Technical Overview

Automation of ELISA Workflows

Automated avian influenza ELISA with Agilent BioTek 406 FX washer dispenser

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Abstract

Enzyme-linked immunosorbent assay (ELISA) is a widely used and powerful technique for the detection of a range of molecules in a variety of settings. The workflows typically consist of repeated cycles of microplate washing, reagent addition, and incubation. Throughput is typically limited when performed manually and can be greatly increased by incorporating automated liquid handling, microplate transfer, and microplate reading. Thus, the required manual processing is limited to the loading of samples and controls onto the microplate, minimizing human error. The Agilent BioTek 406 FX washer dispenser, in combination with the Agilent BenchCel microplate handler and Agilent BioTek Cytation 7 cell imaging multimode reader, can be incorporated into the ELISA workflow to significantly increase a laboratory's assay throughput.

Introduction

ELISA is a widely used and powerful technique in the field of biotechnology and biomedical research. It enables the detection and quantification of various molecules such as proteins, peptides, antibodies, and hormones using specific antibodies and enzymatic reactions. ELISA workflows typically involve multiple steps, including sample preparation, incubation, washing, and detection, which can be time consuming and labor intensive when performed manually.

To overcome these challenges and improve the efficiency and reliability of ELISA assays, automation has emerged as a valuable tool. Automation systems allow for the integration of advanced robotics, liquid-handling and detection devices, and sophisticated software to streamline and optimize the entire ELISA workflow. By automating repetitive tasks and minimizing human error, automation offers several benefits, including increased throughput, enhanced accuracy, and improved data quality. One of the key advantages of using automation in ELISA workflows is the reduction in hands-on time. Automated platforms can perform tasks such as plate transfer, plate washing, and reagent dispensing to increase precision and consistency. This frees up researchers' time and resources, enabling them to focus on other critical aspects of their experiments, such as data analysis and interpretation. Moreover, automation enhances the reproducibility of ELISA results. Manual manipulation introduces inherent variability due to human factors like pipetting errors, inconsistent incubation times, and uneven plate washing. By standardizing and controlling these steps through automation, the likelihood of errors and variability decreases significantly, resulting in more reliable and consistent assay outcomes (Figure 1).

Figure 1. Agilent BioTek 406 FX washer dispenser, Agilent PlateLoc thermal microplate sealer, Agilent BenchCel microplate handler, and Agilent BioTek Cytation 7 cell imaging multimode reader.

Another significant advantage of automation is its ability to increase the throughput of ELISA assays. Automated systems can process multiple samples simultaneously, enabling high-throughput analysis of large sample sets. This capability is particularly valuable in clinical and pharmaceutical research settings, where large-scale screening and profiling are required. Furthermore, automation offers improved data management and traceability. Integrated software systems can automatically record and store data at each step of the ELISA workflow, ensuring accurate and comprehensive documentation. This simplifies data analysis, facilitates experiment reproducibility, and supports compliance with regulatory requirements. Integrating the Agilent BenchCel microplate handler with the Agilent BioTek 406 FX washer dispenser and Cytation 7 cell imaging multimode reader provides a solution to automate the ELISA workflow.

Experimental

Avian influenza virus automated ELISA workflow

Avian influenza (AI) virus affects both domestic and wild birds and can lead to a high mortality rate that could be devastating among larger flocks, such as those found on a domestic chicken ranch or farm. The AI MultiS-Screen Ab Test from IDEXX Laboratories is a screening test for the detection of antibodies to AI in chicken, turkey, duck, ostrich, and goose serum samples. Routine screening of bird flocks for AIV ensures that the flocks are free of disease. The large number of animals that need to be evaluated in flocks requires daily sample processing. During an outbreak, the test volume would be expected to significantly increase, requiring greater resources and potentially overburdening testing facilities. Automation of assay processes can provide time and labor savings in these scenarios.

The various steps and use of the automated dispensers and wash module of the 406 FX used in this study are listed in Table 1. Additional reagents, beyond the contents of a standard ELISA kit may be needed, to account for the priming volumes required for automated liquid handling. It is important to verify that the additional required reagent volumes are available when purchasing validated kits to ensure that reagents with matching lot numbers are acquired.

Table 1. Reagents and dispensers used for the AIV ELISA.

Step	Reagent	Dispenser
1	Wash buffer	Washer manifold
2	Conjugate	Primary peristaltic pump
3	Wash buffer	Washer manifold
4	Substrate	Secondary peristaltic pump
5	Stop solution	Syringe pump A

The overall automated assay workflow is depicted graphically in Figure 2. The 406 FX, BenchCel, and Cytation 7 can provide a solution to help manage increased assay-throughput requirements.

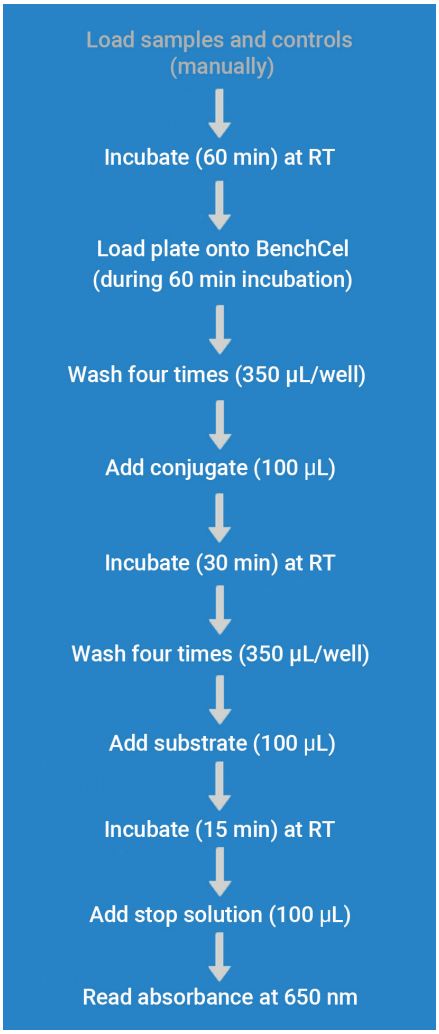

Figure 2. IDEXX AI MultiS-Screen Ab Test procedure. Steps performed by the Agilent BenchCel microplate handler, Agilent BioTek 406 FX washer dispenser, and Agilent BioTek Cytation 7 cell imaging multimode reader.

Materials and methods

The IDEXX AI MultiS-Screen Ab Test (Westbrook, MA) was performed according to the kit instructions, as previously described, with the following modifications.[1] Briefly, undiluted controls (positive and negative) and both undiluted and diluted (1:10 and 1:100) assay controls were pipetted (100 µL) into the assay plate. For this demonstration, five assay plates were loaded at approximately 90-second intervals. The plates were allowed to incubate for 60 minutes while being transferred to the BenchCel stack. At the end of the 60-minute incubation, the Agilent VWorks automation control software program was initiated. The BenchCel workflow was programmed to allow one plate to enter the system every 90 seconds. Plates were transferred to the 406 FX where they were washed five times with 350 µL wash buffer followed by the addition of 100 µL conjugate using the primary peristaltic pump dispenser on the 406 FX. The plates were then allowed to incubate for 30 minutes at room temperature (RT) while being reordered. Constraints were added to BenchCel workflow using the VWorks automation control software to ensure that the appropriate incubation times identified in the assay protocol were adhered to. After incubation, the plates were again transferred by the BenchCel to the 406 FX and washed five times with 350 µL washer buffer followed by the addition of 100 µL substrate solution using the secondary peristaltic pump dispenser. The color development was allowed to proceed for 15 minutes while reordering of plates was performed. After color development, the plates were again moved to the 406 FX using the BenchCel where 100 µL stop solution was added using a syringe pump dispenser. The plates were moved immediately by the BenchCel from the 406 FX to the Cytation 7, where the absorbance was read at 650 nm.

Results and discussion

Control wells are used for assay validation and sample determinations. For the assay results to be valid, the IDEXX AI MultiS-Screen Ab Test requires that the negative control mean at 650 nm absorbance is greater than or equal to 0.600 and the PC:NC ratio is less than 0.5. These values, as well as sample symbols for positive (POS) and (NEG) samples, can be automatically calculated and reported by programming in the appropriate formula in Agilent BioTek Gen5 microplate reader and imager software. As demonstrated in the sample data from plate number 3 (Table 2), the assay results of the AIV assay performed by the automated system meet the validation criteria outlined in the kit instructions.

Table 2. Validation results.

Plate Number 3 Validation			
Parameter	**Value**	**Formula**	**Value**
NC Mean	1.390	IF(0.6≤NEG;1;0)	Valid
PC:NC Ratio	0.366	IF(0.5>POS/NEG;1;0)	Valid

Interpretation of the assay results are based on the S:N ratio (Sample A650)/NC mean) as shown in Table 3.

Table 3. Interpretation formula.

Parameter	Value	Formula
Cutoff NEG	S:N	IF(0.6≤NEG;1;0)
Cutoff POS	S:N	IF(0.5>POS/NEG;1;0)

The output from the cutoff values can be programmed into Gen5 microplate reader and imager software to allow the results to be automatically output based on symbols to signify either negative (NEG) or positive (POS) results. This enables easy interpretation of assay results (Table 4). Highlighted wells correspond to either positive or negative control wells (A1-B2), diluted positive assay controls wells (A6-A7 and B6-B7), or positive assay control wells (D7 and G11). All other wells were buffer controls.

The ability to automate both the liquid handling and plate reading allows for a throughput of up to 10 AI assay ELISA plates. The throughput is limited by the final incubation step requiring 15 minutes for color development before the final reagent addition of stop solution. The timeline of events is shown in the Gantt chart (Figure 3).

Table 4. Example of plate number 3 data output of cutoff (S/N) symbols using Agilent BioTek Gen5 microplate reader and imager software. Cutoff criteria are programmed in the Gen5 software, allowing automated data analysis and output of interpretation to be viewed as symbols for either negative (NEG) or positive (POS) assay results.

Plate Number 3 Data													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
A	**POS**	**POS**	NEG	NEG	NEG	**NEG**	**NEG**	NEG	NEG	NEG	NEG	NEG	Symbols (S/N)
B	**NEG**	**NEG**	NEG	NEG	NEG	**NEG**	**NEG**	NEG	NEG	NEG	NEG	NEG	Symbols (S/N)
C	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Symbols (S/N)
D	NEG	NEG	NEG	NEG	NEG	NEG	**POS**	NEG	NEG	NEG	NEG	NEG	Symbols (S/N)
E	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Symbols (S/N)
F	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Symbols (S/N)
G	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	**POS**	NEG	Symbols (S/N)
H	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Symbols (S/N)

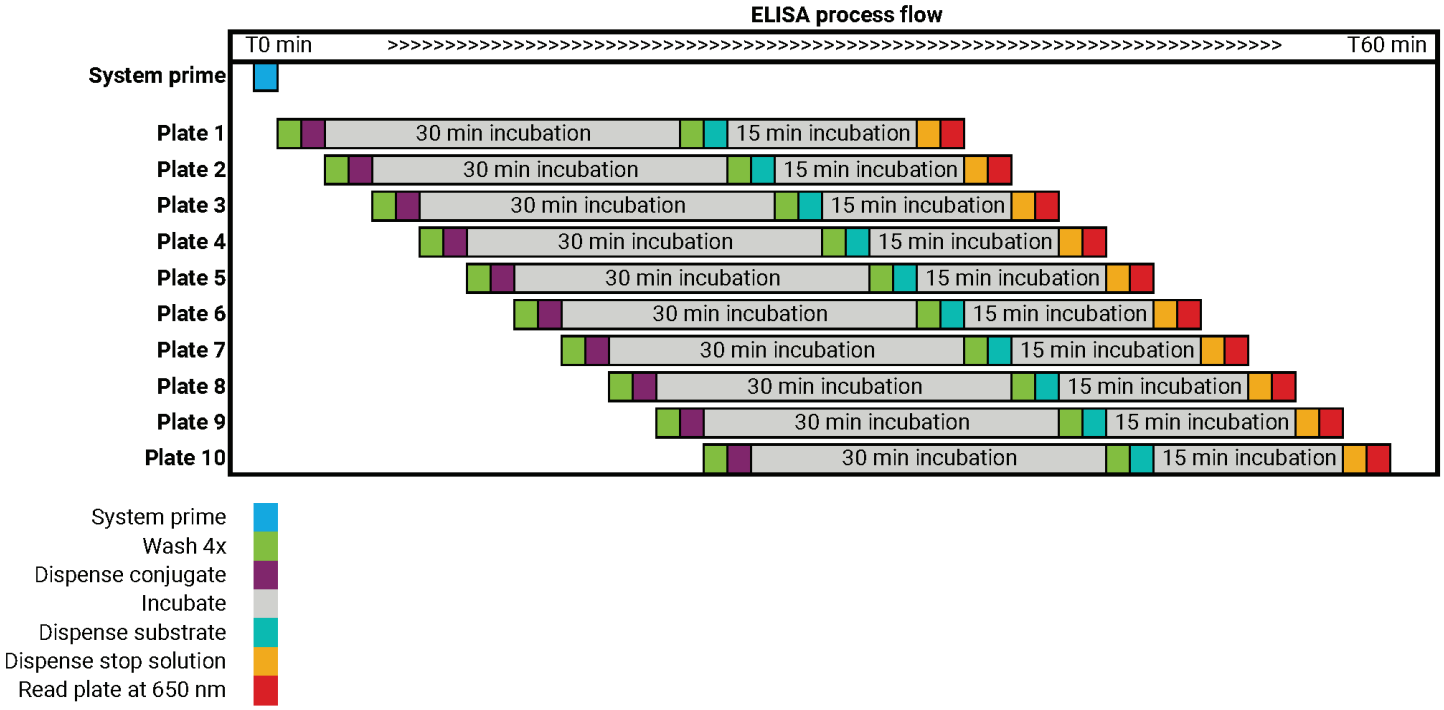

Figure 3. IDEXX AI MultiS-Screen Ab Test workflow. Processes performed by the Agilent BenchCel microplate handler, Agilent BioTek 406 FX washer dispenser, and Agilent BioTek Cytation 7 cell imaging multimode reader are depicted in this Gantt chart for a 10-plate run.

Conclusion

Automation has revolutionized ELISA workflows by providing increased efficiency, accuracy, and throughput. By harnessing robotics, liquid-handling devices, and software integration, researchers can streamline their experiments, minimize variability, and generate reliable data. The use of the Agilent BioTek 406 FX washer dispenser, Agilent BenchCel microplate handler, and Agilent BioTek Cytation 7 cell imaging multimode reader as an automated system for ELISA workflows can provide a solution to help manage increased assay throughput requirements. The use of automation in ELISA assays not only saves time and resources but also opens up new possibilities for accelerating scientific discoveries and advancing biomedical research.

References

1. Held, P. Automated ELISA Liquid Handling with the 406 FX Combination Washer Dispenser. **2023** Publication part number 5994-6052EN. https://www.agilent.com/cs/library/applications/an-automated-ELISA-liquid-handling-406-FX-5994-6052EN-agilent.pdf

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