# Development of rapid diagnostic test kit for pandemic H1N1 2009

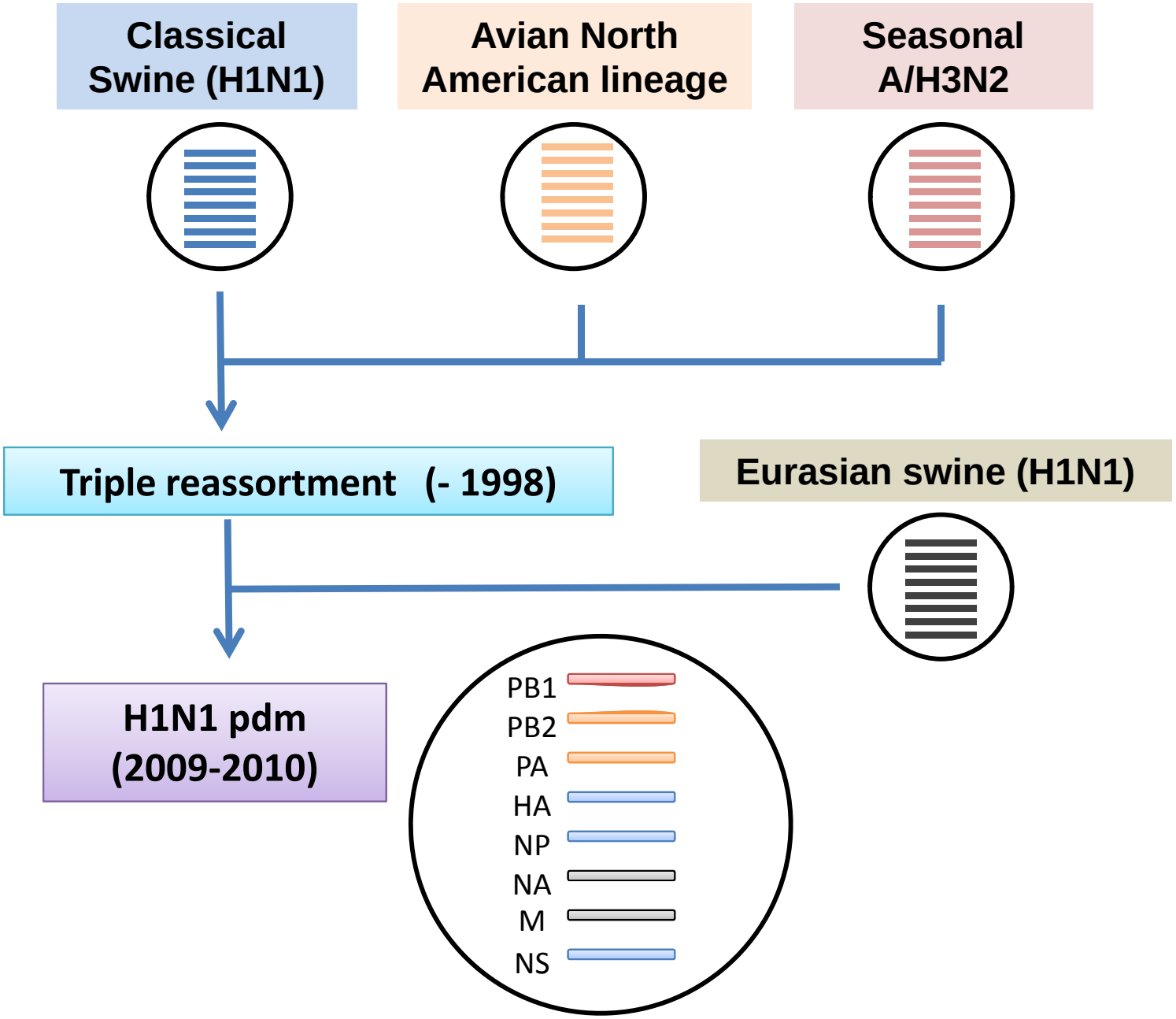
Tadahiro SASAKI

- Department of Virology, Research Institute for Microbial Diseases, Osaka University
- JICA Short Term Expert

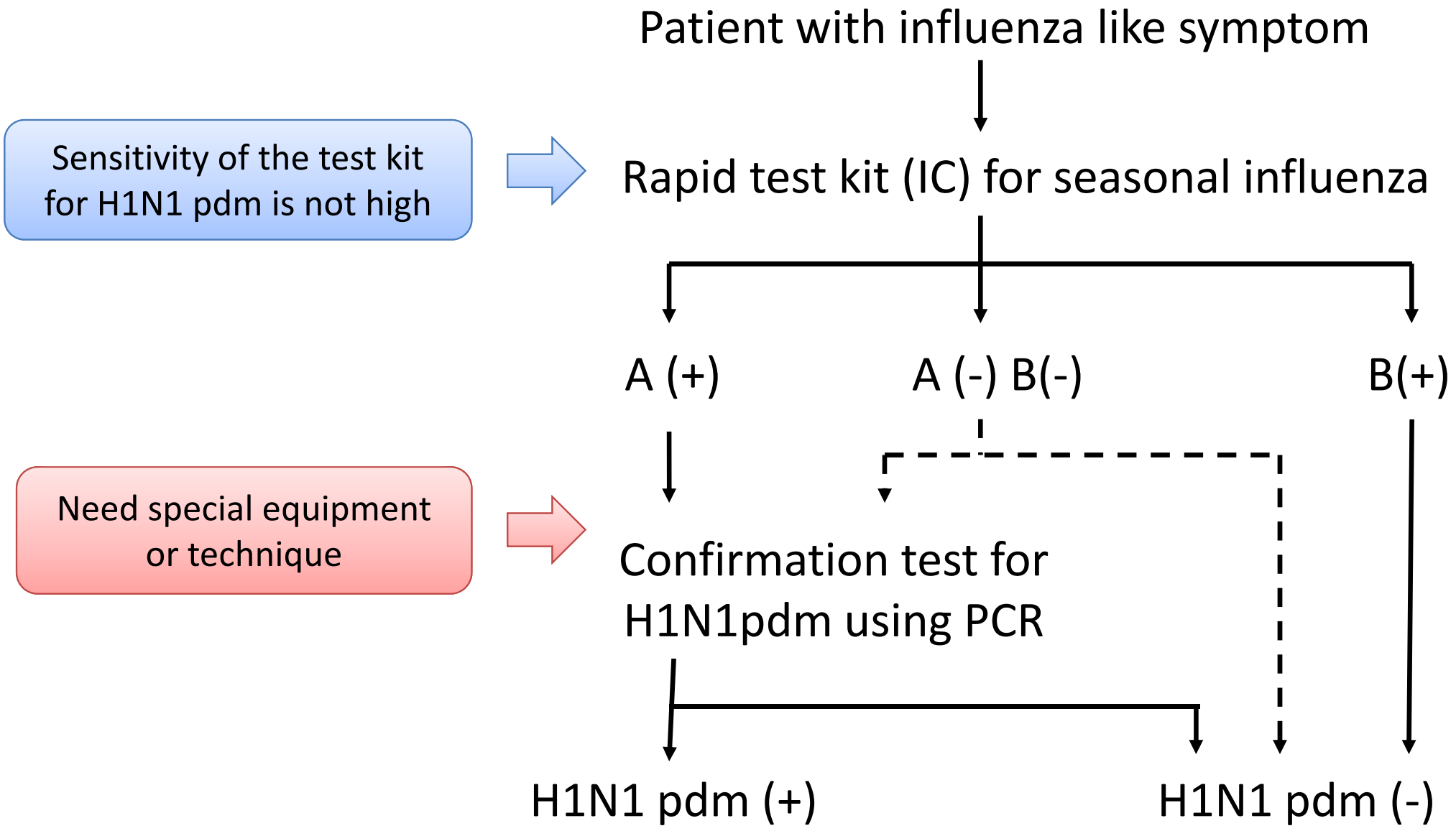
# Influenza A H1N1 2009 pdm (H1N1 pdm)

- First identified at Mexico in April 2009
- WHO declared the pandemic in June 2009
- Confirmed death cases are over 18,097 in the world until May, 2010 (WHO)
- WHO declared H1N1 pdm has moved into post-pandemic period in Aug, 2010

# Characteristics of H1N1 pdm



# Diagnosis algorism of H1N1 pdm

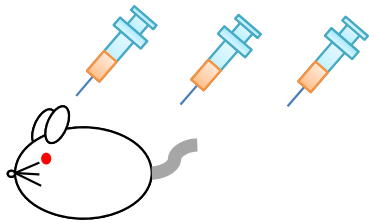


# Aim

- Development of new mouse monoclonal antibodies that can react with H1N1 pdm, but not seasonal influenza A (H1N1 or H3N2) or B viruses.
- Assembly and evaluation of rapid test kit (Immunochromatography kit) for H1N1 pdm detection using the antibodies.

# Outline of the study

**Immunization for mouse with H1N1 pdm (3 times)**



BALB/c (4w, ♀)

**Fusion of splenocyte with mouse myeloma cells (PAI) using polyethyleneglycol1,500**

**Screening for H1N1 pdm or seasonal H1N1 by IFA**

**Selet the hybridoma that react with H1N1 pdm and not react seasonal H1N1**

**Cloning by limiting dilution method**

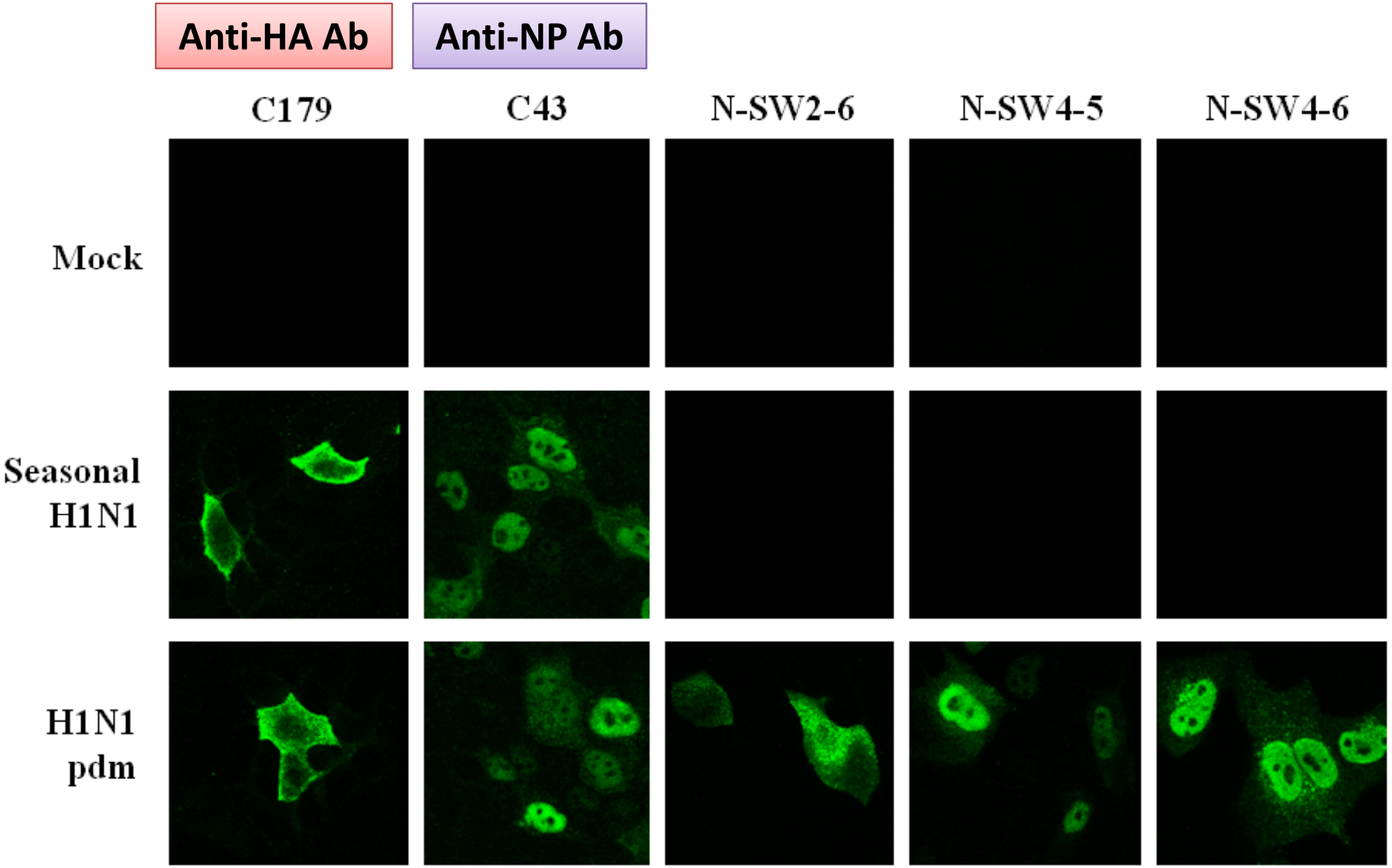
**2nd screening by IFA**

**Hybridoma scale-up**

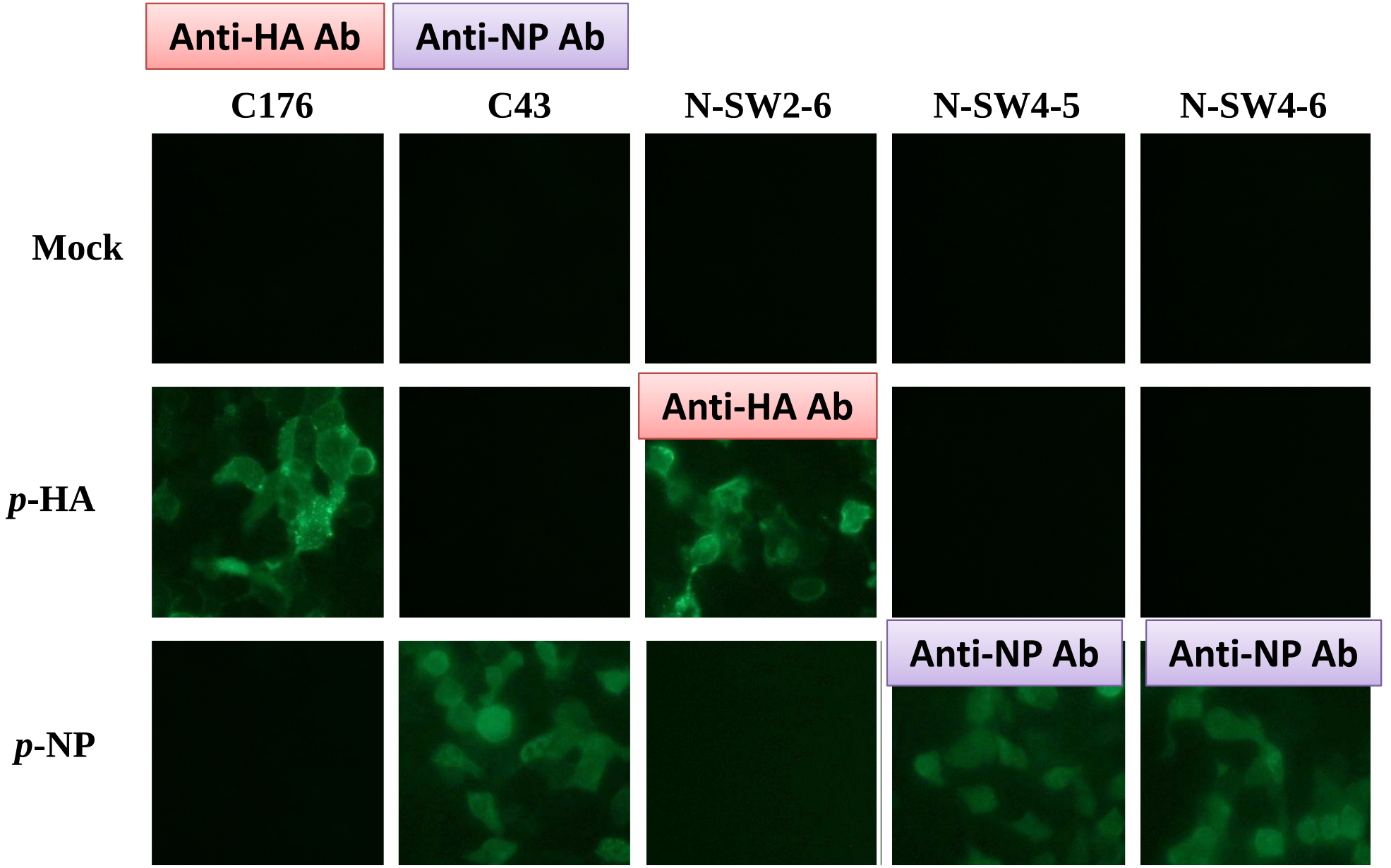
**Characterization of the antibodies**

**Assemble and evaluate rapid test kit using the antibodies**

# H1N1 pdm specific MAbs reactivity



# Target of H1N1 pdm specific MAbs

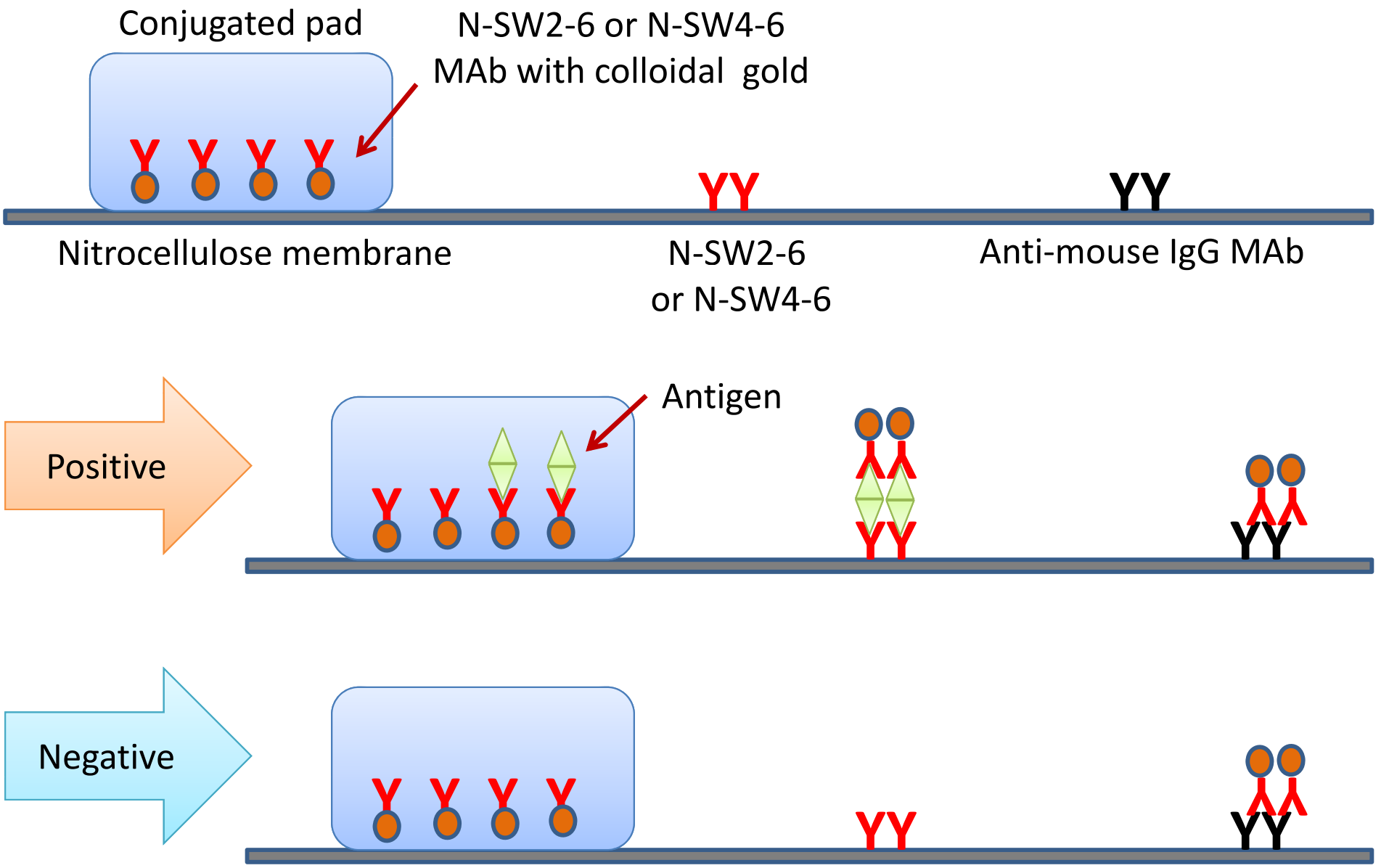


# Isotyping and assembly of rapid test kit

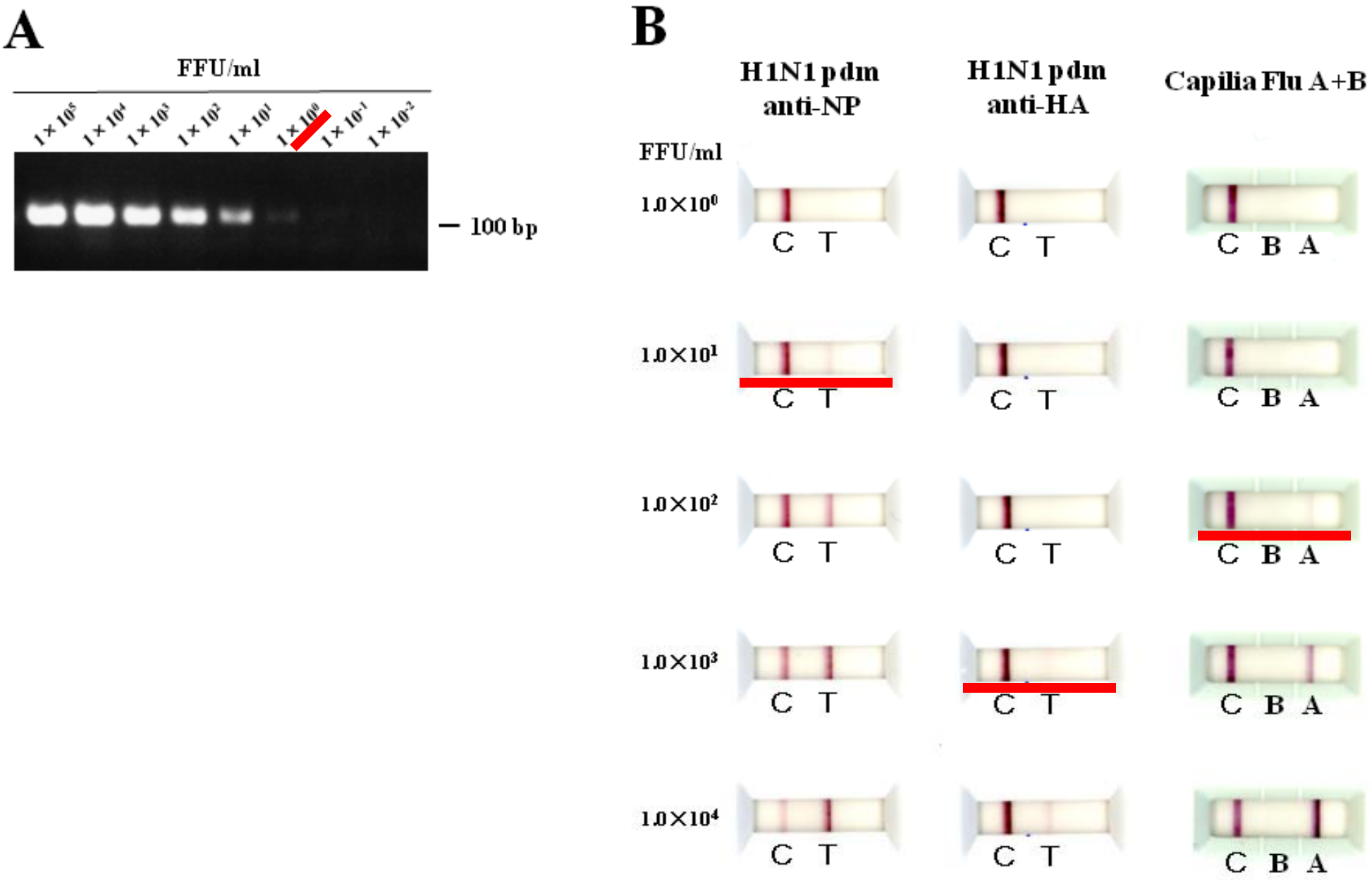
| | Target | Isotyping* | Rapid test kit |
|---|---|---|---|
| N-SW2-6 | HA | IgG1, κ | H1N1 pdm anti-HA kit |
| N-SW4-5 | NP | IgA, κ | |
| N-SW4-6 | NP | IgG1, κ | H1N1 pdm anti-NP kit |

*: Isotyping was conducted by Sigma IsoQuick™ Kit for Mouse Monoclonal Isotyping (Sigma-Aldrich Corporation)

# Immunochromatography (IC)



# Detection limit of the test kit



# Evaluation of rapid test kits

| IC test kit | | RT-PCR (Specimen number) | | | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|---|
| | | Negative | H1N1 pdm | Seasonal A (H1, H3) or B | | |
| H1N1 pdm anti-NP kit | + | 0 | 71 | 0 | 85.5 | 100 |
| | — | 43 | 12 | 42 | | |
| H1N1 pdm anti-HA kit | + | 6 | 41 | 0 | 49.4 | 93.0 |
| | — | 37 | 42 | 42 | | |
| Capilia Flu A+B | + | 1 | 66 | 40 | 84.8 (79.5)* | 97.7 |
| | — | 42 | 17 | 2 | | |

*: % Sensitivity of Capilia kit for H1N1 pdm is shown in parentheses.

# Summary

- We success to obtain three hybridoma cells that the antibodies produced by the cells can react with H1N1 pdm, but not seasonal influenza A (H1N1 or H3N2) or B viruses.
- The target of two antibodies were nucleoprotein and the other was hemagglutinin.
- We assembled two rapid test kit using anti-HA antibody and anti-NP antibody. Anti-NP test kit indicate high sensitivity and specificity.

# Conclusion

- H1N1 pdm anti-NP test kit could be useful for assistance of screening diagnosis and the conformational test for H1N1 pdm at the clinical hospitals.

# Acknowledgement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