Application Note

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# Automated ELISA Liquid Handling Using the Agilent BioTek 406 FX Washer Dispenser

## Semi-automation of the ELISA process

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## Abstract

With a typical ELISA protocol, repeated cycles of microplate washing, reagent addition, and incubation are executed to add specific reagents and to remove unbound material before data collection. When performed manually, this process requires a technician to manage timing and be available to move plates between the washer and multiple dispensers. The Agilent BioTek 406 FX washer dispenser increases laboratory efficiency and productivity. This automation-friendly, multifunctional instrument uses up to two peristaltic pumping systems for reagents, plus up to two highly accurate dual syringe pump dispensers to efficiently manage complex reagent dispensing and washing routines.

## Introduction

The ELISA procedure is a common biochemical assay to detect a variety of analytes in a sample. ELISA typically uses a microplate as a solid surface onto which specific antibodies are bound, allowing the capture and analysis of an infinite number of analytes. It does so by a series of incubations, reagent additions, and wash steps. During incubations, specific molecules are bound to the solid substrate by interactions with the previously absorbed antibodies. Following this binding step, unbound material is removed by the washing step. After the unbound material has been removed, the next reagent—usually a conjugate—is added and allowed to specifically interact. The conjugate is a chimeric molecule containing a specific antibody and an enzyme that are covalently linked together. Again, unbound material is removed and the next reagent, a substrate, is added after. The substrate interacts with the enzymatic portion of the conjugate to produce a colored compound, which can be detected by absorbance, fluorescence, or luminescence, depending on the substrate used. Regardless of the analyte, the same process steps are used in ELISA.

As seen in Figure 1, the 406 FX is a modular system, and is fully programmable from either its built-in touch screen or using Agilent BioTek Liquid Handling Control (LHC) software on an attached PC. The 406 FX is an automated microplate processor that can perform microplate washing steps in 96-, 384-, and 1536-well microplates. In addition to standard wash routines, the 406 FX has built-in cell-washing capabilities. An internal buffer-switching valve allows for the selection of up to four different wash buffers without changing bottles. A built-in sonicator provides the capability for automated cleaning maintenance of the dispense manifold. The device has up to six different reagent dispensers and two separate peristaltic pumps that are capable of dispensing from 1 to 3,000 µL.

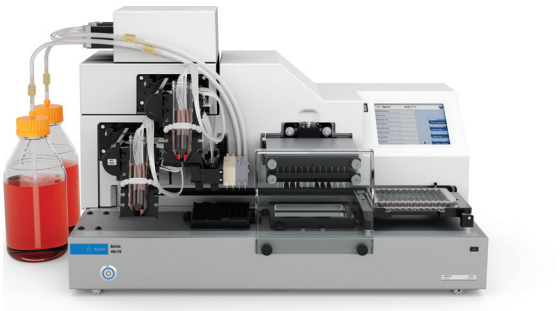

Figure 1. Agilent BioTek 406 FX washer dispenser.

Different cassettes (1, 5, and 10 µL) provide accuracy at different dispense volumes while minimizing priming volume. The 406 FX can also be configured with up to two additional syringe modules, each fitted with two independent syringe pump dispensers that are capable of dispensing from 5 to 3,000 µL. The 406 FX offers plate shaking at three different speeds and is automation compatible.

### Avian influenza virus antibody ELISA

Avian influenza virus (AIV) is a viral disease of domestic and wild birds, which produces a range of responses in the host from being almost asymptomatic to having a high mortality. Because of the variability and severity of symptoms, serological testing offers significant advantages for the detection of infected birds. The IDEXX test kit is a specific screening test for the detection of antibodies to AIV in chicken, turkey, duck, ostrich, and goose serum samples. The basis of the test is that serum from birds 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. After washing to remove unbound material, an anti-AIV monoclonal enzyme conjugate is added (Figure 2).

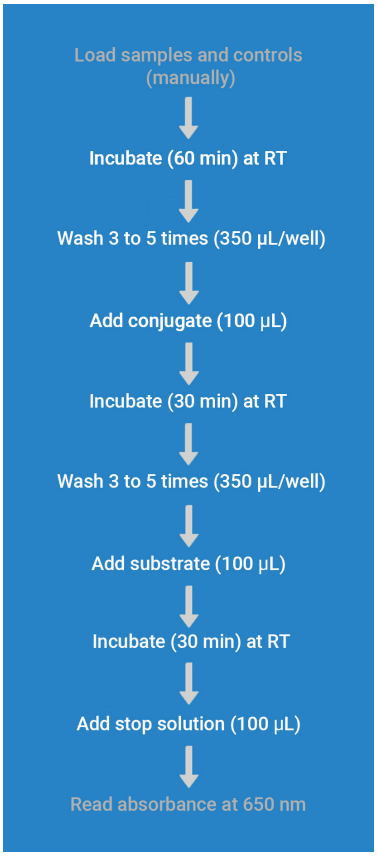


Figure 2. Avian influenza virus antibody assay test procedure. Steps performed by the Agilent BioTek 406 FX washer dispenser are indicated in white.

In the absence of AIV antibodies, the conjugate is free to bind to the AIV antigen coating the plate. Conversely, if there are AIV antibodies present in the sample, the anti-AIV monoclonal antibody conjugate is prevented from binding to the plate. Subsequent color production, which is a function of the amount of captured conjugate, is inversely proportional to the amount of AIV antibodies present in the sample.

The screening of bird flocks for avian influenza is vital to ensure that the flocks are disease free. The sheer number of animals that need to be evaluated under normal circumstances in animal test facilities means that large numbers of samples must be processed daily. In the event of a disease outbreak, the test volume would be expected to increase significantly above normal levels in specific regions. Automation of this process can provide tremendous savings in time and labor. The 406 FX is compatible with different robotic systems including the Agilent BioTek BioStack microplate stacker and the Agilent BenchCel microplate handler.

## Experimental

The multispecies avian influenza antibody test used was an ELISA kit from IDEXX Laboratories (Westbrook, MA) and was performed according to the kit instructions. Briefly, undiluted positive and negative controls and diluted (1:10) samples were pipetted (100 µL) into the assay plate. The plates were allowed to incubate for 60 minutes and then transferred sequentially to the 406 FX, where the plates were washed five times with 350 µL wash buffer followed by the addition of 100 µL conjugate using the peristaltic pump dispenser. The plates were then allowed to incubate for 30 minutes at room temperature. After incubation, the plates were transferred to the 406 FX and washed five times with 350 µL washer buffer followed by the addition of 100 µL substrate solution using the other peristaltic pump dispensers. Color development was allowed for 15 minutes. After color development, the 406 FX added 100 µL stop solution using a syringe pump dispenser (Table 1).

The absorbance of the wells at 650 nm was determined using an Agilent BioTek Epoch 2 microplate spectrophotometer.

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 |

## Results and discussion

Control wells in qualitative ELISAs are used for assay validation and sample determinations. For the assay results to be valid, the IDEXX multispecies AIV assay requires that the negative control mean at 650 nm absorbance be greater than or equal to 0.600 and PC:NC ratio be less than 0.5. As demonstrated in Table 2, the assay results of the AIV assay performed by the 406 FX meet the validation criteria outlined in the kit instructions.

Table 2. Validation results.

| Validation | | | |
|---|---|---|---|
| **Parameter** | **Value** | **Requirement** | **Result** |
| NC Mean | 1.363 | **≥ 0.600** | Pass |
| PC:NC Ratio | 0.140 | **< 0.500** | Pass |

Figure 3 demonstrates that semi-automation can be achieved using the 406 FX washer dispenser. The assay results show both the positive and negative controls within the assay validation criteria. The nature of this assay is that antibodies against the avian influenza virus compete with the assay kits conjugate. This resulted in the absorbance values for the positive controls being lower than the negative controls that did not have the antibodies. As shown in Figure 3, dilution of the positive sample at 1:10 with sample diluent still resulted in a positive determination based on the assay criteria. This dilution was near the detection limit of the assay kit, as further dilution of the positive sample resulted in a negative determination.

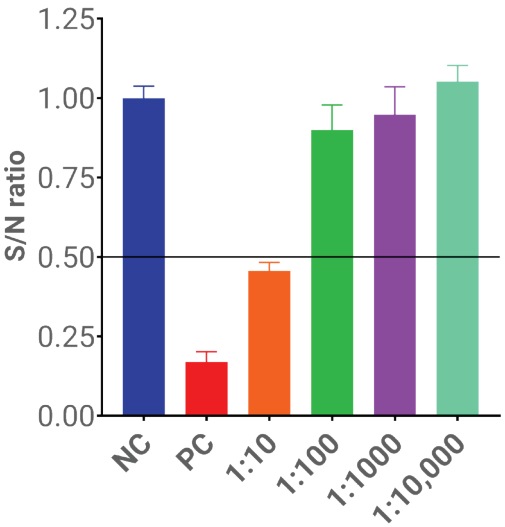


Figure 1. Sample-to-negative ratio for samples and controls of a multispecies AIV ELISA. The horizontal line indicates the cutoff between positive and negative determinations.

Efficient washing along with accurate and precise reagent addition are critical for obtaining the best results with ELISA reactions. These results demonstrate that the 406 FX is capable of both important tasks. LHC software interfaces with the 406 FX to control process order, timing, and incubation delays. This ELISA assay used two different wash steps to remove unbound material, as well as three different reagent additions with intervening incubation steps. Using delay steps in the LHC software for incubation timing allowed walk-away processing with the 406 FX once the diluted samples and controls were pipetted into the assay plate. After the plate processing steps were completed, the plates were manually loaded onto Epoch 2 microplate spectrophotometer.

Laboratory bench space is an asset that is often in short supply. Instruments that are large and bulky, or the requirement for multiple instruments to perform assay functions, leads to crowded and cluttered work areas that decrease productivity. Because the cost of adding new laboratory space is often prohibitive, to improve productivity it is necessary to develop instrumentation that provides multiple functionalities in a compact, single package. The 406 FX washer dispenser provides the same functionality as several instruments in a modest footprint.

The 406 FX is designed to interface with robotic systems. The washer dispenser has an open carrier design that allows for easy microplate placement and retrieval by robotic grippers. Additionally, the carrier can be programmed for plate transfer from either side of the 406 FX. The instrument's low, vertical profile enables easier vertical stacking of hardware. For most ELISA assays, only the 406 FX and an absorbance reader are required. Robotic systems that utilize the 406 FX rather than multiple instruments are less complex, require significantly less space, and are easier to install and maintain.

The 406 FX improves automation efficiency by reducing the number of plate transfer steps required. ELISA procedures typically require the addition of a reagent after a wash step. Using a standard configuration of an automated ELISA system, this would require that the plate be moved from the washer station to the reagent addition station. This plate movement step can be eliminated with the use of the 406 FX, as the washer and dispenser capabilities are integrated into one device. The elimination of redundant plate movement steps can result in considerable time savings through the course of a large assay batch run.

## Conclusion

The Agilent BioTek 406 FX washer dispenser is an ideal multifunctional instrument to manage the fluid handling steps of most conventional ELISA reactions. While this study only demonstrated the Agilent BioTek 406 FX with the IDEXX multispecies AIV ELISA kit, this assay kit serves as a representative of other ELISA tests that could be run. Besides superior assay performance, the 406 FX system provides significant cost savings. The 406 FX does the equivalent of multiple instruments with a price point that is substantially less than their sum and requires significantly less bench space, allowing the vacated bench space to be used for other purposes. In fully automated robotic platforms, the use of a 406 FX rather than the instruments that it replaces is less complex, easier to maintain, and will often lead to direct time savings due to a reduction in required plate movements.

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