MEMORANDUM

TO: Robert Nussbaum
Principal Investigator
University of California, San Francisco

FROM: Sunita Shukla, PhD
Scientific Reviewer
FDA/CDRH/OIR/DCTD

RE: Q140229/S001
DEVICE: ILLUMINA HISEQ
DATED: February 25, 2105
RECEIVED: February 27, 2105
DATE: April 7, 2015

Dear Dr. Nussbaum:

Thank you for submitting Q140229/S001 for our review. This pre-submission seeks FDA input regarding your investigational study protocol proposing to use the Genelex Sequenom Mass Array 4 as an orthogonal confirmation method for next-generation sequencing of pharmacogenetic results. We hope that the input provided with regard to this pre-submission will make it easier for you to prepare for discussions and regulatory requirements pertaining to your current submission.

This feedback represents the best judgment of the Office of In Vitro Diagnostics and Radiological Health staff and consultants. It does not constitute an advisory opinion and does not bind or otherwise obligate or commit the agency to the views expressed, as per 21 CFR 10.85(k). Although pre-submissions and the agency's advice are not decisional or binding on the agency or the applicant, it is FDA's intent to give the best advice possible based on the current information that has been provided.

You have included 2 specific questions within this pre-submission. Our feedback to these questions is provided below. We request that you please prepare an agenda of items that you would like to discuss, based on our feedback below, during our scheduled meeting with you from 11-12 PM EST, on April 10, 2015. Please submit your agenda to us no later than 12 noon EST, April 9, 2015.

Background:

In Q140229/S001, you state that this submission has been submitted to provide more information regarding Genelex, the company that will be orthogonally confirming the pharmacogenetic test results (Genelex will perform a PCR- based assay using Sequenom Mass Array 4 pertaining to CYP2D6, CYP2C19, CYP2C9, CYP3A4, CYP2B6, VKORC1 testing) you will be returning to parents who have consented to have their

primary immunodeficient child's newborn spot sequenced. In an email on 4/1/15, you stated that the Genelex pharmacogenomics result (using buccal swab samples) would be the primary result returned to the subject. In that email you also clarified that you were looking for evidence of a primary immune deficiency in the NGS sequencing result (using dried blood spot samples) but that result would not be returned to the subject.

The variants that will be tested using the Sequenom Mass Array 4 at Genelex are listed below:

CYP2C19: active *1; inactive *2, *3, *4, *5, *6, *7, *8, *12; partially active *9, *10; rapid *17

CYP2D6: active *1, *2; *2A, *35; inactive *3, *4, *5, *6, *7, *8, *10, 11, *12, *14, *15, *19, *20, *36; partially active *9, *17, *29, *41; gene duplications *1, *2, *4, *10,*41

CYP2C9: active *1; inactive *2, *3, *4, *5, *6, *8, *11, *13, *15

VKORC1: high sensitivity 1639G>A

CYP3A4: active *1; partially active *22.

On 3/31/15, you submitted the analytical validation summary data via email from Genelex pertaining to the above noted platform and variants to review as part of Q140229/S001. The data was emailed as the first attachment (CYP2D6, CYP2C19, CYP2C9, CYP3A4, CYP3A5 and VKORC1 v2 Validation Summary) and second attachment (Pharmacogenetic Testing Panel 3 v1.0 ADRA2A, COMT, CYP1A2, CYP2B6, DPYD, F2, F5, GRIK4, HLA-B*57:01, HTR2A, HTR2C, IFNL3, MTHFR, NAT2, OPRM1, SLCO1B1, TPMT, VKORC1 Validation Summary). These data were reviewed with regard to the feedback provided below.

FDA FEEDBACK:

1. UCSF Question: Does Genelex meet FDA orthogonal confirmation standards for this study?

FDA Response: Please see FDA response to #2 below.

2. UCSF Question: Will UCSF need to submit an IDE?

FDA Response: In the current study, you have indicated that results from the Sequenom Mass Array 4 Platform will be used to return information to parents regarding the pharmacogenomics variants noted above. This information will also be included in the subject's medical record. The validation information provided in attachments 1 and 2 does not provide sufficient information to conclude that Genelex meets the requirements as an acceptable orthogonal confirmation method for the following reasons: 1) Genelex did not perform their validation testing using a sufficient number of real buccal swab

samples in order to evaluate the performance of their device using this sample type; 2) the validation testing did not include an accuracy study which compared the Genelex platform to a medically established (Sanger bi-directional sequencing) or FDA cleared assay for the noted variants. We can discuss this in more detail with you in our upcoming teleconference with you on April 10th.

As per 21 CFR 812.2(c) of the IDE regulations, studies exempt from the IDE regulations should not include diagnostic procedures without confirmation by another medically established diagnostic product or procedure. As also noted in feedback provided to you from FDA as part of Q140229 (dated April 2, 2014), a proposal that involves the return of results from an investigational device without confirmation by a reference method, FDA cleared assay, or an acceptable orthogonal confirmation method, is a significant risk study that would require an IDE application. The study may be exempt from IDE requirements if you considered modifications to this proposal, such as confirming all results from the Genelex Sequenom Mass Array 4 with Sanger sequencing or FDA cleared assays (available for 2D6, 2C19, 2C9 and VKORC1), prior to returning results. Your study may also be exempt from IDE requirements if you choose to not return results to parents in the study or if you choose to conduct additional validation testing with the Genelex Sequenom Mass Array 4, so that it could be considered an acceptable orthogonal confirmation method.

As is, your current proposal does not support an IDE exempt investigation and you will need to submit an IDE application. Your IDE application should contain validation studies to demonstrate that the Genelex Mass Array 4 assay is adequately accurate and repeatable using buccal swabs. Please see additional FDA comments below, regarding validation studies that would be needed as part of an IDE application, which can be discussed further during our upcoming teleconference.

Additional FDA Comments:

The comments below were also noted during the review of your pre-submission. You may choose to submit a supplement to this pre-submission with more detailed information regarding your IDE analytical validation plan if you would like Agency feedback before you start your analytical testing.

1. Samples: It appears that several studies in the 2 attachments provided by Genelex use contrived samples, such as Coriell cell lines. Please note that the use of contrived samples do not allow for the evaluation of all potential pre-analytical issues related to the use of buccal samples this study. In addition, the prevalence of some/majority of the polymorphisms evaluated by your device should be high enough that real buccal samples could be obtained. Thus, the analytical validation studies should use all buccal samples and contrived samples should only be used in instances where the prevalence of the polymorphisms is rare and actual samples are difficult to find.

   Furthermore, information pertaining to how the real buccal swab samples were originally collected or stored should be provided, in addition to how they were

collected and stored under the laboratory's validated collection and shipping recommendations. Please note that if banked samples will be used, then then the laboratory should be blinded to the genotype information of the sample.

2. Studies: In attachments 1 and 2, it appears that Genelex provided some information pertaining to several validation studies/results. Please note the following feedback, which is not all inclusive of all potential issues, regarding some of the studies. Please keep the following in mind for any future IDE application:

   A. Accuracy: Sufficient information regarding the analytical validation of the Sequenom Mass Array 4 has not been provided for use with buccal samples. Although cross-platform comparisons were performed using the Mass Array 4 and other platforms, such as Luminex 100 IS, it appears that the accuracy study results generated from the Mass Array 4 for the above noted variants were not compared to a medically established procedure, such as Sanger bidirectional sequencing, therefore FDA is unable to determine the accuracy of the platform.

      In addition, the accuracy and repeatability of your genotyping assay using buccal swab samples should cover all genotypes that your test will evaluate. For an IDE application, the laboratory should test a greater number of samples for the most prevalent genotype (e.g., 10 samples), a smaller set of samples for the intermediate frequency genotpyes (e.g., 5-10 samples), and 1-2 samples for the less prevalent genotypes, if applicable (if no real buccal samples are reasonably available for the least common genotype, then contrived samples, such as cell lines, may be used). Results generated by your test should be compared to results generated by bi-directional sequencing on the same samples.

   B. Limit of Detection (LoD): In the first attachment, the LoD was performed using only Coriell cell lines. In the second attachment, only a few buccal swab samples were tested in the LoD study, while the rest of the samples consisted of Coriell cell lines and blood. Furthermore, in the first attachment, Genelex states that the *LoD Assay performs well for all variants with Coriell DNA concentrations ranging from 8 to 200 ng/well, however in the second attachment, Genelex states that the LoD assay passed for all variants with DNA concentrations ranging from: Coriell DNA 0.3to 160 ng/well; Buccal 0.6- 160 ng/well.* Please note that as part of assay validation, the laboratory should thoroughly assess the lowest input DNA concentrations that would consistently give an acceptable call rate in your desired sample type. The LoD results for the lowest input of DNA appear to be inconsistent between all sample types in both attachments and should be assessed using only buccal swab samples. FDA recommends that the laboratory test a wide range of DNA concentrations and be sure to test enough samples to cover a representative panel of the genotypes that your device evaluates.

   C. Reproducibility: The laboratory should provide data from a multi-day reproducibility study with buccal swab samples that cover a representative panel

of the genotypes that your device evaluates. All steps of the assay from sample extraction to result reporting should be evaluated in this study.

D. Interference: The laboratory should provide sufficient information when testing buccal swabs for potential interferents known to interfere with the performance of your test.

E. Controls: On page 30 of the first attachment, Genelex states the following about their controls: *If all positive controls fail, the tray must be rerun. If both negative controls fail, repeat the entire run. If only one negative control fails and the positive controls pass, the batch may be called.* It is unclear from this procedure why certain criteria are chosen to re-run or not re-run plates. For example, it is unclear if results will be called if only some positive controls fail and others do not and why results may be called even if the negative control fails. The laboratory should investigate all control sample failures prior to calling and reporting results.

F. Discrepant Resolution: On page 26 of attachment 2, Genelex states that *If a discrepancy in the allele calls is noted, call the Supervisor for review. Troubleshooting should be done and the sample will be run an additional time, and may be re-extracted, to resolve the discrepancy.* Please note that FDA considers bidirectional sequencing to be a gold standard reference method, thus re-running a sample that is not compared to bidirectional sequencing is not adequate to determine whether the actual correct result is obtained. All samples that provide discrepant results, not just rare or unusual variants, should be compared to bidirectional sequencing.

If instead of submitting an IDE application, you would like to continue to pursue the orthogonal confirmation route for possible IDE exempt status, we can discuss this further with you in our upcoming teleconference, as additional validation requirements may be needed in this case. If you have any questions regarding this review, please contact Sunita Shukla, at 301-796-6406 or at Sunita.Shukla@fda.hhs.gov.

We look forward to speaking with you in our upcoming meeting on April 10, 2015.

Sunita J. Shukla -S
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Sunita Shukla, Ph.D.

Scientific Reviewer

Denise Johnson-lyles -S

Denise Johnson-Lyles, Ph.D.

Toxicology Branch Chief