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Sensitivity of virus transport medium for isolation of influenza virus

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Abstract

To protect the virus in the specimen from stressors in the environment which can lead to loss of biological properties and ultimately affect the isolation, there is need to use good quality and appropriate transport system to maintain the virus. Certain virus transport media have proven to be effective and convenient for isolation of swine influenza virus using different culture system. This study was designed to know which of the transport medium: Phosphate Buffer Saline-Glycerol Based or Dulbecco's Modified Eagle Medium (D-MEM) will be sensitive for the isolation of swine influenza virus using 10 – 12 days old fertile embryonated hen's egg and Mardin Darby Canine Kidney (MDCK) as the culture systems. A total of 186 nasal swabs were collected from 93 pigs from August to September, 2010 using both PBS-Glycerol and D-MEM as transport medium. Isolation of influenza virus was carried out using conventional cell culture (MDCK) and 10-12day old embryonated hen's eggs. Isolates were identified by Haemagglutination-Inhibition (HAI) test using World Health Organization (WHO) influenza virus reference antisera. PBS-Glycerol Based Transport Medium yielded 15(16.1%) influenza isolates while D-MEM yielded 1 (1.1%) using the two culture systems. PBS-Glycerol Based Transport Medium yielded more isolates which is in line with the fact that a good viral transport medium should allow for survival and diagnosis of viral infections.

Keywords: Isolation, Influenza, Virus, Transport Medium, D-MEM, Glycerol, PBS

Introduction

A risk assessment of the cells used for culture, use of aseptic techniques and a good cell culture practice are essential for survivability of viruses (Coecke et al., 2005). There are various transport media for collection of specimen for virus isolation, out of which D-MEM and PBS-Glycerol can be for specimen collection for isolation of influenza virus from animals (WHO, 2002). Different studies on virus stability show that most viruses are stable in transport medium for 1 to 3 days before processing. Specimen for virus isolation should not be exposed to adverse environmental conditions in order to prevent loss of infectivity and reduction in the titre of the virus present in the specimen. Investigations on the possibility of isolating new pandemic strains of influenza virus (WHO, 2009) highlighted the need for good virus transport medium. Strains of influenza virus are required to be cultured, typed and in state public health laboratories, or at national surveillance laboratories (CDC, 2009), they may go further for molecular detection using real time Polymerase Chain Reaction (PCR). The sensitivity of a culture system for the isolation process can be affected by the choice of transport medium used. Laboratories without commercial transport Medium use minimum Essential Medium (MEM) containing 0% calf serum. Bacteria, yeasts, fungi, molds, mycoplasmas and occasionally protozoa, can all appear as contaminants in tissue culture. Rapidly growing organisms are less problematic as they are often overt and readily detected, whereupon the culture can be discarded. Difficulties arise when the contaminant is cryptic, either because it is too small to be seen on the microscope, e.g., mycoplasma, or slow growing such that the level is so low that it escapes detection. Use of antibiotics can be a common cause of cryptic contaminations remaining undetected (Doyle et al., 1990; Doyle and Bolton, 1994; Hay and Cour, 1997; Freshney, 2005). This study investigated the sensitivity of PBS/Glycerol and

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D-MEM as Virus Transport Medium (VTM) for the isolation of influenza virus using MDCK and 10-12 day old fertile embryonated hen's egg.

Materials and methods

Preparation of Virus Transport Medium

Dulbecco's Modified Eagle Medium (D-MEM) was procured from Stigma. Double distilled water was obtained and D-MEM was reconstituted in the tissue culture section of the Department of Virology, University College Hospital, Ibadan. It was autoclaved and 2mL was aliquoted into cryovials in a Bio-safety level II facility. One litre of PBS /Glycerol was made up of Phosphate Buffered Saline (PBS) and Glycerol in ratio 1:1. The PBS was autoclaved at 121°C at 15pounds pressure for 30 min after which Glycerol was added in a Biosafety cabinet II. The mixture was filtered using 0.22µm filter membrane. Antibiotics were added to the PBS/Glycerol in a Biosafety cabinet II according to WHO, 2002 following standard laboratory procedure [Benzypenicillin 2 x 106 IU/litre; Gentamycin 2mg / litre; Amphotericin B 0.5x106 IU/ litre]. The pH of the medium was taken subsequently and adjusted to a pH of 7.3.

Sterility and Toxicity Test of Viral Transport Medium (VTM)

The reference method used was the Hoechst 33258 staining of Vero cells inoculated with culture supernate and culture using selective broth and agar media (EP, 2006; USFDA, 2005). The inoculated broth and nutrient agar plate with the viral transport medium was incubated for 48 hours and checked for bacterial or fungal growth. Two tissue culture tubes of confluent Rhabdomyoma (RD) cell line were inoculated with the Virus Transport Medium (VTM) and incubated for 24 hours for bacterial growth while another two tissue culture tubes of RD cells not inoculated served as cell controls. To test for toxicity, the VTM was inoculated into monolayer of confluent RD cells and incubated at 37°C for 24 hours and examined under microscope for degenerative changes to the cells. There were also two uninoculated tissue culture tubes which served as cell control.

Study Site

Specimens were collected from pigs at Bodija Abattoir which is a major Abattoir in Ibadan North Local Government Area of Oyo State.

Collection of Specimens

Specimen collection vials were assigned numbers that corresponded to those on the Field Data Collection Sheet. Two nasal swabs were collected from apparently healthy and symptomatic animals. Dry swabs were inserted into the nostrils (left and right) and left in place for a few seconds. This was slowly withdrawn in a rotational motion within the nasopharynx. The tip of each swab was dipped into a vial containing 2mL of the two VTM and part of the applicator stick was broken off and left inside the VTM. A total of 186 specimens were collected from 93 pigs.

Transporting Specimen to the Laboratory

Specimens for virus isolation were placed in ice packs in order to prevent loss of infectivity and transported to the laboratory promptly for processing.

Virus Isolation

Isolation of influenza virus from field specimen was carried out using Mardin Darby Canine Kidney (MDCK) and 10-12 days old embryonated hen's eggs. All virus isolation procedures were carried out in biosafety cabinet level II following standard laboratory procedures (WHO, 2002).

Identification of Virus Isolates

The isolates obtained from the two VTM were identified firstly by Haemagglutination Test (HA) and later followed by Haemagglutination Inhibition Test (HAI) using the WHO reference antisera H1, H3, H1N1 and Influenza B obtained from the Department of Virology, University College Hospital Ibadan, it was magnanimously supplied by the Centre for Disease Prevention and Control (CDC) Atlanta.

Results

Sterility test on the VTM showed that there were no bacterial colonies or fungal hyphae after 48 hours. Also, there was no bacterial or fungal growth in RD cell cultures inoculated with the VTM. Toxicity test showed that the initial preparation of PBS/GlycerolVTM was toxic to the culture cells. These cells were dead and floating while the control cells were not. The pH was determined and found to be acidic, then adjusted to 7.3 with NaHC03. Toxicity test was repeated after pH had been adjusted and found to be appropriate. Using PBS/Glycerol VTM, the MDCK culture system yielded 2 (18.18%) isolates while the 10-12 days old fertile embryonated egg culture yielded 9 (81.81%) isolates as shown in **Table 1**. For D-MEM VTM, the MDCK culture system yielded 6 (40%) isolates while the 10-12 days old fertile embryonated egg culture yielded 9 (60%) isolates as shown in **Table 1**.

Table 1. Haemagglutination (HA) test result comparing the two transport media and viral culture systems

	PBS/Glycerol No (%)	D-MEM No (%)	Total No (%)
MDCK	2 (18.18)	6(40)	8(8.60)
Egg	9 (81.81)	9(60)	18(19.36)
Total	11 (99.99)	15(100)	26 (27.96)

Table 2. Haemagglutination-inhibition (HAI) test result showing number of influenza isolates

	PBS/Glycerol No (%)	D-MEM No (%)	Total No (%)
MDCK	0 (0)	1(100)	1(1.1)
Egg	15(100)	0(0)	15(16.1)
Total	15(100)	1(100)	16(17.2)

Fifteen (93.75%) isolates of influenza virus were obtained using PBS/Glycerol VTM in 10-12 days old fertile embryonated egg as culture system while no isolates of influenza was obtained for culture system of MDCK as shown in **Table 2** after haemagglutination-inhibition (HAI) test has been performed using

the selected WHO influenza reference antisera. On the other hand 1 (6.25%) isolate of influenza virus was obtained using D-MEM as VTM in MDCK culture system while no isolate of influenza virus was obtained in 10-12 days old fertile embryonated egg culture system as shown in **Table 2**.

Discussion

There was no contamination of the transport medium because it was prepared under sterile conditions and antibiotics were added accordingly (WHO, 2002), the Benzypenicillin acted against gram positive bacteria, Gentamycin acted against gram positive bacteria, gram negative bacteria and Mycoplasma, the Amphotericin B destroyed any fungal and yeast contaminants.

The two VTM that were used (Glycerol/PBS and D-MEM) were sensitive for the isolation of swine influenza virus in different culture medium used as supported by WHO, 2002.

Glycerol/PBS based VTM is suitable for egg inoculation than the tissue culture work whereas the D-MEM based VTM is good for tissue culture inoculation than the embryonated egg culture system (WHO, 2002). The result of the study which shows specimen in Glycerol/PBS yielded more isolates in eggs than D-MEM, which is in agreement with previous observation, by WHO, 2002. In contrast specimen placed in D-MEM yielded more isolate of influenza B in eggs than those placed in Glycerol/PBS.

These results indicate that these viral transport media are good for cultural isolation especially Glycerol/PBS if there are possibilities of delay transportation to the laboratory for isolation purpose. Glycerol/PBS viral transport appeared to be the best transport medium for the isolation of the virus studied in this study. The utility of transport medium for the isolation of influenza viruses is of considerably importance.

The addition of antibiotics reduced the contamination rate, as a result of multidrug resistance of bacteria that we have now. This method served encouraging for preparation of VTM in a developing country like this and laboratories where resources are limited.

Therefore, for influenza virus surveillance Glycerol/PBS could be used as transport medium for isolation of influenza viruses.

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