LabAnalysis
HIGH QUALITY CONTROLS

Analysis of virucidal activity over treated surface (internal code 2003197-001)

Scope of work and synthetic method

Scope of work was to test the stability of SARS-CoV-2 virus (inactivated to heat, ATCC VR-1986HK, batch 70035039) over surfaces treated with paints containing “catalyzer”.

Here follows the adopted approach: the treated surfaces have been inoculated with a known amount of virus (7,5 105 genomic copies) and they have been left incubating for a different times (0,1,3 and 6 hours). Thus, the residual virus has been recovered through swab (by swab containing PBS and 70% of ethanol.

Therefore the samples have been processed to extract the viral RNA and measure it through Real-Time PCR, by applying a commercial kit marketed by Generon, specific for the SARS-CoV-2's RdRp gene (COVID 19 (RdRp gene) detection + Process control, PMB00C_M2). The activity has been conducted in triplicate for each sperimental point. As control, in parallel to the surface with “catalyzer”, has been tested a similar surface for texture and shape, but without additive.

Results

The results of the test have been set out in the table below, in which, for each sperimental point, are reported the median Cts (cycle thresholds) for the signal of SARS-Cov-2's RdRp gene. Low values of Ct correspond to a major quantity of viral RNA present over the test-pieces; higher Cts correspond to a minor quantity of viral RNA.

Test over surface	Ct Paint + Catalyzer	Ct “Mock” paint	Reciprocal of $2^{-(\Delta\Delta Ct)}$
T= 0h	31.46	28.69	6.8
T= 1h	31.93	29.42	5.7
T= 3h	32.08	29.44	6.2
T= 6h	32.38	29.97	5.3
Media	31.96	29.38	6.0
Dev Std	0.38	0.53	0.65
RSD %	1.20	1.79	10.80

By analyzing the difference between the Cts of control slides (without catalyzer) and the Cts of examination samples' slides, it is possible to determine the 2 …, indicator of a different expression of the viral RNA over paint with catalyzer compared to the single paint. Such difference can be estimated aproximately 6 times.

This result highlights the virucidal action of the paint with catalyzer. However, it doesn't prove a performance over time of the phenomenon, since this is evident at “time 0” too (as result of the experimental plant, this translates into a virus-paint's period of contact of aproximately 10-15 minutes).

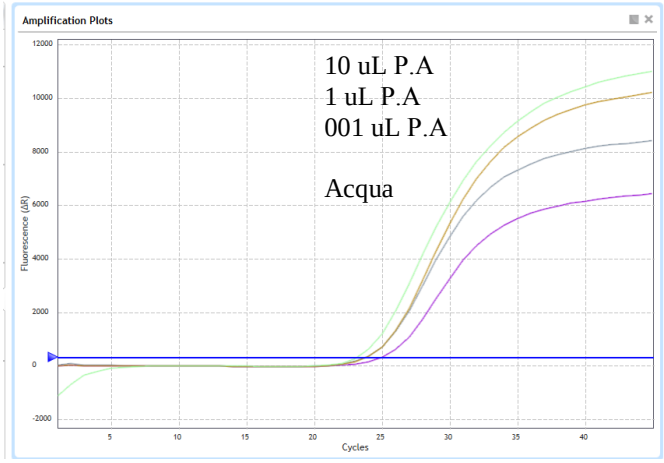
At first glance, this behaviour could be attributed to a reducted efficiency of the virus extraction from the surfaces containing catalyzer or to a potential interference of the catalyzer to the extraction steps of the nucleic material.

With the purpose to further investigate the “catalyzer” action, we have verified the direct effect of the additive ---------, containing about the 40% of Umana's Active Substance.

First of all, it was evaluated the direct effect of the UMANA’s Active Substance over the polymerization reaction. For this purpose, the additive has been added in increasing quantities directly to the mix of reaction (containing the polymerase and the substrata required to the polymerization), together with a positive check for the RdRp gene.

From the amplification curves and the Cts values, it seems clear that the UMANA’s Active Substance increases the efficiency of the polymerization reaction. This evaluation has been executed in presence of different dosages of the RdRp gene’s positive check too.

Hereafter, the raw data obtained are shown, in which it seems clear the increase of the polymerization reaction as consequence of the UMANA's Active Substance (P.A). Therefore, it was not highlighted the inhibiting effect, quite the contrary.



Mix di reazione +	Ct (Level 1)	Ct (Level 2)	Ct (Level 3)	Ct (Level 4)
Acqua	24.00	24.43	25.48	26.32
0,01 uL $_{P.A}$	22.35	23.27	24.48	25.24
1 uL $_{P.A}$	22.33	23.33	24.92	25.30
10 uL $_{P.A}$	20.59	22.72	23.89	24.56

Lastly, it was evaluated the direct effect of the UMANA's Active Substance over the recovery of the virus directly into the solution. The SARS-CoV-2 virus inactivated to heat has been added with an increasing amount of P.A and it was incubated for 0,1,3 or 6 hours in order to simulate the period of contact required for the swabs.

Thus, the samples have been processed for the extraction of the viral RNA and analysed through Real-Time PCR likewise.
The result are reported in the following table:

Prova in soluzione	Ct Controllo (Acqua)	Ct 1 uL P.A	Ct 5 uL P.A
T= 0h	28.53	Absent	Absent
T= 1h	28.12	Absent	Absent
T= 3h	28.59	Absent	Absent
T= 6h	28.45	Absentnte	Absent
Average	28.42	-	-
Dev Std	0.21	-	-
RSD %	0.74	-	-

In response to these data, it is hereby confirmed a strong action of the UMANA's Active Substance (P.A) over the viral material: in presence of P.A no recovery is obtained and no genomic pair is pointed out in solution. Although these test made in solution do not allow a quantification of the virus elimination as consequence of the catalyzer, they withstand the hypotheses previously made, thus suggesting a potential effect of the catalyzer over the recovery of the viral material from the surfaces, which is evident in short periods of contact too. However, these data are insufficient to determinate the catalyzer's exactly mechanism of action over the virus particles (direct action over proteins, over RNA or mechanical seizure).

Conclusions

As stated above, we can conclude that by depositing a known amount of SARS-CoV-2 virus inactived to heat over a surface treated with UMANA paint cointaining catalyzer, the recovery of the nucleic material turns out to be approximately 6 times inferior compared to what happens over a surface similarly treated with a paint not containing catalyzer.

This effect is evident in the period of contact in the order of minutes and it generally remains steady over time.

Such result is corroborated by the fact that not only the UMANA's Active Substance is active directly against the viral genomic material in solution, but also because this additive encourages the efficiency of the polymerization reaction.

The latter leads to assume that the difference of Ct between samples with and without catalyzer is actually underestimated. Thus, the virus elimination could be higher than the value of 6.

The catalitic washable paint over which it was made the first worldwide research project to determine the virucidal degratative activity towards the SARS-Cov-2, the pathogen responsible of the Covid-19 pandemic. The result of the research pointed out that the UMANA's Active Substance has a strong virucidal action against the SARS-Cov-2.
Such effect is evident in the period of contact in the order of minutes and it generally remains steady over time.

Research project on the virucidal ability of UMANA against the SARS-Cov-2, in partnership with LabAnalysis HIGH QUALITY CONTROLS.

CERTIFICATE OF ANALYSIS

ATCC® Number: VR-1986HK™

Lot Number: 70035039

Product Name: Heat Inactivated 2019 Novel Coronavirus

Classification: *Coronaviridae, Betacoronavirus,* SARS-Related Coronavirus 2, Isolate USA-WA1/2020

Volume: 250 µL

Product Format: Frozen heat inactivated virus preparation

Expiration Date: Not applicable

Storage Conditions: - 70°C or colder

Test / Method	Specification	Result
Pre-inactivation titer*	Report results	1.6 x 10^5 $TCID_{50}$/mL
RNA copy number by ddPCR	Report results	3.75 x 10^5 genome copies/µL
Viral inactivation: ≥ 10% of input seed is incubated with host cells under appropriate growth conditions	No viable infecting agent detected by visual observation	Pass

***Titer notes:** Titer was determined prior to heat inactivation in 7 days on Vero E6 cells (ATCC® CRL-1586™) at 37°C with 5% CO_2 as determined by cytopathic effect. Tested on 01APR2020.

Program Manager, ATCC Federal Solutions

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