The HLA-A*02:01 Quickswitch™ Quant kit can be used for determining the biological activity of a cancer vaccine.

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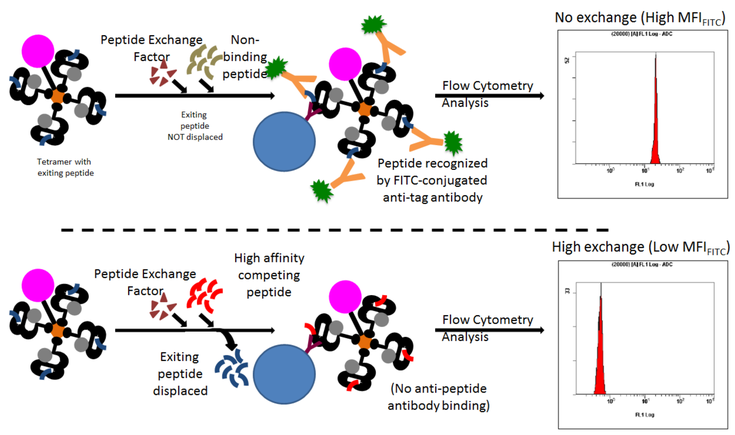
ABSTRACT

We have devised a fast and *user-friendly* assay (QuickSwitch™ Quant) that can both help determine binding of novel peptides to MHC class I molecules and generate new specificity MHC class I tetramers for peptide specific T cell detection. This study aimed to determine whether this kit can also be used for evaluating the biological activity of a vaccine. The tested vaccine was DPX-Survivac, an ovarian cancer vaccine candidate which consists of several survivin peptide antigens that are each restricted to a different human class I allele. We sought to evaluate the specificity and sensitivity of the QuickSwitch™ Quant HLA-A*02:01 Tetramer Kit-PE for assessing the biological activity of SurA2.M, a HLA-A2-restricted peptide, in DPX-Survivac. The complete vaccine also contains non-HLA-A2 restricted peptides, lipids and a polynucleotide adjuvant. We tested the detection of the peptide prepared individually in a buffered solution or in the DPX-Survivac vaccine prepared in an aqueous formulation. Results indicate that peptide exchange rate of SurA2.M is similar whether it is dissolved individually in a buffered solution or mixed with other components of the vaccine. Results also may be dependent on the affinity of the peptide for HLA-A2. Thus, by optimizing a concentration curve using individual peptides, the QuickSwitch™ Quant HLA-A*02:01 Tetramer kit can be used to quantify the concentration of HLA-A2 restricted peptides in simple solutions or more complex formulations.

INTRODUCTION - QuickSwitch™ Quant Assay



The HLA-A2 QuickSwitch™ peptide exchange kit includes a tetramer with an irrelevant exchangeable peptide, a peptide exchange factor, HLA capture beads and a FITC conjugated antibody recognizing the exiting peptide. Flow cytometry analysis of captured tetramers generated after peptide exchange allows to quantify peptide exchange.

METHODS AND MATERIALS

96-well plate QuickSwitch™ Quant Tetramer assay

A. Generation of New Specificity Tetramer Using Peptide Exchange

All individual peptides and DPX-Survivac were reconstituted in water to the indicated concentrations. 500 µL of Quickswitch Tetramer were mixed with 10 µL of Peptide Exchange Factor. 20 µL of Quickswitch/Peptide exchange factor cocktail were added per microwell of a round-bottom 96-well plate for each peptide to be tested. Each well received 0.4 µL of test peptide. The plate was incubated for 4 hours at RT, protected from light.

B. Quantification of Peptide Exchange Using Flow Cytometric Sandwich Immunoassay

20 µL of magnetic capture beads were added to wells of a v-bottom 96-well plate for each tested peptide plus wells for control samples. Each test well received 5 µL from the peptide exchange samples. The plate was shaken for 45 minutes at 550 rpm, room temperature, protected from light. After bead rinse, wells received 25 µL of 1X exiting peptide antibody. The plate was shaken for 45 minutes at 550 rpm, room temperature, protected from light. Beads were rinsed, resuspended in 1x assay buffer and run on a FACSCalibur Flow Cytometer. Single beads were gated on FSC vs. SSC and MFI were measured on 500 events per test. Peptide exchange rate was calculated using a calibration curve generated with MFI values from controls.

T2 HLA-A2 Shift assay

T2 cells are a human cell line that express low levels of HLA-A2 on their surface due to deficiency in TAP protein. Upon incubation with a peptide that can bind to HLA-A2, expression of HLA-A2 on the surface increases as the peptide stabilizes the complex. Immunofluorescent staining of T2 cells using a fluorescein-conjugated monoclonal antibody (anti-HLA-A2) is used to quantify the relative expression of HLA-A2 in vitro after antigen stimulationis. In this study, T2 cells were stimulated with various amounts of peptide for 24 h; HLA-A2 surface expression was detected using BB7.2-PE anti-HLA-A2 antibody quantified using BD FACSCalibur.

IFN- γ ELISPOT

HLA-A2 transgenic mice (HHD, n=6) were vaccinated with DPX-Survivac formulated with decreasing doses of the HLA-A2 restricted peptide SurA2.M. Eight days after vaccination, mice were terminated and spleens collected. Splenocytes were stimulated in an IFN-γ ELISPOT plate with media (background), an irrelevant HLA-A2 restricted peptide or with SurA2.M peptide. Plates were developed after 18 hours and spot forming units (SFU) were quantified using Immunospot Reader (C.T.L.).

Table 1. Peptides used in this study

Name	Sequence	HLA-Restriction	HLA-A2 affinity (nM)
SurA1.T	**FTELTLGEF**	HLA-A1	14587.88
SurA2.M	**LMLGEFLKL**	HLA-A2	25.41
SurA3.K	**RISTFKNWPK**	HLA-A3	24220.66
SurA24	**STFKNWPFL**	HLA-A24	243.83
SurB7	**LPPAWQPFL**	HLA-B7	16536.16
Y9T	**YMLDLQPET**	HLA-A2	21.23
Y10T	**YMLDLQPETT**	HLA-A2	176.26
A16L	**AQYIKANSKFIGITEL**	N/A	22215.71

Table 1. indicates peptides used in this study. Their predicted binding affinity to HLA-A2 was calculated using the IEDB artificial neural network method

RESULTS

Figure 1. Biological activity of the HLA-A2 restricted peptide SurA2.M can be evaluated with the cell based T2 Shift Assay or with the animal based IFN-γ ELISPOT

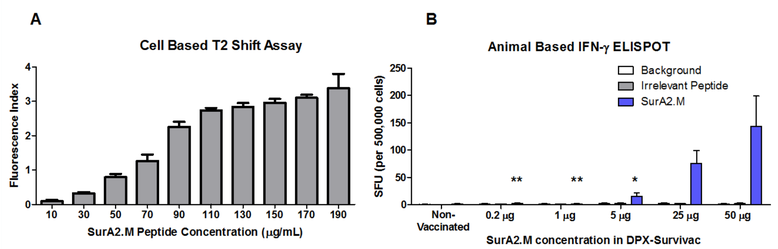


Figure 1A: T2 HLA-A2 Shift Assay results with the HLA-A2 binding peptide SurA2.M. Data are presented as an average ± SEM of three independent experiments and expressed relatively to the unstimulated control. **The response range is within 10 to 110 µg/mL.**

Figure 1B: SurA2.M binds to HLA-A2 and induces IFN-γ secretion by splenocytes of HLA-A2 transgenic mice immunized with DPX-Survivac. Splenocytes were stimulated with media (background), an irrelevant HLA-A2 restricted peptide or with SurA2.M peptide. A dose response was observed. Statistics were performed to compare the response of each dose to the maximum, 50 µg, by 1-way ANOVA followed by Dennett's post-test; *p<0.05, **p<0.01. **IFN–γ ELISPOT can detect 90% degradation of SurA2.M in the vaccine.**

Figure 2. Comparison of the cell free HLA-A2 QuickSwitch™ Quant assay and the cell based HLA-A2 T2 shift assay

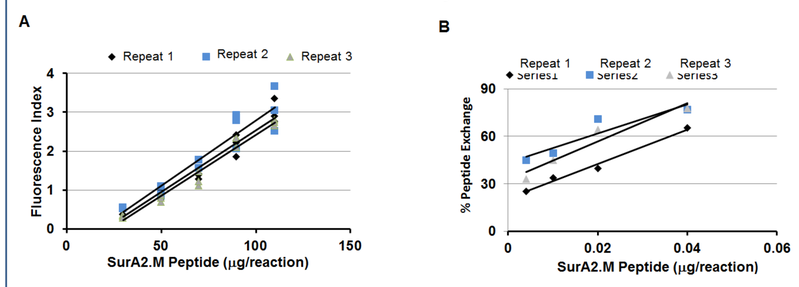


Figure 2A: Quantification of SurA2.M peptide binding to HLA-A2 using the T2 HLA-A2 shift assay. T2 cells were stimulated with the indicated amounts of SurA2.M peptide for 24 h; HLA-A2 surface expression was detected using BB7.2-PE anti-HLA-A2 antibody quantified using BD FACSCalibur. **The T2 Shift assay provides a high degree of linearity when applied to the samples containing 10 µg/reaction to 110 µg/reaction of SurA2.M peptide.**

Figure 2B: Quantification of SurA2.M peptide MHC binding using the HLA-A2 QuickSwitch™ Quant Tetramer assay. Results indicate that the upper limit of quantification is below 0.2 µg/reaction and the response range is within 0.004 µg/reaction to 0.04 µg/reaction. **QuickSwitch™ Quant Tetramer assay provides an acceptable degree of linearity when applied to the samples containing 0.004 µg/reaction to 0.04 µg/reaction of SurA2.M peptide**.

Figure 3. QuickSwitch™ assessment of HLA-A2 binding peptides as individual reagents or as constituents of Survivac vaccine.

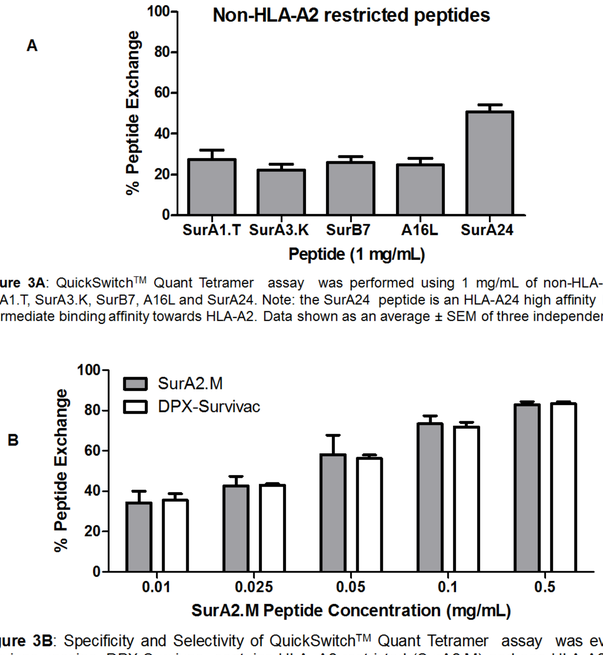


Figure 3A: QuickSwitch™ Quant Tetramer assay was performed using 1 mg/mL of non-HLA-A2 restricted peptides SurA1.T, SurA3.K, SurB7, A16L and SurA24. Note: the SurA24 peptide is an HLA-A24 high affinity binder and displays an intermediate binding affinity towards HLA-A2. Data shown as an average ± SEM of three independent repeats.

Figure 3B: Specificity and Selectivity of QuickSwitch™ Quant Tetramer assay was evaluated using DPX-Survivac vaccine. DPX-Survivac contains HLA–A2 restricted (SurA2.M) and non-HLA-A2 restricted peptides (SurA1.T, SurA3.K, SurA24, SurB7, A16L) in the lipid environment. Results demonstrate that QuickSwitch™ Quant Tetramer assay can be used to evaluate HLA-A2 restricted peptide SurA2.M in the presence of a vaccine matrix. Data shown as an average ± SEM of three independent repeats. Student's t-test was performed to compare the levels of peptide exchange for DPX-Survivac to that for SurA2.M peptide at each concentration level; no statistical differences detected p<0.05.

Figure 4. Peptides of various HLA-A2 affinities assessed with the QuickSwitch™ Quant assay.

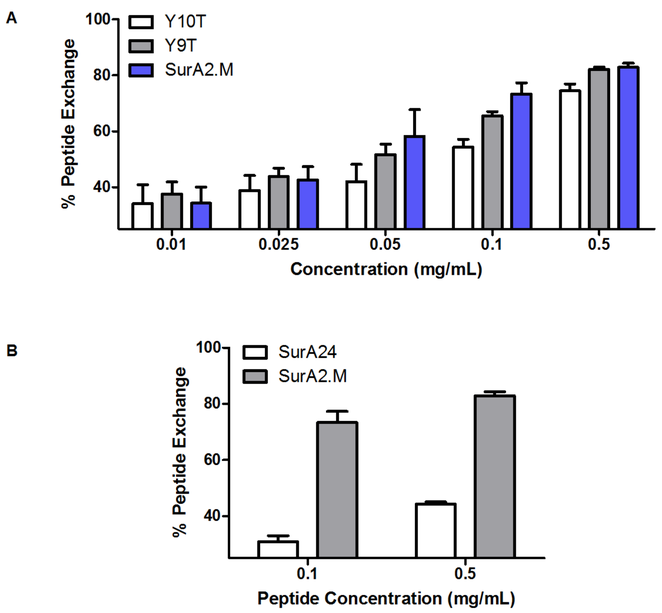


Figure 4A, B: The HLA-A2. QuickSwitch™ Quant Tetramer assay was performed using indicated peptide amounts. Data shown as an average ± SEM of three independent repeats. Predicted binding affinity was calculated using the IEDB artificial neural network method and presented in Table1. **Peptide Y9T binds to HLA-A2 with a higher affinity than Y10T which is reflected on peptide exchange yields across multiple concentrations. Peptide SurA2.M which behaves like peptide Y9T also displays a very high affinity towards HLA-A2. Peptide SurA24, an HLA-A24 high affinity binder, displays an intermediate binding affinity towards HLA-A2, in agreement with the QuickSwitch™ Quant assay data.**

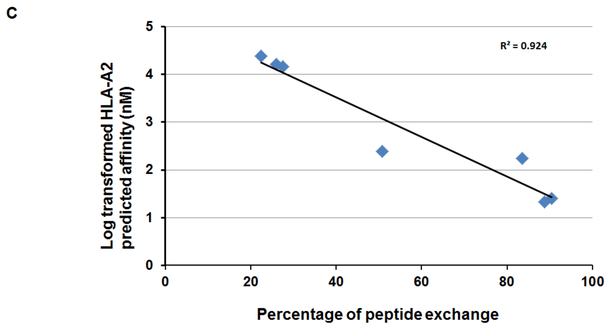


Figure 4C: Log transformed peptide HLA-A2 binding affinities from table 1 were plotted against the measured peptide exchanges at the concentration of 1 mg/mL. **Predicted HLA-A2 binding affinities correlate with values obtained with the QuickSwitch™ Quant assay.**

CONCLUSION

- The HLA-A2 QuickSwitch™ Quant peptide exchange assay can be used to evaluate biological activity of peptide-based vaccines. Similarly to the T2 shift assay, QuickSwitch™ quantifies interactions between HLA-A2 restricted peptide and its receptor, but does this in a cell-free environment.
- Sensitivity of the HLA-A2 QuickSwitch™ Quant is at least two hundred times higher than the HLA-A2 T2 shift assay.
- The HLA-A2 QuickSwitch™ Quant assay permits to accurately quantitate HLA-2 binding peptides in aqueous solutions or mixed with different chemicals in a complex vaccine matrix.
- The QuickSwitch™ Quant Tetramer assay can be used to evaluate peptide affinities toward HLA-A2. Peptide exchange rate mediated and quantified by the HLA-A2 QuickSwitch™ Quant assay is correlated to peptide affinity towards HLA-A2 and is affected by peptide concentration.
- The HLA-A2 QuickSwitch™ Quant peptide exchange assay is user-friendly, fast and can be performed in a high throughput 96 well plate format.

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