**Supplementary data/figures for the article:**

**Viral Transport Media (VTM) pooling to scale-up COVID-19 diagnostics: Resource optimization at population level screening**

Pramod Gautam1, Faraz Ahmad2†, Jyoti Sharma1†, Jasmine Samal2†, Sugandha Singh2, Anwar Alam2, Kiran Kumar Rade3, Nivedita Gupta4, Nasreen Z Ehtesham\*2, Usha Agrawal\*1

1 Cancer Research, Imaging and Bio-Banking Laboratory, ICMR-National Institute of Pathology, New Delhi 110 029, India. 2 Inflammation Biology & Cell Signaling Laboratory, ICMR-National Institute of Pathology, New Delhi 110 029, India. 3 WHO Country Office for India, New Delhi, India. 4 Division of Epidemiology & Communicable Diseases, Indian Council of Medical Research, New Delhi 110 029, India

**†**: These co-authors contributed equally to the work

**\*Co-corresponding Authors:**

Dr. Usha Agrawal, MD, PhD\*

Contact - 011-26165797, 011-26166704, 26198406 (Ext. 435)

E-mail: [ushakaggarwal.nip@gov.in](mailto:ushakaggarwal.nip@gov.in)

Dr. Nasreen Z. Ehtesham, PhD\*

Scientist Emeritus

Contact - 011-26165797, 011-26166704, 26198406 (Ext. 412)

E-mail: [nzehtesham01@gmail.com](mailto:nzehtesham01@gmail.com)

**Email addresses of authors:**

Pramod Gautam (First author): [seepramod77@gmail.com](mailto:seepramod77@gmail.com)

Faraz Ahmad: [farazamu2014@gmail.com](mailto:farazamu2014@gmail.com)

Jyoti Sharma: [jyoti22khandal@gmail.com](mailto:jyoti22khandal@gmail.com)

Jasmine Samal: [samaljasmine@gmail.com](mailto:samaljasmine@gmail.com)

Sugandha Singh: [sugandha583@gmail.com](mailto:sugandha583@gmail.com)

Anwar Alam: [dranwar.iit@gmail.com](mailto:dranwar.iit@gmail.com)

Kiran Kumar Rade: [radek@who.int](mailto:radek@who.int)

Nivedita Gupta: [ngupta@icmr.org.in](mailto:ngupta@icmr.org.in)

Nasreen Z Ehtesham: [nzehtesham@gmail.com](mailto:nzehtesham@gmail.com)

**Supplementary Figure S1.** Flowchart of the experimental work

**Supplementary Table 1**. The table shows the screening stage Ct values of the samples belonging to the four sets.

**Supplementary Figure S2.**The scatter plot and correlation between Ct values for internal control gene only. The plot A compares the Ct values of internal control from 140 μL VTM (NIV kit) at screening stage and individual testing from 560 μL VTM (TaqMan probe-based assay). The plot A shows absence of correlation (R = 0.29, p-value = 0.36). The internal control genes in both the kits are different. It shows that the different input volumes used at the screening stage and individual testing stage doesn’t contribute towards the change in Ct of the internal control gene as the adjusted volumes of other reagent used at the RNA extraction stage. The plot B shows the data comparing the Ct values from 560 μL VTM at the pooled testing stage and Ct values from (140 μL VTM and 560 μL VTM) individual testing stage. It again shows the absence of any correlation (R = -0.18, p-value = 0.46) between Ct values of internal control gene in RT-PCR reaction. This shows that the input volume of the VTM doesn’t contribute towards the change in Ct of the internal control gene.

**Supplementary Figure S3.** Effect of sensitivity/specificity of the kit used, 2nd stage sub-grouping and infection positivity rate on final reduction of expected number of tests in case of pool size of 16. Three different bar-plots refer to the different sensitivity/specificity of kit to be used for pooling (100%, 99% and 95%) on left y-axis. Three points refer to the %age reduction in expected no. of tests on right y-axis. The x-axis refers to the pool design. First three bar-plots are for 2-step pooling and rest are for 4x4 and 8x2 sub-pools for 3 stage pooling plan.

**Supplementary Table 2. Estimation of the reduction in number of diagnostic tests required**. Here we show the plan for pooled testing based on several criteria which affect the final outcome of the approach. Sub-grouping after pooling depends upon several criteria i.e. infection positivity rate, sensitivity and specificity of the kit being used, pooling stages including the final individualized RT-PCR test, initial pool size and 2nd stage sub-grouping to achieve a decent reduction in expected number of tests. This table shows the final expected number of test required per individual factoring all the input variables. The calculation are done a publically available Shiny app based on R statistical software environment (Black et al., 2015)