SOP_QM_MethodVal_A04_1.0_VR_EN	eurofins
Validation Report **Validation of 96er sampling device**	Validation ID: APG20_017 Version: 1.0

Review / Approval

	Name	Date (DD.MM.YYYY)	Signature
Author (Function)	5.1.2e	06.05.2020	5.1.2e
Technical Review (Function)		06.05.2020	
QM/QA Review (Function)		06.05.2020	
Approval BUMA (Function)	5.1.2e	06.05.2020	

1. Introduction

The aim of this validation was to prove the risk of cross contaminations of clinical samples which will be analysed for presence of corona / Covid 19 virus during the swab cutting process. In this process, swabs will be cut on top of a devise, which contains a 96 well plate. The test was set up with 12 test plates and 9 positive controls (10%) on each plate, on the same positions. At all uneven numbered plates, there was no cleaning after stacking the test swabs through the device opening. At all even numbered plates, the device was cleaned after every position movement with Ethanol and RNAse Away.

The results are tested via an RT-qPCR assay that is also used in routine Covid 19 analysis.

2. Scope and limitations

The purpose is to prove that no cross contaminations are happening during the transfer of the swabs into a 96 deepwell plate using the deepwell device.

3. Summary

The use of the "96er sampling device" without cleaning between the sampling steps holds a minimal risk of cross contamination between different wells, no cross contamination was found when the "96er sampling device" was used with cleaning steps between every sampling.

Therefore the procedure with cleaning between the sample processing is the method of choice.

4. Definitions and abbreviations

N/A

5. Responsibilities

Name	Function	Contact details (email, phone)
5.1.2e	5.1.2e	5.1.2e @eurofins.com
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6. Method

6.1 Description of the procedure

The "96er sampling device" is used to cut the tip of a swab (patient sample, dry swab from different suppliers, e.g. with cotton or viscose and with plastic or wooden handle) directly into a single well of the deepwell plate without cross contamination between the wells and therefore the patient samples. See: SOP_APG_SARS-CoV-2 Prozess_1.0

6.2 Equipment and Material

6.2.1 Equipment

Description	Supplier
96er sampling device	D&T engineering GmbH
Lightcycler LC 480II	Roche
Kingfisher	Thermo scientific

Note1: All equipment used in this validation was qualified for the application, released and functional. Test and measuring equipment was calibrated, calibrations were valid.

6.2.2 Materials

Description	Supplier
RNAse away	Roth
Isopropanol	CLN

6.2.3 Reagent and kits

Description	Storage	Supplier
ViroReal Kit SARS-CoV-2 & SARS (Art.Nr.: DHUV02313; Lot: 2004010KSAR)	-20°C bis -15°C	Ingenetix
NucleoMag® Vet (Art.Nr.: 744200.4; Lot.:2003/001)	18°C bis 25°C	Macherey und Nagel

Note 2: All materials, kits and reagents as outlined in 6.2.2 and 6.2.3 used were approved for use. Expiration dates were not exceeded. Kits and reagents were stored according to the manufacturer's instructions in monitored facilities.

6.3 Reference Materials

The reference materials to be used in this validation are listed in the next table.

Reference material	Supplier	Validation Parameter
108 Swabs with positive control RNA of the RT-qPCR Kit	Ingenetix + Puritan	• Accuracy • Repeatability
1044 negative swabs	Puritan	• Accuracy • Repeatability

7. Data Analysis

RNA extracts are analysed with commercial RT qPCR kit ViroReal Kit SARS-CoV-2 & SARS from Ingenetix. According to the manufacturer this kit has a range of Cp values from 23,5 to 31,5 for valid results. Cp above this value would come from very low amounts of RNA and analysis of these samples should be repeated or results should be set on "invalid".

For the validation we set a cut of Cp 35 to call an extract as "positive".

RT qPCR was performed on Roche LightCycler 480 II in 96 well plates and analysis of the data was done with the "Lightcycler® 480 SW 1.5" Software.

Analysis data were exported into a txt. file and converted into an Excel sheet.

8. Results

8.1 Accuracy:

Methodology and samples:

To determine the accuracy of the "96er sampling device" in total 12 plates with the same positive (spiked RNA) and negative samples on identical positions 6 were processed. At plates with even numbers the device was cleaned between each sampling step and plates with uneven numbers weren't cleaned in between the sampling. At one uneven plate (# 5) a malfunction of the internal process control occurred, therefore this plate was declared as invalid.

Acceptance criteria:

All plates should have the same layout in the end (positives and negative signals on the same places) no false negative or false positive samples

Tests and results:

Results are shown in the attachment **VB_APG20_017_96er sampling device_A13** and the raw data in **VB_APG20_017_96er sampling device_A01** + **_A12** and **_A14**

After the first analysis of the 12 plates RT qPCR from selected positions was repeated because of a CP-Value above our threshold of an CP of 35 in first analysis.

In these repeated analyses most of these late signals could not be reproduced except of the extract in one position on plate 7. In this case there was still a signal with CP > 35 (First analysis CP = 36,65, second analysis CP = 36,95).

Some positive control samples in plates 2, 3, 4, 9 and 11 showed no amplification of the ICP but showed amplification of the corona target, therefore they were included in this validation.

The results of the 11 plates show, besides the deviations (see 9.), the expected positive or negative signals in the corresponding positions on the plates, therefore the accuracy is given.

8.2 Repeatability

Methodology and samples:

To determine the accuracy of the "96er sampling device" in total 12 plates with the same positive (spiked RNA) and negative samples on identical positions 6 were processed. At plates with even numbers the device was cleaned between each sampling step and plates with uneven numbers weren't cleaned in between the sampling. At one uneven plate (# 5) a malfunction of the internal process control occurred, therefore this plate was declared as invalid.

Acceptance criteria:

All plates should have the same layout in the end (positives and negative samples on the same places) no false negative or false positive samples

Tests and results:

Results are shown in the attachment **VB_APG20_017_96er sampling device_A13** and the raw data in **VB_APG20_017_96er sampling device_A01** + **_A12** and **_A14**

After the analysis of the 12 plates a few positions were redone because of CP-Values above our threshold of an CP of 35.

After reanalysis the signals are gone except sample F5 on plate 7 (First analysis CP = 36,65, second analysis CP = 36,95).

Overall, it can be seen that there are small differences between the two groups of plates that are processed differently.

In the group of plates with even numbers, where the device was cleaned between sample processing, no positive signals were observed in the first RT qPCR except for a single position (C11) on plate 2 on the positions where negative samples were processed.

In a repeated RT qPCR, the positive signal could not be reproduced on the affected position in plate 2.

In the group of odd-numbered plates, without cleaning of the device was performed between sample processing, 5 signals in total were observed at positions with negative samples.

In a repeated RT qPCR these positive signals could not be reproduced on the affected positions except for one.

Therefore we conclude that a cleaning step between every sample is necessary to avoid artificial signals which are easily overseen and could be reported as false positive.

9. Deviations

Deviations from the validation protocol, rationales for actions taken and an evaluation of their criticality are listed in the following table. All other validation activities were performed in accordance to the validation plan VP_APG20_017; 22.04.2020.

Table 01: Deviations from validation plan

Validation Parameter – Plate - Sample	Requirement from plan	Deviation	Evaluation / Further Actions
Plate 1	Accuracy / repeatability	Well D1 and F1 are interchanged	Uncritical: New position was noted on the RNA-plate sample sheet and could be used for the validation
Plate 5	Accuracy / repeatability	Malfunction of the internal process control	Uncritical. The plate wasn't used for the validation
Plate 6	Accuracy / repeatability	Well D1 and E1 are interchanged	Uncritical: New position was noted on the RNA-plate sample sheet and could be used for the validation
Plate 7	Accuracy / repeatability	Well D1 and E1 are interchanged	Uncritical: New position was noted on the RNA-plate sample sheet and could be used for the validation

All deviations from the validation plan were documented and evaluated concerning their impact on the validation. The deviations assessed as "not critical" are in compliance with the validation plan VP_APG20_017; 22.04.2020.

10. Discussion

Result discussions for the individual validation parameters can be found in the corresponding chapters 8.1 to 8.2.
In summary, the acceptance criteria for all validation parameters are fulfilled.
The use of the "96er sampling device" without cleaning between the sampling steps holds a minimal risk of cross contamination between different wells, no cross contamination was found when the "96er sampling device" was used with cleaning steps between every sampling.

11. Records

Plan and report are stored electronical under the following link:

5.1.2h

The physical documentation is stored in room 205, building 1 second store.

12. References and related documents

SOPs:

SOP_APG_SARS-CoV-2 Prozess_1.0

Validation documents:

VP_APG20_017

RA_APG20_017

13. Attachments

VB_APG20_017_96er sampling device_A01	Raw data Plate 1
VB_APG20_017_96er sampling device_A02	Raw data Plate 2
VB_APG20_017_96er sampling device_A03	Raw data Plate 3
VB_APG20_017_96er sampling device_A04	Raw data Plate 4
VB_APG20_017_96er sampling device_A05	Raw data Plate 5
VB_APG20_017_96er sampling device_A06	Raw data Plate 6
VB_APG20_017_96er sampling device_A07	Raw data Plate 7
VB_APG20_017_96er sampling device_A08	Raw data Plate 8
VB_APG20_017_96er sampling device_A09	Raw data Plate 9
VB_APG20_017_96er sampling device_A10	Raw data Plate 10
VB_APG20_017_96er sampling device_A11	Raw data Plate 11
VB_APG20_017_96er sampling device_A12	Raw data Plate 12
VB_APG20_017_96er sampling device_A13	Raw data of Plates combined in an excel sheet
VB_APG20_017_96er sampling device_A14	Raw data redo Samples