# New molecular approach in the diagnosis of bloodstream infections

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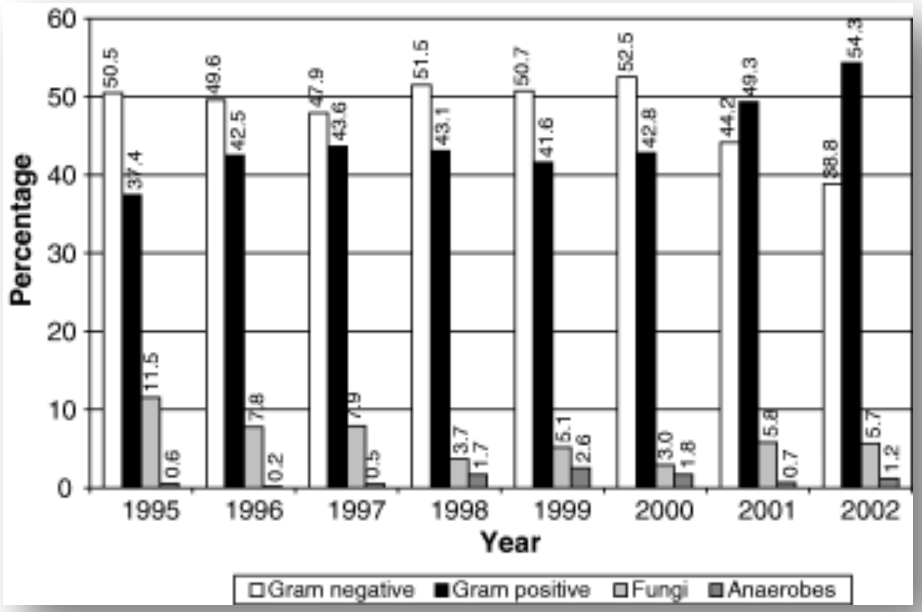
# The most frequent causative agents of sepsis

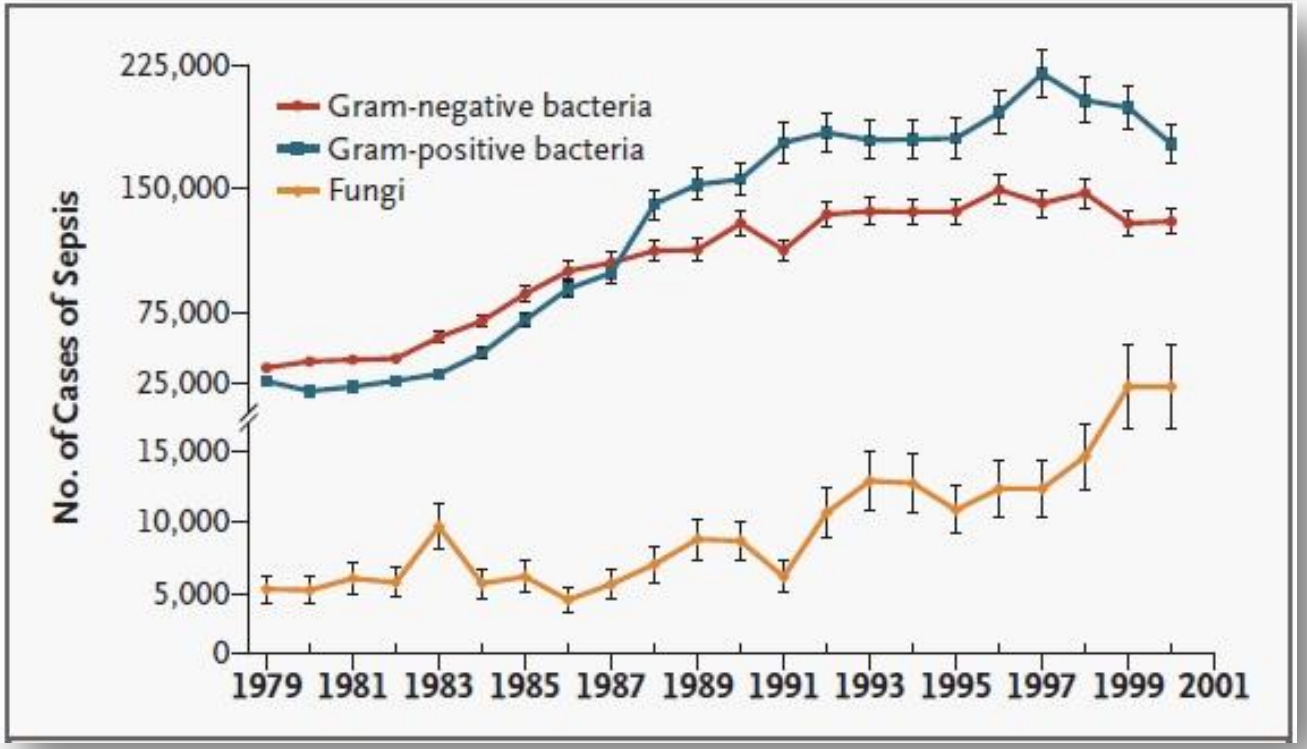



*A. S. Hadziyannis et al., 2003*

| **Pathogenity** | | **Incidence** |
|---|---|---|
| *Candida* | *C. albicans* | 42% |
| | *C. glabrata* | |
| | *C. tropicalis* | |
| | *C. parapsilosis* | |
| | *C. krusei* | |
| | *C. lusitaniae* | |
| *Aspergillus* | | 29% |
| Other mold | | 14% |
| Non identified | | 6% |
| *Cryptococcus* | | 4% |
| Endemic fungi | | 3% |
| *Pneumocystis jiroveci* | | 2% |

*J. Loeffler et al., 2000*

# Numbers of cases of sepsis in the United States, according to the causative organisms

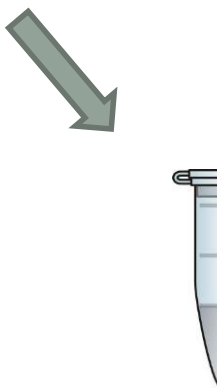


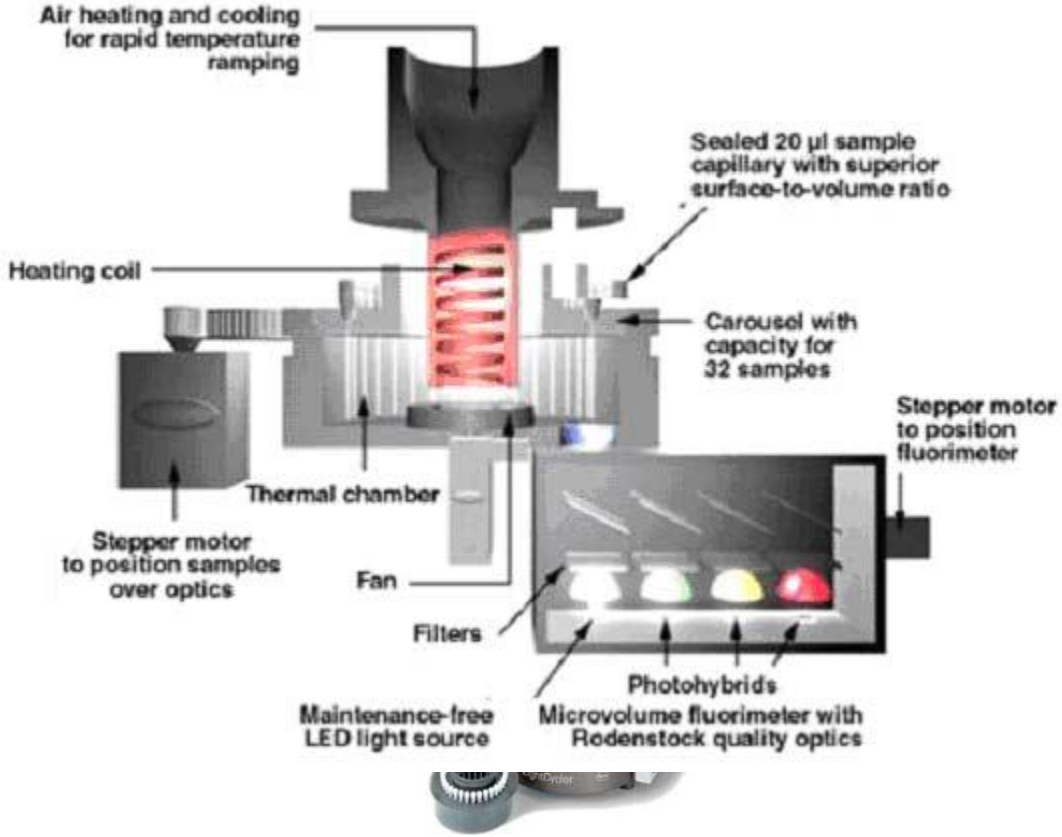

*Greg S. Martin et al., 2003*

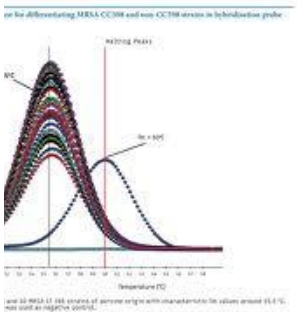
# Recent approaches in the diagnosis of sepsis

| Method | Time (h) | Instrumentation | Cost | Adaptibility |
|---|---|---|---|---|
| Blood culture | 32-48 | + | + | +++ |
| Molecular technics from blood | | | | |
| **1. FISH** | 2-3 | ++ | ++ | + |
| **2. PCR/LCR/NASBA** | 28-34 | ++ | ++ | + |
| **3. Real Time PCR** | 1-3 | ++ | ++ | + |
| Molecular from blood | | | | |
| **1. PCR+sequencing** | 8 | +++ | +++ | + |
| **2. Real Time PCR** | **1-3** | ++ | ++ | **+++** |

# Real Time PCR + Fluorescence Resonance Energy Transfer

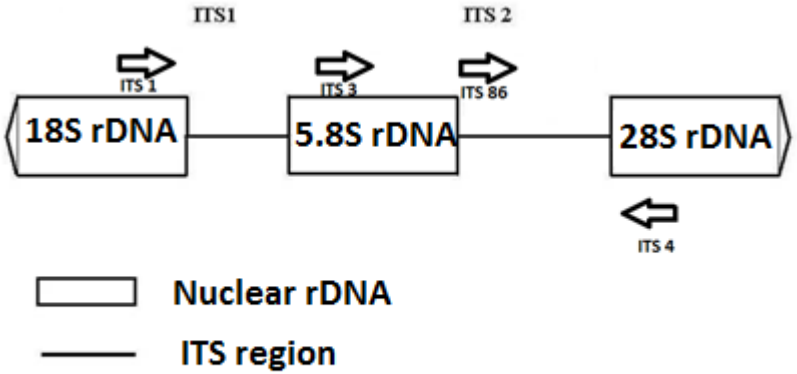




# Differentiation of the fungal and bacterial pathogenes

- Fungal primer

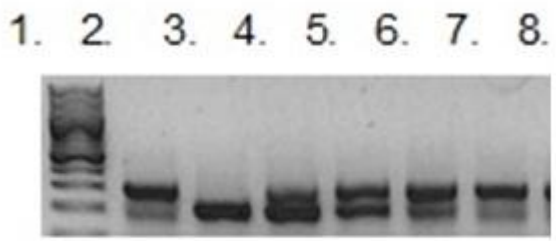



- Bacterial primer

- Primers PLK1 PLK2 are highly conserved in different groups of eubacteria.
- The primers amplify conserved regions of the bacterial 16S rRNA gene.

*Somogyvári et al. 2005*

~300 bp

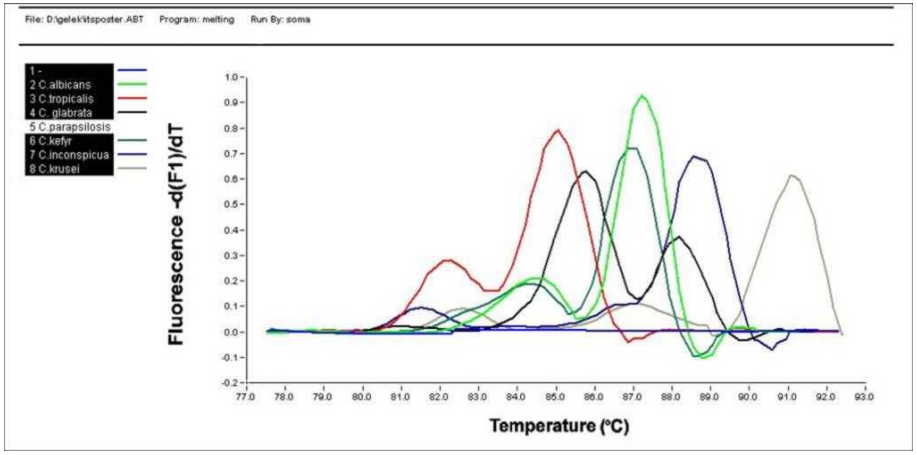



1.100 bp ladder; 2. *C. alb*; 3. *S. aur*; 4. *S.aur+C. alb.*;5. *S.aur 10x+C. alb.*;6.*S.aur 100+C.alb.*; 7. *S.aur 1000x+C.alb.*;8. *S.aur 10000x+C.alb.,*

187 bp

# Melting peaks of the fungal pathogenes

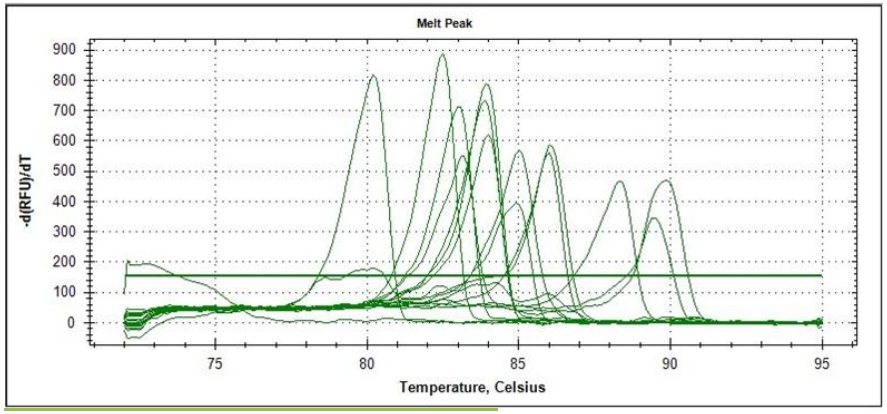
## Melting Point Analysis




**Amplification with ITS86; ITS4 primer pair.**

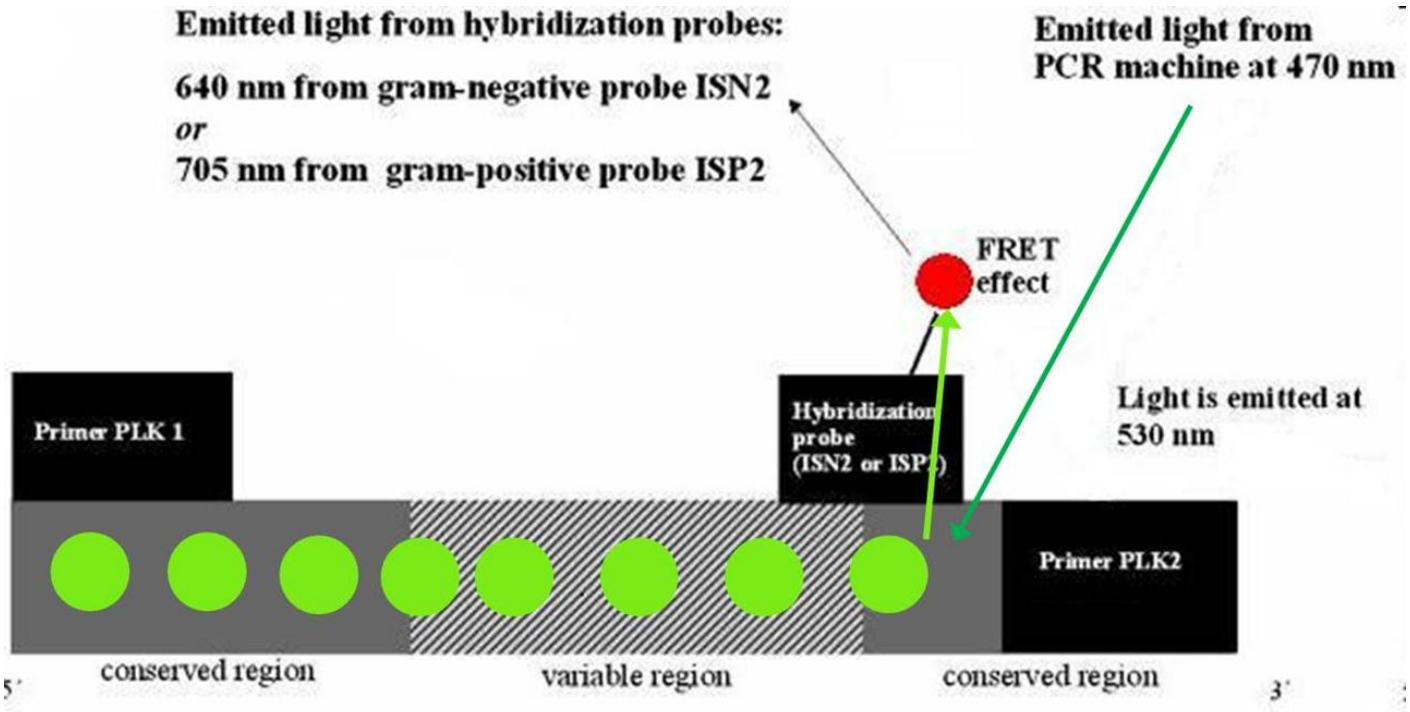
**Ctrl blue; *C. albicans* green; *C. tropicalis* red; *C. glabrata* black; *C. kefyr* dark green; *C. inconspicua* purple; *C. krusei* grey.**

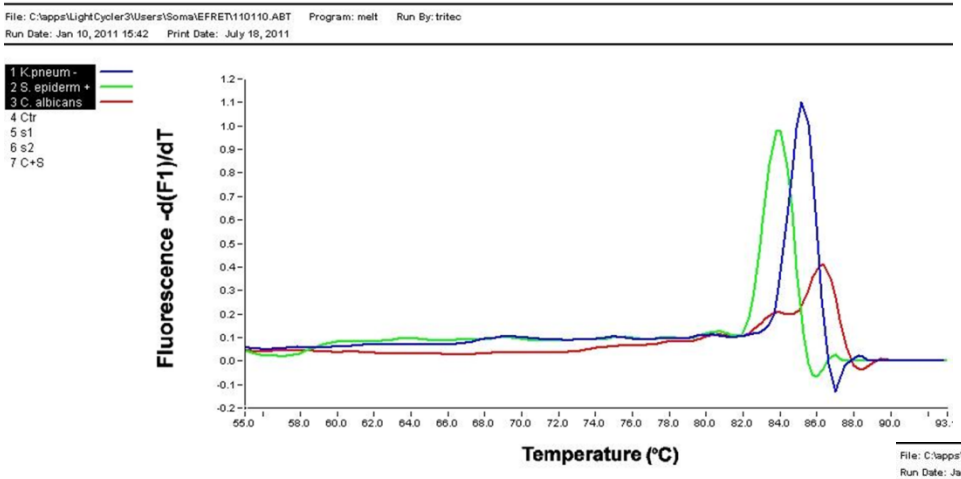
## High Resolution Melting Point Analysis (HRM)



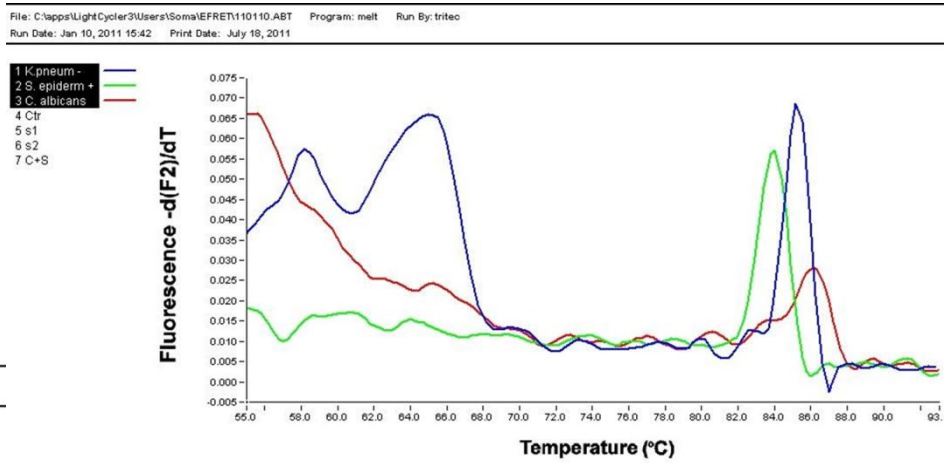

| Fugal strains | Melting Point of the amplicon (°C) ± 0.05 °C |
|---|---|
| *Mucor mucedo* | 80,7 |
| *Candida tropicalis* | 81,8 |
| *C. parapsilosis* | 82,6 |
| *C. guilliermondii* | 82,8 |
| *C. glabrata* | 83,2 |
| *C. dubliniensis* | 83,5 |
| *Cryptococcus neoformans* | 84,1 |
| *C. albicans* | 84,5 |
| *C. lusitaniae* | 85,4 |
| *Fusarium oxysporum* | 85,4 |
| *C. norvegensis* | 86,2 |
| *C. inconspicua* | 86,4 |
| *C. krusei* | 88,6 |
| *Aspergillus niger* | 89,6 |
| *A. flavus* | 90,4 |
| *A. fumigatus* | 90,9 |
| *A. terreus* | ND |

# Gram strain classification with FRET

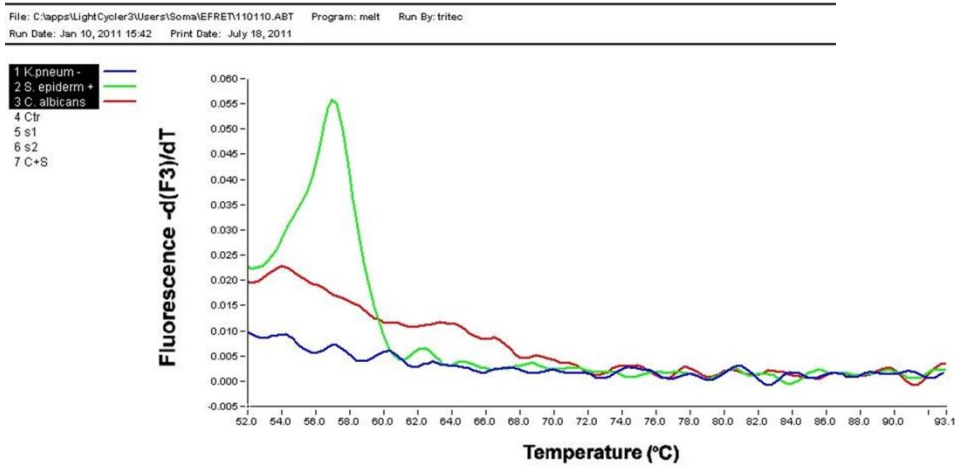





F1 channel - Candida




F2 channel G-




F3 channel G+

# Summary

- We applied the fungal PCR for the detection of the bacterias.
- The sensitivity of the PCR was less than 5 CFU/ml. The multiplex reaction is working with extrem dilution of the templates.
- The Melting Point Analysis and High Resolution Melting Point Analysis were approriate to discriminate the most common pathogen fungus.
- All bacteria tested were correctly classified as gram positive or gram negative with FRET

# Further aims:

- Identification of all bacterial pathogens
- We would like to perform a Real Time PCR without DNA purification from whole blood or serum - and save more time
- Grow up the reliability of the method with internal control.

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NEW SZÉCHENYI PLAN

HUNGARY'S RENEWAL

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# THANK YOU FOR YOUR ATTENTION!

# DZIĘKUJĘ ZA UWAGĘ!