HIV-1 research

New nucleic acid extraction workflow for dried blood spots, to support HIV-1 drug resistance genotyping

Introduction

The Joint United Nations Programme on HIV/AIDS (UNAIDS) has established fast-track targets for globally accelerating HIV prevention to end the AIDS crisis. Cost-effective and robust HIV-1 drug resistance genotyping solutions are urgently needed to meet the UNAIDS 95-95-95 fast-track targets for 2030 [1]. The ability to extract HIV RNA from plasma EDTA and dried blood spot (DBS) samples helps provide flexibility for sample collection in low- and middle-income countries (LMICs). We have created a sample-to-result workflow for HIV-1 drug resistance research that includes RNA extraction from plasma and DBS samples using the Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit for HIV-1 dried blood spots (Cat. No. A53770) and genotyping with the Applied Biosystems™ HIV-1 Genotyping Kit module (Figure 1).

Materials and methods

DBS samples (n = 80) containing various HIV-1 subtypes were lysed using Applied Biosystems™ MagMAX™ Dried Blood Spots Lysis Solution for 30 minutes at room temperature. Each DBS sample was prepared with 100 µL of contrived whole blood that was positive for HIV-1 with a viral load of ≥1,000 copies/mL.

Viral RNA was extracted from the samples using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit for HIV-1 dried blood spots on the Thermo Scientific™ KingFisher™ Flex Purification System. Dried spots of whole blood that was negative for HIV-1 were included to serve as negative controls for extraction and amplification. RNA was also extracted from Thermo Scientific™ AcroMetrix™ HIV-1 RNA controls, which consisted of plasma formulated to mimic naturally occurring human plasma positive for HIV-1. The plasma controls had viral loads of 1,250 copies/mL, 5,000 copies/mL, and 10^6 copies/mL. The following conversion factor was used to calculate the approximate viral load in the Thermo Scientific™ AcroMetrix™ HIV-1 High Control: 1 copy = 1.72 international unit (IU), or 0.58 copies = 1 IU [2]. For the genotyping assay, RNA in the eluates was reverse-transcribed by multiplex RT-PCR and amplified by nested PCR. The nested PCR products were then run on 2% agarose gels to detect bands as described in the user guide for the Applied Biosystems™ HIV-1 Genotyping Kit Amplification Module. The positive call rate was calculated as the number of positive amplified samples divided by the total number of samples, multiplied by 100%. RNA extraction and drug resistance genotyping were performed by three different users.

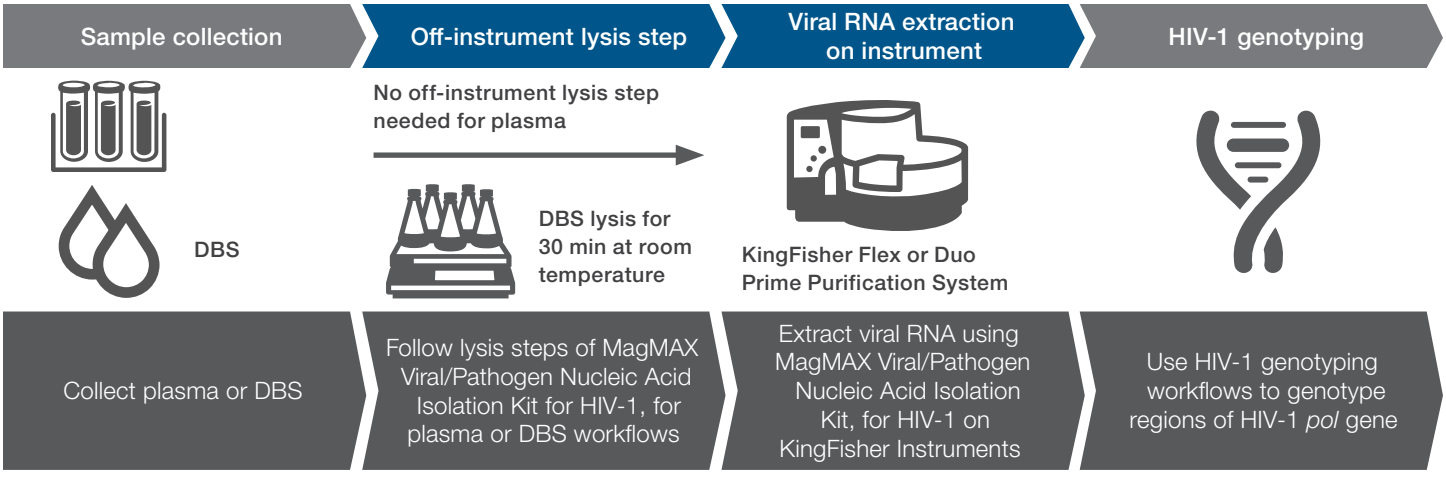


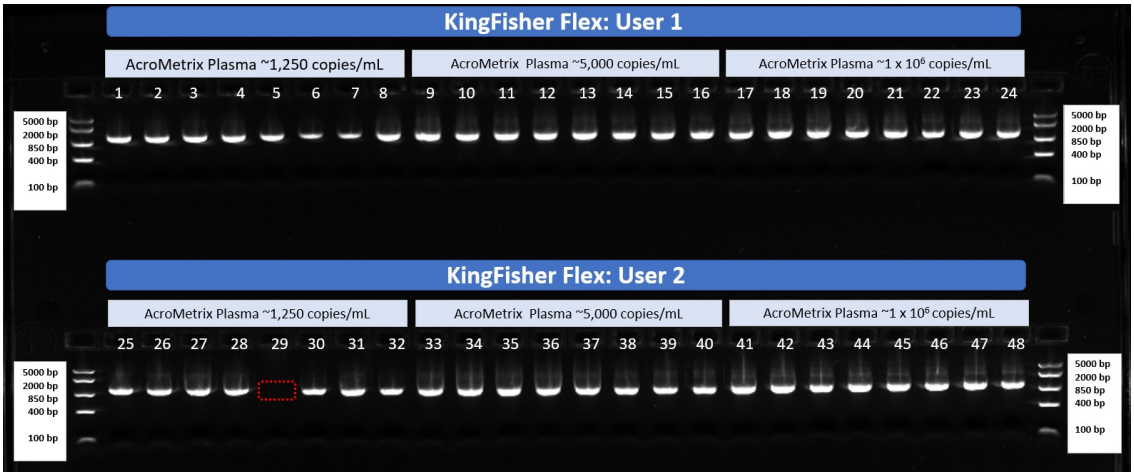
Figure 1. Full workflow for HIV-1 RNA extraction from plasma and DBS samples with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit for HIV-1 dried blood spots.

Results and discussion

The MagMAX Viral/Pathogen Nucleic Acid Isolation Kit enabled successful extraction of amplifiable RNA from HIV-1 subtypes A, B, C, CRF01 AE, and CRF02 AG in plasma at three different viral loads. The average positive call rate was 98% for the plasma controls, all of which had viral loads above 1,000 copies/mL. Table 1 summarizes the positive call rates for all 48 positive control samples with 200 µL inputs. The bands in the gels shown in Figure 2 are the ~1,100 bp nested PCR product containing the protease and reverse transcriptase regions.

Table 1. Positive call rates for known positive plasma controls.

Lane	Viral load (copies/mL)	User	Number of samples amplified	Positive call rate for all samples (%)
1–8	1,250	1	8 out of 8	98% (47 out of 48)
9–16	5,000		8 out of 8	
17–24	10^6		8 out of 8	
25–32	1,250	2	7 out of 8	
33–40	5,000		8 out of 8	
41–48	10^6		8 out of 8	



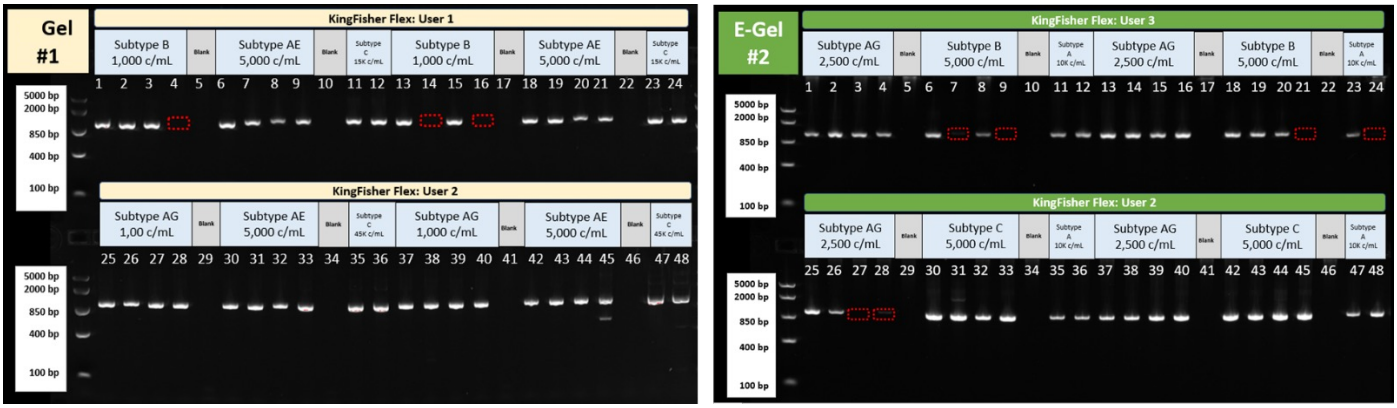
Indicates sample did not amplify.

Figure 2. Agarose gels showing nested amplification products for HIV-1 positive control plasma. HIV-1 RNA was extracted using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit for HIV-1 dried blood spots on the KingFisher Flex Purification System. The nested PCR products were analyzed using the HIV-1 Genotyping Kit Amplification Module.

Of the 80 DBS samples evaluated, 64 had viral loads of >2,000 copies/mL and contained various HIV-1 subtypes. The average positive call rate for these samples was 91% (Table 2). The average positive call rate across all 80 samples, each of which had a viral load of ≥1,000 copies/mL, was 89%. Agarose gels 1 and 2 (Figure 3) show bands at ~1,100 bp, which were the nested PCR product containing the protease and reverse transcriptase regions. Lanes 5, 10, 17, 22, 29, 34, 41, and 46 in both gels were the negative control reactions.

Table 2. Positive call rates for 80 DBS samples extracted with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit for HIV-1 dried blood spots. All 80 samples had viral loads greater than or equal to 1,000 copies/mL.

Lane (gel 1)	HIV-1 subtype	Viral load (copies/mL)	User	Number of samples amplified	Overall positive call rate (%)	Positive call rate for samples with >2,000 copies/mL (%)
1–4, 13–16	B	1,000	1	5 out of 8	89% (71 out of 80)	91% (58 out of 64)
6–9, 18–21	AE	5,000		8 out of 8		
11–12, 23–24	C	15,000		4 out of 4		
25–28, 37–40	AG	1,000	2	8 out of 8		
30–33, 42–45	AE	5,000		8 out of 8		
35–36, 47–48	C	45,000		4 out of 4		
Lane (gel 2)	**HIV-1 subtype**	**Viral load (copies/mL)**	**User**	**Number of samples amplified**		
1–4, 13–16	AG	2,500	3	8 out of 8		
6–9, 18–21	B	5,000		5 out of 8		
11–12, 23–24	A	10,000		3 out of 4		
25–28, 37–40	AG	2,500	2	6 out of 8		
30–33, 42–45	C	5,000		8 out of 8		
35–36, 47–48	A	10,000		4 out of 4		



Indicates sample did not amplify.

Figure 3. Agarose gels showing nested amplification products from DBS samples extracted with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit for HIV-1 dried blood spots. All samples had viral loads of ≥1,000 copies/mL. The bands at approximately 1,100 bp contained the protease and reverse transcriptase targets.

Conclusion

The data obtained in this study indicate that the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit for HIV-1 dried blood spots and the HIV-1 Genotyping Kit Amplification Module are suitable for HIV-1 drug resistance research. Positive call rates of 91% and 98% were observed for DBS samples and positive control plasma, respectively. These products are economical and robust alternatives to existing on-market solutions, and they are specifically designed to meet the needs of LMICs.

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References

1. https://www.unaids.org/sites/default/files/media_asset/201506_JC2743_Understanding_FastTrack_en.pdf
2. https://extranet.who.int/pqweb/sites/default/files/PQDx_0192-0193-0194-0195_v6.pdf

Ordering information

Description	Cat. No.
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit for HIV-1 dried blood spots (DBS)	A53770
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	A42352
AcroMetrix HIV-1 Controls	964003
AcroMetrix HIV-1 Controls (RUO)	950416
HIV-1 Genotyping Kit Amplification Module	A32317
KingFisher Flex Purification System, KingFisher with 96 Deep-Well Head	5400630
KingFisher Duo Prime Purification System	5400110

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