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Letter to the Editor

Importance of sample dilution in the evaluation of the antibody response after SARS-CoV-2 vaccination

Dear Editor,

We read with great interest the recently published article of Tré-Hardy et al. reporting on the time-related changes in the serological response of healthcare workers having received the mRNA-1273 vaccine.[1] Among 205 individuals, 161 (78.5%) were initially seronegative at baseline while 44 (21.5%) already developed antibodies directed against SARS-CoV-2. The antibody response was assessed 2 weeks after the first vaccine injection (T1), 2 weeks after the second vaccine injection (T2) and 3 months after the first injection (T3). The quantitative analysis of the anti-SARS-CoV-2 IgG antibodies directed against the subunits (S1) and (S2) of the virus spike protein was carried out using the LIAISON® SARS-CoV-2 IgG kit (DiaSorin®, Saluggia, Italy). Almost all samples at T1 and all samples at T2 and T3 in the seropositive cohort were above the maximum quantification value of the assay kit, i.e. >400 AU/mL on neat samples. In the discussion, the authors reported that, in previously seropositive subjects ($n = 44$), no drop in antibody between T2 and T3 was observed.

In order to share our experience on that important topic, we would like to present the results we obtained 3 months post-vaccination in the CRO-VAX HCP study (EudraCT registration number: 2020–006,149–21), an ongoing multicenter study in healthcare workers having received BNT162b2, another mRNA vaccine (Pfizer-BioNTech, Mainz, Germany).[2] Among the 200 individuals who were followed up to 3 months, 58 (29%) were seropositive and 142 (71%) were seronegative at baseline.[3] Antibodies against the SARS-CoV-2 receptor binding domain of the S1 subunit of the spike protein (anti-S; Elecsys® anti-SARS-CoV-2 spike quantitative ECLIA, Cobas 801, Roche Diagnostics®, Machelen, Belgium) were measured. As the Roche system permits samples dilution to increase the range of measurement, we diluted our samples 10 or 100 times when signal was out of range according to the manufacturer recommendations. Similar timepoints as Tré-Hardy et al., i.e. baseline, 14 days, 42 days and 3 months, were collected in our cohort and analyzed.

Using neat or 10-fold diluted samples, we did not observe an antibody drop in seropositive individuals between T1, T2 and T3 ($p > 0.05$, Fig. 1), a finding which is similar to that of Tré Hardy et al.[1] However, a highly significant drop in antibody titers was observed at 3 months if a 100-fold dilution was performed ($p < 0.001$; from 16,935 U/mL to 8919 U/mL; a decrease of 47.3%) (Fig. 1). Such dilution factor permits to increase the range of measurement until 25,000 U/mL on the Roche assay. Considering seronegative individuals, a highly significant antibody drop was also shown when a 10- or 100-fold dilution was applied ($p < 0.001$, Table 1). The application of a 10 or 100-fold dilution (depending on the sample) with our kit permits to show an important difference between the previously seronegative and seropositive subjects (1863 U/mL versus 15,856 U/mL at day 42 and 1262 U/mL versus 8919 U/mL at 3 months), a difference which is not observed when neat samples are used (Table 1). Analytical kits that do not allow a wide range of measurement may thus hide a difference of serological response between previously seronegative and seropositive subjects and does not permit to appreciate the drop in antibody titers in both groups (Table 1).

Currently, data about the long-term kinetics of antibodies in vaccinees are scarce. Two studies found an time-dependent antibody decline with the mRNA-1273 vaccine in only 33 and 34 participants while Tré-Hardy et al. followed more than 200 subjects.[4,5] Nevertheless, in previous investigations, sample dilutions were applied to allow a better discrimination between previously seronegative and seropositive subjects.[6–9] Compared to the antibody response observed in past-COVID-19 patients, where none or few samples needed to be diluted,[2,9,10] the antibody response in vaccinees is significantly higher and will certainly require dilutions to obtain the real quantitative value with some assays (i.e. not rounded to the upper limit of measurement).

In conclusion, we agree with Tré-Hardy et al. that a persistent antibody response was observe following the administration of the mRNA vaccine, as observed elsewhere using various assays.[1–4,7] However, the absence of “antibody drop” between T1, T2 and T3 observed in their cohort of previously seropositive could depend on the analytical kit used and the application of a dilution factor in case of signal saturation if such procedure is permitted and documented by the manufacturer. The results of the CRO-VAX HCP study showed that the use of undiluted or diluted samples led to different conclusions regarding the antibody kinetics. The fact that the signal is not saturated with the Roche assay also permit to derived more precise pharmacokinetic models which could latter better predict the probable persistence of antibodies.[3] Such data are important, especially since the question about a third dose has been raised.

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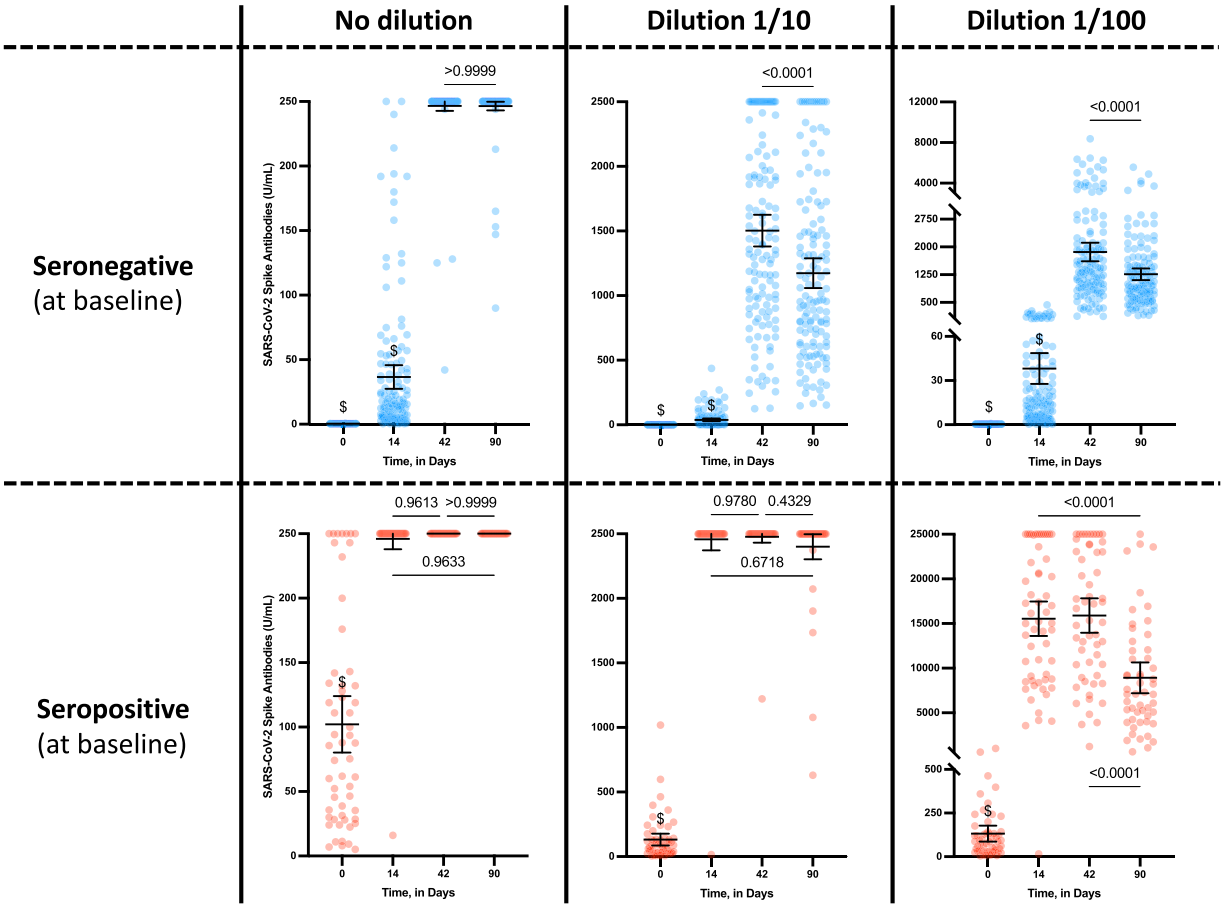

Fig. 1. Evolution of SARS-CoV-2 spike antibodies (U/mL) in previously seronegative (blue) and seropositive individuals (red) according to the time since administration of the first vaccine dose. Means (95% confidence intervals) are shown. Three different representations according to the dilution titers applied are shown (no dilution: up to 250 U/mL; 10-fold dilution: up to 2500 U/mL; 100-fold dilution: up to 25,000 U/mL). All dilutions were automatically performed by the analyzer. Results < 0.4 U/mL (limit of quantification) were rounded to 0.4. $ = statistically different from all other groups (i.e. p <0.0001).

Table 1
Evolution of SARS-CoV-2 spike antibodies (U/mL) in previously seronegative and seropositive subjects according to the dilution factor. Means (95% confidence intervals) are shown.

		Seronegative	Seropositive	*p* value
No dilution	Before first dose	0.40 (0.39–0.41)	102.2 (80.3–124)	<0.0001
(up to 250 U/mL)	14 days	36.6 (27.4–45.7)	246 (238–254)	<0.0001
	42 days	247 (243–251)	250 (250–250)	0.9826
	90 days	246 (243–245)	250 (250–250)	0.9753
Dilution 1/10	Before first dose	0.40 (0.39–0.41)	131.8 (86.1–178)	<0.0001
(up to 2500 U/mL)	14 days	38.2 (27.7–48.6)	2457 (2371–2543)	<0.0001
	42 days	1503 (1380–1625)	2400 (2303–2496)	<0.0001
	90 days	1173 (1057–1288)	2477 (2431–2523)	<0.0001
Dilution 1/100	Before first dose	0.40 (0.39–0.41)	132 (86.1–178)	<0.0001
(up to 25,000 U/mL)	14 days	38.2 (27.7–48.6)	15,540 (13,606–17,473)	<0.0001
	42 days	1863 (1613–2113)	15,856 (13,968–17,824)	<0.0001
	90 days	1262 (1104–1420)	8919 (7201–10,637)	<0.0001

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Julien Favresse*
University of Namur, Department of Pharmacy, Namur Research for Life Sciences, Namur Thrombosis and Hemostasis Center, Rue Saint-Luc, 8 – 5004, Bouge, Namur, Belgium
Clinique Saint-Luc Bouge, Department of Laboratory Medicine, Bouge, Belgium

Jonathan Douxfils
University of Namur, Department of Pharmacy, Namur Research for Life Sciences, Namur Thrombosis and Hemostasis Center, Rue Saint-Luc, 8 – 5004, Bouge, Namur, Belgium
QUALIblood s.a., Namur, Belgium

*Corresponding author.
E-mail address: julien.favresse@slbo.be (J. Favresse)