Context of the request for advice: National Platform bis
Taking into account on one hand the testing needs estimated by Sciensano in the coming period and on the other hand the findings of the Clinical Biology Committee on the available test capacity, the federal platform will be established for the analysis of PCR tests for the detection of SARS-CoV-2. This platform will exist temporarily and in addition to the test capacity in accredited clinical biology laboratories. Therefore, the National Platform bis is set up as an extension of the clinical biology laboratories. Turn-around times (TAT) need to be kept as short as possible and must remain within limits defined in the service level agreements.

Question
The request for advice concerns the storage of the samples within the scope of the National Platform bis. Advice of the RAG is needed on three different aspects concerning the storage of the samples. With each question the trade-off needs to be made between the relevance and the cost of storage.

1. How long should the samples be stored?
2. Should there be a differentiation between positive and negative samples (and non-conform or inconclusive results)?
3. Are there any other guidelines for storing the samples?

Possible reasons for storage of samples
The main reason for storage of samples is the possibility of retesting. Retesting can be done in case of a technical issue. Technical issues can arise before the test result is validated and sent to the prescriber and to Sciensano. Technical issues can be noticed in the days that follow the validation of the result. For this reason, samples can be stored for a few days to a week. This would concern samples with positive and negative and other results.

Another reason for retesting, can be the study of re-infections and the comparison of the viral strains. When the study of re-infections is aimed, long-term storage (months) is necessary. This concerns only positive samples.

Existing guidelines: storage duration
There are no existing guidelines on the duration of storage of samples that have been collected for COVID-19 PCR analyses in Belgium. The WHO or ECDC do not have guidelines about the duration of storage of COVID-19 PCR samples.

Existing guidelines: storage conditions
WHO recommends the storage of dacron or polyester flocked swabs with viral transport medium (VTM) containing antifungal and antibiotic supplements for up to 12 days until analysis at 2-8 °C. If phosphate buffered saline is used instead of VTM, it recommends storage up to 7 days at 4°C. In case other viruses such as influenza should also be tested, the WHO recommends storage of samples for no longer than 5 days at 4-8 degrees, counting from the date of sampling until analyses. We can consider the same storage recommendations for storage, with the aim to re-test in case of technical issues. ECDC recommends that all specimens should be stored at 2-8°C for up to 48 hours after collection. For handling or shipping after 48 hours, storage at -70°C (dry ice) is recommended.

Current practices
The current practices of samples storage vary among laboratories in Belgium. In the set-up of current national platform: every week, there is a pick-up of the samples to be stored at -80°C at the central lab.
The NRC of respiratory viruses currently stores primary samples at -20°C after analysis. Both the samples with a negative and these with a positive result are stored. The duration of storage is at least 6 months (currently all samples are still stored). The extraction eluate is stored during several weeks at -80°C and destroyed after some weeks (for both positive and negative results).

Another laboratory in Brussels report that negative samples are kept during 1 day after the analyses at 2-8°C. The positive samples of this laboratory are stored first at 2-8°C and afterwards at -80°C for a duration of 6 months. The extraction eluates of the positive samples are stored at -20°C for a maximum duration of 6 months.

**Conclusions**

Storage of the samples analysed in the scope of the National Platform bis

The aim of the National Platform bis is to process high throughput volumes of samples. High daily number of samples should be processed. This is not easily compatible with long-term storage of samples. The possibilities of re-infection are already established and documented. (3) Therefore, further studies on that aspect are not considered within the scope of the National Platform bis.

However, to be able to perform re-analysis in case problems are picked up during the quality controls, a minimum storage is required.

*It is recommended that samples are stored for around 1 week with the aim of retesting in case of a technical issue, noticed during or after analysis.* This recommendation is independent of the test result. Samples can be stored at -20°C or at 4°C.

Storage of the primary samples seems the most safe back-up option. However, in order to reduce the burden of storage on the labs, additional processing steps should be avoided. From a logistic point of view, storage volumes should be kept small (especially at low temperatures). Therefore, a pragmatic solution would be to store primary samples for a minimum of 3 days and store the RNA extract in the tubes/microplates as they were used on the machines, at -20°C for around one week.

If pooling will be applied, the samples should be stored longer. If one of the pools in a pooling matrix will turn positive, all individual samples of the whole matrix should be stored for a longer time. This is because the risk for pre-analytic errors is higher in the case of pooling. When the individual results of the unpooled analyses are not in agreement with the pooled results, all samples of the concerning pooling matrix should be re-tested.

Destruction of samples: the national platform bis should state in the SLA with the forwarding laboratories which laboratory will be responsible for the destruction of the samples, including the destruction of non-conform samples.

Storage of the samples outside the scope of the National Platform bis

*It is defined to be within the role of the NRC of respiratory viruses to collect a biobank of viral samples,* but this cannot be exhaustive, seen the long duration and extend of the current epidemic: it only concerns (a subset of) positive samples analyzed within the NRC. Outside the scope for the National Platform bis and the NRC, storage is not mandatory, but it may be interesting to store COVID positive samples for a longer time or store a specific subset of samples in a biobank for research. Hospitals and laboratories can decide about the storage protocols for samples that are analyzed within their laboratory, including the storage duration. Hospitals or institutes that organize studies which require the storage of samples, have to discuss and agree about the modalities of sample storage with the collaborating hospitals and laboratories.

Storage conditions

Samples can be stored at -20°C or at 4°C (between 2-8°C). Storage at 4°C should be limited to maximum 14 days (or 7 days when PBS is used). Ct-values can slightly increase after storage at -20°C. If long-term storage is recommended (months) this is preferably done at -70°C / -80°C, or alternatively can be done at -20°C. Avoid repeated freezing and thawing of specimens.
References

