D7.5 Report on feasibility study for influenza virus characterization, molecular epidemiology studies related to influenza related disease in vaccinated and unvaccinated subjects

DRIVE - 777363

Development of robust and innovative vaccine effectiveness

WP7 – Influenza Vaccine Effectiveness Pilot Studies

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<tbody>
<tr>
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<th>30 March 2019</th>
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<td>V2.0</td>
<td>12 Sept 2019</td>
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¹ Use one of the following codes:
R: Document, report (excluding the periodic and final reports)
DEM: Demonstrator, pilot, prototype, plan designs
DEC: Websites, patents filing, press & media actions, videos, etc.
OTHER: Software, technical diagram, etc.
## Document History

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<td>01 June 2019</td>
<td>First outline</td>
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<td>Final version</td>
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For optimal analysis of strain specific Vaccine Effectiveness studies, a detailed characterization of the viruses detected in both cases and controls is important. The co-circulation of virus types and sub-types, and sometimes of different genetic and antigenic clades within a given subtype (i.e. A(H3N2) viruses during the 2017-18 and 2018-19 seasons) may lead to different VE according to the virus clade responsible for the infection in enrolled patients, and to potential differences in measured VE when this analysis is performed stratified by virus clade. The full genome sequencing of these viruses will provide WHO with a valuable tool to increase the size of the sequence dataset used during the selection of vaccine candidates during Vaccine Composition Meetings (VCM).

Introduction

The Development of Robust and Innovative Vaccine Effectiveness (DRIVE) project is a public-private partnership aiming to build capacity in Europe for estimating brand-specific influenza vaccine effectiveness (IVE). The DRIVE Project, which is funded by the Innovative Medicines Initiative (IMI), was initiated as a response to the changes for licensing of influenza vaccines in Europe. The new guidance on influenza vaccines by the European Medicines Agency (EMA) came into effect in the beginning of 2017. This guidance states that the performance of influenza vaccines should no longer be assessed based on serological assays, but should be based on post-authorization effectiveness studies [1].

In 2017/18, a pilot study was performed for conducting Influenza Vaccine Effectiveness studies through four test-negative design studies (TND) and one register-based cohort study. The tools and processes developed during the pilot season 2017/18, were used and further improved in the 2018/19 season. The main objective of the 2018/19 season was to estimate brand-specific seasonal IVE in Europe by health care setting and age group. During 2018/19 season, nine test-negative design studies (TND), one register-based cohort study and two cohort studies participate in DRIVE.

However, subtype-specific VE estimates may vary by site according to the clade distribution observed by each site may explain these differences (Fig 1 from lannery et al. J Infect Dis. 2016;214(7):1010-1019.)

Fig 1: differences measured in IVE of the A(H3N2) vaccine component according to the infecting clade

<table>
<thead>
<tr>
<th>Genetic Group, Age</th>
<th>Influenza A(H3N2)-Positive Cases, Proportion (%) Vaccinated</th>
<th>Influenza Virus-Negative Controls, Proportion (%) Vaccinated</th>
<th>VE, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ages</td>
<td>939/1817 (51.7)</td>
<td>3866/7078 (54.6)</td>
<td>7 (–5 to 17)</td>
</tr>
<tr>
<td>Genetic group 3C.3b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ages</td>
<td>56/156 (35.9)</td>
<td>3866/7078 (54.6)</td>
<td>44 (16 to 63)</td>
</tr>
<tr>
<td>Genetic group 3C.2a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ages</td>
<td>597/1101 (54.2)</td>
<td>3866/7078 (54.6)</td>
<td>1 (–14 to 14)</td>
</tr>
<tr>
<td>Genetic group 3C.3a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ages</td>
<td>31/55 (56.4)</td>
<td>3866/7078 (54.6)</td>
<td>–48 (–169 to 19)</td>
</tr>
<tr>
<td>Genetic group 3C.3</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>All ages</td>
<td>27/47 (57.5)</td>
<td>3866/7078 (54.6)</td>
<td>1 (–87 to 48)</td>
</tr>
</tbody>
</table>

The integration of virological data in these type of studies is essential to interpret IVE results, particularly when low VE against a specific clade is suspected or when several clades are circulating. The distribution of clades by study site and by influenza period may help to understand the differences between site-specific VE and the changes in VE over the season.

Influenza characterization can be done by sequencing and HIA (Hemagglutination Inhibition Assays). Both methods are used in combination to monitor the antigenic evolution of the influenza viruses. This antigenic evolution of the viruses is a result of the constant emergence of immune escape mutants driven by the immune pressure to which the influenza viruses are exposed, and their high level of evolutionary capacities as a result of the lack of RNA proofreading during replication.

The antigenic evolution is monitored by WHO through the surveillance carried out by the National Influenza Centres, and analyzed by the WHO coordinating centre (WHOcc) the vaccine composition meetings. This detailed surveillance is mandatory, because of the very high annual burden of influenza for which optimal influenza vaccines are required. However, we know that for optimal effectiveness, the HA of the Influenza
vaccines have to be identical to those of the circulating strains. When antigenic differences (mismatches) are observed between the vaccine strain and the circulating viruses, the vaccine effectiveness can be reduced, sometime very significantly. To overcome this problem, the anticipation of virus evolution using tools like antigenic cartography and inference of virus fitness have been used to select the best vaccine candidates and propose vaccine compositions that are more likely matching the circulating strains. Because the selection process has to take place at least 6 months before the vaccination campaign, and 9 months before the actual start of the epidemic, the permanent evolution of the viruses (especially the A(H3N2) component) may become a permanent problem.

A more precise picture of the circulating strains (ie antigenic data, sequence data, clinical description associated to the case can have a positive impact in the strain selection process. For example, the early identification of escape mutants detected from the surveillance or from vaccinated individuals when reduced vaccine effectiveness is observed may be of very important value.

For that purpose, the new generation of sequencing machines (NGS) can be used for the implementation of large-scale molecular characterization of viruses, with detailed information regarding the consensus sequences of the HA and potentially the other gene segments. This technique is of interest, and largely used for A(H3N2) viruses for the identification of escape mutants (in combination with the conventional HIA), and for the description of the trend of their emergence (fig:2 example for the 2019 VCM for the northern hemisphere).

Fig 2: monitoring of the respective proportions of the A(H3N2) clades presented during the feb 2019 VCM.

Last, in cases of vaccine failure, it is theoretically possible to identify in these patients subgroups of minority variants sequences that would reflect, by anticipation, some of the potentially emerging escape mutants that would drive the antigenic evolution of the currently circulating viruses.

A retrospective preliminary study performed on a limited number of hospital cases by the research group of UCBL (Simon B et al, Viruses, 2019) has demonstrated the capacity for NGS and a full genome sequencing strategy to identify a differential genetic sequence between viruses detected from severe and non-severe cases, as well as from vaccinated and non-vaccinated patients. This preliminary needs to be confirmed by larger studies with a robust design. In addition, this study provided some technical information on the threshold to be used when analyzing minority variants (% of variant sequence as compared to the consensus), and the depth of sequencing required to be informative (minimum of reads for each nucleotide position).

Therefore, our aims are (1) to put in place a shared protocol for influenza virus NGS sequencing, and (2) to define how to select a representative sample of DRIVE specimens, to be analysed by NGS during the 2020-2021 studies, in order to identify/describe the viruses sequences, and to identify amino acid changes that can influence the effect of the influenza vaccines. This work should not modify the sampling strategy used for virological surveillance.
Objectives

Our main Objective is to describe, using NGS techniques, the potential genetic heterogeneity of viruses detected during the surveillance conducted by the DRIVE sites, and to identify key influenza virus genotypic evolutions that could affect vaccine effectiveness.

Secondary objectives are:
- to provide real-time data to support the decision for the strain selections carried out during the Vaccine Composition Meeting (VCM);
- to use the high throughput sequencing system available in some site (NGS) to provide sequence data allowing to estimate clade specific VE with pooled influenza data collected among sites participating in DRIVE;
- to provide clues for the antibody-induced antigenic evolution of circulating strains, and to detect if vaccine failure can be either a consequence or a reflect of genetic/antigenic evolution of viruses.

To be informative for the VCM, this analysis has to be done almost in real time, requiring a technical and human resource to perform this analysis (sequencing and big data management), and a system by which these specimens could be analyzed in a time frame adapted for the VCM and analysis of the virus evolution for the relevant stakeholders involved in the strain selection process.

Methods

Both the French National Influenza Reference Laboratory of Lyon, France and the Virology Laboratory in the Genomics & Health Area from FISABIO-Public Health in Valencia, Spain, have a strong experience in virus genetic characterisation by standard (Sanger) and NGS platforms. Before the start of the 2018-19 season, both laboratories prepared a common shared protocol for influenza virus NGS (Annex). Harmonization included materials required, laboratory techniques used between both partners, and the same sequencing platforms. As a proof-of-concept study, both laboratories applied the shared protocol to a limited number of influenza A(H3N2) and A(H1N1pdm09) samples during the 2018-19 season, obtaining satisfactory and comparable results (sufficient sequencing coverage for all the 8 segments of influenza viruses), thus consolidating the feasibility of extending whole-genome NGS sequencing of influenza to DRIVE.

For that purpose, preliminary exploring studies will be carried out by the UCBL lab on some specimens collected during the 2018-19 and 2019-2020 seasons. To meet the objectives of the study we will collect specimens from both vaccinated and non-vaccinated cases. These specimens will be analyzed by NGS sequencing and retrospectively compared to check for the variability of their genetic content. Instead of limiting the analysis to the classical characterization of the hemagglutinin and neuraminidase viral genes, a comprehensive sequencing of the full viral genome will be performed.

In addition, for the 2019-2020 call for IVE studies performed in the context of DRIVE will ask study sites to test the feasibility for doing NGS analysis (to be performed by the UCBL lab) of either HA and NA or WGS in a subset of the ILI/SARI cases recruited in the study (see case definition paragraph). This second, prospective step is required to test the logistics and the technical hurdles for a larger scale of analysis, and to identify sites with all the required technical capacities and know-how. Eventually, these sites will be asked to share their data within the consortium, to allow a multisite integration of the dataset generated during these studies.

Case definition

The ILI/SARI case definition used, and the procedures to identify influenza cases and controls are the ones defined in the DRIVE generic protocols.

Study period

The proportion of strains characterized will vary according to the phase in the influenza season: the proportion will be higher in the early and late phase and lower in the peak of the season. Each site will define the start/end of each influenza phase according to the surveillance indicators used in their site. During this preliminary phase, each site will define the proportion of strains to be characterized (sampling fraction) for each of the phases. Each site will:
- define the influenza phases;
- define the sampling fraction for each phase;
- document the sampling fraction for each phase and for each subtype.

**Sample size**

It is difficult to define the minimum sample size to meet the primary objective of the study. This will depend on several factors, but mainly, on the vaccination coverage among cases and controls. In the first phase of the study, sites will be required to provide a convenient sample of the positive specimens detected in their premises. The study sites will try to characterise the maximum number of strains taking into account the existing resources. Each site will define the proportion (representative of the whole collection if possible) of samples to be characterised.

We recommend to have a higher sampling fraction in the early phase (if possible, characterise all strains) of the influenza season to better understand what is circulating and to be able to provide results for the WHO vaccine strain selection committee that meets around end February each year.

We also recommend a higher sampling fraction in the late phase of the influenza season to detect possible changes in predominant clades.

Subsequently, WP7 will revise the number of strains characterised and included in the analysis. Based on the epidemiological situation, the early VE estimates and the available resources, WP7 will discuss the optimum sampling fraction that would be needed in each study site for the next 2019/20 season.

The random selection should be used to select the viruses included in the study sites (this does not apply to routine virological surveillance). At the end of each time period defined for the selection of strains, study sites will select viruses using the Bernoulli sampling method. This method ensures that each strain has the same probability of being selected. Specific guidelines will be provided to each study site.

**Analysis**

The number (and proportion) of viruses selected, eligible but excluded because they could not be characterized and the reasons for exclusion will be presented, according to the resources of the sites. Cases eligible, selected but not characterized will be described (age, vaccination status, date of onset). Cases excluded due above described reasons will be compared with cases with strains characterized in terms of date of onset, age, vaccination status, chronic diseases.

For each subtype/lineage, the following indicators will be computed in a centralized lab (UCBL):
• number and proportion of viruses selected for characterization;
• number and proportion of viruses sequenced with NGS techniques;
• number and proportion of viruses by clades taking into account the sampling fraction;
• distribution of clades by study site and for the pooled dataset;
• distribution of clades by time (weeks or months).

**Discussion**

The implementation of the first-year study will provide evidence for conducting future real-time monitoring of genetic sequences of circulating viruses detected in the IVE DRIVE studies. It will also be an additional tool for WHO during the vaccine composition meeting.

The second step will be the implementation of this characterization for all DRIVE IVE studies.

**Conclusion**

This work plan will be implemented with the help of the two laboratory structures already in capacity to generate influenza WGS data by NGS within the DRIVE network.
There is a high interest to better understand and capture actual and prospective influenza genetic drifts and to characterize virus variants to support new influenza vaccines development. WHO and ECDC highly recommend and push forward for genetic characterization of influenza strains during seasons to better inform the vaccine recommendation committees and to enhance surveillance for new strains and/or potential pandemic viruses. Fine-tuned antigenic characterisation by monoclonal antibody techniques is used by WHOCC to characterize influenza strains yearly, but these techniques cannot be shared.

Both the French National Influenza Reference Laboratory of Lyon, France and the Virology Laboratory in the Genomics & Health Area from FISABIO-Public Health in Valencia, Spain have a strong experience in virus genetic characterization by standard (Sanger) and Next-Generation sequencing platforms, and will bring-up laboratory support to DRIVE. Harmonization of laboratory techniques used is necessary and possible within the network, according to the proposed following protocol.

Objectives of this study
The main objective is to sequence a significantly relevant sample of strains from the sites during the season, representing the diversity of circulating strains, to capture drifts. The study will also allow for the identification by comparative genomics of differences between circulating viruses and the A(H3N2), A(H1N1)pdm09 and B vaccine strains. Influenza A(H3N2) viruses are of particular interests because they are evolving rapidly and more genetic sequencing data is needed. Instead of limiting the analysis to the classical characterization of the hemagglutinin and neuraminidase viral genes, a comprehensive sequencing of the full viral genome will be performed. This strategy will add value to conventional characterization carried out in most IVE studies, and will help to understand drop in vaccine effectiveness, when antigenic/ genetic variants emerge before or during the Influenza season.

Materials required, procedures, and laboratory protocols.
The protocols following in annex detail the materials required and the laboratory procedures. These protocols are derived from UCB Lyon and FISABIO current procedures. Total nucleic acid from the samples to be included in the study can be extracted by the lab sites using the commercial nucleic acid extraction protocol 1 in annex, or alternatively an equivalent nucleic acid extraction protocol after approval by FISABIO/Lyon. This strategy would eventually facilitate easy shipping of the extracted nucleic acids to FISABIO/Lyon. Another option is to centralize nucleic acid extraction at FISABIO and Lyon: nasopharyngeal swabs in virus transport medium should be then shipped frozen to FISABIO/Lyon by specialized international courier.
I. Introduction

The NucliSENS easyMAG is an automated platform for the isolation (purification and concentration) of total nucleic acids (RNA/DNA) from biological specimens. Used for batch processing of 1-24 samples. The nucleic extraction method is based on Boom Chemistry using magnetic silica particles. Briefly, under high salt conditions, nucleic acid will bind to the silica particles. These silica particles act as solid phase and non-nucleic acid components are removed by several washing steps performed in the NucliSENS easyMAG instrument. Next, nucleic acids are eluded from the silica particles and the silica particles are removed from the extracted specimens by the NucliSENS easyMAG instrument. The resulting eluate contains purified and concentrated total nucleic acids.

II. Specimens Types

Viral transport media (VTM) from swabs collected in VTM. Use a minimum of 200 mL of VTM, up to 1 mL maximum, for final elution in 50 uL of elution buffer. Vortex on high for 10 seconds and brief spin before extraction.

III. Materials, Equipments

NucliSENS easyMAG
NucliSENS easyMAG extraction products:
- NucliSENS Lysis Buffer
- NucliSENS easyMAG Lysis Buffer
- NucliSENS easyMAG Magnetic Silica
- NucliSENS easyMAG Extraction Buffer 1
- NucliSENS easyMAG Extraction Buffer 2
- NucliSENS easyMAG Extraction Buffer 3
- NucliSENS easyMAG Disposables – vessels and pipettes

Multichannel pipettor
1000uL, 100uL, 20uL Pipettors
Aerosol Resistant Tips (ART) 1000uL, 200uL, 20uL
Powder-free gloves
Internal controls (for human DNA and/or viruses)

IV. Worksheet.

Fill the lab worksheet with the sample details (max. 22 samples + one positive + one negative control per run).

GENERAL PRECAUTIONS:

Use Specimen Preparation area; work always in BSC cabinet with gown and frequent glove change. For respiratory viruses, use of a face mask until the sample is neutralized in lysis buffer is highly recommended. Do not allow the NucliSENS easyMAG Lysis Buffer, NucliSENS easyMAG Extraction Buffer 1 or waste from the instrument to come in contact with acidic materials.
Certain reagents contain guanidine thiocyanate. Buffers containing guanidine thiocyanate MUST NOT be mixed with cleaning solutions containing bleach. Liquid waste from extractions procedures contain guanidine thiocyanate MUST NOT be mixed with other laboratory waste. Proper waste disposal is required.

IV. **Procedure**

1. Turn ON easyMAG. Wait for orange light to turn GREEN, then switch ON the easyMAG computer.
2. Perform and record easyMAG Daily Maintenance:
   - Inspect drip tray.
   - Inspect reagent cap filters.
   - Inspect carbon filter.
   - Empty & rinse waste bottle with water.
3. Log-in easyMAG
   - Login name: _______________
   - Password: _________________
4. Click on “reagent” icon
5. Scan reagent stand and scan reagent bottle for all four reagents (A, B, C, D).
6. Click on “Sample” icon
7. **For VTM/Respiratory viruses**
   - Enter Protocol: UTM-Respivir-1ml (or 200ml)
   - Enter Matrix: Other
   - Enter Volume (mL): 1.000 (or 200ml)
   - Eluate (uL): 50 ul (reduce if sample volume is lower)
8. Click on sample ID bar and scan specimen barcodes (or input sample data)
9. Click on “organize run” icon
10. Click on “create run” icon

10. A box will appear with today’s date and run #. Click on OK.
11. Click on to transfer all scanned items onto the worklist.

12. Click on “load run” icon

13. To identify vessels, click on vessel icon

14. Scan position A barcode and then vessel barcode for first 8 samples, continue with position B and C depending on how many samples you have.

15. Click on the silica icon

16. Scan in silica barcode from top of silica box.

17. Highlight all samples by touching screen or with mouse.

18. To apply silica barcode to all samples click on “downward arrow” icon

19. Click on the “progress tab” on third menu bar from top.

20. Print worklist. Select printer icon on right hand vertical menu bar. Pop-up appears, click OK.

21. Click on the “dispense lysis icon”

22. Once the lysis buffer has been dispensed, remove vessels from easyMag and put them in rack and take them to the BSC.

23. For respiratory viruses, add 1 mL of the VTM specimen to the lysis buffer.

24. Vortex internal control (IC). *(Not applicable for this study)*
   
   *For Respiratory viruses, add 10uL of IC to the lysis buffer/specimen mix. For CMV, BKV, HSV, VZV, PARVO, ENTERO, HCV, HBV, HIV, add 5 uL IC. Mix up and down three times.*

25. Incubate at room temperature for 10 minutes.

26. To make silica premix add 550 uL DEPC-PCR grade water to silica (program 1 of pipette)
27. Vortex silica premix before dispensing.

28. Dispense 125 uL silica into Elisa strip (program 2 of pipette)

29. After the 10 incubation period add 100 uL silica premix to vessel (program 3 of pipette)

30. Reload vessels into the easyMAG.

31. Scan position barcode and then vessel barcode (make sure the vessel icon is highlighted).

32. Click on start icon on right hand vertical menu bar.
   A pop-up box will appear asking if you have added the premix silica, click YES.

33. Once the samples have been eluted, transfer the eluate from the vessels to labeled 1.5 ml screw-cap o-ring microtube (use printed label with sample study number). Set pipette at 55uL to remove all of the eluate.

34. If elutes are to be tested same day store eluates at 4°C in the fridge. If PCR / testing is to be performed at a later date, store eluate at -70°C as soon as possible.

V. Quality Control

Failed extraction runs, should be reviewed with senior and/or Charge technologist to establish causes and corrective actions; repeating extraction run maybe necessary.
If the easyMag system becomes inoperable call Biomerieux technical support phone number, provided on each easyMag.

VI. References
    NucliSENS easyMAG User Manual v2.0 ref: 280163
PROTOCOL 2.
Whole-genome amplification protocol
Influenza A (MBT 12/13 modified)

Version: DRAFT Date: 27/06/2019

RT-PCR primers:
(MBTuni-12 [5'-ACGCGTGATCAGCAAAGCAGG-3'] and MBTuni-13 [5'-ACGCGTGATCAGTAGAAACAAGG-3'])

RT-PCR amplification set-up:

☐ Fill-in sample worksheet

<table>
<thead>
<tr>
<th>PCR N°</th>
<th>Sample ID</th>
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<tbody>
<tr>
<td>Sample 1</td>
<td>XxxxxxXxx</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Sample N</td>
<td>Xxxxxx</td>
</tr>
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☐ Make RT-PCR mix (for 50 ul final reaction volume)

<table>
<thead>
<tr>
<th></th>
<th>for 1 sample</th>
<th>for N samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer MBT12 10µM</td>
<td>2 µl</td>
<td></td>
</tr>
<tr>
<td>Primer MBT13 10µM</td>
<td>2 µl</td>
<td></td>
</tr>
<tr>
<td>2 X reaction mix</td>
<td>25µl</td>
<td></td>
</tr>
<tr>
<td>Superscript III RT platinum Taq High Fidelity Enzyme Mix</td>
<td>1µl</td>
<td></td>
</tr>
<tr>
<td>RNASin</td>
<td>0.5 µl</td>
<td></td>
</tr>
<tr>
<td>PCR-grade water</td>
<td>9.5 µl</td>
<td></td>
</tr>
</tbody>
</table>

☐ Add 10 µl of nucleic acid extract
☐ Vortex and spin.
☐ Place in thermocycler

Thermocycler program: (~3.5 hours):

<table>
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<tr>
<th>Temperature</th>
<th>Duration</th>
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<tr>
<td>45°C</td>
<td>1 h</td>
<td></td>
</tr>
<tr>
<td>94°C</td>
<td>2 min</td>
<td></td>
</tr>
<tr>
<td>94°C</td>
<td>30 s</td>
<td></td>
</tr>
<tr>
<td>45°C</td>
<td>30 s</td>
<td>X 5</td>
</tr>
<tr>
<td>68°C</td>
<td>3 min</td>
<td></td>
</tr>
<tr>
<td>94°C</td>
<td>30 s</td>
<td>X 31</td>
</tr>
<tr>
<td>57°C</td>
<td>30 s</td>
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</tr>
<tr>
<td>68°C</td>
<td>3 min</td>
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</tbody>
</table>

References:
PROTOCOL 3.
Whole-genome amplification protocol
Influenza B (Zhou et al. modified)

Version: DRAFT
Date: 27/06/2019

RT-PCR primer cocktail:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>B-PBs-UniF</td>
<td>5’-GGGGGGAGCAGAAGCGGAGC-3’</td>
<td>10 µL</td>
</tr>
<tr>
<td>B-PBs-UniR</td>
<td>5’-CCGGGTATTAGTAGAAACACGAGC-3’</td>
<td>10 µL</td>
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<tr>
<td>B-PA-UniF</td>
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<tr>
<td>B-NP-UniR</td>
<td>5’-CCGGGTATTAGTAGTAACAAGGAGC-3’</td>
<td>6 µL</td>
</tr>
<tr>
<td>B-M-Uni3F</td>
<td>5’-GGGGGGAGCAGAAGCAGCAGCTTT-3’</td>
<td>3 µL</td>
</tr>
<tr>
<td>B-Mg-Uni3F</td>
<td>5’-GGGGGGAGCAGAAGCAGCAGCAGCTTT-3’</td>
<td>3 µL</td>
</tr>
<tr>
<td>B-NS-Uni3R</td>
<td>5’-CCGGGTATTAGTAGTAACAAGGAGGATT-3’</td>
<td>5 µL</td>
</tr>
<tr>
<td>B-NS-Uni3R</td>
<td>5’-CCGGGTATTAGTAGTAACAAGGAGGATT-3’</td>
<td>5 µL</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>84 µL</td>
</tr>
</tbody>
</table>

RT-PCR amplification set-up:

- Fill-in sample worksheet

<table>
<thead>
<tr>
<th>PCR N°</th>
<th>Sample ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Xxxxxxxx</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Sample N</td>
<td>Xxxxxxxx</td>
</tr>
</tbody>
</table>

RT-PCR Amplification mix

<table>
<thead>
<tr>
<th>Reagent</th>
<th>For 1 sample</th>
<th>For n samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNAsin</td>
<td>0.2 µL</td>
<td></td>
</tr>
<tr>
<td>2X RT-PCR Buffer</td>
<td>12.5 µL</td>
<td></td>
</tr>
<tr>
<td>IBV-GA primer cocktail</td>
<td>2 µL</td>
<td></td>
</tr>
<tr>
<td>RT/HiFi enzyme mix</td>
<td>0.75 µL</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15.45 µL</td>
<td></td>
</tr>
</tbody>
</table>

- Vortex and Spin
- Mix 15 µL of reagent mix in each tube and add 10 µl of nucleic acid extract
- Mix gently and spin.
Thermocycler program: (~5h17)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Duration</th>
<th>Cycle number</th>
</tr>
</thead>
<tbody>
<tr>
<td>45°C</td>
<td>60 min</td>
<td>1</td>
</tr>
<tr>
<td>55°C</td>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td>94°C</td>
<td>2 min</td>
<td></td>
</tr>
<tr>
<td>94°C</td>
<td>20 sec</td>
<td>94</td>
</tr>
<tr>
<td>40°C</td>
<td>30 sec</td>
<td>5</td>
</tr>
<tr>
<td>68°C</td>
<td>3 min 30</td>
<td></td>
</tr>
<tr>
<td>94°C</td>
<td>20 sec</td>
<td>45</td>
</tr>
<tr>
<td>58 °C</td>
<td>30 sec</td>
<td></td>
</tr>
<tr>
<td>68 °C</td>
<td>3 min 30</td>
<td></td>
</tr>
<tr>
<td>68°C</td>
<td>10 min</td>
<td>1</td>
</tr>
<tr>
<td>15°C</td>
<td>∞</td>
<td>1</td>
</tr>
</tbody>
</table>

References

Mix 200 ml TBE 5X with 800 ml of ultra-pure water.

NB : Maximum conservation : 1 month

Gel preparation X%
- Mix x g of agarose with 100 ml TBE 1X.
- After heating (600W for 2 min), add 10 µl Syber®Safe DNA gel strain 10 000 X and mix.
- Polymerization for 30 min (1 hour for concentration > 2%).

Amplicons verification
- Add and mix on parafilm 6µl of loading buffer 2X with 6 µl of DNA ladder or 6 µl of amplicons.
- Gently mix.
- Load 10 µl in the wells.
- Do these steps for each samples and the DNA ladder.
- Migration
  - 180V for 50-60 min (4% gel)
  - 200V for 30-35 min (3% gel)
  - 180V for 40-45 min (2% gel)
  - 200V for 20-25 min (1% gel)
- Annotate gel results

<table>
<thead>
<tr>
<th></th>
<th>PM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operator#1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator#2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>PM</td>
</tr>
<tr>
<td>Operator#1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator#2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PROTOCOL 5.
Magnetic bead purification of whole genome amplicons (Influenza A or B)

Version: DRAFT
Date: 27/06/2019

- Vortex the magnetic beads for 30 sec (Macherey Nagel) before use
- Transfer the amplicons in a 1.5ml LoBind tube
- Add 1.8Xµl beads/µl of Amplicons (or 0.6Xµl if size selection needed)
- Vortex 5sec and pulse spin
- Contact: 5 min at room temperature
- Put-on magnet for 3-5min
- Discard the supernatant
- Add 200µl of 70% ethanol
- Put on the magnet and discard the supernatant
- Add and discard ethanol 2 twice
- Completely dry the sample after washing.
- Eluate dried beads in appropriate volume of LowTe
- Mix gently and leave for contact for 5 min
- Put on magnet for 3 min
- Collect the supernatant
- Dose amplicons on a Nanodrop and measure the purity of the extract
- Dilute it if needed before final dosage on a Fluorometer
  - Normalize all concentration at 0.2 ng/µl
Library Preparation Protocol of whole genome amplicons (Influenza A or B).
NEXTERA XT – Illumina

Version: DRAFT  
Date: 27/06/2019

DNA Tagmentation

- Vortex and spin all reagents.
- In each mix well:
  - Add 10 µl of Tagment DNA buffer
  - Add 5µL of amplicons (ADN 0.2ng/µL)
- Homogenize and seal the plate.
- Centrifuge at 280xG for 1 min at room temperature then transfer to a thermal cycler
- Thermocycler program:
  - 55°C for 5 min
  - 10°C ∞
- Add 5µL Neutralization buffer in each well
- Homogenize and seal the plate.
- Centrifuge at 280g for 1 min at room temperature then transfer to a thermal cycler
- Incubation for 5 min at room temperature

Amplification (library recovery PCR)

- Centrifuge i5 and i7 tubes before use
- Put i7 tubes in horizontal range and i5 in vertical
- In each well mix the tagmented sample with
  - 15µL of Nextera PCR Master Mix
  - 5µL of i5 index (white cap)
- Seal the plate
- Centrifuge at 280xG for 1 min at room temperature then transfer to a thermal cycler
- Thermocycler program:
  - 73°C for 3 min
  - 12 cycles of:
    - 95°C for 10 seconds
    - 55°C for 30 seconds
    - 72°C for 30 seconds
  - 72°C pour 5 minutes, then 10°C ∞
- STOPPING POINT ( 10°C ∞ thermos cycler or -4°C)

Library recovery PCR Clean up

- Vortex magnetic bead for 30 secs
- Prepare Ethanol 80%
- Centrifuge at 280xG for 1 min at room temperature then transfer to a thermal cycler
- Mix beads with tagmented DNA according to the following table:

<table>
<thead>
<tr>
<th>Amplicon maximum length</th>
<th>Beads</th>
<th>Volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;300bp</td>
<td>Not recommended</td>
<td>Not recommended</td>
</tr>
<tr>
<td>300-500 bp</td>
<td>1 X vol</td>
<td>90µL</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>&gt;500bp</td>
<td>0.6X vol</td>
<td>30µL</td>
</tr>
<tr>
<td>&gt;500bp (for Miseq)</td>
<td>0.5X (for Miseq) vol</td>
<td>25µL</td>
</tr>
</tbody>
</table>

- Vortex (1800 rpm for 2 min)
- Incubate for 5 min at room temperature
- Put on magnet for 3 min
- Discard the supernatant
- Add 200ul of 80% ethanol
- Put on the magnet and discard the supernatant
- Add and discard ethanol 2 other times
- Completely dry the sample after the final washing step (15 min).
- Eluate dried beads in 52.5 of ReSuspension Buffer
- Mix gently and contact for 2 min
- Put on magnet for 2 min
- Collect 50µL of the supernatant
- STOPPING POINT seal the plate and stock -25°C / -15°C for 1 week maximum

### Normalization

- Prepare 4 mL of 0.1N NaOH
- Prepare 4.4mL of LNA1 (for 96 samples) adding 800uL of LNB1.
- Homogeneise
- Add 45µL of LNA/B1 mix to each well
- Seal the plate
- Centrifuge at 1800 rpm for 30 minutes at room temperature then transfer to a thermal cycler
- Put on magnet for 2 min
- Discard the supernatant
- Add 45µL of LNW1 to each well
- Seal the plate
- Centrifuge at 1800 rpm for 5 minutes at room temperature then transfer to a thermal cycler
- Put on magnet for 2 min
- Discard the supernatant
- Add 45µL of LNW1 to each well (a 2nd time)
- Seal the plate
- Centrifuge at 1800 rpm for 5 minutes at room temperature then transfer to a thermal cycler
- Put on magnet for 2 min
- Discard the supernatant
- Add 30µL of NaOH 0.1N (for elution)
- Seal the plate
- Centrifuge at 1800 rpm for 5 minutes at room temperature then transfer to a thermal cycler
- Add 30µL of LNS1 in each well
- Put on magnet for 2 min
- Collect 30µL of supernatant
- Seal the plate
- Vortex 1000xG for 1 minute
- Pool 5µl of each well in a unique tube
- STOPPING POINT seal the plate and stock -25°C / -15°C for 1 week maximum
PROTOCOL 7.
Quantification of library preps (Kapa qPCR kit)

Version: DRAFT
Date: 27/06/2019

For the first use
- Mix 1 mL of 10X Primer Premix in 5 mL of 2X KAPA SYBR FAST qPCR Master Mix
- Vortex
- Stock in dark (-20°C)

Library dilutions
- Prepare 2 independent dilutions in duplicates, in low bind tubes
at 1/200:

<table>
<thead>
<tr>
<th>Final dilution</th>
<th>1/10</th>
<th>1/100</th>
<th>1/200</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR grade water</td>
<td>45 µl</td>
<td>45 µl</td>
<td>5 µl</td>
</tr>
<tr>
<td>5 µl final library</td>
<td>5 µl 1/10</td>
<td>5 µl 1/100</td>
<td></td>
</tr>
</tbody>
</table>

- at 1/500:

<table>
<thead>
<tr>
<th>Final dilution</th>
<th>1/10</th>
<th>1/100</th>
<th>1/500</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR grade water</td>
<td>45 µl</td>
<td>45 µl</td>
<td>20 µl</td>
</tr>
<tr>
<td>5 µl final library</td>
<td>5 µl 1/10</td>
<td>5 µl 1/100</td>
<td></td>
</tr>
</tbody>
</table>

Mix preparation

For 6 standards:
1 NTC
4 wells per dilution
12 wells for the standards

<table>
<thead>
<tr>
<th>1 tube (µL)</th>
<th>1 library (µL) = 18 wells</th>
<th>X library (µL); X = 13 wells + 4X</th>
</tr>
</thead>
<tbody>
<tr>
<td>X KAPA SYBR FAST qPCR Master Mix + 10X Primer Premix</td>
<td>6</td>
<td>108</td>
</tr>
<tr>
<td>H2O</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>144</td>
</tr>
</tbody>
</table>
For 6 standards:
1 NTC
4 wells per dilution
8 wells for the standard

<table>
<thead>
<tr>
<th></th>
<th>1 tube (µL)</th>
<th>1 library (µL) = 14 wells</th>
<th>X library (µL); X = 10 wells + 4X</th>
</tr>
</thead>
<tbody>
<tr>
<td>X KAPA SYBR FAST qPCR Master Mix + 10X Primer Premix</td>
<td>6</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>H2O</td>
<td>2</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>112</td>
<td></td>
</tr>
</tbody>
</table>

Run preparation

Plan

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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</tr>
<tr>
<td>B</td>
<td>S1</td>
<td>S2</td>
<td>S3</td>
<td>S4</td>
<td>S5</td>
<td>S6</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>C</td>
<td>S1</td>
<td>S2</td>
<td>S3</td>
<td>S4</td>
<td>S5</td>
<td>S6</td>
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<td></td>
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<td></td>
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<tr>
<td>D</td>
<td>1/-1/200</td>
<td>1-1/500</td>
<td>Blanc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>1/-1/200</td>
<td>1-1/500</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>F</td>
<td></td>
<td></td>
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<tr>
<td>G</td>
<td></td>
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<tr>
<td>H</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Add
  - 8µL de mix par well
  - 2µL per standard
  - 2µL per library dilution
  - 2µL per no template control
- Seal the plate
- Centrifuge

Mark at lower-

| DNA Standard 1 | 20 pM |
| DNA Standard 2 | 2 pM  |
| DNA Standard 3 | 0,2 pM|
| DNA Standard 4 | 0,02 pM|
| DNA Standard 5 | 0,002 pM|
| DNA Standard 6 | 0,0002 pM|
Run

- New experiment
- Open Templates and choose the appropriate template file

Program:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Duration</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95°C</td>
<td>5 min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95°C</td>
<td>30 sec</td>
<td>35</td>
</tr>
<tr>
<td>Hybridation/Elongation</td>
<td>60°C</td>
<td>45 sec</td>
<td></td>
</tr>
<tr>
<td>Melt curve analysis</td>
<td>95 - 65 - 95°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooling</td>
<td>40°C</td>
<td>10 min</td>
<td>1</td>
</tr>
</tbody>
</table>

Data analysis

- Report values on spreadsheet «model_input_KAPA.xls »
Run planning
- Connect NextSeq apparatus

Reagent Preparation
- Prepare 1mL of NaOH 0.2M
- De-freeze the cartridge with gentle agitation.
- Prepare the flowcell and dry it if needed

Librairies final denaturation and dilution
- In a Lowbind tube mix
  - 30µL of Pooled Librairies
  - 30µL of NaOH 0.2M
- Vortex and spin
- Incubate 5 min at room temperature
- Add 30µL of TrisHCL (200 mM, pH7)
- Vortex and spin
- Dilution of the denaturated library by adding Xul of HT1
- Vortex and spin
- Stock on ice

Denaturation and Dilution of PhiX control (if needed)
- Dilute PhiX to 4 nM : mix
  - 10uL of PhiX (10nM)
  - 15uL of ReSuspension Buffer
- Vortex and spin
DRIVE 777363 – D7.5

- Stock: 3 months between -25°C and -15°C

Denaturation of the mixture
- In a Lowbind tube mix
  - 5 µL of diluted PhiX
  - 5uL of NaOH 0.2M
- Vortex and spin
- Incubate for 5 minutes at room temperature
- Add 5µL of Tris HCL (0.2M, pH7.0)
- Vortex and spin

_Dilution of denatured PhiX for final mix_
- Add 985µL of HT1 (total volume: 1mL à 20 pM)
- Final dilution (1.8pM in 1.3mL)
  - 117µL of denaturated PhiX (20pM)
  - 1183µL of HT1
- Vortex and spin
- Stock: 2 weeks between -25°C and -15°C

Mix PhiX and Libraries
- In a Lowbind tube mix
  - 1287 µL of the final libraries
  - 13uL of the final PhiX
- Put on ice until sequencing

Cartridge loading
- Clean the n°10 lid
- Pierce the lid using a P1000 cone
- Transfer the whole Phix and Librairies dilution
Sequencing start

- Put on the new flow cell after discarding the used flow cell
- Put on loading then next

- Discard the used reagent and put the empty garbage cartridge

- Discard the used reagent cartridge and insert a new one
- Close and put Next
- Discard the sample reagent cartridge and insert a new one
- Select « load »
- Cartridge check by the system
- Click on next to launch the sequencing
- select « next »

Run monitoring

- Check on screen:
  - Run progress:
  - Q-score distribution
  - Intensity
  - Cluster densité (K/mm3)
  - Cluster passing filter (%)
  - Estimated Yield (Gb)
  - Flow cell image
Following is an example of a sequence data processing and bioinformatic analysis pipeline currently in place at FISABIO-Public Health. This pipeline is used here as a mere reference for a minimum protocol, but can be eventually changed before implementation after consultation/agreement with the Lyon site. The pipeline can be also modified to introduce improvements through the duration of the study.

Sequence processing, mapping and variant calling.

A pipeline mapping of R1 and R2 sequencing reads will be carried out by the bwa mem program (Li, et al., 2009; Li, et al., 2010), and recalibrated using the Picard program https://broadinstitute.github.io/picard/ to generate bam files.

Variants against reference influenza genomes (i.e. from those viral Straits included in the seasonal vaccine) will be called using the freebayes program (Garrison et al., 2009), to generate variant calling tables including frequencies, using following parameters:

**Raw variants** files: SampleName/var/SampleName.freebayes.raw.vcf
- F 0.2 = minimum alternate fraction 20%
- C 2 = minimum alternate count 2
- m 0 = minimum mapping quality 0
- q 0 = minimum base quality 0

**Filtered variants** [-F 0.2 -C 5 -m 30 -q 20] files: SampleName/var/SampleName.freebayes.vcf
- F 0.2 = minimum alternate fraction 20%
- C 5 = minimum alternate count 5
- m 30 = minimum mapping quality 30
- q 20 = minimum base quality 20

Annotation of SNPs/InDels against reference influenza genomes (i.e. from those viral Straits included in the seasonal vaccine) will be carried out using the snpEff program (Cingolani, et al., 2012) to produce annotated files.

References


DRIVE 2019 Annual Forum
Virological characterization, WGS pilot

Task leaders:
F. Xavier López-Labrador
FIDABIO – Public Health, Generalitat Valenciana, Valencia, Spain
CIBER in Epidemiology and Public Health, Instituto de Salud Carlos III, Spain
Bruno Lina
GMR des virus influenza, CHU Hospices Civils de Lyon
Virpath, CIRI, U1111, UMR 5308, ENS, UCBLL, Lyon, France

Influenza viruses
Influenza epidemiology
- Viruses are circulating worldwide
- Evolving constantly
- Type A viruses multiple hosts: human, flock, swine: complex evolution
- The epidemiology is different in the different regions of the world
- The impact is correlated to:
  - the type and subtype
  - the escape to pre-existing immunity
- Disease burden is often underestimated, but much better monitored these last years
- The diagnostic tools are powerful
Protection is mediated by neutralizing antibodies

Leading to antigenic drift: immune escape
As a consequence:

1 - permanent need for change in the influenza vaccine

As a consequence:

2 – possible altered influenza vaccine efficacy
Why sequencing influenza is so important?

Complexity of A/H3N2: HA characterisation

WHO & Cambridge University

Why sequencing influenza is so important?

Evolutionary change ("Antigenic drift") of Human Influenza H3N2:
1968 - 2002

Reference: Fig. 1 A
Koelle et al. Science
22 December 2009.
Whole genome sequencing (WGS) is becoming the new standard in Public Health Microbiology

Reduced accumulation of defective viral genomes contributes to severe outcome in influenza virus infected patients

WHO Scoping paper, 2017

Implementation of Decision WHA70(10)8(b)
Scoping Paper on approaches to seasonal influenza and genetic sequence data under the PIP Framework (“Scoping paper”)

13. In recent years significant progress has been made in sequencing technologies (e.g. next generation sequencing and whole genome sequencing). Some new technologies use GSD rather than physical virus materials in the development of vaccines and other pandemic influenza-related products (“synthetic genomics technologies”).

14. New technologies allow GSD derived from all influenza viruses to be used for an expanding range of purposes. Forward-looking approaches to seasonal influenza vaccines and to GSD in the context of the PIP Framework will thus need to consider the implications of scientific and technological advances that are in progress and those that can be reasonably anticipated.
NGS, a way to determine the consensus sequence and the genetic diversity by WGS directly from clinical specimens

Depth of WGS for the 8 gene segments
WGS allows detecting mutations in internal genes

Rapid spread of influenza A(H1N1)pdm09 viruses with a new set of specific mutations in the internal genes in the beginning of 2015/2016 epidemic season in Moscow and Saint Petersburg (Russian Federation)

Andrey Komissarov, Artem Fabres, Maria Sereginova, Sergey Petros, Yevgeniy Simtsov, Anna Egorova, Maria Plesnova, Elena Ulybysheva, Tamara Musaeva, Darya Davydova, Nadzheda Kuznetsova, Natalia Petrova, Kirill Stypachenko, Elizaveta Severinova, Ekaterina Burpyakova, Kirill Krasnokobedov, Anna Kirillova, Lyudmila Karpenko, Mikhail Grudinin

WGS allows detecting intra-subtype reassortment

Integrating patient and whole-genome sequencing data to provide insights into the epidemiology of seasonal influenza A (H3N2) viruses

Emily J. Robertson, William T. Harvey, Gavin S. Wilks, Samantha J. Stephens, Alexander R. MacLean, Pablo R. Murcia, and Rory N. Gunson

- WGS sequencing identifies intra-subtype reassortants now unadverted by Sanger HA-NA sequencing
- Reassortant genomes may be associated with severe outcomes, antigenic drift
WGS allows detecting intra-subtype reassortment

WGS allows detecting minority antigenic variants

- Influenza viruses have RNA genomes: high mutation rates
- NGS deep sequencing can sample the viral population within each individual.
- Minority variants can be detected prior to the emergence of sustained substitutions later associated to antigenic drift
Implementation of a pilot Influenza WGS study in Lyon, France

Implementation of a pilot Influenza WGS study in Valencia, Spain
Implementation of a pilot Influenza WGS study in Valencia, Spain
From the past 2017-18 season: WGS analyses for identifying strain variation and evolution

DRIVE Influenza WGS pilot

- WP7, D7.5: “Report on feasibility study for influenza virus characterization, molecular epidemiology studies related to influenza related disease in vaccinated and unvaccinated subjects”
- Run on already collected samples from the 2018/19 season
- Using the collected specimens from the study sites we can detect:
  - Substitutions associated to vaccine failure (antigenic drift)
  - Trend of substitutions associated to genetic evolution (clades/subclades)
  - Minority variants detected prior to the emergence of sustained substitutions associated to antigenic drift
- Use pilot to evaluate feasibility for timely feeding GISAID database for WHO
- No funding available, UCBL-Lyon has funding for aprox. 100 samples
- FISABIO-Public Health has funding for 100 additional samples
- Option for sites with ongoing NGS assays to share sequencing data
DRIVE Influenza WGS pilot
Sample sharing
Annex 1
Standard Material Transfer Agreement 1 (SMTA 1)
Standard Material Transfer Agreement within the WHO global influenza surveillance and response system (GISRS)

Shipping: Whatman 903 paper cards
- Used widely for newborn blood collection, HIV, HBV, HCV testing
- Can be used for DNA, RNA storage, transportation at RT, now testing vRNA
- CE-marked, IVD
- Includes slip for sample information
- Inexpensive, easy shipping, many can be placed in an envelope, regular mail.

NGS data sharing: secured ftp site

DRIVE Influenza WGS pilot
Timelines for interim and final reports