ITREMA

IMMUNOASSAY DRUG LEVEL TESTING

ITREMA STANDARD OPERATING PROCEDURE

FOR IMMUNOASSAY DRUG LEVEL TESTING

V1.0 (03 APRIL 2016)

NDLOVU CARE GROUP

UMC Utrecht

ZonMw

RHI

'S REPRODUCTIVE HEALTH & HIV INSTITUTE

Universiteit Utrecht

Radboudumc

WHY DRUG LEVEL TESTING?

- In low- and middle-income countries (LMIC), yearly viral load testing is recommended by the WHO for monitoring of antiretroviral treatment (ART)*
- A detectable viral load implies treatment failure: Major underlying causes are insufficient drug exposure and selection of drug drug resistance
- Point-of-care drug level testing can be useful to objectively assess recent drug intake and prevent unnecessary treatment switching
- Drug level testing can be done in a variety of ways
- ITREMA has opted for qualitative drug level testing as an indicator for recent adherence

* World Health Organisation, Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. 2016

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PRINCIPLES

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HOW DOES AN IMMUNOASSAY WORK?

- Immunoassays consist of two reagents:
  - Reagent 1: An "artificial drug" is coupled to an enzyme called glucose-6-phosphate dehydrogenase (G6PDH). This artificial drug is depicted in a diamond shape with an "H" in the centre (Fig. 1A, B). We will refer to this as "artificial drug-enzyme complex".
  - Reagent 2: An antibody, specific to the artificial drug in reagent 1, and also to the drug that is tested for
- The enzyme coupled to the drug can be eithere active or inhibited
  - When the artificial drug unbound by the antibody (reagent 2), the enzyme is active (Fig. 1A)
  - When the artificial drug is bound by the antibody (reagent 2), the enzyme is inhibited (Fig. 1B)
- The artificial drug-enzyme complex (reagent 1), and the antibody (reagent 2), are added sequentially to patient plasma or serum at set timepoints, allowing for incubation steps in between

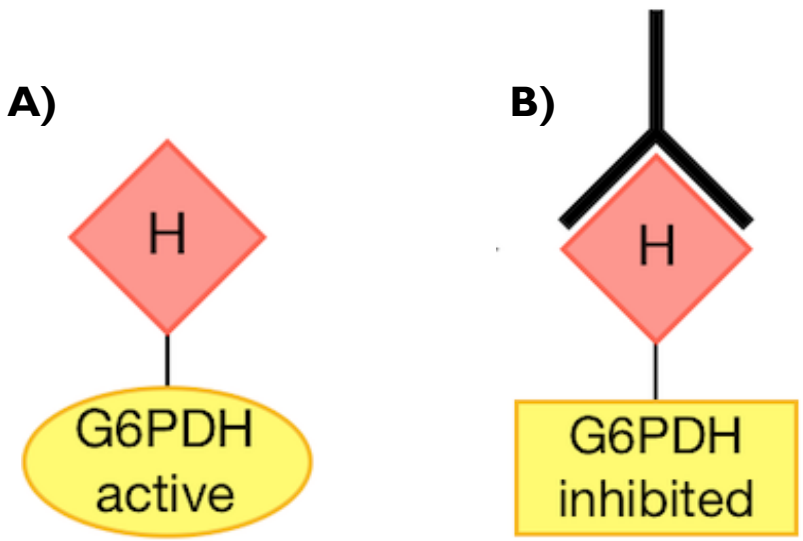


Figure 1A: artificial drug coupled to an active G6PDH
Figure 1B: artificial drug coupled to an inactive G6PDH due to antibody binding

IF THE PATIENT HAS NO DRUG LEVEL

- To the patient plasma, artificial drug-enzyme complex (reagent 1) is added, and then antibody (reagent 2)
- Because the drug of interest is not present in the plasma, the antibody (reagent 2) will only bind to the artificial drug (reagent 1), in such a way that all or most of reagent 1 is bound
- This will result in inhibition of the enzyme in the artificial drug-enzyme complex

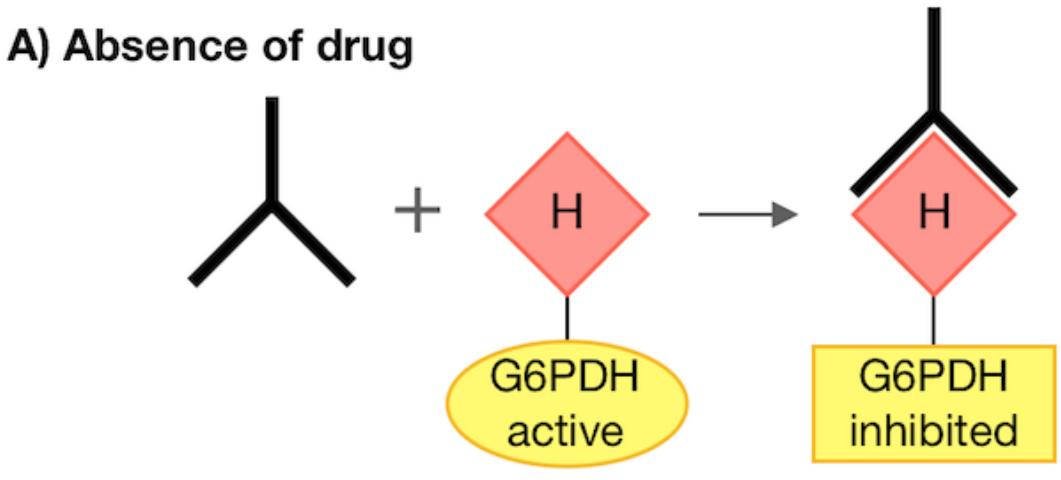


Figure 2A: when there is no drug in the plasma, the antibody will bind the artificial drug (H) and the enzyme G6PDH will be inhibited

IF THE PATIENT DOES HAVE A DRUG LEVEL

- If the patient did take its ART medication, drug will be present in the plasma.
- Therefore, the antibody will not only bind to the artificial drug-enzyme complex, but also to the actual drug in the blood plasma (see the blank diamond in the figure).
- A fraction of the artificial drug-enzyme complex will no longer be bound by the antibody, which will lead to activity of the enzyme.
- The enzyme katalyses a reaction with the formation of NADH from NAD as a side reaction. NADH can be measured spectrophotometrically, because it absorbs light at 340 nm.

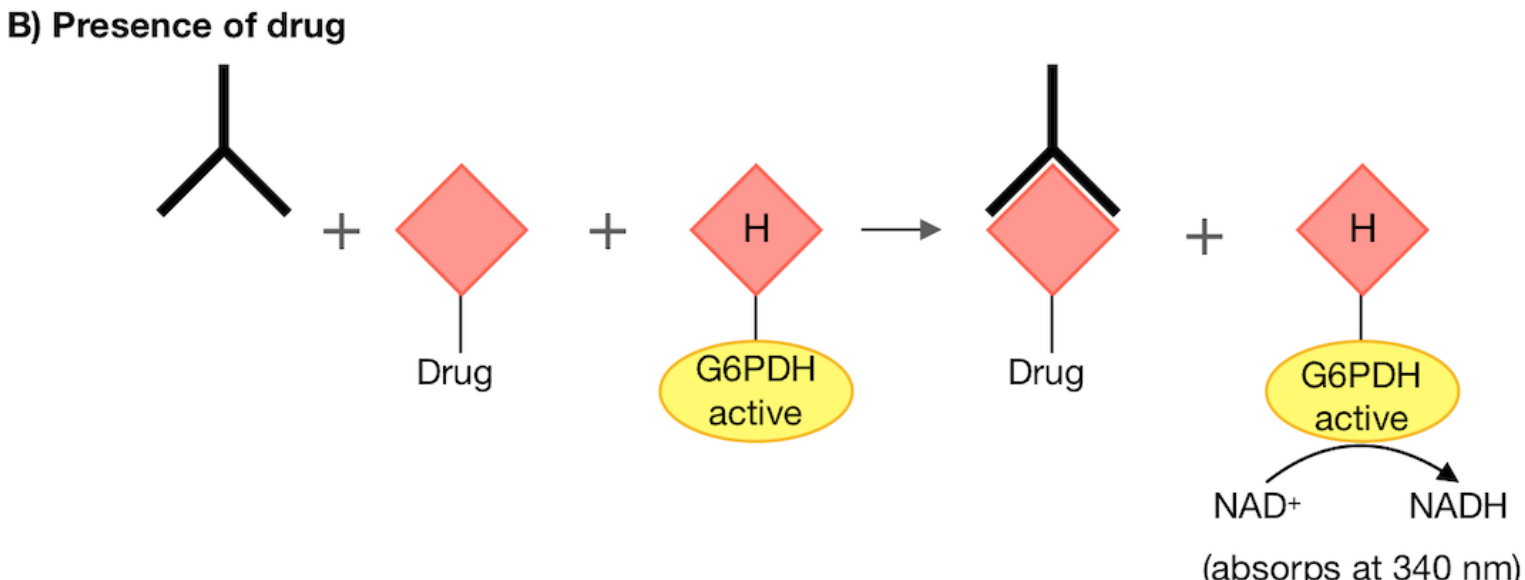
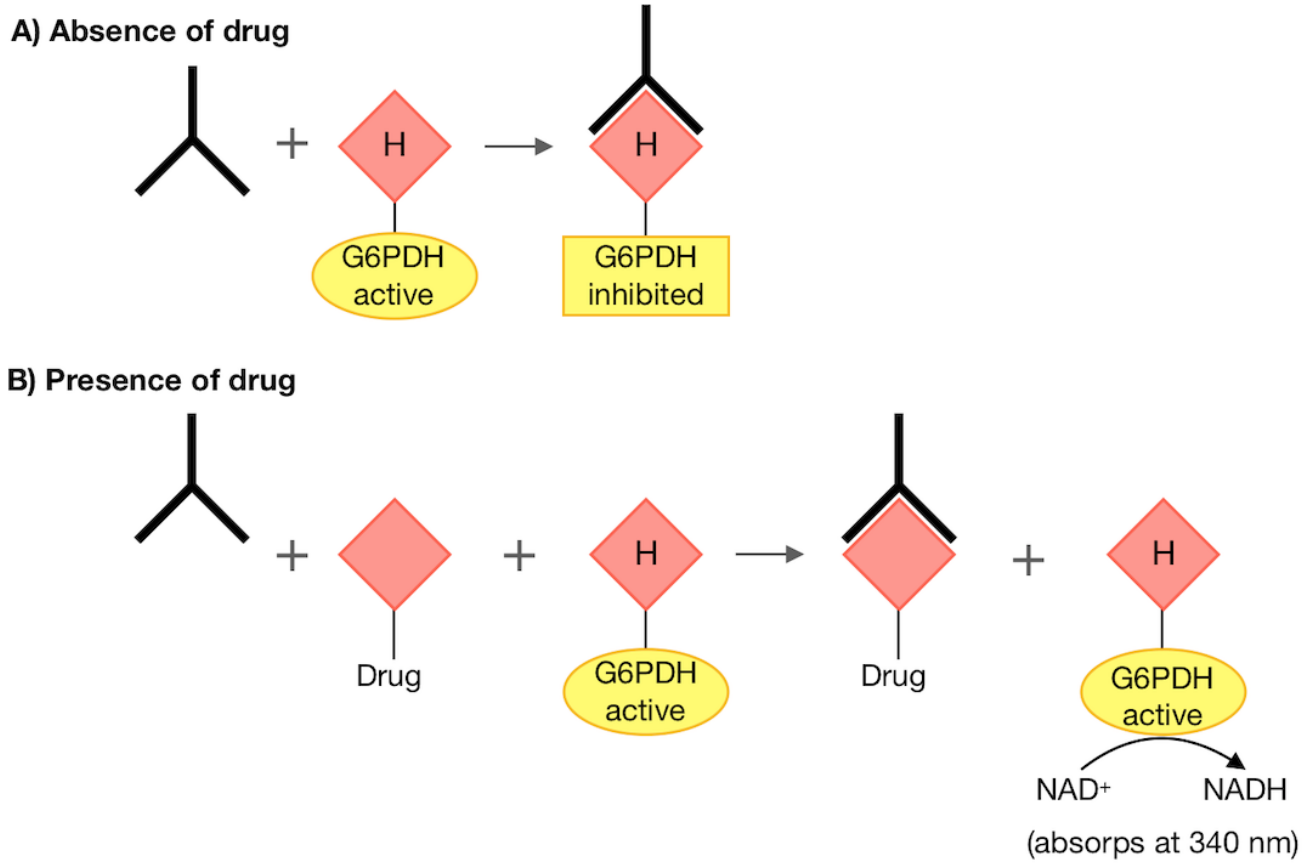


Figure 2B: in the presence of the drug in the blood plasma, competition will occur for antibody binding. The artificial drug, H, will not be bound anymore, which will lead to an active G6PDH. During katalysing its reaction, NADH will be formed, which can be measured

TO SUMMARIZE

- The presence of the drug in the plasma will lead to an increase in NADH
- This is measurable with a spectrophotometer
- Increase in enzyme activity and thus formation of NADH is directly proportional to the drug concentration



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IMMUNOASSAY IMPLEMENTATION

HOW TO IMPLEMENT AN IMMUNOASSAY

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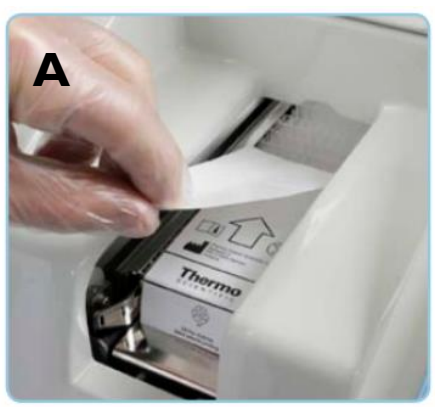
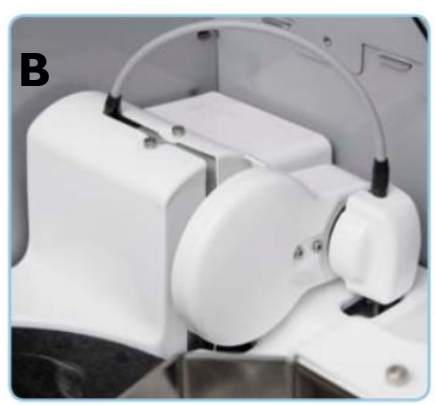
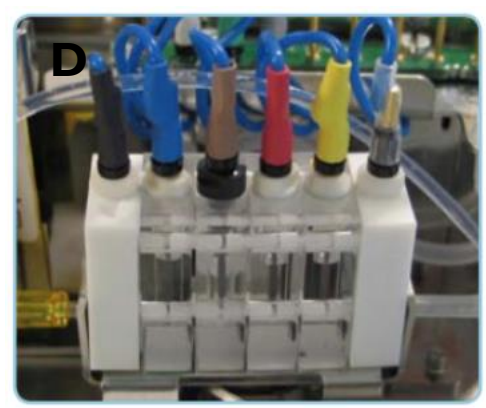
REQUIRED HARDWARE

- EDTA derived input plasma from patients receiving antiretroviral treatment
- Immunoassay are carried out with chemical analyzers. For the ITREMA study, the Thermo Fisher INDIKO Plus ® was used (see figure), but other analyzers are possible as well
- All analyzers have similar basic characteristics and mode of operation







LAB REQUIREMENTS

- Labs should be equipped with:
  - Deionized water
  - Refridgerator, preferably temperature monitored
  - Temperature regulation in the laboratory (chemistry analyzers may be sensitive to changes in temperature)
  - Basic laboratory supplies: Pipette and tips, non-sterile gloves, EDTA-vacutainers
  - Basic safety requirements: Shower, fire extinguisher, biohazardous waste disposal
  - A chemistry analyzer, connected to an uninterrupted power supply
  - Extensions of the chemistry analyzer: Printer, User intervace
  - All the requirements for the chemistry analyzer as stated by the manufacturer (e.g. sample holders, cleaning reagents)
  - Centrifuge capable of separation of blood plasma

PRACTICAL PROCEDURE

EDTA derived input plasma from patients receiving antiretroviral treatment

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Plasma separation (centrifuge)

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Calibration of immunoassay (if required, intervals for calibration are provider-dependent)

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Running of immunoassay on centrifugated plasma, together with QC samples

↓

Controlling of QC samples

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Result interpretation (based on QC results) and reporting

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IMMUNOASSAY DRUG LEVEL TESTING VALIDATION/VERIFICATION

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VALIDATION/VERIFICATION

- New laboratory testing procedures require proper validation or verification in any local setting prior to being used

- The following slides outline a proposal for a basic verification of a previously validated procedure/assay

INTERNAL VERIFICATION

- Run assay on 3 seperate working days
- Perform new calibration per run
- Analyze assay controls in truplicate in case of successful calibration

- Calculate intra assay variation, variation of results within a data set obtained from one experiment
- Calculate inter assay variation, the variation of results obtained from repeated experiments
- 20% relative standard deviation to the mean is allowed

EXTERNAL QUALITY CONTROL

- Low, mid and high assay controls and 10 preselected samples from people living with HIV (PLHIV) receiving ART containing the drug of interested
- Patient samples must be clinically collected samples from PLHIV receiving the assay specific antiretroviral treatment
- Liquid chromatography-coupled mass-spectrometry (LCMS) should be assessed as reference test
- Compare data to available test results performed on LC-MS in Radboudumc
- 20% relative standard deviation to the line of unity is allowed