

Overview

Highly pathogenic Avian Influenza (AI) subtypes are viruses that cause extensive loss of fowl and poultry stocks, and in the case of 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. These demands can be met with carefully planned automation. Two of the biggest obstacles in designing successful automated assay throughput models are managing fluid overages within the confines of the reagent volumes supplied by kits, and processing batches in optimal quantities while staying within the incubation windows of the assay steps. Here we demonstrate the workflow versatility and cost-effective reagent usage of EL406 Combination Microplate Washer/Dispenser using the IDEXX's Mycoplasma Gallisepticum-Synoviae ELISA indirect format kit and Avian Influenza Multi-Species ELISA blocking format kit. Data generated for both a small batch model composed of multiple runs of less than five plates, and a larger batch model integrating the BioStack™ robotic microplate handler 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 usage.

Introduction

Designing efficient automated assay workflows is a challenge for many laboratories. Important considerations include:

- Optimizing reagent volumes used by instrumentation is important to conserve reagents and minimize costs
- “Dead” volumes, especially for assays where bulk reagents are not available
- Strictly defined incubation steps that include specified windows of time and temperature
- Integration of instrument components into automated platform (scheduling software)

The ELISA assay kits utilized for this experiment provide an ideal way to show how a minimal automation investment can result in cost-effective workflow versatility. Using only a BioTek EL406 combination washer/dispenser, and optional BioStack microplate staker, two distinct throughput models were designed for two different IDEXX assays that show the flexibility of these instruments for processing anywhere from small batches of individual plates up to multiple batches that can process 20 or more plates a day.

IDEXX Laboratories Avian Influenza Multi-species and Mycoplasma Gallisepticum-Synoviae Assays

IDEXX FlockChek™ Avian Influenza Multi-species (AI MultiS) ELISA assay utilizes competitive binding to detect antibody to Avian Influenza virus of all subtypes in chicken, turkey, duck, ostrich, and goose serum with high sensitivity and specificity.

IDEXX FlockChek™ Mycoplasma Gallisepticum - Synoviae (Mg/Ms) is a non-competitive enzyme immunoassay for the detection of antibody to Mycoplasma gallisepticum and Mycoplasma synoviae in chicken and turkey serum.

- 5 plate kits with controls and reagents ready to use

- Pre-diluted test serum

- Negative test serum for AI MultiS

- Positive test serum for Mg/Ms

- 96-well microplates coated with respective antigen

- 92 wells / plate: test serum

- 4 wells / plate: controls

- Two automation protocols outlined in Figs 3 and 4

BioTek Instrumentation



Figure 1 – EL406 Combination Washer Dispenser shown with 3 cassettes for peri pump dispensing and the BioStack™ Automated Microplate Processor.

EL406

- Dual-Action™ Manifold independently controls aspiration/dispense
- Peristaltic and syringe pumps: volumes ranging from 1-3000 µL/well
- Peristaltic pumps allow complete recovery of unused reagent for zero “dead volume”
- Mg/Ms assay: three different peristaltic pump cassettes, one for each of the kit reagents
 - optimal reagent usage
 - complete recovery of all unused reagent.
- AI MultiS assay: one 10 µl peristaltic pump cassette for the conjugate dispense, two syringe pumps for the TMB and Stop dispenses

BioStack

- Hands free automation of all washing, incubation and dispense steps

Description of Experiment

Simulation of Work Flow for Mg/Ms Kit

Simulations with blue dye were used to finalize workflows that could gauge assay processing and instrument interface success. The principle limitation to automating the Mg/Ms workflow is reagent conservation. Using steps 1-7 below a workflow comparison between the EL406 automated method and the manual method was performed:

- An empty 96-well microplate was used to tare an analytical balance.
- 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.
- 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 peri pump cassette. Reverse pipetting was used with a multi-channel pipettor to manually dispense 100 µL of the dye in the reagent reservoir to another 96-well microplate.
- Both plates were weighed.
- Both plates were read at 630nm.
- Remaining reagent on the EL406 was purged back to the reagent vessel and measured. Remaining reagent from the manual method was measured.
- Steps 3 through 5 were repeated for the remaining microplates, and step 6 was repeated after the last plates were read.

	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	99.95	1.05
Plate 2	EL406	9.6239	100.25	100	99.75	0.95
	Manual	9.5350	99.32	100	99.32	1.25
Plate 3	EL406	9.7300	101.35	100	98.66	1.43
	Manual	9.6950	100.99	100	99.02	0.93
Plate 4	EL406	9.5896	99.89	100	99.89	1.08
	Manual	9.5230	98.20	100	99.2	0.66
Plate 5	EL406	9.5535	99.52	100	99.52	1.49
	Manual	9.6475	100.49	100	99.51	2.32

Table 1 – Accuracy and precision results for Mg/Ms workflow simulation.

- Results in Tables 1 & 2 demonstrate equivalent performance

Simulation of Work Flow for AI MultiS Kit

Simulations were also 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 MultiS assay and to determine the lowest optimal buffer priming volumes. Results of this simulation were incorporated into the delays and buffer priming values as shown by the final LHC program illustrated by Figure 2 below:

Protocol Steps
Delay 00 hrs 44 min 40 sec
W-Prime 410 mL
P-Prime with 1000 µL High flow rate
BioStack delivers a plate from the input stack
Delay 00 hrs 01 min 20 sec
W/Wash 375 µL for 4 cycles
P-Dispense 100 µL Medium flow rate, 10µL cassette
BioStack returns the plate to the output stack
BioStack restacks the plates
Delay 00 hrs 14 min 00 sec
W-Prime 200 mL
S-Prime syringe A with 8000 µL 2 cycles
BioStack delivers a plate from the input stack
Delay 00 hrs 01 min 20 sec
W/Wash 375 µL for 4 cycles
S-Dispense 100 µL using syringe A without pre-dispense
BioStack returns the plate to the output stack
BioStack restacks the plates
S-Prime syringe B with 8000 µL 2 cycles
BioStack delivers a plate from the input stack
Delay 00 hrs 01 min 50 sec
S-Dispense 100 µL using syringe B without pre-dispense
BioStack returns the plate to the output stack
end of steps

Figure 2 – Integration of BioStack and EL406 to perform throughput Model 2 for the AI MultiS assay.

Design of Automated Workflows

Successful simulations allowed the development of two different automated throughput models

- Throughput Model 1 (Figure 3): small batch model which uses EL406 only
- Throughput Model 2 (Figure 4): higher throughput model suitable for ≥ 5 plate batches

Assay Workflow Model

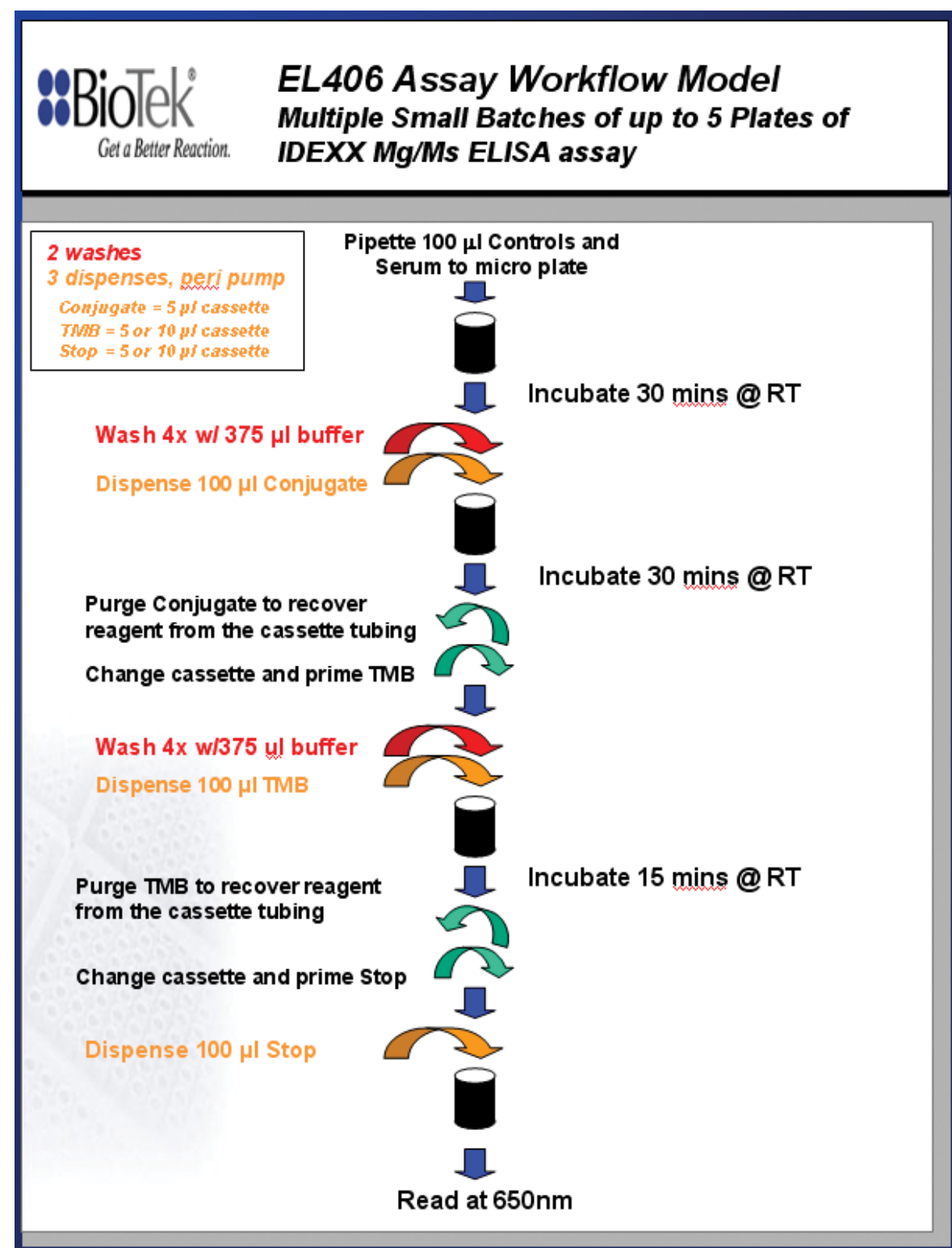


Figure 3 – Throughput Model 1.

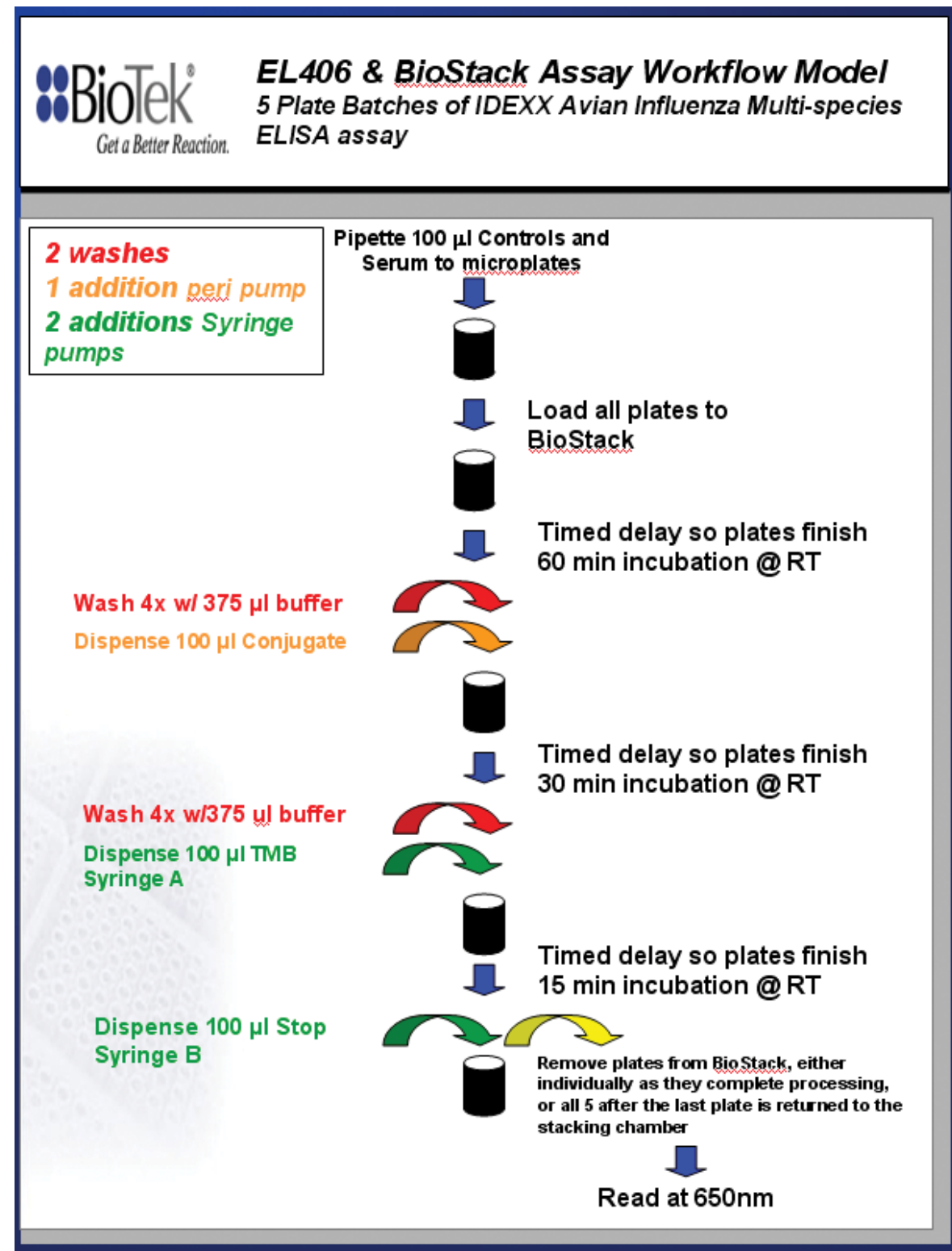


Figure 4 – Throughput Model 2.

Results of Assay Validation

Tables 3 and 4 illustrate the results of the assay validation and sample interpretation. All calculations are performed on the mean OD (650nm). 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.

Assay Validation and Interpretation Criteria	P1	P2	P3	P4	P5	Average P1-P5
NCx >= 0.600	0.668	0.668	0.667	0.663	0.668	0.666
PCx/NCx > 0.075	0.354	0.371	0.306	0.305	0.354	0.338
S/P Ratio >= 0.600 (neg)						
S/P Ratio > 0.500 (POS)	0.889	1.118	1.212	1.278	1.163	1.162

Table 3 – Assay validation and interpretation of results for Mg/Ms.

Assay Validation and Interpretation Criteria	P1	P2	P3	P4	P5	Average P1-P5
NCx >= 0.600	1.648	1.467	1.591	1.511	1.575	1.569
PCx/NCx < 0.500	0.130	0.158	0.139	0.146	0.160	0.144
S/N Ratio >= 0.600 (neg)	1.017	1.018	0.964	1.008	1.015	1.004
S/N Ratio < 0.500 (POS)						

Table 4 – Assay validation and interpretation of results for AI MultiS.

- The data indicates that all plates are well within the defined validation criteria for both assays
- Data provided by Tables 3 & 4 show high correlation of assay performance to comparative QC data provided by IDEXX (not shown)

Data Analysis

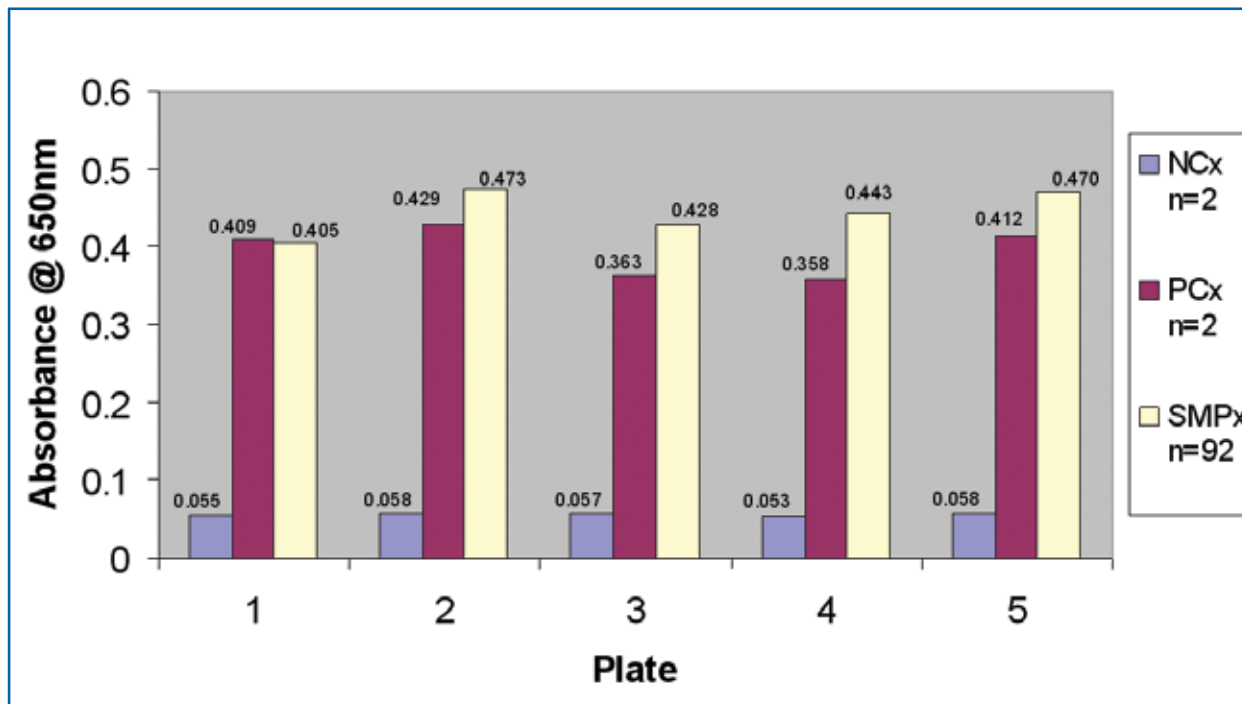


Figure 5 – Mg/Ms assay results by plate.

As shown by the correlation of mean absorbance results of each well group, Figure 5 illustrates satisfactory inter- and intra-batch repeatability for all 5 plates run for Mg/Ms using Throughput Model 1.

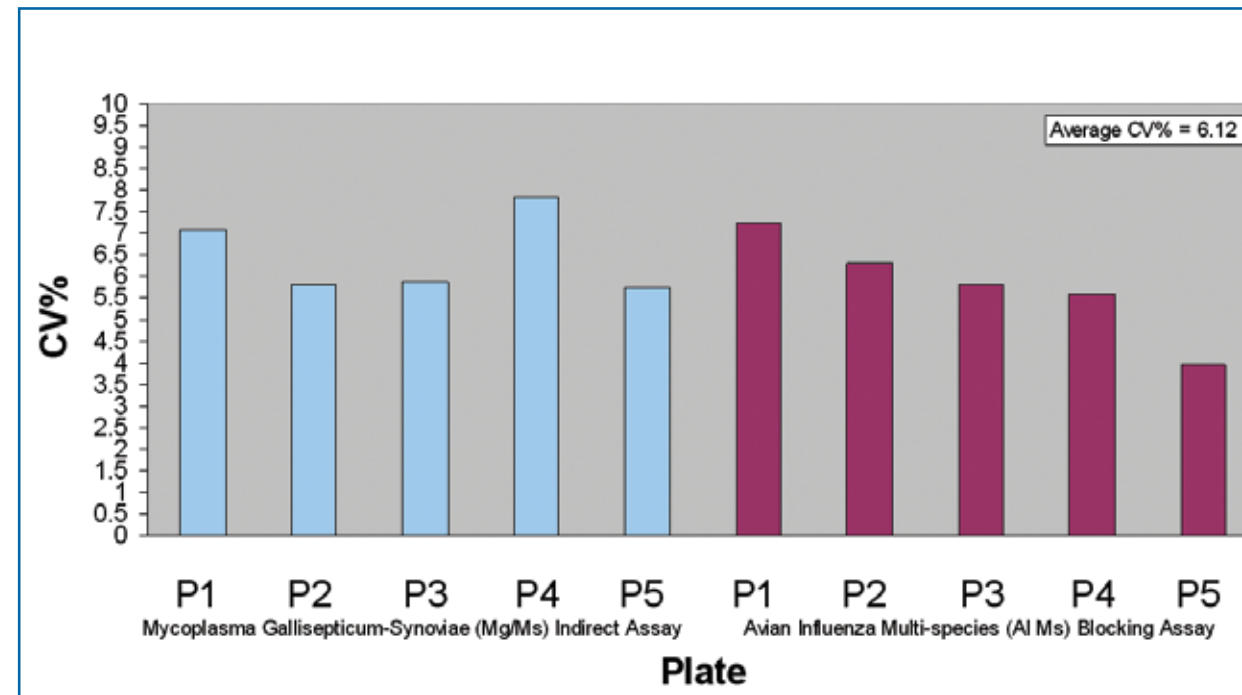


Figure 6 – Combined test serum CV% for all plates of throughput Models 1 and 2.

Figure 6 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 to well variability would include plastic variability, analyst variability, assay variability and washer settings. Acceptable CV% for these assays is < 10%.

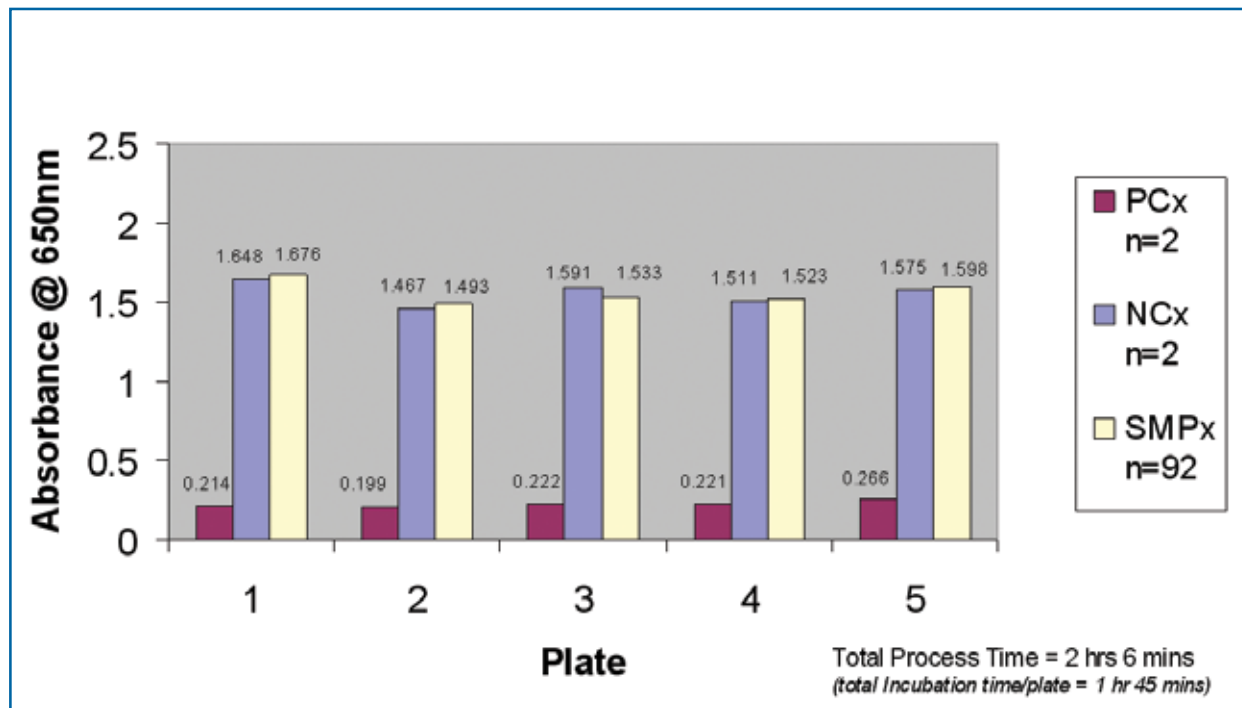


Figure 7 – Avian Influenza multi-species assay results by plate.

Figure 7 illustrates the tight correlation of all data between each of the 5 plates of the Avian Influenza Multi-species assay using an integrated BioStack to demonstrate the viability of Throughput Model 2.

Discussion

Principles of effective automated workflow design are universal to a variety of applications. A few of these principles were incorporated by this experiment to solve some typical workflow challenges, and can easily be adapted to other models:

- 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.
 - Perform simulated throughput models before 'going live' with an assay workflow. Validation of the scheduling is important as validating assay performance on an instrument.
- Investing in automation that is adaptable, easy to use, easy to edit, and easy to upgrade should make any throughput easier to achieve.

Conclusions

- The EL406 is a versatile combination washer/dispenser that achieves reproducible inter- and intra-batch results for a variety of throughputs.
- The EL406 offers a number of alternative setups that assist in optimizing reagent usage while working within standard reagent volumes and incubation windows provided by ELISA assay kits.
- The addition of a BioStack plate staker enhances the time and space saving advantages of the EL406 by providing walk-away throughput options.
- EL406 streamlines multiple tasks using virtually no consumables and saves time and materials on routine maintenance that would be required for multiple single use instruments.
- BioTek instrumentation and IDEXX assays create an adaptable, economical solution for agricultural ELISA screening applications.

1. Bacteria Genomes - MYCOPLASMA SYNOVIAE: Mycoplasma synoviae causes upper respiratory tract infections in chickens and turkeys; http://www.ncbi.nlm.nih.gov/genomes/bacteria/Mycoplasma_synoviae.html

2. Butcher, Gary D., DVM, PhD, Mycoplasma Gallisepticum – A Continuing Problem in Commercial Poultry, Publication VM130, UF IFAS Extension, May 2002, April 2009; <http://edis.ifas.ufl.edu/pdffiles/PS/PS03400.pdf>

3. The Poultry Site Quick Disease Guide, Poultry Diseases Guide - Disease information from The Poultry Site

4. World Health Organization, GAR (Global Alert and Response), Avian Influenza, http://www.who.int/csr/disease/avian_influenza/en/