Application Note

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Versatile, Cost-Effective Automation of Avian Influenza and Mycoplasma Gallispeticum-Synoviae ELISAs

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Abstract

Highly pathogenic Avian Influenza (AI) subtypes are viruses that cause extensive loss of fowl and poultry stocks, and if the H5N1 subtype, can infect humans creating the potential for a global flu pandemic. Mycoplasma Gallisepticum-Synoviae (Mg/Ms) infections also spread rapidly in poultry populations causing widespread loss of production and stock. Ensuring the health of the global agricultural economy and minimizing the probability of a worldwide flu pandemic is placing growing demands on laboratories that routinely test poultry and livestock samples. Two of the biggest challenges to designing well-planned assay throughput models are managing the fluid overages that most automation consumes within the confines of the reagent volumes supplied by many standard ELISA screening kits, and processing batches in optimal quantities while staying within the incubation windows of the assay steps. An experiment was designed using the Agilent BioTek EL406 washer dispenser to showcase workflow versatility and cost-effective reagent use employing two different throughput models, one using the IDEXX Mycoplasma Gallisepticum-Synoviae ELISA indirect format kit, and the other using the IDEXX Avian Influenza Multispecies ELISA blocking format kit. Data generated for both a small batch model composed of multiple runs of fewer than five plates, and a larger batch model integrating the Agilent BioTek BioStack microplate stacker for running batches of five or more plates, demonstrate reliable, reproducible results with an expected level of inter- and intra-batch variability for both assays while offering workflow versatility that optimizes both throughput and reagent use.

Introduction

Designing effective automated assay workflows is a challenge for many laboratories. There can be various obstacles to achieving favorable throughputs. For example, when optimizing reagent volumes used by instrumentation, it is important to conserve reagents, especially target-specific ones that can be costly to manufacture and may not be offered for individual purchase. This presents a significant workflow problem as adding reagent overages or 'dead' volumes required by the automation to the actual volume required to process the assay may total a volume greater than the minimum required reagent provided in the kit.

Strictly defined incubation steps that include specified windows of time and temperature are a typical specification for ELISA kits. Variable incubation times that fall outside of the specification can be a significant factor contributing to reduced assay performance within and between microplates. The shortest incubation time defined by the kit will generally limit the total number of plates that can be effectively processed in a batch without introducing the possibility of assay signal drift from plate to plate. Seamlessly integrating defined incubation windows into a workflow avoids these potential problems.

Using only an Agilent BioTek EL406 washer dispenser and optional Agilent BioTek BioStack microplate stacker, two distinct throughput models were designed for two different IDEXX assays. These throughput models show the flexibility of these instruments for processing small batches of individual plates up to multiple batches that can process 20 or more plates per day. This was done within the necessary incubation windows for the assays without requiring any additional reagent, buffer, or consumables apart from those labeled and provided by the kit, and without the need for any customized instrument accessories. The ELISA assay kits used for this experiment demonstrate how a minimal automation investment can result in cost-effective workflow versatility. The functionality of the instrumentation allows multiple assay-processing combinations, making many of the principles inherent to these versatile, semi-automated approaches adaptable to many laboratory settings.

Experimental

A five-plate kit of both Mycoplasma Gallisepticum- Synoviae (Mg/Ms) and Avian Influenza Multispecies (AI MultiS), along with two batches of prediluted test serum were provided by IDEXX Laboratories. Negative test serum was provided for AI MulitS and positive test serum was provided for Mg/Ms. Test serum was run in replicates of 92 per plate. To validate assay results for both kits, the other four wells of each plate contained two replicates of the assay Negative Control and two replicates of the assay Positive Control.

The assays were performed according to the package insert requirements on 96-well microplates coated with respective antigen. Incubation of the test sample in the coated well allows the antigen-antibody complex to form. A wash step removes unbound material from the wells, and conjugate is added. During a second incubation step, the conjugate binds to any attached antibody in the well (Mg/Ms) or binds directly to the antigen in the absence of antibody (AI MultiS). Unbound conjugate is washed away and enzyme substrate is added. Following substrate incubation, stop solution is added to the wells and the plate is read at 650 nm. Color development is directly related to the amount of antibody to Mg/Ms present in the test sample, or inversely proportional to the amount of anti-AI antibodies in the test sample.

Experiment summary

The experiment was done in two stages. The object of the first stage was to identify potential throughput limitations between the instrument functionality and the provided components and processing requirements of the assay. Then, simulations were designed to finalize workflows that could gauge assay processing and instrument interface success. After incorporating the results of the simulations into the final workflows, the second stage of the experiment was to run the assays using each of the throughput models.

The first simulation was done to determine the best workflow for small batches of fewer than five plates using the Mg/Ms kit. The principal challenge to automating the workflow for this kit was the labeled conjugate volume supplied of 50 mL, which was dispensed 100 µL per well to all 96-wells for five

plates. To effectively automate the assay at this volume, all reagents would have to be conserved following any batch run, including the remaining volume in the reagent trough following the instrument prime. A simulation was designed to compare the EL406 dispense to five plates using 50 mL of blue dye against a manual dispense to five plates using the same volume and solution in two separate batches. Steps 1 through 7 describe the simulation. The results shown by Tables 1 and 2 demonstrate comparable performance between the two dispensing methods.

1. An empty 96-well microplate was used to tare an analytical balance.
2. 50 mL of blue dye solution was added to a Corning 50 mL reagent reservoir, and 50 mL of the same solution was added to a 50 mL conical cylinder for use on the EL406.
3. Following a prime step (this was done for plates 1 and 2 on the EL406 only), 100 µL of the dye was dispensed to each well on the first 96-well microplate using the 5 µL peristaltic pump cassette. Reverse pipetting was used with a multichannel pipettor to manually dispense 100 µL of the dye in the reagent reservoir to another 96-well microplate.
4. Both plates were weighed.
5. Both plates were read at 630 nm.
6. Remaining reagent on the EL406 was purged back to the reagent vessel and measured. Remaining reagent from the manual method was measured.
7. Steps 3 through 5 were repeated for the remaining plates, and step 6 was repeated after the last plates were read.

Table 1. Percent reagent recovery results of Mg/Ms workflow simulation.

	Method	Starting Volume (mL)	Remaining Volume (mL)	Expected Remaining Volume (mL)	% Reagent Recovery	Calculated Reagent Loss per Plate (mL)
Plate 1	EL406	**50**	**39.75**	**40.4**	**98.39**	**0.65**
	Manual	**50**	**39.7**	**40.4**	**98.27**	**0.7**
Plate 2	EL406	**39.75**				**0.24**
	Manual	**39.7**				**0.19**
Plate 3	EL406					**0.24**
	Manual					**0.19**
Plate 4	EL406					**0.24**
	Manual					**0.19**
Plate 5	EL406		**0.4**	**1.35**	**29.63**	**0.24**
	Manual		**0.55**	**1.3**	**42.31**	**0.19**

Table 2. Accuracy and Precision results for Mg/Ms workflow simulation.

	Method	Final Weight (grams)	Actual Volume per Well (µL)	Expected Volume per Well (µL)	% Accuracy	% CV
Plate 1	EL406	**9.6683**	**100.71**	**100**	**99.29**	**0.97**
	Manual	**9.6434**	**100.45**	**100**	**99.55**	**1.05**
Plate 2	EL406	**9.6239**	**100.25**	**100**	**99.75**	**0.95**
	Manual	**9.535**	**99.32**	**100**	**99.32**	**1.25**
Plate 3	EL406	**9.73**	**101.35**	**100**	**98.66**	**1.43**
	Manual	**9.695**	**100.99**	**100**	**99.02**	**0.83**
Plate 4	EL406	**9.5896**	**99.89**	**100**	**99.89**	**1.08**
	Manual	**9.523**	**88.2**	**100**	**99.2**	**0.66**
Plate 5	EL406	**9.5535**	**99.52**	**100**	**99.52**	**3.49**
	Manual	**9.6475**	**100.49**	**100**	**99.51**	**2.32**

The throughput limiting factors for the AI MultiS kit were the final 15-minute incubation and the assay buffer volume. An assay processing simulation was done to test both the timing requirements for processing five plates sequentially using the BioStack within the 60-30-15 incubation profile for the AI MS assay, and to determine the lowest optimal buffer priming volumes so that enough buffer remained to comfortably finish all five plates during a continuous batch run. In this scenario, the two syringe pump dispensers coupled with the peristaltic pump dispensing cassette offered a well-adapted dispense profile for larger throughputs requiring walk-away assay processing. As no user intervention is required to change reagents, the software can access any of the dispensers during assay processing, and the wash step can be programmed to be automatically integrated with the dispense steps. Results of this simulation were incorporated into the delays and buffer priming values as shown by the final Agilent BioTek Liquid Handling Control (LHC) program illustrated by Figure 1.

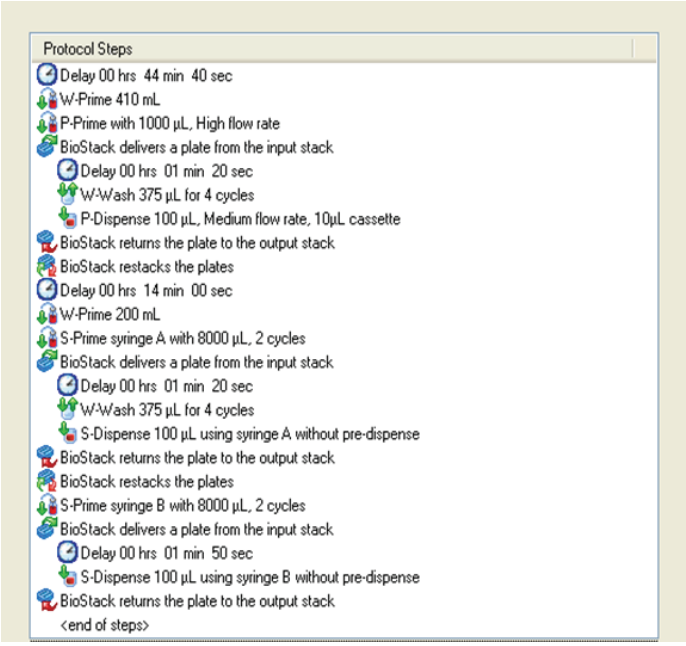

Figure 1. Agilent BioTek LHC integration of Agilent BioTek BioStack microplate stacker and Agilent BioTek EL406 washer dispenser to perform Throughput Model 2 for the Avian Influenza Multispecies assay.

Following the success of the simulations the assays were prepared for testing. The small batch model, illustrated by Figure 2, was run twice, first as a single plate to prove data comparable to the simulation run, and then as a four-plate batch to demonstrate that the cassette change between reagents could be accomplished during the shortest delay between plate processing steps. The aspirate tubing for all three dispense cassettes was placed directly into the respective kit reagent vessel, and reagent remaining in the tubes following the dispense was purged back to the reagent vessel before loading the next cassette. Each reagent had a dedicated cassette, not only to optimize reagent use for the conjugate using a 5 µL cassette to reduce priming volume, but it offered the added advantage of minimizing contamination or carryover between any of the Mg/Ms reagents.

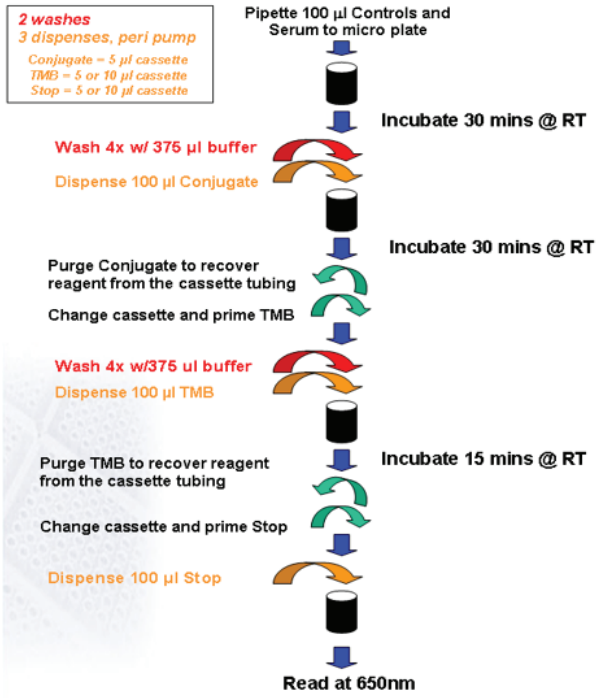

Figure 2. Throughput Model 1. Agilent BioTek EL406 washer dispenser assay workflow model. Multiple small batches of up to five plates of IDEXX Mg/Ms ELISA assay.

Throughput Model 2, illustrated by Figure 3, was designed to provide automated processing of all five plates of the AI MultiS kit, but could also be used to run individual or smaller batches with or without BioStack.

Following preparation of the wash buffer, the EL406 was fitted with the 10 µL peristaltic pump cassette (the 5 µL would be compatible also) and the aspirate tubing was submerged in the conjugate reagent vessel supplied with the kit. The syringe A and B aspirate tubing was submerged into the TMB and Stop reagent vessels, respectively. The final LHC assay programming integrating the BioStack requires 3 to 3.5 minutes to complete the control and sample dispense to each plate. This can be achieved by loading controls and samples to an uncoated microplate, and then using an eight-channel pipettor to dispense to the coated assay plate with a tip change between each column. The plates were then loaded in numeric order into the BioStack 30-plate holder (plate 1 was at the bottom, plate 5 was at the top). The LHC program was started, and the plates were retrieved 2 hours 6 minutes later at the end of the run. Results were generated by reading each plate at 650 nm using the monochromator on an Agilent BioTek Synergy HT microplate reader.

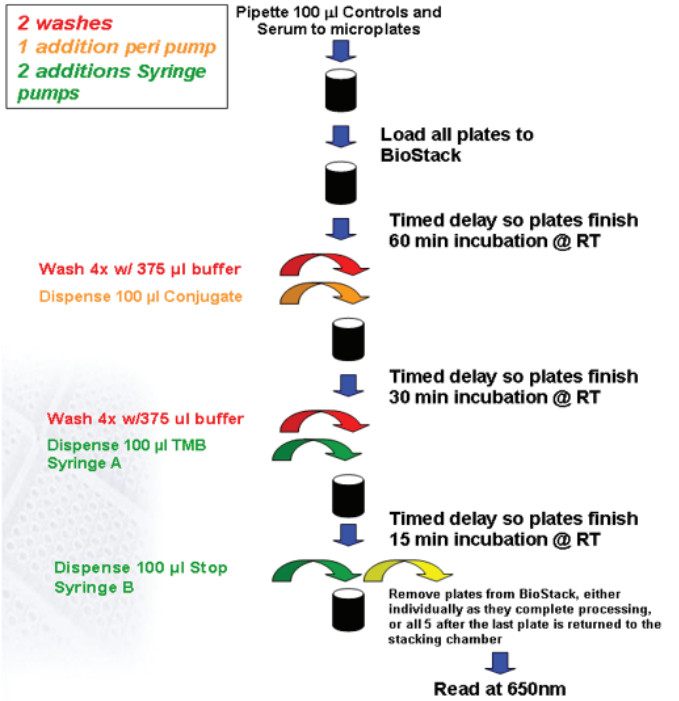

Figure 3. Throughput Model 2. Agilent BioTek EL406 washer dispenser and Agilent BioTek BioStack microplate stacker assay workflow model. Five-plate batches of IDEXX Avian Influenza Multispecies ELISA assay.

Results and discussion

Tables 3 and 4 illustrate the results of the assay validation and sample interpretation. All calculations are performed on the mean OD (650 nm). The samples in this experiment represent 92 replicates of known positive test serum for Mg/Ms and 92 replicates of known negative test serum for AI MultiS. The data indicates that all plates are well within the defined validation criteria for both assays. Data provided by Tables 3 and 4 also showed high correlation of assay performance to comparative QC data provided by IDEXX (not shown).

Table 3. Assay validation and interpretation of results for Mg/Ms. *S/P Ratio = (SMP;x−NC;x)/(PC;x−NC;x)

Assay Validation and Interpretation Criteria	P1	P2	P3	P4	P5	Average P1–P5
NC;x ≤0.150	0.055	**0.058**	**0.057**	**0.053**	**0.058**	**0.056**
PC;x−NC;x >0.075	0.354	**0.371**	**0.306**	**0.305**	**0.354**	**0.338**
S/P Ratio ≤0.500 (Negative)*						
S/P Ratio >0.500 (POS)*	0.989	**1.118**	**1.212**	**1.278**	**1.163**	**1.152**

Table 4. Assay validation and interpretation of results for AI MultiS. *S/N Ratio = SMP;x/NC;x

Assay Validation and Interpretation Criteria	P1	P2	P3	P4	P5	Average P1–P5
NC;x ≥0.600	1.648	**1.467**	**1.591**	**1.511**	**1.575**	**1.558**
PC;x/NC;x <0.500	0.13	**0.136**	**0.139**	**0.146**	**0.168**	**0.144**
S/N Ratio ≥0.500 (Negative)*	1.017	**1.018**	**0.964**	**1.008**	**1.015**	**1.004**
S/N Ratio <0.500 (POS)*						

As shown by the correlation of mean absorbance results of each well group, Figure 4 illustrates proven inter- and intra-batch repeatability for all five plates run for Mg/Ms using Throughput Model 1.

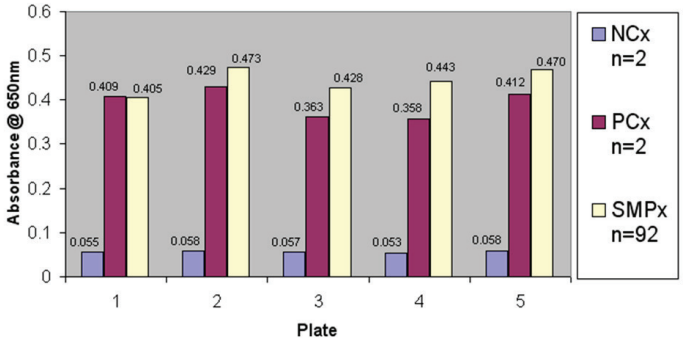

Figure 4. Mg/Ms assay results by plate.

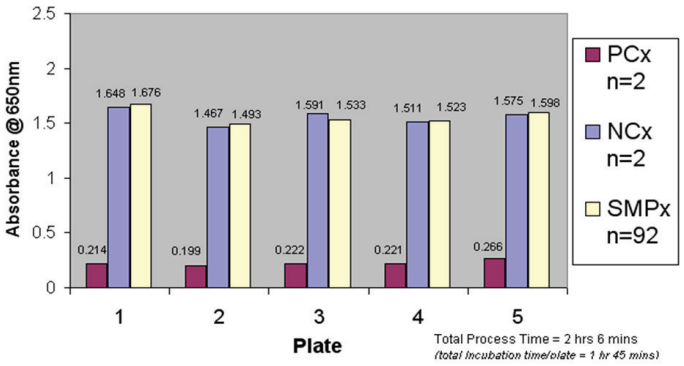

Figure 6. Avian Influenza Multispecies assay results by plate.

Figure 5 illustrates that test serum CV% values for all plates of both assays were within a 2.5% window of variability, with an average CV% for all plates of 6.12%. Factors contributing to well variability would include washer performance, plastic variability, analyst variability, and assay variability. Acceptable CV% for these assays is <10%.

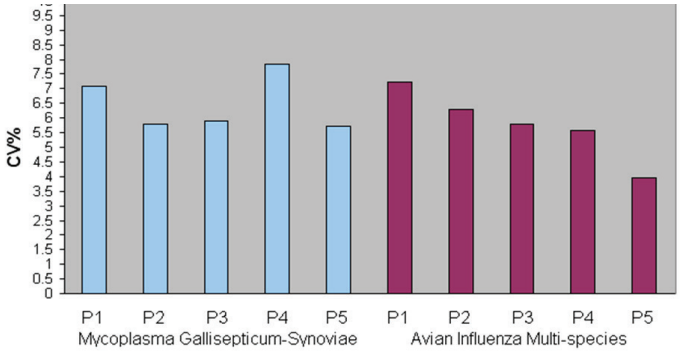

Figure 5. Combined test serum CV% for all plates of Throughput Models 1 and 2.

Figure 6 illustrates the tight correlation of all data between each of the five plates of the Avian Influenza Multispecies assay using an integrated Agilent BioStack microplate stacker to demonstrate the viability of Throughput Model 2.

Conclusion

Principles of effective automated workflow design are universal to any application. A few of these principles were incorporated in this work to solve typical workflow challenges, and can easily be adapted to other throughput models:

1. Look for potential bottlenecks in workflow by understanding possible throughput limitations of the instrumentation in relationship to the requirements of the assay. In the case of the IDEXX Mg/Ms and AI MultiS kits, labeled reagent volumes and incubation specifications for the assays were two areas of greatest challenge to solve using the designated automation.
2. Perform simulated throughput models before 'going live' with an assay workflow. Validation of the scheduling is as important as validating assay performance on an instrument.

The Agilent BioTek EL406 washer dispenser is a versatile combination washer and dispenser that achieves reproducible inter- and intra-batch results for many throughputs, including walk-away assay processing with the addition of an Agilent BioTek BioStack microplate stacker. The EL406 enhances workflow options by combining multiple assay processing tasks using virtually no consumables, offering a number of alternative set-ups that assist in optimizing reagent use and incubation windows, providing flexible software, allowing for optimal scheduling for many batch models, and decreasing downtime from routine maintenance that would be required for multiple single-use instruments. This workflow demonstration of the EL406 in partnership with the IDEXX Mg/Ms and AI MultiS ELISA screening assays is just one example of how the multi-use EL406 can provide throughput solutions that can be adapted to many laboratory settings.

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