Summary of Environmental Risk Assessment of Genetic Engineering Products
Vaxxitek HVT + IBD

I.Introduction

Vaxxitek HVT + IBD vaccine is a vaccine for the control and control of very virulent Infectious Bursal Disease (IBD) and Marek's Disease (MD) diseases in chickens. This vaccine is produced by Merial Select, Inc., USA, which will be distributed by PT Romindo Primavetcom. The content of this vaccine is the HVT + IBD virus (vHVT-013-069) which is a Genetic Modified microorganism Herpesvirus of Turkey (HVT) strain FC 126 with the insertion of the IBD VP2 virus gene. Vaxxitek HVT + IBD PRG vaccine is able to protect against two diseases at once, namely Marek and IBD.

Vaxxitek HVT + IBDV Genetic modified vaccine has been registered and obtained a Certificate of Free Sale in various countries such as Europe (2002), USA (2004), Brazil (2005), EMEA (2005), Argentina (2006), Elsavador (2006), Peru (2006), Venezuela (2006), Bolivia (2007), Columbia (2007), Guatemala (2007), Philippines (2007), Thailand (2007), Ukraine (2007), Canada (2008), Dominica (2008), Ecuador (2008), Marocco (2008), Mexico (2008), Russia (2008), and Malaysia (2013).

Based on Government Regulation No. 21 of 2005 concerning Biosafety of Genetically Engineered Products, and Regulation of the Minister of Environment No. 25 of 2012 concerning Guidelines for Preparation of Genetic Engineering Environmental Risk Analysis, the Biosafety Engineering Genetic Product (TTKH PRG) Technical Team has conducted an environmental safety assessment of Vaxxitek HVT + IBD PRG vaccine. The assessment is based on genetic information and environmental safety information which consists of natural hosts of the parents of GMP microorganisms, genetically engineered traits, genetic stability of PRG microorganisms, ability to spread microorganisms, and possible negative effects of GMO microorganisms for vaccines against the environment, as described below.

II. Information on Microorganisms

II.1. General Description of Microorganisms

The genetic modified microorganisms used to produce the Vaxxitek HVT + IBD vaccine were recombinant Herpes Virus of Turkey strain FC 126 inserted in the VP2 gene IBD virus strain 52/70 Faragher as a donor gene (Aly et al., 2012; Merial Select., 2006).

II.2. Information on Genetic Properties of Microorganisms

Genetic Modified microorganisms are an HVT strain of FC-126, not pathogenic to chickens and can even provide protection against MD. As a VP2 gene donor, the IBD virus strain 52/70 Faragher is inserted in the UL 55 position so it does not inactivate the HVT virus, but expresses VP2 protein. The number of copies of the gene inserted as many as 1 VP2 gene. This expression of the VP2 gene will induce an immune response to the IBD virus. (Bublot., 1999; Analytical Dossier Merial., 1999).

The recombinant HVT + IBD virus seed seed has been tested for genetic stability in vitro on the 10x Chicken Embryo Fibroblast (CEF) and in vivo with 5 and 9 x reverse pass in chicken Specific Pathogen Free (SPF). This can be seen from the test data listed in the report "In Vitro Genetic Stability of the HVT 013-69 Recombinant virus and Invoir Genetic stability of the HVT 013-69 Recombinant virus (Bublot. 1998a; Bublot. 1998b; Analytical Dossier Merial., 1999).

II.3. Genetic Construction Method
Making recombinant VP2 gene donor plasmids

Construction of Plasmid Insertions.
To insert the VP2 gene into the HVT genome an insertion plasmid was arranged for locus 1 intergen. Fragment I which is a BamHI-BamHI HVT genome fragment (FC 126 Strain) in a clone in the BamHI site from pBR322. The result of this clone is plasmid pRD069. The BamHI – EcoRI fragment (2,674 bp) from pRD069 in the sub-clone to pBS II-SK + (Stratagene Inc.) becomes pEL039. Plasmid pEL039 was digested with BamHI and PstI to produce fragment A (992 bp). The 417 bp fragment was obtained from PCR results on pEL039 plasmids using the EL102 Primer and Primer EL161, containing the SalI site. The PCR results were digested with PstI and SalI to produce fragments B (225 bp). Fragment A and fragment B are cloned into pBSII-SK + to produce pEL 077 (4,163 bp). The 923bp fragments obtained and the results of the pEL039 digestion with SacI and BglII were cloned into the SacI-BglII site from pBSII-SK + to pEL 083 (3,848 bp). The 707 bp fragment was obtained from the plasmid PCR pEL039 results by using EL147 Primer and EL162 Primer. The PCR results were cloned into the PCR II (Invitrogen) cloning vector system. BstBI-SalI Fragment (468 bp) was obtained from the results of the recombinant plasmid digestion and cloned to pEL 083 plasmid and produced a plasmid pEL 065 (4,133 bp). FragmenXhoI-SalI (832 bp) was obtained from the results of pEL077 digestion with XhoI and SalI enzymes and cloned to the XhoI-SalI site from pEL065 to produce the intergenic locus 1 insertion plasmid pEL079 (Bublot. 1999).

IE promoter cloning from MCMV

Plasmid pAMB 33 containing IE promoter from MCMV digested with PstI enzyme. The PstI-PstI Fragment (2,286 bp) was isolated and inserted into the pBSII-SK + vector to produce pCD004 (5,250 bp). The pCMVasm (Clontech) plasmid was digested with SalI and SmaI enzymes, producing 3,682 bp fragments containing the LacZ gene and the polyadenylated site of SV40. This fragment was inserted into the SalI-EcoRV site from pBSII-SK + to produce pCD 002 (6,622 bp). Plasmid pCD 004 was digested with HpaI and PstI enzymes to produce a fragment of 1.388 bp, which was then inserted into pCD002 plasmid into pCD009 plasmid (8.004 bp). This plasmid contains an IE promoter from MCMV followed by the LacZ gene and polyodenylation signal from SV40 (Bublot., 1999).

VP2 gene cloning IBD virus

Cloning of VP2 IBDV sequences was carried out by Merial in 1989/1990 in the form of recombinant pEL024 plasmid. The pBSII-SK + recombinant plasmid contains the entire open reading frame of VP2 (to 453 amino acids) flanked by the NotI tread. This IBDV VP2 gene sequence can be accessed from GenBank Accession Number D00869 (NCBI).

Construction of Cassettes for IBDV.

VP2 gene expression plasmid to become a pEL026 plasmid.

This plasmid was digested with XmnI, EcoRI and SalI enzymes to obtain EcoR-SalI fragments (2,442 bp). This fragment was inserted into pBSII-SK into pEL027 plasmid containing VP2 (HCMV) expression tapes, IE promoter, VP2 IBDV gene and SV40 polyadenylation signal (Bublot., 1999).βPlasmid pEL024 was digested with NotI enzyme to obtain the NotI – NotI fragment (1,405 bp) containing all the VP2 genes.

This fragment was inserted into the NotI site and the pCMV

Final donor plasmid construction

Plasmid pEL070 was digested with XmnI, EcoRI and SalI (3,037 bp) enzymes containing MCMV-IE / IBDV-VP2 expression tapes. This fragment was ligated with pEL079 plasmid to produce pEL098 (Bublot., 1999).

Making recombinant virus vHVT 013

The vHVT013 recombinant virus was produced from homologous recombination between plasmid pEL098 and cDNA HVT strain FC 126 through co-transfection into the CEF primary culture (Bublot., 1999).

II.4. Genetic Modification Character

Recombinant HVT + IBD virus is the FC-126 strain Herpesvirus of Turkey (HVT) inserted in the Infectious Bursal Disease (IBD) VP2 strain 52/70 Faragher virus. Genetic modification in the form of IBD VP2 virus insertion into the HVT genome does not affect HVT virulence as its parent and even the VP2 gene protein can protect against IBD infection. Recombinant HVT + IBD is

stable as evidenced by Southern blot and PCR testing after passage in vitro and in vivo (Merial Select, 2006).

II.5. Possible instability of inserted genes that can be transferred to other organisms. The VP2 gene which is inserted into the HVT + IBD recombinant virus is stable in accordance with the explanation contained in point II.2. To find out the possibility of transferring donor genes (VP2 gene) to other organisms, BLAST nucleotide analysis of the IBD VP2 virus strain 52/70 with access number D00869 was carried out. The results of the analysis show that the VP2 gene does not have homology with the sequences of other organisms' genes therefore the transfer of genes to other organisms is not possible.

II.6. Conclusion.
The gene of interest (VP2 gene from IBD virus) inserted into the HVT recombinant virus can be expressed well. This expression of heterologous proteins does not change the virulence properties of HVT and can even provide protection against Marek's disease and IBD. Recombinant HVT + IBD virus contains one copy of the genotypically stable VP2 gene and phenotype. There is no possibility of the spread / transfer of the VP2 gene which is inserted in PRG (HVT + IBD) microorganisms to other organisms.

III. Environmental Safety Information
III.1. Ability to Disseminate Microorganisms
Genetic Modified microorganisms in Vaxxitek HVT + IBD have the same properties as HVT viruses whose parents are not pathogenic and are not excreted in animal excreta so they do not pose a possibility of environmental risks (Goutebroze, 1998 97,361 Research Report Safety, Spread of Vaccinal and Parental Strains from Chicken to Chicken and 98,183 Safety, Spread of Vaccinal and Parental Strains from Chicken to Turkey).

III.2. Information on Host Coverage of Elderly GMO Virus Organisms for Vaccines
The viral parent of HVT + IBD PRG is the FC-126 HVT strain which belongs to the family of Herpesviridae and the subfamily Alphaherpesvirus. Natural hosts of HVT parents are chickens, quails and turkeys.

III.3. Information on Molecular Studies.
The genetic trait that is engineered is the insertion of the IBD VP2 virus gene 52/70 Faragher strain into HVT at UL 55 position. This insertion does not change the virulence properties of HVT and can even protect against Marek's disease and IBD. Recombinant HVT + IBD virus contains one copy of the genotypically stable VP2 gene and phenotype. The transfer of the VP2 gene that has been inserted into the HVT virus is not possible because there is no homology with sequences of the genomes of other organisms so that it is safe for the environment. Genetic modification can be analyzed by one of the Southern Blot methods and / or PCR. While the phenotype of recombinant can be characterized by the method of plaque for double immunofluorescence (Cleuziat, 1999 Phenotypic stability after 3 and 8 passages in vitro) (Cleuziat, 1999).

III.4. Possible negative impacts of vaccine PRG microorganisms on the environment. The BLAST analysis results show that the VP2 gene nucleotides do not have genome sequence homology with other organisms, so it is not possible to transfer these genes to other organisms. Vaxxitek HVT + IBD PRG vaccine does not form spores because the parents of this vaccine are included in the group of viruses. Vaxxitek HVT + IBD PRG virus is not excreted by vaccinated animals so that it does not have the potential to spread to non-target animals. Therefore there is no possibility of risks to the environment.

CONCLUSION

Vaxxitek HVT + IBD vaccine is environmentally safe because it does not risk spreading GMO microorganisms to the environment. This is because PRG microorganisms in the preparation of Vaxxitek HVT + IBD vaccine are not excreted from vaccinated animals into the environment. The phenotype and genotype of PRG microorganisms in the Vaxxitek HVT + IBD vaccine are stable.

TTKH Environmental Security PRG recommends that the proposed Vaxxitek HVT + IBD vaccine be environmentally safe.

If information is found that is not in accordance with current environmental safety data in the future, then the environmental safe status of the Vaxxitek HVT + IBD vaccine needs to be reviewed.

Furthermore, after being determined to be environmentally safe, but the product is proven to pose a risk to human and animal health, the applicant must take control and control measures and destroy Vaxxitek HVT + IBD vaccines in the territory of Indonesia.

The Vaxxitek HVT + IBD vaccine may not be used as a chicken vaccine until it has obtained a safe environmental permit.

REFERENCE

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Ringkasan Pengkajian Keamanan Lingkungan Produk Rekayasa Genetik

Vaxxitek HVT+IBD

I. Pendahuluan

Vaksin Vaxxitek HVT+IBD adalah vaksin untuk pengendalian dan penanggulangan penyakit *Infectious Bursal Disease* (IBD) dan *Marek's Disease* (MD) yang sangat virulen pada ayam. Vaksin ini diproduksi oleh Merial Select, Inc., USA, yang akan diedarkan oleh PT Romindo Primavetcom. Kandungan vaksin ini adalah virus HVT+IBD (vHVT-013-069) yang merupakan jasad renik PRG virus *Herpesvirus of Turkey* (HVT) *strain* FC 126 dengan sisipan gen VP2 virus IBD. Vaksin PRG Vaxxitek HVT+IBD mampu memproteksi terhadap dua penyakit sekaligus yaitu Marek dan IBD.

Vaksin PRG Vaxxitek HVT+IBDV telah terdaftar dan memperoleh *Certificate of Free Sale* di berbagai negara seperti Eropa (2002), USA (2004), Brazil (2005), EMEA (2005), Argentina (2006), Elsavador (2006), Peru (2006), Venezuela (2006), Bolivia (2007), Columbia (2007), Guatemala (2007), Philiphina (2007), Thailand (2007), Ukraina (2007), Canada (2008), Dominica (2008), Equador (2008), Marocco (2008), Mexico (2008), Rusia (2008), dan Malaysia (2013).

Berdasarkan Peraturan Pemerintah No. 21 Tahun 2005 tentang Keamanan Hayati Produk Rekayasa Genetik, dan Peraturan Menteri Lingkungan Hidup No. 25 Tahun 2012 tentang Pedoman Penyusunan Analisis Risiko Lingkungan Produk Rekayasa Genetik, maka Tim Teknis Keamanan Hayati Produk Rekayasa Genetik (TTKH PRG) telah melakukan pengkajian keamanan lingkungan vaksin PRG Vaxxitek HVT+IBD. Pengkajian didasarkan atas informasi genetik dan informasi keamanan lingkungan yang terdiri atas inang alami dari tetua jasad renik PRG, sifat genetik yang telah direkayasa, stabilitas genetik jasad renik PRG, kemampuan penyebaran jasad renik, dan kemungkinan dampak negatif jasad renik PRG untuk vaksin terhadap lingkungan, sebagaimana diuraikan dibawah ini.

II. Informasi Jasad Renik

II.1. Deskripsi Umum Jasad Renik

Jasad renik PRG yang digunakan untuk memproduksi vaksin Vaxxitek HVT+IBD adalah rekombinan *Herpes Virus of Turkey strain* FC 126 yang disisipi gen VP2 Virus IBD *strain* 52/70 Faragher sebagai gen donor (Aly et al., 2012; Merial Select., 2006).

II.2. Informasi Sifat Genetik Jasad Renik

Tetua jasad renik PRG adalah virus HVT *strain* FC-126, tidak patogen pada ayam bahkan dapat memberikan perlindungan terhadap penyakit MD. Sebagai donor gen VP2 adalah virus IBD *strain* 52/70 Faragher yang disisipkan di posisi UL 55 sehingga tidak menginaktivasi virus HVT, akan tetapi mengekspresikan protein VP2. Jumlah kopi gen yang disisipkan sebanyak 1 gen VP2. Ekspresi gen VP2 inilah yang akan menginduksi respon kekebalan terhadap virus IBD. (Bublot., 1999; Analytical Dossier Merial., 1999)

Master seed virus rekombinan HVT+IBD telah diuji stabilitas genetiknya secara *in vitro* pada *Chicken Embryo Fibroblast* (CEF) 10x pasase dan secara *in vivo* dengan 5 dan 9 x pasase balik pada ayam *Specific Pathogen Free* (SPF). Hal ini dapat terlihat dari data pengujian yang tercantum dalam laporan "*In Vitro Genetic stability of the* HVT 013-69 *Recombinant virus* dan

In Vivo Genetic stability of the HVT 013-69 *Recombinant virus* (Bublot., 1998a; Bublot., 1998b; Analytical Dossier Merial., 1999).

II.3. Metoda Konstruksi Genetik

Pembuatan rekombinan plasmid donor gen VP2

Konstruksi Plasmid Insersi

Untuk melakukan insersi gen VP2 ke dalam genom HVT disusun plasmid insersi untuk *locus* 1 *intergen*. Fragmen I yang merupakan fragmen *Bam*HI–*Bam*HI genom HVT (FC 126 Strain) di klon ke dalam *Bam*HI *site* dari pBR322. Hasil klon ini menjadi plasmid pRD069. Fragmen *Bam*HI–*Eco*RI (2.674 bp) dari pRD069 di *sub-clone* ke pBS II-SK+ (Stratagene Inc.) menjadi pEL039. Plasmid pEL039 didigesti dengan *Bam*HI dan *Pst*I untuk menghasilkan fragmen A (992 bp). Fragmen 417 bp diperoleh dari hasil PCR pada plasmid pEL039 dengan menggunakan Primer EL102 dan Primer EL161, mengandung *Sal*I *site*. Hasil PCR ini didigesti dengan *Pst*I dan *Sal*I untuk menghasilkan fragmen B (225 bp). Fragmen A dan fragmen B diklon ke dalam pBSII–SK+ untuk menghasilkan pEL 077 (4.163 bp). Fragmen 923bp yang diperoleh dan hasil digesti pEL039 dengan *Sac*I dan *Bgl*II diklon ke dalam *Sac*I–*Bgl*II *site* dari pBSII–SK+ menjadi pEL 083 (3.848 bp). Fragmen 707 bp diperoleh dari hasil PCR plasmid pEL039 dengan menggunakan Primer EL147 dan Primer EL162. Hasil PCR ini diklon ke sistem vektor kloning PCR II (Invitrogen). Fragmen *Bst*BI-*Sal*I (468 bp) diperoleh dari hasil digesti plasmid rekombinan ini dan diklon ke plasmid pEL 083 dan menghasilkan plasmid pEL 065 (4.133 bp). Fragmen*Xho*I-*Sal*I (832 bp) diperoleh dari hasil digesti pEL077 dengan enzim *Xho*I dan *Sal*I dan diklon ke *Xho*I–*Sal*I *site* dari pEL065 untuk menghasilkan lokus intergen 1 plasmid insersi pEL079 (Bublot., 1999).

Kloning promotor IE dari MCMV

Plasmid pAMB 33 yang mengandung promotor IE dari MCMV didigesti dengan enzim *Pst*I. Fragmen *Pst*I-*Pst*I (2.286 bp) diisolasi dan diinsersikan ke vektor pBSII-SK+ untuk menghasilkan pCD004 (5.250 bp). Plasmid pCMVβ (Clontech) didigesti dengan enzim *Sal*I dan *Sma*I, menghasilkan fragmen 3.682 bp yang mengandung gen *Lac*Z dan tapak poliadenilasi dari SV40. Fragmen ini diinsersikan ke tapak *Sal*I-*Eco*RV dari pBSII-SK+ untuk menghasilkan pCD 002 (6.622 bp). Plasmid pCD 004 didigesti dengan enzim *Hpa*I dan *Pst*I untuk menghasilkan fragmen sebesar 1.388 bp, yang kemudian diinsersikan ke plasmid pCD002 menjadi plasmid pCD009 (8.004 bp). Plasmid ini mengandung promotor IE dari MCMV diikuti dengan gen LacZ dan signal poliodenilasi dari SV40 (Bublot., 1999).

Kloning Gen VP2 virus IBD

Kloning sekuen VP2 IBDV sudah dilakukan oleh Merial tahun 1989 / 1990 dalam bentuk plasmid rekombinan pEL024. Plasmid rekombinan pBSII-SK+ mengandung seluruh *open reading frame* VP2 (menjadi 453 asam amino) yang diapit oleh tapak *Not*I. Sekuen gen VP2 IBDV ini dapat diakses dari *GenBank Accession Number* D00869 (NCBI).

Konstruksi Kaset ekspresi gen VP2 IBDV

Plasmid pEL024 didigesti dengan enzim *Not*I untuk memperoleh fragmen *Not*I–*Not*I (1.405 bp) yang mengandung seluruh gen VP2. Fragmen ini diinsersi ke tapak *Not*I dan plasmid pCMVβ

untuk menjadi plasmid pEL026. Plasmid ini didigesti dengan enzim *Xmn*I, *Eco*RI dan *Sal*I untuk memperoleh fragmen *Eco*R-*Sal*I (2.442 bp). Fragmen ini diinsersi ke dalam pBSII-SK menjadi plasmid pEL027 yang mengandung kaset ekspresi VP2 (HCMV), IE promotor, gen VP2 IBDV dan signal poliadenilasi SV40 (Bublot., 1999).

Konstruksi plasmid donor final
Plasmid pEL070 didigesti dengan enzim *Xmn*I, *Eco*RI dan *Sal*I (3.037 bp) yang mengandung kaset ekspresi MCMV-IE/IBDV – VP2. Fragmen ini diligasi dengan plasmid pEL079 untuk menghasilkan pEL098 (Bublot., 1999).

Pembuatan virus rekombinan vHVT 013
Virus rekombinan vHVT013 dihasilkan dari rekombinasi *homologous* antara plasmid pEL098 dengan cDNA HVT *strain* FC 126 melalui *co-transfection* ke dalam kultur primer CEF (Bublot., 1999).

II.4. Karakter Modifikasi Genetik

Virus HVT+IBD rekombinan adalah virus *Herpesvirus of Turkey* (HVT) *strain* FC-126 yang disisipi gen VP2 virus *Infectious Bursal Disease* (IBD) *strain* 52/70 Faragher. Modifikasi genetik yang berupa penyisipan gen VP2 virus IBD ke genom HVT tidak mempengaruhi virulensi HVT sebagai tetuanya bahkan protein gen VP2 tersebut dapat memproteksi terhadap infeksi IBD. Rekombinan HVT+IBD bersifat stabil yang dibuktikan dengan uji Southern blot dan PCR setelah pasase secara *in vitro* dan *in vivo* (Merial Select., 2006).

II.5. Kemungkinan terjadinya ketidakstabilan gen yang disisipkan sehingga dapat dipindahkan ke organisme lain.

Gen VP2 yang disisipkan ke dalam virus rekombinan HVT+IBD bersifat stabil sesuai dengan penjelasan yang terdapat pada butir II.2. Untuk mengetahui kemungkinan pemindahan gen donor (gen VP2) ke organisme lain, maka telah dilakukan analisis BLAST nukleotida dari gen VP2 virus IBD strain 52/70 dengan nomor akses D00869. Hasil analisis menunjukkan bahwa gen VP2 tersebut tidak memiliki homologi dengan sekuen gen organisme lainnya oleh karena itu pemindahan gen ke organisme lainnya tidak memungkinkan.

II.6. Kesimpulan.

Gen interes (gen VP2 dari virus IBD) yang disisipkan ke dalam virus rekombinan HVT dapat diekspresikan dengan baik. Ekspresi protein heterolog ini tidak merubah sifat virulensi HVT bahkan dapat memberikan perlindungan terhadap penyakit Marek dan IBD. Virus rekombinan HVT+IBD mengandung satu kopi gen VP2 yang bersifat stabil secara genotip dan fenotip. Tidak ada kemungkinan penyebaran / pemindahan gen VP2 yang disisipkan dalam jasad renik PRG (HVT+IBD) ke organisme lain.

III. Informasi Keamanan Lingkungan

III.1.Kemampuan Penyebaran Jasad Renik

Jasad renik PRG dalam Vaxxitek HVT+IBD mempunyai sifat yang sama dengan virus HVT tetuanya yaitu tidak patogen dan tidak diekskresikan ke dalam ekskreta hewan sehingga tidak menimbulkan kemungkinan risiko terhadap lingkungan (Goutebroze, 1998 *Research*

Report 97.361 *Safety, Spread of the Vaccinal and Parental Strains from Chicken to Chicken and 98.183 Safety, Spread of the Vaccinal and Parental Strains from Chicken to Turkey*).

III.2. Informasi Cakupan Inang Organisme Tetua Virus PRG untuk Vaksin

Tetua virus PRG HVT+IBD adalah virus HVT strain FC-126 yang termasuk dalam famili *Herpesviridae* dan subfamili *Alphaherpesvirus*. Inang alami tetua HVT adalah ayam, burung puyuh dan kalkun.

III.3. Informasi Kajian Molekuler.

Sifat genetik yang direkayasa adalah penyisipan gen VP2 virus IBD *strain* 52/70 Faragher ke dalam HVT pada posisi UL 55. Penyisipan ini tidak mengubah sifat virulensi HVT bahkan dapat memberi perlindungan terhadap penyakit Marek dan IBD. Virus rekombinan HVT+IBD mengandung satu kopi gen VP2 yang bersifat stabil secara genotip dan fenotip. Pemindahan gen VP2 yang telah disisipkan ke dalam virus HVT tidak dimungkinkan karena tidak ada homologi dengan sekuen genom organisme lain sehingga aman terhadap lingkungan. Modifikasi genetik dapat dianalisis dengan salah satu dari metoda Southern Blot dan/atau PCR. Sedangkan fenotip dari rekombinan ini dapat dicirikan dengan metoda *plaque for double imunofluorescence* (Cleuziat, 1999 *Phenotypic stability after 3 and 8 passages in vitro*) (Cleuziat., 1999).

III.4. Kemungkinan dampak negatif jasad renik PRG vaksin terhadap lingkungan.

Hasil analisis BLAST menunjukkan bahwa nukleotida gen VP2 tidak memiliki homologi sekuen genom dengan organisme lain, sehingga tidak dimungkinkan transfer gen tersebut ke organisme lain. Vaksin PRG Vaxxitek HVT+IBD tidak membentuk spora karena tetua dari vaksin ini adalah termasuk dalam golongan virus. Virus PRG Vaxxitek HVT+IBD tidak diekskresikan oleh hewan yang divaksin sehingga tidak berpotensi menyebar ke hewan non target. Oleh karena itu tidak ada kemungkinan risiko terhadap lingkungan.

KESIMPULAN

Vaksin Vaxxitek HVT+IBD bersifat aman terhadap lingkungan karena tidak berisiko menyebarkan jasad renik PRG ke lingkungan. Hal ini karena jasad renik PRG dalam sediaan vaksin Vaxxitek HVT+IBD tidak diekskresikan dari hewan yang divaksin ke lingkungan.
Fenotip dan genotip jasad renik PRG dalam vaksin Vaxxitek HVT+IBD bersifat stabil.

TTKH PRG Bidang Keamanan Lingkungan merekomendasikan bahwa vaksin Vaxxitek HVT+IBD yang diajukan adalah aman terhadap lingkungan.

Jika dikemudian hari ditemukan informasi yang tidak sesuai dengan data keamanan lingkungan saat ini, maka status aman lingkungan terhadap vaksin Vaxxitek HVT+IBD perlu dikaji kembali.

Selanjutnya setelah ditetapkan aman lingkungan, namun produk tersebut terbukti menimbulkan risiko terhadap kesehatan manusia dan hewan maka pemohon wajib melakukan tindakan pengendalian dan penanggulangan serta memusnahkan vaksin Vaxxitek HVT+IBD yang berada di wilayah Indonesia.

Vaksin Vaxxitek HVT+IBD tidak boleh digunakan sebagai vaksin ayam sampai memperoleh ijin aman lingkungan.

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