हाफकिन प्रशिक्षण, संशोधन व चाचणी संस्था

महाराष्ट्र शासन अनुदानित सोसायटीज् रजिस्ट्रेशन ॲक्ट १८६० अधिनियमान्वये नोंदणी कृत स्वायत्त संस्था
भारत सरकार, विज्ञान व प्रौद्योगिक मंत्रालय, मान्यताप्राप्त "वैज्ञानिक आणि औद्योगिक संशोधन संस्था"

Haffkine Institute for Training, Research & Testing

An Autonomous Institute of Govt. of Maharashtra Registered under Societies Registration Act 1860
Recognised as "Scientific & Industrial Research Organisation" (SIRO) by Govt. of India, Ministry of Science & Technology.

An ISO 9001: 2008 Certified Organisation

HI/VYR/18/2013
31.10.2013

To,
M/s. Faith Biotech Pvt Ltd
B-56, IInd Floor,
Kiran Ind Estate, Off MG Road
Goregaon (w), Mumbai - 400062
India.

Kind attn: Mr. S. Sundaresan
Sub: Report on the anti-viral efficacy of Sterigen (formerly known as Sterisol) solution

Dear Sir,

Greetings from Haffkine Institute.

Please find enclosed herewith, the report on the anti-viral efficacy of the Sterigen (formerly known as Sterisol) solution sample submitted by you at the Department of Virology.

Hope the results are satisfactory to your requirement.
Assuring you of our best services,

Thanks and regards,

Dr. Abhay Chowdhary
Director

Encl: As above

पत्ता : आचार्य दोंदे मार्ग, परळ, मुंबई - ४०० ०१२. दूरध्वनी क्र. ०२२-२४१६०९४७, २४१६०९६१, २४१६०९६२, फॅक्स - ०२२-२४१६१७८७
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हाफकिन प्रशिक्षण, संशोधन व चाचणी संस्था
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HI/VYR/18/13
Date: 31/10/2013

In-vitro evaluation of the anti-viral activity of the solution sample "Sterigen (formerly known as SteriSol)" submitted by M/s. Faith Biotech Ltd.

Sample label	**Sterigen (formerly known as SteriSol) solution**		
Batch	FI-01	FI-02	FI-03
Date of Sample generation	09-07-2013	25-09-13	01-11-13
Date of Sample submission	10-07-2013	26-09-13	02-11-13
Manufacturer	M/s. Faith Biotech Ltd.		
Sample submitted by	M/s. Faith Biotech Ltd.		
Sample Content	Water plus Sterigen-C (formerly known as SteriSol-C), after passage through a Flow-Through Electrolytic Module		

Test Procedure: The submitted sample was analyzed at 24 hours and 48 hours post collection. Anti-viral efficacy was analyzed as below.

1. **Anti-viral efficacy against Influenza (H1N1) virus:**
   - 1.1. MDCK cells were seeded into 12-well plates at a viable count of 10^3 - 10^4 cells/ml in Minimal Essential Medium with 10% Fetal Bovine serum and appropriate growth factors.
   - 1.2. Two concentrations of Sterigen solution were assayed, Undiluted and 1:10 (diluent – sterile growth medium).
   - 1.3. The above dilutions of Sterigen solution were incubated with equal volume of the H1N1 virus for a period of 5 & 10 minutes.
   - 1.4. These were then inoculated onto the MDCK cells and observed virus induced cytopathic effect.
   - 1.5. Appropriate virus control and sample controls were also incubated along with the test samples.

पत्ता : आचार्य दोंदे मार्ग, परळ, मुंबई - ४०० ०१२. दूरध्वनी क्र. ०२२-२४१६०९४७, २४१६०९६१, २४१६०९६२, फॅक्स - ०२२-२४१६१७८७
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2. **Anti-viral efficacy against Polio virus:**

2.1. MDCK cells were seeded into 12-well plates at a viable count of 10^3 - 10^4 cells/ml in Minimal Essential Medium with 10% Fetal Bovine serum and appropriate growth factors.

2.2. Two concentrations of Sterigen solution were assayed, Undiluted and 1:10 (diluent – sterile growth medium).

2.3. The above dilutions of Sterigen solution were incubated with equal volume of the Polio virus for a period of 5 & 10 minutes.

2.4. These were then inoculated onto the VERO cells and observed virus induced cytopathic effect.

2.5. Appropriate virus control and sample controls were also incubated along with the test samples.

3. **Anti-viral efficacy against Rotavirus:**

3.1. MA-104 cells were seeded into 12-well plates at a viable count of 10^3 - 10^4 cells/ml in Minimal Essential Medium with 10% Fetal Bovine serum and appropriate growth factors.

3.2. Two concentrations of Sterigen solution were assayed, Undiluted and 1:10 (diluent – sterile growth medium).

3.3. The above dilutions of Sterigen solution were incubated with equal volume of the Polio virus for a period of 5 & 10 minutes.

3.4. These were then inoculated onto the MA-104 cells and observed virus induced cytopathic effect.

3.5. Appropriate virus control and sample controls were also incubated along with the test samples.

4. **Hepatitis B Virus:** As there is no suitable in-vitro model for propagation of HBV, the anti-HBV activity was assessed using the HbsAg secretion inhibition assay.

4.1. Briefly, PLC/PRF-5 cells were seeded into 12-well plates at a viable count of 10^3 - 10^4 cells/ml in Minimal Essential Medium with 10% Fetal Bovine serum and appropriate growth factors.

4.2. Two concentrations of Sterigen solution were assayed, Undiluted and 1:10 (diluent – sterile growth medium).

4.3. Two hundred microliters of the samples were added to these cells and incubated for a period of 4 days.

4.4. On the 4th/5th day, the supernatant was aliquoted and the presence of HbsAg was assayed using a commercially available ELISA kit (HepaLisa ULTRA, manufactured by M/s. J. Mitra)

5. **HIV:** Anti-HIV efficacy was determined using the HIV-1 p24 antigen detection assay using a commercially available ELISA kit (M/s. Advanced Biosciences Laboratories Inc.)

5.1. Human Peripheral blood mononuclear cells were seeded in 24 well plates with a viable count of 10^3 - 10^4 cells/ml in RPMI 1640 with 10% Fetal Bovine serum.

5.2. Two concentrations of Sterigen solution were assayed, Undiluted and 1:10 (diluent – sterile growth medium). They were incubated with equal volume of the HIV virus for a period of 5 & 10 minutes.

5.3. This was then inoculated onto the Peripheral Blood Mononuclear cells and observed for a period of 4 to 7 days

5.4. At the end of incubation period, the cell free supernatant was drawn and assayed for p24 antigen presence.

6. **Observation:**

Virus	Concentration	Efficacy after 24 hours		Efficacy after 48 hours	
		5 min	10min	5min	10min
Influenza (H1N1)	Undiluted	+	+	+	+
	1:10 dilution	+	+	-	-
Poliovirus	Undiluted	+	+	+	+
	1:10 dilution	+	+	-	-
Rotavirus	Undiluted	+	+	+	+
	1:10 dilution	+	+	-	-
HBV	Undiluted	+	+	+	+
	1:10 dilution	+	+	-	-
HIV	Undiluted	+	+	+	+
	1:10 dilution	+	+	-	-

+ = antiviral activity, - = no antiviral activity

Inference: Undiluted samples of the Sterigen solution are cytotoxic to the cells, implying that they would be killing the viruses as well by the virtue of viruses being obligate endo-parasites.

The submitted Undiluted sample of Sterigen solution is effective against Polio, Rotavirus, Influenza, HIV and HBV, at a contact time of both 5 minutes and 10 minutes. The efficacy is maintained till 48 hours post collection as assayed in this study. However, the efficacy of a 1:10 dilution of this solution is maintained only for 24 hours post collection.

Dr. Abhay Chowdhary
Director

References: Mossmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay, J Immunol Meth, 1983, 65:55-63.

Disclaimer: This report is issued with an explicit understanding that it would neither be used for the purpose of advertisement nor it would be produced as evidence without prior permission of the undersigned. Further, this report is restricted only to the sample submitted to the Department.