Fluorescence polarization assays: another mainstream in detection of biohazards

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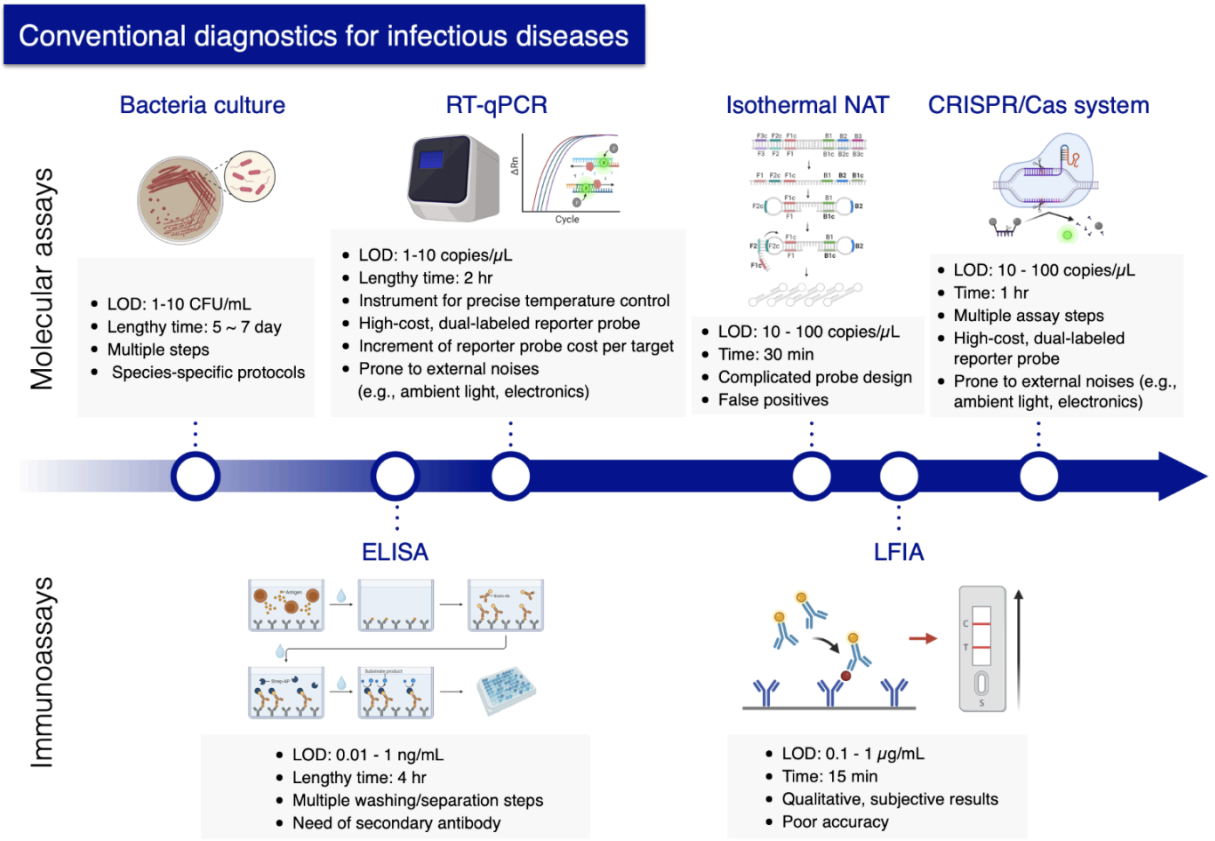

Fig. S1. Overview of conventional diagnostic systems for biohazards. Though being well-established, the conventional molecular/immunological assays have drawbacks in sensitivity, time, procedures, and cost. Especially, the modality of fluorescent readout incurs unstable detection signals and the use of high-cost reporter probe.

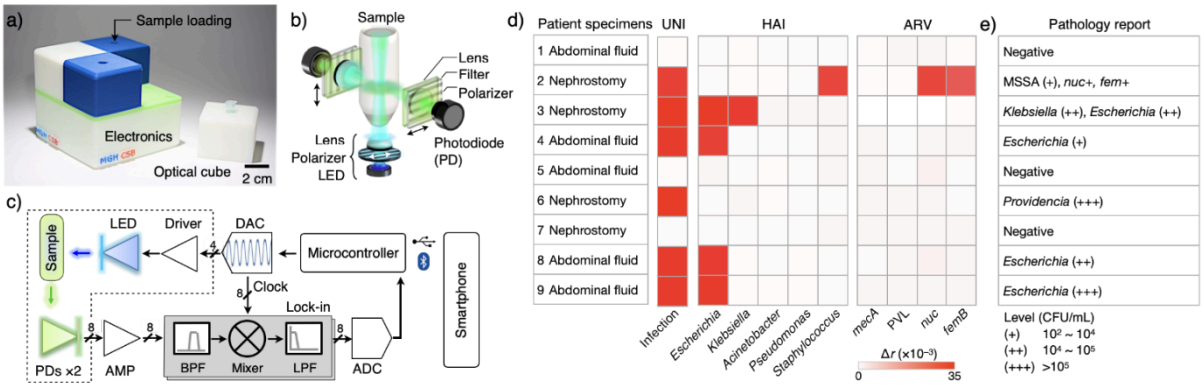

Fig. S2. PAD device for diagnosis of HAIs. NA assay was integrated to a compact FP device for chair-side diagnosis of HAIs. **a)** Photograph of PAD device. It has four optical cubes plugged into the base unit. FP signals measured by the cubes are processed by the base unit. **b)** Inside configuration of the optical cube. The signal acquisition module has two photodiodes, each equipped with an orthogonal polarizer, to deduce FP signal. **c)** Overall circuit diagram. Microcontroller controls the PAD system and is Bluetooth-connected to a smartphone for remote control. The system processes the signal with lock-in amplification method to maximize the signal-to-noise ratio. DAC, digital-to-analog converter; AMP, amplifier; BPF, band-pass filter; LPF, low-pass filter; ADC, analog-to-digital converter. **d)** PAD was applied to the clinical samples to assess their bacterial load (UNI), species (HAI), and resistance/virulence status (ARV). The results matched with those of the pathology report made by culture and qPCR [1]. From [1].

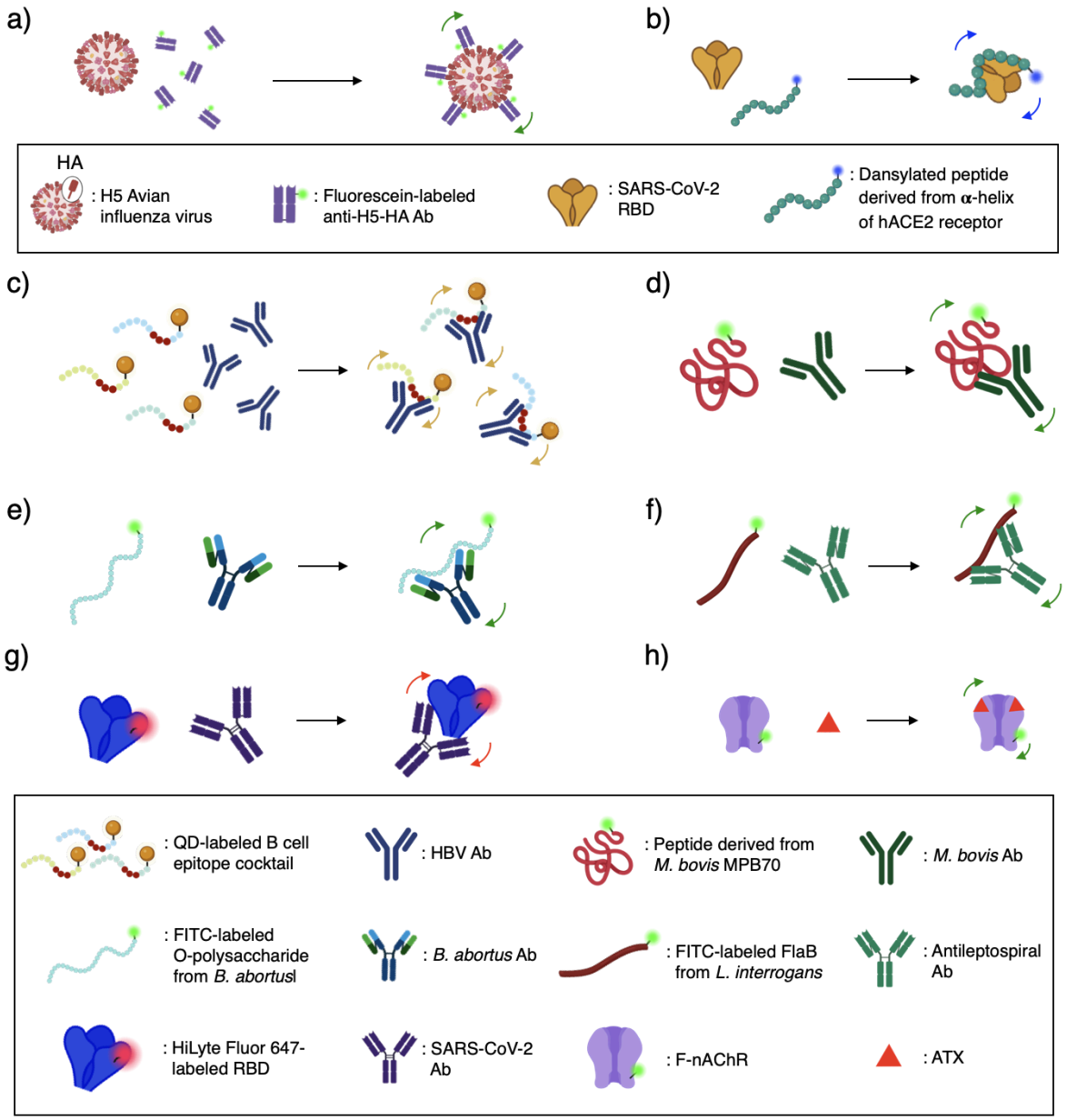

Fig. S3. (a and b) F-labeled Ab is a recognition unit. **a)** Fluorescein-labeled anti-H5-HA Ab detects H5 Avian Influenza virus [2], and **b)** dancylated peptide derived from α-helix of hACE2 receptor detects SARS-CoV-2 RBD protein [3]. **(c and h)** F-labeled pathogen (or its part) is used as a recognition unit for the detection of Ab. **c)** QD-labeled B cell epitope cocktail detects HBV Ab [4]. **d)** Peptide derived from *M. bovis* secretory protein MPB70 detects *M. bovis* Ab [5]. **e)** FITC-labeled O-polysaccharide from *B. abortus* detects *B. abortus* Ab [6]. **f)** FITC-labeled FlaB from *L. interrogans* detects antileptospiral Ab [7]. **g)** HiLyte Fluor 647-labeled RBD protein detects SARS-CoV-2 Ab [8]. **h)** F-nAChR detects its agonist, ATX [9].

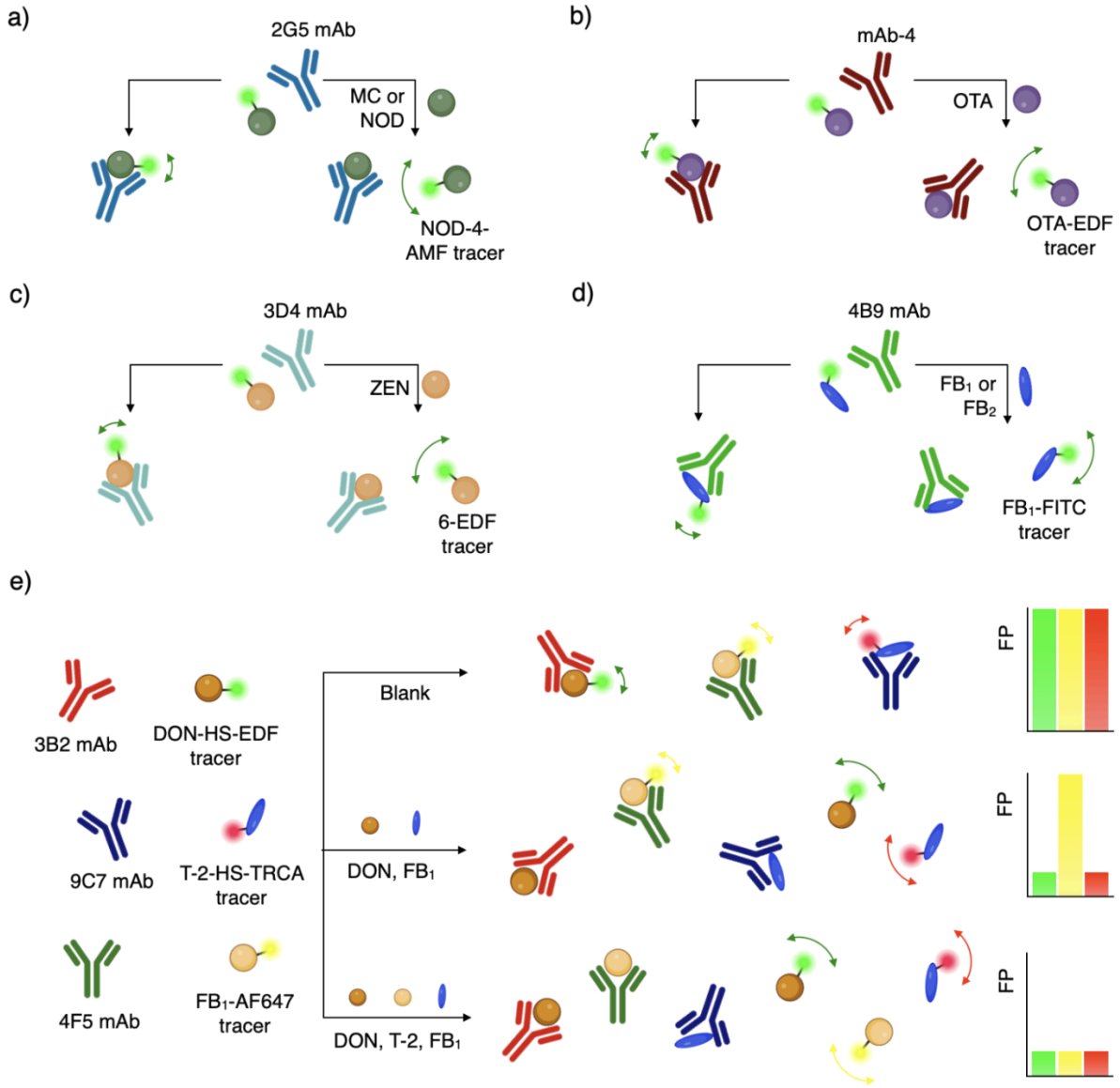

Fig. S4. Toxin sensors based on the competitive FPIA. a) 2G5 mAb and NOD-4-AMF tracer detect MC and NOD [10]. **b)** mAb-4 and OTA-EDF tracer detect OTA [11]. **c)** 3D4 mAb and 6-EDF tracer detect ZEN [12]. **d)** 4B9 mAb and FB1-FITC tracer detect FB1 and FB2 [13]. **e)** In addition, multiple Ab-tracer pairs detect multiple target toxins in parallel. 3B2-mAb with DON-HS-EDF tracer, 9C7 mAb with T-2-HS-TRCA tracer, and 4F5 mAb with FB1-AF647 tracer target DON, T-2, and FB1, respectively, and show independent FP signals at matching wavelengths in response to different target combinations [14]. For the detailed information of Abs and tracers, please refer to the relevant references.

Table S1. Summary of the diagnostic methods for biohazards.

System	Key techniques/ components	Infection	Target[a]	Time (min)	Key metrics (LOD, SV, SP, or AC)[b,c]	Assay characteristics	Reference
PCR	Asymmetric PCR	HBV	Genotype A, B, C, and D	165	2.5 copies/μL	• Multiplex • Cost-effective than multiplex PCR by 20%	[15]
	Asymmetric PCR	Genitourinary infection	16S rRNA gene of *U. parvum*, *U. urealyticum*, *M. genitalium*, and *M. hominis*	165	-	• Multiplex • Cost-effective than multiplex PCR by 20%	[16]
	Asymmetric PCR	HSV	HSV-1/-2, cytomegalovirus, and Epstein-Barr virus	270	0.5 copies/μL SV: 95% SP: 98%	• Multiplex • Cost-effective than multiplex PCR by 20%	[17]
	Template-directed dye-terminator incorporation	HBV	YMDD mutation	325	-	• Point mutation detection	[18]
Enzyme activity Control	Polymerase aptamer	Foodborne diseases	*Salmonella*	150	1 CFU/mL	• Use of a universal reporter probe	[19]
		HAIs	*E. coli, K.pneumoniae,* *A. Baumannii,* *P. Aeruginosa,* *S. Aureus*, Antibiotic resistance and virulence genes (nuc, femB, mecA, PVL)	120	1 CFU/mL	• Use of a universal reporter probe • Integration into a portable FP device	[1]
Isothermal NA amplification	SDA	MTB	MTB	30	10 copies	• -	[20]

	RPA/CRISPR	COVID-19	SARS-CoV-2 N1 and N2 genes	20	3 copies/μL	• Use of a universal reporter probe • One-step, one temperature, one-tube (i.e. all-in-one) • Integration into a portable FP device	[21]
DNA self-assembly	Multivalent hairpin aptamer probe-tethered DNA monolayers	Foodborne diseases	*Salmonella*	45	7.2 CFU/mL	• One-step, one temperature, one-tube (i.e. all-in-one) • Integration into a portable FP device	[22]
	Submicro-hydrogel woven by CHA and STV	Foodborne diseases	SEB	>210	92 pg/mL	• Intervention by magnetic separation and STV addition	[23]
FPIA	Fab fragment	H5 avian influenza	H5-HA	15	2.8 μg/mL	• Integration into a portable FP device	[2]
	Peptide derived from the N-terminal α-helix of hACE2 receptor	COVID-19 and its variants (Delta (B.1.617.2) and Omicron (BA.5))	RBD protein	3	10 fM	• Rapid • Integration into a portable FP device	[3]
	B cell epitopes of HBV surface antigen labeled with CdTe QD	HBV	HBV Ab	15	SV: 84.3% SP: 98,2%	• -	[4]
	Peptide derived from *M. bovis* secretory protein MPB70	*M. bovis*	*M. bovis* Ab	10	-	• -	[5]

	O-polysaccharide from *B. abortus* strain 1119.3	Human brucellosis	Brucellosis Ab	5	SV: 93.5% SP: 96.1%	• -	[6]
	FlaB antigen of *L. Interrogans*	Leptospirosis	*Leptospira* Ab	30	SV: 83.7% SP: 81.2%	• -	[7]
	RBD protein	COVID-19	SARS-CoV-2 Ab	20	AC: 96.5%	• Integration into a portable FP device	[8]
	nAChRs	*Anabaena*	ATX	60	33.3 nM	• -	[9]
Competitive FPIA[d]	2G5 mAb and NOD-4-AMF tracer	Cyanobacteria	MC and NOD	10	0.86 μg/mL (for MC) 0.95 μg/mL (for NOD)	• -	[10]
	mAb-4 and OTA-EDF tracer	*Aspergillus* and *Penicillium*	OTA	10	3 ng/mL	• -	[11]
	3D4 mAb and 6-EDF tracer	*Fusarium*	ZEN	-	12 μg/kg	• Universal to analogs	[12]
	4B9 mAb and FB_1-FITC tracer	*Fusarium*	FB_1 and FB_2	5	157.4 μg/kg (for FB_1) 290.6 μg/kg (for FB_2)	• Universal to analogs	[13]
	3B2 mAb and DON-HS-EDF tracer (for DON), 9C7 mAb and T-2-HS-TRCA (for T-2), and 4F5 mAb and FB_1-AF647 (for FB_1)	*Fusarium*	DON, T-2, and FB_1	30	242.0 μg/kg (for DON), 17.8 μg/kg (for T-2), and 331.5 μg/kg (for FB_1)	• Multiplex	[14]
Aptamer	Multivalent aptamer	RSV	G protein	-	-	•	[24]

	Aptamer loaded with high MW molecule (STV)	Spoilage	*W. viridescens*	>45	80 CFU/mL	•	-	[25]
	Aptamer loaded with high MW molecule (Ab)	*Aspergillus*	AFB1	45	25 pM	•	-	[26]
	Aptamer	*Aspergillus*	AFB1	10	2 nM	•	-	[27]
	Exo III-aided target recycle amplification	*Fusarium*	ZEN	40	0.004 ng/mL	•	-	[28]

[a]*U. parvum*: *Ureaplasma parvum*, *U. urealyticum*: *Ureaplasma urealyticum*, *M. genitalium*: *Mycoplasma genitalium*, *M. hominis*: *Mycoplasma hominis*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *A. baumannii*: Acinetobacter baumannii, *P. aeruginosa*: *Pseudomonas aeruginosa*, *S. aureus*: *Staphylococcus aureus, W. viridescens: Weissella viridescens*

[b]LOD: Limit of detection, SV: Sensitivity, SP: Specificity, AC: Accuracy

[c]Unless otherwise stated, a numerical value indicates the LOD

[d]Please, refer to the relevant references for detailed information of monoclonal Abs (mAbs) and tracers.

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