THHa92

ARC • LNR

*Onderstepoort*
*Veterinary Institute /*
*Veeartsenyunde-Instituut*

*Privaatsak / Private Bag X5*
*Onderstepoort 0110 R S A*

*Tel: (012) 529-9111 (Int: +27 12)*
*Fax: (012) 5656573 (Int: +27 12)*

*E-Mail:postmaster@moon.ovi.ac.za*
*Web site: www.ovi.ac.za*

*AGRICULTURAL RESEARCH COUNCIL*

*LANDBOUNAVORSINGSRAAD*

# *FAX • FAKS*

***To / Aan:*** *Health and Hygiene (Pty) Ltd*

***From / Van:*** *Dr GH Gerdes*

***Fax no / Faks nr*** *011 474 1670*

***Date / Datum*** *10/12/2004*

***Number of pages / Aantal bladsye:*** *(Including cover/Voorblad ingesluit) 1*

***Subject / Onderwerp:*** *Testing against AIV*

## *Message/Boodskap:*

Att: Mr. Temperley

This is a report of results only. A detailed document will follow later.

Testing of two products against AIV was requested.

The test parameters were as follows:-
Products –
F10 General Farm Disinfectant (B/N 050206)
F10 Super Concentrate Disinfectant (B/N 060910)
Concentrations being tested were 1:50 and 1:100 and then 1:250 and 1:500 with the greater dilutions used for the super concentrate. Exposure times were 10mins and 20 mins for both products.

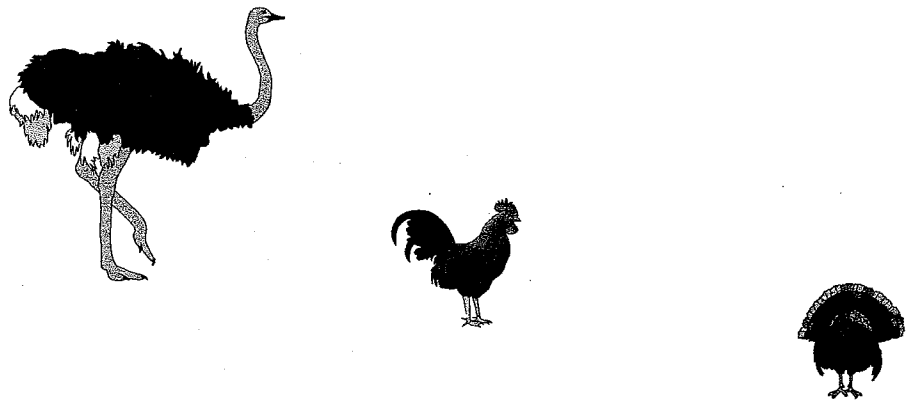
The virus was a recent ostrich influenza isolate subtype H5N2 HPAI which had been adapted to growth in tissue culture. 24 well tissue culture plates were used with all tests conducted in duplicate. A positive negative and disinfectant control were included. The only viral replication seen was in the positive virus control and the products being tested at the concentrations and exposure times given were all successful in inhibiting/preventing virus growth.

The products may be considered effective for use against avian influenza HPAI within the parameters used.

Dr GH Gerdes
For: Research and Technology Manager, OVI
Tel: 012 529 9114
Fax: 012 529 9418
e-mail: OneillM@arc.agric.za

THHa92

# TEST REPORT:
# F10



## *Avian Influenza HPAI*

February 2005

# VIRUCIDAL EFFICACY ASSAY

## REPORT TITLE

10 min./20 minute Inactivation of Avian Influenza by F10

## PRODUCT IDENTITY

F10CL General Farm Disinfectant
(B/N 050206)

F10 Super Concentrate Disinfectant
(B/N 060910)

## AUTHOR

G.H. Gerdes BVSc

## FINAL REPORT

February 14, 2005

## PERFORMING LABORATORY

Agricultural Research Council – Onderstepoort Veterinary Institute (Virology)
Private Bag X05
Onderstepoort, 0110
South Africa

## SPONSER

Health and Hygiene (Pty) Ltd
P.O. Box 347
Sunninghill, 2156
South Africa

# REPORT

## EVALUATION OF VIRUCIDAL EFFICACY OF F10 – TWO FORMULATIONS IN AN INANIMATE SUSPENSION ASSAY AGAINST HPAI AVIAN INFLUENZA SUBTYYPE H5N2

**AIM** : to test the virucidal efficacy of the products at dilutions of 1:50 and 1:100 for F10CL General Farm Disinfectant (F10GFD) and then 1:250 and 1:500 for F10 Super Concentrate Disinfectant (F10SC) with time intervals of 10 and 20 minutes in an inanimate suspension assay.

**BACKGROUND** : the influenza viruses are a large family that infect mammals and birds and are responsible for economically devastating diseases. Infection may result in crippling trade barriers and aggressive stamping out policies in an affected region. The avian influenza viruses, subject of this report are divided into subtypes based on 16 haemagglutinin (H) and 9 neuramidase (N) genes found in various combinations. They are further divided into high and low pathogenic types (HPAI and LPAI) according to defined criteria laid down by the OIE – the World Animal Health body. The subtype HPAI – H5N1 as defined above is a zoonosis passing from bird to human usually with serious consequences. The families are enveloped single-stranded RNA viruses studded with H and N spikes and destruction of the lipid envelope inactivates them. These are respiratory viruses and therefore thermo labile.

**SAMPLE NAME** : F10CL General Farm Disinfectant and F10 Super Concentrate Disinfectant lots B/N 050206 and B/N 060910 respectively.

**INITIATION DATE:** November 22, 2004

**COMPLETION DATE:** December 6, 2004

**SUMMARY OF RESULTS:**

| | |
|---|---|
| Disinfectant: | F10CL General Farm Disinfectant/1:100 and 1:50 in distilled water<br>F10 Super Concentrate Disinfectant/1:500 and 1:250 in distilled water |
| Virus: | HPAI H5N2 |
| Exposure Time: | 10 mins. and 20 mins. |
| Exposure Temperature: | Room temperature |
| Organic Load: | None |
| Efficacy Result: | Virucidal under test conditions |

**MATERIALS:**

1. Virus
The locally isolated AI ostrich liver isolate M78/04 was used. The identity was Ostr. Liv. 2 3AS 2 MDCK (19/10/04) at passage level 3 in eggs and 2 in tissue culture. The H and N identity had been confirmed as H5N2 by the Reference Laboratory at the Central Veterinary Laboratory, Weybridge, U.K.

2. Test cell cultures
The canine kidney line of Madin Darby – MDCK #158 produced in disposable tissue culture titration trays (24 wells) was used.

3. Test Media
Medium used for the cell cultures was Eagles Minimal Essential Medium (E-MEM) supplemented with 10% foetal calf serum (FCS) and Novo-Pen (1mμ/5ml) and Novo-Strep (1G/5ml). Non essential amino acids (NEAE) and Hepes buffer were also added.

4. Disinfectants
F10CL General Farm Disinfectant (B/N 040206) and F10 Super Concentrate Disinfectant (B/N 060910)

**METHOD:**

The AI antigen was pre-titrated and found to have a titer $>10^{-6}$. A working dilution of $10^{-4}$ was decided upon for the test.

A 24 h monolayer of cells in a 24 well sterile plastic testing plate was used. The F10 solutions formed an insoluble protein layer over the cells making microscopic visual assessment impossible. A trial using three different buffers, BLP (Buffered lactose peptone), PBS (Phospho-buffered saline and MEM (Minimal Essential Medium) was set up to determine a suitable diluent for use on tissue culture of the initial F10 in distilled water. There was very little difference between the buffers and it was decided to use a timed exposure, followed by centrifugation using the supernatant obtained to inoculate the plates. The inoculum could not be left on the cells and was replaced by maintenance medium. **(See table 1 and table 2)**

The F10 solutions and the virus in equal parts at the final dilution required was allowed to interact for 10 and 20 mins, and then the procedure of centrifugation outlined above was followed. For the virus control well, distilled water was substituted for F10 and for the disinfectant control well distilled water was substituted for virus. The virus control in a single well was left at room temperature for 20 mins.

The virus was used at a $10^{-4}$ dilution and wells were inoculated in duplicate. There were three controls built into the plate **(see table 3)**

1. A virus control well
2. An uninfected cell control well
3. Disinfectant control wells

**RESULTS:**

24 hrs. post inoculation (p.i.) there was no viral replication (cytopathology). At 48 hrs. the infected control cells showed almost complete cell destruction 3+ which then progressed to 4+. Plates were incubated for 7 days at 37°C and 4-5% $CO_2$ and assessed microscopically every day. After 7 days the plate was discarded. **(see table 4)**

At no time was there any viral cytopathology in the test dilutions or in the uninfected control cells. The only visible cell destruction or virus growth was in the infected control cells not exposed to F10 but infected with virus..

**CONCLUSIONS:**

An exposure time of both 10 mins. and 20 mins. of AI virus to both F10 products at the given dilutions was sufficient to inactivate the virus.
F10 was demonstrated to be virucidal.

**APPENDIX OF TABLES**

**Table 1**

DATE : 1/12/04

CELLS: MDCK #158 (29/11/05)

TEST: F10 + Buffer Trial

| | GFD | | | SUP C | | | |
|---|---|---|---|---|---|---|---|
| 1:25 | | | | | | | 1:100 |
| 1:50 | | | | | | | 1:250 |
| 1:100 | | | | | | | 1:500 |
| 1:200 | | | | | | | 1:1000 |
| | BLP | PBS | MEM | BLP | PBS | MEM | |

GFD – General farm disinfectant
SC – Super Concentrate disinfectant

Table 1 shows the general layout of the buffer trial and table 2 the readings of that trial after the plate was washed.

**Table 2**

| F10 + Buffers | 2/12 | WASH | 3/12 | 6/12 |
|---|---|---|---|---|
| GFD | | | | |
| BLP, PBS, MEM – 1:25 | O | | D | D |
| 1:50 | O | | - | - |
| 1:100 | O | | - | - |
| 1:200 | O | | - | - |
| SC | | | | |
| BLP, PBS, MEM – 1:100 | O | | D | D |
| 1:250 | O | | - | - |
| 1:500 | O | | - | - |
| 1:1,000 | O | | - | - |

D = Degenerated O = Opaque - = Cells unaffected

**Table 3**

DATE : 6/12/04

CELLS: MDCK # 159 (3/12/04)

TEST: F10 + M78/04 $10^{-4}$

| | Test -10 min | | Test – 20 min | | Disinfectant Controls | |
|---|---|---|---|---|---|---|
| F10 GFD | 1:50 | 1:50 | 1:50 | 1:50 | 1:50 | 1:50 |
| | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | Virus Control M78/04 ($10^{-4}$) |
| F10 SC | 1:250 | 1:250 | 1:250 | 1:250 | 1:250 | 1:250 |
| | 1:500 | 1:500 | 1:500 | 1:500 | 1:500 | Cell Control |

GFD – General farm disinfectant
SC – Super Concentrate disinfectant

**Table 4**

| F10 + Virus | 7/12 | 8/12 | 9/12 | 10/12 | 13/12 |
|---|---|---|---|---|---|
| 1:50 | - | - | - | - | - |
| 1:100 | - | - | - | - | - |
| 1:250 | - | - | - | - | - |
| 1:500 | - | - | - | - | - |
| F10 + Water | | | | | |
| 1:50 | - | - | - | - | - |
| 1:100 | - | - | - | - | - |
| 1:250 | - | - | - | - | - |
| 1:500 | - | - | - | - | - |
| Cell Control | Neg | Neg | Neg | Neg | Neg |
| Virus Control | Neg | 3+ | 4+ | 4+ | 4+ |

Table 3 shows the general layout of the test and table 4 the readings obtained on the days mentioned.