

# EUIBIS *N.meningitidis* EQA



May 2007

# Aim of EUIBIS *N meningitidis* EQA



- To optimise and standardise European meningococcal reference laboratory meningococcal strain characterisation and detection to ensure accurate surveillance.

# Design of EUIBIS *N. meningitidis* EQA

- To include all EU member and accession states
- To assess phenotypic characterisation of isolates
- To assess non-culture (PCR-based) detection and characterisation
- To assess molecular characterisation of both isolates and DNA positive (PCR) only material
- To provide safe, simulated clinical samples suitable for DNA extraction and PCR detection
- Utilise expert support of HPA Quality Assurance Laboratory (QAL {UKNEQAS} , Cfl, Colindale, UK)



- **small panels – distributed regularly and frequently**
- **Individual anonymous reports compared to consensus results**

# EUIBIS EQA 1<sup>st</sup> & 2<sup>nd</sup> distributions



## 1<sup>st</sup> distribution Nov05

- X10 isolates for phenotypic (and molecular) characterisation
- To include representatives of serogroups and the major disease-causing lineages (clonal complexes) in Europe
- X4 simulated non-culture samples (for DNA extraction & PCR detection/confirmation assays)
- Allow for molecular characterisation

## 2<sup>nd</sup> distribution Sept06

- X5 isolates for phenotypic (and molecular) characterisation
- X10 simulated clinical samples (for DNA extraction & PCR detection/confirmation assays)
- Assess molecular assay detection & characterisation sensitivity



# Comments on 1<sup>st</sup> distribution - cultures

- **Serogroup: excellent agreement - 17 laboratories reported serogroup**  
13 used serological & 4 used PCR-based methods  
*7861 Non-capsulated organism caused problems*
- **Serotype (*porB*): Good agreement where Mabs available (only 2 labs used molecular *porB* assay)**  
*porB 3-25 vs availability of type 17 Mab?*  
*serotype 21 Mab availability? (*porB* 3-64)*  
*Problem serotypes 14 (80%), 21(53%)*
- **Serosubtype (*porA*): Excellent agreement where Mabs available**  
**Excellent *porA* (VR1 & VR2) agreement (11 labs) and for VR3 in 5 labs**  
*Some evidence of transposition or clerical errors in both phenotypic and genotypic results*  
*problems with VR2- P1.3, P1.14, P1.15 and P1.6 Mab?*

# Comments on 1<sup>st</sup> distribution - MLST



- MLST (ST) results were in excellent agreement for the 9 labs reporting
- 7855 was problematic for 2 labs – reporting clonal complex
- Consider reporting of clonal complex (CC) in subsequent distributions

allows for useful but incomplete results

- 7858 was problematic for 2 labs –
- Different alleles to consensus reported at *pgm* or *gdh* lead to different STs

How close are ST 1881 & 352 to the consensus 275?



# Preparation of simulated non-culture samples for DNA extraction and PCR

- **Standardised saline suspension produced & diluted in a safety cabinet (HPA MRU, Manchester, UK)**
  - Miles & Misera viable cell count estimation
- **Suspension heat-killed (100°C for 5 mins) = Stock suspension**
- **Dilutions of Stock suspension in sterile defibrinated horse blood**
  - Use **HPA MRU** ABI Taqman assays to assess suitable dilutions for EQA panel
- **Frozen Stock suspension sent to **HPA QAL (UKNEQAS)**, Cfl, **London** for specified dilution in defibrinated horse blood, freeze-drying and distribution**
- **Reconstituted on receipt, tested and reported anonymously to **HPA QAL (UKNEQAS)** for joint review by **HPA MRU** to determine consensus - individual reports released**

# 1<sup>st</sup> distribution - simulated non-culture samples – consensus results



No.	<i>concentration</i> <sup>1</sup> (orgs/mL)	Group	Genotype			MLST
			<i>porA</i>	VR1	VR2	
7863 <sup>2</sup>	4.0 x 10 <sup>6</sup>	B	7	16	35	32
7864	0	Negative control	-	-	-	-
7865 <sup>3</sup>	1.6 x 10 <sup>6</sup>	C	5-1	10-8	36-2	11
7866 <sup>4</sup>	9.2 x 10 <sup>5</sup>	B	7-2	4	37	41

➤ X5 labs reported *porA* but only x2 labs MLST

<sup>1</sup> estimated number of viable organisms / mL (HPA MRU)

<sup>2</sup> 7863 = 7853, <sup>3</sup> 7865 = 7862 and <sup>4</sup> 7866 = 7854

# 1<sup>st</sup> distribution - simulated non-culture samples – consensus results



- **Serogroup B:** good agreement  
7863 12/12 labs = B, 7866 10/12 labs = B (but x2 labs reported C & W135)
- **Serogroup C:** good agreement but more problematic  
7865 9/11 = C but x2 labs report B (mix-up/clerical error?)
- **Negative:** Good agreement (7864 7/7 = Neg)  
*but why did x5 labs not report? – could have reported: “not A, B, C, Y or W135” or any combination*
- **X10<sup>6</sup> organisms / mL = good positive case, readily confirmed by most labs using PCR detection**
- **Only x2 labs reported MLST data – labs may have determined alleles at < 7 loci and were therefore unable to assign ST. Consider CC for 2<sup>nd</sup> distribution**

## 2<sup>nd</sup> distribution - cultures



No.	phenotype <sup>1</sup>	<i>porA</i>			<i>porB</i>	MLST	
		VR1	VR2	VR3		ST	CC
8318	A:21:P1.10	5-2	10	37-1	3-60	75	1
8319	C:2a:NT	5-1 <sup>2</sup>	10-8	36-2	2-2	11	11
8320	Y:14:P1.5	5-1	10-4	36-2	3-36	23	23
8321	C:2a:NT	del <sup>3</sup>	del <sup>3</sup>	del <sup>3</sup>	2-2	11	11
8322	B:4:P1.3,6	18-1	3	38	3-1	41	41/44

<sup>1</sup>Phenotype dependent on Mab availability. Problems with serogroup A both phenotypically 20/22 and genotypically 8/12 similarly serogroup Y 12/22 and B & C genotypically confirmed by 13/13. but problems with labs confirmed serogroup genotypically

## 2<sup>nd</sup> distribution – cultures - comments



- **Serogroups B and C:** excellent phenotypic (22/22) and genotypic confirmation (13/13)
- **Serogroup A:** problems with serogroup A both phenotypically 20/22 and genotypically 8/12 – availability of specific serogroup A PCR assay?
- **Serogroup Y:** serogroup Y 12/22 phenotypically and 11/14 genotypically – availability of specific serogroup Y serological reagent and specific PCR availability?

### *porA:*

Notation and reporting of *porA* deletion (8321) caused problems but the (point mutation) stop codon in VR1 (8319) caused relatively few problems.

# Phenotyping-serotyping



**Problems with serotypes:**

**4 (67%), 14 (47%), and 21 (73%)**

## 2<sup>nd</sup> distribution - simulated non-culture samples – consensus results



No.	<i>concentration</i> <sup>1</sup> (orgs/mL)	Genotype					
		Group	<i>porA</i>			MLST	
			VR1	VR2	VR3	ST	CC
8323 <sup>2</sup>	2.5 x 10 <sup>5</sup>	C	5-1	10-4	36-2	50	11
8324 <sup>2</sup>	1.8 x 10 <sup>4</sup>	C	5-1	10-4	36-2	50	11
8325 <sup>2</sup>	2.5 x 10 <sup>3</sup>	C	5-1	10-4	36-2	50	11
8326	1.4 x 10 <sup>6</sup>	W135	5	2	36-2	11	11
8327	1.2 x 10 <sup>6</sup>	A	5-2	10	37-1	75	1
8328 <sup>3</sup>	1.3 x 10 <sup>6</sup>	B	17	16-3	36	136	41/44
8329 <sup>3</sup>	1.3 x 10 <sup>4</sup>	B	17	16-3	36	136	41/44
8330 <sup>3</sup>	2.6 x 10 <sup>3</sup>	B	17	16-3	36	136	41/44
8331	3.2 x 10 <sup>5</sup>	Negative	<i>N.meningitidis</i>	POSITIVE	<i>Str. pneumoniae</i>		
8332	Negative Control						

<sup>1</sup> estimated number of viable organisms / mL (HPA MRU)

<sup>2</sup> 8323, 8324 & 8325 were all dilutions of same organism.

<sup>3</sup> 8328, 8329 & 8330 were all dilutions of same organism.

## 2<sup>nd</sup> distribution - simulated non-culture samples - comments

### Serogroup:

- Good agreement for the strongly positive samples of serogroup B (8328) and C (8323) – confirmed by 18/20 and 16/18 labs respectively.
- Less good agreement with the more dilute samples but considerably better for serogroup C (8325 confirmed by 12/17 labs) than B (particularly 8330 which was confirmed by 8/17 labs)
- Suggests that  $\times 10^3$  organisms/mL may be close to the practical limit of detection
- Serogroups A (8327) and W135 (8326) were less well confirmed probably due to limited availability of the specific assays
- Two incorrect reports could be due to assay specificity
- **Controls - Negative & *Str pneumoniae***: Good agreement regarding *N.meningitidis* assays. A number of labs did not report Negative findings
- For those labs confirming *Str pneumoniae* was a bonus result!



## **2<sup>nd</sup> distribution - simulated non-culture samples – *porA* molecular typing**

***porA*:**

**fewer labs utilising *porA* on non-culture material**

**The more dilute the sample the less chance for *porA* product and sequence results**

**X10<sup>3</sup> organisms/mL may indicate the limit of *porA* typing (8325 4/10 labs VR1 & 4/9 labs VR2 and 8330 3/8 labs VR1 & 2/7 labs VR2) similar for VR3**



## 2<sup>nd</sup> distribution - simulated non-culture samples – MLST molecular typing

- **MLST:** Good agreement for 5 labs with the strong positive material (8323, 8326, 8327 & 8328)
- Problems encountered with the weaker samples (8324, 8325, 8329 & 8330)

Some labs unable to confirm MLST and also examples of “new” alleles due to mis-priming (*see following slides for Lab 30*)

- **Lab 30 (HPA MRU) mis-priming in initial loci amplification resulted in “new” allelic sequences. Repeated sequencing confirmed same result. Resolved post-EQA by repeating initial amplification and the re-sequencing.**

# Methodology-Extraction



**boiling 6 labs**

**QIAamp mini kit 2**

**MagnaPure 2**

**Other 2**

# Methodology Detection



**TaqMan/fluorescence 5 labs**

**Agarose gel electrophoresis (7)**

**(4 real time/Taqman PCR, 16S, ctrA PCR, 5xPCR)**

# Conclusions

- **X2 successful distributions of mixed culture and non-culture material**
- **Safe - No incidents!**
- **Phenotypic characterisation good – some limited access to reagents & different notation/reporting**
- **Genotypic characterisation successful for labs using it**
- **PCR-based detection, confirmation and characterisation was generally successful but demonstrated some potential limitations**

# Future



- **A further EU-IBIS EQA distribution is in preparation**
- **Comments regarding future design and composition to be discussed**
- **Future of EU-IBIS and reference laboratory EQA?**

