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Time to Agree: The Efforts to Standardize Molecular Microbiological Methods (MMM) For Detection of Microorganisms in Natural and Engineered Systems

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ABSTRACT

In the past decade, molecular microbiological methods (MMM) have significantly expanded the understanding of the microbial populations in several environments, including oilfields and associated infrastructure. These methods are now highly regarded as accurate, comprehensive, and useful to aid the optimization of microbial control strategies. The resulting information has helped operators and service companies to better assess the threat of microbiologically influenced corrosion (MIC) and act upon it. Nonetheless, despite finding acceptance in the industry, the results from these methods can greatly vary from lab to lab, due to the lack of a standardized protocol. In this study, we describe the joint effort of an initiative between operators, service companies, 3rd party labs and universities to establish a consensus on how to properly

collect and preserve samples for molecular analysis, and agree on a set of lab protocols to allow comparable results. We show how all the stakeholders used science-based conclusions to decide on the most comprehensive protocols that balances easiness of use in the field and accuracy of results. This industry-wide effort to standardize these methods will have a profound impact on data collection, quality of data and assessment of microbiological threats in the field.

Key words: Microorganisms, qPCR, Next Generation Sequencing, detection, molecular techniques, monitoring

INTRODUCTION

The presence of microorganisms in natural environments (oilfield reservoirs) and engineered systems (flowlines and separation facilities) has been associated with problems such as microbiologically influenced corrosion (MIC), H₂S production, and biofouling.¹ Due to the ubiquitous nature of microorganisms and the threats they pose to the system, monitoring procedures can provide guidance to properly treat the system and minimize the detrimental impact of high accumulation of microorganisms and their activities in these environments.

Although culture-based techniques have been the standard procedure for enumeration and quantification of microorganisms in oilfields², numerous reports in the literature highlight the limitations of these methods to accurately identify the organisms that are harmful to the systems.^{3,4,5}

The implementation of modern molecular techniques, often referred to as Molecular Microbiological Methods (MMM), has significantly improved the microbial monitoring in field samples and has become a common procedure for operators, service companies, universities, and analytical labs. These methodologies have helped to shed light into the complexities of the microbiology in environmental and industrial settings and have dramatically increased the success in the control of problems caused by microorganisms.⁶ For, example, the adoption of 16S rRNA gene sequencing has uncovered numerous microorganisms, such as thiosulfate-reducers and methanogens, that are directly related to microbiological corrosion. Many of these organisms are not readily detected by conventional culture-base methods.^{7, 8}

Nonetheless, despite being highly accurate, sensitive, and providing a wealth of high-quality information, molecular microbiological methods present their own set of challenges to the user. Proper preservation of samples, ability to obtain enough genetic material and data analysis are just a few of the challenges surrounding these methodologies. Numerous reports in the literature have shown that, depending on the choices of protocols, the results can be highly variable, which may negatively impact the value of these methods to the end user.^{9,10} Although standards dealing with analysis of microbial populations in field samples by other methods do exist,^{11,12} the lack of standard operating procedures to perform and analyze either quantitative PCR (qPCR) or DNA Sequencing (Metagenomics, 16S rRNA gene profiling) for field samples is one of the main reasons the full adoption of these techniques has faced some resistance. To overcome this, a NACE Task Group was created in 2018 to elaborate a document that will serve as guidance for any laboratory to perform qPCR and DNA Sequencing in field samples. The Task Group was named TM21465 (formerly TG-561) and started its activity at Corrosion 2018 in Phoenix. The goal of this Task Group is to create a set of protocols that is best suited for analysis of field samples using molecular techniques. The suggested protocols were based on the participants' laboratory experience in each step of the process, and the methodology suggested was experimentally tested. The outcome of the document will be a comprehensive guide that will provide the best quality data and that could be used by different laboratories to generate comparable results.

In this paper, we describe the results of two years of coordinated efforts between different laboratories and subject matter experts and highlight the main findings that will serve as the framework for the standard document.

TASK GROUPS AND DOCUMENT CRAFTING

Subgroups

Volunteers who elected to participate in the drafting of the Standard Document were allocated to 3 different subgroups, each tasked to elaborate parts of the document. The 3 subgroups were named: 1- Sample Collection and Preservation, 2- DNA Extraction Methods and 3- Primer Sets and Data Analysis. Each subgroup elected a Group Leader, tasked to meet with the other members of the subgroup for further discussions, share experimental protocols, collect laboratory data that corroborate their protocols and draft their part of the document.

Compilation of the document and revision sessions

Following the work from the individual subgroups, the write-ups from these subgroups were combined in a single document by the Chair and Vice-Chair. The full document was then shared with all volunteers and revision sessions were scheduled in a timely fashion. These sessions were intended for further discussions, changes, and revisions of the document. Sharing of further experimental findings was allowed during these sessions to enrich the science-based approach to the document. These changes were further incorporated into the document before submitting to the ballot.

RESULTS and DISCUSSION

The development of the NACE TM21465 (formerly TG-561) was kicked off in April 2018, during the NACE 2018 Corrosion Conference in Phoenix, Arizona, USA. The Task Group formed was the result of increasing agreement on the need of a standard for the analysis of oilfield samples using MMM. Forty-eight participants attended the kick-off meeting. During the meeting, 3 subgroups were created, where volunteers were able to sign in to participate in one or more subgroups. The list of all subgroups, volunteers and Group Leaders are shown in **Table 1**.

Table 1– TM214465 Subgroups and Volunteers

Sample Collection and Preservation	DNA Extraction Methods	Primer Sets and Data Analysis
GL: Lisa Gieg	GL: Kathleen Duncan	GL: Nicolas Tsesmetzis
Susmitha Kotu Amanda Harmon Nicole Williamson Herman DeVries Joe Moore Faisal Al-Abbas Bart Lomans Neil Sharma Marc Demeter Gilberto Guerrero Dennis Enning Jaspreet Mand Mary Eid Mohita Sharma	Nicolas Tsesmetzis Sam Rosalina Scott Leleika Marcia Lutterbach Silvia Salgar Maria Clara Pagliaricci Mary Eid Vince Sandifer	Neil Sharma Torben Skovhus Bart Lomans Amanda Harmon Michael Enzien Mohita Sharma Silvia Salgar