Automated Enzyme-linked Immunosorbent Assays by Opentrons Robotic Liquid Handling Systems

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INTRODUCTION

ELISAs are widely used analytic tools for diagnostic or research purposes. This technique requires a high level of consistency in sample handling, which can be achieved by reducing hands-on time to minimize human errors. Fully automated protocols were developed and tested on Opentrons robotic liquid handler OT-2 and Flex to perform commercially available ELISA kits and custom ELISA development.

METHOD

- Assay: Takara Fibronectin (FN) EIA Kit (sandwich ELISA, Fig 1)
- Platform: Opentrons OT-2
- Workflow:
  1. Target Capture
  2. Wash
  3. Detection
  4. Wash
  5. Signal Development

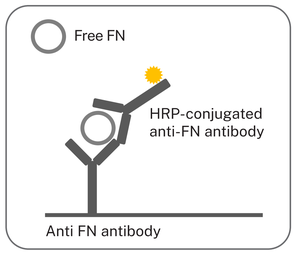


Figure 1. Assay format

- Assay: Tecan Cortisol Saliva ELISA Kit (competitive ELISA, Fig 2)
- Platform: Opentrons OT-2
- Workflow:
  1. Target Capture & Detection
  2. Wash
  3. Signal Development

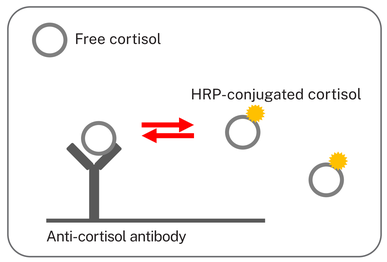


Figure 2. Assay format

- Assay: Cell Sciences SARS-CoV-2 Surrogate Virus Neutralization Test Kit (competitive ELISA, Fig 3)
- Platform: Opentrons OT-2
- Workflow:
  1. Target Capture & Detection
  2. Wash
  3. Signal Development

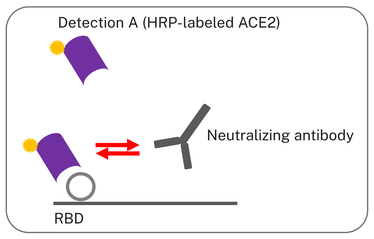


Figure 3. Assay format

- Assay: SARS-CoV-2 Spike RBD-coupled Magnetic Beads (bead-based ELISA, Fig 4)
- Platform: Opentrons OT-2 with Magnetic Module
- Workflow:
  1. Target Capture
  2. Wash
  3. Detection
  4. Wash
  5. Signal Development

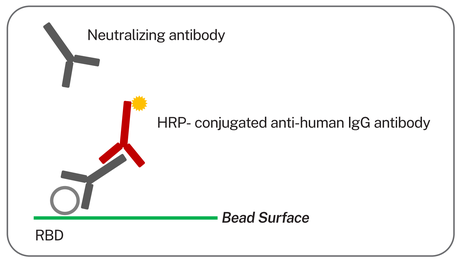
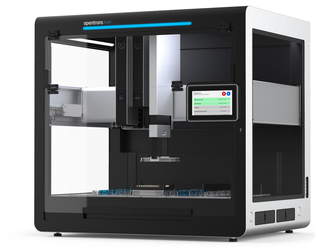


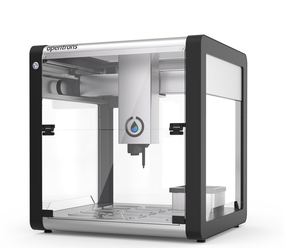
Figure 4. Assay format

- Assay: Assay Development for Cytokine Detection (sandwich ELISA, Fig 5)
- Platform: Opentrons Flex
- Workflow:
  1. ELISA Plate Prep
  2. Wash
  3. Target Capture
  4. Wash
  5. Detection
  6. Wash
  7. Signal Development

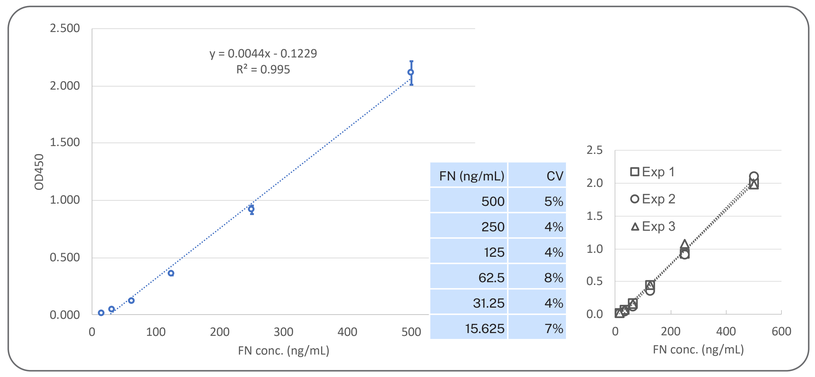
		1	2	3	4	5	6	7	8	9	10	11	12
Sample 1	A	TNFab				IFN ab				uncoated			
Sample 1	B												
Sample 2	C												
Sample 2	D												
Sample 3	E												
Sample 3	F												
blk	G												
blk	H												

Figure 5. ELISA plate design





RESULTS



FN (ng/mL)	CV
500	5%
250	4%
125	4%
62.5	8%
31.25	4%
15.625	7%

Figure 6. Takara Fibronectin (FN) EIA Kit (Fig 1). FN samples were prepared in serial dilutions. The goodness-of-fit for linear regression was determined by plotting the average absorbances (n=3) against the concentrations of FN, and STD and CV obtained (Left). Results from 3 separate tests were plotted to confirm the reproductivity of the assay (Right)

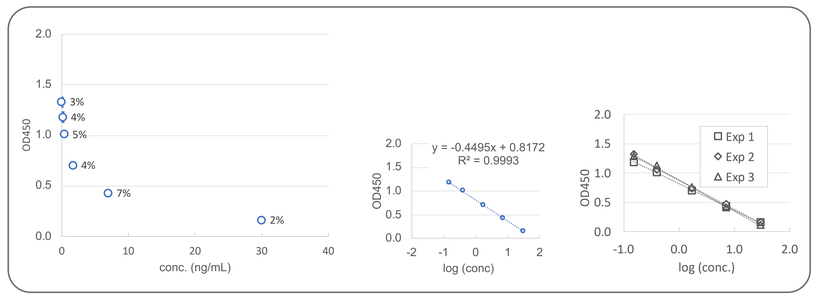
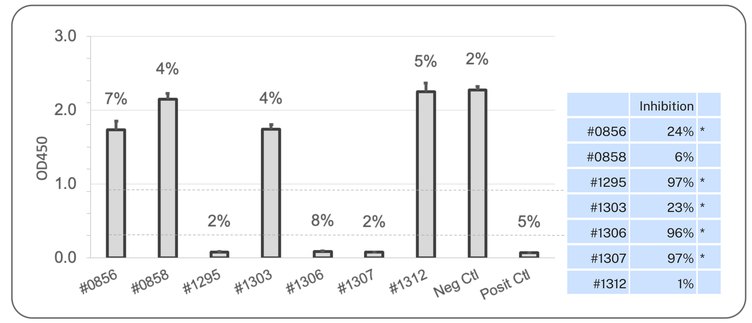
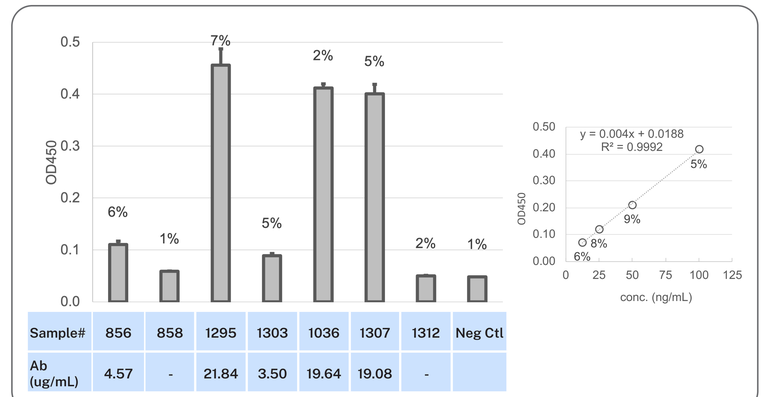


Figure 7. Tecan Cortisol Saliva ELISA Kit (Fig 2). Cortisol samples were prepared in serial dilutions. The goodness-of-fit for linear regression was determined by plotting the average absorbances (n=3) against the concentrations of cortisol, and STD and CV obtained (Left). Results from 3 separate tests were plotted to confirm the reproductivity of the assay (Right)



	Inhibition	
#0856	24%	*
#0858	6%	
#1295	97%	*
#1303	23%	*
#1306	96%	*
#1307	97%	*
#1312	1%	

Figure 8. Cell Sciences SARS-CoV-2 Surrogate (Fig 3). Human serum samples previously tested positive by FDA-certified ELISA (*) were confirmed by the assay (Inhibition > 20%, calculated by Inhibition = [1 – (OD450 value of sample / OD value of negative control)] x 100%, n = 3).



Sample#	856	858	1295	1303	1036	1307	1312	Neg Ctl
Ab (ug/mL)	4.57	-	21.84	3.50	19.64	19.08	-	

Figure 9. SARS-CoV-2 Spike RBD-coupled Magnetic Beads (Fig 4). Serial dilutions of recombinant neutralizing antibody were prepared and tested, and CV calculated (n = 3) (Left). Human serum samples previously tested positive by FDA-certified ELISA were confirmed by the assay (n = 3) (Right).

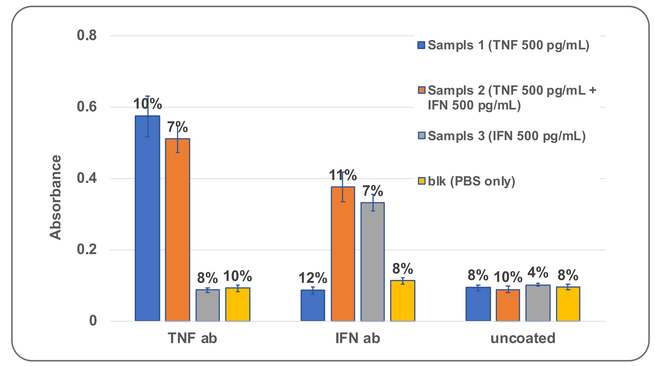


Figure 10. Assay Development for Cytokine Detection (Fig 5). An ELISA assay was developed by coating a 96-well plate with anti-TNF alpha and anti-IFN gamma antibodies and tested by using samples containing these cytokines, and CV calculated (n=6).

CONCLUSION

- The robot can be programmed to perform complete ELISAs from antibody/antigen coating to signal development
- The results demonstrated consistency of sample handling (CV<10%), accuracy (R-squared>0.99) and reproducibility of the assay
- As a typical example, for detection of SARS-CoV-2 antibody, both commercially available 96-well plate-based and in-house bead-based ELISAs were fully automated and data comparable to FDA-certified assays