Environment & Infectious Risks Expertise and Research Unit

Cellule d'Intervention Biologique d’Urgence (CIBU)
[Laboratory for Urgent response to Biological Threats]

A 24/7 RESPONSE TEAM

XXII Actualités du Pharo, 7 octobre 2016, Marseille, France

WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research
Missions and activities

- Respond to "special biological urgencies" which can endanger Public Health:
  - Epidemics
  - Accidents
  - Bioterrorist attacks using biological weapons

- Two modes of intervention:
  - Direct mode
    Independent laboratory upstream of NRCs, conducting training, outbreak intervention, consulting, research, …
  - Indirect mode
    Logistics support (human and material resources) to another laboratory (NRC, WHOCC, …) if it is overloaded with work
Articulation with National Reference Centres

Unidentified agent

Already identified agent

CIBU

Viruses

Bacteria

PIV

PIB

Scientific and technical volunteers

SAM

NRC inside Institut Pasteur

Unusual situation: Support request

Assignment of:
- Technical staff
- Specific equipment

Transfer to the dedicated NRC

NRC outside Institut Pasteur
Operational 24/7

On call/email system
+33 6 86 68 35 53 – cibu@pasteur.fr

Involved structures:
- NRC Rabies
- NRC Listeria
- NRC Anaerobic bacteria & botulism
- NRC Mycology & Antifungal

Permanence by pairs
- 1 scientist
- 1 technician

Total
- 15 trained people

Equipment: Enhanced P3 with Type III safety cabinet
### Intervention activities

Samples analysed during nights, week-ends and bank holidays in 2015 by CIBU

<table>
<thead>
<tr>
<th></th>
<th>MERS</th>
<th>Ebola</th>
<th>Other</th>
<th>Total / Moy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nb</strong></td>
<td>50</td>
<td>10</td>
<td>23</td>
<td>83</td>
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<tr>
<td><strong>Analysed</strong></td>
<td>39</td>
<td>10</td>
<td>23</td>
<td>72</td>
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<tr>
<td><strong>Emergency</strong></td>
<td>39</td>
<td>10</td>
<td>2</td>
<td>51</td>
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<tr>
<td><strong>Urgent patients</strong></td>
<td>21</td>
<td>5</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td><strong>Response time in emergency</strong></td>
<td>8,4</td>
<td>6,5</td>
<td>7,25</td>
<td>7,98</td>
</tr>
</tbody>
</table>
Intervention activities

Characterisation of emerging pathogens and transmission routes – Risk assessment

March – April 2003:
*SARS Coronavirus Vietnam*

April-May 2009:
« Swine » flu A(H1N1)
Intervention activities

Natural disasters – Risk assessment for infectious diseases

January 2004: *Avian influenza A(H5N1) in Cambodia*
Intervention activities

Capacity building – Sampling, Diagnostics & Biosafety

March-April 2014:
Ebola outbreak in Guinea
Intervention activities

Human ressources – Laboratory support

2014-2015: Ebola outbreak in Guinea
Intervention activities

Human ressources – Laboratory support
Intervention activities

Human resources – Laboratory support

Weekly numbers of new Ebola cases (Patients base - source: WHO)
Training activities

Biosafety & Diagnostics

Lab personnel IP laboratory, Red Cross ETC, Macenta, Guinea
Training activities

Biosafety, Diagnostics and Field laboratory set-up

12 scientists and technicians from 6 Western Africa countries participating in an exercise recreating the scenario of a possible emerging epidemic.
## Other supporting activities

<table>
<thead>
<tr>
<th>Institution</th>
<th>Country</th>
<th>qRT-PCR protocol</th>
<th>qRT-PCR positive control</th>
<th>Technical advice</th>
<th>Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gendarmerie royale</td>
<td>Marocco</td>
<td>Yes</td>
<td>Yes</td>
<td>Diag</td>
<td>No</td>
</tr>
<tr>
<td>IP Maroc</td>
<td>Marocco</td>
<td>Yes</td>
<td>Yes</td>
<td>Diag, equipment</td>
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<tr>
<td>IP Cambodge</td>
<td>Cambodia</td>
<td>Yes</td>
<td>Yes</td>
<td>Transport</td>
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<tr>
<td>IP Alger</td>
<td>Algeria</td>
<td>No</td>
<td>No</td>
<td>Diag</td>
<td>No</td>
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<tr>
<td>CPC</td>
<td>Cameroun</td>
<td>Yes</td>
<td>Yes</td>
<td>Diag</td>
<td>No</td>
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<tr>
<td>Centre Médical de l'IP</td>
<td>France</td>
<td>No</td>
<td>No</td>
<td>PPE on and off</td>
<td>No</td>
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<tr>
<td>Institute of Tropical Medicine</td>
<td>Belgium</td>
<td>No</td>
<td>No</td>
<td>BSC III</td>
<td>No</td>
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<tr>
<td>Seychelles Public Health Lab</td>
<td>Seychelles</td>
<td>No</td>
<td>No</td>
<td>Transport</td>
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<td>IP Nouvelle-Calédonie</td>
<td>New Caledonia</td>
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<td>Diag, Transport</td>
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<tr>
<td>IP Laos</td>
<td>Lao PDR</td>
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<td>Yes</td>
<td>Diag, Transport</td>
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<tr>
<td>IP Cayenne</td>
<td>France</td>
<td>Yes</td>
<td>No</td>
<td>Transport</td>
<td>No</td>
</tr>
<tr>
<td>Victoria Hospital</td>
<td>Mauritius</td>
<td>Yes</td>
<td>No</td>
<td>Diag</td>
<td>No</td>
</tr>
<tr>
<td>IP Tunis</td>
<td>Tunisia</td>
<td>No</td>
<td>No</td>
<td>Diag, Transport</td>
<td>No</td>
</tr>
<tr>
<td>HKU-Pasteur Research Pole</td>
<td>Hong Kong</td>
<td>No</td>
<td>No</td>
<td>Diag</td>
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<tr>
<td>IP de Madagascar</td>
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<td>IP Guadeloupe</td>
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<td>P3, BSC III</td>
<td>SPR</td>
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<tr>
<td>IPK</td>
<td>Cuba</td>
<td>No</td>
<td>No</td>
<td>Planned</td>
<td></td>
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</table>
Two main research areas

**Theme 1:** Resistance of human pathogens in the environment
- Study of resistance mechanisms of epidemic-prone viruses outside the host (in aerosols, surfaces,…)

**Theme 2:** Identification & characterisation of human pathogens
- Robust, rapid diagnostic tools for field or point-of-care testing
- Innovative broad-spectrum tools: High-Throughput Sequencing, multiplex serology platform, …
- Molecular eco-epidemiology and large-scale serosurveillance studies (« One Health » Concept)
Resistance of human pathogens in the environment

- Matrix effects (air, water, surfaces, ...)
- Physico-chemical parameters (T, humidity, salinity, ...)
- Virus properties (strain, viral load, ...)

Wild type viruses

Lentiviral Pseudotypes

Recombinant viruses (Reverse genetics)
Diagnostic tools for minimally equipped field- and mobile- laboratories

Isothermal amplification:

- faster
- less expensive
- simple / no instrumentation
- robust
- transportable to the point-of-care
- easy to use and interpret
Diagnostic tools for minimally equipped field- and mobile- laboratories

- Real-time Ebola virus RT-LAMP assay performances compared to real-time RT-PCR Gold Standard assay

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<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>No. of samples</td>
<td>174</td>
</tr>
<tr>
<td>True positive</td>
<td>90</td>
</tr>
<tr>
<td>False negative</td>
<td>0</td>
</tr>
<tr>
<td>True negative</td>
<td>84</td>
</tr>
<tr>
<td>False positive</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100.0%</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(96.0 to 100.0 %)</td>
</tr>
<tr>
<td>Specificity</td>
<td>100.0%</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(95.7 to 100.0 %)</td>
</tr>
</tbody>
</table>

Equivalent clinical performances and 10 x faster time-to-result compared to real-time RT-PCR
Plastic Microcard-format assay

Sample: Blood ~100 µL

Single Use Integrated Device:
- Total lysis
- RNA concentration/purification
- up to 5 RT-LAMP assays

Result: YES / NO

Diagnostic tools for point-of-care testing

Anne-Gaëlle Bourdat
Remco Den Dulk
Mélanie Flaender
Diagnostic tools for point-of-care testing

Paper Microcard-format assay

Lysed sample

Blood

~100 µL

Single Use Integrated Device

Integration of positive and negative controls
Up to 4 simultaneous RT-RPA assays

Result

YES / NO

Laura Magro
Béatrice Jacquelin
Patrick Tabeling
High-throughput broad spectrum tools for differential diagnosis and surveillance

- **Multiplex Arbovirus Immuno-assay**

1. **Incubation steps**
   - Specific serum antibodies IgM / IgG
   - Secondary anti-host antibody (biotinylated)

2. **Wash steps**
   - Streptavidin-R-Phycoerythrin

3. **Reading**

Up to 100 types of coupled bead-sets / well

**Tools:**
- DEN3
- DEN4
- ZIKV
- WNV
- YFV
- ROCV
- CHIK
- MAYV
Transfer and evaluation of the performances of the arboviral MIA

- Zika virus outbreak in French Polynesia
  - 2014

- Zika virus outbreak in Central/South-America
  - 2016
Clinical performances (IgG serology)

Sensitivity: 98.95
Specificity: 95.45

Area under the ROC curve (AUC): 0.978
Standard Error: 0.00975
95% Confidence interval: 0.952 to 0.993
z statistic: 49.046
Significance level P (Area=0.5): <0.0001

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Negative controls N=154
- Healthy blood donor (2009-2011): 41%
- Dengue IgM and/or IgG Positive patient (2009-2011): 39%
- Asymptomatic patient (2015-2016): 8%
- Confirmed / Suspected zika case (acute phase): 7%
- Dengue IgM and/or IgG Positive patient (2015): 4%
- Child with birth defect (genetic cause): 1%

Positive controls N=95
- Confirmed Zika case (qRT-PCR positive): 31%
- Clinical symptoms of Zika virus infection: 19%
- Epidemiological link with confirmed/suspected Zika case: 19%
- Child with birth defect possibly linked to Zika virus infection: 31%
Large scale seroprevalence studies

Data driven mathematical modeling of the shared epidemiology of Zika & other arboviruses in human populations across the globe

- 8 countries
- > 10 000 samples
Large scale seroprevalence studies

Project aims

• Estimate population seroprevalence
• Establish mathematical models to characterize key transmission parameters
• Characterize effects of interaction between arboviruses
• Develop risk maps
• Compare results across testing platforms
Large scale seroprevalence studies

Animal populations: surveillance of reservoirs and «sentinel» hosts

- SBV
- JEV
- WNV, USUV
- EBOV, MARV
- RVFV, WNV
- CHIKV

Institut Pasteur
Contribution to the initial characterisation of the strains of the Zaïre species of *Ebolavirus* by NGS

- Full genome sequences of 88 strains of EBOV
- Sampled from patients in Guinea during the peak of the epidemic diffusion
- Deep sequencing
  - cDNA synthesis,
  - Nextera library construction
  - Illumina sequencing with a HiSeq2000
- Phylogenetic analysis
THE CIBU TEAM

Centre for viral identification

Centre for genotyping and sequencing of pathogens

Centre for bacterial identification
Examples of IgG serological profiles: plasma samples from confirmed Zika cases

**Primary infection**

**Secondary infection (Post-Dengue)**