Biosafety Advisory Council

18-01-2008

Secretariat

O./ref.: WIV-ISP/BAC/2008_SC_634
Email: bac@sbb.ihe.be

Title: Advice of the Belgian Biosafety Advisory Council on the notification **B/BE/07/BVW1** of the company Actogenix for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Context

The notification B/BE/07/BVW1 has been submitted by Actogenix to the Belgian Competent Authority in October 2007 for a request of deliberate release in the environment of genetically modified organisms other than higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The title of the notification is: **"Phase 1b and Phase 2a clinical trials with an hIL-10-expressing *Lactococcus lactis* (*L. lactis*)".** The planned activity concerns clinical trials with the bacterium *Lactococcus lactis* strain MG1363 which has been genetically modified to produce the therapeutic protein human interleukin-10. This GM-medication is developed to reduce the symptoms of patients suffering from inflammatory bowel disease.
The patients will take their treatment at home and shedding/excretion will occur mainly during evacuation of faeces most probably at the patient's home or elsewhere. In consequence the national territory is considered as the wider potential release area of the GM *L. lactis*.

The dossier has been officially acknowledged by the Competent Authority on 05 November 2007 and forwarded to the Biosafety Advisory Council for advice.
Within the framework of the evaluation procedure, the Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Four experts from the common list of experts drawn up by the Biosafety Advisory Council and the Division of Biosafety and Biotechnology (SBB) answered positively to this request. The SBB also took part in the evaluation of the dossier.
The experts and the SBB assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the genetically modified organism for its intended uses, would not raise any problems for the environment, animal

health or human health (people coming in contact with the treated patient and/or with the GMO).

On 17 December 2007, based on a list of questions prepared by the Biosafety Advisory Council, the Competent Authority requested the notifier to provide additional information about the notification. The answers to these questions were received from the notifier on 20 December 2007 and reviewed by the coordinator and the experts. This additional information was considered satisfactory by the scientists in charge of evaluating the dossier.

For the purpose of this evaluation, the following legal basis has been considered:
- Annex II (principles for the risk assessment) and annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient, as well as aspects related to social, economical or ethical considerations, are outside the scope of this evaluation.

In parallel to the scientific evaluation of the notification, the Competent Authority also made the dossier available on its website for the one-month public consultation foreseen in the abovementioned Royal Decree. As a result of this consultation, the Competent Authority forwarded to the Biosafety Advisory Council a list of 3 questions of the public relevant for the environmental and/or public health safety of the GMO.
All these questions were taken into account in the elaboration of the advice of the Biosafety Advisory Council given below. Answers are sent separately to the Competent Authority.

Summary of the Scientific evaluation

1. The characteristics of the donor, the recipient or parental organism

No major risks were identified.

The question of the Biosafety Advisory Council about the antibiotic resistance profile of the strain was adequately answered by the notifier: the strain is sensitive to 4 antibiotics, representative for 4 antibiotic groups (ampicillin, tertracycline, erythromycine, and gentamycine).

2. Information related to the vector

No major risks were identified.

3. Information related to the characteristics of the GMO

No major risks were identified.
According the additional information provided by the notifier, immunogenicity against the GM *L. lactis* will be evaluated in the clinical trial by monitoring for anti-hIL-10 antibodies (surveillance of potential appearance of abnormal immune responses in treated patients). An immunotoxicology study is currently conducted by the notifier in mice and data will be provided for the clinical application .

4. The condition of release

No major risks were identified.

5. The risks for the environment and human health

No major risks were identified.
Accidental contact of immunocompetent persons with the genetically modified *L. lactis* is not expected to have adverse consequences.
The shedding of the GM *L. lactis* in the environment will occur mainly during evacuation of faeces. The sewage system will be the receiving environment. The experts agree with the notifier that the GMO is not able to survive/establish in this environment.

6. The monitoring, control, waste treatment and emergency plans proposed by the applicant

As requested, the notifier completed the initial information with a draft version of the detailed "directions for use" which will be included in the treatment package and will be further explained to the patient. It adequately covers instructions for the safe storage of the study medication, proper procedure for disposal/storage of the used study medication materials, detailed instructions in case of spillage of the product, detailed instructions in the case of accidental ingestion by other individuals. This will limit the chance for anyone except the patient, including immunocompromised persons, to be significantly exposed to the GMO.
Although the patient will be invited to inform his relatives, the notifier does not foresee any direct information to the relatives living close to the patient. The Biosafety Advisory Council agrees that it would be difficult to achieve in the limits of the patient's privacy.

The notifier also gave satisfactory further information about the methods that allow the identification of the bacterial species as well as the genetically modified strain.

Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that the genetically modified *Lactococcus lactis* strain MG1363 engineered to produce the therapeutic protein human interleukin-10 will have any adverse effects on human health or on the environment in the context of the intended clinical trials.

Therefore, the Biosafety Advisory Council issues a **positive advice with the following conditions**:

- The Biosafety Advisory Council should receive a copy of the clinical trial protocol as soon as it has been finalised (including information for the patient).
- The notifier and the investigators must strictly apply the protocol, the biosafety monitoring and, if necessary, the emergency measures as described in the dossier.
- Any protocol amendment, which could have biosafety implications, has to be previously approved by the Competent Authority.
- The notifier is responsible to verify that each investigator has the required authorisations to perform the clinical trial activities inside the hospital (laboratory, pharmacy, hospital room, consultation room...).
- The Biosafety Advisory Council should be informed within 2 weeks when the first patient starts the treatment and the last patient receives the last treatment.
- At the latest six month after the last visit of the last patient included in each trial, the notifier must send the final study report including a report with details concerning the biosafety aspects of the project. This report will at least contain:
    - the number of patients included in the trial
    - the list of all adverse events
    - a report on the accidental releases, if any, of the recombinant *L. lactis*

p.o.

Prof. D. Reheul
President of the Biosafety Advisory Council.

Annex 1: Compilation of comments of experts in charge of assessing the dossier B/BE/07/BVW1 (ref: BAC_2007_MI_621)

Bioveiligheidsraad
Conseil de Biosécurité

4 December 2007

Secretariaat
Secrétariat

O./ref.: WIV-ISP/BAC_2007_MI_621
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Compilation of comments of experts in charge of assessing the dossier B/BE/07/BVW1

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 17 October 2007
Coordinator: Dr. P. Hermans
Experts: Jozef Anné (KUL), Geert Huys (UGent), SBB (WIV/ISP), Liliane Tenenbaum (ULB), Willy Zorzi (ULg)
Domains of expertise of experts involved: Medical microbiology, microbial genetics, molecular genetics, gene therapy, human medicine, epidemiology, immune disorders, gut microbiology, probiotics, lactic acid bacteria, biosafety, workers protection
SBB: Didier Breyer, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **B/BE/07/BVW1** concerns a notification of the company Actogenix for deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 05 November 2007 and concerns clinical trials with the bacterium *Lactococcus lactis* strain MG1363 which has been genetically modified to produce the therapeutic protein interleukin-10. This GM-medication is developed to lessen symptoms and pain in inflammatory bowel disease in humans.

Depending on their expertise, the experts were invited to evaluate the genetically modified organism considered in the notification as regards its molecular characteristics and its potential impact on human health and the environment. The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient are outside the scope of this evaluation.
The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

List of comments received from the experts

1. INFORMATION RELATED TO THE CHARACTERISTICS OF THE DONOR, THE RECIPIENT OR PARENTAL ORGANISM

(e.g. possibility of natural transfer of genetic material to other organisms, pathological, ecological and physiological characteristics, indigenous vectors ...)

Comment 1

MG1363 is a derivative of the natural isolate *L.lactis* National Collection of Dairy Organisms (NCOO) 712. The 5 different plasmids present in NCDO 712, were removed by protoplast-induced curing. One of these plasmids -the 33 MDa plasmid pLP712 - encodes genes for lactose and casein utilization. This plasmid proved essential for normal bacterial growth and acid production in milk; the remaining 4 plasmids appeared to be cryptic.
The removal of the pLP712 plasmid made it impossible for the bacterial strain MG1363 to access nutrients as lactose and caseins, present in milk, that are essential for its growth and providing a source for sugars (glycolysis) and amino acids respectively MG1363 can therefore no longer survive in the natural niche of *L lactis* and is confined to artificially supplemented culture conditions.
This strain is sensitive to a wide array of antibiotics and deficient in factors necessary for conjugative transposition.
L. lactis is commonly found in and added to food products. It is not classified as a hazardous organism and is not able to produce spores. *L.lactis* has a poor capacity of colonization and as such does not colonize the GI tract.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

- On p. 10 of the Technical Dossier (TD), the applicants refer to the scientific name of the recipient as '*Lactococcus lactis* strain MG 1363'. According to currently available taxonomic information, this name should be further specified as '*Lactococcus lactis* subsp. *cremoris* strain MG 1363' as reported by Wegmann et al. (cfr. reference 3). The subdivision at subspecies level should also be mentioned under the subheading '2.Taxonomy'.
- On p. 11 of the TD, under the subheading '6. description of detection and identification techniques', '16sRNA sequencing' should be replaced by '16S RNA gene sequencing'. This remark returns at several positions in the TD.
- On p. 11 of the TD, under the subheading '7. sensitivity, reliability, etc.', the applicants should specify what 'elaborate experience' was described in reference 5, i.e. the development of quantitative PCR assays for the culture-independent monitoring of the GMO strain and its hIL-10 production.
- On p. 12 of the TD, the applicants cite literature to support their claim that *L. lactis* does not colonize the human gut. Given the fact that characteristics such as gastrointestinal survival and colonization capacity are thought to be strain-specific rather than species-specific, I advise to also mention the strain designation for the lactococci used in the studies of Drouault *et al.* (ref. 11) and

Klijn *et al.* (ref. 12). In the first study, a *L. lactis* strain other than MG 1363 was used (IL 1403) whereas in the second study a transconjugant derived from a mutant generated from strain MG 1363 was used. Only in the latter case, relevant information is provided in relation to the physiological characteristics of sAGX0037. In their literature survey, the applicants should not only focus on colonization but also on adhesion, i.e. the initial interaction of bacterial cells with mucus cells that may or may not lead to colonization. In a recent study by Dharmawan *et al.* (2006), adhesion capacity of *L. lactis* IS-16183 in competition with *E. coli* O157:H7 is reported. On the other hand, I would like to stress that results from in-vitro adhesion studies should be interpreted with great caution as reproducibility between studies can be poor.

- On p. 14 of the TD, under the subheading 'd. pathogenicity etc.', the authors claim that *L. lactis* is non-pathogenic largely based on its history of safe use as a food-grade organism. Whereas I do not object against this statement when it concerns application in healthy subjects, I do not support extrapolation of this claim to all clinical subpopulations. In a recent review by Mofredj *et al.* (2007), several historical and new cases of human infections with *L. lactis* are summarized. Importantly, most if not all cases concerned patients with underlying malignancies such as diabetes, cancer, HIV infection etc. Whereas I do not see any immediate reason to disprove of the clinical trials planned with *L. lactis* sAGX0037 in IBD patients, I am in favour of the precautionary principle and would suggest that the applicants take into account this information when members of the patient groups are recruited.
- On p. 15 of the TD, under subheading 'e. antibiotic resistance etc.', the applicants cite ref. 17 to highlight that L. lactis strains are susceptible to a wide range of antibiotics. Like many other properties (see above), however, also antibiotic resistance is strain-specific and should thus be evaluated on a case-by-case basis. In a recent study of Florez et al. (2007), 11/93 L. lactis isolates were shown to exhibit acquired phenotypic resistance to tetracycline and/or streptomycin. For this reason, I recommend that the applicants specifically determine the phenotypic resistance profile of strain sAGX0037 using up-to-date protocols for susceptibility testing as a part of its safety evaluation. The fact that this strain contains the conjugative transposons Tn916 and Tn919 (ref. 15 in the TD) implies that it harbours copies of the ribosomal protection gene tet(M) which confers phenotypic resistance to tetracycline and minocycline. Provided that the tet(M) genes in strain MG 1363 are not silent, this phenotype should thus be reflected in the resistance profile of sAGX0037.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

It has been reported that some bacteriophages can complement thymidylate synthase deficiency (Kozlof et al., 1977) .
Have the notifiers envisaged the possibility that such a complementation could occurs after release of the l.lactis thyA deficient strain in the environment. Do they have arguments to exclude this possibility?

2. INFORMATION RELATED TO THE VECTOR
(e.g. description, sequence, mobilisation ...)

Comment 1

I have evaluated this item and I have no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

I have evaluated this item and I have no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Is the modification of the secretion signal of the hIL10 gene susceptible to confer new epitopes to hIL10? Is there any risk of exacerbated immune response to the secreted IL10? For the patient? For the personal of home relatives who could accidentally come into contact with the OGM?

3. INFORMATION RELATED TO THE CHARACTERISTICS OF THE GMO

3.1. Information related to the genetic modification
(e.g. methods used for the modification, description of the insert/vector construction ...)

Comment 1

After successive steps of genetic modifications (double homologous recombination), the function "expression of hIL-10" was stably integrated in the chromosome of the strain MG1363 by replacing the original WT function (thymidylate synthetase). The Em function of the vector was eliminated during a targeted gene replacement. The removal of the thymidylate synthetase function generated a dependence of sAGX0037 for thymine/thymidine leading to cell dead in absence of these nutriments. It represents a self-limiting system able to eliminate this strain when exposed to non-optimal environments. The GMO sAGX0037 is expected to be non-pathogenic for humans.
The stability of the GMO was tested during 75 generations. One could question if this is sufficient? Ex: What's about the stability of the GMO, if the culture conditions propose sub-bacteriostatic concentrations in some antibiotics?...

Note from SBB:
About the stability of the strain sAGX0037: it is also stated in the technical dossier (Part 1A, p. 23/52) that the sAGX0037 was entered in the notifier collection in January 2007 and has been maintained in culture ever since (monitoring of quality during storage and production).
Remark: The results of a Phase I trial conducted in The Netherlands with a comparable recombinant MG1363 *L. lactis* strain (LL-Thy12; expressing IL-10 and *thyA*-deficient) showed that the GMO was genetically intact after passage of the bacterium through the human gastrointestinal tract.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

3.2. Information on the molecular characteristics of the final GMO

(e.g. number of copies of the transgenes, phenotypic and genetic stability of the transgenes, expression of the new genetic material, re-arrangements in the genome, inclusion or suppression of genetic material ...)

Comment 1

cf. point 3.1.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

On p. 25 of the Technical dossier under the subheading 'f. description of identification and detection techniques etc.', '16sRNA' should read '16S rRNA gene'

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

The notifiers compare the doses of recombinant protein giving side effects in human and the dose measured in the gastrointestinal tract of mice treated with the IL10-expressing L;Lactis. What would be the dose of IL10 expressed by the solution if spilled on the hands of the patient, child, cleaning lady, etc…? Is it still lower than the threshold dose giving side effects?

3.3. Considerations for human, animal or plant health

(e.g. invasiveness and virulence, toxic or allergenic effects, possibility of survival outside of receiving host, other product hazards ...)

Comment 1

I have evaluated this item and I have no questions/comments

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

- Regarding the statements on lack of pathogenicity of the final GMO on p. 26 and p. 27: please see my previous reference to the review of human *L. lactis* infections (Mofredj *et al.,* 2007)
- Regarding the statement on lack of colonization capacity of the final GMO on p. 27: please see remark above on the importance of in-vitro adhesion studies.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Is the modification of the secretion signal of the hIL10 gene susceptible to confer allergenicity to hIL10? Has this been evaluated in mice? It would be a good idea to evaluate this possibility in mice with a humanized immune system. (ex. Firat H, et al., 1999; Geluk A. et al., 2000)

4. INFORMATION RELATING TO THE CONDITION OF RELEASE

(e.g. description of the activity, quantities of GMO to be released, workers protection measures, elimination of any contaminating material in the preparation of the GMO stock, elimination of the GMO at the end of the experiment ...)

Comment 1

At the end of the therapy or in case of problem during the treatment, it is possible to inactivate of the GMO by a standard antibiotic administration.

The release of the GMO by the treated patients is expected in the faeces.
Because of the limited properties of survival in classical environments in absence of a thymine/thymidine addition, the risk exposure and the danger for the environment is very limited in intensity and in time.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

5. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT AND HUMAN HEALTH

5.1. Information on spread ("shedding") of the GMO from the treated patient/animal to other persons/animals or to the environment (including indirect/delayed effects due to vertical transmission to offspring).

(e.g. genetic transfer capability, routes of biological dispersal, target organisms ...)

Comment 1

cf. points 4. and 5.2

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

I would like to see an extended control (eg. up to 3 weeks) for the release of the GMO and/or its DNA.

Comment 4

Has evaluated this item and has no questions/comments.

5.2. Information on possible effects on human health resulting from interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release (carekeepers, patient relatives, immunocompromised people ...).

Comment 1

We could invite to decontaminate the faeces during and also the first days after the end of the treatment in order to avoid an (important, uncontrolled) release of the GMO in the environment. The presence in the neighbouring of sensible people as babies, immunocompromised people, could be problematic if the treated patients have access without hygienic restriction to the same sanitary fittings as these sensible people. In addition to the standard hygienic rules, the notifier could propose different procedures of decontamination with the use description of the different products. Following the caution principle over the GMO use and release, the elimination of the different GMO containers (after use) and the faeces could merit a treatment such as biohazard material ("biohazard bin"…), even if the survival of the GMO and the potential transduction of its genetic material (via phages) are claimed by the notifier as highly unlikely.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Except if I have overlooked it, nothing was mentioned about immunocompromised people. This needs to be addressed

Comment 4

See 3.3

5.3. Information on possible effects on animal health or on the environment.

Comment 1

cf. point 5.2.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

5.4. Information on selective advantages or disadvantages conferred to the GMO compared to the parental organism.

Comment 1

cf. points 1. and 3.1.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

5.5. Information on the possibility of the GMO to reconvert to his wild type form and possible consequences for human health or the environment.

Comment 1

cf. points 1, 2, 4 and 5.2

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

See point 1.

5.6. Information on the possibility of the GMO to exchange genetic material with other micro-organisms and possible consequences for human health or the environment.

Comment 1

cf. points 3.1 and 5.2

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

5.7. Information on the possibility of gene transfer to other organisms and about the selective advantages or disadvantages conferred to those resulting organisms (possible consequences for human health or the environment).

Comment 1

cf. points 3.1 and 4

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

6. INFORMATION RELATED TO THE MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY PLANS PROPOSED BY THE APPLICANT

6.1. Monitoring plan proposed by the notifier and possibility to identify the occurrence of non-anticipated adverse effects.

(adequation between the monitoring plan and risks identified during the risk assessment, when appropriate measures to minimize the potential risks to offspring ...)

Comment 1

The part of the dossier proposed by the notifier, about the monitoring plan and possibility to identify the occurrence of non-anticipated adverse effects, is considered very light. It is necessary to develop this point.

Comment 2

Personnel protection, material decontamination and waste treatment at the hospital are well described in the technical dossier. Those are in compliance with standard biosafety recommendations.
However, the patient will not reside in the hospital during self-administration of the treatment. For this part of the protocol, the notifier mentions the following protective measures: "normal hygienic practices are sufficient".
The notifier should provide the detailed protocol (recommendation/training) given to the patient (and information given to closest living patient's relatives) before starting the treatment.

Comment 3

On p. 44, under the subheading 'monitoring techniques', the applicants state that they have developed methods based on 16S ribosomal genes that uniquely identify the GMO. Although not cited as such, I assume that this refers to the methods described in reference 5. If this is the case, I remark that these methods are designed to detect *L. lactis* at species level but do not allow to discriminate between different strains of this species e.g. between strain MG 1363 and *L. lactis* strains from food intake transiently residing in the intestine. Strain-specific PCR primers have been developed for *L. lactis* subsp. *cremoris*, e.g. Maruo *et al.* (2006) derived strain-specific markers from RAPD fingerprints.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Are there methods to detect the bacteria at patient's home? Is it relevant?

6.2. Information on the waste generated by the activity and its treatment.

(e.g. type of waste, amount ...)

Comment 1

cf. point 5.2

Comment 2

see point 6.1

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

6.3. If applicable, information on the emergency plan(s) proposed by the notifier.

Comment 1

I have evaluated this item and I have no questions/comments

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

6.4 Information related to the identification of the GMO and the detection techniques

(e.g. identification methods and detection techniques, sensitivity, reliability and specificity of the proposed tests ..)

Comment 1

For the detection of hIL-10, an ELISA was proposed with a sensitivity (detection limit) of 8.4 pg/ml of hIL-10. It is perhaps possible to increase this detection limit for the analysis of the environmental matrices, by using of other alternative immuno-detection methods like Real-Time-immuno-PCR.

Note from SBB:
The recommendation to improve the hIL-10 detection limit is probably useful when studying *in situ* / *in vivo* hIL-10 production from treated patient samples (for example in human or animal models)*.
However it could be less relevant in complex environmental matrices (e.g after release in the environment from shedding). Detection (and sequencing) of specific hIL-10 rDNA, measurement of hIL-10 rRNA versus hIL-10 rDNA, and evaluation of 16sDNA/16sRNA ratio by Quantitative PCR from sAGX0037 *L. lactis* strain isolated (on appropriate culture medium) from environmental sample should be more relevant. The notifier describes this approach.

*The SOP describing hIL-10 sandwich ELISA is designed to determine the amount of hIL-10 in the supernatant of recombinant *L. lactis*, in serum and tissue samples.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Alternatively to the development of strain-specific PCR primers (see reference Maruo *et al.*), I can also recommend to determine a DNA fingerprint of the final GMO e.g. using Amplified Fragment Length Polymorphism (AFLP) and/or Pulsed-Field Gel Electrophoresis (PFGE) profiling (Vancanneyt *et al.*, 2006). The availability of such a fingerprint could prove extremely useful at various stages of the trial, e.g. to monitor the quality and temporal stability of different production batches of the lyophilised GMO strain, and to monitor the genetic stability of the GMO during and after the clinical administration to IBD patients.

Comment 4

Are the mentioned methods extensively assessed also for possible inhibition of PCR in "dirty" samples?

Comment 5

See point 6.1

7. OTHER INFORMATION

7.1 Do you have any other questions/comments concerning this notification that are not covered under the previous items?

No other comments.

References

Dharmawan *et al.* (2006) Adhesion Properties of Indigenous Dadih Lactic Acid Bacteria on Human Intestinal Mucosal Surface *Asian-Australian Journal of Animal Sciences* **19(5)**:751-755

Firat H, Garcia-Pons F, Tourdot S, et al. (1999) H-2 class I knockout, HLA-A2.1-transgenic mice: a versatile animal model for preclinical evaluation of antitumor immunotherapeutic strategies. *Eur J Immunol.* 29(10):3112-21

Florez et al. (2007) Antibiotic survey of Lactococcus lactis strains to six antibiotics by Etest, and establishment of new susceptibility-resistance cut-off values. *Journal of Dairy Research* 74(3):262-268

Geluk A, van Meijgaarden KE, Franken KL, Drijfhout JW, D'Souza S, Necker A, Huygen K, Ottenhoff TH (2000) .Identification of major epitopes of Mycobacterium tuberculosis AG85B that are

recognized by HLA-A*0201-restricted CD8+ T cells in HLA-transgenic mice and humans. *J Immunol.* 165:6463-71

Kozloff L.M., Lute M., L. Crosby L.K.. (1977) Bacteriophage T4 Virion Baseplate Thymidylate Synthetase and Dihydrofolate Reductase *Journal of virology*, 23, p. 637-644.

Maruo *et al.* (2006) Monitoring the cell number of Lactococcus lactis subsp. cremoris FC in human feces by real-time PCR with strain-specific primers designed using the RAPD technique. *International Journal of Food Microbiology* **110(1)**:69-76

Mofredj *et al.* (2007) Lactococcus lactis : un pathogène opportuniste? *Médecine et Maladies Infectieuses* **37**:200-207

Vancanneyt *et al.*, (2006) Intraspecific Genotypic Characterization of Lactobacillus rhamnosus Strains Intended for Probiotic Use and Isolates of Human Origin. *Applied and Environmental Microbiology* **72**:5376-5383