How do we measure HIV drug resistance

Monique Nijhuis
Measure HIV drug resistance

Genotypic resistance test vs Phenotypic resistance test
Measure HIV drug resistance

Genotypic resistance test vs Phenotypic resistance test

Examen if HIV has changes (mutations) in the genetic structure
Measure HIV drug resistance

Genotypic resistance test vs Phenotypic resistance test

Examen if HIV has changes (mutations) in the genetic structure

Recombinant phenotypic test (part of the virus with mutations)

Examen if drugs can inhibit the replication of HIV
Measure HIV drug resistance: blood sample

Centrifugation of blood sample
Measure HIV drug resistance: blood sample

Centrifugation of blood sample

HIV

55% Plasma

1% White blood cells & platelets

44% Red blood cells

Genotypic resistance test

Recombinant phenotypic test
Measure HIV drug resistance: blood sample

Centrifugation of blood sample

- 55% Plasma
- 1% White blood cells & platelets
- 44% Red blood cells

HIV-infected cells (alive)

HIV

genotypic resistance test
recombinant phenotypic test

genotypic resistance test
phenotypic resistance test
Measure HIV drug resistance: blood sample

Centrifugation of blood sample

Dried blood spot

HIV-infected cells (alive)

HIV-infected cells (dead)

55% Plasma

1% White blood cells & platelets

44% Red blood cells

genotypic resistance test recombinant phenotypic test

genotypic resistance test

phenotypic resistance test

HIV

genotypic resistance test

recombinant phenotypic test
Genotypic drug resistance test

Plasma → Reverse transcription → Viral cDNA → PCR → Amplified DNA
Genotypic drug resistance test

- Plasma
- DBS cells
- Proviral DNA
- Viral RNA
- Viral cDNA
- Amplified DNA
- Reverse transcription
- PCR
Genotypic drug resistance test

Plasma

DBS cells

Proviral DNA

Viral RNA

Reverse transcription

Viral cDNA

PCR

Amplified DNA

Genetic sequencing

Comparison to wild type reference sequence

ATGGCATCAGAT

ATGGCATCAGAT

ATGGCATCAGAT

Interpretation using a proprietary database

Reporting
Genotypic drug resistance test pro/cons

Pro:
- relatively fast and cheap
- relatively successful on low level viremia samples
Genotypic drug resistance test pro/cons

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Hofstra, personal communication
Genotypic drug resistance test pro/cons

Pro:
- relatively fast and cheap
- relatively successful on low level viremia samples

Con:
- indirect measurement of drug resistance
- need to have a database with known impact of mutations
Interpretation of genotypic resistance

Identification of relevant drug resistance mutations:

- www.iasusa.org
Interpretation of genotypic resistance

Interpret impact of (combinations) of resistance mutations: Algorithms

- www.hivdb.stanford.edu
- www.hiv-grade.de
Genotypic drug resistance test pro/cons

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- relatively successful on low level viremia samples

Con:
- indirect measurement of drug resistance
- need to have a database with known impact of mutations
  - resistance may depend on the interaction of mutations
  - virus background, subtype
  - challenging for new drugs and especially new drug classes

- Phenotypic drug resistance assay (recombinant phenotypic test)
Recombinant phenotypic resistance test

1. Amplified DNA
2. HIV-1 resistance test vector assembly
3. Cell transfection and pseudovirion collection
4. Drug susceptibility testing and quantification
5. Reporting
Recombinant phenotypic resistance test

Amplified DNA

HIV-1 resistance test vector assembly

Cell transfection and pseudovirion collection

Drug susceptibility testing and quantification

Reporting

Drug concentration (log)

Suppression

Fold resistance

IC_{50}

Patient isolate

Wildtype

0%

25%

50%

75%

100%

1

3

5

7

9

11
Genotypic drug resistance test pro/cons

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- Phenotypic drug resistance assay (recombinant phenotypic test)
- Phenotypic drug resistance assay
Measure HIV drug resistance: blood sample

Centrifugation of blood sample

Dried blood spot
Phenotypic drug resistance test

HIV-infected cells (alive) → Virus culture → Amplified DNA → HIV-1 resistance test vector assembly → Cell transfection and pseudovirion collection → Drug susceptibility testing and quantification → Reporting
Phenotypic drug resistance test

Pro:
- direct measurement of drug resistance
- you do not need to know the location of the mutation

Con:
- relatively time consuming and expensive
- clinically relevant levels of drug resistance are not always available
- require specialized laboratory (BSLIII)
Biosafety level III laboratory

double door entry, inward air flow, biosafety cabinets, on site autoclave
Choose your resistance test wisely…

**Genotypic resistance test:**
- known antiretroviral drugs with good interpretation algorithms

**Phenotypic resistance test:**
- virological failure can not be explained based on genotypic resistance profile
- new drugs or new drug classes