ENVIRONMENTAL RISK MANAGEMENT AUTHORITY
NGĀ KAIWHAKATŪPATO WHAKARARU TAIAO

FORM NO2R

Application for approval to

IMPORT INTO CONTAINMENT LOW RISK GENETICALLY MODIFIED ORGANISMS BY RAPID ASSESSMENT

under sections 40 and 42B of the Hazardous Substances and New Organisms Act 1996

Application Title: The import of the live, gene-deleted *E. coli* vaccine Poulvac® *E. coli* into an approved containment facility for export only.

Applicant Organisation: Pacificvet Limited

	IBSC	ERMA NZ
Considered by:		X

ERMA Office use only

Application Code: ☐☐☐☐☐☐☐☐

Formally received:____/____/____

ERMA NZ Contact: ___________________________

Initial Fee Paid: $

Application Status:

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ENVIRONMENTAL RISK MANAGEMENT AUTHORITY
NGĀ KAIWHAKATŪPATO WHAKARARU TAIAO
ERMA
New Zealand

Application for approval to import into containment low risk genetically modified organisms under Section 40 of the Hazardous Substances and New Organisms Act 1996

IMPORTANT

1. An associated User Guide NO2R is available for this form and we strongly advise that you read this User Guide before filling out this application form. If you need guidance in completing this form please contact ERMA New Zealand.
2. This application form only covers the import of low-risk genetically modified organisms that meet Category A and/or B experiments as defined in the *HSNO (Low-Risk Genetic Modification) Regulations 2003.*
3. If you are making an application that includes not low-risk (formerly Category C) organisms, as described in the *HSNO (Low Risk Genetic Modification) Regulations 2003*, then you should complete form NO2G instead of form NO2R (this form).
4. You should periodically check with ERMA New Zealand or on the ERMA New Zealand web site for new versions of this form and only use the most recent version.
5. You can also talk to an Applications Advisor at ERMA New Zealand who can help you scope and prepare your application. We need all relevant information early on in the application process. Quality information up front will speed up the process and help reduce costs.
6. This application form may be used to seek approvals for more than one new organism where the organisms are used in the same project, or have a similar risk profile.
7. Any supporting material that does not fit in the application form must be clearly labelled, cross-referenced, and included as appendices to the application form.
8. Commercially sensitive information must be collated in a separate appendix but referenced in the application. You need to justify why you consider the material commercially sensitive, and make sure it is clearly labelled as such. Confidentiality of material is subject to the provisions of the Official Information Act 1982 and the basis of which is that information should be publicly available unless there is good reason to protect it. Please make yourself familiar with the Official Information Act and with ERMA New Zealand Sheet Number 12 on our website.
9. Applicants must sign the form and enclose the correct application fee (plus GST) if it is submitted to ERMA New Zealand. The initial application fee can be found in our published *Schedule of Fees and Charges*. Please check with ERMA New Zealand staff or the ERMA New Zealand website for the latest schedule of fees. We are unable to process applications that do not contain the correct initial application fee.
10. Unless otherwise indicated, all sections of this form must be completed for the application to be progressed.
11. Please provide an electronic version of the completed application form, as well as sending a **signed hard copy**.

You can get more information by contacting your Institutional Biological Safety Committee or ERMA New Zealand. One of our staff members will be able to help you.

This version of the application form was approved by the Chief Executive of ERMA New Zealand on 28 October 2003.

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Section One – Applicant Details

1.1 Name and postal address in New Zealand of the organisation or private individual making the application

Name > Pacificvet Limited

Postal Address > P.O. Box 16-129, Hornby, Christchurch 8441

Physical Address > 3 Hickory Place, Islington, Christchurch 8042

Phone > 03-349-8438

Fax > 03-349-8863

E-mail > kent@pacificvet.co.nz

1.2 If application is made by an organisation, provide name and contact details of a key contact person at that organisation

This person should have sufficient knowledge to respond to queries and have the authority to make decisions that relate to processing of the application.

Name: Kent W. Deitemeyer
Position: Co-owner & Company Director
Address: Pacificvet Limited, PO Box 16-129, Hornby, Christchurch 8441
Phone: 03-349-8438
Fax: 03-349-8863
E-mail: kent@pacificvet.co.nz

Section Two – Lay Summary of the Application

Provide a short description of the background and aims of the work suitable for lay readers.

In this summary, describe the rationale for the overall project these organisms are to be used in so that people not directly connected with the research can understand why these organisms are to be imported. This explanation is particularly important if the organisms to be imported contain DNA from New Zealand native flora and fauna, or human genes. Summarise the application in clear, simple language that can be understood by the general public. Include a description of the organism(s) to be imported into containment, and any risks and benefits associated with their importation. This summary will be used to provide information for those people and agencies who will be notified of the application (eg Ministry of Agriculture and Forestry, Department of Conservation, Crown Research Institutes) and for members of the public who request information. Do not include any commercially sensitive information in this summary.

- Colibacillosis is a common systemic disease of economic importance in poultry and occurs worldwide. *Escherichia coli* (*E. coli*) infection occurs as an acute fatal septicemia or subacute pericarditis and airsacculitis, as well as perihepatitis, arthritis, and also cellulitis. Among bacterial infections, colibacillosis is very often the first cause of morbidity and mortality in poultry. Large numbers of *E. coli* are maintained in the poultry house environment through fecal contamination. Systemic infection occurs when large numbers of pathogenic *E. coli* gain access to the bloodstream via the respiratory tract or intestine. Bacteremia progresses to septicemia and death, or the infection extends to serosal surfaces, pericardium, joints and other organs. Uncontrolled, avian *E. coli* represents a serious animal welfare concern and risk to public health as it is a zoonotic organism with avian *E. coli* species known to adapt to humans.

- This application seeks to import and then store a live, attenuated, genetically-modified *E. coli* commercial vaccine (Poulvac® *E. coli*) for poultry against colibacillosis in an approved containment facility specifically designed to store veterinary vaccines. The intent is to export the vaccine to the poultry industry in the South Pacific islands. The *E. coli* vaccine is not for use in NZ but is deemed important for protecting NZ poultry genetic stock and their progeny exported to the South Pacific countries where endemic *E. coli* creates colibacillosis in chickens, a disease resulting in serious welfare, biosecurity, public health and economic issues for poultry farms.

- The Poulvac® *E. coli* vaccine contains an *E. coli* strain that has been genetically-modified by the deletion of the *aro*A gene responsible for the biosynthesis of amino acids in the virulent *E. coli* parent strain (The GMO is named *aro*A- PTA-5094). The *aro*A gene-deleted vaccine is capable of triggering a protective immunity in poultry against the infection and disease from wild, virulent *E. coli* bacteria found in the environment. However, because of the *aro*A gene is deleted, the live vaccine bacteria is avirulent and unable to form a self-sustaining population since the vaccine strain has lost the capability to synthesize the amino acids necessary for its survival.

- The vaccine is produced by commercial vaccine companies. It is tested for efficacy, safety, and freedom of contamination by other pathogenic organisms (see Attachment 1 for brochure). The vaccine is in a lyophilised plug contained in hermetically-sealed vials within protective packaging. No vials will be opened in our containment facility. The risk to the New Zealand environment, flora and fauna is

assessed in this application as improbable due to the containment controls, the vaccine containers are never opened, and the vaccine strain is unable to maintain a self-sustaining population.

Prior to exports of the vaccine we will obtain the necessary approvals required to export GMOs from New Zealand.

Section Three –Description of Organism(s) to be Imported

If the application is for importation of more than one organism, information required in this section must be provided separately for each organism. If there are commercial reasons for not providing full information here, alternative approaches should be discussed and agreed by ERMA New Zealand before submitting the application.

3.1 Give a short summary statement of the purpose of this application to be used on ERMA New Zealand's public register. (Maximum of 255 characters). What are the organisms to be imported and what will these organisms be used for? Or why are these organisms being imported?

- To import a commercial, gene-deleted live *Escherichia coli* vaccine (Poulvac® *E. coli*) used in the immunisation of poultry against colibacillosis into an approved PC2 containment facility for future export.

3.2 Give the unequivocal identification of the host organism(s) to be imported.

Please include details (if applicable) on the following:

Latin binomial or appropriate taxonomic classification to uniquely identify the organism, including full taxonomic authority:

- *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919
(Taxonomic Classification of the parent organism referenced from from *Bergey's Manual of Systematic Bacteriology, 2nd Edition*, April 2001, Springer-Verlag:New York)

Common name(s), if any:

- *E. coli*

Type of organism (eg bacterium, virus, fungus, plant, animal, animal cell):

- Bacterium

Taxonomic categories such as family, order, class and division (if applicable):

- Domain: "Bacteria";
- Phylum BXII: *Proteobacteria phy. nov.*;
- Class III: *"Gammaproteobacteria";*
- Order XIII: *"Enterobacteriales";*
- *Family I: Enterobacteriaceae;*
- *Genus XIII: Escherichia;*

Strain(s) and genotype(s), if relevant:

- Wild-type *E. coli* isolate EC34195 (an O78:K80 isolate). This organism is an avian isolate of *E. coli* isolated from a clinical case of avian colibacillosis submitted to the Veterinary Laboratories Agency (VLA), Addlestone, Surrey, UK and serotyped at VLA in 1995. The parent strain was selected for its colonization, invasion, persistence and pathogenicity in one-day-old SPF chicks and by in vitro characterization for its antibiotic sensitivity pattern.

Other information, including presence of any inseparable or associated organisms, and whether a prohibited organism[1] is involved:

- The vaccine is a pure culture of *E. coli aro*A- PTA-5094. As a commercial veterinary vaccine that is regulated by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, each vaccine batch is tested for purity, safety and efficacy. In terms of purity, each vaccine serial batch must be succesfully tested for freedom of any adventitious agents and pathogens. These vaccines are manufactured in the United States in accordance with the US Code of Federal Regulations, Volume 9 (US 9 CFR). Each Master Seed of the vaccine antigen and final vaccine batch are tested for freedom of extraneous viruses, bacteria, mycoplasma and fungi.
- The APHIS batch test report for every batch of vaccine imported into New Zealand is held on file by the applicant in accordance with its Good Manufacturing Practice (GMP) accrediation from the ACVM Group, NZ Food Safety Authority and is subjected to regular audit.

[1] For prohibited organisms, please refer to the Second Schedule of the HSNO Act.

3.3 Information on the host organism(s)

If more than one type of host organism is to be imported this section must be completed separately for each organism. If there are commercial reasons for not providing full information here alternative approaches must be discussed with and agreed by ERMA New Zealand before submitting the application.
Please complete the following table.

	Yes	No
1. Is the organism normally capable of causing disease in humans, animals, plants or fungi? *If yes, you must give the details below and complete sections 4 & 5.1 of this form.*	X	
2. Is the organism a human cell line? *If yes, you must complete section 5.2 of this form and provide details below.*		X
3. Is the organism considered to be native biota? *If yes, you must complete section 5.2 of this form and provide details below.*		X
4. Does the organism contain infectious agents normally able to cause disease in humans, animals, plants, or fungi[2]? *If yes, you must give the details below and complete sections 4 & 5.1 of this form.*		X
5. Does the organism produce dessication-resistant structures, such as spores or cysts, that can normally be disseminated in the air? *If yes, you must give the details below and complete sections 4 & 5.1 of this form.*		X
6. Is the organism characterised to the extent that its main biological characteristics are known?	X	
7. Does the organism normally infect, colonise or establish in humans? *If yes, you must give the details below and complete sections 4 & 5.1 of this form.*		X
8. If the organism is a whole plant or plant tissue[3], is it: **a. allowed to develop reproductive structures?** *If yes, please provide further information on containment in section 4 of this form.* **b. kept in a closed container?**		X
9. Is it a category 1 host organism[4]?		X
10. Is it a category 2 host organism[5]?	X	

Note: If the genetic modification does not involve a category 1 or 2 host organism then the proposed project does not meet the criteria in section 42B(2) of the HSNO Act for the rapid assessment of adverse effects for importation of genetically modified organisms into containment.

[2] For example, mammalian cell lines containing active viruses or infectious agents normally able to cause disease in humans.
[3] For a whole plant or plant tissue to be considered a Category 1 host organism, it must not be allowed to develop reproductive structures **and** must be kept in a closed container.
[4] Refer to section 7(1) of the HSNO (Low-Risk Genetic Modification Regulations) 2003.
[5] Refer to section 7(2) of the HSNO (Low-Risk Genetic Modification Regulations) 2003.

Other details of the host characteristics (including its ability to form a self-sustaining population) if it is a pathogenic microorganism

- The host organism is wild-type *E. coli* isolate EC34195 (an O78:K80 isolate) is an avian isolate of *E. coli* isolated from a clinical case of avian colibacillosis (see section 3.2 for details).

If host was sourced from humans provide details of where the material was obtained from, and whether approval was obtained from an Ethics Committee (if applicable). Be as specific as possible as this information may be needed to decide whether Māori have been appropriately consulted.

- N/A. See above.

If native biota were used as host organism, from where in New Zealand or elsewhere was this material obtained? Be as specific as possible as this information may be needed to decide whether Māori have been appropriately consulted.

- N/A. The parent *E. coli* isolate is not native biota. See above.

3.4 How were the new organism(s) developed?

Provide details of the following:

CONSTRUCTION OF AROA GENE DELETED *E. COLI* MUTANT (*aro*A- PTA-5094 (American Type Culture Collection [ATCC] patent depository accession number PTA-5049)
See References:
- Attachment 2, Patent Notice;

I) Recipient

The parental organism is an avian isolate of *E. coli* isolated from a clinical case of avian colibacillosis submitted to the Veterinary Laboratories Agency (VLA), Addlestone, Surrey, UK and serotyped at VLA in 1995. The parent strain was selected for its colonization, invasion, persistence and pathogenicity in one-day-old SPF chicks and by in vitro characterization for its antibiotic sensitivity pattern. The recipient strain was generated by conjugation between the transformed donor (*E. coli* K12 S17 λ pir harboring PNG101 with aroA harboring 100 bp deletion) and wild-type parent strain (wild-type *E. coli* isolate EC34195).

II) Characterization of the Deletion

The *aroA* gene, which encodes 3-phosphoenolpyruvylshikimate-5-phosphate synthetase, an enzyme of the common aromatic biosynthetic pathway, is located adjacent and promoterdistal to ser C in the ser C- *aroA* operon. Loss of function for the aro A gene in the recipient results in a requirement for aromatic metabolites, including tyrosine, phenylalanine, tryptophan, p -aminobenzoate (PABA) and 2,3-dihydroxybenzoate. The requirement for PABA, a metabolite not found in vertebrate tissues, results in attenuation of *in vivo* growth.

III) Construction of the aro A Deleted *E. coli* Mutant

1. PCR primers are designed incorporating Srf I and Bgl II restriction sites and stop codons to amplify two separate PCR products of approximately 650 bp for the 5' and 3' ends of the aro A gene from the poultry *E. coli* 078 isolate described above.

2. Both PCR products are digested with Bgl II for 2 hours, electrophoresis is run for 1 hour at 100 volts, the bands are excised and the respective bands are purified using SephaGlas bandprep kit.

3. Equal volumes of each purified PCR product are mixed and ligated into pCR2.1.

4. Ligated plasmid harboring aroA are transformed into DH5 α maxi-competent cells and cloning is confirmed by restriction enzyme mapping and PCR.

5. Complete *aro A* gene with deletion from pCR2.1 is excised with Ecor V and SpeI then purified and ligated into a predigested (SpeI) suicide vector (SacB, pKNG101), transformed into competent *E. coli* K12 S17 λ pir and cloning is confirmed by restriction enzyme mapping and PCR.

6. A conjugation is performed between donor (*E. coli* S17 λ pir harboring pKNG101 with *aro*A harboring 100 bp deletion) and wild-type *E. coli* isolate.

7. Colonies appearing after 48 hours incubation at 37°C are subcultured onto minimal media supplemented with gentamicin and streptomycin and aromatic amino acids (20 mg/l of each of DL tryptophan, DL phenylalanine and DL tyrosine). Individual colonies are tested by PCR. Colonies that yielded a wild-type PCR product and mutated PCR product of some 100 bp smaller are retained for further studies.

8. Single crossovers are cultured in LB-G broth supplemented with 10% sucrose at 37°C with gentle agitation for 16 hours. Serial dilutions of the overnight cultures are plated onto LB-G plates supplemented with 10% sucrose and incubated at 37°C for 16 hours.

9. Colonies which grow on the 10% sucrose LB-G plates are subcultured onto each of LB-G, LB-G + gentamicin and streptomycin and minimal and incubated at 37°C for 16 hours. Colonies only growing on the LB-G plates (double crossovers) are subcultured onto 5% sheep's blood agar and maintained at 4°C.

IV) Intermediate Cloning Vector

Suicide vector (SacB, PNG101) was the intermediate cloning vector. Conjugation was performed between donor (S17 harboring PNG101 with *aro A* harboring 100 bp deletion) and wild-type *E. coli* isolate.

PREPARATION OF MASTER SEED

The *E. coli aroA*- strain (constructed in Example 1) is grown on tryptic soy agar plate once and passed 3 times in tryptic soy broth. The culture is distributed into glass vials, sealed and lyophilized.

This is a commercial veterinary vaccine for chickens and has met the U.S. Department of Agriculture's regulation on proving the genetic stability of the vaccine strain through back passage studies as per Veterinary Services Memorandum No. 800.201 U.S.Dated February 22, 2000, "Backpassage Studies". Guidelines for evaluating the genetic stability of Master Seeds for conventional modified live or live recombinant derived vaccines to provide assurance that such vaccine microorganisms will not revert to virulence when administered to the host animal.
Five successive backpassages are required.

Vector system(s) used:

- As outlined above.

Type and source of additional genetic material (i.e. donor DNA):

- No additional genetic material. Only the *aroA* gene deletion of the original *E. coli* strain.

Use of special genetic material: please complete this table by marking the correct box

	Yes	No
Was genetic material derived from organisms capable of causing disease in humans, animals or plants used in developing these GMOs? *If Yes, you **must** provide full details in this section and complete sections 4 & 5.1 of this form.*	X	
Was genetic material from native biota used in developing these GMOs? *If yes, provide details below and complete section 5.2.*		X
Was human genetic material used in developing these GMOs? *Answer Yes if human genetic material in any form was used, ie obtained directly from humans, from a gene bank, synthesised, copied and so on. Please provide details below and complete section 5.2.*		X

If nucleic acids from pathogenic microorganisms were involved, please specify exactly which sequences and species and strain they were derived from. Be as specific as possible as this information will be needed to decide whether this application involves a low-risk genetic modification or is "not low-risk". Include risk group classification (e.g. 1, 2, 3 or 4) if relevant. If the nucleic acid introduced was characterised so that its sequence and gene function are known, please state this and provide verifiable evidnece. Please attach maps of genetic constructs if possible.

- As above.

If native biota were involved, from where in New Zealand or elsewhere was this material obtained? Be as specific as possible as this information may be needed to decide whether Māori have been appropriately informed and/or involved.

- No native biota involved. Only isolated and identified *E. coli* strain from the Veterinary Laboratory Agency, Addlestone, Surrey, UK.

If material was sourced from humans provide details of where the material was obtained from, and whether approval was obtained from an Ethics Committee (if applicable). Be as specific as possible as this information may be needed to decide whether Māori have been appropriately informed and/or involved.

- No human-sourced materials used.

Other details of the development(s) (such as what techniques or experimental procedures were used during the modification of the organism(s), if any unusual manipulations were carried out) **including if the foreign genetic material is expressed and where it is expressed:**

- As above in the explanation of the construction of the organism.

3.5 Identify the category or categories of genetic modification(s) as described in the HSNO (Low-Risk Genetic Modification) Regulations 2003, had the genetic modifications been carried out in New Zealand.

Identify the category of host organism (e.g. 1 or 2), category of genetic modification (e.g. A or B) and explain your characterisation if necessary. This is particularly important for work involving pathogenic microorganisms and viral vectors.

Please complete the table below as it will guide you in your classification of the organism(s) in accordance with the Regulations.

	Yes	No
1. Does this application involve a category 1 host organism? *Copy from question 9 of table 3.3.*		X
2. Does this application involve a category 2 host organism? *Copy from question 10 of table 3.3.*	X	
3. Does the modification increase the pathogenicity, virulence, or infectivity of the host organism to laboratory personnel, the community, or the environment? *An answer of yes to this question may indicate that the development is not low-risk[6]. Please check with your ERMA applications advisor.*		X
4. Does the modification result in the genetically modified organism having a greater ability to escape from containment than the unmodified host organism? *An answer of yes to this question may indicate that the development is not low-risk. Please check with your ERMA applications advisor.*		X
5. Is the organism to be maintained under a minimum of PC1[7] containment?		X
6. Is the organism to be maintained under a minimum of PC2[8] containment? Yes, in an existing, accredited PC2 containment facility.	X	
7. Does the organism conform to the requirements of a Category A genetic modification?[9]		X
8. Does the organism conform to the requirements of a Category B genetic modification?[10]	X	

Explanation of characterisation:

- The *E. coli aro*A- PTA-5094 strain is derived from a Category 2 host pathogenic *E. coli* strain that has undergone a Category B genetic modification. This modification is characterised as a well-

[6] Modifications of category 1 host organisms that result in a host organism that is more pathogenic, virulent or infectious to laboratory personnel, the community, or the environment may be considered low-risk (i.e. Category B) if the increase is pathogenicity or virulence is not considered to be of a higher level than that of a category 2 host organism.

[7, 8] If the answers to both questions 5 and 6 are no, then this application is not considered low-risk and must be submitted to ERMA New Zealand.

[9, 10] If the answers to both questions 7 and 8 are no, then this application is not considered low-risk and does not meet the requirements of section 42B(2) of the HSNO Act for rapid assessment of adverse effects for importation of genetically modified organisms into containment.

defined 100 base pair deletion of the *aro*A gene that disables a virulence factor by creating an inability of the genetically-modified *E. coli* strain to biosynthesize aromatic amino acids thus resulting in a strain that is unable to revert to a wild-type phenotype or generate a self-sustaining population yet retains its immunogenic capacity as a safe, protective vaccine. The modification does not increase the virulence, pathogenicity or infectivity of the host organism.

Under Schedule 1(k) of the Low Risk Regulations, developments involving modifications to pathogenic micro-organisms that result in resistance to antibiotics used for clinical or veterinary treatment of infections caused by that micro-organism are not low risk.

In New Zealand, veterinary infections of *E. coli* are treated with Paracillin (water soluble Amoxycillin) or Apralan (Apramycin in a water soluble or feed additive form). These are used only on prescription from a poultry veterinarian. Oxytetracyclines are avoided due to the concern for *E. coli* resistance to the tetracylines.

Treatment strategies include control of predisposing infections or environmental factors, and early use of antibiotics. Unfortunately, a high frequency of resistance to tetracycline, kanamycin, neomycin, cephalotin, streptomycin and erythromycin has been observed. Many strains are also resistant to several antibiotics. Wide spread sensitivity to ampicillin and chloramphenicol has also been observed.

The genetic modification of the *E. coli* does not result in the microorganism gaining resistance to antibiotics used for clinical or veterinary treatment of infections caused by that micro-organism. Therefore this bacterium is “low-risk”.

3.6 Provide unique name(s) for the new organism(s) to be imported for entering in the public register.

These name(s) should clearly identify the species and strain(s) and genetic modification(s).
For example, “*Escherichia coli* DH5α modified by pBluescript containing cholera toxin gene”

- *Escherichia coli aro*A- PTA-5094 as a vaccine antigen modified by the deletion of the *aro*A gene (American Type Culture Collection [ATCC] patent depository accession number PTA-5049).

3.7 Characteristics of the organism(s) to be imported

Provide information on the known phenotypic characteristics of each organism(s) to be imported. For example, note novel traits conferred by the genetic modification. This information should be relevant to the identification of the risks of the organism (section 5).

- The development of the aroA gene-deleted *Escherichia coli* vaccine for poultry:

  Escherichia coli (*E. coli*) infection occurs as an acute fatal septicemia or subacute pericarditis and airsacculitis, as well as perihepatitis, arthritis, and also cellulitis.

The parental organism is an avian isolate of *E. coli* (wild-type *E. coli* isolate EC34195) isolated from a clinical case of avian colibacillosis submitted to the Veterinary Laboratories Agency (VLA), Addlestone, Surrey, UK and serotyped at VLA in 1995. The parent strain was selected for its colonization, invasion, persistence and pathogenicity in one-day-old SPF chicks and by in vitro characterization for its antibiotic sensitivity pattern.

- Characterization of the Deletion:

  The aroA gene, which encodes 3-phosphoenolpyruvylshikimate-5-phosphate synthetase, an enzyme of the common aromatic biosynthetic pathway, is located adjacent and promoter distal to serC in the serC-aroA operon. Loss of function for the aroA gene in the recipient results in a requirement for aromatic metabolites, including tyrosine, phenylalanine, tryptophan, p-aminobenzoate (PABA) and 2,3-dihydroxybenzoate. The requirement for PABA, a metabolite not found in vertebrate tissues, results in attenuation of *in vivo* growth.

- Inability to form a Self-Sustaining Population:

  In back passage studies, the *aroA* deleted vaccine antigen was unable to survive three back passages due to the inability to generate PABA for continued survival and generate a self-sustaining population in the environment thus eliminating the risk to other vertebrates that may have come in contact with the vaccine antigen.

Section Four – The Proposed Containment System and its Effectiveness

Describe the proposed containment system (physical and operational)

A brief description stating the containment level i.e. PC1 or PC2 and referencing the relevant MAF/ERMA New Zealand containment standard will be sufficient for most projects except those involving work identified for further information in Section 3.3 such as pathogenic microorganisms or organisms that produce dessication-resistant structures normally disseminated by air. For plants, state whether they will be allowed to develop reproductive structures or not and methods to contain them if applicable. For those types of developments identified in section 3.3 and 3.4 please provide further information about the containment system and the ability of the organism(s) to escape from this system and form self-sustaining populations.

- The containment system is in an existing, fully-approved PC2 containment facility currently used for restricted biological products of animal origin with its sole purpose to hold sealed and packaged commercial veterinary vaccines for import, storage, and export only.

- The facility is in full compliance with MAF Biosecurity New Zealand/ ERMA New Zealand Standard Facilities for Micro-organisms and Cell Cultures: 2007.

Risk Potential and Mitigation:

- Historical record: After 15 years of handling, storage, and shipping these vaccines in and from the applicant’s containment and transitional facility, there have been no incidents or accidents that caused a release of vaccine antigen from containment.

- The applicant's current containment and transitional facility manual covers all work procedures, contingency plans, and records keeping.

- There are three possible issues that create the greatest risk for accidental or purposeful release of the vaccine organism from containment:

  - Breakage of vaccine vials
  - Theft or sabotage
  - Mistaken delivery to a domestic (NZ) client

- Mitigation of Breakage: The vaccine vials are only handled twice: on receipt and on dispatch. Due to the secure packaging of vials (hermetically-sealed with rubber stoppers and aluminum seals packed inside a sealed plastic protective container), the potential for breakage is extremely low. These vaccines are lyophilised (freeze-dried). The vaccine antigen is a solid 'plug' of vaccine encased inside the vials requiring reconstitution with non-chlorinated water.

- In case of breakage, the clean-up procedure is based on the using a MAF-approved sanitizer with synergistic disinfectant properties (Ecolab VORTEXX: 7% hydrogen peroxide, 5% peracetic acid, 25% acetic acid) for the published inactivation of *E. coli* based on a PAA dilution of 10-15 ppm for a five minute exposure time with a kill rate of 99.999%). The instructions are to fully immerse broken vials and any exposed packaging materials in a 1:10 dilution (500 ppm) of peracetic acid for no less than three hours. Clean-up procedures are specified including personal hygiene and clothing steps to be taken. The disinfected materials, clothing, gloves, are then quarantined for incineration with a commercial medical waste service. Responsible staff are trained and directions are posted on the transitional facility door.

- Mitigation of theft is controlled by maintaining a thorough security system and processes such as during office hours, entry is controlled through locked doors at both reception and dispatch areas. A sign-in procedure is maintained for visitors.

- New Zealand Fire Services have inspected the premises, have a site map, have keys to the outer doors (but not the containment lockers), and have been informed of the containment facility procedures. The facility is alarmed and monitored for smoke and fire.

- Running stock levels are monitored and traceability of the vaccine stock is in accordance with the NZ Food Safety Authority Good Manufacturing Practise accreditation of the applicant.

- Mistaken delivery is mitigated by GMP rules for opening the locked containment facility, selection and allocation of vaccine to export clients only, checking by two people, sign-off of delivery dockets prior to packing for export in a secure container.

- Export shipments are never packed at the same time as domestic shipments avoiding possible mistakes.

- Potential for spread is mitigated by the fact that the applicant's premises are approximately 4 km from the nearest poultry farm. The applicant's staff has no contact with this farm. Due to the fragility of this

attenuated poultry vaccine, the sealed packaging encased in vials in the form of a solid, freeze-dried plug requiring reconstitution with non-chlorinated water, an accidental release of antigen capable of infecting poultry would be have to concluded to be improbable.

- An active rodent control is carried on the premises (EcoLab) as part of the applicant's GMP accreditation.

Section Five - Identification and Assessment of Adverse Effects and Risks

This section should be completed if use of pathogenic microorganisms, human cells, native or valued flora or fauna as host or donor nucleic acid sources were identified in section 3. It is expected that organisms meeting the low risk requirements will not normally have any *significant* biological risks associated with them, so that an assessment of adverse effects will not normally be required. However, there may still be some adverse effects that need to be identified and assessed. This might include economic, social and cultural adverse effects and other risks not addressed by the *HSNO (Low-Risk Genetic Modification) Regulations 2003*.

5.1 Identification and assessment of non-cultural adverse effects of the organism(s). This section should be filled out for those types of developments identified in section 3.3 and 3.4 that warrant further assessment. Consider and identify the adverse effects of the organism(s) both in and out of containment. Assessment of adverse effects should take account of the containment system in place. If you consider that there are significant residual adverse effects a rapid assessment could be inappropriate. In that event, talk to your IBSC or to ERMA New Zealand. Complete the following sections, but state if they are not relevant to your application.

A. Effects on the environment:

- There is an improbable risk of this vaccine being released into the environment. As a commercial vaccine, it is sealed in vials and cartons and stored in an approved PC2 containment facility for re-export. The vaccine is never opened. Mitigation steps are in place for accidents, sabotage, or theft. As a freeze-dried vaccine, it would require purposeful application in that it would have to be opened, diluted with unchlorinated water, and applied as a spray on veterbrates. Due to its gene-deletion phenotype, it is unable to establish a self-sustaining population.

B. Effects on human health and safety:

- There is an improbable risk of the vaccine to human health or risk to occupational safety. The vaccine is only handled in sealed vials and cartons. Containment steps and precautions mitigate exposure risks. It is important to appreciate that this is a vaccine applied by mass application spray to chickens and has a high safety profile, even for the vaccinators as long as basic hygiene precautions are taken as with any other poultry vaccine that is applied to the target species in the same way in New Zealand poultry (basic respirator, goggles and disposable gloves).

C. Economic effects:

- Highly improbable that there would be any economic effect in the case of an accidental or purposeful release as the vaccine antigen is not capable of generating a self-sustaining population. To the contrary,

this is a vaccine antigen with the sole purpose to provide economic health and welfare benefits to poultry operations as preventive immunological tool where *E. coli* is a risk issue.

D. Other Effects:

- It is highly improbable that sufficient antigen would be released to infect a susceptible host capable of replicating and shedding large amounts of a vaccine organism to other suceptible populations. A release of the vaccine organism outside containment would have to be exceedingly purposeful intention as the vaccine requires careful reconstitution in unchlorinated water and would need to be directly applied to a target species. The vaccine antigen is unable to generate a self-sustaining population as strain dies off rapidly.

5.2 Identification and assessment of potential adverse effects on the relationship of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna and other taonga

If your application involves genetic material from humans, native flora and fauna, or from flora and fauna which it is reasonable to think may be valued by Māori, you should address these issues here. Include details of any consultations with Māori that you have undertaken in relation to this application. If concerns were raised during the consultation process you should consider whether or how you are able to address those concerns. If Māori have residual concerns over the application then it should be referred to ERMA New Zealand and not dealt with by the IBSC.

- There is no additional risk having further Treaty of Waitangi implications that are not already taken into account that affect the interest in maintaining the Precautionary Principle of the HSNO Act for the protection of New Zealand society as a whole, our environment, and our native flora and fauna.

- The vaccine is to be held in an MAF Biosecurity NZ audited and approved PC2 containment and transitional facility designed for holding veterinary vaccines for import, stock, and re-export only. The facility has a 15-year track record of no accidental or intentional release of any organism. The facility is audited by Biosecurity NZ twice yearly .

- There is an improbable risk to native avian species for these reasons:
    1. This an attenuated, avirulent poultry vaccine GMO and are extremely fragile if not maintained under refrigeration.
    2. The organism is incapable of a maintaining a self-sustaining population and dies off.
    3. Exposure to sunlight and heat de-activates these attenuated vaccine antigens within hours.
    4. Even under ideal conditions, these GMOs rapidly deteriorate and require the presence and direct inoculation of a susceptible host capable of replicating the bacterium to survive.
    5. An accidental release of the GMO from the location of the containment facility in an industrial estate in Christchurch. There is negligible risk for the GMOs to be released and even less risk to reach a native avian species.
    6. The vaccine would have to be reconstituted by mixing with unchlorinated water and applied directly to a target species. It is highly improbable that such a sequence of events would take place accidentally and would have to be done with purposeful intention (and done with some care as to not harm the vaccine antigen).

7. The *aro*A gene-deleted *E. coli* vaccine strain does not revert to virulence. Back passage studies that are required for licensure (Master Seed reversion to virulence studies as required by the USDA-APHIS).

Section Six – Additional Information

6.1 Do any of the organism(s) need approvals under any other legislation or are affected by international obligations?

For example, the Animal Welfare Act, or the Convention on International Trade in Endangered Species.

- Yes, the Biosecurity Act, 1993.

6.2 Have any of the new organism(s) in this application previously been considered in New Zealand or elsewhere?

For example, have they been previously considered for development? If yes, please provide details or the relevant approvals.

- Yes, this new organism is a vaccine that is licensed for commercial use by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service for use in chickens. It is used in the United States extensively in broiler and commercial egg layer chicken flocks.

6.3 Is there any additional information that you consider relevant to this application that has not already been included?

- **No.**

6.4 Provide a glossary of scientific and technical terms used in the application.

Antibody: A protein (immunoglobin) that recognises an antigen and facilitates clearing the body of the antigen by either exclusion or elimination mechanisms. Antibodies are produced by plasma cells.

Antibody-mediated immunity: A primary immune response where antibodies are generated specifically against an antigen (e.g. a pathogen) to exclude or eliminate the organism from invading the host and creating disease. Generally associated with the neutralising extracellular antigens.

Antigen: Any substance (usually foreign) that binds specifically to an antibody or a T-cell receptor; often used as a synonym for immunogen, e.g. a virus, a bacteria, or a parasite.

***aro*A gene**: The *aro*A gene encodes the enzyme 3-phosphoenolopyruvylshikimate-5-phosphate synthetase required in a common aromatic biosynthetic pathway. Through the deletion of the *aro*A gene, this function is lost thus the organism requires an external source of aromatic metabolites including tyrosine, phenylalanine, tryptophan, p-aminobenzoate (PABA) and 2,3-dihydroxybenzoate. The requirement for PABA, a metabolite not found in vertebrate tissues, results in the attenuation of the *aro*A gene deleted *E. coli* strains ability to grow in vivo.

Attenuate: To decrease the virulence of a pathogen and render it incapable of causing disease. Vaccines are often attenuated bacteria or viruses that raise protective immune mechanisms without causing harmful infection and disease.

Back-passage: A method of testing the safety and stability of an attenuated live virus or bacteria vaccines whereby the vaccine is re-isolated from vaccinated animals and re-injected into unvaccinated animals to determine if the organism reverts to virulence. A vaccine strain is considered stable if it can maintain its attenuation through five back-passages.

Cell-mediated immunity: A primary immune response mediated by antigen-specific T cells capable of identifying and targeting infected host cells and killing the cell to eliminate the invading intracellular pathogen.

Crossover: Crossover refers to recombination of genetic material as the exchange of chromosome segments between nonsister chromatids in meiosis. Crossing over creates new combinations of genes in the gametes that are not found in either parent, contributing to genetic diversity, and in genetic modification of a bacteria, the creation of an entirely new strain with a well-defined phenotype (e.g. *aro*A deletion in an E. coli strain).

Immunoglobins: See: Antibodies. Denoted by their class type, e.g. IgA, IgM, IgG, etc.

LB-G Plates: Abbreviation for Luria-Bertani agar gel plates used as a common media for growing non-fastidious bacteria and a standard media for the cultivation and maintenance of recombinant E. coli strains.

PCR: see polymerase chain reaction.

Plasmid: A circular DNA that replicates independently of the cell's chromosome. Denoted in genetic modification descriptions as a prefix "p" followed by an identification, e.g. pCR2.1 is the identification of the plasmid carrying the aroA deletion for conjugation.

Polymerase chain reaction (PCR): Used to amplify a sequence of DNA using a pair of primers at each end of the DNA target sequence. The DNA is amplified by DNA polymerase in a three-step reaction cycle: denaturation, primer annealing and polymerisation.

Minimal media: A bacterial growth media containing a minimum of nutrients for bacterial growth which may be adjusted with varying nutrient constituents is used to either select for or against the maintenance and growth of a recombinant strain.

Suicide vector: A plasmid that cannot replicate in a particular host.

6.5 List of appendices. Give the names of any appendices included with this application.

- List of Attachments:

1. Poulvac *E. coli* product brochures (broiler version; layer version).
2. Patent Document: "Avian *E. coli* Vaccine for the Protection Against Colibacillosis" outlining the development and genetic construction of Poulvac® *E. coli* vaccine, live culture

CHECKLIST

Please check the following before submitting your application:

All sections completed	Yes
Appendices enclosed	Yes/ NA*
Confidential information identified and enclosed separately	Yes/NA
Copies of additional references attached	Yes/NA
Application signed and dated	Yes
If application submitted to ERMA New Zealand:	
Initial fee enclosed (incl. GST)	Yes
Electronic copy of application e-mailed to ERMA New Zealand	Yes

*NA – not applicable

Signed: **Date:**

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