MATCH biosystems, S.L. B01642826
Quórum IV - Parque Científico UMH
Avd de la Universidad, s/n
03202 Elche (Alicante, ESPAÑA)

MATCH Candida Dx QUICK PROTOCOL

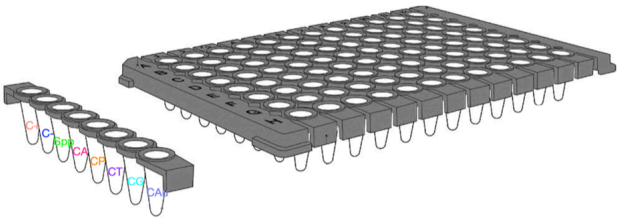
MATERIALS AND EQUIPMENT NEEDED

1. DNA extraction (at least 250 µl)
2. 1x MATCHCAN 01
3. 1x MATCHCAN 02 (pink cap)
4. 1x MATCHCAN 03 (blue cap)
5. 1x MATCH 04 (clear cap)
6. Optical film
7. Vortex
8. Centrifugue
9. Real Time thermocycler

PROTOCOL FOR POSITIVE GENOMIC CONTROL

MATCH Candida Dx comes with a positive (genomic) control (MATCHCAN 03) that simulates a sample with genomic DNA of *Candida albicans.* This control should be used in case there is any doubt that the kit is performing as it should.

1. Add 200 µl of MATCH 04 into the MATCHCAN 03 tube. Vortex and spin down.
2. Add 100 µl of MATCH 04 into the MATCHCAN 02 tube. Vortex and spin down.
3. Add 20 µl of the MATCH 04 tube into the second well of the strip. This is the negative control (NTC). See Figure 1.



Well	Configuration CAu	Configuration CK
A	C +	C +
B	C -	C -
C	Spp.	Spp.
D	CA	CA
E	CP	CP
F	CT	CT
G	CG	CG
H	CAu	CK

Figure 1: MATCHCAN 01 well

4. Add 20 µl of the resuspended MATCHCAN 02 into the first well of the strip. This is the positive control of the kit.
5. Add 20 µl of MATCHCAN 03 into the rest of the wells of the kit.
6. Close the strip with the optical film.
7. Vortex for 15 seconds and spin down.
8. Program your thermocycler as follows:

Table1: Thermal cycling conditions

STEP	TEMPERATURE	TIME	REPEATS
Initial denaturalization	95ºC	3 minutes	1
Denaturalization	95ºC	10 seconds	35
Annealing and extension (plate reading FAM/SYBR channel)	60ºC	25 seconds	
Melt curve (plate reading FAM/SYBR channel)	65-95ºC	Increment of 0.5ºC every 0:05	1

9. Enter the tubes and wait for the program to end to see the results.

PROTOCOL FOR PATIENT SAMPLES

1. Add 100 µl of the DNA extraction into MATCHCAN 02. Vortex and spin down.
2. Add 20 µl of the elution buffer used for the DNA extraction into the C- well (see Figure 1).
3. Add 20 µl of MATCHCAN 02 into the C+ well. We will use this control to see if there is any inhibition.
4. Add 20 µl of the rest of the DNA sample into the rest of the wells.
5. Close the strip with the optical film.
6. Vortex for 15 seconds and spin down.
7. Program your thermocyler as described in table 1.
8. Enter the tubes and wait for the program to end to see the results.

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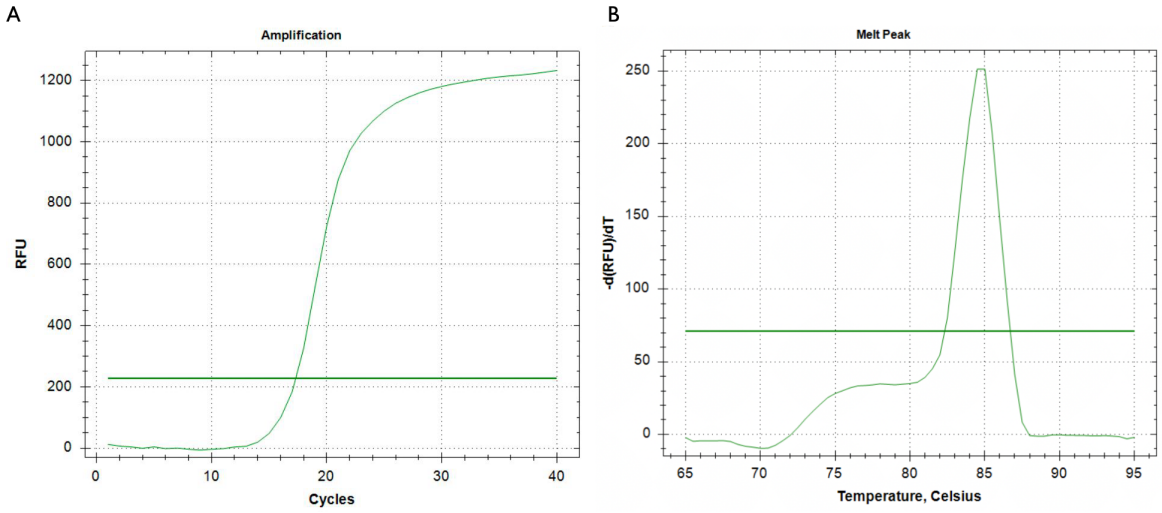
INTERPRETATION OF RESULTS

A reaction in a well is considered positive if a fluorescence signal with a rising curve is observed during amplification (Figure 2) (A), and the melting curve derivative shows a peak between 80°C and 90°C (B). On the contrary, if the amplification curve is not observed or the temperature of melting products doesn't contain at least 1 peak in the defined range, it indicates a negative reaction.

For a test to be valid, it is mandatory that the positive control yields a positive result with a CT value less than 16, and the negative control yields a negative result. If the negative control is reported as positive, it is likely due to a handling error. If a CT greater than 20 is observed in the positive control, it indicates the presence of a PCR inhibitor, and the test is considered invalid. In both cases, it is recommended to retest.

Figure 2: Positive result for the MATCH Candida Dx test



In the following table there are some examples of different interpretations of the MATCH Candida Dx kit.

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Table 2: Different interpretations of the MATCH Candida Dx kit.

CP	CN	Spp	CA	CP	CT	CG	CAu/CK	Interpretation
+	-	-	-	-	-	-	-	Absence of any Candida specie
+	-	+	+	-	-	-	-	Positive for *C. albicans*
+	-	+	-	+	-	-	-	Positive for *C. parapsilosis*
+	-	+	-	-	-	-	-	Positive for another *Candida spp.*
-	-/+							Positive control reported as negative. Probable PCR inhibition
+/-	+	+/-						Negative control reported as positive. Handling error, contamination