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(57) Abstract :
METHODS AND COMPOSITIONS FOR INFECTIOUS RNA, cDNA, AND mRNA OF SARS CORONAVIRUS. ABSTRACT The invention METHODS AND COMPOSITIONS FOR INFECTIOUS RNA, cDNA, AND mRNA OF SARS CORONAVIRUS • is provides a RNA, cDNA of a severe acute respiratory syndrome (SARS) coronavirus, recombinant SARS coronavirus vectors, and SARS coronavirus replicon particles. Also provided are methods of making the compositions of this invention and methods of using the compositions as immunogens and/or vaccines and/or to express heterologous nucleic acids. The invention provides compositions and methods for detecting the presence of SARS-coronavirus, for screening anti-SARS coronavirus agents and vaccines, and for reducing infection with plus-strand RNA viruses such as SARS-coronavirus and also provides a method for detecting replication of severe acute respiratory syndrome coronavirus (SARS-coronavirus) in a sample, comprising detecting the presence SARS-coronavirus sgRNA in a sample. In one example, sgRNA comprises at least a portion of a leader sequence. The method further comprises detecting SARS-coronavirus gRNA. While not intending to limit the method of detection, in one embodiment, the detecting of gRNA and/or sgRNA is by reverse transcriptase PCR, ribonuclease protection assay, and/or by Northern blot. In another embodiment, the method further comprises quantitating sgRNA and/or gRNA. Also provides a method for detecting the presence of severe acute respiratory syndrome coronavirus (SARS-coronavirus) in a sample, comprising: a) providing: (i) a sample; and (ii) cells, wherein said cells support replication of SARS-coronavirus in the absence of substantial cytopathic effect; b) inoculating the cells with the sample to produce inoculated cells; and c) detecting the presence of the SARS-coronavirus in the inoculated cells. In some preferred embodiments, the cells are chosen from but not limited to HEK-293T, Huh-7, Mv1Lu, pRHMK and pCMK.

No. of Pages : 21 No. of Claims : 9

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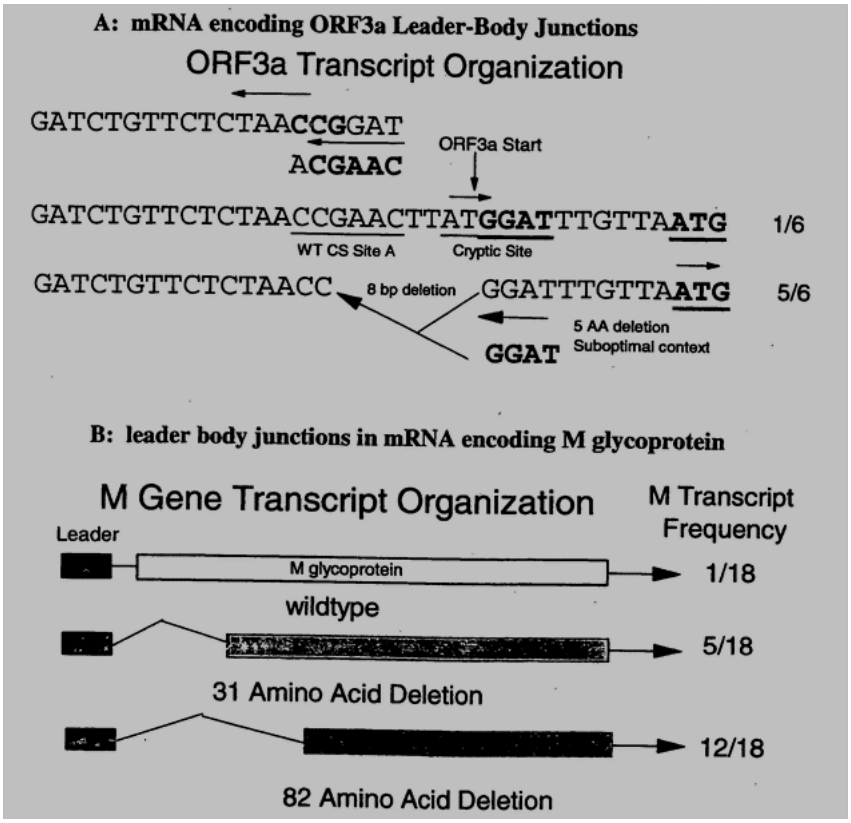

FIG.0.1-A, B: MRNA ENCODING STATUS.

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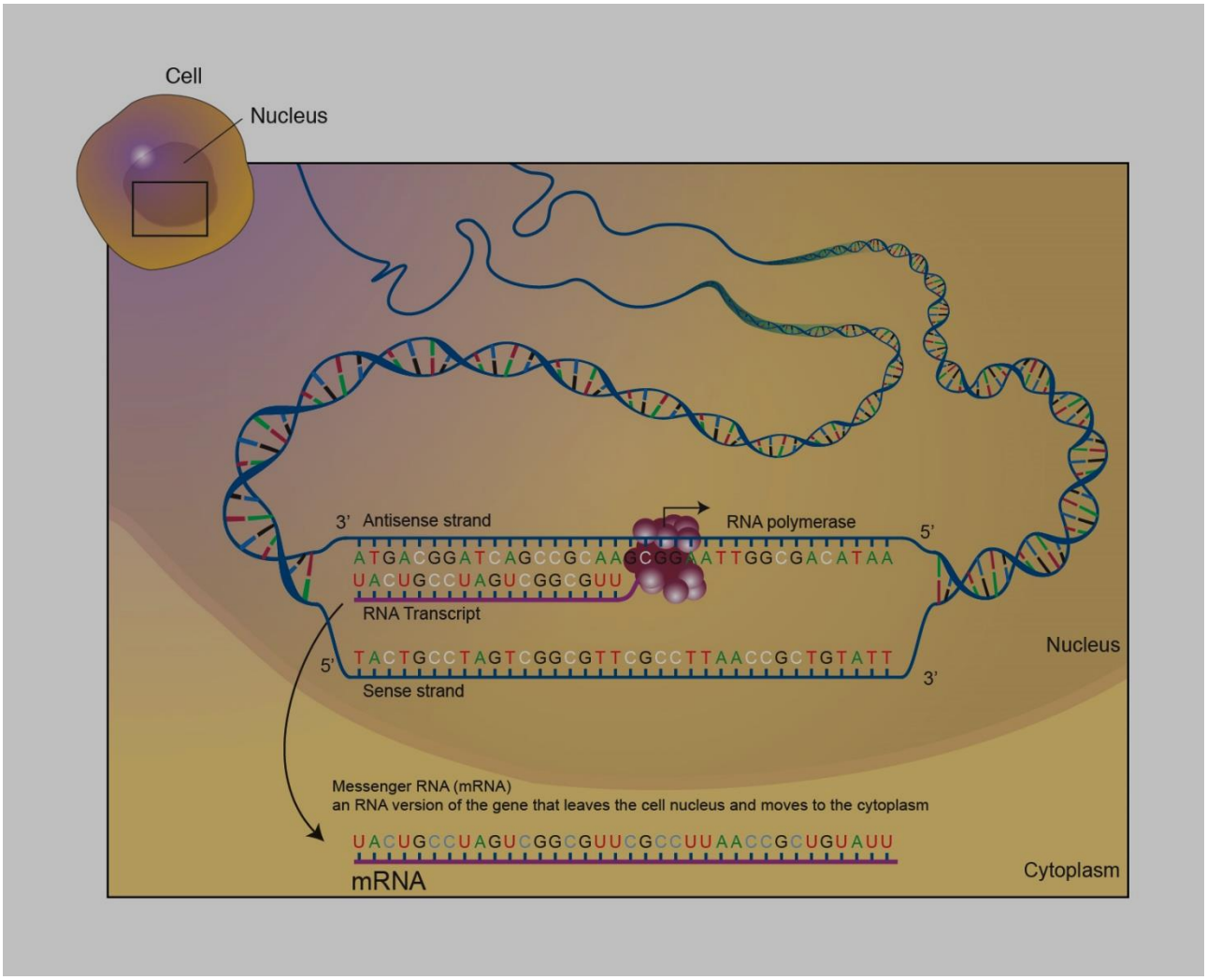

FIG.0.1: MRNA NUCLEUS, CYTOPLASM STATUS.

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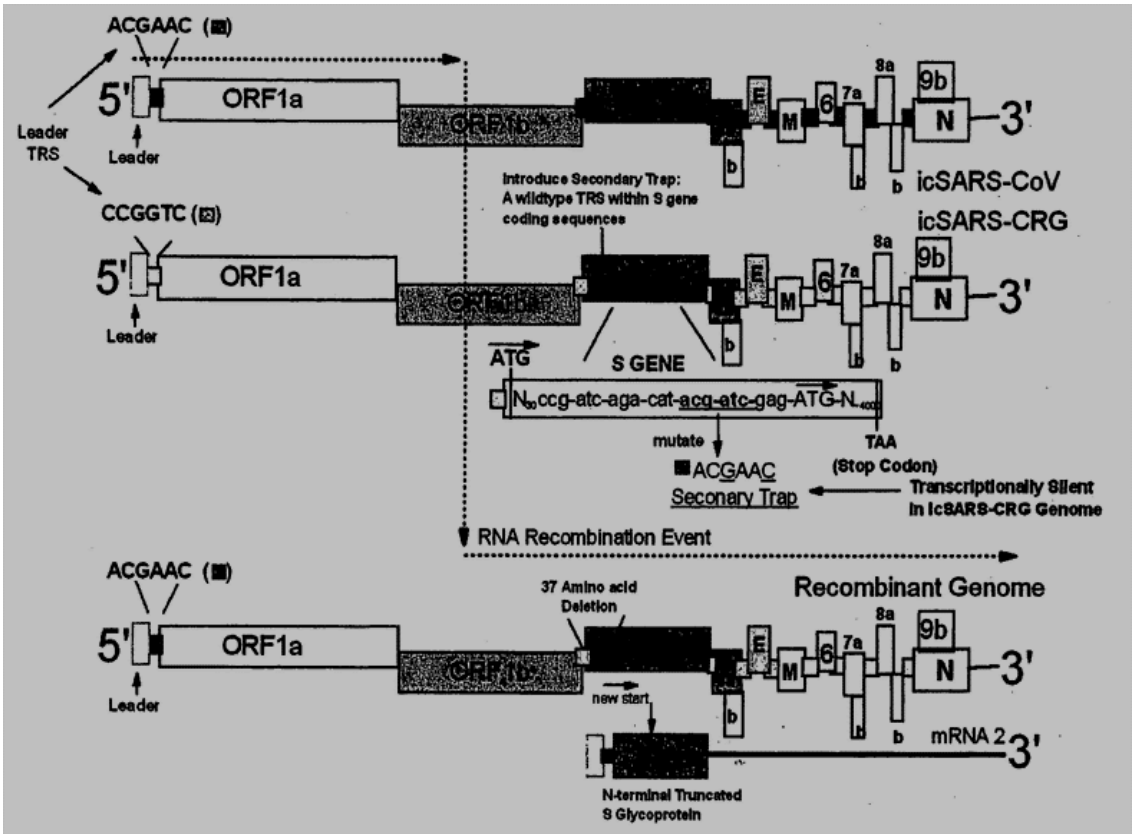

FIG.0.2: RNA RECOMBINANT STATUS.

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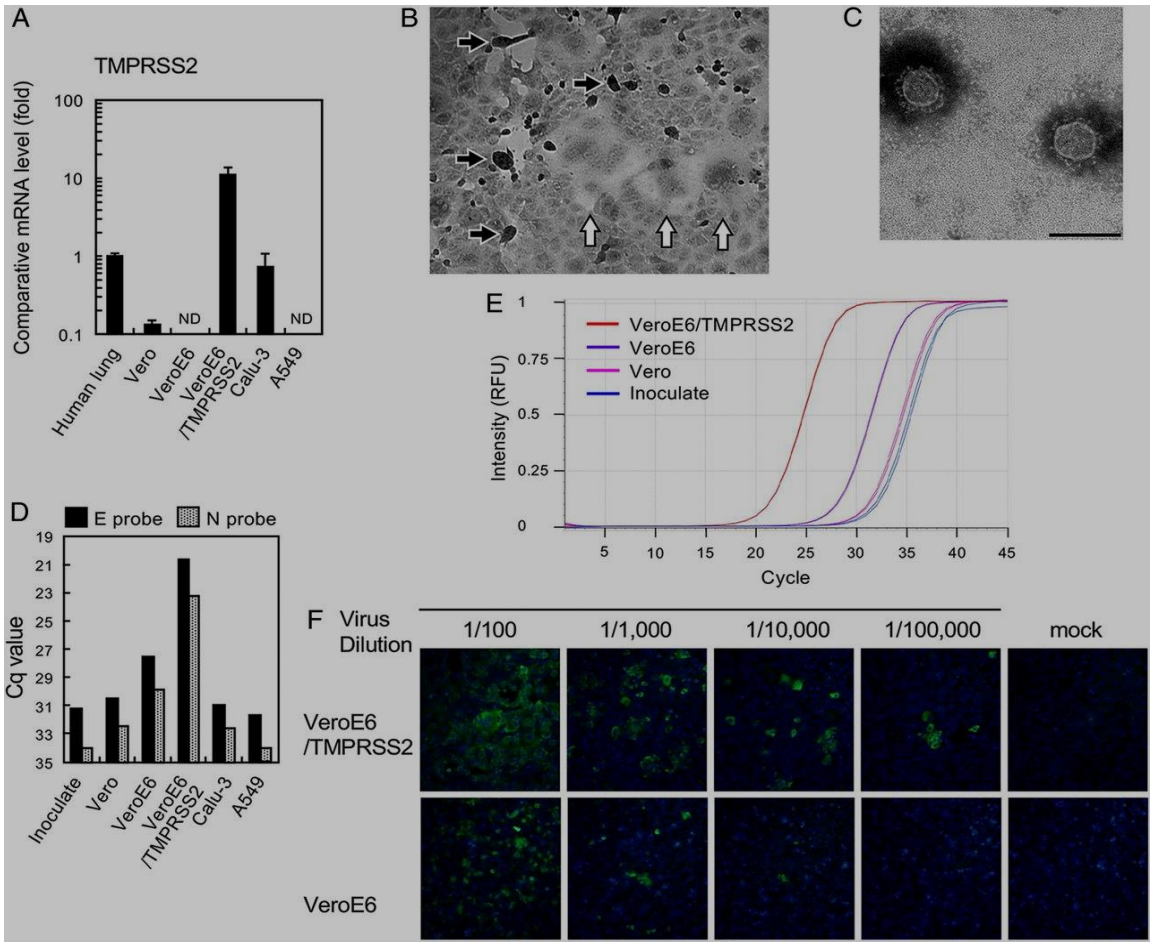

FIG.0.3: ENHANCED ISOLATION OF SARS-COV

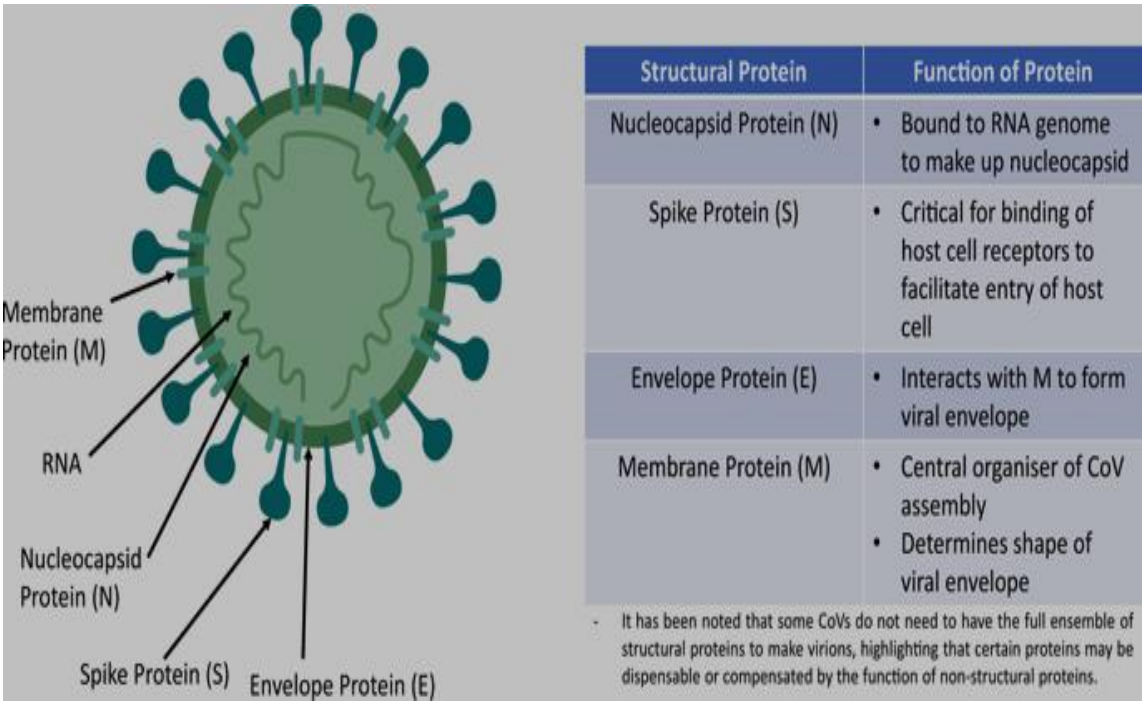

FIG.0.4: REVISITING THE DANGER OF THE CORONAVIRUS.

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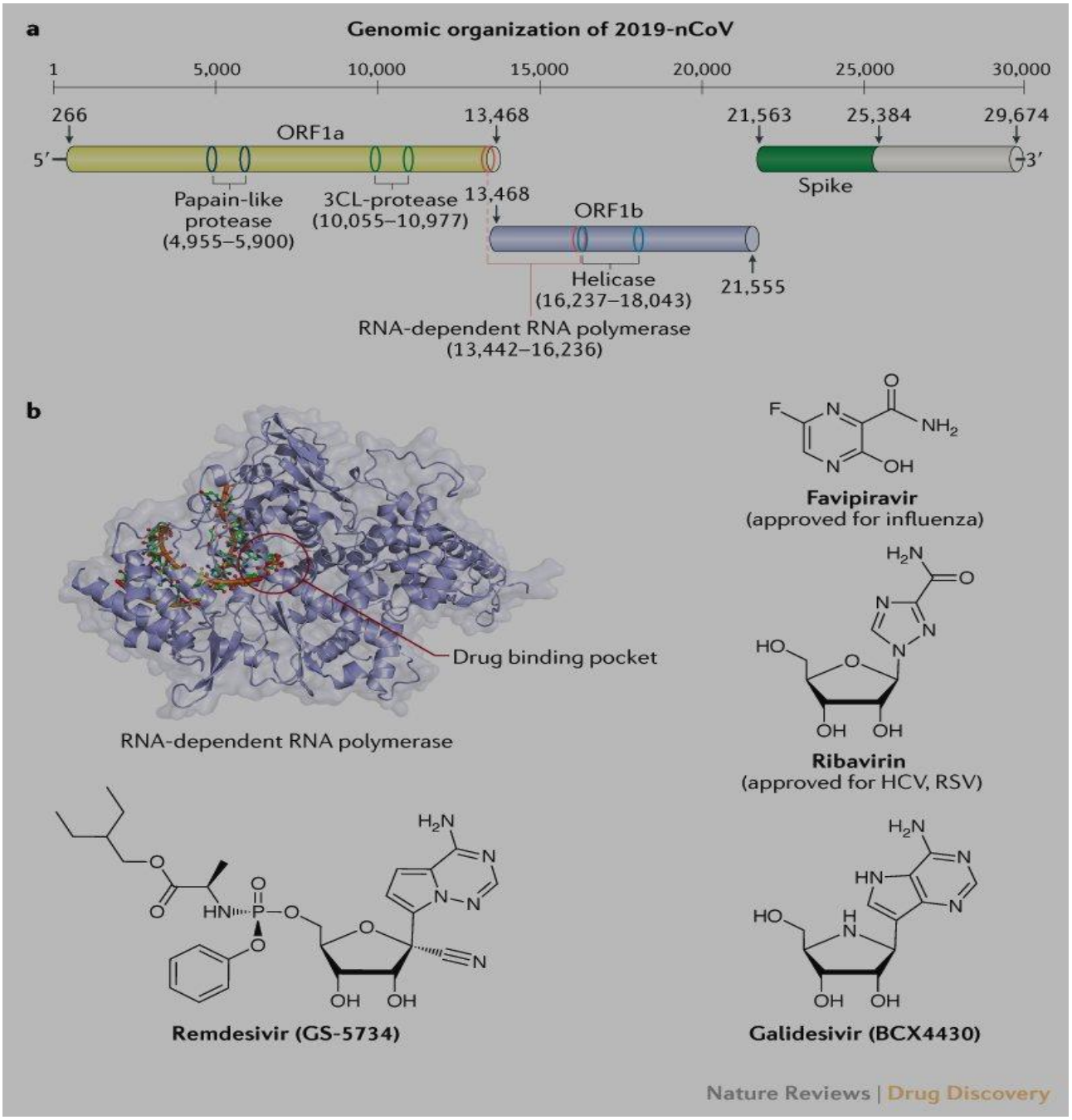

FIG.0.6: THERAPEUTIC OPTIONS FOR THE COVID-19

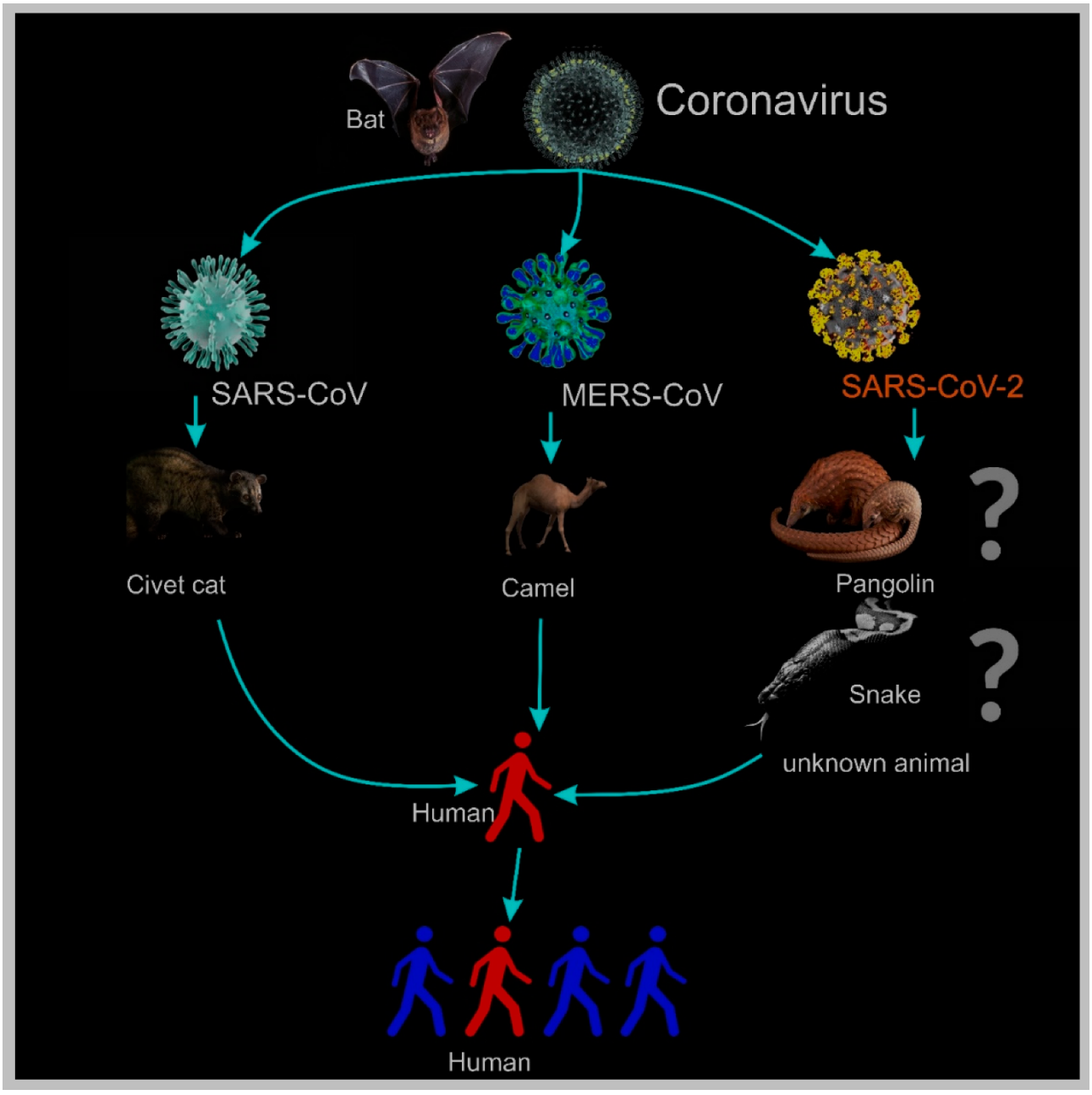

FIG.0.8: HUMAN TO HUMAN FLOW.

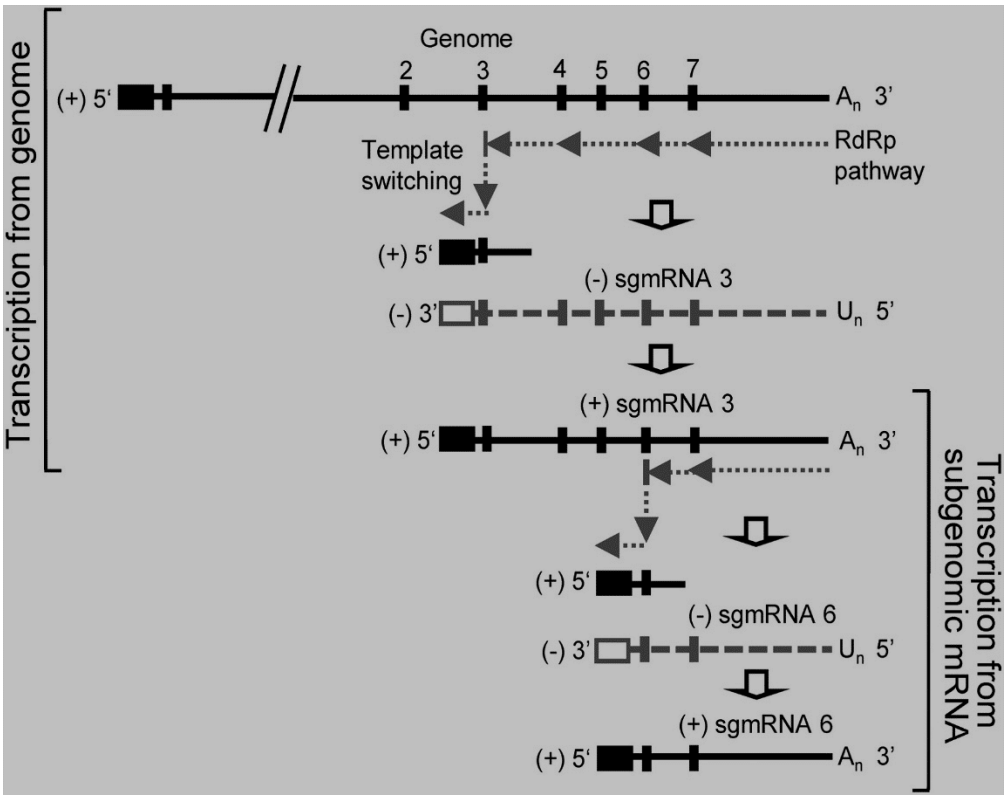

FIG.0.9: RNA APPLICATION STATUS.

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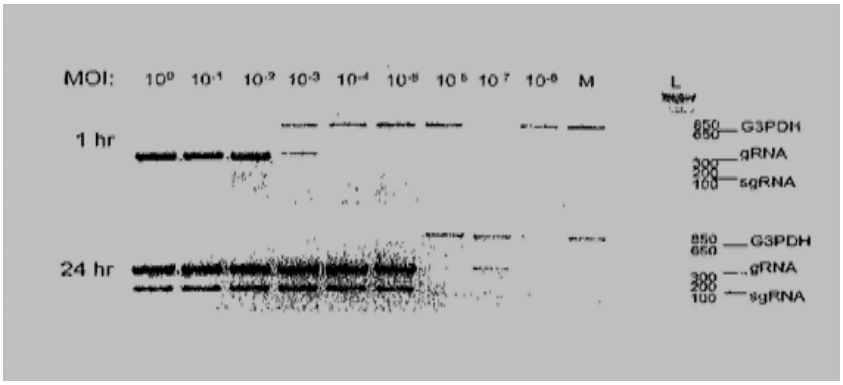

FIG. 1: SHOWS AN EXEMPLARY MULTIPLEX RT-PCR ASSAY FOR THE DETECTION OF SARS-COV REPLICATION.

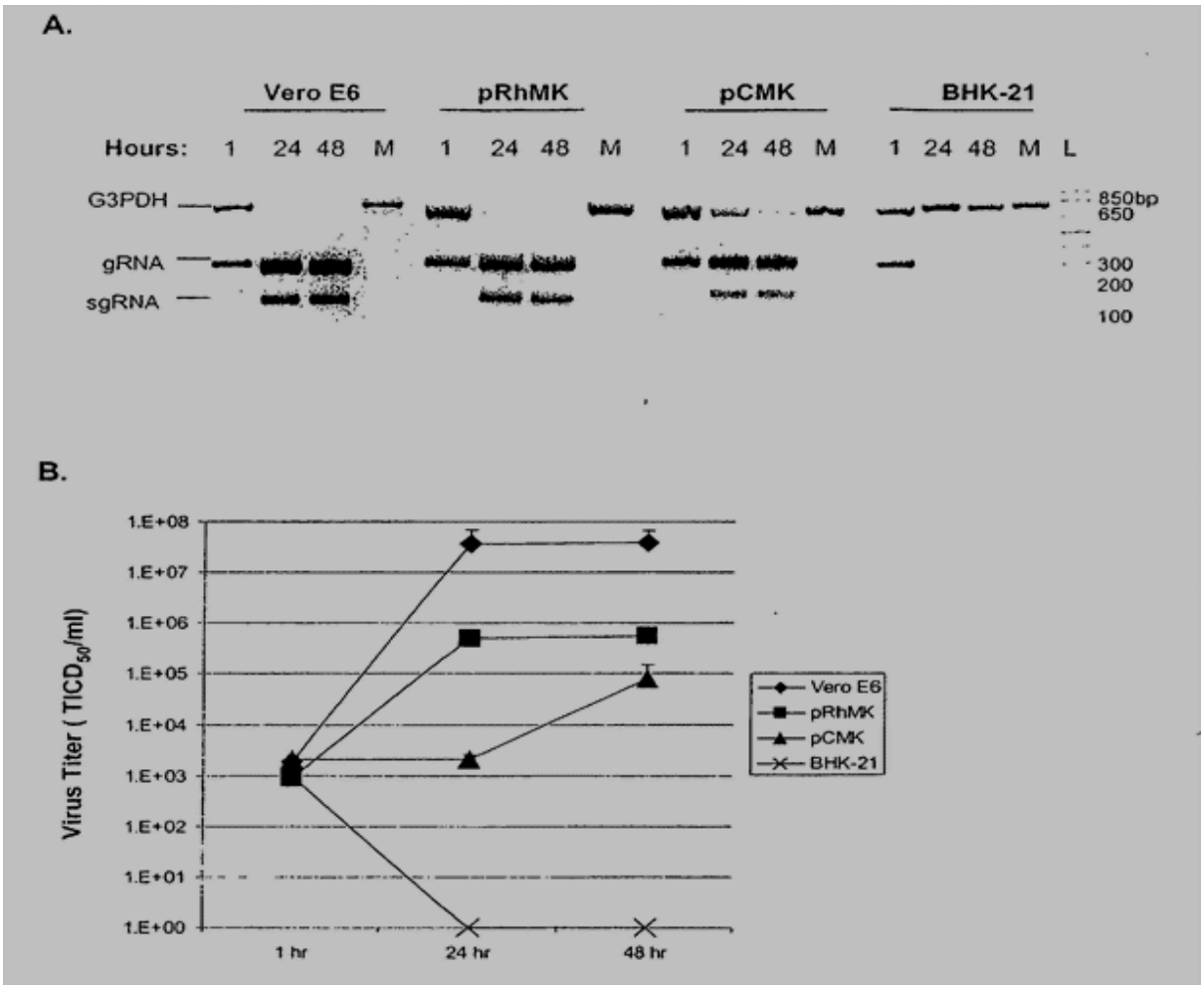

FIG. 2: SHOWS SUSCEPTIBILITY OF MONKEY KIDNEY CELLS TO SARS-COV. (A) AMPLIFICATION OF G3PDH, SARS-COV GRNA AND SGRNA AT 1, 24, 48 H POST-INOCULATION (P.I.). AFRICAN GREEN MONKEY CELLS (VERO E6), PRIMARY RHESUS MONKEY KIDNEY CELLS (PRHMK), PRIMARY CYNOMOLOGUS MONKEY KIDNEY CELLS (PCMK). MOCK INOCULATED CELLS (M) AND BABY HAMSTER KIDNEY CELLS (BHK21) INCLUDED AS NEGATIVE CONTROLS. NEGATIVE IMAGES ARE SHOWN. RESULTS ARE REPRESENTATIVE OF 2 EXPERIMENTS PERFORMED IN DUPLICATE. (B) TITRATION OF CELL SUPERNATANTS IN VERO E6 CELLS ($TCID_{50}$). GRAPH DEPICTS THE AVERAGE OF TWO TO THREE EXPERIMENTS EACH IN TRIPLICATE.

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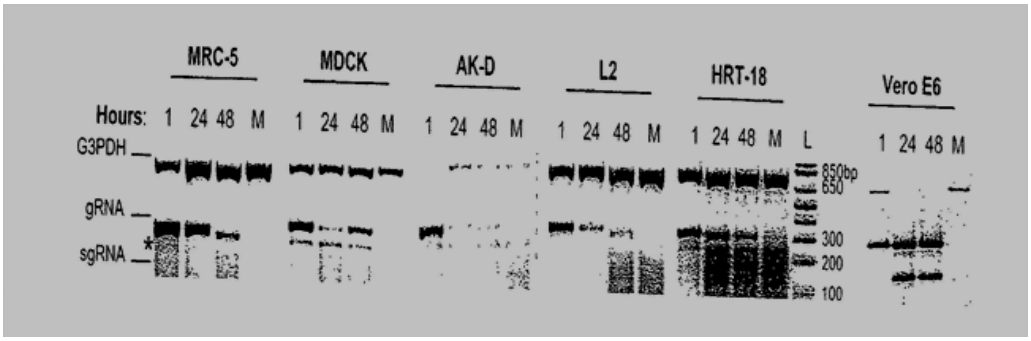

FIG. 3: SHOWS SUSCEPTIBILITY OF CELLS EXPRESSING KNOWN CORONAVIRUS RECEPTORS

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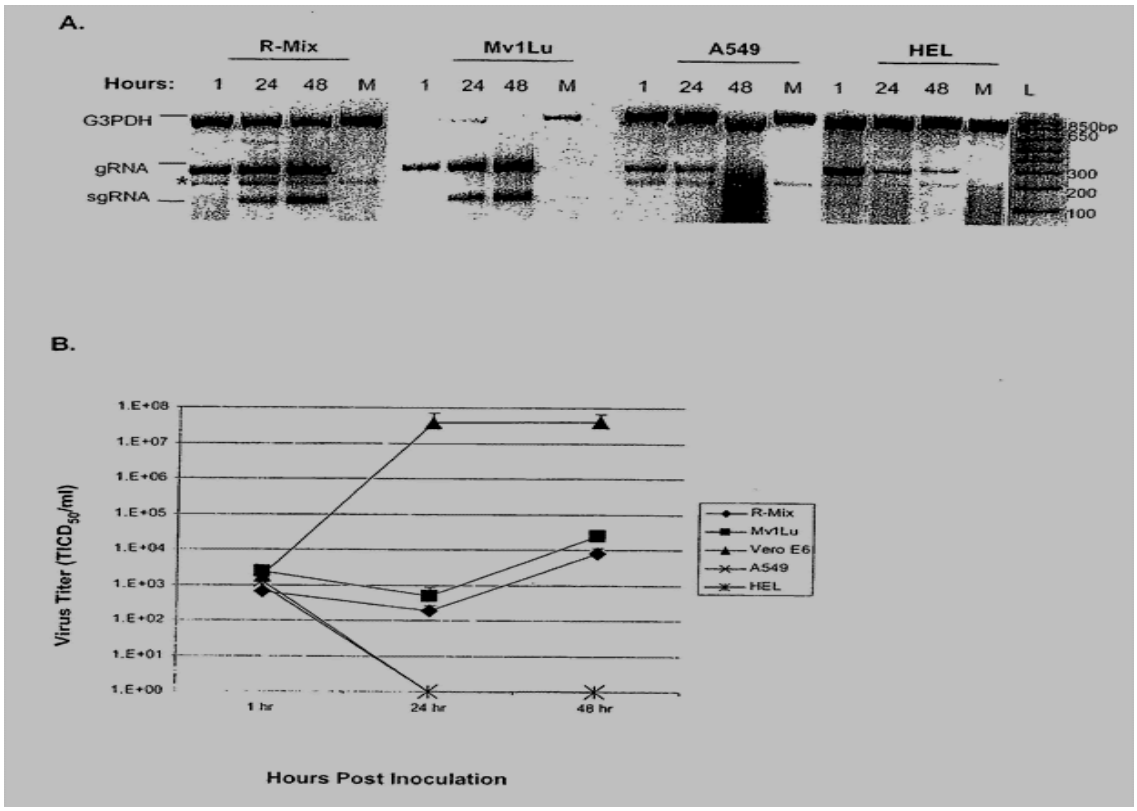

FIG. 4: SHOWS SUSCEPTIBILITY OF CLINICALLY RELEVANT CELLS TO SARS-COV. (A) AMPLIFICATION OF G3PDH, SARS-COV GRNA AND SGRNA AT 1, 24 AND 48 H P.I. MIXED MONOLAYER OF MINK LUNG CELLS AND HUMAN LUNG CELLS (R-MIX), MINK LUNG CELLS (MV1LU), HUMAN LUNG CELLS (A549) AND HUMAN EMBRYONIC LUNG CELLS (HEL). MOCK INOCULATED CELLS INCLUDED AS NEGATIVE CONTROL. NEGATIVE IMAGES ARE SHOWN. FIGURE IS REPRESENTATIVE OF TWO EXPERIMENTS PERFORMED IN DUPLICATE. (B) TITRATION OF CELL SUPERNATANTS IN VERO E6 CELLS ($TCID_{50}$). GRAPH IS AVERAGE OF 2 EXPERIMENTS PERFORMED IN TRIPLICATE.

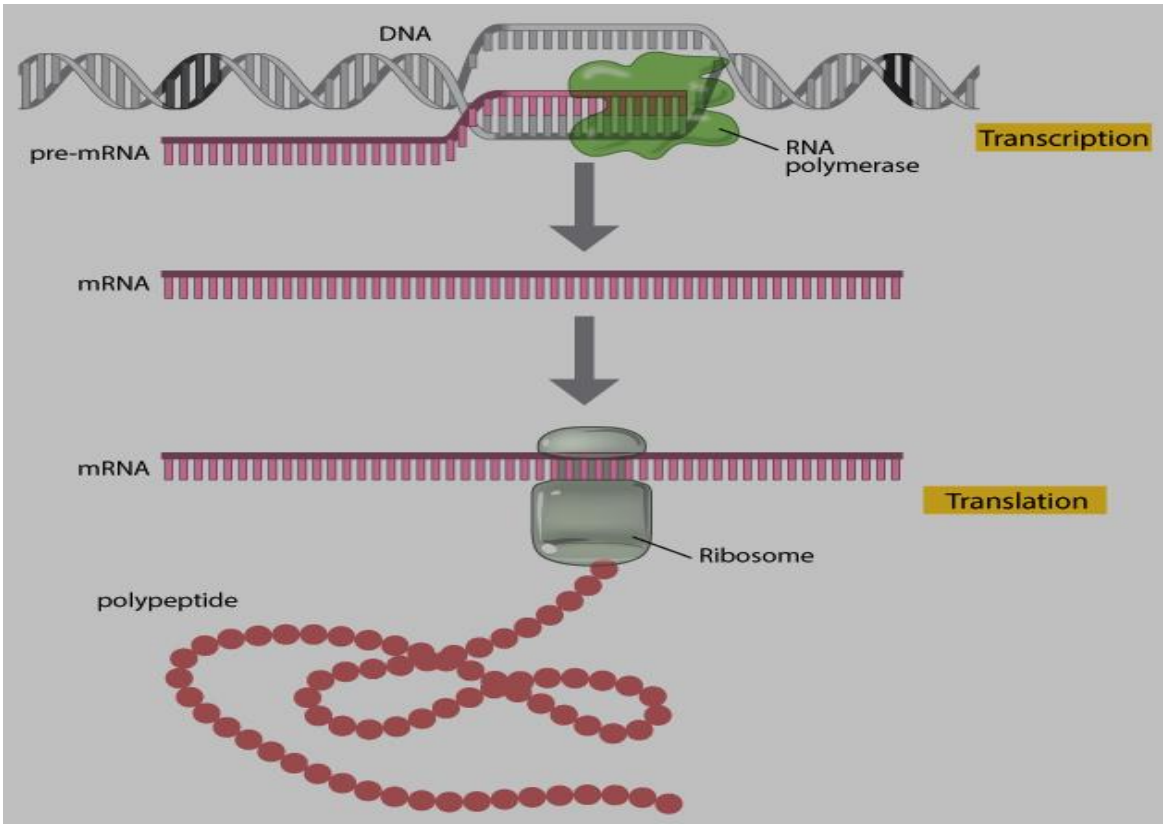

FIG.5: mRNA, DNA PROTEIN STATUS.

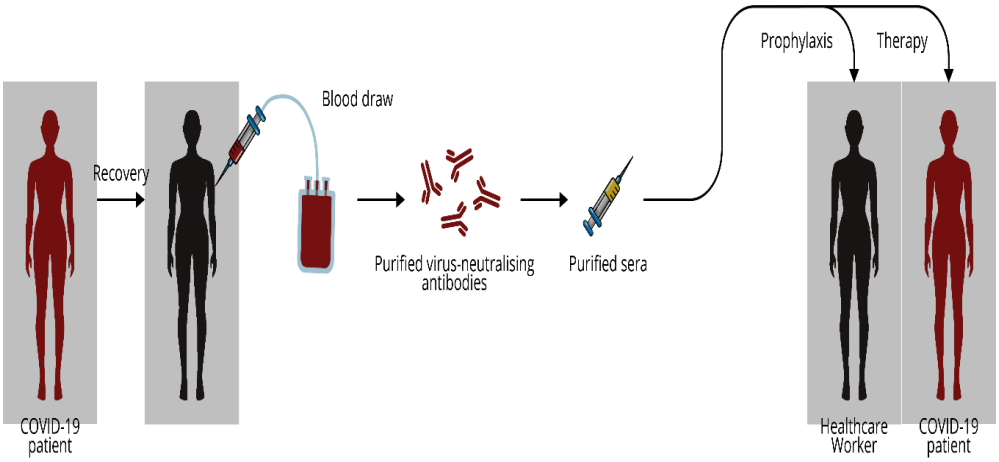

FIG.6: ANTIBODIES CREATION