

for SARS-CoV-2 positive. CSF: transparent appearance, slightly xanthochromic color, coagulation and negative film. Proteins 105 mg / dl, glucose 45 mg / dl, leukocytes 121 mm<sup>3</sup>, erythrocytes 66 mm<sup>3</sup>, PMN 8% and MNN 92%. Negative culture, PCR Herpes Virus negative, Viral load for SARS CoV2 in CSF 3,400 cop / ml and plasma 118,900 cop / ml. Aseptic meningitis is confirmed by SARS-CoV-2. Antiviral and antibiotics are discontinued and Gamma globulin and methylprednisolone are administered. Evolving favorably and egress at 6th day to complete oral steroid treatment for 3 more day.

**Conclusion.** The mechanism by which SARS-CoV2 affects the CNS is still unknown. This findings suggests direct infection can be possible. Although it is also described vascular affection has been found that the Spike protein of the virus binds to ACE-2 receptor present in the cerebral vascular endothelium. Neurological manifestations have been described even without respiratory symptoms. A novel pediatric case with viral load for SARS-CoV-2 in CSF is demonstrated. Importance of detecting SARS-CoV-2 in children with encephalitis, which can progress satisfactorily.

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### 362. Saliva as a Reliable Sample Type for Mass SARS-CoV-2 Testing Strategies

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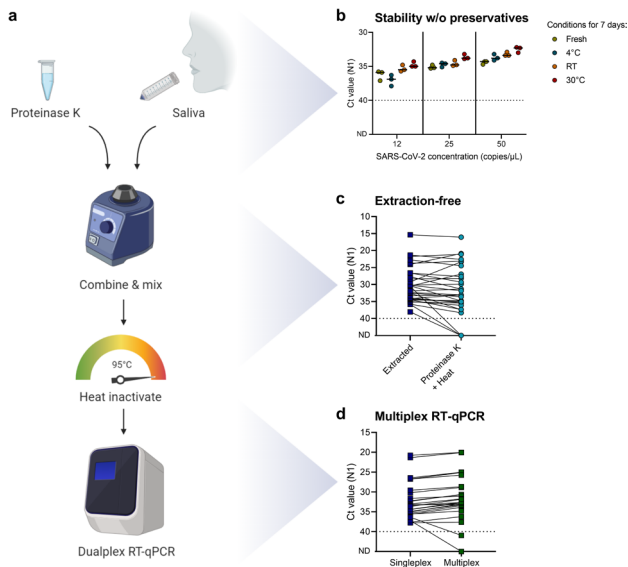
**Session:** P-15. COVID-19 Diagnostics

**Background.** Quickly detecting and isolating individuals positive for SARS-CoV-2 is essential for limiting virus spread. Policy makers rely on the number of active cases to make decisions, and individuals use this information to evaluate risk should they return to public spaces. Robust testing strategies have been plagued with limited authorized diagnostic assays and high test prices, with large-scale implementation hampered by worldwide supply chain issues.

**Methods.** Having identified its potential early in the pandemic, we simplified saliva-based COVID-19 diagnostic testing by (1) not requiring collection tubes with preservatives, (2) replacing nucleic acid extraction with a simple enzymatic and heating step, and (3) testing specimens for SARS-CoV-2 in dualplex RT-qPCR. Moreover, we validated this approach ("SalivaDirect") with reagents and instruments from multiple vendors to circumvent supply chain disruptions.

**Results.** SalivaDirect's simplified protocol does not compromise on sensitivity. In our hospital cohort, we found a high positive agreement (94%) between saliva tested with SalivaDirect and nasopharyngeal swabs tested with a commercial RT-qPCR kit. With the National Basketball Association we tested 3,779 saliva specimens from healthy individuals and detected low rates of invalid (0.3%) and false-positive (< 0.05%) results. Using comparative assays and sample types, we also demonstrated SalivaDirect to efficiently detect SARS-CoV-2 in asymptomatic individuals.

SalivaDirect is a simplified method for SARS-CoV-2 detection



(A) Schematic overview of SalivaDirect workflow depicting the main steps of mixing saliva with proteinase K, heat inactivation, and dualplex qRT-PCR testing. Figure created with Biorender.com. (B) SARS-CoV-2 is stable in saliva for at least 7 days at 4C, room temperature (RT), and 30C without addition of stabilizing

buffers. Spiked-in saliva samples of low virus concentrations (12, 25, and 50 SARS-CoV-2 copies/mL) were kept at the indicated temperature for 7 days and then tested with SalivaDirect. N1 cycle threshold (Ct) values were lower when kept for 7 days at 30C as compared to fresh specimens (Kruskal-Wallis; p = 0.03). Horizontal bars indicate the median. (C) Comparing Ct values for saliva treated with proteinase K and heat as compared to nucleic acid extraction yields higher N1 Ct values without extraction (Wilcoxon; p < 0.01). (D) Testing extracted nucleic acid from saliva with the N1 primer-probe set (singleplex) as compared to a multiplex assay showed stronger N1 detection in multiplex (Wilcoxon; p < 0.01). The dotted line in (B)-(D) indicates the limit of detection.

**Conclusion.** Saliva is a valid alternative to swabs for SARS-CoV-2 screening. Importantly, SalivaDirect enables labs to utilize existing infrastructure, improving test implementation time and requiring limited investment to scale-up to meet mass testing needs. With the safe and reliable self-collection of saliva, our vision is to help provide accessible and equitable testing solutions, especially in low-resource and remote settings.

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### 363. Characteristics of Envelope and Nuclear Gene Expression Patterns in Asymptomatic SARS-CoV-2 Patients: A Single Center Retrospective Observational Study

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**Session:** P-15. COVID-19 Diagnostics

**Background.** Reverse transcription-polymerase chain reaction (RT-PCR) is used for the diagnosis of COVID-19, caused by SARS-CoV-2. RT-PCR is a method that detects the virus by amplifying two regions of the target viral genome, namely the nuclear (N) and envelope (E) encoding sequences. However, no reports have shown a relationship between the symptoms and the gene expression patterns, especially in asymptomatic patients. Herein, we validated the characteristics of E and N gene expression patterns using RT-PCR on samples obtained from asymptomatic COVID-19-positive patients.

**Methods.** In this retrospective cohort study, conducted at Juntendo University Nerima Hospital, Tokyo, Japan, SARS-Cov-2 RT-PCR positive patients whose specimens had been obtained and analyzed by our laboratory technicians from September 1, 2020 to December 31, 2020 were enrolled. For RT-PCR, the LightMix Modular SARS-CoV-2 reagent (TIB MOLBIOL company) was used. After excluding patients who had symptoms, background, demographic, laboratory, and gene expression pattern data were collected from RT-PCR-positive asymptomatic patients. We also investigated patients who met the release criteria of the Center for Disease Control and prevention. Continuous and categorical variables were analyzed, with p < 0.05 set as statistical significance using the student-t test, chi-square test, or Fisher's exact test, respectively.

**Results.** Of 92 RT-PCR-positive asymptomatic patients, 57 comprised the expression E only group (Group E) and 35 comprised the E+N group (Group E+N). Significantly more patients in Group E met the release criteria compared to those in Group E+N [41 (71%) vs 10 (28%), p < 0.001]. Among patients who met the release criteria, those in Group E+N had significantly more immunosuppression [7 (70%) vs 8 (30%), p = 0.004].

Moreover, among the patients who underwent RT-PCR screening, no patients in Group E developed symptoms [0 vs 6 (42%), p = 0.02].

**Conclusion.** The results of this study suggest that RT-PCR-positive asymptomatic patients can be divided into three patterns: pre-symptomatic, gene E+N-positive patients; post-symptomatic covid-19-recovered patients, regardless of gene E and N expression patterns; and false positive, gene E-positive patients.

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### 364. Individualized Prognostics in COVID-19 Facilitated by Computer Recognition of Blood Leukocyte Subsets

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**Session:** P-15. COVID-19 Diagnostics

**Background.** To determine whether CBC differentials of COVID+ inpatients can predict, at admission, both maximum oxygen requirements (MOR) and 30-day mortality.

**Methods.** Based on an approved IRB protocol, CBC differentials from the first 3 days of hospitalization of 12 SARS CoV-2 infected patients were retrospectively extracted from hospital records and analyzed with a privately owned Pattern Recognition Software (PRS, US Patent 10,429,389 E2) previously validated in sepsis, HIV, and hantavirus infections. PRS partitions the data into subsets immunologically dissimilar from one another, although internally similar.