

## Laboratory materials needed for the diagnosis of bacterial meningitis and invasive meningococcal disease by PCR

### PCR equipment

Sub-category	Short Description	Supplier
<b>Equipment: Real-Time PCR machines, validated for the assays</b>	Applied Biosystems™ (ABI) 7500 Fast Dx Real-Time PCR Instrument, with tower computer	ThermoFisher
	Applied Biosystems™ (ABI) 7500 Real-Time PCR Instrument, with tower computer	ThermoFisher
	Applied Biosystems™ (ABI) 7300 Fast Dx Real-Time PCR Instrument, with tower computer	ThermoFisher
	Stratagene™ Mx3005P qPCR Instrument with Laptop and Software	ThermoFisher
	Applied Biosystems™ (ABI) StepOnePlus™ Real-Time PCR System	ThermoFisher
	Applied Biosystems™ (ABI) QuantStudio Real-Time PCR system	ThermoFisher
	Agilent AriaMx Real-time PCR System (Additional items to order: laptop, 4 filters (ROX, HEX, FAM, CY5), calibration plate	Agilent

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Reagents and kits**

<b>Sub-category</b>	<b>Short Description</b>	<b>Specifications</b>	<b>Supplier</b>
<b>Reagents</b>	Mastermix KIT Quanta PerfeCTa qPCR ToughMix Low ROX, 10 x 1.25 mLs	PerfeCTa® qPCR ToughMix®, Low ROX™, QuantaBio	VWR International, LLC
	ABI Taqman RT-q PCR mastermix		ThermoFisher
<b>Kits commercially available</b>	<i>Spn, Nm A, B, C, Y, W (not X), not Hib</i>	Diagenode diagnostics	
<b>Extraction kits (not needed for CSF)</b>	QIAamp DNA Mini Kit:Kit designed for isolation of DNA from biological sources (Genomic, Mitochondrial, Bacterial, Parasite or Viral)	Main sample type: Whole blood, tissue, cells Format: Spin column Processing: Centrifugation or vacuum Elution volume 50-200ul Time to run or prep: 20 minutes Yield 4-30ug	

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### Primers and probes

Sub-category	Target	Primer	RT nucleotide sequence for primer	Probe modification	Supplier
Primers (with control) and Probes	<i>H. influenzae hpd</i>	hpdF822	GGTTAAATATGCCGATGGTGTTG		IDT* or Sigma
		hpdR952	TGCATCTTTACGCACGGTGTA		IDT or Sigma
		Pb896i	TTGTGTACTACTCCGT"TT"GGTAAAAGAACTTGACAC	5' FAM, BHQ1 on "T", 3' SpC6	IDT or Sigma
	<i>N. meningitidis sodC</i>	F351	GCACACTTAGGTGATTTACCTGCAT		IDT or Sigma
		R478	CCACCCGTGTGGATCATAATAGA		IDT or Sigma
		Pb387	CATGATGGCAGCAACAAATCCTGTTT	5' FAM; 3' BHQ	IDT or Sigma
	<i>S. pneumoniae lytA</i>	F373	ACGCAATCTAGCAGATGAAGCA		IDT or Sigma
		R424	TCGTGCGTTTTAATTCCAGCT		IDT or Sigma
		Pb400	TGCCGAAAACGC"TT"TGATACAGGGAG	5' FAM, BHQ1 on "T", 3' SpC6	IDT or Sigma
	<i>Hib bcsC</i>	F192	TTA TAA AAT GTT AGA TCG TGC GGA TAC T		IDT or Sigma
		R359	CCA TAG TGT GTC CAA CGG CTA TAT		IDT or Sigma
		Pb244i	CTT TAT CTT "TT"CA AGT ACG ATT GG	5' FAM, BHQ1 on "T", 3' SpC6	IDT or Sigma
	<i>N.m. A csaB</i>	F2531	AAAATTCAATGGGTATATCACGAAGA		IDT or Sigma
		R2624	ATATGGTGCAAGCTGGTTTTCAATAG		IDT or Sigma
		Pb2591i	CTAAAAG"TT"AGGAAGGGCACTTTGTGGCATAAT	5' FAM, BHQ1 on "T", 3' SpC6	IDT or Sigma
	<i>N.m. B csb</i>	F737	GCTACCCCATTTT CAGATGATTTGT		IDT or Sigma
		R882	ACCAGCCGAGGGTTTATTTCTAC		IDT or Sigma
		Pb839i	AAGAGATGGGYAACAAAC"TT"ATGTAATGTCTTTATTT	5' FAM, BHQ1 on "T", 3' SpC6	IDT or Sigma
<i>N.m. C csc</i>	F478	CCCTGAGTATGCGAAAAAATT		IDT or Sigma	
	R551	TGCTAATCCCGCTGAATG		IDT or Sigma	
	Pb495i	TTTCAATGC"TT" AATGAATACCACCGTTTTTTTGC	5' FAM, BHQ1 on "T", 3' SpC6	IDT or Sigma	

Sub-category	Target	Primer	RT nucleotide sequence for primer	Probe modification	Supplier
Primers (with control) and Probes	<i>N.m. W</i> <i>csw</i>	F857	TATTTATGGAAGGCATGGTGTATG		IDT or Sigma
		R964	TTGCCATTCCAGAAATATCACC		IDT or Sigma
		Pb907i	AAATATGGAGCGAATGATTACAGTAACTATAATGAA	5' FAM, BHQ1 on "T", 3' SpC6	IDT or Sigma
	<i>N.m. X</i> <i>csxB</i>	F173	TGTCCCCAACCGTTTATTGG		IDT or Sigma
		R237	TGCTGCTATCATAGCCGCC		IDT or Sigma
		Pb196	TGTTTGCCACATGAATGGCGG	5' FAM, 3' BHQ1	IDT or Sigma
	<i>N.m. Y</i> <i>csv</i>	F787	TCCGAGCAGGAAATTTATGAGAATAC		IDT or Sigma
		R929	TTGCTAAAATCATTGCTCCATAT		IDT or Sigma
		Pb1099i	TATGGTG" T" ACGATATCCCTATCCTTGCCTATAAT	5' FAM, BHQ1 on "T", 3' SpC6	IDT or Sigma
	Primers Internal control	RNSP human			
	For Multiplex <i>sodC</i> ( <i>Nm</i> species); for <i>Nm W</i> and <i>Nm C</i> ; for <i>Hif</i> and <i>Hid</i>	FAM Probe (Dye)			IDT or Sigma
	For multiplex <i>hpd</i> ( <i>Hi</i> species); for <i>Nm A</i> and <i>Nm Y</i> ; for <i>Hib</i> and <i>Hic</i>	HEX probe (dye)			IDT or Sigma
	For multiplex <i>lytA</i> ( <i>Spn</i> species); for <i>Nm X</i> and <i>Nm B</i> ; for <i>Hia</i> and <i>Hie</i>	Quas670/ CY5 probe (Dye)			IDT or Sigma

\* Integrated DNA Technologies

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### Consumables

Sub-category	Short Description	Specifications	Use	Supplier
<b>Consumables</b>	PCR grade water (2 x 25mL)	Liquid Not DEPC-Treated Deionized Filtered-sterilized Dnase-free Endonuclease-free Exonuclease-free Genomic DNA-free Molecular Biology Grade Rnase-free		Promega
	Tube Cryogenic, 2.0ml	Polypropylene Capacity: 2.0 ml Resistant to temperatures from -190°C to +121°C Sterile DNase/RNase, human DNA and pyrogens free Single use Attached safelock cap Conical bottom with skirt Labeling area	Sterile microtube to store specimen and reagents. This tube is used for long and short storage in freezers or dry ice at -80°C or in liquid Nitrogen at -196°C	VWR

Sub-category	Short Description	Specifications	Use	Supplier
	Microcentrifuge tubes, 1.5ml	Polypropylene Capacity: 1.5 ml Sterile DNase/RNase free Single use Attached safelock cap Conical bottom Labeling area	Sterile micro tube to store specimen, reagents and perform reactions.	VWR
	Qualification test plate (AriaMx)			Agilent
	PCR 96 well reaction plate (AriaMx)	Capacity: 96 well x 0.2ml Format: 96 well plate Color: optical Skirted, 25/box, for AriaMx	Plate to perform 96 different PCR reactions in parallel.	Agilent
	optical adhesive film	Color: Optical Plate compatibility: 384-well plates, 96-well plates Pressure sensitive	Adhesive film to use in combination with PCR microplates. The film helps avoid well-to-well contamination and evaporation.	
	strip caps	AriaMx		
	mask		High-filtration mask to cover the lower half of the face (nose-mouth-chin), sealed for maximum water and air tightness to serve as barrier for airborne pathogens and aerosols of body fluids.	
	disposable lab coat size small			
	disposable lab coat size medium			
	disposable lab coat size large			
	cover for shoes			
	alcohol			
	bleach			

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### Consumables

Sub-category	Short Description	Specifications	Use	Supplier
Laboratory Equipment	Benchtop centrifuge	Microcentrifuge Eppendorf Model 5418 Max RCF 2,000 Rotor: 8x1.5/2.0ml or 4x(8x0.2ml) tube strip	particularly for preparing PCR reactions	Fisher Scientific
	Biosafety hood	Biological Safety Cabinet, Class II, Type A2, Airstream Class II Type A2 BSC, 1.2m	open the sample, inactivate	
	-20 degree freezer		long term storage	
	-80 degree freezer		long term storage	
	Refrigerator		keeping reagents, short-term storage	
	Vortex	Miniature vortex for mixing of specimens and for extractions		Fisher Scientific
	Workstation	Self-contained work area to prevent contamination during PCR manipulations		

### Disclaimer

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