WORLD WIDE CLINICAL LABZ

5575 Conner Ave
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Attestation and Peer Review: Efficacy of Quantitative and Qualitative Testing of Micro/Nanoplastics in Dried Human Blood	
Validation: Plastictox by Arrow Lab Solutions	
Effective Date: 07/09/2024	Lab President: Michael P. Clemmon
Review Date: 07/09/2024	Lab Director: Bin Wei
Determination: APPROVED	

Scope

The scope of this document is to validate the efficacy of quantitative and qualitative detection of microplastics in human blood through Plastictox. The method, procedural explanation, and initial validation were provided under NDA for this attestation to determine efficacy through World Wide Clinical Labs, a CLIA/COLA certified high-complexity laboratory. All testing was performed externally, at SV Biotech, BV, with the documentation submitted to World Wide Clinical Labz for the purposes of this evaluation.

List of Acronyms

LOQ / LLOQ: Lower Limit of Quantitation

ULOQ: Upper Limit of Quantitation

QC: Quality Control

FM: Fluorescent Microscopy

LOD: Limit of Detection

pt/mL : particles per milliliter

CO: Cut-Off Concentration

SOP: Standard Operating Procedure

CV: Calculated Value

NRS: Nile Red Solution

FBS: fetal bovine serum

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Introduction and Purpose

The purpose of this Fluorescent Microscopy (FM) method attestation is to validate the use of Plastictox for the purpose of sample analysis to confirm microplastic concentration at various sizes. Determination of performance specifications ensures that the assay is operating according to expected performance standards and is capable of producing accurate and reliable results in their laboratory and with all personnel.

The following has been produced after viewing the procedures, practices, and acceptance criteria provided by Danielle Vlecken for use by Arrow Lab Solutions to establish performance qualification of microplastic quantitation and qualification on FM instrumentation. The verification activities conform to standard bioanalytical practices for analysis of biological samples.

1. Performance specifications have been verified and documented for each test system.
2. The Lab Director has reviewed the data and determined the acceptability of the test system prior to the transition from R&D to clinically approved testing
3. Testing personnel have performed the validation testing
4. The lab will document all materials, data, steps used in the process, and the results.
5. Records will be retained as long as the method is in use plus two years.

OVERVIEW

This performance qualification verification protocol is conducted using patient dried blood samples for a variety of patient demographics. This verification must be repeated if any changes are made to the SOP or method

The processing of dried blood samples involves staining of the sample, prior to its subjection to the FM method. In order to ensure that the microplastics assay performed in a laboratory is robust and produces precise and accurate results, the Performance Qualification Verification must be completed in the same lab where the patient samples are analyzed. This ensures that all necessary facilities, equipment and supplies are in place and meet the requirements for successful completion of the sample analysis.

EXPERIMENTS

The following experiments will be covered in this document.

- Precision
- Accuracy
- Linearity / Reportable Range
- Lower Limit of Quantitation (LLOQ)
- Matrix Interference

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1. Validation Assessments

1.1 Limit of Detection/Limit of Quantitation

Accuracy/precision batches have been reviewed to establish LLOQ, LOD, and ULOQ.

The LLOQ has demonstrated that a single particle of microplastic at ~1mcm is detectable

Limit of detection (LOD) has been established and has been deemed to be equal to the LLOQ

ULOQ is theoretically infinite, due to the ability to dilute samples. However, any dillution must be listed through annotation on the lab report, as it requires the calculated concentration to be calculated manually.

1.2 Linearity / Analytical Measured Range (AMR) / Calibration Accuracy

The calibration curve consists of five spiked FBS samples. The calibrators were run a total of 2 to 4 times across 3 separate runs. After performing linear regression, the back-calculated values of each calibrator were determined. The mean, standard deviation, and CV have been calculated across all batches for each calibrator level.

Acceptance Criteria: The percent recovery mean were all within +/- 25% of target for each level. Similarly, the inter-day CV were confirmed within 25% at each level.

Contaminant concentrations above the ULOQ will be reported with an annotation for patient samples. A dilution will be performed to get an accurate reading on such a sample, should one arise

1.3 Accuracy and Precision

For intraday precision and accuracy, three QC levels were prepared by an analyst, independently from the calibrators. Each QC level was run across three analytical batches.

For interday precision and accuracy, three QC levels were prepared by an analyst, independently from the calibrators. Each QC level was run across three analytical batches.

Acceptance Criteria: For both studies, the percent average recovery and the percent relative standard deviation for each QC level was calculated. Both intra-day and inter-day percent average recovery and percent relative standard deviation was within +/- 25% or 3 particles of the target for each of the three QC levels.

Similarly, the standard deviation and CV was calculated. Both intra-day and inter-day CV was less than 25% for each of the three levels.

For each analytical batch, blanks are performed for each of the following

-NRS

-NRS in ethanol (1:1)

-Empty filter card

-FBS

1.4 Interference

The blank protocol, which consists of NRS, NRS in ethanol, an empty filter card, and unspiked FBS, determine that none of the matrices or solutions used are capable of producing a false positive

2. Operator Qualification

All validation and samples thus far have been performed by Danielle Vlecken, PhD

Any new operators performing the assay will demonstrate proficiency with the method by running an analytical batch containing two standard curves, QC's, and blanks. Accuracy and precision results shall meet the acceptance criteria described in this document before the operator may perform patient sample analysis.

3. Instrument Qualification

A new FM instrument of the same model and configuration as the system used for this validation study must be qualified specifically for this analytical procedure prior to use for routine patient sample analysis. An analytical batch containing two standard curves, QC's , and blanks will be run on the new system, and on an existing system. Precision, accuracy, linearity, LLOQ, shall meet the acceptance criteria described in the relevant sections of this document. Furthermore, the mean QC values shall be within +/- 25% of the corresponding result from the existing system at each QC level.

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4. Determination

Upon the review of the SOPs, validation, raw data, and Plastictox insert and report, we have deemed the test to be viable for the detection of microplastics in dried human blood samples at the ranges being reported. World Wide Clinical Labs is not responsible for any diagnostic measures taken by individuals upon the receipt of a lab result generated through this test.

5. References

1. https://pubs.acs.org/doi/10.1021/envhealth.3c00052
2. https://pubmed.ncbi.nlm.nih.gov/25867233/
3. https://www.sciencedirect.com/science/article/pii/S0160412022001258
4. https://www.nature.com/articles/srep44501
5. https://pubmed.ncbi.nlm.nih.gov/28340965/

7-9-2024

Lab President Date