

## Identification and Titration of Hydro Pericardium Syndrome Virus (HPSV) by using Modified Counter-Current Immuno-Electrophoresis (MCCIE)

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### Abstract

A rapid, simple, and accurate Modified Counter Current Immuno-electrophoresis (MCCIE) technique was developed and compared with the Reverse Passive Hemagglutination Assay (RPHA) for the titration of HPS virus. The MCCIE test had 100% correlation with the conventional RPHA and correctly titrated 116 samples which were already titrated with RPHA. Instead of horizontal electrophoresis chamber used in counter current Immuno-electrophoresis test, a modification in Counter Current Immuno-Electrophoresis (CCIE) test was made by using 1% melted agarose gel filled in 8 cm long and 3 mm narrow U-shaped glass tubing for the detection as well as quantification & titration of HPS virus. Results of Liver samples of 116 poultry birds including 16 broilers (6 livers were from clinically positive), 50 Desi and 50 commercial layers (adult) obtained through modified counter current Immuno-electrophoresis (MCCIE) were found similar to those found through CCIE and titres obtained through MCCIE were found similar to RPHA tests. The titres in MCCIE test were expressed as appearance of hazy colored precipitation band in the twelve U-shaped tubes. All of the six clinically positive liver samples gave positive results with titres ranging from 1:32 to 1:128. Of these 6 positive, 3 samples had 1:32, 2 samples had 1:64 and 1 sample had 1:128 titres. While none of desi and commercial layer was found to be positive. The results indicated that MCCIE is simple and inexpensive test as compared to CCIE & RPHA to detect and titrate HPS virus in the infected poultry liver samples. All the HPS virus isolates from HPS infected broiler livers and adenovirus group-I obtained from NARC, Islamabad and VRI, Lahore showed MCCIE & RPHA titre (1:64) with specific HPS antibodies.

**Keywords:** Titration; Hydro pericardium syndrome virus (HPSV); Modified counter-current immuno-electrophoresis (MCCIE)

### Introduction

Immunodiffusion is a laboratory technique used to evaluate the binding of an antibody to its antigen. Counter immuno-electrophoresis is similar to immunodiffusion, but with the addition of an applied electrical field across the diffusion medium, usually an agar or polyacrylamide gel. The effect is rapid migration of the antibody and antigen out of their respective wells towards one another to form a line of precipitation, or a precipitin line, indicating binding [1]. Counter-current immuno-electrophoresis was considered as a rapid sensitive method for detecting pneumococcal capsular antigens in sputum and result can be obtained within 45 minutes [2]. Hydro pericardium syndrome is an acute infectious disease of chickens characterized by high morbidity and mortality, distension of pericardial sac along with accumulation of transparent jelly-like fluid of white, amber or occasionally green colour. Multifocal hepatic necrosis and severe involvement of kidneys is also seen. HPS was most exclusively seen in broilers at the age of 3-6 weeks of age. The duration of disease is 10-14 days. Although the specific etiology has yet to be defined, available evidence suggests the condition is caused by a pathogenic group-I adenoviruses. Other agents may serve as potentiators and increase the severity of disease under field conditions [3]. In 1987, this disease was first seen in Karachi near Angara Goth. High mortality and morbidity about 30-60% was recorded [4]. In India, HPS infectious agent was identified in liver homogenates by

using antisera raised in laboratory animal by using Counter immuno-electrophoresis and demonstrated a precipitin line in positive cases [5].

After getting successful identification of HPS virus through Counter Current Immuno-electrophoresis (CCIE) and titration of HPS virus through RPHA test [6] and investigation of Hydro pericardium syndrome antibodies in wild birds through IHA test [7], the titration of HPS virus can also be possible by little modification of CCIE. This article elaborates the modification in CCIE and the use of modified CCIE in the titration of HPS virus and has given useful results for the detection and titration of HPS virus in test samples of liver homogenates and adenovirus group-I from NARC, Islamabad and VRI, Lahore.

### Materials and Methods

#### Raising of hyper immune HPS antiserum

A commercial Hydro pericardium syndrome vaccine (HPS) (Sanna Laboratory, Faisalabad, Pakistan) was purchased from local market and hyper immune antiserum against this vaccine was raised in a laboratory rabbit. Fleetingly, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 ml HPS vaccine was inoculated subcutaneously in the rabbit on alternate days. Ten days after the last injection the rabbit was slaughtered and serum was separated. Processing of poultry livers were triturated separately in normal saline along with antibiotics penicillin and streptomycin, each 1000 µg/ml and the homogenate was centrifuged at 1500 rpm for 5 minutes. After centrifugation, the liver proteins were precipitated by

treating with chloroform @ 1:2 (chloroform: supernatant). The chloroform supernatant was again centrifuged at 1500 rpm for 5 minutes. The supernatant was used for HPS virus [8].

**Modified counter current immuno-electrophoresis test (MCCIE)**

Counter Current Immuno-electrophoresis (CCIE) test was modified to titrate HPS virus as Modified Counter Current-Immuno-electrophoresis (MCCIE). In MCCIE test, U-shaped glass tube of 8 cm long and 3 mm narrow lumen was used. 0.25 ml of 1% melted agarose was filled in the U-shaped tube in such a way that 2-3 cm length of both the arms of U-Shaped tube remained vacant. After the solidification of gel, 10 µl of HPS test antigen (Un-Known) was dispensed over the solidified gel in one arm of U-shaped tube and 10 µl of the known hyper immune serum against HPS antigen in the other arm of U-shaped tube. Platinum wires were pierced 0.25 cm deep in gel of both the arms of U-shaped tube. The platinum wire pierced in arm containing hyper immune serum was connected with anode while platinum wire that pierced in arm containing test HPS antigen was connected with cathode of 3.0 Volts battery. A constant current was applied for 15 minutes and then current was switched off. A circular precipitin band of hazy colour was taken as positive result. In negative

control in place of known antiserum, normal saline was used and no band was seen in the control negative tube. The test was standardised with known antigens from NARC, Islamabad and VRI, Lahore and known antiserum.

**Quantification & titration of HPS antigen with MCCIE test**

For titration of HPS test antigen, two-fold dilutions of HPS test antigen were made in micro titration plate. 10 µl from each of two-fold dilution of antigen was subject to ten U-shaped glass tubes mounted on a stand for twelve U-shaped tubes. In 11<sup>th</sup> U-Shaped tube, hyper immune serum was tested with known HPS antigen (Control Positive) and in 12<sup>th</sup> tube normal saline was used in place of known hyper immune serum (Control Negative). The highest dilution making precipitation band was taken as the titre of HPS antigen [9].

**Results**

Out of 116 liver homogenates, only 6 clinically positive homogenates showed positive results which had titres from 1:32 to 1:128. Out of which, 3 had 1:32, 2 had 1:64 and 1 sample had 1:128 titre (Table 1). These results show that HPS occurs only in broilers and desi or commercial layers are not affected with this disease.

Poultry Birds	Total Livers	Modified Counter Current Immuno-Electrophoresis (MCCIE) Test							
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Broilers (3-6 Weeks)	10+6 <sup>*</sup> =16	-	-	-	-	3	2	1	-
Desi	50	-	-	-	-	-	-	-	-
Layers (Adult)	50	-	-	-	-	-	-	-	-

<sup>\*</sup>Clinically Positive Case

**Table 1:** Prevalence of HPS in domestic poultry.

**Discussion**

A total of 116 poultry liver homogenates including 16 broilers (6 livers were clinically positive), 50 desi and 50 commercial layers, were subjected to Modified Counter Current Immuno-electrophoresis and reverse passive heam-agglutination tests. Titration results of HPS antigen are exactly equal to the findings obtained by Manzoor & Hussain, through RPHA test [6]. CCIE has not been used ever for titration of antigen or antiserum because its design was not capable to titrate antigen or antibodies but CCIE has been modified as MCCIE in such a way that now it can be easily used for the titration of antigens as well as antibody. After making a comparison of results attained through MCCIE with the titres obtained through RPHA by using the same HPS antigen used by Manzoor & Hussain [6] and getting the expected results from the control positive known HPS antigens of NARC, Islamabad and VRI, Lahore it can be straightforwardly declared that MCCIE test can be used for the titration of HPS antigen in place of RPHA test and also for the titration of serum in place of IHA Test (Table 2).

HPS Virus	1:2	1:4	1:8	1:16	1:32	1:64
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Adenovirus group-I (NARC*)	+	+	+	+	+	+
Adenovirus group-I (VRI**)	+	+	+	+	+	-

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**Table 2:** Control positive of MCCIE test.

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