UZ LEUVEN | LABORATORIUMGENEESKUNDE

Evaluation PrimeStore MTM (bioTRADING)

Objective

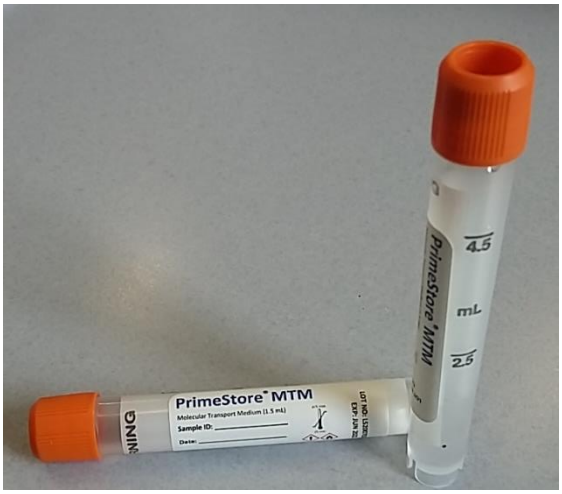
Because of the SARS-CoV-2 pandemic and the need for extra test-capacity, there is a need for a virus inactivation medium for collection and transport of specimens. Samples collected in a virus inactivation medium can be processed for nucleic acid (NA) extraction with pipetting robots and without the need to work in a biosafety cabinet.

We received PrimeStore MTM samples from bioTRADING Benelux b.v.

In our evaluation the stability of SARS-CoV-2 RNA during 96 hours and the compatibility with our NA-extraction platforms was evaluated

PrimeStore MTM

Description tube: 5 ml (CORNING) tube with screw-cap containing 1.5 ml of PrimeStore MTM. The screw-cap is a regular non-pierceable cap and is not able to capture the swab in the tube (no 'capture-cap')



The PrimeStore MTM has been proven to be effective in the inactivation of SARS-CoV-2 (ref. 1)

Study design

The PrimeStore MTM is tested in parallel with UTM transport medium (=golden standard at UZ Leuven).

Using 2 SARS-CoV-2 positive patient samples, spike-in samples representing a positive and a low positive sample are recreated in the PrimeStore MTM and the validated golden standard transport medium. Samples are stored and tested once a day over the course of 5 days by nucleic acid extraction and SARS-CoV-2 PCR (at UZ Leuven, using the MagMAX™ Viral/Pathogen II kit on KingFisher followed by SARS-CoV-2 E gene PCR (adjusted from ref.2)).

Samples were stored for 2 hours at room-temperature until the first measurement and afterwards they were stored at 2-8°C.

Additionally, to evaluate platform compatibility, additionally 3 spike-in samples in the PrimeStore MTM and UTM are tested once on each platform available at UZ Leuven. Due to the use of a spike-in internal control during nucleic acid extraction, a possible reduction in extraction efficiency or the presence of inhibitory compounds can be evaluated.
The following platform were tested:

- MagMAX™ Viral/Pathogen II kit on KingFisher (ThermoFisher Scientific) followed by SARS-CoV-2 E gene PCR (adjusted from ref.2)
- NucliSens extraction on eMAG (BioMérieux) followed by SARS-CoV-2 E gene PCR (adjusted from ref.2)
- Xpert Xpress SARS-CoV-2 analysis on GeneXpert (Cepheid)

Because PrimeStore MTM contains guanidine isothiocyanate it could not be tested with the Panther system and the Aptima SARS-CoV-2 test (Hologic). Due to the lithium dodecyl sulphate based lysis buffer used with Panther, transferring guanidine isothiocyanate containing transport medium to lysis tubes will result in the formation of precipitates. In addition, the Panther system uses large amounts of sodium hypochlorite, resulting in the formation of toxic gas, when mixed with guanidine isothiocyanate.

Resulting Ct-values for SARS-CoV-2 and the internal control for PrimeStore MTM samples are compared with those from the UTM samples and should fall within the range of a 2.0 Ct-difference (= 4-fold difference in input material) for SARS-CoV-2 and a 3.3 Ct-difference for the internal control (IC).
The 2.0 Ct-difference is an often used acceptance-criterion for comparing different methods in the lab at UZ Leuven.
The 3.3 Ct-difference for the internal control is an often used acceptance-criterion for inhibition/extraction efficiency in the lab at UZ Leuven.

Results

Stability of SARS-CoV-2 RNA during 96 hours

Test	**KF + SARS-CoV-2 qPCR**									
Target	**SARS-CoV (E-gene)**					**ZICR (Internal control)**				
Day	1	2	3	4	5	1	2	3	4	5
1 PS	26.2	25.2	25.5	24.0	24.8	30.0	29.0	29.6	28.4	29.1
1 UTM	24.4	24.6	24.1	23.8	24.4	28.8	28.2	27.9	27.7	28.9
2 PS	32.8	30.5	31.2	29.6	30.9	32.0	31.9	32.2	30.2	31.5
2 UTM	30.6	29.9	29.9	29.9	30.3	30.0	30.4	29.9	29.4	30.5
3 PS	neg	neg	neg	neg	neg	32.2	32.0	32.9	30.5	32.0
3 UTM	neg	neg	neg	neg	neg	30.0	30.5	30.6	29.2	30.7

PS= PrimeStore MTM / UTM= reference

1 = positive sample

2 = low-positive sample
3 = negative sample (no spike-in)

Results of the reference (UTM) are colored yellow.
Results that have a Ct difference ≤ 2.0 / 3.3 with the reference are colored green.
Results that have a Ct difference > 2.0 / 3.3 with the reference are colored orange.

All IC-results have a Ct-difference ≤ 3.3 between corresponding samples, indicating the absence of qPCR inhibition and the correct performance of all NA-extractions and qPCRs.

All SARS-CoV-2 results, except 1, have a Ct-difference ≤ 2.0 between corresponding samples. For the low-positive sample on day 1, the Ct-difference is 2.2 but the sample is still positive. The SARS-CoV-2 Ct-values for all samples (PrimeStore MTM and UTM) over a time interval of 96 hours are stable (within 2.0 range, except for the low positive sample on day 1). There is no increase in the Ct-value over time, indicating the stability of SARS-CoV-RNA in the 96-hours time period.

Platform compatibility

3 samples (labeled 4, 5 and 10) in UTM (U) and PrimeStore MTM (PS) were tested.

Results of the reference (UTM) are colored yellow.
Results that have a Ct difference ≤ 2.0 / 3.3 with the reference are colored green.
Results that have a Ct difference > 2.0 / 3.3 with the reference are colored orange.

KingFisher + qPCR:

Test	**KF + SARS-CoV qPCR**			
Target	**E-gene**		**ZICR (IC)**	
Medium	U	PS	U	PS
4	31.7	32.8	30.0	31.6
5	33.8	33.5	30.5	32.0
10	28.0	28.3	30.2	30.8

eMAG + qPCR:

Test	**KF + SARS-CoV qPCR**			
Target	**E-gene**		**ZICR (IC)**	
Medium	U	PS	U	PS
4	32.9	31.2	30.4	30.2
5	34.8	33.9	30.6	30.2
10	26.4	25.9	29.6	29.2

GeneXpert (Xpert Xpress SARS-CoV-2)

Test	Xpert Xpress SARS-CoV-2					
Target	E-gene		N2-gene		SPC (IC)	
Medium	U	PS	U	PS	U	PS
4	33.1	33.6	35.9	36.2	28.3	28.7
5	34.5	35.0	37.2	38.5	28.2	28.9
10(*)	31.9	30.7	34.9	33.6	28.3	28.3

(*) sample 10 in this GeneXpert experiment has a different SARS-CoV-2 concentration than the sample 10 used in the 2 other platforms)

All IC-results have a Ct-difference $\leq$ 3.3 between corresponding samples, indicating the absence of qPCR inhibition and the correct performance of all NA-extractions and qPCRs.

All SARS-CoV-2 results have a Ct-difference $\leq$ 2.0 between corresponding samples, indicating PrimeStore MTM is compatible with the 3 tested platforms.

Conclusion

The SARS-CoV-2 RNA is stable in the PrimeStore MTM over a time period of 96 hours.

PrimeStore MTM is compatible with 3 tested platforms:

- MagMAX™ Viral/Pathogen II kit on KingFisher (ThermoFisher Scientific) followed by SARS-CoV-2 E gene PCR
- NucliSens extraction on eMAG (BioMérieux) followed by SARS-CoV-2 E gene PCR
- Xpert Xpress SARS-CoV-2 analysis on GeneXpert (Cepheid)

References

1. "Inactivation analysis of SARS-CoV-2 by specimen transport media, nucleic acid extraction reagents, detergents and fixatives" S.R. Welch et al. July 2020 (https://doi.org/10.1101/2020.07.08.194613)
2. "Detection of 2019 novel coronavirus (2019-nCoV) by real time RT-PCR" V.M. Corman et al.; Euro Surveill. 2020;25(3):pii=2000045. (https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045)

Belgian Reference center for coronaviruses Leuven, 24-8-2020