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

Proposed new recommendations
(First draft-version public consultation)

NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the Recommendations to assure the quality, safety and efficacy of recombinant hepatitis E vaccines to a broad audience and to improve transparency of the consultation process.

These Recommendations were developed based on the outcomes and consensus of the WHO working group meeting convened in 2017 with participants from national regulatory authorities, national control laboratories, vaccine manufacturers and academia researchers.

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee on Biological Standardization. Written comments proposing modifications to this text MUST be received by 12 February 2018 in the Comment Form available separately and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Department of Essential Medicines and Health Products (EMP). Comments may also be submitted electronically to the Responsible Officer: Dr Dianliang Lei at email: leid@who.int.

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide" (WHO/IMD/PUB/04.1).
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The named authors [or editors as appropriate] alone are responsible for the views expressed in this publication.
Recommendations published by 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.

Introduction

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

The WHO Strategic Advisory Group of Experts (SAGE) on immunization issued a position paper on hepatitis E 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 (ORF 2) capsid protein manufactured in E. coli using recombinant technology. The WHO Global Advisory Committee on Vaccine Safety (GACVS) reviewed this same hepatitis E vaccine in 2014 and concluded that it had an acceptable safety profile (2). In 2016 the 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 document highlights the need for some urgency in addressing all viral hepatitis, but particularly for hepatitis E for which there is only one vaccine approved anywhere in the world.

There have been requests to the WHO from manufacturers and other stakeholders to develop recommendations to ensure the quality, safety and efficacy of hepatitis E vaccines. In response, a series of meetings were convened by WHO to review the current status of development and likely time to licensure of recombinant hepatitis E 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.

Scope

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

The scope of the present document encompasses recombinant hepatitis E vaccines for prophylactic use based on the ORF 2 capsid protein.

This document should be read in conjunction with other relevant WHO guidance, especially those on the nonclinical (5) and clinical (6) evaluation of vaccines. Other WHO guidance should 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, on the characterization of cell banks (7) and on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (8).

General considerations

Hepatitis E virus

HEV is a non-enveloped positive-sense RNA virus of the Hepeviridae family. The single stranded viral genome is 7.2kb in length and contains 3 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 initially classified into four genotypes (genotype 1 to 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. In 2017 a further four genotypes were described, of which genotype 7 may be significant in human infections. Each genotype has been sub-classified into multiple subtypes (10).

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 geographic location, based primarily on differences in circulating HEV genotypes (11-13). A global burden of disease study estimated that HEV genotypes 1 and 2 account for approximately 20.1 million HEV infections, 3.4 million symptomatic cases, 70 000 deaths, and 3000 stillbirths annually (14).
Hepatitis E infection due to genotypes 1 and 2 has been identified in at least 63 countries, of which about half have reported large outbreaks (14). The overall burden of disease due to hepatitis E is greatest in low and middle income countries (LMICs), especially where clean drinking water is scarce, such as Asia and Africa, as faecal contamination of drinking water is a major route of HEV transmission (14). Large outbreaks of hepatitis E have not been reported from developed countries although small clusters of cases associated with foodborne transmission have occurred in Europe and Japan (15). There are also countries with no recorded sporadic disease or outbreak but where serologic 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 from 3 continents (Asia, Africa and North America [Mexico]); these outbreaks were caused mostly by HEV genotype 1. Large waterborne hepatitis E outbreaks frequently occur in the Indian subcontinent (16). In Europe, North America and Australia, cases due to genotype 1 have been reported in returning travelers and their contacts. The distribution of HEV genotype 2 has been focal, with the majority of cases reported from Mexico, Nigeria, Namibia and a few other West African countries (17).

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 person to person, may contribute to the prolongation of outbreaks, particularly in displaced populations (18). Recent large outbreaks have occurred among displaced persons in Sudan, Chad, Niger and Uganda (17, 19-21). 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 <2 years of age (19); however, the cause of death in these children was not verified. As in the outbreak in northern Uganda in 2007, the South Sudan outbreak also started during the rainy season with high attack rates (7.4%) among camp residents and high mortality among pregnant women (10.4%) (20). A sero-survey conducted during this outbreak showed that more than half of residents had no evidence of recent or past HEV infection, suggesting that these persons remained uninfected. Both the Ugandan and South Sudanese outbreaks lasted well over a year, indicating that prevention and control efforts in such outbreaks can be challenging.

Although waterborne HEV outbreaks can result in large numbers of cases over a short period of time, most hepatitis E cases in LMICs likely occur as part of smaller clusters or result from sporadic transmission (22). The risk factors for sporadic hepatitis E are less well understood, although water contamination may play a role.

In industrialized countries, where the disease burden is much lower, zoonotic transmission, mainly through consumption of uncooked or undercooked meat, is a possible mode of transmission and HEV genotype 3 is the predominant genotype (12). Despite the ubiquity of HEV genotype 3 in the swine population, clinically apparent human infections with this genotype have been reported almost entirely in developed countries. The primary
determinants of this pattern of geographic distribution are unclear but could in part reflect
differences in surveillance for HEV cases, since few low-income countries have HEV
surveillance.

In recent years, HEV genotype 4 has been found to circulate in animals in India and China,
and has been found in several European countries; most human cases of hepatitis due to HEV
genotype 4 have been reported in mainland China and Taiwan. The mode of transmission of
HEV genotype 4 is also believed to be mainly through consumption of infected animal meat.

There is no evidence of sexual transmission of HEV (12). Transfusion transmission of HEV
occurs and is well documented; however its contribution to the overall disease burden is
limited (12, 23, 24).

Disease and diagnosis

HEV-infected persons exhibit a wide clinical spectrum, ranging from asymptomatic infection
through acute icteric hepatitis to fulminant hepatitis. In immunocompetent persons,
asymptomatic infection is common 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 increases with the age at the time of infection (25). The incubation
period ranges from 15 to 60 days, with a mean of 40 days (26). Infection with HEV genotype
1 is associated with serious disease more often than other genotypes, and the extent to which
such severe disease occurs with genotypes 2 and 4 is not well documented. Studies in
nonhuman primates have shown a relationship between the dose of viral inoculum and the
host’s immunological response and degree of liver injury (27).

In LMICs, where HEV genotypes 1 and to a lesser extent genotype 2 are the most commonly
identified causes of hepatitis E, the disease mainly affects young adults (aged 15–39 years),
with preponderance in males. During waterborne outbreaks, children may develop severe
hepatitis E due to co-infection with hepatitis A virus (28).

During epidemics, fulminant hepatitis E occurs at a disproportionately high rate among
pregnant women (29-31). The disease is typically most severe during the third trimester of
pregnancy (32, 33). While in the general population mortality from hepatitis E ranges from
0.1% to 4%, among women in the third trimester of pregnancy, it can range from 10% to 50%
(1, 32, 34, 35). The exact mechanism of the high mortality among pregnant women is unclear
(35). The causes of death include fulminant liver failure and obstetric complications
including excessive bleeding (30). During pregnancy, HEV is transmitted from mother to
fetus and results in poor fetal outcomes, including miscarriage, premature delivery, and
stillbirths (29, 16).

HEV genotypes 3 and 4 have been repeatedly reported to cause severe disease as well as
chronic hepatitis E in immunocompromised persons in Europe. Chronic infections do not
occur in otherwise healthy individuals. HEV infection in persons who receive
immunosuppressive treatment following solid organ or bone marrow transplantation and persons with severe immunodeficiency of other origins is associated with risk of progression to chronic hepatitis E (36). 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 (37-39). The clinical manifestation and progression of chronic hepatitis E (lasting >6 months) are variable; some cases progress to significant fibrosis in a relatively short period of time.

Persons with pre-existing chronic liver disease are prone to develop severe hepatitis following HEV infection. Those with advanced liver disease, including cirrhosis, may develop acute hepatic failure when infected with HEV (16). 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 detection of HEV-specific IgM antibodies, recent appearance or several-fold increase in titres of specific IgG antibodies or detection of HEV RNA in blood samples (40). Specific detection of HEV antigen also can be a marker for diagnosis of hepatitis E (41). However, the performance characteristics (sensitivity and specificitity) of currently available commercial assays for anti-HEV antibodies are suboptimal (42-44). In one study that compared 6 different assays, sensitivity of the individual assays ranged from 72% to 98%, and specificity from 78% to 96%; further, the kappa coefficients for agreement between results of various pairs of tests varied from 0.42 to 0.80 (45). This has implications for clinical trials based on serological outcomes and for studies that rely on these serologic tests to estimate burden of disease and previous history of infection. One recent study compared results from a newer diagnostic assay, to the results 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 (46).

**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 persons known to have had hepatitis E during the 1978 outbreak found that 47% had detectable anti-HEV IgG 14 years after infection (47), though the difficulties with interpreting serologic assay results has already been mentioned. A recent study based on 67 months’ serological following-up data and mathematical modelling suggested that naturally acquired anti-HEV IgG will remain detectable in half of the seropositive individuals until 14.5 years (48). In another follow-up study, 100% of persons had measurable anti-HEV IgG 5 years after infection (49). 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 other evidence that naturally-acquired infection does not confer life-long 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 life-long protection, and attack rates are highest among young to middle-aged adults, suggesting that infection during early life may not confer lifetime 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 HEV vaccines have been evaluated in virus challenge studies in non-human primates, up to mid-2017 there had been only one vaccine licensed for human use in any country. This vaccine was licensed in China in December 2011 for use from the age of 16 years. 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 (50-53). After three doses vaccine efficacy was 100% over a 12-month period after the last dose and was 95.5% over 19 months in all subjects who had received at least one dose. There are other vaccines based on HEV capsid protein which are currently in non-clinical or clinical development.

Terminology

The definitions given below apply to the terms as used in these WHO Recommendations. They 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 following terms are used in these recommendations - master cell bank (MCB): a bank of a cell substrate from which all subsequent cell banks used for vaccine production will be derived. The MCB represents a well characterized collection of cells derived from a single tissue or cell; and working cell bank (WCB): a cell bank derived by propagation of cells from an MCB under defined conditions and used to initiate production of cell cultures on a lot-by-lot basis. Also referred to as “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 adsorbed monovalent antigen bulks and may contain VLP antigens from one or multiple HPV types. If prepared from one or more monovalent 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.

**Pool**: a pool of a number of single harvests of the same virus type processed at the same time.

**Seed lot (master, working seed lot)**: a quantity of bacterial 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.

**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.

- **Anti-hepatitis E serum**, 50 units/ampoule, First reference reagent established in 1997 held at NIBSC (95/584), (BS97.1869).


The WHO Catalogue of International Reference Preparations should be consulted for the latest list of appropriate WHO International Standards and reference materials. (http://www.who.int/bloodproducts/catalogue/en/).

National reference for potency assay is under development by the National Institutes for Food and Drug Control, China.

**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 E vaccine is a sterile liquid vaccine preparation that contains purified recombinant capsid protein. Use of the international name should be limited to vaccines that meet the specifications elaborated below.

A.1.2 Descriptive definition
The recombinant hepatitis 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: main principles for pharmaceutical products (54) and Good manufacturing practices for biological products (55) should apply to the production of design, establishment, operation, control and maintenance of manufacturing facilities for recombinant hepatitis E vaccines.

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 (56, 57) and should be approved by 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 characteristics of the parental cells, including bacteria or eukaryotic cells if relevant, should be fully described. The recombinant production strain (parental cell transformed with the recombinant expression construct) should be fully described and information 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. 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, 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 provided. 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 (58).

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, such that the genotype and phenotype are maintained and clearly identifiable. Cell banks should be identified and fully characterized by 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 and approved by the NRA. 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 should be tested for the absence of bacterial and fungal contamination by appropriate tests, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (59), or by a method approved by the NRA, to demonstrate that only the bacterial production strain is present in the MCB and WCB.

A.3.1.2 Other expression systems

If other expression systems are used, characterisation may be based on the WHO Recommendations for the production of Human Papilloma Virus VLP vaccines (60).

A.4 Control of HEV protein production

A.4.1 Microbial purity

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 to feed cells or to induce production or increase 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 antigen 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 antigen 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. If tests for sterility and adventitious agents, as described below in sections A.4.4.2.2 and A.4.4.2.4, are not performed within 24 hours then the samples taken for these tests should be kept at a temperature of -60 °C or below and subjected to not more than one freeze–thaw cycle.

A.4.2.2.2 Sterility tests for bacteria, fungi and mycoplasmas

Harvests from bacterial expression systems could have bacterial contamination. Therefore, a method such as the microbial limits test might be appropriate for addressing culture 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 and fungal contamination by appropriate tests as specified in Part A. section 5.2 of the WHO General requirements for the sterility of biological substances (59).

A.4.2.2.3 Test for identity

Each harvest should be identified as HEV antigen by a suitable assay such as SDS-PAGE, ELISA or other methods. The virus protein may be wholly or partially purified by denaturation and renaturation before testing. The tests should be approved by the NRA. Alternatively, the identity can be confirmed as part of testing of the purified antigen.

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 dimers or 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.

The purified antigen bulk can be stored under conditions shown by the manufacturer to allow it to retain the desired biological activity. 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 HPLC may also be used for purity analysis.

A.5.1.2 Protein content

Each purified antigen bulk should be tested for the total protein content using a suitable method. Alternatively, the total protein content may be calculated from measurement of an earlier purified 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 below) by an appropriate method which is type specific. The ratio of antigen content to protein content may be calculated and monitored for each purified antigen bulk.

International standards and reference reagents for the control of HEV vaccine antigen content are not available. 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 (59), 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 above) may also serve to assess the integrity of the HEV monomer. This test could be eliminated with approval of NRA.

A.5.1.6 Particle size and structure

The protein is expected to aggregate to form particles whose size and structure should be established and monitored. The distribution of particle sizes should be determined. This test may be omitted for routine lot release 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 for routine lot release upon demonstration that the process consistently eliminates the reagent from the purified antigen bulks with the approval 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 by 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 (56).

These tests may be omitted for routine lot release 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 systems.

A.6 Control of adsorbed antigen bulk

In some cases the adsorbed antigen bulk may be filled directly without further dilution or addition of excipients in which case the adsorbed antigen bulk is the final lot.

A.6.1 Addition of adjuvant

The purified HEV antigen may be adsorbed onto an adjuvant such as an aluminium salt or other substance. 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 (59), or by an alternative 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 approval of the NRA.

A.6.3.3 Identity

Each adsorbed antigen bulk should be identified as the appropriate HEV antigen by 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 may be assayed for adjuvant content.

A.6.3.5 Degree of adsorption

The degree of adsorption (completeness of adsorption) of 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 may 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 purified antigen bulk it may be omitted from the testing of the adsorbed antigen bulk.

International standards and reference reagents for the control of HEV vaccine antigen content are not available. Therefore, product-specific reference preparations may be 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, this 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 manner as to avoid contamination of the product. In preparing the final bulk vaccine, any substances such as diluents, stabilizers or adjuvants that are added to the product 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 final bulk, unless otherwise specified, should be approved by the NRA. Where the antigen bulk is the final formulation as the tests below will be performed in Section A.6 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 (59), or by 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 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 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 or if performed on the final lot.

A.7.1.4 Preservative content

The final bulk may 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 with an appropriate in vivo or in vitro 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 for detection of antibodies in in vivo tests and the analysis of data should be approved by the NRA.
vaccine potency should be compared with that of a reference preparation; the NRA should
determine the limits of potency and approve the reference preparation used.

Manufacturers should establish a product-specific reference preparation that is traceable to a
lot of vaccine, or bulks used in the production of such a 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 acceptable procedure for replacing reference vaccines should be in place
(61, 62).

A.7.1.6 Osmolality

The osmolality of the final bulk may 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 the WHO Good manufacturing
practices for pharmaceutical products and for biological products (54, 55) 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,
unless otherwise specified, should be approved by the NRA.

A.9.1 Inspection of containers

Every container in each final lot should be inspected visually or mechanically, and those
showing abnormalities (e.g. improper sealing, clumping or the presence of particles) should
be discarded and recorded for each relevant abnormality. A limit should be established for the
percentage of containers rejected.

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 (59), or by 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 approval 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 amebocyte 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 Protein/antigen content

The protein/antigen content should be determined.

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 approval of the NRA.

A.9.11 Potency

A potency test should be carried out on each final lot as outlined in Part A, section A.7.1.5, if such a test has not been performed on the final bulk.

A.9.12 General safety (innocuity) test

The need to test the final lots of the HEV vaccine for unexpected toxicity (also known as abnormal toxicity) should be agreed with the NRA.

Some countries no longer require this test (63).

A.10 Records

The requirements given in the WHO Good manufacturing practices for pharmaceutical products and for biological products (54, 55) should apply.

A.11 Retained samples

The requirements given in the WHO Good manufacturing practices for pharmaceutical products and for biological products (54, 55) should apply.

A.12 Labelling

The requirements given in the WHO Good manufacturing practices for pharmaceutical products and for biological products (54, 55) should apply, with the addition of the following information.

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 potency per dose;
- the number of doses, if the product is issued in a multiple-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;
- any special dosing schedules.
A.13 Distribution and transport

The requirements given in the WHO Good manufacturing practices for pharmaceutical products and for biological products (54, 55) should apply. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (64).

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 (65). 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 adsorbed antigen bulks.

Accelerated stability tests may be undertaken to give additional information on the overall characteristics of a vaccine, and may also aid in assessing comparability when the manufacturer plans 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 (65). 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 vaccine should be ensured that the minimum 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 in a controlled temperature chain for a defined period of time, subject to approval by the NRA.

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, 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

Nonclinical evaluation of hepatitis E vaccines should be based on the WHO guidelines on nonclinical evaluation of vaccines (5). Details of the design, conduct, analysis and evaluation of nonclinical studies are available in the WHO guidelines (5). Further guidance on the general principles for nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines can be found in a separate WHO guidelines document (8).

Prior to 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 that are based on the ORF2-encoded viral capsid protein.

B.1 Strategy for cloning and expressing the gene product

The viral genome contains 3 open reading frames. Of these, ORF2 codes for the viral capsid protein which is the target of neutralizing antibodies against HEV (66-68).

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: (i) the construction, genetics and structure of the expression vector; (ii) the origin and identification of the gene that is being cloned; and (iii) potential retrovirus-like particles in, and genetic markers for, mammalian cell-based expression systems. The physiological measures used to promote and control the expression of the cloned gene in the host cell should be described in detail.

Data should be provided to demonstrate the stability of the expression system during storage of the WCB and 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 instance,
involving rearrangements, deletions or insertions of nucleotides – must be documented. The
NRA should approve 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 sodium dodecyl sulfate polyacrylamide gel electrophoresis
(SDS-PAGE), MALDI-TOF, HPSEC and binding activity to monoclonal antibodies.
Immunogenicity of the protein should be analyzed in an appropriate animal.

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
Immunogenicity of the vaccine should be evaluated in relevant animal species that respond
well to vaccine antigen (e.g., rodent, rabbit, swine, or non-human primates) (69).
Immunogenicity data can provide initial insights into immunological characteristics of the
vaccine antigen and are useful for evaluation of the vaccine formulation and underlying
protective mechanisms and justification for including an adjuvant in a vaccine.

The nonclinical passive immunization studies in non-human primates (NHPs) and human
epidemiology studies indicate that the humoral immunity is likely the primary effector
mechanism that directly mediates protection against HEV. Clear correlation between the
serum IgG responses to vaccine antigen and protection has indeed been demonstrated in
clinical (70) and nonclinical (71) studies conducted with recombinant hepatitisE capsid-based
vaccines. On this basis, it is recommended that evaluation of vaccine immunogenicity should
include 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
as per intended posology. For an adjuvanted vaccine, the advantage of adjuvant included in vaccine should be demonstrated by 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. Several animal models are known to be experimentally permissive to infection by human HEV (69), including swine (genotypes 3 and 4), rabbit (genotype 4), and different species of NHPs such as cynomolgus macaques (genotypes 1 and 2) and rhesus macaques (genotypes 1-4). The challenge studies conducted in NHP demonstrated protective immunity of the hepatitis E vaccines, which are subsequently proven to be efficacious in humans (51, 70). The susceptibility of NHP to human genotypes 1 and 2 makes it a primary model for challenge studies with these two HEV genotypes.

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

The design of challenge studies may vary, depending on platforms with which vaccine is produced. Vaccination of animals is usually conducted as per the intended posology, and challenge of animals pursued at the time when vaccinated animals develop peak protective responses. In general challenge via intravenous route is acceptable because 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 endpoints used to define protection should be specified in study protocol and should include:

- Infection marker such as HEV RNA in stool and serum at serial time points, and/or
- Hepatitis of histopathological evidence using liver biopsy, and
- Biochemical parameter of ALT change at serial time points.

In addition, passive immunization studies in animal models involve passive transfer of human vaccinee’s antisera to naïve animals, followed by HEV virus challenge might be useful for estimation of a specific IgG titer associated with protection.

B.3.3 Cross-neutralisation protection of different genotypes

The genetic differentiation of HEV strains is determined by 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 (genotype 1 to 4). These four genotypes share a single serotype based on their immuno reactivity. Therefore, HEV vaccine with recombinant pORF2s derived from a given genotype is expected to provide cross-genotype protection against all 4 genotypes. Results from preclinical and clinical studies have substantiated this notion. In preclinical animal models, the same protection was observed on animals challenged with different genotypes of HEV after immunization of recombinant
pORF2 derived from distinct genotypes. Purcell et al. (72) and Li et al. (50) demonstrated that immunization with recombinant pORF2 derived from genotype 1 HEV was able to protect against genotype 1, 2, 3 and 4 HEV infection in HEV NHP models. The recombinant pORF2 derived from genotype 4 HEV provided cross protective effects in genotype 1 and 4 HEV infection on a non-human primate model (73). More recently, results from an HEV rabbit model suggested that recombinant capsid proteins derived from genotype 1 cross protects against genotype 4 HEV infection (74, 75).

Furthermore, in clinical trials conducted in China and in Nepal, recombinant pORF2 protein vaccine derived from genotype 1 HEV sequences showed protection for acute hepatitis caused by genotype 1 and 4 HEV infection (52, 70, 75).

From the biochemical analysis, a cross-genotype and neutralizing epitope, as recognized by mAb 8G12, was identified between genotype 1 and 4 HEV using the recombinant capsid proteins (76). The mAb 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, partially, rationalize the cross-genotype protection of vaccines with antigens derived from a given type (such as based on genotype 1).

To date, clinical data on cross-protection from completed clinical trials with recombinant HEV capsid-based vaccines are still limited. Potency of different vaccines may differ and the degree of cross-protection may be vaccine specific. Therefore, if cross-protection is claimed, challenge studies should be conducted in appropriate animal models to evaluate cross-protection against heterologous viruses.

B.3.4 Biodistribution studies

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

B.4 Toxicology studies

Toxicology studies should be undertaken in accordance with the WHO guidelines on nonclinical evaluation of vaccines (5). Such studies should be performed with 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 the GLP principles, prior to initiation of early human clinical trials. Because the target population for the hepatitis E vaccines includes women of childbearing age, GLP-compliant reproductive and developmental toxicity studies are also required at a late stage of clinical development.

In general, toxicity evaluation in one relevant species is justified. The route and dosing regimen should reflect the intended clinical use. For evaluation of developmental toxicity, dosing regimen should consider one or two doses prior to mating so that pregnant animals
and embryo and fetus 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 recommendation in WHO Guidelines on nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines should be followed (8).

If a novel cell substrate (i.e. 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 (77). General expectations for vaccine clinical development programmes, including the assessment of safety and immunogenicity, approaches to dose-finding and the design of efficacy trials are covered in detail in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (78) and are 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 section C.3); and
- Serological and virus detection assays for the laboratory confirmation of acute HEV infection in vaccine efficacy trials (see section C.4).

Sponsors should also consult section 5.3.3 of the Guidelines on clinical evaluation of vaccines: regulatory expectations (78).

C.2.1 Serological assays
C.2.1.1 Functional antibody
Currently there is no well-established assay for measuring anti-HEV virus neutralizing antibody. There is no efficient HEV cell infection model so that a direct measurement of anti-HEV neutralizing antibody is not feasible. Neutralizing antibody has been estimated using methods such as real-time PCR or an immunofluorescence foci assay (IFA) to detect virus but these methods are not standardised 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 the IFA and with anti-HEV IgG using sera from HEV-infected and vaccinated macaques (79).

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 at least the same as, or a truncated version of, that in the vaccine. It is recommended that the quantitation of anti-HEV IgG should be referenced to the WHO standard (80) sera 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 purposes of detecting acute infection with HEV there are commercial assays available to detect 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 (e.g., 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 for detection). Comparative studies have shown that there are considerable differences in the sensitivity and specificity of commercially available assays, with even more variability for IgM compared to IgG assays (81, 82). The assays used to detect and quantify anti-HEV antibody in suspected cases of HEV 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 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 Standards (genotype 3a and 3b strains; (83). Assays with different targets (e.g., which 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 (84), 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

HEV vaccines will be used mainly or exclusively in regions with relatively high rates of clinically apparent infections but 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 HEV 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-naïve subjects. Therefore, it is important that the primary series is selected based on 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, 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 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 re-vaccination

In the absence of an immune correlate of protection, it is recommended that the possible need for re-vaccination is not based solely on waning antibody levels. There should be planned long-term follow-up for HEV 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 re-vaccination 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-randomised to receive further doses at pre-defined intervals after completion of a primary series. The immune responses to additional doses could be compared with the post-primary responses 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 HEV vaccine to protect against a range of wild-type strains covering the four main genotypes of HEV may vary depending on the vaccine construct and it is important that this is investigated in nonclinical studies (see section B.3.3.3).

In clinical trials in which vaccine-elicted 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 HEV. If there are marked differences observed in IgG antibody when measured using vaccine vs. 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 conduct efficacy trials.

C.3.2 Special populations

Thus far, the efficacy of hepatitis E vaccination has been demonstrated in healthy adults aged between 16 and 65 years, most of whom were aged less than 45 years. There may be interest in the use of HEV vaccines in younger age groups, in subjects at particular risk of developing severe or fulminant hepatitis (e.g. during pregnancy and in pre-existing liver disease) and in 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 to extend its use to various special populations based on safety and immunogenicity data. For example:

- In much of the world the peak age incidence for a clinically apparent primary infection is in early or mid-adulthood. 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 use of the adult regimen or alternative dose regimens in specific paediatric age sub-groups.

- Section 5.6.4 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (78) discusses the evaluation of the safety and immunogenicity of vaccines during pregnancy. Protection against hepatitis E disease by vaccination during pregnancy, as opposed to vaccinating women before or between pregnancies, would require the development of a vaccine that is able to elicit antibody levels that are likely to be protective (e.g. 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 the condition. Vaccine regimens should be supported by immune responses documented in specific subgroups that are representative of the intended target populations.

C.4 Efficacy

C.4.1 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 this guidance there is one vaccine against hepatitis E that is licensed in one country (See General Considerations)(51, 52);

- The licensed vaccine is not widely used and it is not included in national immunisation programmes so that 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 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 a direct comparison of immune responses can be conducted. 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 randomised into the trial). 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 Guidelines on clinical evaluation of vaccines: regulatory expectations (78).

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 (i.e. symptomatic) HEV infection of any genotype (see section C.4.2.4).

- It is not required that efficacy is shown against asymptomatic HEV infection. With the exception of immunodeficient subjects, who may develop chronic infection with possible sequelae, asymptomatic infection is not of any clinical significance.

- It is not required that vaccine efficacy trials are 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 based on available public health disease surveillance data and/or on pre-trial evaluations of epidemiology conducted by the sponsor. In two prior efficacy trials (51-53, 70) HEV genotypes that caused clinically apparent infections in the control (placebo) groups were limited in accordance with strains circulating at 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

Based on the peak age incidence of hepatitis E it is likely that vaccine efficacy trials will target 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:

- Enrolling adults 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 the 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 who are considered to be seronegative based on a threshold determined by the assay used. This may allow for a smaller sample size to be randomised into the trial due to an expected higher attack rate but 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 endpoint

In accordance with the recommended primary objective, the primary endpoint 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.

Clinical features for the case definition

The clinical features that trigger subjects to present to study site staff or to a local designated healthcare facility for laboratory investigations for acute hepatitis E should be selected to capture 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 of malaise, fatigue, anorexia or 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.

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
collection 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 alanine aminotransferase (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 an elevated total serum bilirubin in the first sample.

The confirmation of HEV as causative of the clinical picture may be based on one or more of:
- 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, with sampling continuing for up to 12 weeks if HEV is not confirmed by other
  parameters;
- Detection of HEV RNA in blood or in stool, which are usually short-lived, but may
  persist for longer in stool compared to blood.

To avoid missing cases, protocols should plan for appropriately timed repeat specimens (e.g.
at 2-6 weeks after the first sample) in those 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, repeat testing is conducted after a short
interval.

Samples obtained at first presentation should also be tested to detect acute infection or
establish co-infection with other hepatitis viruses that can cause the same clinical picture,
including:
- IgM against hepatitis A virus
- Hepatitis B surface antigen and core antigen
- Antibody against hepatitis C

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
are also retested for evidence of hepatitis A, B and C to document any possible co-infections
(the most likely co-infecting virus being hepatitis A due to similar modes of transmission).

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 or not there is evidence of co-infection with other
hepatitis viruses, as follows:
• In subjects who completed the vaccination series within pre-determined visit windows, if more than one dose is required; and
• With symptom onset 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 there are pre-defined secondary analyses that 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 randomised and regardless of adherence to study visit windows;
- Cases that occurred at any time after the last dose received (i.e. 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, there may be 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 co-infection with other hepatitis viruses, whether acute (most likely co-infection with hepatitis A) or chronic (i.e. 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 pre-defined timeframe in which a sufficient number of cases is 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. If longer-term follow-up within such a trial is not considered to be feasible then 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.

C.5 Safety
The evaluation of the safety of candidate hepatitis E vaccines should be in accordance with the recommendations made in section 7 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (78). If the primary series consists of several 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 some special considerations for vaccine safety depending on the vaccine construct (e.g. in the case of a live attenuated or live viral vectored vaccine or use of a new adjuvant) and the intended target population (e.g. 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 in immunogenicity trials did not give rise to any major concerns, it may be acceptable that a full assessment of safety (i.e., including detailed documentation of local and systemic reactogenicity as well as all unsolicited adverse events) could be confined to a randomised 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 (85) and Guidelines for independent lot release of vaccines by regulatory authorities (86) should apply. These recommendations specify that no new biological substance should be released until consistency of batch 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 (87). For control purposes, the relevant International Standards currently in force should be obtained for the purpose of calibrating national, regional and working standards (88). 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 (86).

A protocol for the manufacturing and control of recombinant hepatitis E vaccines, based on the model 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

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The second draft was prepared by the drafting group after public consultation with incorporate the comments received from public consultation into the text as appropriate. ……

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47. Khuroo MS. Seroepidemiology of a second epidemic of hepatitis E in a population that had recorded first epidemic 30 years before and has been under surveillance since then. Hepatol Int. 2010 Feb 3;4(2):494–499.


84. additional genomic sequencing, which published data suggest may be targeted to a specific region (78), is currently required to determine the HEV (sub)-genotype


Appendix 1. Model summary protocol for the manufacturing and control of recombinant hepatitis E vaccines

The following 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 approval of the NRA.

It is possible that a 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/should 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/should 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: ______________________
Products license (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**
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**

Name of the culture medium: _______________________
Date of inoculation: _______________________
Temperature of incubation: _______________________

Control of bacterial purity

Methods: _______________________
Result: _______________________
Date: _______________________

Date of harvest: _______________________
Volume of harvest: _______________________
Yield (mg/ml): _______________________
Volume after filtration: ______________________

Bulk:

Final Bulk

Identification (Lot number): ______________________
Date of manufacture/blending: ______________________
Volume: ______________________
mg per ml: ______________________

<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>

Preservative content

Method: ______________________
Date of test: ______________________
Result: ______________________

Adjuvant content

Method: ______________________
Date of test: ______________________
Result: ______________________

Degree of adsorption?

Method: ______________________
Date of test: ______________________
Result: ______________________

Sterility test

Tests for bacteria and fungi

Method: ______________________
Media: ______________________
Number of containers tested: ______________________
Volume of inoculum per container: ______________________
Volume of medium per container: ______________________
Temperatures of incubation: _______________________

Date of start of test: _______________________

Date of end of test: _______________________

Result: _______________________

5

Potency test

8 Final bulk

9 Final lot

(To be completed as Part A is finalized)
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 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;
- 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 __________________________________________

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.
5 WHO Technical Report Series, No. 000, Annex 0.
9 Evaluation of the summary protocol, independent laboratory testing and/or procedures specified in a defined document etc., as appropriate.¹