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

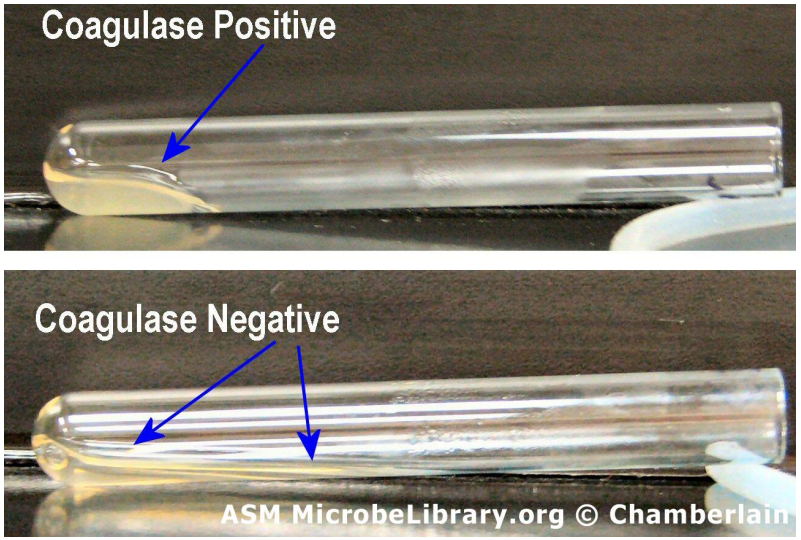
Methods of bacterial ID

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

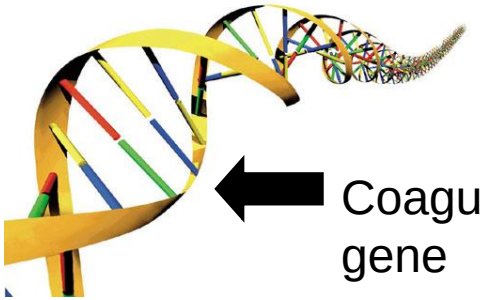
- Genotypic
  - Detects the genetic code of bacteria (DNA)
  - Not influenced by gene expression

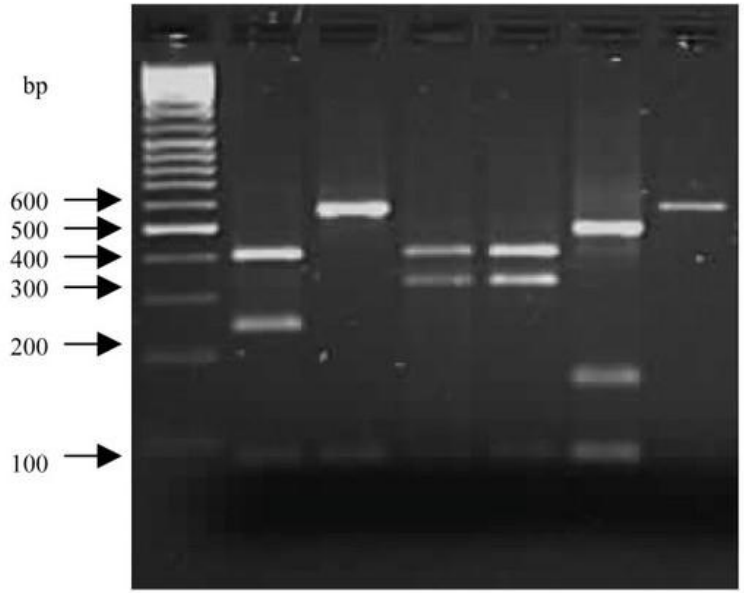
Eg coagulase for staphylococcal ID

Å Phenotypic test

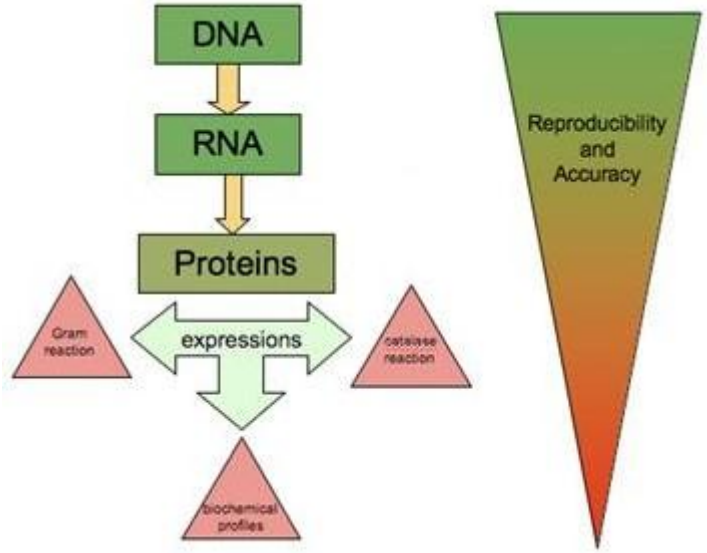


Å Genotypic test





Biochemical ID: Pros and cons

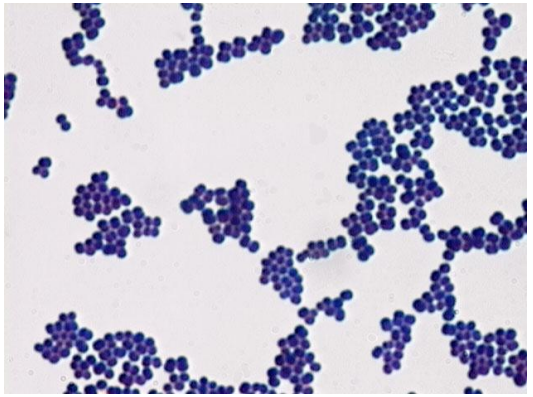


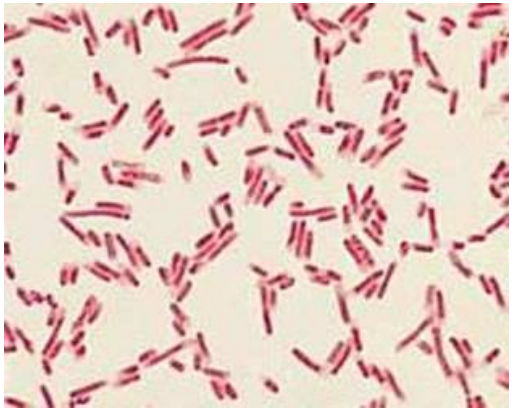
- Pros
  - Cheap
  - Experience with use++
  - Does not require expertise
  - Potentially fast TAT (range: seconds to overnight)

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

Type of phenotypic ID

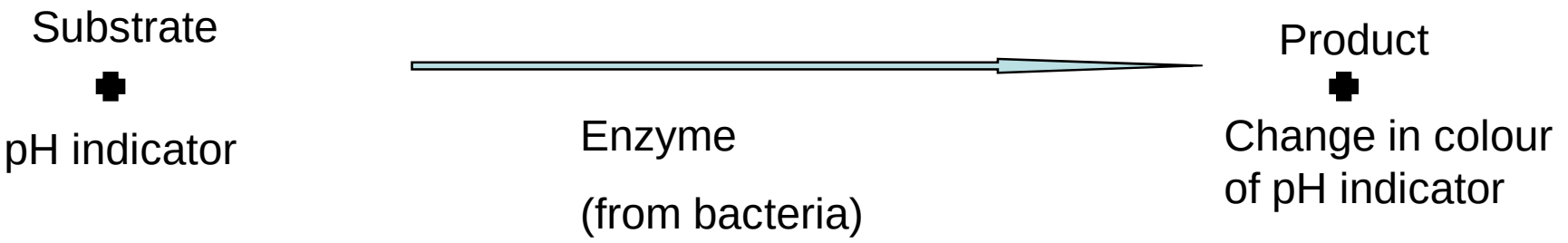
- Appearance
  - Macroscopic
  - Microscopic (eg gram stain, rod vs coccus)
- Growth requirement/rate
  - Media
  - Atmospheric gases
  - Temperature
- Smell
- Motility
- Hemolysis on blood agar
- **Biochemical tests**





(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 indictors

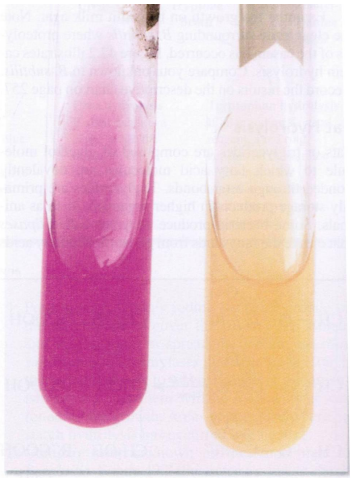


FIGURE 42.4 Urease test. Tube on the left is positive (*Proteus*); tube on the right is negative. © The McGraw-Hill Companies/Auburn University Photographic Service

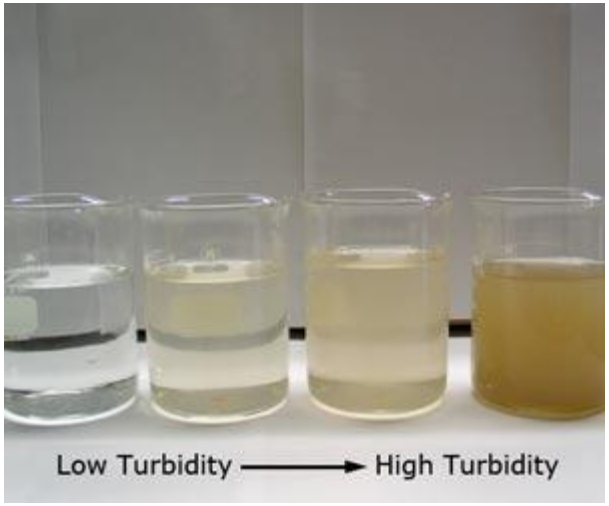
Å Colour changes occur at different pHs for different indicators

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



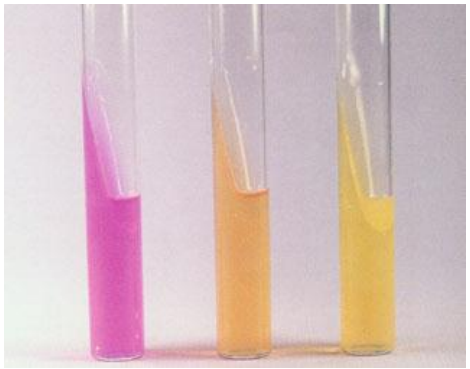
Positive and Negative controls

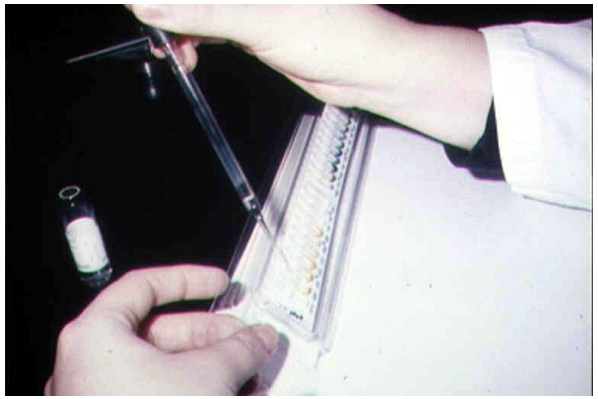
- Positive control: bacteria with known +ve test result
- Negative control: bacteria with known -ve test result
- If either or both of the controls fail, then the test is not valid

-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

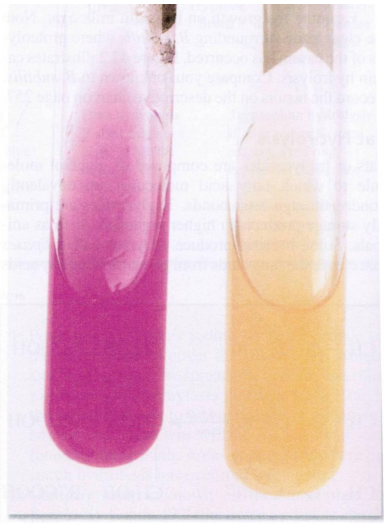
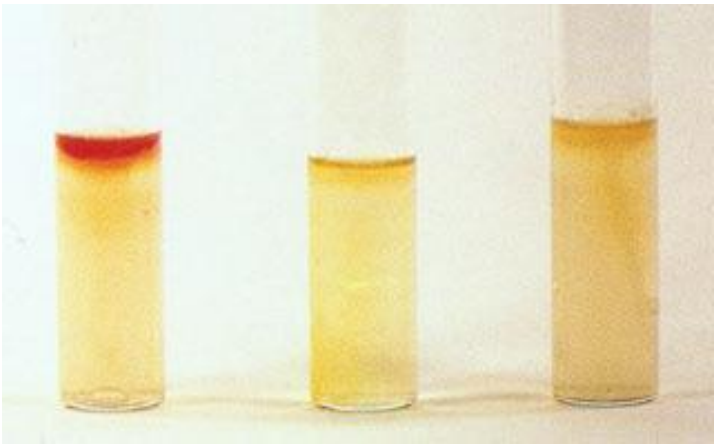


FIGURE 42.4 Urease test. Tube on the left is positive (*Proteus*); tube on the right is negative. © The McGraw-Hill Companies/Auburn University Photographic Service

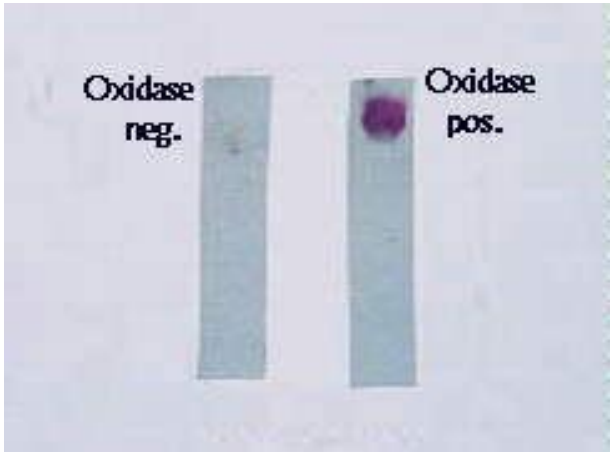
Indole

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



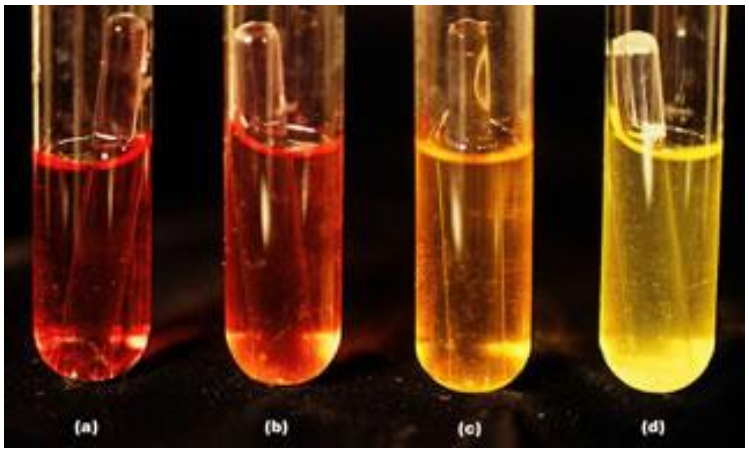
Oxidase

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



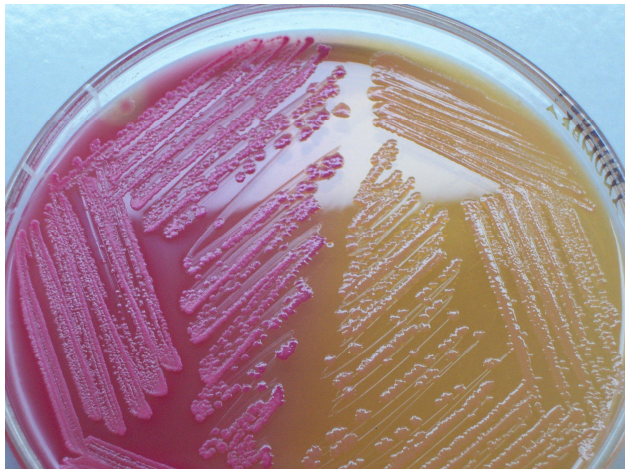
Glucose fermentation

- 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

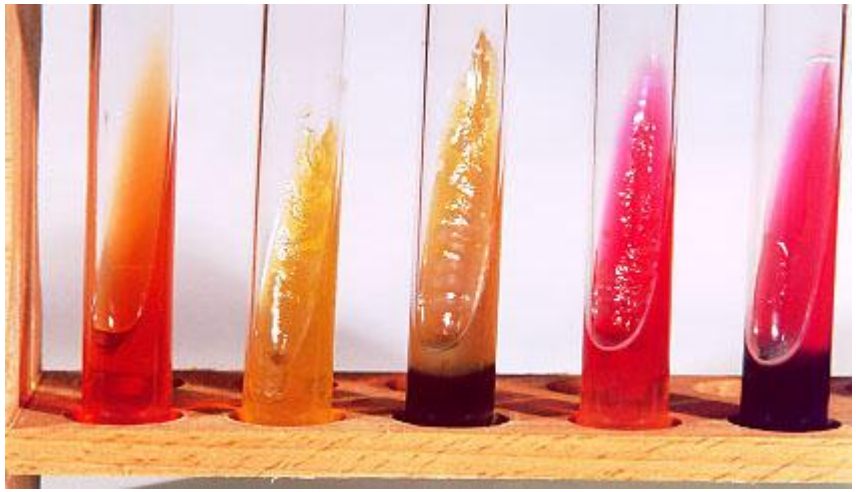


Nitrate

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

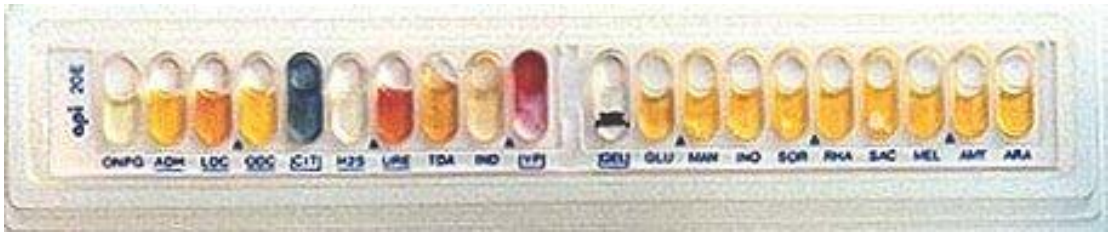
TSI slope

- 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

- Minituarized biochemical reactions in >20 wells
- Takes 2-24 hrs
- Reaction profile (ñbiocodeò) 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-omithine	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

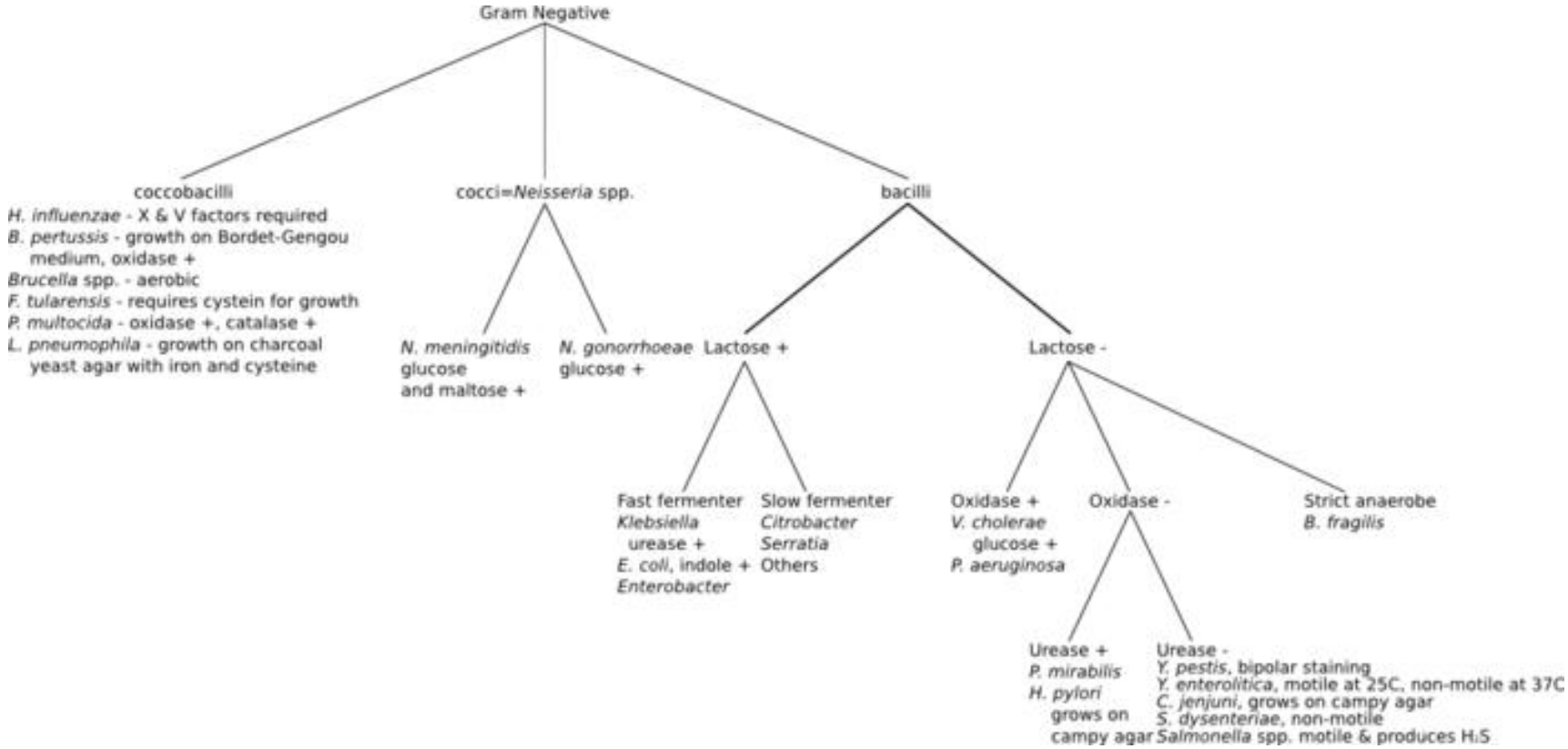
Diagnostic algorithms for bacterial ID

- Primary tests allow genus level ID (enterobacteriacae, 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

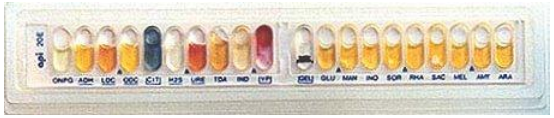
	Indole	Methyl red	Voges Proskauer	Citrate	Urease
E.coli	+	+	-	-	-
Enterobacter	-	-	+	+	-
Klebsiella pneumoniae	-	-	+	+	+
Salmonella	-	+	-	+	-
Shigella	-	+	-	-	-
Proteus mirabilis	-	+	-	+/-	+

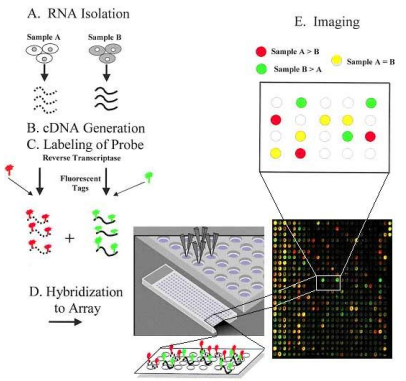
Example 2 of diagnostic algorithm



Changes in biochemical tests for ID: past and future

1970 1980 2000 2010





- 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