

OHEJP PROJECT OUTCOMES

METASTAVA

Standardisation and validation of metagenomics methods for the detection of foodborne zoonoses, antimicrobial resistance and emerging threats.





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WHAT IS METAGENOMICS AND WHY IS IT IMPORTANT?

Metagenomics is the study of genetic material within samples such as soil, food or human and animal clinical samples. This genetic material could be that of bacteria, viruses, fungi or parasites. The study of the genetic material can either be done completely randomly (“shotgun metagenomics”), targeting nucleic acids of all potential microorganisms, or targeting particular genes (e.g. for bacterial identification) or using particular enrichment methods to focus on particular groups of microorganisms (e.g. viruses).

Metagenomics has resulted in significant advances in the understanding of microbes, their evolution, their diversity in the environment and their contribution to animal and human disease. Many research groups across the globe routinely use metagenomics, and it is increasingly used to identify possible causes of unexplained disease outbreaks, in addition to being used to complement routine diagnostic evaluation, and to study the role of microbial communities in health and disease.

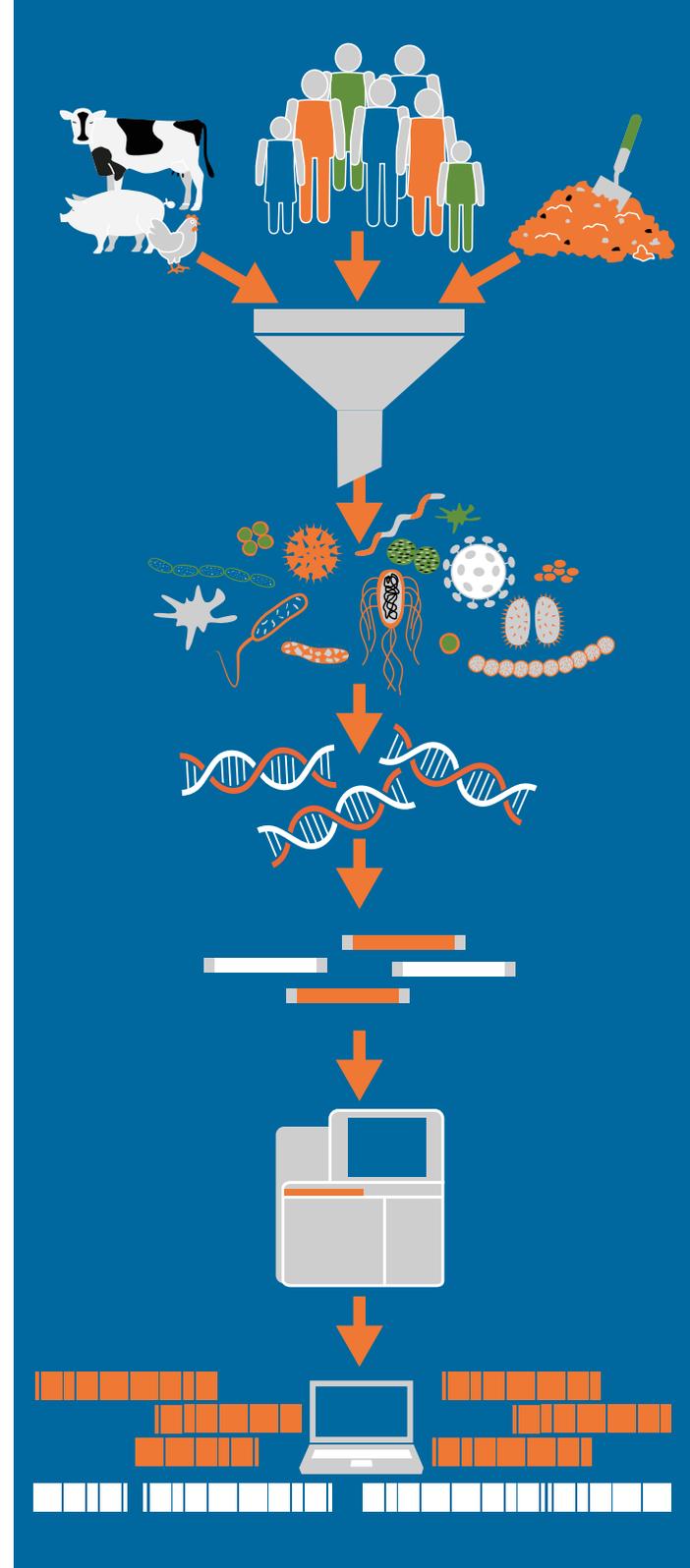
Shotgun metagenomic methods include sequencing of all nucleic acids in a sample in an attempt to identify pathogens or microbial communities. It is a powerful tool to identify microorganisms that cannot be cultured in the laboratory or to identify novel viruses. These methods have started to evolve from being used in research settings to being used in diagnostic settings, thus posing technology and application challenges as their scope changes.

For effective results the scientific community needs to work together to standardise and validate metagenomic methods and their interpretation for the detection of microorganisms that are implicated in foodborne zoonoses, antimicrobial resistance and emerging microbiological threats, and can be used in all pillars of One Health.

The One Health EJP uses its unique position to facilitate a collaborative approach between institutes to deliver important multisectoral research to attain optimal health and wellbeing outcomes for humans, animals and the environment. We bring together 44 acclaimed European scientific institutes working together on 29 research projects to address potential and existing risks that originate at the animal-human-environment interface.

There are a number of projects in the Consortium that are using metagenomics in their One Health research. These projects include [METASTAVA](#), [TELE-Vir](#), [FARMED](#) and [PARADISE](#).

The METASTAVA project addressed gaps in knowledge by investigating how to ensure that the diagnostic use of metagenomic methods for pathogen detection were accessible to public health, veterinary health and food laboratories.





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WHAT IS THE METASTAVA PROJECT?

The METASTAVA project is a small transnational research project consisting of five One Health EJP partner institutes across five European countries: Sciensano in Belgium, National Veterinary Institute (SVA) in Sweden, The French Agency for Food, Environmental and Occupational Health and Safety (ANSES) in France, The Friedrich Loeffler Institute (FLI) in Germany and Wageningen University & Research (WUR) in the Netherlands, and with Erasmus Medical Center (EMC) involved as an active contributor. This partnership of public and veterinary health and food safety institutes provides a unique platform to bring together expertise from different health sectors to ensure that standardisation of methods, quality control and correct data interpretation can be achieved across the One Health domains.

At the start of the project, several important knowledge gaps were identified, including the need for:

- the development of a set of reference data for the model pathogens, representing common sample types
- the development of harmonised work flows for generation and analysis of metagenomic data fitting to a defined diagnostic scope for model pathogens
- the development of a validation protocol for metagenomic diagnostics.

The key microorganisms of interest in this project include hepatitis E virus (HEV), norovirus (NoV), zoonotic pox viruses, antibiotic resistant bacteria and Shigatoxigenic *E. coli* (STEC). These microorganisms were used as model pathogens for developing the methods and reference datasets. The METASTAVA project provides exploratory analytical validation data, data on methodology reproducibility, exogenous spike-in control materials for metagenomics and data generation and analysis methods for these key microorganisms.

Using a range of microorganisms that have human, animal and environmental health implications enabled the METASTAVA project to build a community of medical, veterinary and food diagnostic scientists in public institutions who are in the process of implementing metagenomics.

The One Health approach is a powerful way to facilitate the standardisation of methods for generating and analysing metagenomic data from pathogen and sample combinations that are relevant to all three health domains. In doing this, a guidance document to support the informed methodological choices for metagenomic diagnostic approaches, quality assurance and correct interpretation of results can be created to support best practice.



METASTAVA
Project Leader:
Steven Van Borm



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Recently, a large number of high quality review papers have addressed the potential and current hurdles of diagnostic metagenomics, and several research groups and different consortia have addressed aspects of method standardisation and workflow validation.

In the context of this research and development landscape, the METASTAVA project has created a guidance document to support the use of metagenomics for diagnostic purposes. The document, **Key considerations for the implementation of high throughput sequencing based metagenomics (mNGS) in diagnostic clinical, food and veterinary labs: a no-nonsense pointer**, is a document designed to bring together the best resources available on metagenomics to help guide those interested in diagnostic metagenomics, or those currently using the techniques in their labs. It contains a comprehensive list of global research initiatives which provide guidelines and standards for the use of diagnostic metagenomics, in addition to a list of metagenomics review papers which highlight the advantages and drawbacks of the metagenomic approach. Finally, it provides some guidance for the informed design of metagenomic experiments as an informed and critical scientific reflection about all steps involved in diagnostic metagenomics is important.



These guidelines provide a useful resource for scientists using, or planning to implement diagnostic metagenomics, in addition to stakeholders with direct links to:

- Ongoing research efforts in the metagenomics field
- Relevant review papers
- Guidelines for the quality assurance of metagenomics assays
- Guidelines for the correct interpretations and follow-up of metagenomic finding

As a result of the experiences learned from METASTAVA project's research, it is clear that rational design of metagenomic studies that incorporate smart quality control metrics and quality assurance approaches to reduce technical uncertainties are required, in addition to informed and critical interpretation of metagenomic data. The guidance document produced aims to provide those wanting to use diagnostic metagenomics with the tools needed to create informed experimental design and to appropriately interpret their data. By referencing existing guidelines and ongoing research efforts, the METASTAVA guidance document provides food for thought to inform best practise in a diagnostic environment.



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The metagenomics research conducted by the METASTAVA project can be applied to many different research and diagnostic settings. This is highlighted by the diversity of the publications from the project, and the ability to apply this research directly to the COVID-19 pandemic. Read more in the list below:

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Evaluation of a commercial exogenous internal process control for diagnostic RNA virus metagenomics from different animal clinical samples.

Van Borm, S., Fu, Q., Winand, R., Vanneste, K., Hakhverdyan, M., Höper, D., Vandenbussche, F. (2020).

Journal of Virological Methods, 283.

DOI: <https://doi.org/10.1016/j.jviromet.2020.113916>.

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COVID-19 in health-care workers in three hospitals in the south of the Netherlands: a cross-sectional study.

Sikkema, RS., Pas, SD., Nieuwenhuijse, DF., O'Toole, A., Verweij, J., van der Linden, A., Chestakova, I., Schapendonk, C., Pronk, M., Lexmond, P., Bestebroer, T., Overmars, RJ., van Nieuwkoop, S., van den Bijllaardt, W., Bentvelsen, RG., van Rijen, MML., Buiting, AGM., van Oudheusden, AJG., Bram M Diederens, BM., Bergmans, AMC., van der Eijk, A., Molenkamp, R., Rambaut, A., Timen, A., Kluytmans, JAJW., Oude Munnink, BB., van den Bergh, MFQK., Marion P G Koopmans, MPG. (2020). *The Lancet Infectious Diseases*, 20(11), p1273-1280.

DOI: [https://doi.org/10.1016/S1473-3099\(20\)30527-2](https://doi.org/10.1016/S1473-3099(20)30527-2)

Monitoring SARS-CoV-2 circulation and diversity through community wastewater sequencing.

Izquierdo Lara, RW., Elsinga, G., Heijnen, L., Oude Munnink, BB., Schapendonk, CME., Nieuwenhuijse, D., Kon, M., Lu, L., Aarestrup, FM., Lycett, S., Medema, G., Koopmans, MPG., Miranda de Graaf, M. (2020). *medRxiv*

DOI: <https://doi.org/10.1101/2020.09.21.20198838>

Increased viral read counts and metagenomic full genome characterization of porcine astrovirus 4 and Posavirus 1 in sows in a swine farm with unexplained neonatal piglet diarrhea.

Van Borm, S., Vanneste, K., Fu, Q., Maes, D., Schoos, A., Vallaey, E., Vandenbussche, F. (2020). *Virus Genes*, 56, 696–704.

DOI: <https://doi.org/10.1007/s11262-020-01791-z>

Detection of Norovirus Variant GII.4 Hong Kong in Asia and Europe, 2017–2019.

Martin Chi-Wai Chan, Sunando Roy, Joseph Bonifacio, Lin-Yao Zhang, Preeti Chhabra, Jenny C.M. Chan, Cristina Celma, Mary Ann Igoy, Sin-Leung Lau, Kirran N. Mohammad, Jan Vinjé, Harry Vennema, Judith Breuer, Marion Koopmans, Miranda de Graaf. (2021). Detection of Norovirus Variant GII.4 Hong Kong in Asia and Europe, 2017-2019. *Emerg Infect Dis*. 27(1): 289–293.

DOI: <https://doi.org/10.3201/eid2701.203351>



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Each of the One Health EJP projects creates a unique network of institutes across Europe with wide ranging expertise to achieve objectives using a cohesive One Health approach. We bring together expertise in medical, veterinary and environmental health scientific communities and use integrated approaches to solve complex global issues.

The METASTAVA project worked collaboratively to achieve it's aims and to produce impactful scientific outcomes which could benefit scientists and policy makers across the globe.

The One Health EJP members of this project were from the following institutes:

- Sciensano, Belgium
- National Veterinary Institute (SVA), Sweden
- Friedrich-Loeffler-Institut (FLI), Germany
- French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France
- Wageningen University and Research (WUR), The Netherlands

Project partner from outside of the One Health EJP:
Erasmus Medical Center (EMC), The Netherlands



- Sciensano, Belgium
- National Veterinary Institute (SVA), Sweden
- Friedrich-Loeffler-Institut (FLI), Germany
- French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France
- Wageningen University and Research (WUR), The Netherlands
- Erasmus Medical Center (EMC), The Netherlands



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