InActiv Blue® – virus transport medium

A safe alternative for transport of infectious patient samples, including swabs and saliva

Current test strategies for COVID-19 face a common challenge: lab operators might get infected with SARS-CoV-2 when handling test tubes containing patient samples taken from the nose or throat of COVID-19 patients. Our concern for occupational health risk has resulted in the development of InActiv Blue®: a new type of liquid-filled tube to transport naso- or oropharyngeal swabs or saliva from the healthcare professional to the test lab for molecular analysis (RT-qPCR).

KEY FEATURES AND BENEFITS OF INACTIV BLUE®

- complete inactivation of SARS-CoV-2: abrogates the infectious potential of collected patient material **within 1 minute**
- safe and stable preservation of RNA up to 30 days when samples are stored at 2-25 °C and up to 8 days when stored at 37 °C
- perfect stability of SARS-CoV-2 RNA upon multiple freeze-thaw cycles of patient samples
- suitable for molecular detection of wide range of respiratory viruses and bacteria (multiplex PCR)
- no cold chain required
- blue color as visual pipetting control
- independent validation with a wide range of common RT-qPCR platforms
- production and testing of each batch according to the highest quality standards in ISO 13485 certified labs
- CE marked by FertiPro
- documented proof of compatibility with saliva as alternative sample type

NEWEST FINDING

Nasopharyngeal swabs from individuals with suspected respiratory tract infection can be stored in InActiv Blue® medium for subsequent multiplex PCR testing. Test results demonstrate that the medium is compatible with BioFire FilmArray Respiratory 2.1 (RP2.1) plus Panel analysis and allows determination of all viruses and bacteria in this panel, including: adenovirus type 1 and 31, influenza B, parainfluenza type 1, 2, 3 and 4; coronavirus OC43, NL63, 229E and HKU1; rhinovirus 1A, Influenza A subtype H3 and H1N1; MERS-CoV, RSV A, human metapneumovirus 8, Bordetella pertussis, Chlamydia pneumoniae, Mycoplasma pneumoniae, and Bordetella parapertussis.

VIRUS INACTIVATION PERFORMANCE

Quick and complete inactivation of pathogens in infectious samples reduces the risk of accidental infections through unsafe laboratory practices

InActiv Blue® completely inactivates SARS-CoV-2 within 1 minute (≥ 5 log TCID reduction) and has been proven equally effective for the inactivation of other viruses such as viruses of the Orthopoxvirus genus (e.g. vaccinia virus as surrogate for monkeypox virus). The medium is even capable to inactivate the more resistant non-enveloped viruses such as norovirus and parvovirus.

The virus inactivation performance is evidenced through extensive testing by an independent laboratory (FARAH Research Center, Department of Veterinary Pathology, University of Liège, Belgium). This lab routinely applies the TCID50 method that is worldwide the most widely used assay to determine detect virus inactivation. In a TCID50 assay, a sample that contains a high concentration of virus is serially diluted and each dilution is placed on replicated cultures of susceptible adherent cells in wells of a flat-bottomed plate. Infected cultures are incubated, after which wells are scored positive or negative, based on the presence or absence of virally induced cytopathology. Complete virus inactivation by InActiv Blue® is evidenced by 100% cell survival upon exposure to virus stock applied at a dilution that results in ≥ 50% cytopathic effects in control (non-activating) medium.

We guarantee that each newly produced batch of InActiv Blue® is subject to the SARS-CoV-2 virus inactivation performance test before release to the market. Furthermore, the InActiv Blue® buffer formulation is extremely heat stable, an important feature for your applications (equal performance upon long-term exposure of InActiv Blue® at 55 °C).

INACTIV BLUE® AS A RELIABLE MEDIUM TO DETECT SARS-COV-2 RNA, INFLUENZA A/B & RSV A/B

Several clinical labs have demonstrated excellent comparative RT-qPCR test performance of InActiv Blue® against UTM. A summary of results is outlined in Figure 1-3.

Brief overview of test set-up: patient samples in standard transport medium of (nasopharyngeal) swabs that were previously tested positive for SARS-CoV-2, influenza A/B or RSV A/B, are diluted in InActiv Blue® or standard medium. Samples were subsequently stored at room temperature and retested after 1 day to determine the PCR quantification cycle (Cq) for SARS-CoV-2, influenza A/B or RSV A/B respectively. Figure 1-3 indicate a perfect concordance over a broad range of Cq values.

Figure 1: Correlation of Cq SARS-CoV-2 positive samples in InActiv Blue® and UTM (n= 160)

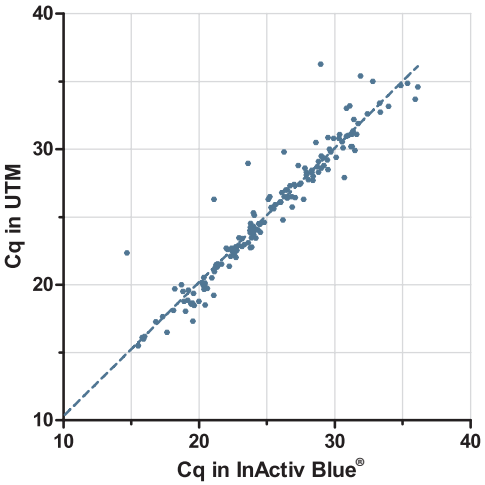


Figure 2: Correlation of Cq influenza A/B positive samples in InActiv Blue® and UTM (n= 43)

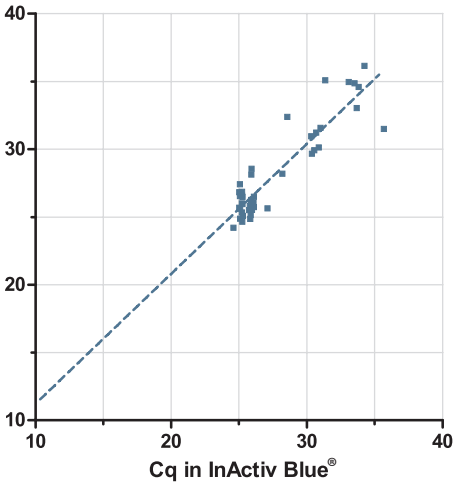
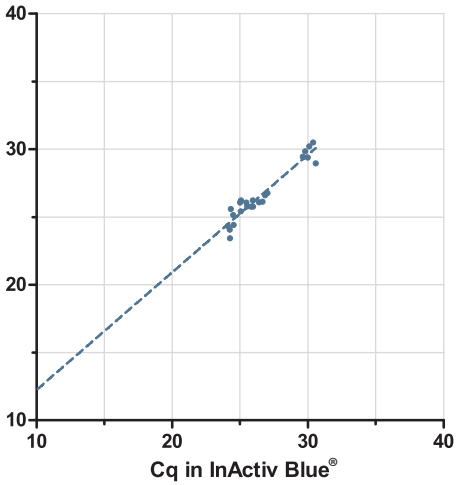


Figure 3: Correlation of Cq RSV A/B positive samples in InActiv Blue® and UTM (n= 24)



STABILITY OF SARS-COV-2 RNA ENSURED

Storage of samples up to 30 days

Since (unexpected) delays in transport and testing can occur, it is important that a virus transport medium will ensure RNA stability and detectability for at least one week. As tested in two independent labs, InActiv Blue® medium was found to perfectly stabilize viral RNA for at least 8 days when samples are stored between 2-37 °C (Figure 4-6). Furthermore, sample RNA stability is ensured up to 30 days when stored between 2-25 °C as indicated in Figure 6.

Brief overview of test set-up: patient samples in standard transport medium of (nasopharyngeal) swabs that were previously tested positive for SARS- CoV-2, were diluted in InActiv Blue® or standard medium on day 0. Samples were subsequently stored at room temperature (RT), 4 °C or at 37 °C and retested on day 1, day 4 , 8 and 30 to determine the PCR quantification cycle (Cq) for SARS-CoV-2. The figures below indicate the Cq values obtained for a total of 119 samples tested in 2 different laboratories.

Figure 4: Stability of SARS-CoV-2 RNA in InActiv Blue® stored at RT for 8 days, compared to storage in UTM (n=95)

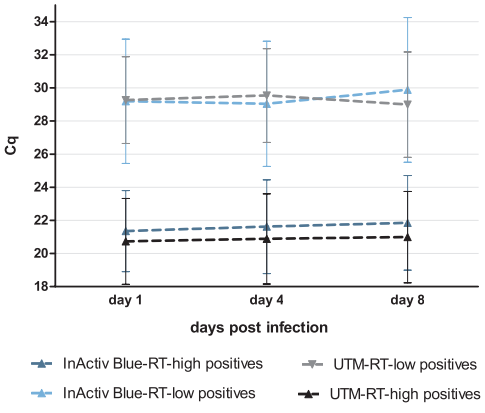


Figure 5: Stability of SARS-CoV-2 RNA in InActiv Blue® stored at 37 °C for 8 days (n= 12)

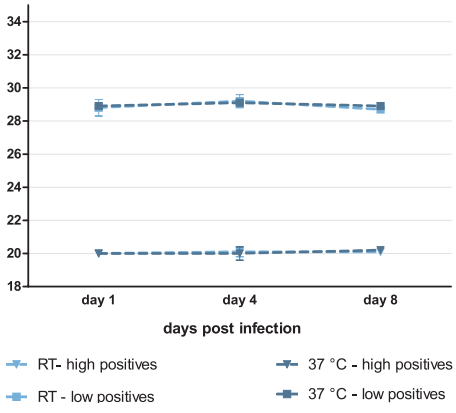
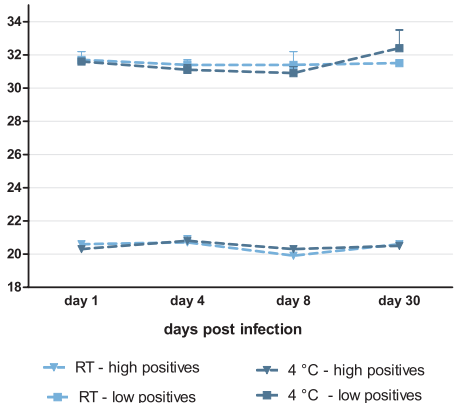
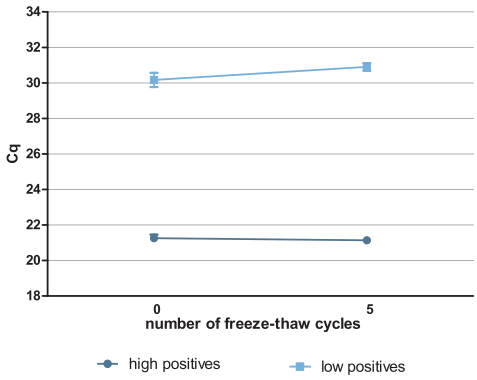


Figure 6: Stability of SARS-CoV-2 RNA in InActiv Blue® stored at 4 °C and RT for 30 days (n=12)



Freeze-thaw stability of samples

Figure 7: Freeze-thaw stability (n=6)



InActiv Blue® medium perfectly supports your needs when samples must be stored for a longer time. Test data demonstrate that the stability of viral RNA is not affected by repeated freeze-thaw cycles.

Brief overview of test set-up: patient samples in standard transport medium of (nasopharyngeal) swabs that were previously tested positive for SARS-CoV-2, were diluted in InActiv Blue® on day 0. These samples were subsequently tested on day 0 and following 5 freeze-thaw cycles performed over 6 days. Figure 7 shows that the Cq values remain stable!

STABILITY OF INFLUENZA A/B – RSV A/B RNA

Additional data collected in collaboration with Sciensano confirm that InActiv Blue® is equally able to stabilize RNA of other common respiratory viruses: influenza A/B and RSV A/B.

Brief overview of test set-up : patient samples in standard transport medium of (nasopharyngeal) swabs that were previously tested positive for respectively influenza A/B or RSV A/B, were diluted in InActiv Blue® or standard medium on day 0. These samples were subsequently tested on day 1, 2, 3, 4, 7 and 8. Figure 8 illustrates that Cq values remain stable (8 days tested), Figure 9 shows that Cq values are similar (or even more sensitive) in InActiv Blue® medium compared to standard UTM medium.

Figure 8: Stability of influenza and RSV RNA in InActiv Blue® stored at RT for 8 days (n=7)

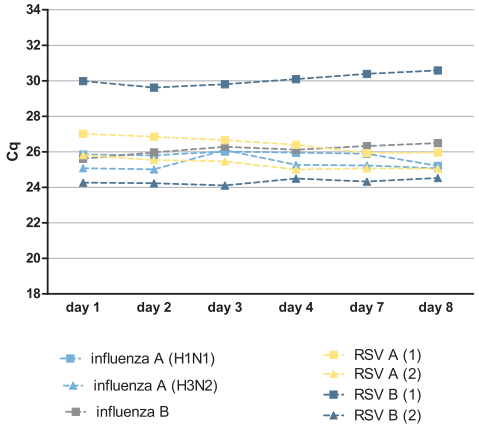
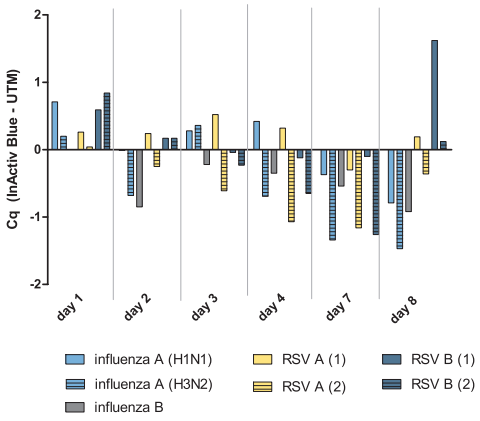
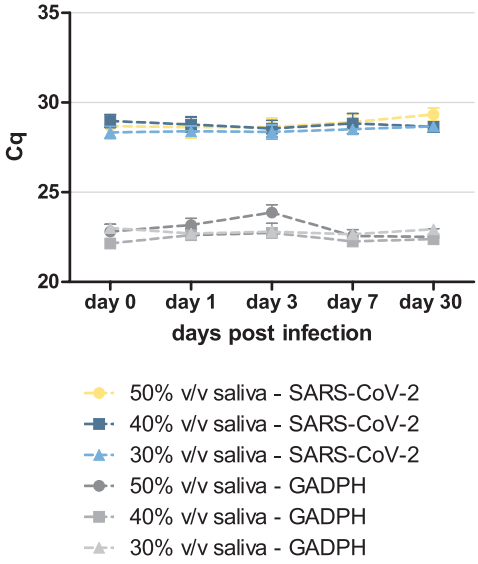


Figure 9: Equal or better performance of InActiv Blue® compaired to UTM



USE OF SALIVA SAMPLES STORED IN INACTIV BLUE®

Figure 10:



Tests on saliva can play an important role to better control the COVID-19 pandemic. Massive use of preventive screening saliva tests can help to identify patients that spread the disease without having symptoms. Data below indicates that SARS-Co-V-2 RNA remains stable for at least 30 days at room temperature in saliva samples stored in InActiv Blue®.

Brief overview of test set-up: 11 healthy donors were refrained from eating, drinking, smoking and using chewing gum for at least 30 min prior to saliva collection. In total, 54 ml of saliva was pooled and vortexed to prepare different dilutions of saliva in InActiv Blue® ranging from 30% to 50% saliva (v/v). On day 0, all tubes were spiked with viral-like particles to obtain a final low concentration of 100 000/ml. One set was stored at room temperature, the other set in the fridge at 4 °C. The PCR quantification cycle (Cq) for SARS-CoV-2 RNA was determined for all conditions on day 0, day 1, day 3, day 7 and day 30. Figure 10 shows that saliva is also a suitable specimen type for diagnostic testing on SARS-CoV-2: viral RNA of the E-gene and a human endogenous gene (GADPH) remains perfectly stable for at least 30 days. No difference is found between samples stored at RT or 4 °C (data not shown).

See Jonckheere et al. (2022, J of Pediatrics, Perinatology and child Health) for clinical study performance of InActiv Blue® and saliva.

COMPATIBILITY WITH PLATFORMS

While labs are responsible for validating downstream RNA extraction and molecular testing, a non-exhaustive list of platforms that have successfully diagnosed patient samples stored in InActiv Blue® is available below:

instrument	*RNA extraction*	*RT-qPCR*
Abbott Alinity M	Alinity m Sample Prep Kit 1 #09N18-001	Alinity m SARS-CoV-2 AMP kit #09N78-090
CFX96 (Bio-Rad)	Real-Prep Viral DNA/RNA kit (BioSewoom)	STANDARD M nCoV Real-Time Detection kit (SD BIOSENSOR #11NCO10)
CFX96 Deep Well Real Time PCR detection System (Bio-Rad) Maelstrom 9600 (Tanbead)	Tanbead Nucleic Acid Extraction Kit (96) #W665A10	Allplex TM 2019 nCov Assay RP4520D59
CFX96 Deep Well Real Time PCR detection System (Bio-Rad) Microlab STARlet IVD (Seegene/Hamilton)	STARMag96x4 viral DNA/RNA 200c kit #EX00013C	Allplex TM 2019 nCov Assay RP4520D59
CFX384 (Bio-Rad #1855485)	Zymo Research's Quick-RNA 96 #R1053	Bio-Rad iTaq one-step RT-qPCR mix #1725141
	Norgen Biotek's Total RNA Purification 96-well Kit #24370	Bio-Rad iTaq one-step RT-qPCR mix #1725141
	magtivio's MagSi-NA Pathogens #MDKT00210960	Bio-Rad iTaq one-step RT-qPCR mix #1725141
Chemagic 360 Janus qPCR Janus reformatter (G3) Perkin Elmer QuantStudio7flex	Chemagic Viral DNA/RNA Kit special H96 #CMG-1033-S	Sars-CoV-2-RT-qPCR Reagent kit #3501-0010
EMAG (Biomérieux) Stratagene MX3000-3005 (Agilent)	NUCLISENS easyMAG (Biomérieux #280130-35; #280146; #200292)	Superscript III Platinum One-Step qRT-PCR system (Invitrogen #11732-088)
Hamilton Starlet	96X4 viral DNA/RNA 200 C kit #EX00013C	Allplex SARS-CoV-2 #RV10248X
Hamilton Starlet/Tanbead	OptiPure Viral Auto Plate #W665A10	Allplex SARS-CoV-2 #RV10248X
Lightcycler 480 (Roche)	MagnaPure 96 and Viral NA Small volume kit 0654388001	LightMix Modular Sarbecovirus SARS-CoV2 PCR kit #50-0776-96
cobas 6800 system (Roche)	cobas omni reagent for sample preparation	cobas SARS-CoV-2: #09175431190 cobas Utility Channel with probes and primers for SARS -CoV-2 E gene
Kingfisher FLEX (Thermofisher) Quantstudio 5 (Thermofisher)	Thermofisher's MagMAX Viral/Pathogen II kit #A48383	TaqPath COVID-19 CE-IVD RT-PCR kit #A48067
Kingfisher FLEX (Thermofisher) Quantstudio 7 (Thermofisher)	Thermofisher's MagMAX Viral/Pathogen II kit #A48383	TaqPath COVID-19 CE-IVD RT-PCR kit #A48067
cobas Liat PCR system (Roche)	COBAS SARS-CoV-2	COBAS SARS-CoV-2
Lightcycler 96 (Roche)	magtivio's MagSi-NA Pathogens #MDKT00210960	Bio-Rad iTaq one-step RT-qPCR mix #1725141
BioFire FilmArray (Biomérieux)	BioFire Respiratory 2.1 plus Panel (Biomérieux)	

KNOWN LIMITATION:
do not use with platforms that use bleach decontamination, such as Hologic's Panther System!
Please contact us for alternatives.

LEARN MORE ABOUT INACTIV BLUE?

Mail to: **info@inactivblue.com**
We are happy to answer all your questions!

BIBLIOGRAPHY

Darnell, Subbarao, Feinstone and Taylor (2004). J Virol Methods 121(1): 85-91
Darnell and Taylor (2006). Transfusion 46(10): 1770-1777
Jonckheere et al. (2022). J of Pediatrics, Perinatology and child Health 6: 042-053