

Biochemical identification of bacteria

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Outline

- “ Phenotypic vs genotypic tests
- “ Pros and cons of biochemical tests
- “ Basis of biochemical tests
- “ Examples of biochemical test
- “ Diagnostic algorithms
- “ The future of biochemical identification tests

Methods of bacterial ID

“ Phenotypic

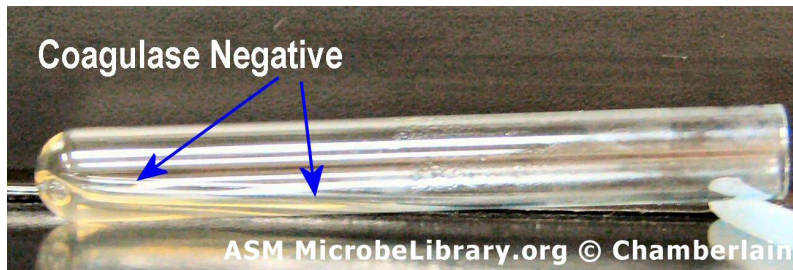
- . Detects the physical properties of bacteria
- . Influenced by gene expression
- . Includes biochemical tests

“ Genotypic

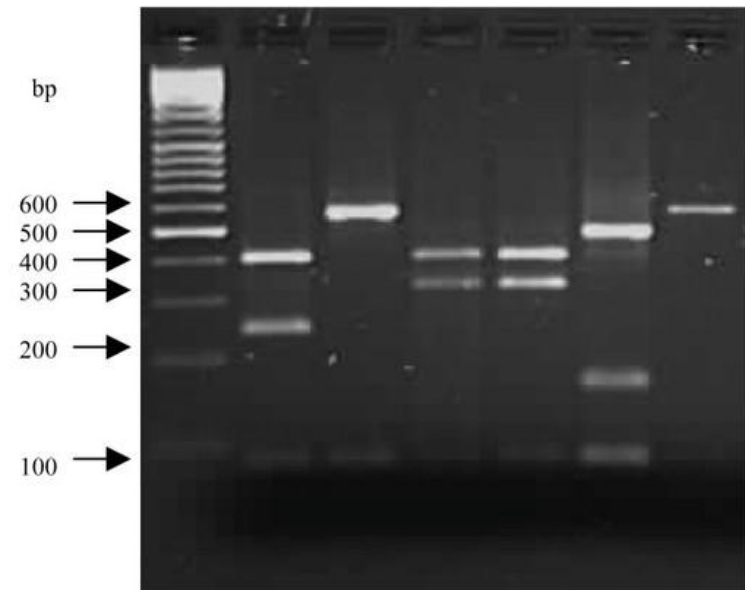
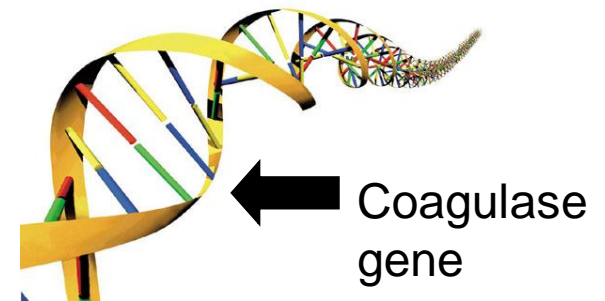
- . Detects the genetic code of bacteria (DNA)
- . Not influenced by gene expression

Eg coagulase for staphylococcal ID

” Phenotypic test



” Genotypic test



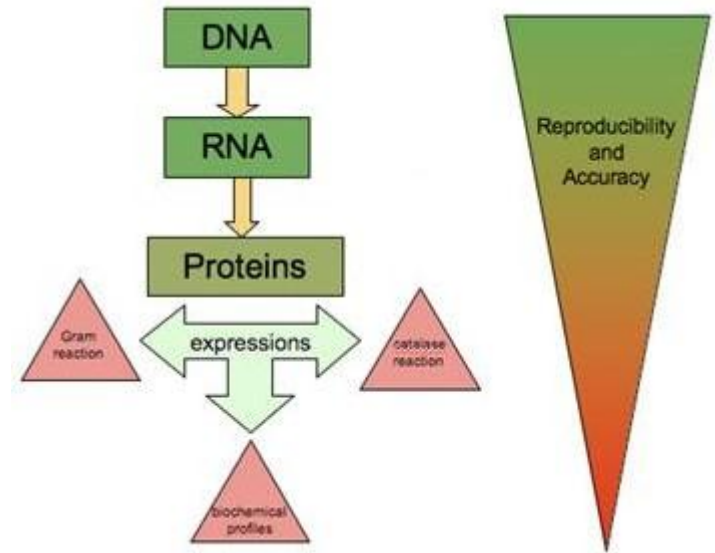
Biochemical ID: Pros and cons

“ Pros

- . Cheap
- . Experience with use++
- . Does not require expertise
- . Potentially fast TAT (range: seconds to overnight)

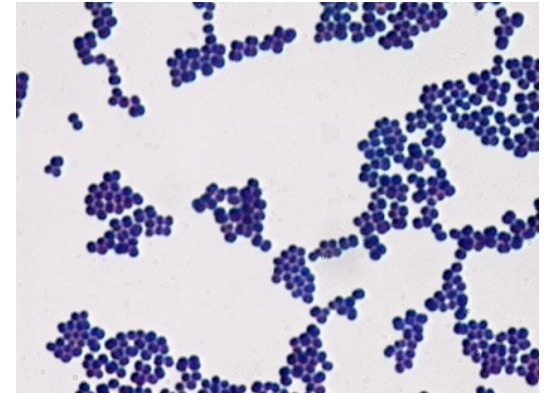
“ Cons

- . Biosafety risk (live organisms)
- . Less accurate, less discriminatory
- . Phenotype may be unstable
 - “ Eg inducible (ie influenced by gene expression)
- . Not possible if organism is slow growing or fastidious
- . Subjective interpretation (less reproducible)



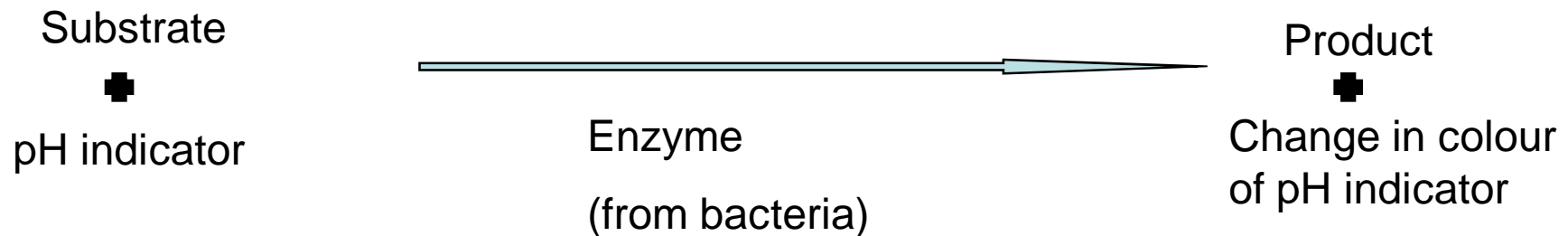
Type of phenotypic ID

- “ Appearance
 - . Macroscopic
 - . Microscopic (eg gram stain, rod vs coccus)
- “ Growth requirement/rate
 - . Media
 - . Atmospheric gases
 - . Temperature
- “ Smell
- “ Motility
- “ Hemolysis on blood agar
- “ **Biochemical tests**



(See lecture on % Culture characteristics for bacterial identification+)

Basis of biochemical tests



“ Important features

- . Standardisation of method
- . standardised amount of bacteria used for test (=inoculum)
- . +ve and . ve controls

pH indicators

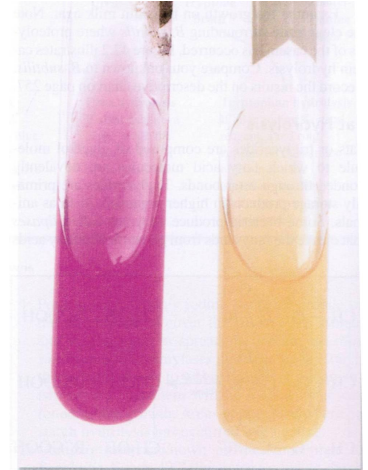


FIGURE 42.4 Urease test. Tube on the left is positive (Proteus); tube on the right is negative. © The McGraw-Hill Companies/Auburn University Photographic Service

“ Colour changes occur at different pHs for different indicators

“ pH Indicator	pH range	Change from acid to alkaline
“ Methyl red	4-6	red to yellow
“ Andrades	5-8	pink to yellow
“ Bromescol blue	5-6	yellow to purple
“ Phenol red	6-8	yellow to red

Standardisation of the inoculum

“ Examples of solid phase:

- . Loop size (eg 1microL, 10microL)

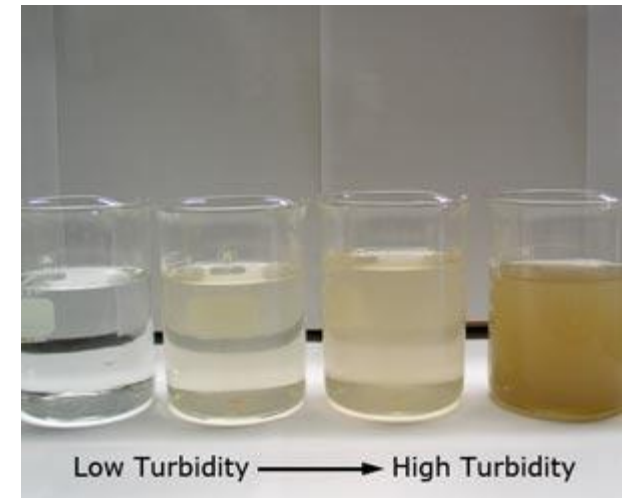


“ Examples of liquid phase

- . Turbidity of fluid

“ The ability of particles in suspension to refract and deflect light rays

- . Optical density
- . Nephelometry



Positive and Negative controls

- “ Positive control: bacteria with known +ve test result
- “ Negative control: bacteria with known -ve test result
- “ If either or both of the controls fail, then the test is not valid



-ve
control

+ve
control

test
isolate

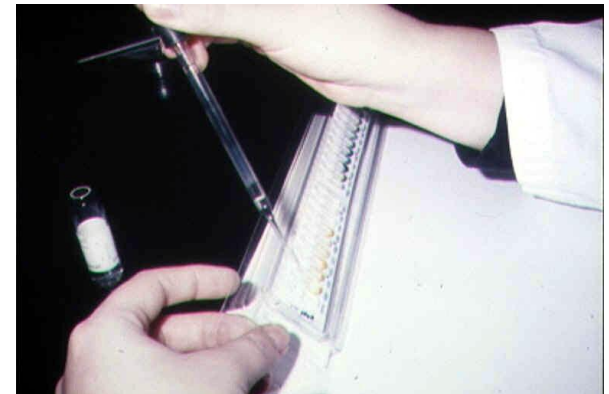
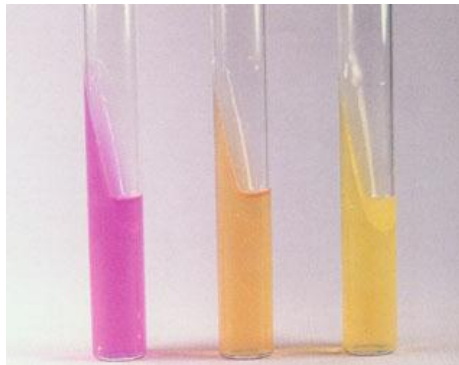


Types of biochemical ID methods

“ Manual vs automated

- . Automated systems have the advantage of automated reading which improves speed, consistency and removes subjective error.

“ In house vs commercial



Examples of common biochemical tests used for ID of gram negative bacteria

- “ Urease
- “ Indole
- “ Oxidase
- “ Glucose fermentation
- “ Lactose fermentation
- “ Nitrate

Urease

- “ Detects hydrolysis of urea to ammonia by urease enzyme
- “ Ammonia causes an increase in pH which is detected by the pH indicator (orange → pink)
- “ Urease +ve bacteria:
 - . Proteus
 - . Klebsiella

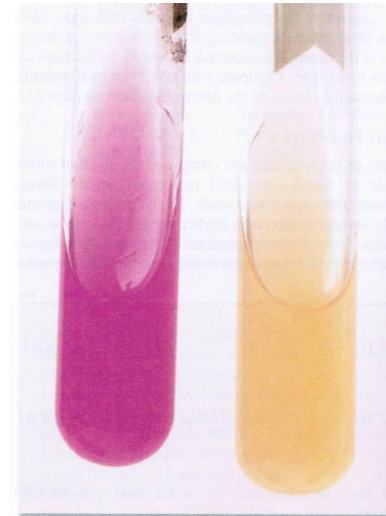
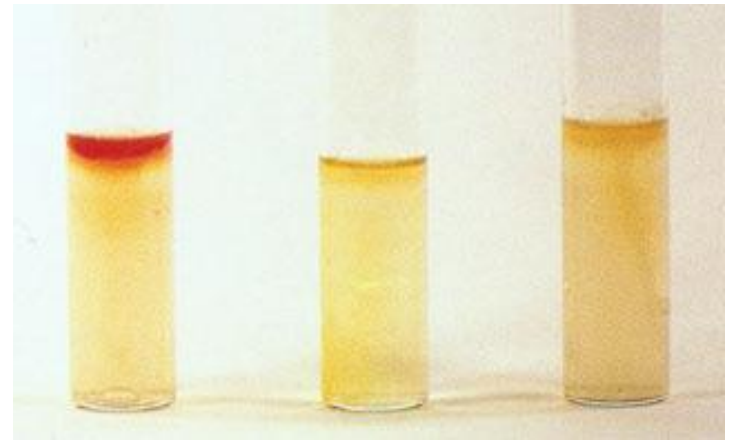


FIGURE 42.4 Urease test. Tube on the left is positive (*Proteus*); tube on the right is negative. © The McGraw-Hill Companies/Auburn University Photographic Service

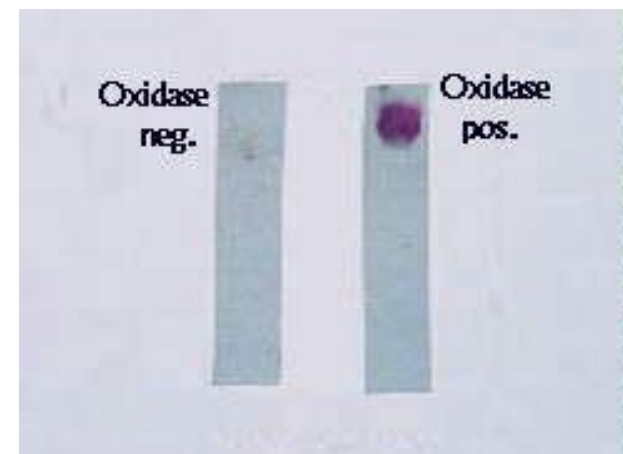
Indole

- “ Detects indole production from tryptophan, which produces a colour change in combination with dimethylaminobenzaldehyde (clear to red)
- “ Indole +ve bacteria:
 - . E.coli
 - . Citrobacter



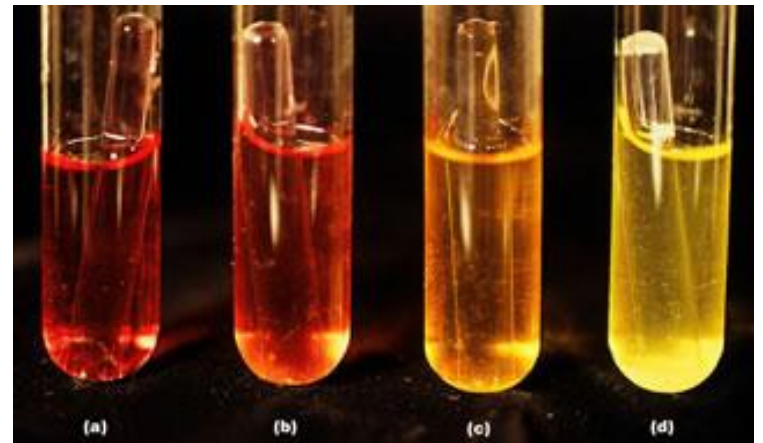
Oxidase

- “ Detects cytochrome oxidase enzyme that converts dimethylphenyldiamine to indophenol blue (clear to blue)
- “ Oxidase +ve bacteria:
 - . Pseudomonas
 - . Vibrio



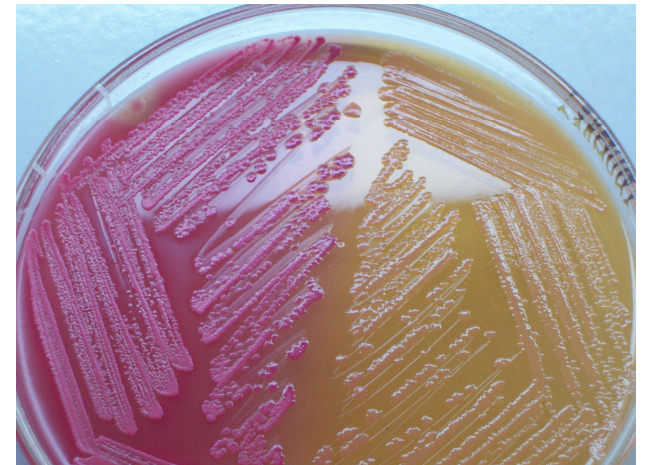
Glucose fermentation

- “ Detects ability of bacteria to ferment glucose to pyruvic acid using the Embden Meyerhof pathway
- “ Detected by phenol red pH indicator (red/alkaline to yellow/acid)
- “ Bacteria that ferment glucose:
 - . E.coli
 - . Proteus



Lactose fermentation

- “ Detects ability of bacteria to ferment lactose to glucose then to pyruvic acid using the Embden Meyerhof pathway
- “ Detected by phenol red pH indicator (red/alkaline to yellow/acid)
- “ Bacteria that ferment glucose:
 - . E.coli
 - . Klebsiella



Nitrate

- “ Detects nitrate reductase enzyme which converts nitrate to nitrite.
- “ Nitrite then revealed by addition of naphthylamine and sulfonic acid to form diazonium dye (clear to red)
- “ Nitrate +ve bacteria:
 - . E.coli
 - . Klebsiella



TSI slope

- “ Incorporates multiple substrates and pH indicators into 1 tube
- “ By streaking bacteria onto surface and stabbing it into media, both aerobic and anaerobic conditions are generated



API

- “ Minutuarized biochemical reactions in >20 wells
- “ Takes 2-24 hrs
- “ Reaction profile (%biocode+) compared to an on-line database of >20000 isolates
- “ Commerical test



	Tests	Active ingredients	Reactions/enzymes
1	ONPG	2-nitrophenyl-bD-galactopyranoside	b-galactosidase
2	ADH	L-arginine	Arginine DiHydrolase
3	LDC	L-lysine	Lysine Decarboxylase
4	ODC	L-ornithine	Ornithine Decarboxylase
5	CIT	Trisodium citrate	Citrate utilization
6	H2S	Sodium thiosulphate	H2S production
7	URE	Urea	Urease
8	TDA	L-tryptophane	Tryptophane deaminase
9	IND	L-tryptophane	Indole production
10	VP	Sodium pyruvate	Acetoin production(Voges Proskauer)
11	GEL	Gelatine	Gelatinase
12	GLU	D-glucose	Fermentation/oxidation (Glucose)
13	MAN	D-mannitol	Fermentation/oxidation (Mannitol)
14	INO	Inositol	Fermentation/oxidation (Inositol)
15	SOR	D-sorbitol	Fermentation/oxidation (sorbitol)
16	RHA	L-rhamnose	Fermentation/oxidation (rhamnose)
17	SAC	D-sucrose	Fermentation/oxidation (saccharose)
18	MEL	D-melibiose	Fermentation/oxidation (melibiose)
19	AMY	Amygladin	Fermentation/oxidation (Amygladin)
20	ARA	L-arabinose	Fermentation/oxidation (arabinose)

Automated Biochemical ID systems

“ Examples:

- . Vitek
- . Biolog
- . Pheonix
- . Autoscan Walkaway



“ Varying capacity for:

- . Number of specimens they can handle
- . Size/extent of comparative database
- . Interfacing with lab data program
- . Turn around time
- . Capacity for ID to species level



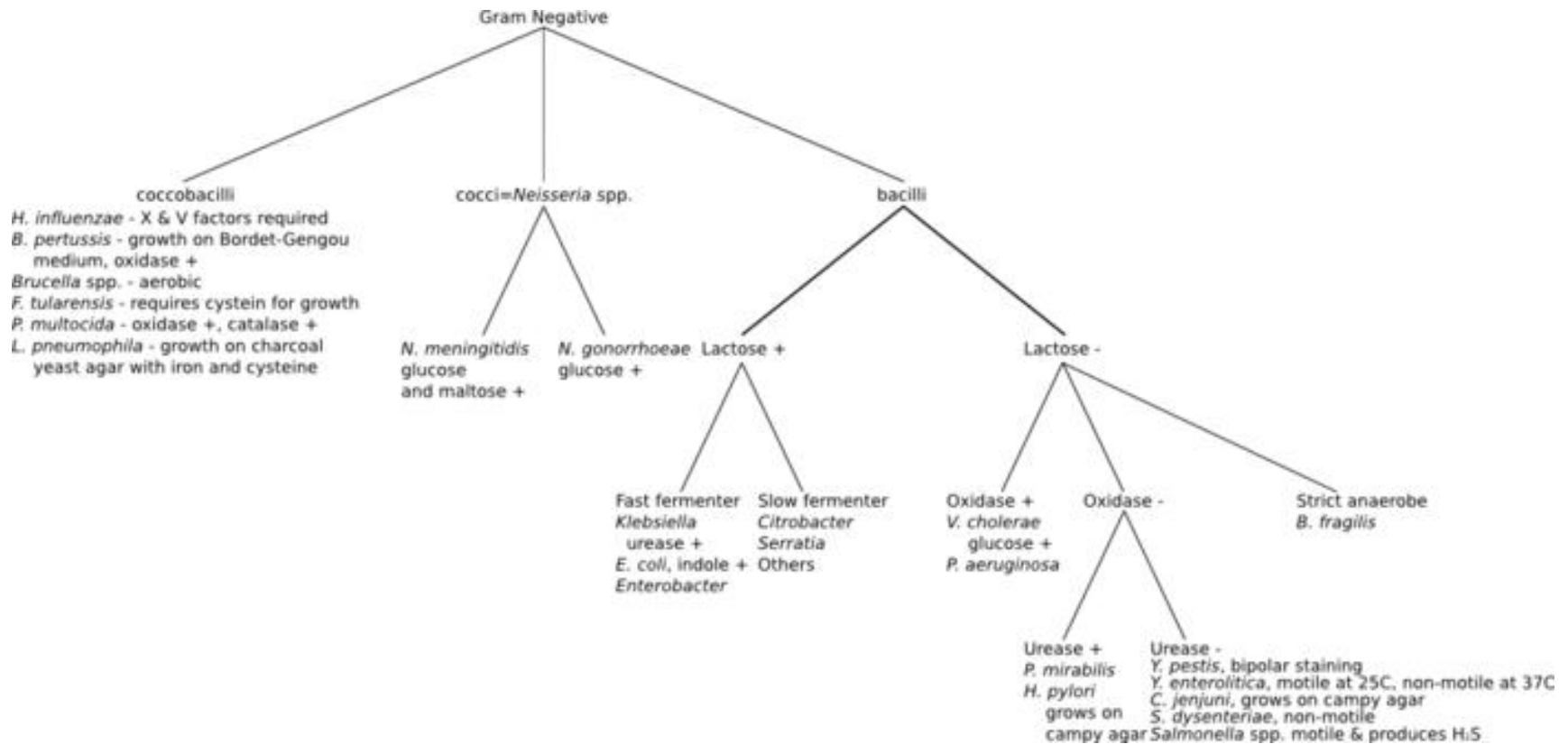
Diagnostic algorithms for bacterial ID

- “ Primary tests allow genus level ID (enterobacteriaceae, ~~non~~non-glucose fermenters+, HACEK, etc)
 - . Gram stain
 - . Culture morphology
 - . Basic biochemical tests
 - “ Eg Oxidase, indole, urease tests, etc
- “ Species level identification requires more complex, second line tests

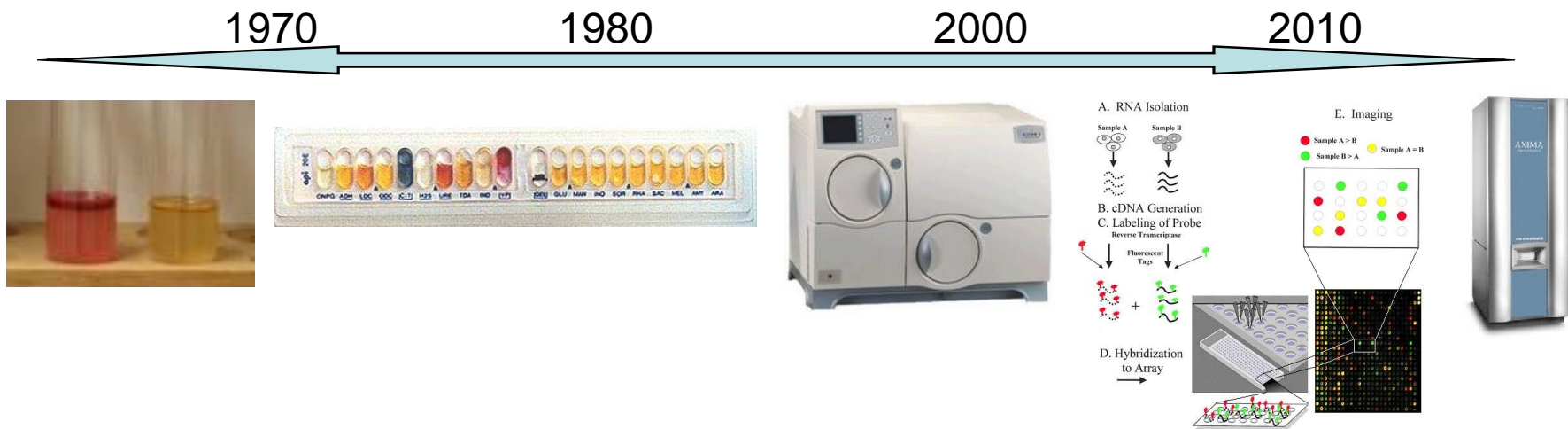
Example 1 of diagnostic algorithm

	Indole	Methyl red	Voges Proskauer	Citrate	Urease
E.coli	+	+	-	-	-
Enterobacter	-	-	+	+	-
Klebsiella pneumoniae	-	-	+	+	+
Salmonella	-	+	-	+	-
Shigella	-	+	-	-	-
Proteus mirabilis	-	+	-	+/-	+

Example 2 of diagnostic algorithm



Changes in biochemical tests for ID: past and future



- “ Increased automated and minituarisation
- “ Increasingly replaced by genotypic tests
- “ Is identification necessary: could we manage with susceptibility testing alone?

Conclusions

- “ Biochemical tests remain critical to bacterial identification
- “ Need to understand the principles of the common/primary tests
- “ Biochemical tests have limitations
- “ In the future they will increasingly be replaced by genotypic tests