

*Supplementary Information*

**Rapid Identification of *Candida* Species in Candidemia Directly from Blood Samples  
Using Imperfect Match Probes**

Yoshitsugu Higashi<sup>1†</sup>, Hideki Niimi<sup>2\*†</sup>, Ippei Sakamaki<sup>1</sup>, Yoshihiro Yamamoto<sup>1</sup> and Isao Kitajima<sup>2</sup>

1. Department of Clinical Infectious Diseases, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, Toyama 930-0194, Japan
2. Department of Clinical Laboratory and Molecular Pathology, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, Toyama 930-0194, Japan

\*correspondence to:

Hideki Niimi, M.D. Ph.D. E-mail address: hiniimi@med.u-toyama.ac.jp

Department of Clinical Laboratory and Molecular Pathology, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama,  
2630 Sugitani, Toyama 930-0194, JAPAN

Phone: (+81)-76-434-7759, Fax: (+81)-76-434-7759

†Both authors contributed equally to this work

**Table S1. Sequence homology between the IM Q-probes and the target regions of the eight kinds of *Candida* species reported in the DNA Data Bank of Japan**

<i>Candida</i> species	accession No.	IM Q-probe 1		
		sequence (5'→3')	probe position	Mismatch
		CTTTCCTTCTGGGTAGCCATTT		
<i>C. albicans</i>	AF114470	CTTTCCTTCTGGGTAGCCATTT	585-607	0
	AY497744	CTTTCCTTCTGGGTAGCCATTT	548-570	0
	JN940588	CTTTCCTTCTGGGTAGCCATTT	617-639	0
	JN941105	CTTTCCTTCTGGGTAGCCATTT	690-712	0
<i>C. glabrata</i>	AB094140	CTTTCCTTCTGGCTA <b>ACCCCAA</b>	686-708	6
	AY046237	CTTTCCTTCTGGCTA <b>ACCCCAA</b>	670-692	6
	AY083231	CTTTCCTTCTGGCTA <b>ACCCCAA</b>	577-599	6
	KT229542	CTTTCCTTCTGGCTA <b>ACCCCAA</b>	664-686	6
<i>C. parapsilosis</i>	AB030915	CTTTCCTTCTGGCTAGCC <b>TTTT</b>	647-669	2
	AY055857	CTTTCCTTCTGGCTAGCC <b>TTTT</b>	690-712	2
	JQ008832	CTTTCCTTCTGGCTAGCC <b>TTTT</b>	619-641	2
	KF255835	CTTTCCTTCTGGCTAGCC <b>TTTT</b>	448-470	2
<i>C. tropicalis</i>	EF412966	CTTTCCTTCTGGCTAGCC <b>TTTT</b>	647-669	2
	EU034726	CTTTCCTTCTGGCTAGCC <b>TTTT</b>	690-712	2
	JQ008834	CTTTCCTTCTGGCTAGCC <b>TTTT</b>	617-639	2
	KT449837	CTTTCCTTCTGGCTAGCC <b>TTTT</b>	623-645	2
<i>C. krusei</i>	AB053239	CTTTCCTTCTGGCTAGCC <b>CTCG</b>	659-681	4
	EF550360	CTTTCCTTCTGGCTAGCC <b>CTCG</b>	625-647	4
	JF274497	CTTTCCTTCTGGCTAGCC <b>CTCG</b>	638-660	4
	KU147485	CTTTCCTTCTGGCTAGCC <b>CTCG</b>	420-442	4
<i>C. lusitaniae</i>	AY497762	CTTTCCT <b>CCTCCTCTTAGCAAT</b>	527-549	12
	FJ176816	CTTTCCT <b>CCTCCTCTTAGCAAT</b>	650-672	12
	JN941111	CTTTCCT <b>CCTCCTCTTAGCAAT</b>	674-696	12
	JQ698900	CTTTCCT <b>CCTCCTCTTAGCAAT</b>	639-661	12
<i>C. dubliniensis</i>	AY497766	CTTTCCTTCTGGCTAGCCATTT	548-570	1
	AY669334	CTTTCCTTCTGGCTAGCCATTT	119-141	1
	MF045510	CTTTCCTTCTGGCTAGCCATTT	148-170	1
	FM992695	CTTTCCTTCTGGCTAGCCATTT	1864058-1864080	1
<i>C. guilliermondii</i>	AB054282	CTTTCCTTCTGGCTA <b>ACCATTC</b>	666-688	3
	AY227020	CTTTCCTTCTGGCTA <b>ACCATTC</b>	624-646	3
	HG798649	CTTTCCTTCTGGCTA <b>ACCATTC</b>	668-690	3
	KJ126853	CTTTCCTTCTGGCTA <b>ACCATTC</b>	691-713	3

<i>Candida species</i>	accession No.	IM Q-probe 2		
		sequence (5'→3')	probe position	Mismatch
<i>C. albicans</i>	AF114470	TGGAATAATAGAATAGGACGTTATGGTTC	679-708	0
	AY497744	TGGAATAATAGAATAGGACGTTATGGTTC	642-671	0
	JN940588	TGGAATAATAGAATAGGACGTTATGGTTC	711-740	0
	JN941105	TGGAATAATAGAATAGGACGTTATGGTTC	784-813	0
<i>C. glabrata</i>	AB094140	TGGAATAATGAATAGGACGTT-TGGTTC	795-823	2
	AY046237	TGGAATAATGAATAGGACGTT-TGGTTC	779-808	2
	AY083231	TGGAATAATGAATAGGACGTT-TGGTTC	686-715	2
	KT229542	TGGAATAATGAATAGGACGTT-TGGTTC	774-803	2
<i>C. parapsilosis</i>	AB030915	TGGAATAATAGAATAGGACGTTATGGTTC	741-770	0
	AY055857	TGGAATAATAGAATAGGACGTTATGGTTC	790-819	0
	JQ008832	TGGAATAATAGAATAGGACGTTATGGTTC	719-748	0
	KF255835	TGGAATAATAGAATAGGACGTTATGGTTC	544-573	0
<i>C. tropicalis</i>	EF412966	TGGAATAATAGAATAGGACGTTATGGTTC	738-767	0
	EU034726	TGGAATAATAGAATAGGACGTTATGGTTC	782-811	0
	JQ008834	TGGAATAATAGAATAGGACGTTATGGTTC	707-736	0
	KT449837	TGGAATAATAGAATAGGACGTTATGGTTC	715-744	0
<i>C. krusei</i>	AB053239	TGGAATAATAGAATAGGACGC-ATGGTTC	751-779	2
	EF550360	TGGAATAATAGAATAGGACGC-ATGGTTC	717-745	2
	JF274497	TGGAATAATAGAATAGGACGC-ATGGTTC	730-758	2
	KU147485	TGGAATAATAGAATAGGACGC-ATGGTTC	512-540	2
<i>C. lusitaniae</i>	AY497762	TGGAATAATAGAATAGGACGC-ATGGTTC	618-646	2
	FJ176816	TGGAATAATAGAATAGGACGC-ATGGTTC	741-769	2
	JN941111	TGGAATAATAGAATAGGACGC-ATGGTTC	766-794	2
	JQ698900	TGGAATAATAGAATAGGACGC-ATGGTTC	730-758	2
<i>C. dubliniensis</i>	AY497766	TGGAATAATAGAATAGGACGTTATGGTTC	642-671	0
	AY669334	TGGAATAATAGAATAGGACGTTATGGTTC	213-242	0
	MF045510	TGGAATAATAGAATAGGACGTTATGGTTC	242-271	0
	FM992695	TGGAATAATAGAATAGGACGTTATGGTTC	1864151-1864180	0
<i>C. guilliermondii</i>	AB054282	TGGAATAATAGAATAGGACGTTATGGTTC	773-802	0
	AY227020	TGGAATAATAGAATAGGACGTTATGGTTC	731-760	0
	HG798649	TGGAATAATAGAATAGGACGTTATGGTTC	775-804	0
	KJ126853	TGGAATAATAGAATAGGACGTTATGGTTC	798-827	0

<i>Candida species</i>	accession No.	IM Q-probe 3		
		sequence (5'→3')	probe position	Mismatch
<i>C. albicans</i>	AF114470	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	758-790	3
	AY497744	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	721-753	3
	JN940588	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	791-823	3
	JN941105	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	864-896	3
<i>C. glabrata</i>	AB094140	GCATCAGTATTCATTGTCAGAGGTGAAATTC	874-906	3
	AY046237	GCATCAGTATTCATTGTCAGAGGTGAAATTC	858-890	3
	AY083231	GCATCAGTATTCATTGTCAGAGGTGAAATTC	765-797	3
	KT229542	GCATCAGTATTCATTGTCAGAGGTGAAATTC	852-884	3
<i>C. parapsilosis</i>	AB030915	GTATCAGTATTCAGTAGTCAGAGGTGAAATTC	820-852	4
	AY055857	GTATCAGTATTCAGTAGTCAGAGGTGAAATTC	863-895	4
	JQ008832	GTATCAGTATTCAGTAGTCAGAGGTGAAATTC	792-824	4
	KF255835	GTATCAGTATTCAGTAGTCAGAGGTGAAATTC	621-653	4
<i>C. tropicalis</i>	EF412966	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	819-851	3
	EU034726	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	862-894	3
	JQ008834	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	789-821	3
	KT449837	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	795-827	3
<i>C. krusei</i>	AB053239	GCATCAGTATTCAGTCGTCAGAGGTGAAATTC	830-862	3
	EF550360	GCATCAGTATTCAGTCGTCAGAGGTGAAATTC	796-828	3
	JF274497	GCATCAGTATTCAGTCGTCAGAGGTGAAATTC	809-841	3
	KU147485	GCATCAGTATTCAGTCGTCAGAGGTGAAATTC	591-623	3
<i>C. lusitaniae</i>	AY497762	GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	696-718	2
	FJ176816	GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	819-851	2
	JN941111	GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	844-876	2
	JQ698900	GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	808-840	2
<i>C. dubliniensis</i>	AY497766	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	722-754	3
	AY669334	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	293-325	3
	MF045510	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	322-354	3
	FM992695	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	1864232-1864264	3
<i>C. guilliermondii</i>	AB054282	GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	853-885	2
	AY227020	GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	811-843	2
	HG798649	GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	855-887	2
	KJ126853	GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	878-910	2

The base sequence differences between the IM Q-probes and the target regions are shown

in red.

**Table S2. The T<sub>m</sub> value database of the eight kinds of *Candida* species**

<i>Candida</i> species	T <sub>m</sub> degree (°C)		
	IM Q-probe 1	IM Q-probe 2	IM Q-probe 3
<i>C. albicans</i>	62.0	65.5	59.4
<i>C. glabrata</i>	-	55.0	57.8
<i>C. parapsilosis</i>	53.5	65.5	55.6
<i>C. tropicalis</i>	53.7	65.6	59.4
<i>C. krusei</i>	53.3	59.2	59.1
<i>C. lusitaniae</i>	-	59.3	62.4
<i>C. dubliniensis</i>	58.2	65.6	59.3
<i>C. guilliermondii</i>	50.3	65.4	62.2

Using the mean value of triplicate T<sub>m</sub> value measurements, we constructed a preliminary database of the eight kinds of *Candida* species, all of which are detected in our hospital.

**Table S3. Validation of the measurement errors among 10 samples using the same *Candida albicans* DNA template in the same trial**

Sample	Tm degree (°C)		
	IM Q-Probe 1	IM Q-Probe 2	IM Q-Probe 3
1	61.8	65.0	59.3
2	62.0	65.3	59.5
3	62.0	65.3	59.5
4	62.0	65.3	59.5
5	62.2	65.3	59.5
6	62.2	65.5	59.5
7	62.2	65.5	59.5
8	62.2	65.5	59.5
9	62.2	65.5	59.5
10	62.2	65.5	59.7
mean	62.1	65.37	59.5
SD	0.14	0.16	0.09
CV	0.002	0.002	0.002
Max	62.2	65.5	59.7
Min	61.8	65.0	59.3
Range	62.0 ± 0.2	65.25 ± 0.25	59.5 ± 0.2

**Table S4. The specificity with D values (calculated using the registered Tm values) among the eight kinds of *Candida* species in the database**

<i>Candida</i> species in the database	most similar <i>Candida</i> species	D Value
<i>C. albicans</i>	<i>C. dubliniensis</i>	3.11
<i>C. glabrata</i>	<i>C. lusitaniae</i>	0.21
<i>C. parapsilosis</i>	<i>C. tropicalis</i>	2.98
<i>C. tropicalis</i>	<i>C. parapsilosis</i>	2.98
<i>C. krusei</i>	<i>C. dubliniensis</i>	3.71
<i>C. lusitaniae</i>	<i>C. glabrata</i>	0.21
<i>C. dubliniensis</i>	<i>C. albicans</i>	3.11
<i>C. guilliermondii</i>	<i>C. tropicalis</i>	4.38

**Table S5. Validation of the limit of identification in triplicate trials using the same**

***C. albicans* DNA template**

CFU/ PCR Tube	Trial	Tm degree (°C)			D Value	Identification results
		IM Q-probe 1	IM Q-probe 2	IM Q-probe 3		
88	1st	62.5	66.5	60.0	0.374	<i>C. albicans</i>
	2nd	62.8	66.5	60.0	0.283	<i>C. albicans</i>
	3rd	62.7	66.0	60.0	0.141	<i>C. albicans</i>
1.75	1st	62.5	65.8	59.7	0.163	<i>C. albicans</i>
	2nd	63.0	66.5	60.0	0.327	<i>C. albicans</i>
	3rd	62.5	66.0	60.0	0.08	<i>C. albicans</i>
1.2	1st	62.7	66.0	59.8	0.22	<i>C. albicans</i>
	2nd	63.0	66.0	60.0	0.37	<i>C. albicans</i>
	3rd	63.0	66.0	60.0	0.37	<i>C. albicans</i>
0.88	1st	62.8	-	-	-	Not identified
	2nd	63.0	-	60.0	-	Not identified
	3rd	-	-	60.0	-	Not identified

CFU, colony forming unit

-: not detected



**Table S6. Sequence homology between the IM Q-probes and fungal target regions**

<i>Candida</i> species		Freq. (%)	IM Q-probe 1 (5'→3') CTTTCCTTCTGGGTAGCCATT	mismatch (bp)
<i>Candida albicans</i>	(AF114470)	42.1	CTTTCCTTCTGGGTAGCCATT	0
<i>Candida glabrata</i>	(AY083231)	26.7	CTTTCCTTCTGGCTA <b>ACCCAA</b>	6
<i>Candida parapsilosis</i>	(AB030915)	15.9	CTTTCCTTCTGGCTAGCC <b>TTTT</b>	2
<i>Candida tropicalis</i> * <sup>1</sup>	(KT449837)	8.7	CTTTCCTTCTGGCTAGCC <b>TTTT</b>	2
<i>Candida krusei</i>	(AB053239)	3.4	CTTTCCTTCTGGCTAGCC <b>CTCG</b>	4
<i>Candida lusitaniae</i>	(KU147480)	1.1	CTTTCCT <b>CCTCCTTAGCAAT</b>	12
<i>Candida dubliniensis</i>	(AY497766)	0.9	CTTTCCTTCTGGCTAGCCATT	1
<i>Candida guilliermondii</i> * <sup>2</sup>	(AY497770)	0.4	CTTTCCTTCTGGCTA <b>ACCATT</b>	3
<i>Candida fermentati</i> * <sup>2</sup>	(AY553853)	0.8	CTTTCCTTCTGGCTA <b>ACCATT</b>	3
<i>Candida inconspicua</i>	(AB053243)		CTTTCCTTCTGGCTAGCC <b>TCG</b>	4
<i>Candida kefyri</i>	(AB054675)		CTTTCCTTCTGGCTA <b>ACCTGT</b>	4
<i>Candida nivariensis</i>	(AY727047)		CTTTCCTTCTGGCTA <b>ACCCAA</b>	6
<i>Candida norvegensis</i>	(AB053237)		CTTTCCTTCTGGCTAGCC <b>TCC</b>	5
<i>Candida orthopsilosis</i> * <sup>1</sup>	(HQ215535)		CTTTCCTTCTGGCTAGCC <b>TTTT</b>	2
<i>Candida pelliculosa</i>	(AB054562)		CTTTCCTTCTGGCTA <b>ACCTGT</b>	5
<i>Candida sake</i>	(AB013529)		CTTTCCTTCTGGGT <b>AACCTTC</b>	3
<i>Candida zeylanoides</i> * <sup>2</sup>	(AB013509)		CTTTCCTTCTGGCTA <b>ACCATT</b>	3

<i>Candida</i> species		Freq. (%)	IM Q-probe 2 (5'→3') TGGAATAATAGAATAGGACGTTATGGTTC	mismatch (bp)
<i>Candida albicans</i>	(AF114470)	42.1	TGGAATAATAGAATAGGACGTTATGGTTC	0
<i>Candida glabrata</i>	(AY083231)	26.7	TGGAATAAT <b>GGAATAGGACGTT</b> -TGGTTC	2
<i>Candida parapsilosis</i>	(AB030915)	15.9	TGGAATAATAGAATAGGACGTTATGGTTC	0
<i>Candida tropicalis</i> * <sup>1</sup>	(KT449837)	8.7	TGGAATAATAGAATAGGACGTTATGGTTC	0
<i>Candida krusei</i>	(AB053239)	3.4	TGGAATAATAGAATAGGAC <b>G</b> -ATGGTTC	2
<i>Candida lusitaniae</i>	(KU147480)	1.1	TGGAATAATAGAATAGGAC <b>G</b> -ATGGTTC	2
<i>Candida dubliniensis</i>	(AY497766)	0.9	TGGAATAATAGAATAGGACGTTATGGTTC	0
<i>Candida guilliermondii</i> * <sup>2</sup>	(AY497770)	0.4	TGGAATAATAGAATAGGACGTTATGGTTC	0
<i>Candida fermentati</i> * <sup>2</sup>	(AY553853)	0.8	TGGAATAATAGAATAGGACGTTATGGTTC	0
<i>Candida inconspicua</i>	(AB053243)		TGGAATAATAGAATAGGACGTTATGGTTC	0
<i>Candida kefyri</i>	(AB054675)		TGGAATAAT <b>GGG</b> ATAGGACGT <b>-TTGGTTC</b>	4
<i>Candida nivariensis</i>	(AY727047)		TGGAATAAT <b>GGAATAGGACGT</b> - <b>TTGGTTC</b>	3
<i>Candida norvegensis</i>	(AB053237)		TGGAATAATAGAATAGGACGTTATGGTTC	0
<i>Candida orthopsilosis</i> * <sup>1</sup>	(HQ215535)		TGGAATAATAGAATAGGACGTTATGGTTC	0
<i>Candida pelliculosa</i>	(AB054562)		TGGAATAAT <b>GGAATAGGACGT</b> - <b>TTGGTTC</b>	3
<i>Candida sake</i>	(AB013529)		TGGAATAATAGAATAGGACGTTATGGTTC	0
<i>Candida zeylanoides</i> * <sup>2</sup>	(AB013509)		TGGAATAATAGAATAGGACGTTATGGTTC	0

<i>Candida</i> species		Freq. (%)	IM Q-probe 3 (5'→3') GCATCAGTAATCAGTTGTCAGAGGAGAAATTC	mismatch (bp)
<i>Candida albicans</i>	(AF114470)	42.1	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	3
<i>Candida glabrata</i>	(AY083231)	26.7	GCATCAGTATTCATTGTCAGAGGTGAAATTC	3
<i>Candida parapsilosis</i>	(AB030915)	15.9	GTATCAGTATTCAGTAGTCAGAGGTGAAATTC	4
<i>Candida tropicalis</i> * <sup>1</sup>	(KT449837)	8.7	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	3
<i>Candida krusei</i>	(AB053239)	3.4	GCATCAGTATTCAGTCGTCAGAGGTGAAATTC	3
<i>Candida lusitanae</i>	(KU147480)	1.1	GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	2
<i>Candida dubliniensis</i>	(AY497766)	0.9	GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	3
<i>Candida guilliermondii</i> * <sup>2</sup>	(AY497770)	0.4	GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	2
<i>Candida fermentati</i> * <sup>2</sup>	(AY553853)	0.8	GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	2
<i>Candida inconspicua</i>	(AB053243)		GCATCAGTATTCAGTCGTCAGAGGTGAAATTC	3
<i>Candida kefyr</i>	(AB054675)		GCATCAGTATTCATTGTCAGAGGTGAAATTC	3
<i>Candida nivariensis</i>	(AY727047)		GCATCAGTATTCATTGTCAGAGGTGAAATTC	3
<i>Candida norvegensis</i>	(AB053237)		GCATCAGTATTCAGTCGTCAGAGGTGAAATTC	3
<i>Candida orthopsilosis</i> * <sup>1</sup>	(HQ215535)		GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	3
<i>Candida pelliculosa</i>	(AB054562)		GCATCAGTATTCATTGTCAGAGGTGAAATTC	3
<i>Candida sake</i>	(AB013529)		GTATCAGTATTCAGTTGTCAGAGGTGAAATTC	3
<i>Candida zeylanoides</i> * <sup>2</sup>	(AB013509)		GCATCAGTATTCAGTTGTCAGAGGTGAAATTC	2

The base sequence differences between the IM Q-probes and the target regions are shown in red.

\*<sup>1</sup>*C. tropicalis* and *C. orthopsilosis* cannot be distinguished using the IM Q-probe method.

\*<sup>2</sup>*C. guilliermondii*, *C. fermentati* and *C. zeylanoides* cannot be distinguished using the IM Q-probe method.

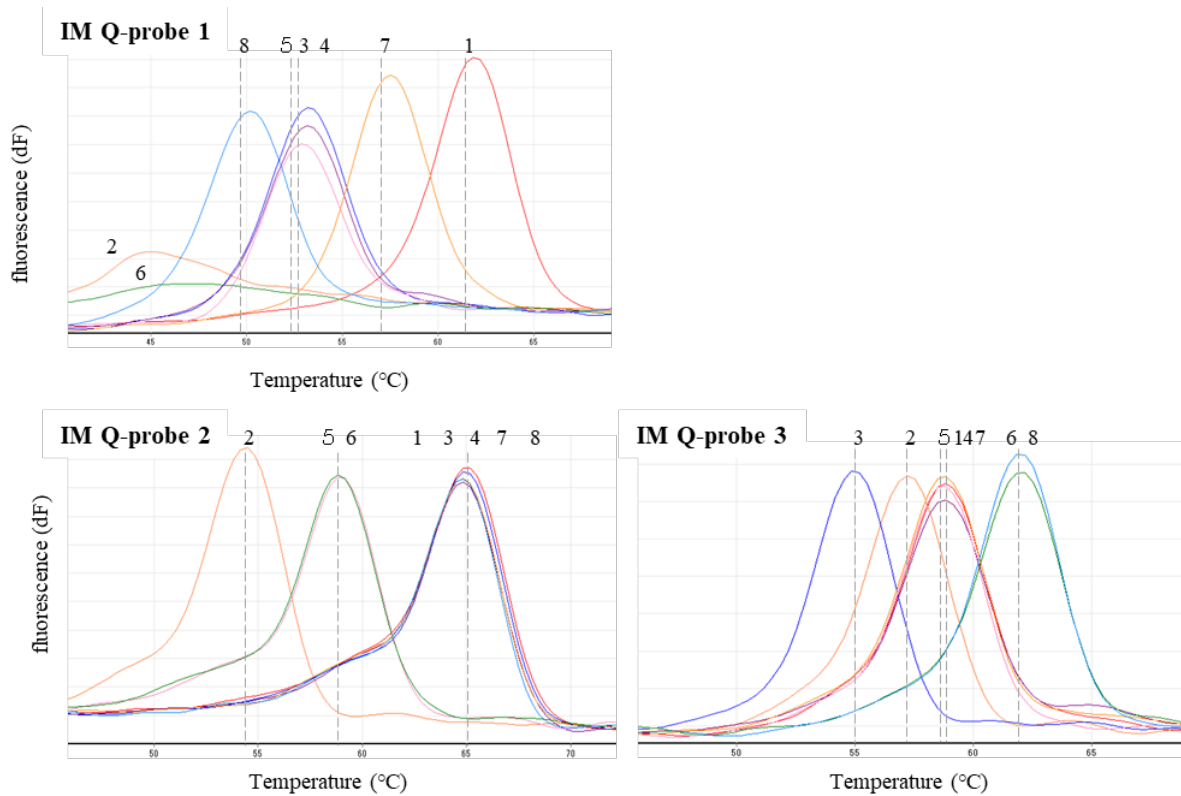
Freq. = The frequency of *Candida* species causing candidemia<sup>10</sup>

**Table S7. A Tm value analysis of genus *Aspergillus* and genus *Cryptococcus* using the IM Q-probe method**

Fungi	Tm degree (°C)			most similar <i>Candida</i> species	D Value
	IM Q-probe 1	IM Q-probe 2	IM Q-probe 3		
<i>Aspergillus fumigatus</i>	-	56.7	55	<i>Candida glabrata</i>	3.18
<i>Cryptococcus neoformans</i>	-	56.5	45.2	<i>Candida glabrata</i>	9.97

-: not detected

**Figure S1. T<sub>m</sub> value variations among eight kinds of *Candida* species with each IM Q-probe**



The T<sub>m</sub> value variations depend on the number and position of the probe-target mismatches.

- 1) *C. albicans*, 2) *C. glabrata*, 3) *C. parapsilosis*, 4) *C. tropicalis*, 5) *C. krusei*, 6) *C. lusitaniae*,  
 7) *C. dubliniensis*, 8) *C. guilliermondii*