



## Brief report

## TRANSVAC workshop on standardisation and harmonisation of analytical platforms for HIV, TB and malaria vaccines: 'How can big data help?'



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## ABSTRACT

High-throughput analyses of RNA and protein expression are increasingly used for better understanding of vaccine-induced immunity and protection against infectious disease. With an increasing number of vaccine candidates in clinical development, it is timely to consider standardisation and harmonisation of sample collection, storage and analysis to ensure results of highest quality from these precious samples. These challenges were discussed by a group of international experts during a workshop organised by TRANSVAC, a European Commission-funded Research Infrastructure project. The main conclusions were: Platforms are rarely standardised for use in preclinical and clinical studies. Coordinated efforts should continue to harmonise the experimental set up of these studies, as well as the establishment of internal standards and controls. This will ensure comparability, efficiency and feasibility of the global analyses performed on preclinical and clinical data sets.

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**Abbreviations:** dcRT-MLPA, dual colour reverse transcription multiplex ligation-dependent probe amplification; EC, European Commission; ELISA, enzyme linked immunosorbent assay; FP, Framework Programme; HIV, human immunodeficiency virus; HVTN, HIV Vaccine Trials Network; IMI, innovative medicines initiative; PBMC, peripheral blood mononuclear cell; QA, quality assurance; QC, quality control; RNA, ribonucleic acid; RT-PCR, real-time polymerase chain reaction; SOP, standard operating procedures; TB, tuberculosis.

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## 1. Introduction

Global molecular analyses are exploited to enhance our understanding of novel vaccination strategies. High-throughput technologies, including microarray analyses and RNA deep sequencing, allow genome-wide profiling of gene expression within different study groups. Similarly, targeted assays enable study of the expression of a dedicated number of genes [e.g. dual colour reverse transcription multiplex ligation-dependent probe amplification (dcRT-MLPA) assay], cell-expressed molecules (e.g. flow cytometry) or secreted molecules (multiplex assays).

Expectations of data output from these analyses in vaccine trials are high, and it is hoped that through the systematic analysis of biomarkers using modern bioassays, predictive biomarkers, which can be used as (surrogate) markers of clinical endpoints or of adverse events, can be identified. In tuberculosis (TB), human immunodeficiency virus (HIV), and malaria, few approaches are on the pathway for use with clinical trial samples for prediction of both vaccine efficacy and vaccine safety. However, molecular analytical tools are providing first hints regarding mechanisms underlying protection against, or susceptibility to, developing clinical disease [1–3]. Since there are now a number of vaccine candidates in phase II/III clinical trials in the TB, HIV, and malaria arenas, it is timely to consider standardisation and harmonisation of sample collection, storage and molecular analysis to ensure highest quality data from these precious samples.

In order to discuss these challenges a workshop was organised by TRANSVAC, a European Commission (EC)-funded project coordinated by the European Vaccine Initiative. The aim of the workshop was to define and implement a process supporting the harmonisation of operational procedures for the profiling and the assessment of novel vaccine candidates, novel vaccine formulations, and/or novel routes of administration. Through internal research activities in the field of HIV, TB, and malaria, and through the supply of services to 24 projects, including free access to adjuvants, animal models, microarray analysis, and assays/standards, the TRANSVAC partners have contributed to harmonisation of protocols. These efforts, which took place between 2009 and 2013, were discussed at the TRANSVAC workshop.

## 2. Overview from data sets obtained with different technologies, diseases and species, and in different clinical settings

### 2.1. Genome-wide high-throughput technologies

To obtain meaningful data sets from preclinical studies and clinical trials, standardisation and harmonisation of sample collection, storage and analysis are crucial. Results performed with three genome-wide high-throughput technologies (Agilent Technologies and Affymetrix transcriptome platforms, as well as Illumina sequencing platform) were presented [4,5]. While sample collection and pre-processing of the samples (e.g. RNA isolation, labelling for microarray analysis and library generation for sequencing) are well standardised, analysis was confounded by different influences, including the nonhuman primate sub-species analysed, the health history of study participants, and by differences in the sources of RNA (e.g. cell-free nucleic acids and platelet RNA, both derived from different types of blood cells).

It was concluded that essential factors for studies involving microarrays are (i) group sizes, (ii) timepoints of measurement (including multiple pre-vaccination time points to account for inter-individual variation), (iii) strength of vaccine-induced responses, (iv) nature of test samples, and (v) quality of test samples.

Previous studies have found that, depending on sequencing depth, next-generation sequencing platforms can be more comprehensive than microarrays in detecting expression differences and have no hybridisation bias [6,7], but are computationally more complex and time consuming. Nevertheless, computational bioinformatics' analyses are essential for both techniques to obtain meaningful data and to compare data sets, and can best be embedded at the research group level [8,9].

### 2.2. Other technologies

Global analysis of metabolites (metabolomics) in serum has proven its value for biomarker design in infectious diseases including monitoring of vaccine trials [7].

Flow cytometric analysis and/or mass cytometric analysis of cells or cell-bound proteins can be used as predictive biomarkers for disease outcome and response to immune interventions [10]. These approaches seem to be more powerful than conventional methods, such as ELISA and Luminex, with key features like a short sample processing time, low blood amounts required per condition to be tested, the possibility to process both stimulated or non-stimulated samples, and the use of fresh samples which reduces the artefacts and loss of sensitivity due to cryopreservation. Important issues to guarantee reliability of the obtained data are standardisation of sample preparation, transport and storage, inter-test variation (occurring when large numbers of samples are processed by a single operator on a single day), data acquisition, and appropriate quality controls (QCs) (e.g. acceptable percentage of dead cells, minimum number of analysed events, reference controls). In the field of cancer immunotherapy, harmonisation and standardisation of T-cell immunoassays (e.g. ELISpot and intracellular cytokine staining) has proven to be feasible on an international scale with great success [11].

Growth inhibition assays are increasingly used in TB and malaria. For TB, whole blood or PBMC-based tests utilising a liquid culture system for detection of mycobacterial growth have shown promise and are currently being assessed for use in early phase vaccine clinical trials [12,13].

As an alternative to array-based platforms, assays have been designed that offer specific, robust, affordable and practical bio-profiling platforms. The dcRT-MLPA assay is a RT-PCR-based gene expression profiling method, which represents a valid alternative to perform intermediate sized multiplex screens [1,3] once a tailored signature has been composed, e.g., based on information from unbiased genome-wide expression analysis. The assay setup ensures high assay sensitivity and avoids the limitations of multiplex PCR and the costly aspects of genome-wide platforms such as microarrays and RNA sequencing.

### 2.3. Integration of epidemiological data

It is becoming increasingly obvious that type of samples used (e.g. whole blood, PBMC, serum, plasma and urine), age of the individuals, or environmental factors (e.g. the circadian rhythm of the subjects including the number of sleep hours) can have a great impact on host responses [14]. It is thus important to carefully monitor epidemiological data from clinical trial study participants to draw adequate conclusions, when analysing the data.

In the context of clinical trials, systems biology combines clinical and epidemiological data with all transcriptional, proteomic, metabolomic and immunological data gathered [8,9,15–19]. There are three interlinking components of systems biology: experimental design, biological questions and data analysis. This results in a data set that ultimately needs to be validated before it can be usefully applied. Tools are available that can greatly reduce data

complexity and help in the identification of biomarkers, but oversimplification may lead to loss of insight into pathomechanisms.

A major bottleneck remains the difficulty to sustain a highly controlled environment in phase I clinical trials, during the time period between vaccination and the expected “operation” time of the vaccine. Moreover, to fully correct for all the parameters influencing the data, sampling schedules including a high number of critically chosen samples and time points are needed, but are frequently ignored due to time and cost restrictions. A trade-off thus has to be found between the amount of data that can be obtained and the means and know-how available to analyse the collected data.

#### 2.4. Comparison of data from different preclinical/clinical trials

A number of EC Framework Programme (FP) 6 and FP7 projects (i.e. TBVAC/NEWTBVAC, ADITEC, Euroneut41, OPTIMALVAC and EMVDA), and the IMI project BioVacSafe have contributed to standardisation of different protocols and SOPs, in order to allow comparison of readouts between different clinical trial sites. While strict reporting forms are well advanced [20–22], bottlenecks are time frame differences and investigator-specific protocols.

A different approach is to centralise all immunological readouts. The HIV Vaccine Trials Network (HVTN, Dr. Julie McElrath) is the quintessential example of a centralised infrastructure driving and executing the analysis of vaccine-induced immune responses in large clinical trials. HVTN has centralised use of qualified and validated immune assays, of common reagents, and of archived specimens, as well as collaborations and infrastructures including advanced planning. A centralised lead laboratory is responsible for quality assurance (QA)/QC and the repository of samples, while specialised working groups take care of protocols, support and QC of specimen [22]. Notable trials that were evaluated by HVTN were the HIV-1 STEP and RV144 trials [23,24].

### 3. Recommendations and conclusions

Only few global analysis platforms are fully standardised to inform and allow informative use in preclinical studies and clinical trials through which licensure could be obtained. Coordinated efforts between different disease networks should continue to achieve standardisation of immunological and global platforms that will allow their effective use in a clinical setting, their use for biomarker discovery and validation, and their use in generating data sets that can be compared between different platforms and across different preclinical settings and/or different clinical trials.

The main challenges to be overcome when performing global analyses can be grouped into the following:

- I. Definition of study group sizes and numbers in order to compare studies.
- II. Recommendations for sample collection and storage for future analysis wherever possible according to generally agreed-upon recommendations.
- III. Understanding of the influence of the environment on host responses. Not all variables can be taken into account when performing clinical studies and it is important to identify the parameters of greatest influence and to harmonise their monitoring.
- IV. Establishment of internal standards and controls to ensure comparability, efficiency and feasibility of molecular and immunological profiling.

These challenges require international collaboration, which could be led by the Systems Biology Platform of the European

Vaccine Research and Development Infrastructure, in association with regulatory agencies and representatives of the World Health Organisation. The process of harmonising methodology, sample and data collection, and the analysis of data will benefit from previous experiences in ADITEC and BIOVACSAFE European projects, together with the NIAID-sponsored Systems Biology for Infectious Diseases Research Program. The working parties should agree on core recommendations and a strategic action plan to address these priorities. If funding for European Vaccine Research and Development Infrastructure materialises in 2015, a pilot phase will be launched for structuring global analyses of infectious diseases with high public health importance, such as AIDS, tuberculosis, malaria, and influenza.

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