

Test Report: Treated absorbent surface test. HUMAN INFLUENZA A VIRUS (H1N1)

Test Laboratory

BluTest Laboratories Ltd

Identification of sample

Name of the product
Manufacturer
Date of Delivery
Storage Conditions
Active substances

DUO MAX-TREATED TISSUE PAPER

DUO TECH LTD, 11-12 QUEENS SQUARE, BRISTOL, BS1 4NT
March 2 2010
Room temperature
Unknown

Project:

BT-DUO-006

Test Method and its validation

Method

See SOP

Neutralizer

EMEM + 0.125 w/v % bovine serum albumin (Fraction V)

Experimental Conditions

Period of analysis
Product diluent used
Product test concentrations
Appearance product dilutions
Contact time
Test temperature
Interfering substance
Stability of mixture
Temperature of incubation
Identification of virus

March 5 – 9 2010
N/A
5.0% impregnated tissue
N/A
 $t = 2 \text{ min} \pm 10 \text{ s}$
 $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$
3.0g/l bovine serum albumin
Precipitate absent throughout the test
 $35^{\circ}\text{C} \pm 1^{\circ}\text{C} + 5\% \text{ CO}_2$
Influenza A (H1N1) (TC Adapted) (ATCC- VR-1469)/MDCK cells

Standard operating procedure.

1. Strains: Influenza virus A H1N1 (ATCC VR-1469).
2. Sample: Circular discs with a diameter of 35 mm are cut in three ply facial tissue paper as provided. Five discs of the treated sample and four discs of untreated sample are cut. Samples are handled wearing gloves.
3. A set of untreated discs (four) and a set of treated discs (five) are placed in separate labelled 90 mm diameter polystyrene petri dishes and transferred to a class II Safe 2020 cabinet (GU-EQ-005).
4. The lids of the petri dishes are removed and the samples exposed to UV-light, using a standard disinfection cycle for 60 minutes.
5. Use sterile forceps to turn over the discs in the petri-dishes without removing them from the cabinet and repeat the UV light exposure.
6. Aseptically load each set of discs into the wells of a sterile 6-well polystyrene tissue culture plate.
7. Prepare MDCK cell culture in advance by inoculating 0.6×10^4 cells/0.1 mls EMEM + 5% FBS into wells in rows A to F of a sterile plastic 96-well tissue culture plate. Incubate at 37°C, 5.0% CO₂ overnight.
8. The next day remove the medium and wash the cells twice in sterile phosphate buffered saline (without calcium and magnesium) and replace the medium in each well with 0.1 mls of influenza medium (EMEM + 0.125% BSA + 10 mM HEPES + 1 µg/ml TPCK-treated trypsin).
9. Aseptically transfer 0.9mls of influenza base medium (EMEM + 0.125% BSA + 10 mM HEPES) into each well of a 96-well deep-well block.
10. Mix 1.8mls of virus suspension with 0.2 mls of either 0.3g/100mls (low soil load) or 3.0g/100mls (high soil load) of sterile bovine serum albumin.
11. At timed intervals add 0.1 mls of the viral suspension/BSA mix onto the untreated and treated tissue paper samples (allow at least 30 seconds between each application). This volume of suspension should saturate the tissue disc.
12. Inoculate four untreated tissue paper samples and four treated tissue paper samples.
13. Incubate the samples for the contact time and then add 0.9 mls of sterile influenza base medium.
14. Compress the tissue paper to release the liquid and mix the liquid
15. Transfer 0.1 ml to the appropriate well of the 96-well block, in either row 1 or row 7 for each sample. (10^{-2} dilution)
16. Neutralizer control (NC). Add 0.1 mls of virus suspension to a treated tissue paper sample, then immediately add 0.9 mls of influenza base medium. Transfer 0.1 mls to an appropriate well in the block in either row 1 or row 7.
17. Serially dilute the samples by transfer of 0.1 mls through wells in row 1 to row 6 or from wells in row 7 to row 12 of the deep well block. Discard and replace pipette tips between each transfer.
18. Transfer a column of wells containing the dilution series for each sample into 6 replicate columns of the tissue culture plate, transferring 0.1 ml to each well.
19. Place plates into a container to reduce desiccation from the plates and incubate at 35°C, 5.0% CO₂ for 2 days.
20. Determine the number of infected cell cultures for each sample dilution series and use the karber formula to calculate the tissue culture infectious dose₅₀ (TCID₅₀).
21. Take log₁₀ values and determine the mean log reduction by mean log₁₀ TCID₅₀ untreated – mean log₁₀ TCID₅₀ treated.

Conclusion. DuoMax-treated tissue paper, when saturated with a suspension of influenza A virus (H1N1) ATCC-VR-1469 under high soil conditions resulted in a log₁₀ reduction of 3.67 (>5000 fold reduction) in 2.0 minutes at 20°C.



Dr Chris Woodall
Director
BluTest Laboratory
Glasgow, UK
9 March 2010

DISCLAIMER

BluTest (BT) has performed the Testing detailed in this report using reasonable skill and care and that BT has used reasonable endeavours to carry out the Testing [in accordance with a modified EN 14476 protocol]. All forecasts, recommendations and results contained in any report to the Company shall be submitted in good faith. However, other than as expressly set out in this report, no warranty is given (i) in relation to the Testing or the use(s) to which any results or deliverables produced in the course of the Testing are or may be put by the Company or their fitness or suitability for any particular purpose or under any special conditions notwithstanding that any such purpose or conditions may have been made known to BT or (ii) that the intended results or deliverables from the Testing can be achieved or (iii) that the Company can freely make use of the results or the deliverables without infringing any third party intellectual property rights and the Company will be deemed to have satisfied itself in this regard. BT shall have no liability (which is hereby excluded to the fullest extent permissible by law) in respect of any loss, liability or damage, including without limitation any indirect and/or consequential loss such as loss of profit or loss of business, market or goodwill, that the Company may suffer directly or indirectly as a result of or in connection with: (i) the performance of the Testing, except for direct loss arising from a breach of the foregoing warranties; (ii) the use of any materials, samples or other information provided by the Company for use in the Testing; and (iii) the Company's reliance upon or use of any results or deliverables provided as part of the Testing. The total liability of BT shall not exceed the sums paid to BT for the performance of the Testing.