

# CNRL Laboratory Protocol



## **Inactivation of influenza virus with high pathogenic potential for antiviral susceptibility testing**

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## Inactivation procedure with 1% Triton X-100

### Materials:

1. Tissue culture supernatant with influenza virus (cleared from cell debris by 5 min 1500 rpm centrifugation)
2. Triton® X-100, BDH Chemicals Ltd., Cat No 306324N, Poole, United Kingdom, 10% in infection medium (DMEM, + antibiotics, + NEAA, without FCS)
3. 15 ml tubes

**REMEMBER**, WITH VIRUSES WITH HIGH PATHOGENIC POTENTIAL THIS PROCEDURE HAS TO BE CARRIED OUT AT BSL-3 LEVEL

### Procedure:

1. Pipette 9 parts culture supernatant and 1 part 10% Triton X-100 in the 15 ml tube
2. Close the tube firmly and mix thoroughly on vortex mixer for 15 sec
3. Incubate for 1 hour at room temperature
4. Clean tube following own BSL-3 procedures and transport to BSL-2 level laboratory
5. Store at -80°C until testing

## Validation

Inactivation of viruses by 1% Triton X-100 has been validated with seasonal A(H3N2) influenza virus and low pathogenic avian influenza A(H7N3) virus. After removal of Triton X-100 the inactivated material has been inoculated on MDCK cells. After 3 ten-day passages no cytopathological effect was found and no viral RNA and mRNA were detected in the supernatant or cell fraction.

Antiviral susceptibility determination for neuraminidase inhibitors has been tested with inactivated seasonal A(H1N1) and A(H3N2) influenza viruses. At 1% Triton X-100 the NA activity of a tissue culture supernatant with influenza virus was approximately doubled (Figure). Although the NA-activity was doubled, the IC<sub>50</sub> values were similar.

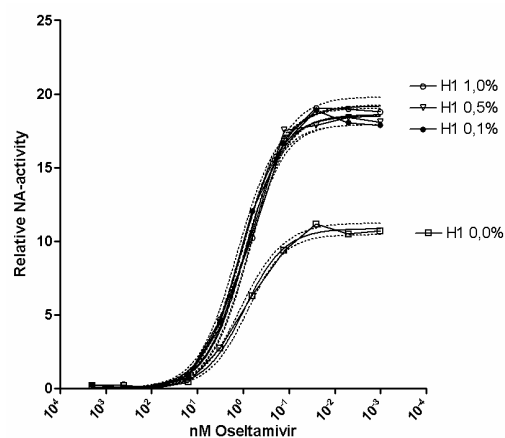


Figure. Effect of different Triton X-100 concentrations on NA activity and IC<sub>50</sub> of seasonal influenza virus A(H1N1).

The procedure has also been validated with A(H1N1)pdm09 virus. See reference.

Jonges M, Liu WM, van der Vries E, Jacobi R, Pronk I, Boog C, Koopmans M, Meijer A, Soethout E. Influenza virus inactivation for studies of antigenicity and phenotypic neuraminidase inhibitor resistance profiling. *J Clin Microbiol.* 2010 Mar;48(3):928-40.