophilized Pellet	Synthetic RNA encapsulated in a phage protein envelope Lyophilized Swab	Inactivated Whole Virus Lyophilized Pellet	Inactivated Whole Virus Lyophilized Swab
Kit Configuration	1 vial of dried synthetic RNA & 1 vial (1.5ml) of molecular standard water	1 vial of dried synthetic RNA & 1 vial (1.5ml) of molecular standard water	5 individually packaged lyophilized pellets & 5 vials (1.5ml each) of molecular standard water	5 individually packaged swabs	5 individually packaged lyophilized pellets & 5 vials (1.5ml each) of molecular standard water	6 individually packaged swabs
Storage	2-25 degrees C	2-25 degrees C	2-25 degrees C	2-25 degrees C	2-25 degrees C	2-25 degrees C
In Use Stability	Aliquots can be stored at -20 degrees C Thawed aliquots are single-use	Aliquots can be stored at -20 degrees C Thawed aliquots are single-use	5 days hydrated at 4 degrees C Multi-use	5 days hydrated at 4 degrees C Single-use	5 days hydrated at 4 degrees C Multi-use	5 days hydrated at 4 degrees C Single-use
Purpose	Quality control for amplification/detection	Quality control for amplification/ detection	Quality control for extraction, amplification and detection	Quality control for extraction, amplification and detection	Quality control for extraction, amplification and detection	Quality control for extraction, amplification and detection
Approximate Copies per vial /swab	1.10E + 06	1.10E + 06	1.00E + 05	1.00E + 05	1.00E + 05	1.00E + 05

SYNTHETIC Helix Elite™ SARS-CoV-2 Controls

Items

- Nucleocapsid (N) Gene Targets N1, N2, N3
  - Cat #**HE0060S**
- N, RdRp, E and S gene Targets
  - Cat #**HE0061S**

Format

- 1 vial of dried synthetic RNA, 1 vial of molecular standard water for rehydration
- Target Concentration: $1.10x10^6$ copies/vial
- Shipping and storage at 2-25°C
- After rehydration can be stored at -20ºC
- CE-IVD

ENCAPSULATED SYNTHETIC Helix Elite™SARS-CoV-2 Controls

Items

- **Cat # HE0062S SARS-CoV-2 Process Control (Pellet)**
- **Cat # HE0063S SARS-CoV-2 Process Control (Swab)**

Format

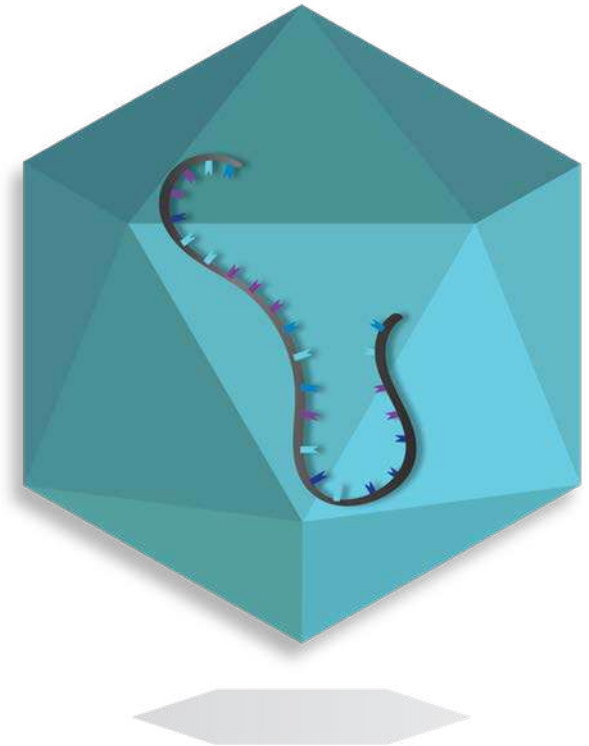
- Ready to use single use pellet or swab
- Synthetic RNA transcripts encapsulated in a phage protein envelope
- Includes human lung epithelial cells
- Orf1ab/RdRP, S (spike), E (envelope), M (membrane Protein), N (nucleocapsid) transcripts
- Target Concentration: $1.10x10^6$ copies/pellet or swab

Configuration

- Storage 2-25ºC
- 5 individual pellets and a vial of molecular grade water OR 5 individual swabs
- Biosafety Level 1

Encapsulated Format

- RNA transcripts encased in bacteriophage coat proteins that form a barrier between the transcripts and the environment
- The protein coat completely encapsulates the RNA, which blocks it from being degraded by nucleases (RNases, etc)
- Naked RNA is fragile and easily degraded, but encapsulated or protected RNA is stable



INACTIVATED Helix Elite™ SARS-CoV-2 Whole Virus Controls

Item

- HE0065N Inactivated SARS-CoV-2 Whole Virus (Pellet)

Format

- Chemically Inactivated whole SARS-CoV-2/USA/WA1/2020 isolate
- Contains human lung epithelial cells
- Target Concentration: $1.10x10^5$ copies/pellet

Configuration

- Storage 2-25ºC
- Biosafety Level 1
- 5 individually packaged pellet and 5 vials of molecular grade water

INACTIVATED Helix Elite™ SARS-CoV-2 Whole Virus Controls

Item

- Catalog Number HE0066NS Inactivated SARS-CoV-2 Whole Virus (Swab)

Format

- Chemically Inactivated whole SARS-CoV-2/USA/WA1/2020 isolate
- Contains human lung epithelial cells
- Target Concentration: $1.10x10^5$ copies/swab

Configuration

- Storage 2-25ºC
- Biosafety Level 1
- 6 individually packaged swabs

HE0065N and HE0066NS are composed of:

- SARS-CoV-2 isolate SARS-CoV-2 USA/WA1/2020
- Human lung epithelial cells
- Lyophilized in a patient-relevant matrix

SARS-CoV-2 Inactivation and Verification

- Live virus is chemically inactivated
- Verified inactivated by a validated-endpoint dilution assay. Ten independent viral samples are inoculated into Vero E6 cells at a multiplicity of infection (MOI) of 1. The inoculated cell cultures were observed for signs of cytopathic effect (CPE) for 5 days to confirm virus inactivation.
- For lot release and to confirm no virus growth, all inactivated virus must demonstrate no visible CPE over 5 days after challenging Vero E6 cells.

Flu/RSV/SARS-CoV-2 CONTROL PANEL

Format

- Catalog Number 8246
- Quick dissolve inactivated swabs

Configuration

- Contains 6 positive controls and 6 negative controls
- Shipping and storage at 2-25°
- In-Use Stability: 5 hours at 25º
- CE-IVD
- BSL1

Flu/RSV/SARS-CoV-2 CONTROL PANEL

Positive Control:

Human Lung Epithelial Cells, A549 (ECACC 86012804)
Influenza A (H3N2) Virus EL-13-03
Influenza A (H1N1) Virus Strain California/04/09 NCPV 0905242v
Influenza B Virus Strain Hong Kong/5/72 EL-14-03
Respiratory Syncytial Virus A Strain Long EL-07-02
SARS-CoV-2, Isolate USA/WA1/2020

Negative Control:

Coxsackievirus B1 NCPV 0812141v
Human Lung Epithelial Cells, A549 (ECACC 86012804)

NEW: RESPIRATORY CONTROL PANEL (22 TARGETS)

Format

- Catalog Number 8247
- Quick dissolve inactivated pellets

Components

- Contains 6 positive controls and 6 negative controls
  - Shipping and storage at 2-25°
  - In-use Stability: 6 hours at 25ºC
  - CE-IVD
  - BSL1

NEW: RESPIRATORY CONTROL PANEL (22 TARGETS)

Positive Control:

Adenovirus Type 6 NCPV 0011056v
Bordetella parapertussis Gibson GL0300
Bordetella pertussis Gibson GL0301
Chlamydophila pneumoniae Microbix EL-46-02
Coronavirus 229E NCPV 0310051v
Coronavirus HKU1 surrogate Microbiologics MB0066
Coronavirus NL63 surrogate Microbiologics MB0065
Coronavirus OC43 surrogate Microbiologics MB0068
Coronavirus OC43 surrogate Microbiologics MB0067
Human Metapneumovirus surrogate Microbiologics MB0059
Human Rhinovirus NCPV 0111101v
Influenza A Microbix EL-13-03
Influenza A subtype H1 Microbix EL-13-02
Influenza A subtype H1-2009 NCPV 0905242v
Influenza A subtype H3 NCPV 0405271
Influenza B NCPV 0910043v
Mycoplasma pneumoniae NCTC 10119
Parainfluenza Virus 1 Microbiologics MB0112
Parainfluenza Virus 2 NCPV 0401115v
Parainfluenza Virus 3 Microbix EL-10-02
Parainfluenza Virus 4a surrogate Microbiologics MB0052
Respiratory Syncytial Virus NCPV 0709161v
SARS CoV-2 Microbiologics MB0167

Negative Control:

Blank, only contains media components

Questions?