WHITE PAPER

Lateral flow testing in an antimicrobial resistance pandemic

Antimicrobial resistance (AMR) is the biggest global threat to public health, with at least 1.27 million deaths attributed to antibiotic resistant bacteria[1]. Amongst those associated most with deaths include third-generation cephalosporin-resistant and carbapenem-resistant *Enterobacterales*, which cause life threatening infections with limited treatment options. Intervention strategies are crucial in combatting AMR and include improving detection to better inform appropriate antibiotic prescribing and infection control methods that prevent the spread and dissemination.

During the COVID-19 pandemic, we saw a multitude of lateral flow diagnostics brought to the market and into people's homes, which raised awareness of how such a simple, easy to use diagnostic could be used to curtail the spread of infectious disease. We discuss in this whitepaper the use of lateral flow devices in combatting the 'silent pandemic' of AMR.

What are lateral flow devices?

Lateral flow immunoassay (LFIA) tests are hand held diagnostics that confirm the presence or absence of analytes (such as pathogen antigens) using different porous materials and antibodies that are coupled with labels to generate a visual result.

On one end of the LFIA strip is an absorbent pad whereby the sample is added. Migration begins along to a conjugate pad where the target analyte, if present, binds to mobile monoclonal antibodies containing a label such as colloidal gold. Capillary pressure causes the analyte-antibody complex to migrate further along the strip to a nitrocellulose membrane containing immobilised monoclonal antibodies that capture the analyte of interest to generate a visible test line. A control line is also present that contains antibodies that bind to the labelled antibodies to show the test is working correctly.

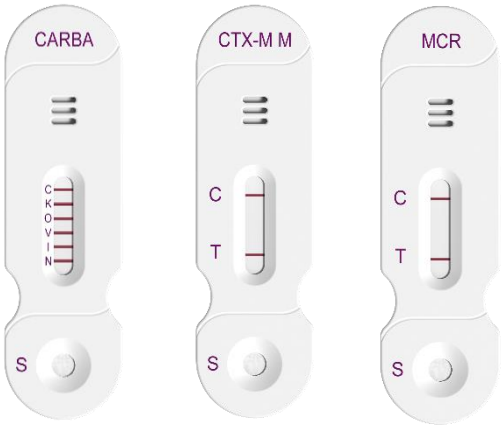
How can they be used to combat AMR?

In a routine microbiology workflow, detection of antimicrobial resistance is achieved through antibiotic sensitivity testing (AST) methods and/or direct identification of antibiotic resistance mechanisms. The latter is particularly important, as antibiotic susceptibility does not always correspond to the presence or absence of resistance mechanisms which, undetected, can lead to inappropriate antibiotic use and spread of resistance. The quicker the detection of resistance mechanisms, the faster appropriate treatments and infection control measures can be implemented.

Resistance mechanisms can be detected using phenotypic methods that directly detect proteins conferring resistance or antibiotic hydrolysis, or genotypic assays that detect resistance genes. Although phenotypic methods are simpler to use, require less expertise and more cost effective compared to molecular approaches, they are generally labour intensive and more time consuming, slowing the time to result.

In recent years, lateral flow tests have revolutionised AMR testing by overcoming the shortfalls of existing phenotypic methods, providing an economic, simple and rapid means to detect antibiotic resistance.

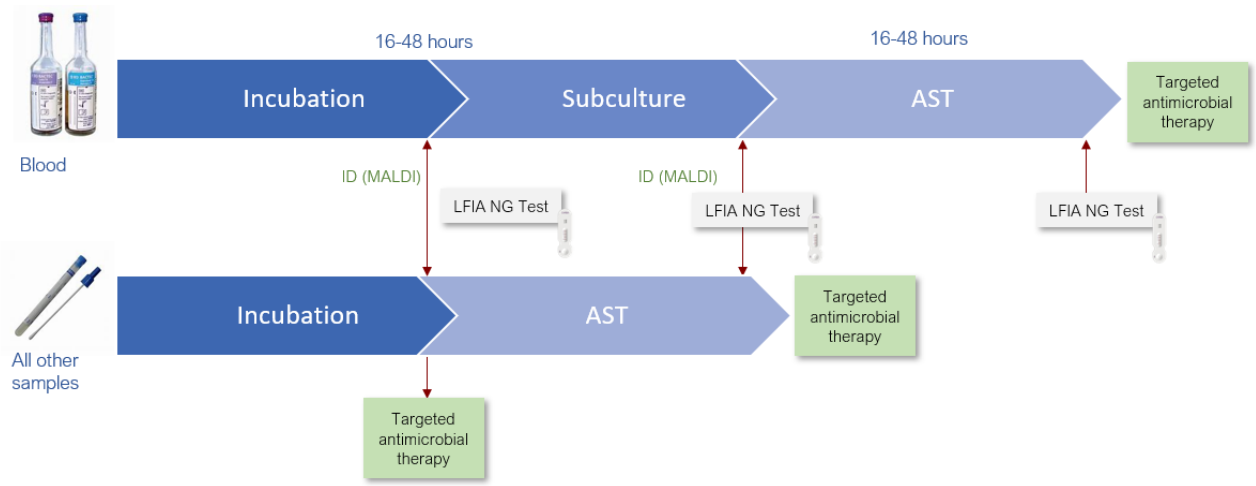
Una Health Ltd provides several LFIA's that detect various antibiotic resistance mechanisms; NG-Test® CARBA, NG-Test® CTX-M-Multi and NG-Test® MCR-1. Using patented technology that enables highly sensitive and specific multiplexing, these resistance tests can be used to confirm the presence of resistance enzymes that confer resistance to carbapenems, third-generation cephalosporins and colistin respectively.



The Standards for Microbiological Investigations (SMI) recommends the use of immunochromotographic tests for the confirmation of carbapenemases from screening or clinical samples[2]. The tests can be used to detect resistance mechanisms direct from plate culture in just 15 minutes, detecting resistance 16-48 hours earlier than waiting for antibiotic sensitivity testing (AST) results.

Similarly, NG Test CTX-M-M can be used as a confirmatory method for detection of CTM-M enzymes, the most prevalent enzyme of extended-spectrum β-lactamases in the UK, from Enterobacteriaceae isolates resistant to indicator cephalosporins but susceptible to carbapenems from screening or clinical samples.

LFIAs can also be used to detect resistance markers directly from blood culture. A positive blood culture prepared with a rapid method can be rapidly screened following MALDI identification using NG-Test Carba 5 or CTX-M-15, making it a cost effective algorithm[3].



Use of LFIAs in the microbiological workflow for detecting AMR. LFIAs can be used in screening direct from solid agar or positive blood culture, following a rapid preparation method, enabling targeting antimicrobial therapy to start sooner. They can also be used for confirming resistance mechanisms following AST.

In addition to saving time in the microbiological workflow through the direct detection of resistance markers from plate culture samples or positive blood culture, studies have also found LFIAs to be more time efficient compared to molecular methods (less than 30 mins vs. over one hour)[4,5].

Supporting evidence

There are over 100 publications on NG Test LFIA performance, including comparison studies to other rapid tests and molecular methods. The table below summarises performance based on recent studies, showing high sensitivity and specificity.

Product	Sensitivity	Specificity	Reference
NG-Test® CARBA	100%	100%	Diego et al.(2022)[6]
	99.1%	100%	Saito et al. (2022)[7]
	100%	99%	Huang et al. (2022)[8]
	100%	99.9%	Zhu et al. (2021)[9]
	98.5%	100%	Jenkins et al. (2020)[10]
	100%	100%	Han et al (2021)[11]
NG-Test® CTX-M-Multi	100%	100%	Bernabeu et al (2020)[12]
	100%	100%	Bianco et al (2020) [13]
	100%	99.6%	Fang et al. (2023)[14]
	98.8%	100%	Cendejas et al (2022)[15]
	91.6%	100%	Comini et al (2022)[3]
NG-Test® MCR-1	100%	99%	Fenwick et al.(2020)[16]

For more publications, visit (include link to our website with the NG publication PDF)

To find out more, contact our team at Una Health at enquiries@unahealth.co.uk or visit https://unahealth.co.uk/

References

1. Study on global AMR burden published in The Lancet – AMR Conference (amr-conference.com)
2. UK SMI B 60: detection of bacteria with carbapenem hydrolysing β lactamases (carbapenemases) (publishing.service.gov.uk)
3. Evaluation of a diagnostic algorithm for rapid identification of Gram-negative species and detection of extended-spectrum β-lactamase and carbapenemase directly from blood cultures | Journal of Antimicrobial Chemotherapy | Oxford Academic (oup.com)
4. Application of a multiplex immunochromatographic assay for rapid identification of carbapenemases in a clinical microbiology laboratory: performance and turn-around-time evaluation of NG-test Carba 5 - PubMed (nih.gov)
5. Comparison of five methods for detection of carbapenemases in Enterobacterales with proposal of a new algorithm - Clinical Microbiology and Infection
6. Comparative Evaluation of Phenotypic Synergy Tests versus RESIST-4 O.K.N.V. and NG Test Carba 5 Lateral Flow Immunoassays for the Detection and Differentiation of Carbapenemases in Enterobacterales and Pseudomonas aeruginosa - PubMed (nih.gov)
7. Evaluation of NG-Test CARBA 5 for the detection of carbapenemase-producing Gram-negative bacilli - PubMed (nih.gov)
8. Evaluating NG-Test CARBA 5 Multiplex Immunochromatographic and Cepheid Xpert CARBA-R Assays among Carbapenem-Resistant Enterobacterales Isolates Associated with Bloodstream Infection - PubMed (nih.gov)
9. Carbapenemase detection by NG-Test CARBA 5-a rapid immunochromatographic assay in carbapenem-resistant Enterobacterales diagnosis - PubMed (nih.gov)
10. Evaluation of NG-Test Carba 5 for Rapid Phenotypic Detection and Differentiation of Five Common Carbapenemase Families: Results of a Multicenter Clinical Evaluation (nih.gov)
11. Evaluation of the Immunochromatographic NG-Test Carba 5, RESIST-5 O.O.K.N.V., and IMP K-SeT for Rapid Detection of KPC-, NDM-, IMP-, VIM-type, and OXA-48-like Carbapenemase Among Enterobacterales - PubMed (nih.gov)
12. A Lateral Flow Immunoassay for the Rapid Identification of CTX-M-Producing Enterobacterales from Culture Plates and Positive Blood Cultures - PubMed (nih.gov)
13. Evaluation of the NG-Test CTX-M MULTI immunochromatographic assay for the rapid detection of CTX-M extended-spectrum-β-lactamase producers from positive blood cultures - PubMed (nih.gov)
14. Evaluation of a Lateral Flow Immunoassay for Rapid Detection of CTX-M Producers from Blood Cultures - PubMed (nih.gov)
15. Evaluation of a lateral flow immunoassay to detect CTX-M extended-spectrum β-lactamases (ESBL) directly from positive blood cultures for its potential use in Antimicrobial Stewardship programs (nih.gov)
16. Evaluation of the NG-Test MCR-1 Lateral Flow Assay and EDTA-Colistin Broth Disk Elution Methods To Detect Plasmid-Mediated Colistin Resistance among Gram-Negative Bacterial Isolates (nih.gov)