

임상 미생물 검사

2019. 5. 9

길병원 진단검사의학과
서 일 혜



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- II. 세균배양 검사
- III. 면역진단 검사
- IV. 분자진단 검사



I. 검경 및 염색

1. 현미경 검사

- 아직 배양되지 않은 미생물에 대한 기초 정보제공

1) 광학 현미경

- 1,000-2,000배 확대
- 0.2mm 관찰 가능
- 세균 관찰

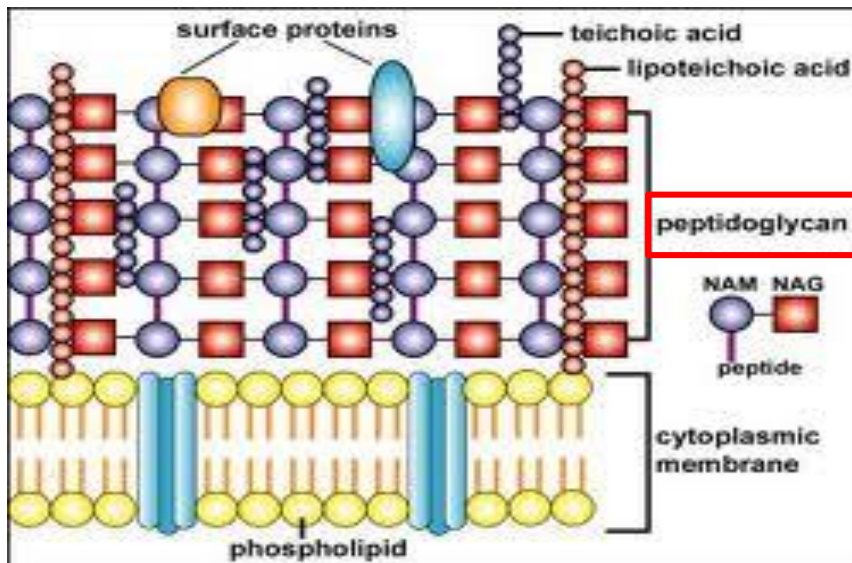
2) 전자현미경

- 10,000,000배 확대
- 0.2nm-50pm 관찰 가능
- 바이러스 관찰

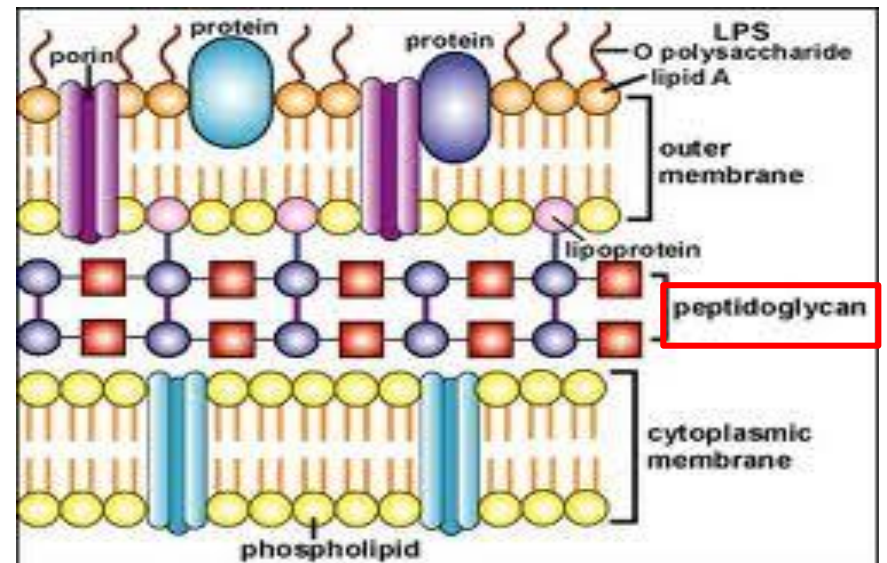


2. 염색

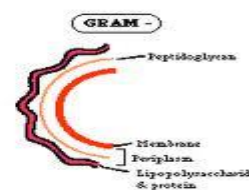
1) 그람 염색: 세균의 세포벽 (cell wall) 구조의 차이




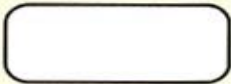





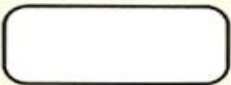


그람 양성균



그람 음성균



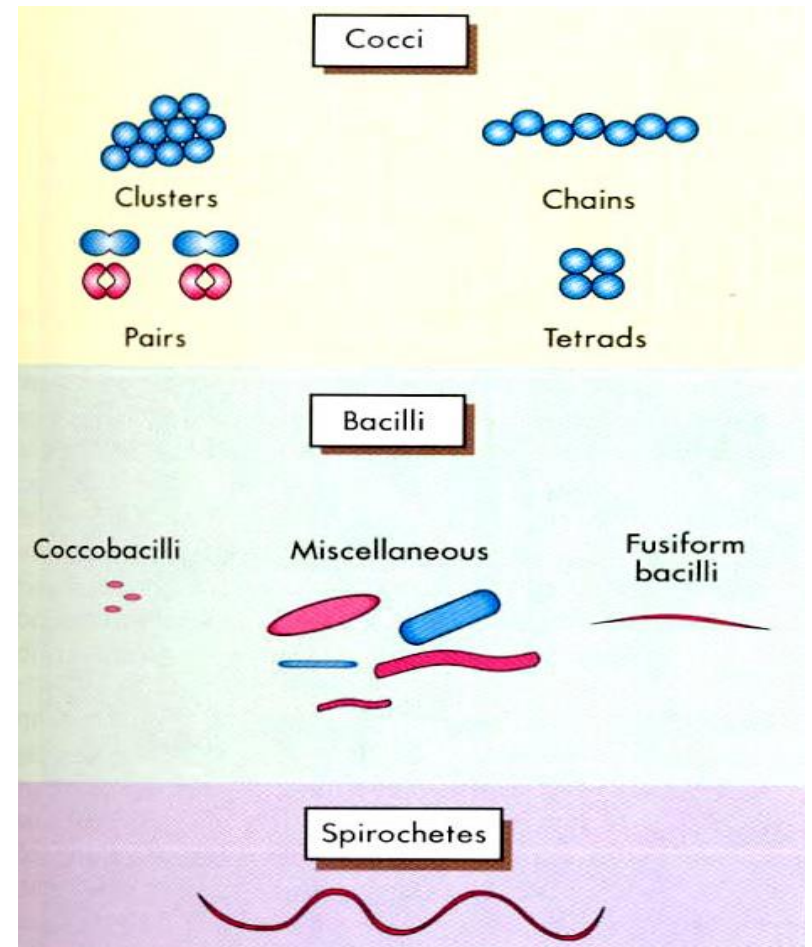


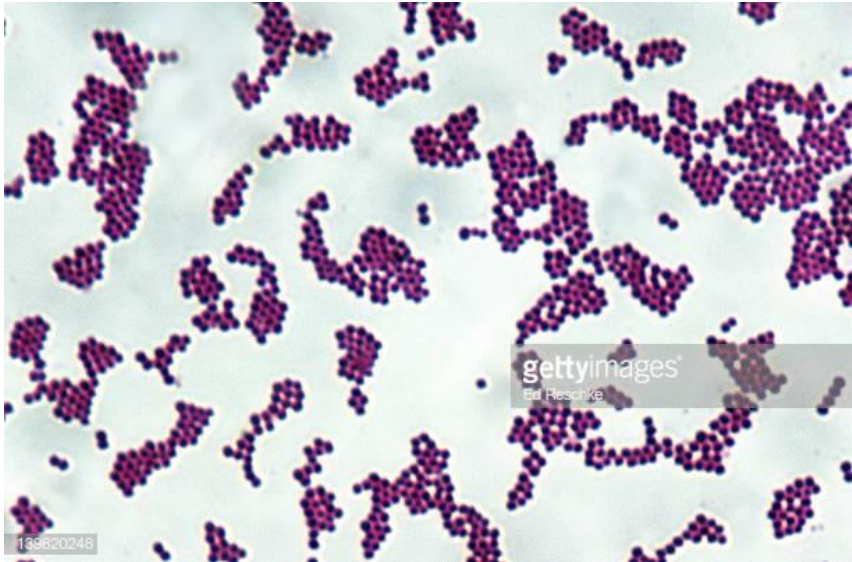
Gram ⁺ bacteria	Steps for staining	Gram ⁻ bacteria
1 	Cells on slide	
2  Stain purple	Primary stain (crystal violet)	 Stain purple
3  Remain purple	Mordant (Gram's iodine)	 Remain purple
4  Remain purple	Decolorizer, (alcohol and/or acetone)	 Become colorless
5  Remain purple	Counterstain (safranin)	 Stain pink



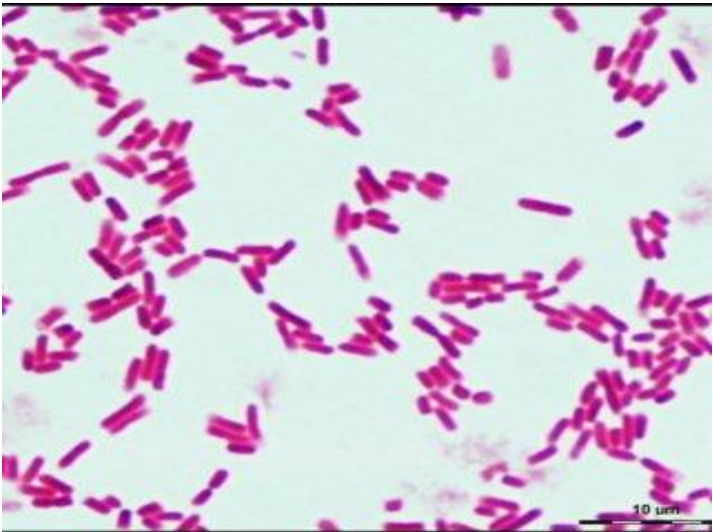
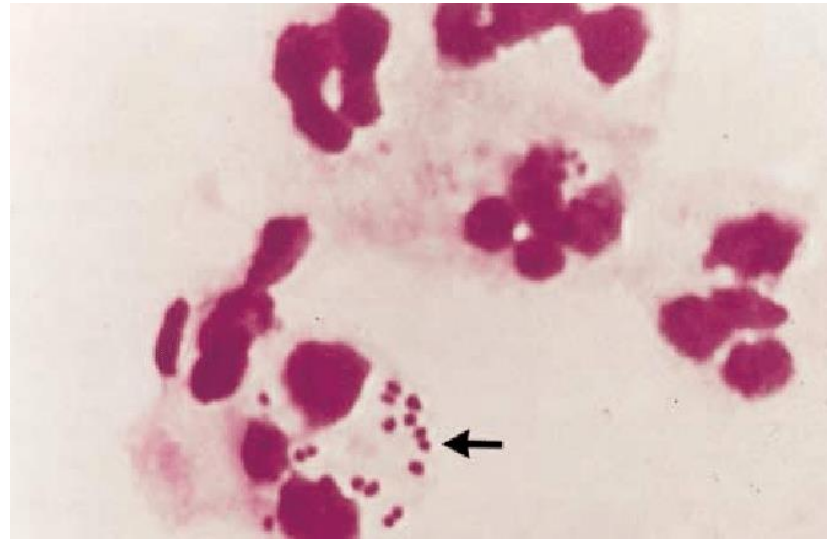
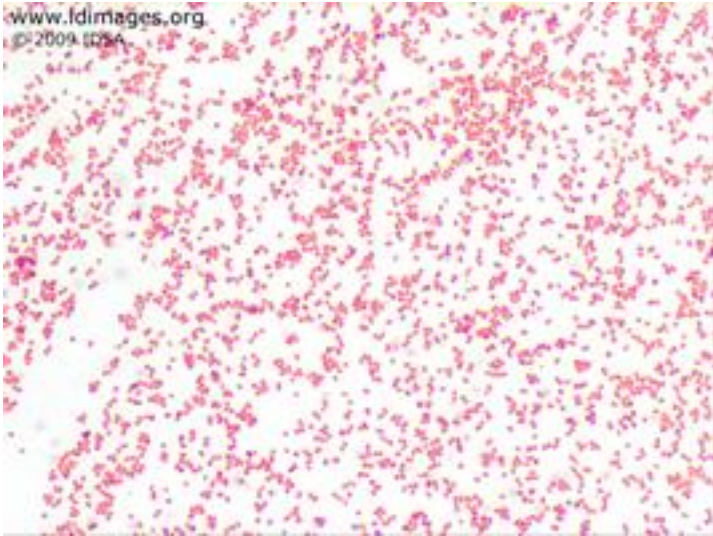
● 그람염색 결과판독

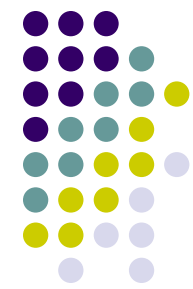
<p>그람양성 알균</p> <ul style="list-style-type: none"> - clusters - chain - diplococci 	<p>그람양성 막대균</p> <ul style="list-style-type: none"> - no spore - spore
<p>그람음성 알균</p> <ul style="list-style-type: none"> - diplococci 	<p>그람음성 막대균</p> <ul style="list-style-type: none"> - coccobacilli - bacilli - fusiform - spirachetes





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2) 항산성(Acid-fast) 염색

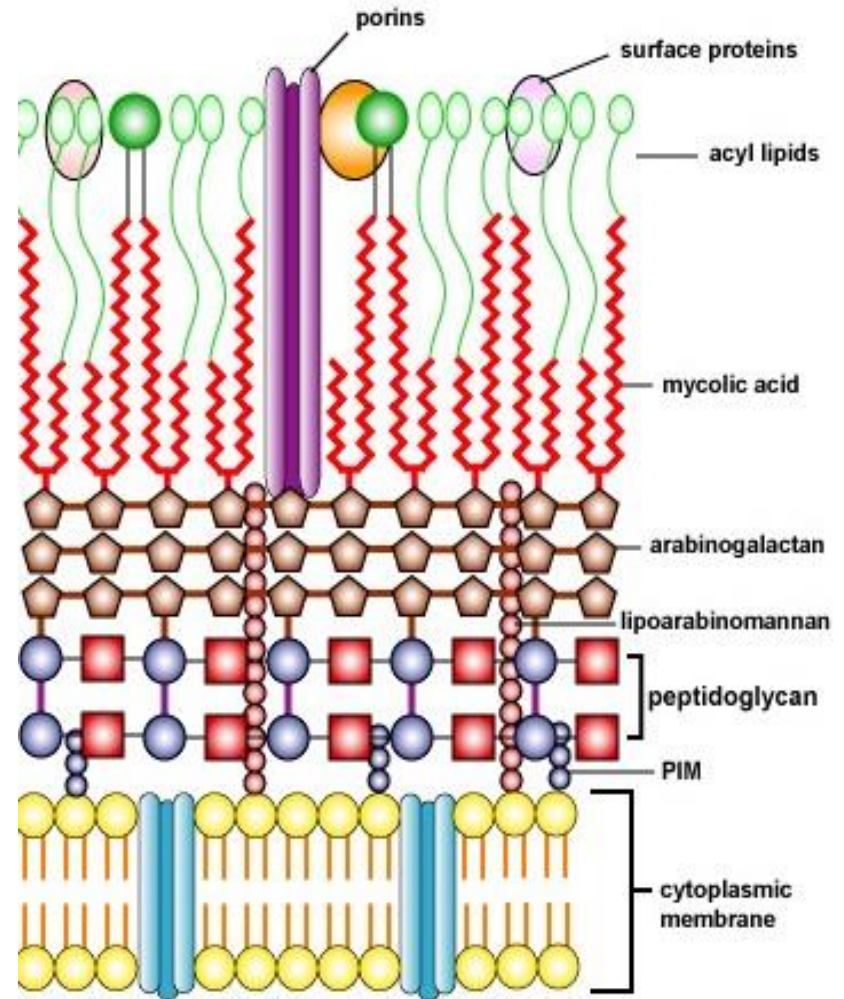
- 마이코박테리아(Mycobacteria)

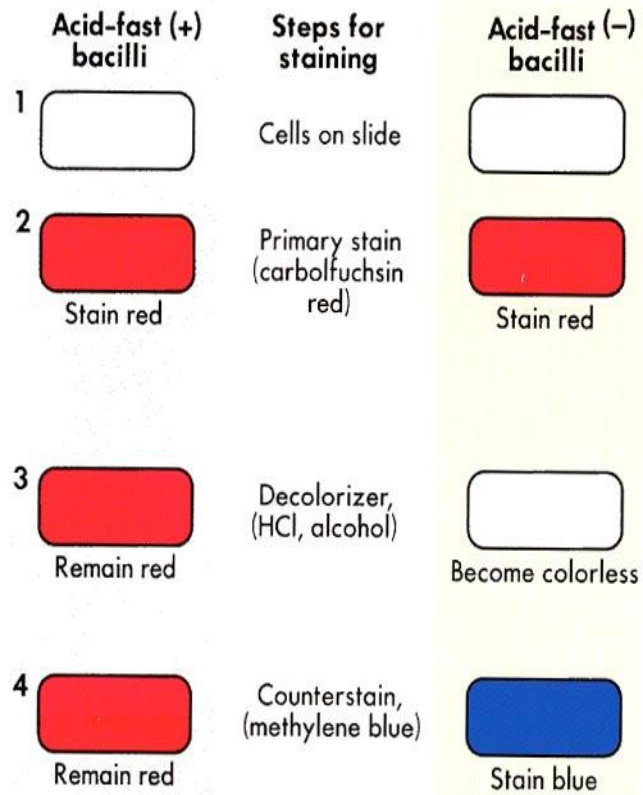
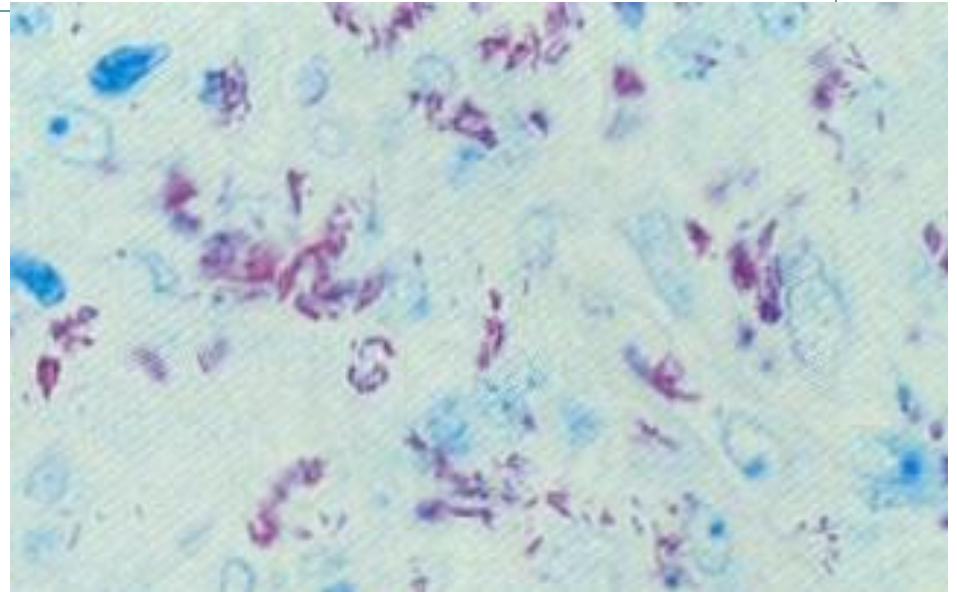
두꺼운 세포벽 (mycolic acids)

-> 산, 알칼리 등에 저항성

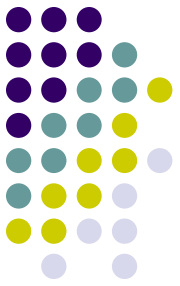
-> 열, 자외선에 취약

- Ziehl-Neelsen 염색법
- Auramine rhodamine 형광 염색법





결과보고(CDC)	항산균 수
Trace	1-2/300 field
1+	1-9/100 field
2+	1-9/10 field
3+	1-9/1 field
4+	> 9 / 1 field



II. 세균 배양검사

- 세균을 증식 분리하는 과정

- 순수 배양균

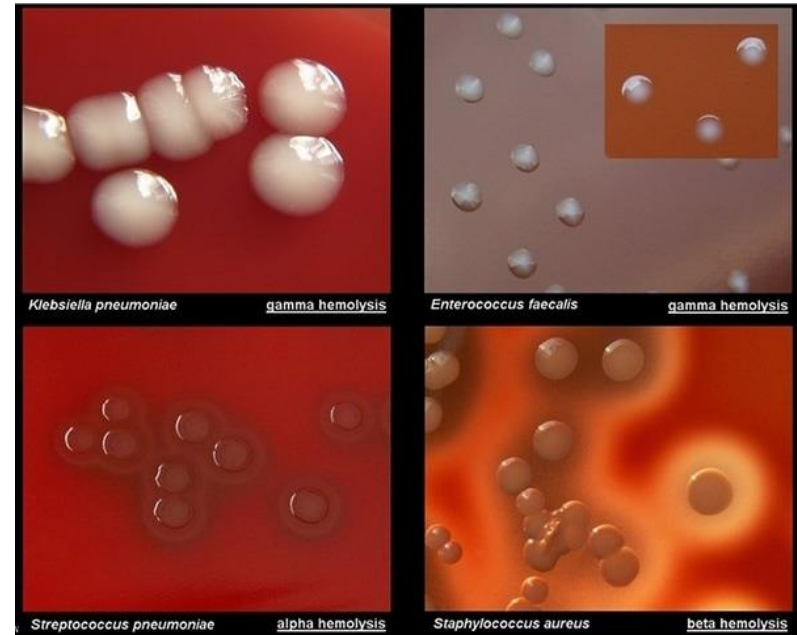
집락의 특징

생화학적 성상

그람염색

혈청학적 반응

유전자 분석



- 균종 동정(Identification) & 감수성 검사 시행



1. 배지

- Blood agar 배지
- Chocolate 배지
- MacConkey 배지
- Salmonella-Shigella 배지
- TCBS 배지



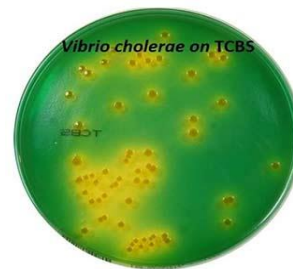
Escherichia coli



Salmonella



Shigella



Vibrio cholerae on TCBS Agar



Vibrio parahaemolyticus on TCBS Agar

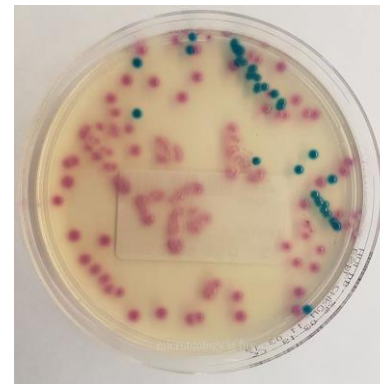
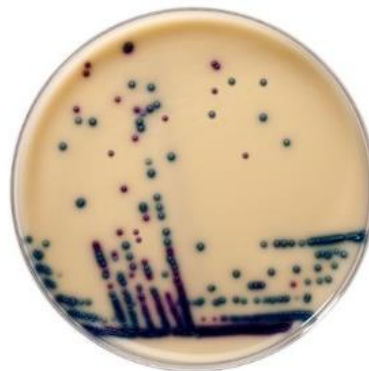


- Thioglycollate 배지
- Brain-Heart infusion 배지
- CHROM 배지

VRE

CRE

C. difficile ID



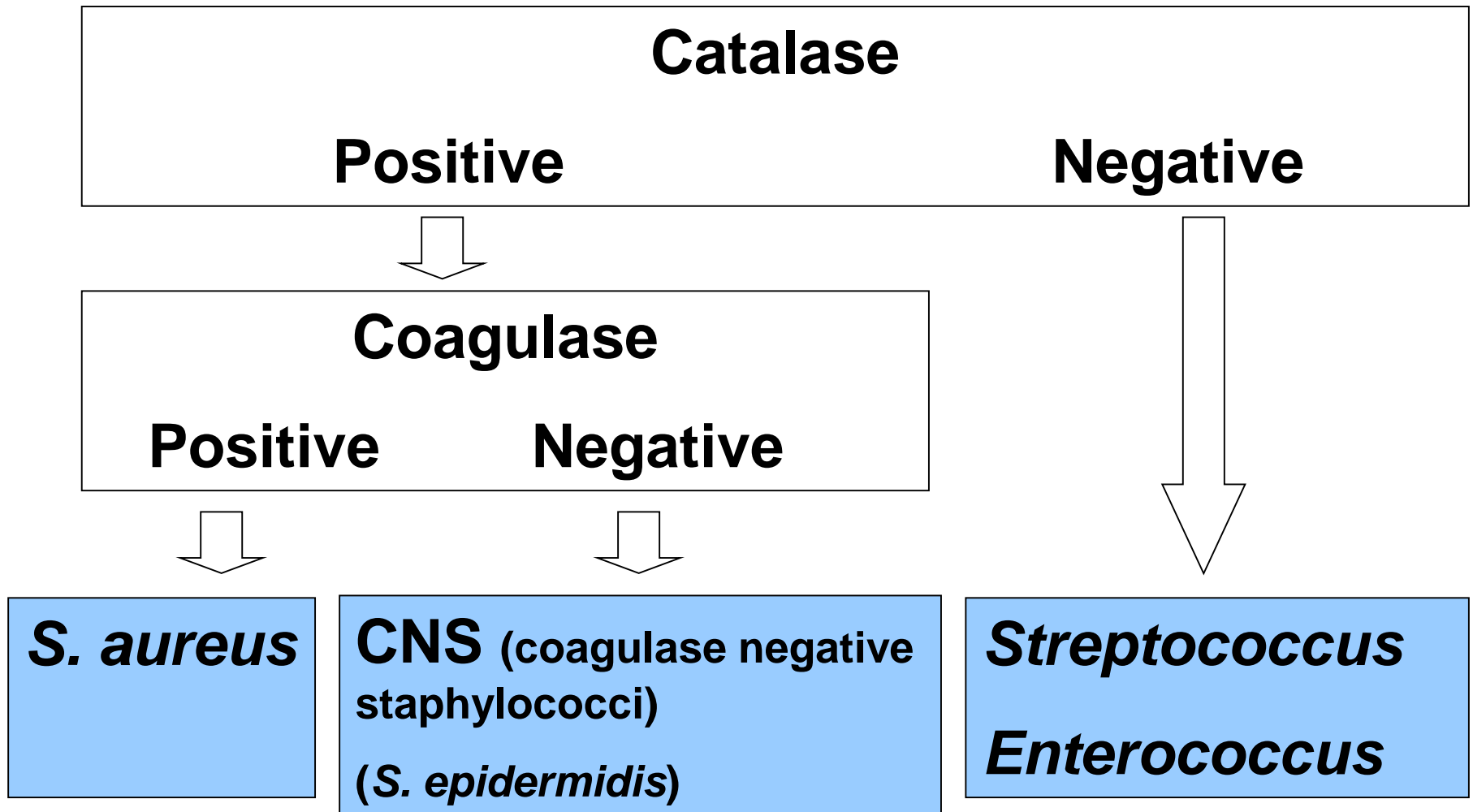


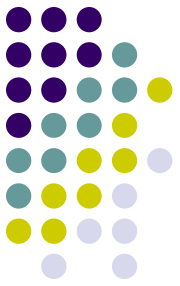
2. 배양 환경

- 산소성 배양: 일반 배양(78%N₂, 21%O₂, 0.04%CO₂, 기타)
- 5-10% CO₂ 배양: CO₂ 배양기, candle jar
- 미산소성(microaerophilic) 배양: 5%O₂, 10%CO₂, 85%N₂
- 무산소성 배양: 10% H₂, 10%CO₂, 80%N₂

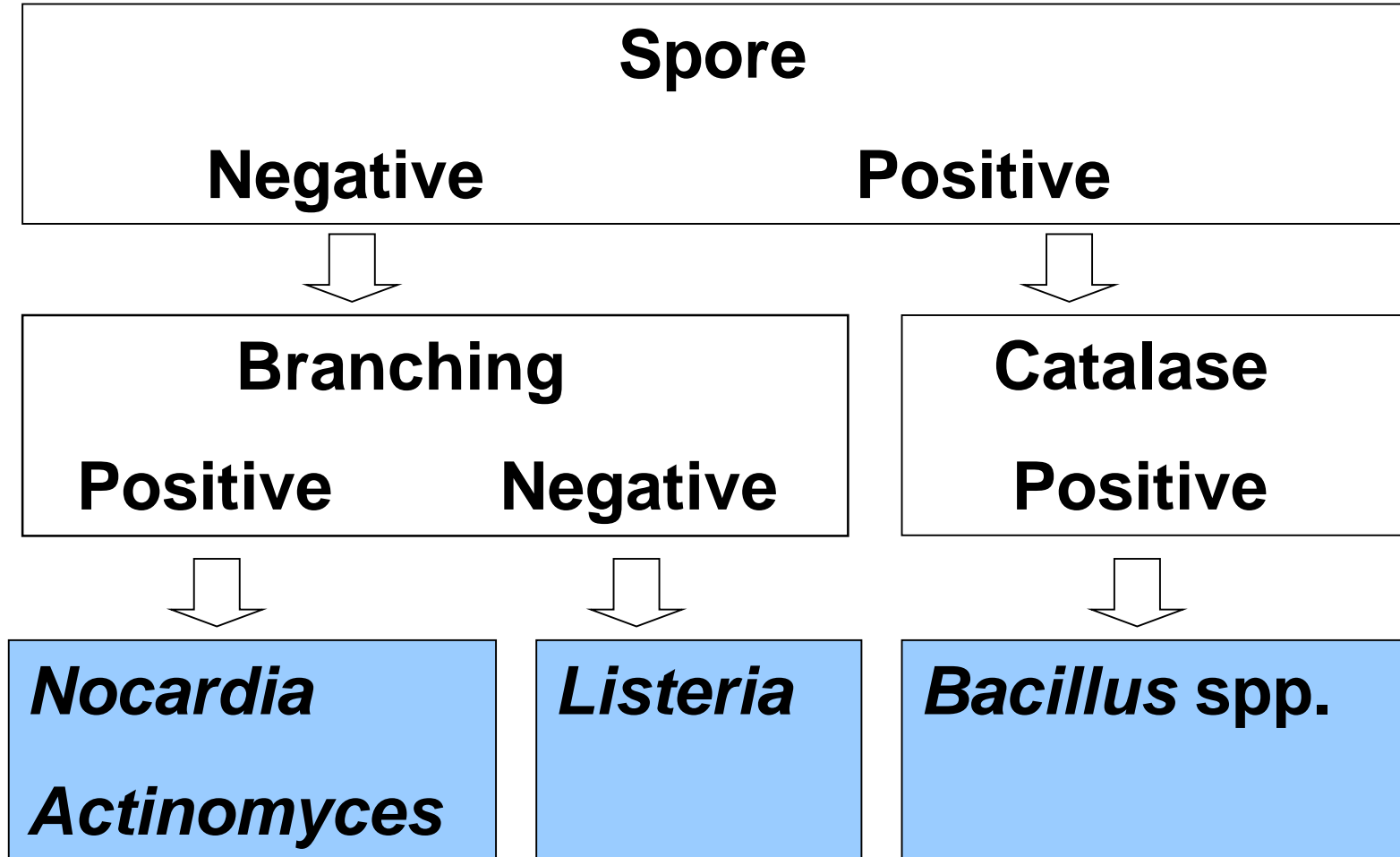
3. 기본 동정법

그람양성 알균(Gram positive cocci)



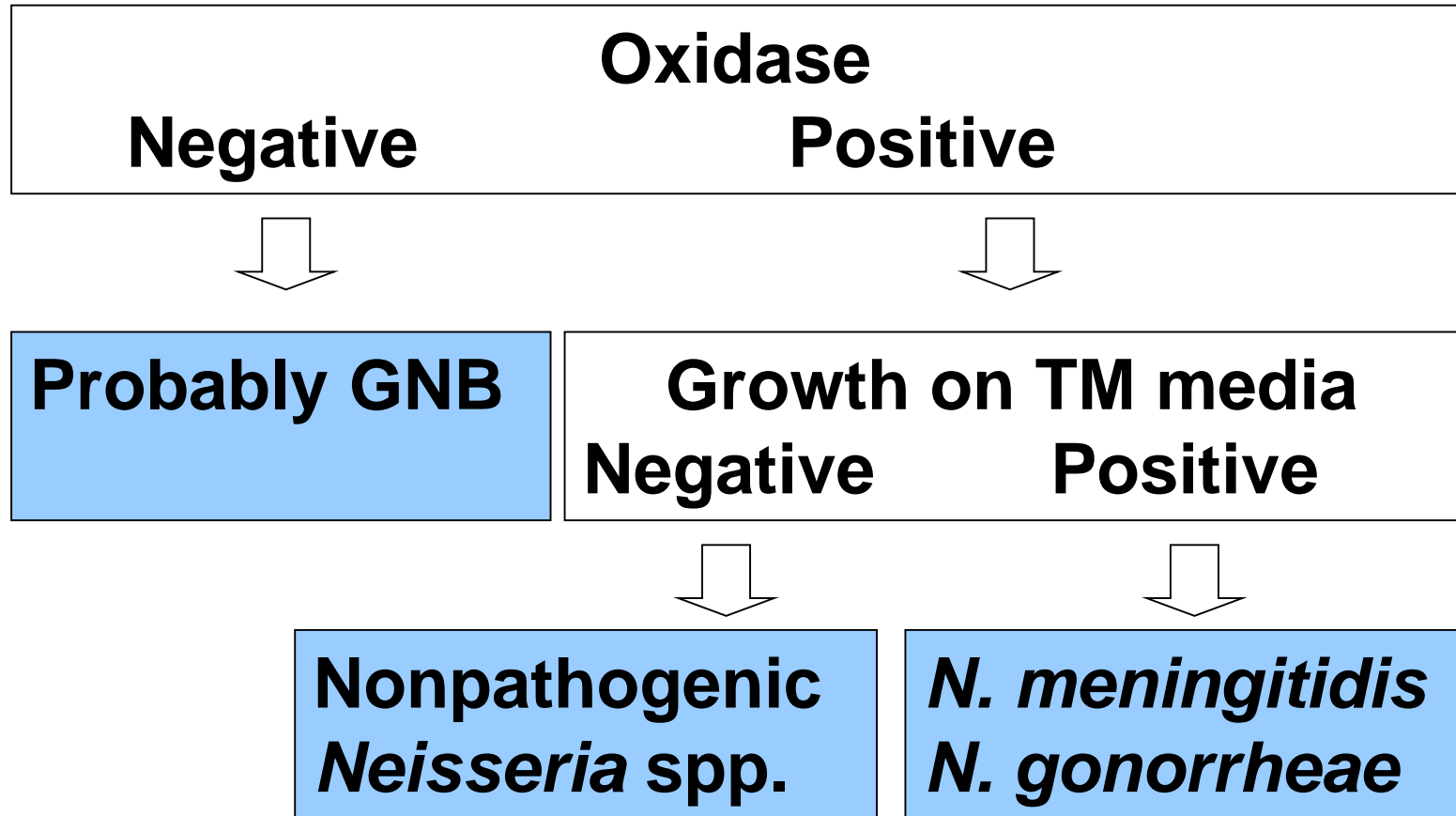


그람양성 막대균(Gram positive bacilli)





그람음성 알균(Gram negative cocci)

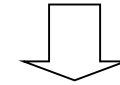
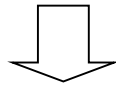




그람음성 막대균(Gram negative bacilli)

Fermentative

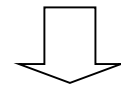
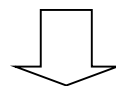
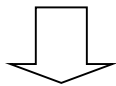
Nonfermentative



**Oxidase
Negative**

Positive

**Oxidase
Negative**



Enterobacteriaceae
E.coli
Klebsiella
Enterobacter
Serratia
Proteus
Citrobacter

Vibrio
Aeromonas
Plesiomonas

Pseudomonas
Burkholderia
Cryseobacterium
Alcaligenes

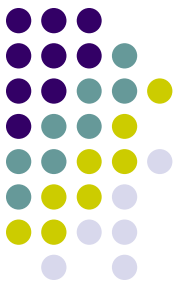
Acinetobacter
Stenotrophomonas
Chryseomonas



4. 자동화 장비에서의 생화학적 분석

- 다양한 생화학적 시험 결과
 - > reference data와 비교하여 동정
- 균동정 검사의 정확성: 장비의 데이터베이스에 기초
 - 16S rRNA 유전자 염기서열분석, DNA교잡법의 발달
 - > 균명의 변화, 새로운 종의 발견

ex) *Clostridium difficile* -> *Clostridioides difficile* (*C. difficile*)



III. 면역진단 검사

- 검사원리: 항원-항체 반응을 이용해 검체 내 항원 또는 항체를 검출
- 민감도와 특이도 높으면서 비교적 저렴
- 이용범위
 - 배양이 어렵거나 오래 걸리는 경우
 - 위험도가 높아 배양을 못할 때
 - 항균제 등의 투여로 배양이 안 되는 경우
 - 감염의 경과나 치료효과를 평가



1. 면역반응

1) Primary response (1차 항체 반응)- 처음감염

- 면역반응 곡선

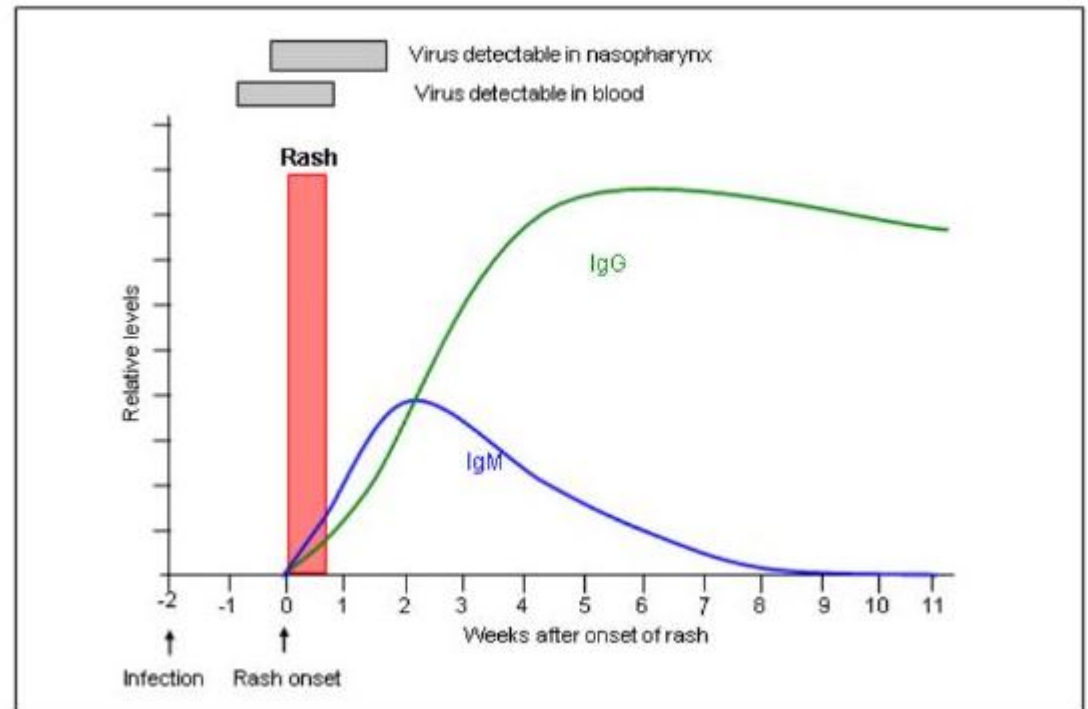
lag (window) phase

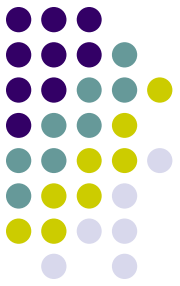
log phase

plateau phase

decline phase

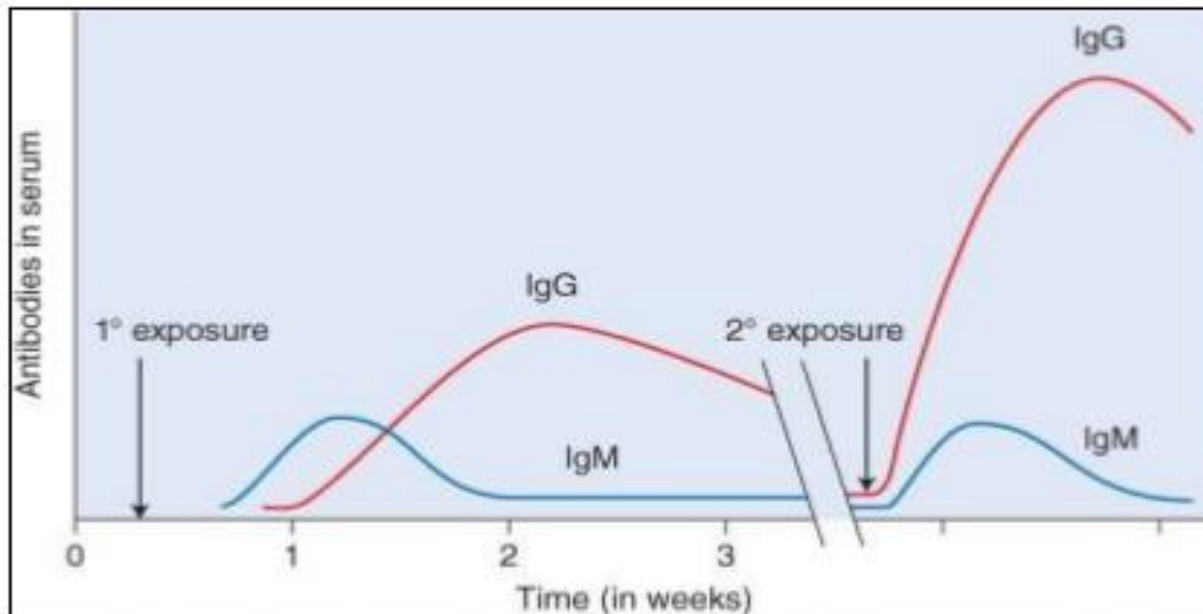
- IgM -> IgG





2) Secondary response (이차 항체 반응): 재감염

- lag phase 단축
- IgG 항체가 일차적으로 증가





2. 면역검사의 해석

1) IgM 항체검사

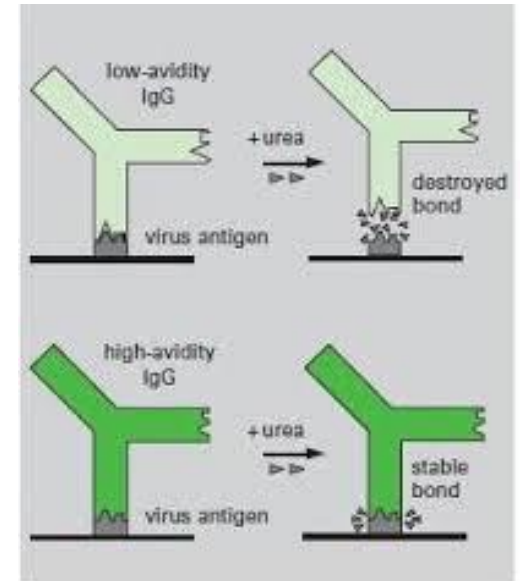
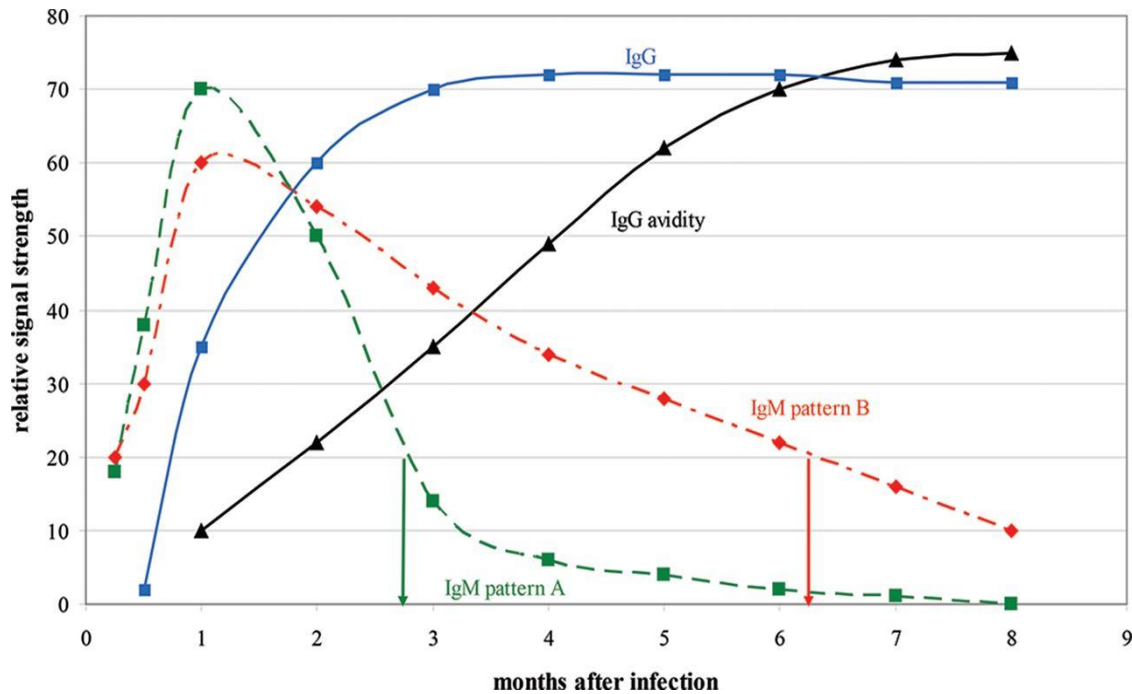
- 최근 감염 진단에 유용
 - 단, *M. pneumoniae*, *T. gondii*, CMV, HAV 감염 등에서 해석 주의
- 위양성 or 위음성 주의

2) IgG 항체검사

- 최근 or 과거 구별 안됨
- 급성기 vs. 회복기(2-3주 후) 역가 증가로 급성감염 입증



- **IgG avidity (항원-항체 결합력) 검사: 높으면 최근 감염 배제**





3. 면역진단 검사법의 종류

- 침강법
- 응집법
- 면역형광법(Immunofluorescence assay, IFA)
- 효소면역측정법(Enzyme immunoassay, EIA)
- 화학발광면역측정법(Chemiluminescence immunoassay, CLIA)
- 방사선면역측정법(Radioimmunoassay, RIA)
- Western blot immunoassay
- 신속면역검사(Rapid immunoassay)



IV. 분자진단 검사

1. 검사법

1) 중합효소연쇄반응(Polymerase chain reaction)

- RT-PCR
- Nested PCR
- Real-time PCR
- Multiplex PCR

2) 염기서열분석(Sequencing)

- Sanger sequencing
- Pyrosequencing
- Next-generation sequencing (NGS)



2. 전통적으로 동정하기 어려운 균 동정

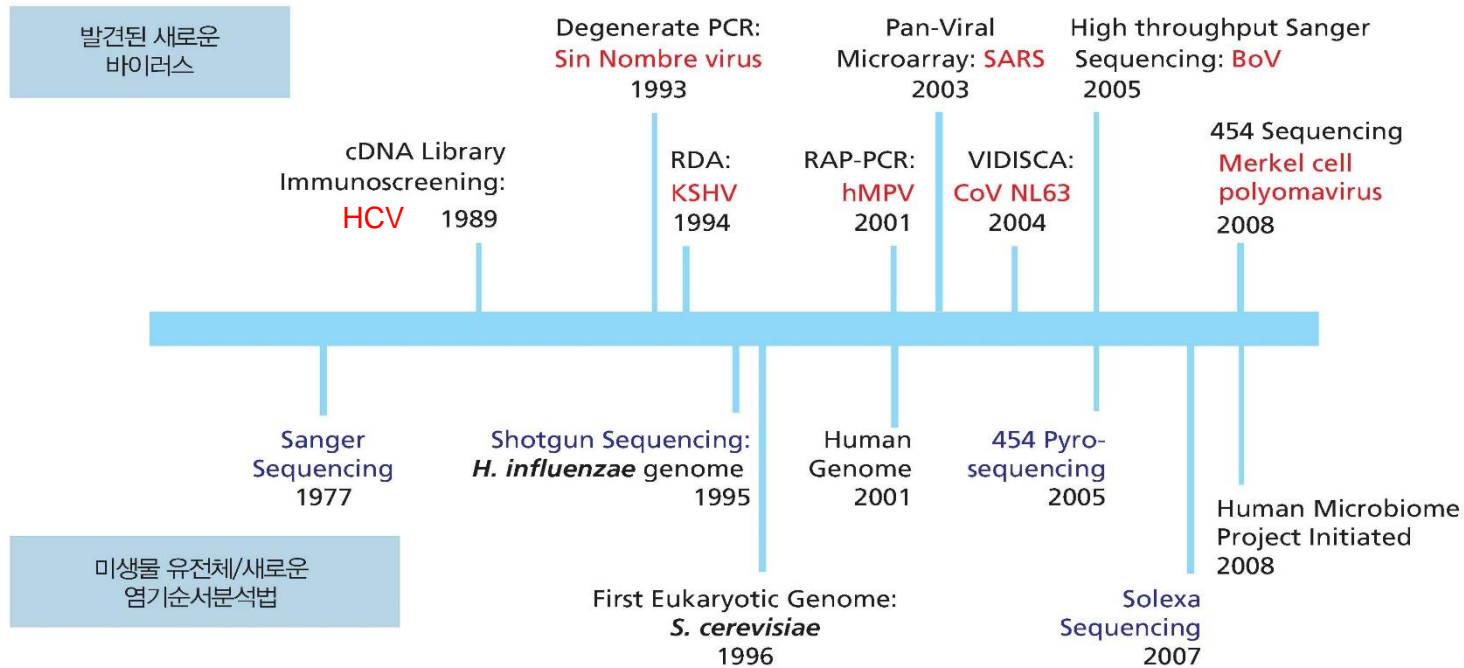


그림 89-1. 분자진단검사 방법의 발전과 이를 이용한 새로운 병원성 바이러스의 발견

HCV, hepatitis C virus; KSHV, Kaposi's sarcoma-associated herpesvirus; hMPV, human metapneumovirus; SARS, severe acute respiratory syndrome coronavirus; CoV NL63, human coronavirus NL63; BoV, human bocavirus; RDA, representational differential analysis; RAP-PCR, random arbitrarily primed PCR; VIDISCA, virus discovery cDNA-AFLP (amplified restriction fragment length polymorphism)



3. 검체를 이용한 분자진단 검사

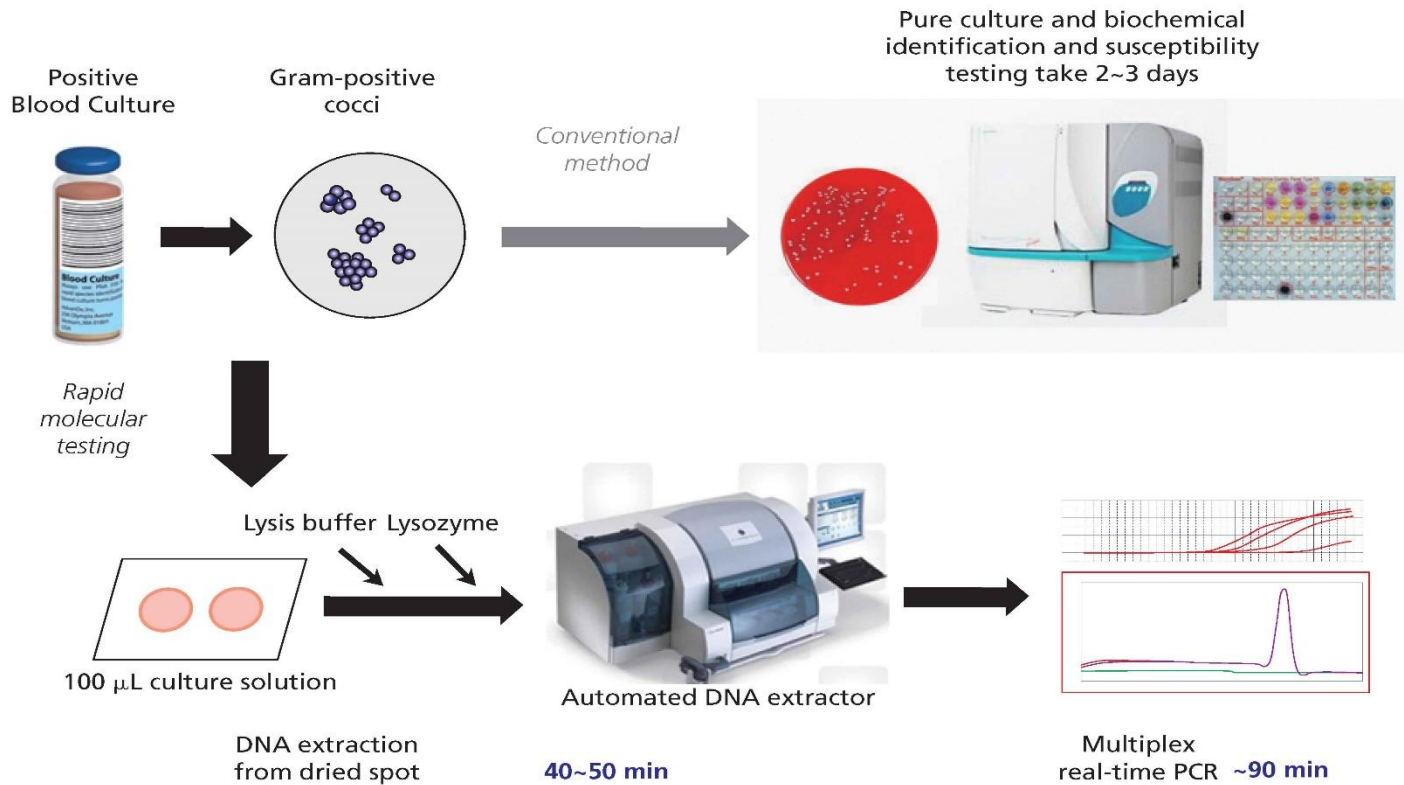
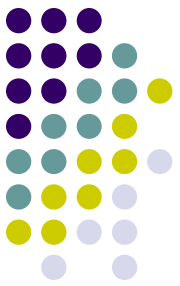


그림 51-2. 혈액배양병에서 직접 핵산을 추출하여 다중핵산증폭검사를 시행하는 과정



4. 균주를 이용한 분자진단 검사

- 세균동정: 16S rRNA Sequencing
- 진균동정: Internal transcribed spacer (ITS1, ITS2)

large-subunit rRNA D2 Sequencing

- 결핵 및 비결핵 항산균 검출 및 동정
- 약제내성 검사



5. 의료관련 감염과 분자진단 검사

1) 다제내성균 신속검출

- **MRSA : *mecA* or *SCCmec***
- **VRE: *vanA*, *vanB***
- **CPE: *KPC*, *NDM*, *OXA*, *IMP*, *VIM***

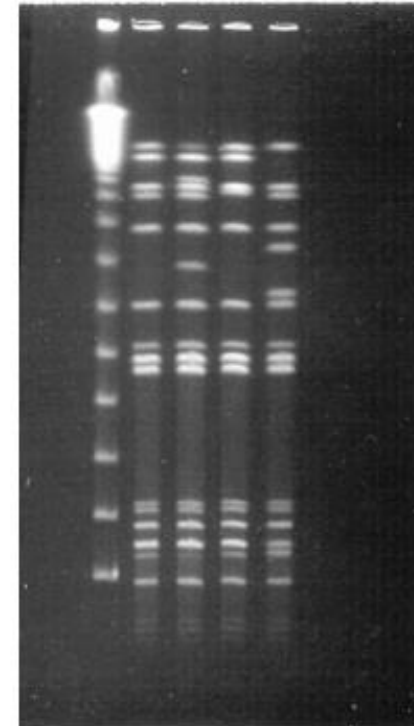
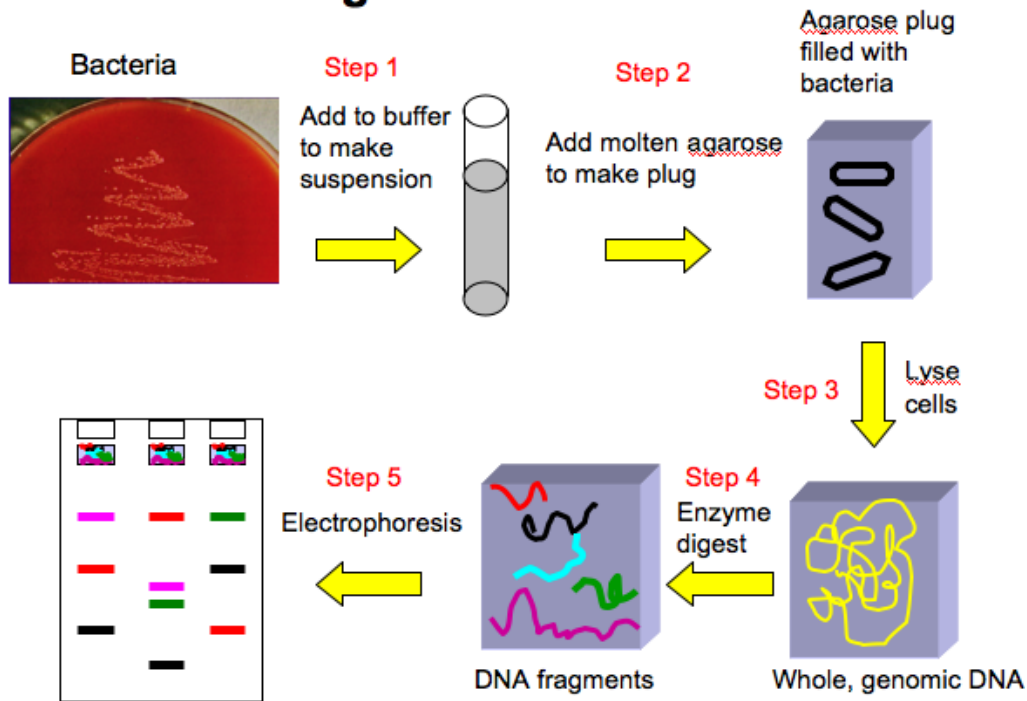
2) 균주형별 분류(strain typing)- 역학 감시

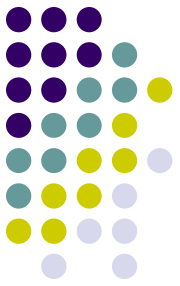
- **PFGE (pulse-field gel electrophoresis)**
- **RFLP (restriction fragment length polymorphism)**
- **MLST (multilocus sequence typing)**



PFGE (pulse-field gel electrophoresis)

Figure 1 - PFGE





MLST (multilocus sequence typing)

