Supplementary Materials

Rapid detection of azole-resistant *Aspergillus fumigatus* in clinical and environmental isolates using lab-on-a-chip diagnostic system

Running title: CMOS-based LoC detection of resistant *A. fumigatus*

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Table S1. Information of 30 clinical and environmental samples.

Sample	Source	Country	Allele
C78	Environmental	Republic of Ireland	TR34/L98H
C80	Environmental	Republic of Ireland	TR34/L98H
C85	Environmental	Republic of Ireland	TR34/L98H
C86	Environmental	Republic of Ireland	TR34/L98H
C92	Environmental	Republic of Ireland	TR34/L98H
C284	Environmental	USA	TR34/L98H
C287	Environmental	USA	TR34/L98H
C288	Environmental	USA	TR34/L98H
C133	Clinical	London	TR34/L98H
C134	Clinical	London	TR34/L98H
C143	Clinical	London	TR34/L98H
C144	Clinical	London	TR34/L98H
C342	Environmental	London	TR34/L98H
C160	Clinical	England	TR34/L98H/T289A
C161	Clinical	England	TR34/L98H/T289A
C79	Environmental	Republic of Ireland	TR46/Y121F/T289A
C87	Environmental	Republic of Ireland	TR46/Y121F/T289A
C91	Environmental	Republic of Ireland	TR46/Y121F/T289A
C93	Environmental	Republic of Ireland	TR46/Y121F/T289A
C95	Environmental	Republic of Ireland	WT
C96	Environmental	Republic of Ireland	WT
C285	Environmental	USA	WT
C286	Environmental	USA	WT
C238	Environmental	South Africa	WT
C241	Environmental	India	WT
C242	Environmental	India	WT
C243	Environmental	India	WT
C244	Environmental	Australia	WT
C245	Environmental	Australia	WT
C249	Environmental	South Africa	WT

Table S2. The primer sequences of F1P (F1C+F2) and loop primer used for developing TR46 specific LAMP assay. The introduced mismatches are shown in bold.

FIP	Primer sequence (5'->3')
Set1	TAATTAGGCAACTTTCAT-TCCGGATGTGTGCTGAGCC**a**AA**g**GAAAGTTGTCTAG
Set2	TAATTAGGCAACTTTCAT-TCCGGATGTGTGCTGAGC**aa**AATGAAAGTTGTCTAG
Set3	TAATTAGGCAACTTTCAT-TCCGGATGTGTGCTGAGC**aa**AA**g**GAAAGTTGTCTAG
Set4	TAATTAGGCAACTTTCAT-TCCGGATGTGTGCTGAG**aaa**AA**g**GAAAGTTGTCTAG
Set5	TAATTAGGCAACTTTCAT-TCCGGATGTGTGCTGA**a**CC**a**AA**g**GAAAGTTGTCTAG

Loop primer	Sequence (5'->3')
LF	CGGCTCAGCACACATCCGGACCG
LB	ACCATACACCCTAACTCATACTACGG

Table S3. Detection and quantification of azole-resistance *A. fumigatus* in clinical and environmental samples by TR-LAMP mutant specific assays and the AsperGenius® resistant assay in testing 30 clinical and environmental isolates.

¥TTP: Time-to-positive.

αFor sample 87 one of the TR46 markers (Y121F) did not provide a definitive melt temperature (shown in bold), suggesting nonspecific amplification.

Sample	WGS	TR-LAMP assay				AsperGenius® resistant assay							
		TR34 mutation detection		TR46 mutation detection		TR34 mutation detection				TR46 mutation detection			
		TTP¥ (min)	Estimated concentration (copies/reaction)	TTP¥ (min)	Estimated concentration (copies/reaction)	YELLOW555		FAM		TEXAS Red		Cy5	
						Tm (°C)	TR34	Tm (°C)	L98H	Tm (°C)	T289A	Tm (°C)	Y121F
C78	TR34/L98H	12.30 (SD=±0.05)	8.78E+04	-	-	66.62	+	65.99	+	63.92	-	64.57	-
C80	TR34/L98H	12.72 (SD=±0.14)	5.63E+04	-	-	66.98	+	66.15	+	63.68	-	64.67	-
C85	TR34/L98H	12.99 (SD=±0.10)	4.33E+04	-	-	66.82	+	66.39	+	64.10	-	65.15	-
C86	TR34/L98H	12.04 (SD=±0.08)	1.15E+05	-	-	66.80	+	66.18	+	63.70	-	64.50	-
C92	TR34/L98H	12.65 (SD=±0.13)	6.06E+04	-	-	66.82	+	66.19	+	63.91	-	64.16	-
C284	TR34/L98H	12.77 (SD=±0.05)	5.34E+04	-	-	66.84	+	66.00	+	63.52	-	64.16	-
C287	TR34/L98H	12.29 (SD=±0.05)	8.87E+04	-	-	66.71	+	66.08	+	63.80	-	64.24	-
C288	TR34/L98H	12.52 (SD=±0.11)	6.96E+04	-	-	66.72	+	66.10	+	63.41	-	64.05	-
C133	TR34/L98H	13.84 (SD=±0.18)	1.73E+04	-	-	66.86	+	66.42	+	63.92	-	64.77	-
C134	TR34/L98H	14.52 (SD=±0.07)	8.42E+03	-	-	66.79	+	66.36	+	63.85	-	64.70	-
C143	TR34/L98H	13.55 (SD=±0.11)	2.33E+04	-	-	67.00	+	66.36	+	63.26	-	64.71	-
C144	TR34/L98H	14.90 (SD=±0.15)	5.63E+03	-	-	67.21	+	66.55	+	63.26	-	64.51	-
C160	TR34/L98H	14.49 (SD=±0.11)	5.69E+03	-	-	66.81	+	66.56	+	63.25	-	64.51	-
C161	TR34/L98H	13.76 (SD=±0.25)	1.88E+04	-	-	66.85	+	66.22	+	63.49	-	64.33	-
C342	TR34/L98H	14.48 (SD=±0.20)	8.78E+03	-	-	66.75	+	66.11	+	63.18	-	64.24	-
C79	TR46/Y121F/T289A	-	-	13.86 (SD=±0.20)	2.90E+05	64.92	-	60.34	-	67.12	+	69.15	+
C87α	TR46/Y121F/T289A	-	-	13.98 (SD=±0.15)	2.55E+05	64.40	-	60.78	-	62.88	+	63.52	-
C91	TR46/Y121F/T289A	-	-	14.01 (SD=±0.14)	2.48E+05	64.82	-	61.60	-	67.25	+	68.89	+
C93	TR46/Y121F/T289A	-	-	16.03 (SD=±0.06)	3.15E+04	64.63	-	61.59	-	67.05	+	68.91	+

C95	WT	-	-	-	-	65.69	-	60.83	-	63.13	-	64.38	-
C96	WT	-	-	-	-	64.03	-	60.96	-	63.07	-	64.12	-
C285	WT	-	-	-	-	64.83	-	60.74	-	63.25	-	64.52	-
C286	WT	-	-	-	-	64.46	-	60.78	-	63.1	-	64.36	-
C238	WT	-	-	-	-	64.51	-	61.85	-	63.35	-	64.41	-
C241	WT	-	-	-	-	64.57	-	62.12	-	63.61	-	64.47	-
C242	WT	-	-	-	-	64.82	-	60.75	-	63.65	-	64.51	-
C243	WT	-	-	-	-	64.54	-	60.86	-	63.38	-	64.44	-
C244	WT	-	-	-	-	64.95	-	60.86	-	63.37	-	64.43	-
C245	WT	-	-	-	-	64.55	-	60.87	-	62.99	-	64.25	-
C249	WT	-	-	-	-	64.74	-	60.85	-	62.97	-	63.97	-

Table S4. List of all the accession numbers of the *cyp51A* gene nucleotide sequences used in this study (https://www.ncbi.nlm.nih.gov/genbank/; last accessed August 25, 2020)

Accession number			
MF070878.1	KX788503.1	KX788552.1	KX788533.1
MF070879.1	KX788504.1	KX788553.1	KX788536.1
MF070883.1	KX788505.1	KX788554.1	KX788538.1
MF070884.1	KX788506.1	KX788555.1	KX788539.1
EU626235.1	KX788507.1	KX788486.1	KX788540.1
MF070870.1	KX788508.1	KX788487.1	KX788541.1
MF070873.1	KX788510.1	KX788488.1	KX788542.1
MF070875.1	KX788511.1	KX788489.1	KX788543.1
MF070876.1	KX788512.1	KX788491.1	KX788544.1
MF070877.1	KX788513.1	KX788492.1	KX788545.1
MF070880.1	KX788514.1	KX788493.1	KX788551.1
MF070881.1	KX788515.1	KX788494.1	KP725003.1
KX788509.1	KX788516.1	KX788495.1	
KJ210331.1	KX788517.1	KX788496.1	
KX159724.1	KX788518.1	KX788497.1	
MF070871.1	KX788519.1	KX788498.1	
MF070882.1	KX788520.1	KX788499.1	
KP725004.1	KX788521.1	KX788500.1	
AF338659.1	KX788522.1	KX788501.1	
KP725002.1	KX788523.1	KX788502.1	
KX788490.1	KX788524.1	KX788556.1	
KX788534.1	KX788525.1	KX788557.1	
KX788535.1	KX788526.1	KX788558.1	
KX788537.1	KX788527.1	KX788559.1	
KX788546.1	KX788528.1	KX788560.1	
KX788547.1	KX788529.1	KX788561.1	
KX788548.1	KX788530.1	KX788562.1	
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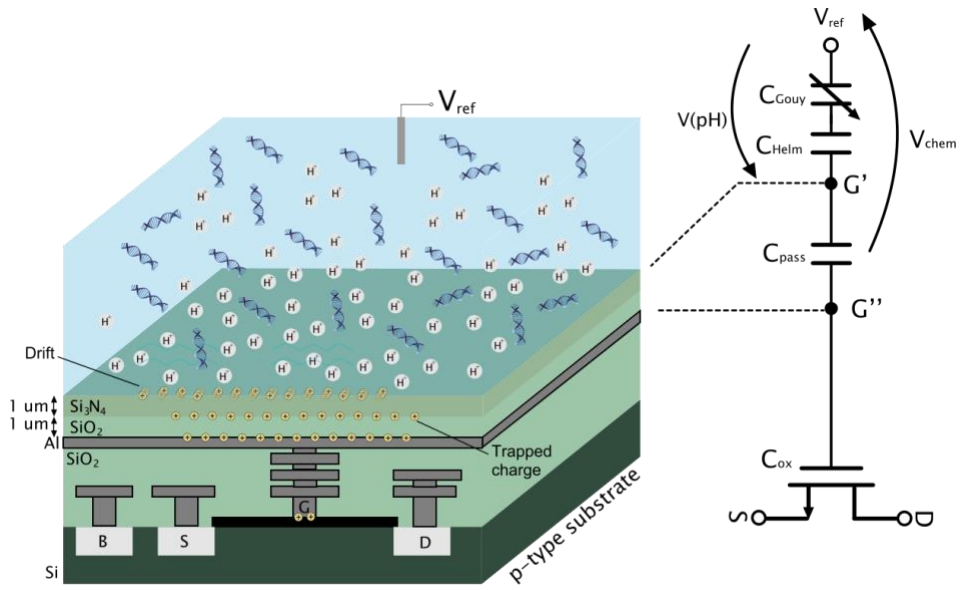


Fig. S1. Structure of the ISFET in unmodified CMOS technology and equivalent macromodel.

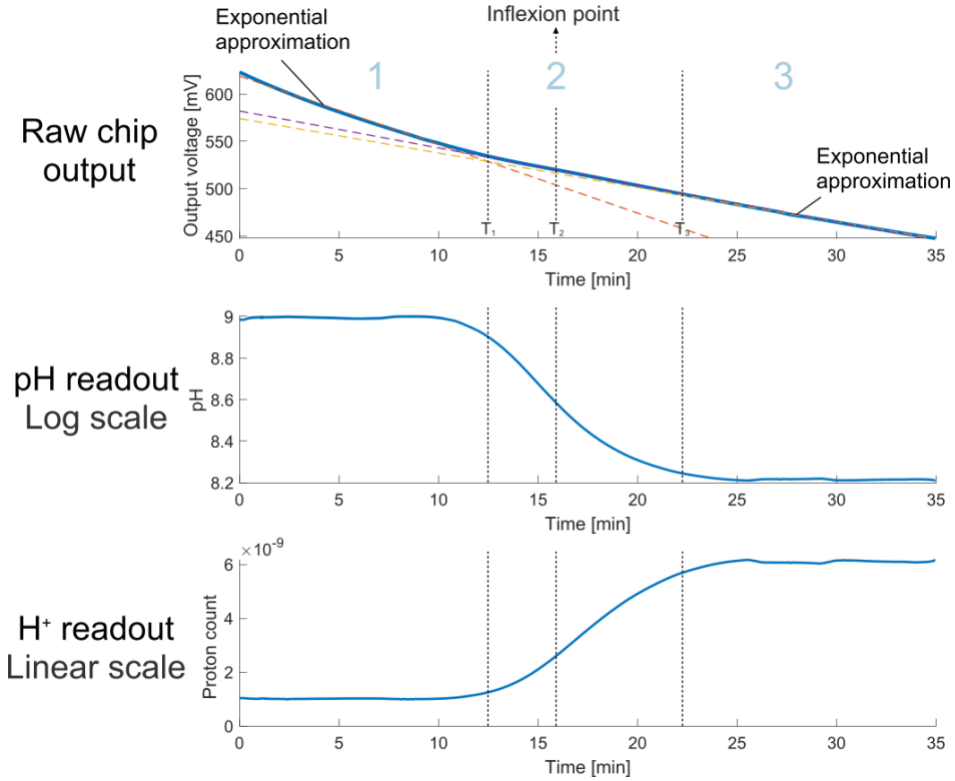


Fig S2. Processing algorithm to extract the amplification curve from the averaged sensor outputs.

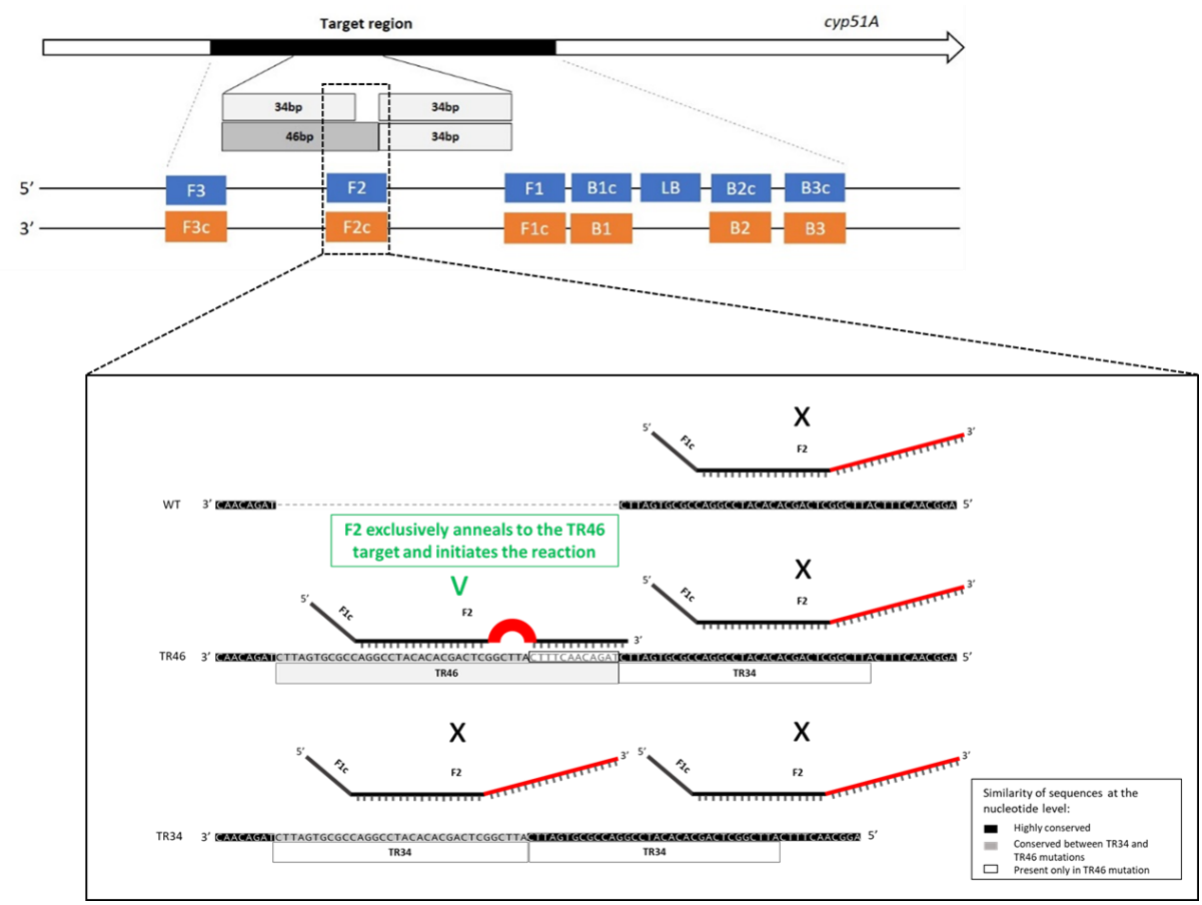


Fig. S3. Schematic diagram of *cy51A* gene representing the position of TR46-LAMP assay and the specific location of F2 primer. The magnified region shows the alignment of the TR34 (MF070870.1), TR46 (MF070879.1), and WT (MF070881.1) sequences for *A. fumigatus*. To emphasize the priming location and composition of the FIP, F2 and F1c are shown in black and red lines: the black lines shows the exact matches and the red semi-circle highlights the introduced mismatches at 3' of F2. Due to the mismatches introduced in F2, a yes/no one step reaction is established to differentiate TR46 mutants from WT and TR34 mutants.

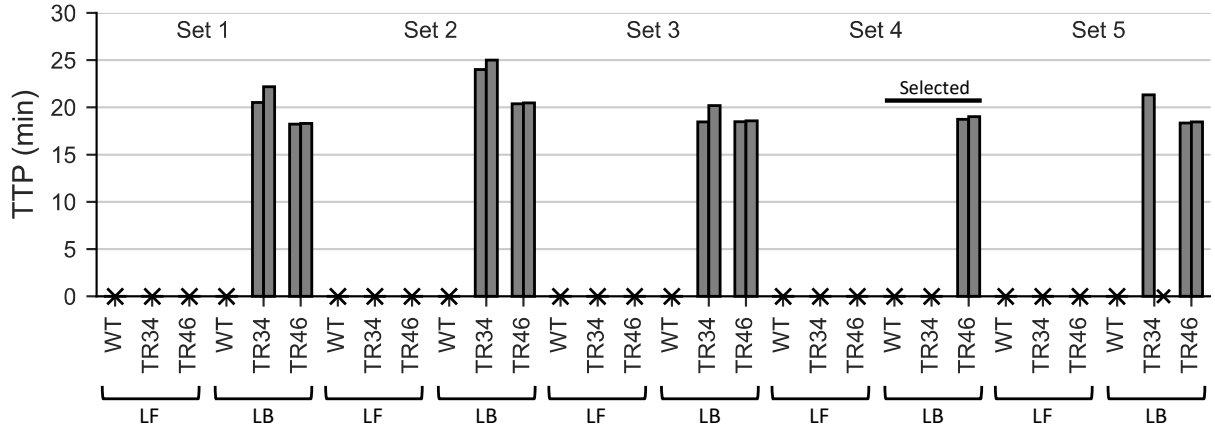


Fig. S4. TR46-LAMP primers screening. Assay optimization was performed using 10 copies per reaction in duplicates. Set 1 to 5 contain different combination of mismatches in F2. Details of F2, LF and LB sequences is shown in Table S2.

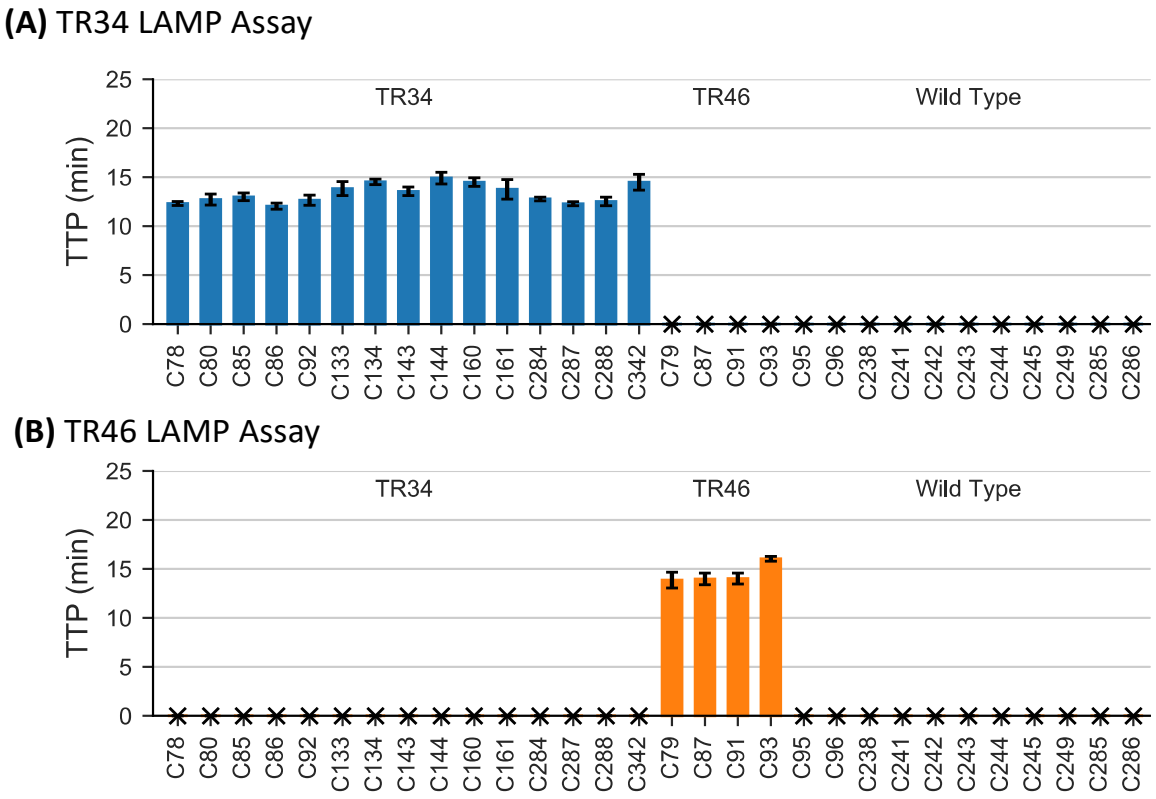


Fig. S5. Detection of azole-resistance *A. fumigatus* in clinical and environmental samples by TR-LAMP mutant specific assays. (**A**) TR34 LAMP assay. (**B**) TR46 LAMP assay. Bars indicate the mean TTP in minutes. Error bars indicate 4 standard deviations across triplicates. Black crosses in