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High-performance liquid chromatographic total particles quantification of retroviral vectors pseudotyped with vesicular stomatitis virus-G glycoprotein

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Abstract

A novel and rapid method for the total particles quantification of murine leukemia virus derived retroviral vectors pseudotyped with vesicular stomatitis virus-G glycoprotein was developed using high performance liquid chromatography. Virus particles were detected by absorbance at 260 nm and quantified using a calibration curve generated from highly purified and concentrated viral stock characterized by negative stain electron microscopy. The method requires Benzonase® digestion and concentration of the supernatant prior to analysis. The virus eluted in 12.55 min at a flow rate of 1 mL/min in 20 mM Tris–Cl, pH 7.4 + 1.1 M NaCl. The limits of detection and quantification of this assay were 4.71×10^8 and 1.57×10^9 viral particles/mL, respectively. Linearity was between 3.0×10^9 and 1.0×10^{11} viral particles/mL with a correlation coefficient of 0.9923 and a slope of 6×10^{-6}. The assay precision was < 5% and <10% for intra- and inter-day analysis, respectively. This assay was used for the total particles quantification of a 7-day, large-scale perfusion culture production of a retroviral vector grown in 293 cells expressing the β-galactosidase gene.

Keywords: Retroviral vector; Vesicular stomatitis virus-G glycoprotein

1. Introduction

Retroviral vectors (retrovectors) are the most widely used system in gene transfer clinical trials because of their ability to deliver and sustain long term gene expression. Retroviruses belong to the class of ss RNA enveloped viruses, spherical in shape, with an average size of 120 nm. They contain ∼62% protein, ∼36% lipid, ∼2% carbohydrate and 2% RNA [1]. The usual low titer yields obtained with the wild type retrovirus were greatly improved with the pseudotyping of its envelope with the G-protein of the vesicular stomatitis virus (VSV-G) [2] enabling concentration up to 100-fold by high speed ultracentrifugation with good recovery [2–4]. Currently, the scale of retroviral vectors being produced is sufficient only for ex vivo cell and gene therapy trials. It has been estimated that for in vivo applications, much higher virus titers (10^7–10^{14}) are required to obtain a therapeutic effect [1,5]. To achieve this goal, significant work has been dedicated to improve producer cells and develop processes for large-scale production. For example, adaptation of the 293GPG packaging cell line to suspension culture greatly improved the virus production at a larger scale [6]. To facilitate the optimization of conditions during the development of large-scale production and downstream processes, rapid quantification assays of virus particles concentration are needed. To date, the most commonly used methods to quantify the concentration of retrovirus particles (retroparticles) are titration and electron microscopy (EM). Titration methods which measure infectious particles rely mostly on the infection of permissive cells taking several days before results are obtained. EM measures the total particles concentration but

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fails to distinguish between infectious and non-infectious particles. These methods to quantify total retroparticles as well as immunostaining, reverse transcriptase (RT) and polymerase chain reaction (PCR) [7–10] are not suitable for routine quantification assays since they are labor intensive and quite often non-specific. Due to the instability of most retrovectors, a rapid assay to quantify total particles independent of infectivity allows immediate action when necessary without subjecting the virus under different environmental conditions. This led us to the development of an anion exchange chromatography method that specifically and efficiently resolved the virus from the rest of the contaminants in a total analysis time of 23 min.

In this paper, we present the results obtained during the development of the method and the characterization of the analytical parameters. Also, its applicability during a large-scale production is demonstrated.

2. Experimental

2.1. High-performance liquid chromatography (HPLC)

A HPLC Alliance system was used (Waters, Milford, MA) equipped with a 2690 separation module, in-line degasser, 996 photodiode array detector (PDA) and a Millennium32 software for data acquisition and peak integration. A UNOQ anion exchange polishing column (4.6 mm × 10 mm, Bio-Rad, Hercules, CA) was used to isolate the virus from the rest of the matrix components. The solutions used were always filtered through a 0.45 μm membrane prior to use. Mobile phases were as follows: Solution A: 0.1 M Tris–Cl, pH 7.4, Solution B: 2 M NaCl in Milli-Q water and Solution C: Milli-Q water. Prior to sample injection, the column was always equilibrated with 5 column volumes (CV) of 20% A, 80% C and a buffer blank (20 mM Tris–Cl, pH 7.4) was injected to ensure a flat baseline. Afterwards, 200 μL of the sample were injected and the run started with 20% A, 25% B, 55% C (v/v). The virus eluted with a linear salt gradient from 0.9 to 1.1 M NaCl in 20% A. Following virus elution, the column was regenerated with 1.6 M NaCl in 20% A and re-equilibrated with 5 CV of 20% A, 0% B, 80% C for the next injection.

2.2. Samples preparation

Prior to analysis, virus supernatants were digested with 200 U/mL of Benzonase® (EM Science, Hawthorne, NY) and concentrated 25-fold by centrifugation. Briefly, 20 μL of Benzonase® working solution (10,000 U/mL in 20 mM Tris, 2 mM $MgCl_2 \cdot 6H2O$ + 20 mM NaCl) was added to 1 mL of virus sample and incubated for 1 h at RT with slow shaking. The digested samples were concentrated by centrifugation at 5000 × *g*, for 1.0–2.5 h at 4 °C (Sorvall RC 3B Plus) using a Macrosep centrifugal device with a molecular weight cut-off (MWCO) of 300 K (Pall Life Sciences, Ann Arbor, MI). Prior to injection, all samples were filtered through a 0.45 μm GHP Acrodisc 13 mm membrane (Pall Life Sciences, Ann Arbor, MI). For concentrated virus, dilution was performed in 20 mM Tris–Cl, pH 7.4 to obtain a value that would fall within the linearity range.

2.3. In-house standard

The in-house standard used was a retrovector pseudotyped with VSV-G encoding the fusion of thymidine kinase and green fluorescent protein produced in 293 cells (293GPGTK-GFP) and purified by size exclusion chromatography (SEC) as described elsewhere [4]. The purification process is summarized as follows; virus supernatants were concentrated by ultracentrifugation in 25% sucrose cushion; digested with Benzonase®, chromatography purified and concentrated/diafiltered by ultrafiltration. The total particles concentration expressed as viral particles/mL (VP/mL) of the purified virus was quantified by negative stain electron microscopy (NSEM) at the Institut Armand Frappier (IAF, Laval, Que., Canada) according to methods described previously [11,12].

2.4. Calibration curve

The total particles concentration obtained by NSEM was used to generate the HPLC calibration curve. Several concentrations of the in-house standard covering the linearity range of the curve were diluted in 20 mM Tris–HCl, pH 7.4. The calibration curve was constructed by plotting the peak area response at absorbance 260 nm (*y*-axis) against the VP/mL (*x*-axis). The concentration of the unknown was quantified according to the standard linear regression equation.

2.5. Assay linearity, limit of detection (LOD), limit of quantification (LOQ), and precision

The performance criteria of the developed method was characterized according to a method described previously [13]. The assay linearity was determined by injecting several concentrations of the purified virus and the correlation coefficient and slope were determined. To determine The LOD and LOQ of the assay, the noise was first determined. The three lowest concentrations of the established linear curve were injected in six replicates and the standard deviations (S.D.) of each were calculated. The mean of the S.D. was calculated and divided by the slope of the linear curve. The LOD and LOQ were determined by multiplying the assay noise by a factor of 3 and 10, respectively. The method's precision was determined by six (6) repeat injections of a concentrated virus sample at 3×10^{10} VP/mL for five consecutive days. The percentage of relative standard deviation (% R.S.D.) for the six repeat consecutive injections per day were calculated for intra-assay precision; the mean and S.D. for the six repeat injection during five consecutive days were calculated

for percentage of R.S.D. for the inter-assay precision determination.

2.6. Virus titer determination

Virus titer determination was performed on 293GPGTK-GFP by expression of GFP using the fluorescence activated cell sorting (FACS, XL-MCL, Beckman Coulter, Brea, CA) analysis as described elsewhere [4]. For the retrovector encoding the β-galactosidase gene (293GPG Lac-Z) expression of Lac-Z was determined by the colorimetric method [14] using a fluorometer (FL600, BioTek Instruments, Winooski, VT). Briefly, 143B cells were plated in a 96-well plate at 1.1×10^4 cells/well and allowed to adhere overnight in Dulbecco's modified Eagle's medium (DMEM, GIBCO-BRL, Gaithesburg, MD) + 10% heat inactivated fetal bovine serum (FBS) at 37 °C, 5% CO_2. The next day, the spent medium was replaced with the virus samples appropriately diluted in DMEM, 10% FBS + 8 μg polybrene. The following day, 100 μL of DMEM + 10% FBS were added and further incubated for a total of 48 h. The cells were washed, lysed, and the β-galactosidase activity was determined by absorbance at 405 nm. Unless otherwise specified, all samples and unknowns were assayed in triplicate.

2.7. Large scale production of a retrovector

Large-scale production of 293GPG Lac-Z was performed using the perfusion culture [6]. Briefly, inoculations were performed at 0.4×10^6 cells/mL and grown DMEM +10% FBS + tetracycline. Perfusion was started at 0.6 volume–volume per day (VVD) for 2 days. Once the cells reached a density of 0.98×10^6/mL, the tetracycline containing medium was removed and the cells collected. The cells were resuspended in media containing no tetracycline and re-introduced back into the bioreactor with the perfusion rate increased to 2 VVD. Virus supernatants were collected at 5.5 L/day each day post tetracycline withdrawal and quantified for total particles concentration using the newly developed method.

3. Results and discussion

3.1. Virus retention time, confirmation of identity and separation from matrix components

The confirmation of the virus identity was determined by the retention time of the highly purified in-house standard, 293GPGTK-GFP (Fig. 1A). The virus (indicated by an arrow) eluted at 12.55 min with a NaCl concentration of 1.1 M in 20 mM Tris–Cl, pH 7.4. Nucleic acid standards, DNA and RNA were analysed to determine if they interfere with the virus elution. Fig. 1B shows a human 293 DNA (ATCC, Manassas, VA) eluted as split peaks at 7.32 and 7.52 min, respectively with a NaCl concentration of 0.9 M. Fig. 1C shows a ribosomal RNA 16S + 23S from *E. coli* (Molecular Probes, Eugene, OR) eluted at 7.46 min with a NaCl concentration similar to the DNA. Both standards were eluted earlier than the virus. Preliminary work on the development of the method was focused on the efficient separation of the virus from the rest of the matrix components, particularly nucleic acids because DNA is one of the two known major contaminants in

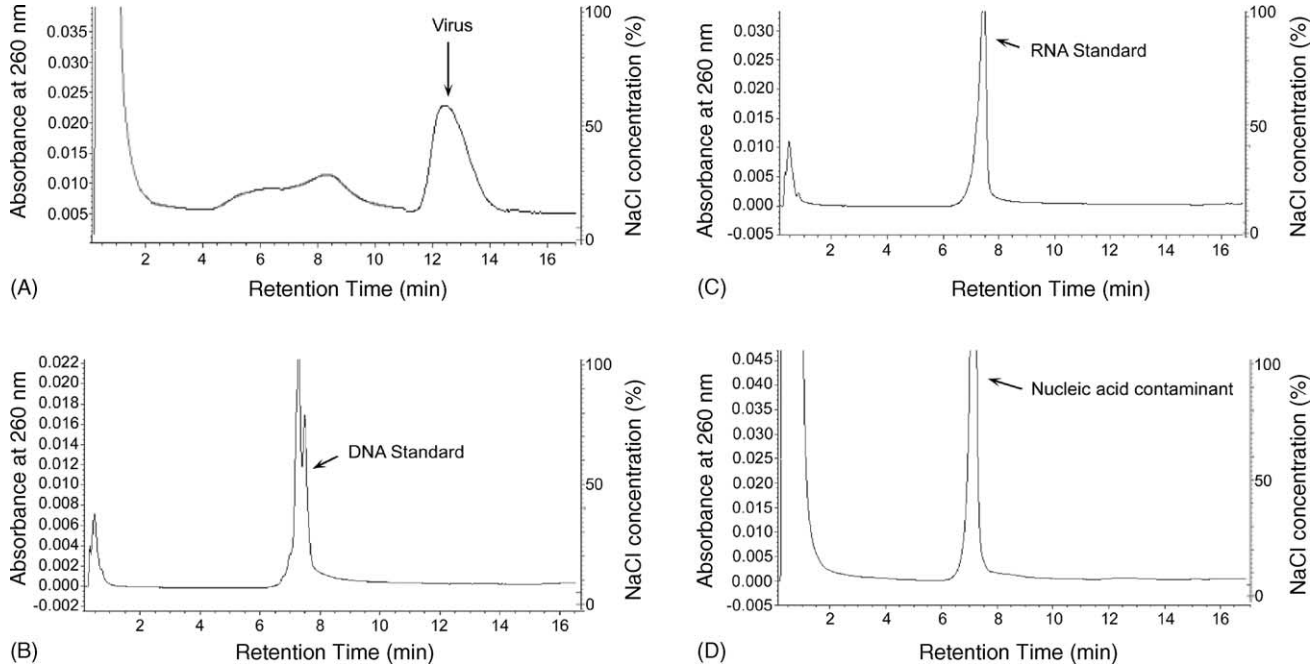

Fig. 1. HPLC chromatograms of (A) size exclusion chromatography purified in-house standard, 293GPGTK-GFP, (B) 293 human DNA standard, (C) ribosomal RNA 16S and 23S from *E. coli* and (D) supernatant from an uninfected 293 producer cell.

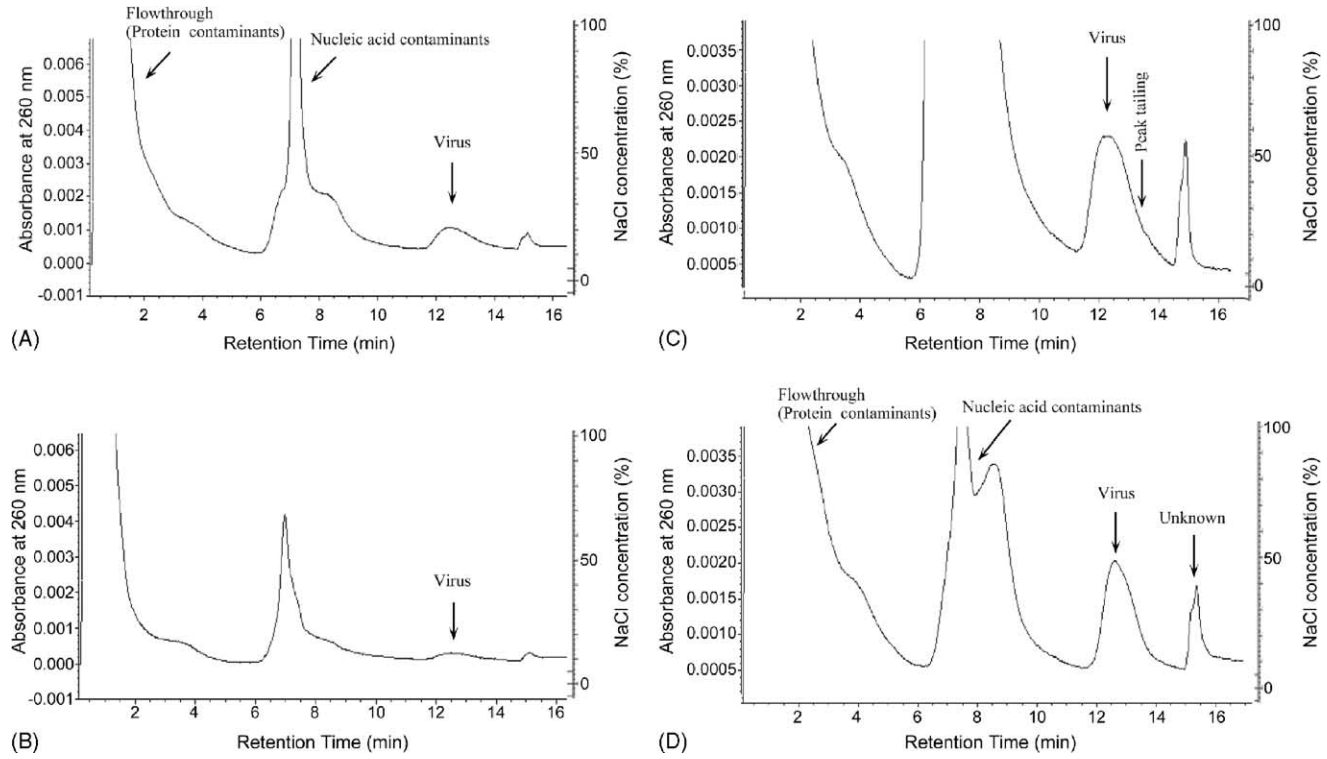

Fig. 2. HPLC chromatograms of 293GPGTK-GFP supernatant, (A) without Benzonase® digestion, (B) with Benzonase® digestion. Concentrated virus (C) without Benzonase® digestion and (D) with Benzonase® digestion.

retroviral vector preparations [15]. Nucleic acids are large molecules having the same charge as the virus and there is a possibility that they might co-elute. The presence of a large amount of nucleic acids (indicated by an arrow) in the virus preparation was demonstrated with non-infected and infected 293 producer cells shown in Figs. 1D and 2A, respectively. The other major contaminant known to be present in retroviral vector preparations is bovine serum albumin (BSA) derived from the culture media eluted in the flowthrough at the beginning of the analysis (Fig. 2A–D). Moreover, based on the profile of the non-infected 293 cells (Fig. 1D), it was shown that there were no peaks co-eluting with the virus. Consequently, the method showed a good separation performance and is suitable for the analysis of crude viral preparations. Most of the methods currently used for total particles quantification suffer from non-specificity since they do not have the ability to isolate the virus. This non-specificity leads to inaccuracy because of the presence of interfering species present in the sample matrix. EM for example, although widely accepted by regulatory agencies for the enumeration and quantification of endogenous retroviral particles in biological preparations [16] is not a suitable method for virus quantification in supernatants because accuracy of this method is dependent on the virus homogeneity [17].

3.2. Sample digestion and concentration

As discussed above, nucleic acids particularly DNA are one of the major known contaminants of retroviral vector preparations and it has been suggested that under particular conditions, VSV-G complexes with DNA [18]. Although the virus was specifically eluted from the rest of the matrix components, the occurrence of this complexation could result in the overestimation of the total particles since DNA absorbs strongly at 260 nm. To eliminate the possibility of VSV-G-DNA complexation, Benzonase® digestion of samples prior to analysis was necessary. A representative elution profile of a non-digested and digested supernatant from production Day 4 is shown in Fig. 2A and B, respectively. Without digestion, a clearly detectable virus peak (indicated by an arrow) eluted at 12.50 min (Fig. 2A). However, upon digestion, the virus peak (indicated by an arrow) was significantly reduced and almost non-detectable (Fig. 2B). This observation suggests that the virus peak was contaminated with non-viral DNA that formed a complex with VSV-G corroborating a previously published report [18]. The presence of large amounts of nucleic acid contaminants (DNA + RNA) in the preparation is attributed to the inherent nature of the production process. During the later days of virus production, the toxic effect of VSV-G results in cell death, leading to lysis that releases not only cellular DNA but also contaminants derived from the cell membrane and cytosol. It was also observed that without digestion, filtration of the sample was difficult due to an increase in viscosity. The digestion of the supernatant, resulted in the virus peak response to be below the assay's limit of quantification (Fig. 2B). To be able to use this method for quantification, a pre-concentration step was necessary prior to analysis. We performed a 10-

Table 1
Peak response area of a 10- and 25-fold concentrated virus at different days of production

Production day	10-fold concentrated virus	25-fold concentrated virus
4	N/A	27594 ± 1335
5	N/A	35983 ± 1699
6	N/A	52523 ± 675
7	34583 ± 761	83004 ± 1765
8	43708 ± 633	109963 ± 3267
9	54190 ± 300	135857 ± 5028
10	45322 ± 714	115078 ± 4587

Values presented are mean ± S.D. of triplicate analysis, N/A: baseline.

and 25-fold concentration using the Macrosep ultracentrifugal device with the supernatants of a 7-day virus production (4–10 days post induction). Table 1 presents the results obtained. For the 10-fold concentration, although production Days 7–10 had quantifiable responses, production Days 4–6 did not. While with the 25-fold concentration, all the supernatants from the 7-day production yielded quantifiable peak area responses. The amount of time required to concentrate the supernatants up to 25× varied from 1 to 2.5 h for the different days of production; production Day 4, 1 h; Days 5 and 6, 1.1 h; Days 7 and 8, 2 h; and Days 9 and 10, 2.5 h (Table 2). The differences in the amount of time to concentrate the virus at each day of production was attributed mainly to the composition of the sample matrix. In the earlier days of production, there were less virus and less contaminants, while for the later days, virus production was at its maximum and more contaminants were present due to cell lysis. The necessity to digest the concentrated virus with Benzonase® is demonstrated in Fig. 2C and D, without and with digestion, respectively. Digestion in this manner was performed with the virus supernatant before concentration. Upon digestion (Fig. 2D), the virus peak area (indicated by an arrow) was reduced by ∼30% compared to the non-digested virus (Fig. 2C). Moreover, with the non-digested virus, a noticeable peak tailing (Fig. 2C, indicated by an arrow) was observed which disappeared upon digestion (Fig. 2D). All of the above observations demonstrate the need to digest and concentrate the virus supernatant prior to analysis.

3.3. In-house standard and NSEM

The requirement of a highly pure virus for use as an in-house standard for the determination of total particles concentration by NSEM was obtained by SEC as described elsewhere [4]. This SEC purified virus preparation was characterized for purity by SDS-PAGE and infectivity by FACS analysis [4]. The purity profile of the in-house standard is shown in Fig. 1A with the virus indicated by an arrow. By HPLC, it was demonstrated that the in-house standard was highly purified from the concentrated virus (Fig. 2D) that was used as starting material for the purification. By comparison, the nucleic acid and the flowthrough peaks (both indicated by arrows, Fig. 2D) were significantly reduced and the unknown peak eluted right after the virus was eliminated after the purification (Fig. 1A). The inaccuracy of NSEM was minimized by measuring more homogeneous samples after removal of the contaminants. The total particles concentration of the purified virus obtained by this method was shown consistently between 5×10^{10} and 3×10^{11} VP/mL. Structural and morphological characterization showed that 93–97% are intact particles with size range between 100 and 120 nm. The concentration obtained by this method was used to generate the calibration curve for the developed method.

3.4. Assay linearity range, limit of detection, limit of quantification and precision

For the determination of assay linearity, LOD and LOQ, a SEC purified in-house standard was used while a concentrated virus was used for the determination of the assay intra- and inter-precision. To determine the linearity of the calibration curve, the retrovirus total particles concentration was plotted as a function of the peak area response at absorbance 260 nm. Calibration samples were within a range of 3×10^9 to 1×10^{11} VP/mL and were injected in triplicate. We were not able to go beyond 3×10^{11} VP/mL for the determination of linearity since this was the highest concentration we could obtain with the in-house standard. The linearity of the calibration curve was obtained between 3×10^9 and 1×10^{11} VP/mL with a correlation coefficient (R^2) of 0.9923, slope of 6.06×10^{-6} and intercept of 2.06×10^4. Standard errors were 2.18×10^{-7} and 1.05×10^4 for the slope and the

Table 2
Time requirement for the 25-fold concentration of virus supernatants at different days of production

Production day	Initial volume (mL)	Volume of samples left at different hours of concentration (mL)					
	Before concentration	0.5 h	1.0 h	1.10 h	2.0 h	2.5 h	Concentration-fold
4	15	2.0	0.59	N/A	N/A	N/A	25
5	15	2.0	0.98	0.59	N/A	N/A	25
6	15	2.0	0.96	0.59	N/A	N/A	25
7	15	2.0	1.15	0.95	0.59	N/A	25
8	15	3.0	2.00	1.00	0.59	N/A	25
9	15	4.0	2.50	1.49	0.80	0.59	25
10	15	3.0	2.00	1.41	0.70	0.59	25

Table 3
Determination of the assay's limit of detection and limit of quantification

Injection	Peak area response at absorbance 260 nm		
	3×10^{9a}	5×10^{9a}	7×10^{9a}
1	5323	16740	27827
2	5055	16001	30901
3	5066	16890	29835
4	5134	19777	31567
5	5099	19885	31443
6	5156	19990	32091
Mean	5139	18214	30611
S.D.	98	1855	1564

Mean $\pm$ S.D. = 942
Assay noise = $942/6.06 \times 10^{-6} = 1.55 \times 10^8$ VP/mL
Limit of detection = $1.55 \times 10^8 \times 3 = 4.65 \times 10^8$ VP/mL
Limit of quantification = $1.55 \times 10^8 \times 10 = 1.55 \times 10^9$ VP/mL

[a] Total retroparticles concentration (VP/mL)

intercept, respectively. A R.S.D. of <5% were obtained for all triplicate injections.

A summary of the assay's LOD and LOQ determination are shown in Table 3. The assay noise was 1.55×10^8 VP/mL. LOD, which can be defined as the minimum value of the virus that can be detected under the established conditions was calculated by multiplying the assay noise by a factor of 3 was 4.65×10^8 VP/mL. LOQ which is defined as the lowest concentration of the virus that can be quantified with an acceptable level of precision was calculated by multiplying the assay noise by a factor of 10 was 1.55×10^9 VP/mL. The intra- and inter-assay precision determined by injection of six replicates of 3×10^{10} VP/mL for five consecutive days were < 5% and < 10%, respectively. The summary of results for precision determination is presented in Table 4. As previously discussed, existing methods to quantify total retrovirus particles suffers from inaccuracy. The method developed showed good precision even in crude virus preparation as in the case of the concentrated virus containing significant amount of contaminants.

Table 4
Determination of the assay's intra- and inter-assay precision

Injection	Peak area response at absorbance 260 nm				
1	70539	78939	79939	74200	79380
2	69916	79916	79916	78300	75465
3	68441	77496	75410	71050	78525
4	70417	70489	75957	77600	78660
5	65652	72598	73562	77150	81045
6	66569	77596	79562	76100	76230
Intra-assay precision					
Mean	68589	76172	77391	75733	78218
S.D.	2080	3756	2765	2702	2057
% R.S.D.	3.03	4.93	3.57	3.57	2.63
Inter-assay precision					
Mean = 75221					
S.D. = 3835					
% R.S.D. = 5.10					

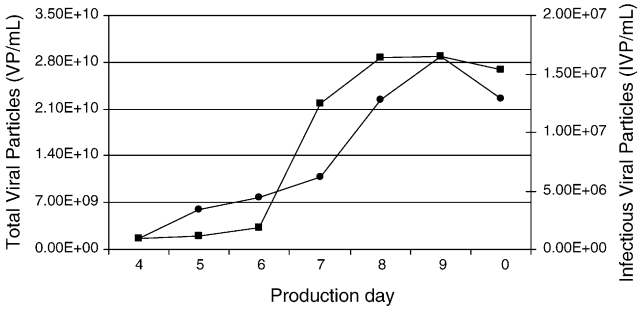

Fig. 3. HPLC total particles quantification of a 7-day large-scale perfusion culture production of a retroviral vector expressing the β-galactosidase gene grown in 293 cells. (■) Infectious viral particles (IVP/mL). (●) Total viral particles (IVP/mL).

3.5. Production monitoring

We applied the developed method for the quantification of total particles of a 7-day (Days 4–10 post induction) large-scale perfusion culture production of a retrovector grown in 293 cells expressing the β-galactosidase gene. As shown in Fig. 3, a slow linear increase in the total particles concentration was observed from Day 4 to Day 7 at 1.69×10^9 to 1.29×10^{10} VP/mL. Then it significantly increased to 2.23×10^{10} VP/mL on Day 8, reaching a maximum total particles production of 2.88×10^{10} VP/mL on Day 9. As of Day 10, the concentration started to decline. The successful monitoring and supervision of the production was instrumental in designing and documenting this process. The perfusion process allowed continuous harvesting and daily batch downstream processing of virus supernatants minimizing exposure to variable environmental conditions. It is widely documented that most retroviral vector preparations are unstable when subjected to different changes in the environmental conditions such as pH and temperature leading to loss of infectivity [19,20]. Although titration assays are not suitable for routine quantification assays, they are the only means to determine if a virus is infective. The infectivity or most commonly known as titer of the virus expressed as infectious viral particles (IVP) per mL (IVP/mL) was determined on all the virus supernatants for the 7-day production and correlated with the total particles concentration. The virus titer on Days 4–6 remained at a constant level of 10^6 IVP/mL. On Day 7, the titer significantly increased to 1.25×10^7 IVP/mL reaching a maximum on Days 8 and 9 at 1.65×10^7 and 1.64×10^7 IVP/mL, respectively. The titer started to decline on Day 10. The titer results showed a similar trend with the total particles results. A strong correlation was obtained between the infectious and total particles having reached the maximum production on Day 9 then declined on Day 10. The only noticeable difference was the significant increase in titer on Day 7 which was unobserved for the total particles. Based on the results obtained, it was estimated that the infectious particles were lower by a factor of 3–4 logs than the total

particles. The ratio obtained here correlates well with the ratio obtained by several investigators who found that during the production of retrovirus, virus like particles (VLPs) which are non-infectious are also produced due to several factors [21–24]. They found that these VLPs have the same physico-chemical properties as the retroparticles except that either they are deficient in genomic RNA or they lack the viral protease to fully process the immature to mature virion. Interestingly, these findings corroborate with the HPLC results we obtained indicating these VLPs co-elute with the infectious virus. This supports the high total particles to infectious particles ratio observed here. With this assay we were able to estimate the virus purity by taking the ratio at absorbance 260 and 280 nm which would also give an indication of the presence of contaminants. The ratio obtained ranged between 1.11 and 1.18. The ratios we obtained by this method were lower than the ratio obtained with a sucrose gradient purified virus, 1.20–1.24 estimated by spectrophotometry [25,26]. The higher ratios obtained with the latter method indicate the high amount of DNA in the preparation probably because the sucrose gradient method does not separate the DNA from the virus due to the similarity in densities.

4. Conclusion

The developed method has the advantage over existing methods such as EM, immunostaining, RT and PCR because it is rapid, specific and suitable for the quantification of the retroviral particles even in crude preparations. The virus efficiently resolved from the rest of the matrix components in a total analysis time of 23 min. Because this is a specific method, inaccuracy leading to overestimation was overcome. Compared to existing methods, results can be obtained in hours instead of days making it suitable as a routine quantification assay. The method requires Benzonase® digestion and concentration of the supernatant prior to analysis due to the large amounts of nucleic acid contaminants and low levels of virus, respectively. By this method, estimation of the relative purity of the virus was also possible which could be used as a tool during the entire downstream processing of a retroviral vector.

This method could facilitate the development of processes for the large-scale production of retroviral vectors and can be applied for the quantification of all VSV-G pseudotyped retrovectors expressing different genes.

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References

[1] S.T. Andreadis, C.M. Roth, J.M. Le Doux, J.R. Morgan, M.L. Yarmush, Biotechnol. Prog. 15 (1999) 1.
[2] J.C. Burns, T. Friedmann, W. Driever, M. Burrascano, J.K. Yee, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 8033.
[3] J. Galipeau, H. Li, A. Paquin, F. Sicilia, G. Karpati, J. Nalbantoglu. Cancer Res. 59 (1999) 2384.
[4] J. Transfiguracion, D.E. Jaalouk, K. Ghani, J. Galipeau, A. Kamen, Hum. Gene Ther. 14 (2003) 1139.
[5] G. Braas, P.F. Searle, N.K. Slater, A. Lyddiatt, Bioseparation 6 (1996) 211.
[6] K. Ghani, A. Garnier, H. Coelho, J. Transfiguracion, P. Trudel, A. Kamen, Sixth Conference on Protein Expression in Animal Cells, Mont-Tremblant, Quebec, Canada, 7–11 Septmber 2003.
[7] E. Tjotta, O. Hungnes, B. Grinde, J. Virol. Methods 27 (1990) 169.
[8] B.A. Arnold, R.W. Hepler, P.M. Keller, Biotechniques 25 (1998) 98.
[9] C. de Wit, C. Fautz, Y. Xu, Biologicals 28 (2000) 137.
[10] K. Brorson, Y. Xu, P.G. Swann, E. Hamilton, M. Mustafa, C. de Wit, L.A. Norling, K.E. Stein, Biologicals 30 (2002) 15.
[11] R. Alain, F. Nadon, C. Seguin, P. Payment, M. Trudel, J. Virol. Methods 16 (1987) 209.
[12] R. Alain, Microsc. Today 97 (1997) 20.
[13] J. Transfiguracion, A. Bernier, N. Arcand, P. Chahal, A. Kamen, J. Chromatogr. B Biomed. Sci. Appl. 761 (2001) 187.
[14] J.R. Morgan, J. Lee, R.G. Tompkins, M.L. Yarmush, Biotechnol. Prog. 10 (1994) 441.
[15] J. Chen, L. Reeves, N. Sanburn, J. Croop, D.A. Williams, K. Cornetta, Virology 282 (2001) 186.
[16] E.W. Milne, Bioprocess J. 2 (2003) 65.
[17] S. Schidlovsky, in: R.C. Gallo (Ed.), Structure of RNA Tumor Viruses: Recent Advances in Cancer Research: Cell Biology, Molecular Biology and Tumor Virology, CRC Press, Boca Raton, FL, 1977, p. 189.
[18] T. Okimoto, T. Friedmann, A. Miyanohara, Mol. Ther. 4 (2001) 232.
[19] K. Ye, H.K. Dhiman, J. Suhan, J.S. Schultz, Biotechnol. Prog. 19 (2003) 538.
[20] S.T. Andreadis, D. Brott, A.O. Fuller, B.O. Palsson, J. Virol. 71 (1997) 7541.
[21] A. Wu, H. Liu, H. Xiao, J.A. Conway, E. Hunter, J.C. Kappes, EMBO J. 16 (1997) 5113.
[22] G. Gao, S.P. Goff, J. Virol. 72 (1998) 5905.
[23] M. Shehu-Xhilaga, M. Crowe, J. Mak, J. Virol. 75 (2001) 1834.
[24] K.M. Felsentein, S.P. Goff, J. Virol. 66 (1992) 6601.
[25] M. McGrath, O. Witte, T. Pincus, I.L. Weissman, J. Virol. 25 (1978) 923.
[26] O.N. Witte, I.L. Weissman, Virology 61 (1974) 575.