Preparation Of High Potent Measles Haemagglutinating Antigen From Wild Type Measles Virus In Ibadan, Nigeria

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Abstract: This study reports the preparation of preparation of high potent measles haemagglutinating antigen from wild type measles virus in Ibadan, Nigeria. Measles haemagglutinating antigen was prepared indigenously from isolates obtained from two children infected with measles at the Oluyoro Catholic Hospital (OCH) Ibadan, with titres of 7.15 and 9.5 $TCID_{50}$. The isolates were then adapted to Vero cell line after eight serial passages. Stock isolates were then prepared in three bateches following polyethylene glycol between 80 concentration method. The haemagglutination activity (HA) of the three batches of the measles HA antigen were then tested with 4% washed monkey RBC in a 96 well V shaped bottom microtitre plate alongside with commercial measles HA antigen lot no. 733031. Batch 1 antigen gave a titre of 1:640 while commercial antigen titre was 1:320, Batch 2 antigen titre dropped one fold to give 1:320 with commercial antigen titre 1:320, and untreated measles virus gave a titre of about 1:10. Cell controls were also set up to validate the results of this study. Batch 3 HA antigen titre rose back to 1:1280 while commercial antigen gave 1:320 and untreated measles virus 1:10. Twenty-seven (27) field samples were collected from the Otunba Tuwashe Children's emergency ward (OTCHEW) of the University College Hospital (UCH), Ibadan. They were collected based on the criteria determined by past history of measles infection, vaccination history, and manifestation of other exanthematous illness. The age range of children tested was between 7 months and 15 years. An actual adult individual infected with acute measles was also tested using paired sera. All samples were tested using batch 1 antigen. The findings of the field study showed that 5 subjects with clinical history of measles tested positive to the indigenous prepared high potent measles haemagglutinating antigen with titres ranging from 1:32 to 1:128, while 6 subjects with history of vaccination had titres also ranging from 1:32 to 1:128. Four subjects with other exanthematous illness also tested positive with titres of 1:32 and 1:64. Specificity and sensitivity tests of the measles HA antigen using 4 known measles unexposed and unvaccinated subjects and 5 known measles infected subjects gave a specificity and sensitivity of 1, this showed that the antigen was highly specific and equally sensitive. The result from the adult measles patient also confirmed the efficacy of our measles HA antigen giving an acute phase serum titre of 1:16 and convalescence phase titre of 1:128, this showed a 4-fold rise in titre which is diagnostic of measles infection. The findings of this study are of a significant practical impact and confirm that an indigenous prepared antigen can give a higher titre than the commercially prepared antigens. Thus, if the indigenous prepared antigen is further refined and produced massively, it will be highly cost effective especially in field for sero-monitoring and surveillance.

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Introduction:

Measles virus (MV) is an enveloped virus belonging to the genus Morbillivirus of Family Paramyxoviridae (Norrby, 1992; Fields *et al*., 1996; Jin *et al*., 1998; Griffin, 2007). It has a non-segmented, negative sense, single stranded RNA as its genome (). The RNA genome contains 15,894 nucleotides encoding six structural and two nonstructural proteins. These are the nucleoprotein (N); phosphoprotein (P); matrix protein (M); fusion protein (F); haemagglutinin (H) and large polymerase (L). The nonstructural proteins V and C are derivatives of the viral P mRNA (Takeuchi *et al*., 2002). It is highly related to Rinderpest virus and more distantly related to Cannine Distemper virus group in antigenic structure (WHO, 2007). Measles virus has been considered as a monotypic antigenically stable virus, genomic variations with minor antigenically stable variations have also been observed in both haemagglutinin (H) and the fusion (F) protein (Kingele et al., 2000). MV is the agent of acute illness called measles that has been reported as highly contagious and universal in occurrence (Palevsky, 2002). Measles produces significant illness, death, and disability (Pan American Health Organization, 2005); and infects approximately 40 million people resulting in nearly 1 million deaths

annually in developing countries (Oldstone, 1998). It has been reported as a major cause of childhood morbidity and mortality in Nigeria; 212,183 and 168,107 cases were recorded in 2000 and 2001 respectively (WHO, 2001). For instance in 2005, Adamawa State, Nigeria experienced 3,974 cases and 238 measles-deaths (Dubray *et al*., 2005). WHO, AFRO (2004) reported measles as one of the top five causes of death in children less than five years of age in many African countries. Measles disease is usually characterized by fever of 38°C or more; maculopapular rash of about 3 days or more; with one or a combination of coryza, cough, conjunctivitis and Koplik spots in the oral mucosa of measles' victims (Palevsky, 2002). It is spread through respiration (contact with fluids from an infected person's nose and mouth, either directly or through aerosol transmission), and is highly contagious, 90% of people without immunity sharing a house with an infected person will catch it. Measles is one of the vaccine-preventable diseases (Washington State Department of Health, 2006) hence one of the Expanded Programme on Immunization (EPI) – target diseases for eradication (WHO, 1999). The MV is monotypic and essentially without animal reservoir (Norrby *et al*., 1995), as a result measles control and eradication should be within reach of aggressive vaccination campaign. Moreover the virus is antigenically stable making the vaccine derived from the 1954 Edmonston isolate provide protection against all MVs (Rota *et al*. 1994); and interrupt its transmission from infected to immune individuals. The industrialized nations of the world (e.g. the US) have used the live attenuated MV vaccine to eradicate transmission of indigenous measles strains (WHO, 1990; Benenson, 1990).

Measles is caused by measles virus (MV) in humans; it is a hyper acute and highly infectious disease characterized by a maculopapular rash, fever, cough, coryza, conjunctivitis, koplik-spots (Koplik, 1869). It causes high mortality in poor populations, particularly among children less than 5 years of age. Case fatality rates in developing countries estimated to vary between 2%-6% and as high as 30% during outbreaks (Anon, 1989). Measles and its complications are responsible for more children deaths world wide than all other childhood vaccine preventable diseases combined and more than any other single specific agent. This is best illustrated by Christensen's Greenland study in 1953. Although other primate species may be infected through contact with human, no animal reservoir of measles virus has been identified (Albrech *et al*., 1977, 1980).

Molecular epidemiology of MV entails genetic characterization of the wild-type (wt) virus; and it is an important component of measles surveillance (WHO, 2003). Measles surveillance and epidemiological investigation is able to identify the source and transmission pathways of the virus. This is most beneficial when the change in viral genotypes over time in a particular region can be observed, because the information from the genetic analyses can document the interruption of transmission of endemic measles. Consequently, molecular characterization of wt MVs has become a valuable tool for the evaluation of the effectiveness of measles control and elimination programmes. WHO therefore recommended that viral surveillance be conducted during all phases of measles control and that virological activities be expanded to provide an accurate description of the global distribution of measles genotypes.

Immunization programs world wide now present greater than 1.5 million deaths from measles in developing countries. Yet approximately 1 million children continue to die each year from measles- a preventable and potentially eradicable disease (Tanin *et al*., 1994). Clinical diagnosis of measles infection in children is straight forward and often times easy and the laboratory is often not consulted before a definitive diagnosis is made. However, this is often confused with manifestation of other exanthematous diseases like dengue fever, varicella and rubella which shows similar clinical manifestations. As such if therefore necessary to do a differential laboratory diagnosis particularly in measles endemic countries such as Nigeria. Laboratory diagnosis however, is confirmatory and this can be done using direct virus isolation in cell culture using acute phase PBMC of infected patients inoculated into SLAM Vero or B958/B95[a] cell lines. Characteristic cytopathic effect (CPE) can be visualized after about 6 to 8 days of virus culture (Ono *et al*., 2001).

Serology forms the gold standard for confirming suspected cases due to its high sensitivity for measles (WHO, 2007). Serological diagnosis which is the most widely used and commonly seen is done by detection of measles IgM or IgG antibody by enzyme linked immunosorbent assay (ELISA) using recombinant MV proteins as capture antigen; complement fixation test or haemagglutination inhibition test (HA/HI). Laboratory diagnosis can also be done through Reverse transcriptase-PCR or virus isolation.

The HI test is the most widely acceptable test in most developing countries like Nigeria. Haemagglutination inhibition test has long for a long time a gold standard for laboratory diagnosis of measles infection (WHO, 2007). This is because it is very sensitive and specific and easily performed in any laboratory. The ELISA is another sensitive test which is also used for diagnosis of measles virus infection but it is more expensive and require the use of special equipment not found in many laboratory in developing countries like Nigeria (WHO, 2007; Adu Personal communication, 2007).

Neutralization titre of any given serum measured by serological tests like Neutralization test and Heamagglutination inhibition assays (WHO, 2007) has been shown to correlate well with the degree of protection against MV and titres of sera from human vaccines have been demonstrated to be lower against some wild type viruses than vaccine strains whilst sera from late convalescents neutralized both wild and vaccine strains efficiently (Tamin *et al*., 1994; Klingele *et al*., 2000).

The HA/HI test is not only relevant in the diagnosis of measles infection but is also very important for seromonitoring of measles infection. Therefore, a readily available measles HA antigen will be a very valuable and useful tool for monitoring the progress of measles seroconversion following immunization in Nigeria. To perform the HA/HI test a potent measles antigen is therefore needed. Up till now, this measles antigen is still being imported at very expensive amount into Nigeria. Also to import the foreign brands of this measles antigen into Nigeria takes s very long time and often a time, they arrive having lost their potency or very close to their expiry date. Previous attempts to produce indigenous available measles antigen in our laboratory by sonication had not been too successful because of the very low titre of the antigen (Adu Personal Communciation, 2007).

While many developed countries have successfully controlled measles, and are currently in measles elimination phase (CDC, 2005); Nigeria, in tune with global goal, is on measles control goal (i.e. morbidity and mortality reduction). As a result, it recently concluded a nationwide accelerated measles vaccination campaign in June /July, 2006. As one of the leading cause of childhood morbidity and mortality, the need to effectively control and eventually eradicate measles cannot be overemphasized. Therefore, this current study seek to isolate a Nigeria strain of measles virus; to use the Nigeria isolates to prepare an indigenous measles antigen for laboratory diagnosis and seromonitoring of measles infection in Nigeria, using the polyethylene glycol ether concentration method, and to compare the indigenously produced measles antigen with an imported commercial measles antigen from Japan.

MATERIALS AND METHODS:

The whole blood samples used for this study were collected from children in the Otunba Tuwashe Children's emergency ward (OTCHEW) of the University College Hospital (UCH), Ibadan. Physical examination of suspected measles cases were carried out by respective hospital pediatrician/clinician. The subjects were assessed for fever, maculopapular skin rash, as well as, oral lesion to detect Koplik's spot. The nose and eyes will visually be examined too. Children with a recent history of measles infection, children presenting with symptoms of any other exanthematous illness other than measles virus infection and children confirmed to have received their complete measles vaccination were selected for this study. Two to five milliliters (2-5ml) of blood were collected from twenty-seven children between the ages 7 months and 15 years of age and one adult showing rash not more than 4 days after the appearance of rash.

PREPARATION OF SAMPLES: The lymphocytes isolation from whole blood was carried out as described by Amersham (the manufacturer of Ficoll-Paque^{TM}). At room temperature, 2ml freshly drawn blood collected in EDTA-treated tube was mixed with 2ml minimum essential medium (MEM) in a clean 15ml plastic centrifuge tube. With a Pasteur pipette, these were mixed. To an empty centrifuge tube, 3 ml Ficoll-paque^{TM} was added. On this, the 4 ml dilute blood was carefully layered without mixing; this tube was screw-capped and centrifuged at 400 x g for 30 minutes at room temperature. With another pipette, the upper layer of centrifuged fluid was removed, following which the lymphocyte layer was also removed carefully and dispensed into 6 ml MEM for onward transport on ice packs to Virology Laboratory, Ibadan. All samples were properly labeled to identify each patient, location and date. All samples were stored always at -20°C until ready for use.

PREPARATION OF CELL CULTURE: A T_{25} flask containing confluent monolayer of B95^{a} cell line grown in RPMI 1640 growth medium was provided by tissue culture unit of the Laboratory. The monolayer was washed twice with 5mls PBS/EDTA and layered with 0.25ml Trypsin varsene to dislodge the cells. 3mls of Growth medium was used to stop the react on of the Trypsin-Varsene when it was evident that the cells had already detached. The confluent monolayers of cells were splitted into a T_{75} flask supplemented with Growth medium and grew until it reached confluency. The confluent monolayer of cells in the T75 flask was later splitted into 4 large flasks (T_{150}) and grew to form a confluent monolayer.

VIRUS ISOLATION: The isolated lymphocytes were cultured in confluent monolayer of B95^{a} cell line grown in RPMI 1640 growth medium. Two days after inoculation, the medium was changed to RPMI 1640 maintenance medium. Cells were observed daily for the characteristic measles cytopathic effects (CPE) of stellate bodies and few syncitia started showing up on the 5th day after inoculation. On the 7th day, 90% CPE was observed in the flasks and they were frozen. Cells showing CPE were repassed in confluent monolayer of B95^{a} cell line to increase the titre of the virus.

ADAPTATION OF MEASLES VIRUS IN VERO CELL LINE: Virus isolated in B95[a] cell lines were passed for about 8 passages in Vero cell line for adaptation, following the adaptation, stock measles virus for antigen preparation were then prepared in T150 tissue culture flasks.

PREPARATION OF MEASLES HAEMAGGLUTINATING ANTIGENS: After adaptation of wild type virus in Vero cell, four large flasks (T150) containing confluent monolayer of Vero cells were inoculated with 0.5ml vaccine strain of Measles Virus (MV) using MEM supplemented with 10% fetal calf serum (FCS). Two days after inoculation, the medium was changed to 2% maintenance medium and further incubated for another 3 days. Cells were observed daily for the characteristic measles cytopathic effects (CPE) of stellate bodies and few syncitia started showing up on the 5th day after inoculation. On the 7th day, 80% CPE was observed in the flasks, the virus was harvested and frozen. After about three cycles of freeze thawing, the resultant cell culture fluid was then clarified by centrifuging at low speed 1,500 rpm for 10 minutes. The supernatant fluids were then concentrated with 5ml of Polyethylene glycol (PEG) 6000 and spun at 4000 rpm for 60 minutes. The supernatants were decanted after which 1ml of PBS was added to each tube and contents of all the tubes were pooled together in a single 50ml centrifuge tube. The virus concentrate was further treated with 5µl of Tween 80 (Detergent) with vigorous shaking. Half volume of Ether was then added and held in the cold temperature for about 10 minutes. The treated virus was further centrifuged at 3000 rpm for 20 minutes. Nitrogen gas was then passed through the antigen solution to remove any residual ether, after which the measles antigen was carefully collected from the interphase. The antigens were aliquotted in cryovials (0.5ml each) and stored always at -20°C. The Harvested Antigens were later tested for Haemaglutination activity.

HAEMAGLUTINATION INHIBITION (HI) TESTS

Haemagglutination Assay: The haemagglutination test was carried out in 96 well V-bottom microtitre plates (Corning Inc. USA) using 50µl droppers and diluters. The assay was carried out in an end-point procedure. A two-fold serial dilutions was performed using our indigenous prepared HA antigen as well as commercial HA antigen starting from 1:10 to 1:2560. Fifty microlitre (50µl) of 0.4% suspension of washed monkey RBC were added to each well and the plates were incubated at room temperature for 1 h before readings were taken. The HA titre was recorded as the highest dilution of the antigen showing 50% haemagglutination. This was used to calculate the 4HA unit used for the HI test.

Pretreatment of Serum Samples: Fifty microlitre (50µl) of each serum samples were dispensed into labeled 1 ml ependorf tube. Fifty microlitre (50µl) PBS and 150µl (25% W/V) kaolin suspension was then added to each tube. Kaolins functions to remove non-specific inhibitors from the sera. The solution was mixed gently and incubated at room temperature for 20 minutes. This was then centrifuged at 1200 rpm for 5 minutes at 4°C.

Haemagglutination Inhibition: Rows of 96 well microtitre plates were numbered each according to the serial number of the test sera. 25µl of the diluent PBS was added to all the wells from the second to the twelfth wells of every row. 50ul of the Kaolin treated sera was added to the first wells of each row after which 25µl was taken out of the first wells and diluted down to the tenth wells of each row. The last 25µl was thrown away while wells 11 and 12 were left for RBC control. 25µl of the 4HA Measles antigen were added to the first down to tenth well of each row and incubated on bench for 1 hour for the Antigen/Antibody reaction to take place. After this, 25µl of 1% RBC indicator was added to all the wells including the PBC controls and incubated for another 1 hour on bench. The serum titre was expressed as the highest dilution of the serum showing complete inhibition of haemagglutination. Also, on a separate plate designated as control plate, Positive serum control and Back titration of the 4HA Antigen was done. 25µl of PBS was added to the wells 2-12 while 50µl of 4HA Antigen was added to well with 25µl of it taken and diluted down to 10. 1% monkey RBC was added as indicator. A serum known to have a titre of 1:320 was used in the Positive serum control. 25ul of the serum was added to wells 1 to 10 with wells 11-12 left for RBC control. 25ul of MV antigen (4HA) was added to wells 1 to 10 and incubated for 1 hour on bench after which 25ul of monkey RBC 1% was added to all the wells on the Positive control Row. It was incubated on bench for another 1 hour. The level of significance between variables were calculated using the Chi-Square Statistical Method

Specificity and Sensitivity Test: This was carried out with on the indigenous MV HA antigen using four known sera samples collected from previously unexposed and unvaccinated children as well as on five children with clinically manifested measles infection. The results were then read as positive or negative depending on the agglutination pattern.

RESULTS:

Measles virus was successfully isolated in B95[a] cell line from lymphocytes of two infected children. The titres of the MVs were 7.15 and 9.5 $TCID_{50}$. These B95[a] viruses were also successfully adapted to the Vero cell after 8 serial passages. The titre of the prepared batch 1 antigen was 1:640. This was higher than the titre 1:320 of the commercial antigen (Table 1). The second batch of prepared measles virus HA antigen gave a one fold drop in titre of 1:320 compared to batch 1 HA antigen. The commercial MV HA antigen remained 1:320 while the titre for the untreated measles virus stood at 1:10 (Table 1). The titre for the batch 3 antigens stood at 1:1280 while untreated virus titre was 1:10 as shown in Table 1. There was a remarkable increase in HA activity of the PEG/Tween 80 treated virus to untreated MV virus. The commercial antigen titre was 1:320, which is one dilution lower than the treated batch 3 viruses (Table 1).

Table 1: Batch 1, 2 and 3 Measles Virus HA Antigen HI Tests

TITRES	Batch 1 Indigenous MV HA 2	Commercial MV HA Antigen	Batch 2 Indigenous MV HA 2 Antigen	Untreated MV HA antigen	Commercial MV HA Antigen	Batch 3 Indigenous MV HA 2 Antigen	Untreated MV HA antigen	Commercial MV HA Antigen
1: 10	+	+	+	+	+	+	+	+
1: 20	+	+	+	-	+	+	-	+
1: 40	+	+	+	-	+	+	-	+
1: 80	+	+	+	-	+	+	-	+
1: 160	+	+	+	-	+	+	-	+
1: 320	+	+	+	-	+	+	-	+
1: 640	+	-	-	-	-	+	-	-
1:1280	-	-	-	-	-	+	-	-
1:2560	-	-	-	-	-	-	-	-

Key: + = Agglutination - = No Agglutination

The arithmetic mean of the 3 batches of indigenous measles HA antigen titre, the arithmetic mean of the titre of commercial HA antigen and the arithmetic mean of the titre untreated measles virus titre were determined and shown in Table 2. Table 2 also shows the specificity and the sensitivity of the PEG HA antigen using known sera samples.

Table 3 shows the HI for the field samples in the different categories of subjects used in this study. A total of 29 sera samples were tested for measles virus HI Antibody (27 children ages 7 months to 15 years and paired samples from an adult patient). Out of these 20 (69%) sera showed HI antibody of which 7 (24.1%) sera had titres 1: 32; 4 (13.8%) subjects had 1:64 while 2 (6.9%) subjects had 1:4 and 1:128 respectively (Table 3). This shows that the test antigen is highly specific being able to detect actual infections with wild measles virus. Four of the vaccinated subjects had a titre of 1:32 and 2 vaccinated subjects had a titre of 1:64. This shows the ability of the antigen to detect antibodies to both vaccine strain of measles virus. The sensitivity of our test antigen is seen in the fact that 5 (25%) subjects with no documented clinical history showed varying level of HI antibody titres with the highest being 1:8 and lowest 1:2. This shows that even when immune response is poor or in case of wrong diagnosis, our test antigen is sensitive enough to detect the HI antibodies even at a very low antibody titre.

Table 2: Mean, Specificity and Sensitivity of Measles Virus HA Antigens

	Indigenous MV HA Antigen	Commercial MV HA Antigen	Untreated MV HA Antigen
Mean	746.67	320	10
Specificity	1	1	1
Sensitivity	1	1	1

Table 3: Distribution Of Hi Antibody Titres among the Subjects Using Batch 1 PEG Treated Measles Virus HA Antigen

Titre	No Tested	No. Positive (%)	Known Negative Subjects	No Documented Clinical History of MV Infection	Subjects with Other Exanthematous Illness	Measles Vaccinated Subjects (%)	Recent Measles Infected Subjects (%)
1: 2	29	1 (3.5)	-	1	-	-	-
1: 4	29	2 (6.9)	-	2	-	-	-
1: 8	29	3 (10.3)	-	2	1	-	-
1: 16	29	1 (3.5)	-	-	1	-	-
1: 32	29	7 (24.1)	-	-	2	4	1
1: 64	29	4 (13.8)	-	-	-	2	2
1:128	29	2 (6.9)	-	-	-	-	2
1:256	29	-	-	-	-	-	-
Total	**29**	**20(69.0)**	**0**	**5 (25.0)**	**4 (20.0)**	**6 (30.0)**	**5 (25.0)**

The HI of the paired sera from an adult patient with recent measles virus infection showed a 4-fold rise in titre between acute phase sera (1:16) and convalescence phase sera (1:128) (Table 4). This further shows the specificity and efficacy of our PEG/Tween 89 concentrated MV HA antigen as a potential diagnostic agent.

Table 4: HI of an Adult Subject with Recent Measles Infection

Stage of Infection	HI Titre Using Batch 1 HA Antigen
Acute Phase	1.16
Convalescence Phase	1.128

DISCUSSION:

The Haemagglutinin inhibition (HI) test is a relatively easy and inexpensive test and is very useful in detecting antibodies against viruses that possess haemagglutinin surface glycoprotein such as in measles, rubella and influenza. It requires small amount of antigen and it has been found that 41 antibodies corresponding strongly to virus neutralization potential. HI antibody titres have been found to correlate with level of protection from wild type virus (Obi et al., 1996). Measles is endemic in Nigeria (Adu *et al.,* 1996) hence Nigerian children are expected to be born with a high level of maternally acquired antibodies which declines rapidly at age 6 months till the child begins to develop its own antibody at age 9 months (Babaniyi *et al.,* 1995).

Minimum protective antibody titres vary from one part of the world to the other due to differences in measles epidemiologic pattern in different regions (WHO, 1996), however, in an endemic area like Nigeria, the required minimum protective antibody titre is 1: 40 (Anon, 1995). In Nigeria, the measles virus immunization is at the age of 9 months. This is a one dose, one time programme and the children are expected to be protected through life. In a country like Nigeria where the MV is endemic and all the risk factors for measles outbreak are abundant, a one dose, one time vaccination programme may not be able to protect the child for life. In this work, attempt is therefore made to look at the MV immune status of secondary school children from ages between 10 and 23 years from three secondary schools in Ibadan irrespective of their vaccination status because it was very difficult to ascertain their vaccination records due to lack of proper record-keeping habit of Nigerians.

The HI test in also very useful in seroepidemiology and seromonitoring of important diseases such as measles and influenza. Over the years antigen used for measles HI diagnosis was being imported even till date. Various brands from various countries are prepared from circulating strains of measles virus peculiar to our own geographical region. Also, in most cases when there is an outbreak of measles, the time it takes for these HA antigens to be imported and sent to the laboratories for usage is very long, and laboratory investigations have to be delayed for this reason. Therefore, a potent measles HA antigen was prepared using an isolate obtained from an acutely infected patient with a local measles virus strain in Ibadan. Various workers have prepared potent measles HA antigens (Norrby *et al*., 1962; Filczak and Korbecki, 1978).

Three batches of measles HA antigens were prepared using PEG 6000 and Tween 80 treatment (Norrby and Enders, 1962; Filczak and Korbecki, 1978). PEG was found to increase HA activity following concentration at 80% in rubella virus and in measles virus (Norrby and Enders, 1962). Concentrated virus was further treated with Tween 80 and ether (Norrby and Enders, 1962).

Batch 1 HA antigen was tested for HA activity alongside with imported commercial measles HA antigen lot no. 733031. Batch 1 antigen titre was one fold higher than commercial antigen titre having 1:640 and 1:320 respectively. This high titre might be due to the fact that harvested tissue culture fluid was stabilized with 2% FCS before PEG concentration. Batch 2 HA antigen was not stabilized with 2% FCS before PEG concentration. Batch 2 HA antigens was one fold lower in titre (1:320) when compared to batch 1 HA antigen (1:640). This shows that the stabilizing effect of serum on pretreatment of measles virus haemagglutnin protein. It has also been demonstrated that ether treatment brings about degradation of virus particles, resulting in release of a free, small haemagglutinin (Norrby and Enders, 1962; Filczak and Korbecki, 1978). This could be seen in the high disparity between titres of untreated MV isolated with a titre of about 1:10 and Batch 2 MV HA antigen with a titre 1:320. The finding of this study also shows that the treatment with Tween 80 had a protective effect during ether treatment with a resultant increase in HA titre.

From the field test, 5 subjects with clinical history of measles tested positive with a low titre of 1:32 and 2 subjects with high titre of 1:128. When compared with the titre obtained in vaccinated subjects with titres 1:32 and 1:64 respectively, it can be deduced that HI antibody levels in vaccinated is lower than the minimum titre required for protection against infection of wild MV which is 1:88. The specificity and sensitivity of the indigenous prepared antigen was 1. This shows that this indigenous prepared MV HA antigen is very specific and sensitive in detecting antibodies to MV HA.

Another interesting discovery in this study is that 4 subjects having other exanthematous illness tested positive with titres ranging from 1:8 to 1:32. This

shows that these subjects were actually exposed to MV but lower titres were seen as a result of masking of measles infection by other infectious agents with similar etiology. This could explain why infections in many patients may remain silent or subclinical due to coinfection with other viral infections of similar etiology.

Paired sera samples of an adult patient with a recent measles infection showed an acute phase titre of 1:16 and convalescence titre of 1:128 with batch 1 MV HA antigen. This confirms the patients to be positive to MV infection. The finding of this study demonstrates the efficacy of PEG/Tween 80 treated MV HA antigen as a potential agent for MV antibody detection. This is of a significant practical impact because this is the first time an indigenously produced MV antigen to be prepared. This current study is important because the indigenously produced antigen prepared from our own local circulating wild MV strain will be very useful in the diagnosis of wild MV and serve a s a useful tool in the seromonitoring of vaccinated and unvaccinated children. It is therefore hoped that an extensive field trial across the geo-political zones will be carried out to further test the efficacy, specificity and sensitivity of our indigenous MV HA antigen. If this is successfully done, recommendations can be made to the Federal Ministry of Health on the use of our indigenous antigen for seromonitoring of measles susceptible children across the nation. Also, it can be further developed on an industrial scale which will attract more economic development and enhanced foreign exchange through exportation of this antigen to other neighbouring African countries endemic to MV.

It is also hoped that if the extensive survey is carried out with our indigenously produced antigen, the data generated will go a long way in assisting the success of the current measles eradication campaign. This test antigen, if put to judicious use will go a long way in assessing the success or failure of the current measles elimination campaign by showing seroconversion levels across the age groups of the children being immunized with the current measles vaccine.

In conclusion, HI antibody testing has long being the predominant assay for testing of neutralizing or protective level of immunity to MV in various subjects. In Nigeria, seroprevalence studies have been hampered because of the non-availability and high cost of imported HA antigen. The findings of this study are of a significant practical impact and confirm that an indigenous prepared antigen can give a higher titre than the commercially prepared antigens. Thus, if the indigenous prepared antigen is further refined and produced massively, it will be highly cost effective especially in field for seromonitoring and surveillance.

The PEG/Tween 80 concentration method of preparing a cheap and potent HA antigenic could also be employed in preparing HA antigen to other viruses that bear the haemagglutinin protein e.g Rubella and Influenza. If this can be achieved it will go a long way in buffering our public health campaigns against childhood killer diseases e.g. measles and other illness that could be life threatening e.g. Influenza A (H5N1). Therefore, Virology Laboratories should be equipped with adequate test facilities to monitor post vaccination seroconversion among subjects. Second round of supplementary immunization should be administered to vaccines. Their should be further investigation through extensive field trials with our indigenously prepared measles HA antigen for the sole purpose of utilizing if positive results are obtained as a sole diagnostic agent for measles seroprevalence studies. Organized private sector and government agencies involved in providing health. Care products e.g. pharmaceutical companies and biotech industries should be intimated about the potential usefulness of this MV HA antigen so as to enhance mass production of indigenous MV antigen for local utilization and export. The PEG/Tween 80 treatment should be employed in preparing other HA antigens from related viruses that utilize their haemagglutinating property e.g. influenza for production of local antigen for local use. The results obtained from the extensive field studies to be carried out, will serve as important epidemiological tool for our public health agencies.

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