Application Note

Automation of the IDEXX Avian Influenza Virus Antibody Test Kit Using Individual Components and a Twister II from BioTek

Modular Robotics Systems

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The screening of chicken flocks for Avian Influenza Virus provides an important role in insuring that bird flocks are disease free. The sheer numbers of animals that need to be tested under normal circumstances in many animal test facilities requires large numbers of samples be processed daily. In the event of a disease outbreak the test-volume would be expected increase many fold above the current levels in specific regions. The ability to automate that the process represents tremendous savings in time and manpower. Here we describe the BioTek robotic system used to automate the IDEXX Avian Influenza Virus Antibody Test Kit.

Introduction

Avian Influenza Virus (AIV) is a viral disease of domestic and wild birds, which has a range of responses from almost asymptomatic to very high mortality. This disease is caused by orthomyxoviruses, which are Type A influenza viruses. While there are 14 known subtypes, based on surface hemagglutinins and 9 based on neuraminidases, only types H5 and H7 are associated with significant losses.

The IDEXX test kit is a specific screening test for the detection of antibody to AIV in chicken serum samples. The basis of the test is that serum from chickens exposed to AIV antigens will contain specific anti-AIV antibodies, which can then be captured on a test plate coated with AIV antigens through an antigen-antibody complex. This complex is then detected using an anti-chicken IgG conjugate, followed by the addition of a color generating substrate. The assay is assessed by the measurement of the absorbance of each well.

The screening of flocks for Avian Influenza Virus provides an important role in insuring that bird flocks are disease free. The sheer number of animals, chickens in particular, that are tested for AIV as a result of statutory requirements or good business practices has resulted in increasingly larger numbers of test samples. In the event of a disease outbreak the test volume would be expected to increase many fold from the current level. Traditionally these tests have been performed manually. Samples are diluted and reagents added with the use of multichannel pipettes. Microplates are moved one at a time to plate washers and plate readers by hand. Besides requiring a great deal of time and effort, these manual interventions are tedious, repetitive and prone to error.

These factors justify the use of automation for this assay. Because of the current and potential throughput needs, the system needs to be scalable, yet relatively small in size, as the physical space of the laboratory is limited. The throughput requires routine processing of five plates per day with larger runs of up to 15 plates per day, possible. Because the laboratory's assay menu is subject to change, the system needs to be modular in nature and have the ability to be easily reprogrammed or reconfigured for alternative use. And of course budget constraints require that the system be of modest financial cost. Here we describe the robotic system from BioTek Instruments that was used to automate the Avian Influenza Antibody Test kit from IDEXX.

Figure 1. Front View of Robotic Avian Influenza Test System.

The robotic system employed utilizes a Caliper Twister II rotating robotic arm/gripper to move microplates to and from each station. In addition to the Twister II, a Precision XS pipetting station (BioTek) was used to dilute samples and transfer them to the kit's assay plate. Plate washes were carried out using an ELx405 96-well microplate washer (BioTek), while reagents were added using three MicroFill dispensers (BioTek) each dedicated to a specific reagent. Room temperature incubations were carries out using a LPX44 (Liconic) plate hotel and absorbance measurements were made using a PowerWave XS microplate spectrophotometer (BioTek).

The robotic system was configured using a modular 3-piece table system. Each section has interconnected power, serial, and USB communication cables that allow for easy system integration. The center table housed the Twister II robot and two storage pods with three microplate stacks each (Figures 2 and 3). Both of the end tables utilized dual surfaces that allowed for the vertical stacking of instrumentation. One of the end tables was configured with the LPX44 plate hotel and one of the MicroFill dispensers on the top surface, with the remaining two MicroFill dispensers on the lower level. The second table had the Precision XS pipettor on the upper surface and a PowerWave reader and the ELx405 washer on the lower surface. In addition a slot was cut into the top surface to allow used pipette tips from the Precision to be discarded into a collection can located below.

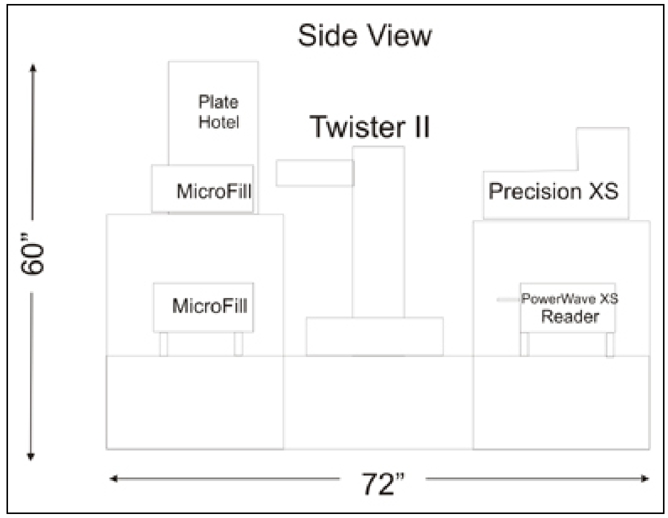

Figure 2. Side View Schematic of Robotic Avian Influenza Test System.

The table system had a number of features that improved the utility of the system. Removable skins on the bench units allows for easy access to instrumentation and cabling when required, but prevented exposure during the run and allowed for a clean uncluttered look when installed (Figure 1, 5, and 6). Grommet holes were cut into the shelves to allow power and communication cables easy passage. In addition a waste slot was cut to allow for used tips from the pipettor to fall into a hazardous waste container. The tables were configured with locking casters that allowed for the system to be moved when necessary.

In conjunction with instrument-specific software adapters, ILink-Pro software interfaces with all of the instrumentation of the system to move supplies, initiate instrument specific tasks, as well as coordinate plate scheduling. Using the graphical interface of the ILink Pro software a method was defined that moved necessary predefined supplies from storage racks to the required process locations as needed and returned them to storage or dumped them into a waste container at the end of the run. This resulted in a 45-step process (Figure 4) for each plate. Incubation and process-times were also provided in order for the system scheduler to most efficiently interweave several plates (Figure 7).

Avian Flu Procedure

1. Manually transfer samples from tubes to sample plate
2. Dilute samples 1:500
3. Transfer of samples and controls to assay plate
4. Incubate at RT for 30 minutes
5. Wash assay plate 5 times with 350 µL of water
6. Add 100 µL of Conjugate Solution
7. Incubate at RT for 30 minutes
8. Wash assay plate 5 times with 350 µL of water
9. Add 100 µL of Substrate Solution
10. Incubate at room temperature for 15 minutes
11. Add 100 µl of Stop solution
12. Measure absorbance at 650 nm

Supplies were loaded onto the system before each batch run of the assay. Sample plates with undiluted serum samples, empty dilution plates, nested pipette tips and assay plates were placed into storage stacks of the robot system. The reagent specific MicroFill dispensers supply bottles were filled with the appropriate amount of reagent, while the ELx405 washer supply bottle was loaded with water and all of the instruments were primed.

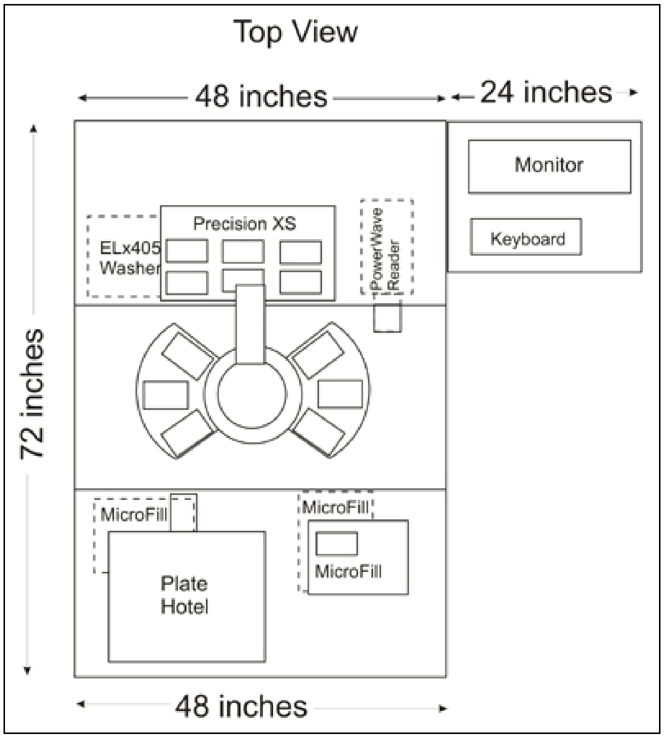

Figure 3. Top View Schematic of Robotic Avian Influenza Test System.

In the Avian Influenza method the Twister II robot was responsible for the movement of all of the materials. To start the AIV assay, a sample plate, two empty dilution plates, and an assay plate were moved from the stack storage to the Precision XS pipettor. The Precision XS then made the necessary sample dilutions and transferred the diluted samples to the assay plate. In addition, undiluted controls were transferred from tubes to the assay plate. Once the plate was loaded the robot moved the assay plate from the Precision XS to the microplate incubator and the plate incubated for 30 minutes. Used pipette tip racks were moved to waste, while the sample and dilution plates were moved back to the storage stacks, freeing the Precision XS to process the next sample plate. After the completion of the incubation, the assay plate was moved to the ELx405 washer, where it was washed 5 times with 350 µL of water. The washed plate was then moved to one of the MicroFill dispensers, 100 µL of conjugate was added and the plate moved to the incubator for the second 30-minute incubation. After the second incubation, the plate was again moved to the washer and washed, followed by movement to the second MicroFill, where 100 µL of substrate reagent was added. After the addition of substrate, the plate was moved to the incubator for 15 minutes to allow for color development. The plate was then moved to the third MicroFill, where 100 µL of stop reagent was added. The assay plate was then moved to the PowerWave XS reader and the absorbance at 650 nm measured, the data recorded and the plate moved back to storage.

Run	Inst	Instruction Description	Time	Duration
		Avian Flu		
1	2	Robot.Move : Sample PLate from ILinkStorage(1) to Precision(4)	00:00:00	00:00:30
1	3	Robot.Move : TipBox1 from ILinkStorage(5) to Precision(2)	00:00:30	00:00:30
1	4	Robot.Move : Dilution plate from ILinkStorage(3) to Precision(6)	00:00:60	00:00:30
1	5	Precision.SetRunFolders.	00:01:30	00:00:02
1	6	Precision.LoadProgram.First Dilution.pgm	00:01:32	00:00:02
1	7	Precision.Run.	00:01:34	00:04:30
1	8	Robot.Move : TipBox1 from Precision(2) to Waste(1)	00:06:04	00:00:30
1	9	Robot.Move : TipBox2 from ILinkStorage(5) to Precision(2)	00:06:34	00:00:30
1	10	Robot.Move : Dilution plate 2 from ILinkStorage(4) to Precision(3)	00:07:04	00:00:30
1	11	Precision.SetRunFolders.	00:07:34	00:00:02
1	12	Precision.LoadProgram.Second Dilution.pgm	00:07:36	00:00:02
1	13	Precision.Run.	00:07:38	00:04:30
1	14	Robot.Move : TipBox2 from Precision(2) to Waste(2)	00:12:08	00:00:30
1	15	Robot.Move : TipBox3 from ILinkStorage(5) to Precision(2)	00:12:38	00:00:30
1	16	Robot.Move : Assay PLate from ILinkStorage(2) to Precision(1)	00:13:08	00:00:30
1	17	Robot.Move : Dilution plate from Precision(6) to ILinkStorage(4)	00:13:38	00:00:30
1	18	Precision.SetRunFolders.	00:14:08	00:00:02
1	19	Precision.LoadProgram.Controls Transfer.pgm	00:14:10	00:00:02
1	20	Precision.Run.	00:14:12	00:02:30
1	21	Precision.SetRunFolders.	00:16:42	00:00:02
1	22	Precision.LoadProgram.Samples Transfer.pgm	00:16:44	00:00:02
1	23	Precision.Run.	00:16:46	00:04:30
1	24	Robot.Move : Assay PLate from Precision(1) to Incubator(1)	00:21:16	00:00:30
1	26	Robot.Move : Dilution plate 2 from Precision(3) to ILinkStorage(4)	00:50:46	00:00:30
1	27	Robot.Move : Sample PLate from Precision(4) to ILinkStorage(6)	00:51:16	00:00:30
1	28	Robot.Move : Assay PLate from Incubator(1) to Washer(1)	00:51:46	00:00:30
1	29	Robot.Move : TipBox3 from Precision(2) to Waste(3)	00:52:16	00:00:30
1	30	Washer.Process.Wash 5Times	00:52:46	00:01:30
1	31	Robot.Move : Assay PLate from Washer(1) to MicroFill_1(1)	00:54:16	00:00:30
1	32	MicroFill_1.RUNPROGRAM.Add100.	00:54:46	00:00:30
1	33	Robot.Move : Assay PLate from MicroFill_1(1) to Incubator(1)	00:55:16	00:00:30
1	35	Robot.Move : Assay PLate from Incubator(1) to Washer(1)	01:24:46	00:00:30
1	36	Washer.Process.Wash 5Times	01:25:16	00:01:30
1	37	Robot.Move : Assay PLate from Washer(1) to MicroFill_2(1)	01:26:46	00:00:30
1	38	MicroFill_2.RUNPROGRAM.Add 100.	01:27:16	00:00:30
1	39	Robot.Move : Assay PLate from MicroFill_2(1) to Incubator(1)	01:27:46	00:00:30
1	41	Robot.Move : Assay PLate from Incubator(1) to MicroFill_3(1)	01:42:46	00:00:30
1	42	MicroFill_3.RUNPROGRAM.Add 100.	01:43:16	00:00:30
1	43	Robot.Move : Assay PLate from MicroFill_3(1) to KC4Reader(1)	01:43:46	00:00:30
1	44	KC4Reader.Read.Read 650.prt	01:44:16	00:00:60
1	45	Robot.Move : Assay PLate from KC4Reader(1) to Incubator(1)	01:45:16	00:00:30

Figure 4 Avian Flu Automated Method Steps. At run-time the 45 discreet steps for each assay plate of the AIV test method are scheduled for processing. Based on the timing requirements and constraints these steps are interweaved to provide the most efficient use of time by the ILink-Pro software.

Discussion

The basis of the Avian Influenza Virus antibody detection assay is a technology known as ELISA. The strength of this assay technology is that while the number of analytes that can be assayed is virtually limitless, the process of doing so does not change significantly. As such, the instrumentation employed for one assay can easily be used for other analytes.

Figure 5. Left Side View of System. The Twister II robotic arm/gripper and storage racks are seen in the center section, while the Precision XS is seen on the top level of one of the end-unit tables.

The modular robotic assay system designed to automate the Avian Influenza Virus antibody detection assay from IDEXX can be reconfigured for many other assays. Additional instrumentation can be added to increase throughput as needed. For example if assays that require the addition of a fourth reagent are employed, another MicroFill dispenser can be added as a resource to the system.

Figure 6. Right Side View of System. The Twister II robotic arm/gripper and storage stacks are seen, along with the LPX44 plate hotel and computer console.

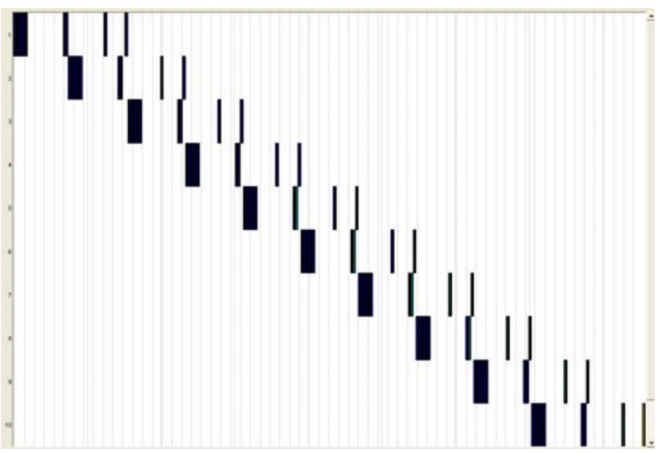

Figure 7. Gantt Chart of a 10-plate run. The process timing of 10 plates of the IDEXX AIV assay are indicated as a time line. Initiation of each plate is controlled by ILink-Pro software in order to avoid instrument-timing conflicts. Dark bars indicate the time of the process steps; with the incubation times are indicated open periods between each process steps

Here we have demonstrated the automation of the Avian Influenza Antibody detection assay from IDEXX using a modular robotic system from BioTek Instruments. While we have only automated one assay, the flexibility of the robotic system allows many other assays, particularly ELISA assays to be automated. The ability to reprogram and reconfigure existing instrumentation along with the ability to add other instruments provides flexibility for additional assays. While the Precision pipettor was only used to perform sample dilutions and transfer samples and controls to the assay plate, it can also be used to add reagents. By aspirating and dispensing with disposable tips from reagent troughs small amounts of precious reagents can be pipetted. Routines for the Precision XS can be programmed using Precision Power software. The MicroFill dispensers can be used with different reagents and/or different volumes. The ELx405 washer is also programmable via software, and can be configured with an optional valve module that allows for the switching of buffers without changing any bottle fittings.