



# L'approche syndromique dans le diagnostic des méningites/encéphalites

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Dans une **approche diagnostique** classique face à un patient malade, le médecin effectue:

**Systematiquement :**

Une anamnèse

Un examen clinique

**Fréquemment:**

Des examens complémentaires

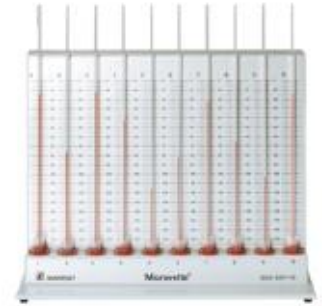
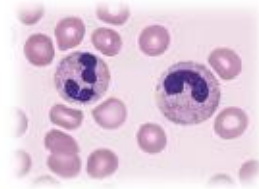




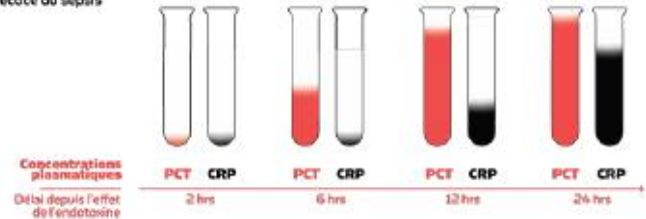
# Tests diagnostiques

## Tests **d'orientation** vers le diagnostic

- Formule sanguine
- Vitesse de sédimentation
- Bandelette urinaire
- Protéine C réactive
- Procalcitonine
- Rx du thorax



Le dosage de la PCT permet un diagnostic précoce du sepsis





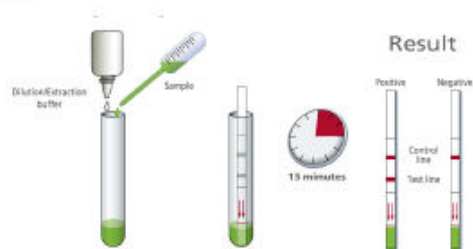
# Tests diagnostiques

Test diagnostique centré sur **un** pathogène

- Influenza
- Virus respiratoire syncytial
- HIV
- Streptocoque gr A
- TBC, Malaria...



Détection Ag/Ac



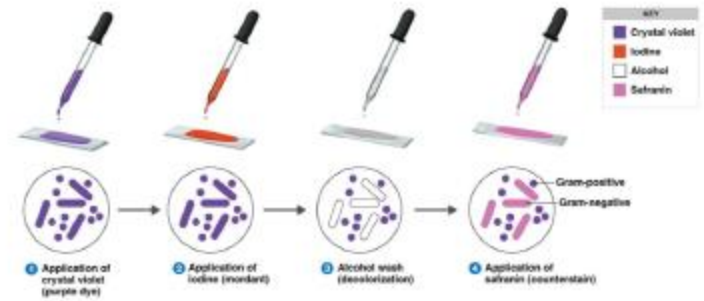
PCR



# Tests diagnostiques

Test diagnostique centré sur **plusieurs** pathogènes

- Coloration de Gram

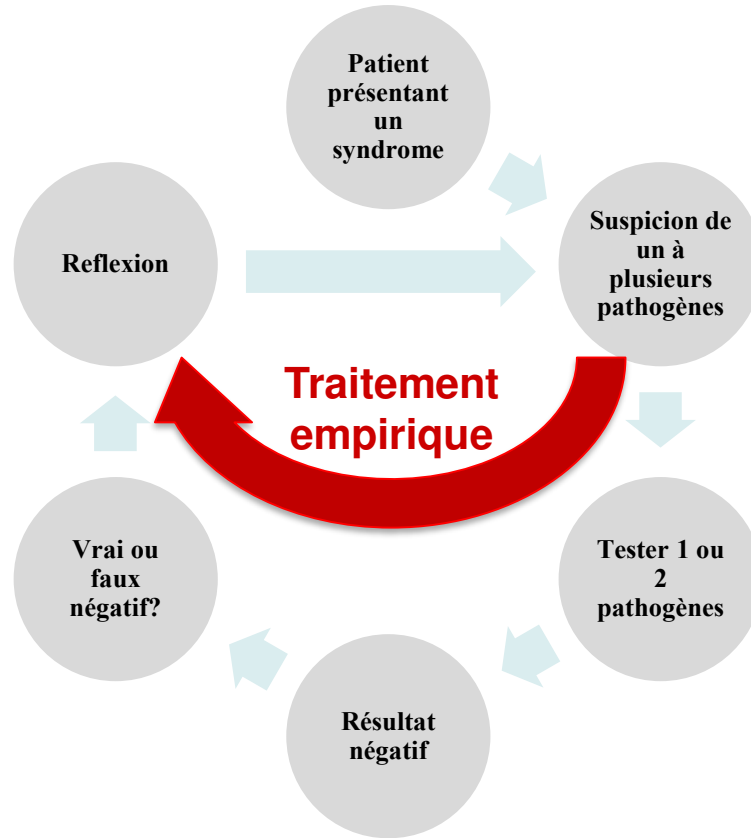


- Culture de liquide biologique



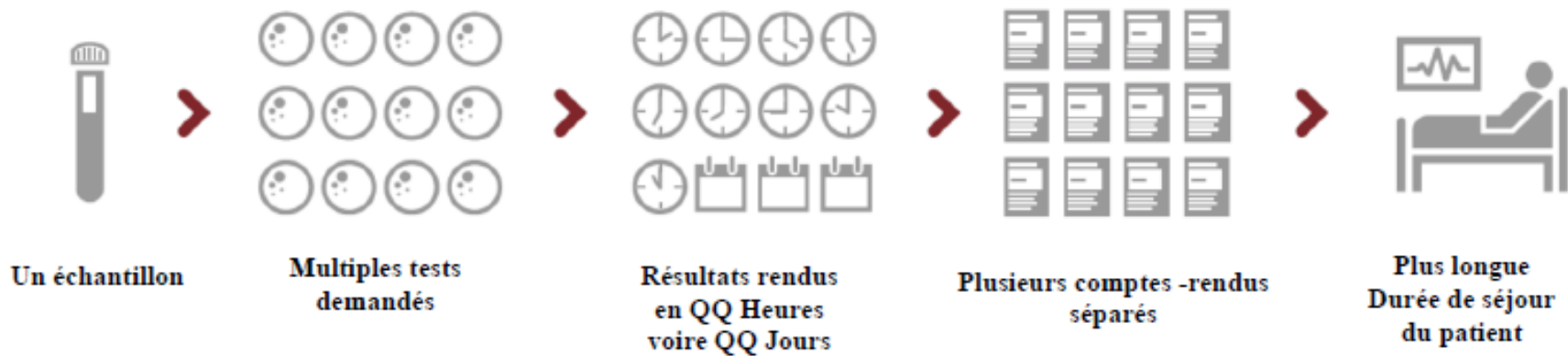


# Constat





# Approche classique





# Approche syndromique



**L'approche syndromique** consiste en la recherche simultanée de différents pathogènes pouvant être impliqués dans une infection, lorsqu'un patient présente des symptômes cliniques non spécifiques d'un agent.

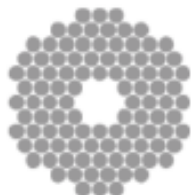


The right test, the first time.

**Don't Guess. Know.**



# Approche syndromique



test complet

Résultats rendus  
en Une Heure

Plusieurs résultats dans un  
seul compte-rendu

Améliore la gestion  
du patient



The right test, the first time.

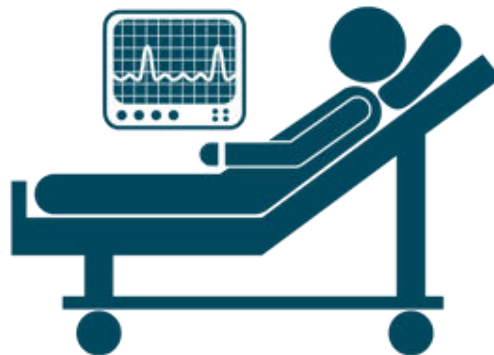
**Don't Guess. Know.**



Des décisions parfois urgentes

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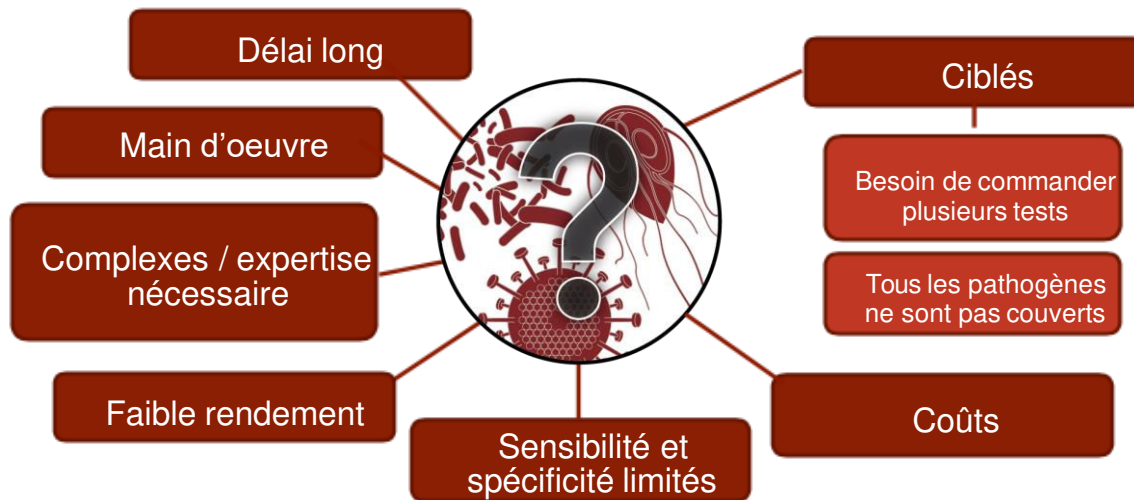
FilmArray™



The right test, the first time.



## Des outils diagnostics variés





**Fast Multiplex PCR  
FilmArray**

**“Batched” Multiplex PCR**

**Detecting co-  
infections**

**High  
Sensitivity  
and  
specificity**

**Detecting key  
nosocomial  
pathogens**

**Fast PCR “Simplexes”**

**Easy**

**Rapid results**

**Immunoassay Tests**

Fast PCR “Simplexes” (Cepheid,  
Alere, Idylla....)

“Batched” Multiplex PCR (Seegene,  
Fast Track, PathoFinder, ...)

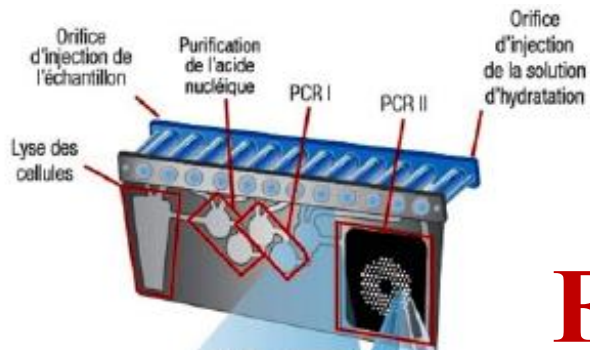


# Un système, une solution intégrale



**Multiplex PCR**

**2 min. de temps de preparation  
1h pour l'établissement du  
rapport final**



# FilmArray



Insérer la cassette dans la station de chargement

Injecter la solution d'hydratation

Ajouter le tampon de l'échantillon et l'échantillon

Injecter l'échantillon

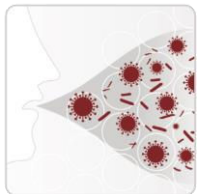
Insérer la cassette dans l'instrument et lancer l'analyse



# Un système. Plusieurs applications.

FilmArray™

## Respiratory Panel

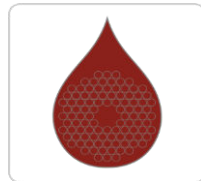


20

targets

- 3 bacteria
- 17 viruses

## Blood Culture Identification Panel

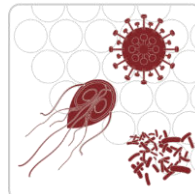


27

targets

- 19 Bacteria
- 5 yeast
- 3 antibiotic resistance genes

## Gastrointestinal Panel



22

targets

- 13 bacteria
- 5 viruses
- 4 parasites

## Meningitis Encephalitis Panel



15

targets

- 6 bacteria
- 8 viruses
- 1 fungus



# The Fastest Way to Better Results

- **Facile** – Deux minutes de temps de préparation
- **Rapide** – Résultat en une heure environs
- **Complet** –
  - RP- 20 cibles (17 viruses & 3 bacteria)
  - BCID- 27 cibles (19 bacteria, 4 yeast & 3 antibiotic resistance genes)
  - GI- 22 cibles (13 bacteria, 4 parasites & 5 viruses)
  - ME- 14 cibles (1 yeast, 6 bacteria & 7 viruses)
- **Système fermé**– Risque de contamination réduit
- **Diagnostic moléculaire**– Gain en sensibilité et spécificité



# Meningitis / Encephalitis

15 pathogènes

## Bactéries:

*E. Coli K1*  
*H. influenzae*  
*L. monocytogenes*  
*N. meningitidis*  
*S. agalactiae*  
*S. pneumoniae*

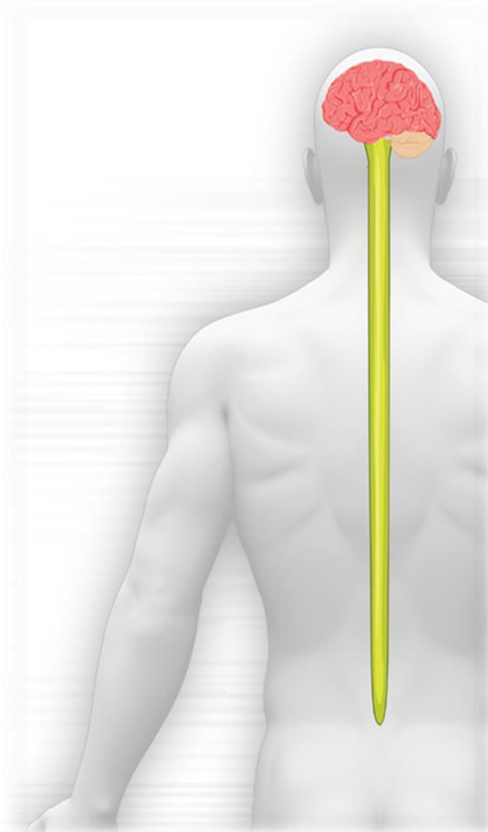
## Virus:

Cytomegalovirus (CMV)  
Enterovirus  
Epstein-Barr virus (EBV)  
Herpes simplex type 1 (HSV-1)  
Herpes simplex type 2 (HSV-2)  
Human herpesvirus 6 (HHV-6)  
Parechovirus  
Varicella zoster virus (VZV)

## Levure:

*Cryptococcus neoformans / gattii*

*Echantillon : Liquide céphalo- rachidien*





# Panel Respiratoire

20 pathogènes

## Virus

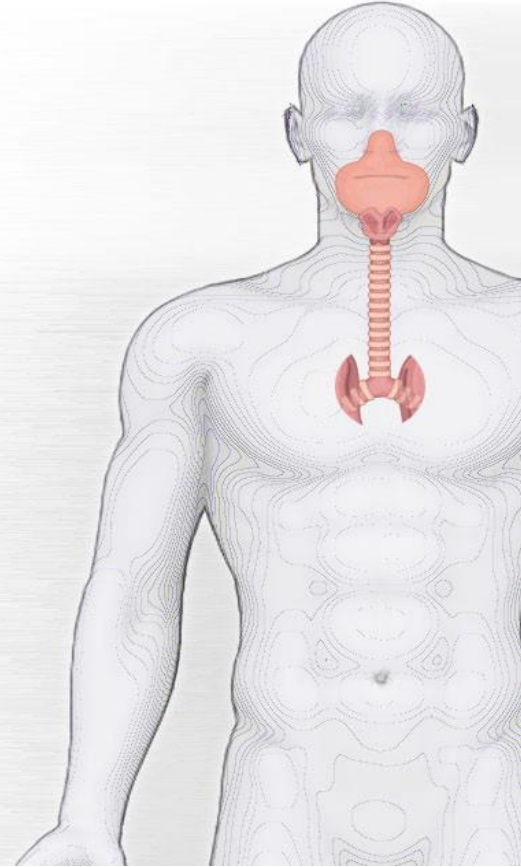
Adenovirus  
Coronavirus 229E  
Coronavirus HKU1  
Coronavirus OC43  
Coronavirus NL63  
Human Metapneumovirus  
Human Rhinovirus/  
Enterovirus  
Influenza A  
Influenza A/H1  
Influenza A/H1-2009  
Influenza A/H3  
Influenza B

Parainfluenza 1  
Parainfluenza 2  
Parainfluenza 3  
Parainfluenza 4  
RSV

## Bactéries

*Bordetella pertussis*  
*Chlamydomphila pneumoniae*  
*Mycoplasma pneumoniae*

***Echantillon: Ecouvillons naso-pharyngés***





# Panel Hémoculture (BCID)

27 pathogènes

## Bactéries Gram + :

*Enterococcus spp.*  
*L. monocytogenes*  
*Staphylococcus*  
*S. aureus*  
*Streptococcus spp.*  
*S. agalactiae (Group B)*  
*S. pyogenes (Group A)*  
*S. pneumoniae*

## Marqueurs de résistance:

*mecA*  
*Van A/B*  
*KPC*

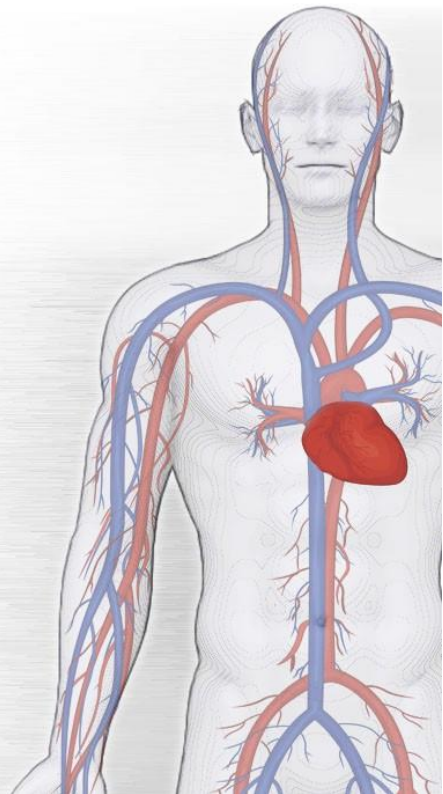
## Bactéries Gram - :

*A. baumannii*  
*Enterobacteriaceae*  
*Enterobacter cloacae*  
Complex  
*E. coli*  
*H. influenzae*  
*K. oxytoca*  
*K. pneumoniae*  
*N. meningitidis*  
*P. aeruginosa*  
*Proteus*  
*S. marcescens*

## Levures:

*C. albicans*  
*C. glabrata*  
*C. krusei*  
*C. parapsiiosis*  
*C. tropicalis*

*Echantillon : Hémoculture*  
*Positive*





# Panel GastroIntestinal

22 pathogènes

## Bactéries:

*Aeromonas*  
*Campylobacter*  
*Clostridium difficile* (Toxin A/B)  
*Plesiomonas shigelloides*  
*Salmonella*  
*Vibrio*  
*Vibrio cholerae*  
  
*Yersinia enterocolitica*

## E. coli / Shigella

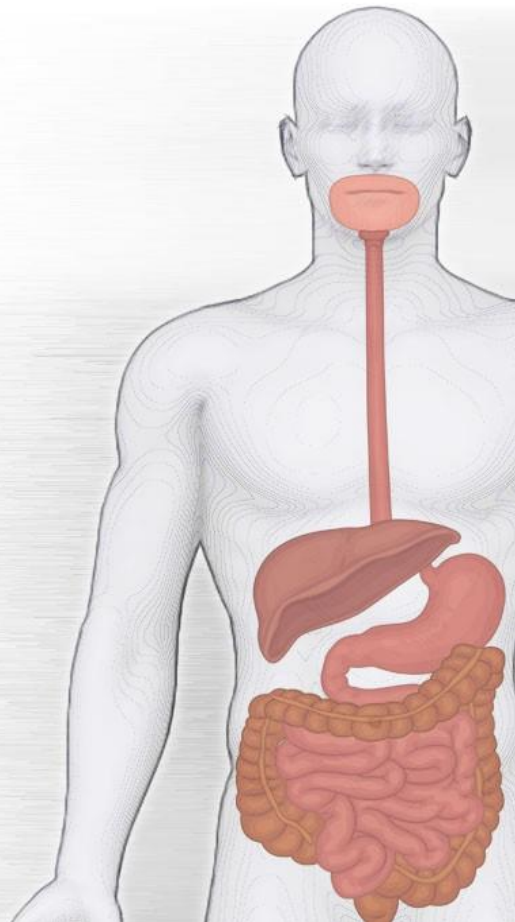
*E. coli* O157  
Enteroaggregative *E. coli* (EAEC)  
Enteropathogenic *E. coli* (EPEC)  
Enterotoxigenic *E. coli* (ETEC)  
Shiga-like toxin-producing *E. coli* (STEC)  
*Shigella*/Enteroinvasive *E. coli* (EIEC)

## Parasites:

*Cryptosporidium*  
*Cyclospora cayetanensis*  
*Entamoeba histolytica*  
*Giardia lamblia*

## Virus:

Adenovirus F 40/41  
Astrovirus  
Norovirus GI/GII  
Rotavirus A  
Sapovirus



FilmArray™

*Echantillon : Selles resuspendues dans du Cary*



# Pneumonia panel



Pneumonia

27 pathogens

## **Bacteria:**

*Acinetobacter calcoaceticus-baumannii* complex  
*Serratia marcescens*  
*Proteus spp.*  
*Klebsiella pneumoniae*  
*Enterobacter spp.*  
*Escherichia coli*  
*Haemophilus influenzae*  
*Moraxella catarrhalis*  
*Pseudomonas aeruginosa*  
*Staphylococcus aureus*  
*Stenotrophomonas maltophilia*  
*Streptococcus pneumoniae*  
*Klebsiella oxytoca*  
*Streptococcus pyogenes*  
*Streptococcus agalactiae*  
*Legionella spp.*  
*Mycoplasma pneumoniae*  
*Chlamydia pneumoniae*

## **Viruses:**

Influenza A  
Influenza B  
Respiratory Syncytial virus  
Human Rhinovirus/Enterovirus  
Human Metapneumovirus  
Parainfluenza virus  
Adenovirus  
Coronavirus  
MERS-coV

- **Semi-Quantitative Panel: Infection vs Colonization**
- **Samples: BAL, Sputum**

7 resistance genes

## **Antibiotic Resistance Markers:**

*mecA/C* – MREJ (MRSA)  
KPC (Carbapenem resistance)  
NDM (Carbapenem resistance)  
Oxa48 like (Carbapenem resistance)  
*ctx-M* (Penicillin, 3rd Gen. Cephalosporin resistance) - ESBL  
VIM (Carbapenem resistance)  
IMP (Carbapenem resistance)



# Interprétation facile

**FilmArray**  
Meningitis / Encephalitis (ME) Panel - IVD

BIO F I R E

www.BioFireDx.com

<b>Run Summary</b>	Sample ID: HHV6DNA15C3	Run Date: 18 May 2015 6:34 PM
<b>Detected:</b> Human herpesvirus 6	<b>Controls:</b> Passed	
<b>WARNING:</b> The FilmArray ME Panel does not distinguish between latent and active CMV and HHV-4 infections. Detection of these viruses may indicate primary infection, secondary reactivation, or the presence of latent virus. Results should always be interpreted in conjunction with other clinical, laboratory, and epidemiological information.		

**Result Summary**

**Bacteria**

Not Detected  
Not Detected  
Not Detected  
Not Detected  
Not Detected

**FilmArray**  
BCID Panel

BIO F I R E

www.BioFireDx.com

<b>Run Summary</b>	Sample ID: SDY_9621_LED_50_6	Run Date: 29 May 2013 3:41 PM
<b>Organisms Detected:</b> Enterobacteriaceae Klebsiella pneumoniae	<b>Controls:</b> Passed	
<b>Applicable Antimicrobial Resistance Genes:</b> KPC + Detected		
<b>Result Summary - Interpretations</b>		
<b>Antimicrobial Resistance Genes</b>		
✓ Detected	KPC (carbapenem-resistance gene)	
⊕ N/A	mecA (methicillin-resistance gene)	
⊕ N/A	vanA/B (vancomycin-resistance genes)	
NOTE: Antimicrobial resistance data does not include mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.		
<b>Gram Positive Bacteria</b>		

**FilmArray**  
GI Panel

BIO F I R E

www.BioFireDx.com

<b>Run Summary</b>	Sample ID: 009196-03-0790	Run Date: 27 Sep 2013 12:03 PM
<b>Detected:</b> <i>Giardia lamblia</i>	<b>Controls:</b> Passed	
<b>Result Summary</b>		
<b>Bacteria</b>		
Not Detected	Campylobacter	
Not Detected	Clostridium difficile toxin A/B	
Not Detected	Plesiomonas shigelloides	
Not Detected	Salmonella	
Not Detected	Vibrio	
Not Detected	Vibrio cholerae	
Not Detected	Yersinia enterocolitica	
<b>Diarrheagenic E. coli/Shigella</b>		
Not Detected	Enteraggregative E. coli (EAEC)	
Not Detected	Enteropathogenic E. coli (EPEC)	
Not Detected	Enterotoxigenic E. coli (ETEC) /stx	
Not Detected	Shiga-like toxin-producing E. coli (STEC) stx1/stx2	
⊕ N/A	E. coli O157	
Not Detected	Shigella/Enteroinvasive E. coli (EIEC)	
<b>Parasites</b>		
Not Detected	Cryptosporidium	
Not Detected	Cyclospora cayentensis	
Not Detected	Entamoeba histolytica	
✓ Detected	<i>Giardia lamblia</i>	
<b>Viruses</b>		
Not Detected	Adenovirus F 40/41	
Not Detected	Astrovirus	
Not Detected	Norovirus GI/GII	
Not Detected	Rotavirus A	
Not Detected	Sapovirus	

<b>Run Details</b>	Pouch: GI Panel v2.1	Protocol: Stool FA v2.3
<b>Run Status:</b> Completed	Serial No.: 00788640	<b>Operator:</b> John Madison (jm)
<b>Lot No.:</b> 133813		<b>Instrument:</b> ITI FA "FA1315"

**FilmArray**  
Respiratory Panel

BIO F I R E

www.BioFireDx.com

<b>Run Summary</b>		
<b>Sample ID:</b> RYNAND0232	<b>Run Date:</b> 21 Jun 2013 7:34 PM	
<b>Detected:</b> Influenza A H1-2009 Respiratory Syncytial Virus	<b>Controls:</b> Passed	
<b>Equival:</b> None		

<b>Result Summary</b>		
Not Detected	Adenovirus	
Not Detected	Coronavirus 229E	
Not Detected	Coronavirus HKU1	
Not Detected	Coronavirus NL63	
Not Detected	Coronavirus OC43	
Not Detected	Human Metapneumovirus	
Not Detected	Human Rhinovirus/Enterovirus	
✓ Detected	Influenza A H1-2009	
Not Detected	Influenza B	
Not Detected	Parainfluenza Virus 1	
Not Detected	Parainfluenza Virus 2	
Not Detected	Parainfluenza Virus 3	
Not Detected	Parainfluenza Virus 4	
✓ Detected	Respiratory Syncytial Virus	
Not Detected	Bordetella pertussis	
Not Detected	Chlamydomphila pneumoniae	
Not Detected	Mycoplasma pneumoniae	

<b>Run Details</b>		
<b>Pouch:</b> Respiratory Panel v1.7	<b>Protocol:</b> NPS v2.0	
<b>Run Status:</b> Completed	<b>Operator:</b> KMRAP	
<b>Serial No.:</b> 00345787z	<b>Instrument:</b> ITI FA "FA2004"	
<b>Lot No.:</b> 114612		



**Run Information**

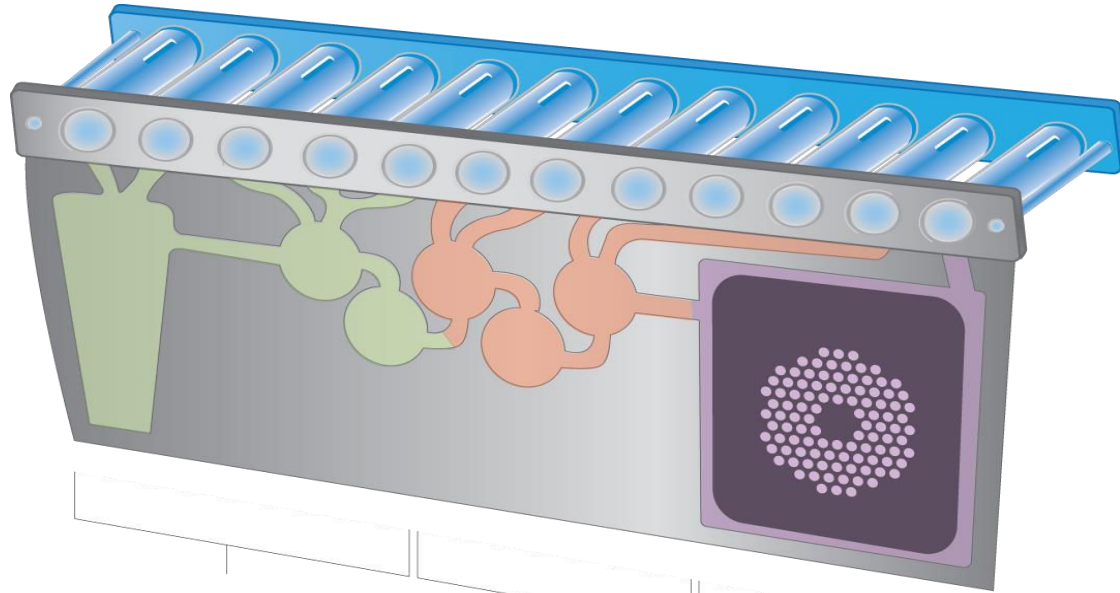
Sample ID	123-xyz-sampID	Run Date	05 Sept 2017 8:00 AM
Protocol	BAL v2.0	Serial No.	01234567
Pouch Type	Pneumo v2.0	Lot No.	123456
Controls	Passed	Operator	Jane Doe (JD)
Run Status	Completed	Instrument	FA1234

**Result Summary**

		Bacteria			
	Bin*	10 <sup>4</sup> copies/mL	10 <sup>5</sup> copies/mL	10 <sup>6</sup> copies/mL	≥ 10 <sup>7</sup> copies/mL
Not Detected	<i>Acinetobacter calcoaceticus-baumannii</i> complex				
✓ Detected	<i>Enterobacter aerogenes</i> ≥ 10 <sup>7</sup> copies/mL				
✓ Detected	<i>Enterobacter cloacae</i> complex 10 <sup>5</sup> copies/mL				
Not Detected	<i>Escherichia coli</i>				
Not Detected	<i>Haemophilus influenzae</i>				
Not Detected	<i>Klebsiella oxytoca</i>				
✓ Detected	<i>Klebsiella pneumoniae</i> group ≥ 10 <sup>7</sup> copies/mL				
Not Detected	<i>Moraxella catarrhalis</i>				
Not Detected	<i>Proteus</i> spp.				
Not Detected	<i>Pseudomonas aeruginosa</i>				
Not Detected	<i>Serratia marcescens</i>				
Not Detected	<i>Staphylococcus aureus</i>				
Not Detected	<i>Streptococcus agalactiae</i>				
Not Detected	<i>Streptococcus pneumoniae</i>				
Not Detected	<i>Streptococcus pyogenes</i>				
Antimicrobial Resistance Genes					



# The FilmArray Pouch



Reagent  
Storage

Chemical  
Circuit  
Board

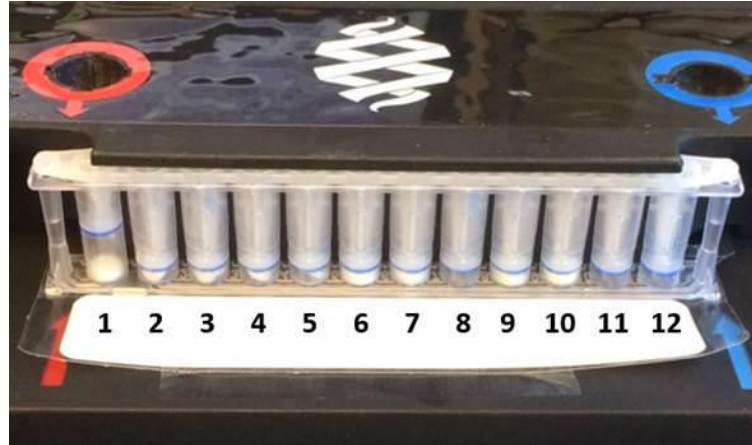
Sample  
Extraction &  
Preparation

1<sup>st</sup> Stage  
Multiplex  
PCR

2<sup>nd</sup> Stage PCR



# FilmArray Reagent Storage



Well 1: RNA or DNA process control

Wells 2-4: wash buffer

Well 5: nucleic acid elution buffer

Well 6: reverse transcription/first stage PCR master mix

Well 7: dilution buffer

Well 8: empty

Wells 9-10: PCR2 master mix with LCGreen Plus+

Wells 11 and 12: empty (well twelve is for any overflow for the second stage PCR reagents)



# Sample Prep

## Hydration Solution:

- Molecular grade water
- Used to hydrate the pouch

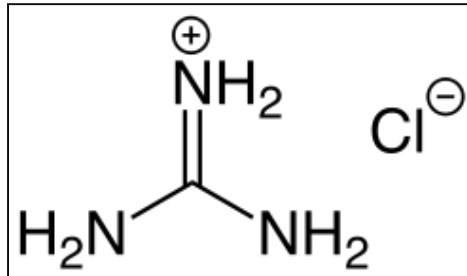




# Sample Prep - Lysis

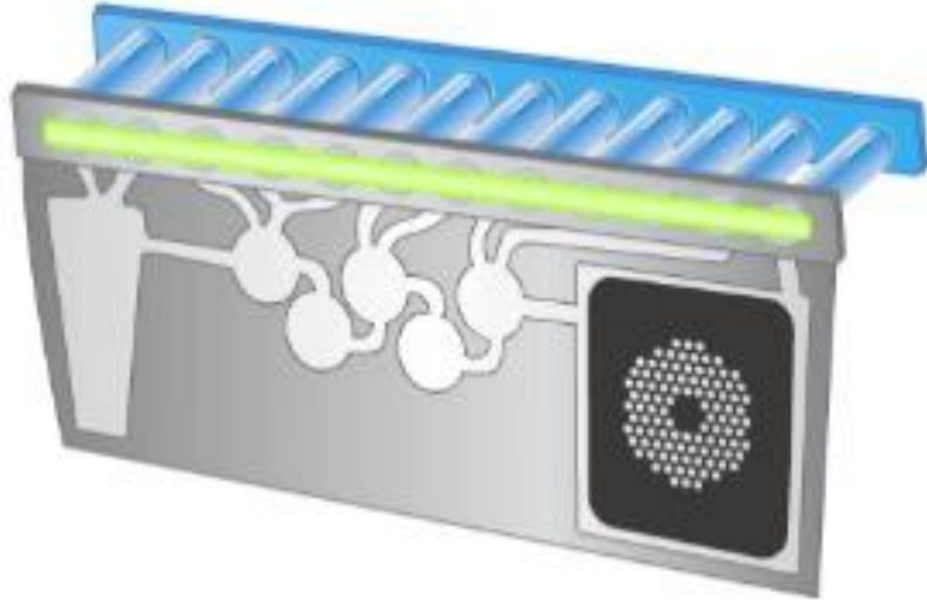
## Sample Buffer:

- Breaks open virus particles
- Weakens cell walls
- Contains Guanidinium HCL and detergent
  - One of the most **chaotropic** substances known to man
  - Disrupts and denatures macromolecules.

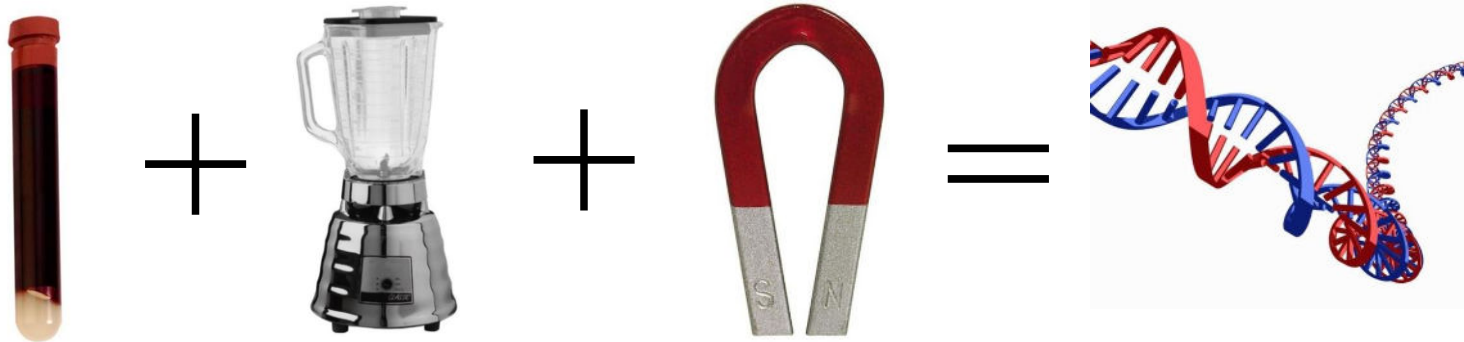
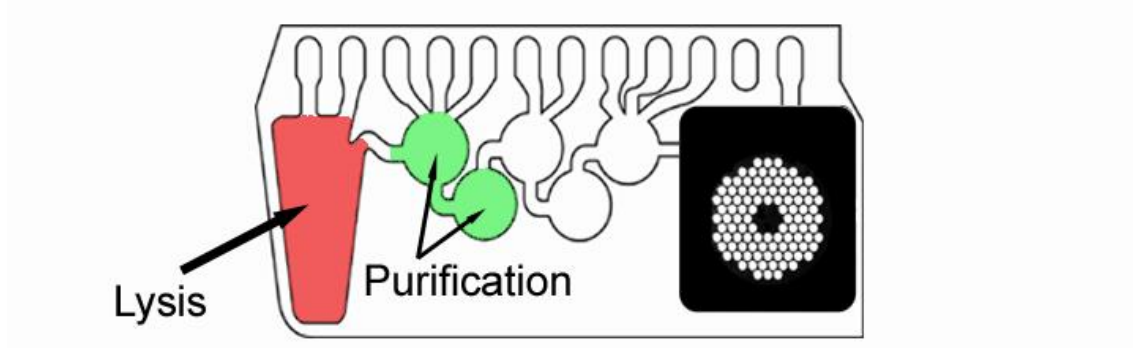




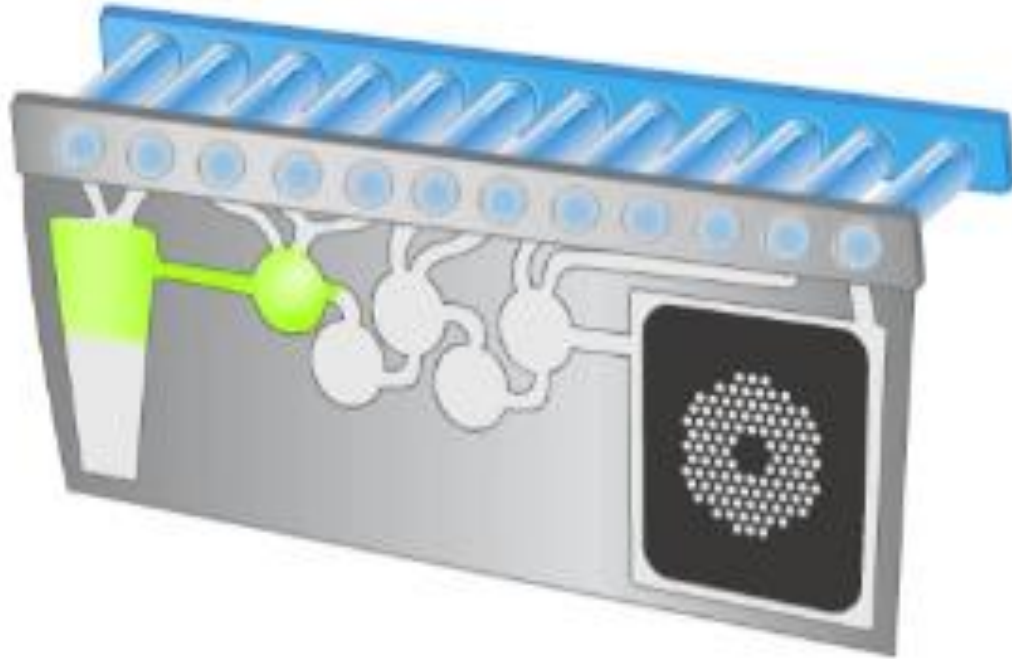
# Seal Bar and Sample Plunged



# Integrated Sample Prep



# Bead Beating

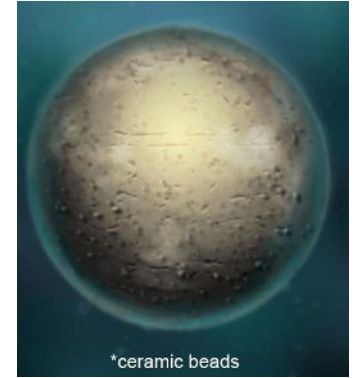




# Sample Prep - Lysis

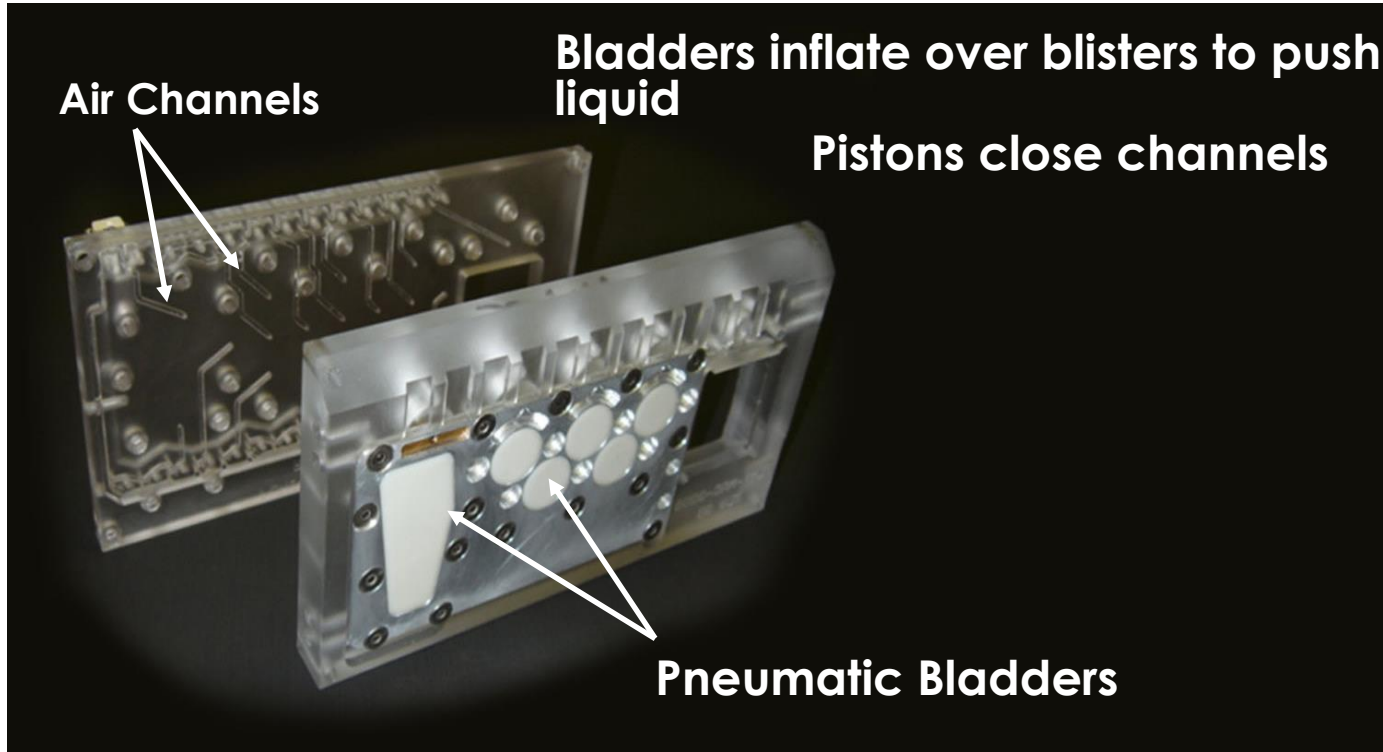
## Bead Beating

- Uses Ceramic Beads
- Bead Beater Motor – 10,000 RPMs
  - Strikes lysis blister – makes an annoying sound
- Physically breaks open cell walls
- Although it makes a noise sonication is NOT involved in breaking the cells



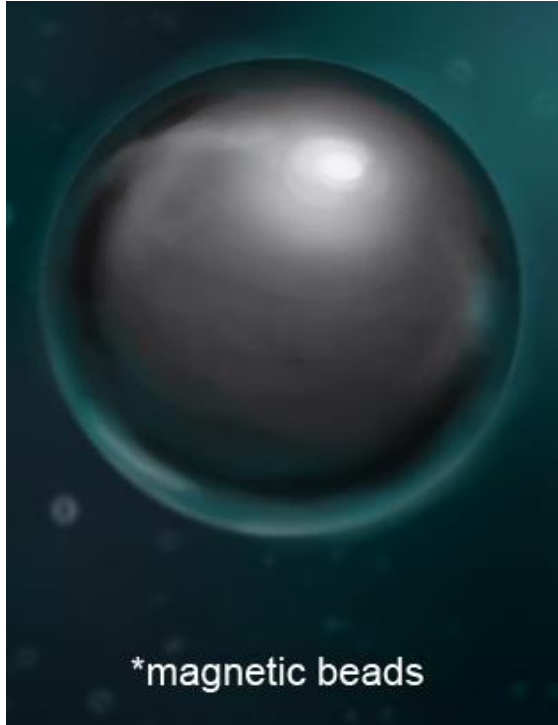


# Liquid Manipulation





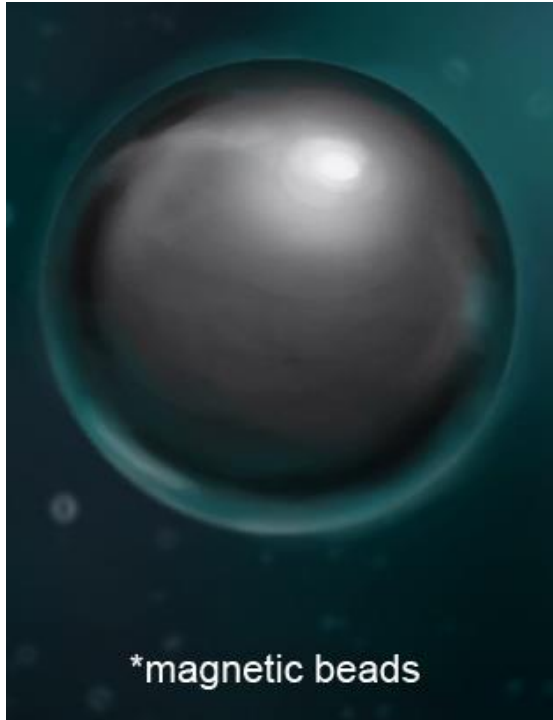
# Sample Prep – Purification of Nucleic Acids



## Magnetic Beads:

- Iron sphere coated with silica
  - Silica bears a positive charge
  - Nucleic acids bear a negative charge
  - Magnetic force is not used to bind or move DNA

# Sample Prep – Purification of Nucleic Acids



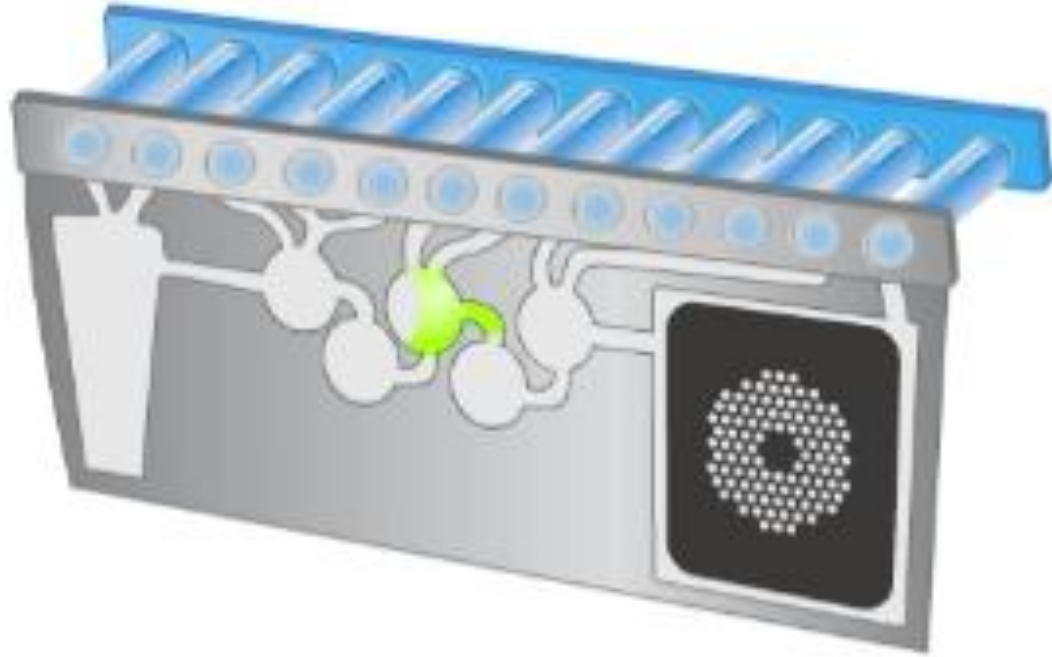
## Magnetic Beads:

- Density approximately 1.05
  - They slowly sink

## Ceramic Beads:

- Density = 8.03
  - They sink fast
- When the bead beater stops all the ceramic beads sink and the magnetic beads remain in solution
- The solution is then moved to the purification blister
- A magnet holds the beads in place for the next wash steps

# PCR Stage

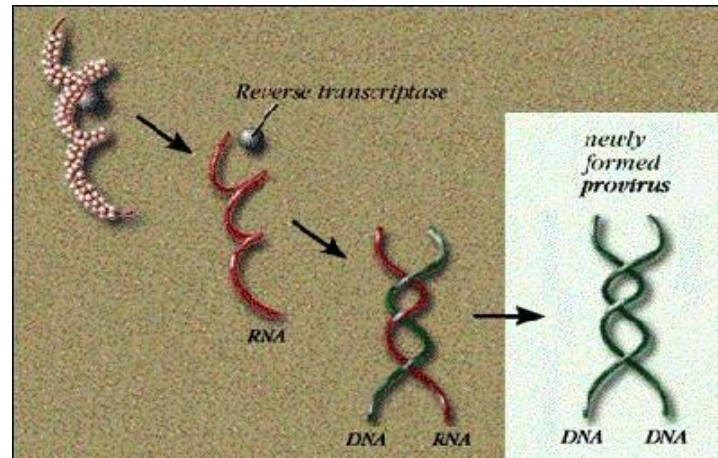




# Converting RNA to DNA

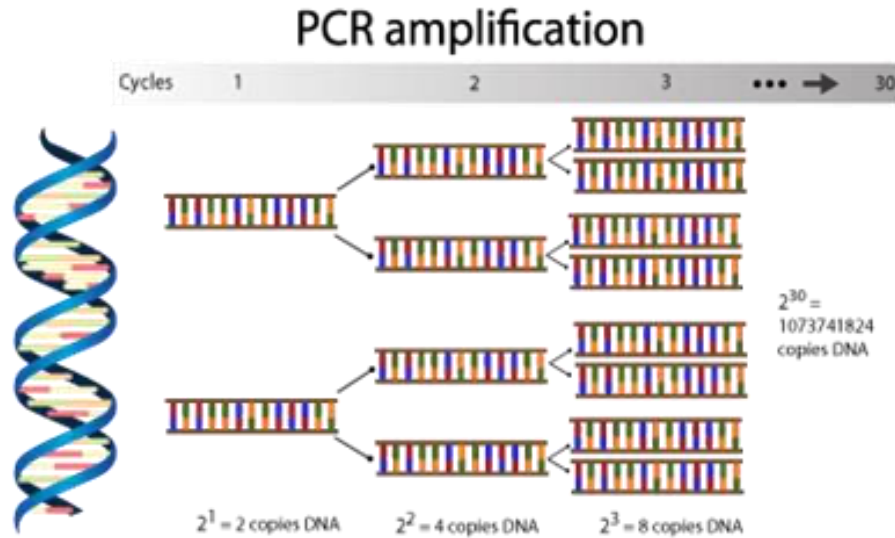
## Reverse Transcription

- Conversion d'ARN en ADN





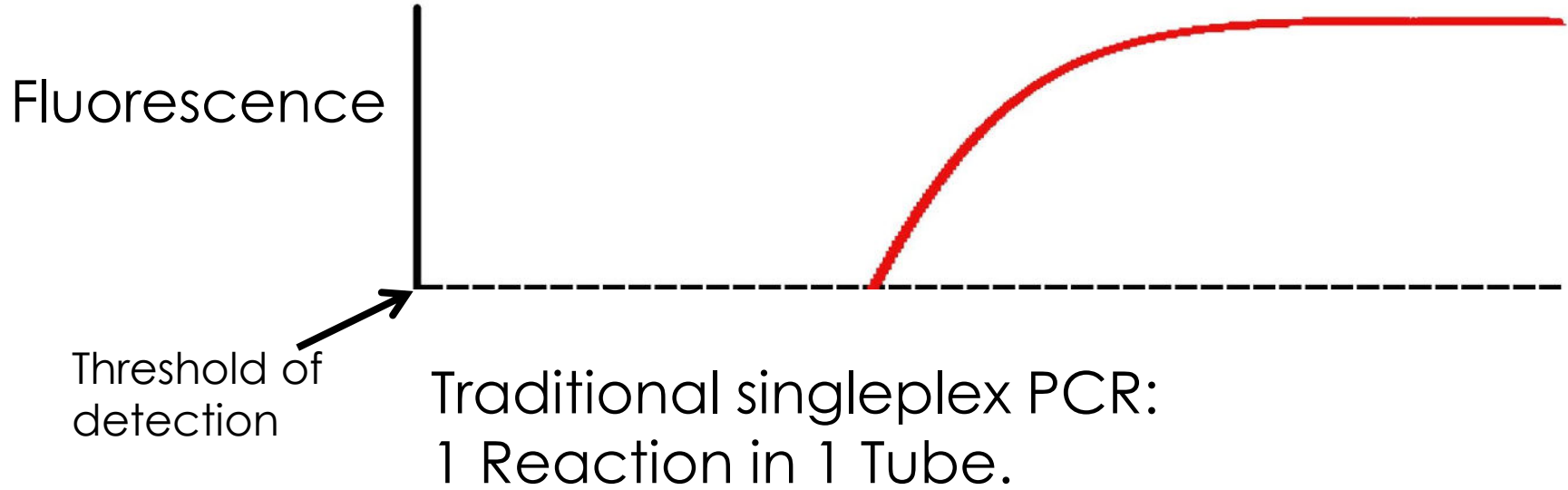
# Amplification



Chain Reaction, copies from copies produced

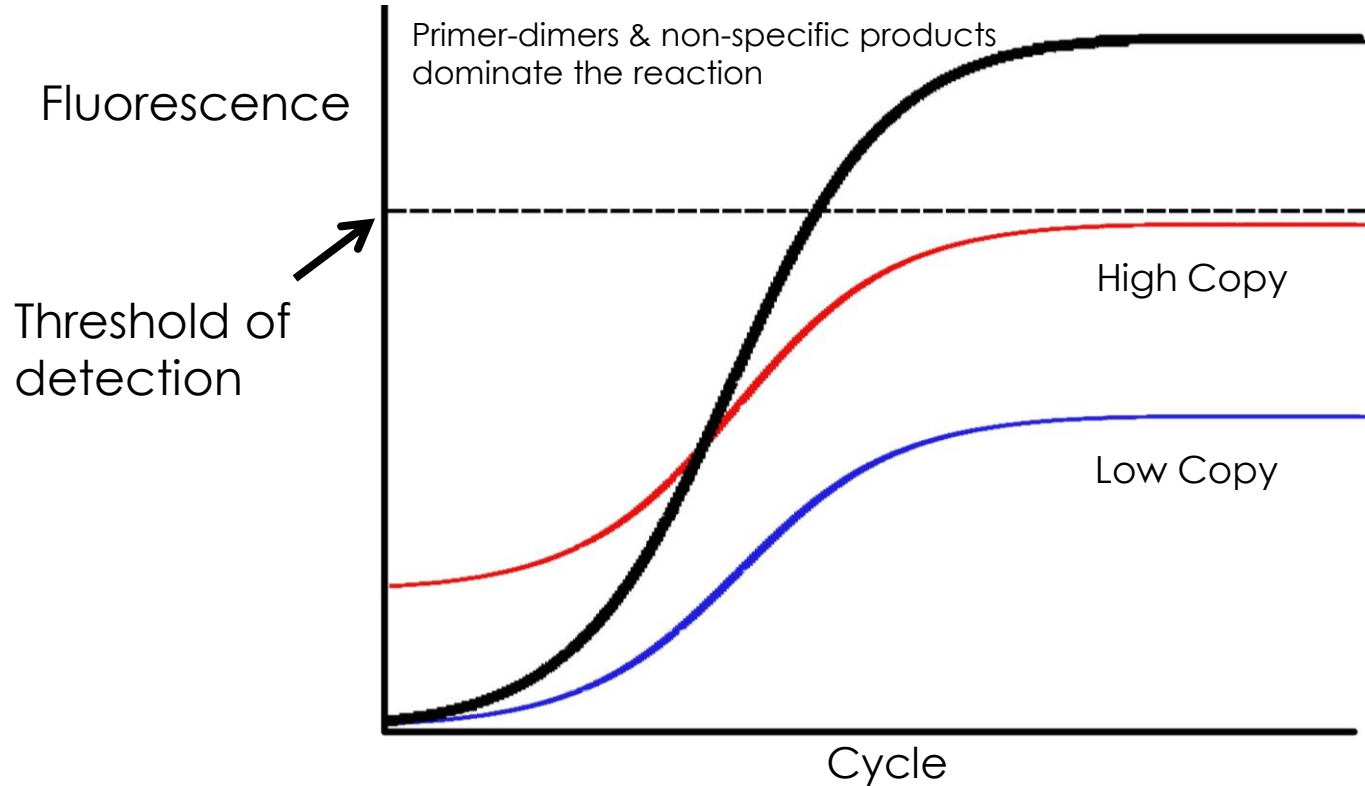


# Challenge of Multiplex PCR



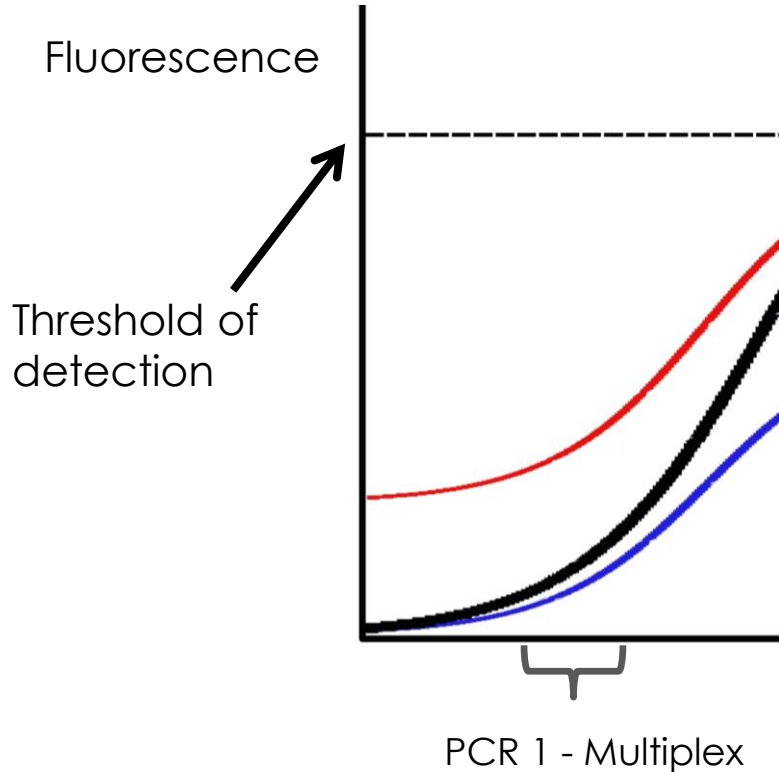


# Challenge of Multiplex PCR





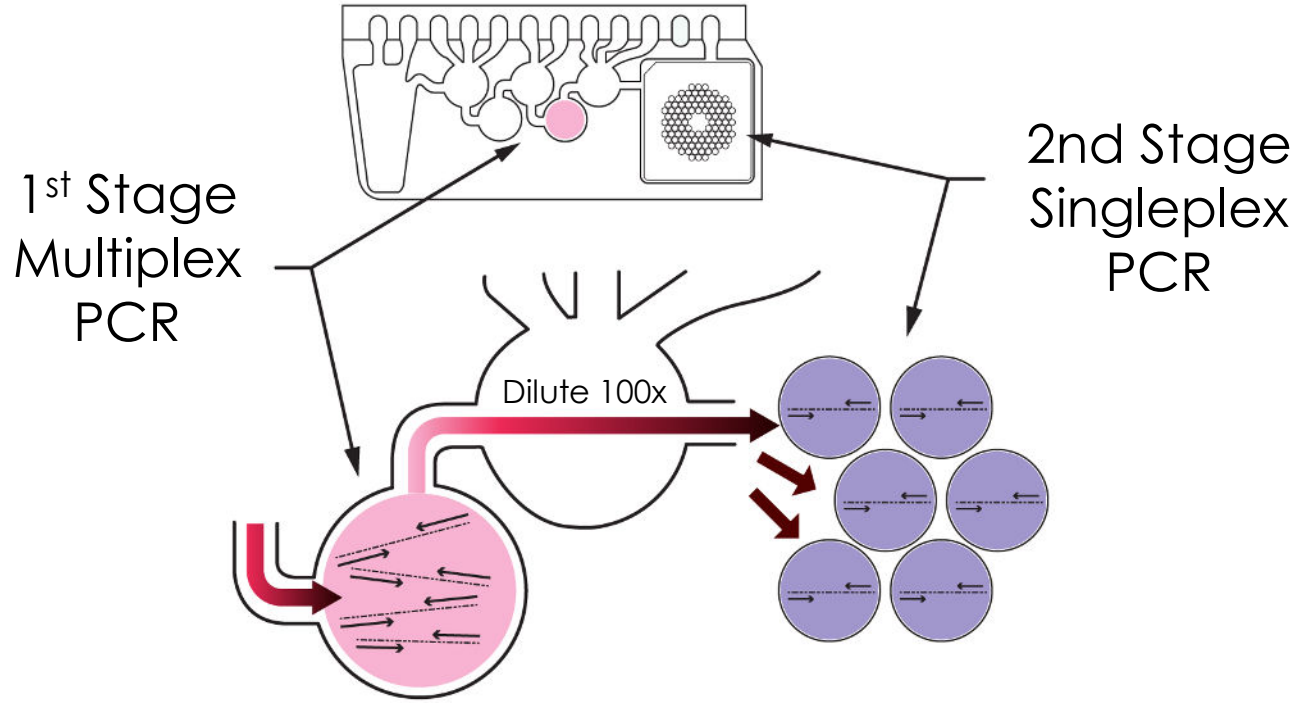
# The FilmArray – How it Works



Multiplex PCR is halted before competitive effects become problematic.

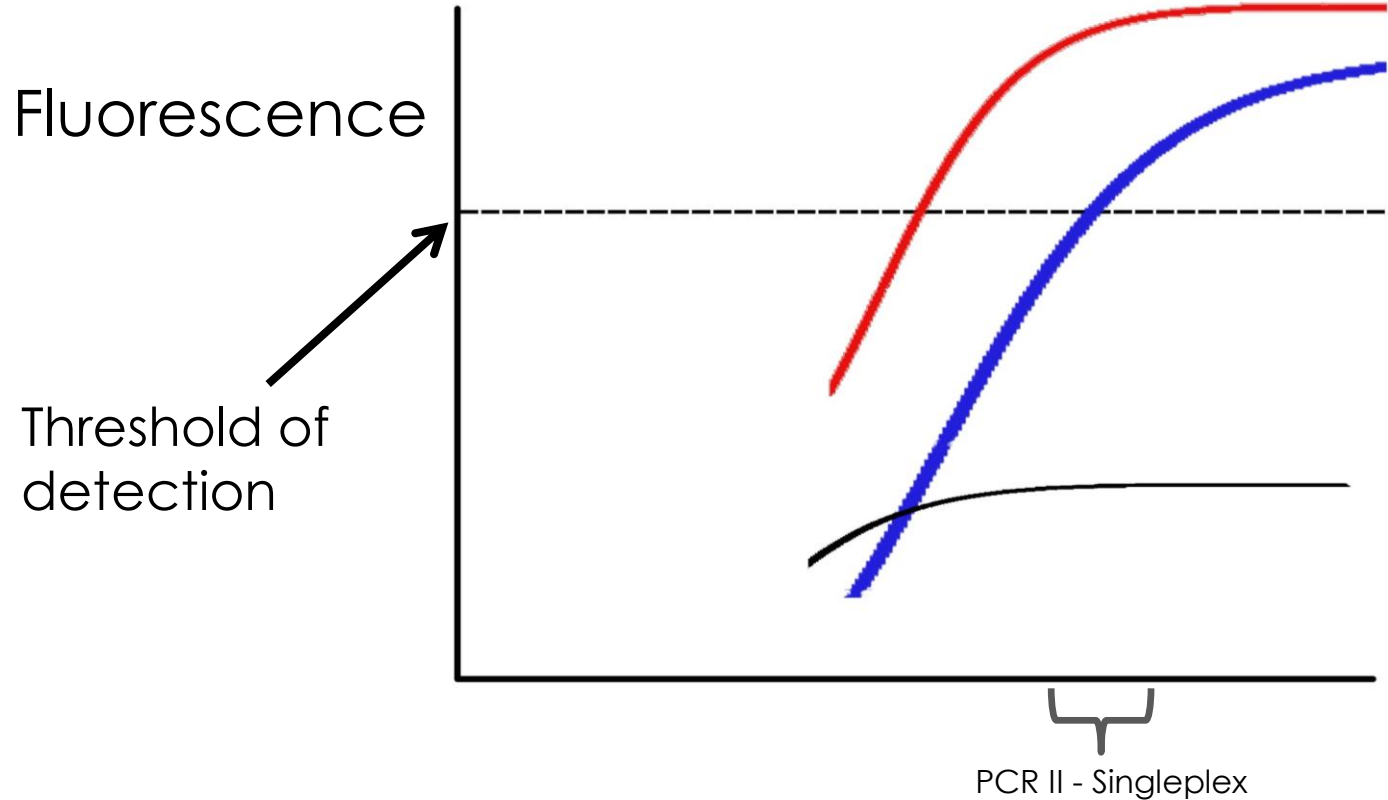


# Nested Multiplex PCR (nmPCR)





# The FilmArray – How it Works





# Amplification - nmPCR

## 1<sup>st</sup> Stage PCR:

- 1 large (140  $\mu$ L) reaction
- Massively Multiplexed
  - 36 primer pairs (RP)
- Occurs in 2 blisters
- 1 peltier device uniformly heats both blisters
- 27 cycles
- No detection

## 2<sup>nd</sup> Stage PCR:

- 102 (1  $\mu$ L) reactions
- Nested singleplex reaction
  - 1 primer pair in each array well
- Occurs in 102 wells in the array
- 1 peltier device uniformly heats entire array
- 30 cycles
- Melting analysis using LC Green Plus Detection



# Detection

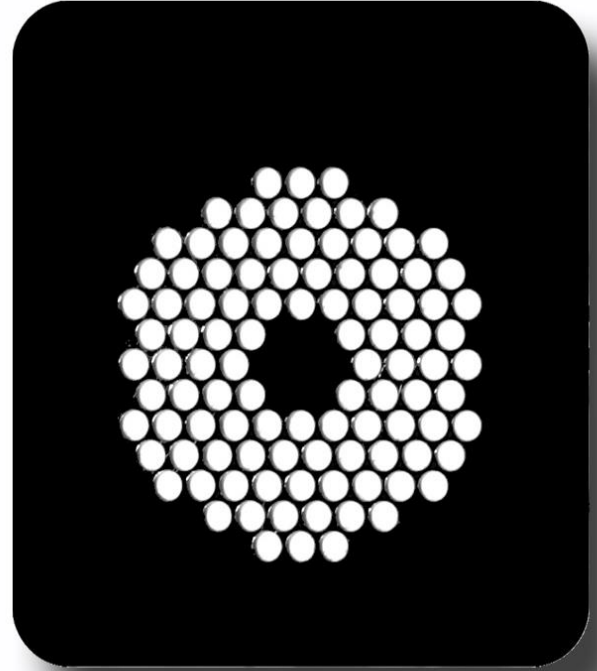
## LC Green Plus

- Double stranded DNA binding dye.
- Invented at BioFire Diagnostics.
- Analogous to SYBR Green.
- Fluoresces when bound to double stranded DNA.
- Does not fluoresce when free floating in solution.
- Not amplicon specific.
- Melting analysis used to confirm product.



# Automated Results Analysis

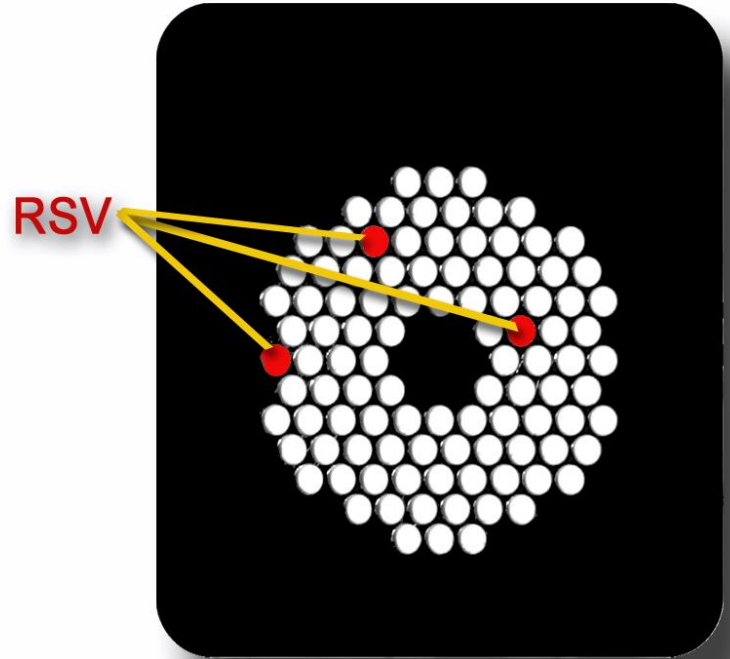
- 102 individual 2<sup>nd</sup> stage PCR wells
- Each well contains one reaction
- Melt curves generated for each well





# Automated Results Analysis

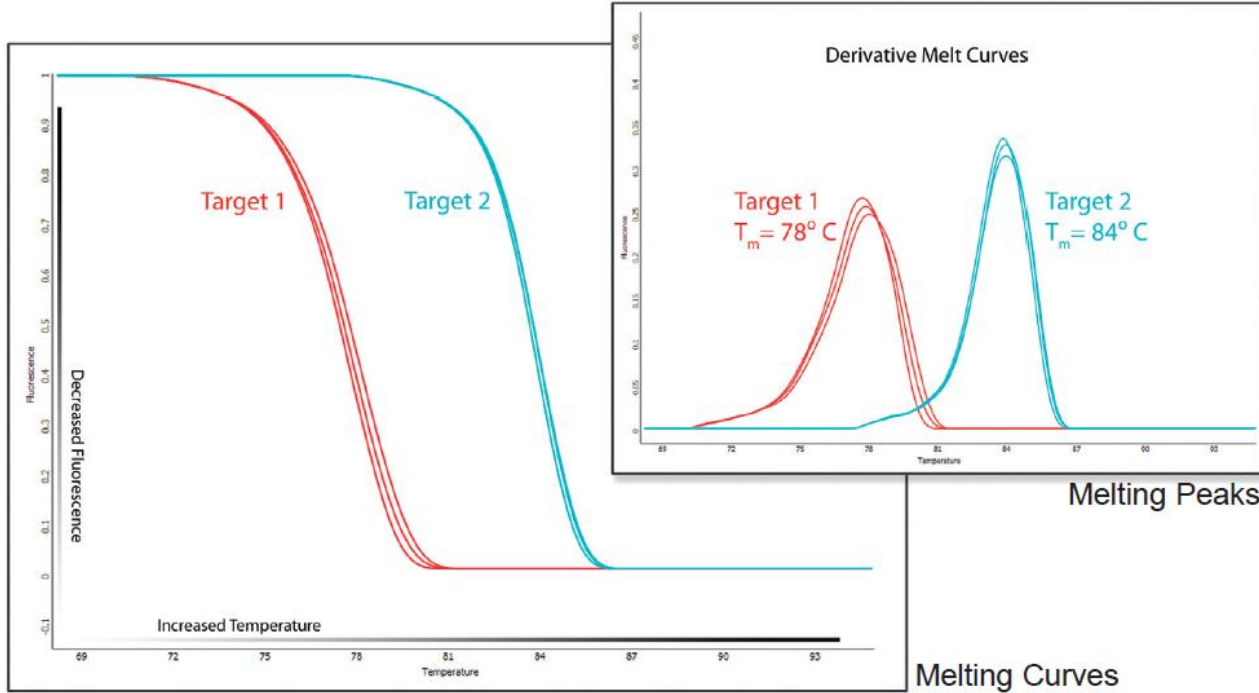
- All targets tested in triplicate.





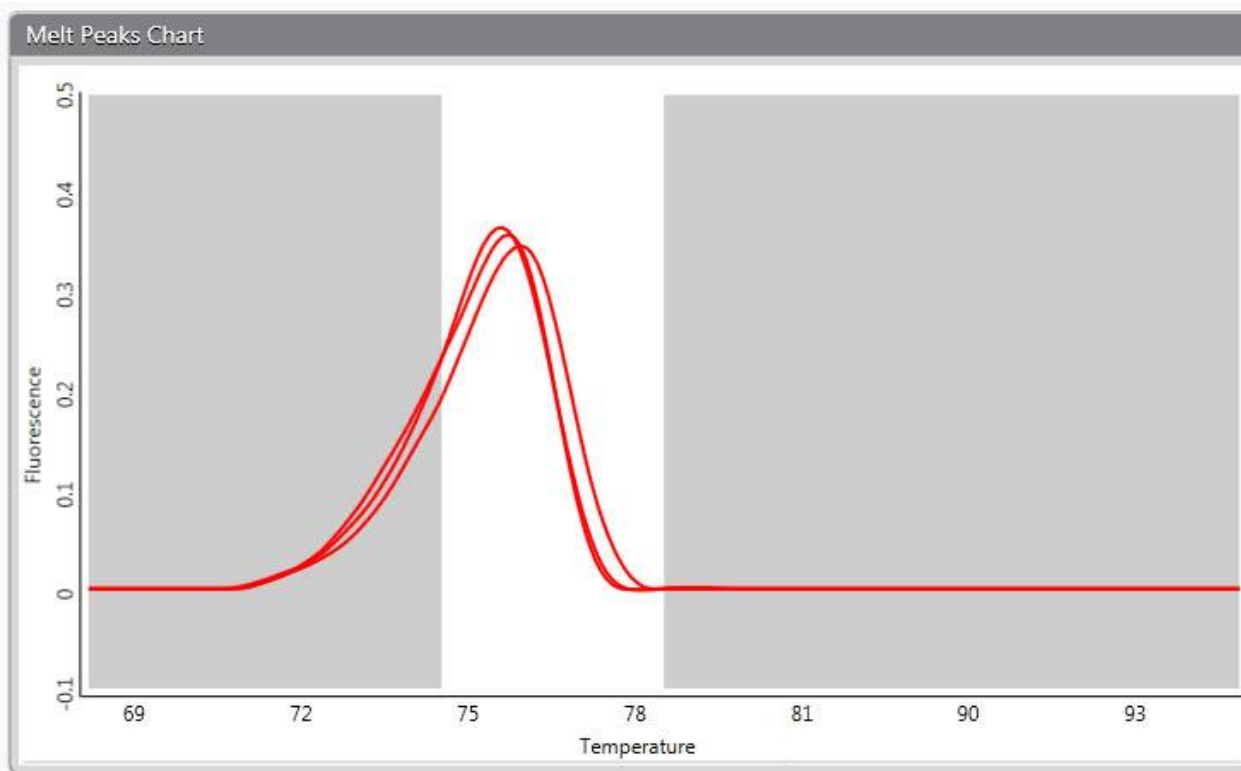


# Melt Curve Analysis





# Interpretation of Results





# When Is An Assay Positive

Replicate Similarity Calls	Assay Call
There is at least one pair of replicates with positive melt detector calls, melt peaks in range, and a similarity score that does not exceed the similarity limit	Positive
Otherwise	Negative

## 3 criteria

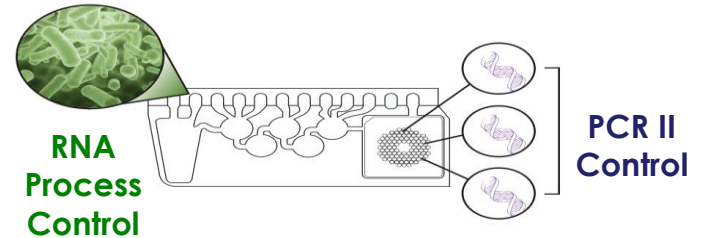
- 1) 2 out of 3 triplicates must have a melting peak.
- 2) Melting peaks must be in the melting window.
- 3) Melting peaks must be significantly similar to each other.

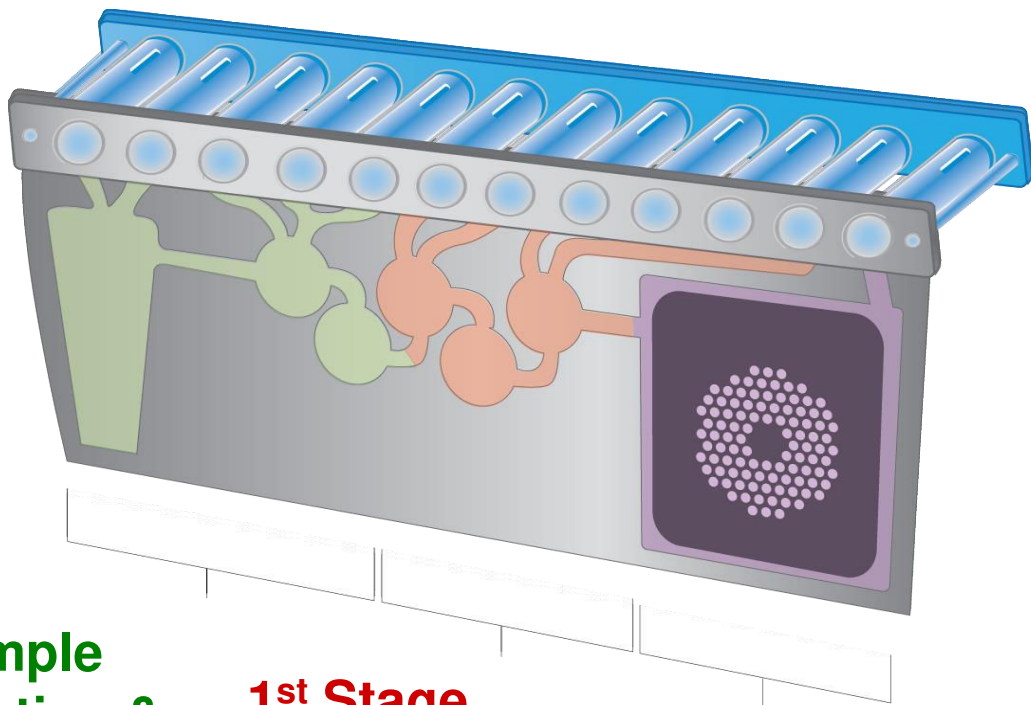


# Internal Controls

## Integrated in the pouch (user does not need to add)

- RNA Process Control
  - Schizosaccharomyces pombe organism
  - Controls for every step inside the pouch:
    - Lysis, Extraction, Purification, Reverse Transcription, PCR I, PCR II, and Detection
- PCR II Control
  - Synthetic DNA template spotted on the array
  - Controls only for PCR II





**Reagent  
Storage**

**Chemical  
Circuit  
Board**

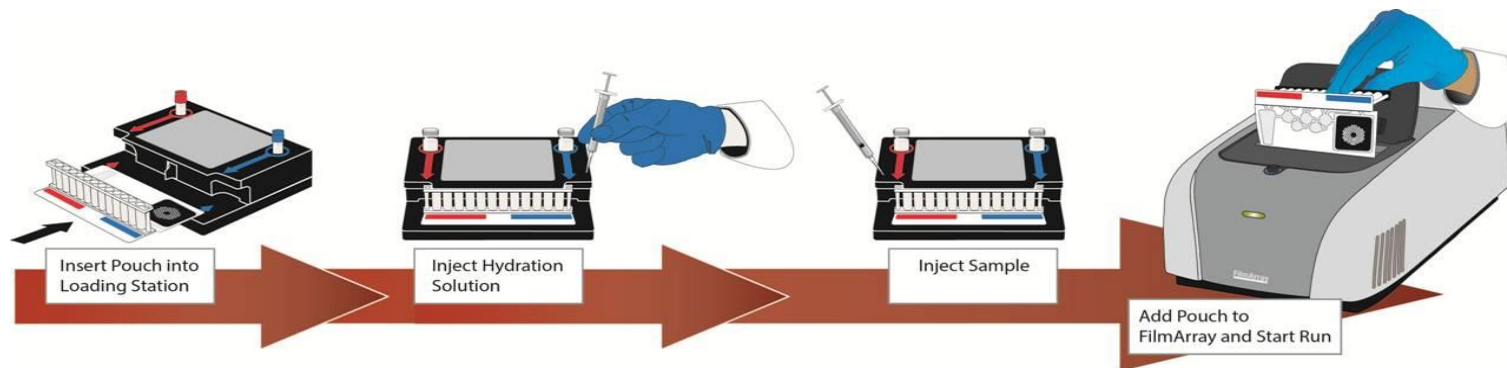
**Sample  
Extraction &  
Preparation**

**1<sup>st</sup> Stage  
Multiplex  
PCR**

**2<sup>nd</sup> Stage  
PCR**



# Easy & Fast sample preparation



**Simple:**  
2 minutes for sample preparation

**Easy:**  
No precise pipetting required

**Fast:**  
Run time of 45-65 min.



# ***L'approche syndromique FilmArray pour des meilleures perspectives de diagnostic***

## **Une meilleure prise en charge des patients**

(prévention des infections nosocomiales, adaptation thérapeutique, temps de séjour réduit...)

- Peu de manipulations pré-analytique (simplicité)
- Rapidité des résultats (environ 1 heure)
- Une certaine exhaustivité dans la recherche de pathogènes
- Excellente sensibilité et spécifique

Nécessité d'une collaboration étroite entre le médecin et le biologiste pour définir l'indication au test et l'interprétation du résultat



**IM Alliance**  
Intégrateur de solutions médicales



**MÉRIEUX  
UNIVERSITÉ**



# L'engagement historique d'une famille au service de la médecine et de la santé publique dans le monde

- Une tradition pasteurienne : Marcel Mérieux a travaillé avec Louis Pasteur en 1894.

- Un engagement depuis 4 générations : Depuis la création de l'Institut Mérieux, les générations se sont succédées pour développer son héritage.



**Marcel Mérieux**  
1894 - Étudiant de **Louis Pasteur**



**Marcel Mérieux**  
1897 - Création de l'Institut Mérieux



**Dr Charles Mérieux**  
1937 - Dr Charles Mérieux prend les rênes du laboratoire



**Dr Alain Mérieux** **Alexandre Mérieux**  
1963 - Création de bioMérieux



**DON'T GUESS.**



**KNOW.**



Syndromic infectious disease testing in about an hour.