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Quality assuranced of the recombinant hepatitis B vaccine produced at the GMP standard Hepatitis B Vaccine Plant, DMR (LM) (2003-2006)

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For effective control of hepatitis B viral infection in Myanmar, the GMP standard Hepatitis B (HB) Vaccine Plant was established in 2003. In this plant, the recombinant hepatitis B vaccine has been produced in compliance with the WHO GMP recommendations since 2004. The major purpose of applying for the WHO GMP is to achieve a high quality product. The quality of the product is individually or collectively influenced by a variety of factors such as men (personnel), machines, materials and methods used in manufacturing processes. In this descriptive study, quality assurance of the recombinant hepatitis B vaccine was assessed by monitoring the following systems in the plant; documentation, training, audit, preventive maintenance, calibration, validation, clean environment, raw material and product control, in-process control, change control and investigation of deviation and customer's complaint , and annual product review. It was found that the location, layout design and facilities of the plant building and premises were in accordance with WHO GMP recommendations. The standard organization set-up and effective quality management system had already been established. Production processes and quality control testing were regularly carried out by qualified and trained staff under strict aseptic technique in well controlled clean rooms by using established batch processing records and SOPs, standard materials and chemicals, and well calibrated and validated equipment. The starting raw materials, intermediate and finished products passed the specifications of quality control tests recommended by WHO. The results of the phase I and phase III clinical trials carried out on 17 adult volunteers and 134 neonates (target population) respectively showed the safety and 100 % immunogenicity of the vaccine. Therefore, recombinant HB vaccine, locally produced at the HB vaccine plant, Department of Medical Research (Lower Myanmar) has been proven to be a high quality product , and found to be totally safe, immunogenic and capable of protecting against HB viral infection. It is also considered to be the WHO GMP acceptable quality product for use in global immunization programme.

INTRODUCTION

Hepatitis B (HB) viral infection is an important health problem worldwide because the infection can cause significant liver diseases. Myanmar is hyperendemic for HB viral infection as research studies have shown that the HB carrier rate is 10-12 % and infection rate, 35-65 % [1, 2]. Recombinant hepatitis B(HB) vaccines using recombinant DNA technology entered the market and were registered at US FDA in 1986 [3]. In

Myanmar, the contract for the Hepatitis B Vaccine Plant Project under EDCF loan of US$ 12.6 million was signed in 2001 between (DMR, LM) and Samsung /CJ Corporation, Republic of Korea, for the development of yeast-derived recombinant hepatitis B vaccine in Myanmar. According to the contract, the supplier has to build a WHO Good Manufacturing Practice (GMP) standard HB Vaccine Plant scheduled to produce 5 million doses of yeast (*Hansenula polymorpha*) derived recombinant hepatitis B vaccine annually. The construction of the plant and test production of vaccines was completed in April 2003 and April 2004 respectively. All steps of manufacturing and quality control testing were carried out in accordance with WHO GMP recommendations. Clinical trials on adult volunteers and neonates were successfully carried out in 2005 and 2006 respectively, showing safety and immunogenicity in vaccine recipients. The major purpose of applying for the WHO GMP in this Plant is to achieve and ensure the high quality of the product. The quality of the product is individually or collectively influenced by four basic elements of GMP; men (personnel), machines, materials and methods used in the vaccine plant. These elements are controlled by the established quality management in the Plant. In this study, quality management system applied in the GMP standard DMR HB Vaccine Plant was systematically assessed with the aim of ensuring that the recombinant HB vaccine produced at the Plant is a high quality product acceptable by WHO for use in the global immunization programme.

MATERIALS AND METHODS

This is a descriptive study carried out at the GMP standard HB Vaccine Plant where recombinant HB vaccines are produced and controlled to the quality standard recommended by WHO GMP. The quality of vaccine is ensured by controlling the quality management system, starting from the receipt of raw materials, through in-process products to the release of finished products. In this study, quality assurance of the recombinant HB vaccine was assessed by monitoring the following systems in the Plant; documentation, training, audit, preventive maintainance, calibration, validation, clean environment, raw material and product control, in-process control, change control, investigation of deviation/customer's complaint and annual product review.

RESULTS

The DMR (LM) HB Vaccine Plant is located at the corner of No 7 Main Road and Kha Yay Pin Road, Hlegu Township, Yangon Division, 40 kilometers away from Yangon downtown area. Figure1. illustrates the lay-out design of the main building in the HB Vaccine Plant with having flow patterns of personnel, material, process, product, waste, and air pressure.

Educational qualification and specialties of the 20 scientists working in the Plant are shown in Table 1. Thirteen scientists (65%) hold post graduate degrees and 18 scientists (90%) had vaccine-related training abroad.

Table 1. Educational qualification and specialties of 20 scientists working in the Plant

Qualification / Specialities	No. of Scientists (%) n=20
Post graduate degree	13 (65%)
PhD	4 (20%)
MMed Sc/ MSc	9 (45%)
MBBS	12 (60%)
BSc	9 (45%)
Diploma	1 (20%)
Training abroad	18 (90%)
Service ≥ 15 years	15 (75%)
Medical Doctor	9 (45%)
Microbiologist	6 (30%)
Biochemist	2 (10%)
Molecular Biologist	1 (5%)
Pathologist	1 (5%)
Biologist	3 (15%)
Chemist	3 (15%)
Economist	1 (5%)
Engineer	4 (20 %)

Fig.1. Lay-out design of the main building in the HB Vaccine Plant, Hlegu Township

Table 2 shows the in-process control results of intermediate products and purified bulks of different production lots showing the consistency of the production processes.

Table 2. Consistency of in-process products and purified bulks in production process

	Lot no.			
OD of seed culture at 24 hours	R.02	R.03	R.04	R.05
Weight of cell cake after 120 hours Fermentation (gm/ litre)	15.9	14.93	15.59	15.25
Protein concentration of purified bulk (mg/ml)	137.6	140.8	138.8	130.0
	0.75	0.68	0.65	0.58

Table 3 depicts the results of the quality control testing of water for injection (WFI) and waste water eluted from waste water treatment system in the Plant. The results of WFI and waste water testing were found be within the expected specifications and acceptable for pharmaceutical use and safety to the environment respectively. Results of the Quality Control testing of the in-process and finished products are summarized in Table 4, passing the respective specifications recommended by WHO.

Table 3. Quality control testing of water for injection (WFI) and waste water

Test items	Specifications/ Results	Interval of testing
WFI		
pH	5 – 7	Daily
Conductivity	≤ 1.3µsm/cm at 25˚C	Daily
Total organic carbon(TOC)	≤ 500 PPB (µg/L)	Daily
Endotoxin	≤ 0.25 EU/ml	Daily
Sterility(Microbial count)	< 1 CFU/10ml	Daily
Waste water		
Temperature	< 40˚C	Daily
pH	6.5-7.5	Daily
Biochemical oxygen demand	< 40 mg/L	Every 4 month
Chemical oxygen demand	< 30 mg/L	/
Dissolved solids	< 15 mg/L	/
Suspended solids	< 1000 mg/L	/

Table 5 summarizes the findings of environmental monitoring performed in clean rooms of production areas. The results

measured were found to be within the specifications as recommended by WHO GMP.

DISCUSSION

The WHO defines Quality Assurance (QA) as a wide- ranging concept covering all matters that individually or collectively influence the quality of a product. It is the totality of the arrangement made with the object of ensuring that pharmaceutical products are of quality required for their intended use.

Table 4. Quality control testing of the in-process and finished products of recombinant HB vaccines

No.	Test items	Specification / results
	Purified Bulk / Final Bulk	
1	Protein content	The measured value
2	Polysaccharide content	Not more than 10 µg / 100 µg of Protein
3	Lipid content	Not more than 10 µg/100 µg of Protein
4	Pyrogenicity	The summed increase temp is not more than 1.3˚C
5	Sterility	No evidence of the presence of bacteria or fungi
6	Antigen content	The measured value
7	Purity	
	Tween 20 content	Not more than 50 µg/100 µg of Protein
	Bromide contents	Not more than 50 µg/100 µg of Protein
8	Host derived DNA	Not more than 100 ng/ dose
9	Identification(SDS PAGE)	Identified at about 24 KDa
	Finished Product	
1	Characteristics	Whites turbid solution
2	pH	5.9-6.9
3	Sterility	No evidence of the presence of bacteria and fungi
4	Abnormal toxicity	At least 80% of mice survive the test
5	Thimerosal content	0.007-0.012 w/v %
6	Protein contents	Not more than 40 µg/ml
7	Aluminum content	Not more than 1.25 mg/ml
8	Potency	The lower value of 95% confidence limit is not less than 1.0
9	Filled volume	Average and individual as specified
10	Leak test	No leak is detected
11	Foreign matter	No foreign matter is detected
12	Test for glass container	Not more than 2 ml
13	Pyrogenicity	The summed increase temp is not more than 1.3˚C

GMP is that part of QA which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization. The aim of GMP is to build quality into the product. QA therefore incorporates GMP and other factors such as those of Good Laboratory Practice (GLP) and Good Clinical Practice (GCP). The manufacture of biological products are undertaken in accordance with the basic principles of GMP [4, 5, 6, 7, 8]. In this study, regarding the location and design of the building, it is acceptable for GMP since the plant is not near to public housing, and operating rooms were used with materials of highest standard so that cleanliness can be easily performed and maintained.

Table 5. Environmental monitoring of clean rooms in production areas of the Plant

Types of clean room	Specifications/ Results		
	Class 100*	Class 10,000	Class 100,000
Test items			
Temperature	20˚- 22˚C	20˚- 22˚C	20˚- 22˚C
Humidity	50 ± 5 %	50 ± 15 %	50 ± 20 %
Pressured differential	1.2 -1.5 mm water	1.2 - 1.5 mm water	ND
Air velocity	0.3–0.45 m/sec	ND	ND
Air changes	300 –400 /hr	30 – 40 / hr	20 – 30 / hr
Air direction	Unidirectional & parallel	ND	ND
Lighting	100–150 foot candles	100	100
Noise level	< 60 decibel	< 60 decibel	< 60 decibel
Particle counts (of ≥ 0.5µm)	< 100/ cuft	< 10,000/ cuft	< 100,000/cuft
Microbial counts	< 1 CFU	< 5 CFU	< 50 CFU

* Environmental monitoring was carried out daily during production period and once a week at rest . ND = Not defined

Positive pressure rooms were used to process sterile products and negative pressure was used in septic rooms where infectious materials were handled. Heating, ventilation, air conditioning (HVAC) and lighting were designed to maintain a satisfactory temperature and relative humidity to minimize contamination and to take into account the comfort of the personnel working in protective clothing.

Regarding the organizational structure and the personnel, there were 3 separated divisions under the plant Director ; Production, Quality Control and Quality Assurance which are independent of each other in accordance with WHO GMP recommenddation.. There was also an adequate number i.e a total of 89 scientists and technicians who are aware of the principles of GMP. They are highly qualified with necessary training and practical experience. Their specific duties are recorded in written description and there are no gaps or unexplained overlaps in the responsibilities. During the year under study, 24 lectures on GMP and related topics were given regularly followed by evaluation tests at the Plant. Yearly routine medical check-up of all staff were also performed for detection of infectious diseases.

Daily cleaning of machines and equipment is done by operators at the Plant. Maintenance of a total of 261 equipment and machines is carried out by engineers from the Plant and by the Europ. Continents Co., Myanmar every 6 months. Calibration of 1020 gauges and displays is carried out by engineers from SEOHO Corporation, Republic of Korea once a year. Validation of 39 major equipment, processes, tests and systems including HVAC, water treatment system, Media fill test is performed by QA personnel from the Plant once a year. Equipment used for validation work is scheduled to be recalibrated once a year in the international standard substitute for calibration. Registries of all machines and equipment are also kept systematically in the documentation room as recommended by WHO GMP. Documentation is an essential part of QA system and is the key for operating the Plant in compliance with GMP requirements. No work procedure or tests are performed without established standard operating procedures (SOPs) and Batch Processing Records (BPRs) at the Plant. In addition, in-process quality control testing at every step of the vaccine production process starting from raw materials , through intermediate products to finished products are carried out to meet the specifications recommended by WHO [9]. The consistency of the results of production lots were observed in this study [10]. During the years under study, 249 SOPs, 12 BPRs, 16 certificates of analysis (COA), and considerable numbers of essential protocols, reports, results and records were prepared, checked and approved. These documents were systematically kept in the documentation room with limited access. A total of 6 internal GMP audit and 2 external GMP audit were carried out by QA personnel of the Plant and authorized persons of Myanmar FDA respectively during the study period. Any change regarding men (personnel), machines, materials and methods used in the plant were strictly controlled and recorded to prevent undesirable effects on product quality. Any deviation from normal processes or test results occurring during the vaccine manufacturing and testing procedures, were thoroughly investigated by QA personnel. The products were recommended to be passed or failed according to the findings. The plant also has an established SOP to investigate customers' complaint, followed by retesting of retention samples, report and product recall if and when necessary. Routine annual product reviews are carried out at the end of each year for documenting any changes and deviation, and for improvement of product quality and yields. The results of the phase I and phase III clinical trials carried out on 17 adult volunteers and 134 neonates (target population) respectively showed total safety and 100 % immunogenicity [11, 12].

Regarding the materials, all chemicals, reagents, buffers and glass vials used at the Vaccine Plant are analytical grade and purchased only from qualified and reputed suppliers. Before use in production process, all incoming materials are serially processed on receipt, lot-in, quarantine, sampling, testing followed by release or rejection. Finished products (vaccine vials) issued from the production division are also subjected to the process of quarantine,

sampling, testing, submission to FDA for lot release followed by ware-housing and distribution. In this study, it was found that there was a well established quality management system in the Plant, systematically and effectively conducted by the Quality Assurance team, controlling men, machines, materials and methods involved in the whole manufacturing process starting from the receipt of raw materials to the release of finished products (vaccines) to ensure high quality product. The production processes and quality control testing in this Plant are consistently carried out in well controlled clean rooms by qualified and trained personnel under strict aseptic technique, using established SOPs, BPRs, standard chemical and tested materials, and yearly calibrated and validated equipment appropriate to their specifications recommended by WHO GMP guidelines. The vaccine on clinical trial has been found to be totally safe, immunogenic and capable of protecting against HB viral infection. Therefore, recombinant HB vaccine locally produced at the HB Vaccine Plant, Department of Medical Research (LM) is recommended to be a high quality product, and it is also considered to be the WHO GMP acceptable quality product for use in the global immunization programme.

REFERENCES

1. Khin Maung Tin, TinHtut, Hla Myint &Tun Khin. Prevalence of hepatitis A and B in Burma. *Proceedings of the First Conference of Medical Specialities* 1981; 26-31.
2. Khin Pyone Kyi, Than Swe, Khin Mya Lwin, Kyin Yee Oo & Soe Lwin. Prevalence of Hepatitis B infection in health care personnl. *35th Burma Medical Conference, Abstracts* 1988.
3. Maynard JE. Hepatitis B: global importance and need for control. *Vaccine* 1990; 8: 18-20.
4. WHO Good Manufacturing Practices for pharmaceutical products; main principles. *WHO Technical Report Series* 2003; 908.
4. Quality Assurance of Pharmaceuticals. WHO Good Manufacturing Practices and Inspection. A Compendium of guidelines and related materials. 1999.
5. A WHO guide to Good Manufacturing Practice Requirements. Part 1. Standard operating procedures and master formulae. Department of vaccines and other biologicals WHO, Geneva 1999.
6. A WHO guide to Good Manufacturing Practice Requirements. Part 2. Validation Department of vaccinea and other biologicals WHO,. Geneva 2001.
7. Peine IC. Quality Assurance Compliance: Procedures for Pharmaceutical and Biotechnology Manufacturer, Interpharm Press, USA 1994.
8. Khin Pyone Kyi, Moh Moh Htun, Khin Khin Aye, Ti Kyi Win, Khin May Oo, Win Aung *et al.* Purification of Hepatitis B Surface Antigen Expressed in *Hansenula polymorpha* Master Cell Bank. *Myanmar Health Research Congress. Programme and Abstracts* 2005:20.
9. Moh Moh Htun, Khin Khin Aye, Ti Kyi Win, Phyu Phyu Khine, Kyin Kyin San, Win Maw Tun *et.al*. Analysis of cell growth of Recombinant HBsAg expressed Hansenula polymorpha and consistency of yicld of HBsAg protein in the different batches of Working Cell Banks. *Myanmar Health Research Congress. Programme and Abstracts* 2006; 45.
10. Myat Phone Kyaw, Kyaw Moe, Myo Khin, *et a*l. Evaluation in healthy adults, the safety of recombinant hepatitis B vaccine produced at hepatitis B Vaccine plant. Department of Medical Research (LM). *Myanmar Health Research Congress. Programme and Abstracts* 2006; 3.
11. Myat Phone Kyaw, Khin Pyone Kyi, Myo Khin, Moh Moh Htun, *et.al* Safety and Immunogenicity of DMR Recombinant Hepatitis B Vaccine. *Myanmar Health Research Congress. Programme and Abstracts* 2007; 6.