K983762

A 510(k) summary of the NucliSens® CMV pp67 assay

510(k) Summary

NucliSens® CMV pp67

(a)(1) **The submitter's name, address, telephone number, a contact person, and the date the summary was prepared;**

Submitter's Name: Organon Teknika Corporation

Submitter's Address: 100 Akzo Avenue, Durham, North Carolina, 27712, USA

Submitter's Telephone: (919) 620-2373

Submitter's Contact: Ron Sanyal Ron Sanyal

Date 510(k) Summary Prepared: September 10, 1999

(a)(2) **The name of the device, including the trade or proprietary name if applicable, the common or usual name, and the classification name, if known;**

Trade or Proprietary Name: NucliSens® CMV pp67

Common or Usual Name: Nucleic acid amplification-based assay for CMV RNA

Classification Name: Cytomegalovirus serological reagents

(a)(3) **An identification of the legally marketed device to which the submitter claims substantial equivalence;**

Device Equivalent to: CMV Brite™ CMV Antigenemia Detection Test Kit

(a)(4) **A description of the device.**

Device Description:

The NucliSens® CMV pp67 assay is a nucleic acid amplification-based qualitative assay to be used in conjunction with the NucliSens®™ Reader for the detection of human cytomegalovirus (HCMV) pp67 mRNA in EDTA anticoagulated whole blood from adult transplant donors and HIV infected individuals. It is intended as an aid in the diagnosis of active (acute or reactivated) HCMV infection. This product is not intended for use in screening of blood or plasma donors.

The NucliSens® CMV pp67 assay is comprised of four separate stages:

a. Nucleic acid release
b. Nucleic acid isolation
c. Nucleic acid amplification
d. Nucleic acid detection

a. Nucleic acid release

The sample is added to NucliSens® Lysis Buffer containing guanidine thiocyanate and Triton X-100. Viral particles and cells present in the sample are disintegrated; RNases and DNases present in the sample are inactivated. Nucleic acids are released.

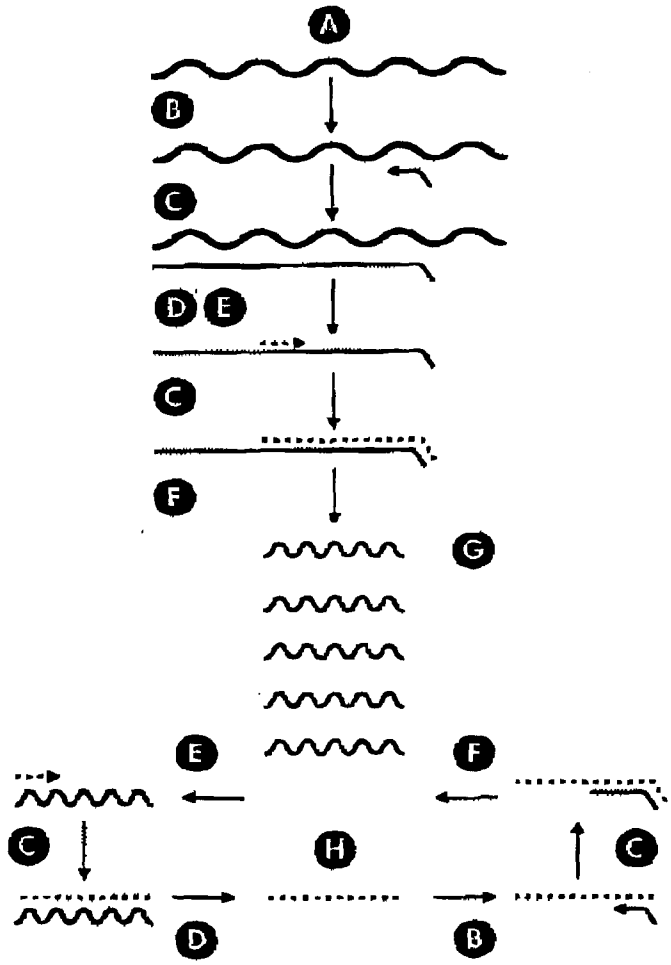
b. Nucleic acid isolation

Under high salt conditions, nucleic acids in NucliSens® Lysis Buffer are bound to silicon dioxide particles.[13-17] These Silica particles act as a solid phase. Unbound non-nucleic acid components are removed by several washing steps. Finally, bound nucleic acid is eluted from the solid phase. Prior to the addition of Silica particles, HCMV System Control (SC) RNA molecules are added to the specimen in Lysis Buffer in a known number. SC RNA is added for validation of the test procedure.

c. Nucleic acid amplification

HCMV mRNA expressed from the UL65 open reading frame on the viral genome and encoding a phosphorylated matrix tegument protein of 67 kDa (pp67 mRNA) is converted to a double-stranded DNA molecule by the concerted action of three enzymes and two oligonucleotides[18]. One of these oligonucleotides contains a 5'-terminal T7 RNA polymerase promoter sequence in addition to a stretch of nucleotides that is complementary to a sequence on the HCMV pp67 mRNA. The second oligonucleotide encompasses a short sequence which is identical to a segment of the mRNA and is located upstream of the region where the T7 promoter-containing oligonucleotide can anneal. Together, these oligonucleotides define a part of the HCMV pp67 mRNA which is converted to a DNA intermediate with a functional T7 promoter. This process starts with hybridization of the oligonucleotide that contains the T7 RNA polymerase binding site to the target RNA (Primer 1; Figure 3.3). Avian Myeloblastosis Virus Reverse Transcriptase (AMV-RT) elongates the oligonucleotide, creating a cDNA copy of the RNA template and forming a RNA/DNA hybrid. The RNA portion of this hybrid is hydrolyzed by RNase H, leaving single-stranded DNA to which the second oligonucleotide can anneal (Primer 2; Figure 3.3), thereby again forming a substrate suitable for reverse transcriptase extension. This extension finally reveals a double-stranded DNA intermediate with a transcriptionally active T7 promoter portion. Recognizing the now functional promoter, T7 RNA polymerase produces multiple copies of RNA transcripts which are antisense to the original target RNA sequence. Each newly synthesized antisense RNA molecule can act as a template by itself and again be converted to a DNA intermediate with a functional T7 promoter in a way similar to the original target RNA, except that the oligonucleotide primers anneal in reverse order because the newly generated RNA molecules are opposite in orientation to the original target and the resulting DNA intermediate is only partly double-stranded. Again, many RNA copies are generated from each RNA target that reenters the reaction resulting in exponential synthesis of RNA products.

By the same process and making use of the same oligonucleotides, multiple copies of RNA can be generated from the SC RNA that was added to each sample in the nucleic acid extraction procedure. This SC RNA contains the same segment of viral RNA as defined by the oligonucleotides in addition to a unique sequence by which it can be distinguished from the viral mRNA.



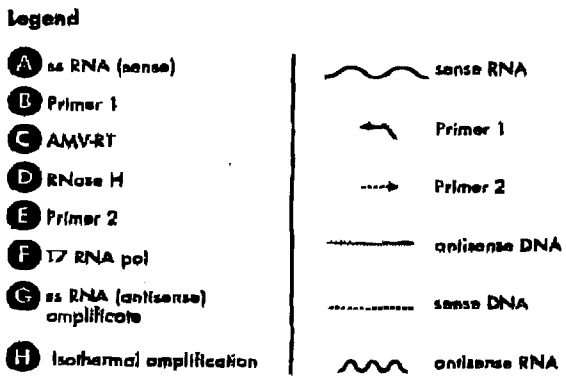


Figure 3.3. NASBA amplification principle

d. Nucleic acid detection

Following NASBA-based amplification, single-stranded HCMV pp67 mRNA-derived amplicons can be detected by a nucleic acid hybridization procedure employing the electrochemiluminescence (ECL) principle.[19] Amplicons are captured by hybridization to an oligonucleotide immobilized on paramagnetic beads (oligo-beads). To differentiate between

amplicons derived from the HCMV pp67 mRNA (wild type or WT RNA) or from the SC RNA, aliquots of the amplification reaction are added to two hybridization solutions which, in addition to the oligo-beads contain a Ruthenium chelate-labeled oligonucleotide probe that is specific for either the WT RNA or the SC RNA. Detection is achieved by adding tripropylamine (TPA) as a substrate to the hybridization reaction mixtures followed by automated analysis in a NucliSens® Reader. In the instrument's detection chamber, the paramagnetic beads carrying the amplicon/probe complexes are captured onto the surface of an electrode by means of a magnet. Voltage applied to this electrode triggers the ECL reaction in which light is produced by cyclical oxidation-reduction of the Ruthenium metal ion in the presence of TPA. Results are recorded as ECL counts by the NucliSens® Reader. The presence of HCMV pp67 mRNA in a sample is reflected by the WT RNA ECL signal. The WT RNA ECL signal and the ECL signal for SC RNA are used to determine the validity of the test.

NucliSens® CMV pp67 controls

System Control

The qualitative detection of HCMV pp67 RNA using the NucliSens® CMV pp67 assay is based on the co-amplification of sample RNA together with a second target RNA sequence referred to as System Control RNA (SC RNA). As the SC RNA is added to the sample during the lysis stage, the use of the system control itself functions as a significant quality control for the isolation, amplification, and detection stages of the assay to assure proper reporting of an individual specimen. Without proper isolation, amplification and detection of the system control, no or a low signal for the HCMV pp67 mRNA would be reported as an invalid test instead of a negative result.

As the SC is added to the patient specimen and competes with the WT for amplification resources, a high concentration of WT might overwhelm the SC resulting in a large WT ECL signal and a low SC ECL signal. This does not have a severe impact on the assay performance because strongly positive samples will still be called positive. If the specimen is negative, the overwhelming does not occur and the SC signal is high and the WT signal is low. In the presence of inhibitors or interfering substances, both signals would be low resulting in an invalid assay. The use of SC RNA in the NucliSens® CMV pp67 assay allows the user to determine conditions that potentially may result in a false negative result without affecting the assay's ability to detect positives.

Since the assay makes use of an internal System Control RNA (SC RNA) which is added to each individual sample, inhibition testing is being performed on every sample[15-17]. Factors inhibitory to the amplification of the CMV mRNA will also inhibit amplification of the SC-RNA which contains the same primer recognition sites as the viral target mRNA. For the interpretation of a test result, if the sum of the ECL counts for the WT probe and the ECL counts for the SC probe is greater than or equal to 10,000, no inhibition has taken place, and the ECL signal for the WT probe can be used to elucidate whether the original whole blood sample is positive or negative for the presence of CMV pp67 mRNA.

Test run controls

In addition to the System Control (provided in the kit), which functions as an internal control at the individual sample level, additional CMV pp67 mRNA positive and negative controls 1Xntrolß

control, the whole blood should additionally contain CMV-infected cells, e.g. from a spike of *in vitro* cultured cells infected with a strain of HCMV. Once added to NucliSens® Lysis Buffer, the external controls should be treated identically as the test specimens, including the addition of SC RNA prior to entering the nucleic acid isolation procedure.

By virtue of the external positive control lysis of cells containing CMV pp67 mRNA in whole blood specimens can be monitored, in addition to proper isolation, amplification, and detection

of the authentic CMV pp67 mRNA. The negative control serves to monitor contamination, both of the EDTA-anticoagulated human whole blood used for the external controls and of the reagents and materials used during the test procedure.

a)(5) **A statement of the intended use of the device.**

Device Intended Use: The NucliSens® CMV pp67 assay is a nucleic acid amplification-based qualitative assay to be used in conjunction with the NucliSens® Reader for the detection of human cytomegalovirus (HCMV) pp67 mRNA in EDTA anticoagulated whole blood from adult transplant donors and HIV infected individuals. It is intended as an aid in the diagnosis of active (acute or reactivated) HCMV infection. This product is not intended for use in screening of blood or plasma donors.

(a)(6) A summary of the technological characteristics of the new device in comparison to those of the predicate device.

The similarities and/or differences between Organon Teknika's NucliSens® CMV pp67, the Biotest Diagnostics Corporation's CMV BRITE™ Antigenemia and typical CMV cell culture are compared in Table a.6.

TABLE a.6

PARAMETERS	ORGANON TEKNIKA CORPORATION NucliSens™ CMV pp67	BIOTEST DIAGNOSTICS CORPORATION CMV Brite™ Antigenemia	Typical CMV Cell Culture
TYPE OF ASSAY	Qualitative Assay	Qualitative Assay	Qualitative Assay
PANEL	Microbiology (83)	Microbiology (83)	Microbiology (83)
CATEGORY	Nucleic Acid Amplification-Based *In Vitro* Assay	The CMV Brite test kit uses the application of a two monoclonal antibodies (C10/C11) cocktail in an indirect immunofluorescence staining of cytospin preparations of peripheral blood leukocytes.	Cell culture innoculation
INTENDED USE	This test is intended as an aid to detect an active CMV infection. The NucliSens™ CMV pp67 is a nucleic acid amplification-based *in vitro* qualitative assay for detection of the presence of human cytomegalovirus (HCMV) pp67 mRNA in anticoagulated whole blood. It is intended as an aid in the diagnosis of active (acute or reactivated) HCMV infection. This product is not intended for use in screening of blood or plasma donors.	This test is intended as an aid to detect an active CMV infection. The CMV Brite test kit is intended for the qualitative detection of cytomegalovirus (CMV) lower matrix protein pp65 by indirect immunofluorescence using microscopy in isolated peripheral blood leukocytes obtained from EDTA and heparin anticoagulated human peripheral blood. The detection of CMV pp65 in human peripheral blood cells aids in the diagnosis of acute or reactivated CMV infection. This product is not FDA cleared (approved) for use in testing (i.e. screening) of blood or plasma donors.	This test is intended as an aid to detect an active CMV infection. Cell culture is intended for the qualitative detection of the presence of HCMV in leukocytes from whole blood. It aids in the diagnosis of HCMV infection.
SAMPLE	Anticoagulated whole blood.	Isolated peripheral blood leukocytes obtained from EDTA and heparin anticoagulated human peripheral blood.	Leukocytes from whole blood

(b)1) A brief discussion of the nonclinical tests submitted, referenced, or relied on in the premarket notification submission for a determination of substantial equivalency.

Not applicable.

(b)(2) A brief discussion of the clinical tests submitted, referenced, or relied on in the premarket notification submission for a determination of substantial equivalency.

b.2.1 Interfering substances

Elevated levels of lipids, bilirubin and hemoglobin in specimens did not interfere with the detection of CMV pp67 RNA by the NucliSens® CMV pp67 assay. The presence of antinuclear antibodies had no effect on the performance of the assay. Specimens known positive for rheumatoid factor gave no false positive or false negative results. Specimens from multiparous women gave no false positives or false negatives.

Table b.2.1 Interfering Substances

Specimen Type	Unspiked Negative Results/Total	Spiked with CMV RNA Positive Results/Total
Icteric (up to 3.8 mg/dL)	10/10	10/10
Hemolyzed	10/10	10/10
Lipemic (up to 2,400 mg/dL)	10/10	10/10
Antinuclear Antibodies	10/10	10/10
Rheumatoid Factor	10/10	10/10
Multiparous Women	10/10	10/10

The use of EDTA as an anticoagulant does not interfere with the performance of this assay.

b.2.2 Analytical specificity and sensitivity

b.2.2.1 Analytical specificity : CMV strains and cross-reactivity

The NucliSens® CMV pp67 is capable of detecting the Towne, C87, Davis, ESP, and AD169 strains. Cells were infected with laboratory strains AD169, Towne, C87, Davis and ESP until the appearance of cytopathic effects (CPE). Nucleic acid isolated from the infected cells was analyzed with the NucliSens® CMV pp67 assay. HCMV pp67 mRNA could be amplified from all reference strains tested. In clinical and analytical studies, the NucliSens® CMV pp67 assay was performed upon patients from different geographic areas of North America and Europe. Longitudinal series of specimens were collected from these patients with episodes of HCMV infection as confirmed by conventional culture and/or high levels of antigenemia. From all of these episodes of confirmed HCMV infection, at least one or more specimen(s) tested positive in the NucliSens® CMV pp67 assay. Therefore, the NucliSens® CMV pp67 assay is capable of detecting pp67 mRNA of CMV strains encountered in patients originating from different geographical areas.

The specificity of the NucliSens® CMV pp67 assay was evaluated by testing a panel of specimens from individuals exhibiting the following infections or diseases, but known negative for the HCMV virus. These specimens were tested without spike to assess possible false positive type reactions and after being spiked with an aliquot of HCMV RNA to assess possible false negative type reactions.

Table b.2.2.1 Analytical Specificity: Cross-reactivity

Specimen Type	Unspiked Negative Results/Total	Spiked with HCMV RNA Positive Results/Total
HIV-1	10/10	10/10
HTLV-I/II	8/8	8/8
Herpes Simplex Virus Type I	10/10	10/10
Herpes Simplex Virus Type II	10/10	10/10
Epstein Barr Virus	10/10	10/10
Varicella Zoster Virus	10/10	10/10
Respiratory Syncytial Virus	10/10	10/10
Systemic Lupus Erythematosus	10/10	10/10

In addition, four cell lines, each infected with a related Herpes virus, were tested with the NucliSens® CMV pp67 assay to check for possible cross-reactivity. There was no cross-reactivity of the assay with these viruses:

Human Herpes Virus 6A
Human Herpes Virus 6B
Human Herpes Virus 7
Human Herpes Virus 8.

EDTA anticoagulated whole blood samples were inoculated with the following common human bacteria flora:

Escherichia coli
Bacillus cereus
Staphylococcus epidermidis
Psuedumonas fluorescens.

Two 0.1 ml aliquots of each contaminated sample were added to Lysis Buffer. CMV mRNA spike was added to one of the two Lysis Buffer tubes. The specimens were then processed with the NucliSens® CMV pp67 assay. No evidence of interference by the bacteria flora with the performance of the assay was observed.

b.2.2.2 Analytical specificity : whole blood donors

The analytical specificity of the NucliSens® CMV pp67 assay was assessed in a volunteer whole blood donor population by evaluating specimens from 100 donors. No specimen was reported positive for HCMV by the NucliSens® CMV pp67 assay.

Of the one hundred (100) specimens, fifty (50) were from HCMV seronegative whole blood donors and fifty (50) were from HCMV seropositive whole blood donors. The fifty seropositive specimens were positive by a HCMV EIA assay. These fifty specimens were tested with both the NucliSens® CMV pp67 assay and an FDA cleared CMV pp65 antigenemia assay. No specimen was found positive by either assay. Relative to the CMV pp65 antigenemia assay in a whole blood donor population, the overall agreement was 100%.

Table b.2.2.2 Comparison of NucliSens® CMV pp67 to CMV pp65 Antigenemia in a Population of Seropositive Blood Donors

		CMV pp65 Antigenemia		
		Positive	Negative	Total
NucliSens® CMV pp67	Positive	0	0	0
	Negative	0	50	50
	Total	0	50	50

b.2.2.3 Analytical sensitivity

To assess the performance of the NucliSens® CMV pp67 assay with specimens representing active CMV infection, patients at risk for HCMV infection were evaluated at sites located in New England, in the Northeast and on the West Coast regions of the United States (Table b.2.2.3). Fifty-one (51) clinical specimens that were positive for HCMV in cell culture or in a FDA cleared CMV pp65 antigenemia assay or in both were evaluated with the NucliSens® CMV pp67 assay. The NucliSens® CMV pp67 assay reported a positive result for 43 of the specimens whereas CMV pp65 antigenemia assay tested positive for 46 specimens and culture for 40. Relative to antigenemia and/or culture positive, overall agreement was 84.3% (43/51).

Table b.2.2.3 Comparison of NucliSens® CMV pp67 to Antigenemia and/or Culture in a CMV infected population

		Antigenemia and/or Culture		
		Positive	Negative	Total
NucliSens® CMV pp67	Positive	43	0	43
	Negative	8	0	8
	Total	51	0	51

b.2.2.4 Reproducibility

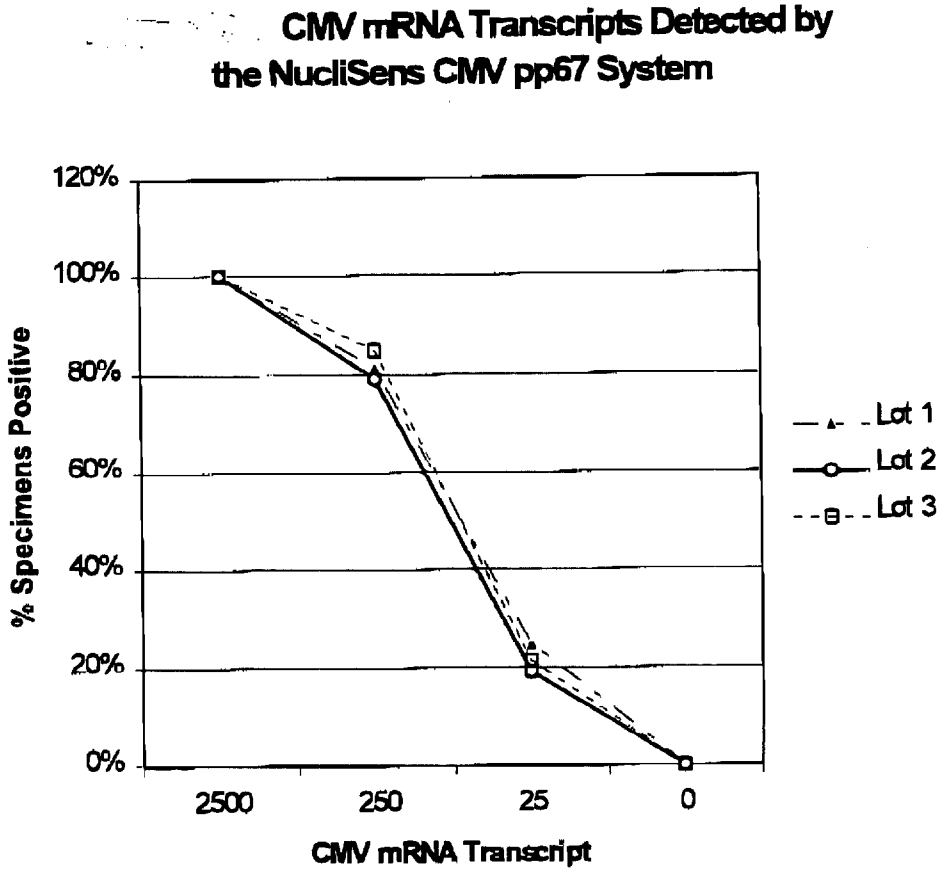
The following numbers of results were obtained when performing multiple tests of the same 4 samples at 3 sites with each of the same 3 lots.

Table b.2.2.4. a. Reproducibility of the NucliSens® CMV pp67 assay

Site 1									
	Lot A		Lot B		Lot C		Totals		
Specimen (RNA molecules/input)	Tech.1	Tech.2	Tech.1	Tech.2	Tech.1	Tech.2	Positive	%	Expected
1 (2500)	8/8	8/8	8/8	8/8	8/8	8/8	48/48	100%	100%
2 (250)	8/8	8/8	7/8	6/8	5/8	3/8	37/48	77%	93%
3 (25)	2/8	3/8	1/8	2/8	2/8	3/8	13/48	27%	13%
4 (0)	0/8	0/8	0/8	0/8	0/8	0/8	0/48	0%	0%
	37/64=58%		32/64=50%		29/64=45%		98/192=51%		
Site 2									
	Lot A		Lot B		Lot C		Totals		
Specimen(RNA molecules/input)	Tech.1	Tech.2	Tech.1	Tech.2	Tech.1	Tech. 2	Positive	%	Expected
1 (2500)	8/8	4/4	8/8	8/8	8/8	8/8	44/44	100%	100%
2 (250)	8/8	3/4	6/8	7/8	5/8	6/8	35/44	80%	93%
3 (25)	0/8	0/4	2/8	0/8	2/8	4/8	8/44	18%	13%
4 (0)	0/8	0/4	0/8	0/8	0/8	0/8	0/44	0%	0%
	23/48=48%		31/64=48%		33/64=52%		87/176=49%		
Site 3									
	Lot A			Lot B			Lot C			Totals		
Specimen (RNA molecules/input)	Tech.1	Tech.2	Tech.3	Tech.1	Tech.2	Tech.3	Tech.1	Tech.2	Tech.3	Positive	%	Expected
1 (2500)	8/8	4/4	4/4	8/8	6/6	2/2	8/8	4/4	4/4	48/48	100%	100%
2 (250)	7/8	2/4	4/4	7/8	6/6	2/2	6/8	3/4	4/4	41/48	85%	93%
3 (25)	0/8	0/4	1/4	2/8	2/6	0/2	2/8	2/4	1/4	10/48	21%	13%
4 (0)	0/8	0/4	0/4	0/8	0/6	0/2	0/8	0/4	0/4	0/48	0%	0%
	30/64=47%			35/64 = 55%			34/64=53%			99/192=52%		

Multiple logistic regression indicated that the probability of detection is not affected by lot or test site differences.

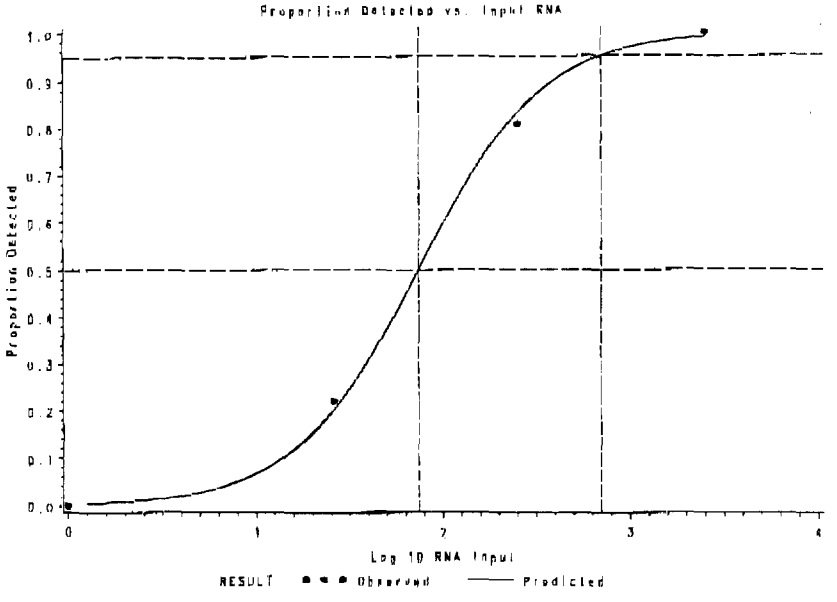
Figure **b.2.2.4.b** presents the above information graphically.



b.2.2.5 Limit of detection

The data shown in Table **b.2.2.4.a.** were used to estimate reliable detection limits. The following figure shows the fit of a logistic regression model to the observed proportions as a function of input copy number.

Figure b.2.2.5 Logistic regression of observed detection rates for different concentrations of CMV pp67 RNA transcript using the NucliSens® CMV pp67 assay



The analysis indicated a 95% response rate with 700 input RNA molecules with 95% fiducial limits ranging from 480 to 1158 RNA molecules.

b.2.2.6

In the analytical studies as presented in sections 9.1, 9.2, and 9.3, a total of 1427 samples have been analyzed. In 99.3 % of the cases (1417 samples) a valid test result was obtained. Only 10 samples (0.7%) revealed an invalid test result.

b.2.2.7 Clinical performance

b.2.2.7 .a. Transplant patient population

In a bone marrow and solid organ transplant population of 50 patients, the performance of the NucliSens® CMV pp67 assay was compared to cell culture and a pp65 antigenemia assay. Multiple specimens were collected over an extended period of time from these patients as part of a longitudinal study conducted in Italy to monitor for the presence of HCMV infection after transplantation. Only results from specimens for which all three assays revealed

a valid test result were considered. The percentage of invalid test results for the NucliSens® CMV pp67 assay in this study was 2.3%. In Table **b.2.2.7.a.**, the results of this study are summarized. If either antigenemia or culture were found positive for a specimen, that particular specimen was regarded as CMV positive. If both antigenemia and culture were negative, the state of nature for that specimen was considered CMV negative. In the table, for each patient enrolled in the study, the number of CMV positive and CMV negative specimens is indicated based on the combined antigenemia/culture results and based on the NucliSens® CMV pp67 assay.

Table b.2.2.7.a. Numbers of Positive and Negative Tests with Antigenemia and/or Culture and with NucliSens® CMV pp67 in Serial Testing of 50 Patients in a Transplant Population

Patient No.	Positive Tests Antigenemia or Culture	Negative Tests Antigenemia and Culture	Positive Tests NucliSens®	Negative Tests NucliSens®	Patient No.	Positive Tests Antigenemia or Culture	Negative Tests Antigenemia and Culture	Positive Tests NucliSens®	Negative Tests NucliSens®
1	7	5	5	6	26	6	5	4	7
2	4	7	1	10	27	5	2	3	3
3	4	20	3	10	28	1	5	1	4
4	4	11	1	11	29	2	10	1	7
5	5	8	3	10	30	3	4	1	6
6	6	5	1	9	31	1	10	0	10
7	5	8	2	9	32	4	5	0	8
8	12	5	5	6	33	2	12	0	8
9	10	9	7	8	34	6	18	0	14
10	5	9	4	8	35	1	7	0	7
11	13	8	7	11	36	1	20	0	9
12	10	11	1	13	37	2	12	0	10
13	22	3	14	8	38	2	14	0	10
14	7	21	6	12	39	2	10	0	8
15	5	7	3	7	40	0	12	0	8
16	9	15	3	12	41	0	10	0	10
17	6	8	6	7	42	0	11	0	8
18	5	6	5	6	43	0	12	0	9
19	3	6	2	6	44	0	17	0	10
20	13	9	4	16	45	0	14	0	7
21	9	11	5	7	46	0	11	0	9
22	6	8	2	9	47	0	11	0	9
23	10	6	8	7	48	0	15	0	9
24	5	5	5	5	49	0	13	0	7
25	4	5	5	4	50	0	18	0	10

Agreement between the NucliSens® CMV pp67 assay results and the combined antigenemia/culture results was calculated on a per subject basis (Table b.2.2.7.b.) and on a per test basis (Table b.2.2.7.c.). For the analysis on a per subject basis (Table b.2.2.7.b.), a similar algorithm was employed as for the individual specimens (i.e. combined antigenemia/culture versus NucliSens® CMV pp67) and a patient was considered CMV positive if any specimen for that patient was found positive during the course of the study.

Table b.2.2.7.b. Agreement of NucliSens® CMV pp67 Assay with Antigenemia and/or Culture on a Per Subject Basis in a Transplant Population

		Antigenemia and/or Culture*		
NucliSens® CMV pp67		+	-	Total
	+	30	0	30
	-	9	11	20
	Total	39	11	50

* Represents the result of any positivity over course of study of serial specimens from 50 patients

Overall Agreement =100 (41/50) = 82%
Sensitivity = 100(30/39) = 77% (95% C.I. using 'exact' method: 60.7 to 88.9%)
Specificity = 100(11/11) = 100% (95% C.I. using 'exact' method: 71.5 to 100%)
Positive Predictive Value = 100(30/30) = 100%
Negative Predictive Value = 100(11/20) = 55%

Table b.2.2.7.c. Agreement of NucliSens® CMV pp67 Assay with Antigenemia and/or Culture on a Per Test Basis in a Transplant Population

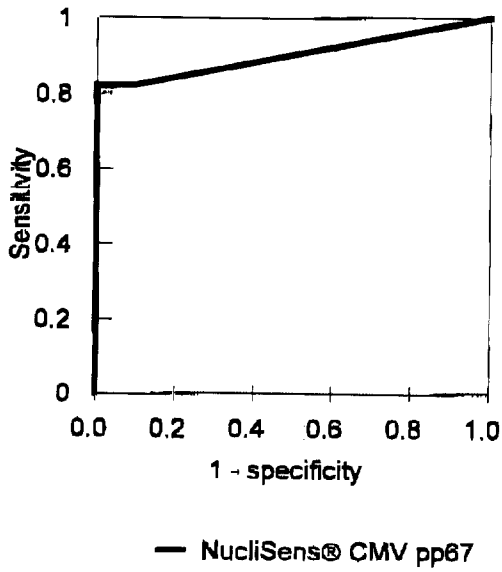
		Antigenemia and/or Culture*		
NucliSens® CMV pp67		+	-	Total
	+	73	19	92
	-	73	203	276
	Total	146	222	368

* Represents multiple determinations from serial specimens from 50 patients where all tests had valid responses

Overall Agreement =100 (73+203)/368 = 75%

Using the combined antigenemia/culture results as the true state of nature, a Receiver Operator Characteristic (ROC) curve was constructed for the NucliSens® CMV pp67 assay on a per subject basis (Figure **b.2.2.7.d.**). The estimated area under the curves as a diagnostic measure for the discriminatory capacity of the assay for specimens catagorized as CMV positive or CMV negative in this way, was 0.902 with a standard error of 0.042 for the per subject analysis.

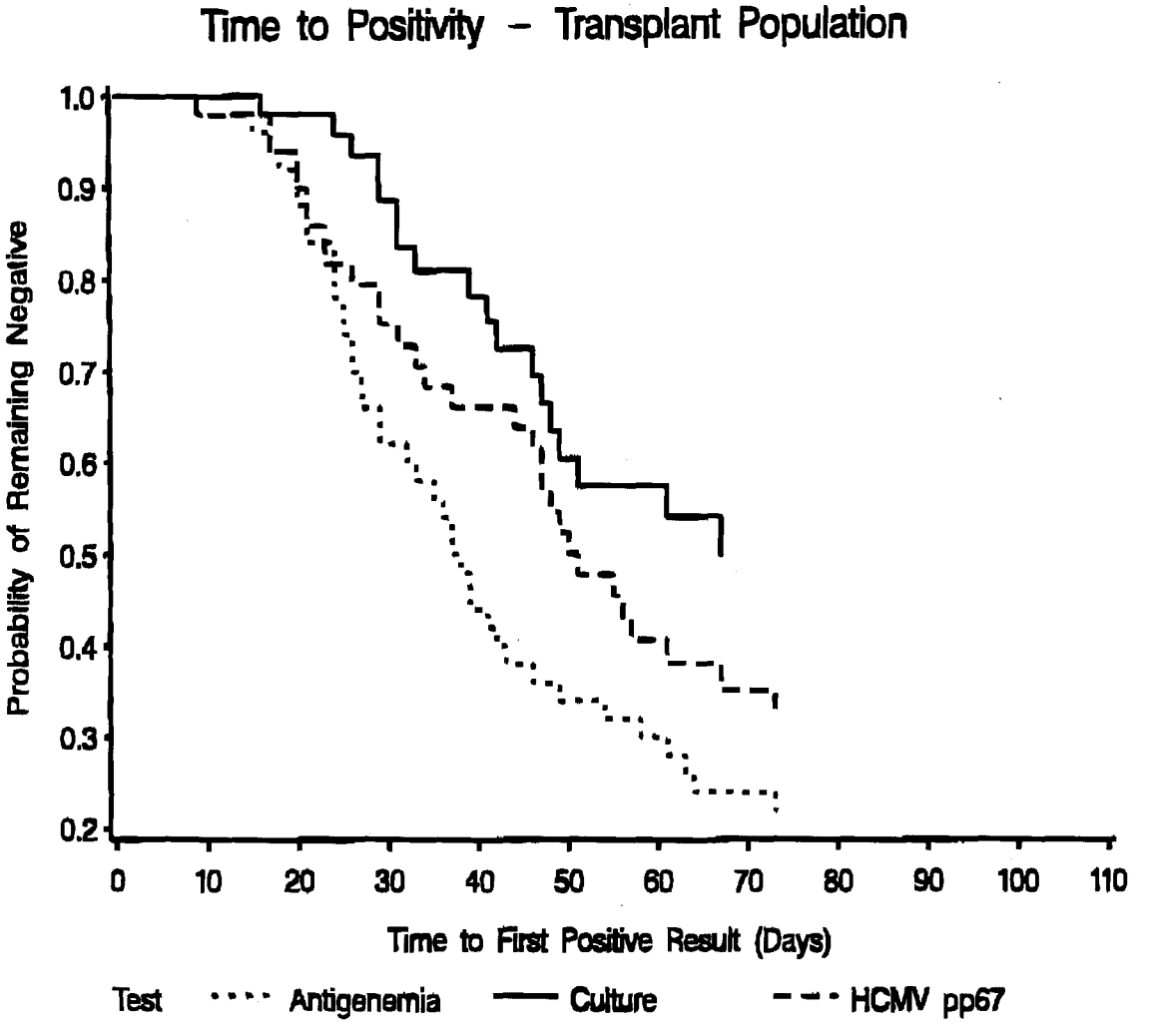
Figure b.2.2.7.d. ROC Curve of NucliSens® CMV pp67 Assay with Antigenemia and/or Culture as Truth on a Per Subject Basis in a Transplant Population.



Time to Positivity in a Transplant Population

To investigate which test first revealed a positive result in the course of time, a time to positivity analysis was carried out. For this analysis, results for antigenemia and culture were considered separately. The Kaplan-Meier curve shown in Figure **b.2.2.7.e** plots the probability that a test remains negative versus time. Median times to first positive result for antigenemia, culture, and NucliSens® CMV pp67 were 38, 67, and 51 days, respectively.

Figure b.2.2.7.e.



b.2.2.8 HIV-1 Infected population

In a HIV-1 infected population of 50 individuals, the performance of the NucliSens® CMV pp67 assay was compared to cell culture and a pp65 antigenemia assay. Multiple specimens were collected over an extended period of time from these individuals as part of a longitudinal study conducted in the Netherlands to monitor for the presence of HCMV infection or disease during routine clinic visits. Only results from specimens for which all three assays revealed a valid test result were considered. The percentage of invalid test results for the NucliSens® CMV pp67 assay in this study was 1.6%. In Table b.2.2.8.a, the results of this study are summarized. If either antigenemia or culture were found positive for a specimen, that particular specimen was regarded as CMV positive. If both antigenemia and culture were negative, the state of nature for that specimen was considered CMV negative. In the table, for each patient enrolled in the study, the number of CMV positive and CMV negative specimens is indicated based on the combined antigenemia/culture results and based on the NucliSens® CMV pp67 assay.

Table b.2.2.8.a Numbers of Positive and Negative Tests with Antigenemia and/or Culture and with NucliSens® CMV pp67 In Serial Testing of 50 Patients in a HIV Infected Population

Subject	Positive Tests Antigenemia or Culture	Negative Tests Antigenemia and Culture	Positive Tests NucliSens®	Negative Tests NucliSens®	Subject	Positive Tests Antigenemia or Culture	Negative Tests Antigenemia and Culture	Positive Tests NucliSens®	Negative Tests NucliSens®
1	3	5	0	8	26	2	4	0	6
2	7	3	7	3	27	7	3	1	9
3	8	5	6	5	28	4	1	1	4
4	10	2	2	10	29	3	21	1	23
5	3	8	0	11	30	2	7	3	6
6	0	9	0	9	31	0	8	0	8
7	10	12	6	16	32	0	6	0	6
8	3	21	1	22	33	3	6	1	8
9	4	5	2	6	34	5	0	1	4
10	2	6	0	8	35	6	2	5	3
11	6	9	5	10	36	3	4	0	7
12	0	11	0	10	37	5	4	3	6
13	1	6	0	7	38	0	6	0	6
14	1	2	0	3	39	1	2	1	2
15	0	20	0	19	40	4	3	4	3
16	0	6	0	6	41	1	13	1	12
17	9	6	4	11	42	5	7	2	10
18	3	2	2	3	43	1	18	1	18
19	2	6	1	6	44	4	3	1	6
20	3	2	1	4	45	2	7	1	8
21	2	6	1	7	46	7	3	2	8
22	7	16	5	18	47	7	1	6	2
23	3	3	0	5	48	0	16	1	15

24	0	4	0	4	49	10	4	9	5
25	2	9	0	11	50	7	0	6	1

Agreement between the NucliSens® CMV pp67 assay results and the combined antigenemia/culture results was calculated on a per subject basis (Table **b.2.2.8.b**) and on a per test basis (Table **b.2.2.8.c**). For the analysis on a per subject basis (Table **b.2.2.8.b**), a similar algorithm was employed as for the individual specimens (i.e. combined antigenemia/culture versus NucliSens® CMV pp67) and a patient was considered CMV positive if any specimen of the patient was found positive during the course of the study.

Table b.2.2.8.b Agreement of NucliSens® CMV pp67 Assay with Antigenemia and/or Culture on a Per Subject Basis in a HIV-1 Infected Population

	Antigenemia and/or Culture*			
NucliSens® CMV pp67		+	-	Total
	+	32	1	33
	-	9	8	17
	Total	41	9	50

* Represents the result of any positivity over course of study of serial specimens from 50 patients

Overall Agreement =100 (40/50) = 80%
Sensitivity = 100(32/41) = 78% (95% C.I. using 'exact' method: 62.4 to 89.4%)
Specificity = 100(8/9) = 89% (95% C.I. using 'exact' method: 51.8 to 99.7%)
Positive Predictive Value = 100(32/33) = 97%
Negative Predictive Value = 100(8/17) = 47%

Table b.2.2.8.c Agreement of NucliSens® CMV pp67 Assay with Antigenemia and/or Culture on a Per Test Basis in a HIV-1 Infected Population

	Antigenemia and/or Culture*			
NucliSens® CMV pp67		+	-	Total
	+	76	11	87
	-	95	268	363
	Total	171	279	450

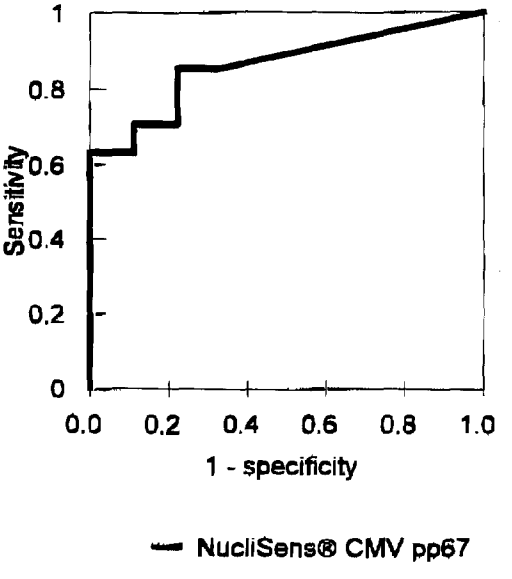
* Represents multiple determinations from serial specimens from 50 patients where all tests had valid responses

Overall Agreement =100 (268+76)/450 = 76%

Using the combined antigenemia/culture results as the true state of nature, a ROC curve were constructed for the NucliSens® CMV pp67 assay on a per subject basis (Figure **b.2.2.8.d.**). The estimated area under the curve as a diagnostic measure for the discriminatory capacity of the assay for specimens

catagorized as CMV positive or CMV negative in this way, was 0.862 with a standard error of 0.054 for the per subject analysis.

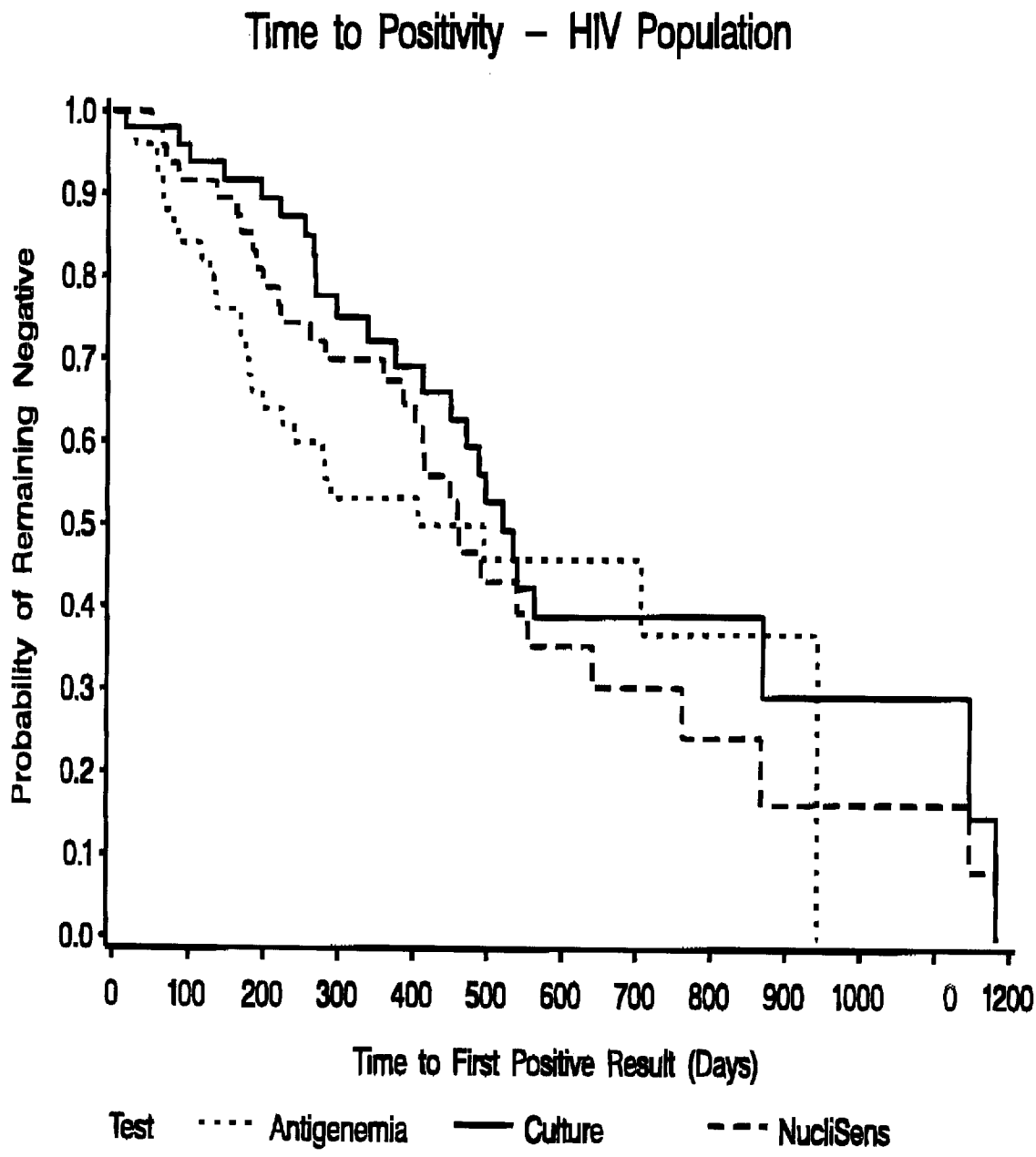
Figure b.2.2.8.d. ROC Curve of NucliSens® CMV pp67 Assay with Antigenemia and/or Culture as Truth on a Per Subject Basis in a HIV-1 Infected Population is shown in the following figure.



Time to Positivity in a HIV Infected Population

To investigate which test first revealed a positive result in the course of time, a time to positivity analysis was carried out. For this analysis, results for antigenemia and culture were considered separately. The Kaplan-Meier curve shown in Figure b.2.2.8.e. plots the probability that a test remains negative versus time. Median times to first positive result for antigenemia, culture, and NucliSens® CMV pp67 were 405, 519, and 458 days, respectively.

Figure b.2.2.8.e



(b)3) The conclusions drawn from the nonclinical and clinical tests that demonstrate that the device is as safe, as effective, and performed as well or better than the legally marketed device identified in (a)(3).

The conclusions drawn from the clinical tests demonstrate that the NucliSens™ CMV pp67 assay is as safe, as effective, and performed as well as the legally marketed CMV Brite™ CMV Antigenemia Detection Test Kit and typical cell culure in the diagnosis of active HCMV infection.

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

SEP 15 1999

Mr. Ron Sanyal, M. Pharm., CQE, RAC
Regulatory Affairs Administrator
Organon Teknika Corporation
100 Akzo Avenue
Durham, North Carolina 27712

Re: K983762
Trade Name: NucliSens® CMV pp67
Regulatory Class: II
Product Code: LIN
Dated: July 1, 1999
Received: July 2, 1999

Dear Mr. Sanyal:

We have reviewed your Section 510(k) notification of intent to market the device referenced above and we have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (Premarket Approval), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 895. A substantially equivalent determination assumes compliance with the Current Good Manufacturing Practice requirements, as set forth in the Quality System Regulation (QS) for Medical Devices: General regulation (21 CFR Part 820) and that, through periodic QS inspections, the Food and Drug Administration (FDA) will verify such assumptions. Failure to comply with the GMP regulation may result in regulatory action. In addition, FDA may publish further announcements concerning your device in the Federal Register. Please note: this response to your premarket notification submission does not affect any obligation you might have under sections 531 through 542 of the Act for devices under the Electronic Product Radiation Control provisions, or other Federal laws or regulations.

Under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88), this device may require a CLIA complexity categorization. To determine if it does, you should contact the Centers for Disease Control and Prevention (CDC) at (770)488-7655.

This letter will allow you to begin marketing your device as described in your 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and additionally 809.10 for in vitro diagnostic devices), please contact the Office of Compliance at (301) 594-4588. Additionally, for questions on the promotion and advertising of your device, please contact the Office of Compliance at (301) 594-4639. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers Assistance at its toll free number (800) 638-2041 or at (301) 443-6597 or at its internet address "http://www.fda.gov/cdrh/dsmamain.html"

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Division of Clinical Laboratory Devices
Office of Device Evaluation
Center for Devices and Radiological Health

Enclosure

510(k) NUMBER (IF KNOWN): K983762

DEVICE NAME: NucliSens® CMV pp67

INDICATIONS FOR USE:

Then NucliSens® CMV pp67 assay is a nucleic acid amplification-based qualitative assay to be used in conjunction with the Nuclisens® Reader for the detection of human cytomegalovirus (HCMV) pp67 mRNA in EDTA anticoagulated whole blood from adult transplant donors and HIV infected individuals. It is intended as an aid in the diagnosis of active (acute or reactivated) HCMV infection. This product is not intended for use in screening of blood or plasma donors.

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

for Lu D. Dubois, Ph.D.

(Division Sign-Off)
Division of Clinical Laboratory Devices
510(k) Number K983762

Prescription Use ✓ OR Over-The Counter- Use _____
(Per 21 CFR 801.109) (Optional Format 1-2-96)