DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

Summary Basis for Regulatory Action

Date: March 26, 2012

From: Pradip Akolkar, Ph.D., Scientific Lead, BLA Review Committee

BLA/ STN#: 125394/0

Applicant Name: Avioq, Inc.

Date of Submission: November 12, 2010

Complete Response letter: September 22, 2011

Response to CR letter received: September 26, 2011

Additional information received: October 21, 2011

MDUFMA Goal Date: March 27, 2012

Trade Name: Avioq® HTLV-I/II Microelisa System

Proper Name: Human T-Lymphotropic Virus Types I & II (HTLV-I and HTLV-II/ Enzyme Immuno Assay (EIA)/Lysate)

Indication: To detect antibodies to HTLV-I (anti-HTLV-I) and/or antibodies to HTLV-II (anti-HTLV-II) in human serum and plasma specimens

Recommended Action: Approval

Office Signatory Authority: Jay S. Epstein, M.D.
Director, OBRR /CBER

Signatory Authorities Action:

X **I concur with the summary review.**

□ **I concur with the summary review and include a separate review to add further analysis.**

□ **I do not concur with the summary review and include a separate review.**

Material Reviewed/ Consulted - List of Specific documentation used in developing the SBRA

Clinical Review	Pradip Akolkar
Preclinical Review	Krishnakumar Devadas, Bharat Khurana
Statistical Review	Showjen Lee
CMC Review/ Facilities	Krishnakumar Devadas and Martha O'Lone
Establishment Inspection Report	Pradip Akolkar, Krishnakumar Devadas, Martha O'Lone, Jennifer Schmidt, Deborah Trout, and Esra Toussaint
Lot Release Review	Leslyn Aaron
Software and Instrumentation	Diane Gubernot
Labeling and Promotion	Dana Martin
OCTGT Review	Elizabeth Lybarger

1. Introduction

In December 2009, Avioq, Inc. acquired from bioMerieux, Inc. certain intellectual property rights and the associated Biologics License Application and supplements submitted to FDA for commercializing the Vironostika® HTLV-I/II Microelisa System. The Vironostika® HTLV-I/II Microelisa System was licensed by FDA in 1998 for the *in vitro* qualitative detection of antibodies to Human T-Lymphotropic Virus Type I (HTLV-I) and/or Human T-Lymphotropic Virus Type II (HTLV-II) in human serum or plasma. It is intended for blood donor screening to prevent transmission of HTLV-I and HTLV-II to recipients of cellular blood components, and as an aid in clinical diagnosis of HTLV-I or HTLV-II infection and related diseases. The Vironostika® HTLV-I/II Microelisa System was on the market from 1998 to 2008, when bioMerieux discontinued the product and requested that its license be revoked. Therefore, Avioq is manufacturing the previously licensed assay. As a result, FDA agreed that Avioq could perform limited studies to demonstrate the comparability of the test manufactured in their facility to the one manufactured by bioMerieux.

The validation of the Avioq® HTLV-I/II Microelisa System was performed at Avioq's own manufacturing facility in Research Triangle Park, NC. Avioq hired several key employees from bioMerieux who were integrally involved in R&D, manufacturing, QA and QC of the originally licensed product. Avioq also acquired the same manufacturing equipment from bioMerieux, which was installed in the Avioq facility and will be used to manufacture the HTLV-I/II test.

Intended Use: The Avioq® HTLV-I/II Microelisa System is a qualitative enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to Human T-Lymphotropic Virus Type I (HTLV-I) and/or Human T-Lymphotropic Virus Type II (HTLV-II) in human serum or plasma. It is intended for screening individual human donors, including volunteer donors of whole blood and blood components, and other living donors for the presence of anti-HTLV-I/HTLV-II, and for use as an aid in clinical diagnosis of HTLV-I or HTLV-II infection and related diseases. It is also intended for use in testing serum and plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating. It is not intended for use on cord blood specimens. In addition to being used as a Manual assay, the assay is also intended for use with the ORTHO® Summit System (OSS) for screening blood donors.

2. Background

HTLV-I, a human type C retrovirus, has been etiologically associated with Adult T-Cell Leukemia (ATL), and additionally with a demyelinating neurologic disorder termed Tropical Spastic Paraparesis, and/or HTLV-I Associated Myelopathy (TSP/HAM). Antibodies to HTLV-I are found with high frequency in persons affected with these disorders. More recently, HTLV-I infection has been shown to be associated with B- and T-cell chronic lymphocytic leukemia (CLL), multiple myeloma, some cases of non-Hodgkin's lymphoma (NHL), polymyositis, arthritis, Kaposi's sarcoma, uveitis, strongyloidiasis and mycosis fungoides. HTLV-I is endemic in some Caribbean countries, Southern Japan, and possibly in some areas of Africa. In the United States, HTLV-I has been identified in ATL patients, intravenous drug abusers, and in healthy individuals.

HTLV-II, a related virus, is endemic in Amerindian tribes, and a high rate of HTLV-II seropositives has been observed among intravenous drug abusers. Although the pathogenecity of HTLV-II is less

well established than HTLV-I, the first reported patients with HTLV-II infections presented with an atypical T-cell variant of hairy cell leukemia suggesting a viral etiology. In more recent observations HTLV-II infection has been associated with large granular lymphocyte leukemia (LGL), leukopenic chronic T-cell leukemia, T-prolymphocytic leukemia, mycosis fungoides and chronic neurodegenerative diseases like myelopathy, and spastic ataxia. Antibodies to HTLV-II are significantly cross-reactive to HTLV-I antigens. Transmission of HTLV-I and HTLV-II infections to transfusion recipients of infected cellular blood products is well documented. Other known modes of transmission include breast milk, sexual contact, and sharing of contaminated needles and syringes by intravenous drug abusers. Perinatal transmission is suspected but remains unproven.

PRINCIPLE OF THE TEST

The Avioq® HTLV-I/II Microelisa System is an enzyme-linked immunosorbent assay in which the solid phase (Microwells) is coated with a purified HTLV-I viral lysate, a purified HTLV-II viral lysate, and a recombinant HTLV-I p21E antigen.

With the addition of a diluted test sample containing antibodies to either HTLV-I or HTLV-II, complexes are formed by the interaction of the antibodies in the sample and the solid phase antigens. Following incubation, the sample is aspirated and the well is washed with buffer. Subsequently, anti-human immunoglobulin (goat) conjugated with horseradish peroxidase (HRP) is added which binds the antibody-antigen complex during a second incubation. Following a wash and incubation with tetramethylbenzidine (TMB) substrate, a blue color is produced. The enzyme reaction is stopped by the addition of a sulfuric acid solution which changes the color to yellow. The amount of antibody present in the sample is proportional to color development.

3. Chemistry, Manufacturing and Controls (CMC)

Components of Avioq® HTLV-I/II Microelisa System

The Avioq® HTLV-I/II Microelisa System is comprised of the following components:

- **HTLV-I/II Microelisa Strips** – Twelve per holder, each containing 8 wells coated with inactivated HTLV-I viral lysate, a recombinant HTLV-I antigen (rp21E), and inactivated HTLV-II viral lysate; contained in a foil pouch with silica gel desiccant.
- **EnzAbody for HTLV-I/II** (EnzAbody Concentrate) – Horseradish Peroxidase Conjugated Goat Anti-human Immunoglobulin, ~0.06% w/w or 30µg; lyophilized with goat serum, sucrose, and non-fat dry milk.
- **EnzAbody Diluent** – Phosphate buffered saline containing 10% goat serum and non-ionic surfactants. Preservatives: 0.2% gentamicin sulfate and 0.02% cinnamaldehyde.
- **Sample Diluent** – Phosphate buffered saline containing 10% goat serum, non-ionic surfactants, sodium chloride, 0.14% bovine serum albumin, non-fat dry milk and amaranth dye. Preservative: 0.03% (w/v) bromonitrodioxane.
- **TMB Solution** – Citric acid containing 0.03% tetramethylbenzidine•2HCl.
- **Peroxide Solution** – Citric acid/sodium citrate buffer containing 0.04% urea peroxide.
- **Negative Control Serum** – Human serum with protein stabilizers; nonreactive by FDA licensed tests for antibodies to HTLV-I, HTLV-II, HIV-1, HIV-2, HCV, and nonreactive for HBsAg and HIV-1 -(b)(4)- Ag. Preservative: 0.05% (w/v) bromonitrodioxane.

- **HTLV-I Positive Control Serum** – Inactivated human serum with protein stabilizers and Amaranth red dye; reactive for antibodies to HTLV-I; nonreactive by FDA licensed tests for antibodies to HIV-1, HIV-2, HCV, and nonreactive for HBsAg and HIV-1 -(b)(4)- Ag. May cross-react with HTLV-II antigen. Preservative: 0.05% (w/v) bromonitrodioxane.
- **HTLV-II Positive Control Serum**– Inactivated human serum with protein stabilizers and Patent blue dye; reactive for antibodies to HTLV-II; nonreactive by FDA licensed tests for antibodies to HIV-1, HIV-2, HCV, and nonreactive for HBsAg and HIV-1 -(b)(4)- Ag. May cross-react with HTLV-I antigen. Preservative: 0.05% (w/v) bromonitrodioxane.
- **Wash Buffer Concentrate (**Product number 259879), provided as an accessory to the kit.

Manufacturing Quality Control

Avioq® HTLV-I/II Microelisa System kit components such as viral lysate, HTLV-I positive serum, HTLV-II positive serum, and negative plasma are obtained from outside vendors. Avioq has established specifications and acceptance criteria for all incoming material.

Several components for the Avioq HTLV-I/II Microelisa System are manufactured by Avioq Inc. in their facility at Research Triangle Park, NC. Acceptance criteria and specifications have been established for all kit components. Components are assembled into kits, each lot of which is subjected to a final performance test with an in-house panel of samples containing varying titers of HTLV-I and HTLV-II antibodies and CBER Lot Release Panels for HTLV-I and HTLV-II. Meeting the established performance parameters is required for release of each test kit lot by Avioq.

CBER Lot Release

Each new lot of a donor screening test typically is subject to lot release by testing at CBER using panels developed for that purpose.

Three lots of Avioq® HTLV-I/II Microelisa System were received and tested in the Division of Biological Standards and Quality Control (DBSQC). CBER Reference Panels were used to evaluate kit function (CBER HTLV-I Reference Panel 18 and CBER HTLV-II Reference Panel 15). All three lots demonstrated the expected reactivity on the CBER Reference Panels. The following is a list of the lots submitted in support of approval:

Lot Number	Expiration Date	Lot Release Determination
11018	10/12/2012	Pass
11019	10/12/2012	Pass
11020	10/15/2012	Pass

Establishment Description

Avioq, Inc. will manufacture the Avioq® HTLV-I/II Microelisa System at the following location:
104 T. W. Alexander Drive, ---(b)(4)----
Research Triangle Park, NC 27709.

All manufacturing activities take place in ----------(b)(4)---------- is a newly registered facility. Manufacturing activities at this facility will be approved under the BLA for the Avioq® HTLV-I/II Microelisa System. Avioq has stated during teleconferences that they are not developing or manufacturing any other products at this time. Avioq will use their QC test equipment in ---(b)(4)-- for this test kit.

Avioq conducted Installation Qualification/Operational Qualification of the facility electrical service, vacuum, process chilled water and compressed air.

Facilities Review

The review of facility and equipment related issues identified concerns about the lack of information and validation of manufacturing processes, equipment, and cleaning procedures. These issues were noted in the CBER Complete Response letter. The sponsor has responded adequately to each of CBER's comments.

Facility Inspection

Avioq, Inc., FEI # 3008376326
104 T.W. Alexander Drive, ---(b)(4)---
Research Triangle Park, N.C. 27709

Two pre-license inspections of Avioq's new facility were performed prior to licensure.

The first FDA inspection of Avioq, Inc.'s new Research Triangle Park, NC facility was conducted on May 2-6, 2011. The initial inspection included a comprehensive review of the documentation of the quality systems, facility, equipment (qualification, cleaning and process validation), batch records, packaging and labeling, materials control, production steps and process controls in the manufacture of Avioq's HTLV-I/II test kit. During the inspection the team observed all processing steps: formulation, filling, lyophilization, plating, labeling, bulk formulation, and filling. The firm was issued a FDA form-483 with 32 observations and the inspection team recommended a follow-up pre-license inspection prior to approval of the BLA.

The second pre-license inspection was conducted on January 10-12, 2012. This inspection included observation of manufacturing activities such as thawing, mixing, filling, lyophilization, and kitting. During this follow-up inspection, corrective actions for the 483 observations from the first pre-license inspection were verified. The inspection resulted in an issuance of a FDA form-483 with three observations. Avioq has now provided adequate information to close out the three observations.

Environmental Assessment

Avioq submitted to the BLA a request for a categorical exclusion from an Environmental Assessment under 21 CFR §25.31(c). The sponsor believes this application meets the categorical exclusion criteria and to their knowledge no extraordinary circumstances exist. The Agency agrees with this conclusion and an environmental assessment is not warranted.

Instrument and Software

Instrument: Avioq and Ortho-Clinical Diagnostics (OCD) will launch the Avioq® HTLV-I/II Microelisa System on the Ortho Summit System (OSS) instrument, just as the Vironostika® HTLV-I/II Microelisa System was used on this automated system. There is no formulation change in the Avioq® HTLV-I/II Microelisa System compared to the originally licensed Vironostika® HTLV-I/II Microelisa System. The assay procedures for the Avioq assay are identical to the Vironostika assay.

Software: Likewise, there is no significant change in the OSS software except for minor changes to accommodate changes in the microwell plate dimensions (changes from 12 wells/strip to 8 wells/strip) and in the assay trade name.

Although the changes in the OSS software (HTLV ORTHO® Assay Protocol Disk [OAPD]) are considered to be minor, Avioq performed a study to validate the Avioq® HTLV-I/II Microelisa System OAPD on OSS. This study was to ensure that the Avioq® HTLV-I/II Microelisa System will maintain the same level of performance when used on the OSS instrument. The results from this external field study were submitted as an amendment to the BLA and support the launch of the Avioq® HTLV-I/II Microelisa System on the OSS upon licensure. OCD formally notified Avioq of the changes made to the OAPD and will retain the information on software changes in the Design History File associated with the OSS.

Review Issues

The following issues were identified during the course of the review:

Review Issue 1: Avioq had not provided a complete list of all materials used in the assay, with the specifications and acceptance criteria. This was provided in response to an information request. The issue was resolved.

Review Issue 2: Avioq had not provided complete validation of the lyophilizer used in the manufacturing of the EnzAbody Reagent. FDA agreed that the determination of the eutectic point of the lyophilized EnzAbody Reagent could be completed after the approval of the BLA. Avioq will perform a 100% inspection of their lyophilized EnzAbody concentrate lots prior to determining its eutectic point. The issue was resolved.

Review Issue 3: Avioq had not completed antimicrobial effectiveness studies. After discussion with the DMPQ reviewer, Avioq initiated the remaining studies. The results of these studies were submitted as an amendment to the BLA. The issue was resolved.

4. Comparison of the Performance of the Avioq HTLV-I/II Microelisa System (Manual Method) to the Vironostika HTLV-I/II Microelisa System (Manual Method)

The original assay, the Vironostika® HTLV-I/II Microelisa System, was licensed in 1998, and Avioq obtained from BioMerieux the rights to manufacture the assay in their own facility using the same processes and raw materials (including the biologics) under the name of the Avioq® HTLV-I/II Microelisa System. Avioq will be using the performance characteristics reported for the Vironostika® HTLV-I/II Microelisa System in the package insert of the Avioq test. Therefore, studies were conducted to compare the performance of the Avioq test to the performance reported in the package insert of the Vironostika® HTLV-I/II Microelisa System (no Vironostika test kits were available to conduct a head-to-head study).

A. Reproducibility

In order to demonstrate the reproducibility of the Avioq® HTLV-I/II Microelisa System, a panel consisting of HTLV-I and HTLV-II antibody-positive specimens with varying degrees of reactivity (four HTLV-I and four HTLV-II) and two negative specimens were tested with each of the three validation kit lots over a four-day period by two analysts. Each sample was tested in quadruplicate on each of the four days. The total %CV for the positive specimens using the three validation lots ranged from 8.9 – 19.7% (n=96) compared to the total %CV range for the positive specimens of 11.1 – 14.7% for Vironostika® HTLV-I/II assay (n=288, 3 sites, 3 lots, 2 operators, 4 days and testing performed in quadruplicate). The studies performed by Avioq demonstrated that the reproducibility of the Avioq® HTLV-I/II Microelisa System is comparable to that of the Vironostika® HTLV-I/II Microelisa System.

Table 1: Summary of Reproducibility Study for the Avioq® HTLV-I/II Microelisa System (Manual Method)

				Total				Inter-Assay		Intra-Assay	
Panel ID	**Status**	**N**	**Mean S/C**	**SD**	**%CV**	**S/C Lower 95% CI**	**S/C Upper 95% CI**	**SD**	**%CV**	**SD**	**%CV**
HTLV-I S1	Pos	96	3.10	0.276	8.9	3.04	3.15	0.244	7.9	0.136	4.4
HTLV-I S2	Pos	96	2.92	0.300	10.3	2.85	2.98	0.246	8.4	0.176	6.0
HTLV-I S3	Pos	96	2.50	0.312	12.5	2.44	2.56	0.259	10.4	0.180	7.2
HTLV-I S4	Pos	96	2.18	0.226	10.4	2.13	2.22	0.190	8.7	0.126	5.8
HTLV-I S5[a]	Neg	96	0.25	0.023	9.2	0.24	0.25	0.020	8.0	0.012	4.8
HTLV-II S1	Pos	96	3.29	0.561	17.1	3.17	3.40	0.482	14.7	0.298	9.1
HTLV-II S2	Pos	96	3.15	0.569	18.1	3.03	3.26	0.537	17.1	0.210	6.7
HTLV-II S3	Pos	96	2.46	0.486	19.7	2.36	2.56	0.462	18.8	0.170	6.9
HTLV-II S4	Pos	96	2.22	0.364	16.4	2.14	2.29	0.300	13.5	0.214	9.6
HTLV-II S5[a]	Neg	96	0.23	0.020	8.7	0.22	0.23	0.016	7.2	0.012	5.4

[a] Specimen negative for antibodies to HTLV-I and HTLV-II

B. Specificity

The estimated clinical specificity of the Vironostika® HTLV-I/II Microelisa System was determined in studies conducted to support licensure of the original product. The results of those studies are shown in Table 2 (data taken from the original package insert).

Table 2: Estimated Clinical Specificity in Whole Blood and Plasma Random Donor and Source Plasma Populations for the Vironostika® HTLV-I/II Microelisa System (Manual Method)

Number Tested		Non-reactive	Repeatedly Reactive	Repeatedly Reactive (%)	Repeatedly Reactive 95% Confidence Limits[a] (%)		Supplemental Test Positive[b]	Estimated Specificity (%)[c]	Specificity 95% Confidence Limits[a] (%)	
1315	Serum Site 1	1314	1	0.08	0.004	0.334	0	99.92	99.58	99.99
3754	Serum Site 2	3753	1	0.03	0.002	0.117	0	99.97	99.85	99.99
1255	Plasma Site 1	1255	0	0.00	0.000	0.153	0	100.00	99.71	100.00
3812	Plasma Site 2	3809	3	0.08	0.020	0.204	0	99.92	99.77	99.98
1279	Source Plasma Site	1278	1	0.08	0.004	0.344	0	99.92	99.57	99.99
11415	**Overall**	**11409**	**6**	**0.05**			**0**	**99.95**	**99.89**	**99.98**

a = Confidence limits for specificity were calculated using the exact method.

b = A positive result in these studies was defined by the presence of antibodies to two gene products (gag, p19 and/or p24 and env, gp46 and/or 61/68) using a research use Western blot and/or RIPA.

Additional supplemental tests and HTLV-I and HTLV-II type differentiation were performed using the following research use assays: reactivity to the recombinant or native gp46-I or gp46-II peptides on a Western blot, HTLV-I and HTLV-II peptide EIAs, HTLV-I and HTLV-II IFA, and/or PCR (using specific primers to the tax and pol regions).

c = $\frac{\text{(Number Screened – Number Repeatedly Reactive) x 100}}{\text{(Number Screened – Number Confirmed Positive)}}$

In order to evaluate whether the estimated specificity of the Avioq test is comparable to that of the originally licensed Vironostika test, serum (n = 1000) and plasma (n = 1000) samples of unknown status from low risk populations (blood donors) were tested with all 3 validation lots. Each lot was used to test similar numbers of specimens. Of the 2000 specimens tested, two were repeatedly reactive (see Table 3). Both specimens were negative by research use HTLV-I and HTLV-II IFA and Western blot. Therefore, the specificity of the Avioq assay observed in this study was 1998/2000 = 99.90% (95% CI 99.44 – 100%), compared to 11,409/11,415 = 99.95 % (95% CI of 99.89 – 99.98%) for the Vironostika assay. The in-house studies performed by Avioq demonstrated that the specificity of the Avioq® HTLV-I/II Microelisa System is comparable to that of the Vironostika® HTLV-I/II Microelisa System using the manual method.

Table 3: Estimated Specificity of the Avioq® HTLV-I/II Microelisa System in Random Blood Donors (Manual Method)

	Number Tested	Non-reactive	Repeatedly Reactive	Supplemental Test Positive	Estimated Specificity (%)	Specificity 95% Confidence Limits	
Serum	1000	999	1	0	99.90	99.44	100.00
Plasma	1000	999	1	0	99.90	99.44	100.00
Overall	2000	1998	2	0	99.90	99.44	100.00

C. Sensitivity

The estimated sensitivity of the Vironostika® HTLV-I/II Microelisa System was determined in studies conducted to support licensure of the original product. The results of those studies are shown in Table 4 (data taken from the original package insert).

Table 4: Reactivity with Supplemental Test HTLV-I, HTLV-II, and Vironostika® HTLV-I/II Antibody Positive Specimens (Manual Method)

Group	Supplemental Test Result[a]	No. Tested	No. Repeatedly Reactive with Licensed HTLV-I Tests	No. Repeatedly Reactive with Vironostika® HTLV-I/II
Adult T-Cell Leukemia	HTLV-I	47	47	47
Tropical Spastic Paraparesis	HTLV-I	43	43	43
Nasopharyngeal Lymphoma	HTLV-I	1	1	1
Intravenous Drug Abusers	HTLV-I HTLV-II	5 95	5 94c	5 95
Hospital Patients[b]	HTLV-I HTLV-II	107 38	107 38	107 38
Blood Donors	HTLV-I HTLV-II HTLV-I/II	146 138 16	146 138 16	146 138 16
TOTAL		**636**	**635**	**636**

[a] A positive result in these studies was defined by the presence of antibodies to two gene products (gag, p19 and/or p24 and env, gp46 and/or 61/68) using Western Blot and/or RIPA.

Additional supplemental tests and HTLV-I and HTLV-II type differentiation were performed using the following research use assays: reactivity to the recombinant or native gp46-I or gp46-II peptides on a Western Blot, HTLV-I and HTLV-II peptide EIAs, HTLV-I and HTLV-II IFA, and/or PCR (using specific primers to the tax and pol regions).

[b] Asymptomatic and some symptoms indicative of HTLV disease.

[c] The licensed HTLV-I test missed one IVDA (signal/cutoff values 0.8, 0.9, 0.9) that was indeterminate by Western Blot (p21 only) and typed as HTLV-II by PCR.

In order to evaluate whether the sensitivity of the Avioq test is comparable to that of the originally licensed Vironostika test, a panel of 200 seropositive serum or plasma repository samples (100 HTLV-I and 100 HTLV-II) was tested on all three validation lots (Table 5). All specimens were previously found to be repeatedly reactive on an FDA licensed HTLV-I/II donor screening test (either ----------(b)(4)----------) and confirmed positive for HTLV-I/II antibodies with a research use supplemental test (WB, IFA, RIPA). Eighty-seven percent of these specimens were from US blood donors and none were previously tested using the original licensed Vironostika assay. The estimated sensitivity of the assay observed in this study was 200/200 = 100% (95% CI 98.17 – 100%) compared to 636/636 = 100% (95% CI of 99.97 – 100%) for the Vironostika® HTLV-I/II assay. This in-house study performed by Avioq demonstrated that the estimated sensitivity of the Avioq® HTLV-I/II Microelisa System is comparable to that of the Vironostika® HTLV-I/II Microelisa System using the manual method. The wider 95% confidence interval for the Avioq® HTLV-I/II Microelisa System can be attributed to the smaller sample size in the study.

Table 5: Reactivity of the Avioq® HTLV-I/II Microelisa System with HTLV-I/II Seropositive Repository Samples

Number Tested	Number Repeatedly Reactive	Number Non-reactive	Estimated Sensitivity (%)	Sensitivity 95% Confidence Limits (%)	
200	200	0	100.00	98.17	100.00

5. Comparison of the Performance of the Avioq® HTLV-I/II Microelisa System (Manual Method) to the Automated Avioq® HTLV-I/II Microelisa System (on OSS)

The performance data presented in the BLA for the Avioq assay were obtained using the manual testing method. However, in order for the assay to be used efficiently in a blood donor screening setting, an automated system is necessary. The Vironostika® HTLV-I/II assay was used in blood establishments on the OSS instrument using assay-specific software marketed by OCD. To address this, Avioq submitted an IND (-(b)(4)-) protocol in collaboration with OCD to evaluate the Avioq test on the OSS instrument. These studies performed by OCD involved testing a panel of HTLV-I and HTLV-II positive specimens, as well as normal blood donor specimens, using both the manual testing method and on the OSS instrument. The results of the studies conducted, using the approved protocol, were submitted as an amendment to the BLA (STN 125394/0.7). These studies were intended to demonstrate that the performance characteristics of the Avioq® HTLV-I/II Microelisa System on the OSS instrument are comparable to that of the manual assay.

A. Reproducibility

The reproducibility of the Avioq® HTLV-I/II Microelisa System on the OSS instrument was performed using a panel of 10 specimens tested in duplicate (four HTLV-I positives, four HTLV-II positives, and two negatives). The study was conducted at two sites on a total of three instruments twice per day for four days using one validation lot of the assay kit and compared to testing this panel using the manual method using the same validation lot. The results of this study are summarized in Table 6.

Table 6: Reproducibility Study for the Avioq® HTLV-I/II Microelisa System on OSS (Automated Method)

				Total				Inter-Center		Intra-Center	
Panel ID	**Status**	**N**	**Mean S/C**	**SD**	**%CV**	**S/C Lower 95% CI**	**S/C Upper 95% CI**	**SD**	**%CV**	**SD**	**%CV**
HTLV-I S1	Pos	96	4.54	0.375	8.3	4.47	4.62	0.305	6.7	0.226	5.0
HTLV-I S2	Pos	96	4.03	0.366	9.1	3.95	4.10	0.288	7.1	0.232	5.8
HTLV-I S3	Pos	96	3.63	0.325	8.9	3.57	3.70	0.253	7.0	0.208	5.7
HTLV-I S4	Pos	96	2.84	0.283	10.0	2.78	2.89	0.218	7.7	0.184	6.5
HTLV-I S5[a]	Neg	96	0.26	0.052	20.3	0.25	0.27	0.045	17.5	0.028	10.8
HTLV-II S1	Pos	96	5.86	0.421	7.2	5.77	5.94	0.323	5.5	0.276	4.7
HTLV-II S2	Pos	96	5.75	0.347	6.0	5.68	5.82	0.270	4.7	0.224	3.9
HTLV-II S3	Pos	96	4.95	0.463	9.4	4.85	5.04	0.385	7.8	0.268	5.4
HTLV-II S4	Pos	96	4.22	0.332	7.9	4.16	4.29	0.277	6.6	0.189	4.5
HTLV-II S5[a]	Neg	96	0.13	0.028	20.7	0.13	0.14	0.023	17.0	0.016	12.1

[a] Specimen negative for antibodies to HTLV-I and HTLV-II

The reproducibility study demonstrated that the total variability for the positive specimens ranged from 6% to 10% using the OSS method compared to 8.9 to 19.7% for the positive specimens using the manual method (see Table 1).

B. Analytical Sensitivity

To demonstrate that the analytical sensitivity of the Avioq® HTLV-I/II Microelisa System on the OSS instrument is comparable to the manual method, eight dilution panels (two-fold serial dilutions of four HTLV-I and four HTLV-II antibody-positive samples down to the assay cutoff value by the manual method) were tested using both the manual and automated methods. To evaluate the correlation of the two methods, Deming's regression analysis was performed. This analysis showed that there was a high correlation for the S/CO ratio for both formats, with a correlation coefficient of 0.94. To demonstrate the equivalence of the S/CO ratio between the two methods, a paired t-test was performed. The overall mean S/CO for the manual method was 3.143 (n=459) compared to an overall mean of 4.198 (n=459) on the OSS instrument. Although this difference in S/CO ratio is statistically significant, these studies demonstrated that the assay on the OSS instrument did not have diminished analytical sensitivity.

C. Clinical Sensitivity

A panel of 100 blood donor specimens that were repeatedly reactive on an FDA licensed assay (----------(b)(4)----------) was tested using the Avioq® HTLV-I/II Microelisa System using both methods (manual method and automated method on the OSS instrument). Ninety-five (95) of the specimens were repeatedly reactive (100% concordance) using both assay methods (manual and automated). The S/CO ratio of the 95 specimens detected by the Avioq assay ranged from 1.058 – 8.400 for the manual method and 1.280 – 7.403 for the automated method on OSS; the correlation coefficient for the S/CO ratio was 0.94. Twenty-nine (29) of these specimens were positive for antibodies to HTLV-I and 46 were positive for antibodies to HTLV-II on a research use supplemental test, and 20 were positive for antibodies to HTLV-I/II (not typed).

Five (5) of the 100 specimens in the panel (1 HTLV-I, 2 HTLV-II and 2 HTLV-I/II) were non-reactive using the Avioq assay. Additional confirmatory testing on these specimens showed that none were positive on a supplemental test. It should be noted that these specimens were previously subjected to five freeze/thaw cycles prior to testing; data obtained by Avioq indicates that samples may be frozen and thawed once with no loss of reactivity.

The study conducted using the 95 confirmed positive specimens gave assurance that the sensitivity of the Avioq assay was not altered when the assay was run on the automated testing system. However, FDA asked Avioq for additional data from testing using specimens that were not subjected to more than one freeze/thaw cycle, in keeping with the specimen restrictions identified in the package insert. Avioq submitted a postmarketing commitment ----------(b)(4)----------
---

D. Clinical Specificity

Blood donor specimens (n = 16,339) were tested at two sites (----------(b)(4)---------- [n =12,249] and at OCD [n = 4090]). The results of this testing are shown in Table 7.

Table 7: Summary of Clinical Specificity Results for the Avioq HTLV-I/II Microelisa System on OSS (Automated Method)

	At OCD Site			At -(b)(4)- Site			All Sites/Lots Combined
	Lot 10003	Lot 10004	Lot 10005	Lot 10003	Lot 10004	Lot 10005	

Number Tested	1366	1366	1358	4160	4080	4009	16,339
Non-Reactive	1364	1366	1358	4157	4078	4007	16,330
Initially Reactive	2	0	1	3	3	2	11
Repeatedly Reactive	2	0	0	3	2	2	9
Confirmed Positives	0	N/A	N/A	0	0	0	0
Total False Positives	2			7			9
Total Tested	4,090			12,249			16,339
Estimated Specificity	**99.95%**			**99.94%**			**99.94%**
95 % CI	**99.82%-99.99%**			**99.88%-99.98%**			**99.90%-99.97%**
Overall Estimated Specificity	**99.95%**						
95% CI	**99.89% to 99.98%**						

Of the 16,339 blood donor specimens tested, nine were repeatedly reactive. All nine were classified as false positive based on the results of testing using a research use Western blot (OCD specimens) or IFA (-(b)(4)- specimens). The confidence intervals for the estimated specificity for the Avioq assay for all three lots at each site and for all sites and lots combined overlap with that for the manual Vironostika assay (95% CI 99.89 – 99.98%). The overall estimated specificity of the Avioq® HTLV-I/II assay on OSS was 16,330/16,339 = 99.94% (95% CI: 99.90 - 99.97%) compared to 11,409/11,415 = 99.95% (95% CI: 99.89 - 99.98%) using the manual method.

These studies demonstrated that the performance of the Avioq® HTLV-I/II Microelisa System on the OSS instrument is comparable to that using the manual testing method.

6. Labeling

The package insert for the Avioq® HTLV-I/II Microelisa System will be the same as that used for the Vironostika HTLV-I/II Microelisa System, except that the name of the assay will be changed and the Intended Use statement will include a statement that the assay is also intended for use with the ORTHO Summit System (OSS) for screening blood donors and additional data to support this statement. The assay kits manufactured at Avioq were evaluated to demonstrate that the performance of the Avioq HTLV-I/II Microelisa system is comparable to the performance of the Vironostika HTLV-I/II Microelisa System as reported in the bioMerieux package insert. Therefore, the performance characteristics of the assay will remain the same as those reported in the bioMerieux package insert.

The intended use of the original Vironostika HTLV-I/II test does not include use in testing living donors (other than blood donors). This statement was revised in the package insert for the Avioq® HTLV-I/II Microelisa System to include other living donors and for use in testing serum and plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating. The revised statement was reviewed and approved by the Office of Cellular, Tissue and Gene Therapies (OCTGT/CBER) reviewer.

7. Recommendations

a. Recommended Regulatory Action

Based on the review of the information submitted in the BLA, all of the review issues have been resolved. The review committee recommends that this BLA be approved.

b. Recommendation for Postmarketing Activities

----------(b)(4)----------

