Aurora
ILLUMINATING SOLUTIONS

# Validation Report: VERSA Mini PCR Workstation
## Reverse Transcription of Avian Flu RNA and Amplification of cDNA & Detection of H5N1

## I. Objectives

1. To ensure stability of RNA (highly thermolabile and degradatively sensitive) during liquid-handling procedures
2. To automate reverse transcription of H5N1 viral RNA (positive control)
3. To check automated distribution of three separate pre-made master mixes
4. To allow amplification of determinants from three different genes of viral RNA
5. To determine the handling of very sensitive nucleic acid like RNA
6. To check for cross contamination or false positive/negative amplicons resulting from automated operation (if any)

## II. Materials & Methods

The automation of the H5N1 kit supplied by Mediagnost, Reutlingen, Germany) was set up as follows:

1. Tips (1000 µL, and 20 µL) were placed at deck position 8 and 1, respectively. Tip changer sequences were performed to change tip for each reagent and DNA sample.
2. The vials of the lyophilized reaction mixes Rx-Mix A (for identification of A-type of the influenza virus), Rx-Mix H5 (for identification of hemagglutinin gene HA 5), and Rx-Mix N1 (for identification of neurminidase gene NA 1) provided in the kit were placed in the appropriate slots on the cooling block at 4C.
3. Reverse transcriptase, *taq* DNA polymerase, sterile nuclease free water, viral RNA, and negative controls were incubated in appropriate slots of the cooling block.
4. The viral RNA sample and the negative controls were placed at deck position 2.
5. A 96-well PCR plate was placed at deck position 5.
6. The protocol sequences were carried with VERSA Mini PCR Workstation.
7. The Rx-Mixes were reconstituted from lyophilized reaction mixes by adding 470 µL of nuclease-free water to each of the Rx-Mix vials with the workstation and mixed with pipetting action of the workstation.
8. 100 µL Aliquots of each reaction mix were transferred into vials placed on the cooling block for preparation of the working master mixes.

9. To each of the above-said vials containing aliquoted Rx Mix, reverse transcriptase (2 µL), and *taq*-polymerase (0.8 µL) were added.
10. To set up 25 uL reactions, 22.5 µL from Rx-Mix A was distributed by the workstation into wells of the PCR plate designated in the automation sequence. Similarly, the same volume from Rx-Mix H5, and Rx-Mix N1 was distributed.
11. Viral RNA (2.5 µL) was then added to the appropriate wells of the above-said PCR plate containing the reaction mixes and enzymes. Similarly, the same volume of the negative controls was also added to the respective wells of the PCR plate. Pipetting action was used for mixing.
12. The entire automated operation was carried inside the HEPA Hood to avoid air-borne contamination.
13. Reverse transcription and amplification were carried on MyCycler (BioRad Labs) as shown in Table 1.
14. The amplified product was detected with ethidium bromide on agarose gel (3%).

**Table 1**: Conditions for reveres transcription and cDNA amplifications

| **Step** | **Sub-step** | **Temperature ℃** | **Duration Min** |
|---|---|---|---|
| Reverse Transcription | | 42 | 60 |
| Initial Denaturation | | 94 | 3 |
| Cycling 35X | | | |
| | Denaturation | 94 | 1 |
| | Annealing | 55 | 1 |
| | Extension | 72 | 1 |
| Final elongation | | 72 | 5 |

## III. **Results**

The results were interpreted from the location of the bands of respective amplicon in the agarose gel. The presence of 107, 191, and 192 bp bands from the respective reaction mixes indicated the successful reverse transcription of the viral RNA into cDNA (Figure 1).

The presence of amplicons also indicated the successful amplification from the respective genes of the influenza virus thus confirming the presence of its particular traits (genes) for determining its identity. The negative controls did not show any band that also indicated that no cross contamination had occurred in the automated process.

The figures 2, 3, and 4 available on the brochure of the kit vendor is also presented for comparison.

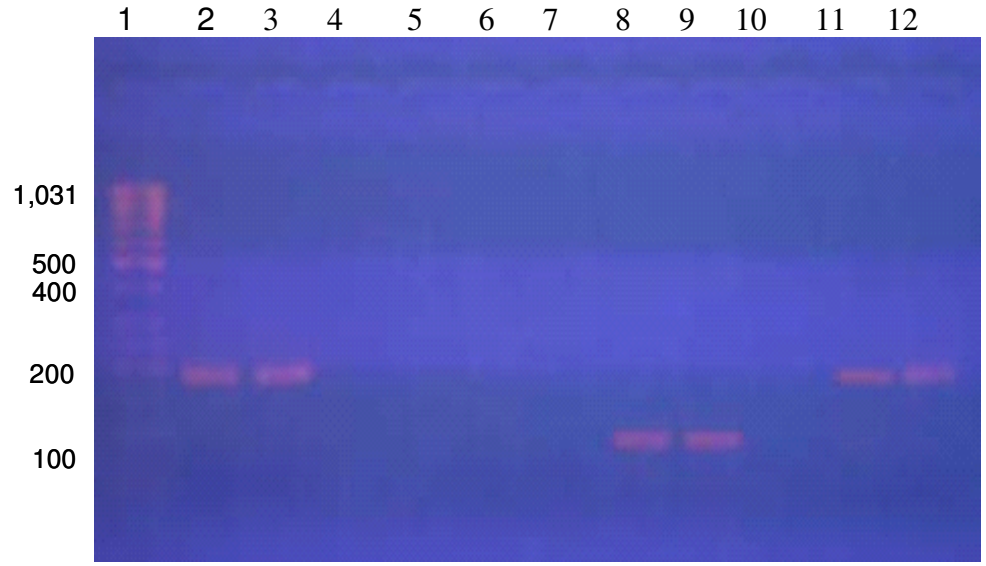


**Figure 1:** Agarose (3%) Gel, lane # 1 (DNA Ladder, 50bp-1 kb), lane 2-3 (Influenza type A amplicon 191bp), lane 4-5 (Negative control for A), lane 6-7 (Negative control for N1), lane 8-9 (N1 amplicon, 107bp), lane 10 (Negative control for H5), and lane 11-12 (H5 amplicon, 192bp).

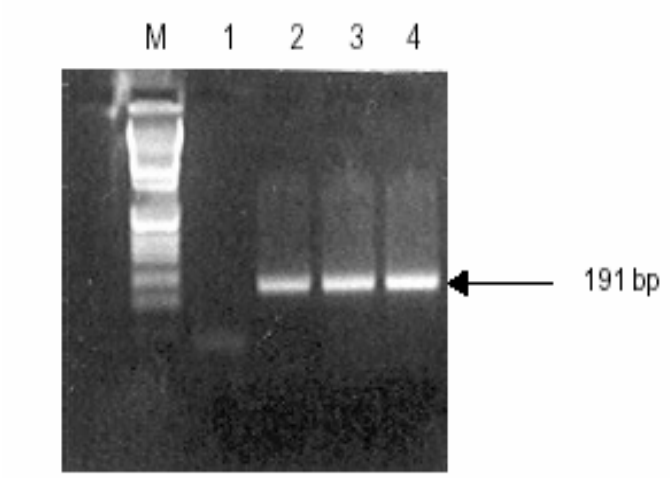


**Figure 2**: Reverse transcription and amplification with Rx-Mix A (presented by the kit vendor). Lane M (DNA standard), 1 (Negative control, $dH_2O$), 2 (Influenza A PR/H1N1), 3 (Patient serum), and 4 (Influenza A /H5N1).

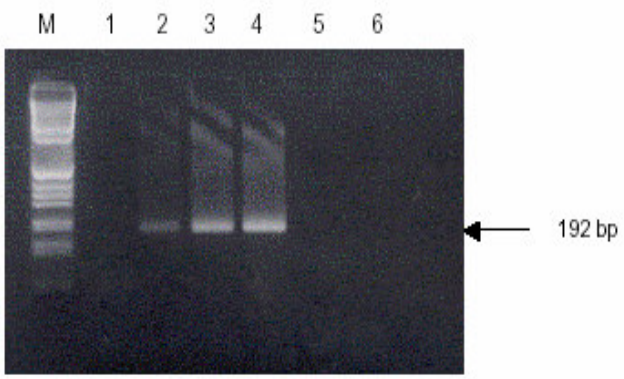


**Figure 3**: Reverse transcription and amplification with Rx-Mix N5 (presented by the kit vendor). Lane M (DNA standard), 1 (Negative control, $dH_2O$), 2 (Influenza A /H5N1, $10^{-2}$), 3 (Influenza A /H5N1, $10^{-1}$), 4 (Influenza A /H5N1), 5 (Influenza A PR /H1N1) , and 6 (Patient serum).

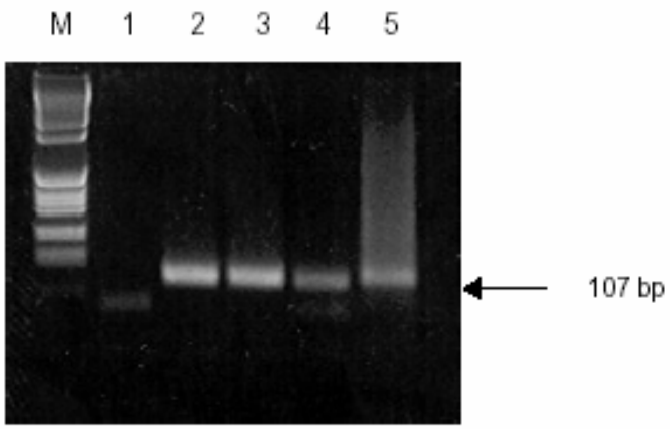


**Figure 4**: Reverse transcription and amplification with Rx-Mix N1 (presented by the kit vendor). Lane M (DNA standard),1 (Negative control, $dH_2O$), 2 (Influenza A PR /H1N1), 3 (Influenza A /H5N1), 4 (Influenza A /H5N1, $10^{-1}$), 5 (Serum patient)

## IV. **Conclusion**

1. Processes automated
   a. Reverse transcription of positive control avian flu RNA (supplied in the kit) to synthesize cDNA.
   b. PCR carried on cDNA for influenza A type, H5, and N1 amplicons using specific mastermixes supplied in the kit.
2. The results were consistent and clean, indicating accurate and precise volume delivery of samples.
3. No false positive or negative results indicate no cross contamination of the samples.

## V. **Reference**

Product manual: H5N1 Detection Kit for the detection of Influenza A/H5N1. Mediagnost, Germany.

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