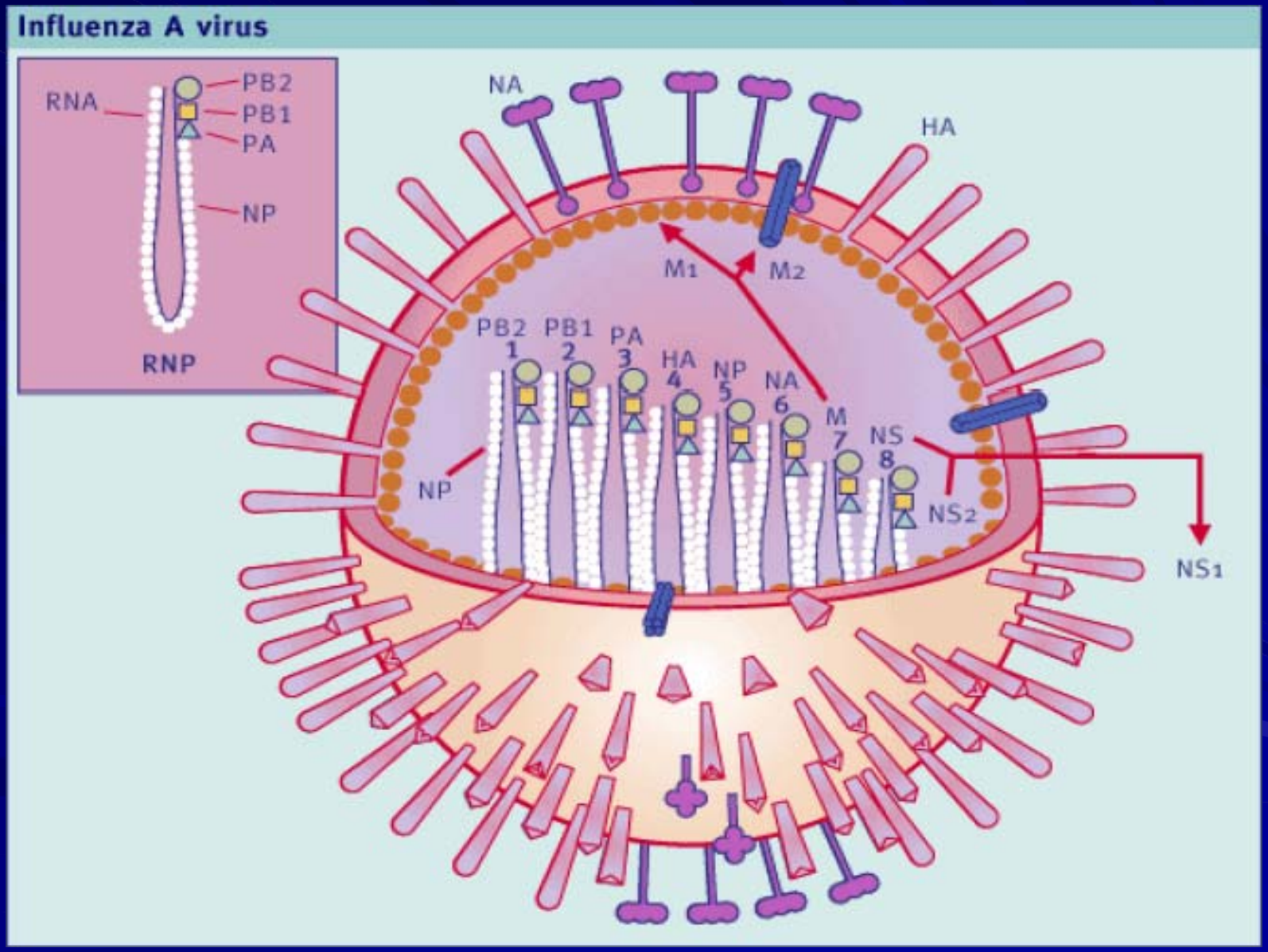
Εργαστηριακή Διάγνωση Γρίπης Μοριακή διάγνωση ή όχι ?

Εθνικόν και Καποδιστριακόν Πανεπιστήμιον Αθηνών

ΣΠΑΝΑΚΗΣ ΝΙΚΟΛΑΟΣ
Αναπλ. Καθηγητής ΜΙΚΡΟΒΙΟΛΟΓΙΑΣ, ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΔΟΜΗ ΙΩΝ ΓΡΙΠΗΣ



ΓΟΝΙΔΙΑ ΙΩΝ ΓΡΙΠΗΣ

Influenza A		
RNA segment	Encoded protein	
	Name	Function
1	PB2	polymerase
2	PB1	polymerase
3	PA	polymerase
4	**HA,H**	hemagglutinin
5	NP	nucleoprotein
6	**NA, N**	neuraminidase
7	M1, M2	matrix (membrane) proteins
8	NS1,NS2	Nonstructural proteins

Μέθοδοι ανίχνευσης και δείγματα

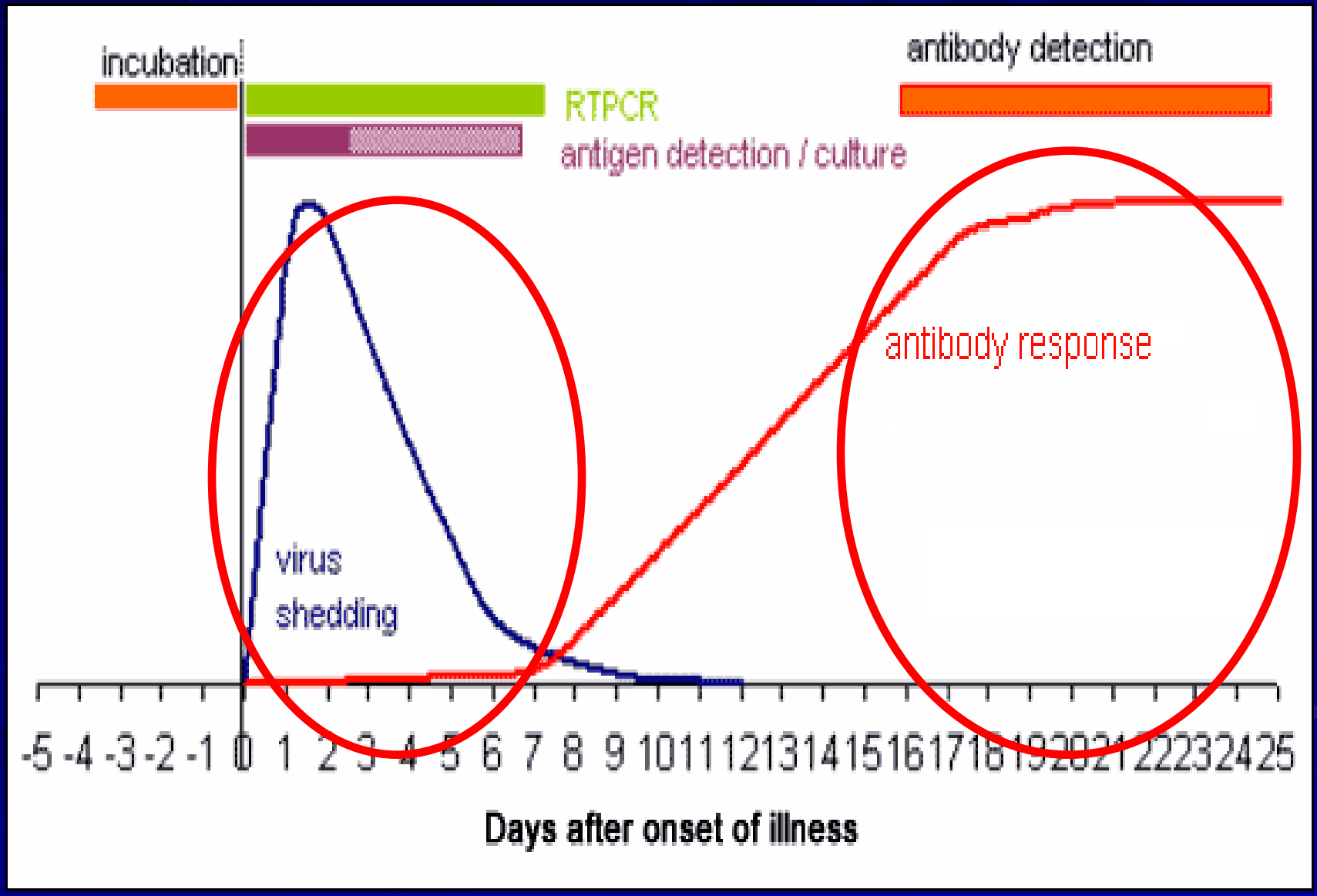
Δοκιμασίες σε δείγματα αναπνευστικού:

- Μέθοδοι PCR (Μοριακές μέθοδοι)
- Ταχεία ανίχνευση αντιγόνων (Rapid Test)
- Απομόνωση ιού σε καλλιέργεια
- Ανοσοφθορισμός

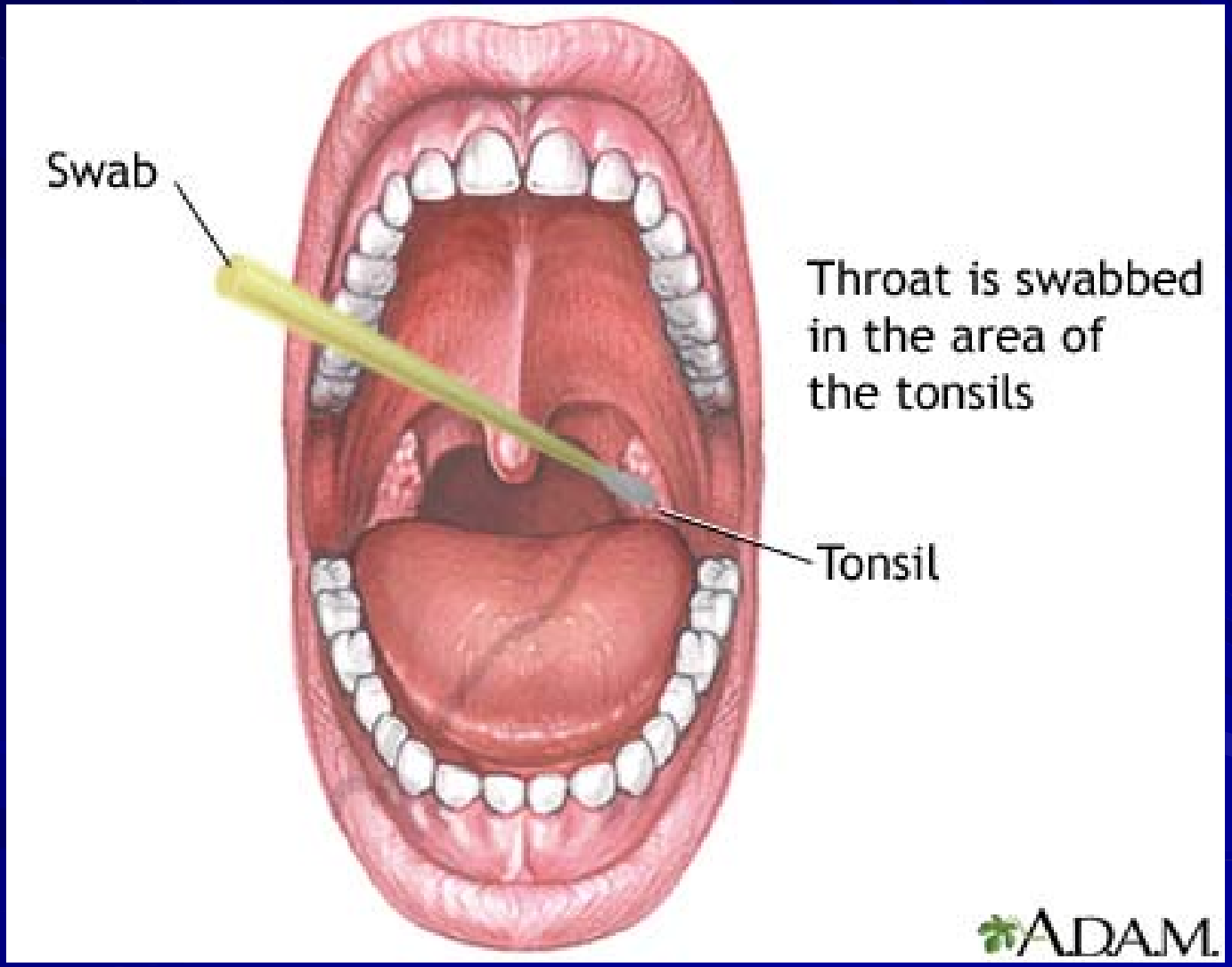
Δοκιμασίες σε ορό αίματος:

- Ανίχνευση ειδικών αντιγόνων και αντισωμάτων
- Μέθοδοι PCR (Μοριακές μέθοδοι)

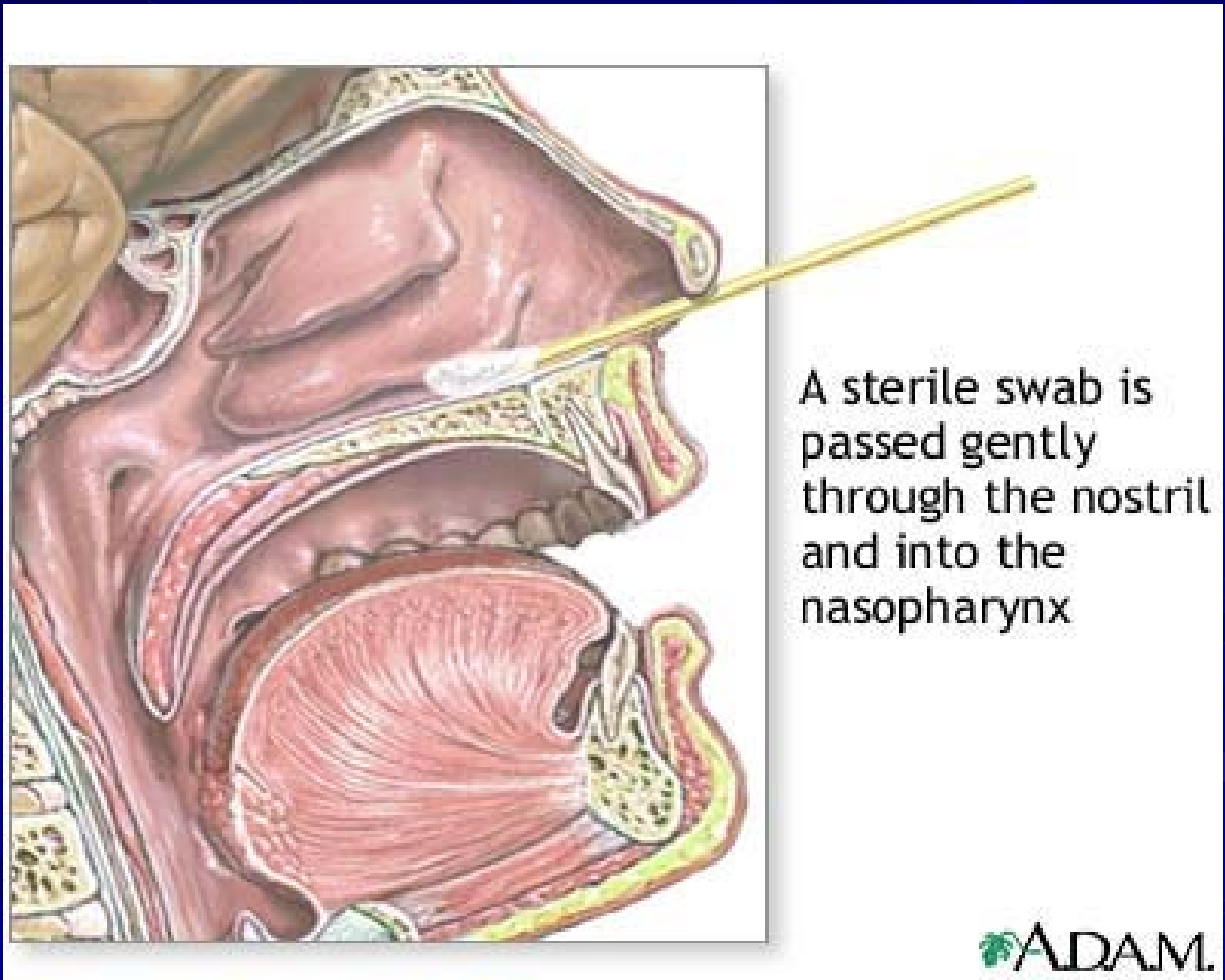
Πότε συλλέγουμε δείγματα



Φαρυγγικό επίχρισμα



Ρινοφαρρυγγικό επίχρισμα



http://www.nlm.nih.gov/medlineplus/ency/imagepages/9687.htm

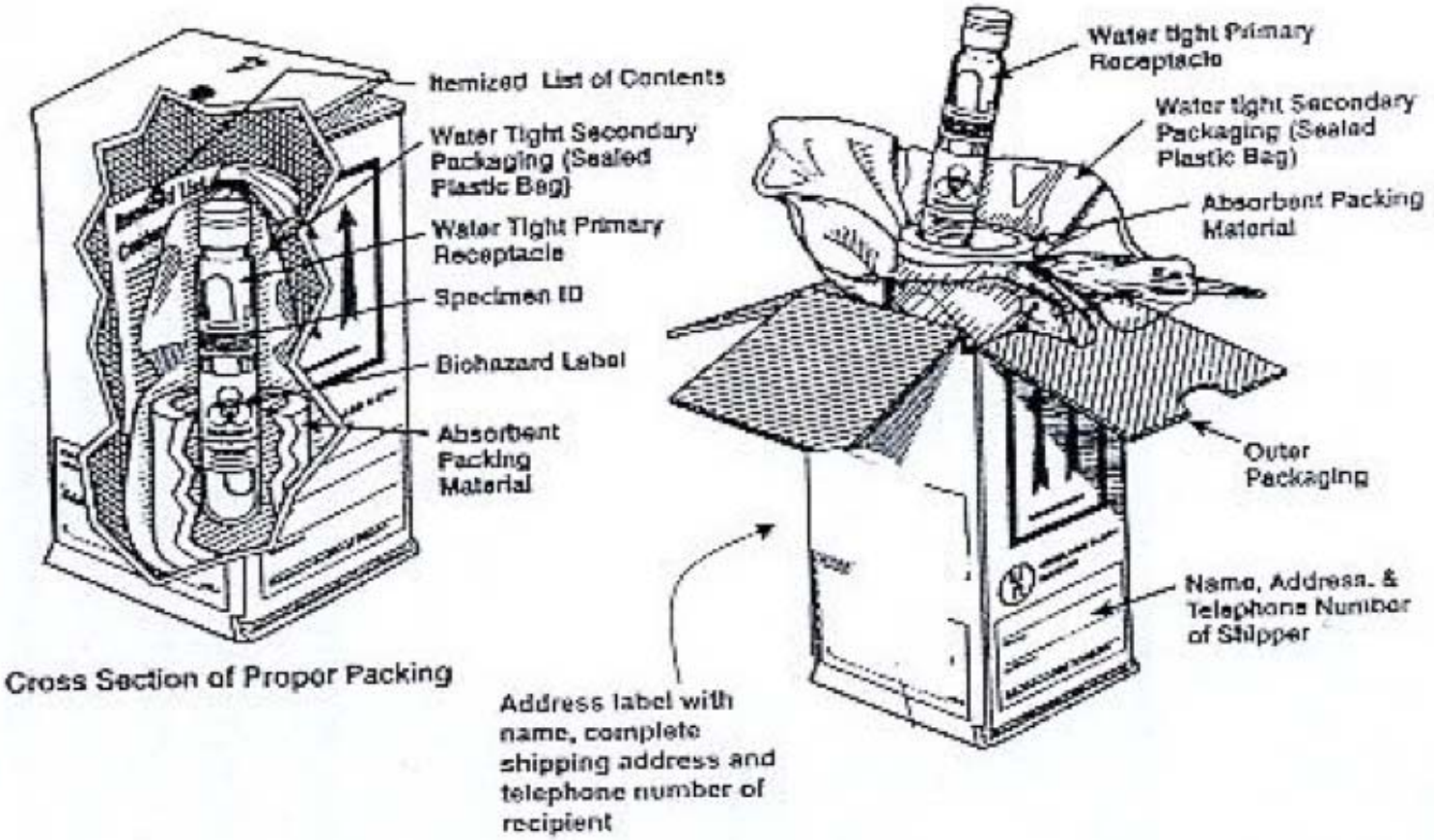
Συλλογή – Αποθήκευση – Μεταφορά δειγμάτων

- Προτιμούμε τους στειλεούς με πλαστικό ρύγχος και συνθετικό άκρο – ΟΧΙ ΒΑΜΒΑΚΙ.
- Για καλλιέργεια του ιού τοποθετούμε σε ειδικό σωληνάριο με υγρό καλλιέργειας

Συλλογή – Αποθήκευση – Μεταφορά δειγμάτων

- Οι στειλεοί που προορίζονται για άμεση ανίχνευση (είτε rapid test είτε PCR) φυλάσσονται max. 24ώρες στους 4°C.
- Οι στειλεοί που προορίζονται για καλλιέργεια πρέπει να τοποθετηθούν σε ειδικό καλλιεργητικό υγρό και να αποσταλούν ΑΜΕΣΩΣ στο ειδικό εργαστήριο.

Συλλογή – Αποθήκευση – Μεταφορά δειγμάτων



The labeling for contents should include the words:

"UN 3373 Diagnostic Specimens"

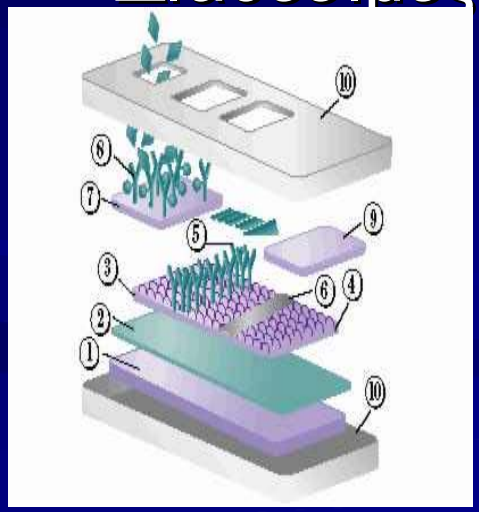
Δοκιμασίες ταχείας ανίχνευσης (rapid tests)

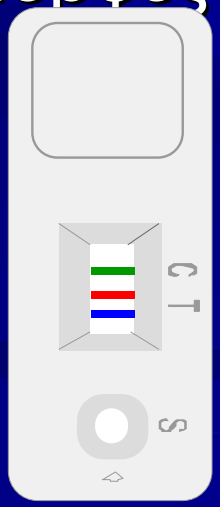
- Ταχεία ανίχνευση – Πολλά διαθέσιμα εμπορικά test.
- Χαμηλή ευαισθησία (max 70%) και ειδικότητα (max. 90%)
  - ΨΕΥΔΩΣ ΘΕΤΙΚΑ αποτελέσματα μπορεί να παρατηρούνται στην αρχή και στο τέλος μιας επιδημίας
  - ΨΕΥΔΩΣ ΑΡΝΗΤΙΚΑ αποτελέσματα προκύπτουν στο μέσο μιας επιδημίας

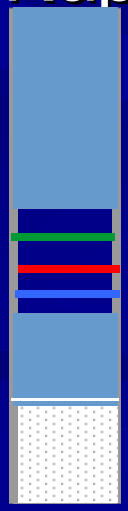
ΠΡΟΣΟΧΗ: ΔΕΝ ΠΑΡΕΧΕΤΑΙ Η ΔΥΝΑΤΟΤΗΤΑ ΤΥΠΟΠΟΙΗΣΗΣ, ΑΝΙΧΝΕΥΣΗ ΜΟΝΟ Α ή Β

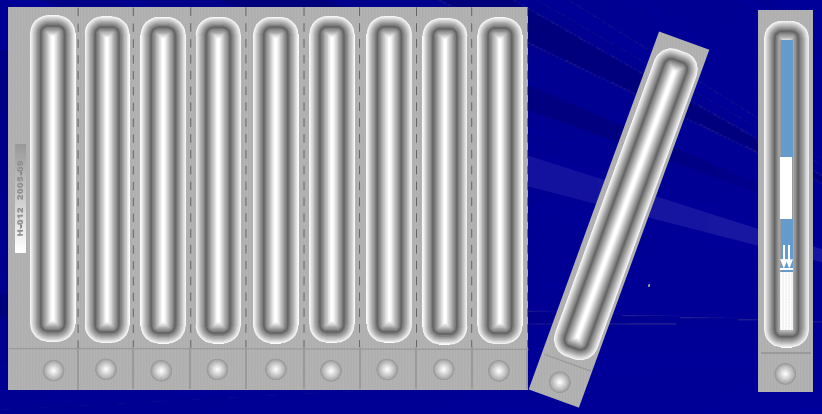
Δοκιμασίες ταχείας ανίχνευσης

- Δείγματα από το ανώτερο αναπνευστικό που συλλέγονται εντός 3-4 ημερών από την έναρξη των συμπτωμάτων.
- Ανοσοχρωματογραφικές τεχνικές
  - Καθηλωμένα αντισώματα έναντι αντιγόνων του ιού
- Διαθέσιμες μορφές : Κάρτα, Ταινία, Σωληνάριο









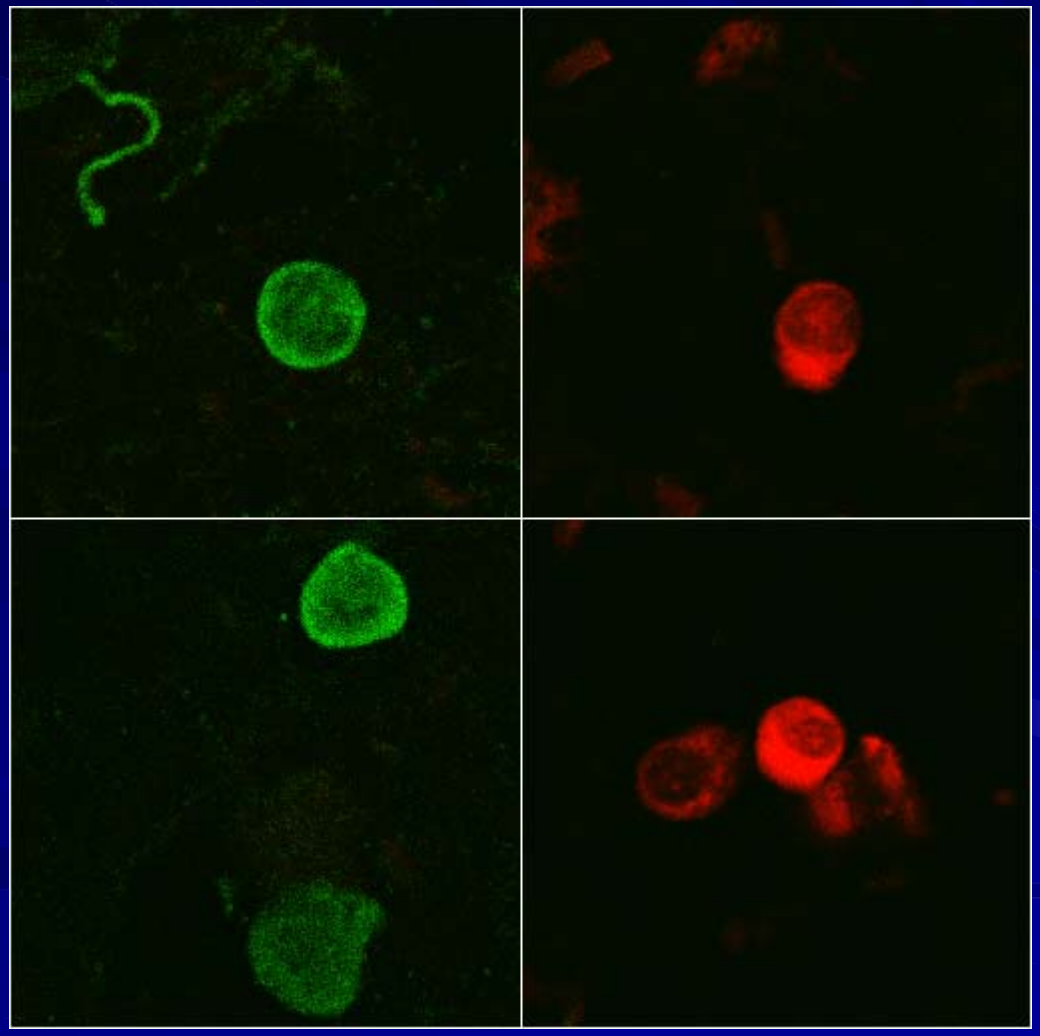
Ανοσοφθορισμός

- Ανοσοφθορισμός (DFA ή IFA)
  - Διάκριση influenza A και B.
  - Περιορισμένη τυποποίηση ιών
- Δύσκολη ερμηνεία των αποτελεσμάτων
- Απαιτεί εξειδικευμένο εξοπλισμό (μικροσκόπιο φθορισμού)
- Εμπειρία στην αξιολόγηση
- Καλύτερα αποτελέσματα σε σύγκριση με τις μεθόδους ταχείας ανίχνευσης

Έμμεσος ανοσοφθορισμός από τραχειακό έκπλυμα

Anti-H5

Anti-H3

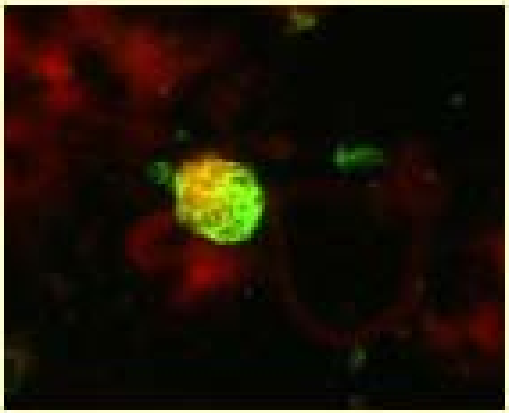


Anti-A/NP

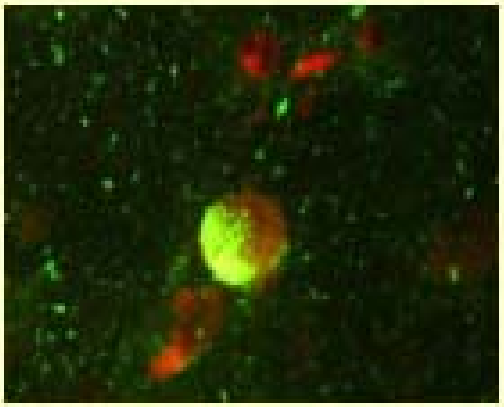
Anti-B

Από: World Bank Training, Alexander Klimov, CDC

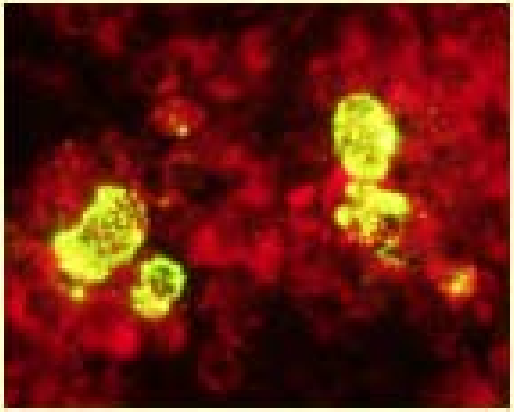
Ανοσοφθορισμός – ανίχνευση πολλών αναπνευστικών ιών ταυτόχρονα



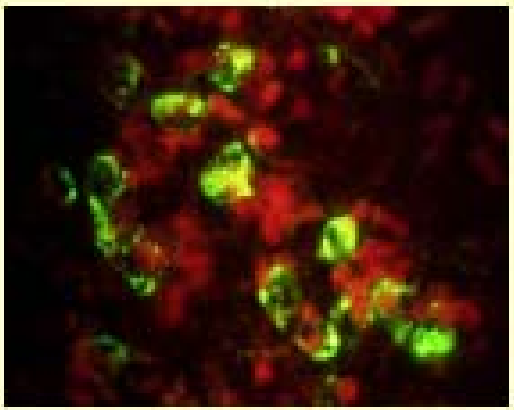
Flu A



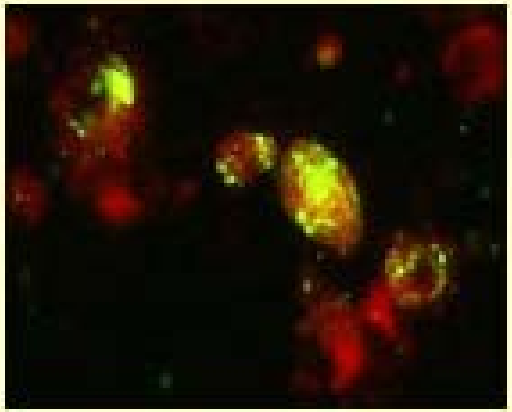
Flu B



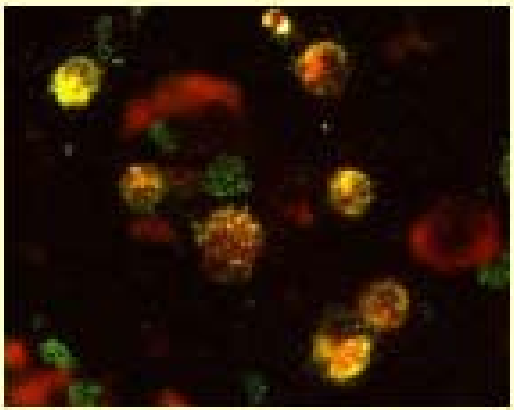
PIV1



PIV3



RSV



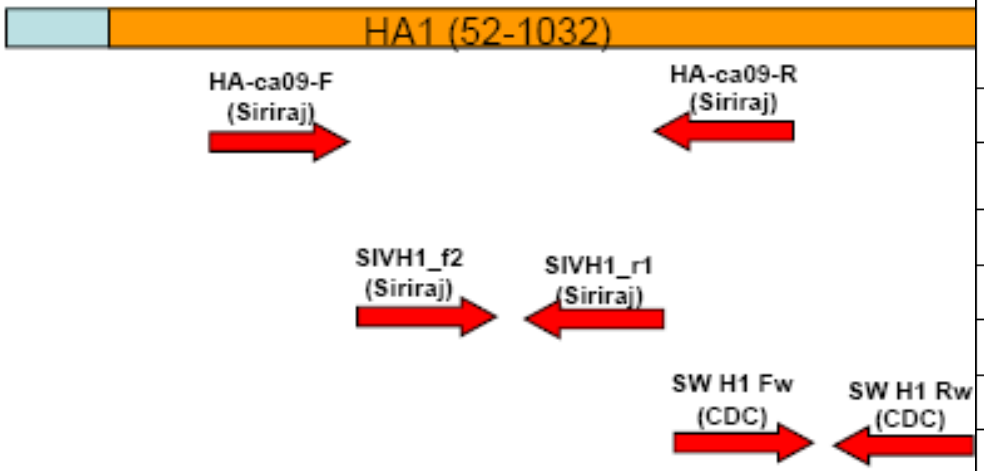
Adenovirus

Ιικές κυτταροκαλλιέργειες

- Ιική κυτταροκαλλιέργεια
  - Η απομόνωση των ιών γρίπης είναι εφικτή με ιική κυτταροκαλλιέργεια, αλλά γίνεται σε χρονικό διάστημα που δεν προσφέρεται για έγκαιρη κλινική διάγνωση (2-3 ημέρες τουλάχιστον)
  - Πραγματοποιείται μόνο σε εξειδικευμένα εργαστήρια – Κέντρα αναφοράς.
  - Προσφέρεται για τυποποίηση
  - Μεγάλο κόστος
    - Συνεχής καλλιέργεια και συντήρηση ευκαρυωτικών κυττάρων
    - Εξειδικευμένα όργανα και υλικά

Μοριακές Τεχνικές (RT-PCR, Real Time RT-PCR)

- Μοριακή ανίχνευση
  - Με Real-time RT-PCR με χρήση γενικών εκκινητών (primers) που ενισχύουν τμήμα του γονιδιώματος (πρωτεΐνη Μ) για τους τύπους Α και Β και ειδικούς εκκινητές που ενισχύουν τα γονίδια Η1 και Η3 των ιών γρίπης τύπου Α για τη διάκριση μεταξύ εποχικής και πανδημικής γρίπης



Influenza A		
RNA segment	Encoded protein	
	Name	Function
1	PB2	polymerase
2	PB1	polymerase
3	PA	polymerase
4	**HA,H**	hemagglutinin
5	NP	nucleoprotein
6	**NA, N**	neuraminidase
7	M1, M2	matrix (membrane) proteins
8	NS1,NS2	Nonstructural proteins

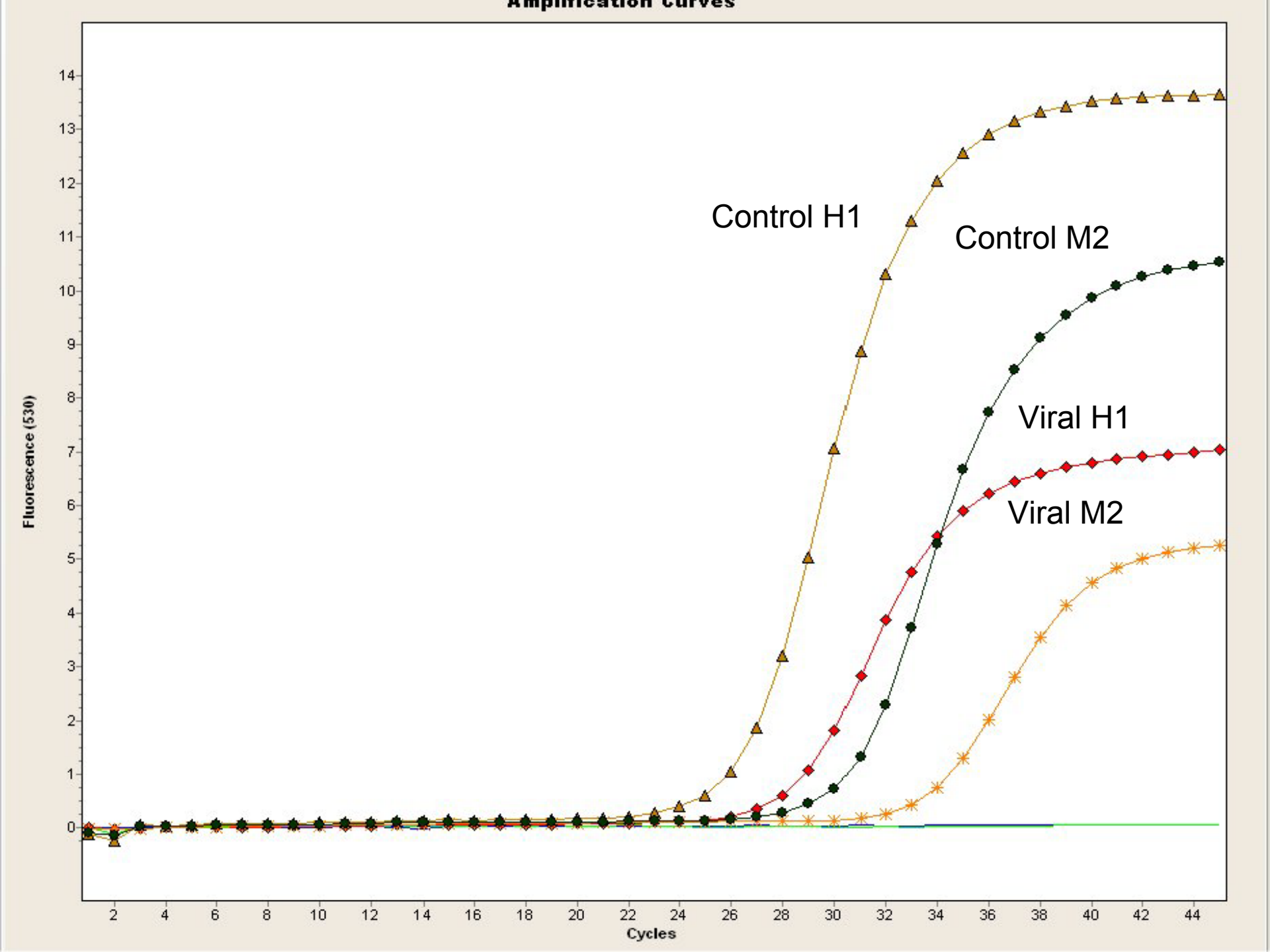

Amplification Curves
Fluorescence (530)
Cycles
Control H1
Control M2
Viral H1
Viral M2

Table 1: Influenza Virus Testing Methods

Method[1]	Types Detected	Acceptable Specimens[2]	Test Time	CLIA Waived[3]
Viral tissue cell culture (conventional; yields live virus)	A and B	NP[4] swab, throat swab, NP[2] or bronchial wash, nasal or endotracheal aspirate, sputum	3-10 days	No
Rapid cell culture (shell vials; cell mixtures; yields live virus)	A and B	As above	1-3 days	No
Immunofluorescence, Direct (DFA) or Indirect (IFA) Florescent Antibody Staining [antigen detection]	A and B	NP[4] swab or wash, bronchial wash, nasal or endotracheal aspirate	1-4 hours	No
RT-PCR[5] (singleplex and multiplex; real-time and other RNA-based) and other molecular assays [influenza viral RNA or nucleic acid detection]	A and B	NP[4] swab, throat swab, NP[2] or bronchial wash, nasal or endotracheal aspirate, sputum	Varies (Generally 60 minutes-8 hours)	No
Rapid Molecular Assay [influenza viral RNA or nucleic acid detection]	A and B	NP[4] swab, nasal aspirate, wash, swab	<30 minutes[7]	Yes/No[7]
Rapid Influenza Diagnostic Tests[6] (antigen detection)	A and B	NP[4] swab, (throat swab), nasal wash, nasal aspirate	<30 min.	Yes/No

Figure 1: Guide for considering influenza virus diagnostic tests for individual patients when influenza viruses are circulating in the community[1]

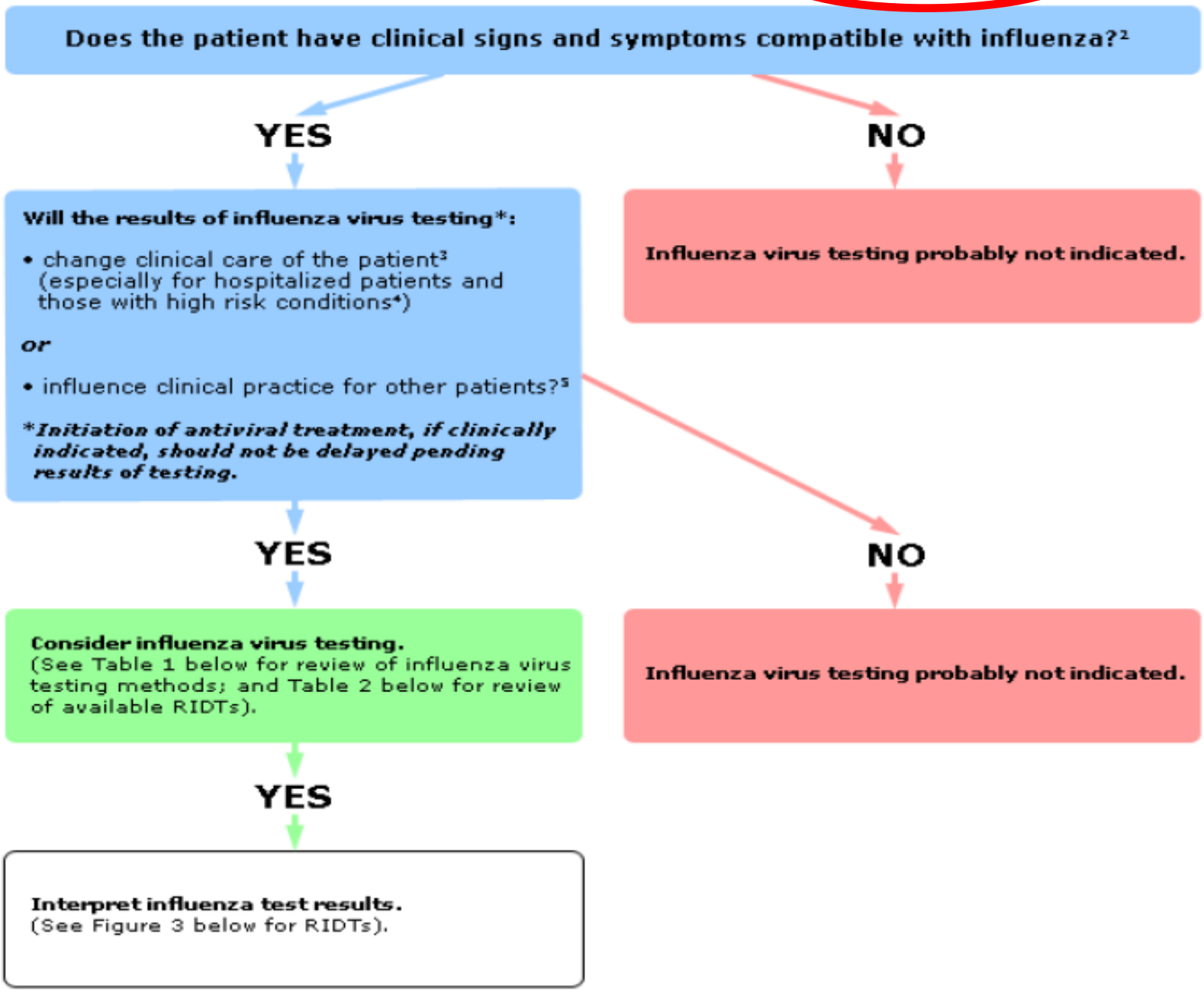


Figure 2: Guide to use of influenza virus diagnostic tests in investigating outbreaks in institutional or other closed settings[1]

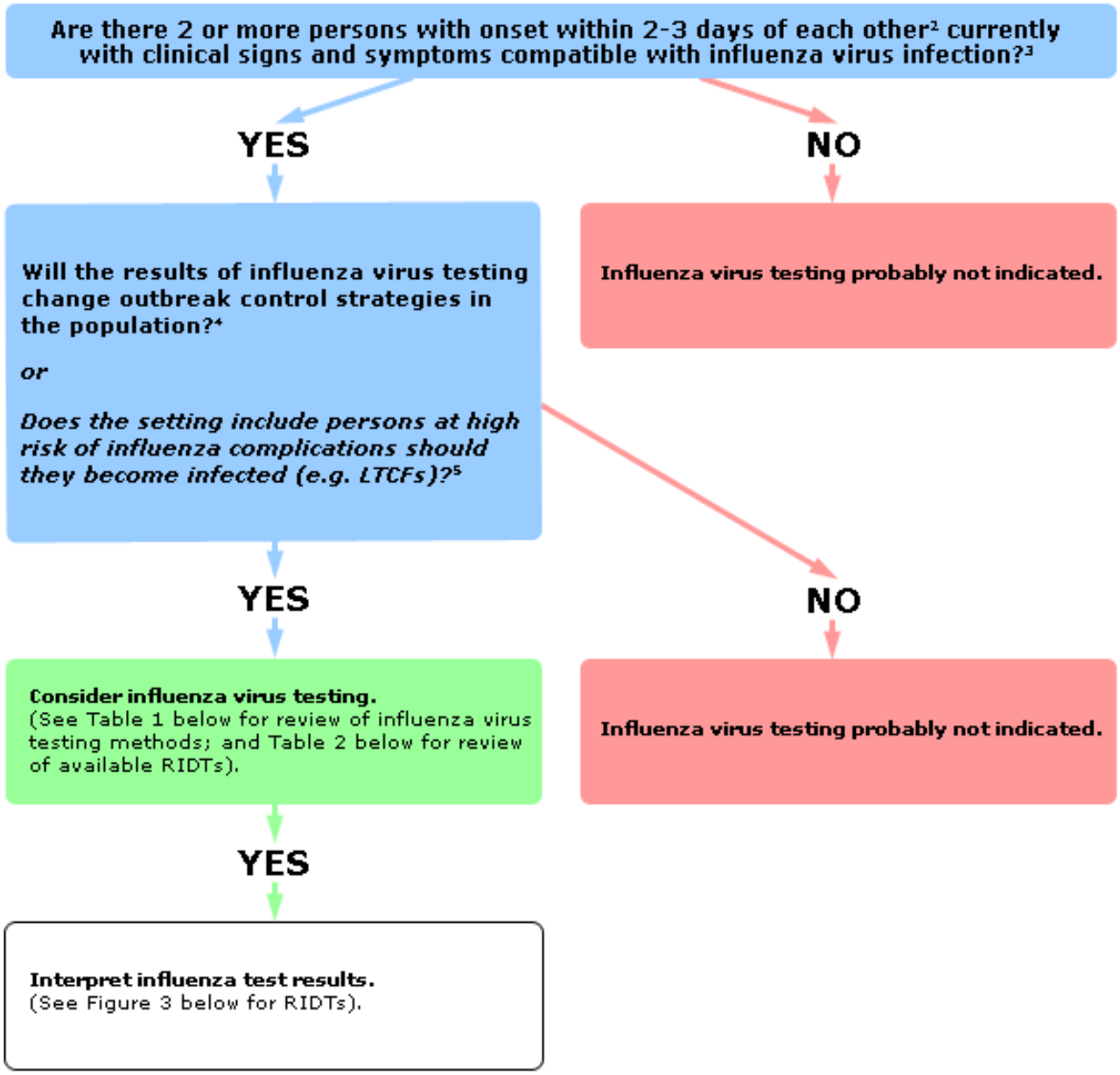
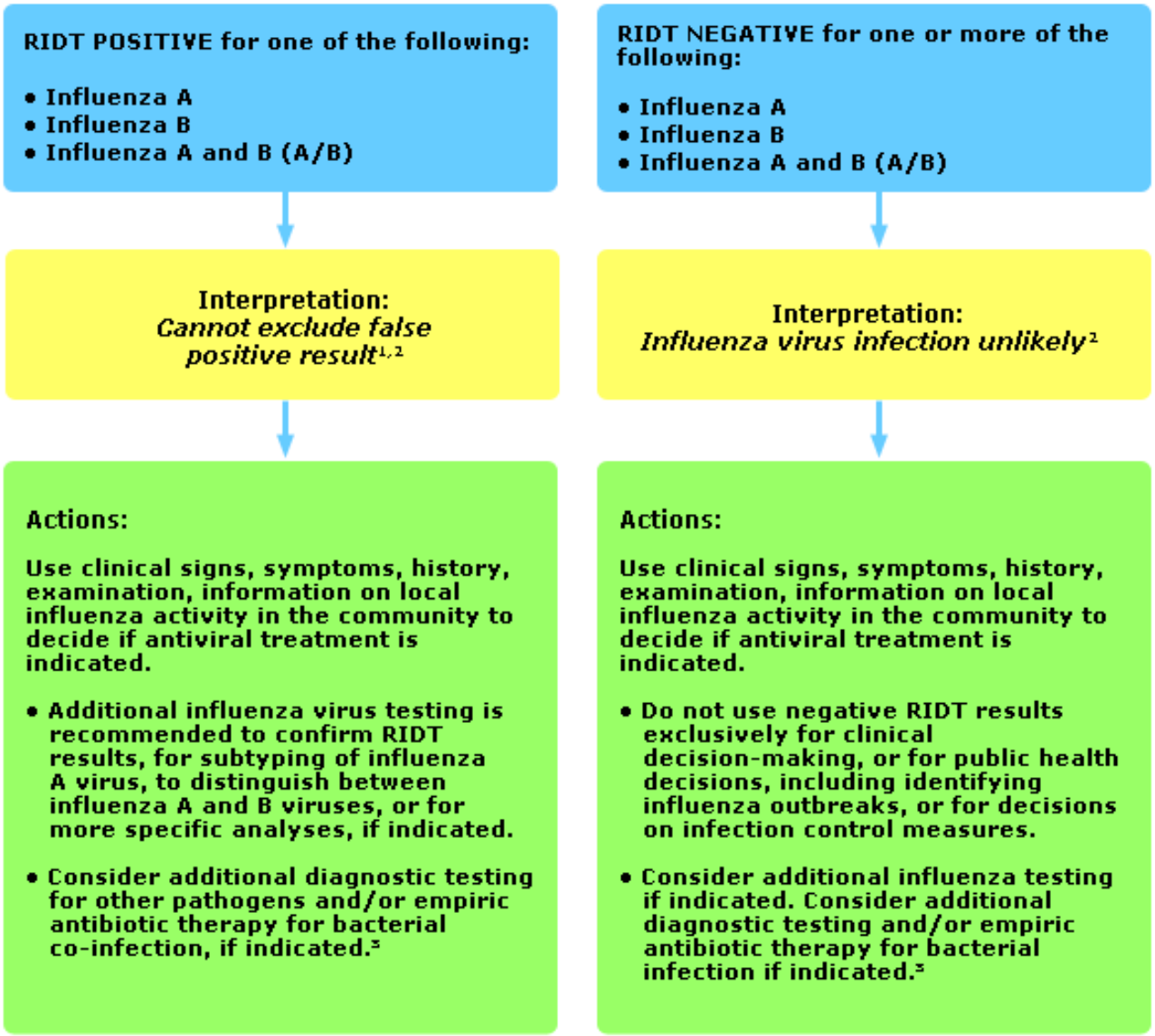


Figure 3: Algorithm to assist in the interpretation of RIDT results and clinical decision-making during periods *when influenza viruses are circulating* in the community[1]

Figure 4: Algorithm to assist in the interpretation of RIDT results and clinical decision-making during periods *when influenza viruses are not circulating* or influenza activity is low in the community[1]



Clinical Considerations of Testing When Influenza Prevalence is Low

When influenza prevalence is relatively low, the positive predictive value (PPV) is low and false-positive test results are more likely. By contrast, when influenza prevalence is low, the negative predictive value (NPV) is high, and negative results are more likely to be true.

If Influenza Prevalence is...	And Specificity is...	Then PPV is...	False Pos. rate[1] is...
VERY LOW (2.5%)	MODERATE (80%)	VERY LOW (6-12%)	VERY HIGH (88-94%)
VERY LOW (2.5%)	HIGH (98%)	LOW (39-56%)	HIGH (44-61%)
MODERATE (20%)	MODERATE (80%)	LOW (38-56%)	HIGH (44-62%)
MODERATE (20%)	HIGH (98%)	HIGH (86-93%)	LOW (7-14%)

Clinical Considerations of Testing When Influenza Prevalence Is High

When influenza prevalence is relatively high, the NPV is low and false-negative test results are more likely. When influenza prevalence is high, the PPV is high and positive results are more likely to be true.

If Influenza Prevalence is...	And Sensitivity is...	Then NPV is...	False Neg. rate[2] is...
MODERATE (20%)	LOW (50%)	MODERATE (86-89%)	MODERATE (11-14%)
MODERATE (20%)	HIGH (90%)	HIGH(97-99%)	LOW (2-3%)
HIGH (40%)	LOW (50%)	MODERATE (70-75%)	MODERATE (25-30%)
HIGH (40%)	HIGH (90%)	HIGH (93-94%)	LOW (6-7%)

Use of RIDTs in Clinical Decision-making

RIDTs may be used to help with diagnostic and treatment decisions for patients in clinical settings, such as whether to prescribe antiviral medications. However, due to the limited sensitivities, negative results of RIDTs do not exclude influenza virus infection in patients with signs and symptoms suggestive of influenza. Therefore, antiviral treatment should not be withheld from patients with suspected influenza, even if they test negative by RIDT and further influenza testing of respiratory specimens by molecular assays may be indicated. More information about Antiviral Drugs and recommendations on their use.

Testing is not needed for all patients with signs and symptoms of influenza to make antiviral treatment decisions (See Figures 1-4). Once influenza activity has been documented in the community or geographic area, a clinical diagnosis of influenza can be made for outpatients with signs and symptoms consistent with suspected influenza, especially during periods of peak influenza activity in the community.

^ Top of Page

Use of RIDTs for Public Health Purposes to Detect Influenza Outbreaks

RIDTs can be useful to identify influenza virus infection as a cause of respiratory outbreaks in any setting, but especially in institutions (i.e., nursing homes, chronic care facilities, and hospitals), cruise ships, summer camps, schools, etc. Positive RIDT results from one or more ill persons with suspected influenza can support decisions to promptly implement infection prevention and control measures for influenza outbreaks. However, negative RIDT results do not exclude influenza virus infection as a cause of a respiratory outbreak because of the limited sensitivity of these tests. Testing respiratory specimens from several persons with suspected influenza will increase the likelihood of detecting influenza virus infection if influenza virus is the cause of the outbreak. Public health authorities should be notified promptly of any suspected institutional outbreak and respiratory specimens should be collected from ill persons (whether positive or negative by RIDT) and sent to a public health laboratory for more accurate influenza testing by molecular assays and viral culture.

Hospitalized patients

Influenza testing is recommended for hospitalized patients with suspected influenza. Molecular assays such as RT-PCR are recommended for testing hospitalized patients, especially if RIDTs are used and yield negative results. However, empiric antiviral treatment should be initiated as soon as possible for hospitalized patients with suspected influenza without the need to wait for any influenza testing results (see Antiviral Drugs, Information for Health Care Professionals). Antiviral treatment should not be stopped based on negative RIDT results given the limited sensitivities of RIDTs. Infection prevention and control measures should be implemented immediately upon admission for any hospitalized patient with suspected influenza even if RIDT results are negative (see Prevention Strategies for Seasonal Influenza in Heath Care Settings). Respiratory specimens can be tested for influenza by immunofluorescence, RT-PCR or viral culture. Serology for influenza should not be performed for clinical management. Clinicians should understand that negative results of influenza testing do not exclude influenza virus infection, especially if the time from illness onset to collection of respiratory specimens is more than 3 days, or if upper respiratory specimens were tested and the patient has lower respiratory tract disease. If influenza is suspected, testing of clinical specimens collected from different respiratory sites can be done (e.g., upper and lower respiratory tract) and can be collected on more than one day to increase likelihood of influenza virus detection; intubated patients should have endotracheal aspirate specimens tested if influenza is suspected, but not yet confirmed.

Detection of influenza virus infection and prompt implementation of infection prevention and control measures is critical to prevention of nosocomial influenza outbreaks. When there is influenza activity in the community, clinicians should consider influenza testing, including viral culture, for patients who develop signs and symptoms of influenza while they are in a health care facility. This should be done as part of a broader surveillance strategy for influenza as discussed in Prevention Strategies for Seasonal Influenza in Heath Care Settings.

Suspected influenza institutional outbreaks

For suspected influenza outbreaks in institutions, respiratory specimens should be collected from patients with suspected influenza as early as possible once the outbreak is suspected (See Figure 2). The use of influenza molecular assays is preferred. If RIDTs are used in these settings, clinical specimens should also be sent for influenza testing by viral culture and RT-PCR to provide detailed information on specific influenza A virus subtypes and strains, and antiviral susceptibility data and to verify RIDT test results. Active daily surveillance for suspected influenza illness and collection of specimens from patients with suspected influenza should continue through at least 2 weeks after implementation of control measures to assess effectiveness of the measures and to monitor for potential emergence of antiviral resistance. See Prevention Strategies for Seasonal Influenza in Heath Care Settings.

Use in Detecting Novel Influenza A Cases

- Molecular assays, such as RT-PCR, are designed to accurately identify influenza A and B viral RNA by using conserved gene targets. Some assays will detect influenza A or B viruses but will not determine the influenza A virus subtype, and thus will not be able to indicate if the infection is due to a novel influenza A virus. Novel influenza A viruses are antigenically and genetically distinct from currently circulating influenza A viruses among humans and usually represent zoonotic transmission from avian or swine species to humans.
- Some FDA-cleared devices can not only detect influenza A or B viruses, but also can identify influenza A hemagglutinin genes, allowing for determination of some or all of the seasonal influenza A virus subtypes [i.e., A(H1N1)pdm09 or A(H3N2)]. These assays will not only identify the currently circulating influenza A virus strains, but also may identify viruses that are detected as influenza A for which no subtype could be identified. These "unsubtypables" may represent novel influenza A virus infections.
- Clinicians and laboratorians using molecular assays that are capable of detecting all currently circulating seasonal influenza A virus subtypes [i.e., A(H1N1)pdm09 or A(H3N2)], and who identify an "unsubtypable" result (i.e., influenza A with no subtype detected), should contact their state or local public health laboratory immediately for additional testing to determine if the infection is due to a novel influenza A virus.

ΣΥΜΠΕΡΑΣΜΑΤΑ

- Η απάντηση στην ερώτηση «Μοριακός Έλεγχος ή όχι» δεν είναι Μονολεκτική.
- Ο ταχείες αντιγονικές δοκιμασίες είναι χρήσιμες στον καθορισμό της έναρξης μιας επιδημίας τοπικού ή υπερτοπικού χαρακτήρα. Λόγω της χαμηλής τους ευαισθησίας απαιτείται να έχουν αυξηθεί τα περιστατικά με γρίπη.
- Αν χρησιμοποιηθούν (επί έλλειψης διαθεσιμότητας μοριακών μεθόδων) σε περιόδους επιδημίας για τη διάγνωση περιστατικών και την απόφαση χορήγησης θεραπείας ή λήψης άλλων μέτρων θεραπείας, τότε το θετικό αποτέλεσμα είναι πραγματικό και αξιολογείται.
- Σε κάθε άλλη περίπτωση απαιτείται Μοριακός Έλεγχος που μπορεί να μας εξασφαλίσει σε ποσοστό άνω του 95% σωστή και έγκαιρη διάγνωση ανεξάρτητα από το αν βρισκόμαστε σε περίοδο επιδημίας ή όχι.
- Τα σοβαρά περιστατικά που νοσηλεύονται ήδη ή βρίσκονται στις ομάδες υψηλού κινδύνου για την ανάπτυξη σοβαρής νόσου από influenza ή των επιπλοκών της, ΟΠΩΣΔΗΠΟΤΕ πρέπει να ελέγχονται με Μοριακό Έλεγχο, πχ. Νοσηλευόμενοι σε ΜΕΘ, ΜΑΦ, βρέφη, νεογνά, ανοσοκατεσταλμένοι, κ.λπ.
- Όλες οι υπόλοιπες τεχνικές χρησιμοποιούνται από εξειδικευμένα κέντρα για τη μελέτη και την παρακολούθηση της εποχικής γρίπης και συμβάλλουν στην βελτίωση των μεθόδων πρόληψης, επιτήρησης, και αντιμετώπισης της επιδημίας.