COMMISSIE
COGEM
GENETISCHE MODIFICATIE

VISITING ADDRESS:
A. VAN LEEUWENHOEKLAAN 9
3721 MA BILTHOVEN

POSTAAL ADDRESS:
POSTBUS 578
3720 AN BILTHOVEN

TEL.: 088 689 2777
INFO@COGEM.NET
WWW.COGEM.NET

To the Minister for the Environment and Public Transport
A.A. Aartsen
P.O. box 20901
2500 EX The Hague

DATE	19 August 2025
REFERENCE	CGM/250819-01
SUBJECT	Advice Explanation of the COGEM formula for free vector particles

Dear Minister,

The so-called COGEM formula, which is used to determine if additional safety measures are required for gene therapy studies, among other things, can sometimes be challenging for applicants to use. This advisory report provides an update and further explanation of the formula.

Summary:
In biomedical or clinical research, cells are often genetically modified using lentiviral or retroviral vectors, which are genetically modified (GM) viruses. These GM viruses can integrate their genome into a cell's genome and incorporate a gene of interest. Although genetically modified cells often undergo various cultivation and washing steps after treatment with GM vectors, 'free' vector particles (particles not taken up by the cell) may remain present. The presence of these particles poses a potential risk to third parties if they are accidentally exposed to them. Depending on the number of particles present, measures may be necessary to prevent exposure. The number of remaining lentiviral or retroviral vector particles after cultivation and washing can be determined experimentally or theoretically. An experimental approach is preferred but not always possible. In such cases, the 'COGEM formula' can be applied.
COGEM has noted that the COGEM formula is not always applied correctly. For some applicants, it also appears to be unclear how the formula's outcome (the reduction ratio) relates to the number of free vector particles. To clarify the formula's use, COGEM provides a more detailed explanation in this advisory report and outlines its limitations.

The attached report contains COGEM’s advice and considerations.

Yours sincerely,

Prof. dr. ing. Sybe Schaap
Chair of COGEM

c.c.

- Y. de Keulenaar, MA, Head of the GMO Office
- Ministry of Infrastructure and Water Management, Environmental Safety and Risks Directorate, Directorate-General for the Environment and International Affairs

Advice Explanation of the COGEM formula for calculating free lentiviral or gammaretroviral vector particles after ex vivo transduction

COGEM advice CGM/250819-01

1. Background information

Replication-deficient viral vectors derived from lentiviruses (mainly HIV-1) and gammaretroviruses (mainly MoMuLV) are frequently used in biomedical and clinical research for the genetic modification of cells. These vectors integrate their genome into the host cell genome. After transduction, the genetically modified (GM) cells often undergo various culture and washing steps, partly with the aim of removing the excess of so-called 'free' vector particles (i.e. not internalised in the cell). There are various risks associated with the exposure of third parties to these remaining vector particles. When integration of the vector genome occurs in cells of the unintended recipient, tumour suppressor genes may be disrupted or proto-oncogenes may be activated, which can lead to cancer (oncogenesis). Potentially undesirable effects may also occur due to expression of the transgene used.

Laboratory staff may be exposed to free vector particles during their work in laboratories (under contained use). However, if staff members become unintentionally infected with these particles, they cannot spread them to third parties because these vectors lack the genetic information essential for replication. Based on occupational health and safety perspectives, COGEM has previously recommended additional containment measures when the risk of exposure to free vector particles is high.[1]

Replication-deficient lentiviral or gammaretroviral vectors are widely used in clinical gene therapy studies to produce genetically modified cells, such as CAR T cells. For this purpose, cells (typically blood or stem cells) are harvested from a patient or donor and genetically modified outside the body. Even after washing or culture steps, free vector particles may remain in the suspension with GM cells after transduction. If free infectious vector particles are present in the medical product, they can spread to third parties (e.g. family members or bystanders) in the event of an incident involving blood-to-blood contact with the patient. Exposure of third parties to these vector particles could result in unintended transduction, which poses potential health risks. Depending on the number of infectious vector particles remaining in the medical product (the GM cells) after the cultivation and washing steps, additional measures may be necessary to minimise the risk of transmission of free vector particles from the patient to third parties.

Although experimental determination of the number of remaining free vector particles after cultivation and washing is preferred, the COGEM formula can offer a solution when an experimental approach is infeasible.[2,3] The COGEM formula offers the most accurate estimate of the number of remaining lentiviral or gammaretroviral vector particles. The formula was initially developed for risk assessments of laboratory work involving replication-deficient lentiviral vectors pseudotyped with the glycoprotein of the vesicular stomatitis virus (VSV-G), primarily with adherent cells.[2] However, the formula is now also used to assess applications for clinical studies with ex vivo transduced cells.[4,5,6,7] The COGEM formula was amended in 2020 after various parameters from the formula were experimentally validated on behalf of COGEM.[3,8]

In recent years, COGEM has advised on a large number of clinical studies involving ex vivo lentiviral or retroviral transduced cells. In several cases it has become apparent that the COGEM formula has not been applied correctly. As a result, it was not possible to determine how many free vector particles might still be present. Furthermore, it seems that some applicants do not immediately understand how the calculated reduction ratio relates to the number of remaining vector particles. This advisory report therefore provides further clarification on the use of the COGEM formula, incorporating the latest adjustments.[3,9,10]

2. Explanation of the COGEM formula

The result of the COGEM formula is expressed as the reduction ratio. The reduction ratio is determined by several parameters: the natural decay of the vector particles (half-life); inactivation, or loss of infectivity, due to the presence of trypsin; and loss of particles due to washing the cultured cells.

However, the formula <u>does not</u> take into account the decrease in free vector particles due to their uptake by cells (i.e. transduction), as this depends heavily on the experimental conditions and the transduction efficiency of the cells used.

The COGEM formula and an explanation of the variables and units used can be found below:

$$Reduction\ ratio\ = \frac{wash^{W} \times (wash \times tryp)^{I} \times 2^{(F \times T)}}{N_i}$$

wash: The reduction achieved through the washing steps. As the formula was developed for work with lentiviral vectors in the laboratory, it is based on the washing of adherent cells in culture dishes. The value of this variable depends on various factors, including the size of the culture dishes used. When washing a culture dish with a diameter of 10 cm (to which 10 ml of medium is added), the constant value '20' can be used, which was experimentally determined and corresponds to a 95% reduction in the volume of the medium (and thus the free vector particles present).[3] In the case of a 35 mm culture dish (to which 3 ml of medium is added), the experimentally determined constant value of '50' can be used.[3] Depending on the culture dishes used, the formula is as follows:

- Culture dish with a diameter of 10 cm: $Reduction\ ratio\ = \frac{20^{W} \times (20 \times tryp)^{I} \times 2^{(F \times T)}}{N_i}$
- Culture dish with a diameter of 35 mm: $Reduction\ ratio\ = \frac{50^{W} \times (50 \times tryp)^{I} \times 2^{(F \times T)}}{N_i}$

Different values may be used when the experimental setting deviates from the method described above (i.,e when a different culture dish format is used, a different method is used to wash cells, when cells in suspension are used instead of adherent cells, or when a different kind of culture system –other than culture dishes– is used), and when experimental data on these values are available or adequately justified.

W: The number of washing steps performed.

***tryp*[a]:** The reduction achieved through washing cells with trypsin. Trypsin is a proteolytic enzyme that is widely used to detach adherent cells in cell cultures. However, it can also cleave envelope proteins from lentiviral particles, thereby inactivating the vector particles. The formula includes a washing step with trypsin as an additional inactivation factor.[b] The aforementioned study showed that this value depends heavily on the pseudotyping protein used. For lentiviral vectors pseudotyped with different envelope proteins, the values determined in the study are listed below.[3] These values may differ for pseudotyped retroviral vectors.

Reduction factor in the inactivation of pseudotyped lentiviral vectors by trypsin (*tryp*)							
VSV-G	*Measles*	*GALV*	*Rabies*	*RD114*	*4070A*	*10A1*	*MULV*
1	4	79	2	110	4	1	10

VSV-G: glycoprotein of vesicular stomatitis virus, Measles: glycoprotein of measles virus, GALV: glycoprotein of Gibbon ape leukaemia virus, Rabies: glycoprotein of rabies virus, RD114: glycoprotein of feline endogenous RD114 retrovirus, 4070A: glycoprotein of Moloney MULV 4070A retrovirus, 10A1: glycoprotein of Moloney MULV virus strain 10A1, MULV: glycoprotein of murine leukaemia virus.

I: The number of inactivation washing steps with trypsin.

F: The natural decay of the vector particles. F is calculated by dividing 24 hours by the vector particle's half-life in hours. This value depends heavily on the pseudotyping protein used. The following values have been determined experimentally for lentiviral vectors pseudotyped with different envelope proteins.[3]

Reduction factor (F) based on the half-life of pseudotyped lentiviral vectors:								
Pseudotyping protein	*VSV-G*	*Measles*	*GALV*	*Rabies*	*RD114*	*4070A*	*10A1*	*MULV*
Value of F	0,7	1,7	2,9	1,6	1,1	0,7	1,7	1,3
Half life (in hours)	34,7	14,1	8,3	15,4	21,2	36,6	13,8	18,6

VSV-G: glycoprotein of vesicular stomatitis virus, Measles: glycoprotein of measles virus, GALV: glycoprotein of Gibbon ape leukaemia virus, Rabies: glycoprotein of rabies virus, RD114: glycoprotein of feline endogenous RD114 retrovirus, 4070A: glycoprotein of Moloney MULV 4070A retrovirus, 10A1: glycoprotein of Moloney MULV virus strain 10A1, MULV: glycoprotein of murine leukaemia virus.

These values may differ for pseudotyped retroviral vectors. Depending on the pseudotyping and experimental conditions, different half-lives have been reported in the literature for gammaretroviral vectors.[c,11,12,13,14,15,16]

T: The culture time in days (24 hours) after transduction.

---

a. Originally, inactivation by human complement was also included in the formula as an inactivation factor. However, the research report showed that the effectiveness of complement inactivation depends on too many variables to be included as a constant factor in the formula.

b. Originally, a reduction factor of 10 was used in the formula. Combined with a washing step in a 10 cm culture dish, this resulted in the value 20 (wash) x 10 (tryp) = 200 in the original formula.[2]

c. Reported half-lives for pseudotyped retroviral (MoMuLV) vectors at 37 °C include: VSV-G: 4.5 ± 1 hour[11], 4070A: 4-14 hours[13,15], GALV: 10-13 hours[13,15], 10A1: 14 hours[13], RD114: 6- >24 hours[13,15]. It should be noted that these values apply to the specific conditions under which the experiments were conducted. These may differ from the conditions used for the relevant licence application.

N_i: The original number of infectious vector particles in the inoculum (in previous advisory reports represented as C_i). If data are available on the reduction in the number of particles due to transduction, this can be taken into account in the value for the inoculum in the COGEM-formula.

3. Relationship to the number of free particles

COGEM noticed that confusion can sometimes arise regarding the relationship between the result of the COGEM formula, the reduction ratio, and the number of free infectious vector particles present. The term 'reduction ratio' may be the cause of this confusion. The number of free infectious vector particles is equal to 1/reduction ratio. In other words, the number of free vector particles can be calculated directly by reversing the numerator and denominator in the COGEM formula:

$$Number\ of\ free\ particles = \frac{N_i}{wash^W \times (wash \times tryp)^I \times 2^{(F \times T)}}$$

For clinical studies involving ex vivo retro- and lentiviral transduced cells, COGEM has recommended a specific threshold value for vector particles. This threshold value was recently adjusted to 500 free vector particles (equivalent to a reduction ratio of 0.002)[10] and is used to determine whether additional measures are required to minimise the risk of spreading lentiviral and retroviral vector particles. If this threshold is exceeded, the following generic control measures must be applied:

- After administration of the medicinal product, the insertion site should be disinfected using an adequate method for inactivating any remaining cells and vector particles;
- Following administration of the medicinal product (ex vivo transduced cells), the patient should remain in the hospital for at least 16 hours to ensure that the appropriate hospital hygiene measures can be observed. The patient, medical personnel and visitors should be informed about protocols and safety measures concerning the care of wounds and infected material during the first 16 hours after administration of the medicinal product. If the applicant can make a plausible case that the vector particles have already been sufficiently cleared from the patient's body in less than 16 hours, this period may be shortened accordingly.

4. Limitations of the formula

The COGEM formula provides the best possible approximation of the number of residual vector particles present. However, it should be noted that the parameters used depend on the specific experimental conditions used, meaning that the actual number of free vector particles may differ.

The formula was originally intended for cultures with adherent cells. In addition, the half-life and degree of inactivation by trypsin have only been determined for pseudotyped lentiviral vectors. Experimental determination of the number of free infectious lentiviral or retroviral vector particles is therefore preferred over applying the COGEM formula.

If experimental determination is not possible, the COGEM formula offers the best approximation of the number of free vector particles present, despite its limitations. Confirming the values of the parameters in the formula for the specific conditions of the experiment or clinical study helps to provide a more accurate estimate of the number of vector particles present.

References

1. COGEM (2021). Heroverweging inschaling werkzaamheden met replicatiedeficiënte lenti- en gammaretrovirale vectordeeltjes onder Ingeperkt Gebruik. [In Dutch] COGEM advice CGM/210218-01
2. COGEM (2009). Inschaling van laboratoriumwerkzaamheden met lentivirale vectoren. [In Dutch] COGEM advice CGM/090331-03
3. Dautzenberg IJC & Hoeben RC (2020). The COGEM formula revisited. Experimental validation of the reduction ratio formula for free lentiviral particles. COGEM report CGM 2020-01
4. COGEM (2019). Generieke milieurisicobeoordeling van klinische studies met ex vivo retro- en lentiviraal getransduceerde cellen. [In Dutch] COGEM advice CGM/190729-01
5. European Commission. Advanced therapies. https://health.ec.europa.eu/medicinal-products/advanced-therapies_en (visited: 21 juli 2025)
6. Loket Gentherapie (RIVM). Aanvraagformulier Retro/lentivirale vectoren zonder deeltjes – VoV. https://www.loketgentherapie.nl/aanvraagformulier-retrovirale-vectoren-vov [In Dutch] (visited: 21 juli 2025)
7. Loket Gentherapie (RIVM). Aanvraagformulier Lentivirale vectoren en SIN virale partikels - VoV https://www.loketgentherapie.nl/aanvraagformulier-cellen_LLV-SIN_met_deeltjes-vov [In Dutch] (visited: 21 juli 2025)
8. Dautzenberg IJC *et al*. (2020). The stability of envelope-pseudotyped lentiviral vectors. Gene Ther. 28: 89-104
9. COGEM (2020). Generiek advies over de milieurisicobeoordeling van klinische studies met ex vivo lentiviraal getransduceerde cellen: aanwezigheid van vrije vectordeeltjes in het medisch product. [In Dutch] COGEM advice CGM/200507-01
10. COGEM (2025). Advies Heroverweging grenswaarde aanwezige vrije lenti- en retrovirale vectordeeltjes bij klinische studies met ex vivo getransduceerde cellen. [In Dutch] COGEM advice CGM/250520-01
11. Higashikawa F & Chang L (2001). Kinetic analyses of stability of simple and complex retroviral vectors. Virology 280: 124-131
12. Andreadis ST *et al*. (1997). Moloney murine leukemia virus-derived retroviral vectors decay intracellularly with a half-life in the range of 5.5 to 7.5 hours. J. Virol. 71: 7541-7548
13. Uckert W *et al*. (2000). Efficient gene transfer into primary human CD81 T lymphocytes by MuLV-10A1 retrovirus pseudotype. Hum. Gene Ther. 11: 1005-1014
14. Ghani K *et al*. (2007). Generation of a high-titer packaging cell line for the production of retroviral vectors in suspension and serum-free media. Gene Ther. 14: 1705-1711
15. Ghani, K *et al*. (2009). Efficient human hematopoietic cell transduction using RD114- and GALV-pseudotyped retroviral vectors produced in suspension and serum-free media. Hum. Gene Ther. 20: 966-974
16. Carmo M *et al*. (2009). Stabilization of gammaretroviral and lentiviral vectors: from production to gene transfer. J. Gene Med. 11: 670-678