NAATWORK NEWS
Advancing molecular diagnostics
2014 Vol. 2

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# LETTER FROM THE EDITOR

## REAL-TIME PROGRESS

**How many times have you heard and read about the importance of "an early and accurate diagnosis?" Molecular diagnostics have turned this long-elusive goal into a reality for many gastrointestinal, nosocomial, respiratory and other infections. Ingrid Op den Buijs and Jeroen van den Bovenkamp of the Stichting PAMM in the Netherlands explain in this NAATWORK News how a real-time MRSA diagnosis (as opposed to a three-day wait) avoids the cost and distress of mandatory patient isolation. Dave Thomas offers specific numbers: switching to the BD MAX™ Enteric Bacterial Assay for diarrheal screening has saved the Hampshire Hospitals NHS Foundation Trust between £450,000 and £800,000 and an estimated 2000 isolation bed days. Professor Neil Woodford describes the role that molecular assays play in containing the spread of carbapenem-resistant organisms, one of the top public health priorities in Europe. Dr. Martin Altwegg, on the other hand, notes that we should not lose sight of the flip side of this need for speed. For tests that don't require an immediate result, centralization can offer improved efficiency. As we investigate current trends in this issue of NAATWORK News, we are already looking forward to new breakthroughs such as next-generation sequencing. Molecular is on the move. Enjoy your reading.**

*Elodie Kazek*

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NAATWORK CAFE

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**NAAT** UPDATE

# ESGMD: NAATWORKING IN MAASTRICHT

**An interview with Professor Dr. Martin Altwegg**



In March 2014, the European Study Group on Molecular Diagnostics (ESGMD) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) held its first postgraduate education course in Maastricht, the Netherlands. Entitled "Principles of Molecular Microbiological Diagnostics" and hosted by ESGMD chairman Professor Dr. Paul Savelkoul, the three-day event brought together novices and experts in molecular diagnostics.

Professor Dr. Martin Altwegg, Head of Microbiology/Molecular Biology at Bioanalytica AG, Lucerne, Switzerland, is one of the founding members and currently a board member of ESGMD. He talked to **NAAT**WORK News about this successful first post-graduate event and the current state of molecular diagnostics.

"We welcomed about 100 people in Maastricht. For us, that was a good number that allowed for smooth interaction and easy access to experts. Our goal was accessibility, also in terms of content. The program was broad by design so that we could reach both PCR novices and experts with a general overview of the PCR technologies, solutions and standards available today. Even though we didn't set out to push scientific or technological advances, there was something to learn for everyone. For example, I came across next-generation sequencing insights that were new to me."

"ESGMD started in 1995 as a joint project with Professor Dr. Jan Verhoef of the University of Utrecht. The group's mission really has not changed much since. We study all aspects of the use of molecular techniques for the diagnosis of infectious diseases. We also want to increase technical knowledge and share expertise among laboratories. In addition, we support joint research projects between labs, especially for rare diseases with limited availability of clinical specimens. Finally, we want to promote the acceptance of molecular diagnostics as a standard practice where appropriate. We have made great strides. Today, most labs use some molecular testing. In the beginning, there was just a few of us working with 'home brew' assays that required in-depth microbiology and molecular biology expertise. Commercial tests have since made PCR accessible to a large group of professionals. The integration and automation we are seeing today have also contributed greatly to our field's progress."

"Of course, it all started with the initial invention of PCR, followed by the development of thermocyclers. Next, improvement and automation of nucleic acid extraction techniques and the introduction of real-time PCR decreased total turnaround time and reduced the contamination issues inherent to traditional extraction and amplification techniques. Another, more recent, milestone for our discipline was next-generation sequencing. Today, and even more so in the future, I expect big advances in highly multiplexed assays. In addition, there are new solutions to better handle differences in urgency. On the one hand, we are in the midst of a move towards near-patient or even point-of-care testing for infections such as diarrhea (Noroviruses, *Clostridium difficile*) or respiratory tract infections (*Mycoplasma pneumoniae, Bordetella pertussis*, viruses), which demand rapid treatment and/or isolation of the patient to contain the spread of the disease. On the other hand, we can improve efficiency and reduce costs through the centralization of tests that don't require an immediate response."

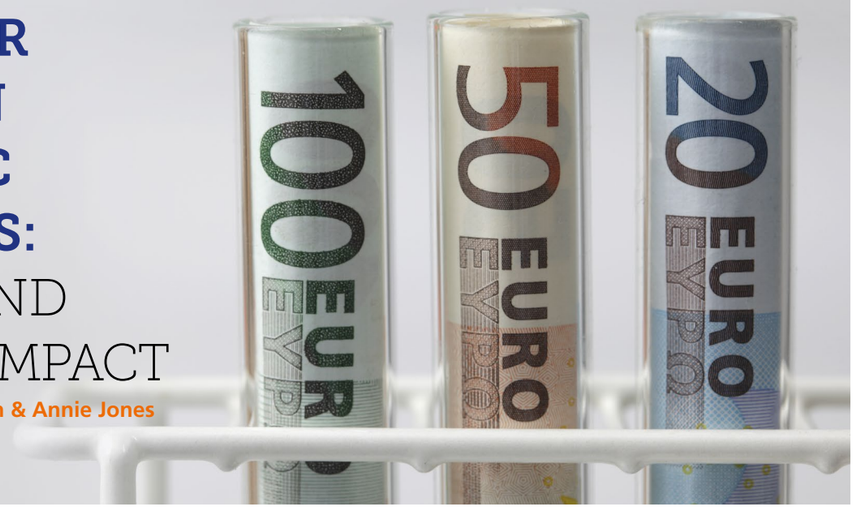
**More information about the ESGMD Molecular Diagnostics Study Group:**
**www.escmid.org/research_projects/study_groups/molecular_diagnostics/**

**All ESCMID members can join the ESGMD Molecular Diagnostics Study Group. To apply for a membership:**
**www.escmid.org/research_projects/study_groups/molecular_diagnostics/esgmd_membership_application/**

NAAT TRENDS

# MOLECULAR DETECTION OF ENTERIC PATHOGENS: CLINICAL AND ECONOMIC IMPACT

Dave Thomas, Nicki Hutchinson & Annie Jones




Infectious intestinal disease (IID) is a common condition. There are an estimated 17 million cases of IID reported annually in the UK. Surveillance data from the Health Protection Agency between 1992 and 2000 on more than 5000 outbreaks of IID in England and Wales showed that 27% of outbreaks occurred in the hospital setting. Current UK guidelines recommend the isolation of patients admitted to hospital with diarrhea until laboratory results are available using a syndromic approach to testing. However, between 4% and 70% of patients in hospital outbreaks of IID test negative for all pathogens, and failure to rapidly identify these patients represents a considerable drain on resources. The unnecessary isolation of patients without an infectious cause of their diarrhea and delay in obtaining an accurate diagnosis are factors contributing to the associated costs.

Historically, the principal diagnostic modalities for IID have included culture, microscopy and antigen-based tests. Culture methods are slow and often have low sensitivity when antibiotics are used. Antigen testing assays are costly and only exist for a limited number of pathogens. As causative pathogens can be viral or bacterial, IID is well suited for automated multiplex molecular testing, which has an emerging role in the diagnosis of diarrheal disease.

In conjunction with molecular testing for Norovirus and *Clostridium difficile*, a decision was made in 2013 to introduce the BD MAX™ Enteric Bacterial Assay for the screening of diarrheal admissions in the Hampshire Hospitals NHS Foundation Trust. The Trust serves a population of approximately 600,000 across Hampshire and parts of West Berkshire. Between 2012 and 2013, 59,000 patients received planned inpatient or day care treatment by the Trust.

In the winter months, the Trust screens all diarrheal admissions. Patients are isolated until laboratory results are available. The decision to introduce the system was based on the fact that the system is fully automated, has a time-to-result of less than three hours and includes the common major bacterial pathogens associated with IID: *Salmonella spp, Campylobacter spp, Shigella spp* (including *S. dysenteriae* serotype 1 strains containing the stx gene), Enteroinvasive *Escherichia* coli (EIEC) and Shiga toxin-producing *Escherichia* coli (including O157). The addition of the BD MAX™ Enteric Panel to the screening of diarrheal admissions to the hospital during the winter months has enabled results to be made available to the clinical staff within three hours. Patients testing negative can be admitted to general wards freeing up isolation beds. Over the first year of testing, it is estimated that more than 2000 isolation bed days have been saved. It is estimated that a hospital bed costs between £225 to £400 per day with an estimated cost saving with the reduction in isolation beds to the Trust of between £450,000 and £800,000. Accurate diagnosis of IID means appropriate antibiotics can be started quickly if clinically indicated, and it improves antibiotic stewardship. In many cases, rapid diagnosis allows patients to be discharged to home or community care more quickly, again easing the pressure on hospital beds. Reducing the amount of time that patients spend in isolation has many other benefits, including reducing anxiety and improving the overall patient experience.

*Dave Thomas, General Microbiology Lab Manager,*
*North Hampshire Hospital NHS Foundation Trust, UK*

**A longer version of this article previously appeared in Hospital Healthcare Europe; 2014; 8.**



NAAT INTERVIEW

# AN EXPERT VIEW ON THE MATURE MOLECULAR LAB

**An interview with Ingrid Op den Buijs, Stichting PAMM, the Netherlands**

Stichting PAMM is a large regional pathology and medical microbiology lab in the Netherlands. It serves four hospitals and approximately 800,000 potential patients. The lab, which over the years has developed an established expertise in molecular diagnostics, has been working with the BD MAX™ since 2011. Ingrid Op den Buijs was appointed as a dedicated BD MAX™ research specialist under the supervision of Jeroen van den Bovenkamp and has published a number of studies and posters in the last three years.

"We started using molecular techniques on targets that are difficult or impossible to grow. That is why *Clostridium difficile* and Norovirus screening came first. When we validated the BD MAX™ *Clostridium difficile* kit, we found twice as many positives compared to the immunoassay tests we had been using. Switching to PCR on the BD MAX™ as our primary *Clostridium difficile* test thus made absolute sense. We do a secondary neutralization test to check whether the toxin is active. We also moved to molecular MRSA screening to improve turnaround time. Dutch guidelines mandate strict isolation for suspected MRSA patients, unless you can have a diagnosis within 24 hours. Molecular MRSA screening offered a time-to-result of less than a day as opposed to two to three days, as well as increased sensitivity. As for future developments, we are now wrapping up two research studies, one on an assay to support the diagnosis of Tuberculosis and one for atypical *Pneumoniae* pathogens."

"In terms of diagnostics, the hospital performs mass STD and fecal tests on high-throughput equipment. The BD MAX™ we typically use for targets with a relatively low occurrence of 200 per week or less. For *Clostridium difficile* and Norovirus screening, we use BD assays. But about 80 percent of our assays are developed in-house."

"The time-saving potential of the BD MAX™ was clear from the beginning. But even though efficiency was the reason for getting the BD MAX™, I find its sensitivity compared to conventional methods such as microscopy and immunoassay tests even more convincing. There are also operational advantages. One is the elimination of the need to batch samples, reducing turnaround time for urgent cases. Another benefit is its full automation, freeing lab techs from repeated manual interventions."

"In the future, I would love to introduce more genotyping. Right now, we use it for VRE. The goal is to expand that to other targets, for example Tuberculosis. Transferring more tests to the BD MAX™ will allow the lab staff to focus on genotyping. When I started, we did a lot of conventional PCR. Then we went to real-time PCR and the increases in speed that brought, and now with the BD MAX™ you have a machine that is not only quick but also fully automated. It has all happened so fast. I think sequencing is going to be the next push forward."

**REVIEW IT!** In May 2014, Ingrid Op den Buijs presented her research "Mycobacterium tuberculosis complex: Development and evaluation of the detection of Mycobacterium tuberculosis complex on the BD MAX™ system in a European multicentre study" at BD's "Meet the Expert" event at ECCMID 2014 in Barcelona. View the poster: http://www.pamm.nl/fileadmin/media-archive/corporate-new/Bestanden/Over_PAMM/Publicaties/ECCMID_2014_MTB_Final_JB.pdf

BD MAX™ UPDATE

**In May 2014, BD launched its BD MAX™ Enteric Parasite Panel at ECCMID. The new panel allows for a targeted syndromic detection approach for gastroenteritis diagnosis. It complements the BD MAX™ Enteric Bacterial Panel and the Diagenode Enteric Viral Panel.**

**In June 2014, the BD MAX™ Open System (OSR) suite was extended with reagents for TNA extraction and amplification. As a result, both RNA and DNA pathogens can be extracted and detected from the same sample.**

**A substantial June 2014 BD MAX™ software upgrade has introduced more user options, including complete LIS connectivity, interpretation algorithms for user-developed protocols, and much more. For more information, please contact your application specialists.**

NAAT TRENDS

# LABORATORY DETECTION OF CARBAPENEM-RESISTANT ORGANISMS: THE EMERGING ROLE OF MOLECULAR ASSAYS TO DEFINE CARBAPENEMASE PRODUCERS

**Professor Neil Woodford**

Carbapenem-resistant organisms (CRO) and carbapenemase producers are considered an international public health threat. With only a few antimicrobial treatment options available and a relatively empty drug pipeline, acquired carbapenemases are the most important Gram-negative resistance challenge for the next five to ten years. Rapid detection through molecular techniques can help limit the impact of carbapenemases, decreasing the time to confirm likely resistance. A quick diagnosis of infected and colonized patients allows for implementation of infection control practices and adjustment of empiric therapy.

Research shows carbapenem resistance is on the rise across Europe. The majority of these resistant organisms are assumed to produce one of the "Big Five" carbapenemases: KPC, NDM, OXA-48, VIM and IMP. The increasing occurrence of carbapenemase producers is of particular interest because they are typically multi drug-resistant and consequently very difficult to treat.

## DETECTION

Stopping the spread of carbapenemase producers is challenging because of the difficulty in detecting them. The presence of a carbapenemase gene does not always translate into grossly elevated MICs for the carbapenems, yet the organism can spread and transfer its resistance gene to other isolates. On the other hand, there are organisms that show increased MICs of carbapenems, yet do not possess a carbapenemase gene but have a combination of ESBL/AmpC and porin loss.

Molecular diagnostics decrease the time to confirm probable resistance, enabling rapid adjustment of empiric therapy, and allow timely implementation of infection control measures. Molecular assays also play an increasing role in defining carbapenemase producers from other carbapenem-resistant organisms.

In order to identify a CRO, a test that challenges the isolated organism versus one or more carbapenems must be performed. Antimicrobial Susceptibility Testing (AST) – quantitative (MICs in agar or broth), qualitative (e.g. disk testing) or by automated systems – provides key information.

A recent algorithm by EUCAST advises that confirmatory tests for carbapenemase production should be untertaken for any Enterobacteriaceae isolate with a meropenem MIC >0.12 mg/L or a disk inhibition zone <25 mm. Aside from its clinical value, extensive AST provides insights on the isolate phenotype. Analysis by experts can be useful to postulate possible responsible mechanisms. Rapid phenotypic tests like the Carba NP and derivatives, and MALDI-ToF provide a fast qualitative answer on presence/absence of a carbapenemase gene starting from isolated colonies or, in some cases, from clinical samples.

## DIAGNOSIS

Rapid diagnostic methods are crucial for identifying infected/colonized patients in order to initiate appropriate patient management, implement infection control procedures and prevent onward transmission. From MALDI-ToF, real-time PCR, isothermal amplification and next-generation sequencing, a growing number of screening tests are available and are becoming increasingly user-friendly.

These techniques allow for increasing confirmation of carbapenem resistance mechanisms in regional or diagnostics laboratories rather than only in national reference labs. This will improve patient management and help limit transmission and spread of these organisms. Still, each type of test has its pros and cons and has a different coverage of the "Big Five", so it is important to choose one with the broadest coverage possible. For instance, coverage of all the alleles in IMP and VIM families is particularly cumbersome and not all commercial kits cover all the OXA-48 variants.



NAAT TRENDS

Too little is known about patient dynamics within hospital networks. Rapid identification and appropriate management of "high- risk patients" will be critical in order to contain a growing Gram-negative crisis. There is an urgent need for large-scale colonization studies, and cost-benefit analysis for implementing such screening on admission.

A coordinating role for national reference labs remains essential, not least for setting local, regional and national experiences into appropriate international context, and also by acting as national hubs that in turn can function as spokes in international networks.

*Professor Neil Woodford, Head of the Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit at Public Health England (PHE), UK*

**WATCH IT!** The above article is a summary of a webinar Professor Woodford gave on April 17, 2014. Watch the complete webinar: http://laboratory-manager.advanceweb.com/Webinar/Webinar-Archives/Laboratory-Detection-of-Carbapenem-Resistant-Organisms-The-Emerging-Role-of-Molecular-Assays-to-Define-Carbapenemase-Producers.aspx

NAAT EDUCATION

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- Keeping up with an evolving pathogen… Are mecA genes the only reason for MRSA?
- Laboratory detection of carbapenem-resistant organisms – the emerging role of molecular assays to define carbapenemase producers
- Antimicrobial stewardship versus CRE: the war continues
- The use of molecular diagnostic methods to detect fecal pathogens and their role in hospital care
- Screening for *Chlamydia trachomatis, Neisseria gonorrhoeae,* and *Trichomonas vaginalis* – "The Three Amigos"
- New strategies for accurate Influenza point-of-care testing

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