

Metadata file for

Perrine Robin, Laura Barnabei, Stefano Marocco, Jacopo Pagnoncelli, Daniele Nicolis, Chiara Tarantelli, Agatino Christian Tavilla, Roberto Robortella, Luciano Cascione, Lucas Mayoraz, Céline M. A. Journot, Mounir Mensi, Francesco Bertoni, Igor Stefanini Sandrine Gerber-Lemaire. A DNA biosensors-based microfluidic platform for attomolar real-time detection of unamplified SARS-CoV-2 virus.

General – Chemistry

Detailed procedures and analyses of each experiments are stored as pdf files in the folder “Labbook - Chemistry” in the format “PRcoatXXX” with XXX indicating the name of the batch.

Fluorescence experiments allowing DNA quantification at the surface of the slides are stored as xcl files in the folder “Surface characterization” and in the subfolder “Fluorescence-characterizations”. As general rule, the name of the xcl file is PRcoatXXX-quantification, with XXX matching the name of the batch written in the Labbook.

XPS measurements of the wide spectrum and the resolved spectrums of N 1s, C 1s, Si 2p, O 1s and P 2p energies can be found in the subfolder “XPS” of the folder “Surface characterization” as xcl format.

General - Biology

Detailed RNA extraction data, along with experimental procedures and test of different extraction kits are present in the excel folder called “RNA extraction”, that can be found in the folder “Extraction data”. Two different sheets of that folder show two different tests of extraction. One sheet shows the experimental validation of the chosen kit through qPCR. The format is in xlsx.

Experimental procedures conducted by qPCR to assess the validity of the probes designed are present in the folder “Computational data”, in the file “SARS-CoV2 test of primers”. Raw data hybridization together with images revealing the fluorescence are present both in the excel file called “Raw Data Hybridization” as well as in the power point called “raw data figures manuscript” that are present in the folder “Fluorescence imaging”. The format is in xlsx and ppt.

General - Microtechnique

Procedures of RNA detection can be found in the subfolder “Labbooks-Detection” of the folder “Labbooks”, in a pdf named “Laboratory_Report_SUPSI”. Collected data and scripts are in the folder “Fluorescence detection”, sorted in folders depending on the type of slides studied. Each folder contains 3 subfolders:

Raw_data: every file generated from the experiment is in csv format and is saved in this folder.

Script: the Matlab script that elaborates the csv files. There is also an html copy of the Matlab script in the folder “html”.

Output: the folder where the Matlab script saves data/images.

More specific resume can be found in the folder «Fluorescence detection» detailing names of script and data.

Main manuscript

Table 1 – Selected probes for SARS-CoV-2 detection

Five probes targeting highly conserved regions of SARS-CoV-2 were selected based on their specificity, GC content, secondary structure and stability of the probe-target duplex estimated by the thermodynamic model. Data can be found in the folder “Computational data”.

Figure 3 – SARS-CoV-2 RNA detection with S-PP1 slides

The slides **S-PP1** used for the virus detection experiment are from the batch PRcoat89. Details about the production of the slides used for these experiments can be found in the Labbook pdf file “PRcoat89” in the subfolder “Labbooks-Chemistry”. Hybridization of the probes present in the slides and the viral SARS-CoV2 RNA was performed at 50°C for 10 minutes in presence of Sybr green I (1:500), a procedure detailed in the pdf file “Laboratory_Report_SUPSI” in the subfolder “Labbooks-Detection”. The copies/μL of the virus that has been tested were: 50-25-12-6-3-1 copies/ μL. RNA of MERS as well as blank (water) in presence of the Sybr green I were used as a negative control. Raw data are present in the file called “Raw data hybridization”. Results are shown as the mean ± SD of two independent experiments, that can be find in the respective subfolders “merge”, “S-PP1_1” and “S-PP1_2” in the folder “Fluorescence detection”. Detail about the script to generate the figure can be found in the file “resume merge”.

Figure 4 – Immunofluorescence detection of SARS-CoV-2 RNA with S-PP1 slides

The slides used for the virus detection experiment are from the batch PRcoat89. Details about the production of the slides used for these experiments can be found in the Labbook pdf file “PRcoat89” in the subfolder “Labbooks-Chemistry”. Hybridization procedures are explained in the folders S-PP1_1 and S-PP1_2. Details bout the procedure can be found in the pdf file “Laboratory_Report_SUPSI” in the subfolder “Labbooks-Detection”. After the hybridization took place, the slides have been later analyzed using Cytation Cell Imaging Multi-Mode Reader. Images are present in the file called “Raw data hybridization”, in the folder “Fluorescence imaging”.

Supporting Information

Figure S1 – Specificity of PP1 probe

The specificity of the probe was determined using the qPCR methodology. The primers used were 5' TGAAGCAAGGTGAAATCAAGGA 3' and 5' AACAGCAAGAAGTGCAACGCCAAC 3' (as probe PP1). The samples tested were the RNA with SARS-CoV2 that resulted to be positive. To show the specificity of the probes RNA of MERS virus as well as RNA from mammalian cells has been used. Cycle threshold of the qPCR has been analyzed for the results. Results can be found in the file “SARS-CoV2 test of primers”, in the folder “Computational data”.

Table S1 – Quantity of surface-immobilized ssDNA probe on S-PP1 and S-Suc slides

Quantification of DNA on **S-PP1** slides was performed with the batches PRcoat48, PRcoat60, and PRcoat64. The negative controls refer to the batches PRcoat47, PRcoat48 and PRcoat64. Details about the production of these batches can be found in Labbook pdf files, and results of the fluorescence can be found in the xcl files in the subfolder “Fluorescence-characterizations” in the folder “Surface characterization”.

Figure S2 – Representative standard curve for the quantification of Cy3-complementary PP1 strand liberated from a surface

The representative curve for Cy3-tagged DNA calibration was obtained during PRcoat48 quantification and plot with Origin software. Details about the fluorescence experiment can be found in the subfolder “Fluorescence-characterizations” in the folder “Surface characterization”.

Table S3 – Relative quantification of C, N, O and S detected via XPS for amino-modified slides S-NH₂, succinic-modified slides S-Suc and DNA-functionalized slides S-PP1.

For XPS analysis, the **S-NH₂** and **S-Suc** slides were from the PRcoat33 batch, and **S-PP1** from the batch PRcoat47. Details about the production of these batches can be found in Labbook pdf files. The relative concentrations of the elements were measured via XPS using CASA XPS software. A linear background was applied to all of them. The XPS data can be found in the subfolder “XPS” in the folder “Surface characterization” as xcl format.

Figure S3 – XPS spectra of P 2p region

For XPS analysis, **S-NH₂** (a) and **S-Suc** (b) slides were from PRcoat33 batch, DNA-functionalized slides **S-PP1** (c) was from the batch PRcoat47 and called PRcoat47-positive, and S-Suc slides incubated with NH₂-PP1 probes without coupling agent (d) was from PRcoat47 and called PRcoat47-negative. Details about the production of these batches can be found in Labbook pdf files. The XPS data can be found in the subfolder “XPS” in the folder “Surface characterization” as xcl format.

Figure S4 – XPS spectra of C 1s region

For XPS analysis, **S-NH₂** (red) and **S-Suc** (green) slides were from PRcoat33 batch. DNA-functionalized slides **S-PP1** (blue) refers to the batch PRcoat47 and called PRcoat47-positive. Details about the production of these batches can be found in Labbook pdf files. The XPS data can be found in the subfolder “XPS” in the folder “Surface characterization” as xcl format.

Figure S5 – XPS spectra of N 1s

For XPS analysis, **S-NH₂** (red) and **S-Suc** (green) slides were from PRcoat33 batch. DNA-functionalized slides **S-PP1** (blue) refers to the batch PRcoat47 and called PRcoat47-positive. Details about the production of these batches can be found in Labbook pdf files. The XPS data can be found in the subfolder “XPS” in the folder “Surface characterization” as xcl format.

Table S4 – Quantification of surface immobilized PP1 DNA probe on S-PP1 slides after 10 days of storage at 4°C in different media

Slides used for stability assay are from the batch PRcoat48. Details about the production of these batches can be found in Labbook pdf files. Fluorescence results can be found in the xcl file name “PRcoat 48-stabilitytest” in the folder “Surface characterization”, subfolder “Fluorescence-characterizations”.

Figure S7 – RNA extraction performed using different concentration of human saliva mixed with Lucigen Quick Extract DNA kit (1:1).

RNA concentration was assessed using the nanodrop 2000/2000c spectrophotometer. The kit used for RNA extraction was the Lucigen. Extraction has been made with the kit stored at -20°C, as manufacturer suggestion, at 4°C and at Room Temperature. Especially the storage at room temperature was important for future use of the device in the everyday life. SARS-CoV2 virus was mixed with human saliva (tested negative for the virus) at 1:1. Different concentrations were analyzed. The raw data can be found in the excel file called “RNA extraction”, in the folder “Extraction data”.

Table S6 – Quantity of surface-immobilized MB-PP1 probes on S-MB-PP1 and S-Suc slides

Quantification of DNA on **S-MB-PP1** slides and their respective negative controls was performed with the batches PRcoat87 and PRcoat96. Details about the production of these batches can be found in Labbook pdf files, and results of the fluorescence can be found in the xcl files in the subfolder “Fluorescence-characterizations” in the folder “Surface characterization”.

Figure S8 – Comparison of the sensing properties of S-PP1 and S-MB-PP1 slides

Slides used were from batch PRcoat89 for **S-PP1** slides and PRcoat99 for **S-MB-PP1** slides. Details in the production process can be found in Labbook pdf files PRcoat89 and PRcoat99. Details about fluorescence evaluation can be found in the pdf file “Laboratory_Report_SUPSI” in the folder “Labbooks”. Details about the scripts and collected data can be found in the directory MB-PP1 for **S-MB-PP1** evaluation, and S-PP1_1 for the slides **S-PP1**.

Figure S9 – Difference between S-MB-PP1 samples and baseline

Slides used were from batch P PRcoat99 for **S-MB-PP1** slides. Details in the production process can be found in Labbook pdf files PRcoat99. Details about fluorescence evaluation can be found in the pdf file “Laboratory_Report_SUPSI”. Details about the scripts and collected data can be found in the directory MB-PP1 for **S-MB-PP1** evaluation.