# Quality Control Rapid Response in Pre-Pandemic Preparedness Showcasing a Synthetic H5N1 Genetic Template

A. Alagic [1], T. Gerbaba [1], Q. Aguda[1], P. Zhelev [1], S. Rivers [1], M. Luscher [1]

[1] Microbix, Mississauga, Ontario, CANADA

ADLM Association for Diagnostics & Laboratory Medicine B-206

## BACKGROUND

The SARS pandemic highlighted a critical challenge: limited access to well-characterized patient samples, which restricted the ability to cross-validate diagnostic assays. Furthermore, the lack of External Quality Assessment (EQA) programs prevented laboratories from benchmarking their performance against peers. Recent cases of H5N1 transmission to humans underscore the urgent need for rapid response capabilities in pandemic preparedness. To support this, Microbix utilized synthetic biology to develop a full-process, whole-genome H5N1 cDNA control. This control is designed for use in assay design, verification, validation, and continuous Quality Control (QC)

## METHODS

Microbix used a synthetic biology approach to design and develop a whole genome H5N1 cDNA control based on published H5N1 isolate sequences. This whole genome H5N1 cDNA control was confirmed by Nanopore sequencing, quantified by digital PCR, and formulated on swabs and in liquid format. Both formats were confirmed by testing inhouse on the Cepheid Gene Xpert® SARS/Flu/RSV assay, and additionally the liquid formats were provided for an EQA pilot.

## RESULTS

### SEQUENCE ANALYSIS of MICROBIX"S H5N1 CONTROL

| ISOLATE | A | MBX | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|---|
| A | | 99% | 96% | 96% | 97% | 98% | 97% | 96% | 94% | 96% |
| MBX | 99% | | 96% | 96% | 97% | 97% | 97% | 96% | 94% | 96% |
| B | 96% | 96% | | 97% | 97% | 97% | 94% | 93% | 91% | 93% |
| C | 96% | 96% | 97% | | 97% | 97% | 94% | 93% | 91% | 93% |
| D | 97% | 97% | 97% | 97% | | 97% | 94% | 94% | 92% | 93% |
| E | 98% | 97% | 97% | 97% | 97% | | 96% | 95% | 93% | 95% |
| F | 97% | 97% | 94% | 94% | 94% | 96% | | 93% | 91% | 93% |
| G | 96% | 96% | 93% | 93% | 94% | 95% | 93% | | 93% | 95% |
| H | 94% | 94% | 91% | 91% | 92% | 93% | 91% | 93% | | 94% |
| I | 96% | 96% | 93% | 93% | 93% | 95% | 93% | 95% | 94% | |

Figure 1. Microbix's H5N1 control sequence similarity to representative H5N1 clinical isolates.

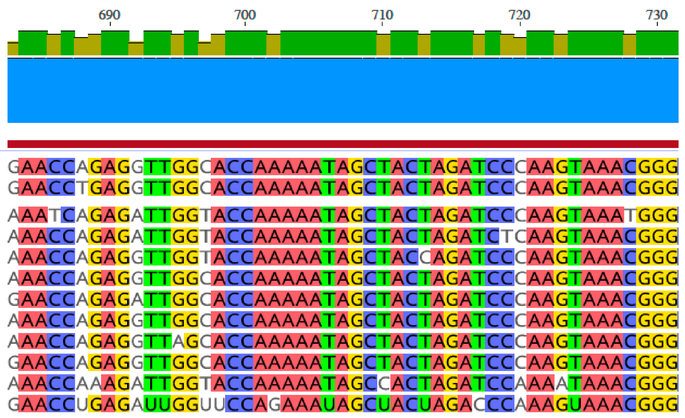


Figure 2. Alignment of Microbix's H5N1 control sequence with representative H5N1 clinical strains

### CONTROL QUANTITATION

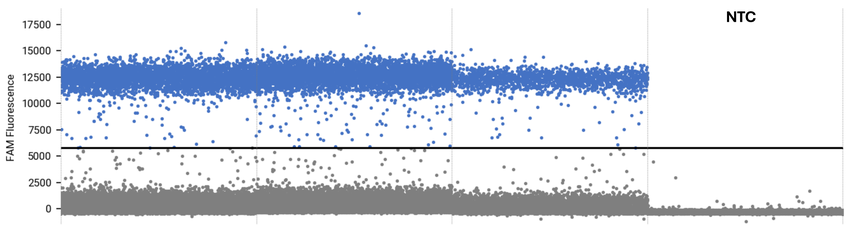


Figure 3. Quantification by dPCR of Microbix's H5N1 SWAB control (elution with nuclease-free water)

.

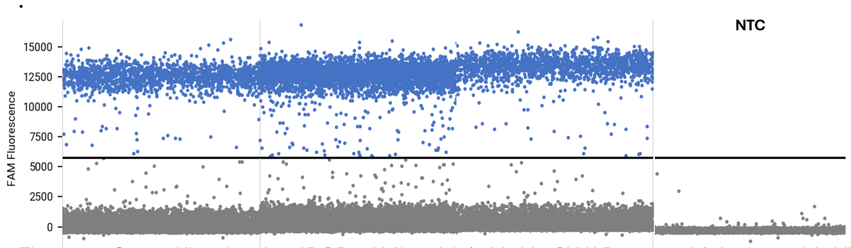


Figure 4. Quantification by dPCR of Microbix's H5N1 SWAB control (elution with UTM).

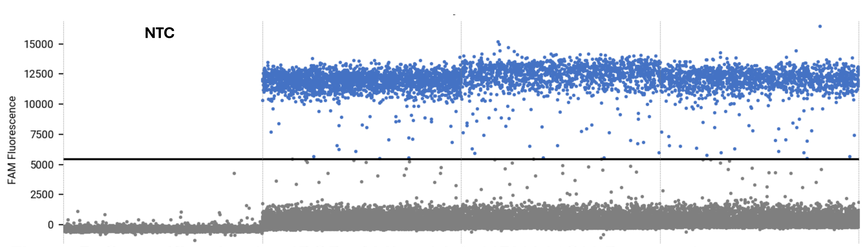


Figure 5. Quantification by dPCR of Microbix's H5N1 LIQUID control.

**SWAB CONTROL CONCENTRATION**

**1 x $10^6$ COPIES/ML**

**LIQUID CONTROL CONCENTRATION**

**6 x $10^5$ COPIES/ML**

### INTERNAL VALIDATION

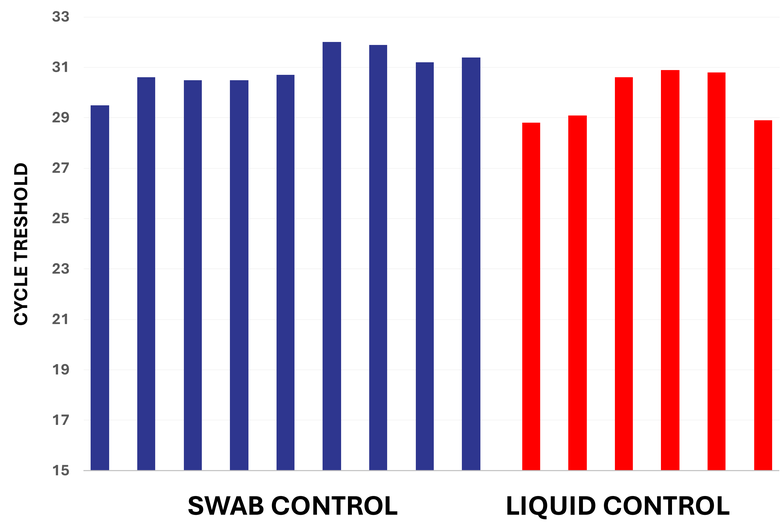


Figure 6. In-house verification testing results on Cepheid Xpert CoV-2/FLU/RSV Plus assay

### EXTERNAL VALIDATION: EQA (PT) PILOT

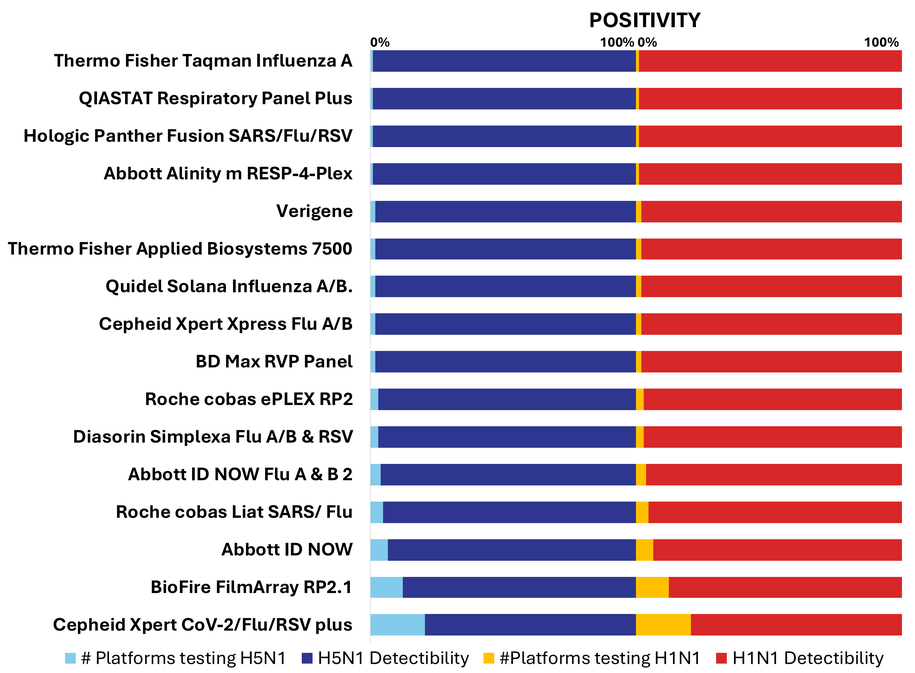


Figure 7. Reactivity of Microbix's H5N1 in EQA pilot with labs using different diagnostic assays reporting positivity for Microbix's H5N1 and H1N1 controls

## CONCLUSIONS

1. Full genome, quantified H5N1 cDNA control was developed by synthetic biology approach
2. Control tested in 75 labs, using 16 different qPCR assays
3. Can be used for typing assay development, assay verification/validation and EQA schemes
4. Control. Is non-infectious, stable and available in swab and liquid format
5. Microbix's Platform approach can be utilized in pandemic and health emergencies as a rapid response to provide reference materials

## AKNOWLEDGMENTS

We acknowledge the following EQA partners for providing the data used in this poster.

American Proficiency Institute

Please see related API's poster A-268 titled "Evaluation of NAAT Recovery of Highly Pathogenic Avian Influenza A (H5N1) Clade 2.3.4.4b from Novel Proficiency Samples".

## CONTACT

Pavel Zhelev, Director, Product Management
Email: pavel.zhelev@microbix.com

MICROBIX

Download poster by scanning this QR code