Study to evaluate the currently available commercial agar plated media for determination of MICs in *N. gonorrhoeae*.

Why perform this study

Pre 2014, most UK laboratories used the BSAC disc diffusion method to susceptibility test *N. gonorrhoeae* isolates. Testing of *N. gonorrhoeae* using the BSAC disc diffusion method had many problems, the main one being that the recommended media (IsoSensitest + NAD, ISON) failed to support up to 20% of isolates tested. EUCAST, along with Gonococcal reference laboratories, have performed extensive development of a disc diffusion method for *N. gonorrhoeae*. Unfortunately, no disc diffusion method was found to provide accurate results, which correlate with MICs.

Since moving to EUCAST disc diffusion methods and breakpoints, UK laboratories follow the advice given for *N. gonorrhoeae*, which is to perform an MIC test. Most laboratories use gradient strips for MIC determination because of their ease of use and reliable results. When using gradient strips laboratories should use the manufacturers' instructions to perform the test, including choice of media. However, currently, not all media is available in the UK.

This study aimed to evaluate the commercial media available in the UK for MIC determination; assessing the ability to grow standard isolates including WHO control isolates plus isolates which have been described as "poor growers".

What we did

The testing was performed in both Specialist Antimicrobial Chemotherapy Unit (SACU) Public Health Wales (PHW) and Antimicrobial resistance in sexually transmitted infections (AMRSTI), Public Health England (PHE). Primary cultures were prepared on Oxoid GC+vitox in AMRSTI and on Oxoid Chocolate agar in SACU.

Initially, five control strains (WHO F, G, K, L & M) were assessed for viability on the six agar plates in both laboratories, with five "poor grower" isolates tested alongside in AMRSTI; then MICs were determined for ceftriaxone, cefixime and azithromycin using gradient strips (Etest, BioMerieux).

The six commercially available agar plates were:

Oxoid (Thermo Fisher): Chocolate vitox (PO5090A), GC vitox (PO0982A) & GC non-selective with lysed blood (PB1052A).

Becton Dickinson: GC Chocolate (254060) & Mueller Hinton Chocolate (254035).

E&O Labs: Mueller Hinton Chocolate (PP2280).

Inocula were prepared using two methods: as per manufacturer's instructions, a 0.5 McFarland in Mueller Hinton Broth in SACU but prepared to 0.5 McFarland in saline in AMRSTI. The latter a common choice in busy diagnostic laboratories.

To assess viability, agar plates were inoculated using a sterile swab, then plates incubated onto the six agar plates as if for the gradient strip method. No strips were applied and plates were incubated in CO2 at 35±1°C for 20-24h. Plates were then prepared in the same manner and ceftriaxone, cefixime and azithromycin gradient strips applied and incubated accordingly.

After the initial viability testing, the main study involved ceftriaxone and azithromycin MICs determined for forty eight isolates collected for the Gonococcal Resistance to Antimicrobial Surveillance Programme (GRASP), by both laboratories, on the six commercial agar plates. Primary culture was on Oxoid GC + vitox in both laboratories. Two control strains were tested alongside: WHO G & K and inocula were prepared as per initial viability study.

For these isolates, the MICs determined in both laboratories were compared with MICs from the reference method, as performed in the GRASP surveillance study. Categorical agreement was also calculated.

Results

Viability

In each laboratory, each of the control isolates and "poor grower" isolates were scored for viability in terms of appearance and weight of growth on each agar plate. The MICs for ceftriaxone, cefixime and azithromycin MICs on all agar plates combined were also scored along with the ease of reading in each laboratory. At this stage, MICs were not compared per agar plate as the number of strains tested was too low.

The scores for each agar plate were combined to achieve an overall total for good growth and ease of reading of MICs.

Table 1: Combined scores for weight of growth and for ease of gradient strip MIC reading.

Commercial agar plate	Good Growth	Gradient Strip Ease of Reading
Oxoid: GC + vitox	**291**	**232**
Oxoid: GC chocolate	239	195
Oxoid: GC non selective agar with lysed blood	196	194
Becton Dickinson: GC chocolate	**279**	**205**
Becton Dickinson: Mueller Hinton chocolate	260	200
E&O labs: Mueller Hinton chocolate	269	199

There were two agar plates which scored the highest for both weight of good growth and for ease of gradient strip MIC reading (seen in bold of Table 1).

A comparison of the ceftriaxone, cefixime and azithromycin MICs for the five control strains on all agar plates and between laboratories showed only good concordance; 56% of MICs were with 1 $\log_2$ dilution. Generally, MICs were 1 $\log_2$ dilution lower in SACU compared with AMRSTI. These lower MICs were thought to be due to the primary culture of isolates on chocolate agar (SACU) compared with GC+ vitox (AMRSTI).

Main study

Agreement (within 1 log2 dilution) of MICs for ceftriaxone and azithromycin can be seen in Table 2. MICs were compared initially between SACU and AMRSTI then between both labs and the MIC performed as part of the GRASP study; using the reference method of agar dilution.

Good MIC agreement (>85%) was seen between both laboratories, on most agars for both agents tested. In particular two agar plates performed the best for both agents: Oxoid GC + vitox and Becton Dickinson GC chocolate. When comparing MICs from both laboratories to the GRASP MIC, again Oxoid GC + vitox and Becton Dickinson GC chocolate agar performed the best, with MIC agreement of 94% (azithromycin & ceftriaxone) and 92% (azithromycin & ceftriaxone) respectively.

Table 2: Percentage MIC agreement of MICs on six commercial agar plates

Commercial agar plate	AZITHROMYCIN		CEFTRIAXONE	
	SACU/ AMRSTI	SACU/ AMRSTI/ GRASP	SACU/ AMRSTI	SACU/ AMRSTI/ GRASP
Oxoid: GC + vitox	98	94	98	94
Oxoid: GC chocolate	n/a*	100*	n/a*	47*
Oxoid: GC non selective agar with lysed blood	86	74	94	67
Becton Dickinson: GC chocolate	98	92	94	92
Becton Dickinson: Mueller Hinton chocolate	87	80	92	67
E&O labs: Mueller Hinton chocolate	n/a*	78*	n/a*	84*

* No data from AMRSTI

The azithromycin MICs performed on Oxoid GC chocolate in SACU and GRASP had 100% agreement (not tested in AMRSTI) however agreement for ceftriaxone was poor (47%).

Percentage categorical agreement for both agents in both laboratories (excluding GRASP) on all agar plates was excellent (>98%), table 3.

Table 3: Percentage categorical agreement on six agar plates.

Commercial agar plate	AZITHROMYCIN		CEFTRIAXONE	
	SACU/ AMRSTI	SACU/ AMRSTI/ GRASP	SACU/ AMRSTI	SACU/ AMRSTI/ GRASP
Oxoid: GC + vitox	100	100	100	100
Oxoid: GC chocolate	98	98	100	100
Oxoid: GC non selective agar with lysed blood	100	98	100	67
Becton Dickinson: GC chocolate	100	100	100	100
Becton Dickinson: Mueller Hinton chocolate	100	100	100	100
E&O labs: Mueller Hinton chocolate	100	100	100	100

The main study was then extended to include ciprofloxacin MICs (Etest, BioMerieux), performed for all 50 isolates on the two best commercial agar plates (Oxoid GC + vitox and Becton Dickinson GC chocolate) in both laboratories.

For ciprofloxacin, the MIC agreement between laboratories on both agar plates was excellent (100%) with 98% categorical agreement (Table 4). There was one isolate which had an MIC near to the breakpoint (EUCAST version 10).

Table 4: Percentage agreement and categorical agreement for ciprofloxacin on two agar plates

Commercial agar plate	MIC AGREEMENT		CATEGORICAL AGREEMENT	
	SACU/ AMRSTI	SACU/ AMRSTI/ GRASP	SACU/ AMRSTI	SACU/ AMRSTI/ GRASP
Oxoid: GC + vitox	100	100	98	98
Becton Dickinson: GC chocolate	100	100	98	98

Conclusions

Susceptibility testing of *N. gonorrhoeae* has always been problematic, with the main issue poor growth on susceptibility testing agar. Poor or slow growth during susceptibility testing can lead to false susceptible results, a very major error. In this study we evaluated six different recommended commercially available media for susceptibility testing *N. gonorrhoeae*.

Two agar plates, Oxoid GC + vitox and Becton Dickinson GC chocolate, performed best for supporting good growth of “poor grower” isolates and WHO control strains. These two agar plates also performed best when reading MIC strip ellipses; scoring higher than all other agars.

The same two agar plates exhibited the highest MIC agreement for ceftriaxone and azithromycin in inter-laboratory comparison but also when compared with the GRASP MIC, performed by the reference method (agar dilution). Other agar plates performed well when comparing between the two laboratories but agreement with the reference method was lower.

Categorical agreement for ceftriaxone and azithromycin on most agar plates was excellent, both between the two laboratories and compared with the GRASP data. The Oxoid GC non selective agar with lysed blood plates performed less well; whilst inter-laboratory categorical agreement was 100%, this reduced to 98% for azithromycin and 67% for ceftriaxone when compared with GRASP data.

Ciprofloxacin MIC agreement and categorical agreement, between the two laboratories and compared with GRASP MICs, was excellent.

Next steps

Both SACU and AMRSTI are currently evaluating ceftriaxone, azithromycin, cefixime and ciprofloxacin gradient strips on the two best performing agar plates using strips from both manufacturers (BioMerieux and Liofilchem).