

BACKGROUND

- Rapid scale-up of testing capacity + testing strategy linked to contact tracing => massive roll-out of PCR assays
- Dynamic of viral shedding during the course of infection: **demand to report cycle threshold (Ct) values**
 - Large variability across different methods and between laboratories
 - Affected by a number of factors (e.g. sampling method, matrix, extraction method, ...)
- ⇒ **need to report beyond a qualitative test result – chosen to move towards a semi-quantitative approach**
- **National harmonization effort to report SARS-CoV-2 PCR positive test results:**
 - More details to clinicians with respect to the stage of infection
 - Indirect potential link with infectivity, although interpretation still requires a clinical and/or serological context

MATERIALS

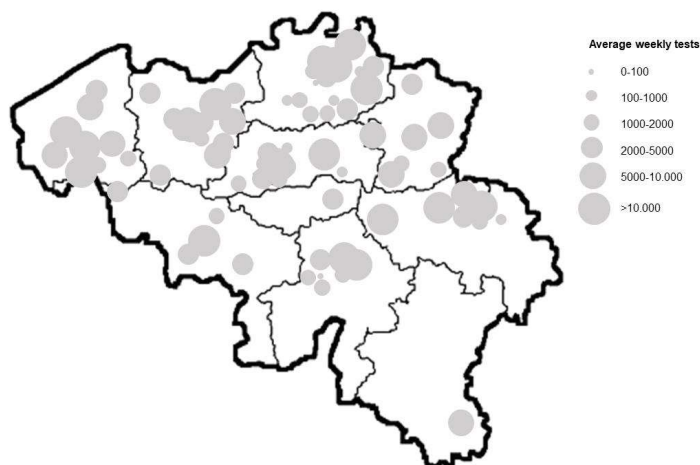
- **Proposal to harmonize the reporting of SARS-CoV-2 PCR positive test results in Belgium:** drafted by NRC UZ/KU Leuven
- **Four categories of positivity based on RNA copies/ml:**

Category of SARS-CoV-2 positivity	SARS-CoV-2 viral load (RNA copies/ml)	Interpretation with respect to infectivity
Very strongly positive	$\geq 10^7$	patient is contagious
Strongly positive	$\geq 10^5 - < 10^7$	patient is probably contagious
Moderate positive	$\geq 10^3 - < 10^5$	patient is potentially contagious, unless there is clinical and/or serological evidence of an old, cleared infection
Weakly positive	$< 10^3$	patient is probably not or no longer contagious if there is also clinical and/or serological evidence of an old, cleared infection

- **Pre-quantified SARS-CoV-2 control material:**
 - Heat-inactivated supernatant of SARS-CoV-2 positive cell culture: 9.04 log copies/ml – classification WGS to 20A (B.1.160)
 - Shipped on dry ice in February 2021 to 124 laboratories performing COVID-19 PCRs in routine diagnostics
 - Recommendations how to set up a standard curve to define and implement thresholds for the PCR assay(s) in use

RESULTS

Figure 1: Data shared by 91 participating labs: 86.1% of testing activity of Belgium (number of PCR assays performed by the 91 labs on a weekly basis vs overall number of tests on national level).



- **Ranking order of SARS-CoV-2 genes targeted in PCR assays in Belgium:** N > E > RdRp > spike > ORF1ab > ORF8 > Nsp2
- Advice provided on the gene recommended for reporting

Table 1: Overview of Ct values and SD for the 17 PCR assays meeting inclusion criteria (only listing the proposed target gene to use for semi-quantitative reporting). SD values exceeding 1 in red.

PCR assay	Labs	SARS-CoV-2 gene	Mean Ct value (SD)		
			10^7	10^5	10^3
Abbott RealTime	3	RdRp/N	6,2 (0,34)	12,7 (0,37)	19,1 (0,50)
Alinity m	6	RdRp/N	15,0 (0,65)	21,9 (0,68)	28,9 (0,75)
Allplex	15	E	17,0 (0,49)	24,3 (0,72)	31,5 (1,02)
Allplex multiplex	7	RdRp	15,3 (0,47)	22,5 (0,63)	29,7 (0,89)
Aries RUO	7	ORF1ab	18,3 (0,54)	25,3 (0,73)	32,4 (1,06)
BioGX BD MAX	3	N1	16,4 (0,71)	23,4 (0,56)	30,4 (0,42)
Cobas	4	E	17,6 (0,50)	24,0 (0,51)	30,4 (0,55)
GeneFinder	6	E	18,9 (0,37)	26,1 (0,61)	33,3 (0,85)
NeuMoDx	2	N	15,7 (0,09)	22,6 (0,10)	29,4 (0,29)
PerkinElmer	2	ORF1ab	16,4 (1,82)	22,7 (1,63)	28,9 (1,44)
R-DiaSARS-CoV-2	3	E	17,6 (0,39)	23,9 (0,44)	30,3 (0,68)
RealStar	2	S	16,3 (1,38)	23,1 (1,69)	30,0 (2,00)
ELITe MGB	2	ORF8	17,8 (0,86)	24,6 (0,94)	31,3 (1,02)
Plus ELITe MGB	2	RdRp/ORF8	16,8 (0,66)	23,8 (0,71)	30,9 (0,76)
TaqPath	17	N	14,5 (0,69)	21,3 (0,78)	28,1 (0,95)
Xpert	7	E	16,7 (0,49)	23,4 (0,51)	30,1 (0,54)
Xpert multiplex	9	N2/E	16,5 (0,57)	23,2 (0,92)	29,9 (1,35)

- **Top 5:** Allplex (Seegene), Xpert (Cepheid), Taqpath (Thermo Fisher), Aries (Luminex) & GeneFinder (Osang Healthcare)

CONCLUSION

Thanks to this national harmonization effort, many Belgian laboratories currently report SARS-CoV-2 positive PCR results in the same semi-quantitative manner, distinguishing four categories of positivity based on RNA copies/ml, to clinicians and to healthdata.be (feeding the national contact tracing system), improving infection control measures.