ORIGINAL ARTICLE

Ultrasound enhanced detection of individual meningococcal serogroups by latex immunoassay

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J Clin Pathol 2002;**55**:37–40

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Accepted for publication 31 July 2001

Aims: To examine A, C, Y, and W135 *Neisseria meningitidis* serogroup characterisation by ultrasonic standing wave enhanced latex agglutination tests (USELATs) of clinical samples. In addition, to determine USELAT enhancement of detection sensitivity for the individual antigens compared with conventional card latex agglutination tests (LATs).

Methods: Wellcogen (Abbott Murex), Slidex meningite kit 5 (bioMerieux), and Pastorex (Sanofi) kits and beads coated in house with antibodies to Y and to W135 alone were tested. Positive control antigens consisted of A and C polysaccharide preparations and the Pastorex Y/W135 kit sample. The limiting concentrations of antigen detection were determined by USELAT and by LAT. Thirty five clinical samples (plasma), previously characterised by the polymerase chain reaction (PCR) and culture, were tested by USELAT and, when sample volume allowed, by LAT.

Results: USELAT enhancement of control antigen detection ranged from 16 to 128 fold for the different latex systems. Enhancements for the different control antigens were comparable between kits. USELAT tests of clinical (A/C/Y/W135) samples (n = 15) with the Wellcogen (A/C/Y/W135) and Slidex meningite (A/C/Y/W135) kits showed comparable specificities. A set (n = 22) of Y and W135 samples gave 18, 19, and 17 positive results for Wellcogen (A/C/Y/W135), Pastorex (A/C/Y/W135), and in house beads (Y/W135), respectively. Positive USELAT PCR and culture results were concordant. A typical sensitivity for the commercial kits was 80% (Wellcogen).

Conclusions: USELAT identified serogroups for 80% of samples, whereas LATs identified only 40%. The USELAT detection of the A, C, Y, and W135 antigen serogroups showed comparable enhancement for the kits tested. The commercial availability of latex beads coated with antibody to the Y and W135 serogroups would expedite their identification.

Neisseria meningitidis is classified into 13 serogroups based on the capsular polysaccharide produced. The ability to confirm meningococcal disease rapidly and to distinguish between the serogroups of *N meningitidis* commonly associated with meningococcal disease (groups A, B, and C) is of importance for optimal clinical management of cases and contacts. Serogroups B and C are predominant in Europe, whereas serogroup A infection is most common in sub-Saharan Africa and China, where large scale epidemics affecting up to 1000 cases/100 000 population occur.[1] In the spring of 2000, outbreaks of disease were caused by serogroup W135 brought into several countries by pilgrims returning from Mecca,[2] and increased rates of serogroup Y infections have occurred in the USA over recent years.[3]

Traditionally, laboratory confirmation involves the identification of meningococci by culture and microscopy techniques. Culture takes several days, and may be hindered by the antimicrobial treatment, which, for optimal effect, is recommended to be given to patients without waiting for microbiological specimen collection. The polymerase chain reaction (PCR) is an increasingly important and sensitive technique for the detection and serogroup characterisation of meningococci.[4 5] In England, Wales, and Northern Ireland, the Public Health Laboratory Service Meningococcal Reference Unit (PHLS MRU), uses a sialyltransferase (siaD) PCR assay to identify serogroup B and C infection for those samples where meningococcal DNA has been detected using the ctrA PCR assay.[4–6] Serogroup Y and W135 PCR assays[7] have been developed by the PHLS MRU and have recently been modified by applying Taqman methodology (M Guiver, personal communication, 2000).

"Culture takes several days, and may be hindered by the antimicrobial treatment"

Latex agglutination tests (LATs) can be used to detect meningococcal polysaccharide but are of practical value only when used on cerebrospinal fluid.[8] Slidex meningite kit 5 (bioMérieux), the Wellcogen bacterial antigen kit (Abbott Murex Biotech, Dartford, UK), and the Pastorex kit (Sanofi Diagnostics Pasteur) are readily available for this purpose.

The application of non-cavitating standing wave ultrasound increases the sensitivity of different LATs by up to 2000 fold,[9] in part because of the increased rate of particle collision as antibody coated beads are forced into the pressure nodal regions.[10] The confirmation of infection in sera and cerebrospinal fluid of patients with clinically diagnosed meningococcal disease (serogroups B or combined A/C/Y/W135) has previously been reported to rise fivefold using ultrasound when compared with conventional LAT.[11]

We extended the evaluation of the ultrasound enhancement approach, already applied to serogroup B detection,[11] to identify a wider range (A/C/Y/W135) of individual *N meningitidis* serogroups from patient specimens. Commercially available and in house latex reagents were used in our study.

Abbreviations: CAMR, Centre for Applied Microbiological Research; LAT, latex agglutination test; MRU, Meningococcal Reference Unit; PCR, polymerase chain reaction; PHLS, Public Health Laboratory Service; siaD, sialyltransferase; USELAT, ultrasonic standing wave enhanced latex agglutination test

MATERIALS AND METHODS

Agglutination test kits

The components of the Wellcogen bacterial antigen kit included one suspension of latex particles with a combination of adsorbed antibodies for the detection of *N meningitidis* combined serogroups A/C/Y/W135 and another latex suspension with *N meningitidis* serogroup B/*Escherichia coli* K1 antibodies. The Slidex meningite kit 5 included three separate suspensions of latex particles for the detection of *N meningitidis* serogroups A, C, and B/*E coli* K1 capsular polysaccharide. The Pastorex kit included four suspensions of latex particles, three for the individual detection of *N meningitidis* serogroups A, C, and B/*E coli* K1 individually and one for combined Y/W135 capsular polysaccharide antigen detection.

Suspensions of latex particles for individual detection of *N meningitidis* serogroups Y and W135 were prepared in house. Particle suspensions were separately coated with Y and W135 agglutinating sera (Abbott Murex Biotech), essentially as described by Jenkins *et al*,[12] with the exception that the glycine buffered saline (pH 8.0) contained 0.17M glycine and 0.1M NaCl.

Antigens

Neisseria meningitidis serogroup A and B polysaccharides of known concentration were obtained from the Centre for Applied Microbiological Research (CAMR; Porton Down, UK). Serogroup C polysaccharide was available in deoxyacetylated (CAMR) and acetylated forms (bioMérieux). The two polysaccharides are denoted here as C^{O-} and C^{O+} for the deoxyacetylated and acetylated forms, respectively. The detection limits were determined, in duplicate, from the dilution series of the known A, B, C^{O-}, and C^{O+} polysaccharide concentrations. Positive antigen controls for Y and W135 were obtained from a combined antigen preparation supplied with the Pastorex kit.

Ultrasound enhancement

Details of the ultrasound test procedure, the laboratory voltage generating equipment, the standing wave field, and the interpretation of agglutination patterns have been described previously.[12 13] Ultrasound enhanced latex agglutination tests (USELATs) were performed using 1 mm^2 internal dimension glass microcells (Vitrocom, Mountain Lakes, New Jersey, USA), rather than circular glass capillaries, because recent work favours their use.[14] It was noted that to remove non-specific aggregation after sonication, more disruption was required for the Slidex meningite kit (10 stirs) compared with the Wellcogen and Pastorex kits (three to four stirs).

Identification of clinical samples

The two sample sets comprised 35 clinical samples (obtained from PHLS MRU, Manchester, UK) and were specifically selected specimens in which *N meningitidis* serogroups had been determined using PCR and culture techniques.[4 5] The first set of samples (n = 15) was selected principally to determine whether the ultrasound enhanced bioMérieux kit could discriminate between serogroups A and C. Samples were tested blind using *N meningitidis* A and C latex suspensions (Slidex meningite), the Wellcogen combined ACYW135 latex suspension and, if a test with the latter was positive, Wellcogen negative control latex (containing latex beads coated with unrelated antibody) to rule out the possibility of non-specific activity. (Negative control latex reagent is not supplied with the Slidex meningite kit.) When the sample volume allowed, ultrasound enhanced positive samples were also screened, using the conventional test card method. The second set of samples (n = 20) was tested with both the standard and ultrasound enhanced tests, using the Pastorex and Wellcogen kits, in addition to the laboratory made Y and W135 specific latex beads. Samples found positive using the Pastorex and Wellcogen reagents were tested using the corresponding kit negative control latex reagents.

RESULTS AND DISCUSSION

Table 1 shows the limits for detection (expressed as the lowest "positive control" antigen concentration required to give detectable agglutination when compared with a phosphate buffer control) of *N meningitidis* serogroups A, B, C^{O-}, and C^{O+}, for both the Slidex meningite and Wellcogen kits. The sensitivities of the standard test card systems were comparable for the two test latex sources. Ultrasound increased the sensitivity for serogroup detection in both kits by 16 to 64 fold. Ultrasound enhancement was not more than twofold different (corresponding to a single test dilution step) for the two sources of test latex.

An analysis of 15 meningococcal laboratory confirmed positive serum samples from 10 patients was performed using the Slidex meningite A and C latex reagents, and the Wellcogen A/C/Y/W135 latex reagent (sample volume limitation did

Table 1 Ultrasound enhancement of *Neisseria meningitidis* serogroup detection using Slidex meningite and Wellcogen latex agglutination reagents

	Standard test card limit (ng/ml)†		Ultrasound enhanced limit (ng/ml)		Ultrasonic improvement	
Serogroup*	Slidex meningite	Wellcogen	Slidex meningite	Wellcogen	Slidex meningite	Wellcogen
A	30.50	61.00	0.48	0.95	×64	×64
B	244.00	244.00	15.25	7.60	×16	×32
C^{O-}	244.00	244.00	7.60	7.60	×32	×32
C^{O+}	244.00	490.00	15.25	15.25	×16	×32

*Serogroup C polysaccharide was detected in the deoxyacetylated (C^{O-}) and and acetylated forms (C^{O+}); †expressed as the lowest antigen concentration (ng/ml) to yield a positive agglutination.

Table 2 Serogroup detection from meningococcal laboratory confirmed samples using standard and enhanced Wellcogen and Slidex meningite latex agglutination kits

Serogroup*	Total*	Slidex meningite A LAT	USELAT	Slidex meningite C LAT	USELAT	Murex A/C/Y/W135 LAT	USELAT
A	4	1 (3 ND)	4	–	–	4	4
C	9	–	–	1 (1 ND)	7	1	6
Y	2	–	–	–	–	2	2

*As confirmed by polymerase chain reaction and culture.
LAT, latex agglutination test; ND, not determined owing to insufficient sample; USELAT, ultrasonic standing wave enhanced latex agglutination test.

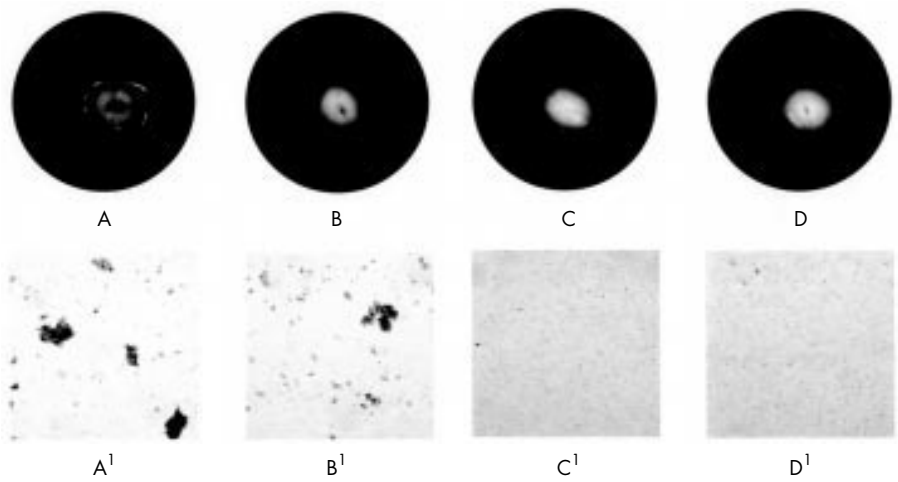

Figure 1 Wellcogen *Neisseria meningitidis* card (A–D) and ultrasound enhanced (A^1–D^1) tests of samples (W135 serogoup by PCR and culture) from two patients. One sample gave a positive result on test card (A) and with ultrasound (A^1) whereas in tests with control latex it was negative (C, C^1). The other sample was negative on test card (B) whereas ultrasound produced agglutination (B^1). Its tests with control latex were negative (D, D^1).

not allow testing with the Pastorex kit here). Results (table 2) showed significant enhancement of serogroup detection using ultrasound, particularly in the specimens from patients with meningococcal serogroup C infection. Polysaccharide antigen detection results by latex tests were all concordant with PCR and culture methods. Ultrasound enhancement detected 11 and 10 of 13 group A or C samples with the Slidex meningite and Wellcogen kits, respectively.

Figure 1 compares the agglutination results for two clinical samples (both serotyped W135 by PCR and culture) using the Wellcogen *N meningitidis* latex agglutination kit with test card and with ultrasound. The first sample shows agglutination with test latex on the test card (fig 1A) and with ultrasound (fig $1A^1$). The test droplet for the second clinical sample (fig 1B) had the same appearance as its test card control—it showed no agglutination (fig 1D). However, ultrasound enhancement showed agglutination in this sample (fig $1B^1$) when compared with its sonicated control (fig $1D^1$). No agglutination was detected for either patient sample test procedure with control latex (fig 1C,D and C^1, D^1).

Ultrasound enhanced the Pastorex kit serogroup sensitivity for Y/W135 antigens 128 fold over the standard test card procedure (table 3). Ultrasound enhancement for the in house serogroup Y and W135 specific latex particles was 16 fold (table 3). Serogroup Y and W135 detection from 22 meningococcal laboratory confirmed clinical samples using the Pastorex and Wellcogen kits and the in house coated particles showed concordance with PCR and culture methods, in addition to improved detection upon application of ultrasound (table 4). Comparable totals of 19 and 18 of 22 laboratory confirmed Y or W135 samples were detected using ultrasound by the Pastorex and Wellcogen kits, respectively, whereas the in house separate Y and W135 kits detected 17 of these.

Serogroup detection using the combined serogroup Wellcogen reagent was significantly enhanced with the application of ultrasound. From a total of 35 laboratory confirmed samples, 28 were correctly serogrouped using ultrasound enhancement compared to 14 by the standard test card procedure. The most pronounced enhancement of detection by the

Table 3 Ultrasound enhancement of *Neisseria meningitidis* serogroup detection using the Pastorex reagent and serogroup Y and W135 specific latex particles coated in house

Latex reagent	LAT	USELAT	Ultrasonic improvement
Pastorex Y/W135	1/16	1/2048	×128
In house Y	1/16	1/256	×16
In house W135	1/2	1/32	×16

Detection limits are the highest dilution (lowest concentration) of positive antigen control (Pastorex kit) at which agglutination could be detected on a test card compared with phosphate buffer.
LAT, latex agglutination test; USELAT, ultrasonic standing wave enhanced latex agglutination test.

Table 4 Serogroup detection from meningococcal laboratory confirmed samples using standard and enhanced Wellcogen, Pastorex, and in house latex agglutination kits

		Wellcogen		Pastorex		In house		In house	
Serogroup*	Total*	A/C/Y/W135 LAT	USELAT	Y/W135 LAT	USELAT	Y LAT	USELAT	W135 LAT	USELAT
Y	3	3	3	2	3	3	3	–	–
W135	19	6	15	10 (2 ND)	16	–	–	1 (5 ND)	14

*As confirmed by polymerase chain reaction and culture.
LAT, latex agglutination test; ND, not determined owing to insufficient sample; USELAT, ultrasonic standing wave enhanced latex agglutination test.

Wellcogen kit was observed for serogroups C and W135. Similarly, both the Pastorex and the in house serogroup detection kits had increased serogroup detection on application of ultrasound, although by a lower factor.

The ability to detect antigen in serum or plasma by ultrasound with higher sensitivity than that of conventional agglutination, while retaining the speed of the latter test, offers a distinct advantage. Usually, in the UK, patient samples are dispatched to an accredited central laboratory for PCR analysis. The rapid ultrasonic procedure could provide serogrouping information during the delay incurred through sample transit. It could also provide information outside normal laboratory hours if an experienced operator is available.

> "The ultrasound enhanced Slidex meningite kit is a relatively low cost, quick, and simple technique with a modest training requirement"

The ability of the ultrasound enhanced Slidex meningite kit to identify serogroups A and C from clinical samples may have particular relevance for its use outside the UK. Serogroup A is the major cause of large epidemics of meningococcal meningitis in the African meningitis belt.[15] Serogroup C makes up most of the remainder of cases, although there is also recent evidence of W135 disease.[16–18] Prompt diagnosis and initiation of vaccination campaigns are needed to control these sometimes devastating epidemics. PCR is currently not a practical method of diagnosis in many regions of Africa because it is an expensive and exacting technique.[11] The ultrasound enhanced Slidex meningite kit is a relatively low cost, quick, and simple technique with a modest training requirement. This reproducible[12] technique has the potential to provide local diagnosis and outbreak epidemiology. The optimal exploitation of this facility requires that coated latex for the separate detection of Y and W135 should be made available commercially.

The ability to distinguish some of the serogroups that historically have been less frequently encountered is becoming more important in situations where performing routine culture represents a challenge. It is noteworthy that recently in the USA there has been an increase in the numbers of meningococcal infections caused by serogroup Y,[3] which is now responsible for 26% of cases there. As shown in our study, USELAT kits that include individual meningococcal serogroup specific reagents allow the rapid identification of serogroups such as A, Y, and W135, which may be important if current trends continue.

ACKNOWLEDGEMENTS

This work was supported by the Meningitis Research Foundation.

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Take home messages

- Ultrasound enhancement increased the identification of serogroups with the use of latex agglutination tests from 40% to 80%
- Enhancement was greatest for serogroup C
- Comparable enhancement was seen for all the kits tested for the detection of the A, C, Y, and W135 antigen serogroups
- The commercial availability of latex beads coated with antibody to the Y and W135 serogroups would expedite their identification

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