Molecular Diagnostics for Bacterial Meningitis

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Outline

- IMPORTANCE OF RAPID DIAGNOSTIC TESTS FOR BACTERIAL MENINGITIS
- CURRENT LAB DIAGNOSTICS
- LAB DEVELOPED rt-PCR TESTS
- FUTURE MOLECULAR DIAGNOSTICS
- CULTURE FOR OTHER SURVEILLANCE PROGRAMS

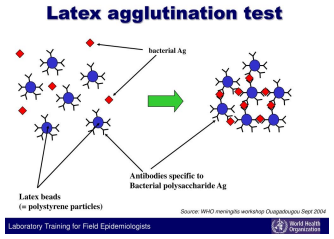
Importance of Rapid Diagnostics for Bacterial Meningitis

- Bacterial Meningitis is a life-threatening disease
- Rapid detection of meningitis pathogens is critical for case management, surveillance, and outbreak investigations
- Limitations of existing diagnostic tests prevent their wide usage
  - Culture: low recovery rate due to antibiotic use prior to specimen collection, improper storage/transport conditions etc.
  - Subcellular detection methods: polysaccharide antigen or nucleic acid-based
- Needs for development of next generation rapid tests

Current Lab Diagnostics for Bacterial Meningitis

Latex agglutination tests

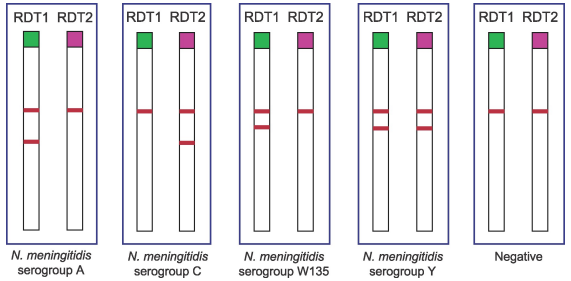
- Target various meningitis pathogens but not all meningococcal serogroups
- Rapid (< 20 mins)
- High cost
- Cold chain for storage/distribution
- Performance may vary
  - lab verification: 33-100% sensitivity; 93-100% specificity
  - field evaluation: 69-80% sensitivity; 81-94% specificity



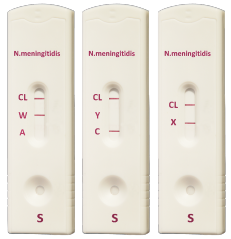
Immunochromatographic tests

- Sp and all meningococcal serogroups except B
- Rapid (<15 mins)
- Cassette format expensive
- Cold chain for storage/distribution
- High sensitivity and specificity

Duplex Dipstick



MeningoSpeed



Latex agglutination tests

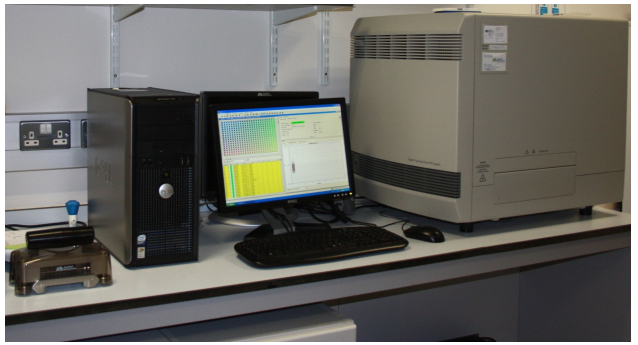
Kit	Target pathogens	Specimen Type	Time	No. of Tests per Kit	Cost per Kit	Limitations
Wellcogen™ Bacterial Antigen Kit	group B *Streptococcus* , *H. influenzae* serotype b, *S. pneumoniae*, *N. meningitidis* serogroups A, C, Y or W, *N. meningitidis* group B/ *E.* coli K1	Blood culture Plate culture[±] CSF, Serum urine	3 mins	30	$1967[+]	• Not all meningitis pathogens are covered • Cold chain required • Operation by trained/experienced staff • Cross-reactivity with other bacterial species
Pastorex™ Meningitis	*N. meningitidis* groups A, C, Y or W, *E.* coli K1, *H. influenzae* serotype b, *S. pneumoniae*, group B *Streptococcus*	Blood culture CSF, Serum urine	5 to 10 mins	25	$384[≠]	
BD Directigen™ Meningitis Latex Test System	*H. influenzae* serotype b, *S. pneumoniae*, *N. meningitidis* groups A, B, C, Y or W, *E. coli* K1, group B *Streptococcus*	Blood culture CSF[*], serum[*] urine	15 to 20 mins	90	$2464[+]	
Phadebact™ CSF Test (require -80°C incubator)	*S. pneumoniae, H. influenzae serotype b, N. meningitidis serogroup A, B, C, Y, W and S. agalactiae*	CSF, Serum Urine	10-15 mins	20	1242	

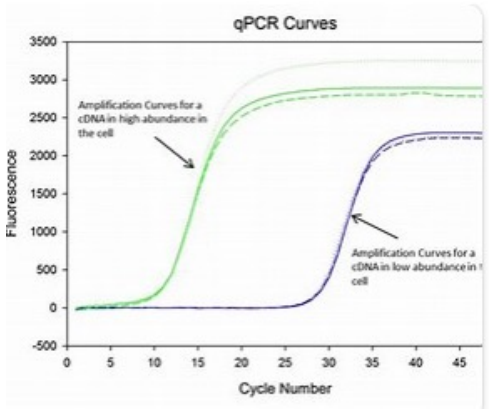
Immunochromatographic tests

Test	Target	Specimen Type	Time	No. of Tests per Kit	Cost per kit	Limitations
CERMES Duplex Dipstick	*N. meningitidis* serogroup A, C, Y, and W	CSF	10 to 15 mins	N/A	N/A	• Not all Nm serogroups are detected • Sp serotypes are not detected • Not all meningitis etiological agents are detected • Large specimen volume required for test
NmX dipstick	*N. meningitidis* serogroup X	CSF, Bacterial suspension in PBS	10 to 15 mins	N/A	N/A	
BioSpeedia MeningoSpeed	*N. meningitidis* serogroups A, C, Y, W, and X	CSF	3 to 10 mins	20	$545	
BioSpeedia PneumoSpeed	*S. pneumoniae*	CSF, urine	3 to 15 mins	20	$136	
Abbott™ BinaxNow® AgCard	*S. pneumoniae*	CSF, urine	15 mins	22	$374	

PCR-based tests

- Relatively fast (1-5 hrs)
- Sensitive/specific for pathogen detection
- High throughput and rapid data reporting
- Multiple platforms available
- Certain tests implemented in a number of African countries





- High cost
- Decentralization to regional/district levels may be challenging
- Require technical trainings and lab infrastructure (freezer, fridge, separate rooms etc)
- Requires effective transport systems for specimen referral

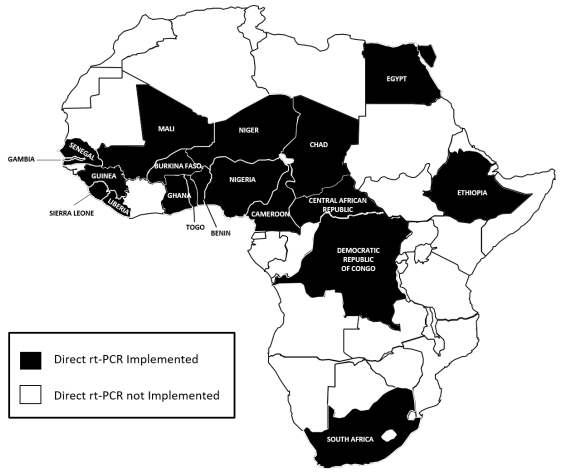
Examples of PCR-based tests

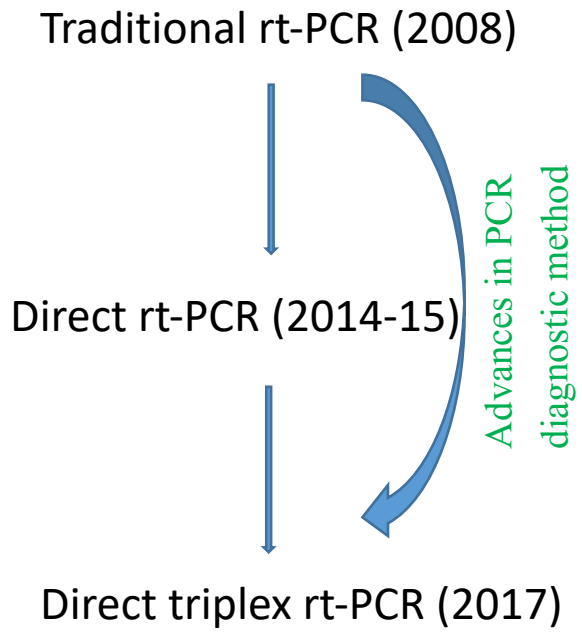
Test	Platform	Targets	Specimen Type	Availability	Instrument	Time
M-DiaSero (Belgium)	Traditional rt-PCR, monoplex	*Neisseria meningitidis* (Serogroups A, B, C, W and Y)	Blood, CSF, Swab	EU/S. Africa	ABI, Bio-Rad, Qiagen, LC480	Up to 4 hrs
HG Meningococcus (Ireland)	LAMP	*Neisseria meningitidis* (Serogroups A, B, C, E, W, X, Y, and Z)	Blood, CSF, Swab, Direct CSF	EU	LAMP instrument	<60 mins
Real-time PCR Detection Kit for Neisseria Meningitidis (China)	Traditional rt-PCR, multiplex	*Neisseria meningitidis* (Serogroups A, B, C, E, W, X, Y, and Z)	CSF	China	N/A	N/A
CDC rt-PCR	Direct PCR, triplex	*Neisseria meningitidis* (Serogroups A, B, C X, W, and Y)	CSF, serum		ABI, AriaMx	2 hrs

Laboratory Developed Real-Time PCR Tests (CDC)

rt-PCR for the detection of meningitis pathogens and capsular genotypes

- High sensitivity/specificity (>95%)
- Implemented in a number of countries
- Procurement of key reagents through IRR, an online ordering system that supports 45 countries
- EQA program in place to ensure data quality
  - WHO PT/CDC Retesting

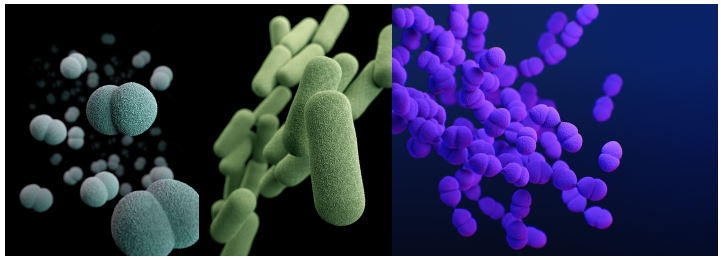




Specimen types:

- CSF for direct rt-PCR
- CSF, blood and other body fluids for traditional rt-PCR

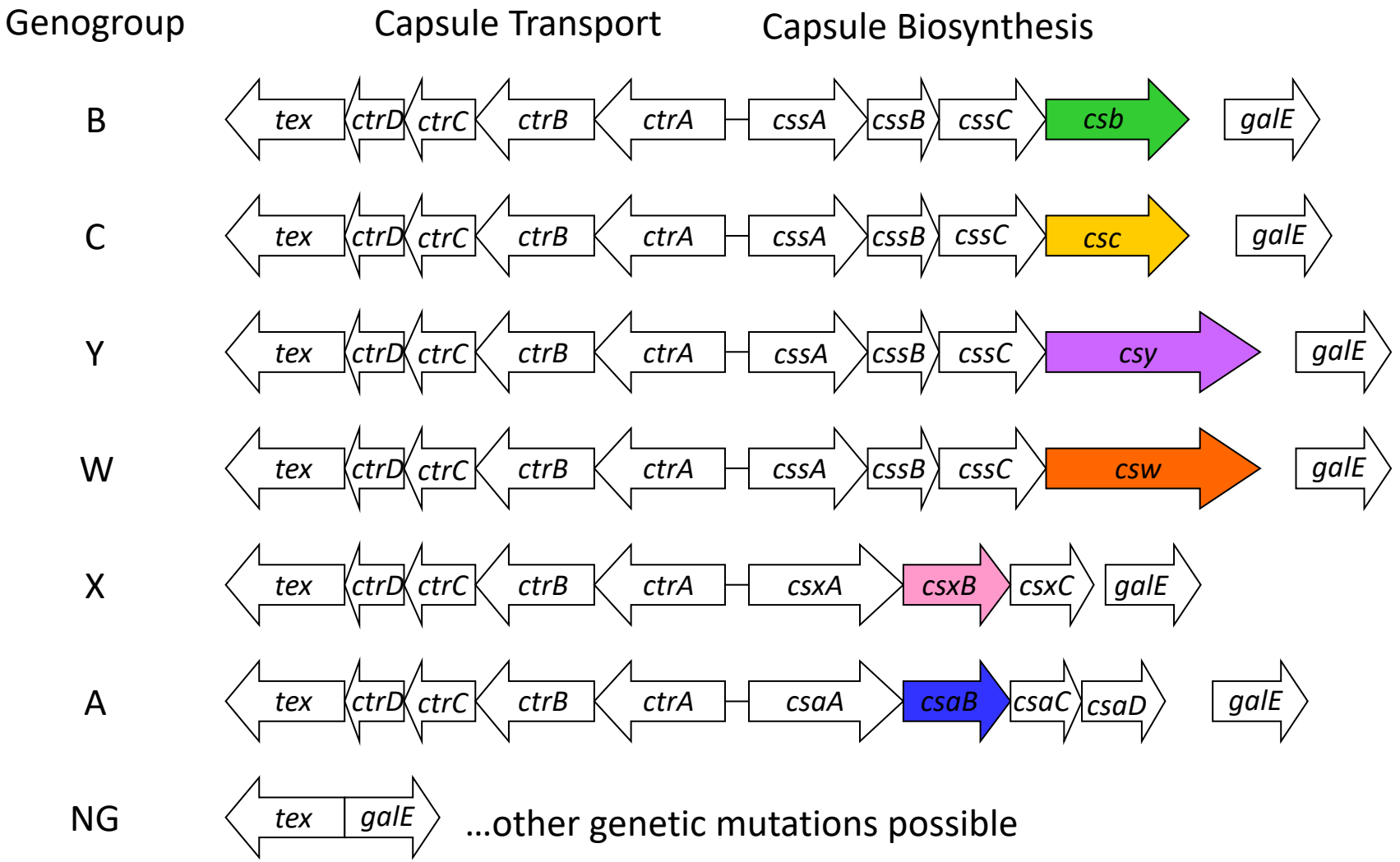
Species-specific real-time PCR tests



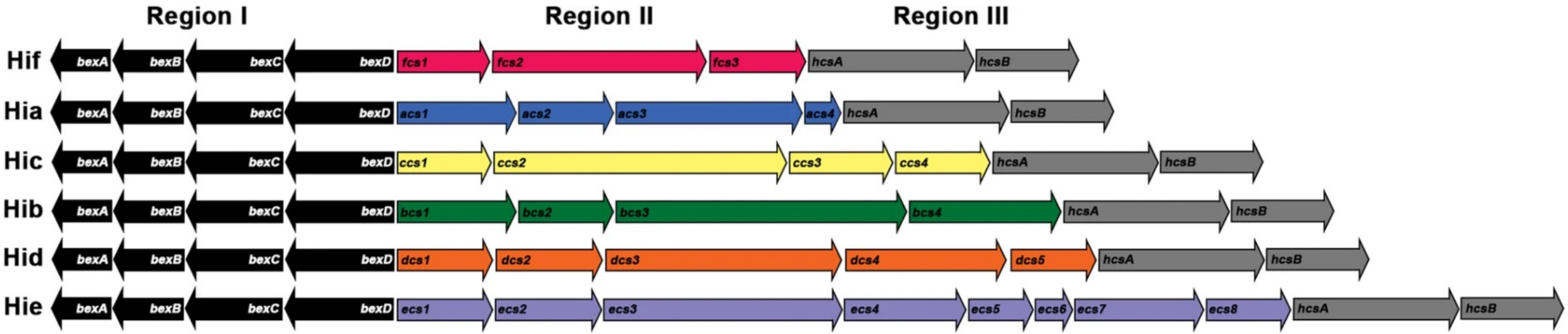
Target Pathogen	Gene function	Gene/amplicon size (bp)	Strain covered
Neisseria meningitidis	*sodC*-Superoxide dismutase	560/127	Groupable and non-groupable
Haemophilus influenzae	*hpd*-Haemophilus protein D	1095/151	Typable and non-typable
	phoB-DNA binding response regulator*	696/106	Typable and non-typable (*hpd*-)
Streptococcus pneumoniae	*lytA*-autolysin	957/51	Typable and non-typable

*also known as phosphate regulon transcriptional regulatory protein
(two component system DNA-binding response regulator [Haemophilus influ - Protein - NCBI (nih.gov)

Capsule biosynthesis genes as targets for Nm serogrouping

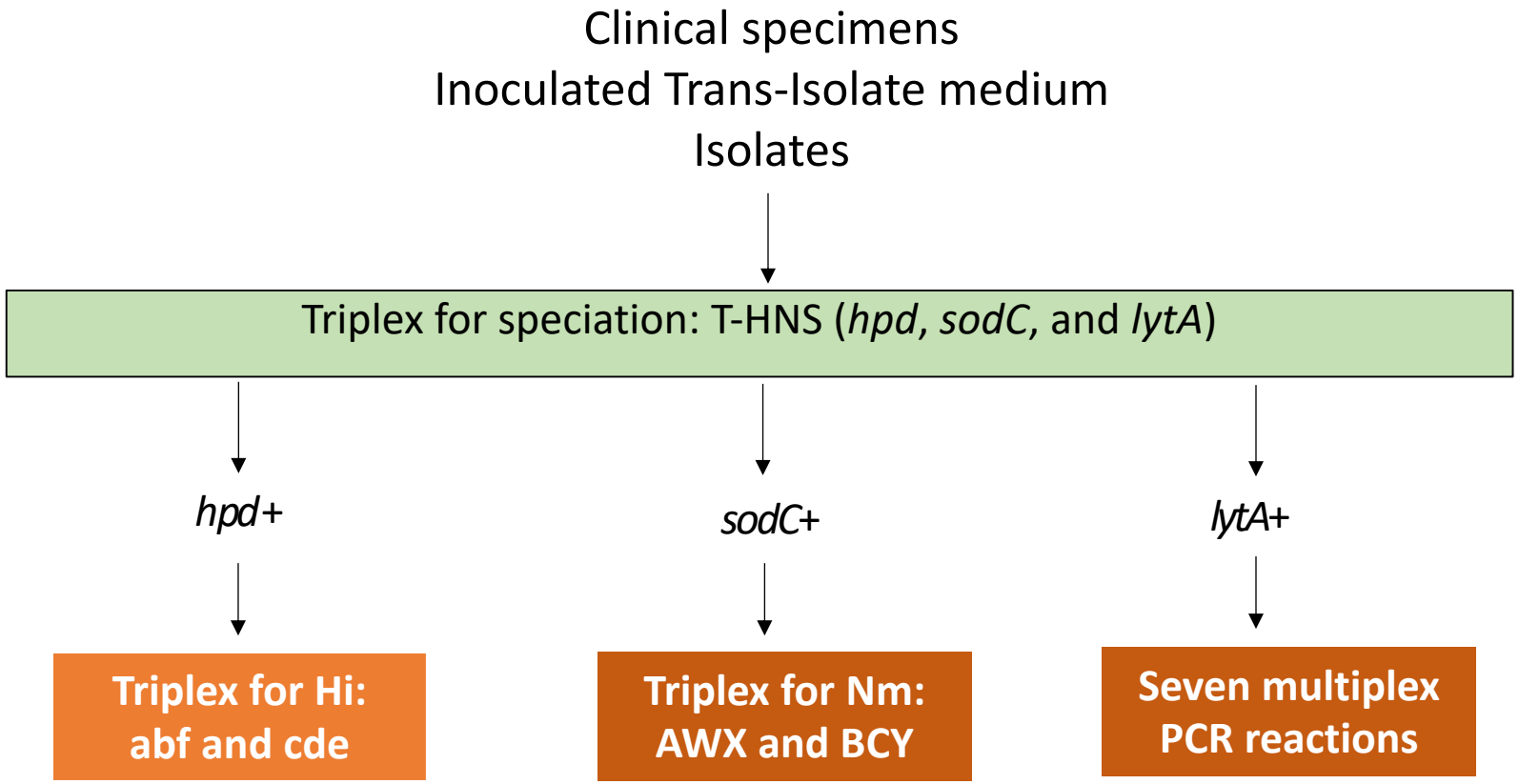


Hi capsule locus



Genomic characterization of Haemophilus influenzae: a focus on the capsule locus | BMC Genomics | Full Text (biomedcentral.com)

rt-PCR testing algorithm



Future Molecular Diagnostics for Bacterial Meningitis

Next generation rapid tests: a key objective for defeating meningitis by 2030

Use case 3

- Affordable multiplex diagnostic test to identify pathogens
- Hospital settings
- Inform treatment plans

https://www.who.int/health-topics/meningitis#tab=tab_1

Timelines for use case 3 development

2017: A call for action: global vision to defeat meningitis

2018: Expert group meeting: discussed IVD tests development and use cases

2020: Use case 3 TPP finalized

2020-21: Use case 3 landscape and Market analysis by PATH

2021: Identification of potential platforms for use case 3

2022-24: Development and validation of potential RDTs

Exploring multiplex molecular diagnostic tests

Portable real-time PCR platforms

Platform	Features	Run Time	Power	Estimated Instrument Co
Q-POC (UK)	Cassette Multiplex (up to 40 targets)	10-30 minutes	Battery powered	$3000
Anitoa Maverick compact qPCR system (US)	4-8 wells Multiplex (up to 4 targets)	~30 minutes	10V, battery backup option	$3500-6000
Coyote Mini8 Plus Real-Time PCR System (Germany)	8 wells Multiplex (up to 2 targets)	<2 hours	12V Battery pack	$6000-$8000
Bio molecular Systems Mic qPCR (Australia)	48 wells Multiplex (up to 4 targets)	~25 minutes	100-240V	$15000
Q160 Mini Real-Time PCR System (China)	16 wells Multiplex (up to 2 targets)	1-2 hours or less	85-265V	$4600
Handheld real-time PCR device (prototype)	4 wells	~35 minutes	?	?

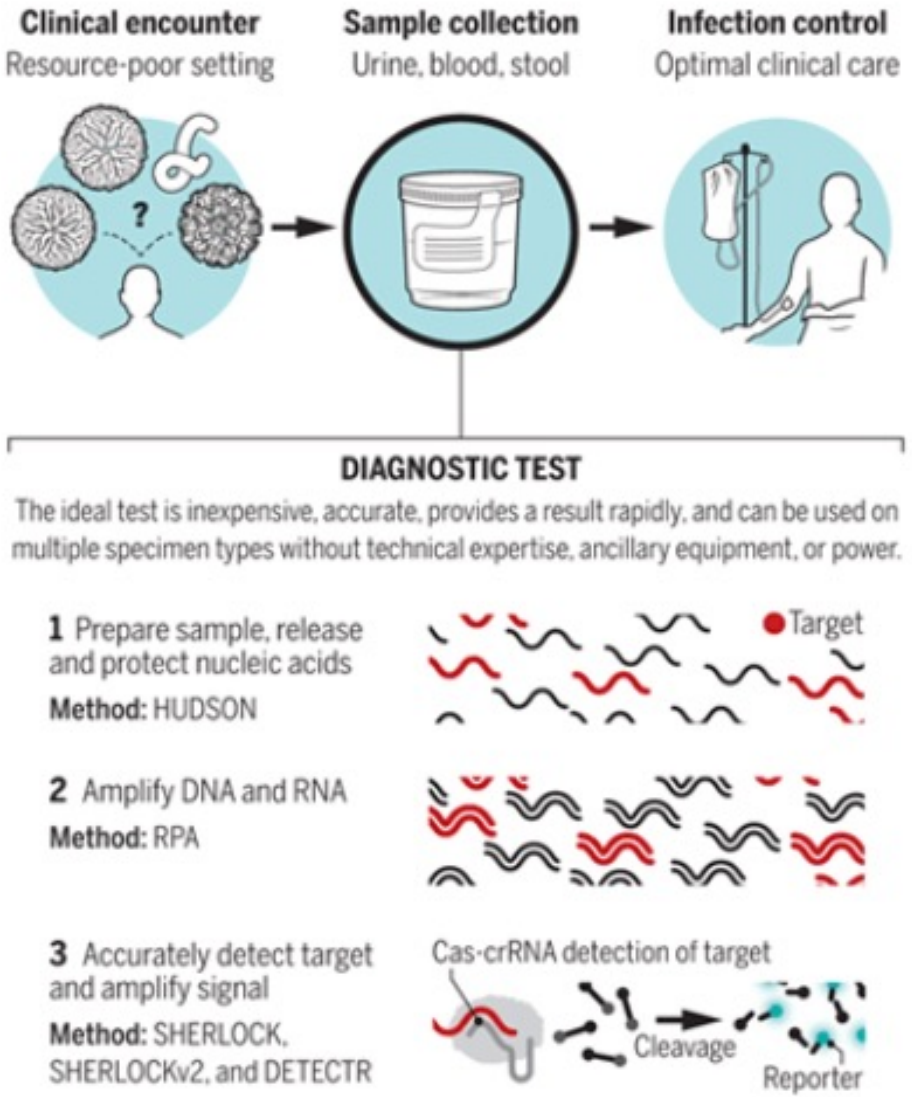
- Highly sensitive and specific
- Affordable
- Small/compact device
- Battery powered
- Multi-pathogen detection
- Expandable-able to include serogroup/serotype specific targets or gene targets associated with AMR

Other potential diagnostic platforms

- Metagenomic next generation sequencing
  - Growing interest in past 10 years
  - Used for detection of bacterial and viral pathogens
  - Various platforms (MinION, Illumin supported platforms, Ion Torrent, BGISEQ etc) and analysis tools available
  - Higher cost and lower sensitive compared to PCR-based tests
  - Targeted approaches offer better sensitivity, reduced cost, and decreased complexity of bioinformatic analysis

Other potential diagnostic platforms

- CRISPR-based detection system
  - Rely on Cas protein, an endonuclease that cleave complementary sequences
  - Cleavage induces nonspecific cleavage of single stranded DNA or RNA, which can be modified with reporter/quencher, allowing signal detection
  - Applied to viral pathogen detection



[Next-generation diagnostics with CRISPR | Science (sciencemag.org)]

Culture for other surveillance programs

- Culture remains valuable for surveillance programs such as AMR and genomic surveillance
  - Bacterial isolates are still needed for antimicrobial susceptibility testing and whole sequencing with the existing technologies
- AMR surveillance
  - Monitor AMR trends and emerging AMR in healthcare settings
  - Inform AMR prevention and control strategies
- Genomic surveillance
  - Monitor hyperinvasive, epidemic-prone, or high transmissible strains
  - Trace meningococcal spread and transmission during outbreak setting

Thank you for your attention