Future of Molecular Diagnostics in Clinical Microbiology

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Diagnostic Microbiology is changing!

Treat patients efficiently

Treat patients efficiently

Control the antimicrobial resistance

Prudent (restricted) use of antibiotics

Prudent (restricted) use of antibiotics

Fast and specific diagnostics

Prudent (restricted) use of antibiotics

Clinical impact of diagnostics

Fast and specific diagnostics

Diagnostic impact on treatment:
("window of opportunity")

- either VERY fast
- or VERY high impact

Diagnostic Challenges

- **Reducing Mortality - sepsis**
- **Point-Of-Care Testing**
- **Choice of Technology**

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Reducing Mortality

- **Initiation of efficient antimicrobial therapy correlates to survival of severe infectious diseases – e.g. sepsis**
- **Current gold standard diagnostics are neither fast nor sensitive**

Diagnostic tools in Sepsis

- **Blood culture**
- **Molecular diagnostics**
- **Early sepsis markers**
- **Clinical decision software**

Diagnostics in Sepsis
Blood culture

- **Gold standard**
- **Proven technology**
- **Advantage:**
  - **Species diagnostics**
  - **AST**
- **Disadvantage:**
  - **False negatives (e.g. antibiotics, difficult-to-grow…)**
  - **Time delay**
  - **Improvement?**

Diagnostics in Sepsis
Molecular Diagnostics: DNA

- **Microbial DNA ("DNAemia"):**
  - **Live microorganisms**
  - **Dead microorganisms**
  - **Phagocytized microorganisms**

Roche Septi*Fast*

Multicenter evaluation compared to blood culture
(Bergamo, Copenhagen, Frankfurt, London, Lyon)

Septi*Fast* Panel

Gram (-)

- Escherichia coli
- Klebsiella pneumoniae/oxytoca
- Serratia marcescens
- Enterobacter cloacae/aerogenes
- Proteus mirabilis
- Pseudomonas aeruginosa
- Acinetobacter baumannii
- Stenotrophomonas maltophilia

Gram (+)

- Staphylococcus aureus
- CoNS (S. epidermidis/haemolyt.)
- Strep. pneumoniae
- Strep. spp
- Enterococcus faecium
- Enterococcus faecalis

Fungi

- Candida albicans, tropicalis & parapsilosis
- Candida krusei & glabrata
- Aspergillus fumigatus

Gorm Lisby

SeptiFast - all sites

- **558 episodes (from 359 patients) with corresponding BC and Septi*Fast***
- **183 episodes with one or both systems positive**
- **96 BC isolates in 96 episodes**
- **186 Septi*Fast* isolates in 144 episodes**
- **232 isolates in 183 episodes with combined testing**

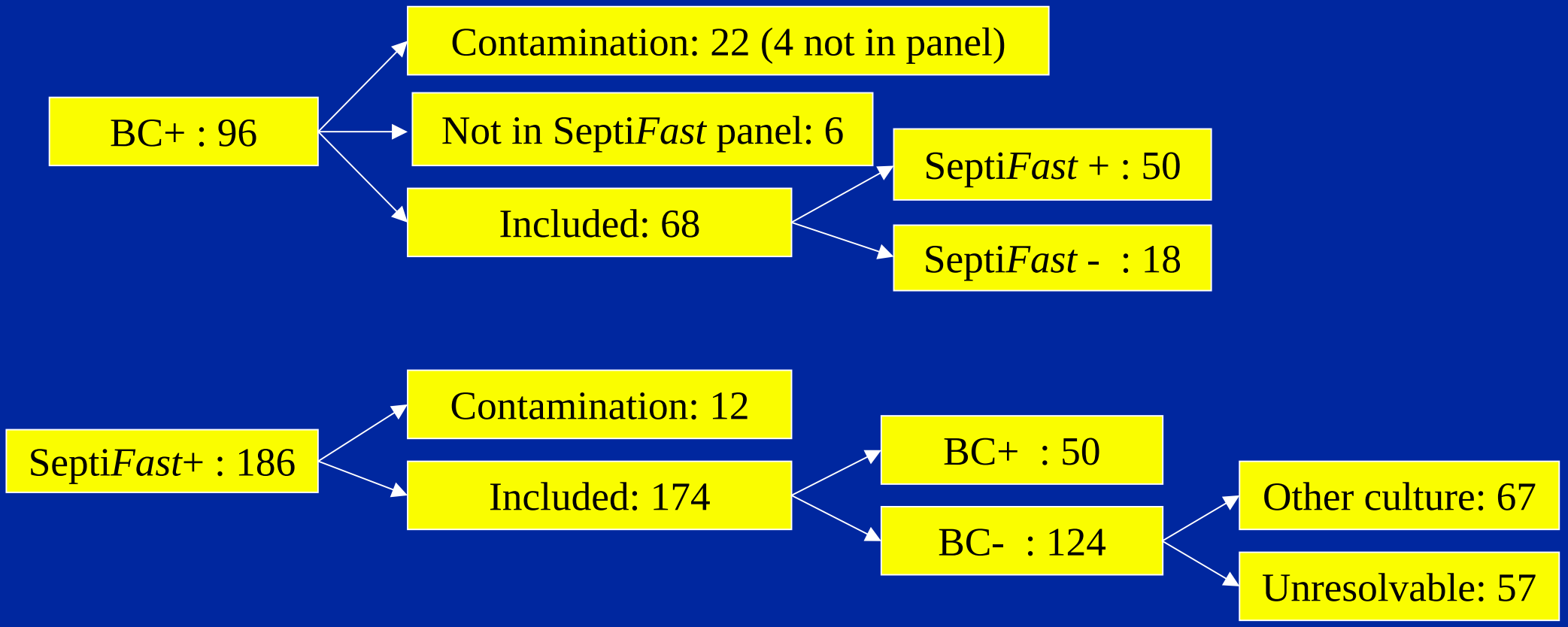
Low level contamination & Invalid PCR

- **Septi*Fast*: Low level contamination**
  - **Coagulase negative Staphylococci 57 episodes**
  - **Streptococci spp 2 episodes**
  - **Total low level contamination rate 10.6%**

- **Septi*Fast*: Invalid PCR**
  - **Negative Internal control (70 episodes) 12.5%**

All sites - isolate information

558 episodes with corresponding BC and Septi*Fast*
183 episodes with one or both systems positive



Pathogens detected

Pathogens	number of isolates detected by			
	SF only	BC only	Any system	Both systems
Total	124	24	198	50
Staphylococcus aureus	20	0	32	12
Escherichia coli	16	1	27	10
Candida albicans	13	2	17	2
Aspergillus fumigatus	12	0	12	0
Klebsiella pneumoniae / oxytoca	10	1	11	0
Stenotrophomonas maltophilia	10	0	12	2
Streptococcus spp.	9	2	16	5
Enterococcus faecium	8	4	14	2
Enterobacter aerogenes / cloacae	8	0	8	0
Enterococcus faecalis	7	2	14	5
Pseudomonas aeruginosa	5	1	6	0
Staphylococcus spp. (coagulase-negative)	2	1	11	8
Streptococcus pneumoniae	2	1	3	0
Candida parapsilosis	2	3	7	2
Serratia marcescens	0	0	1	1
Candida tropicalis	0	0	1	1
Other bacteria (not in PCR panel)	0	6	6	0

The truth

How do we define “the truth”:

Do we trust all Septi*Fast* results
(“Septi*Fast*” friendly truth)

or

Only the culture-confirmed Septi*Fast* positives
(“Acceptable truth”)

One kind of truth

"Septi*Fast* friendly truth"

All positive uncontaminated Septi*Fast* (174)
OR Septi*Fast* negative but BC positive (18)
OR BC positive but not in Septi*Fast* panel (6)

Sensitivity BC: 37.4%
Sensitivity Septi*Fast*: 87.9%

Another kind of truth

“Acceptable truth”

Positive in both systems (50)

OR Septi*Fast* positive but BC negative but confirmed by other culture (67)

OR uncontaminated BC positive but Septi*Fast* negative OR BC positive but not in Septi*Fast* panel (24)

Sensitivity BC: 52.5%

Sensitivity Septi*Fast*: 83.0%

Diagnostics in Sepsis
Molecular Diagnostics: DNA

- **Healthy individuals do not contain microbial DNA in the bloodstream (above cut-off)**

- **Presence of microbial DNA in the blood:**
  - **Information concerning effectiveness of current (empiric) antibiotic therapy**
  - **Information regarding site of focus**

Diagnostic Technology
Molecular Sepsis Diagnostics – who will get it?

- **Not all patients getting BC will get Molecular**
- **Subset of patients needs to be defined**
  - **Early sepsis markers**
  - **Clinical SIRS / sepsis**
  - **Computer based decision system**
- **Indications will later be expanded**

Diagnostic Challenges

- Reducing Mortality - sepsis
- **Point-of-Care Testing**
- Choice of Technology

Point-of-Care Testing

- **A scary scenario?**
- **What will happen to my lab?**
- **How can I influence the development?**

Point-of-Care Testing
Turn-Around-Time

- **Turn-Around-Time (TAT)**
  - **Can be directly influenced by the lab**
- **And what about *total* turn-around-time (*T*-TAT)?**
  - **Sampling (2 min -> 4 hrs)**
  - **Transport (10 min -> 24 hrs)**
  - ***Laboratory TAT (24 hrs -> 2 min)***
  - **Action upon result at bedside (10 min -> never!)**

Point-of-Care Testing

- **Will dramatically improve *T*-TAT:**
  - **No delay due to transport**
  - **Impact on patient treatment is high**
- **No doubt important in future diagnostics, but…..**

Point-of-Care Testing
Problems

- **No “professional” guidance**
- **Severe vs. “trivial” disease**
  - **ID/Clin micro vs. all MD´s – e.g. HIV vs. Strep A**
- **Hospital vs. out-patient clinic**
- **Sensitivity vs. specificity**
  - **Consequences of false positives– e.g. Chlamydia vs. Strep A**
- **AST and resistance**
  - **Interpretation – e.g. UTI vs. Strep A**
  - **Surveillance of resistance?**

Point-of-Care Testing
Future

- **Important “piece of the diagnostic puzzle”**
- **POC used wrong:**
  - **Excessive use of antibiotics**
  - **Increased resistance**
- **POC used right:**
  - **Decreased *T*-TAT**
  - **Controlled use of antibiotics**
  - **Control of resistance**

Point-of-Care Testing
Future Organization

- **Organization of Clinical Microbiology:**
  - **Current: Referral → Local**
  - **Future: Referral → Centralized → Local → POC**

Diagnostic Challenges

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Choice of Technology

Molecular Target/Signal Amplification

- **Today: PCR**
- **Tomorrow: ?**

Choice of Technology

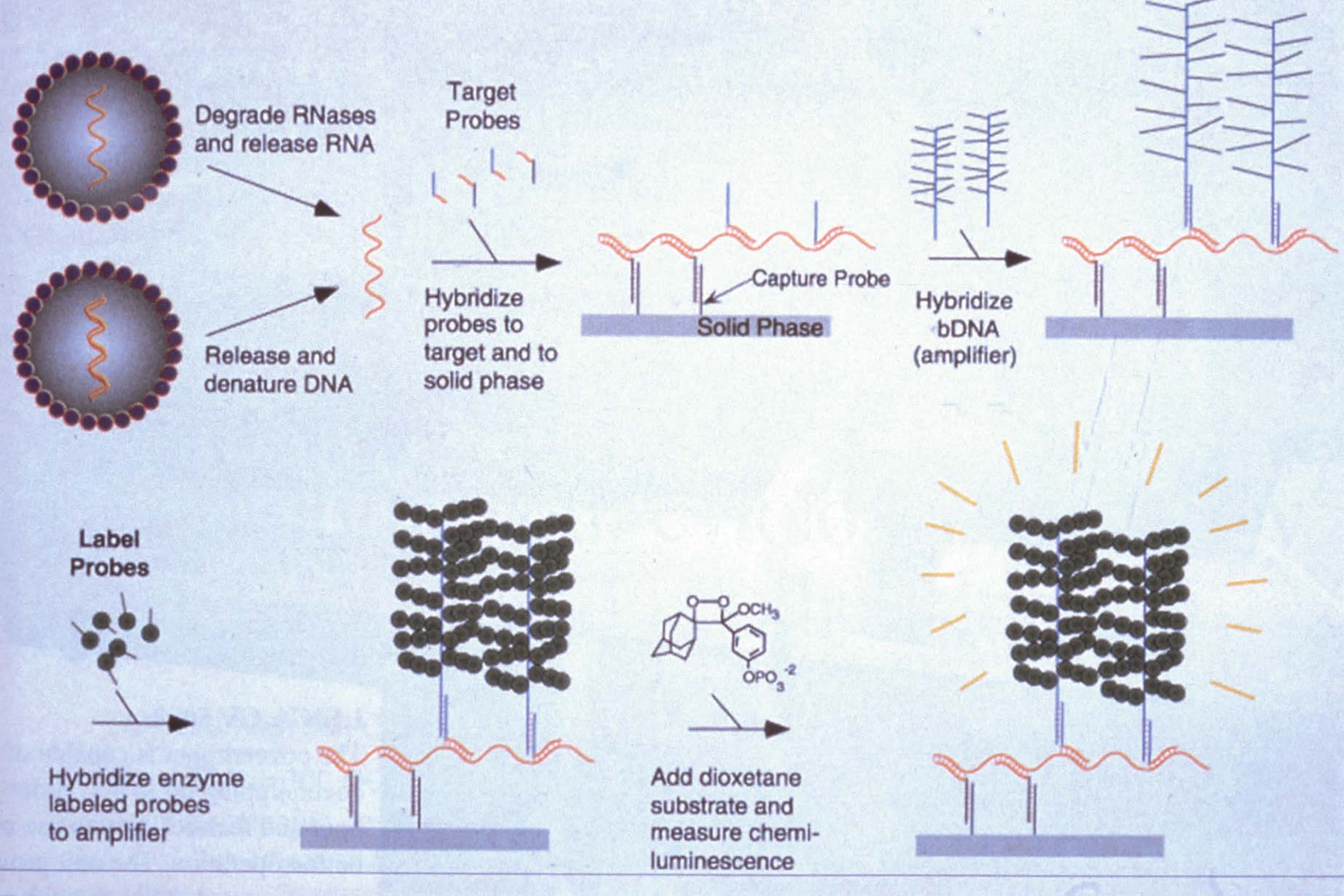
Molecular Target/Signal Amplification

- **Why amplify target?**
- **Because we have to !**

Choice of Technology

Future of Amplification

- **Signal Amplification**


Degrade RNases and release RNA
Release and denature DNA
Target Probes
Hybridize probes to target and to solid phase
Capture Probe
Solid Phase
Hybridize bDNA (amplifier)
Label Probes
Hybridize enzyme labeled probes to amplifier
OCH3
OPO3-2
Add dioxetane substrate and measure chemi-luminescence

Choice of Technology

Future Platforms

- Lab-in-a-well
- Lab-on-a-bead
- Lab-in-a-tube
- Lab-on-a-chip

Choice of Technology

Nucleic Acids

- **DNA or RNA**
- **or Artificial Nucleic Acids**

Choice of Technology

Nucleic Acids - Applications

- **Conservation of genetic information** (>3,500,000 yrs – success)
- **Controlling genetic activity** (>3,500,000 yrs – success)
- **Diagnosis** (approx 20 yrs – limited success)
- **Treatment** (not yet applied)

Choice of Technology

Natural Nucleic Acids - Limitations

- DNA double helix structure needs to uncoil
  - DNA-RNA binding must be reversible

Choice of Technology

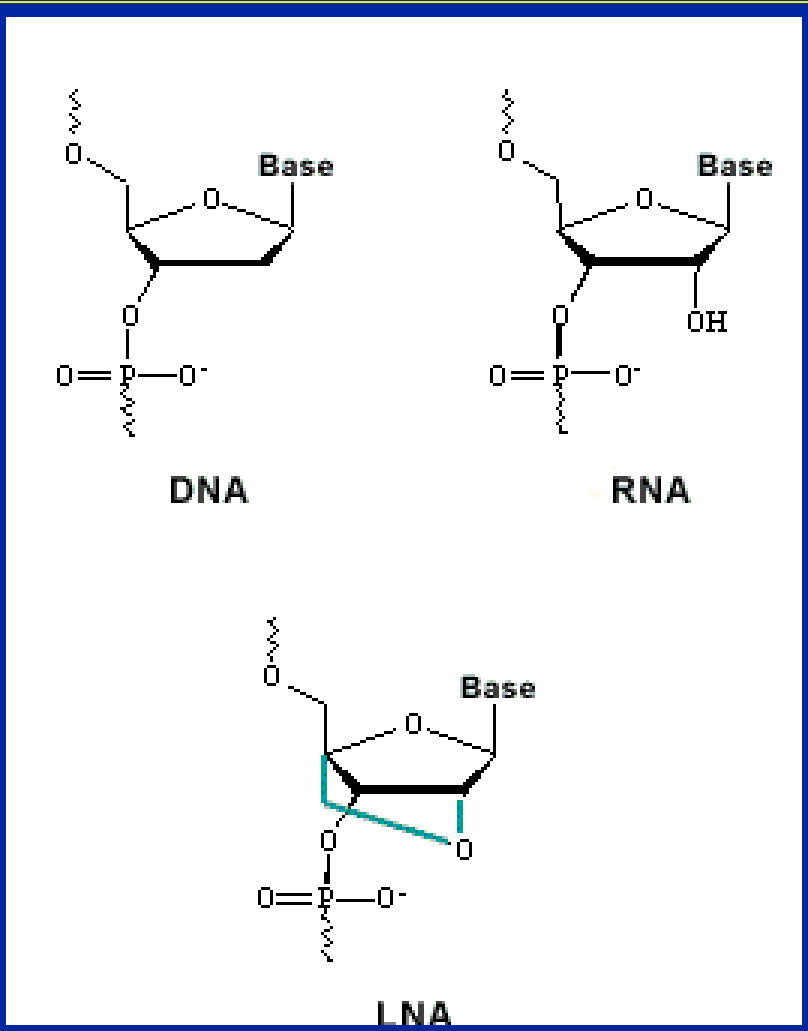
Nucleic Acids - Future

Artificial Nucleic Acids

(PNA, LNA, TINA, XNA)

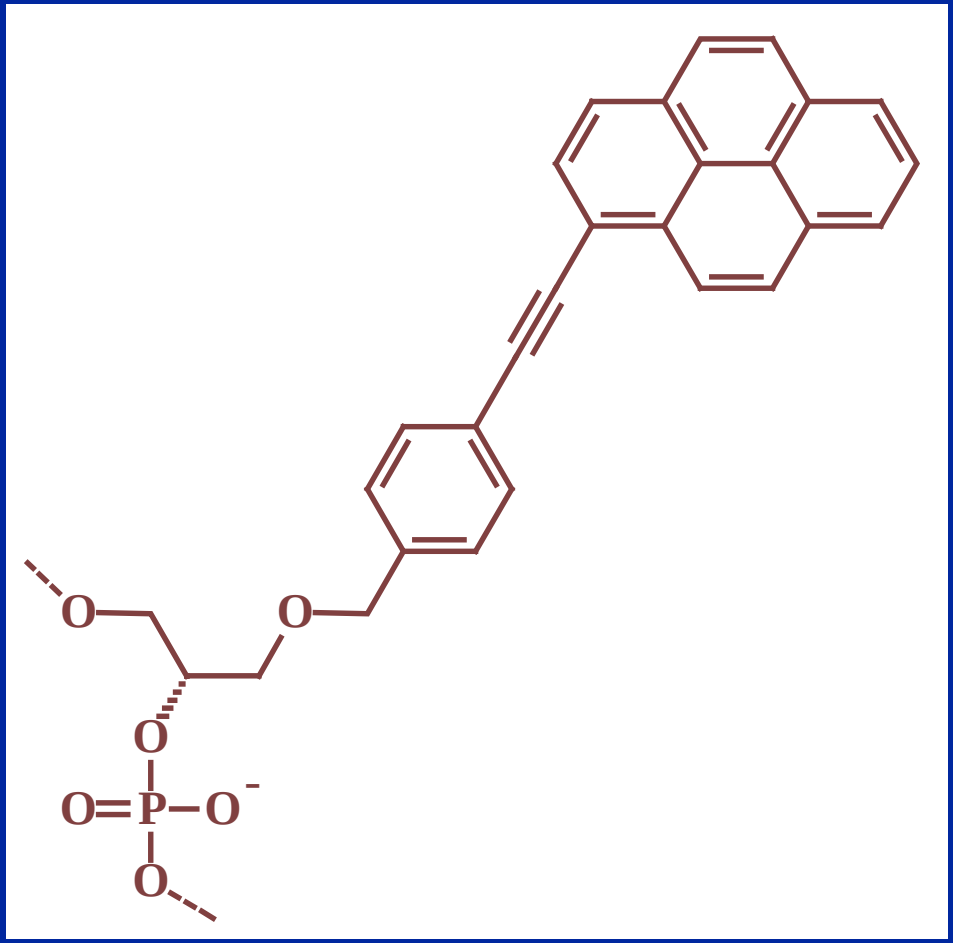
Choice of Technology

Nucleic Acids - Future



Choice of Technology

Nucleic Acids - Future



Molecular Diagnostics in the Future
Conclusions

- **Will improve speed and sensitivity**
- **Will reduce mortality of severe infections**
- **Will advance Point-Of-Care testing**
- **Target amplification will have a limited time-slot**
- **Signal amplification will prevail**
- **Artificial NA´s will revolutionize molecular testing**

Molecular Diagnostics in the Future
Conclusions

Crucial for fast and specific Microbiology

Crucial for control of Antimicrobial Resistance