Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Commentary

Definitions for coronavirus disease 2019 reinfection, relapse and PCR re-positivity

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Introduction

By the beginning of November 2020, almost 50 million cases of coronavirus disease 2019 (COVID-19) had been reported worldwide, with over 35 million people defined as recovering from the disease [1]. According to the Centers for Disease Control and Prevention (CDC), updated on 10 September 2020, there were no confirmed reports to date of a person being reinfected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) within 3 months of the initial infection [2]. In the absence of solid human data, Rhesus monkeys were challenged 28 days after first SARS-CoV-2 infection with same virus strain and did not establish reinfection [3]. However, on 21 September 2020 the European CDC issued a report addressing SARS-CoV-2 reinfection, citing several case studies and calling for a case definition [4].

Defining reinfection, relapsed infection and recurrence of positive (re-positive) nucleic acid detection might have clinical and epidemiological implications for treatment and infection control measures, respectively. In this commentary, we aimed to provide such definitions, including a microbiologically confirmed definition of reinfection, as well as clinical and epidemiological ones.

Reinfections are observed with many respiratory viruses, including human coronaviruses. Reinfections with respiratory viruses may be due to weak or waning initial immune response (e.g. respiratory syncytial virus), reinfection with another genotype/species (e.g. rhinoviruses) or the high variability of the viruses (e.g. influenza virus). Following the SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV) epidemics, specific antibodies were detected in survivors up to 24 and 34 months, respectively. The negligible risk of a second exposure to these viruses has not allowed for a clinical determination as to the immunity against reinfection [5,6]. For endemic coronaviruses, immunity was shown to be temporary, lasting from several months to a few years, and reinfection has been reported after experimental and natural infection [7]. It is assumed that the immune response following a natural viral infection is incomplete and reinfections are

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possible [8]. Currently tested COVID-19 vaccine platforms such as RNA and viral vector vaccines are designed to elicit antibody and T-cell responses, whereas subunit vaccines are more restricted to antibody responses, precluding the effect of active effector T cells and memory T cells. However, it is one of the subunit vaccine candidates that shows so far the highest neutralizing antibody titres and it is still unclear if a future COVID-19 vaccine will provide sustained and sterilizing immunity.

SARS-CoV-2 PCR re-positivity and COVID-19 relapse within 90 days after symptom onset

Several publications have described cases of re-positive SARS-CoV-2 PCR following negative tests and clinical convalescence, or recurrent symptoms, compatible with COVID-19, along with a positive RT-PCR, in recovering patients. These cases were described in a time frame of up to 90 days from acute illness, and may represent persistent/fluctuant viral shedding with persistent or recurring clinical illness, rather than true reinfection. Other viral infections or bacterial/fungal superinfections may also explain a coinciding symptomatic episode in the presence of remnant SARS-CoV-2 RNA in some of these occurrences [9]. In addition, weakly re-positive PCR (cycle threshold (Ct) values > 35) results probably do not reflect true active infection, but rather non-replicating virus [10].

The clinical course of illness among hospitalized COVID-19 patients might be prolonged in patients with severe and critical disease. The median hospitalization duration in a large series from China was reportedly 22 days among survivors, with viral shedding for a median of 20 days and as long as 37 days [11]. In other reports, viral shedding from upper respiratory specimens has been detected up to 12 weeks. According to CDC, positive PCR during a 90-day time frame probably represents prolonged shedding rather than reinfection. However, replication-competent virus has only rarely been detected up to 10–20 days, and even then mostly in cases of severe COVID-19, although even longer times have been reported for patients in an immunocompromised state [2].

In addition to prolonged shedding, re-positive PCR tests have been described in 14% of discharged patients [12]. An investigation of 285 re-positive cases by the Korean CDC demonstrated neutralizing antibodies in all patients, negative cultures in all 108 tested and Ct values > 30 in 68 (89.5%) of 76 tests [13]. Careful analysis of viral load is essential to interpret all these studies and to enable comparison between results, they should be expressed in standardized copy numbers not Ct values.

COVID-19 reinfection

In August 2020, To et al. described an asymptomatic patient from Hong Kong with a positive SARS-CoV-2 PCR test from a sample collected 142 days after a first symptomatic COVID-19 episode. The second sample was collected at airport screening following travel to Europe. Whole-genome sequencing showed a virus from a different clade/lineage. Seroconversion of SARS-CoV-2 IgG occurred only during the second episode. This case was summarized by the authors as reinfection of SARS-CoV-2 [14]. Two other cases of possible reinfection were reported from the USA. In these cases, a second more severe illness presented ~2 months following a first confirmed COVID-19 episode in patients with subsequent household exposure. Genome sequencing identified several potential variations between the viruses in the separate episodes, and the cases were concluded to be reinfections [15,16].

Both humoral and cell-mediated immunity are necessary to elicit protective immunity against SARS-CoV-2 reinfection. Whether most infected persons mount sufficient and sustained immune memory responses, such as neutralizing antibodies and memory T cells, remains unknown. Long-term studies evaluating persistence of SARS-CoV and MERS-CoV-specific IgG antibody responses more than 1 year after infection found that antibody titres were waning over time. However, the presence of antibodies does not necessarily equal protective immunity, especially if the neutralizing activity of the antibodies is not known or is known to be low or absent [17]. Specifically for SARS-CoV-2, some earlier studies reported rapid waning of antibody responses, attenuated after 90 days. However, a recent longer follow-up study showed that antibody titres remain stable over 4 months [18].

Several different clades and subclades of SARS-CoV-2 have been described, with prevalence varying depending on the geographical location. Thousands of distinct variants of the virus, consisting of various mutations, were reported, including over 400 variants in the spike protein. It is possible that immune pressure will select for novel strains that are able to evade immune responses to a previous infection [19].

It is not clear whether reinfection might be a more severe or milder event. A more severe event may occur as the result of antibody-dependent enhancement, or it may depend upon the virulence of the strain and its inoculum [15,16]. Observations of more severe cases may also reflect a selection bias towards testing symptomatic cases [20]. On the other hand, even a non-sterilizing immune response could be protective against severe disease. A milder event of reinfection was described by To et al., explained by priming of the adaptive immune response during the first episode [14].

We cannot expect to be able to invest the same efforts to prove reinfections in three scenarios: a definite proof that the phenomenon exists; proof in patients, in a clinical situation; and proof for epidemiological purposes. For each of these purposes, we might need another definition.

The European CDC has proposed a flow chart for assessing reinfection in previously confirmed COVID-19 cases [4]; however, the assessment includes unavailable tests, such as whole-genome sequencing and phylogenetic analysis. For practical reasons, we propose here a laboratory definition for proven SARS-CoV-2 reinfection, and separately, we propose clinical and epidemiological definitions (see Table 1 for summary):

SARS-CoV-2 reinfection

Confirmed diagnosis of reinfection

A confirmed diagnosis of reinfection will require:

(a) Confirmation of a true first episode—description of the viral load of the first episode is necessary (Ct values > 35 might imply possible contamination rather than true infection). Re-testing of the original specimen is indicated whenever possible.

(b) Proof of a reinfection with two positive SARS-CoV-2 RT-PCR tests with Ct < 35 (or proof of replicating virus by cell culture or detection of subgenomic RNA) at different time-points.

Plus:

(c) Confirmation of infection with two different phylogenetic strains by high-throughput sequencing, corresponding to local epidemiology (proof of two distinct virus variants with any sequence variation between the two episodes).

Plus:

At least one, and ideally two, negative RT-PCR tests, on two different specimens collected between the first and second episodes, should be documented.

For clinical practice: reinfection may be defined as clinical recurrence of symptoms compatible with COVID-19, accompanied by positive PCR test (Ct < 35), more than 90 days after the onset of the primary infection, supported by close-contact exposure or

Table 1
Coronavirus disease 2019 reinfection, relapse and PCR re-positivity definitions

Variable	Confirmed reinfection	Clinical reinfection	Epidemiological reinfection	Relapse/ reactivation	Repositivity
Clinical symptoms	Characteristic clinical symptoms[a]	Characteristic clinical symptoms[a]	Asymptomatic/ symptomatic	Characteristic clinical symptoms[a]	Asymptomatic
PCR	Positive	Positive	Positive	Positive	Positive
Viral culture (should one be performed)	Positive	Positive	Positive	Positive	Negative
Time frame from original infection	>90 days[b]			<90 days	<90 days
Isolation measures	Recommended	Recommended	Recommended	Should be considered	Not recommended
Additional findings	Viral RNA sequencing from both episodes show different strains	Epidemiological risk factor (known exposure or outbreak setting), no other cause	Epidemiological risk factor (known exposure or outbreak setting)	No new exposure, area of low community spread	—

[a] Clinical manifestations characteristic of coronavirus disease 2019 (COVID-19).
[b] Could be considered in the event of under 90 days if recovery proven by two consecutive negative PCR tests and current known COVID-19 exposure.

outbreak settings, and no evidence of another cause of infection. In the presence of epidemiological risk factors (i.e. significant exposure), reinfection should be considered during the first 90 days, if clinical symptoms of the first episode resolved and two PCR tests were negative before the new episode. Viral culture, if collected, is expected to be positive.

Serology does not play a factor in the reinfection definition and could be either positive or negative after the first infection. A negative serology indicates either the absence of potent detectable immune response or antibody waning. Positive serology indicates that neutralizing antibody titres were not sufficient to eliminate the viral inoculum or that the infecting virus is substantially different to the first infection and is not recognized by the antibodies.

For epidemiological purposes: reinfection could be defined as any positive RT-PCR test (Ct values < 35) more than 90 days from first episode, regardless of symptoms. Since confirmation by genotypic assays is time and resource consuming, any case of suspected reinfection should be considered for isolation. Regarding RT-PCR re-positivity within 90 days, further studies performing genotypic assays of first- and second-episode specimens are needed to define reinfection during this period.

COVID-19 relapse

COVID-19 relapse (also described in the literature as 'recrudescence' or 'recurrence' or 'reactivation')—clinical recurrence of symptoms compatible with COVID-19—accompanied by positive/persisting RT-PCR within 90 days of primary infection, and supported by the absence of epidemiological exposure or another cause of the illness. Viral culture may be positive. Demonstration of same strain by whole-genome sequencing could definitively differentiate this entity from reinfection (confirmed relapse). Hence, further research is needed to decide whether these cases should be considered for isolation. An inflammatory syndrome, as a result of inappropriate immune response, has been suggested as an alternative explanation by Gousseff et al. [9]. However, as the authors concluded, positive PCR for all patients and positive viral cultures for some, support viral disease.

SARS-CoV-2 RT-PCR re-positivity

SARS-CoV-2 RT-PCR re-positivity describes positive RT-PCR following negative tests in an asymptomatic patient up to 90 days from the first episode. These cases probably do not represent replicative virus and do not necessitate isolation. Low viral load is usually a feature of re-positivity.

The next few months will probably reveal the extent of reinfections. Fortunately, to date, it seems that reinfection is a rare event. Further research is needed to provide more accurate definitions in terms of time frames, genetic changes definitions and serological status. In addition, infection control measures should be considered for cases of reinfection and relapse.

Transparency declaration

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Author contributions

All authors contributed to the writing the manuscript and reviewed the final version.

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