PERFORMANCE EVALUATION OF THE SOLVO MDQ-KIT

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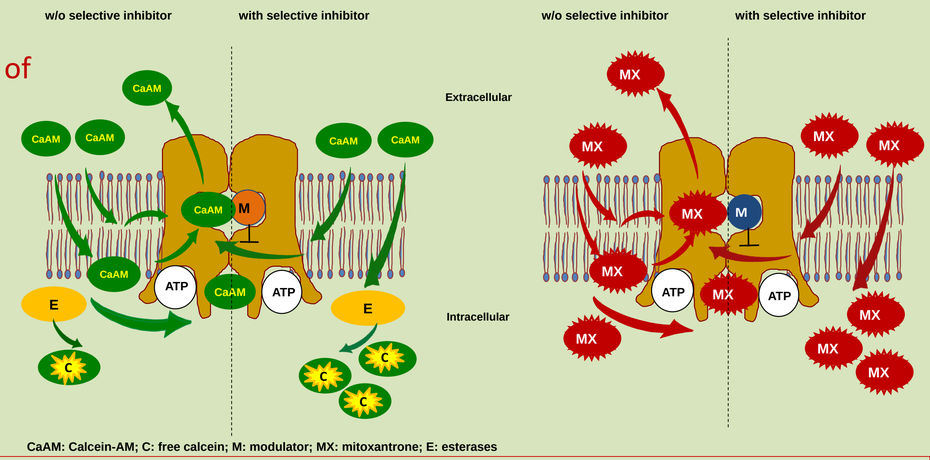
BACKGROUND:

The multidrug resistance (MDR) usually results from the expression of ATP-binding cassette (ABC) transporters, such as the ABCB1 (MDR1 or P-gp), ABCC1 (MRP1), and ABCG2 (MXR or BCRP) which are known to function as drug efflux pumps. MDR is believed to be a major barrier to successful chemotherapy in cancer patients; however, neither the genetic polymorphisms nor the mRNA/protein expression levels correlate closely with the functional activity and studies using the methods above have given conflicting and inconsistent results.

On the other hand, although the functional methods separately gave promising results, standardization and reproducibility of these tests failed to conform with values required from routine diagnostic methods. MultiDrugQuant (MDQ) kit was developed as an improved functional assay system, which can measure the MDR activity of the three, clinically most relevant efflux transporters, such as MDR1, MRP1 and BCRP in living tumor cells.

Dye efflux assays applied in the kit (Figure 1.) are based on determining fluorescence intensity differences using a flow cytometer after short *in-vitro* incubation of the cell suspension with a fluorescent dye such as the calcein-acetoxymethyl ester (calcein AM) for MDR1 and MRP1 with or without the addition of selective inhibitors of MDR1 and MRP1. The BCRP arm of the MDQ kit utilizes mitoxantrone as dye and Ko134 as BCRP-specific inhibitor.

Figure 1. Principle of dye efflux assays



CaAM: Calcein-AM; C: free calcein; M: modulator; MX: mitoxantrone; E: esterases

AIM:

Purpose of the present study was to carry out the laboratory validation and the multicenter performance evaluation of the MDQ-kit according to the requirements to be applied for routine diagnostic methods.

MATERIALS & METHODS:

- Validation of the SOLVO MDQ kit was carried out according to the standards EP10-A3 and EP5-A2 of the Clinical Laboratory Standards Institute (CLSI, former NCCLS) in three university clinical centers in Hungary.
- The reference intervals were determined according to the CLSI guideline C28-A2 on CD3+ lymphocytes of a reference population of 120 healthy volunteers (from 18 to 74 years) at 90% confidence.
- Mononuclear cells were separated from K_3-EDTA tubes using Ficoll gradient and tested at 2–5 10^6/ml within 6 hours after specimen collection.
- The testing laboratories used different flow cytometers, such as BD FACSCalibur, (Becton-Dickinson), and Beckman-Coulter FC500 (Beckman-Coulter).
- The SOLVO MDQ kit was used strictly following the manufacturers instructions. The activity of the multidrug transporter (MAF) was calculated from the difference between the mean fluorescent intensity of cells w/o the specific inhibitors, respectively.

$MAF_{Total} = 100 \times (F_{max} - F_o)/F_{max}$

$MAF_{MRP1} = 100 \times (F_{MRP} - F_o)/F_{max}$

$MAF_{MDR1} = MAF_{Total} - MAF_{MRP1}$

$MAF_{BCRP} = 100 \quad (F_{MX} - F_0)/F_{MX}$

F_{max}/F_{MX}: calcein/mitoxantron fluorescence with inhibitor 1/3

F_o: fluorescence without inhibitor

F_{MRP1}: calcein fluorescence with inhibitor 2

- In order to determine the inaccuracy and for comparative measurements between the laboratories, the MDQ assay was performed on control HL60 cells as well as on selected cell lines with high activity of one of the transporters: HL60/MDR1+, HL60/MRP1+ and PLB/BCRP+.
- Results on different flow cytometers were compared applying CD3 (clone: SK7) or CD19 (clone: SJ25CI) or CD45 (clone: 2D1) monoclonal antibodies for gating the population of interest. All the antibodies were purchased from Becton-Dickinson.
- Robustness of the method was assessed carrying out the test at different concentrations of the fluorescent dyes (10–100 % of the original) and inhibitors (50–150 % of the original) using a Partec CyFlow space flow cytometer (Partec GmbH, Münster, Germany).

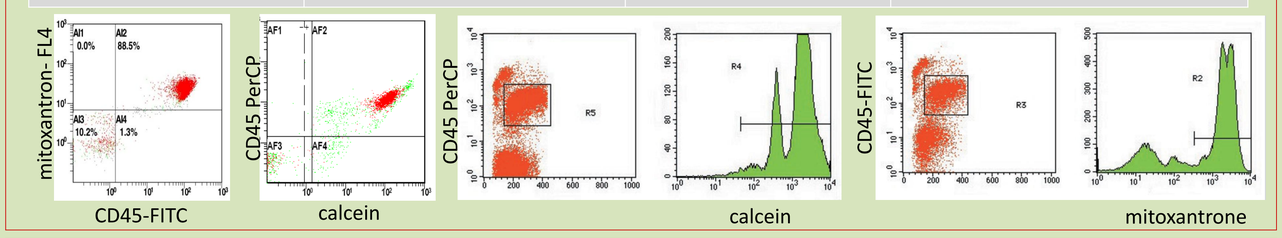
The validation study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki and has been approved by the national as well as institutional ethical committees.

RESULTS

STANDARDIZATION OF FLUORESCENCE-BASED METHOD

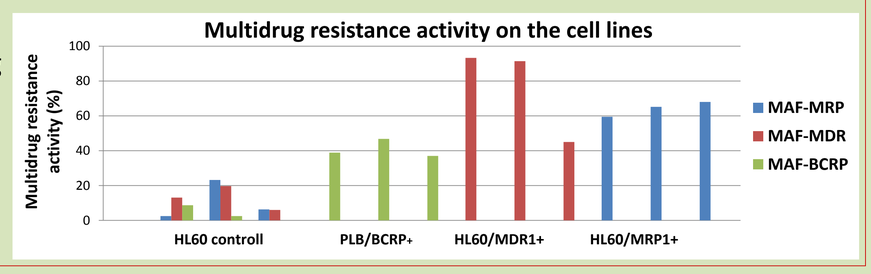
Compatible fluorescent-labelled antibodies enable the gating of cell populations of interest in the calcein- and mitoxantrone-assays. The MAF values were found feasible in controls (left plots) as well as in AML samples (right plots)

Transporter(s)	Dye/Substrate	Channel	Compatible Fluorochromes
MDR1-MRP1	Calcein	FL1	PerCP, PerCP Cy5.5
BCRP	mitoxantron	FL4	FITC



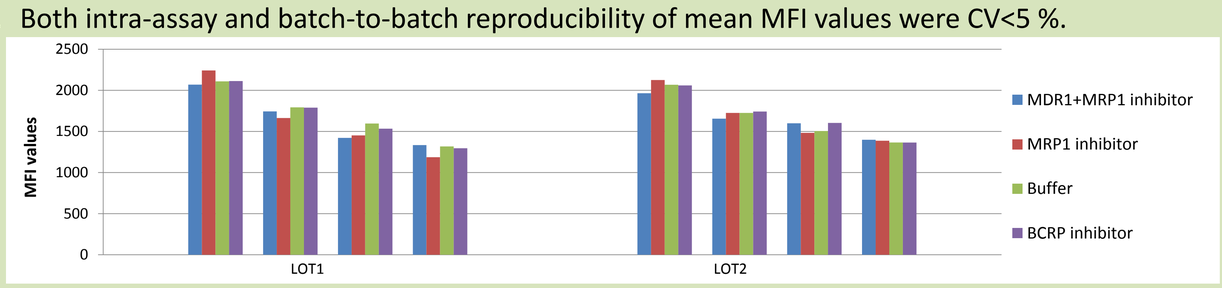
ACCURACY

Measurements carried out using control HL60 cells and ABC transporter overexpressing (HL60-MDR1+; HL60/MRP1+; PLB/BCRP+) cell lines in three university clinical laboratories.

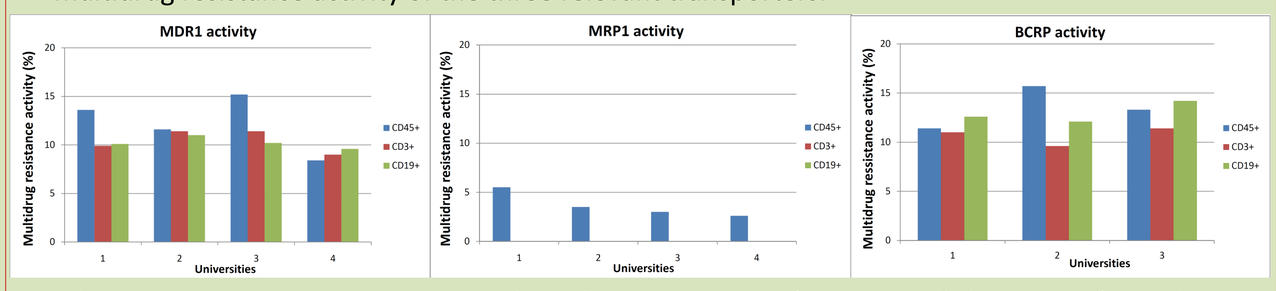


REPRODUCIBILITY AND ROBUSTNESS

1. Both intra-assay and batch-to-batch reproducibility of mean MFI values were CV<5 %.



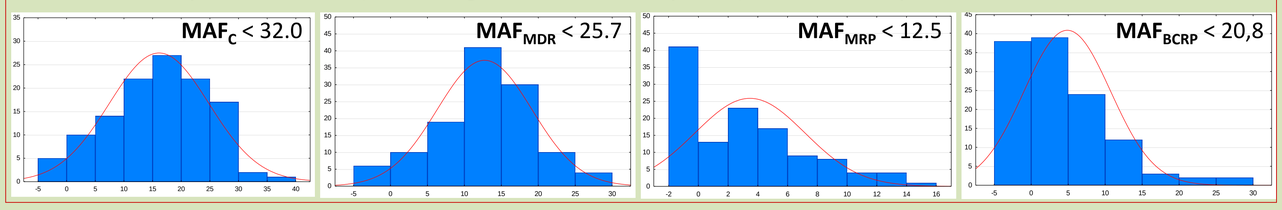
2. Inter-laboratory reproducibility between universities were shown good correlation regarding to multidrug resistance activity of the three relevant transporters.



3. Robustness: Various concentrations of the fluorescent dyes (10–100% of the original) or inhibitors (50–150%) resulted only in negligible differences in the MAF values (data not shown).

REFERENCE VALUES

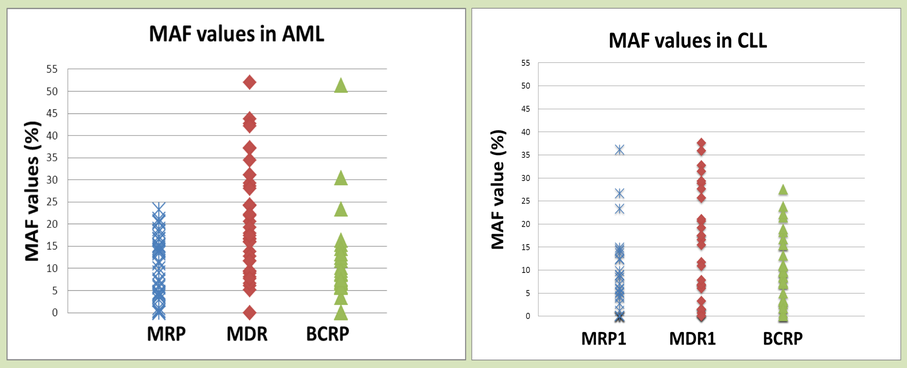
Reference intervals determined at 90% confidence level on CD3+ lymphocytes in a population of 120 healthy individuals (mean age=43.2 14.77 years) without any medication:



TESTING LEUKEMIC SAMPLES USING THE SOLVO MDQ-kit

Preliminary MDQ results measured on the CD45dim cells of 39 AML and on the CD19+ cells of 31 CLL patients.

Both in the AML and CLL groups, MDR transporter activities were often elevated significantly.



CONCLUSION

- The MDQ-kit provides quantitative results on the activity of the three clinically relevant multidrug resistance transporters, such as the MDR1, MRP1 and BCRP.
- Designed as an easy to use and robust, routine flow cytometric assay to predict therapy resistance in haematologic malignancies and autoimmune diseases.
- Both in the AML and CLL groups, MDR transporter activities were often elevated significantly.

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