



Optimal throughput and safety
in RT-qPCR **CoVID-19** testing

DEEP **LIFE**
medical



INTRODUCTION

There are two means for definitive testing for CoVID-19 disease:

1. Test for antibodies in blood using field kits,
2. RT-qPCR Test for the virus antigen by identifying its exact genetic code (RNA) in the sample.

Deep Life offer best of class in both types of test.

This White Paper addresses the second of these two: laboratory RT-qPCR analysis.

This paper describes how a dozen steps in the conventional “one-step” RT-qPCR are replaced by just two, reducing substantially the lab resources needed to process samples and perform the analysis. Deep Life's InstaSafe[®], FastStream[®] and EasyFlow[®] RT-qPCR CoVID-19 test kits deliver this latest advance in CoVID-19 testing.

WHY CHOOSE RT-qPCR AND WHY THE DEEP LIFE KITS?

Most countries choose to run both types of test: antibody and RT-qPCR. Each have their role.

Deep Life antibody field tests are fast, taking just 10 minutes from blood sample to result. The Deep Life antibody kits are also accurate: independently validated to be within 98% of the accuracy of PCR. Antibody tests are even lower cost than RT-qPCR. With these three advantages of antibody tests, why choose RT-qPCR? The reason is antibody kits have two limitations, namely:

1. It takes between 3 and 7 days from the instant of infection before the patient will have enough antibodies for an antibody test to be positive. The test is always positive before the patient shows symptoms, but there is this time lag where infection can be spread before the test is positive.
2. A second limitation of antibody tests is that some patients who have had the virus, but since recovered, will show positive for a period of time, giving a false positive.

The RT-qPCR test determines conclusively whether the patient is currently infected, without either of these drawbacks, but it is a more complex and expensive process. RT-qPCR is the reference against which all other tests are measured. The drawback is while antibody tests can be performed anywhere, RT-qPCR requires a laboratory and is slow: that limits throughput. The Deep Life RT-qPCR kits address that issue by greatly improving RT-qPCR test safety and throughput.

RT-qPCR TECHNOLOGY FOR VIRAL IDENTIFICATION

Quantitative Polymerase Chain Reaction (qPCR) is a technique that amplifies a selected segment of DNA using photo-luminescent markers. It is an extremely sensitive method: a single strand of nucleic acids can be amplified billions of times in a thermo-cycler (qPCR machine) such as that shown on the right, and detected.

qPCR enables the laboratory to detect with certainty if a specific genetic sequence is present in a sample.

The “RT”-qPCR is short for Reverse Transcriptase which is added to the sample mix to enable the qPCR technique to be applied to viruses. Viruses are made from a single strand of RNA, rather than double stranded DNA. The Reverse Transcriptase adds the complementary strand to the viral RNA.



THE RT-qPCR PROCESS FOR COVID-19 TESTING

The RT-qPCR test for CoVID-19 infection is a test for the exact genetic code of the virus that causes CoVID-19 disease: the SARS-CoV-2 virus. The test three main steps:

1. Take a nasopharyngeal swab, transport it to the lab, and release the viral genetic material into a solution by adding a precise amount of viral release reagents.
2. Combine the sample with the raw materials for genetic amplification, a marker to highlight the replicated DNA, Probes and primers specific to the CoVID-19 RNA code to cut the DNA at the desired locations and DNA Polymerase to replicate the DNA.
3. Run the prepared sample on a thermo-cycler. The thermo-cycler amplifies the amount of genetic material exponentially, to enable even the smallest amount to be detected using a green light within the cycler that illuminates the fluorescent markers.

With each run of the thermo-cycler, a positive and negative validation sample is also processed. This provides quality control to ensure that all process steps have been performed correctly.

In addition to the positive and negative tests, the Deep Life RT-qPCR include internal validation markers, to ensure that every single sample and every single batch has been processed properly.

The Deep Life RT-qPCR CoVID-19 test kits are a collaboration between three companies, to deliver the best of class in a combined package spanning from sample collection right through to analysis. The focus has been to simply and speed up the sample preparation and processing, so a lab can handle ten times the number of tests that would be possible using the conventional process. This paper explains how those gains have been achieved.

The Deep Life kits are in packs of 250, suitable for automation of the entire test process, or for manual use.

STEP 1: FROM SAMPLE TO THE START OF RT-qPCR

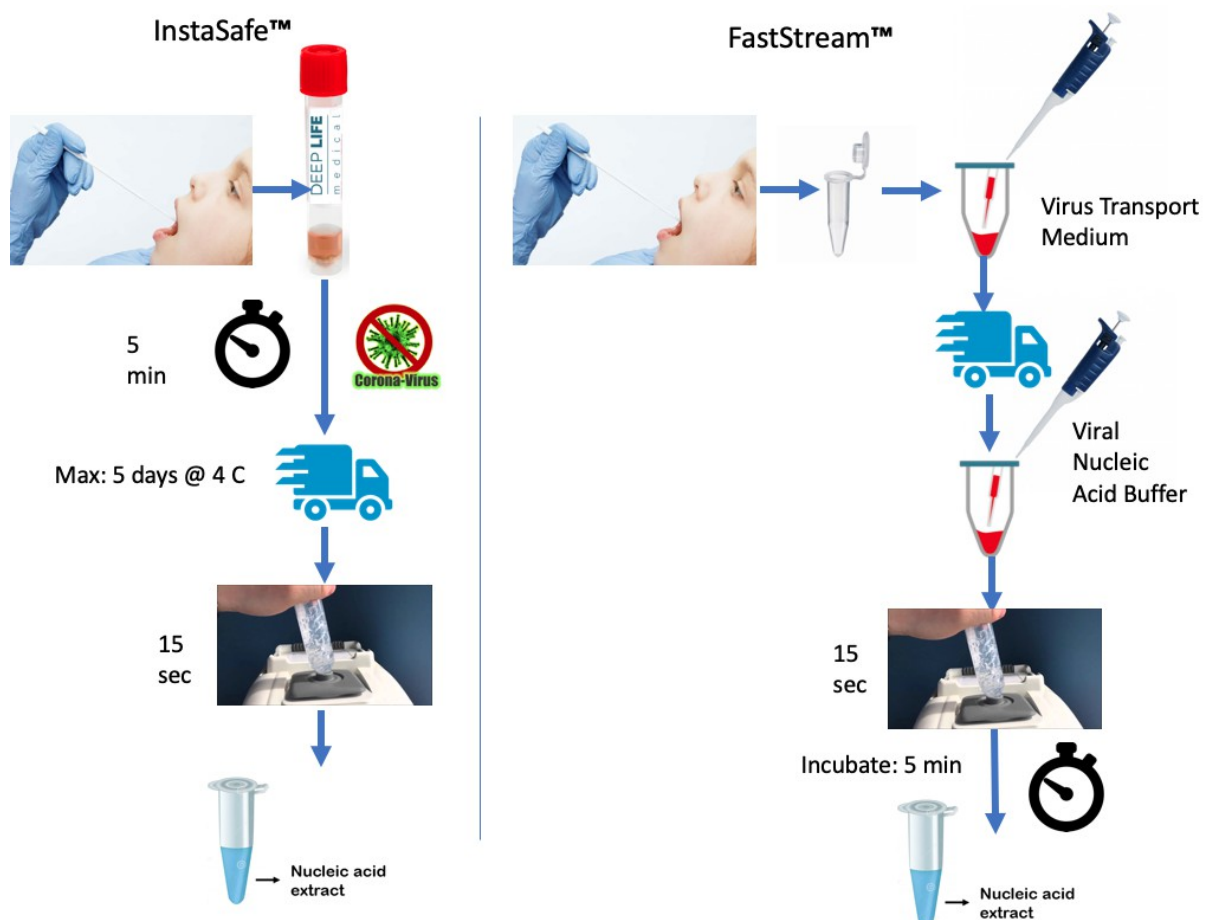
The conventional process involves taking a sample, such as a nasopharyngeal swab or mucus sample, add it to a transport medium, then transport it to a lab where a series of up to six stages in a Biosafety level 3 section are needed before the sample is ready for RT-qPCR.

The Deep Life InstaSafe® sample preparation kit is transport tube that delivers two huge improvements in this process.

1. InstaSafe® kits are transport tubes that have the viral release agents in them already. This means when the sample is added, the nucleic components are released and within five minutes the sample is no longer infectious. It can be handled safely with the minimum of biosafety measure, improving safety for lab staff and reducing the load on resources.
2. The sample is ready immediately for RT-qPCR as soon as it arrives in the lab as the viral load has already been released.

An alternative is the Deep Life FastStream® nucleic acid release kits. These enable the lab to handle nasopharyngeal swab, nasopharyngeal aspirate/lavage, bronchoalveolar lavage, oropharyngeal swab and sputum samples from within a hospital or that have been transported using conventional transport medium. FastStream® goes from sample to the start of qPCR within 5 minutes.

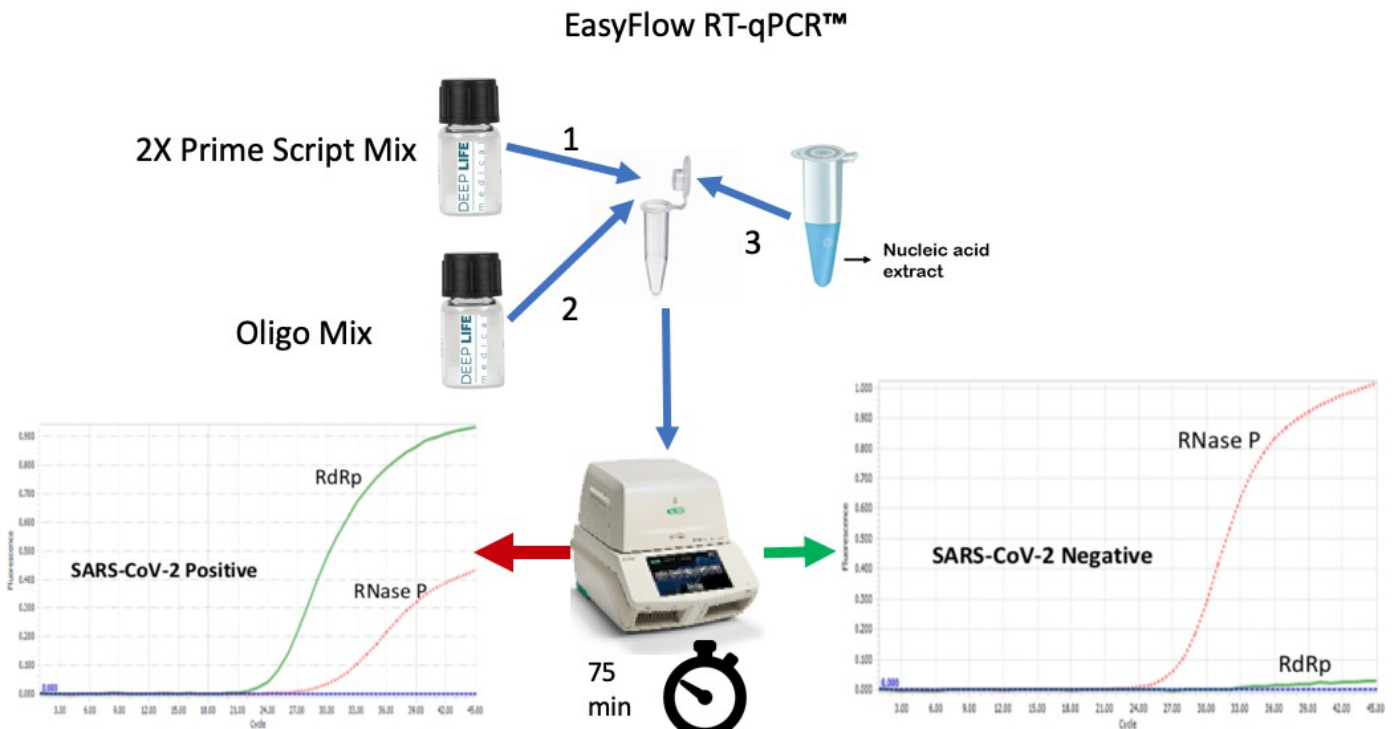
The InstaSafe® and FastStream® sample preparation kits on their own removes as much as 40 minutes from the normal process flow, as well as removing six steps with their time, cost and risk of human error.



This process produces 200uL of prepared nucleic acid sample ready for RT-qPCR.

STEP 2: EASYFLOW™ RT-qPCR KIT FOR CoVID-19

The Deep Life EasyFlow® RT-qPCR test kit contains the biochemistry to amplify and detect the CoVID-19 virus, with the absolute minimum of stages.



The EasyFlow® RT-qPCR kit contains just two mixes that need to be combined with the sample, before placing it in the thermo-cycler: the Oligo Mix and 2X Prime Script Mix.

- ◆ The Oligo mix contains CoVID-19 detectors and an internal control (RNase P gene). Internal control is another form of control that takes place inside every sample tube. It enables the comparison of each reaction to a reference reaction.
- ◆ The other component of the kit is the 2X Prime Script Mix. This mixture contains reaction buffer, Reverse Transcriptase to produce cDNA, raw materials for the DNA amplification and DNA polymerase to perform the DNA amplification.

The EasyFlow® RT-qPCR process uses just two addition steps. The whole process from start to finish is:

1. Take a PCR sample tube
2. Add 5uL of the Prime Script mix
3. Add 2.5uL of the Oligo mix
4. Add 2.5uL from the prepared nucleic acid sample
5. Vortex 30 seconds
6. Incubate 5 minutes at room temperature.

This is vastly simpler and faster than conventional one-step or two-step RT-qPCR processes, where dry powders have to be reconstituted, up to 7 different mixes have to be eluted with the sample, and extracts aliquoted into different solutions, with up to 30 individual actions needed.

STEP 3: PCR THERMOCYCLING AND READING RESULTS

The thermo-cycler runs a reaction volume of 10uL of the RT-qPCR preparation with the following program:

| Number of Cycles | Temperature | Duration |
|------------------|-------------|----------|
| 1 | 45°C | 15 min |
| 1 | 95°C | 3 min |
| | 95°C | 5 sec |
| 45 | 55°C | 35 sec |
| FAM/HEX read | | |

This is a total of 48 minutes, plus any PCR machine transition time, the fastest PCR process for CoVID-19 currently available.

QUALITY CONTROL

Each Deep Life EasyFlow® RT-qPCR kit contains three QA cross-checks.

1. POSITIVE CONTROL

Positive control is an important step in all PCR applications. The aim of positive control is to verify that the test/equipment is indeed capable of detecting the desired strands. A test tube with the desired genetic fragments to be detected is introduced alongside the test samples and goes through the process. The outcome of the positive control should be positive otherwise the experiment should be discarded. A negative outcome is indicative of machine error, process error or that the components of the kit have spoiled. All Deep Life kits include positive control.

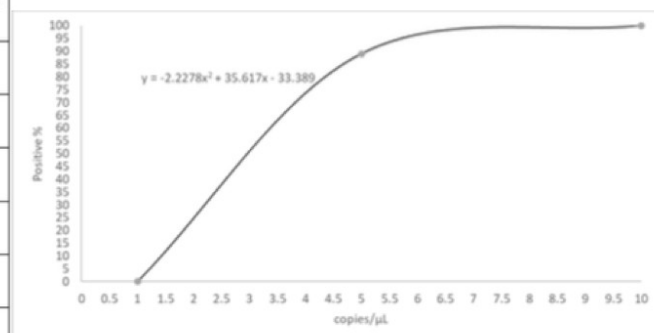
2. NEGATIVE CONTROL

Negative control is also an important step in PCR applications. The aim of negative control is to verify that there has been no contamination in the samples or process. The negative control is treated like a sample (without sample addition) and should yield a negative result at the end of the process. A positive outcome indicates that the process has been contaminated with virus genetic material and the results cannot be trusted. All Deep Life kits include negative control.

3. INTERNAL CONTROL

The EasyFlow® Oligo mix includes a RNase-P internal control that enables the operator to check every single sample has been processed correctly. This is effectively a second measurement channel added into the sample processed and detected simultaneously with the test sample.

| SARS-CoV-2 /reaction | Cq values | | | | | | | | | Positive % |
|----------------------|-----------|-------|-------|-------|-------|-------|--------|-------|-------|------------|
| | Bio-Rad | | | Roche | | | Qiagen | | | |
| 10 ⁴ | 25.22 | 25.61 | 25.53 | 25.24 | 25.28 | 25.23 | 25.99 | 26.11 | 26.02 | 100 |
| 10 ³ | 28.63 | 29.04 | 28.95 | 28.68 | 28.67 | 28.65 | 29.39 | 29.54 | 29.44 | 100 |
| 10 ² | 32.05 | 32.44 | 32.38 | 32.07 | 32.09 | 32.06 | 32.82 | 32.96 | 32.88 | 100 |
| 10 ¹ | 35.46 | 35.87 | 35.8 | 35.51 | 35.48 | 35.48 | 36.22 | 36.39 | 36.3 | 100 |
| 5 | 36.57 | 37.23 | 37.01 | 36.92 | 37.59 | 36.69 | 37.31 | 37.52 | 38.12 | 89 |
| 1 | 38.88 | NA | NA | NA | NA | 38.89 | NA | NA | NA | 0 |



SUMMARY

Deep Life Medical supply the full spectrum of CoVID-19 detection kits, including those using antibody and RT-qPCR technologies. Both types of test are accurate, but differ in the role they support. This paper has focused on the Deep Life RT-qPCR products for clinical laboratories: a sister paper covers antibody test kits for field use.

The scale of CoVID-19 testing around the world has enabled qPCR tests for the underlying SARS-CoV-2 virus to be optimised and streamlined, reducing hours of processing down to minutes. The Deep Life Medical RT-qPCR flow has been engineered to be as easy and fast to use as possible while minimising the potential for infection, cross-contamination and human error.

To deliver the optimised RT-qPCR flow, Deep Life supply 3 products: a laboratory uses Instasafe® or FastStream® to process the samples, then EasyFlow® RT-qPCR to perform PCR processing and analysis. The benefits of these kits over earlier generation flows are:

1. Deep Life CoVID-19 RT-qPCR kits have been designed for a process flow with the least amount of human intervention, to relieve personnel from workload and reduce consumables. This increases the throughput of laboratories, reduces the chances of human error and enables technicians to start testing with minimal training.
2. With the InstaSafe® transport tubes, the collected samples are preprocessed automatically during transport (in 5 minutes) as the transport medium is a nucleic acid extraction mix, and not a viral support medium. This means the laboratory receives samples that are not contagious. Risk of the samples infecting people in route or in the lab are eliminated, and the level of biosecurity in the lab can be relaxed. The biosecurity need only ensure there is no cross-contamination between samples, or between prep and PCR zones.
3. Both InstaSafe® and FastStream® eliminates 30 minutes of sample processing. As the process is simpler and shorter, a greater number of tests can be performed in the laboratory by the same number of technicians.
4. The EasyFlow® RT-qPCR process is the shortest currently available with just two mixes to combine with the sample and no reconstitution of dried material, no complex elutions and no extractions (alliquots). EasyFlow® cuts a 40 minute process down to five minutes.

These benefits together are a leap forward, increasing sample throughput substantially and deliver the optimum in safety for laboratory CoVID-19 testing.

Datasheets and supply information on these products are available from your local agent. The products are generally in stock and ship immediately.

InstaSafe, FastStream and EasyFlow RT-qPCR, are trademarks of Deep Life Medical Lda. All rights reserved. Refer to product datasheets for latest process information. Manufacture is by an EN 13485:2016 registered partner company. All claims for performance and accuracy have been verified by independent State ILAC accredited laboratories. Patents pending.

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