Institut Pasteur de Lille

Service of experience to hygiene hospitalization

THE ACTUAL PROGRESS OF THE PRODUCT NOCOLYSE AGAINST VIRAL ACTIVITY

According to the norm of NFT72 180

Report IPL no.: 161103

This report is specially for the product mentioned under trial only.

I.Material and Methods:

Identification of samples

Name and description of product : NOCOLYSE .
Commendatory: Oxypharm -917,rue Marcel Paul- ZA des Grands Godets - 94508 CHAMPIGNY SUR MARNE Cédex .
Date of reception in the laboratory :04/08/2003

PH :5

Aspect of product :uncolored.

1) Material :

a) Product used :
The product Nocolyse used in 1/10^{e}

b) Medium used:
Medium used for the cellular culture
-Cells VERO ATCC 81 CCL : EAGLE MEM medium + amino acids+ serum of calf fetus + glutamine+ gentalline 8µg/ml.

-Cells KB : EAGLE MEME medium + serum of calf fetus+ glutamine + gentalline 8µg/ml.

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c) Cells references :

The three used cells are from the collection of national cultures of microorganisms [CNCM Institut Pasteur de Lille].

1. Enterovirus Polio 1 , souche Sabin, cultivated on cells VERO.
2. Adenovirus human type 5 , cultivated on cell KB.
3. Orthopox virus of cultivated vaccine on cell VERO.

The cells were conservated on congelation to -80°C with unique dozes for utilization .

The determination of the cytopathique effect (ECP) in the next conditions:

After 5 days at 37°C.

Titration Technique on cells in suspension

In all deep containers (300µl) of micro plaque, put 150 µl from the culture media in the first row of 8 containers, then add 50 µl of titrated viral suspension. After homogeneity, pass 50 µl from one container to another (except the last row which help us notice the cells) this leads to 4 to 4 dilution after repeating the dilution process 8 times.

Finally, put in all containers a 100 µl from the cell suspension on the culture media, contains 10^5 cells per milliliter .

Observation should be under microscope (during 5 days) after incubation at 37 °C and atmosphere rich in 5% of CO_2.

The titration using this method is similar to that of FISHER and YATES using the table of WYSHAK and DETRE. We obtain the estimated number of infected units per milliliter .

2) Methods

Determined technique according to the primary trial :

Separation technique of product-virus, filtration on LH_2O gel.

Preliminary procedures :

a) Preparation of viral suspension

Take 10^7units of titrated infectious viral suspension per milliliter which contains 2% embryonic calf serum for Enterovirus Polio I and Orthopoxvirus of vaccine. 10^7 UI/ml for Adenovirus human type V.

Concentration of viral suspension:

- Enterovirus Polio I = 1.25mg/ml
- Orthopoxvirus (vaccine)= 1.14mg/ml
- Adenovirus human type V= 0.9mg/ml

Titration of virus:

- Enterovirus Polio I = log 7.34
- Orthopoxvirus (vaccine)= log 7.32
- Adenovirus human type V= log 6.98

b) Determination of the subcytotoxic concentration:

The product NOCOLYSE and the molecular filtrate were diluted from 1 to 10^{-6} in PBS Dulbecco.

Culture of cells in suspension during experiment:

- Put 0.1ml of culture media (2% serum of calf fetus) in all plates on board.
- In the rows next to the first 4 plates, add 0.05ml for every dilution of disinfectant.
- The last row of 4 plates gets 0.05ml of untreated saline, sterile without disinfection.
- Add 0.1ml of suspension to culture media containing 10^5 cells/ml
- The board should be covered and incubated at 37°C and atmosphere rich in 5% CO_2.

The subcytotoxic effect was noticed after incubation period equal to the longest time of cultivating the suspension under studied system that was 5 days.

c) Observing the elimination of viral effect by filtration through gel:

This observation aims to ensure that the filtrate of the product has eliminated any viral activity. Put the filtrate, obtained by molecular filtration, in contact with suspended viruses for an hour at 4°C.

Carry out a titration of viral suspension treated by the same conditions in PBS.

Results of preliminary procedures:

a) Subcytotoxic dilution of NOCOLYSE and of filtrate on LH_2O gel

On VERO	Pure	$d = 10^6$	1/5	$d = 10^4$	1/10	$d = 10^4$
On KB		$d = 10^6$		$d = 10^4$		$d = 10^4$
On VERO	Filtrate (pure)	$d = 10^3$	Filtrate (1/5)	$d = 10^3$	Filtrate (1/10)	$d = 10^2$
On KB		$d = 10^3$		$d = 10^3$		$d = 10^2$

b) Observing the elimination of viral effect after filtration:

Concentration filtered product (%) 1/10

VIRUS	VIRAL TITRANT IN LOG (UI/ML)		
	Virus + filtrate	Control virus + PBS	T. Virus
Poliovirus	Log 7.34	Log 7.23	Log 7.33
Vaccine	Log 7.71	Log 7.33	Log 7.32
Adenovirus	Log 7.32	Log 7.32	Log 6.98

Validity of essay: conformed.

Essay in details:

For each virus under investigation, prepare 2 tubes

One for test (R) contains 100µl viral suspension and 900 µl of product.

One as control (T) contains 100 µl viral suspension and 900 µl saline.

After homogeneity (Vortex), put the tubes in double water bath in certain temperature (20°C for disinfectants).

After incubation periods for 15, 30 and 60 minutes measured precisely, viral effect is evaluated through filtration over gel.

Concept

Samples R15, R30, R60 and T60 were filtered through Sephadex LH_2O that keeps the disinfectant and passes the virus. The titrant of viral filtrate was compared to that of viral suspension treated under the same conditions.

The un-oxidizable plates were covered firmly. These plates contain movable tube where the open end has a filter made of polyester cut to fit the tube.

Sephadex suspension

Prepare Sephadex suspension LH_2O (Pharmacia France) by mixing 22g with 100 ml of PBS. It will be sterilized in 30 minutes at 120°C. After autoclaving, leave it at the temperature of the piece before distributing it: 25 ml per each un-oxidizable, sterilized plate.

Centrifuge at 1000g for 10 minutes.

Operation protocol

Samples R15, 30, 60 and T60 were put on the upper part of Sephadex column. All of them were centrifuged at 1000g for 10 minutes in a centrifuge of refrigerating preference at +4°C. Obtain the sterile filtrate. The obtained volume was 1 ml.

Explanation of results:

The viricidal activity of a disinfectant is determined by the concentration and the minimum time of contact that leads to reducing the titrant of 3 infectious viruses by 4 decimal logarithms.

Conditions of essay			
NOCOLYSE Concentration : 1/10e			
Time of contact	Enterovirus Polio I In log (UI/ML)	Adenovirus V	Vaccine
15 minutes	Log 3.48	Log 5.01	Log 4.57
30 minutes	Log 3.09	Log 3.75	Log 3.1
60 minutes	Log 3.05	Log 3.06	Log 2.95
Control – 60 minutes	Log 7.06	Log 7.06	Log 7.06

CONCLUSION

The product NOCOLYSE is active at concentration 1/10 after 60 minutes of contact for Enterovirus Polio I, Orthopoxvirus (vaccine), and Adenovirus human type V according to the norm AFNOR NF T 72 180.

Lille, le 19 Novembre 2003

Responsible technique
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p.o

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