

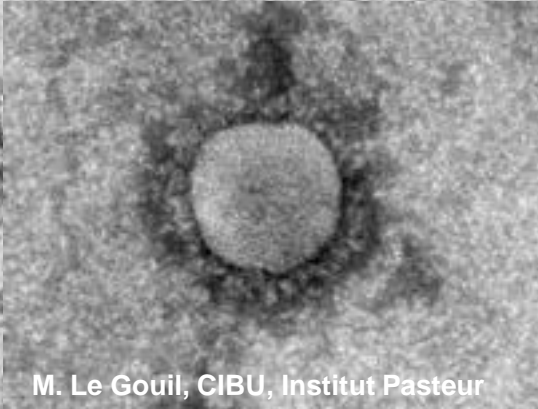
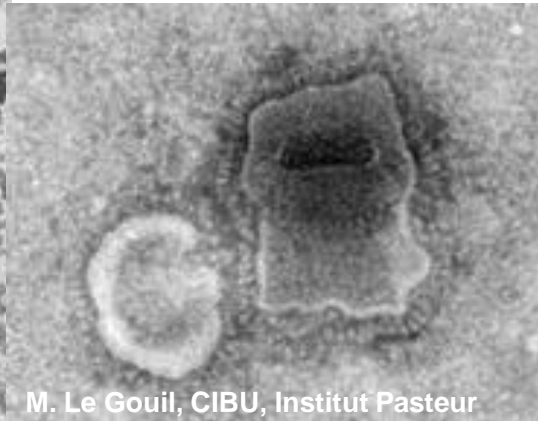
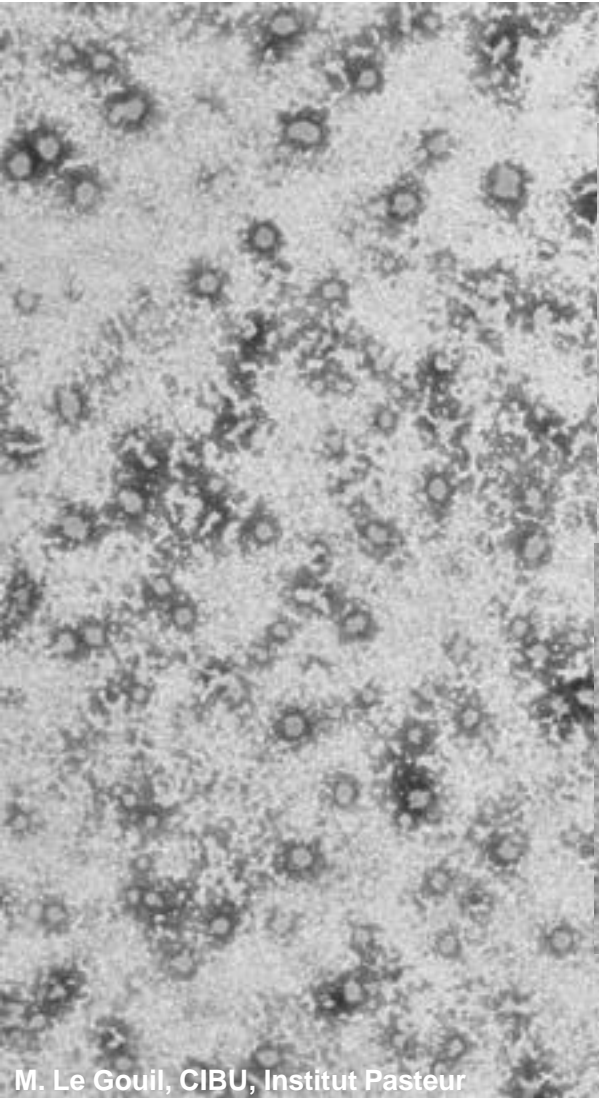
SARS-CoV 2

Human

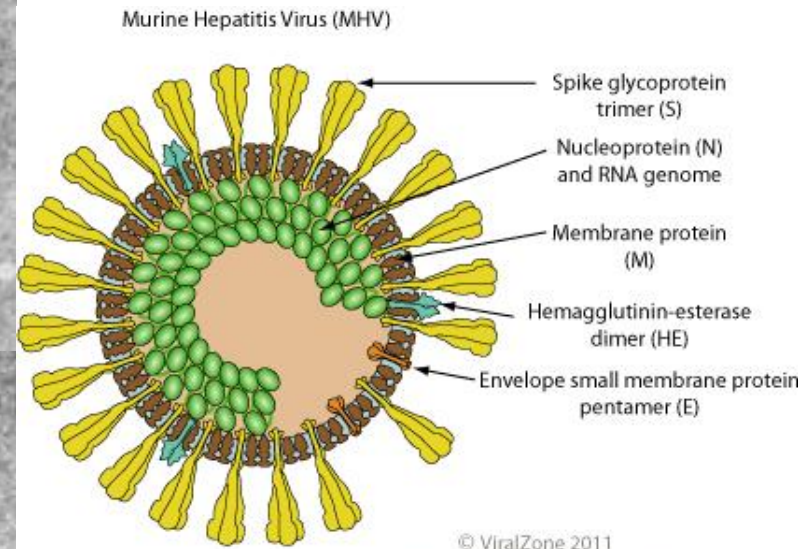
Diagnostics

**Jean-Claude MANUGUERRA, MediLAB Secure,
Institut Pasteur (IP), France**

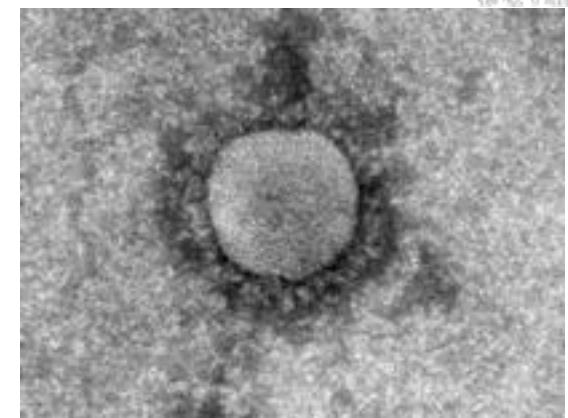
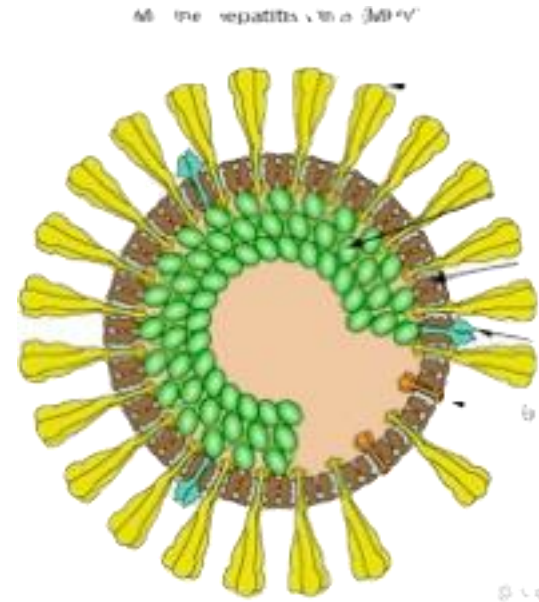
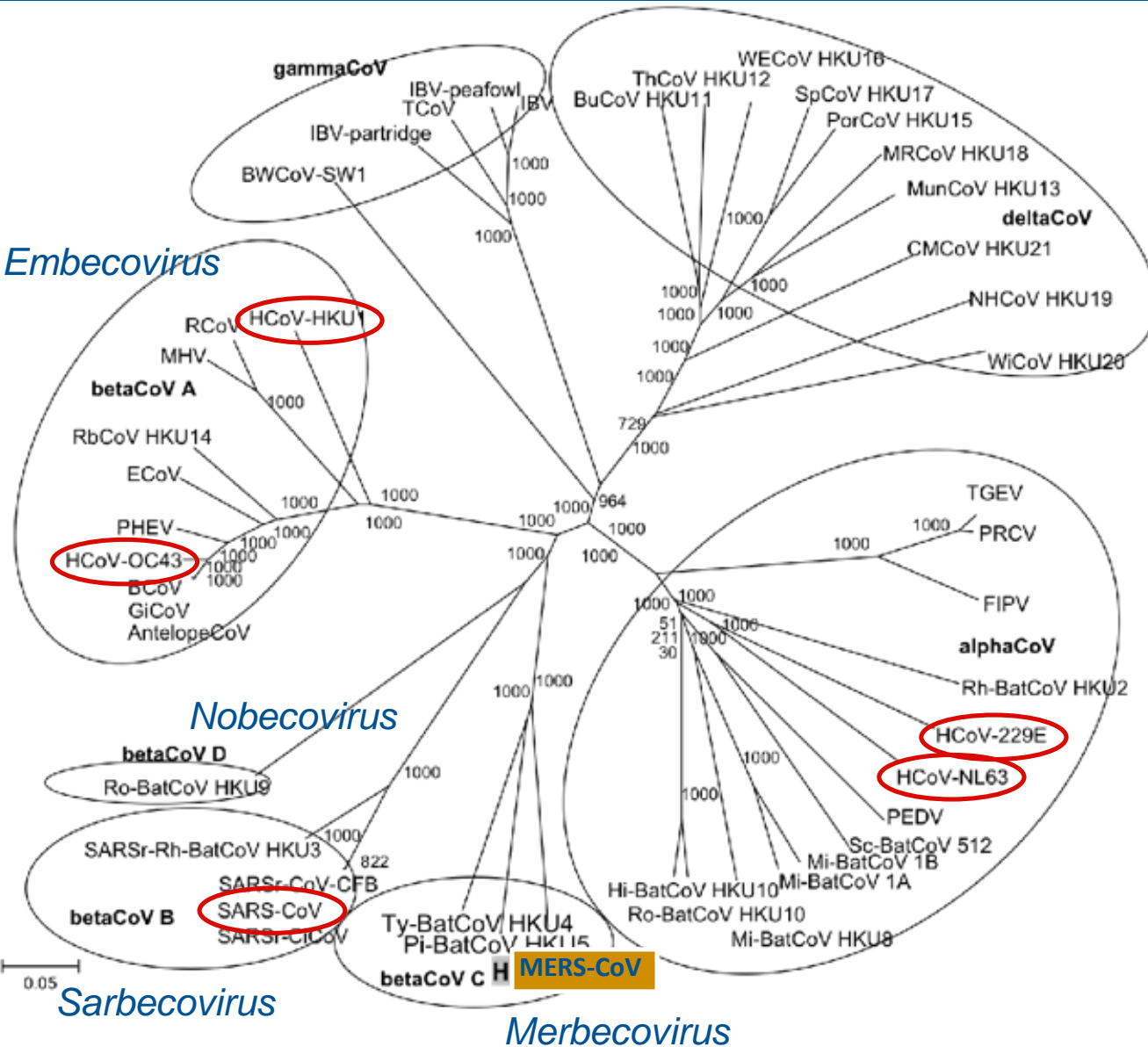
Morphostructure of coronaviruses



MERS-CoV (FRA2)



Coronaviridae



Current taxonomy of coronaviruses

ICTV

Google Custom Search

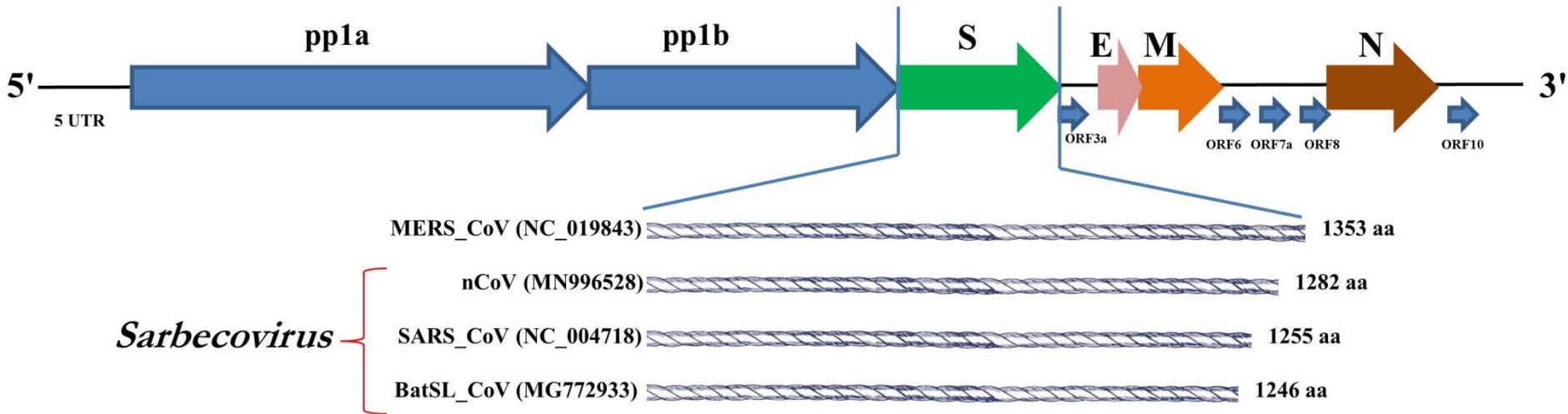
Home Information Taxonomy Files Discussions Study Groups Meetings ICTV Report Login/Join

-	Phylum: <i>Negamaviricota</i>	Realm: <i>Riboviria</i>	2 subphyla	history
-	Subphylum: <i>Haploviricotina</i>	Phylum: <i>Negamaviricota</i>	4 classes	history
-	Class: <i>Chunqiuviricetes</i>	Subphylum: <i>Haploviricotina</i>	1 order	history
+	Order: <i>Muvirales</i>	Class: <i>Chunqiuviricetes</i>	1 family	history
-	Class: <i>Milneviricetes</i>	Subphylum: <i>Haploviricotina</i>	1 order	history
+	Order: <i>Serpentovirales</i>	Class: <i>Milneviricetes</i>	1 family	history
-	Class: <i>Monjiviricetes</i>	Subphylum: <i>Haploviricotina</i>	2 orders	history
+	Order: <i>Jingchuvirales</i>	Class: <i>Monjiviricetes</i>	1 family	history
+	Order: <i>Mononegavirales</i>	Class: <i>Monjiviricetes</i>	11 families	history
-	Class: <i>Yunchangviricetes</i>	Subphylum: <i>Haploviricotina</i>	1 order	history
+	Order: <i>Goujianvirales</i>	Class: <i>Yunchangviricetes</i>	1 family	history
-	Subphylum: <i>Polyploviricotina</i>	Phylum: <i>Negamaviricota</i>	2 classes	history
-	Class: <i>Ellioviricetes</i>	Subphylum: <i>Polyploviricotina</i>	1 order	history
+	Order: <i>Bunyavirales</i>	Class: <i>Ellioviricetes</i>	12 families	history
-	Class: <i>Insthoviricetes</i>	Subphylum: <i>Polyploviricotina</i>	1 order	history
+	Order: <i>Articulavirales</i>	Class: <i>Insthoviricetes</i>	2 families	history
-	Order: <i>Nidovirales</i>	Realm: <i>Riboviria</i>	7 suborders	history
+	Suborder: <i>Abnidovirineae</i>	Order: <i>Nidovirales</i>	1 family	history
+	Suborder: <i>Amidovirineae</i>	Order: <i>Nidovirales</i>	1 family	history
-	Suborder: <i>Comidovirineae</i>	Order: <i>Nidovirales</i>	1 family	history
-	Family: <i>Coronaviridae</i>	Suborder: <i>Comidovirineae</i>	2 subfamilies	history
+	Subfamily: <i>Letovirinae</i>	Family: <i>Coronaviridae</i>	1 genus	history
-	Subfamily: <i>Orthocoronavirinae</i>	Family: <i>Coronaviridae</i>	4 genera	history
+	Genus: <i>Alphacoronavirus</i>	Subfamily: <i>Orthocoronavirinae</i>	12 subgenera	history
-	Genus: <i>Betacoronavirus</i>	Subfamily: <i>Orthocoronavirinae</i>	5 subgenera	history
+	Subgenus: <i>Embecovirus</i>	Genus: <i>Betacoronavirus</i>	4 species	history
+	Subgenus: <i>Hibecovirus</i>	Genus: <i>Betacoronavirus</i>	1 species	history
+	Subgenus: <i>Merbecovirus</i>	Genus: <i>Betacoronavirus</i>	4 species	history
+	Subgenus: <i>Nobecovirus</i>	Genus: <i>Betacoronavirus</i>	2 species	history
+	Subgenus: <i>Sarbecovirus</i>	Genus: <i>Betacoronavirus</i>	1 species	history
+	Genus: <i>Deltacoronavirus</i>	Subfamily: <i>Orthocoronavirinae</i>	4 subgenera	history
+	Genus: <i>Gammacoronavirus</i>	Subfamily: <i>Orthocoronavirinae</i>	2 subgenera	history
+	Suborder: <i>Mesnidovirineae</i>	Order: <i>Nidovirales</i>	2 families	history

Cellular receptors of coronaviruses

	Apha		Embeco		Sarbeco	Merbeco	Gamma
Virus	TGEV 229E	NL63	BcoV OC43	MHV	SARS- CoV	MERS-CoV	IBV
Receptor	Neu6GC Neu5Ac APN	ACE2	Neu5.9Ac	Neu5.9Ac Neu4.5 Ac CEACAM 1	ACE2	DPP4	Neu5Ac

The genome of SARS_CoV 2



Emerging novel Coronavirus (2019-nCoV) - Current scenario, evolutionary perspective based on genome analysis and recent developments

Yashpal Singh Malik , Shubhankar Sircar, Sudipta Bhat, Khan Sharun, Kuldeep Dhama , Maryam Dadar, Ruchi Tiwari & Wanpen Chaicumpa; <https://doi.org/10.1080/01652176.2020.1727993>

SARS-CoV 2 Diagnostics : Technics and Biosafety

- **SARS-CoV 2 antigen detection** →
 - ✓ Not widespread as yet
 - ✓ Possible cross-reaction with other coronaviruses
- **Isolation of Virus in Cell culture** (Caco-2, Vero, MRC5, LLC-MK2 cells) →
 - ✓ Slow
 - ✓ Manipulation require **BSL3** facility
- **Molecular detection : qRT-PCR**
Many assays currently used →
 - ✓ Fast
 - ✓ Well defined
 - ✓ WHO “approved”



Risk assessment necessary
briefly: all manipulation of non-inactivated
samples must be done under **BSL-3** biosafety
procedures (mandatory for virus culture) or “BSL2
+ respiratory protections” for molecular biology.

SARS-CoV 2 Diagnostics : Technics and Biosafety



Patient specimens from suspected or confirmed cases should be transported as **UN3373**, “Biological Substance Category B”.

Viral cultures or isolates should be transported as Category A **UN2814**, “infectious substance, affecting humans”

Laboratory biosafety guidance related to coronavirus disease 2019 (COVID-19): interim recommendations (12th Feb 2020)

SARS-CoV 2 Diagnostics :

Collection of specimens for laboratory diagnosis

- Collect blood cultures for bacteria that cause pneumonia and sepsis, ideally before antimicrobial therapy. DO NOT delay antimicrobial therapy to collect blood cultures.
- Collect specimens from **BOTH** the **upper** respiratory tract (URT; nasopharyngeal and oropharyngeal) **AND lower** respiratory tract (LRT; expectorated sputum (*To treat with mucolytic N-acetylcysteine (Digest-EUR®)*), endotracheal aspirate, or bronchoalveolar lavage) for 2019-nCoV testing by RT-PCR. Clinicians may elect to collect only LRT samples when these are readily available (for example, in mechanically ventilated patients).
- Serology for diagnostic purposes is recommended only when RT-PCR is not available

Clinical management of severe acute respiratory infection when Novel coronavirus (2019-nCoV) infection is suspected: Interim Guidance (WHO) (20200128)

Molecular assays to diagnose SARS-CoV 2

Several assays that detect the 2019-nCoV have been and are currently under development, both *in-house* and commercially. Some assays may detect only the novel virus and some may also detect other strains (e.g. SARS-CoV) that are genetically similar.

In-house developed molecular assays

Some groups shared their protocols which can be accessed below (Summary table and link to the protocols). In some cases, the groups will be willing to send reagents or reagent mixes prepared in their laboratories, with or without associated fees. It is strongly recommended to contact the developer if you need further assistance. The list is not exhaustive and is being updated regularly.

Summary table of available protocols

Country	Institute	Gene targets
China	China CDC	ORF1ab and N
Germany	Charité	RdRP, E, N
Hong Kong	HKU	ORF1b-nsp14, N
Japan	National Institute of Infectious Diseases, Department of Virology III	Pancorona and multiple targets, Spike protein
Thailand	National Institute of Health	N
US	US CDC	Three targets in N gene

[China CDC Primers and probes for detection 2019-nCoV \(24 January 2020\)](#)

[Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR – Charité, Berlin Germany \(17 January 2020\)](#)

[Detection of 2019 novel coronavirus \(2019-nCoV\) in suspected human cases by RT-PCR – Hong Kong University \(23 January 2020\)](#)

[PCR and sequencing protocol for 2019-nCoV - Department of Medical Sciences, Ministry of Public Health, Thailand \(Updated 28 January 2020\)](#)

[PCR and sequencing protocols for 2019-nCoV- National Institute of Infectious Diseases Japan \(24 January 2020\)](#)

[US CDC Real-Time RT-PCR Panel for Detection 2019-Novel Coronavirus \(28 January 2020\)](#)

[US CDC panel primer and probes– U.S. CDC, USA \(28 January 2020\)](#)

<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance>

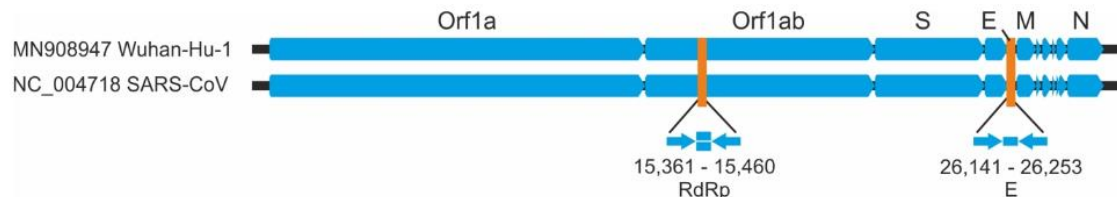
Molecular assays to diagnose SARS-CoV 2

Diagnostic detection of 2019-nCoV by real-time RT-PCR-Protocol and preliminary evaluation as of Jan 17, 2020

Victor Corman, Tobias Bleicker, Sebastian Brünink, Christian Drosten Charité Virology, Berlin, Germany

All assays can use SARS-CoV genomic RNA as positive control. Synthetic control RNA for 2019-nCoV E gene assay is available via EVAg. Synthetic control for 2019-nCoV RdRp is expected to be available via EVAg from Jan 21st onward.

First line screening assay: E gene assay
Confirmatory assay: RdRp gene assay

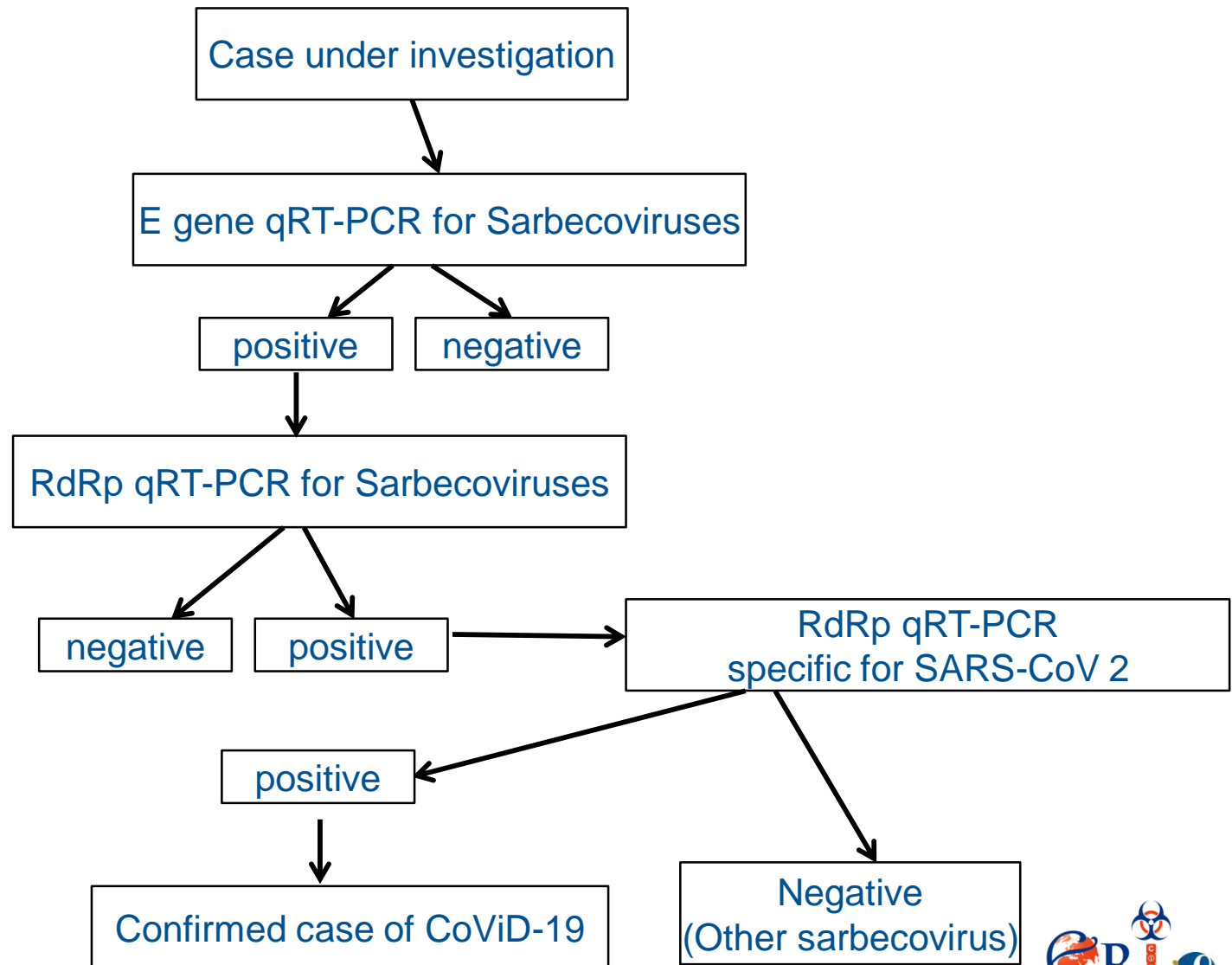


<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance>

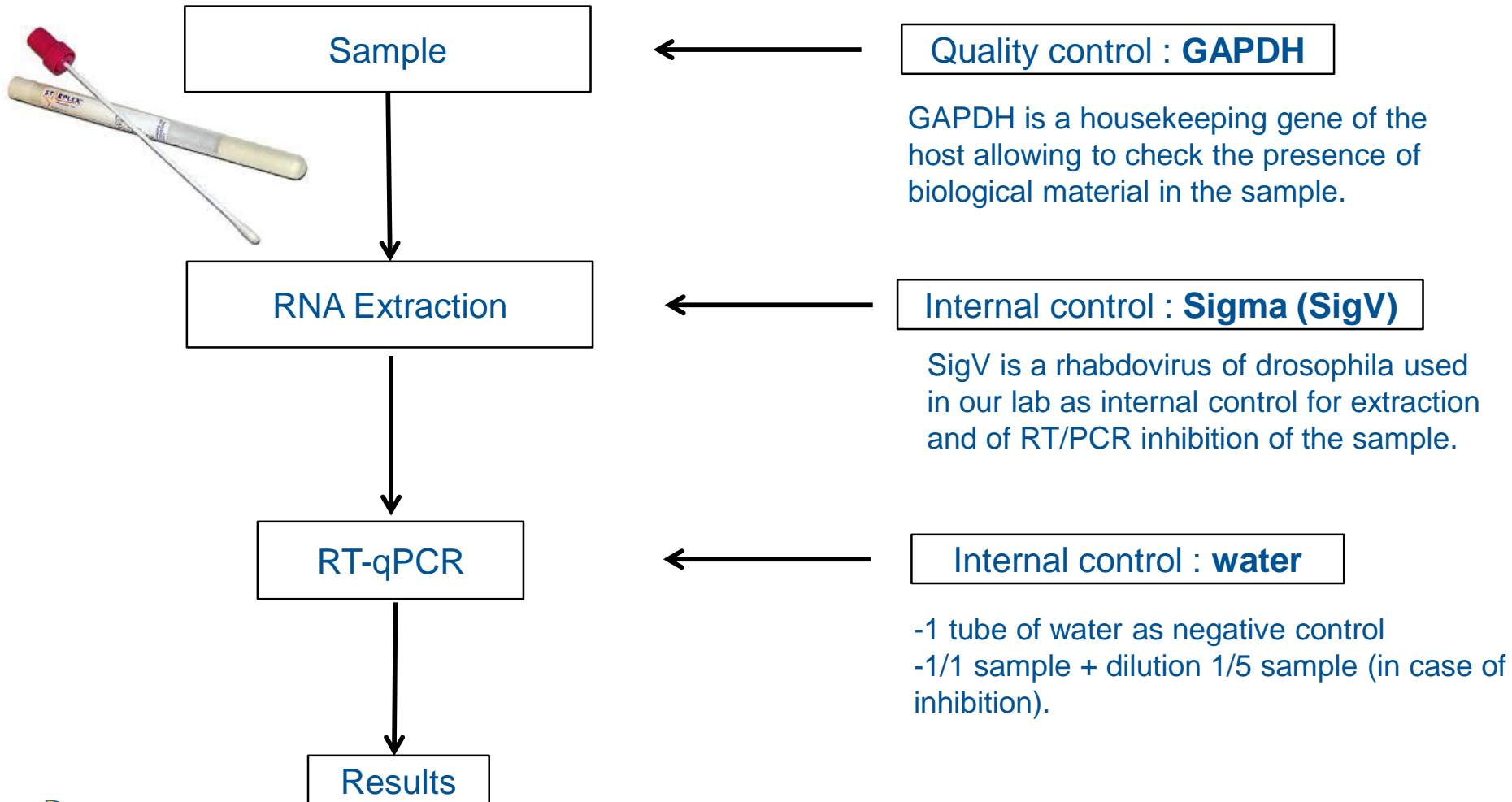
Figure 1 relative positions of amplicon targets on SARS-CoV and 2019-nCoV genome. ORF: open reading frame; RdRp: RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to SARS-CoV, NC_004718.

Algorithm for cases under investigation for SARS-CoV 2 infection for the Corman et al. assays

Algorithm as of
17th Jan 2020



SARS-CoV 2 Diagnostics : Quality Controls



Acknowledgements



Unité de recherche et d'expertise Environnement et risques infectieux
Cellule d'Intervention Biologique d'Urgence
(Laboratory for Urgent response to biological threats)
Institut Pasteur

Charlotte Balière, Justine Basset, **Christophe Batéjat**, Nicolas Berthet, **Valérie Caro**, Valérie Choumet, **Maxence Feher**, **Frédéric Fichenick**, **Quentin Grassin**, **Charlotte Guy**, **Damien Hoinard**, **Aurélia Kwasiborski**, **Anne Le Flèche**, India Leclercq, **Fabienne Lomprez**, **Jean-Claude Manuguerra**, **Guillain Mikaty**, Nathalie Pardigon, **Mathias Vandenberg**, **Jessica Vanhomwegen**.

Thank you for your attention!