Safety of Grifols Plasma Derivatives with Regard to 2019 Coronavirus

The 2019 Coronavirus

Coronaviruses (CoVs) are enveloped RNA viruses that are distributed broadly among humans and other mammals, as well as birds, and cause respiratory, enteric, hepatic and neurologic disease (1). In 2019, a coronavirus was identified in Wuhan, China, that was associated with cluster of pneumonia cases. The World Health Organization has designated the respiratory disease associated with this coronavirus as COVID-19 (2). The virus, initially called 2019-nCoV, has officially been named severe acute respiratory syndrome coronavirus 2, or SARS-CoV-2 (2,3), based on taxonomic similarity to an earlier coronavirus.

SARS-CoV-2 is a β-coronavirus in the family *Coronaviridae*. It is generally spherical in shape, with a diameter ranging from 50 to 200 nm, and possesses a single positive-sense RNA genome (4). SARS-CoV-2 is the third coronavirus in the past two decades to cross species and to infect humans. The previous two coronaviruses to jump species were the severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 (5).

COVID-19 Disease

Common symptoms at onset of illness after infection are fever, cough and myalgia or fatigue (6). Infection may lead to pneumonia and can result in severe and even fatal respiratory diseases such as acute respiratory distress syndrome (4).

While animals are the source of the virus, this virus is now spreading from one person to another (human-to-human transmission). There is currently limited epidemiological information to determine how easily and sustainably this virus spreads between people. Person-to-person spread occurs via respiratory droplets from people coughing, sneezing or direct contact (7).

The incubation period (i.e. the time between exposure to the virus and onset of symptoms) is currently estimated at 1 to 14 days (5,7), but it remains unclear whether those without symptoms can spread the virus. Estimating the extent to which asymptomatic or pre-symptomatic transmission occurs is an urgent priority as it has implications for public health and hospital infection control (8).

It is important to note that the fatality rate from pneumonia-related disease is much lower in COVID-19 (less than 3%) than was observed with SARS (~10%) or has been seen with MERS (~40%) (5). Information on case severity and the effectiveness of control measures continues to develop; as of 27 February 2020 82,132 cases of COVID-19 and 2,801 deaths have been reported worldwide (9). The vast majority of cases are in China.

SARS-CoV-2 and Blood or Blood-Components

There is currently little information about the presence of SARS-CoV-2 in blood or blood-components of infected people; however, the presence of SARS-CoV-2 RNA has been reported at low level in plasma from a small portion (15%) of hospitalized patients showing clinical symptoms (6). It is important to note that the presence of RNA is not synonymous with the presence of infectious virus.

Routine donor screening measures that are already in place should prevent individuals with clinical respiratory infections from donating. For example, blood donors must be in good health

and have a normal temperature on the day of donation (10). In addition to the aforementioned health screening measures, source plasma donors are repeat donors, so if any indication of illness is detected, previous plasma donations in the inventory hold period of not less than 60 days can be identified and removed.

While blood-borne transmission cannot be theoretically discarded, respiratory viruses, in general, are not known to be transmitted by blood transfusion (10). No transmission by blood has been documented for SARS-CoV-2, nor have there been any documented or reported cases of transmission of MERS-CoV or SARS-CoV by blood or blood products (11).

Inactivation/removal of Coronaviruses

Coronaviruses are sensitive to physicochemical conditions that serve to inactivate the virus. These conditions include heat (12,13,14), pH (15), inactivation by chemical agents such as detergents (14), organic chemicals, formaldehyde, oxidizing agents, and methylene blue treatment (16).

Thus, the specific steps with inactivation capacity of enveloped viruses in Grifols plasma protein manufacturing processes, such as pasteurisation (17), heat treatment and solvent/detergent treatment (18), will have similar efficacy against an enveloped virus such as SARS-CoV-2.

Also, manufacturing steps that serve to purify the product by partitioning, such as precipitation by PEG or by Cohn-process cold ethanol fractionation (19), along with dedicated steps with virus removal capacity, including nanofiltration (20), provide, by independent mechanism of action, additional clearance capacity for viruses such as SARS-CoV-2.

Virus Clearance Capacity of Grifols Manufacturing Processes for Enveloped Viruses

Grifols manufacturing processes include specific steps to inactivate or remove a theoretical potential viral load in the plasma manufacturing pool.

The capacity of the manufacturing processes to inactivate and/or remove viruses has been evaluated in virus safety studies. These studies were performed using relevant or models of human viruses that exhibit a wide range of physico-chemical properties that might challenge the virus clearance capacity of the process. In these studies Grifols included the validation of the capacity to inactivate/remove flaviviruses, such as bovine viral diarrhea virus (BVDV) and/or West Nile virus (WNV), for all its products. SARS-CoV-2 shares physico-chemical characteristics with flaviviruses; both being lipid-enveloped RNA viruses with medium size. Accordingly, it is expected that SARS-CoV-2 is inactivated or removed with the same efficacy as these model viruses.

Through virus clearance capacity studies, the steps that have been shown to be especially effective against enveloped viruses are:

Pasteurization: this heat treatment at 60°C for 10 hours is considered a highly effective treatment to inactivate viruses without or (especially) with a lipid envelope.

Solvent-Detergent treatment: this is a very effective and very robust method to inactivate lipid-enveloped viruses. The solvent-detergent mixture damages the lipid envelope of the virus preventing virus replication.

Freeze Drying and Heat treatment: (80ºC, 72 hours): This step in the production process has been demonstrated to be very effective against enveloped viruses.

Low pH Incubation: Incubation at low pH is an effective step for enveloped virus inactivation.

Precipitation and Filtration: Precipitation, such as by polyethylene glycol (PEG) or by cold ethanol (Cohn fractionation) followed by filtration has been shown to be an effective method of removal for enveloped viruses.

Nanofiltration: The mechanism of action for virus removal by these filters is through retention of particles larger than the filter pore. The nanofilters used in the various manufacturing processes all have an effective pore size of 35 nm or less. As SARS-CoV-2 is much larger (50-200 nm), nanofiltration will effectively remove this virus. Removal of other enveloped viruses with similar characteristics in the range of 40 to 200 nm at laboratory scale experiments has been demonstrated (20).

It is also important to note that all Grifols manufacturing processes include at least two steps with effective (≥4 log) clearance capacity for enveloped viruses.

Summary

There have been no reported transmissions of enveloped viruses by plasma protein therapies since the introduction of specific steps with inactivation/removal capacity for enveloped viruses in more than 20 years of widespread clinical use.

No case of SARS-CoV-2 transmission associated with blood transfusion has been reported. Furthermore, as stated by the US FDA, respiratory viruses, in general, are not known to be transmitted by blood transfusion (10). In the hypothetical case of SARS-CoV-2 being present in a plasma manufacturing pool, this virus would be easily and efficiently inactivated or removed by the steps performed during the manufacturing processes. Thus, the current data suggests that the coronavirus responsible for COVID-19 disease, SARS-CoV-2, does not pose a threat to the safety of Grifols plasma products.

The data presented here should alleviate concerns about the safety of plasma derivatives with regard to SARS-CoV-2. Nevertheless, Grifols will continue to be vigilant as the COVID-19 situation develops and to follow the recommendations of Public Health authorities.

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