



Fast & efficient processing of ELISA assays

using Tecan's HydroSpeed™ plate washer and Infinite® F50 absorbance reader

Caution: This note describes a combination of automation and reagent kit that has not been cleared or approved by regulatory authorities including the United States Food and Drug Administration. Consult your local regulatory authority prior to implementation of automation for any IVD application.

Introduction

This application note describes the outcome of a successful evaluation study of Tecan's new HydroSpeed plate washer and Infinite F50 absorbance reader using a HBs Ag assay. This assay is used as an example of a qualitative, one step 'sandwich' type enzyme immunoassay for the detection of hepatitis B viral surface antigen (HBs Ag) in human serum or plasma (1).

Hepatitis B is an infectious disease, caused by the hepatitis B virus (HBV), which infects the human liver causing an inflammation. Transmission of the virus occurs by exposure to infected blood or other infected body fluids, and about one third of the world's population is infected with the HBV. The HBs Ag assay typically allows to detect the HBs Ag in human serum or plasma, as this is the first marker to appear after infection, and can be detected two or three weeks before the first clinical and biological symptoms of the disease (1). Because hepatitis B represents a serious transfusion hazard,

the detection of HBV is one of the most important routine clinical tests performed worldwide.

It is crucial to detect the HBs Ag at the time of blood donation to prevent it being transmitted during a transfusion.

Tecan's new HydroSpeed plate washer offers advanced features for a range of applications including vacuum filtration, magnetic bead separation and washing of ELISA- and cell-based assays in 96- & 384-well plate format. Reliable operation is provided by the HydroSpeed's automated Anti-Clogging™ function, which prevents needle blockage during idle time between plates, typically caused by crystallization of the wash buffer.

The Infinite F50 absorbance reader is Tecan's state-of-the-art, 8-channel absorbance microplate reader that provides not only accurate, reproducible and fast measurements, but sets

new standard in compact and innovative design. Together with Magellan™, Tecan's easy-to-use control and data analysis software, the Infinite F50 is ideal for a variety of ELISA applications.

The HydroSpeed plate washer and the Infinite F50 absorbance reader are a perfect combination for ELISA-based assays, offering high throughput capacity and accurate data acquisition/analysis, fulfilling the major requirements for clinical and research laboratories.

Materials and methods

Instruments

- HydroSpeed plate washer equipped with the 96-HT or 96-indexing wash head for ELISA and cell washing
- Infinite F50 absorbance reader

Microplate

- NUNC® MaxiSorp™ 96-well strip plate (provided with the kit)

Reagents

- HBs Ag ELISA kit

Assay procedures

The HBs Ag ELISA detects a small part of the surface antigen of the hepatitis B virus at low sample concentrations and uses an alkaline wash buffer and tetramethylbenzidine (TMB) for color detection. Please refer to the assay description for detailed information and an explanation of the principle of this ELISA assay (1).

The assay was performed according to the manufacturer's assay manual, using the kit's negative and positive controls in addition to 15 positive and 76 negative patient serum samples (figure 1). All washing steps were performed with the HydroSpeed plate washer equipped with the 96-HT wash head or the 96i wash head, and the readout was performed with the Infinite F50 absorbance reader. For detailed information on the washing program and the measurement settings, please refer to figure 2 and table 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC1	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Pos
B	NC2	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
C	NC3	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg
D	NC4	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg
E	PC1	Neg	Pos	Neg								
F	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Neg	Neg
G	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
H	Pos	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Pos

Figure 1: Plate layout. NC1-4 = negative control; PC1 = positive control; Pos = positive patient samples; Neg = negative patient samples.

Measurement settings and wash program

Measurement parameters	
Plate definition file	NUN96ft.pdfx
Wavelength	450 nm
Reference wavelength	620 nm

Table 1: Measurement parameters for Infinite F50 absorbance reader.

Program Parameter:

Program name:	ELISA_HBsAg_96	Wash head:	96
Program mode:	Plate	Aspiration rate:	4
		Perform tip prime:	No

Plate parameter:

Plate name:	NUNC MaxiSorb_ELISA		
Aspiration position 1:	2000 µm	Dispense Y offset:	2200 µm
Aspiration position 2:	0 µm	Bottom position:	3500 µm
Aspiration position 3:	-2000 µm	Overflow position:	14800 µm
Aspiration position 4:	2000 µm	Bottom form:	Flat

PROGRAM BEGIN 1 ELISA_HBsAg_96

CYCLE 1 Number of Cycles: 5

WASH Mode: Crosswise, Z-Position aspiration: BOTTOM, Z-Position wash: OVERFLOW, Channel: 1, Volume: 800 µl, Head speed: 10 mm/s, Wash rate: 280 µl/s, Aspiration rate: 4, Aspiration time: 4 s

CYCLE 2 Number of Cycles: 1

WASH Mode: Crosswise, Z-Position aspiration: BOTTOM, Z-Position wash: CUSTOM, Z-Position wash value: 4.0 mm, Channel: 1, Volume: 500 µl, Head speed: 10 mm/s, Wash rate: 280 µl/s, Aspiration rate: 4, Aspiration time: 4 s

FINAL ASPIRATE Mode: Crosswise, Z-Position: BOTTOM, Time: 5 s, Head speed: 10 mm/s, Aspiration rate: 4

PROGRAM END

Figure 2: Wash program for the HydroSpeed plate washer equipped with the 96-HT wash head. For the 96i wash head the same program with a wash rate of 180 µl/s was used.

Results and discussion

Interpretation of assay results (according to the kit manual):

1. All negative control values (NC1 - NC4) must give an absorbance value ≤ 0.08 OD
2. Each positive control must give an absorbance value ≥ 1.0 OD
3. Cut-off value: NCmean + 0.05
4. Calculation of ratio: OD of sample / OD of cut-off
 Ratio > 1: sample is positive
 Ratio < 0.9: sample is negative
 Ratio > 0.9 and < 1: sample is negative but has to be retested

negative patient samples were correctly detected, and no false positive or false negative results occurred. None of the samples needed retesting. Results for the 96i wash head are not shown, but were comparable to the data listed above.

Note: All positive patient samples were used without pre-dilution of the probe; therefore raw data values are displayed as 'Overflow' (OD > 4). As a consequence these wells were not marked red, which is the typical indication of positive samples.

Conclusion

The results presented in this application note clearly demonstrate that the HydroSpeed plate washer and the Infinite F50 absorbance reader are well suited for fast & efficient processing of ELISAs, such as a HBs Ag assay. The HydroSpeed plate washer provides outstanding washing performance, enabling critical samples to be handled, such as the undiluted serum probes used in this study. The 96-channel wash head provides the HydroSpeed plate washer with the capability to perform high throughput ELISA washing.

This matches the concept of the Infinite F50 absorbance reader, which is equipped with an 8-channel absorbance optic that enables ultra-fast absorbance measurements of less than 20 seconds for a 96-well plate.



Figure 3: Magellan - raw data.



Figure 4: Magellan - cut-off results.

Figures 3 and 4 show the raw data and final cut-off results obtained from Magellan software using the HydroSpeed plate washer with the 96-HT wash head. All negative controls show absorbance values below 0.08 OD, and all positive controls show absorbance values above 1.0 OD. All positive and

Literature

(1) www.biorad.com

List of Abbreviations

HBs Ag	surface antigen of Hepatitis B virus
NC	negative control
Neg	negative patient samples
PC	positive control
Pos	positive patient samples
TMB	tetramethylbenzidine
OD	optical density
HT	high throughput

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