

La problématique [rappel]

- **Assurer la sécurité des manipulateurs et de l'environnement**
- Niveau de confinement : NSB3 (L3)
- Poste de sécurité microbiologique : PSM 2 – **PSM 3**
- Habillement : **EPI**
- Activités "standards" L3 :
 - ✓ Mycobactéries
 - ✓ (Rétrovirologie)

➤ **Activités ESR : agents hautement pathogènes**

- ✓ BioTox : exercices - Alertes
- ✓ Maladies émergentes
 - 1) Epidémies identifiées (*Nbre de cas...*)
 - 2) Emergences, agent **identifié**
 - 3) Cas graves isolés, agent **non identifié**
 - 4) Découverte fortuite...

Organisations
&
problématiques
différentes

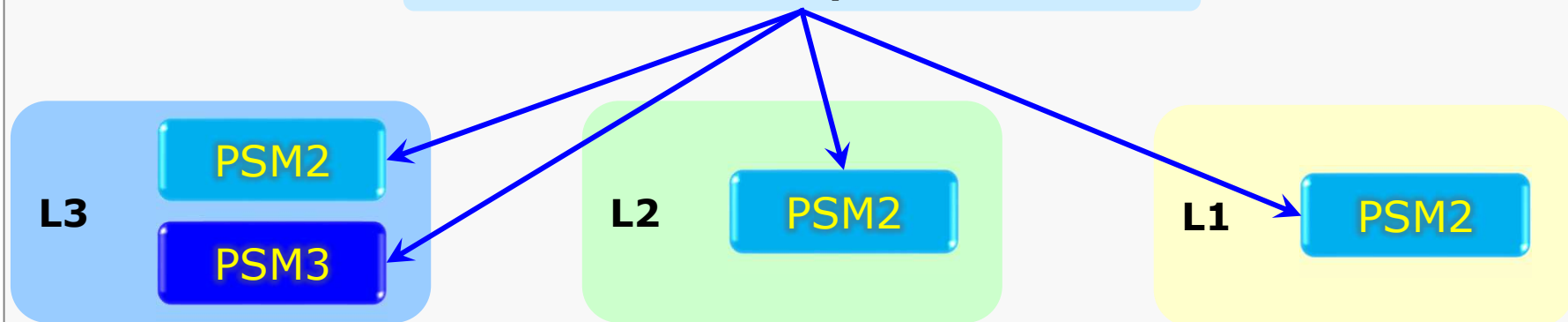
Organisation matérielle complexe

Service clinique
Diagnostic d'urgence ?

Acheminement du prélèvement

Réception du prélèvement

Orientation du prélèvement



Equipe polyvalente
Equipe dédiée
Astreintes
Disciplines (Hémato, Bioch., Microbio....)

*Maintien
des
Compétences !*

Stratégie diagnostique

Objectifs ?

✓ Exclusion ? – Exhaustif ?

Confirmation par CNR

Diagnostic "classique"
bactériologique

Approche moléculaire

"Classique"

"Rapide"

✓ Monoplex
✓ Multiplex

Exhaustif
Lent
Expertise requise
Précautions

Extraction

- manuelle
- automatisée

Amplification

- Cibles
- Maison/commerciales
- **Validation**
- Contrôles

Commerciales
"tout-en-un"

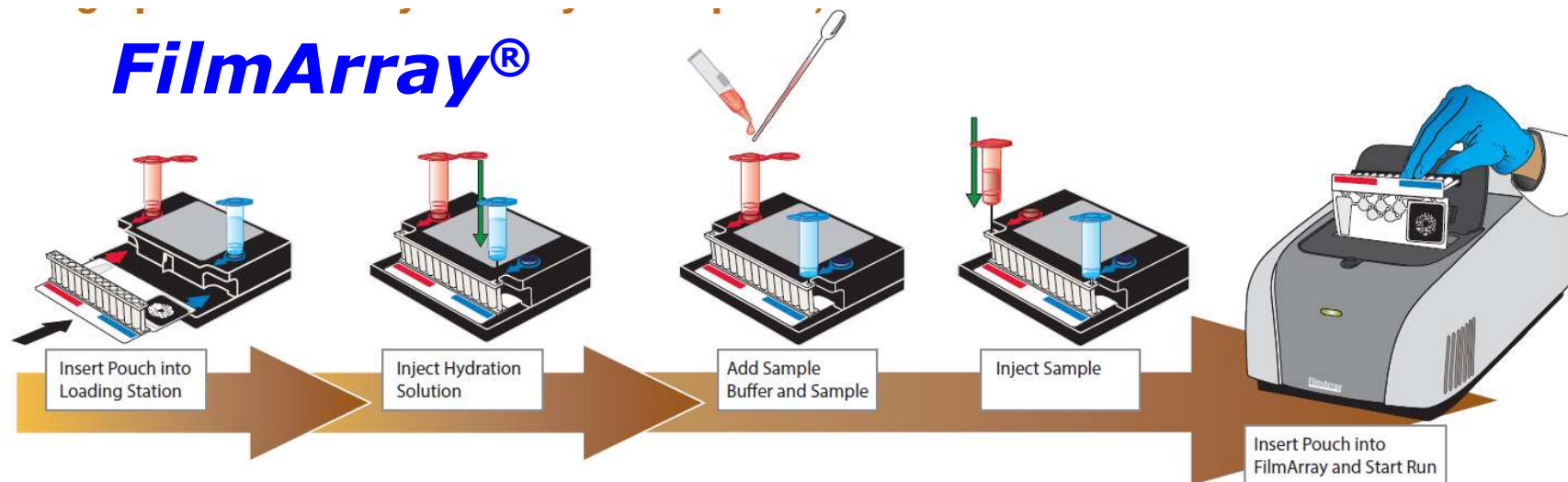
- Ciblées
- Syndromiques

3 - 4h
Expertise requise
Matériel
Adaptable (évolution cibles)
Hétérogénéité

Rapide !
Simple
Matériel dédié
Cibles "figées"
Homogénéité

POTENTIEL INFECTIEUX DU MATÉRIEL
AUX DIFFÉRENTES ÉTAPES ?

FilmArray®



- ✓ *Bacillus anthracis*, 3 Targets
- ✓ *Brucella species*, 2 Targets
- ✓ *Burkholderia mallei / pseudomallei*
- ✓ *Coxiella burnetii*, 2 Targets
- ✓ *Francisella tularensis*, 2 Targets
- ✓ *Rickettsia*, 2 Targets
- ✓ *Yersinia pestis*, 2 Targets
- ✓ Botulinum toxin gene
- ✓ Ricin toxin gene
- ✓ Ebola virus
- ✓ Marburg virus, 2 Targets
- ✓ Variola virus
- ✓ Orthopox genus viruses, 2 Targets
- ✓ EEE virus
- ✓ VEE virus, 2 Targets
- ✓ WEE virus

TABLE 1 Analytes detected by the FilmArray RP2

Analyte	Change relative to RP ^a
Viruses	
Adenovirus	Updated primers ^b , additional assays
Coronavirus 229E	Updated primers
Coronavirus HKU1	Not modified
Coronavirus NL63	Not modified
Coronavirus OC43	Updated primers
Human metapneumovirus	Updated primers
Human rhinovirus/enterovirus	Updated primers
Influenza A virus	Updated primers
H1	Updated primers
H1-2009	Not modified
H3	Updated primers
Influenza B virus	Not modified
Middle East respiratory syndrome coronavirus (MERS-CoV)	New
Parainfluenza virus 1	Updated primers
Parainfluenza virus 2	Updated primers
Parainfluenza virus 3	Updated primers
Parainfluenza virus 4	Updated primers
Respiratory syncytial virus	Updated primers
Bacteria	
<i>Bordetella parapertussis</i> (IS1001)	New
<i>Bordetella pertussis</i> (ptxP)	Not modified
<i>Chlamydia pneumoniae</i>	Not modified
<i>Mycoplasma pneumoniae</i>	Updated primers

GeneXpert®



Biosafety recommendations for laboratories conducting diagnostic testing for EVD with appropriate biosafety BSL3/BSL4 facilities

- Virus isolation should be done only in a maximum containment BSL4 laboratory. Ensure safe and secure handling and storage of the virus isolates and other specimens from accidental or deliberate release.
- The inactivation of specimens, depending on the detection protocol used, should be performed under BSL3 conditions.
- For non-inactivated samples, RT PCR and enzyme-linked immunosorbent assay (ELISA) testing can be performed at a BSL3 laboratory.
- If samples have been inactivated (i.e. cell lysis) RT PCR and ELISA testing can be performed at a BSL2 laboratory.

WHO. Laboratory Guidance for the Diagnosis of Ebola Virus Disease. www.who.int/resources/publications/ebola/laboratory-guidance

Specimen Collection, Transport, and Storage

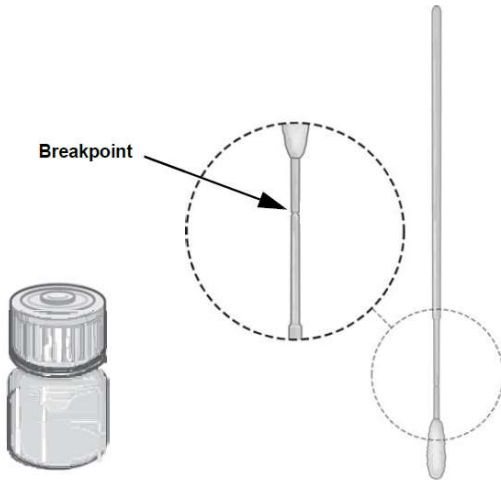
Whole Blood Collection

Collect whole blood specimens by venipuncture in EDTA tubes per the manufacturer's instructions for use. A minimum of 100 μ L of whole blood is required for the Xpert Ebola Assay.

Immediately proceed with the sample preparation step to ensure that the Ebola virus gets inactivated.

Sample Preparation

Venous Whole Blood collected in EDTA-tubes: Open the lid of the Sample Reagent bottle. Transfer 0.1 ml blood by placing the swab (SWAB/E-50) in the EDTA tube and allow it to absorb blood for at least 30 seconds, transfer the sample by inserting the prepared swab into the Sample Reagent bottle (see Figure 1). Hold the swab by the stem and align the small groove against the rim of the tube. Break off the swab by bending to one side.



Réflexions...

Organisation locale

- ✓ Personnel formé
 - Maintien de l'habilitation
 - Exercices
- ✓ Circuit du prélèvement
 - Réception – Orientation
 - Jour/nuit/week-end/fériés...
- ✓ Matériel
 - L3, L2 – PSM3, PSM2
 - Accès aux tests "rapides"
 - Accès aux panels syndromiques
- ✓ Objectifs
 - Diagnostic ? Exclusion ?
 - Exhaustivité / Rapidité
- ✓ Confirmation / Infirmer du résultat
 - CNR (circuit d'envoi)
 - **Homogénéisation des techniques !**