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Inactivation of Human Coronavirus OC43 (hCoV-OC43) by 'Green Up ABV', a Liquid Detergent Preparation

1. Aim:

To test the capability of the liquid detergent preparation 'Green Up ABV' for human coronavirus OC43 (hCoV-OC43) inactivation, by cell viability assay.

2. Samples:

One bottle of 1 liter liquid detergent preparation, 'Green Up ABV', was supplied by the customer. Upon sample receipt at hylabs the sample was stored at room temperature.

3. Related Documents:

Hy Laboratories Ltd. SOP No. 11-035- "General Maintenance in the Tissue Culture Laboratory".

4. Materials:

4.1. Human HCT8 cells (Colon; ATCC, Cat # CCL-244)

4.2. L-Alanyl-L-Glutamine Solution (200 mM; Biological Industries, Cat # 03-022-1B)

4.3. Penicillin-Streptomycin Solution (Biological Industries, Cat # 03-031-1B)

4.4. Fetal Bovine Serum (FBS; Biological Industries, Cat # 04-127-1A)

4.5. RPMI-1640 Medium (ATCC, Cat # 30-2001)

4.6. Human coronavirus OC43 (hCoV-OC43; stock titer: 1.12×10^7 $TCID_{50}$/ml)

4.7. Consumables: Filter tips (Axygen, Cat. MTXF1250), sterile 2 ml Microcentrifuge tubes (SARSTEDT Cat. 72.690), Minisart syringe filter (0.2 µm; Sartorius, Cat # 17597-K)

5. Methods and experimental procedures:

5.1. Cytotoxicity test:

5.1.1. Sample preparation: HCT8 cells (4.1) were grown in RPMI-1640 medium (4.5) supplemented with 2mM L-Alanyl-L-Glutamine (4.2), 1% Penicillin-Streptomycin (4.3) and 10% FBS (4.4), in an incubator at 37^{O}C and 5% CO_2. 24 hours prior to the experiment cells were plated in a 96-well plate, at a final amount of 10,000 cells per well, and grown as described above. On the day of the experiment Green Up ABV (100% in original sample; section 2) was filter sterilized through a Minisart syringe filter (0.2µm filter; 4.7). Next, Green Up ABV was diluted to four (4) final concentrations as follows: 15%, 10%, 7.5%, 5%, all performed in RPMI-1640 medium (4.5) supplemented with 2mM L-Alanyl-L-Glutamine (4.2), 1% Penicillin-Streptomycin (4.3) and 10% FBS (4.4). due to immediate lysis and death of all cells in all dilutions of Green Up ABV, the experiment was repeated as follows:

In the repeated cytotoxicity test: HCT8 cells (4.1) were grown in RPMI-1640 medium (4.5) supplemented with 2mM L-Alanyl-L-Glutamine (4.2), 1% Penicillin-Streptomycin (4.3) and 10% FBS (4.4), in an incubator at 37^{O}C and 5% CO_2. 24 hours prior to the experiment cells were plated in a 96-well plate, at a final amount of 10,000 cells per well, and grown as described above. On the day of the experiment Green Up ABV (100% in original sample; section 2) was filter sterilized through a Minisart syringe filter (0.2µm filter; 4.7). Next, Green Up ABV was diluted to 5% final concentration, followed by five (5) additional serial 10-fold dilutions. All six (6) dilutions performed in RPMI-1640 medium (4.5) supplemented with 2mM L-Alanyl-L-Glutamine (4.2), 1% Penicillin-Streptomycin (4.3) and 10% FBS (4.4).

5.1.2. Experimental procedure: on the day of the first experiment, the cell growth medium was removed and 200µl of each of the four (4) Green Up ABV dilutions prepared (5.1.1) were added, in triplicates, to the cells instead of the removed medium. The cells in all wells were immediately lysed, indicating high cytotoxic effect of the Green Up ABV preparation.

In the repeated cytotoxicity test: on the day of the experiment, the cell growth medium was removed and 200µl of each of the six (6) Green Up ABV dilutions prepared (5.1.1) were added, in triplicates, to the cells instead of the removed medium.

In parallel, in the same 96-well plate, three (3) additional wells (triplicate) were used as negative control (NC) for the viability assay, in which the media was replaced by 200µl sterile RPMI-1640, containing no Green Up ABV.

Following media replacement in the HCT8 96-well plate, cells were incubated for 5 days at 35^{O}C and 5% CO_2, and monitored every 24 hours under the microscope. Cell viability was determined by MTT assay on day five (5) of incubation.

5.1.3. MTT viability assay: on day 5 of the test, the growth medium was removed from each well. Next, 5 mg/ml MTT compound (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, in PBS) was diluted 1:7.5 in EMEM, and 150µl of the diluted MTT were added per well as replacement medium. The plate was then incubated for 2 hours at 35^{O}C and 5% CO_2. Following incubation, medium + MTT was removed, and 100µl DMSO were added per well. The plate was incubated for 15 minutes at room temperature following DMSO addition, and was read by SPECTRAFluor Plus plate reader (Tecan) at 560nm. MTT assay results are presented in Table 1.

5.2. Green Up ABV antiviral activity experiment:

5.2.1. Sample preparation: 24 hours prior to the experiment HCT8 cells were plated in a 96-well plate, at a final amount of 10,000 cells per well, and grown as described in section 5.1.1. On the day of the experiment Green Up ABV (100% in original sample; section 2) was filter sterilized through a Minisart syringe filter (0.2µm filter; 4.7). Next, Green Up ABV was diluted to 5% final concentration in RPMI-1640 medium (4.5) containing hCoV-OC43 (4.6). The medium + virus used for dilution was supplemented with 2mM L-Alanyl-L-Glutamine (4.2), 1% Penicillin-Streptomycin (4.3) and 2% FBS (4.4). The final titer of hCoV-OC43 in the dilution was $1.12x10^6$ $TCID_{50}$.

5.2.2. Experimental procedure: the mixture containing 5% Green Up ABV + hCoV-OC43 (5.2.1) was incubated in an Eppendorf tube, at room temperature, for 60 seconds. Immediately following incubation the mixture was diluted: 1:1,000 and 1:10,000, the cell growth medium was removed and 200µl of each of the two (2) dilutions prepared were added, each in a triplicate, to the cells instead of the removed medium.

In parallel, in the same 96-well plate, seven (7) additional samples (each in a triplicate) were prepared to serve as reference curve for assaying cell viability of the Green Up ABV-treated samples following the above described experimental procedure. For the calibration curve, in each triplicate of wells the cell media was replaced (in the same manner as above) by 200µl per well of 7 serial 10-fold dilutions of hCoV-OC43 (starting at $1.12x10^6$ $TCID_{50}$, and ending at $1.12x10^0$ $TCID_{50}$). Three (3) additional wells (as triplicate) were used as negative control (NC) for the calibration curve viability assay, in which the media was replaced by 200µl sterile RPMI-1640, containing no hCoV-OC43.

Following media replacement in the HCT8 96-well plate, cells were incubated for 6 days at 35^{O}C and 5% CO_2, and monitored every 24 hours under the microscope. Cell viability was determined by MTT assay on day six (6) of incubation (the period required for complete cell death in the calibration curve samples).

5.2.3. MTT viability assay: on day 6 of the experiment, the growth medium was removed from each well. Next, the procedure as described in section 5.1.3 was performed. MTT assay results are presented in Table 2.

6. Results and conclusion:

6.1. Cytotoxicity test results (as presented in Table 1): in the initial assay concentrations of 15% - 5% of Green Up ABV preparation were applied to HCT8 cells, resulting in immediate cell lysis and death. We therefore performed a repeated cytotoxicity assay diluting the Green Up ABV preparation to: 5% - 0.00005%. In the repeated assay we observed cell death when dilutions 5% - 0.05% of Green Up ABV were applied, and with dilutions 0.005% and below, there was no observed cell death. Based on these results we have decided to apply to HCT8 cells, in the Green Up ABV antiviral activity experiment, the two concentrations: 0.005% and 0.0005% following an initial hCoV-OC43 treatment with 5% Green Up ABV as the tested antiviral treatment (i.e to dilute the incubation mixture by 1,000 and by 10,000 following incubation and apply it onto the cells for the viability assay).

6.2. Green Up ABV antiviral activity experiment (MTT assay results as presented in Table 2):

The results displayed indicate that Green Up ABV preparation damages hCoV-OC43 infectivity, and can reduce its $TCID_{50}$ 100-fold (2 log reduction as indicated in Table 2) following 60 seconds of incubation with 5% of the preparation.

6.3. In conclusion, in this study, 5% Green Up ABV preparation was tested for its ability to hamper the infectivity of hCoV-OC43. To that end, we employed a direct method assaying cells viability following their infection by 5% Green Up ABV-treated virus. Results indicate that the tested treatment indeed reduces, by 2-log, the viral load capable of infecting HCT8 live cells.

Table 1: Cytotoxicity test results - % cell viability upon treatment with Green Up ABV

% Green Up ABV	% Viability
5	11.7
5.00E-01	9.7
5.00E-02	42.7
5.00E-03	66.3
5.00E-04	89.8
5.00E-05	92.6
NC	100

NC: negative control (i.e cells incubated with RPMI-1640 medium only)

% Viability: indicating viable cells per sample. Cell viability per each sample was calculated as percentage of the average MTT result of each triplicate, from the average of NC wells MTT results, which was regarded as representing 100% cell viability.

Table 2: Green Up ABV antiviral activity experiment - MTT assay results

% Green Up ABV (as diluted following incubation with hCoV-OC43)	% Viability	Calculated viral $TCID_{50}$	Viral log reduction
5.00E-03	89	$1.12x10^4$	2
5.00E-04	111	$1.12x10^4$	2

% Viability: indicating viable cells per sample. Cell viability per each sample was calculated as percentage of the average MTT result of each triplicate, from the average of standard curve NC wells MTT results, which was regarded as representing 100% cell viability.

Calculated viral $TCID_{50}$: was assessed for each sample compared to the reference curve performed in triplicates of 7 serial 10 fold dilutions (5.2.2), and normalized to the initial viral $TCID_{50}$ during the 60 seconds of incubation with Green Up ABV prior to dilution of the mixture.

Viral log reduction: was calculated by dividing the initial viral $TCID_{50}$ during the 60 seconds of incubation with Green Up ABV (i.e $1.12x10^6$), by the calculated viral $TCID_{50}$ in each sample.

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Reviewed by:	**Dr. Maya Amichay**	**(Name & Sign)**	DATE:	**09.09.2020**