# EVALUATION OF GENOMERA® CDX SYSTEM FOR INFLUENZA AND RSV INFECTIONS

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## BACKGROUND

### EPIDEMIOLOGY OF RESPIRATORY VIRUSES

- Influenza A (FluA), influenza B (FluB) and respiratory syncytial viruses (RSV) are viral pathogens, transmitted by aerosols. These viruses cause respiratory diseases in humans of all ages, and are responsible for burden worldwide[1].
- These viruses commonly have overlapping clinical symptoms which complicates the differentiation between these infections[2].
- Rapid immunoassays and antigen tests have poor sensitivity. Sensitive molecular methods are required for reliable diagnosis[3,4].

### GENOMERA CDX™ SYSTEM

- GenomEra® CDX is a molecular diagnostics platform consisting in an integrated thermal cycler and a time-resolved fluorometer [5].
- The Instrument is used to run analyte-specific, ready-to-use GenomEra® Test Chips. Each chip contains a sample processing control (SPC) which is included in the sample preparation tube.
- The assay sequence lasts approximately 70 minutes and ends with a report of the results.

## OBJECTIVES

**Evaluation of the GenomEra® CDX system performances using the GenomEra FluA/B+RSV assay (FluA/B, or RSV-A/-B) on clinical respiratory specimens**

## METHODS

**Clinical respiratory samples**

- 114 nasal swabs (NS)
- 131 nasal -throat swabs (NTS)
- 54 nasopharyngeal aspirates (NPA)



**Genomera ® CDX System**

Thermal lysis – RT-qPCR (FluA, FluB, RSV-A, and RSV-B)

**Reference Method**

Automatic extraction (Nimbus®) RT-qPCR

**Seegene Allplex® Respiratory panel 1** on CFX96® (BioRad) (FluA, FluB, RSV-A and RSV-B)

**For discrepant results** (BioMérieux, France)

- Automatic Extraction on Easymag®
- RT-qPCR **Influenza A/B and RSV R-Gene®** kits

## RESULTS

### Clinical performances study

| Viral Target | True positive | False positive | True negative | False negative | Sensitivity % (CI95) | Specificty % (CI95) | PPV % (CI95) | NPV % (CI 95) | Accuracy % (95% CI) |
|---|---|---|---|---|---|---|---|---|---|
| **FluA** (n=300*) | 63 | 1 | 234 | 1 | 98.4 (91.6-100) | 99.6 (97.7-100) | 98.4 (89.9-99.8) | 99.6 (97.1-99.9) | **99.3 (97.6-99.9)** |
| **FluB** (n=300**) | 0 | 1 | 299 | 0 | - | 99.7 (98.2-100) | - | 100 | **99.7 (98,2-100)** |
| **RSV-A** (n=300***) | 27 | 0 | 270 | 1 | 96.4 (81.7-99.9) | 100 (98.6-100) | 100 | 99.6 (97.5-100) | **99.7 (98.1-100)** |
| **RSV-B** (n=300***) | 42 | 1 | 252 | 1 | 97.7 (87.7-99.9) | 99.6 (97.8-100) | 97.7 (85.6-99.7) | 99.6 (97.3-99.9) | **99.3 (97.6-99.9)** |

**Figure 1. Evaluation of clinical performances of GenomEra FluA/B+RSV assay.** PPV : positive predictive value. NPV: negative predictive value. For FluA, one sample out of the 300 tested samples remained inconclusive after verification(*). For FluB, one sample out of the 300 tested showed FluB borderline result, even after verification (**). For RSV, two samples out of the 300 tested showed inconclusive results for RSVA and four for RSVB (***).

### Inclusivity study.

**Figure 2. Determination of the LoD in clinical specimens.**

| Virus | Strains | Ct Concentration (Seegene) | 200-400 X Concentration | 20-40 X Concentration | 2-4 X Concentration | 0.2-0.4 X Concentration | 0.02-0.04 X Concentration | 0.002-0.004 X Concentration | 0.0002-0.0004 X Concentration |
|---|---|---|---|---|---|---|---|---|---|
| Flu B | B/Malaysia/2506/2004 (Victoria) | 34,5 | | | | | | | |
| | B/Malaysia/2506/2004 (Victoria) | | | | | | | | |
| | B/Wisconsin/1/2013 | | | | | | | | |
| | B/Brisbane/60/2008 | | | | | | | | |
| | B/Florida/4/2006 | | | | | | | | |
| Flu A H1 | A/H1N1 - A/Solomon Islands/3/2006 | 37,5 | | | | | | | |
| | A/H1N1 - A/New Caledonia/20/99 | | | | | | | | |
| | A/H1N1 - A/California/7/2009 | | | | | | | | |
| Flu A H3 | A/H3N2 - A/Hong Kong/4801/2014 | 37,5 | | | | | | | |
| | A/H3N2 - A/Victoria/210/2009 | | | | | | | | |
| | A/H3N2 - A/Brisbane/10/2007 | | | | | | | | |
| RSV-A | Sample 1 | 25 | | | | | | | |
| | Sample 2 | | | | | | | | |
| | Sample 3 | | | | | | | | |
| | Sample 4 | | | | | | | | |
| | Sample 5 | | | | | | | | |
| RSV-B | Sample 1 | 24,5 | | | | | | | |
| | Sample 2 | | | | | | | | |
| | Sample 3 | | | | | | | | |
| | Sample 4 | | | | | | | | |
| | Sample 5 | | | | | | | | |

Perfect detection of the target viral strain was found for a Ct value of:
i/ 34.6 (33.3-33.5) for FluA $H_1N_{1pdm09}$
ii/ 34.9 (34.4 - 35.4) for FluA $H_3N_2$
iii/ 34.0 (32.3-34.9) for FluB
iv/ 31.4 (29.2-33.8) for RSV-A
v/ 28.8 (27.3-30.2) for RSV-B

➢ **According to Amplirun RNA concentrations, LoD values (indicated with a red bar)corresponded respectively to: 0.1 copies/µl (FluAH1) ; 10 copies/µl (FluAH3); 100 copies/µl (RSV-A) and 1,000 copies/µl (FluB and RSV-B)**

### Cross-reactivity study

**Figure 3. Determination of the analytical specifity with other pathogens.** Ct/C°: Ct value or microorganism concentration (McF=MacFarland)

| Microorganism | Sample | Ct/C° | FluA | FluB | RSV A | RSV B |
|---|---|---|---|---|---|---|
| *Mycoplasma pneumoniae* | Clinical | 26,8 | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Corynebacterium jeikeum* | Culture | 1 McF | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Escherichia coli* | Culture | 1 McF | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Haemophilus influenzae* | Culture | 1 McF | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Lactobacillus sp.* | Culture | 1 McF | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Moraxella catarrhalis* | Culture | 1 McF | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Pseudomonas aeruginosa* | Culture | 1 McF | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Staphylococcus aureus* | Culture | 1 McF | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Staphylococcus epidermidis* | Culture | 1 McF | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Streptococcus pneumoniae* | Culture | 1 McF | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Streptococcus pyogenes* | Culture | 1 McF | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Mycobacterium tuberculosis* | Extract | / | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| *Chlamydia pneumoniae* | Clinical | 32,3 | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |

| Microorganism | Sample | Ct/C° | Flu A | Flu B | RSV A | RSV B |
|---|---|---|---|---|---|---|
| Rhinovirus | Clinical | 21,2 | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| Coronavirus OC43 | Clinical | 16,4 | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| Coronavirus 229E | Clinical | 19,3 | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| Métapneumovirus | Clinical | 20,5 | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| Parainfluenzae 3 | Clinical | 20,2 | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| Entérovirus | Clinical | 24,5 | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| Adénovirus | Clinical | 17,2 | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |
| Parainfluenzae 1 | Clinical | 26,1 | NEG | NEG | NEG | NEG |
| | | | NEG | NEG | NEG | NEG |

- Cross reactivity was evaluated on highly concentrated culture (bacterial strain) or clinical samples (viral strains),
- Fourteen bacteria were tested (incl. *Mycobacterium tuberculosis* after lysis).
- Eight viral strains were tested in clinical samples with high Ct values (16.4 to 26.0).
- **No cross-reactivity could be observed on duplicates for all viral targets.**

## INTERPRETATION AND CONCLUSION

### A very easy-to-use, fast and powerful technology that could be applied to clinical complicated situations

- With 1 false negative and 5 false positive (including one IBV) results, performances for Influenza detection are very good but remain perfectible
  - Modification of the threshold to optimize results : no False positive results
  - **Perfect specificity and almost perfect sensitivity (Accuracy : 99.3% and 99,7% for IAV and IBV respectively)**
- With 1 false negative result, performances for RSV detection are comparable to our reference method :
  - near perfect **specificity and sensitivity (Accuracy : 99.7% and 99.3% for RSV-A and RSV-B respectively)**

### A multicenter study has to be performed to validate comparison with different analytical platforms

- **Work-in-progress**
  (collaboration between CHU Poitiers, France;
  Pärnu Hospital, Estonia;
  FIMLAB, Jyväskylä, Finland; Vaasa Central Hospital, Finland;
  Turku University Hospital, Finland)

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