HIV-1 genotyping

Dried blood spots for HIV-1 RNA integrase genotyping research: a useful sample alternative to plasma for resource-limited and remote regions

Keywords
HIV genotyping, HIV drug resistance testing, HIV genotyping surveillance, HIV public health, HIV research

Introduction

In resource-limited and remote settings, HIV-1 drug resistance (HIVDR) threatens the efforts of the UNAIDS toward their Fast-Track goal of eradicating HIV-1 infection by 2030. Fast-Track 95-95-95 targets state that "by 2030, 95% of people living with HIV know their status, 95% of people who know their status receive treatment, and 95% of people on treatment have a suppressed viral load enabling a stronger immune system" [1]. Currently, HIVDR genotyping assays are used to identify, monitor, and research mutations in the protease (PR) and reverse transcriptase (RT) regions of the HIV-1 genome. However, with the expanding use of integrase inhibitors in resource-limited and remote settings, there is an additional requirement to monitor drug resistance mutations in the integrase (IN) region.

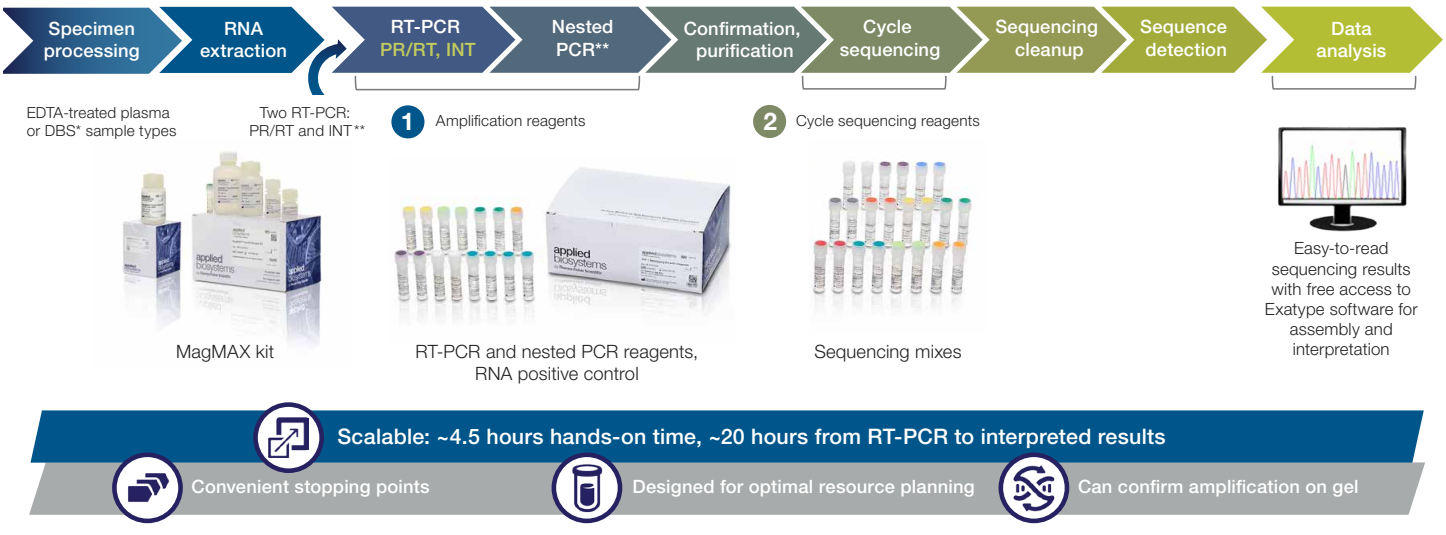
To meet the 95-95-95 goals, HIV-1 genotyping research on PR and RT has been established starting with plasma samples, but many research and surveillance laboratories are also using samples collected as dried blood spots (DBS). Thermo Fisher Scientific proposed a full workflow for researching mutations in the HIV-1 viral RNA genome in the application note entitled "**New nucleic acid extraction workflow for dried blood spots, to support HIV-1 drug resistance genotyping**". This workflow uses the Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, for HIV-1 dried blood spots (DBS), for the extraction of RNA from dried blood spot and plasma samples, which is then followed by gene amplification with HIV-1 genotyping. The workflow proposal includes nucleic acid extraction from plasma and DBS samples for sequencing analysis. The new Applied Biosystems™ HIV-1 Genotyping Kit with Integrase includes all three gene targets of the HIV-1 polymerase region (PR/RT and IN). In addition, the kit meets the previous performance of the Applied Biosystems™ HIV-1 Genotyping Kit workflow with extraction using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, for HIV-1 DBS, while increasing amplification scope.

Here we evaluate a new workflow for researching genomic mutations in the PR, RT, and IN regions of the HIV-1 *pol* gene in RNA extracted from DBS. In resource-limited and remote settings, using DBS for HIV-1 genotyping is ideal because of its low cost and ease of shipment and storage without the requirement for cold-chain transport.

Materials and methods

RNA was extracted from 46 contrived DBS specimens using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, for HIV-1 DBS, on the Thermo Scientific™ KingFisher™ Flex Purification System, KingFisher with 96 Deep-Well Head (Figure 1). The specimens spanned HIV-1 subtypes A, B, C, D, F, G, CRF01_AE, CRF02_AG, and CRF06_cpx and had viral loads ranging from 2,380 copies/mL to 220,251 copies/mL (Table 1). The RNA samples were tested with three unique reagent lots of the HIV-1 Genotyping Kit with Integrase (RUO). Amplification and sequencing were conducted on the PR/RT and IN gene targets using the Applied Biosystems™ Veriti™ 96-Well Thermal Cycler and the Applied Biosystems™ 3500xL Genetic Analyzer for Resequencing & Fragment Analysis, respectively (Figure 2). Each unique DBS sample was amplified with three different individual lots of reagents, resulting in a total of 138 amplified samples. The percentage of samples providing interpretable sequencing results was assessed at the end of the assay workflow using Exatype™ software (Hyrax Biosciences).

Figure 1. Reagents provided in the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, for HIV-1 DBS, and the HIV-1 Genotyping Kit with Integrase.



* DBS samples must be processed within 2 weeks of collection under ambient storage conditions.
** Sample is split for PR/RT and INT testing at the RT-PCR stage.

Figure 2. Workflow for the HIV-1 Genotyping Kit with Integrase.

Results

PR/RT and IN amplification

Amplification of PR/RT and IN was assessed after the nested PCR step using agarose gel electrophoresis (both amplicons have an expected size of ~1.1 kb on an agarose gel). Overall, a total of 134 samples showed positive amplification results for PR/RT and IN, resulting in a 97% (134 of 138) amplification positivity rate. Table 1 indicates percent amplification observed across all three lots tested for PR/RT and IN by HIV-1 subtype.

Table 1. PR/RT and IN amplification results for DBS specimens.

Subtype	Unique samples (N)	Total samples (N x 3 reagent lots)	Viral load min (copies/mL)	Viral load max (copies/mL)	PR/RT (N)	IN (N)
A	4	12	7,780	37,875	100%	100%
B	7	21	2,380	62,500	90% (19)	86% (18)
C	6	18	5,704	13,400	100%	100%
D	4	12	9,273	143,000	100%	100%
F	7	21	4,702	107,375	100%	100%
G	5	15	2,960	220,251	100%	100%
CRF01_AE	4	12	10,000	63,375	92% (11)	100%
CRF02_AG	5	15	5,646	105,160	100%	93% (14)
CRF06_cpx	4	12	3,078	9,050	100%	100%

PR/RT and IN sequencing

10 RNA samples were chosen for sequencing and subsequent Exatype software analysis to determine the drug resistance mutations in the PR/RT and IN gene regions. All of the samples generated interpretable sequencing results. Table 2 lists the genomic mutations identified from the DBS specimens sequenced with the RUO HIV-1 Genotyping Kit with Integrase (3 reagent lots, 30 samples total).

Table 2. Mutations identified in the PR/RT and IN gene regions from DBS specimens.

Reverse transcriptase						Integrase
M41L	A98G	V106I	M184L	T215L	K219E	G163K
D67N	K101E	V179E	M184V	T215S	K219N	
K70R	K103N	Y181C	T215F	T215Y	H221Y	

Conclusions

The HIV-1 Genotyping Kit with Integrase (RUO) enabled consistent amplification of various HIV-1 subtypes from DBS for all three HIV-1 target regions across three different individual assay lots. When combined with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, for HIV-1 DBS, HIV-1 drug resistance research is simple, time-saving, cost-efficient, and streamlined.

Authors

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Reference

1. UNAIDS (2015) Understanding Fast-Track: Accelerating action to end the AIDS epidemic by 2030. **https://www.unaids.org/sites/default/files/media_asset/201506_JC2743_Understanding_FastTrack_en.pdf**

Ordering information

Product	Cat. No.
Reagents	
HIV-1 Genotyping Kit with Integrase	A55120
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, for HIV-1 dried blood spots (DBS)	A53770
Instruments	
KingFisher Flex Purification System, KingFisher with 96 Deep-Well Head	5400630
3500xL Genetic Analyzer for Resequencing & Fragment Analysis	4440463

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