Annex 2

Recommendations to assure the quality, safety and efficacy of recombinant hepatitis E vaccines

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Recommendations published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes recommendations for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these WHO Recommendations are made only on condition that such modifications ensure that the product is at least as safe and efficacious as that prepared in accordance with the recommendations set out below. The parts of each section printed in small type are comments or examples intended to provide additional guidance to manufacturers and NRAs.
Abbreviations

ALT  alanine aminotransferase
DNA  deoxyribonucleic acid
ECBS  WHO Expert Committee on Biological Standardization
ELISA  enzyme-linked immunosorbent assay
GACVS  WHO Global Advisory Committee on Vaccine Safety
GLP  good laboratory practice(s)
HEV  hepatitis E virus
HIV  human immunodeficiency virus
HPLC  high-performance liquid chromatography
HPSEC  high-performance size-exclusion chromatography
IFA  immunofluorescence foci assay
IgG  immunoglobulin G
IgM  immunoglobulin M
LMIC  low- and middle-income countries
MALDI-TOF  matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MCB  master cell bank
NAT  nucleic acid amplification technique
NCL  national control laboratory
NRA  national regulatory authority
ORF2  open reading frame 2
PCR  polymerase chain reaction
RNA  ribonucleic acid
SAGE  WHO Strategic Advisory Group of Experts on Immunization
SDS-PAGE  sodium dodecyl sulfate polyacrylamide gel electrophoresis
TEM  transmission electron microscopy
ULN  upper limit of normal
WCB  working cell bank
Introduction

Hepatitis E virus (HEV) is a major cause of sporadic and epidemic hepatitis, and is found worldwide. The highest seroprevalence rates are observed in regions where low standards of sanitation increase the risk of virus transmission (1).

The WHO Strategic Advisory Group of Experts on Immunization (SAGE) issued a position paper in 2015 which reviewed existing evidence on the burden of hepatitis E and on the safety, immunogenicity, efficacy and cost-effectiveness of a hepatitis E vaccine that was first licensed in China (1). This vaccine contains the HEV open reading frame 2 (ORF2) capsid protein, corresponding to amino acids 368–606 of ORF2, manufactured in Escherichia coli using recombinant technology. The WHO Global Advisory Committee on Vaccine Safety (GACVS) had reviewed this same hepatitis E vaccine in 2014 and concluded that it had an acceptable safety profile (2). In 2016, WHO published its Global health sector strategy on viral hepatitis 2016–2021 (3), which addresses hepatitis A, B, C and E. Hepatitis E is probably the most neglected of the four. This strategy document highlights the urgent need to address all viral hepatitis, including hepatitis E for which only one vaccine is approved anywhere in the world and for which no effective therapies exist.

Following requests from manufacturers and other stakeholders for WHO to develop Recommendations to assure the quality, safety and efficacy of recombinant hepatitis E vaccines, a series of meetings was convened by WHO to review the current status of development and likely time to licensure of such vaccines (4). These meetings were attended by experts from around the world involved in the research, manufacture, regulatory assessment and approval, control-testing and release of hepatitis E vaccines. Participants were drawn from academia, national regulatory authorities (NRAs), national control laboratories (NCLs) and industry.

Purpose and scope

These WHO Recommendations provide guidance to NRAs and manufacturers on the manufacturing processes, and nonclinical and clinical evaluations, needed to assure the quality, safety and efficacy of recombinant hepatitis E vaccines.

The document encompasses recombinant hepatitis E vaccines for prophylactic use based on the ORF2 capsid protein.

The document should be read in conjunction with other relevant WHO guidance, especially on the nonclinical (5) and clinical (6) evaluation of vaccines. Other WHO guidance should also be considered, including – as appropriate to the vaccine – guidance on the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization
of cell banks (7) and on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (8).

Terminology

The definitions given below apply to the terms as used in these WHO Recommendations. These terms may have different meanings in other contexts.

**Adventitious agents:** contaminating microorganisms that may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses that have been unintentionally introduced into the manufacturing process.

**Cell bank:** a collection of containers containing aliquots of a suspension of cells from a single pool of uniform composition, stored frozen under defined conditions (typically −60 °C or below for yeast or bacteria and in liquid nitrogen for insect or mammalian cell lines). The terms master cell bank (MCB) and working cell bank (WCB) are used in these Recommendations. An MCB is a bank of a cell substrate representing a well-characterized collection of cells derived from a single tissue or cell, and from which all subsequent cell banks used for vaccine production will be derived. A WCB is a cell bank derived by propagation of cells from an MCB under defined conditions, and is used to initiate production of cell cultures on a lot-by-lot basis. The WCB is also referred to as a “manufacturer’s working cell bank” in other documents.

The individual containers (for example, ampoules or vials) should be representative of the pool of cells from which they are taken and should be frozen on the same day following the same procedure and using the same equipment and reagents.

**Cell substrate:** cells used to manufacture a biological product.

**Final bulk:** the formulated vaccine present in a container from which the final containers are filled. The final bulk may be prepared from one or more purified antigen bulks. Mixing should result in a uniform preparation to ensure that the final containers are homogenous.

**Final filled lot:** a collection of sealed final containers of finished vaccine that is homogeneous with respect to the risk of contamination during the filling process. All of the final containers must therefore have been filled from a single vessel of final bulk in one working session. Also referred to as “final lot” or “final product” in other documents.

**Purified antigen bulk:** the processed, purified antigen that has been prepared from either a single harvest or from a pool of single harvests. It is the parent material from which the final bulk is prepared.

**Recombinant DNA technology:** technology that joins together (that is, recombines) DNA segments from two or more different DNA molecules that are inserted into a host organism to produce new genetic combinations. It is also
referred to as “gene modification” or “genetic engineering” because the original gene is synthetically altered and changed. These new genes, when inserted into the expression system, form the basis for the production of recombinant DNA-derived protein(s).

Seed lot (master, working seed lot): a quantity of bacterial, viral or cell suspension that has been derived from one strain, has been processed as a single lot and has a uniform composition. It is used to prepare the inoculum for the production medium.

Single harvest: the biological material prepared from a single production run before further downstream processing.

Single harvest pool: a pool of a number of single harvests of the same virus type processed at the same time.

**General considerations**

**Hepatitis E virus**

HEV is a non-enveloped positive-sense RNA virus of the Hepeviridae family. The single-stranded viral genome is 7.2 kb in length and contains three open reading frames. Of these, ORF2 codes for the viral capsid protein which is the target of neutralizing antibodies against HEV (9). HEV isolates were classified into four human genotypes (genotypes 1–4) such that the nucleic acid variation of ORF2 between different genotypes is more than 20%; however, the four genotypes form a single serotype based on their immune reactivity. Different genotypes differ by more than 8.8% in highly conserved ORF1 and ORF2 amino acid sequences (10). The genotypes have been subdivided further into numerous subtypes – though the underlying criteria are controversial (11, 12). Nevertheless, HEV strains that infect humans belong to one currently identifiable serotype, with marked serological cross-reactivity as well as evidence for cross-protection in non-human primates and in humans (13). New genotypes of HEV (that is, genotypes 5–8), with limited information on their pathogenicity in humans and their cross-reactivity with human genotypes 1–4, were reported during the development of these WHO Recommendations (14).

**Epidemiology**

Almost all of the information available on the epidemiology of HEV concerns genotypes 1–4. HEV genotypes 1 and 2 primarily infect humans, whereas genotypes 3 and 4 mainly infect mammalian animals with occasional cross-species transmission to humans.

The epidemiology and clinical presentation of HEV infection vary greatly by geographical location, due primarily to differences in circulating HEV genotypes (15–17). A global burden of disease study estimated that in 2005 HEV
genotypes 1 and 2 accounted for approximately 20.1 million HEV infections, 3.4 million symptomatic cases, 70 000 deaths, and 3000 stillbirths (18).

Hepatitis E infection due to genotypes 1 and 2 has been identified in at least 63 countries, of which around half have reported large outbreaks (18). The overall burden of disease due to hepatitis E is greatest in low- and middle-income countries (LMIC), especially where clean drinking-water is scarce, as faecal contamination of drinking-water is a major route of HEV transmission (18). Although there is no evidence of large outbreaks of hepatitis E occurring in developed countries, small clusters of cases associated with foodborne transmission have occurred in Europe and Japan (19). There are also countries with no recorded sporadic disease or outbreaks but where serological evidence of past HEV infection has been reported, suggesting that HEV infection may be endemic.

Waterborne hepatitis E outbreaks have been reported from at least 30 countries on three continents – Africa, Asia and North America (Mexico) – and have been caused chiefly by HEV genotype 1. Large waterborne hepatitis E outbreaks frequently occur in the Indian subcontinent (20). In Australia, Europe and North America, cases due to genotype 1 have been reported in returning travellers. Determining the distribution of HEV genotype 2 has proved difficult, with most cases reported from Mexico, Namibia, Nigeria and several other West African countries. However, such infections seem rare with only a few cases reported to date (21–23).

In recent years, there have been numerous outbreaks caused by HEV genotype 1 in camps for internally displaced persons and refugees in Africa. There is some evidence that other modes of transmission (including from person to person) may contribute to the prolongation of outbreaks, particularly in displaced populations (24). Recent large outbreaks have occurred among displaced persons in Chad, Niger, Sudan and Uganda (21, 25–27). The first serologically confirmed outbreak documented in Africa occurred among Angolan refugees in Namibia in 1983. During a recent outbreak in northern Uganda, a high mortality rate was recorded among children under 2 years of age (25); however, the cause of death in these children was not verified. As was the case in northern Uganda, an outbreak in South Sudan also started during the rainy season, with high disease attack rates (7.4%) observed among camp residents, and high levels of mortality recorded among pregnant women (10.4%) (26). A sero-survey conducted during this outbreak showed that more than half of the camp residents had no evidence of current or past HEV infection, suggesting that these individuals remained uninfected. Both the Ugandan and South Sudanese outbreaks lasted well over a year, indicating that the implementing of prevention and control efforts during such outbreaks can be challenging.
Although waterborne hepatitis E outbreaks can result in large numbers of cases over a short period of time, most hepatitis E cases in LMIC probably occur within smaller clusters or result from sporadic transmission (28). The risk factors for sporadic hepatitis E are less well understood, although water contamination may play a role.

In developed countries, where the hepatitis E disease burden is much lower, zoonotic transmission, primarily through the consumption of uncooked or undercooked meat, is a potential mode of transmission; with HEV genotype 3 being the predominant genotype (16). Despite the ubiquity of HEV genotype 3 in the domestic pig population, clinically apparent human infections with this genotype have been reported almost entirely in developed countries.

In recent years, HEV genotype 4 has been found to circulate in animals in China, India and several European countries; with most human cases of hepatitis due to HEV genotype 4 having been reported in China. The main mode of transmission of HEV genotype 4 is also believed to be the consumption of infected pork and contact with domestic pigs.

There is no evidence of the sexual transmission of HEV (16). Although transfusion-associated HEV transmission occurs and is well documented, its contribution to the overall disease burden is limited (16, 29, 30).

**Disease and diagnosis**

HEV-infected individuals exhibit a wide clinical spectrum, ranging from asymptomatic infection through acute icteric hepatitis to fulminant hepatitis. Asymptomatic infection is common in immunocompetent individuals and the disease presentation is often mild. The ratio of symptomatic to asymptomatic infection has been estimated to range from 1:2 to 1:10 or more in outbreak settings and may be dependent on age at infection. HEV infection occurs in children and the probability of symptomatic disease increases with age (1, 31). The incubation period ranges from 15 to 60 days, with a mean of 40 days (32).

Although infection with HEV genotype 1 is associated with serious disease more often than infection with other genotypes, the extent to which such severe disease occurs with genotypes 2 and 4 is not well documented. Studies in non-human primates have shown a relationship between the dose of viral inoculum and the host’s immunological response and degree of liver injury (33).

In LMIC, where HEV genotypes 1 and, to a lesser extent, 2 are the most commonly identified causes of hepatitis E, the disease mainly affects young adults (for example, those aged 15–39 years), with a preponderance in males. During waterborne outbreaks, children may develop severe hepatitis E due to coinfection with hepatitis A virus (34).

Fulminating hepatitis E occurs at a disproportionately high rate among pregnant women during epidemics (35–37), the disease being typically most
severe during the third trimester of pregnancy (38, 39). While mortality from hepatitis E ranges from 0.1% to 4% in the general population, it can range from 10% to 50% among women in the third trimester of pregnancy (1, 38, 40, 41). The mechanism underlying the high mortality rate among pregnant women is unclear (31). Causes of death include fulminant liver failure and obstetric complications, including excessive bleeding (36). HEV can be transmitted from mother to fetus during pregnancy, resulting in poor fetal outcomes that include miscarriage, premature delivery and stillbirths (20, 35).

HEV genotypes 3 and 4 have been repeatedly reported to cause severe disease as well as chronic hepatitis E in immunocompromised people in China and Europe. Chronic infections do not occur in otherwise healthy individuals. HEV infection in those who receive immunosuppressive treatment following solid organ or bone marrow transplantation, and in those with severe immunodeficiency of other origins, is associated with risk of progression to chronic hepatitis E (42). HIV-infected patients are not at higher risk for HEV infection; the number of acute infections reported in these populations is low and very few chronic cases have been reported (43–45). The clinical manifestation and progression of chronic hepatitis E (lasting > 6 months) are variable with some cases progressing to significant fibrosis in a relatively short period of time.

Recently, a single case of chronic infection with camelid HEV (which is HEV genotype 7) was reported in a patient who had undergone liver transplantation and who regularly consumed camel meat and milk – suggesting that this genotype might infect humans via foodborne zoonotic transmission. It was reported that immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against new HEV genotypes can be detected by HEV genotype 1 antigen (46).

Individuals with pre-existing chronic liver disease are prone to developing severe hepatitis following HEV infection. Those with advanced liver disease, including cirrhosis, may develop acute hepatic failure when infected with HEV (20). The burden of HEV-induced acute liver failure in patients with pre-existing chronic liver disease is unknown.

Laboratory diagnosis of recent HEV infection is based on the detection of HEV-specific IgM antibodies, the recent appearance or several-fold increase in titres of specific IgG antibodies or the detection of HEV RNA in blood samples (47). Specific detection of HEV antigen can also be a marker for the diagnosis of hepatitis E (48). However, the performance characteristics (sensitivity and specificity) of some currently available commercial assays for anti-HEV antibodies are suboptimal (49–55). In one study that compared six different assays the sensitivity of the individual assays ranged from 72% to 98%, and specificity from 78% to 96%; furthermore, the kappa coefficients for agreement between the results of various pairs of tests varied from 0.42 to 0.80 (56). This
has implications for clinical trials based on serological outcomes and for studies that rely on these serological tests to estimate the burden of disease and previous history of infection. One recent study compared the results obtained using a newer diagnostic assay to the results obtained from the assay used in the original study of seroprevalence in rural Bangladesh and found that the newer assay showed much higher seroprevalence in the population (57).

**Immune response to natural HEV infection**

Past HEV infection is characterized by the presence of serum IgG antibodies directed against the viral capsid protein, which may confer protection against reinfection. However, the protective IgG antibody concentration is not known and the duration of protection following natural infection is uncertain. In Kashmir, serological follow-up of 45 individuals known to have had hepatitis E during the 1978 outbreak found that 47% had detectable anti-HEV IgG 14 years after infection (58) – though the difficulties in interpreting serological assay results mentioned above should be noted. A recent study based on 67 months of serological follow-up data and mathematical modelling suggested that naturally acquired anti-HEV IgG will remain detectable in half of seropositive individuals for 14.5 years (59). In another follow-up study, 100% of people had measurable anti-HEV IgG 5 years after infection (60). However, the subjects studied were living in hyperendemic areas where the possibility of multiple re-exposures and natural boosting cannot be ruled out.

There is some evidence that naturally acquired HEV infection does not confer lifelong immunity. For example, even in endemic areas, the prevalence of anti-HEV IgG in the population does not reach the very high levels observed for hepatitis A which does confer lifelong protection, and attack rates are highest among young to middle-aged adults, suggesting that infection during early life may not confer lifelong protection, or that infections usually occur later in life. In addition, outbreaks recur in countries where previous epidemics would be expected to have resulted in a level of population immunity sufficient to prevent future outbreaks. The duration of protection conferred by naturally acquired antibodies has important implications for long-term vaccine efficacy.

**Vaccines against HEV**

Although many experimental hepatitis E vaccines have been evaluated in virus challenge studies in non-human primates, other animal models or clinical trials, only one vaccine has been licensed for human use as of mid-2018. This vaccine was licensed in China in December 2011 for use in people aged 16 years and over. It is based on a 239-amino-acid recombinant HEV peptide, corresponding to amino acids 368–606 of ORF2 which encodes the capsid protein of genotype 1
HEV (61–64). Vaccine efficacy after three doses was 100% over a 12-month period after the last dose, and 95.5% over 19 months in all subjects who had received at least one dose. Other vaccines based on the HEV capsid protein are currently in nonclinical or clinical development.

**International reference materials**

Subsequent sections of this document refer to WHO reference materials that may be used in laboratory or clinical evaluations. Key standards used in the control of hepatitis E vaccines include the following:

- A WHO reference preparation for antibodies to hepatitis E virus is available for the standardization of diagnostic tests for use in seroprevalence studies and for assessing immunity. This WHO interim Reference Reagent for hepatitis E virus antibody, human serum (code 95/584) was established by the WHO Expert Committee on Biological Standardization (ECBS) in 1997 and was assigned a unitage of 50 units/ampoule (65). The preparation is held and distributed by the National Institute for Biological Standards and Control (NIBSC), Potters Bar, the United Kingdom.

- WHO international reference preparations for hepatitis E virus RNA are also available. These standards are suitable for the calibration of in-house or working standards for use in the amplification and detection of hepatitis E virus RNA. The First WHO International Standard for hepatitis E virus RNA for NAT-based assays was established by the ECBS in 2011 and was assigned a unitage of 250 000 IU/ml (66). The First WHO International Reference Panel for hepatitis E virus genotypes for NAT-based assays (code 8578/13) contains 11 members and was established by the ECBS in 2015 (67). These two preparations are held and distributed by the Paul-Ehrlich-Institut (PEI), Langen, Germany.

- A product-specific national reference for use in potency assays is under development by the National Institutes for Food and Drug Control (NIFDC), China.

The WHO Catalogue of International Reference Preparations should be consulted for the latest list of appropriate WHO international standards and other reference materials. See: http://www.who.int/bloodproducts/catalogue/en/.
Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name should be “recombinant hepatitis E vaccine”. The proper name should be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that meet the specifications elaborated below.

A.1.2 Descriptive definition

The recombinant hepatitis E vaccine is a sterile liquid vaccine preparation that contains purified recombinant capsid protein of hepatitis E virus. The protein may be formulated with a suitable adjuvant. Such vaccines are for prophylactic use.

A.2 General manufacturing recommendations

The general manufacturing recommendations contained in WHO good manufacturing practices for pharmaceutical products: main principles (68) and WHO good manufacturing practices for biological products (69) should apply to the design, establishment, operation, control and maintenance of manufacturing facilities for recombinant hepatitis E vaccines. The addition of any excipient should be justified, including preservative.

A.3 Control of source materials

A.3.1 Cells for antigen production

The use of any type of cell should be based on a cell bank system (7, 70) and should be approved by, and registered with, the NRA. The maximum allowable number of passages or population doublings from the MCB to production level should be approved by the NRA.

A.3.1.1 Recombinant cells for production

The history and characteristics of the parental cells, including bacteria or eukaryotic cells if relevant, should be fully described. The recombinant vaccine production strain (parental cell transformed with the recombinant expression construct) should be fully described and information should be given on the results of any adventitious agent testing required, and on the homogeneity and accuracy of the inserted sequence (including copy number per cell) for the MCB and WCB. Plasmid retention should be demonstrated as part of process validation. A full description of the biological characteristics of the host cell and expression strategy should be given. This should include genetic markers of
the host cell, the construction, genetics and structure of the expression system, induction method, DNA sequencing of the insert and the origin and identification of the HEV sequence that is being cloned. The complete sequence of the entire construct should be determined, including control elements, and should be provided as part of the validation of the production process. The molecular and physiological measures used to promote and control the expression of the cloned HEV sequence in the host cell should be described in detail (71).

Cells must be maintained in a state that allows for recovery of viable cells without alteration of genotype (for example, frozen in liquid nitrogen). The cells should be recovered if necessary in selective media so that the genotype and phenotype are maintained and clearly identifiable. Cell banks should be identified and fully characterized using appropriate tests.

Data (for example, on plasmid restriction enzyme mapping, nutritional requirements or antibiotic resistance, if applicable) that demonstrate the genetic stability of the expression system during passage of the recombinant WCB up to and beyond the passage level used for production should be provided to, and approved by, the NRA as part of the validation of the production process. Any instability of the expression system occurring in the seed culture or after a production-scale run should be documented. Stability studies should also be performed to confirm cell viability, maintenance of the expression system etc. after retrieval from storage. These studies may be performed as part of their routine use in production or may involve samples being taken specifically for this purpose.

A.3.1.1.1 Tests on recombinant bacteria MCB and WCB

MCBs and WCBs (bacterial expression system) used for production should be tested to demonstrate that only the bacterial production strain is present in the MCB and WCB, and that contaminating bacteria and fungi are absent.

A.3.1.2 Other expression systems

If expression systems other than bacterial systems are used, characterization may be based on the WHO Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines (72), Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7) and Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (71).

MCBs and WCBs (animal cell culture system) used for production should be tested for the absence of bacterial, fungal and mycoplasmal contamination by appropriate tests, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (73) and its 1995 amendment for mycoplasma (74).
A.4 Control of HEV protein production

A.4.1 Microbial purity

The microbial purity of recombinant bacterial cultures should be monitored in each fermentation vessel at the end of the production run by methods approved by the NRA.

Any agent added to the fermenter or bioreactor with the intention of feeding cells or of inducing production or increasing cell density should be approved by the NRA. No antibiotics should be added at any stage of manufacturing unless approved by the NRA.

A.4.2 Control of single harvests

A.4.2.1 Storage and intermediate hold times

After the production run, the cell suspension or the product partially purified from it (for example, by preparation of inclusion bodies) should be maintained under conditions shown by the manufacturer to retain the desired biological activity. Hold times should be approved by the NRA.

A.4.2.2 Tests on single harvest or single harvest pool

If appropriate, tests may be conducted on a single antigen harvest or on a pool of single antigen harvests depending on the production strategy. The protocol should be approved by the NRA.

A.4.2.2.1 Sampling

Samples required for the testing of antigen harvests should be taken immediately on harvesting or pooling and before further processing. Tests for sterility and adventitious agents, as described below in sections A.4.2.2.2 and A.4.2.2.4 respectively, should preferably be performed within 24 hours. If these tests are not performed within 24 hours, the samples taken for these tests should be stored at an appropriate temperature. Where mammalian or insect cell expression systems are used, samples should be stored at −60 °C and subjected to no more than one freeze–thaw cycle. For other systems in which the infectivity of adventitious agents will not need to be preserved, an appropriate temperature should be chosen. Moreover, evidence should be provided that the freezing process does not affect the viability of the adventitious agents putatively present in the sample.

A.4.2.2.2 Tests for bacteria, fungi and mycoplasmas

Harvests from bacterial expression systems could have bacterial contamination. Therefore, a method such as the microbial limits test may be appropriate for addressing microbial purity. Such testing should be approved by the NRA.
For non-bacterial production systems, each single antigen harvest or single harvest pool should be shown to be free from bacterial, fungal and mycoplasmal contamination by appropriate tests, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (73) and its 1995 amendment for mycoplasma (74).

A.4.2.2.3 Test for identity

Each harvest should be identified as HEV antigen by a suitable assay such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), enzyme-linked immunosorbent assay (ELISA) or other methods. The tests should be approved by the NRA. Alternatively, the identity can be confirmed as part of testing of the purified antigen.

A.4.2.2.4 Tests for adventitious agents if insect or mammalian cells are used in production

Each single harvest or single harvest pool should be tested for adventitious viruses in cell cultures selected for their appropriateness to the origin and passage history of the insect cell substrate and recombinant baculovirus or the mammalian cell substrate. These cell cultures should include, as a minimum, a monkey kidney cell line and a human cell line. Antisera used for the purpose of neutralizing the recombinant baculovirus should be free from antibodies that may neutralize adventitious viruses and should preferably be generated by the immunization of specific-pathogen-free animals with an antigen made from a source (other than the production cell line) which has itself been tested for freedom from adventitious agents. The inoculated indicator cells should be examined microscopically for cytopathic changes. At the end of the examination period, the cells should also be tested for haemadsorbing viruses.

Additional testing for specific adventitious viruses may be performed (for example, using polymerase chain reaction (PCR) amplification techniques).

A.5 Control of purified antigen bulk

The purification process can be applied to a single antigen harvest, part of a single antigen harvest or a pool of single antigen harvests, and should be approved by the NRA. The maximum number of harvests that may be pooled should also be approved by the NRA. Adequate purification may require several purification steps based on different biophysical and/or biochemical principles and may involve disassembly and reassembly of particles. The entire process (sequence of process steps) used for the purification of the final antigen bulk should be appropriately validated and should be approved by the NRA. Any reagents added during the purification processes (such as DNase) should be documented and their removal adequately validated and tested for, as appropriate (see section A.5.1.7).
The purified antigen bulk can be stored under conditions shown by the manufacturer to allow it to retain the critical quality attributes. Intermediate hold times should be approved by the NRA.

### A.5.1 Tests on the purified antigen bulk

Purified antigen bulks should be subjected to the tests listed below. Some tests may be omitted if performed on the adsorbed antigen bulk. All quality control release tests and specifications for purified antigen bulk, unless otherwise specified, should be validated by the manufacturer and approved by the NRA.

#### A.5.1.1 Purity

The degree of purity of the purified antigen bulk, and levels of residual host-cell protein and DNA, should be assessed by suitable methods. One suitable method for analysing the proportion of potential contaminating proteins is SDS-PAGE under reducing denaturing conditions. The protein bands within the gel should be identified by sensitive staining techniques and quantified by densitometric analysis. Other suitable methods such as high-performance liquid chromatography (HPLC) may also be used for purity analysis.

#### A.5.1.2 Protein content

Each purified antigen bulk should be tested for total protein content using a suitable method.

The total protein content may be calculated from measurement of an earlier purification process intermediate.

#### A.5.1.3 Antigen content

The antigen content should be measured on the purified antigen bulk or the adsorbed antigen bulk (see section A.6.3.7) using an appropriate method. The ratio of antigen content to protein content may be calculated and monitored for each purified antigen bulk.

International standards and reference reagents are not available for the control of hepatitis E vaccine antigen content. Therefore, product-specific reference preparations should be developed and used.

#### A.5.1.4 Sterility tests for bacteria and fungi

Each purified antigen bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (73), or by a method approved by the NRA. Alternatively, this test can be performed on the related adsorbed antigen bulks if the purified bulk is not stored prior to adsorption.
A.5.1.5 **Percentage of intact monomer**

The integrity of the HEV protein should be carefully monitored at least in the early stages of process validation and should be assessed by suitable methods. The purity assay (see section A.5.1.1) may also serve to assess the integrity of the HEV protein monomer. This test could be omitted, subject to the agreement of the NRA.

A.5.1.6 **Particle size and morphology**

The protein is expected to form particles of heterogeneous size; the size and morphology of the particles should be assessed and monitored. The distribution of particle sizes should be determined as a parameter of process control. This test may be omitted once consistency of production has been established, with the agreement of the NRA.

Suitable methods for assessing particle size include dynamic light scattering, size-exclusion chromatography–high-performance liquid chromatography (SEC–HPLC) and transmission electron microscopy (TEM). A reference preparation should be included for comparison.

A.5.1.7 **Tests for reagents used during purification or other phases of manufacture**

A test should be carried out to detect the presence of any potentially hazardous reagents used during manufacture, using a method(s) approved by the NRA. This test may be omitted upon demonstration that the process consistently eliminates the reagent from the purified antigen bulks, subject to the agreement of the NRA.

A.5.1.8 **Tests for residual host-derived material**

Where a eukaryotic expression system is used, the amount of residual host-cell DNA derived from the expression system should be determined in each purified antigen bulk using suitably sensitive methods. The level of host-cell DNA should not exceed the maximum level agreed with the NRA, taking into consideration issues such as those discussed in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7).

These tests may be omitted upon demonstration that the process consistently inactivates the biological activity of the residual DNA or reduces the amount and size of the contaminating residual DNA present in the purified antigen bulks, subject to the agreement of the NRA.

Levels of residual protein from the host cell should be determined for all expression systems.
A.5.1.9  **Test for viral clearance**

When an insect or mammalian cell substrate is used for the production of antigens, the production process should be validated in terms of its capacity to remove and/or inactivate adventitious viruses – as described in the Q5A guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (75). This testing is performed during vaccine manufacturing development or as part of process validation.

If a replicating viral vector such as a baculovirus is used, the production process should be validated for its capacity to eliminate (by removal and/or inactivation) residual recombinant virus.

A.6  **Control of adsorbed antigen bulk**

In cases where the adsorbed antigen bulk is further modified by dilution or addition of excipients to generate the final bulk, the considerations described below apply. Where the adsorbed bulk is filled directly without further modification it is the final bulk and this section does not apply (see instead section A.7).

A.6.1  **Addition of adjuvant**

The purified HEV antigen may be adsorbed onto an adjuvant such as an aluminium salt or other substance. Both the adjuvant and the concentration used should be approved by the NRA.

A.6.2  **Storage**

Until the adjuvanted antigen bulk is formulated into the final bulk, the suspension should be stored under conditions shown by the manufacturer to allow it to retain the desired biological activity. Hold times should be approved by the NRA.

A.6.3  **Tests on adsorbed antigen bulk**

All tests and specifications for adsorbed antigen bulk, unless otherwise specified, should be approved by the NRA.

A.6.3.1  **Sterility tests for bacteria and fungi**

Each adsorbed antigen bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (73), or using a method approved by the NRA.

A.6.3.2  **Bacterial endotoxins**

Each adsorbed antigen bulk should be tested for bacterial endotoxins using a method approved by the NRA.
If it is inappropriate to test the adsorbed antigen bulk, the test should be performed on the purified antigen bulk prior to adsorption, subject to the agreement of the NRA.

A.6.3.3  **Identity**  
Each adsorbed antigen bulk should be identified as the appropriate HEV antigen using a suitable method. The test for antigen content may also serve as the identity test. This test may be omitted if it is performed on the finished product.

A.6.3.4  **Adjuvant concentration**  
Adsorbed antigen bulk should be assayed for adjuvant content.

A.6.3.5  **Degree of adsorption**  
The degree of adsorption (completeness of adsorption) of the antigen to the adjuvant should be assessed, if applicable. This test may be omitted upon demonstration of process consistency, subject to the agreement of the NRA.

A.6.3.6  **pH**  
The pH value of the adsorbed antigen bulk should be monitored until production consistency is demonstrated, subject to the agreement of the NRA.

A.6.3.7  **Antigen content**  
The antigen content of the adsorbed antigen bulk should be measured using appropriate methods. If this test is conducted on the purified antigen bulk, it may be omitted from the testing of the adsorbed antigen bulk. International standards and reference reagents are not available for the control of hepatitis E vaccine antigen content. Therefore, product-specific reference preparations should be developed and used.

A.7  **Control of final bulk**  
The antigen concentration in the final formulation should be sufficient to ensure that the dose is consistent with that shown to be safe and effective in human clinical trials. Should an adjuvant be added to the vaccine formulation, the adjuvant and the concentration used should be approved by the NRA.

The operations necessary for preparing the final bulk should be conducted in such a way as to avoid contamination of the product. In preparing the final bulk vaccine, any substances that are added to the product (such as diluents, stabilizers or adjuvants) should have been shown to the satisfaction of the NRA not to impair the safety and efficacy of the vaccine at the concentration used.
The final bulk should be stored under conditions shown by the manufacturer to allow it to retain the desired biological activity until it is filled into containers.

A.7.1 **Tests on the final bulk**

All tests and specifications for the final bulk should be approved by the NRA, unless otherwise specified. Where the antigen bulk is the final formulation, the tests below will be performed in accordance with section A.6 above at the level of the adsorbed antigen bulk and not repeated here.

A.7.1.1 **Sterility tests for bacteria and fungi**

Each final bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (73), or using a method approved by the NRA.

A.7.1.2 **Adjuvant content**

Each final bulk should be assayed for adjuvant content.

Where aluminium compounds are used, the aluminium content should not exceed 1.25 mg per single human vaccine dose.

Tests for adjuvant content on the final bulk may be omitted if conducted on each final lot derived from the final bulk.

A.7.1.3 **Degree of adsorption**

The degree of adsorption (completeness of adsorption) of the antigen to the adjuvant in each final bulk should be assessed, if applicable, (for example, if the adjuvant is aluminium salts).

This test may be omitted upon demonstration of process consistency subject to the agreement of the NRA, or if performed on the final lot.

A.7.1.4 **Preservative content**

The final bulk should be tested for the presence of preservative, if added. The method used and the permitted concentration should be approved by the NRA.

A.7.1.5 **Potency**

The potency of each formulated final bulk before filling should be assessed by an appropriate in vivo method. If an in vivo potency test is used to test final fill lots, this test may be omitted on the formulated final bulk before filling. The methods used for antibody detection in the in vivo test and for the analysis of data should be approved by the NRA. The vaccine potency should be compared with that of a reference preparation approved by the NRA.
In vitro methods such as ELISA may be developed to assess potency. With the approval of the NRA, the in vitro assay may replace the in vivo assay when appropriately validated and when consistency of production is demonstrated.

Manufacturers should establish a product-specific reference preparation that is traceable to a specific lot of vaccine, or to bulks used in the production of a specific lot, which has been shown to be efficacious in clinical trials. The performance of this reference vaccine should be monitored by trend analysis using relevant test parameters and the reference vaccine should be replaced when necessary. An established procedure for replacing reference vaccines should be in place (76, 77).

A.7.1.6 Osmolality
The osmolality of the final bulk should be tested. The osmolality test may be omitted if performed on the final lot.

An alternative test (for example, freezing point) may be used as a surrogate measure for ionic strength/osmolality.

A.8 Filling and containers
The requirements concerning filling and containers given in WHO good manufacturing practices for pharmaceutical products: main principles (68) and WHO good manufacturing practices for biological products (69) should apply to vaccine filled in the final form.

Care should be taken to ensure that the materials of which the container and, if applicable, the transference devices and closure are made do not adversely affect the quality of the vaccine.

Manufacturers should provide the NRA with adequate data to prove the stability of the product under appropriate conditions of storage and shipping.

A.9 Control tests on the final filled lot
The following tests should be performed on each final filled lot (that is, in the final containers). Unless otherwise justified and authorized, the tests should be performed on labelled containers from each final filled lot by means of validated methods approved by the NRA. All tests and specifications, including methods used and permitted concentrations, should be approved by the NRA, unless otherwise specified.

A.9.1 Inspection of containers
Every container in each final lot should be inspected visually or mechanically, and those showing abnormalities (for example, improper sealing, clumping or presence of particles) should be discarded and recorded for each relevant
abnormality. A limit should be established for the percentage of containers rejected to trigger investigation of the cause, potentially resulting in batch failure.

A.9.2 Appearance
The appearance of the vaccine should be described with respect to its form and colour.

A.9.3 Identity
An identity test should be performed on at least one container from each final lot, using a validated method approved by the NRA. The potency test may serve as the identity test.

A.9.4 Sterility tests for bacteria and fungi
Each final lot should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (73), or using a method approved by the NRA.

A.9.5 pH and osmolality
The pH value and osmolality of the final lot should be tested. The osmolality test may be omitted if performed on the final bulk. The osmolality test may also be omitted for routine lot release upon demonstration of product consistency, subject to the agreement of the NRA.

An alternative test (for example, freezing point) may be used as a surrogate measure for ionic strength/osmolality.

A.9.6 Preservatives
Each final lot should be tested for the presence of preservative, if added.

A.9.7 Test for pyrogenic substances
Each final lot should be tested for pyrogenic substances. Where appropriate, tests for endotoxin – for example, the limulus amoebocyte lysate (LAL) test – should be performed. However, where there is interference in the test (for example, from the adjuvant) a test for pyrogens in rabbits should be performed.

A suitably validated monocyte-activation test may also be considered as an alternative to the rabbit pyrogen test.

The test is conducted until consistency of production is demonstrated, subject to the agreement of the NRA.
A.9.8  **Adjuvant content**
Each final lot should be assayed for adjuvant content, if applicable. Where aluminium compounds are used, the aluminium content should not exceed 1.25 mg per single human vaccine dose.

A.9.9  **Extractable volume**
For vaccines filled into single-dose containers, the extractable content should be checked and shown to be not less than the intended dose.

For vaccines filled into multi-dose containers, the extractable content should be checked and should be shown to be sufficient for the intended number of doses.

A.9.10  **Degree of adsorption**
The degree of adsorption to the adjuvant (completeness of adsorption) of each antigen present in each final vaccine lot should be assessed, if applicable, and the limit should be approved by the NRA.

This test may be omitted for routine lot release upon demonstration of product consistency, subject to the agreement of the NRA.

A.9.11  **Potency**
A potency test should be carried out on each final lot as outlined above in section A.7.1.5. However, if the in vivo potency test has been performed on the final formulated bulk, the test on the final lot may be omitted, subject to the agreement of the NRA.

A.10  **Records**
The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles (68) and WHO good manufacturing practices for biological products (69) should apply.

A.11  **Retained samples**
The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles (68) and WHO good manufacturing practices for biological products (69) should apply.

A.12  **Labelling**
The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles (68) and WHO good manufacturing practices for biological products (69) should apply. In the case of recombinant hepatitis E
vaccines, the label on the carton, the container or the leaflet accompanying the container should state:

- that the vaccine has been prepared from recombinant bacterial cells, or another expression system;
- the genotype of the HEV antigen present in the preparation;
- the protein/antigen content and potency per dose;
- the number of doses, if the product is issued in a multi-dose container;
- the name and maximum quantity of any residual antibiotic present in the vaccine;
- the name and concentration of any preservative added;
- the name and concentration of any adjuvant added;
- the name and concentration of any other excipient added;
- the temperature recommended during storage and transport;
- the expiry date;
- the name of the manufacturer;
- the lot/batch number; and
- any special dosing schedules.

A.13 Distribution and transport

The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles (68) and WHO good manufacturing practices for biological products (69) should apply. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (78).

A.14 Stability testing, storage and expiry date

A.14.1 Stability testing

Adequate stability studies form an essential part of vaccine development. Current guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines (79). Stability testing should be performed at different stages of production – namely on single antigen harvests or single harvest pools, purified antigen bulk, final bulk (whenever materials are stored before further processing) and final lot. Stability-indicating parameters appropriate to the stage of production should be defined or selected. A shelf-life should be assigned to all in-process materials during vaccine production –
particularly intermediates such as single antigen harvests, purified antigen bulk and final bulk.

The stability and expiry date of the vaccine in its final container, maintained at the recommended storage temperature up to the expiry date, should be demonstrated to the satisfaction of the NRA using final containers from at least three final lots made from different purified antigen bulks.

Accelerated stability tests may be undertaken to provide additional information on the overall characteristics of a vaccine and may also aid in assessing comparability should the manufacturer decide to change aspects of manufacturing.

The formulation of vaccine antigens and adjuvant (if used) must be stable throughout the shelf-life of the vaccine. Acceptable limits for stability should be agreed with the NRA. Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile (79). Data should be provided to the NRA in accordance with local regulatory requirements.

The final stability-testing programme should be approved by the NRA and should include an agreed set of stability-indicating parameters, procedures for the ongoing collection and sharing of stability data, and criteria for rejecting vaccines.

A.14.2 Storage conditions

The final lot should be kept at 2–8 °C. If other storage conditions are used they should be fully validated and approved by the NRA. The storage conditions used should ensure that the minimum vaccine potency specified on the label of the container or package will be maintained after release and until the end of the shelf-life, provided that the vaccine is stored under the recommended conditions. During storage, adsorbed vaccines should not be frozen.

If a vaccine has been shown to be stable at temperature ranges higher than the approved 2–8 °C range, it may be stored under extended controlled temperature conditions for a defined period, subject to the agreement of the NRA (80).

A.14.3 Expiry date

The expiry date should be based on the shelf-life supported by stability studies and should be approved by the NRA. The expiry date should be based on the date of blending of final bulk, the date of filling or the date of the first valid potency test on the final lot.

Where an in vivo potency test is used, the date of the potency test is the date on which the test animals are inoculated.
Part B. Nonclinical evaluation of recombinant hepatitis E vaccines

The nonclinical evaluation of hepatitis E vaccines should be based on the WHO guidelines on nonclinical evaluation of vaccines (5) which provide details on the design, conduct, analysis and evaluation of nonclinical studies. Further guidance on the general principles for the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines can be found in separate WHO guidelines (8).

Prior to the clinical testing of any new hepatitis E vaccine in humans there should be extensive product characterization, proof-of-concept studies, immunogenicity studies and safety testing in animals. The extent of nonclinical evaluation will depend on the complexity of the vaccine formulation on a case-by-case basis. The following specific issues should be considered in the context of the development of recombinant hepatitis E vaccines based on the ORF2-encoded viral capsid protein.

B.1 Strategy for cloning and expressing the gene product

The HEV genome contains three open reading frames. Of these, ORF2 codes for the viral capsid protein which is the target of neutralizing antibodies against HEV (81–83).

A full description should be given of the biological characteristics of the host cell and expression vectors used in production. This should include details of: (a) the construction, genetics and structure of the expression vector; (b) the origin and identification of the gene that is being cloned; and (c) potential adventitious retrovirus-like particles (and/or their genetic markers) in mammalian cell-based expression systems. The physiological measures used to promote and control the expression of the cloned gene in the host cell should also be described in detail.

Data should be provided to demonstrate the genetic stability of the expression system beyond the passage level used for production. Any instability of the expression system occurring in the seed culture or after a production-scale run (for example, involving rearrangements, deletions or insertions of nucleotides) must be documented. NRA approval should be obtained for the system used.

B.2 Product characterization and process development

Rigorous identification and characterization of recombinant DNA-derived vaccines is required as part of the application for marketing authorization. The ways in which these products differ chemically, structurally, biologically or immunologically from the naturally occurring antigen must be fully documented. Such differences could arise during processing at the genetic or post-translational level, or during purification.
The expressed protein should be characterized by biochemical, biophysical and immunological methods such as SDS-PAGE, isoelectric focusing, circular dichroism, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), N-terminal sequencing, high-performance size-exclusion chromatography (HPSEC) and binding activity to monoclonal antibodies. The immunogenicity of the protein should be analysed in an appropriate animal model.

It is crucially important that vaccine production processes are appropriately standardized and controlled to ensure consistency in manufacturing, and in the collection of nonclinical data that may indicate potency and safety in humans. The extent of product characterization may vary according to the stage of development. The vaccine lots used in nonclinical studies should be adequately representative of the formulation intended for use in clinical investigation and, ideally, should be the same lots as those used in clinical trials. If this is not feasible, the lots used in nonclinical studies should be comparable to clinical lots with respect to physicochemical characteristics, stability and formulation.

B.3 Pharmacodynamic studies
B.3.1 Immunogenicity studies

The immunogenicity of the vaccine should be evaluated in relevant animal species that respond well to the vaccine antigen (for example, rodent, rabbit, swine or non-human primate) (84). Immunogenicity data can provide initial insights into the immunological characteristics of the vaccine antigen and are useful in evaluating the vaccine formulation and underlying protective mechanisms, and in justifying the inclusion of an adjuvant.

Nonclinical passive immunization studies in non-human primates and human epidemiology studies indicate that humoral immunity is probably the primary effector mechanism that directly mediates protection against HEV. Indeed, clear correlation between serum IgG responses to vaccine antigen and protection has been demonstrated in clinical (85) and nonclinical (86) studies of recombinant hepatitis E capsid-based vaccines. On this basis, it is recommended that the evaluation of vaccine immunogenicity should include an assessment of serum anti-HEV IgG antibodies.

Immunogenicity studies should establish a dose–response relationship by testing different doses of vaccine antigen. Ideally, immune responses are assessed after each dose of vaccine in line with the intended posology. For an adjuvanted vaccine, the advantage conferred by the adjuvant should be demonstrated on the basis of serological data – with or without additional elucidation of cellular immune response depending upon the adjuvant used.
B.3.2 Challenge studies

The protective effect of vaccine antigen should be evaluated in an appropriate animal model. Examples of animal models that are known to be experimentally permissive to infection by human HEV include swine (genotypes 3 and 4), rabbit (genotype 4), and different species of non-human primates such as cynomolgus macaques (genotypes 1 and 2) and rhesus macaques (genotypes 1–4) (84). Challenge studies conducted in non-human primates have demonstrated the protective immunity of hepatitis E vaccines which have subsequently been shown to be efficacious in humans (62, 85).

The animals used should be HEV-naive. The naive status of animals at baseline should be confirmed by the absence of detectable anti-HEV total IgG antibody in sera and absence of detectable HEV RNA in faeces and in sera. The virus used for animal challenge studies should correspond to the wild-type virus strain from which the vaccine antigen is derived.

The design of challenge studies may vary, depending on the platforms used to produce the vaccine. The vaccination of animals is usually conducted in accordance with the intended posology, with the subsequent challenge made when vaccinated animals develop peak protective responses. In general, challenge via the intravenous route is acceptable as transmission through the oral route is less efficacious. The challenge dose should be sufficiently high to ensure the establishment of reliable infection and/or histopathological hepatitis. Important end-points used to define protection should be specified in the study protocol and should include:

- infection marker such as HEV RNA in stool and serum at serial time points; and/or
- histopathological evidence of hepatitis using liver biopsy; and
- biochemical parameter of alanine aminotransferase (ALT) change at serial time points.

In addition, passive immunization studies in animal models that involve the transfer of antisera from human vaccinees to naive animals followed by HEV challenge might be useful in estimating a specific IgG titre associated with protection.

B.3.3 Cross-neutralization protection against different genotypes

The genetic differentiation of HEV strains is based on whether the nucleic acid variation of ORF2 between two viruses is more than 20%. According to this criterion, human HEV isolates are classified into four genotypes (genotypes 1–4). These four genotypes share a single serotype based on their immunoreactivity and cross-neutralization (87, 88). Therefore, hepatitis E vaccine based on recombinant
ORF2 derived from a given genotype is expected to provide protection against all four HEV genotypes. Results from preclinical and clinical studies have substantiated this expectation. In preclinical animal models the same protection was observed in animals challenged with different genotypes of HEV following immunization with recombinant ORF2 protein derived from a single genotype. For example, Purcell et al. (89) and Li et al. (61) demonstrated that immunization with recombinant ORF2 protein derived from genotype 1 HEV was able to protect against genotype 1, 2, 3 and 4 HEV infection in non-human primate models. In addition, a vaccine based on recombinant ORF2 protein derived from genotype 4 HEV provided cross-protection against genotypes 1 and 4 HEV infection in a non-human primate model (90). Studies conducted in an HEV rabbit model have indicated that recombinant capsid proteins derived from genotype 1 HEV cross-protects against genotype 4 HEV infection (91, 92).

Furthermore, during clinical trials conducted in China and Nepal, a recombinant ORF2 protein vaccine derived from genotype 1 HEV sequences was found to protect against acute hepatitis caused by genotypes 1 and 4 HEV infection (63, 85, 92).

Based on biochemical analysis of the recombinant capsid proteins, a cross-genotype and neutralizing epitope (as recognized by monoclonal antibody 8G12) was identified in both genotypes 1 and 4 HEV (93). The monoclonal antibody 8G12 was shown to block the binding of naturally acquired antibodies in human and animal sera. The presence of these “8G12-like” antibodies or the epitopes recognized by these antibodies could be regarded as a partial demonstration of cross-genotype protection elicited by vaccines based on antigens derived from a given type.

To date, only limited data on cross-protection are available from completed clinical trials with recombinant HEV capsid-based vaccines. If cross-protection against heterologous viruses is claimed then challenge studies should be conducted in appropriate animal models to evaluate the potential for such cross-protection.

**B.4 Biodistribution studies**

Pharmacokinetic studies are not required for recombinant human hepatitis E vaccines. If a novel excipient (including a novel adjuvant) is included in the vaccine then a biodistribution study should be considered (8).

**B.5 Toxicology studies**

Toxicology studies should be undertaken in accordance with the WHO guidelines on nonclinical evaluation of vaccines (5). Such studies should be performed using the final vaccine formulation in relevant animal species and should reflect the
intended clinical use of the vaccine (5). Repeated-dose toxicity and local tolerance should be evaluated in relevant species following good laboratory practice (GLP) principles, prior to the initiation of early human clinical trials. Because the target population for hepatitis E vaccines includes women of childbearing age, GLP-compliant reproductive and developmental toxicity studies are also required.

In general, toxicity evaluation in one relevant species is justified. The route and dosing regimen used should reflect the intended clinical use. For the evaluation of developmental toxicity, the dosing regimen should consider one or two doses prior to mating so that pregnant animals, embryos and fetuses are exposed to a maximal vaccine response during the critical window of organogenesis.

If the vaccine is formulated with a novel adjuvant, nonclinical toxicology studies should be conducted as appropriate for the adjuvant concerned, and the recommendations provided in the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines should be followed (8).

If a novel cell substrate (that is, a substrate that has not been previously licensed or used in humans) is used for the production of a hepatitis E vaccine, safety aspects (such as potential immune responses elicited by residual host-cell proteins) should be investigated in a suitable animal model. Such studies should be undertaken particularly if the final product contains an adjuvant that might enhance responses to low levels of residual proteins.

Part C. Clinical evaluation of recombinant hepatitis E vaccines

C.1 Introduction
Clinical trials should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (94). General guidance on vaccine clinical development programmes is provided in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (6) and is not repeated here. This section addresses issues for clinical development programmes that are specific to, or of special concern for, vaccines intended to prevent clinically apparent infections with HEV.

C.2 Assays
This section considers:

- serological assays for establishing the baseline serostatus of trial subjects and evaluating the humoral immune response to vaccination (see also section C.3); and
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Serological and virus detection assays for laboratory confirmation of acute hepatitis caused by HEV infection in vaccine efficacy trials (see also section C.4).

Sponsors should also consult section 5.3.3 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (6).

C.2.1 Serological assays

C.2.1.1 Functional antibody

Currently there is no well-established assay for measuring anti-HEV neutralizing antibody. Since there is no efficient HEV cell infection model, a direct measurement of anti-HEV neutralizing antibody is not feasible. Neutralizing antibody has been estimated using methods such as real-time PCR (95) or an immunofluorescence foci assay (IFA) to detect virus, but these methods are not standardized or suitable for processing large numbers of sera and each has its drawbacks. Sponsors are encouraged to develop high-throughput assays for anti-HEV neutralizing antibody. For example, a potential high-throughput neutralization assay based on recombinant HEV capsid particles has been compared with IFA and with the measurement of anti-HEV IgG using sera from HEV-infected and vaccinated macaques (95).

C.2.1.2 Total binding antibody

For the purposes of estimating the immune response to vaccination, sponsors may choose to develop in-house anti-HEV IgG assays in which the antigen used to coat the wells is the same as – or at least a truncated version of – that used in the vaccine. It is recommended that the quantitation of anti-HEV IgG should be referenced to the WHO standard sera (96) as part of the validation of the assay. Using the selected assay methodology, a cut-off value should be identified and justified for distinguishing seronegative and seropositive sera.

For the detection of acute infection with HEV, commercial assays are available for detecting HEV-specific IgG, IgM, IgA and total immunoglobulin. These commercial assays vary considerably in their use of synthetic or recombinant antigen, viral strain origin and genotype, viral gene product(s) and detection method – for example, anti-HEV IgM antibody detection commonly uses a μ chain capture ELISA whereas IgG antibody detection usually involves a direct antigen-coating ELISA with secondary enzyme conjugated antibody. Comparative studies have shown considerable differences in the sensitivity and specificity of commercially available assays, with even more variability for IgM compared to IgG assays (52, 53). The assays used to detect and quantify anti-HEV antibody in suspected cases of hepatitis E in efficacy trials
must be adequately justified, taking into account what is known about their performance characteristics.

C.2.2 Virus detection assays

Appropriate HEV RNA or antigen detection assays are required to confirm the presence of the virus in blood and/or stools of suspected cases of hepatitis E (see section C.4). Commercial quantitative PCR assays are available along with WHO HEV RNA international reference preparations for genotype 3a and 3b strains (66, 67, 97). Assays with different targets (for example, assays that target ORF2 or the ORF2/3 overlapping region) have been shown to have different performance characteristics. The ability of assays to detect and quantify HEV RNA from specific genotypes should be taken into account when selecting the method to be used in trials.

In vaccine efficacy trials it is recommended that HEV should be identified at least to genotype level for all PCR-positive cases. The fragments that are amplified by real-time PCR are usually less than 100 nucleotides in length and are located on conserved parts of the genome. Therefore, additional genomic sequencing (which published data suggest may be targeted to a specific region) is currently required to determine the HEV (sub-)genotype. Sponsors should provide full details of the methodology applied and appropriate controls should be used.

C.3 Immunogenicity

C.3.1 Formulation, dose and regimen

C.3.1.1 Primary series

Hepatitis E vaccines will be used mainly or exclusively in regions with relatively high rates of clinically apparent infections. However, pre-vaccination testing for HEV serostatus will not be feasible in routine use. In naturally primed individuals (not all of whom may have detectable pre-vaccination anti-HEV IgG) the first dose of hepatitis E vaccine may elicit large increments in antibody due to an anamnestic response. In contrast, multiple doses of the same vaccine may be required to achieve similar antibody levels in HEV-naive subjects. Consequently, it is important that the primary series should be selected on the basis of the immune responses observed in subjects who are seronegative – including seronegative subjects who are unlikely to have been naturally primed.

In the absence of an established immune correlate of protection for HEV, the selection of the vaccine dose and regimen may be based on reaching an antibody plateau response unless this is precluded by concerns over reactogenicity. It is desirable that immunogenicity studies should explore the minimum number of doses and the shortest dose interval(s) required to achieve a plateau immune response.
C.3.1.2  Need for revaccination

In the absence of an immune correlate of protection, it is recommended that the possible need for revaccination is not based solely upon waning antibody levels. There should be planned long-term follow-up for hepatitis E cases in vaccine efficacy trials, and/or data should be collected from vaccine effectiveness studies to determine waning protection against clinically apparent HEV infection (see section C.4.2.7).

In anticipation that revaccination may be necessary to maintain protection, it is recommended that the immune response to additional doses of vaccine is assessed. For example, subjects enrolled into an immunogenicity trial could be sub-randomized to receive further doses at predefined intervals after completion of a primary series. The immune response to additional doses could be compared with the post-primary response of the same individuals and/or compared with the response to a single dose administered to previously unvaccinated and seronegative control subjects.

C.3.1.3  Cross-protection

The ability of a candidate hepatitis E vaccine to protect against a range of wild-type strains covering the four main HEV genotypes may vary according to the vaccine construct. It is important that this should be investigated in nonclinical studies (see section B.3.3).

In clinical trials in which vaccine-elicited antibody is determined against the antigen in the vaccine (see section C.2.1.2), it is recommended that IgG is also measured using antigens derived from a range of wild-type HEVs. If marked differences are observed in IgG antibody when measured using vaccine versus non-vaccine antigens and/or by HEV genotype, it would be of particular interest to assess whether a similar effect is observed for functional antibody levels. In addition, depending on the range of investigations already completed, it may be appropriate to conduct additional nonclinical studies to evaluate the possible implications of the findings for protection before proceeding to the conducting of efficacy trials.

C.3.2  Special populations

Thus far, the efficacy of hepatitis E vaccination has been demonstrated in healthy subjects aged 16 years and above – most of whom have been under 45 years of age. There may be interest in the use of hepatitis E vaccines in younger age groups and/or subjects at particular risk of developing severe or fulminant hepatitis (for example, during pregnancy and in individuals with pre-existing liver disease) and/or immunodeficient subjects who are at risk of developing chronic HEV infection. If a vaccine has already been shown to be efficacious in healthy adults
it may be possible, on the basis of safety and immunogenicity data, to extend its use to various special populations. For example:

- There may be interest in completing primary vaccination before the period of greatest risk – in which case, safety and immunogenicity data should be generated to support the use of appropriate regimen(s) in specific paediatric age subgroups.
- Section 5.6.4.2 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (6) discusses the evaluation of vaccine safety and immunogenicity during pregnancy. Protection against hepatitis E disease by vaccination during pregnancy (as opposed to the vaccination of women before or between pregnancies) would require the development of a vaccine that could elicit antibody levels that are likely to be protective (for example, similar to those observed in adults enrolled in vaccine efficacy trials) after a single dose or after two doses administered within a short interval.
- Subjects with pre-existing liver disease and immunodeficient subjects may have very variable immune responses to vaccination depending on the underlying cause and specific nature of their condition. Vaccine regimens should be supported by immune responses documented in specific subgroups that are representative of the intended target populations.

C4 Efficacy
C4.1 Requirement for a demonstration of vaccine efficacy
It is currently recommended that the protective efficacy of a candidate vaccine against clinically apparent HEV infection should be evaluated in a pre-licensure vaccine efficacy trial. The following considerations apply:

- At the time of preparing these WHO Recommendations there is one vaccine against hepatitis E that is licensed in one country (See General considerations) (62, 63).
- This licensed vaccine is not widely used and it is not included in national immunization programmes. As a result, the use of a control group that does not receive vaccination against hepatitis E is possible.
- In jurisdictions in which a licensed vaccine is available, it is possible that individual NRAs may consider that licensure can be based on a trial that evaluates the efficacy of the candidate vaccine relative to that of the licensed vaccine in a population similar to that in which the efficacy of the licensed vaccine was established.
The lack of an immune correlate of protection against hepatitis E does not rule out immunobridging a candidate vaccine to a licensed vaccine that has been shown to be efficacious. However, this approach is possible only if both vaccines contain the same antigen(s) so that immune responses can be compared directly. In addition, the demonstration of efficacy of the first licensed vaccine was confined to HEV genotypes 1 and 4 and it is not known whether the protective immune response may vary between genotypes. Furthermore, the baseline seropositivity rate of the population in which efficacy was demonstrated was estimated at 47% (based on data from less than one tenth of the total subjects randomized into the trial) (63). It cannot be assumed that the point estimate of vaccine efficacy would be applicable to populations with very different pre-vaccination seropositivity rates.

Taking these considerations into account, the focus of this section is on clinical development programmes that include vaccine efficacy trials in which the control group does not receive vaccination against hepatitis E. Most of the recommendations are also applicable to trials in which the control group receives a licensed vaccine against hepatitis E. Clinical programmes leading to licensure based on immunobridging are not addressed in this guidance. The general principles to consider are discussed in sections 5.6.2 and 6.3.3 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (6).

C.4.2 Considerations for efficacy trial design

C.4.2.1 Primary objective

The primary objective will be to demonstrate that the candidate vaccine protects against clinically apparent (that is, symptomatic) HEV infection caused by any genotype (see section C.4.2.4).

- It is not required for efficacy to be shown against asymptomatic HEV infection. With the exception of immunodeficient subjects (who may develop chronic infection with possible sequelae) asymptomatic infection is of no clinical significance.
- It is not required for vaccine efficacy trials to be powered to demonstrate genotype-specific efficacy (see section C.4.2.2).

C.4.2.2 Trial sites

Efficacy trials will be conducted in endemic areas in which the estimated attack rate for clinically apparent HEV infection is sufficient to complete enrolment into an adequately powered vaccine efficacy trial within a reasonable time frame. Sites may be chosen on the basis of available public health disease-surveillance
data and/or pre-trial evaluations of epidemiology conducted by the sponsor. In two prior efficacy trials (62–64, 85), HEV genotypes that caused clinically apparent infections in the control (placebo) groups were limited to strains circulating at the trial sites in the years in which they were conducted. Sponsors are encouraged to consider selecting sites in a range of geographical areas in which different genotypes are circulating and/or to conduct separate vaccine efficacy trials in regions with different genotype distributions.

C.4.2.3 Subject selection criteria

Because of the peak age incidence of hepatitis E it is likely that vaccine efficacy trials will target adolescents and adults. An upper age limit may be set depending on the age-specific attack rates.

In endemic areas, the adult population will include a variable proportion of subjects who are seropositive for IgG against HEV. Options for subject selection include the following:

- Adults could be enrolled without knowledge of their baseline serostatus for HEV – which is the usual approach in vaccine efficacy trials conducted in endemic regions. This provides an assessment of the benefit of vaccination over and above the level of pre-existing protection against HEV infection due to natural exposure. The proportion of subjects enrolled who are seropositive at the time of enrolment should be estimated retrospectively by determining anti-HEV IgG in samples obtained from all, or a randomly selected subset of, subjects prior to vaccination. This information is helpful when considering extrapolation of the estimate of vaccine efficacy observed to other regions not included in the trial.

- A possible alternative approach would be to pre-screen subjects for anti-HEV IgG and to enrol only those considered to be seronegative on the basis of a threshold determined by the assay used. This may allow for a smaller sample size to be randomized into the trial due to an expected higher attack rate; however, it is also possible that seronegative adults are less likely to encounter HEV compared to seropositive adults of a similar age range and resident in the same region due to differences in their living conditions. Therefore, detailed knowledge of the local epidemiology of hepatitis E should be taken into account before choosing this approach.

C.4.2.4 Primary end-point

In accordance with the recommended primary objective, the primary end-point should be clinically apparent acute hepatitis that is confirmed to be due to HEV.
Sponsors could consider appointing an independent data-adjudication committee to review the data and determine which subjects meet the case definition to be counted in the primary analysis.

C.4.2.4.1 Clinical features for the case definition

The clinical features that trigger subjects to present to study site staff or to a local designated health-care facility for laboratory investigations for acute hepatitis E should be selected with the aim of capturing as many cases as possible while limiting unnecessary investigations. On this basis it is reasonable to define a possible case of acute viral hepatitis requiring laboratory investigation as an illness presenting with any, or a minimum number of, signs and symptoms – including malaise, fatigue, anorexia, right upper quadrant tenderness for longer than 3 days or any duration of jaundice or dark urine. Additional symptoms that could be considered include any abdominal pain, nausea or vomiting that persists for at least 3 days and for which there is no known likely explanation.

C.4.2.4.2 Laboratory confirmation of acute hepatitis E infection

It is recommended that the laboratory confirmation of acute hepatitis E cases should be conducted in a designated central laboratory. If more than one central laboratory is necessary for practical reasons, it is essential that the laboratories use identical methodologies, and consideration should be given to testing a randomly selected subset of samples at each laboratory to assess concordance.

There is variability in the onset and duration of elevated ALT levels in serum, detectable HEV RNA in serum or stool, the appearance of anti-HEV IgM and the appearance of (or detectable increase in) anti-HEV IgG in relation to the first appearance of symptoms. An increase in ALT to at least 2.5-fold the upper limit of normal (ULN) based on the local or central laboratory normal range should lead to investigations to determine whether HEV is the causative agent. If the first sample does not show a ≥ 2.5-fold elevation in ALT, the test should be repeated after approximately 1–2 weeks for any subject with jaundice, persistent symptoms or elevated total serum bilirubin in the first sample.

The confirmation of HEV as causative of the clinical picture should be based on any two or more of the following:

- IgM against HEV – which is often detectable at the time of onset of clinical symptoms but may peak after 1–2 weeks, and in some cases remains detectable for several months;
- at least a 4-fold rise in anti-HEV IgG between the first sample and a convalescent sample;
- detection of HEV RNA in blood or in stool – which is usually short-lived but which may persist for longer in stool than in blood.
To avoid cases being missed, protocols should plan for appropriately timed repeat specimens to be collected (for example, at 2–6 weeks after the first sample) from individuals with elevated ALT. Since HEV RNA is most likely to be detectable early in the course of a clinical illness, it is recommended that samples are obtained as early as possible and, if negative, that repeat testing is conducted after a short interval.

Samples obtained at first presentation should also be tested to detect acute infection with HEV or coinfection with other hepatitis viruses that can cause the same clinical picture, including testing for:

- IgM against hepatitis A virus
- hepatitis B virus surface antigen and anti-core IgM
- antibody against hepatitis C virus and/or hepatitis C virus RNA.

If the first sample is negative for evidence of acute infection with hepatitis A, B, C or E viruses and further samples are indicated to rule out hepatitis E, it is recommended that these should also be retested for evidence of hepatitis A, B and C viruses to document any possible coinfections.

C.4.2.5 Primary, secondary and other analyses

In a vaccine efficacy trial, it may be permissible that the primary analysis includes only confirmed acute hepatitis E cases – whether there is evidence of coinfection with other hepatitis viruses or not – as follows:

- in subjects who completed the vaccination series within predetermined visit windows, if more than one dose is required; and
- with symptom onset occurring more than a defined period after the only or final dose of the series that takes into account what is known about the timing of the post-dose anti-HEV IgG peak.

This approach gives the most optimistic estimation of vaccine efficacy. If the primary analysis is confined to cases counted as described above it is essential that predefined secondary analyses are carried out to estimate vaccine efficacy based on confirmed cases of clinically apparent HEV infection defined and counted as follows:

- all cases in subjects who received at least one assigned dose as randomized and regardless of adherence to study visit windows;
- cases that occurred at any time after the last dose received (that is, counted from the day of dosing) in those who completed the assigned number of doses;
- cases that occurred after each sequential dose, depending on the number of doses in the series and counted from the day of dosing.
Vaccine efficacy should be explored according to HEV genotype if this is feasible, depending on the numbers of cases that occur due to individual genotypes.

It is recommended that an additional analysis should explore any differences in clinical or laboratory features (including severity) between cases that occur in the candidate vaccine group and the control group (whether the control group receives placebo or a licensed vaccine against hepatitis E). The analysis should take into account whether the severity observed in individual subjects could reflect coinfection with other hepatitis viruses – whether acute (most likely coinfection with hepatitis A) or chronic (that is, acute hepatitis E occurring in subjects who have chronic hepatitis B or C infection).

C.4.2.6 Case ascertainment

It is recommended that an active case-ascertainment strategy is used throughout the time frame of a vaccine efficacy trial. This is essential at least up to the time of the primary analysis, which may be conducted after a specific number of total cases has been accumulated or after a predefined period in which a sufficient number of cases are expected to occur to estimate vaccine efficacy.

C.4.2.7 Duration of protection

While the primary analysis may lead to licensure, it is recommended that trials continue to use active case ascertainment to follow up subjects for several years to provide data on waning vaccine protection without unblinding of treatment assignment at the level of the individual. These data can be reported at some time after licensure of the vaccine and may point to the need for further doses to be administered at intervals to maintain protection.

C.4.2.8 Vaccine effectiveness

The need for vaccine effectiveness studies should be established at the time of licensure.

If longer-term follow-up within a pre-licensure trial is not considered to be feasible, the duration of vaccine protection should be investigated within a vaccine effectiveness study and/or as part of routine disease surveillance conducted by public health authorities. Furthermore, the efficacy of the vaccine against individual genotypes should be explored as part of a vaccine effectiveness study and/or during routine disease surveillance.

C.5 Safety

Evaluation of the safety of candidate hepatitis E vaccines should be undertaken in accordance with the recommendations made in section 7 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (6). If the
primary series consists of several vaccine doses it is important to document whether reactogenicity increases with sequential doses. Additionally, the safety of post-primary doses should be evaluated. There may be special considerations for vaccine safety depending on the vaccine construct and the intended target population (for example, if the vaccine is proposed for administration during pregnancy).

If a candidate vaccine is evaluated in a large pre-licensure trial, and if the safety profile documented during immunogenicity trials did not give rise to any major concerns, it may be acceptable for a full assessment of safety (that is, including detailed documentation of local and systemic reactogenicity, as well as all unsolicited adverse events) to be confined to a randomized subset of the total subjects. Serious adverse events should be documented in all subjects enrolled at all trial sites.

**Part D. Recommendations for NRAs**

**D.1 General recommendations**

The general recommendations for NRAs and NCLs given in the WHO Guidelines for national authorities on quality assurance for biological products (98) and WHO Guidelines for independent lot release of vaccines by regulatory authorities (99) should apply. These recommendations specify that no new biological substance should be released until consistency of lot manufacturing and quality has been demonstrated.

The detailed production and control procedures – as well as any significant changes in them that may affect the quality, safety and efficacy of recombinant hepatitis E vaccines – should be discussed with and approved by the NRA (100). For control purposes, the relevant international reference preparations currently in force should be obtained for the purpose of calibrating national, regional and working standards (101). The NRA may obtain from the manufacturer the product-specific or working reference to be used for lot release.

Consistency of production has been recognized as an essential component in the quality assurance of recombinant hepatitis E vaccines. In particular, the NRA should carefully monitor production records and quality control test results for clinical lots, as well as for a series of consecutive lots of the vaccine.

**D.2 Official release and certification**

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO Recommendations (99).

A summary protocol for the manufacturing and control of recombinant hepatitis E vaccines, based on the model summary protocol provided in Appendix 1 and signed by the responsible official of the manufacturing
establishment, should be prepared and submitted to the NRA in support of a request for the release of a vaccine for use.

A Lot Release Certificate signed by the appropriate NRA official should then be provided if requested by a manufacturing establishment and should certify whether or not the lot of vaccine in question meets all national requirements and/or Part A of these WHO Recommendations. The certificate should provide sufficient information on the vaccine lot. The purpose of this official national release certificate is to facilitate the exchange of vaccines between countries and should be provided to importers of the vaccines. A model NRA Lot Release Certificate is provided in Appendix 2.

Authors and acknowledgements

The first draft of these WHO Recommendations was prepared by a WHO drafting group comprising Dr E. Gurley, International Centre for Diarrheal Diseases Research, Bangladesh and Johns Hopkins University, the USA; Dr P. Minor, National Institute for Biological Standards and Control, the United Kingdom; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, the United Kingdom; Dr Y. Sun, Paul-Ehrlich-Institut, Germany; Dr R. Wagner, Paul-Ehrlich-Institut, Germany; Dr Y. Wang, National Institutes for Food and Drug Control, China; Dr Q. Zhao, Xiamen University, China; and Dr D. Lei, World Health Organization, Switzerland, taking into consideration the discussions and consensus reached during a WHO working group meeting to develop WHO recommendations to assure the quality, safety and efficacy of recombinant hepatitis E vaccines, held in Geneva, Switzerland, 11–12 May 2017 and attended by: Dr I. Ciglenecki, Médecins Sans Frontières International, Switzerland; Mr S. Gao, Xiamen Innovax Biotech Co. Ltd, China; Dr E. Gurley, International Centre for Diarrheal Diseases Research, Bangladesh and Johns Hopkins University, the USA; Mr B. Huang, Xiamen Innovax Biotech Co. Ltd, China; Dr N. Kamar, Toulouse University Hospital, France; Mrs D. Kusmiaty, National Quality Control Laboratory of Drug and Food, Indonesia; Dr P. Minor, National Institute for Biological Standards and Control, the United Kingdom; Dr M. Li, China Food and Drug Administration, China; Dr A. Lommel, Paul-Ehrlich-Institut, Germany; Dr J. Lynch, International Vaccine Institute, Republic of Korea; Dr K. Maithal, Zydus Cadila Healthcare Ltd, India; Dr C. Ngabéré, Ministry of Public Health, Chad; Dr S. Phumiamorn, Institute of Biological Products, Thailand; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, the United Kingdom; Dr H. Iftikhar Qureshi, Pakistan Health Research Council, Pakistan; Dr J.W.K. Shih, Xiamen Innovax Biotech Co. Ltd, China; Mr B. Shrestha, Walter Reed/AFRIMS Research Unit Nepal, Nepal; Dr D. Steele, Bill & Melinda Gates Foundation, the USA; Dr E. Teshale, Centers for Disease Control and Prevention, the USA; Dr R. Wagner, Paul-Ehrlich-Institut, Germany; Dr Y. Wang, National
Institutes for Food and Drug Control, China; Dr X. Wu, National Institutes for Food and Drug Control, China; Dr C. Young, Health Canada, Canada; Dr J. Zhang, Xiamen University, China; Dr Q. Zhao, Xiamen University, China; and Dr P. Duclos, Dr G. Enwere, Dr I. Knezevic, Dr O. Lapujade, Dr D. Lei and Ms C.A. Rodriguez-Hernandez, World Health Organization, Switzerland.

The resulting document was then posted on the WHO Biologicals website for a first round of public consultation from November 2017 to February 2018. A second draft was then prepared by Dr E. Gurley, Johns Hopkins Bloomberg School of Public Health, the USA; Dr P. Minor, Consultant, the United Kingdom; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, the United Kingdom; Dr Y. Sun, Paul-Ehrlich-Institut, Germany; Dr Q. Zhao, Xiamen University, China; Dr R. Wagner, Paul-Ehrlich-Institut, Germany; Dr Y. Wang, National Institutes for Food and Drug Control, China; and Dr D. Lei, World Health Organization, Switzerland, incorporating comments received from regulatory authorities, manufacturers and academia as appropriate.

A third draft was then prepared by Dr E. Gurley, Johns Hopkins Bloomberg School of Public Health, the USA; Dr P. Minor, Consultant, the United Kingdom; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, the United Kingdom; Dr Y. Sun, Paul-Ehrlich-Institut, Germany; Dr Y. Wang, National Institutes for Food and Drug Control, China; Dr Q. Zhao, Xiamen University, China; and Dr D. Lei World Health Organization, Switzerland, taking into consideration comments received during an informal consultation held in Beijing, China, 18–19 April 2018 and attended by: Dr E. Gurley, Johns Hopkins Bloomberg School of Public Health, the USA; Mr D. Minh Hung, Ministry of Health, Viet Nam; Dr M. Li, China Food and Drug Administration, China; Dr Z. Liang, National Institutes for Food and Drug Control, China; Dr J. Martin, National Institute for Biological Standards and Control, the United Kingdom; Dr P. Minor, Consultant, the United Kingdom; Dr C. Ngabéré, Ministry of Public Health, Chad; Dr S. Phumiamorn, Ministry of Public Health, Thailand; Dr H. Ifitikhar Qureshi, Pakistan Health Research Council, Pakistan; Dr J. Shin, WHO Regional Office for the Western Pacific, Philippines; Mr Y. Tang, WHO Country Office, China; Mr B. Shrestha, Walter Reed/AFRIMS Research Unit Nepal, Nepal; Dr R. Simalango, National Agency of Drug and Food Control, Indonesia; Dr D. Steele, Bill & Melinda Gates Foundation, the USA; Dr Y. Sun, Paul-Ehrlich-Institut, Germany; Dr E. Teshale, Centers for Disease Control and Prevention, the USA; Dr Y. Wang, National Institutes for Food and Drug Control, China; Dr H. Wahu Triestano Wibowo, National Agency of Drug and Food Control, Indonesia; Dr X. Wu, National Institutes for Food and Drug Control, China; Dr M. Xu, National Institutes for Food and Drug Control, China; Dr X. Yao, China Food and Drug Administration, China; Dr J. Zhang, Xiamen University, China; Dr Q. Zhao, Xiamen University, China; and Dr D. Lei, World Health Organization, Switzerland; representatives from industry: Dr H. Chandra, Cadila
Healthcare Limited, Vaccine Technology Center, India; Ms W. Weidan Huang, Xiamen Innovax Biotech Co. Ltd, China; Dr B. Huang, Xiamen Innovax Biotech Co. Ltd, China; and Dr J.W.K. Shih, Xiamen Innovax Biotech Co. Ltd, China.

The resulting document was then posted on the WHO Biologicals website for a second round of public consultation from July to September 2018, and the document WHO/BS/2018.2348 was subsequently prepared by Dr D. Lei, Dr P. Minor, Dr M. Powell and Dr Y. Sun with contributions from other drafting group members and from Professor J. Zhang, Xiamen University, China, incorporating comments received from regulatory authorities, manufacturers and academia as appropriate.

Further changes were subsequently made to document WHO/BS/2018.2348 by the WHO Expert Committee on Biological Standardization.

References


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Appendix 1

Model summary protocol for the manufacturing and control of recombinant hepatitis E vaccines

The following summary protocol is intended for guidance. It indicates the information that should be provided as a minimum by the manufacturer to the NRA. Information and tests may be added or deleted/omitted as necessary with the approval of the NRA.

It is possible that a summary protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO recommendations for a particular product should be provided in the protocol submitted.

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet (package insert) that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it must also be accompanied by a Lot Release Certificate from the NRA of the country in which the vaccine was produced or released, stating that the product meets national requirements as well as the recommendations in Part A of this document.

Summary information on final lot

International name: 
Trade name/commercial name: 
Product licence (marketing authorization) number: 
Country: 
Name and address of manufacturer: 
Name and address of licence holder, if different: 
Final packaging lot number: 
Type of container: 
Number of containers in this final lot: 
Final container lot number: 
Date of manufacture: 
Nature of final product (adsorbed): 
Preservative and nominal concentration: 
Volume of each single human dose: 
Number of doses per final container: 
Summary of the composition (include a summary of the qualitative and quantitative composition of the vaccine per human dose, including any adjuvant used and other excipients):

Shelf-life approved (months):

Expiry date:

Storage condition:

The following sections are intended for recording the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production. If any test has to be repeated, this must be indicated. Any abnormal result must be recorded on a separate sheet.

**Detailed information on manufacture and control**

**Starting materials**

The information requested below is to be presented on each submission. Full details on master and working seed lots and cell banks are requested upon first submission only and whenever a change has been introduced.

Identity of seed lot strain used for vaccine production:

Reference number of seed lot:

Date(s) of reconstitution (or opening) of seed lot ampoule(s):

**Single harvests used for preparing the bulk**

Lot number(s):

Volume(s) of fermentation paste, storage temperature, storage time and approved storage period:

Name of the culture medium:

Date of inoculation:

Temperature of incubation:

Control of bacterial purity

Method:

Specification:

Date:

Result:
Date of harvest: __________________________
Volume of harvest: __________________________
Yield (mg/ml): __________________________
Volume after filtration: __________________________

Identity test
Method: __________________________
Specification: __________________________
Date: __________________________
Result: __________________________

Test for bacteria and fungi
Method: __________________________
Media: __________________________
Volume inoculated: __________________________
Date of start of test: __________________________
Date of end of test: __________________________
Result: __________________________

Test for Mycoplasmas (if applicable)
Method: __________________________
Volume inoculated: __________________________
Date of start of test: __________________________
Date of end of test: __________________________
Result: __________________________

Test for adventitious agents (if applicable)
Method: __________________________
Specification: __________________________
Date: __________________________
Result: __________________________

Control of purified antigen bulk
Lot number of purified bulk: __________________________
Date of purification: __________________________
Volume(s), storage temperature, storage time
   and approved storage period: __________________________

Purity
Method: __________________________
Specification: __________________________
Date: ____________________________________________
Result: __________________________________________

**Protein content**
Method: ____________________________________________
Specification: _______________________________________
Date: ____________________________________________
Result: __________________________________________

**Antigen content**
Method: ____________________________________________
Specification: _______________________________________
Date: ____________________________________________
Result: __________________________________________

**Sterility test for bacteria and fungi**
Method: ____________________________________________
Media: _____________________________________________
Volume inoculated: ___________________________________
Date of start of test: _________________________________
Date of end of test: _________________________________
Result: __________________________________________

**Percentage of intact monomer**
Method: ____________________________________________
Specification: _______________________________________
Date: ____________________________________________
Result: __________________________________________

**Particle size and morphology**
Method: ____________________________________________
Specification: _______________________________________
Date: ____________________________________________
Result: __________________________________________

**Test for reagents used during purification or other phases of manufacture (if relevant)**
Method: ____________________________________________
Specification: _______________________________________
Date: ____________________________________________
Result: __________________________________________
Test for residual host-cell protein (if relevant)
Method: 
Specification: 
Date: 
Result: 

Test for residual host-cell DNA (if applicable)
Method: 
Specification: 
Date: 
Result: 

Test for viral clearance (if relevant)
Method: 
Specification: 
Date: 
Result: 

Control of adsorbed antigen bulk (if applicable)
Lot number of adsorbed antigen bulk: 
Date of adsorption: 
Volume(s), storage temperature, storage time and approved storage period: 

Sterility test for bacteria and fungi
Method: 
Media: 
Volume inoculated: 
Date of start of test: 
Date of end of test: 
Result: 

Bacterial endotoxin
Method: 
Specification: 
Date: 
Result: 

Identity test
Method: 
Specification: 
Annex 2

Date: ____________________________
Result: __________________________

**Adjuvant content**
Method: __________________________
Specification: ______________________
Date: ____________________________
Result: __________________________

**Degree of adsorption**
Method: __________________________
Specification: ______________________
Date: ____________________________
Result: __________________________

**pH**
Method: __________________________
Specification: ______________________
Date: ____________________________
Result: __________________________

**Antigen content**
Method: __________________________
Specification: ______________________
Date: ____________________________
Result: __________________________

**Control of final bulk**
Identification (lot number): ____________________________
Date of manufacture/blending: __________________________
Volume(s), storage temperature, storage time
and approved storage period __________________________

<table>
<thead>
<tr>
<th>Blending:</th>
<th>Prescription (SHD)</th>
<th>Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEV antigen (mg):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjuvant:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preservative (specify):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others (salt):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final volume (ml):</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Sterility tests for bacteria and fungi

Method: ________________________________
Media: ________________________________
Volume inoculated: ____________________
Date of start of test: __________________
Date of end of test: ____________________
Result: ______________________________

Adjuvant content

Method: ________________________________
Specification: _________________________
Date: ________________________________
Result: ______________________________

Degree of adsorption

Method: ________________________________
Specification: _________________________
Date: ________________________________
Result: ______________________________

Preservative content

Method: ________________________________
Specification: _________________________
Date: ________________________________
Result: ______________________________

Potency test

In vivo assay (may be performed at final bulk stage)

Species, strain, sex and weight specifications: ________________________________
Number of mice tested: ________________________________
Dates of vaccination, bleeding: ________________________________
Date of assay: ________________________________
Lot number of reference vaccine and assigned potency: ________________________________
Vaccine doses (dilutions) and number of animals responding at each dose: ________________________________
ED$_{50}$ of reference and test vaccine: ________________________________
Potency of test vaccine (with 95% fiducial limits): ________________________________
If an in vitro assay is used
   Method:  
   Specification:  
   Date:  
   Result:  

Osmolality test
   Method:  
   Specification:  
   Date:  
   Result:  

Control of final lot
Lot number:  
Date of filling:  
Type of container:  
Filling volume:  
Number of containers after inspection:  
Number and percentage of containers rejected:  

Appearance
   Method:  
   Specification:  
   Date:  
   Result:  

Identity test
   Method:  
   Specification:  
   Date:  
   Result:  

Sterility tests for bacteria and fungi
   Method:  
   Media:  
   Volume inoculated:  
   Date of start of test:  
   Date of end of test:  
   Result:  
Osmolality test
Method: 
Specification: 
Date: 
Result: 

pH
Method: 
Specification: 
Date: 
Result: 

Preservative content
Method: 
Specification: 
Date: 
Result: 

Test for pyrogenic substances
Method: 
Specification: 
Date: 
Result: 

Adjuvant content
Method: 
Specification: 
Date: 
Result: 

Extractable volume
Method: 
Specification: 
Date: 
Result: 

Degree of adsorption
Method: 
Specification: 
Date: 
Result: 
**Potency test**

*In vivo assay (may be performed at final bulk stage)*

Species, strain, sex and weight specifications: 

Number of mice tested: 

Dates of vaccination, bleeding: 

Date of assay: 

Lot number of reference vaccine and assigned potency: 

Vaccine doses (dilutions) and number of animals responding at each dose: 

ED$_{50}$ of reference and test vaccine: 

Potency of test vaccine (with 95% fiducial limits): 

*If an in vitro assay is used*

Method: 

Lot number of reference and assigned potency: 

Specification: 

Date: 

Result: 

**Certification by the manufacturer**

Name of Head of Quality Control (typed) 

*Certification by the person from the control laboratory of the manufacturing company taking overall responsibility for the production and quality control of the vaccine.*

I certify that lot no. of recombinant hepatitis E vaccine, whose number appears on the label of the final containers, meets all national requirements and/or satisfies Part A$^1$ of the WHO Recommendations to assure the quality, safety and efficacy of recombinant hepatitis E vaccines.$^2$

Signature 

Name (typed) 

Date 

---

$^1$ With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.

Certification by the NRA

If the vaccine is to be exported, attach the NRA Lot Release Certificate (as shown in Appendix 2), a label from a final container and an instruction leaflet for users.
Appendix 2

Model NRA Lot Release Certificate for recombinant hepatitis E vaccines

Certificate no. ________________________

The following lot(s) of recombinant hepatitis E vaccine produced by _______ in ________________, whose lot numbers appear on the labels of the final containers, meet all national requirements and Part A of the WHO Recommendations to assure the quality, safety and efficacy of recombinant hepatitis E vaccines and comply with WHO good manufacturing practices for pharmaceutical products: main principles, WHO good manufacturing practices for biological products and Guidelines for independent lot release of vaccines by regulatory authorities.

The release decision is based on ________________________________

The certificate may include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
- type of container used;
- number of doses per container;

---

1 Name of manufacturer.
2 Country of origin.
3 If any national requirements have not been met, specify which one(s) and indicate why the release of the lot(s) has nevertheless been authorized by the NRA.
4 With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.
9 Evaluation of the summary protocol, independent laboratory testing and/or procedures specified in a defined document, and so on as appropriate.
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date) and expiry date;
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate;
- certificate number.

The Director of the NRA (or other appropriate authority)

Name (typed) __________________________
Signature __________________________
Date __________________________