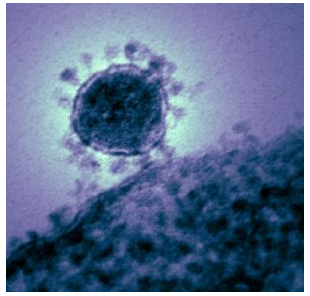


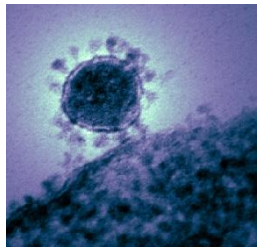
IL NUOVO CORONAVIRUS 2019-NCOV: STATO DELL'ARTE

Istituto Superiore di Sanità, Roma
29 gennaio 2020

Maria R. Capobianchi
National Institute for Infectious Diseases
«Lazzaro Spallanzani» IRCCS
Roma

World Health Organization

WHO Collaborating Center for clinical care, diagnosis, response and training on Highly Infectious Diseases



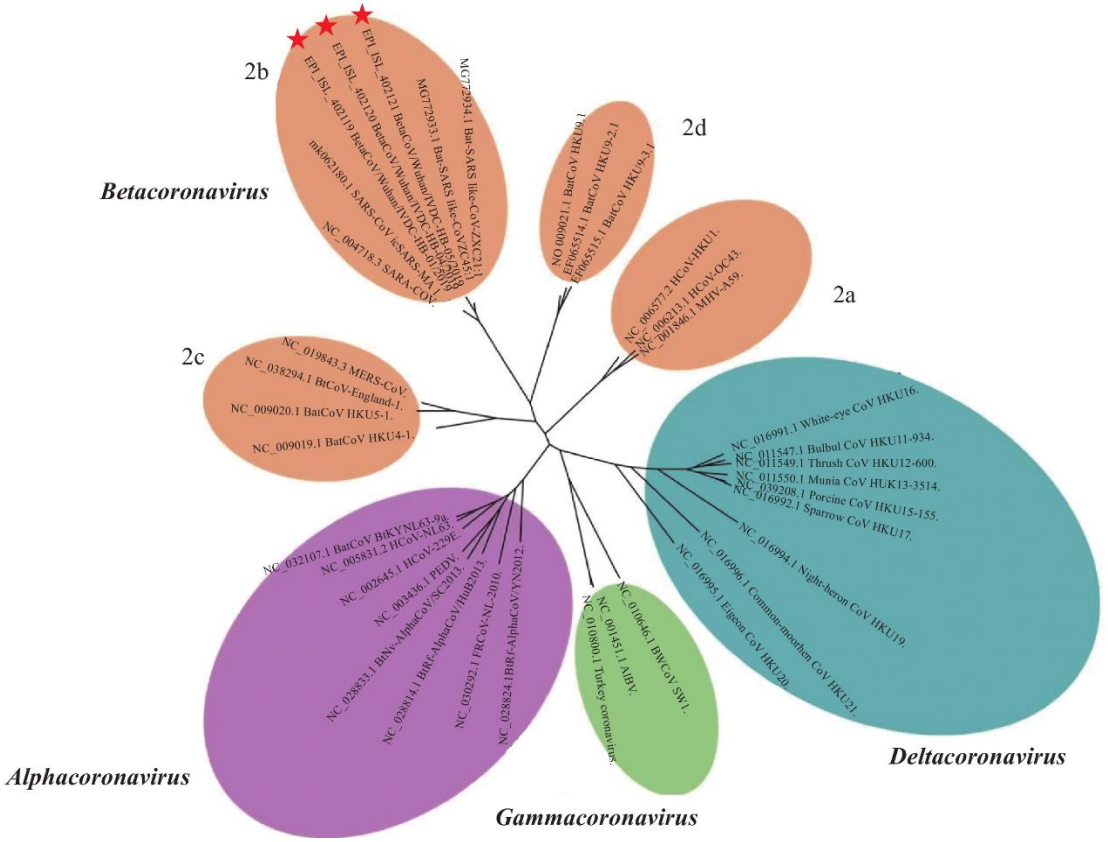
ProMED INTERNATIONAL SOCIETY FOR INFECTIOUS DISEASES

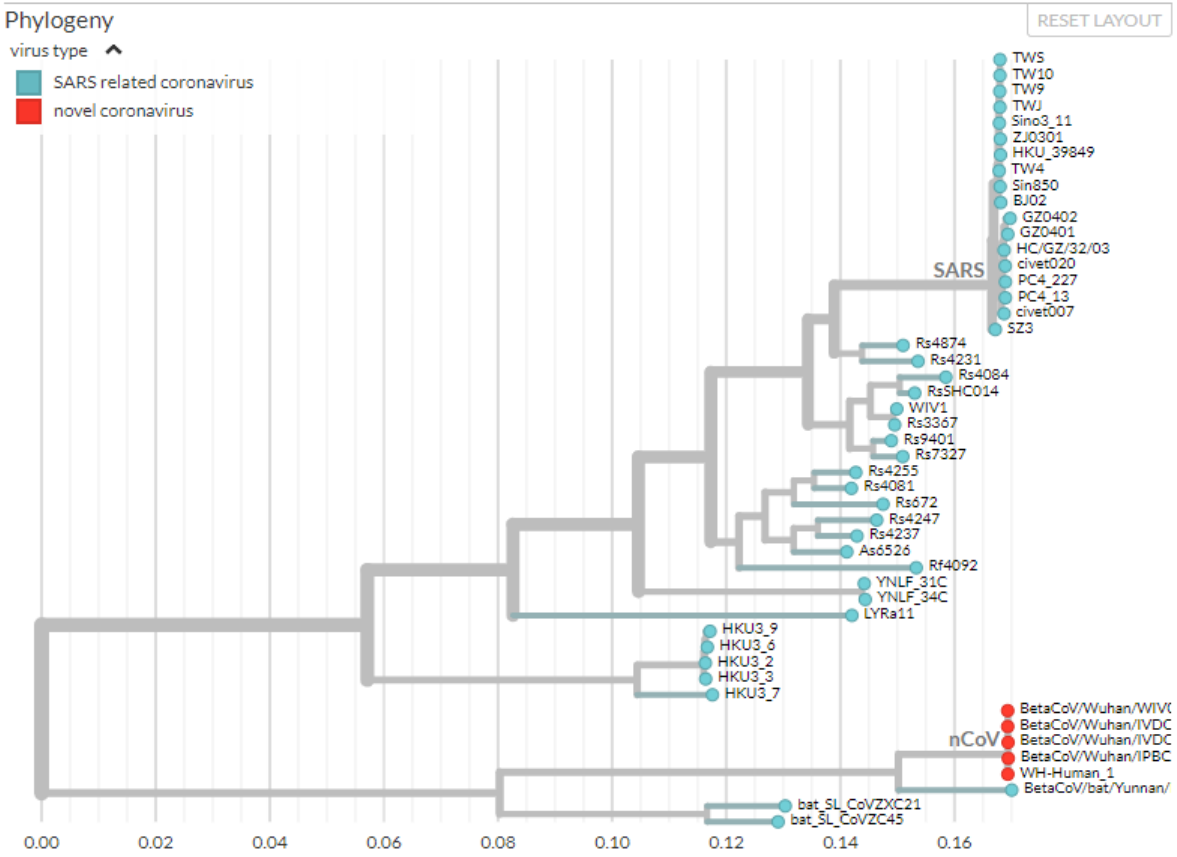
Published Date: 2019-12-30 23:59:00
Subject: PRO/AH/EDR> Undiagnosed pneumonia - China (HU): RFI
Archive Number: 20191230.6864153

ProMED INTERNATIONAL SOCIETY FOR INFECTIOUS DISEASES

Published Date: 2020-01-05 18:15:37
Subject: PRO/AH/EDR> Undiagnosed pneumonia - China (HU) (03): updates, SARS, MERS ruled out, WHO, RFI
Archive Number: 20200105.6872267

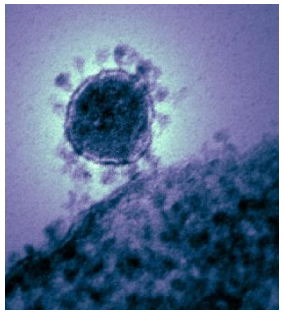
- On 9 January 2020, China CDC reported that a novel coronavirus (2019-nCoV) had been detected as the causative agent for 15 of the 59 pneumonia cases.
- On 10 January 2020, the first novel coronavirus **genome sequence** was disclosed



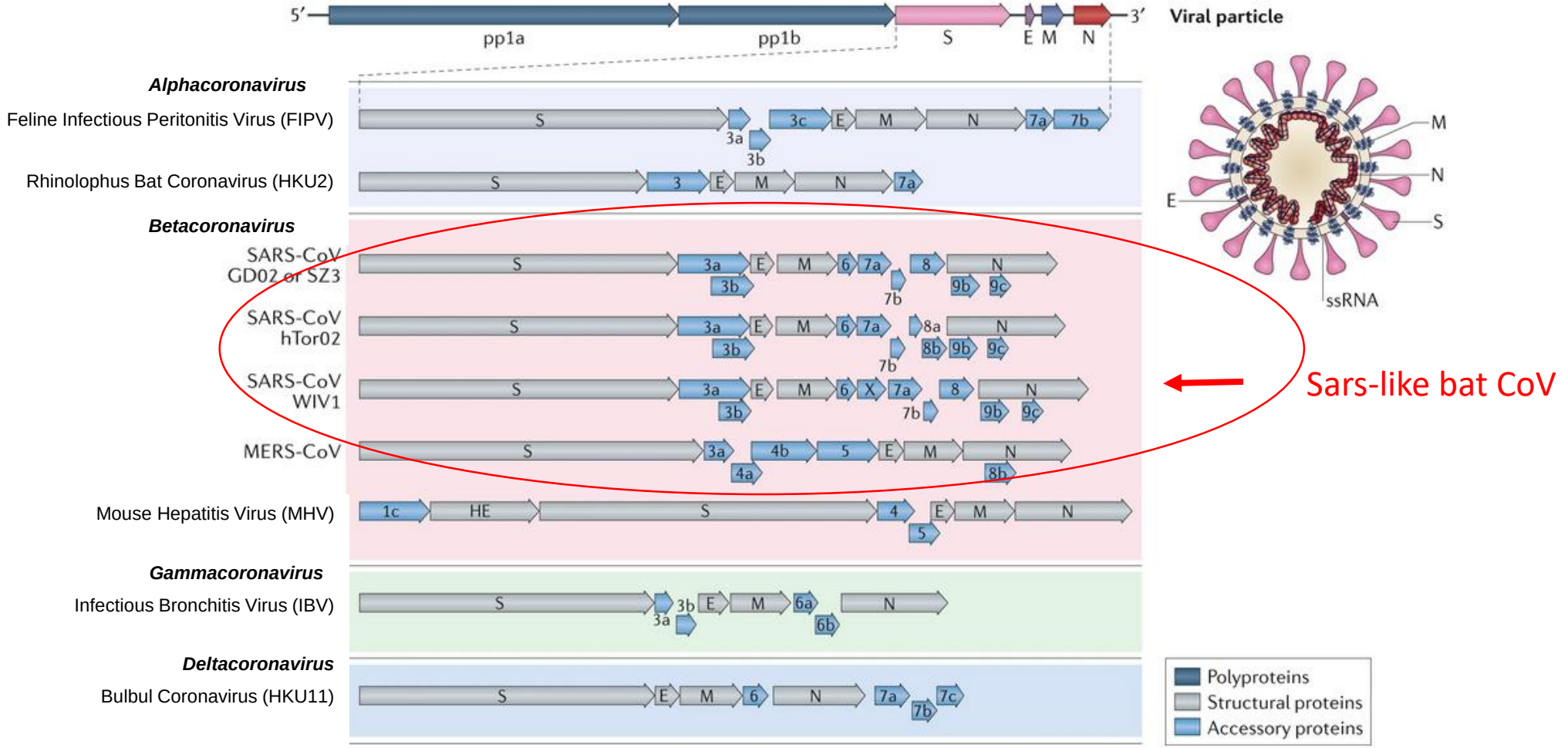


Tan et al. China CDC Weekly. 2020, 2(4): 61-62.

https://nextstrain.org

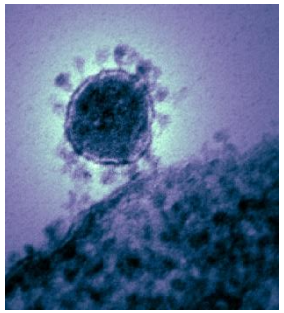


Genomes, genes and proteins of different coronaviruses



- Enveloped spherical particles, 100–160 nm diameter.
- Positive-sense, single-stranded RNA (ssRNA), size 27–32 kb
- 5‘: **nonstructural proteins** (polyprotein pp1ab: 16 proteins involved in genome transcription and replication)
- 3‘: **structural proteins** (envelope glycoproteins spike (S), envelope (E), membrane (M) and nucleocapsid (N))
- Accessory genes species-specific and dispensable for virus replication.

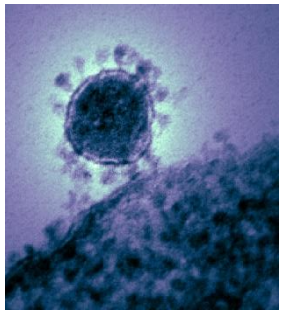
Cui J et al. Nature Reviews Microbiology vol. 17 | March 2019



DIAGNOSI vs CONFERMA

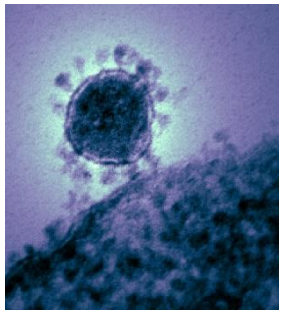
Come per tutte le infezioni caratterizzate da potenziale diffusivo, l'identificazione dei casi segue un percorso pre-definito.

- La diagnosi (patient oriented)
- Conferma (surveillance oriented)



Difficoltà del laboratorio

- Necessità, specie in assenza di un sospetto clinico ben indirizzato, di una diagnosi che tenga conto di altre possibili agenti eziologici (diagnosi differenziale)
- Scarsità/assenza di kit specifici commercialmente disponibili, validati (non CE)
- Necessità di sviluppare, spesso in fretta, metodi nuovi e, in assenza di standard di riferimento, di sviluppare metodi di conferma
- Scarsità di materiali di riferimento per lo sviluppo e la validazione dei metodi diagnostici
- Necessità di partecipare ai network internazionali per poter accedere a: metodi, materiali/agenti, EQA, assistenza/conferme dei risultati
- Necessità di laboratori ad elevato biocontenimento (definire il gruppo di rischio)?

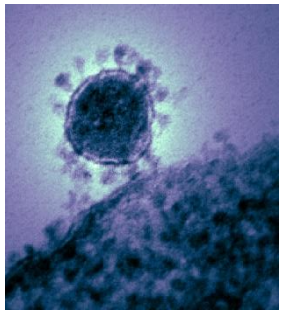


Cosa è cambiato

- L'approccio sindromico per mettere al centro il paziente e non il patogeno (pannelli)
- Per molti dei patogeni emergenti continuano ad essere disponibili solamente test «home made», ma l'industria adesso è più coinvolta.
- I test di screening sono accessibili ad un più ampio numero di laboratori
- La Real Time PCR ha soppiantato nelle procedure di screening le tecniche colturali, ma queste in associazione a PCR classiche+sequenziamento genomico e analisi filogenetica, continuano ad essere indispensabili per l'identificazione di nuovi agenti o per la conferma dei primi casi

Cosa non è cambiato

- L'approccio alla diagnostica differenziale non può che essere basato sulla **stratificazione dei rischi**
- Necessità di definire i **livelli di biosicurezza** adeguati alla gestione dei campioni in base al/i patogeni



Recommendations for specimen collection

Global Surveillance for human infection with novel coronavirus (2019-nCoV)

Interim guidance

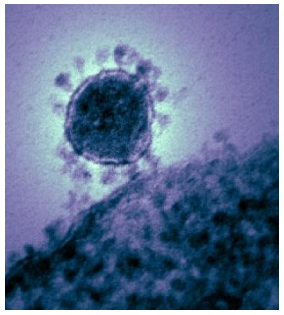
21 January 2020

WHO/2019-nCoV/SurveillanceGuidance/2020.3

World Health Organization

- **Lower respiratory specimens** likely have a higher diagnostic value than upper respiratory tract specimens for detecting 2019-nCoV infection (sputum, endotracheal aspirate, or bronchoalveolar lavage)

- If patients do not have signs or symptoms of lower respiratory tract disease or if the collection is not possible, upper respiratory tract specimens such as a nasopharyngeal aspirate or **combined nasopharyngeal and oropharyngeal swabs** should be collected.



Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases

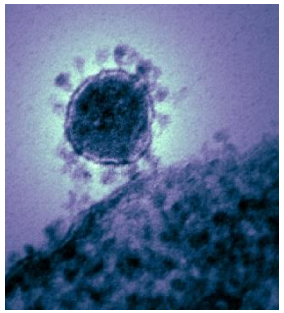
Interim guidance
17 January 2020

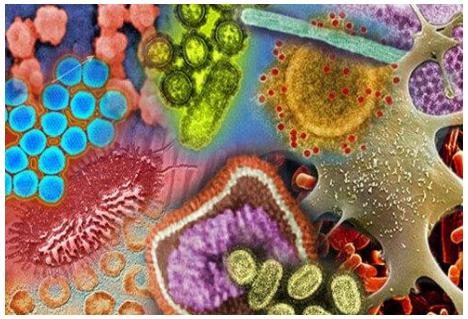
WHO/2019-nCoV/laboratory/2020.3

World Health Organization

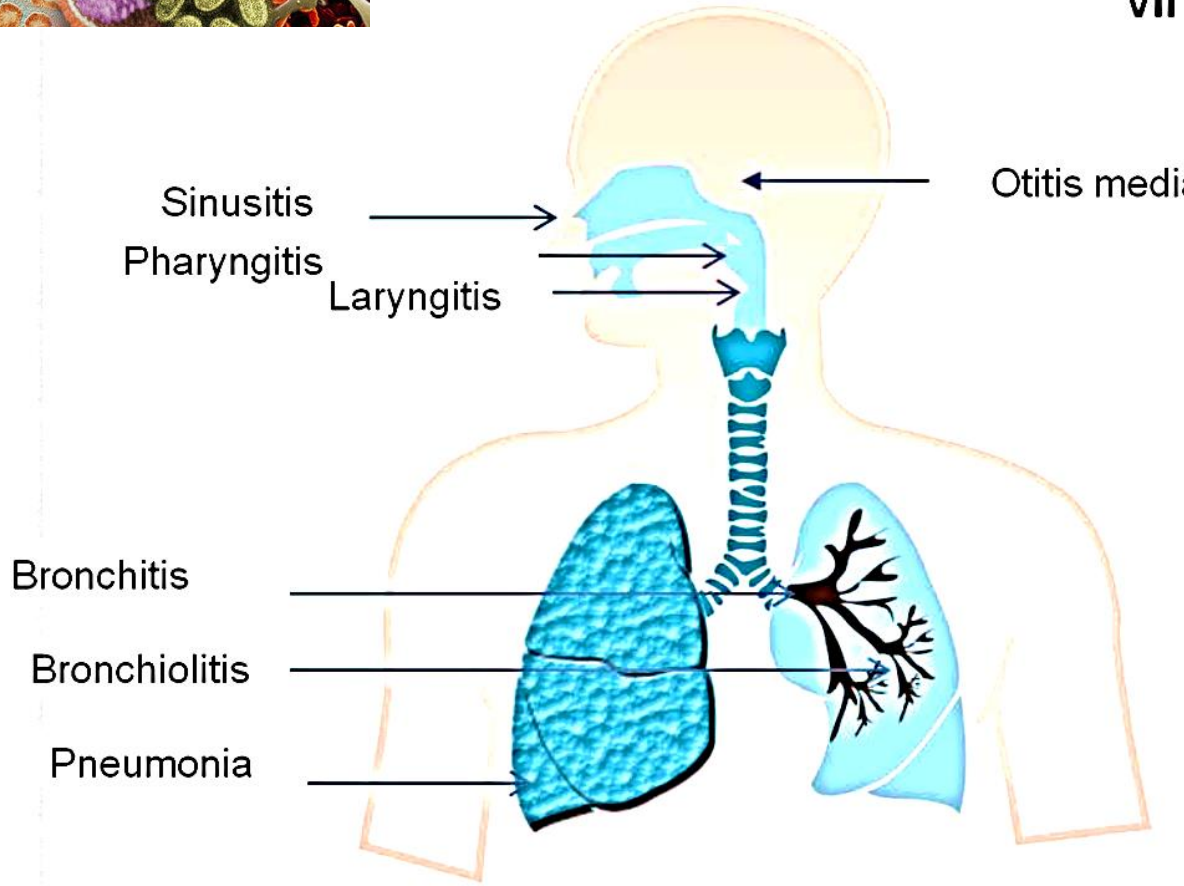
Table 2. Tests to be performed in expert laboratories for patients meeting the case definition

Test	Type of sample	Comments
In laboratories that have validated broad coronavirus RT-PCR assays it is advised to check the primers against the published 2019-nCoV sequence and check if primers are overlapping and have the capacity to detect the 2019-nCoV. On a positive results sequencing should be performed to determine the precise virus detected (e.g. on an amplicon of a non-conserved region).	Respiratory sample	Collect on presentation. Done by an expert laboratory.
NAAT for 2019n-CoV when it becomes available (assays currently under validation)	Respiratory sample	Collect on presentation. Done by an expert laboratory until validation has been finalized.
Whole genome sequencing	Respiratory sample	Collect on presentation. Done by an expert laboratory.
Serology, broad corona virus serology on paired samples if available.	Serum	Paired samples necessary for confirmation, the first sample collected in week 1 of illness and the second collected 3-4 weeks later. If a single serum sample can be collected, collect at least 3 weeks after onset of symptoms. Done by expert laboratory until more information on performance of available assays.





Diagnosi differenziale

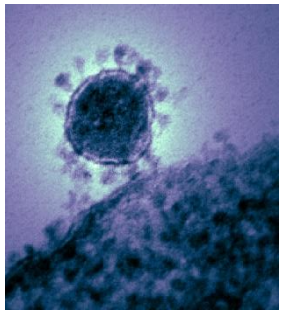


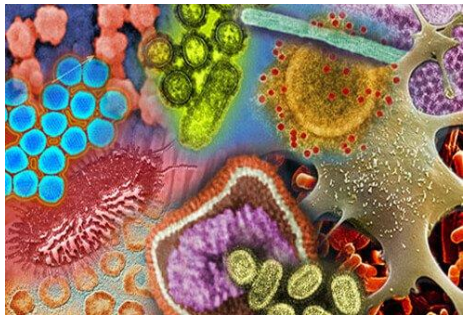
Viruses that infect the upper respiratory tract

Rhinovirus
Coronavirus
Influenza virus
Parainfluenza virus
Respiratory Syncytial virus
Herpesvirus
Adenovirus
Bocavirus
Coxsackivirus

Viruses that infect the lower respiratory tract

Influenza virus
Parainfluenza virus
Respiratory Syncytial virus
Adenovirus
Bocavirus
Metapneumovirus





Diagnosi differenziale

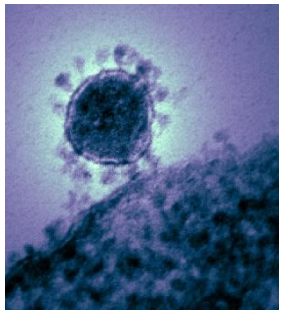
Ampia scelta, pannelli multiplex di varia composizione
TAT: tempistica dettata dalle policy di laboratorio (batching e frequenza sedute analitiche)

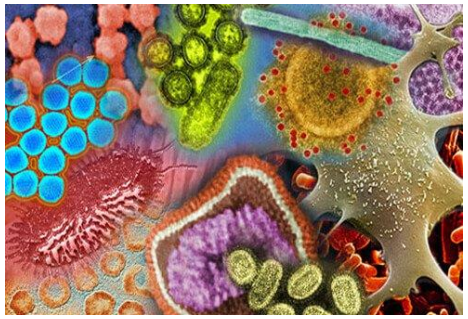
TABLE 1 | Commercially available nucleic acid amplification assays for respiratory pathogens[a].

Test	Manufacturer	Technology	Targets	Sample type[b]	Turnaround time	Complexity classification
NxTAG® respiratory pathogen panel	Luminex Corporation	Real-time RT-PCR	Multiplex Panel (20 targets)	NPS	~4 h	High
eSensor® respiratory viral panel (RVP)	GenMark Diagnostics	Multiplex microarray, competitive DNA hybridization	Multiplex Panel (14 targets)	NPS	~8 h	High
Verigene® RP flex	Luminex Corporation	RT-PCR & and microarray hybridization	Multiplex Panel (16 targets)	NPS	~2 h	Moderate
ePlex® respiratory pathogen (RP) panel	GenMark Diagnostics	RT-PCR	Multiplex Panel (17 targets)	NPS	~2 h	Moderate
FilmArray® respiratory panel (RP)	BioFire Diagnostics, Inc.,	Nested multiplex RT-PCR	Multiplex Panel (20 targets)	NPS	~1 h	Moderate
FilmArray® respiratory panel 2 (RP2)	BioFire Diagnostics, Inc.,	Nested multiplex RT-PCR	Multiplex Panel (21 targets)	NPS	~45 min	Moderate
FilmArray® respiratory panel® (RP) EZ	BioFire Diagnostics, Inc.,	Nested multiplex RT -PCR	Multiplex Panel (14 targets)	NPS	~1 h	Waived
Lyra® parainfluenza virus assay	Quidel Corporation	Real-time RT-PCR	Parainfluenza virus types 1, 2, and 3	NS, NPS	~4 h	High
Lyra® RSV + hMPV assay	Quidel Corporation	Real-time RT-PCR	RSV, hMPV	NS, NPS	~4 h	High
Simplexa™ flu A/B & RSV Kit	Focus Diagnostics, Inc.,	Real-time RT-PCR	Flu A, Flu B, and RSV	TS	<4 h	High
Panther fusion paraflu assay	Hologic, Inc.,	Real-time RT-PCR	Parainfluenza 1, 2, and 3	NPS	~2.5 h	High
Panther fusion AdV/hMPV/RV assay	Hologic, Inc.,	Real-time RT-PCR	Adenovirus, hMPV, and Rhinovirus	NPS	~2.5 h	High
ARIES® flu A/B & RSV assay	Luminex Corporation	Real-time PCR	Flu A, Flu B, and RSV	NPS	<2 h	Moderate
ARIES® bordetella assay	Luminex Corporation	Real-time PCR	*B. pertussis, B. parapertussis*	NPS	<2 h	Moderate
Simplexa™ flu A/B & RSV direct	Focus Diagnostics, Inc.,	Real-time RT-PCR	Flu A, Flu B, and RSV	NPS	<2 h	Moderate
Xpert® flu/RSV XC	Cepheid	Real-time RT-PCR	Flu A, Flu B, and RSV	NPS, NA, and NW	<1 h	Moderate
Solana RSV + hMPV assay	Quidel Corporation	Isothermal RT-helicase-dependent amplification (HDA)	RSV, hMPV	NS, NPS	~45 min	Moderate
Illumigene® mycoplasma direct DNA amplification assay	Meridian Bioscience, Inc.,	Loop-mediated isothermal DNA amplification (LAMP)	*Mycoplasma pneumoniae*	NPS, TS	<1 h	Moderate
Illumigene pertussis DNA amplification assay	Meridian Bioscience, Inc.,	Loop-mediated isothermal DNA amplification (LAMP)	*Bordetella pertussis*	NPS	<1 h	Moderate
Xpert® xpress Flu/RSV	Cepheid	Real-time RT-PCR	Flu A, Flu B, and RSV	NPS, NA, and NW	~30 min	Waived
Xpert® xpress flu	Cepheid	Real-time RT-PCR	Flu A, Flu B	NPS, NA, and NW	~30 min	Waived
cobas® Liat influenza A/B & RSV assay	Roche Molecular Diagnostics	Real-time RT-PCR	Flu A, Flu B, and RSV	NPS	~20 min	Waived
cobas® Liat influenza A/B assay	Roche Molecular Diagnostics	Real-time RT-PCR	Flu A, Flu B	NPS	~20 min	Waived
Alere i influenza A & B 2 test	Abbott Laboratories	Isothermal nucleic acid amplification	Flu A, Flu B	NPS, NS	<15 min	Waived
Alere i RSV	Abbott Laboratories	Isothermal nucleic acid amplification	RSV	NPS, NPS in VTM	<15 min	Waived

[a]FDA 510(k) Premarket Notification (FDA, 2018a). Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm; [b]NPS, nasopharyngeal swab; NS, nasal swab; NA, nasal aspirate; NW, nasal wash; TS, throat swab. [c]Waived for direct NS, NPS, and NA/NW specimens; Moderate for NS and NA/NW specimens eluted in transport media.

Das S, et al. Front. Microbiol 2018





Diagnosi differenziale

- Pannelli molecolari multiplex, rapidi ed automatizzati
- No estrazione separata
- Campioni singoli

} TAT ridotto a poche ore

Bacterial

- *Mycoplasma pneumoniae*
- *Legionella pneumophila*
- *Bordetella pertussis*

Viral

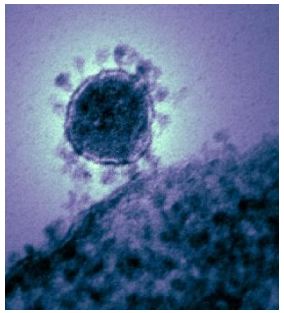
- *Influenza A*
- *Influenza A subtype H1N1/2009*
- *Influenza A subtype H1*
- *Influenza A subtype H3*
- *Influenza B*
- *Coronavirus 229E*
- *Coronavirus HKU1*
- *Coronavirus NL63*
- *Coronavirus OC43*
- *Parainfluenza virus 1*
- *Parainfluenza virus 2*
- *Parainfluenza virus 3*
- *Parainfluenza virus 4*
- *Respiratory Syncytial virus A/B*
- *Human Metapneumovirus A/B*
- *Adenovirus*
- *Bocavirus*
- *Rhinovirus/Enterovirus*

Altri patogeni riscontrati nella diagnosi differenziale

- Rino/entero
- Influenza A /H3 e H1)
- Influenza B
- Micoplasma pneumoniae
- Legionella
- CoV 229E

Bacteria (semi quantitative)	Antibiotic Resistance Genes
Acinetobacter calcoaceticus-baumannii complex	**ESBL**
Enterobacter cloacae	CTX-M
Escherichia coli	
Haemophilus influenzae	**Carbapenemases**
Klebsiella aerogenes	KPC
Klebsiella oxytoca	NDM
Klebsiella pneumoniae group	Oxa48-like
Moraxella catarrhalis	VIM
Proteus spp.	IMP
Pseudomonas aeruginosa	
Serratia marcescens	**Methicilin Resistance**
Staphylococcus aureus	mecA/mecC and MREJ
Streptococcus agalactiae	
Streptococcus pneumoniae	
Streptococcus pyogenes	

Atypical Bacteria (Qualitative)	Viruses
Legionella pneumophila	Influenza A
Mycoplasma pneumoniae	Influenza B
Chlamydia pneumoniae	Adenovirus
	Coronavirus
	Parainfluenza virus
	Respiratory Syncytial virus
	Human Rhinovirus/Enterovirus
	Human Metapneumovirus
	Middle East Respiratory Syndrome Coronavirus (MERS-CoV)*
	* MERS-CoV will only be available on the Pneumonia Panel *plus*



Risk assessment: Outbreak of acute respiratory syndrome associated with a novel coronavirus, Wuhan, China;
first update 22 Jan 2020

ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

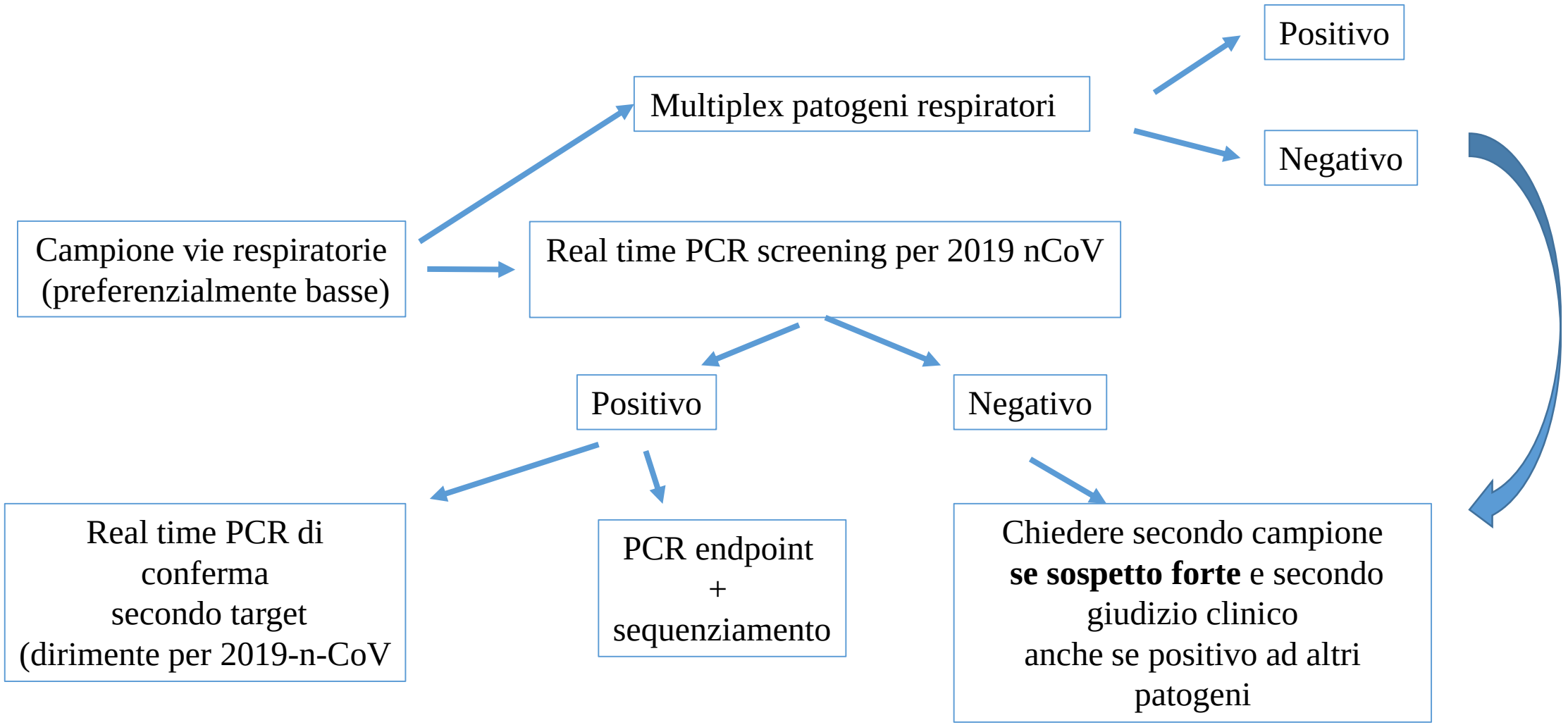
Diagnosi differenziale

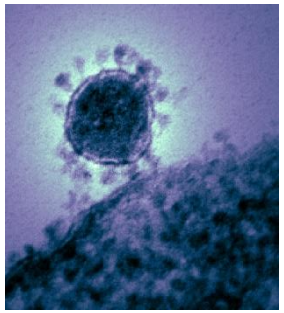
I sintomi più comuni consistono in febbre, tosse secca, mal di gola, difficoltà respiratorie. Sembra che il virus possa causare sia una forma lieve, simil-influenzale, che una forma più grave di malattia. E' indispensabile eseguire la diagnosi differenziale nei confronti dei più comuni patogeni pneumotropi.

Ma….

«A positive test result for another respiratory pathogen does not rule out a 2019-nCoV infection, as little is currently known about co-infections»

ALGORITMO DIAGNOSTICO per la ricerca molecolare diretta





Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected

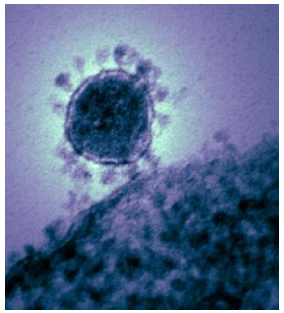
Interim guidance

January 2020

World Health Organization

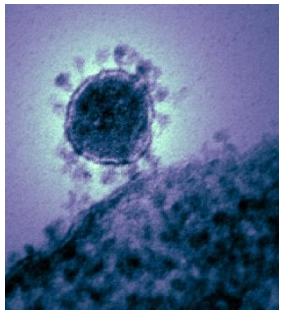
WHO/2019-nCoV/IPC/v2020.1

Le indicazione dell'OMS riguardo le misure di protezione da adottare nel laboratorio per la manipolazione dei campioni clinici raccomandano di utilizzare il livello di biosicurezza 2 associato alle precauzioni standard ed al corretto utilizzo dei dispositivi di protezione individuale, ponendo particolare attenzione alla protezione delle vie respiratorie nei casi particolari in cui ci fosse il rischio di generare aerosol, in questi casi è bene utilizzare un filtrante respiratorio FFP2.



Considerazioni sui livelli di biosicurezza

- La ricerca dell'acido nucleico virale viene effettuata con metodi molecolari
- I tamponi di lisi utilizzati per l'estrazione degli acidi nucleici (es. AVL + etanolo) inattivano l'infettività virale
- L'aggiunta del tampone di lisi al campione clinico deve essere effettuata ad un livello di biosicurezza commisurato alla valutazione del rischio; cappa BSCII
- L'isolamento del virus va effettuato in laboratorio a livello di biosicurezza 3
- La sieroconversione viene valutata attualmente mediante metodi non commerciali, il cui allestimento richiede la disponibilità di colture virali, e quindi di laboratorio a livello di biosicurezza 3

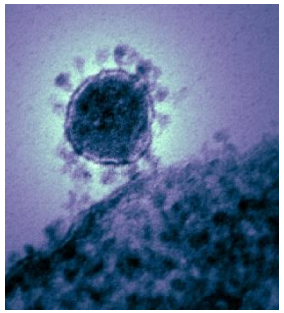


Risk assessment: Outbreak of acute respiratory syndrome associated with a novel coronavirus, Wuhan, China; second update 26 Jan 2020

Options for response might change when more epidemiological and clinical data become available.

Testing guidance for 2019-nCoV in the EU/EEA

- ECDC has developed a guidance document Laboratory testing of suspect cases of novel coronavirus (2019-nCoV) using RT-PCR for the EU/EEA Member States.



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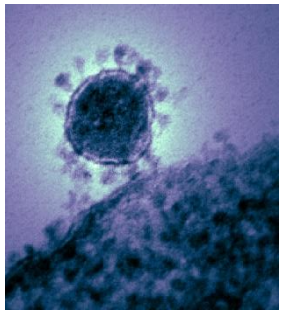
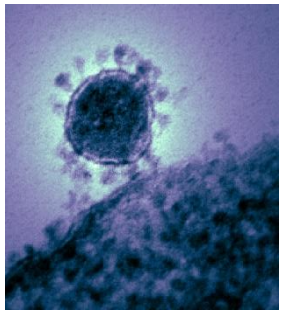
Testing guidance for 2019-nCoV in the EU/EEA

- ECDC has developed a guidance document Laboratory testing of suspect cases of novel coronavirus (2019-nCoV) using RT-PCR for the EU/EEA Member States.
- Laboratories are advised to implement molecular tests specific for 2019-nCoV, the RT-PCR test developed at the Institute of Virology, Charité, Berlin, or the RT-PCR test developed at the School of Public Health, Hong Kong University, both published on WHO's webpage.
- Synthetic positive controls can be obtained via the European Virus Archive global (EVAg) catalogue. ECDC plans to support the participation in an external quality assessment that will be offered through EVAg.
- Any positive test should be confirmed by a second RT-PCR test targeting a different 2019-nCoV gene.



Strategie diagnostiche

- Fuori dalle aree epidemiche:
  screening con 1 test seguito da conferma con un secondo test con target diverso e da caratterizzazione mediante sequenziamento (1 gene all'inizio e successivamente WGS)
- Nei luoghi di epidemia:
  screening con 1 test con target diverso e conferma con un secondo test solo per i casi dubbi.
  Utilizzo del WGS per scoprire cluster epidemici a supporto delle indagini epidemiologiche



The 2014 Ebola virus outbreak in West Africa highlights no evidence of rapid evolution or adaptation to humans

Until more genomic sequence data from this outbreak are obtained, **it is not possible to say more about the likelihood that this lineage will adapt to human populations**.

Taken together, continued genomic surveillance of the epidemic, especially from animal reservoirs sampled during the outbreak and across the geographical range, combined with epidemiological investigation and clinical recognition, are required to trace the history epidemic.

Li, X. et al. Sci. Rep. 6, 35822; 2016