# Loop-mediated isothermal amplification for the early diagnosis of invasive meningococcal disease in children

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## ABSTRACT

**Background** Rapid molecular diagnostic testing has the potential to improve the early recognition of meningococcal disease (MD). The aim of this study was to report on the diagnostic test accuracy of point-of-care loop-mediated isothermal amplification (LAMP) in the diagnosis of MD.

**Design** Data were collected prospectively from three UK emergency departments (ED) between November 2017 and June 2019. Consecutive children under 18 years of age attending the ED with features of MD were eligible for inclusion. The meningococcal LAMP test (index test) was performed on a dry swab of the child's oropharynx. Reference standard testing was the confirmation of invasive MD defined as positive *N. meningitidis* culture or PCR result from a sterile body site (blood or cerebrospinal fluid).

**Results** There were 260 children included in the final analysis. The median age was 2 years 11 months and 169 (65%) children were aged 5 years or younger. The LAMP test was negative in 246 children and positive in 14 children. Of the 14 children with positive LAMP tests, there were five cases of invasive MD. Of the 246 children with negative LAMP tests, there were no cases of invasive MD. The sensitivity of LAMP testing was 1.00 and the specificity was 0.97. The negative and positive predictive values were 1.00 and 0.36, respectively. The positive likelihood ratio was 28.3.

**Discussion** Non-invasive LAMP testing using oropharyngeal swabs provided an accurate fast and minimally invasive mechanism for predicting invasive MD in this study.

**Trial registration number** NCT03378258.

### What is already known on this topic?

- Invasive meningococcal disease occurs when *Neisseria meningitidis* crosses from the respiratory mucosa and invades the host. Even in resource-rich settings, invasive meningococcal disease carries an approximate 5%–10% risk of mortality.
- Recent advances in molecular diagnostics have resulted in the development of loop-mediated isothermal amplification (LAMP) technology. LAMP is a form of rapid nucleic acid amplification that has several advantages over traditional molecular diagnostic techniques such as PCR including rapidity, amount of equipment required and tolerance of biological fluids (which facilitates direct testing of clinical material).

### What this study adds?

- A meningococcus LAMP test can be performed using oropharyngeal swabs with results available within one hour. The test was highly sensitive and specific for identification of invasive meningococcal disease. The LAMP test performed favourably when compared with conventional tests such as C reactive protein, white cell counts and neutrophil count.

## INTRODUCTION

Invasive meningococcal disease (MD) occurs when *Neisseria meningitidis* (*N. meningitidis*) crosses from the respiratory mucosa and invades the host.[1] *N. meningitidis* is a gram-negative diplococcus that colonises the nasopharynx and oropharynx of humans.[1 2] In most people, carriage is asymptomatic but in a minority, the bacteria cross the mucosa and enter the blood.[1] Once *N. meningitidis* crosses from the naso/oropharynx to the blood, it can multiply rapidly with resultant bacteraemia and an associated cytokine storm.[1] This leads to increased host vascular permeability, organ dysfunction and disseminated intravascular coagulation.[1] *N. meningitidis* may also cross the blood-brain barrier resulting in meningitis. Even in resource-rich settings, invasive MD carries an approximate 5%–10% risk of mortality.[1–5]

The early recognition of MD is challenging in children because during the prodrome phase, MD often presents with symptoms and signs that are indistinguishable from self-limiting infections.[5–7] When there is diagnostic uncertainty, clinicians may perform tests such as a full blood count, C reactive protein (CRP) and procalcitonin to identify those at highest risk,[5 7] but these conventional tests lack the necessary sensitivity to rule out MD.[5] This leads to many children receiving unnecessary parenteral

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antibiotics (just in case) while others are incorrectly reassured and discharged home.[5 7]

Recent advances in molecular diagnostics have resulted in the development of loop-mediated isothermal amplification (LAMP) technology.[8 9] LAMP is a form of rapid nucleic acid amplification that use specific looped primers and strand displacing DNA polymerase.[9] LAMP has several advantages over traditional molecular diagnostic techniques such as PCR including rapidity, amount of equipment required and tolerance of biological fluids (which facilitates direct testing of clinical material).

A commercially available CE-marked LAMP test has been developed for the rapid detection of the *ctrA* gene present within all serogroups of *N. meningitides* and can be performed on a range of specimen types including blood, cerebrospinal fluid (CSF) and naso/oropharyngeal swabs.[8 9] Data collected during the development of this LAMP test suggested that it has the potential to outperform traditional testing for MD identification in children,[8 9] and its performance accuracy is equivalent on naso/oropharyngeal samples and blood testing.[10] Naso/oropharyngeal swabs are easy to collect and require minimal DNA extraction (heating for 5 min) as opposed to blood which requires phlebotomy and a more complicated DNA extraction process.[8 9] However, nasal carriage of meningococci, rates of which vary across populations and age, may result in a positive test and unnecessary treatment.[8 10–12] Such carriage is uncommon in infancy and early-childhood (<5%), but increases steadily to a peak in adolescence (approximately 25%).[11–14]

The primary objective of this study was to evaluate the diagnostic accuracy of LAMP testing of oropharyngeal swabs for identifying invasive MD in children and to compare LAMP testing with conventional tests such as CRP and white cell counts (WCC).

## METHODS

The data for this diagnostic accuracy study come from the Petechiae in Children (PiC) study,[15] a mixed method prospective, multicentre cohort study. The full protocol is available as an open access publication.[15] The PiC study was designed and reported in line with the Standards for Reporting Diagnostic accuracy studies (STARD) statement.[16]

### Participants

Consecutive children under 18 years of age attending emergency departments (ED) with a reported or recorded fever (≥38°C) and features of meningococcal sepsis/meningitis were eligible for inclusion. The case report form (online supplementary material) included 29 possible features of meningococcal sepsis/meningitis based on NICE guidance and based on a published review conducted by the research team.[1 5] Children with pre-existing conditions predisposing to non-blanching rash including haematological malignancy, idiopathic thrombocytopenic purpura, coagulopathy and active Henoch-Schonlein purpura were excluded. Participants for this analysis were enrolled between 9 November 2017 and 30 June 2019 at three EDs in the UK.

### Test methods

The index test employed in the study was the commercially available HG Direct Swab meningococcus LAMP test 'HG-MENDS' (HiberGene Diagnostics, Ireland). This assay consists of reaction strips containing lyophilised reaction mix comprised of specific primers targeting a conserved region of the *N. meningitidis ctrA* gene, an exogenous bacteriophage sequence used as assay extraction control together with strand displacing polymerase enzyme, an intercalating dye and all other necessary reagents and buffer. Testing was performed on dry Nylon FLOQSwabs product code 519C (Copan diagnostics, Italy) of the child's oropharynx. Swabs were collected by ED clinical staff during the initial assessment of the child and placed in supplied HG elution buffer (HiberGene Diagnostics, Ireland) denatured at 95°C for 5 min with a 50 µL aliquot analysed immediately as per the manufacturer's instructions for use (http://www.hibergene.com/products/hg-meningococcus/). All reactions were performed in the ED using a supplied HG Swift LAMP instrument (HiberGene Diagnostics, Ireland) which interprets generated fluorescent signals in real time. The total run time for each sample was 40 min. At the end of each run, HG swift reported one of three results—positive, negative or invalid.

The reference standard for invasive MD was positive culture or PCR for *N. meningitidis* from a sterile body site (blood or CSF). Reference standard testing was performed at accredited NHS hospital laboratories by individuals blinded to the results of the index test. In all instances, LAMP testing was performed prior to the results of the reference standard testing being available.

### Follow-up

Researchers at each site checked attendance records at 7 days to monitor for any unplanned reattendances by study participants. Participating institutions also cross-referenced enrolled children with notifications of MD from their hospital laboratory to Public Health England or the Public Health Agency Northern Ireland. These processes ensured that all cases of MD were recorded, including any who had been discharged without treatment. In situations where the child was enrolled but discharged home without reference standard testing, they were assumed to not have MD provided they met the following criteria:

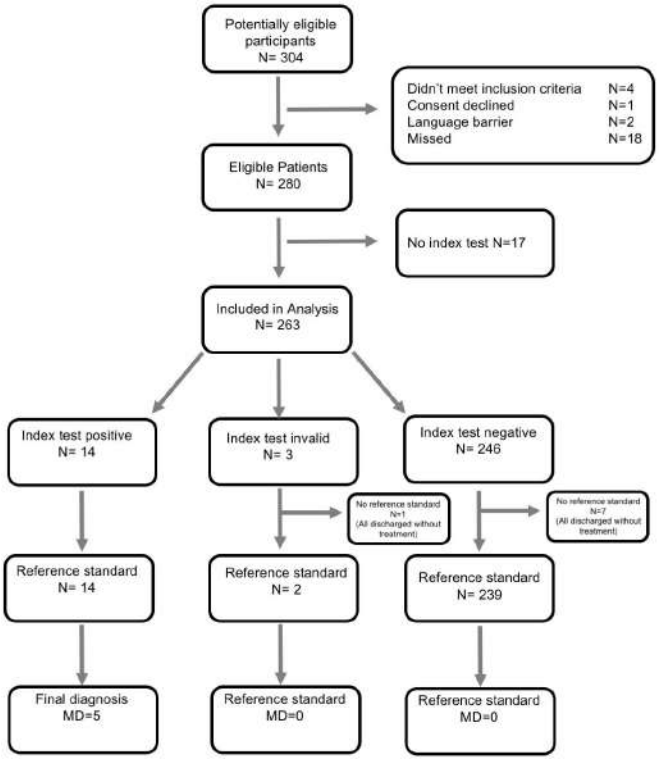


**Figure 1** Flow of patients through the study. MD, meningococcal disease.

**Table 1** Summary for the 260 study participants seen in emergency departments with suspected meningococcal disease (n and (%) unless otherwise stated)

| Characteristic | |
|---|---|
| Age (months; median (range)) | 35 (1–156) |
| Male sex | 142 (54.6) |
| Vaccination status | |
| Vaccinations up-to-date | 253 (97.3) |
| Meningococcal B vaccinated | 150 (57.7) |
| Meningococcal C vaccinated | 179 (68.8) |
| Index tests | |
| LAMP | 260(100) |
| C reactive protein | 260(100) |
| White cell counts | 260(100) |
| Reference standard | |
| Culture/PCR | 253 (97.3) |
| Presumed negative | 7 (2.7) |
| Outcomes | |
| Received parenteral antibiotics at first presentation | 120 (46.2) |
| Admitted to hospital | 142 (54.6) |
| Admitted to PICU | 4 (1.5) |
| Deaths | 1 (0.4) |
| Confirmed meningococcal disease | 5 (1.9) |

LAMP, loop-mediated isothermal amplification; PICU, paediatric intensive care unit.

1. They were not subsequently diagnosed with MD within 7 days of discharge.
2. They did not have a MD notification to public health.

## Analysis

The study population was described in terms of demographic characteristics with median age and gender. Simple descriptive statistics (total number and proportion) were used to describe vaccination status, parenteral antibiotic use, admission to hospital, admission to intensive care units and survival. The diagnostic accuracy of LAMP testing was reported as sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and negative/positive likelihood ratios (LHR) with 95% CI. In situations where the LAMP test provided an invalid result, the test result was excluded from analysis.

## Sample size calculation

As per the published protocol, we calculated that we would need 203 LAMP-negative patients to estimate an NPV of 95% or greater with CIs of ±3%. Disease prevalence was estimated at 15% or lower, based on preparatory work in our centre and other epidemiological studies, and we anticipated a combined refusal of consent and dropout rate of 10%. Accounting for these factors, we calculated that we would need to recruit 250 participants.

## Consent model

Due to the potentially life-threatening nature of MD, we used research without prior consent (RWPC),[17] described in detail elsewhere.[15 18] All participants were invited to provide consent at the earliest appropriate opportunity once the clinical condition had stabilised (typically within 24 hours of enrolment).

## Data management

Study data were collected and managed using REDCap (Research Electronic Data Capture). The initial REDCap case report form (CRF1) was used to contemporaneously record data regarding the initial clinical assessment and results of LAMP testing. The second CRF was completed 7 days after discharge and was used to record laboratory results, length of stay and other aspects of care not susceptible to recall bias (Copies of the CRFs are available in the online supplementary material.

## Public and patient involvement (PPI)

There has been public and patient involvement with the PiC study from outset. The PPI group assisted in the design of the PiC study including the protocol, study information and RWPC methodology. The lead of the PPI group sat on the trial oversight committee and received regular updates regarding progress and was a coauthor of the published PiC protocol.[15] PPI group members have promoted the study locally and nationally through appearances on local and national radio and television.

## Office for Research Ethics Committees (OREC) and local Research Governance

The study was approved by both the Northern Ireland OREC (Project ID 224660, OREC ID 17/NI/0169) and by the Belfast Health and Social Care Trust Research Governance.

## Study registration

The PiC study was registered at https://www.clinicaltrials.gov (trial registration: NCT03378258) on 19 December 2017.

# FINDINGS

A total of 304 consecutive children were screened for inclusion in the study across the three sites, of which 24 were ineligible; 18 of these were screened after hospital admission and laboratory testing, four did not meet inclusion criteria, two could not provide consent due to unavailability of translators and one declined consent. Of the 280 participants, 17 did not have LAMP testing performed due to a lack of time and/or trained staff, and

**Table 2** Summary of children with confirmed meningococcal disease

| Diagnosis | Blood culture | Blood PCR | Age | Received meningococcal B vaccine | Received meningococcal C vaccine | Admitted to intensive care | Survival |
|---|---|---|---|---|---|---|---|
| *N. meningitidis B* | Negative | *N. meningitidis B* | 4 years 10 months | No | Yes | Yes | Yes |
| *N. meningitidis B* | Negative | *N. meningitidis B* | 4 years 10 months | No | Yes | Yes | Yes |
| *N. meningitidis B* | Negative | *N. meningitidis B* | 4 years 7 months | Yes | Yes | No | Yes |
| *N. meningitidis B* | *N. meningitidis B* | *N. meningitidis B* | 3 months | Yes | No | Yes | No |
| *N. meningitidis B* | Negative | *N. meningitidis B* | 3 years 1 month | Yes | Yes | No | Yes |

**Table 3** Diagnostic accuracy of LAMP testing on oropharyngeal swabs for predicting meningococcal disease

| Index test | Meningococcal disease | | Sensitivity (95% CI) | Specificity (95% CI) | Positive predictive value (95% CI) | Negative predictive value (95% CI) | Positive likelihood ratio (95% CI) | Negative likelihood ratio (95% CI) |
|---|---|---|---|---|---|---|---|---|
| | Present | Absent | | | | | | |
| LAMP positive | 5 | 9 | 1.00 (0.46 to 1.00) | 0.97 (0.93 to 0.98) | 0.36 (0.14 to 0.64) | 1.00 (0.98 to 1.00) | 28.3 (14.9 to 53.8) | N/A |
| LAMP negative | 0 | 246 | | | | | | |

LAMP, loop-mediated isothermal amplification.

three had invalid results, leaving 260 patients for inclusion in the final analysis. The three invalid results occurred in children without MD (figure 1). Of the analysis population of 260, 245 were enrolled in the lead study site, 12 from the second site and 3 from the third site. Reference standard testing was performed in 253 participants; none of the seven children without reference standard testing received parenteral antibiotics, all were diagnosed with 'viral illness' by the discharging clinician and none re-presented within 7 days of discharge.

A total of 142 participants (54.6%) were male, and the median age was 2 years, 11 months (range 1 month–13 years, 11 months), with 169 (65%) aged 5 years or younger. A total of 253 (97.3%) were appropriately vaccinated for age according to the UK vaccination schedule; 150 (57.7%) had received meningococcal B vaccination and 179 (68.8%) meningococcal C vaccination. Parenteral antibiotics were given to 120 (46.2%), and 142 (54.6%) were admitted; four were admitted to a paediatric intensive care unit and one died. There were five (1.9%) confirmed cases of invasive MD (tables 1 and 2), all of which had positive blood PCR for *N. meningitidis*. One case was confirmed by both culture and PCR for *N. meningitidis* in blood (table 2).

The LAMP testing of oropharyngeal swabs was positive in 14 children, of whom five had invasive MD (table 2). All five cases of invasive MD were recruited from the Royal Belfast Hospital for Sick Children. Of the 246 with negative LAMP tests, there were no cases of invasive MD. The sensitivity of LAMP testing was 1.00 (95% CI 0.46 to 1.00) and the specificity was 0.97 (95% CI 0.93 to 0.98). The NPV and PPV were 1.00 (95% CI 0.98 to 1.00) and 0.36 (95%CI 0.14 to 0.64), respectively. The positive LHR was 28.3 (95% CI 14.9 to 53.8); the negative LHR was incalculable due to there being no false negatives in this cohort (table 3). In all instances, the LAMP test result was available within 40 min. The oropharyngeal swabs used to perform LAMP testing underwent additional laboratory testing for meningococcal *ctrA* gene using PCR. Of the 260 analysed oropharyngeal specimens, seven (2.7%) tested positive for *N. meningitidis* and 253 tested negative. The seven positive test results included all of the cases of invasive MD.

All of the 260 children included in the final analysis had data relating to their measured CRP levels, total WCC and neutrophil counts recorded. The diagnostic accuracy of these tests is shown in table 4. The LAMP test performed better than CRP, WCC or neutrophil counts in this population with a significantly higher specificity ($p<0.0001$) for all.

## INTERPRETATION

This study found the LAMP test can be performed using oropharyngeal swabs in the ED, as a point-of-care test, with results available within the hour. The invalid rate was low (n=3; 1.1%) and the LAMP test was highly sensitive and specific for identification of invasive MD. The LAMP test performed favourably when compared with conventional tests such as CRP, WCC and neutrophil count in this cohort.

Although vaccination programmes have made MD uncommon in the UK, a small but significant number of children develop invasive MD each year.[19 20] Differentiating children with MD from those with a self-limiting viral infection is increasingly difficult on clinical grounds alone. Existing biomarkers such as CRP, WCC and neutrophil counts are of little additional help to clinicians given their inaccuracy for predicting MD.[5 21] The findings of the parent study from which the data in this analysis are drawn (the PiC study) further support this, demonstrating

**Table 4** Diagnostic accuracy of CRP, abnormal white cell count (<5000/uL or >15 000/uL) and elevated neutrophil count (>10 000/uL) at predicting meningococcal disease

| Test | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) | Positive LHR (95% CI) | Negative LHR (95% CI) |
|---|---|---|---|---|---|---|
| CRP>6 mg/L | 1.00 (0.46 to 1.0) | 0.29 (0.23 to 0.35) | 0.03 (0.01 to 0.06) | 1.00 (0.94 to 1.00) | 1.4 (1.3 to 1.5) | NA |
| CRP≥10 mg/L | 1.00 (0.46 to 1.0) | 0.39 (0.33 to 0.45) | 0.03 (0.01 to 0.07) | 1.00 (0.95 to 1.00) | 1.6 (1.5 to 1.8) | NA |
| CRP≥20 mg/L | 1.00 (0.46 to 1.0) | 0.54 (0.47 to 0.60) | 0.04 (0.01 to 0.10) | 1.00 (0.96 to 1.00) | 2.2 (1.9 to 2.5) | NA |
| CRP≥50 mg/L | 0.60 (0.17 to 0.93) | 0.78 (0.73 to 0.83) | 0.05 (0.01 to 0.15) | 0.99 (0.96 to 0.99) | 2.8 (1.3 to 6.0) | 0.5 (0.2 to 1.5) |
| Abnormal white cell count | 0.60 (0.17 to 0.93) | 0.67 (0.61 to 0.73) | 0.03 (0.01 to 0.11) | 0.99 (0.95 to 1.00) | 1.8 (0.9 to 3.9) | 0.6 (0.2 to 1.7) |
| Elevated neutrophil count | 0.40 (0.07 to 0.83) | 0.73 (0.67 to 0.78) | 0.03 (0.00 to 0.11) | 0.98 (0.95 to 1.00) | 1.5 (0.5 to 4.4) | 0.8 (0.4 to 1.7) |

CRP, C reactive protein; LHR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

that an abnormal WCC, neutrophil count or elevated CRP were all poorly specific for MD (0.67, 029 and 0.73), respectively.[22] These specificities were significantly lower than that of the LAMP test (specificity 0.97, p<0.0001).

In summary, the LAMP test was as sensitive as CRP at a cut-off of 6 mg/L and more specific than an abnormal WCC or an elevated neutrophil count for the detection of MD. The LAMP test conferred additional benefits for patients, including a quicker time to result than conventional microbiology testing and it would be entirely plausible for a child to have their throat swabbed at triage and results available within the hour.

Further research to fully ascertain the best use of LAMP testing within the diagnostic pathway is required but potential uses include as an adjuvant to blood culture and PCR techniques to rapidly identify those children with likely MD and tailor antimicrobial therapy.

## LIMITATIONS

This study has some limitations. First, the majority of participants were under 5 years of age, a group which has low carriage rates of *N. meningitidis* in the naso/oropharynx. This will have overinflated the specificity of the LAMP test due to fewer false positive results than would be seen with higher carriage rates. Our findings should, therefore, be applied with caution to populations with higher carriage rates, such as adolescents and young adults, and its performance accuracy is worthy of further study in this age group.

There were seven recorded false positive results (positive on LAMP testing but negative by PCR using the same swab); it is unclear if the difference in results was due to issues with handling samples (ie, genuine false positives) within the ED or due to a greater sensitivity of LAMP testing compared with conventional PCR. Previous studies have suggested that molecular amplification techniques using LAMP technology may offer a greater sensitivity than conventional PCR techniques.[10] Further research to determine if LAMP testing in the ED results in a greater number of false positives than LAMP testing in a laboratory setting could help to determine the ideal location and service model for LAMP testing. One potential solution could be colocated laboratories within the ED able to perform rapid tests including some molecular diagnostic tests.

The presented data are based on very few cases (n=5) of confirmed invasive MD, resulting in wide 95% CIs for sensitivity. This was despite the selection of eligibility criteria designed to include only participants with symptoms or signs suggestive of MD. Further study in a high prevalence setting would therefore be helpful to further illuminate the performance accuracy of LAMP as a rule-out test in isolation. Based on this study alone, with only five cases of MD, the LAMP test could not be used as a rule-out test.

Finally the majority of the LAMP testing occurred in a single large tertiary children's hospital. While it is likely that this centre is similar to other large city secondary and tertiary care centres, the dominance of one centre increases the inherent risk of bias within the study and makes the results more difficult to generalise. A prospective multicentre study involving a range of site types, and a greater number of older children and adolescents, is required to fully understand the clinical utility of the LAMP test.

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**Contributors** TW, MDL, DR, J-AM, DF, JM and MS conceived and designed the study. TW co-ordinated the running of the study including data management and site training. MC and BP were involved with the conduct of the study including data collection. KW provided study design expertise and designed the approach to consent.

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**Competing interests** JM Holds share options in HiberGene Diagnostics Ltd.

**Patient consent for publication** Not required.

**Ethics approval** The Northern Ireland Research Ethics Committee (REC Reference—17/NI/0169) and the Belfast Health & Social Care Trust Research Governance (Reference 16 201MS-SW) approved the protocol.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available in a public, open access repository. All of the individual participant data collected during this study will be available (including data dictionaries) on the Queen's University Belfast data repository. The full study protocol is available as an open access publication.

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