LABORATORY VALIDATION OF THE MULTIDRUGQUANT™ ASSAY 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), ABCCL (MRP1), and ABCG2 (MXR or BCRP) which are known to function as drug efflux pumps. Although it is believed to be a major barrier to successful chemotherapy in cancer patients, neither the genetic polymorphisms nor the mRNA or 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 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 calcin-acetoxymethyl ester (calcine AM) for MDR1 and MRP1 with or without the addition of selective inhibitors of MDR1 and MRP1 (Figure 1A, B). The BCRP arm of the MDQ kit utilizes mitoxantron as dye and Ko134 as BCRP-specific inhibitor.

PRINCIPLE OF CALCEIN ASSAY

Figure 1.

A permanent human cell line, which is a subclone of the transfectant
- MDR1 (BCRP): mitoxantron resistant
- MDR2 (ABCG2): mitoxantron sensitive
- MRP1: rhodamine 123 sensitive

AIM:
Purpose of the study was the laboratory validation of the MultiDrugQuant-kit prior to the performance evaluation in acute myeloid leukaemia (AML) and chronic lymphocytic leukaemia (CLL).

MATERIALS & METHODS:
Validation of the SOLVO MDQ kit was carried out according to the standards EP10-A3 and EPS-A2 of the Clinical Laboratory Standards Institute (CLSI, former NCCCLS) in three university clinical centers in Hungary.

- Mononuclear cells were separated from K2-ACTED tubes using Ficoll gradient and tested at 2– 10^6/ml within 6 hours after specimen collection.
- In order to evaluate another preanalytical procedure, CPT tubes (Becton Dickinson) containing sodium-citrate anticoagulant was applied and compared to the technique described above.
- The testing laboratories used different flow cytometers, such as BD FACS Calibur, (Becton-Dickinson), 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_{max} = 100 × [F_{max} - F_{i}]/F_{max}
- MAF_{min} = 100 × [F_{max} - F_{i}]/F_{max}
- MAF_{BCRP} = 100 × [F_{max} - F_{i}]/F_{max}
- MAF_{BCRP} = 100 × [F_{max} - F_{i}]/F_{max}

In order to determine the accuracy and for comparative measurements between the laboratories, the MDQ assay was carried out 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/MDR+.

RESULTS

STANDARDIZATION OF FLUORESCENCE-BASED METHOD
Compatibility of fluorochromes with the calcine- and mitoxantron-assays were assessed, in order to gate the cell population of interest using marked antibodies. The multidrug resistance activity values were found comparable and eligible based on the standardized method.

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

ACCUacy
Measurements carried out using control HL60 cells and ABC transporter overexpressing (HL60-MDR+, HL60/MRP+, PLB/MDR+) cell lines in the three different laboratories.

REPRODUCIBILITY

1. Both intra-assay and batch to batch reproducibility were CV<5 %.

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

Testing of the methods for target cells from normal peripheral blood
Multidrug resistance transporter activities were measured on the different cell population with CD3+/CD19+/CD45 moAbs.

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.
- Recently, clinical trial have been started in malignant haematological diseases (AML, CLL) and in autoimmune disorders to assess the correlation between the transporter activity and the therapeutic response as well as to evaluate the predictive value of the biomarker.

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