Molecular Diagnostics: The Future of Clinical Microbiology

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BD Diagnostic Systems

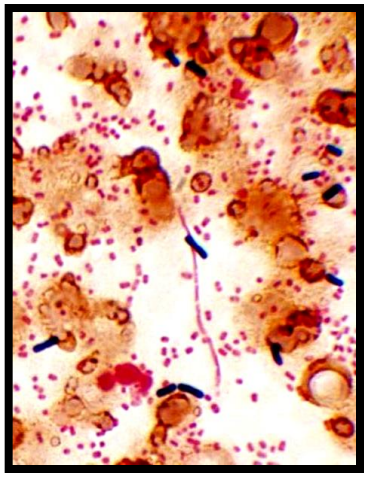
BD DIAGNOSTICS

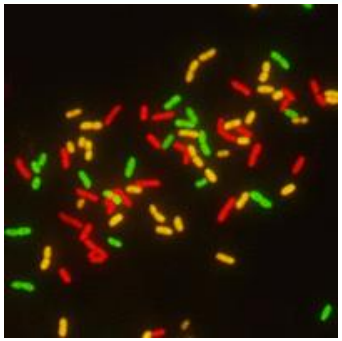
BD
Helping all people live healthy lives

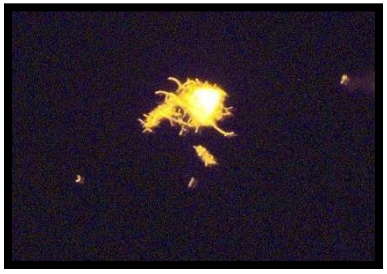
Clinical Diagnostics

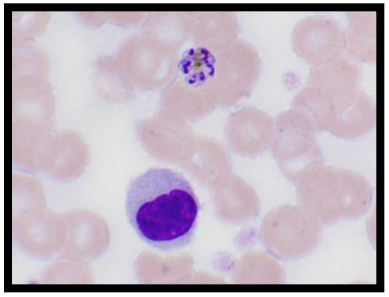
- Microscopy
- Culture
- Antigen Detection
- Antibody Detection
- Molecular Diagnostics

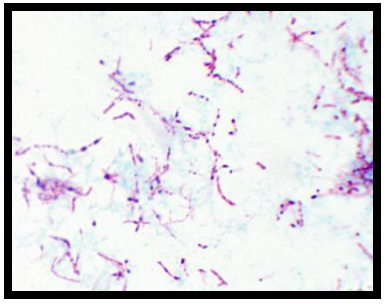
Microscopy

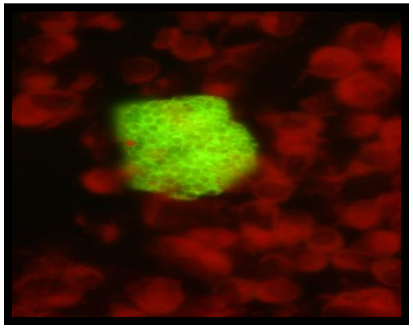


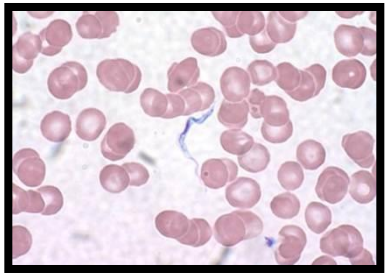


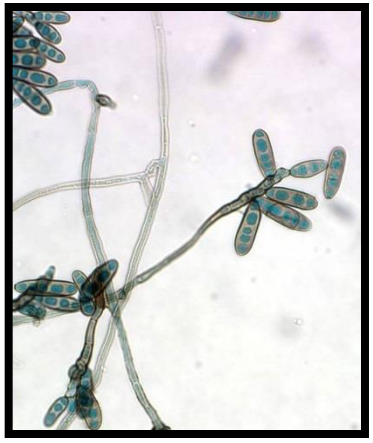


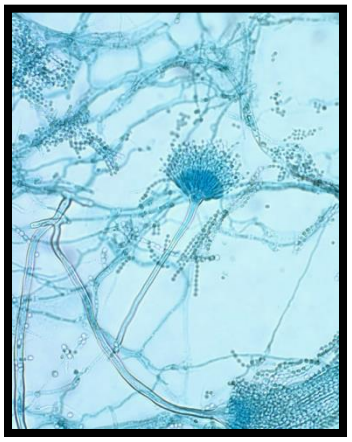


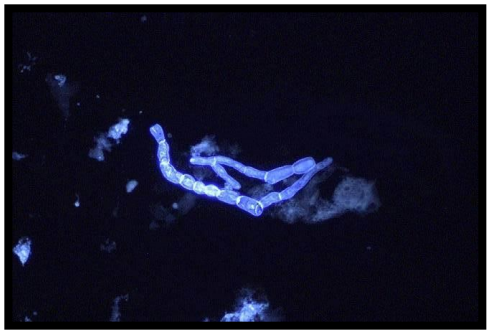


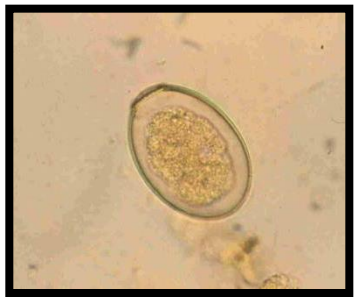












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Microscopy

Techniques:

- Contrasting stains
- Differential stains
- Immunofluoresent stains

Advantages:

- Rapid assessment of specimens and associated pathogens
- Diagnostic test of choice for certain organism groups
- Specific identification of key pathogens

Disadvantages:

- Relatively insensitive
- Subjective interpretation

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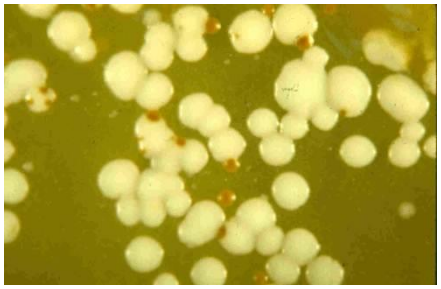
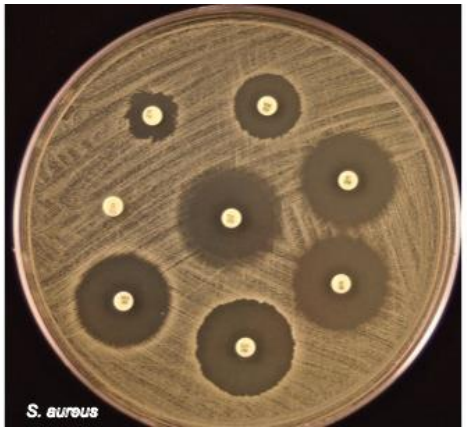
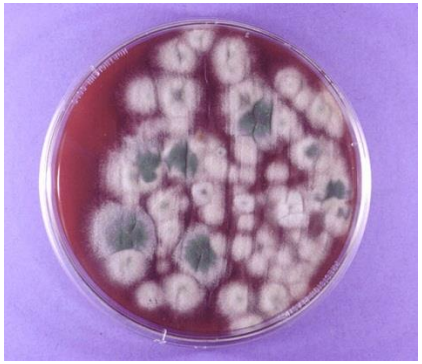
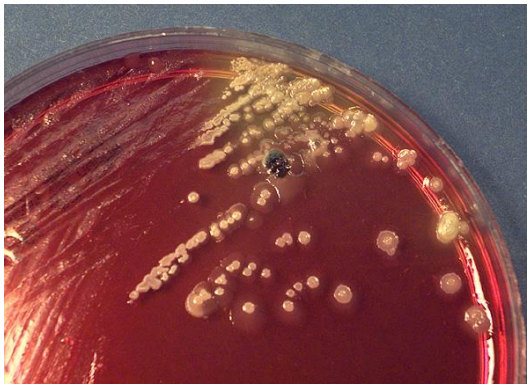
Culture









Culture

Techniques:

- Use of enriched, differential, and selective culture media, as well as tissue culture cell monolayers
- Used for all major groups of bacteria, fungi and viruses, as well as some parasites

Advantages:

- Isolation of pathogens that can provide a definitive diagnosis
- Defines relative proportion of pathogenic and nonpathogenic organisms
- Organism available for ASTs

Disadvantages:

- Slow time to results
- Only able to detect organisms that grow in culture

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Antigen Tests

- Antigen tests used for diagnosis of bacterial, fungal, viral and parasitic infections
- Useful for rapid results
- Inexpensive POC tests
- Not technically demanding and high volume tests performed on automated platforms
- Analytical performance (sensitivity, specificity, predictive value) improving but still inferior to culture and molecular diagnostics
- Provides clinical value but frequently must be confirmed by alternative diagnostic tests

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Antibody Tests

- Primarily used for screening patients for immunity to specific diseases or past exposure to pathogens
- Antibody tests used less commonly for diagnosis of acute infections; requires demonstration of elevated antibody levels or change in levels
- Improvements in culture techniques and introduction of molecular testing have decreased the value of serology for diagnosis of active infections

Molecular Diagnostics: Historical

- Commercial assays performed on large platforms
- High volume assays, such as blood screening for viral agents and STD testing
- Microbiology lab developed tests were primarily restricted to research labs or academic settings
- Testing generally was expensive and required specialized technical expertise and testing facilities

Molecular Diagnostics

Dedicated Nucleic Acid Extraction

bioMerieux easyMAG

Roche MagNA Pure LC

Hamilton Microlab Starlet

Qiagen
QiaSymphony

Dedicated Real-time PCR

Roche LightCycler

Qiagen
Rotor-Gene

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Integrated Extraction & Amplification Systems

BD
Viper XTR

- Closed Systems
- Floor standing

Hologic/GenProbe

Panther

Tigris

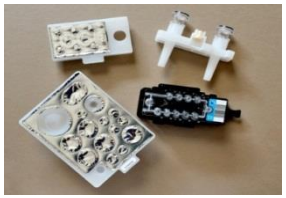
- Closed Systems
- Floor standing

BD

Integrated Extraction & Amplification Systems

BioFire Film Array

Nanosphere Verigene



Cepheid GeneXpert

BD MAX™ System

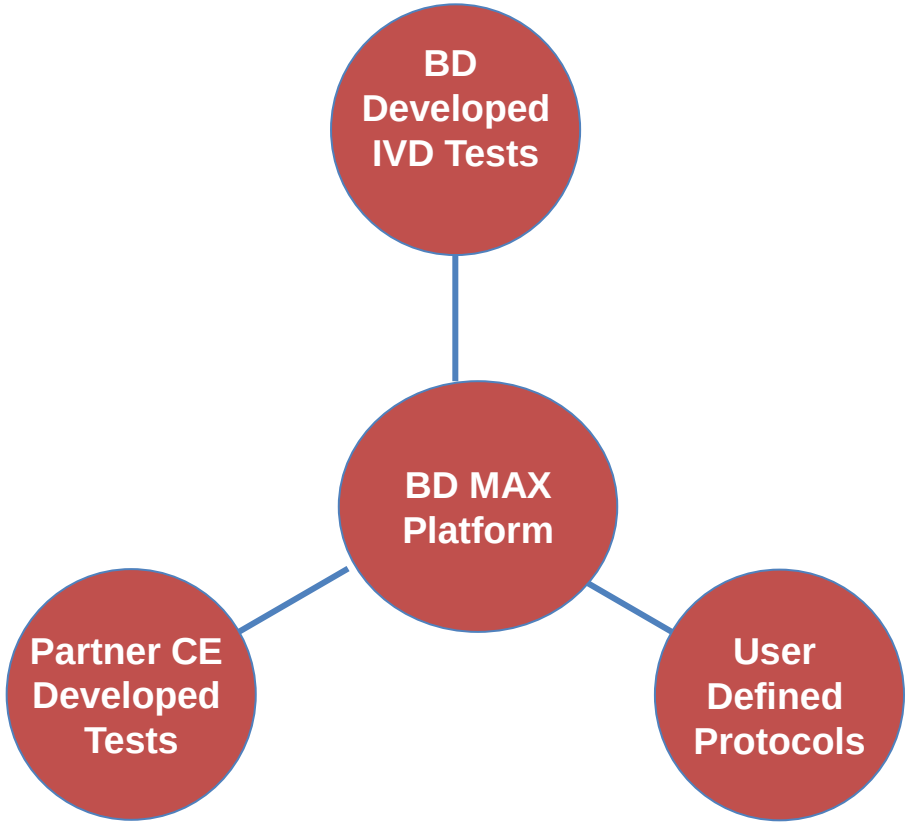
Integrated Extraction & Amplification Systems

BD MAX™ System

- **Broad IVD and LDT menu**
- **Open system**

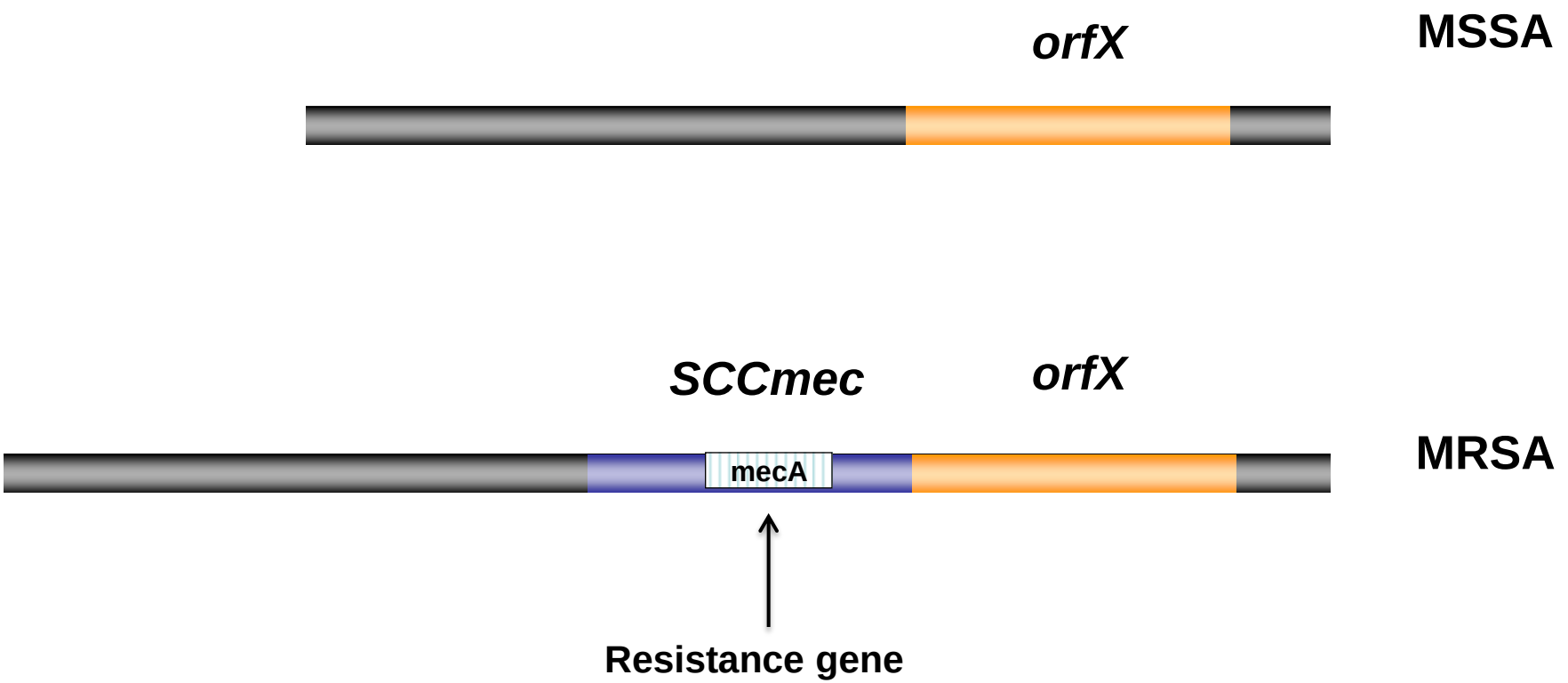
A New Model for Molecular Assay Development

- **BD R&D**
  - 6 teams of 8-10 scientists currently work on assay development
  - 1 team of 6-8 support open system assay development
- **Commercial partners**
  - Diagenode
  - BioGX
  - Others
- **Clinical partners**
  - EU MAX Expert User group
  - US MAX Expert User group
  - Research groups

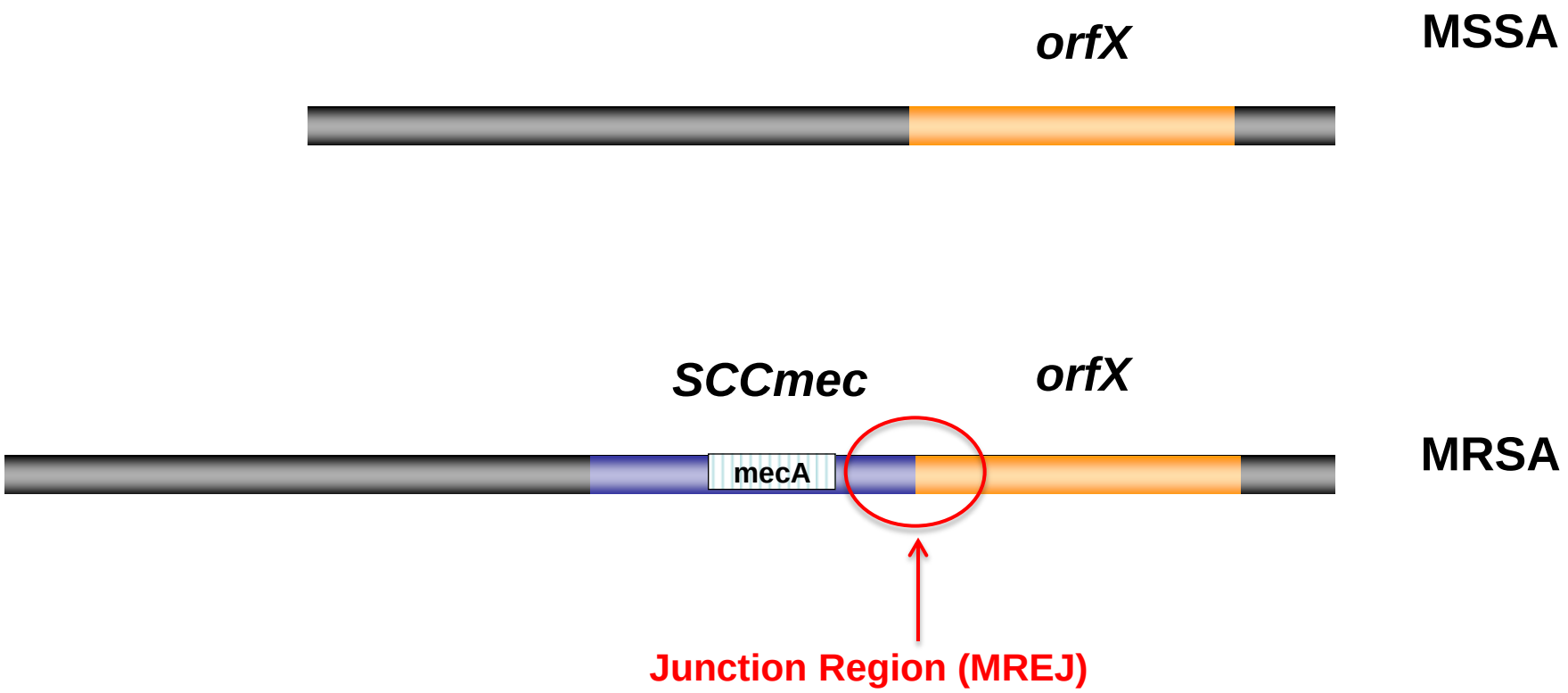


MRSA: Making a Good Assay Better

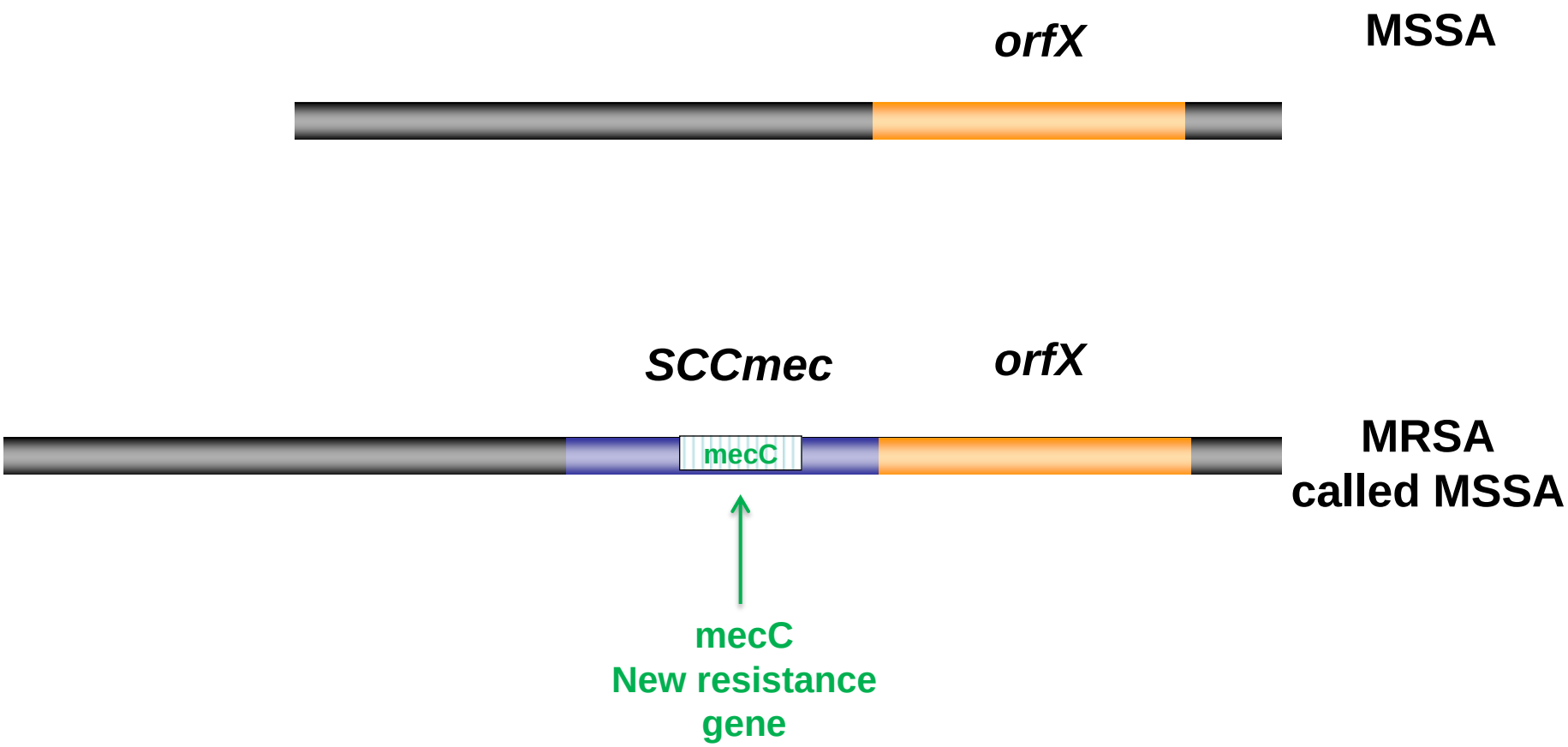
MRSA: mecA Resistance Gene



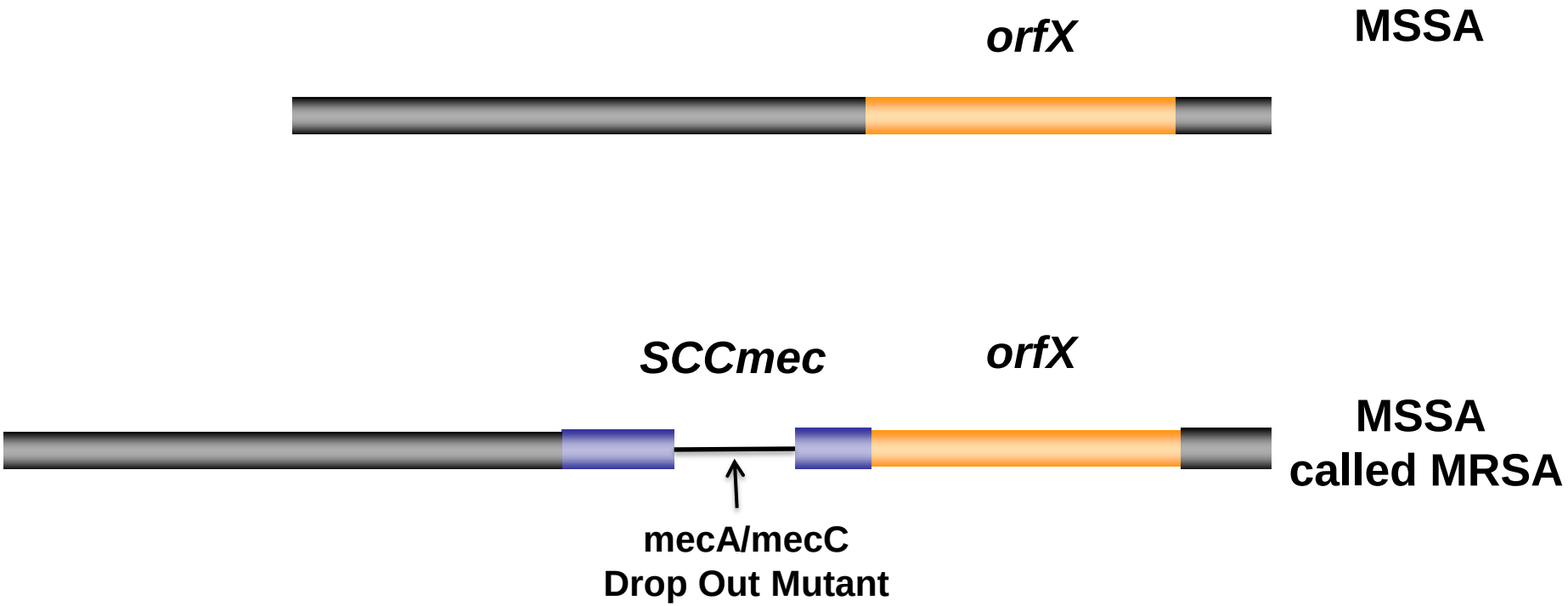
MRSA: mecA Resistance Gene
MRSA: Junction Region Target



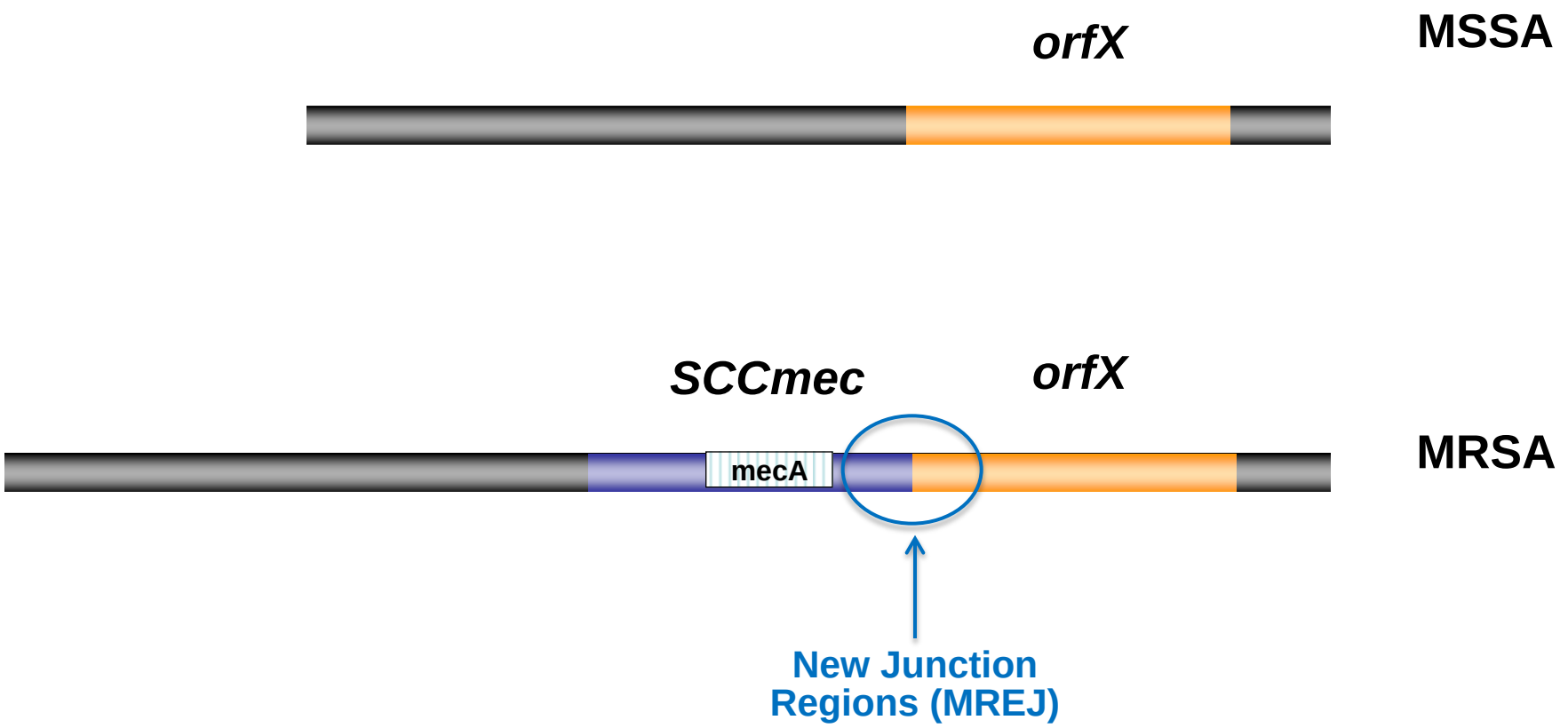
MRSA: New Resistance Genes



MRSA: Drop Out Mutants



MRSA: New Junction Region Targets



Next Generation MAX StaphSR and MRSA-XT will detect a broader range of MRSA variant strains

	MREJ Types detected
Current MAX MRSA	i ii iii iv v vii
Next Gen MAX StaphSR and MAX MRSA-XT	i ii iii iv v vi vii ix xiii xiv xxi

- Detects both mecA and mecC genes
- Detects mecA and mecC dropout mutants
- Detects newly discovered MREJ strains

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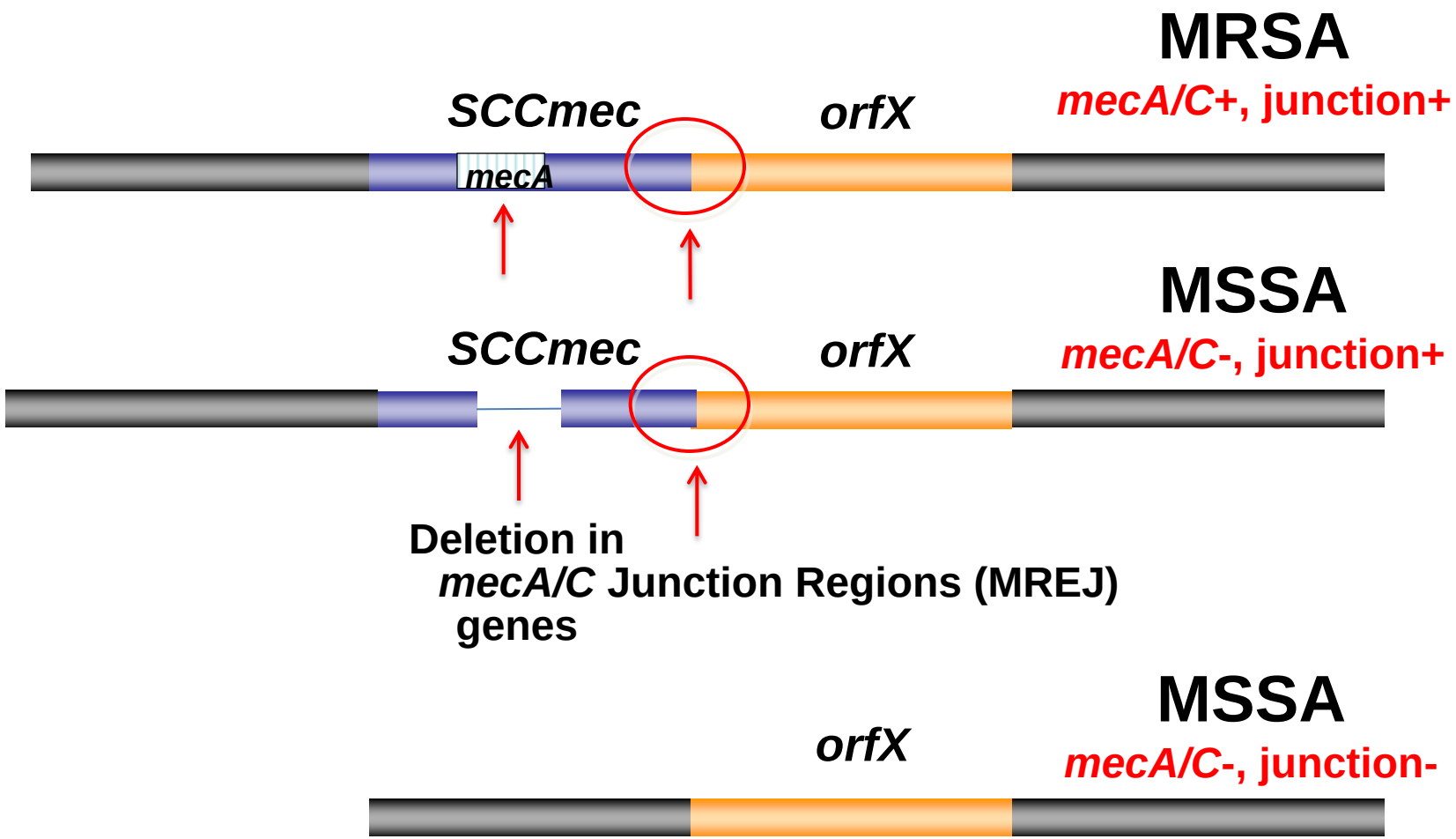
New MAX MRSA XT and StaphSR Assays Are More Sensitive and Specific



Development of Syndromic Menu

- Clinical approach to menu development is – develop comprehensive syndromic solutions
- Enteric diseases
  - Clostridium difficile (CE/IVD)
  - Bacterial panel – Salmonella, Shigella, Campylobacter, STEC (CE/IVD)
  - Viral panel – Norovirus, Rotavirus (CE)
  - Parasites – Cryptosporidium, Giardia, E. histolytica (CE)
  - Uncommon enteric bacterial pathogens
- Assay development
  - Detect pathogens not previously assayed
  - Improve detection over existing assay methods

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Clostridium difficile Diagnostic Tests

Diagnostic Method	Target	Sensitivity (%)	Specificity (%)	Turnaround Time
Cell cytotoxicity culture assay	Toxin	70-80	90-95	Days
Glutamate dehydrogenase	Common antigen	70-90	<90	Hours
Enzyme immunoassay	Toxin	40-70	>97	Hours
Toxigenic culture	Toxin	>95	95-97	Days
PCR	Toxin	>95	>97	Hours

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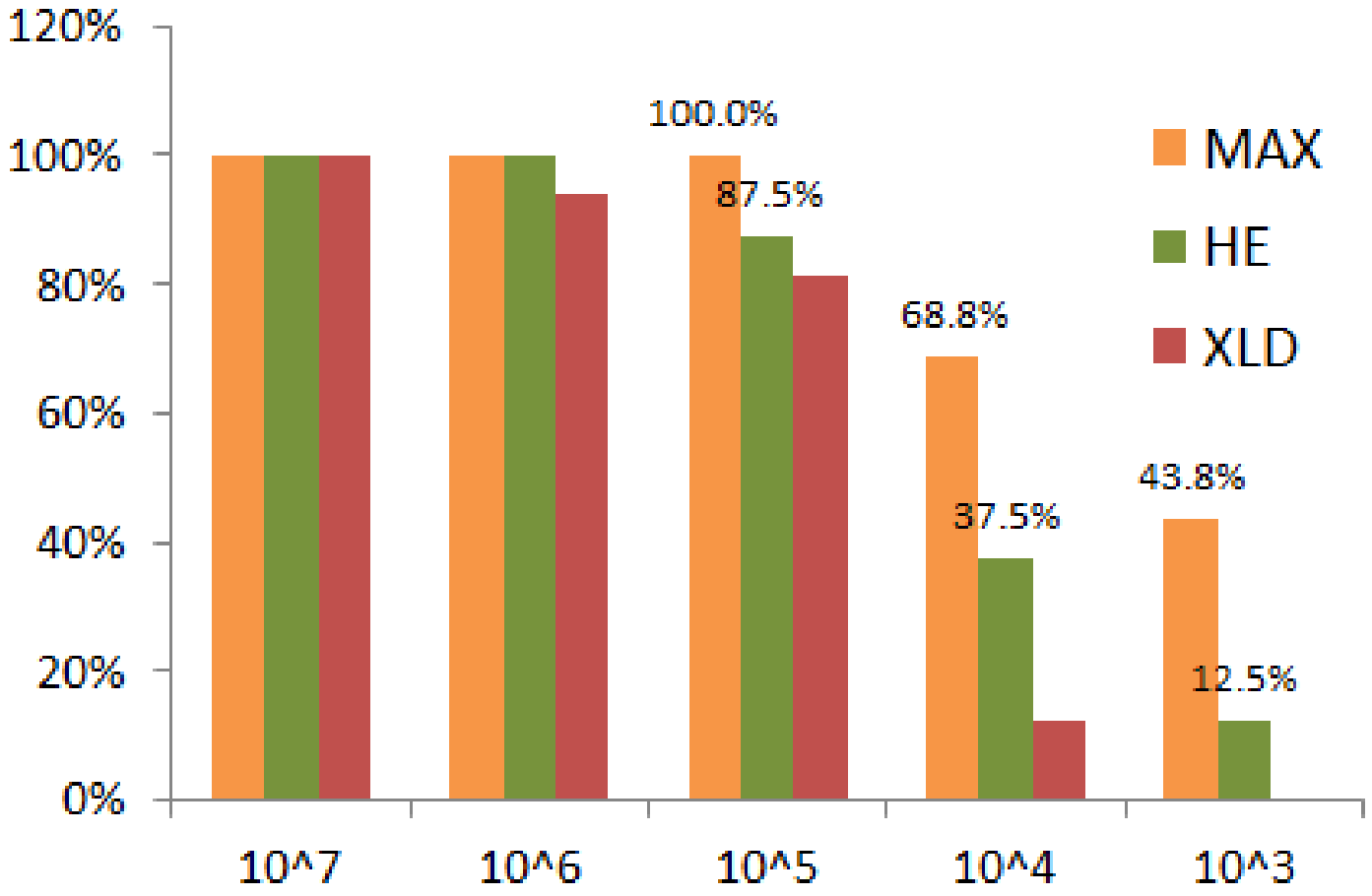
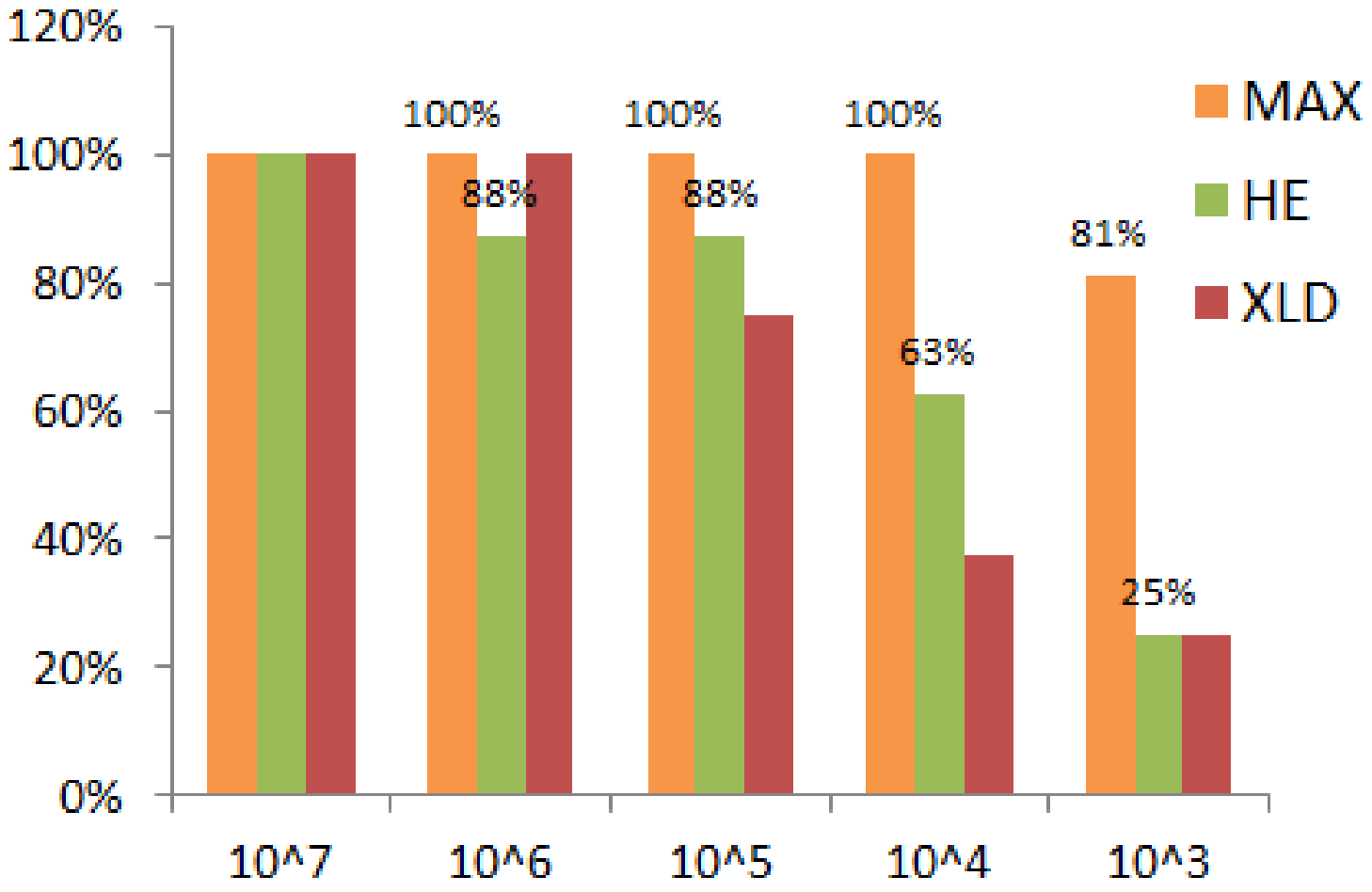
BD MAX Limit of Detection

Salmonella spp.



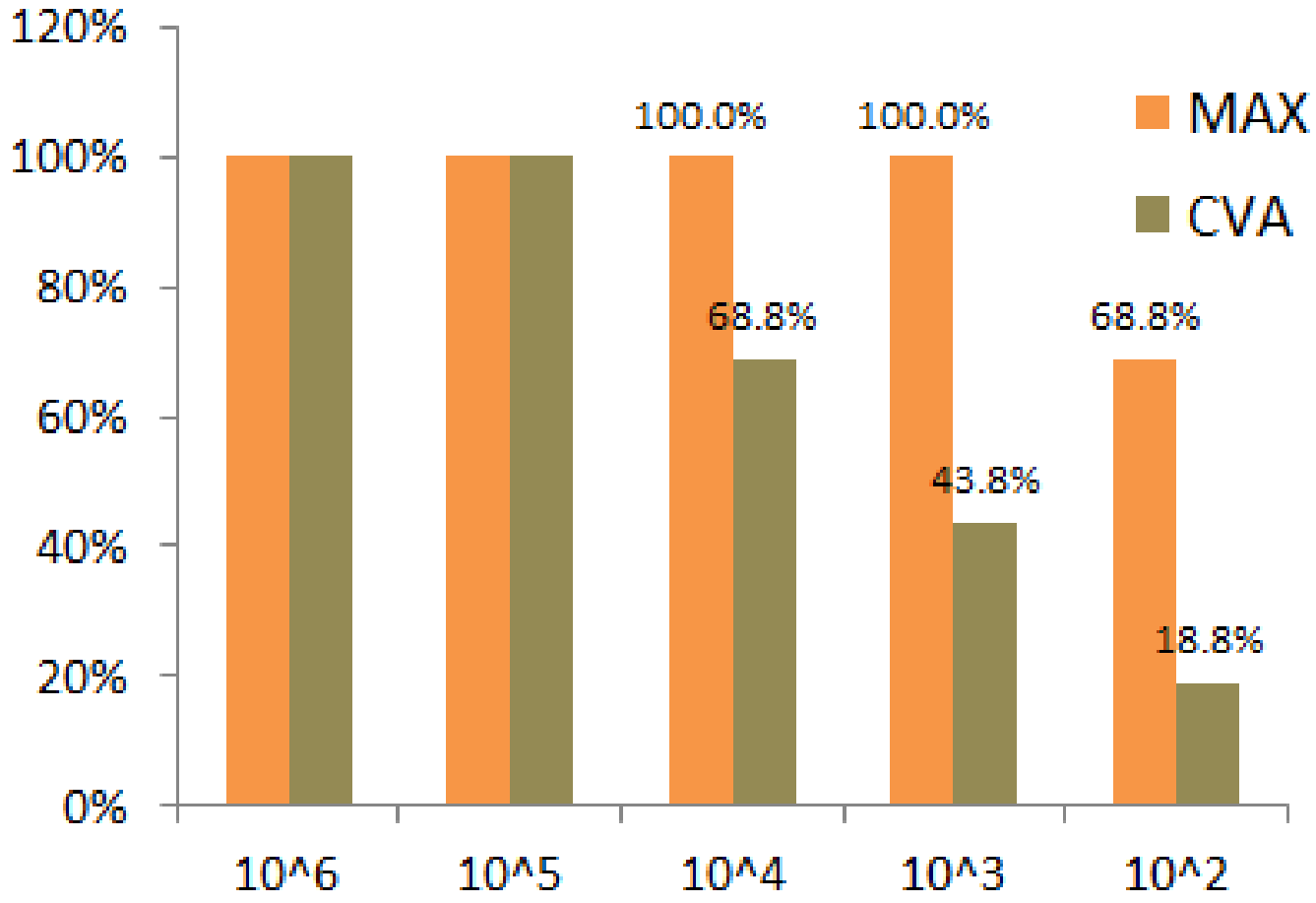
BD MAX Limit of Detection

Shigella spp.

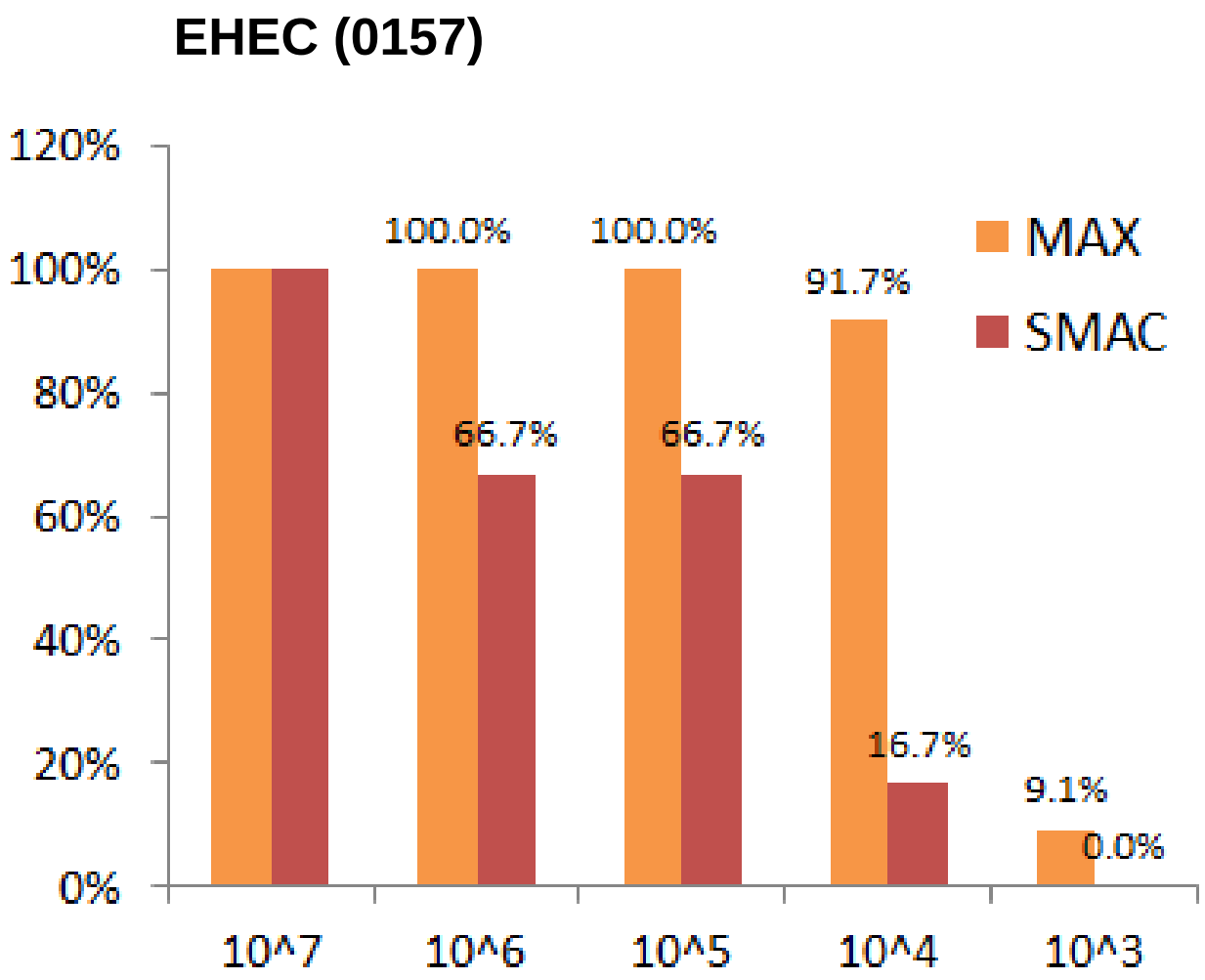


BD MAX Limit of Detection

Campylobacter spp.



BD MAX Limit of Detection



Global Medical Problems

Problem
Outbreaks with carbapenem-resistant bacteria
Bacterial meningitis outbreak in Chile
Coronavirus outbreak in Middle East

2011 Outbreak with Multidrug Resistant Gram-Negative Bacteria at the NIH Clinical Center

- On June 13 a patient from NYC was admitted into the NIH Clinical center ICU. She was colonized with a MDR A. baumannii and a KPC strain and was immediately placed on enhanced contact isolation in a private room. She was discharged 1 month later.
- On August 5 a second patient was found to be infected with the same strain of KPC.
- A total of 18 patients became infected, with 11 deaths including 7 patients whose deaths were directly attributed to this organism.
- This outbreak illustrates the difficulty in controlling, by traditional infection control practices, essentially untreatable multidrug resistant gram-negative bacteria.

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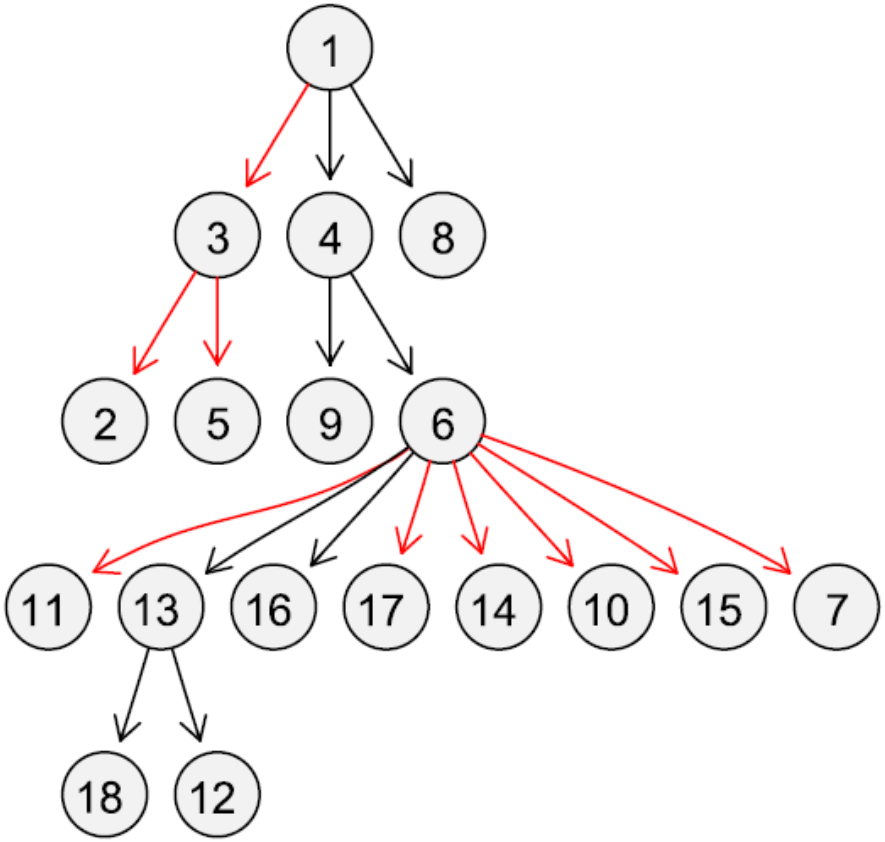
Tracking a Hospital Outbreak



- Traditional epidemiology approaches under-estimated transmission
- Successful intervention required a combination of comprehensive screening and rigorous infections control practices.

Carbapenemase Assay

Developer	Assay Targets
BD R&D	KPC, NDM-1, Oxa-48
Dalpke et al	Panel 1: KPC; VIM-2 IMP-1,-2; GES Panel 2: NDM; OXA-23,-48; VIM-1
CheckPoint	KPC; NDM; Oxa-48; VIM
BioGX	KPC; NDM; OXA-48; VIM/IMP

2012-13 Neisseria meningitidis Outbreak in Chile

- On Sunday Dec 16 a woman traveling on LAN flight from Santiago, Chile to Punta Cana, Dominican Republic developed symptoms of meningitis and died of an overwhelming infection with Neisseria meningitidis. All 200 passengers on this flight were given prophylactic antibiotics.
- By April 2013, 178 cases of meningococcal meningitis were reported in Chile.
- Most infections were with serogroup W135 and were localized in the Metropolitan Region (includes Santiago) and the adjoining Valparaiso Region.

BD MAX™ Open System Group (OSG)

- A multiplex bacterial meningitis assay for N. meningitidis, S. pneumoniae, and H. influenzae was developed in 1 month
- Based in Baltimore MD R&D Systems Engineering:
  - Composed of Manager, Senior Scientists and Engineers
- Objective: Provide advanced applications support to customers and MAX partners
- Offers scientist-scientist collaboration for customers developing LDTs, performs technical feasibility testing in-house

2012-13 New Coronavirus Outbreak in Middle East

- Between April 2012 and May 2013, 49 cases of human infection with a new coronavirus originating in the Middle East (Middle East Respiratory Syndrome Coronavirus; MERS-CoV) were reported with 26 deaths (53%) due to severe acute respiratory disease.
- Cases were reported from Saudi Arabia (37), Jordan (2), as well as the UK (4), France (2), Tunisia (2) and Germany (2).
- MERS-CoV is related to bat viruses; however, no animal reservoir has been identified. Until recently, limited person-to-person spread was documented in close contacts and healthcare personnel.
- Most coronavirus infections are mild respiratory disease but MERS-CoV and SARS-CoV cause severe acute respiratory syndromes with an associated high mortality.

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MERS-CoV Assay

- Developed by clinical partners (Hendrickson and Bose, Medical College of Wisconsin)
- Primers, probes, and plasmid control designed for RNA-dependent RNA polymerase gene using 3 published genome sequences for MERS-CoV.
- Clinical testing performed with 20 spiked positive samples and 56 negative clinical samples. Assay with 100% sensitivity and specificity.
- Development and validation time: 2 weeks

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Global Problems – Molecular Solutions

Problem	Solution
Outbreaks with carbapenem-resistant bacteria	Multiplex assay for KPC, NDM-1, Oxa-48
Bacterial meningitis outbreak in Chile	Multiplex assay for N. meningitidis, S. pneumoniae, H. influenzae
Coronavirus outbreak in Middle East	Screening assay and confirmatory assay for MERS-CoV

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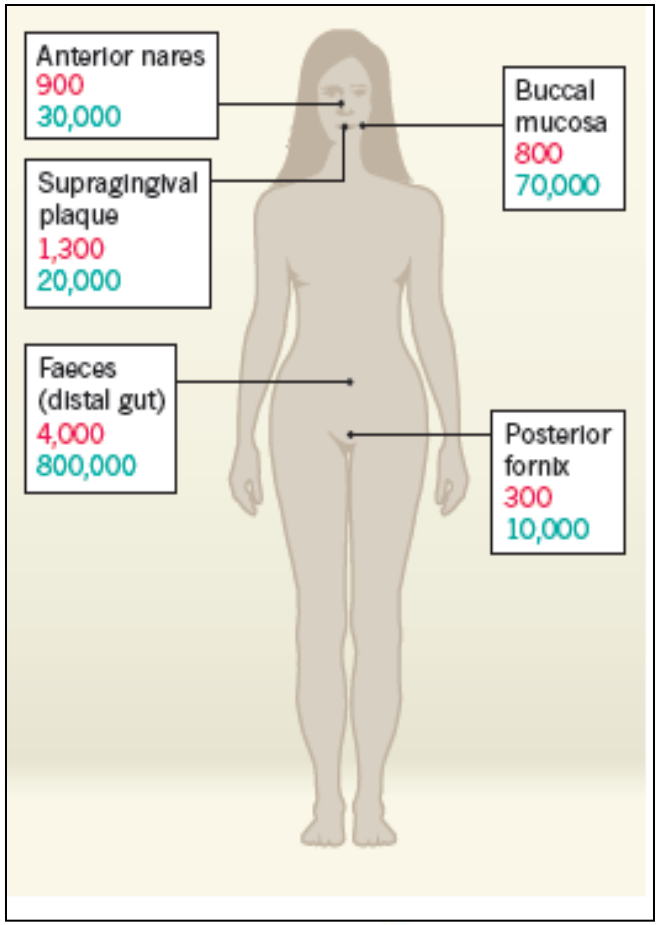
Summary

- BD MAX, BioFire Film Array, Cepheid GeneXpert, Nanosphere Verigene and Hologic-GenProbe Panther and Tigris are examples of integrated systems of nucleic acid extraction, amplification, and analysis.
- BD MAX is a unique open system for simultaneously performing multiplex assays developed by BD, commercial partners, and clinical partners.
- The ability to develop novel assays (such as for the diagnosis of multidrug-resistant bacteria, bacterial meningitis, MERS-CoV) enables diagnostic labs to respond rapidly to emerging infectious diseases

Human Microbiome

- Human Genome Project (1990-2003) – 13 year project to map the 22,000 genes in the human genome
- Human Microbiome Project (2005-2010) – 5 year project to map the 8 million unique genes in the bacteria that populate our bodies
- Most bacteria on the body are critical for our health (“bacteria are good not bad”)
- Although there is **taxonomic heterogeneity** (most of us have a unique population of bacteria), there is **functional redundancy** (we share common gene functions)



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Microbiome and Intestinal Health and Disease

- The intestinal microbiome is characteristically diverse and functions in close balance to:
  - protect against colonization with pathogens
  - detoxify potential poisons
  - produce energy and nutrients by digestion of food
  - maintain mucosal and systemic immunity
- **Clostridium difficile diarrhea –** under the influence of antibiotics or disease, the microbiome shifts from a complex flora to a predominance of toxin-producing C. difficile.
- **Crohn's disease** and **inflammatory bowel disease –** distinct, characteristic changes of microbiota that alter regional metabolism and mucosal immunity.

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What Impact Will Microbiome Research Have on the Clinical Microbiology Laboratory?

- **Antibiotic susceptibility testing**: genome sequencing can identify known resistance genes but cannot determine expression or previously unrecognized resistance markers.
- **Microbial identification**: sequencing is unlikely to replace mass spectrometry for organism identification but can identify the composition of a population of organisms, virulence potential of microbes, and be used as the definitive typing method.
- **New approach to laboratory diagnostics**: laboratory testing could shift from diagnosis of disease to prediction of disease by monitoring shifts in the microbiome species or metabolic function.
- **Technical challenges:** preanalytical processing, sequencing, and data analysis are daunting but success will revolutionize diagnostics.

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Development of Syndromic Menu

- Syndromic menu
  - Comprehensive multiplex assay
  - Targeted multiplex assays
- Comprehensive assays
  - Broad menu of analytes – but
  - Expensive
  - Decreased analytical performance
- Targeted assays
  - Requires clinical assessment of patient and development of differential diagnosis
  - Faster assay development and regulatory clearance

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