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Rapid bead-based enrichment of human immunodeficiency virus (HIV)

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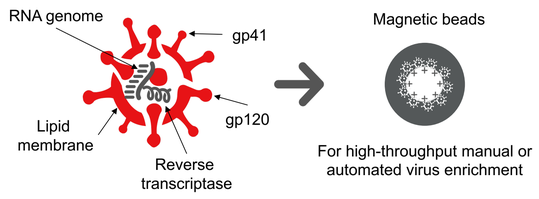
Abstract

Enrichment of viruses is essential in viral research and analysis. A range of isolation and enrichment methods including ultracentrifugation and filtration are used—methods that require expensive instruments, are time consuming, or may result in low yield. Here we demonstrate a short and simple virus isolation method using Invitrogen™ Dynabeads™ Intact Virus Enrichment for HIV, while maintaining an active reverse transcriptase (RT) enzyme. Retaining an active RT enzyme is a fundamental requirement for all retroviruses to replicate. HIV has a very high mutation rate, so measuring RT activity to determine the level of replication has the advantage of eliminating problems associated with mutations that affect immunological detection (e.g., surface antigens) as the virus must always retain an active RT enzyme. Here we have used assay technology from Cavidi AB that measures the RT-dependent production of DNA from a synthetic RNA template.

Virus enrichment



Introduction

A very fast protocol for HIV enrichment in <15 minutes is possible, enabled by the rapid binding kinetics provided by magnetic beads contained in the Dynabeads Intact Virus Enrichment product. Here we describe a viral load assay technology consisting of an HIV isolation step, followed by a RT reaction, and finally detection of RT activity by luminescence enzyme-linked immunoassay (LEIA). In the isolation step, we leveraged the negatively charged HIV virus binding to the strongly positively charged Dynabeads Intact Virus Enrichment beads. These beads were used for HIV enrichment from plasma samples to capture a functional and active RT enzyme located inside the virus. The RT was released into the lysate by a detergent-containing lysis buffer while the virus was present on the bead surface. The RT enzyme was collected and used to perform RT-mediated transcription of a synthetic RNA template. The amount of RT activity was measured in a quantitative RT assay, and the activity was proportional to the amount of replication-competent virus in the plasma sample.

In summary, the viral load assay was characterized by precision and specificity, with minimal interference from anti-retroviral treatments, endogenous substances, and disease-related factors.

Binding kinetics

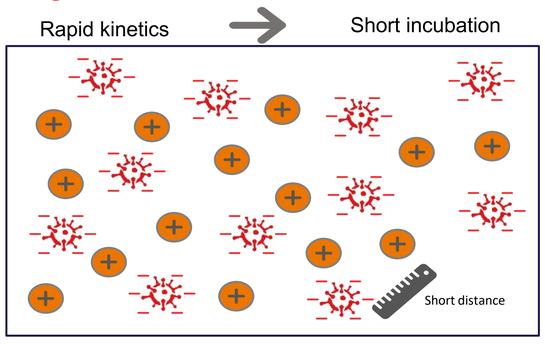


Figure 1. One of the key features of Dynabeads magnetic beads used in any enrichment protocol is the rapid binding kinetics. The proximity of the beads to the targets in the solution translates into short incubation times and very fast protocols.

Charge-based isolation and release

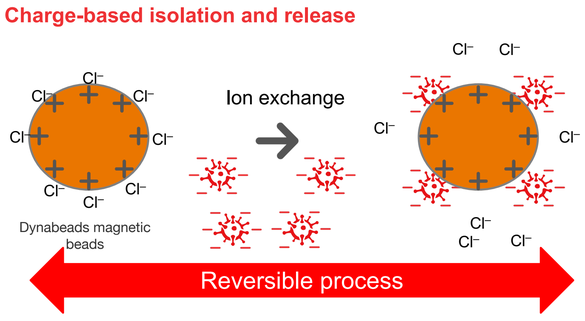


Figure 2. For enrichment of negatively charged viruses such as HIV, a positively charged Dynabeads Intact Virus Enrichment product was used. Virus added to the beads bound to the bead surface within 5 minutes.

Results

RT assay technology from Cavidi AB

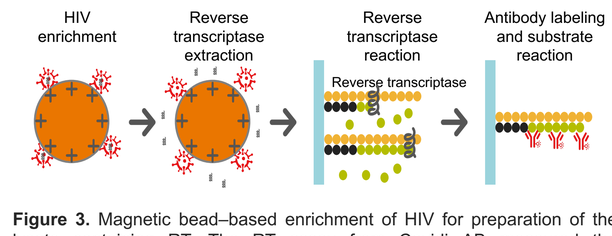
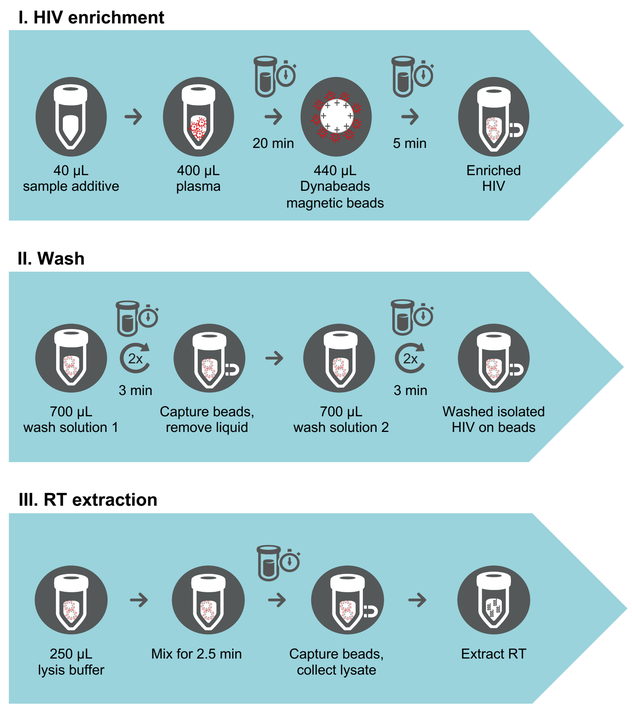


Figure 3. Magnetic bead–based enrichment of HIV for preparation of the lysate containing RT. The RT assay from Cavidi AB measured the production of DNA from a synthetic RNA template by reverse transcription. The RT reaction was performed in 96-well microplates with protein A–coated wells. The RT-synthesized product was detected by LEIA. The RT activity (RTa) measured is proportional to the amount of replication-competent virus in the starting sample. (Source: Cavidi AB)

Workflow—preparation of RT extract



Intra-assay and inter-assay precision

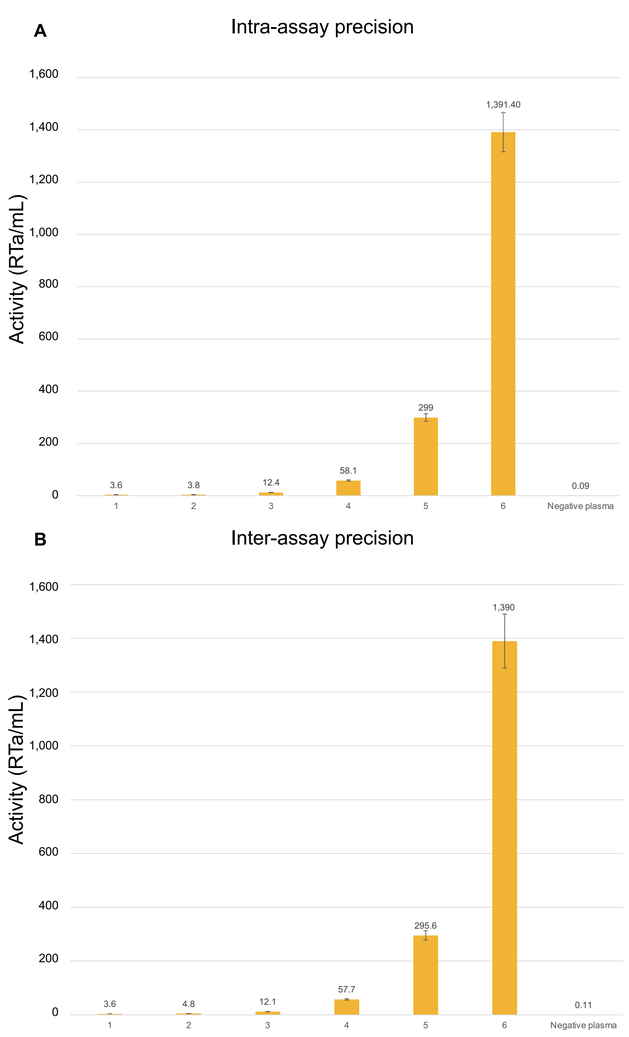


Figure 5. (A) Data from test case analyzing precision with three different lots of reagents, three different instruments, and three different operators. The spiked samples were used to compose a precision testing panel containing six different HIV-1–positive samples in quadruplicate and one HIV-negative EDTA-plasma sample also in quadruplicate, for a total of 28 samples. A precision testing panel run uses one 28-sample panel with samples placed in a random fashion within each run during separation to minimize position-dependent effects. The same plate layout is used for all runs. **(B)** The inter-assay data shown is from one instrument and three different lots of reagents, six runs per lot, with each sample in quadruplicate in the run.

Viral load assay

Targeted RTa/mL	Average RTa/mL (triplicate)	Log-transformed values	
		x-axis	y-axis
1,500	1,317	3.18	3.12
600	514	2.78	2.71
160	138	2.20	2.14
60.0	53.6	1.78	1.73
16.0	12.8	1.20	1.11
5.0	3.4	0.70	0.53
2.0	1.6	0.30	0.21
1.0	0.7	0.00	–0.14

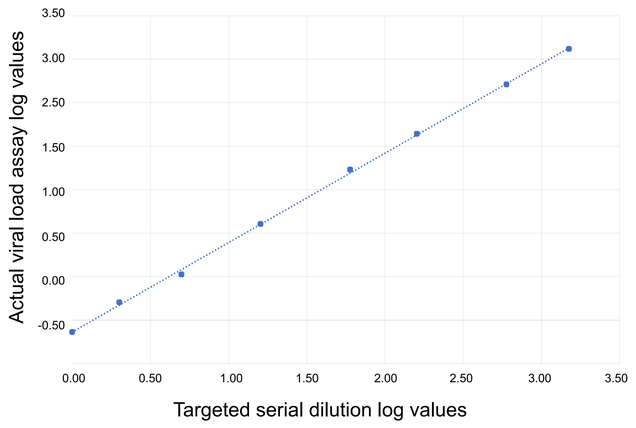


Figure 6. The range of HIV-binding was analyzed for serial dilutions of high-titer HIV-positive plasma, with the targeted viral load values in RTa/mL in the table. Similar results were obtained with other plasmas, and the range was reproducibly around 2–1,500 RTa/mL. At higher viral loads, the signal starts to saturate the plate reader detector and finally reaches the point where no further signal increase is possible. (Source: Cavidi AB)

Specificity

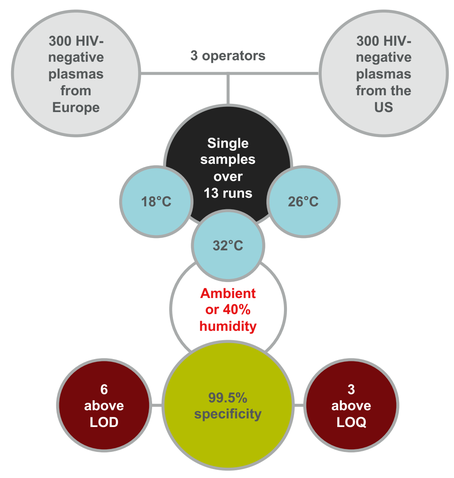


Figure 7. 600 HIV-negative EDTA-plasma samples were sourced from hospital donors, 300 from Europe and 300 from the US. Samples were run as single samples distributed over 13 runs. Tests were performed by 3 different operators under varying environmental conditions, 18°C, 26°C, and 32°C, at ambient or 40% humidity. The analysis determined the number of samples that had a viral load value above the previously determined limit of detection (LOD) or above the previously determined limit of quantification (LOQ). The LOD for six samples was above the previously determined value, and the LOQ for three of those samples was above the previously determined limit. Only the three samples with LOQ values above the previously determined value would have been reported as having a viral load. (Source: Cavidi AB)

Antiviral interference

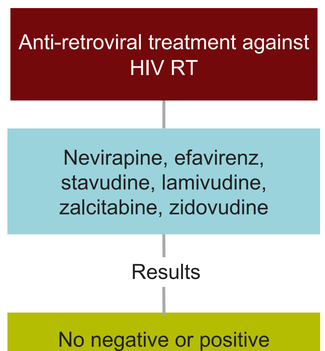


Figure 8. Three different types of interference were analyzed: drugs, endogenous substances, and disease plasmas. Each type of interference could affect binding of HIV to the beads or interfere with the RT assay leading to underestimation of HIV viral load in the sample or create a false-positive titer leading to overestimation of HIV viral load. Testing was performed according to EP07 interference testing [1]. Drugs were tested at a concentration of 3x physiological C_{max} according to EP37 [2]. For drugs not included in EP37, C_{max} values were obtained from the literature. Vehicle controls were included in each run for all the vehicles used in that run. Target concentration was 5 RTa/mL. Each drug was spiked in triplicate. Each drug was also spiked in duplicate in negative samples to test for false positives. Nevirapine, efavirenz, stavudine, lamivudine, zalcitabine, and zidovudine were used to test for interference with HIV antiviral treatments. Other substances were also tested but not shown: atorvastatin, fluconazole, levothyroxine, tamoxifen, enfuvirtide, acetaminophen, acetylsalicylic acid, cyclosporine, doxycycline HCl, ibuprofen, levodopa, methyldopa, metronidazole, clindamycin, gabapentin, tetracycline, amphotericin B, phenylbutazone, rifampicin, theophylline, ampicillin NA, acetylcysteine, diphenhydramine, pamidronate, azithromycin, dexamethasone, furosemide, ciprofloxacin, and clarithromycin. (Source: Cavidi AB)

Other interference

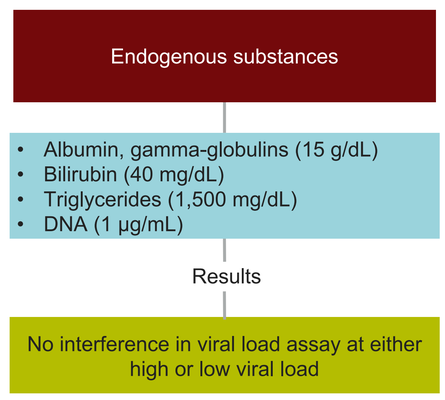


Figure 9. The impact of each endogenous substance was tested on samples spiked to an RTa level close to the clinical decision point (4 ± 2 RTa/mL) and at a high RTa level (5 RTa/mL and 100 RTa/mL). The substances were tested in triplicate for both RTa spiking levels. Controls were triplicate samples with spiked-in water or vehicle. (Source: Cavidi AB)

Disease interference

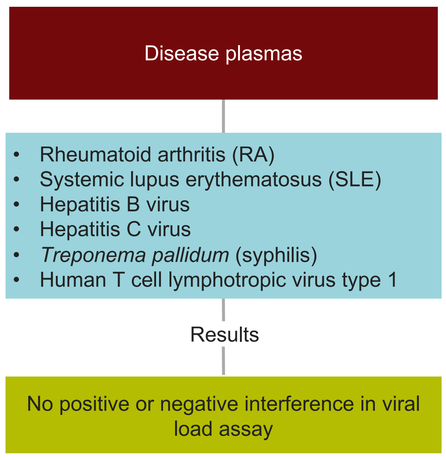


Figure 10. Disease or other medical conditions may cause plasma to contain factors that interfere with the HIV viral load assays. Both false-positive signals and inhibition of true-positive signals are possible. The impact of each type of plasma was tested on samples spiked to a target concentration of 5 RTa/mL. Each condition was tested with undiluted plasma from 10 HIV-negative individuals. Each sample was tested in duplicate for false-positive signals and in triplicate, spiked with HIV, to test for inhibition. For all 10 plasmas, the average difference in viral load between samples with and without added disease plasma should be 0.27 logarithmic units and for 9/10 individual plasmas the difference should be 0.30 logarithmic units. (Source: Cavidi AB)

Materials

Dynabeads Intact Virus Enrichment (Cat. No. 10700D).

Conclusions

Here we demonstrated a fast and easy method for enrichment of HIV from donor samples used in a downstream viral load assay that was developed by Cavidi AB. Using the Dynabeads Intact Virus Enrichment beads enabled a fast and easy enrichment of HIV in only 15 minutes, followed by collection of the active RT enzyme used in the viral load assay.

References

1. Clinical and Laboratory Standards Institute. EP07: Interference testing in clinical chemistry. https://clsi.org/media/2235/ep07ed3_sample.pdf.
2. Clinical and Laboratory Standards Institute. EP37: Supplemental tables for interference testing in clinical chemistry. https://clsi.org/media/2236/ep37ed1_sample.pdf.

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