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To the Minister for the Environment
Ms V.L.W.A. Heijnen
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DATE	31 January 2023
REFERENCE	CGM/230131-01
SUBJECT	Research project on detection methods for replication-competent lentiviral and retroviral vectors

Dear Mrs Heijnen,

Attenuated lentiviruses and retroviruses are often used in biomedical, genetic, and clinical research because these viruses are able to integrate their genetic material into the genome of the host cell, giving them the ability to insert genes into cells. These viruses are attenuated by removing a large part of the viral genome. The attenuated viruses (hereafter called lentiviral or retroviral vectors) are still able to integrate their genome into the host genome, but they are no longer capable of forming new virus particles: they are replication-defective. However, the spontaneous formation of a replicating virus (i.e. replication-competent virus (RCV)) during the manufacture of these vectors cannot be ruled out. As RCVs can spread, they present a risk during activities involving lentiviral and retroviral vectors.

COGEM commissioned a study into the methods that can be used to identify the presence of RCVs during the production or use of lentiviral and retroviral vectors. The results of this study are described in the attached research report, '**Detection of replication competent virus formation during production and use of lenti- and gammaretroviral vectors'** (CGM 2023-01). The study consisted of an extensive literature review and a questionnaire-based survey of stakeholders on their practical experiences with RCV testing.

The research report contains a good inventory of the currently available RCV assays, with information on their suitability and limitations, the available data on validation of the assays, and the positive and negative controls used. The report distinguishes between 'structural assays', which are based on detecting protein sequences of the RCV (immune assays or molecular assays), and 'functional assays', which reveal the biological activity of any RCVs present (cellular assays). A major consideration regarding the structural assays mentioned in the report is that a positive result does not necessarily mean that a functional RCV has in fact been formed, because the structural

components that the assay identifies may also be present in the replication-defective vector particles. When using functional assays, care should be taken to consider the possibility that an infection in the indicator cell line used, such as the presence of endogenous retroviruses, can lead to a false positive outcome. Using multiple tests may provide a more accurate indication of the reliability of the outcome.

A key finding in the report is that performing an amplification phase involving serial passaging on sensitive cell cultures before the RCV assay can increase the sensitivity of the tests and reduce the likelihood of a false positive. During this step the number of (interfering) replication-defective vector particles is reduced because these can only infect once and the number of any RCVs present is increased because of their replication competence. However, this process is time-consuming.

The report states that limited data are available for validation of RCV assays and that the available data are hard to compare. Furthermore, when an assay detects no RCVs, it is still possible that RCVs are present, but the quantity is too small to detect (below the limit of detection). The likelihood of a false negative outcome can be reduced by carrying out an amplification phase.

In the questionnaire survey, various organisations that produce retroviral and lentiviral vectors or use them in clinical or other research were asked to provide information on the RCV assays that they used. Twenty organisations were approached, of which seven completed the survey. Four of these said that they carry our RCV testing.[1] Two of these organisations use the vectors for clinical applications; the other two produce vectors or transduced cells for more fundamental research purposes. Based on the questionnaire results, the authors of the report conclude that RCV testing is mainly performed when the vectors are produced for clinical applications. When these types of vectors are used for clinical applications, the European Medicines Agency (EMA)[2] and the GMO legislation[3] require RCV assays to be carried out to rule out the possibility of replication capacity being restored during manufacture by recombination or complementation. However, certain vectors that are manufactured in an advanced production system in which the likelihood of RCV being formed is negligible[4] are often exempted from mandatory testing. Laboratories where more fundamental research is carried out only perform RCV testing when this is mandatory under the GMO legislation for the environmental risk assessment. RCV assays may also be carried out when the situation requires it. For example, one organisation reported that RCV tests are only performed if unexpected cell death is observed during the production of the vectors.

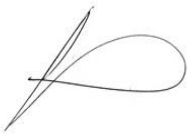
1. The other three organisations do not carry out RCV tests because this is not mandatory for the environmental risk assessment, for example because they work with 3rd generation SIN lentiviral vectors or because the activities are not carried out at a lower containment level.
2. European Medicines Agency (EMA, 2018). Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products. EMA/CAT/80183/2014
3. Good Practice on the assessment of GMO-related aspects in the context of clinical trials with human cells genetically modified by means of retro/lentiviral vectors. Version 3 https://www.loketgentherapie.nl/documenten/good-practice-on-assessment-of-gmo-related-aspects-in-context-of-clinical-trials-with-0
4. Such as SIN lentiviral vectors manufactured with a 2nd or 3rd generation production system. There have been no reports in the scientific literature of the presence of RCV during production with these systems. COGEM has previously advised that an RCV tests is not necessary: COGEM advice CGM/090331-03

The organisations use different tests to detect RCV. All of them use an amplification phase before the RCV assay. One organisation mentioned that they sometimes detected RCVs when producing gammaretroviral vectors. The RCV was formed in the presence of endogenous retroviral sequences in production cells derived from mice.[5]

From the research report, COGEM concludes that a wide range of RCV assays are available and that each has its own advantages and disadvantages. COGEM advises including an amplification phase before testing to reduce the likelihood of both false positive and false negative outcomes. COGEM also stresses the importance of validation and the need to use suitable positive and negative controls for the correct interpretation of the assay. It therefore advises using only validated assays for permit applications.

COGEM notes that laboratories and companies that work with lentiviral or retroviral vectors and perform RCV testing often do not make information on RCV detections and the controls used publicly available. These data are needed to obtain a better understanding of whether or not RCV testing is necessary for lentiviral and retroviral vectors and production systems. COGEM points out that these data can influence the environmental risk assessment of these vectors and that making this information more freely available would help to improve the authorisation procedures. COGEM therefore advises the Ministry of Infrastructure and Water Management to consult with the industry about sharing this information.

Yours sincerely,



Professor Sybe Schaap
Chair of COGEM

c.c.
- Y. de Keulener, head of the GMO Office
- Ministry of Infrastructure and Water Management, Environmental Safety and Risks Directorate, Directorate-General for the Environment and International Affairs

5. COGEM has previously pointed out that the presence of endogenous retroviruses (ERVs) can increase the risk of RCV formation: COGEM (2021). COGEM advice CGM/210218-01