Comprehensive Virological Analysis for Evidence Based Risk of SARS-CoV2 Transmission to Clinical Staff During Surgery.

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Our recent review of the literature (doi:10.20944/preprints202003.0451.v2) shows current knowledge of SARS-CoV-2 tissue distribution beyond the respiratory tract is poor. Problems include: i) narrow range of sample types analysed (focusing on stool and blood) ii) use of non-validated PCR assays of unknown specificity/sensitivity, iii) overinterpretation of results from standard PCRs to infer infectious virus is present. The last point is particularly important, with many researchers failing to realise that all of the standard SARS-CoV-2 virus-specific PCRs in common use cannot differentiate between infectious virus and non-infectious neutralised virus or non-infectious fragments of viral RNA abundantly shed during infection. Recent work highlights this point: stool samples yielding strong PCR signals completely lacked infectious SARS-CoV-2 virus (Wolfel et al. Nature 2020) and thus cannot transmit infection.

The risk to clinical staff during clinical procedures such as laparotomy is therefore unclear. Data from conventional PCR assays alone is likely to over-estimate risk, limiting patient access to essential surgery. We will address this critical knowledge gap by applying a range of high-quality virological techniques to diverse sample types, thereby **providing robust data to support evidence-based practice**. Central to our approach is assay-1, our UKAS accredited PCR assay that detects and quantifies SARS-CoV2 RNA that we will apply to each of our samples**. Crucially, we now have the ability to differentiate between infectious and non-infectious virus** using assays currently at a late stage of development at Birmingham. Assay-2 is a viral subgenomic PCR assay which, unlike conventional PCRs, specifically detects active virus infection. Assay-3 is viral nucleocapsid detection by immunohistochemistry. Assay-4 is a virus culture assay which directly detects infectious virus in fresh samples.

We have access to a diverse range of tissue types which will be tested using the above assays. We have ethical approval to obtain multiple tissues from post mortems. Importantly these include needle biopsies *and* open post mortems, allowing us to investigate viral infection heterogeneity within different organs (noted during COVID-19 autopsies). The deceased include patients from ICU

but also community deaths thus spanning a range of time periods post-initial SARS-CoV2 infection. These tissues will be fixed, but encapsidated viral RNA is stable and can readily be detected by our PCR and viral nucleocapsids detected by immunohistochemistry. These samples are complemented by fresh surgical samples from COVID-19 patients undergoing essential emergency surgery. Overall, tissues analysed will include skin, fat, muscle, fascia, peritoneal fluid and tissues of the female reproductive tract.