

P99000082953

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September 24, 1999

Secretary of State  
Division of Corporations  
State of Florida  
409 East Gaines  
Tallahassee, FL 32399  
Attn: Doug Spittler

300002998053--6  
-09/27/99--01141--002  
\*\*\*\*\*70.00 \*\*\*\*\*70.00

Re: Merger of Safe Water Technologies, Inc. (Florida) into Centrex, Inc. (Oklahoma)

Gentlemen:

Enclosed herewith are 2 copies of the Articles of Merger in which Safe Water Technologies, Inc., a Florida corporation, merged into Centrex, Inc., an Oklahoma corporation, with the Oklahoma corporation being the surviving corporation. Also enclosed for your information is a copy of the Agreement and Plan of Merger as executed by the parties along with a copy of the Certificate of Merger issued by the Oklahoma Secretary of State. Also enclosed is the \$70.00 filing fee, \$35 for each constituent corporation which is a party to the Merger. Please file the Articles of Merger and return one filed stamped copy to me in the enclosed self-addressed envelope. If you have any questions, please call me as soon as possible.

*Was NOT Attached  
DO NOT Need*

Very truly yours,

*Frederick K. Slicker*  
Frederick K. Slicker

FKS/dmp  
Enclosures

HA1125\_CENTREXMMerger- FL sec state cover ltr SWT 9-15-99wpd.wpd

FILED  
99 SEP 27 AM 8:40  
SECRETARY OF STATE  
TALLAHASSEE, FLORIDA

*Merger*

V. SHEPARD OCT 5 1999

ARTICLES OF MERGER  
Merger Sheet

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MERGING:

SAFE WATER TECHNOLOGIES, INC., a Florida corporation, P99000082953

INTO

**CENTREX, INC.**, an Oklahoma corporation not qualified in Florida.

File date: September 27, 1999

Corporate Specialist: Velma Shepard

Account number: 300002998053

Account charged: 70.00

FILED  
99 SEP 27 AM 8:40  
SECRETARY OF STATE  
TALLAHASSEE, FLORIDA

**ARTICLES OF MERGER**

TO THE SECRETARY OF STATE OF THE STATE OF FLORIDA, 409 EAST GAINES STREET,  
TALLAHASSEE, FLORIDA 32299:

These Articles of Merger are being filed pursuant to Section 601.117 of the Florida General Corporation Laws. In lieu of filing an executed Agreement and Plan of Merger, the Surviving Corporation hereby states and certifies as follows:

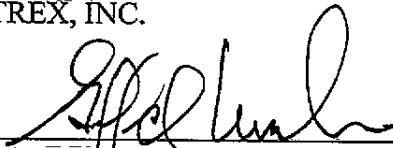
1. The names and states of incorporation of each of the Constituent Corporations are:

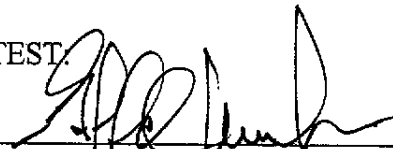
<u>NAME OF CORPORATION</u>	<u>STATE OF INCORPORATION</u>
Centrex, Inc.	Oklahoma
Safe Water Technologies, Inc.	Florida

2. An Agreement and Plan of Merger has been approved, adopted, certified, executed and acknowledged by each Constituent Corporation, in accordance with the provisions of Section 1082 of Title 18 of the Oklahoma Statutes and Section 601.1107 of the Corporation Laws of Florida. The Board of Directors and Shareholders of the Surviving Corporation approved the Merger September 13, 1999; and the Board of Directors and Shareholders of Safe Water Technologies, Inc. approved the Merger on September 22, 1999 and September 22, 1999, respectively.
3. The name of the Surviving Corporation is Centrex, Inc.
4. The Certificate of Incorporation of the Surviving Corporation is not being changed by reason of the Merger.
5. The executed Agreement and Plan of Merger is on file at the principal place of business of the Surviving Corporation at 8908 S. Yale, Suite 409, Tulsa, OK 74137-3545.
6. A copy of the Agreement and Plan of Merger will be furnished on request and without cost to any shareholder of any Constituent Corporation.
7. The authorized capital of Safe Water Technologies, Inc. is 10,000,000 shares of common stock, par value \$.001 per share, and 950,000 shares are issued and outstanding, all of which voted for the Merger.

IN WITNESS WHEREOF, the Surviving Corporation has caused these Articles of Merger to be executed by its President and attested by its Secretary this 24<sup>th</sup> of Secretary, 1999.

CENTREX, INC.

By:   
Gifford Mable, President

ATTEST.  
By:   
Gifford Mable, Secretary

## AGREEMENT AND PLAN OF MERGER

This Agreement and Plan of Merger ("Agreement") is entered into by and among CENTREX, INC., an Oklahoma corporation ("CENTREX"), SAFE WATER TECHNOLOGIES, INC., a Florida corporation ("SWT"), and UTEK CORPORATION, a ~~Florida~~ corporation ("UTEK").

Delaware

WHEREAS, UTEK is the majority shareholder of SWT; and

WHEREAS, Dr. Luis Garcia-Rubio is the inventor and principal investigator who has discovered and has developed an on-line spectroscopic and turbidimetric technique for detecting, classifying and counting micrororganisms, such as cryptosporidium and giardia, in water solutions and related technology, processes and procedures ("Invention") covered by US Patent Application described in Schedule 1, as to which formal notice of allowance has been received but not yet published ("Patent Applications"); and

WHEREAS, the University of South Florida ("USF") initially owned the Invention and Patent Applications, but USF has assigned all its rights, titles and interests in the Invention to the University of South Florida Research Foundation ("USFRF"); and

WHEREAS, SWT has acquired an exclusive worldwide License Agreement ("License") with USFRF, a copy of which is attached hereto as an exhibit, to practice patents covering the Invention and to manufacture, market and commercialize products covered by the Invention; and

WHEREAS, the parties desire to provide for the terms and conditions upon which SWT will merge into CENTREX in a statutory merger ("Merger") in accordance with 18 Oklahoma Statutes, Section 1082 of the Oklahoma General Business Corporation Act ("Oklahoma Act") and Section 607.1107 of the Corporation Law of Florida ("Florida Act"), upon consummation of which the assets and business of SWT will be owned by CENTREX, all liabilities and obligations of SWT will become the liabilities and obligations of CENTREX, and all issued and outstanding shares of capital stock of SWT will be exchanged for common stock of CENTREX; and

WHEREAS, for federal income tax purposes, it is intended that the Merger qualify as a tax-free reorganization within the meaning of Section 368(a)(1)(A) of the Internal Revenue Code of 1986, as amended ("Code").

NOW, THEREFORE, in consideration of the premises and for other good and valuable consideration, the receipt, adequacy and sufficiency of which are hereby acknowledged, the parties agree as follows:

ARTICLE I  
THE MERGER

1.01. The Merger

(a) Agreement to Merge. Subject to filing evidence of the Merger with the Secretary of State of Oklahoma, the jurisdiction of the Surviving Corporation and the terms and conditions of this Agreement, at the Effective Time, as defined below, SWT shall be merged with and into CENTREX in accordance with the provisions of this Agreement and the Oklahoma Act; the separate corporate existence of SWT shall cease; and CENTREX shall continue as the surviving corporation ("Surviving Corporation"). The constituent corporations ("Constituent Corporations") to the Merger are CENTREX and SWT. The name of the Surviving Corporation, CENTREX, INC., shall not be changed by reason of the Merger.

(b) Effective Time. The Merger shall become effective ("Effective Time") upon filing of a Certificate of Merger substantially in the form attached as Exhibit A ("Certificate of Merger") with the Secretary of State of the State of Oklahoma in accordance with applicable provisions of the Oklahoma Act.

(c) Appointment of Service Agent. CENTREX hereby irrevocably appoints the Secretary of State of the State of Florida as its agent to accept process in Florida in any proceeding for the enforcement of any obligation of any Constituent Corporation in Florida as well as for the enforcement of any obligation of the Surviving Corporation arising from or by reason of the Merger, including any suit or other proceeding to enforce appraisal rights of any shareholder of SWT. CENTREX designates that all such process received by the Secretary of State of Florida shall be sent to CENTREX at 8908 South Yale, Suite 409, Tulsa, Oklahoma 74137-3545.

(d) Effect of the Merger. At the Effective Time, all rights, powers, privileges, franchises, licenses and permits of the Constituent Corporations, and all property, real, personal and mixed, shall be vested in the Surviving Corporation; and all debts, duties, liabilities and claims of every kind, character and description of the Constituent Corporations shall be debts, duties, liabilities of and claims against of the Surviving Corporation and may be enforced against the Surviving Corporation to the same extent as if such debts, duties, liabilities of and claims against had been incurred by it originally. All rights of creditors of the Constituent Corporations and all liens upon property of any Constituent Corporation shall be preserved unimpaired and shall not be altered in any way by reason of the Merger.

1.02. Conversion of Stock. At the Effective Time, by virtue of the Merger and without any action on the part of the shareholders of the Constituent Corporations:

(i) Each of the 950,000 shares of SWT that are issued and outstanding at the Effective Time shall be converted into 1 share (or 950,000 shares in the aggregate) of common stock of the Surviving Corporation, as follows:

<u>SWT Shareholder</u>	<u>No. of Shares</u>
UTEK	684,000 shares
USFRF	190,000 shares
Ms. Flo Hassell	76,000 shares

(ii) All issued and outstanding options, warrants or other rights to acquire any capital stock of SWT at the Effective Time shall by reason of the Merger and without action on the part of the holders of any such rights be automatically canceled for all purposes; and

(iii) Each share of common stock of CENTREX issued and outstanding at the Effective Time and each right to receive a share of common stock of CENTREX upon the satisfaction of any conditions outstanding at the Effective Time shall remain issued and outstanding and shall not be effected in any manner by reason of the Merger.

1.03. Effect of Merger.

(a) Rights in SWT Cease. At and after the Effective Time, the holder of each certificate of common stock of SWT shall cease to have any rights as a shareholder of SWT. All dividends or other distributions with respect to SWT common stock prior to the Effective Time shall be payable to the shareholders of SWT without interest upon surrender of certificates representing SWT common stock.

(b) Closure of SWT Stock Records. From and after the Effective Time, the stock transfer books of SWT shall be closed, and there shall be no further registration of stock transfers on the records of SWT.

1.04. Certificate of Incorporation of the Surviving Corporation. The Certificate of Incorporation of the Surviving Corporation shall not be changed by reason of the Merger.

1.05. Bylaws of the Surviving Corporation. The Bylaws of the Surviving Corporation shall not be changed by reason of the Merger.

1.06. Directors of the Surviving Corporation. The directors of the Surviving Corporation immediately after the Effective Time shall be the persons named in Exhibit B until each of their respective successors is duly elected and qualified.

1.07. Officers of the Surviving Corporation. The officers of the Surviving Corporation immediately after the Effective Time shall be the persons set forth in Exhibit B until each of their respective successors is duly elected and qualified.

1.08. Closing. The Closing of the Merger shall take place at the offices of Frederick K. Slicker, 8908 S. Yale, Suite 410, Tulsa, Oklahoma 74137-3545 at 5:00 p.m. local time upon execution of the Agreement.

## ARTICLE II REPRESENTATIONS AND WARRANTIES

2.01. General Representations and Warranties of SWT and UTEK. SWT and UTEK represent and warrant to CENTREX that the facts set forth below are true and correct:

(a) Organization. SWT is a corporation duly organized, validly existing and in good standing under the laws of the State of Florida, is qualified to do business as a foreign corporation in each other jurisdiction in which the conduct of its business or the ownership of its properties require such qualification, and has all requisite power and authority to conduct SWT's business and operate properties.

(b) Authorization. The execution of this Agreement and the consummation of the Merger and the other transactions contemplated hereby have been duly authorized by the Board of Directors and Shareholders of SWT; no other corporate action on its part is necessary in order to execute, deliver, consummate and perform its obligations hereunder; and SWT has all requisite corporate and other authority to execute and deliver this Agreement and consummate the transactions contemplated hereby.

(c) Capitalization. The authorized capital of SWT consists of 10,000,000 shares of common stock, par value \$.001 per share; at the date hereof 950,000 shares of its common stock are issued and outstanding and owned by the shareholders listed in Paragraph 1.02 above, and no shares were held in its treasury. All issued and outstanding shares of common stock of SWT have been duly and validly issued and are fully paid and non-assessable shares and have not been issued in violation of any preemptive or other rights of any other person or any applicable laws. There are no outstanding options, warrants, commitments, calls or other rights or agreements requiring it to issue any shares of SWT common stock or securities convertible into shares of the common stock of SWT to anyone for any reason whatsoever.

(d) Binding Effect. The execution, delivery, performance and consummation of the Merger and the transactions contemplated hereby will not violate any obligation to which SWT is a party and will not create a default hereunder; and this Agreement constitutes a legal, valid and binding obligation of SWT, enforceable in accordance with its terms, except as the enforcement may be limited by bankruptcy, insolvency, moratorium, or similar laws affecting creditor's rights generally and by the availability of injunctive relief, specific performance or other equitable remedies.



(e) Litigation Relating to this Agreement. There are no suits, actions or proceedings pending or to the knowledge of SWT or UTEK threatened which seek to enjoin the Merger or the transactions contemplated by this Agreement or which, if adversely decided, would have a materially adverse effect on the business, results of operations, assets, prospects, the Patent Applications, the License, the results of the operations of SWT.

(f) No Conflicting Agreements. Neither the execution and delivery of this Agreement nor the fulfillment of or compliance by SWT and UTEK with the terms or provisions hereof will result in a breach of the terms, conditions or provisions of, or constitute a default under, or result in a violation of, the corporate charter or bylaws of SWT, the Patent Applications, the License, the Research Agreement, or any agreement, contract, instrument, order, judgment or decree to which either UTEK or SWT is a party or by which UTEK or SWT or any of its assets is bound, or violate any provision of any applicable law, rule or regulation or any order, decree, writ or injunction of any court or governmental entity which materially affects its assets or business.

(g) Consents. No consent from or approval of any court, governmental entity or any other person is necessary in connection with execution and delivery of this Agreement by SWT or UTEK or performance of the obligations of SWT or UTEK hereunder or under any other agreement to which SWT or UTEK is a party; and the consummation of the transactions contemplated by this Agreement will not require the approval of any entity or person or prevent the termination of the Patent Applications, the License, or any other material right, privilege, license or agreement relating to SWT or its assets or business.

(h) Title to Assets. SWT will at Closing have good and marketable title to its assets (tangible and intangible), free and clear of all liens, claims, charges, mortgages, options, restrictions, security agreements and other encumbrances of every kind or nature whatsoever, including the duly executed and delivered License.

(i) The Patent Applications and the License.

(i) To the knowledge of UTEK and SWT, the Patent Applications listed in Schedule 1 are valid and are in full force and effect to the extent the Patents have been granted; the Patent Applications which are pending are being prosecuted in good faith with diligence; USFRF has received formal notice of acceptance but patents have not yet been published or formally issued; and neither UTEK nor SWT has any reason to believe that patents will not be issued pursuant to the Patent Applications; and

(ii) To the knowledge of UTEK and SWT, the Invention does not and will not infringe the intellectual or other rights of another. This representation and warranty is not a representation or warranty that

there are no infringing intellectual rights of any other but is a representation and warranty only that neither SWT nor UTEK has any knowledge thereof; and CENTREX acknowledges that neither UTEK nor SWT has conducted an independent investigation to determine whether the Invention infringes the rights of any other party or that the Invention itself is marketable; and

- (iii) The Invention is owned by USFRF, which has all right, power, authority, ownership and entitlement to file, prosecute and maintain in effect the Patent Applications with respect to the Invention listed in Schedule 1 hereto; and
- (iv) Dr. Luis Garcia-Rubio is the only inventor of the Invention, and he has assigned all of his rights, titles, and interests in the Invention to USFRF; and
- (v) The License is in full force and effect and is legal, valid, binding and enforceable in accordance with its terms.

(j) Liabilities of SWT. SWT has no assets, no liabilities of any kind, character or description except those created by the License, and except for \$ \_\_\_\_ in actual out-of-pocket expenses incurred in the Patent Applications.

(k) Condition of Tangible Assets. All of the tangible assets of SWT have been operated in accordance with customary operating practices generally acceptable in its industry to which and have been maintained and are in good working order and repair in the ordinary course of business, subject only to reasonable and ordinary wear and tear.

(l) Financial Statements. The unaudited financial statements of SWT attached as Schedule 2.01(l) as of the Closing will present fairly its financial position and the results of its operations on the dates and for the periods shown therein; provided, however, that interim financial statements are subject to customary year-end adjustments and accruals that, in the aggregate, will not have a material adverse effect on the overall financial condition or results of its operations. SWT has not engaged in any business not reflected in its financial statements. There have been no material adverse changes in the nature of its business, prospects, the value of assets or the financial condition since the date of its financial statements. There are no outstanding obligations or liabilities of SWT except as specifically set forth in the SWT financial statements, including the obligation to maintain the Patent Applications and any patents issued pursuant thereto or in a schedule attached hereto and specifically agreed to by CENTREX.

(m) Taxes. All returns, reports, statements and other similar filings required to be filed by SWT with respect to any federal, state, local or foreign taxes, assessments, interests, penalties, deficiencies, fees and other governmental charges or impositions have been timely filed with the appropriate governmental agencies in all jurisdictions in which such tax returns are required to be filed; all such tax returns properly reflect all liabilities of SWT for taxes for the periods, property or events covered thereby; and all taxes, whether or not reflected on those tax returns, and all taxes claimed to be due from SWT by any taxing authority, have been properly paid, except to the extent contested in good faith by appropriate proceedings and reserves have been established in its financial statements to the full extent if the contest is adversely decided against it. SWT has not received any notice of assessment or proposed assessment in connection with any tax returns, SWT has not extended or waived the application of any statute of limitations of any jurisdiction regarding the assessment or collection of any taxes. There are no tax liens (other than any lien which arises by operation of law for current taxes not yet due and payable) on any of its assets. There is no basis for any additional assessment of taxes, interest or penalties. SWT has made all deposits required by law to be made with respect to employees' withholding and other employment taxes, including without limitation the portion of such deposits relating to taxes imposed upon SWT.

(n) Absence of Certain Changes or Events. SWT has not:

- (i) Sold, encumbered, assigned or transferred any of its material assets or its interest in the Patent Applications or the License or any other material asset; or
- (ii) Amended or terminated the License or the Patent Applications; or
- (iii) Suffered any material damage, destruction or loss; or
- (iv) Received notice or has knowledge of any material adverse effect on the Patent Applications or the License or any other material asset or liability of SWT; or
- (v) Made any commitments or agreements for capital expenditures or otherwise; or
- (vi) Entered into any transaction or made any commitment not disclosed to CENTREX; or
- (vii) Agreed to take any of the actions set forth in this paragraph.

(o) Material Contracts. A complete and accurate copy of all material agreements, contracts and commitments of the following types, whether written or oral to which it is a party or is bound, has been provided to CENTREX and such agreements are in full force and effect without amendment. In addition:

- (i) There are no outstanding unpaid promissory notes, mortgages, indentures, deeds of trust, security agreements and other agreements and instruments relating to the borrowing of money by or any extension of credit to SWT; and
- (ii) There are no outstanding operating agreements, lease agreements or similar agreements by which SWT is bound; and
- (iii) The complete and executed License and the Patent Applications with all schedules, exhibits and amendments related thereto and all material correspondence with the patent authorities relating thereto have been provided to CENTREX; and
- (iv) There are no outstanding licenses to or from others of any intellectual property and trade names; and
- (v) There are no outstanding contracts or commitments to sell, lease or otherwise dispose of any of the property of SWT.

(p) Compliance with Laws. SWT is in compliance with all applicable laws, rules, regulations and orders promulgated by any federal, state or local governmental body or agency relating to its business and operations. SWT owns all franchises, licenses, permits, easements, rights, applications, filings, registrations and other authorizations which are necessary for it to conduct business, all of which are valid and in full force and effect, and SWT is in full compliance therewith.

(q) Litigation. There is no suit or action or any arbitration, administrative, legal or other proceeding of any kind or character, or any governmental investigation pending or threatened against SWT, the Patent Applications, the License or affecting its assets or business, and there is no factual basis therefor. There are no pending or threatened actions or proceedings before any court, arbitrator or administrative agency which would, if adversely determined, individually or in the aggregate, materially and adversely affect its assets or business.

(r) Employees. SWT has no employees. SWT is not a party to or bound by any employment agreement or any collective bargaining agreement with respect to any of the employees.

(s) Employee Benefit Plans. There are no employee benefit plans in effect, and there are no outstanding or unfunded liabilities to employees of SWT.

(t) Books and Records. The books and records of SWT are complete and accurate in all material respects, fairly present its business and operations, have been maintained in accordance with good business practices, and accurately reflect in all material respects its business, financial condition and liabilities.

(u) No Broker's Fees. Neither UTEK nor SWT has incurred any finder's, broker's, investment banking, financial, advisory or other similar fees or obligations.

(v) Full Disclosure. All representations or warranties of UTEK and SWT are true, correct and complete in all material respects on the date hereof and shall be true, correct and complete in all material respects as of the Closing as if they were made on such date. No statement made by SWT herein or in the exhibits and schedules hereto or any document delivered by SWT or on its behalf pursuant to this Agreement contains an untrue statement of material fact or omits to state all material facts necessary to make the statements therein not misleading in any material respect.

2.02. General Representations and Warranties of CENTREX. CENTREX represents and warrants to UTEK and SWT that the facts set forth are true and correct:

(a) Organization. CENTREX is a corporation duly organized, validly existing and in good standing under the laws of the State of Oklahoma, is qualified to do business as a foreign corporation in each other jurisdiction in which the conduct of its business or the ownership of its properties require such qualification, and has all requisite power and authority to conduct its business and operate properties.

(b) Authorization. The execution of this Agreement and the consummation of the Merger and the other transactions contemplated hereby have been duly authorized by the Board of Directors and Shareholders of CENTREX; no other corporate action on its part is necessary in order to execute, deliver, consummate and perform its obligations hereunder; and it has all requisite corporate and other authority to execute and deliver this Agreement and consummate the transactions contemplated hereby.

(c) Capitalization. The authorized capital of CENTREX consists of 45,000,000 shares of common stock, par value \$.001 per share, of which 7,000,000 shares are issued and outstanding immediately, and 5,000,000 shares of Preferred Stock, none of which is issued and outstanding. All issued and outstanding shares of common stock of CENTREX have been duly and validly issued and are fully paid and non-assessable shares and have not been issued in violation of any preemptive or other rights of any other person or any applicable laws. There will be no outstanding options, warrants, commitments, calls or other rights or agreements requiring it to issue any shares of CENTREX common stock or securities

convertible into shares of its common stock to anyone for any reason whatsoever immediately after the Effective Time, except that CENTREX has adopted an incentive stock option plan and has reserved for issuance thereunder up to 3,000,000 shares, none of which has been currently granted.

(d) Binding Effect. The execution, delivery, performance and consummation of the Merger and the transactions contemplated hereby will not violate any obligation to which CENTREX is a party and will not create a default hereunder; and this Agreement constitutes a legal, valid and binding obligation of CENTREX, enforceable in accordance with its terms, except as the enforcement may be limited by bankruptcy, insolvency, moratorium, or similar laws affecting creditor's rights generally and by the availability of injunctive relief, specific performance or other equitable remedies.

(e) Litigation Relating to this Agreement. There are no suits, actions or proceedings pending or to its knowledge threatened which seek to enjoin the Merger or the transactions contemplated by this Agreement or which, if adversely decided, would have a materially adverse effect on its business, results of operations, assets, prospects or the results of its operations of CENTREX.

(f) No Conflicting Agreements. Neither the execution and delivery of this Agreement nor the fulfillment of or compliance by CENTREX with the terms or provisions hereof will result in a breach of the terms, conditions or provisions of, or constitute a default under, or result in a violation of, its corporate charter or bylaws, or any agreement, contract, instrument, order, judgment or decree to which it is a party or by which it or any of the assets is bound, or violate any provision of any applicable law, rule or regulation or any order, decree, writ or injunction of any court or governmental entity which materially affects its assets or business.

(g) Consents. No consent from or approval of any court, governmental entity or any other person is necessary in connection with its execution and delivery of this Agreement and performance of the obligations of CENTREX hereunder or under any other agreement to which CENTREX is a party; and the consummation of the transactions contemplated by this Agreement will not require the approval of any entity or person in order to prevent the termination of any material right, privilege, license or agreement relating to CENTREX or its assets or business.

(h) Title to Its Assets. CENTREX has good and marketable title to its assets (tangible and intangible), free and clear of all charges, claims, liens, mortgages, options, restrictions, security agreements and other encumbrances of every kind or nature whatsoever.

(i) Condition of Tangible Assets. All of its tangible assets have been operated in accordance with customary operating practices generally acceptable in its industry to which and have been maintained and are in good working order and repair in the ordinary course of business, subject only to reasonable and ordinary wear and tear.

(j) Financial Statements. The unaudited financial statements of CENTREX attached as Schedule 2.02(j) present fairly its financial position and the results of its operations on the dates and for the periods shown therein; provided, however, that interim financial statements are subject to customary year-end adjustments and accruals that, in the aggregate, will not have a material adverse effect on the overall financial condition or results of its operations. CENTREX has not engaged in any business not reflected in its financial statements. There have been no material adverse changes in the nature of its business, prospects, the value of assets or the financial condition since the date of its financial statements. There are no material outstanding obligations or liabilities of CENTREX except as specifically set forth in the CENTREX financial statements.

(k) Taxes. All returns, reports, statements and other similar filings required to be filed by it with respect to any federal, state, local or foreign taxes, assessments, interests, penalties, deficiencies, fees and other governmental charges or impositions have been timely filed with the appropriate governmental agencies in all jurisdictions in which such tax returns are required to be filed; all such tax returns properly reflect all liabilities of it for taxes for the periods, property or events covered thereby; and all taxes, whether or not reflected on those tax returns, and all taxes claimed to be due from it by any taxing authority, have been properly paid, except to the extent it has contested in good faith by appropriate proceedings and adequate reserves have been established in its financial statements to the full extent if the contest is adversely decided against it. CENTREX has not received any notice of assessment or proposed assessment in connection with any tax returns. CENTREX has not extended or waived the application of any statute of limitations of any jurisdiction regarding the assessment or collection of any taxes. There are no tax liens (other than any lien which arises by operation of law for current taxes not yet due and payable) on any of its assets. CENTREX has no knowledge of any basis for any additional assessment of taxes. CENTREX has made all deposits required by law to be made with respect to employees' withholding and other employment taxes, including without limitation the portion of such deposits relating to taxes imposed upon it.

(l) Absence of Certain Changes or Events. CENTREX has not:

- (i) Sold, encumbered, assigned or transferred any of its material assets for less than fair consideration; or
- (ii) Amended or terminated any material agreement; or
- (iii) Suffered any material damage, destruction or loss; or

- (iv) Received notice or have knowledge of any material adverse effect on its material assets; or
- (v) Made any commitments or agreements for capital expenditures; or
- (vi) Entered into any transaction other than in the ordinary course of business consistent with past practice, except that CENTREX has made an offer regarding Food Technology Services, Inc. ("FTS") in conjunction with and in full cooperation with UTEK, which proposal has not been acted upon by FTS; and
- (vii) Agreed to take any of the actions set forth in this paragraph.

(m) Material Contracts. A complete and accurate copy of all material agreements, contracts and commitments of the following types, whether written or oral to which it is a party or is bound, has been provided to UTEK:

- (i) All material promissory notes, mortgages, indentures, deeds of trust, security agreements and other agreements and instruments relating to the borrowing of money by or any extension of credit to it; and
- (ii) All material operating agreements and lease agreements; and
- (iii) All material licenses to or from others of any intellectual property and trade names.

(n) Compliance with Laws. CENTREX is in compliance with all applicable laws, rules, regulations and orders promulgated by any federal, state or local governmental body or agency relating to its business and operations. CENTREX owns all franchises, licenses, permits, easements, rights, applications, filings, registrations and other authorizations which are necessary for it to conduct business, all of which are valid and in full force and effect, and it is in full compliance therewith.

(o) Litigation. There is no suit, action or any arbitration, administrative, legal or other proceeding of any kind or character, or any governmental investigation pending or threatened against it affecting its assets or business, and there is no factual basis therefor. There are no pending or threatened actions or proceedings before any court, arbitrator or administrative agency which would, if adversely determined, individually or in the aggregate, materially and adversely affect its assets or business.

(p) Employees. CENTREX has 5 part-time employees, four of whom are employed but do not receive cash compensation. CENTREX has no written agreements with its employees.



(q) Employee Benefit Plans and Arrangements. CENTREX has no full-time employee benefit plans in effect, and CENTREX has no unfunded liabilities to employees.

(r) Books and Records. The books and records of CENTREX are complete and accurate in all material respects, fairly present its business and operations, have been maintained in accordance with good business practices, and accurately reflect in all material respects its business and financial condition.

(s) No Broker's Fees. CENTREX has incurred no finder's, broker's, investment banking, financial, advisory or other similar fee in connection with this Agreement.

(t) Full Disclosure. All representations or warranties of CENTREX are true, correct and complete in all material respects on the date hereof and shall be true, correct and complete in all material respects as of the Closing as if they were made on such date. No statement made by it herein or in the exhibits and schedules hereto or any document delivered by it or on its behalf pursuant to this Agreement contains an untrue statement of material fact or omits to state all material facts necessary to make the statements therein not misleading in any material respect.

2.03. Investment Representations of SWT Shareholders. SWT, for itself and for its shareholders, represents and warrants to CENTREX that:

(a) General. Each shareholder has such knowledge and experience in financial and business matters as to be capable of evaluating the risks and merits of an investment in the shares ("Shares") of common stock of CENTREX issuable pursuant to the Merger. Each shareholder is able to bear the economic risk of the investment in the Shares, including the risk of a total loss of the investment in the Shares. The acquisition of the Shares is for each shareholder's own account and is for investment. Except as permitted by law, each shareholder has no present intention of selling, transferring or otherwise disposing in any way of all or any portion of the Shares. All information that each shareholder has supplied to CENTREX in connection with this Agreement is true and correct. Each shareholder acknowledges that an investment in the Shares involves a very high degree of risk. Each shareholder has conducted all investigations and due diligence concerning CENTREX which it deems appropriate, and each shareholder has found all such information obtained fully acceptable. Each shareholder is knowledgeable about the prospects, business, financial condition, operations and possible acquisitions of CENTREX. Each shareholder has had an opportunity to ask questions of the officers and directors of CENTREX concerning the Shares and the business and financial condition of and prospects for CENTREX, and the officers and directors of CENTREX have adequately answered all questions asked and made all relevant information requested available to each shareholder. Each shareholder understands that success of CENTREX is dependent upon CENTREX's receipt of funds necessary to provide working capital, which may not occur. Each shareholder understands

and agrees that the following restrictions and limitations are applicable to the purchase, resale and distribution of the Shares pursuant to applicable securities laws.

(b) Stock Transfer Restrictions.

- (i) Each shareholder is aware that it must bear the full economic risk of an investment in the Shares of CENTREX for an indefinite period of time, because the transaction in which the Shares are being issued has not been registered under the Securities Act of 1933, as amended ("Securities Act"), or the securities laws of any state; and, therefore, the Shares cannot be sold, pledged, transferred or otherwise disposed of unless registered under applicable securities laws or an exemption from registration is available. Each shareholder further understands that only CENTREX can take action to register the Shares, and the cost of registration is prohibitive.
- (ii) A legend will be placed on the certificates representing the common stock of CENTREX in substantially the following form:

**NOTICE OF TRANSFER RESTRICTIONS**

**The shares evidenced by this Certificate have been acquired for investment only and have not been registered under the Securities Act of 1933, as amended, or the securities laws of any state. The Shares may not be sold, transferred, pledged or otherwise disposed of without the receipt of an opinion of counsel acceptable to CENTREX that no such registration is required.**

- (iii) Stop transfer instructions have been placed in CENTREX's transfer records with respect to the Shares to insure that any transfer or disposition thereof is in full compliance with applicable law. Each shareholder agrees that CENTREX may refuse or delay transfer of the Shares or impose other restrictions on the transfer of the Shares if CENTREX is not satisfied that the transfer is lawful. However, CENTREX acknowledges and agrees that this determination must be made within a reasonable time; and if CENTREX finds the transfer is satisfactory and permitted by applicable law, CENTREX will not refuse or delay the transfer.

**ARTICLE III  
INDEMNIFICATION**

3.01 By UTEK. UTEK agrees to indemnify, defend and hold harmless CENTREX and its shareholders, directors, officers, employees, agents and representatives and their respective successors and assigns against and in respect of any cost, damage, expense

(including reasonable legal fees and actual expenses), liability or loss incurred or suffered by any of them resulting from or arising out of the: (i) breach, inaccuracy, misrepresentation or untruth of any representation or warranty, or the nonfulfillment of any agreement or covenant of UTEK contained in this Agreement or in any document delivered by UTEK or SWT to CENTREX pursuant hereto; and (ii) any action, assessment, claim, demand, proceeding or suit incident to any of the foregoing. The liability of UTEK hereunder may be satisfied by the return to CENTREX of shares of CENTREX common stock issued pursuant hereto valued at the fair market value on the date the breach is discovered to the extent of the breach.

3.02 By CENTREX. CENTREX agrees to indemnify and hold harmless UTEK and its directors, officers, employees, agents and representatives and their respective successors and assigns against and in respect of any cost, damage, expense (including reasonable legal fees and actual expenses), liability or loss incurred or suffered by any of them resulting from or arising out of: (i) the breach, inaccuracy, misrepresentation or untruth of any representation, warranty, or the nonfulfillment of any agreement or covenant of CENTREX contained in this Agreement or in any document delivered by it to UTEK pursuant hereto; and (ii) any action, assessment, claim, demand, proceeding or suit incident to any of the foregoing.

3.03 Costs. The indemnification rights and obligations of a party hereto shall include the right to receive and the duty to pay and reimburse the indemnified party all its reasonable costs and expenses incurred in the enforcement of its rights hereunder.

3.04 Survival of Representations and Warranties.

(a) By UTEK. The representations and warranties made by UTEK shall survive for a period of 3 years after Closing, and thereafter all such representations and warranties shall be extinguished, except with respect to claims then pending for which specific notice has been given during such 3 year period. UTEK shall have liability and responsibility for the surviving representations and warranties made by it herein, notwithstanding any due diligence investigation or examination by CENTREX.

(b) By CENTREX. The representations and warranties made by CENTREX shall survive for a period of 3 years after Closing, and thereafter all such representations and warranties shall be extinguished, except with respect to claims then pending for which specific notice has been given during such 3 year period. CENTREX shall have liability and responsibility for the surviving representations and warranties made to CENTREX, notwithstanding any due diligence investigation or examination by UTEK.

3.05 Limitations on Liability. Notwithstanding any other provision herein to the contrary, neither party hereto shall be liable to the other party for any cost, damage, expense, liability or loss under this indemnification provision until after the sum of all amounts

individually when added to all other such amounts in the aggregate exceeds \$5,000, and then such liability shall apply only to matters in excess of \$5,000.

3.06 Rights of Indemnitors. The indemnified party shall notify the indemnifying party of the assertion or commencement of such action, claim or proceeding within a reasonable period of time or, if citation or service of process has been made, within 15 days thereafter. The indemnified party may, at its option and at its sole expense, participate in the defense of and contest any such action, claim or proceeding; provided, however, the indemnified party shall at all times also have the right to participate fully therein. If the indemnifying party, within a reasonable time after receiving such notice, fails to participate, the indemnified party shall have the right, but shall not be obligated, to undertake the defense of the action, claim or proceeding for the account of and at the risk of the indemnifying party; provided, however, in the event that the indemnified party shall determine to compromise or settle (exercising its judgment in good faith) any such action, claim or proceeding, the indemnified party shall be required to give the indemnifying party 15 days' notice of such determination after its receipt of actual notice of the claim. The indemnified party shall then be entitled to compromise or settle the action, claim or proceeding for the account of and at the risk of the indemnifying party; provided, however, the settlement shall be effective without the consent of both the indemnifying and indemnified parties, which consent shall not be reasonably withheld. The parties agree that any indemnified party may join any indemnifying party in any action, claim or proceeding brought by a third party, as to which any right of indemnity created by this Agreement would or might apply, for the purpose of enforcing any right of the indemnity granted to such indemnified party pursuant to this Agreement.

3.07 Additional Rights. Any right of indemnity of any party pursuant to this Agreement shall be in addition to and shall not operate as a limitation on any other right to indemnity of such party pursuant to this Agreement, any document or instrument executed in connection with the consummation of the transaction contemplated hereby or otherwise.

#### ARTICLE IV ARBITRATION

In the event a dispute arises with respect to the interpretation or effect of this Agreement or concerning the rights or obligations of the parties hereto, the parties agree to negotiate in good faith with reasonable diligence in an effort to resolve the dispute in a mutually acceptable manner. Failing to reach a resolution thereof, either party shall have the right to submit the dispute to be settled by arbitration under the Commercial Rules of Arbitration of the American Arbitration Association. The parties agree that all arbitrations shall be conducted in Tulsa, Oklahoma, unless the parties mutually agree to the contrary. The cost of arbitration shall be borne by the party against whom the award is rendered or, if in the interest of fairness, as allocated in accordance with the judgment of the arbitrators. All

awards in arbitration made in good faith and not infected with fraud or other misconduct shall be final and binding.

#### ARTICLE V MISCELLANEOUS

No party may assign any right or obligation hereunder the Agreement without the written consent of the other parties hereto. No permitted assignment shall relieve a party of its obligations under this Agreement without the separate written consent of the other parties. This Agreement shall be binding upon and enure to the benefit of the parties and their respective permitted successors and assigns. Each party agrees that it will comply with all applicable laws, rules and regulations in the execution and performance of its obligations under this Agreement. This Agreement shall be governed by and construed in accordance with the laws of the State of Oklahoma. This document constitutes a complete and entire agreement among the parties with reference to the subject matters set forth herein. No statement or agreement, oral or written, made prior to or at the execution hereof and no prior course of dealing or practice by either party shall vary or modify the terms set forth herein without the prior consent of the other parties hereto. This Agreement may be amended only by a written document signed by the parties. Notices or other communications required to be made in connection with this Agreement shall be delivered to the parties at the address set forth below or at such other address as may be changed from time to time by giving written notice to the other parties. This Agreement may be executed in multiple counterparts, each of which shall constitute one and a single Agreement.

#### ARTICLE VI PIGGYBACK REGISTRATION RIGHTS

CENTREX covenants and agrees that if it files with the Securities and Exchange Commission an underwritten registration statement on SEC Form SB-1 or SB-2 or Form S-1 or its equivalent, which includes the offer of shares owned by shareholders of CENTREX, CENTREX will use its best efforts to include some or all of the shares of CENTREX common stock issued to and then held by UTEK pursuant to this Agreement. If the underwriters include any selling shareholder shares, UTEK shall be permitted to include some or all of its CENTREX shares on a pro rata basis to the extent and upon the same terms and conditions as other CENTREX shareholders are permitted to have their CENTREX shares included in the proposed offering. If the underwriters do not permit for any reason the inclusion of selling shareholder shares in the offering, UTEK shares shall also not be included. It is the expressed intent of this Article that UTEK be treated exactly the same as any other selling CENTREX shareholder in connection with any underwritten offering of CENTREX common stock, no better and no worse. If CENTREX proposes an underwritten offering, CENTREX will give UTEK 15 days' prior written notice thereof, and UTEK shall give CENTREX notice within 10 days thereafter of UTEK's desire as to the number of shares, if any, that UTEK desires to

include in the offering. CENTREX will notify the lead underwriters of UTEK's desire, and CENTREX will include UTEK shares in accordance with this Article. As a condition of including any UTEK shares in the offering, UTEK shall (i) sign all underwriting agreements, representations, warranties, certificates and other papers as the underwriters require of UTEK and other CENTREX shareholders whose shares are to be included in the offering; (ii) pay pro rata all costs of the offering to the same extent as other CENTREX selling shareholders are required to pay; and (iii) take all other actions and do all other things as are required of other selling shareholders. Failure of UTEK to respond within 10 days after notice of CENTREX's intention to file an underwritten offering shall constitute a waiver of the rights set forth in this Article.

IN WITNESS WHEREOF, the parties have caused this Agreement to be duly executed by a duly authorized officer this 17<sup>th</sup> day of September, 1999.


CENTREX, INC.

By:   
Gifford M. Mabie, President

SAFE WATER TECHNOLOGIES, INC.  
CANCER DIAGNOSTICS, INC.

By:   
Dr. Uwe Reischl, President

UTEK Corporation

By:   
Dr. Clifford M. Gross, Chief Executive Officer

**Exhibit B**  
**to**  
**Agreement and Plan of Merger**

Directors

Gifford M. Mabie

Officers and Key Consultants

Rhonda Vincent, Director of Finance  
Frederick K. Slicker, General Counsel  
Thomas R. Coughlin, Jr., Medical Director  
Vicki L. Pippin, Director of Administration

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**SCHEDULE 1**

**DESCRIPTION OF THE INVENTION**





US005616457A

United States Patent [19]  
García-Rubio

[11] Patent Number: 5,616,457  
[45] Date of Patent: Apr. 1, 1997

[54] METHOD AND APPARATUS FOR THE  
DETECTION AND CLASSIFICATION OF  
MICROORGANISMS IN WATER

[75] Inventor: Luis H. García-Rubio, Temple Terrace,  
Fla.

[73] Assignee: University of South Florida, Tampa,  
Fla.

[21] Appl. No.: 385,539

[22] Filed: Feb. 8, 1995

[51] Int. Cl.<sup>6</sup> ..... C12Q 1/00; C12Q 1/04;  
C12Q 1/02; G01N 31/00

[52] U.S. Cl. .... 435/4; 435/34; 435/29;  
435/41; 435/42; 435/9; 436/10; 436/164;  
210/745; 73/61.71; 73/64.43

[58] Field of Search ..... 435/4, 34, 29,  
435/41, 42, 9; 436/10, 164; 210/745; 73/61.71,  
64.43

[56] References Cited

U.S. PATENT DOCUMENTS

2,863,354	12/1958	Diehl et al.	435/34
3,954,615	5/1976	Shelef	435/4
4,066,362	1/1978	Carter	435/34
4,220,858	9/1980	Ikeguchi et al.	422/55
4,602,348	7/1986	Hart	435/4

4,855,061	8/1989	Martin	422/55
4,957,624	9/1990	Peranio	435/4
5,030,419	9/1991	Ellis et al.	422/55
5,032,261	7/1991	Pyper	435/4
5,084,165	1/1992	Wang et al.	422/55
5,164,085	11/1992	Spokoiny et al.	435/4
5,242,602	9/1993	Richardson et al.	422/55
5,277,802	1/1994	Goodwin	435/4
5,303,026	4/1994	Strobl et al.	422/55
5,403,497	4/1995	Schultz	435/4

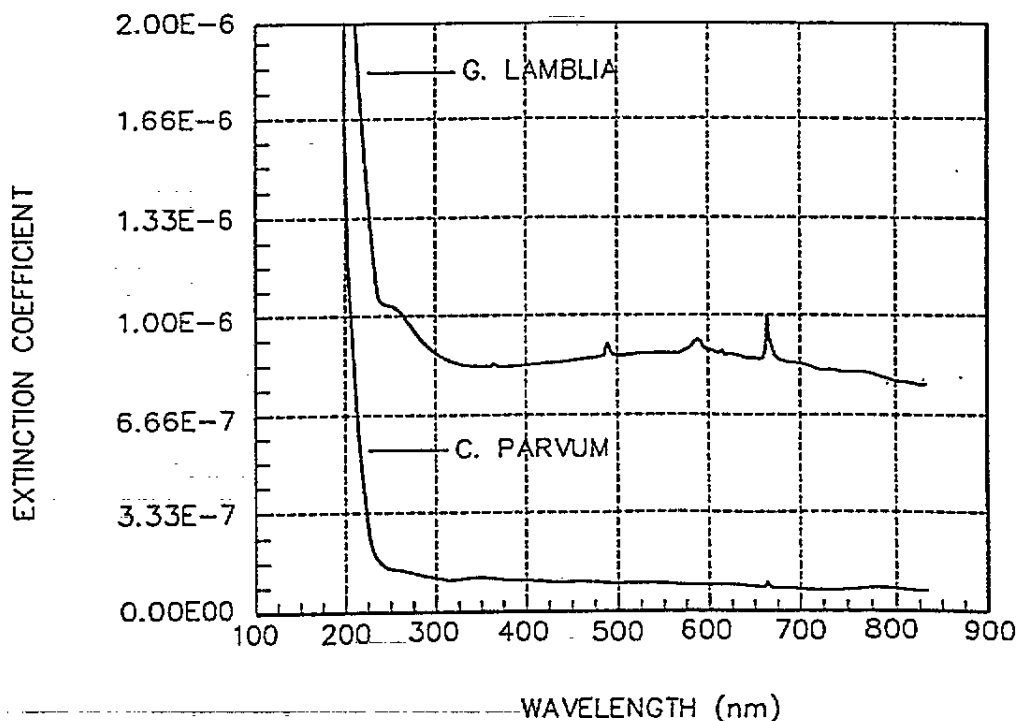
Primary Examiner—John Kight  
Assistant Examiner—Louis Leary  
Attorney, Agent, or Firm—Allen, Dyer, Doppelt, Milbrath &  
Gilchrist, P.A.

[57] ABSTRACT

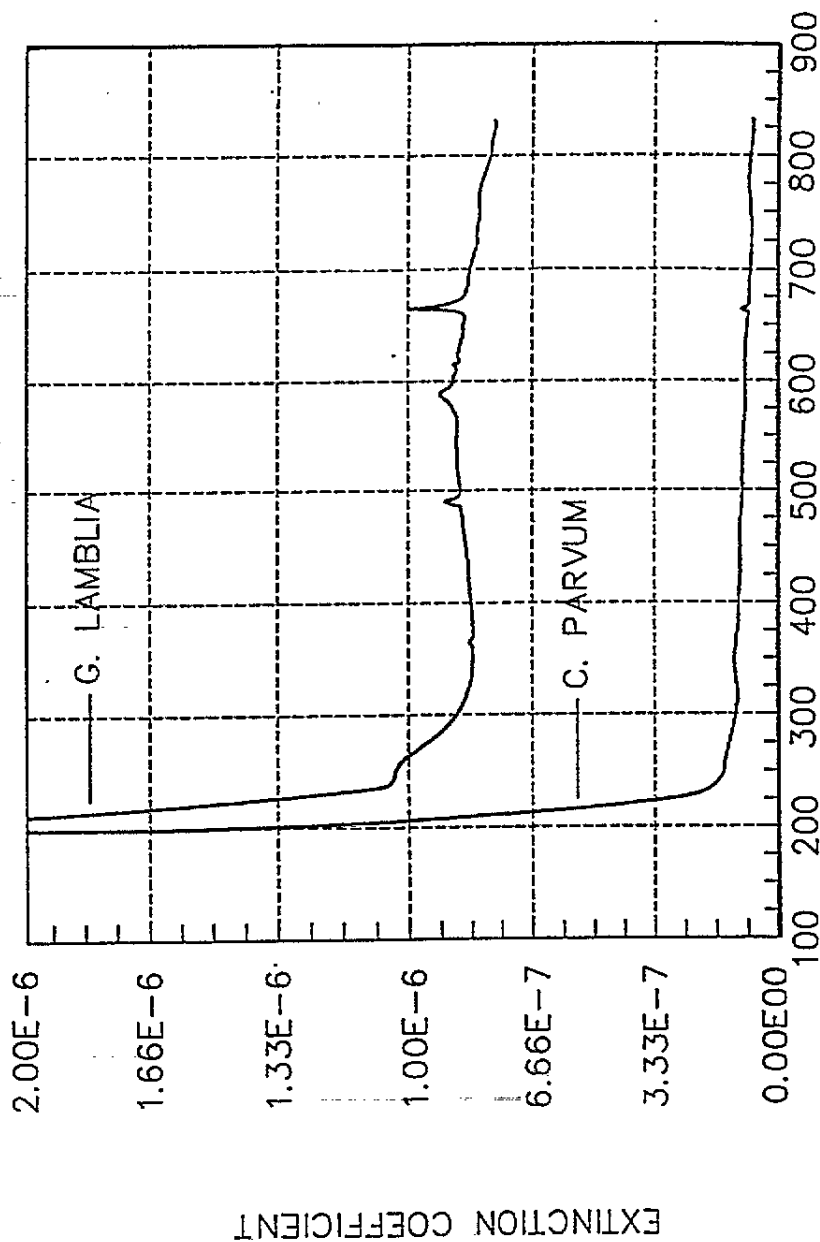
The method and apparatus for detecting the presence of an organism in a sample of liquid involves collecting an extinction spectrum of liquid sample, deconvoluting the spectrum to obtain a particle size distribution for the sample, comparing the spectrum and the particle size distribution with, respectively, a control spectrum and a control particle size distribution for the organism, and determining from the comparisons whether the organism to be detected is present in the sample. The apparatus and method permit on-line real-time quantitative classification, identification, and viability assessment of an organism such as a harmful microorganism in a water supply line.

8 Claims, 6 Drawing Sheets

C. PARVUM AND G. LAMBLIA  
EXTINCTION SPECTRA



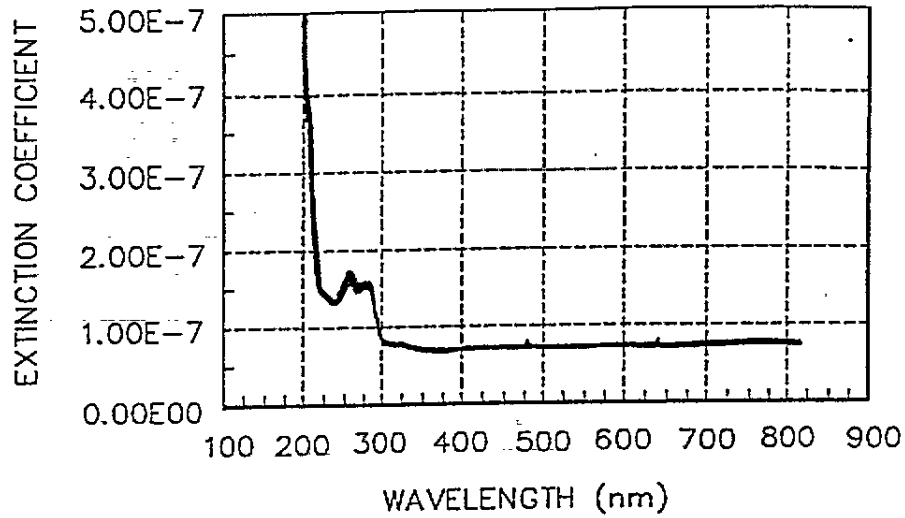
C. PARVUM AND G. LAMBLIA  
EXTINCTION SPECTRA



WAVELENGTH (nm)

FIG. 1

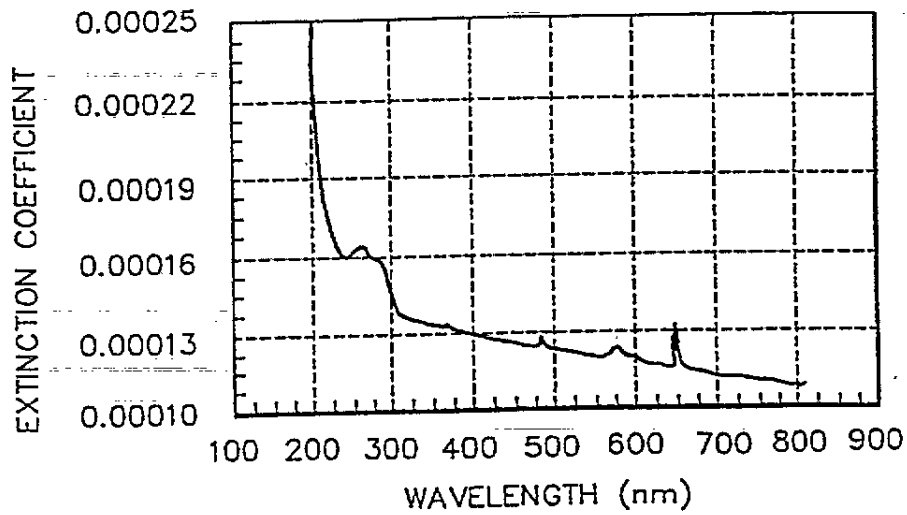
C. PARVUM WITH FITC



EXTINCTION SPECTRA OF CRYPTOSPORIDIUM PARVUM  
INDIRECTLY STAINED WITH ab-FITC

FIG. -2.

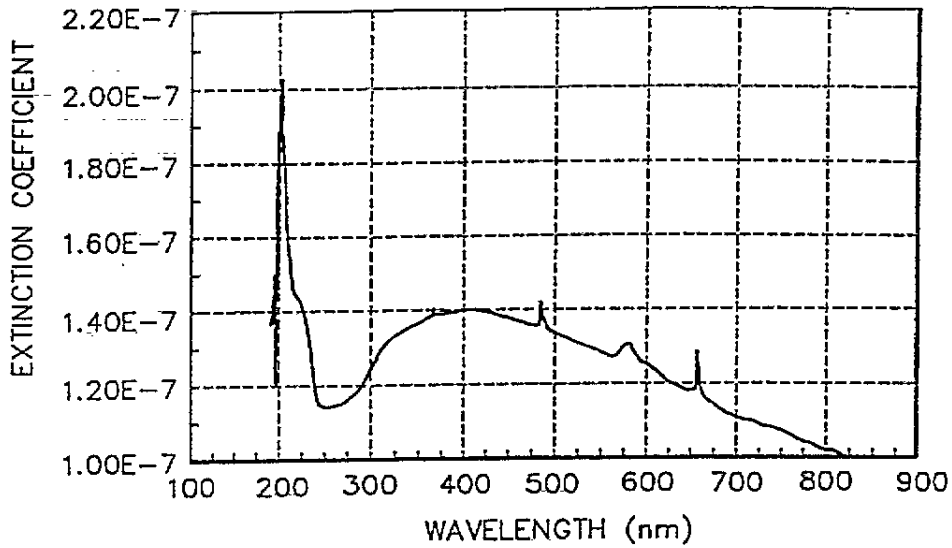
GIARDIA LAMBLIA WITH FITC



EXTINCTION SPECTRA OF GIARDIA LAMBLIA  
INDIRECTLY STAINED WITH ab-FITC

FIG. 3.

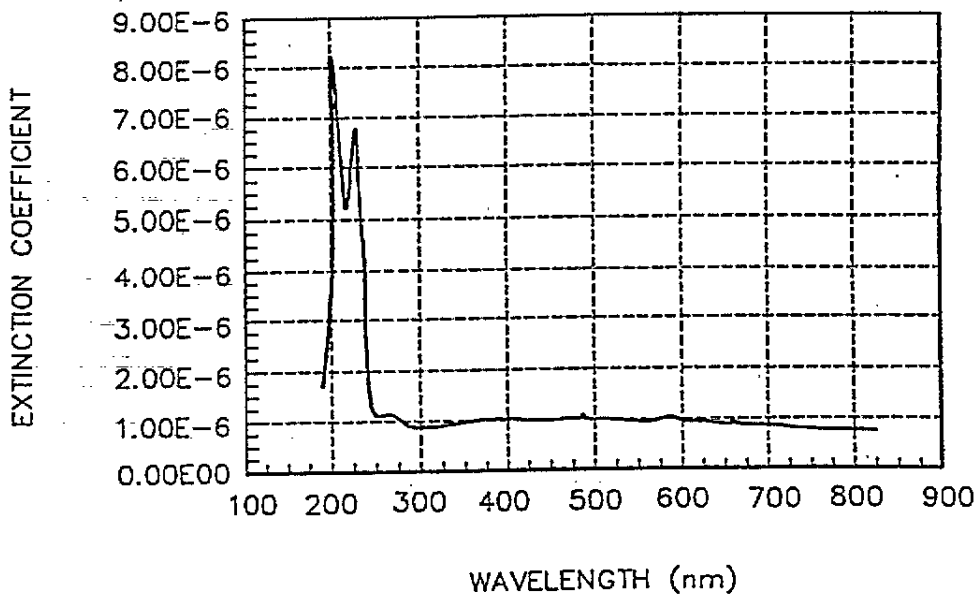
C. PARVUM WITH RHODAMINE



EXTINCTION SPECTRA OF CRYPTOSPORIDIUM PARVUM DIRECTLY STAINED WITH  $\alpha$ b-RHODAMINE

FIG. 4.

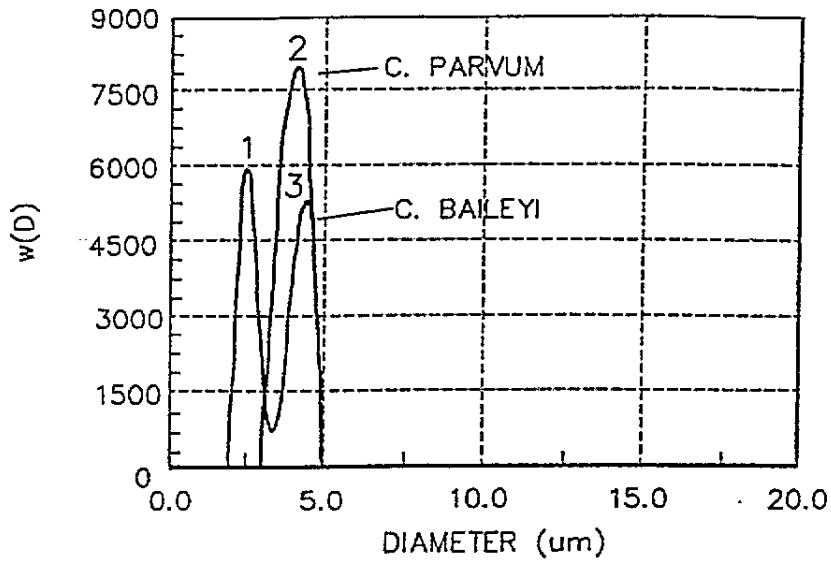
GIARDIA LAMBLIA WITH RHODAMINE



EXTINCTION SPECTRA OF GIARDIA LAMBLIA DIRECTLY STAINED WITH  $\alpha$ b-RHODAMINE

FIG. 5.

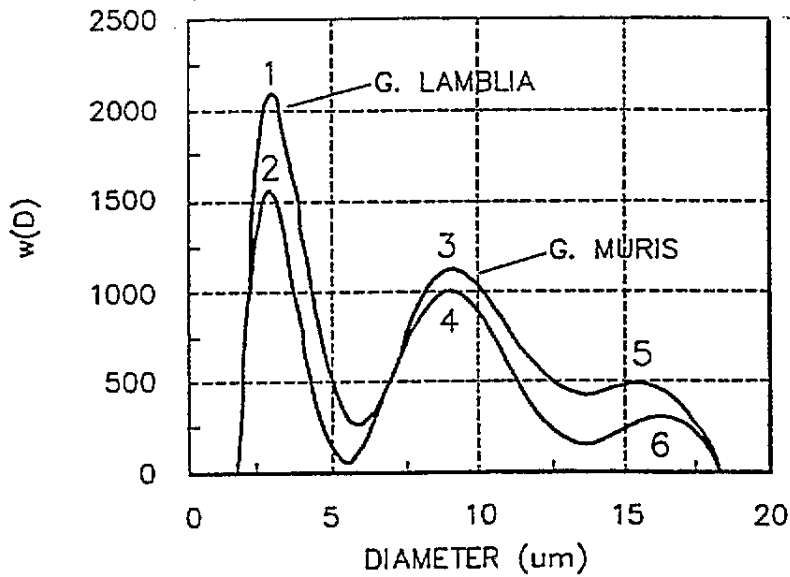
CRYPTOSPORIDIUM WEIGHT BASED PSD



WEIGHT BASED PARTICLE SIZE DISTRIBUTION OF C. PARVUM AND C. BAILEYI.

FIG. 6.

GIARDIA WEIGHT BASED PSD



WEIGHT BASED PARTICLE SIZE DISTRIBUTION OF G. LAMBLIA AND G. MURIS.

FIG. 7.

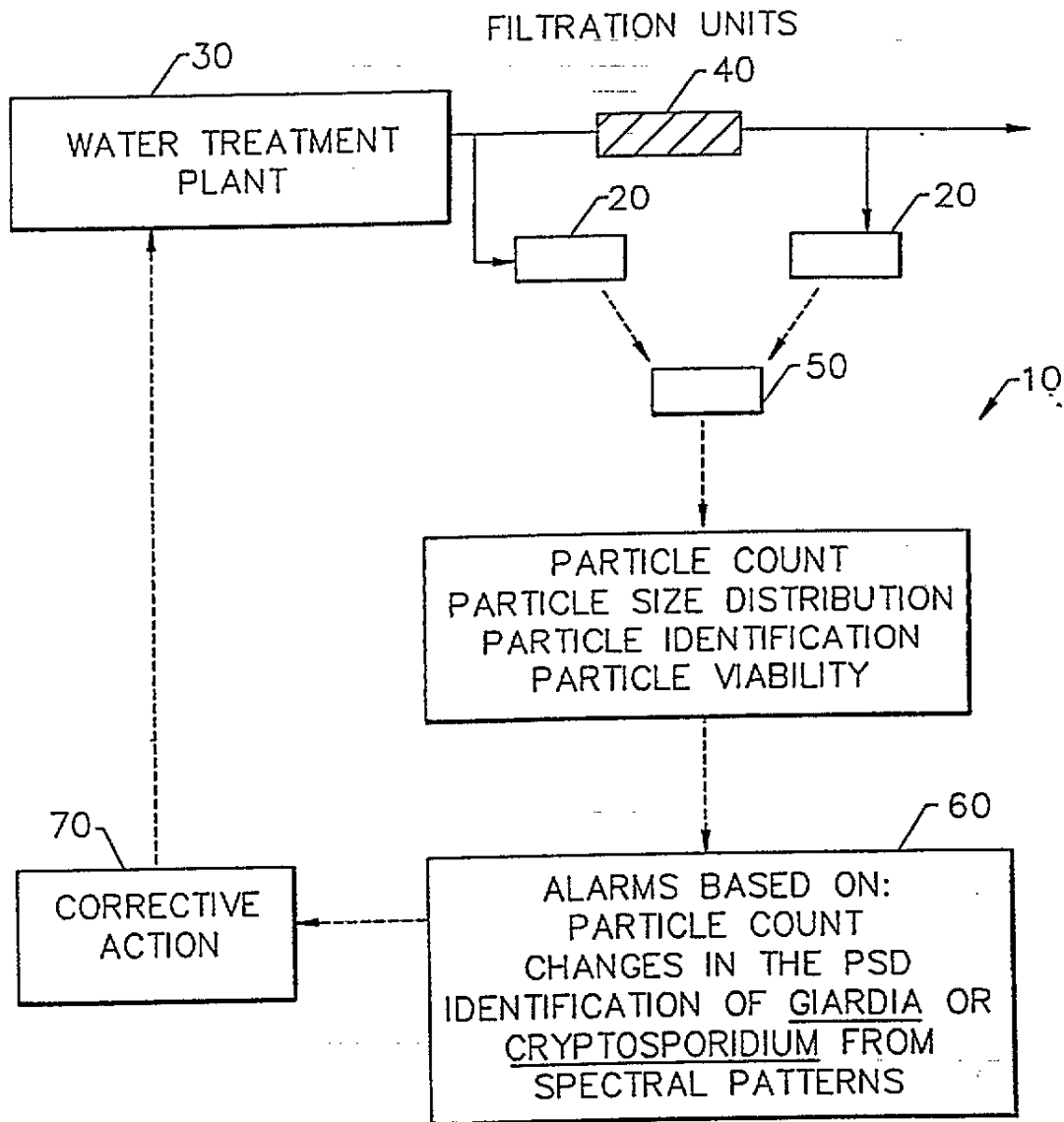


FIG. 8.

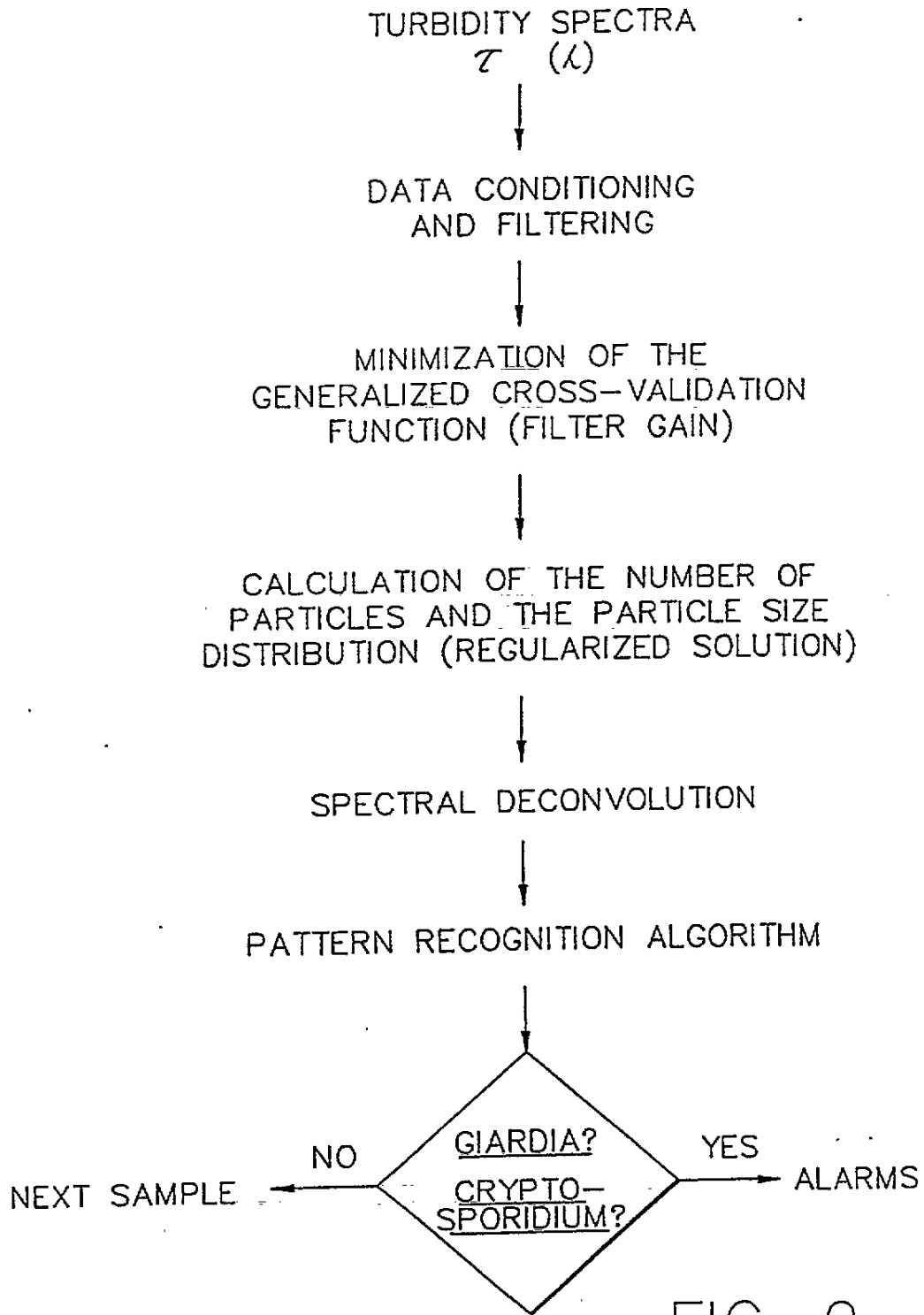


FIG. 9.

## METHOD AND APPARATUS FOR THE DETECTION AND CLASSIFICATION OF MICROORGANISMS IN WATER

### BACKGROUND OF THE INVENTION

#### 1. Field of the Invention

The present invention relates to the detection of organisms in liquids, and, more particularly, to the spectrophotometric detection of microorganisms in aqueous solutions.

#### 2. Description of Related Art

Water quality is an extremely important environmental issue, particularly as regards the quality of drinking water. The number and size of the particulate matter suspended in drinking water is continuously monitored after the filtration units in water treatment facilities, specifically to detect the presence of microorganisms such as enteroviruses, protozoa, and bacteria capable of presenting serious health hazards.

The enteric protozoa *Cryptosporidium* and *Giardia* are known to cause waterborne diseases, even when present in fairly dilute concentrations. *Giardia* is the most frequently identifiable agent responsible for such diseases in the United States; *Cryptosporidium* has caused outbreaks in the United Kingdom as well as the United States and is now recognized as being one of the most disinfectant-resistant waterborne pathogens known. These occurrences have emphasized the need for rapid detection techniques or contaminants in source and treated water.

Currently there is no on-line instrumentation capable of detecting, counting, and classifying specific microorganisms. The technology known in the art requires that the particulate matter suspended in the water be concentrated and then detected with the use of microscopic techniques. Such laboratory techniques include immunofluorescent assay (IFA), polymerase chain reaction (PCR), flow cytometry (FC), and cell sorting. IFA technology may be used in conjunction with microscopy for identification following labeling with specific antibodies. The limitations associated with IFA include long analysis times, an inability to detect viability and to distinguish between species, and low sensitivity. The disadvantages of PCR include environmental interference, long analysis times, and an inability to quantify organisms. FC has the disadvantages of high instrumentation costs, high level of training of personnel required, an inability to distinguish between species, and small sample volumes.

In industrial settings, detection methods include turbidity and particle counting. Turbidity is known as a technique for evaluating filter efficiency and water quality and can be used on line. Standard turbidity measurements respond to both particle size and number; therefore, they do not distinguish between the two. Liquid-borne particle counters (LPC) illuminate a very small sample volume for analysis and have not traditionally been used for on-line applications. Although LPC cannot differentiate between species, if coupled with adequate sampling strategies, particle counters can be used effectively for on-line applications.

As is known from spectroscopy theory, a measure of the absorption of a solution is the extinction coefficient, which also provides a measure of the turbidity. Spectra in the visible region of the electromagnetic spectrum reflect the presence of metal ions and large conjugated aromatic structures double-bond systems. In the near-uv region small conjugated ring systems affect absorption properties. However, suspensions of very large particles are powerful scatterers of radiation, and in the case of microorganisms, both

light scattering and absorption effects are sufficiently strong to permit quantitative detection and classification. It is therefore known to use uv/vis spectroscopy to monitor purity, concentration, and reaction rates of such large molecules.

Many attempts have been made to estimate the PSD and the chemical composition of suspended particles using optical spectral extinction (turbidity) measurements. However, previously used techniques require that either the form of the PSD be known a priori or that the shape of the PSD be assumed. The present inventor has applied standard regularization techniques to the solution of the turbidity equation and has demonstrated correct PSDs of a large variety of polymer lattices, protein aggregates, silicon dioxide particles, and microorganisms.

### SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a spectroscopic and turbidimetric technique for the identification and classification of microorganisms in a liquid medium.

It is a further object to provide on-line instrumentation capable of detecting, counting, and classifying particulates in an aqueous medium.

It is an additional object to provide such instrumentation having at least 2 nanometer resolution.

It is another object to provide a technique capable of detecting the viability of microorganisms present in a liquid medium.

It is yet a further object to provide a monitoring technique for drinking water to prevent the passage of waterborne pathogens into the drinking water supply.

These and other objects are addressed by the apparatus and method of the present invention for detecting the presence of an organism in a sample of liquid. The method comprises the steps of collecting an extinction spectrum over a predetermined range of wavelengths of the sample of liquid and then deconvoluting the spectrum to obtain a particle size distribution for the sample. The wavelength range generally comprises the entire ultraviolet/visible (uv/vis) range, from 180 to 900 nm. In a particular embodiment, however, the spectrum is collected over the wavelength range of 400 to 820 nm. Previously used systems have used a single frequency to classify organisms, whereas in the present invention the whole spectrum is solved to obtain a self-consistent solution. The spectrum and the particle size distribution are then compared with, respectively, a control spectrum and a control particle size distribution for the organism. From these comparisons it can be determined from the comparisons whether the organism to be detected is present in the sample.

An additional embodiment of the method, used for particle counting applications, further comprises the step of determining the quantity of the organism to be detected in the sample from the particle size distribution.

Yet another embodiment entails determining the viability of the organism to be detected in the sample from the particle size distribution.

The apparatus of the present invention comprises means for performing the above-listed steps. In a particular embodiment, the spectrum collecting means comprises a spectrophotometer.

The features that characterize the invention, both as to organization and method of operation, together with further



objects and advantages thereof, will be better understood from the following description used in conjunction with the accompanying drawing. It is to be expressly understood that the drawing is for the purpose of illustration and description and is not intended as a definition of the limits of the invention. These and other objects attained, and advantages offered, by the present invention will become more fully apparent as the description that now follows is read in conjunction with the accompanying drawing.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows extinction spectra for *Cryptosporidium parvum* and *Giardia lamblia*.

FIG. 2 shows extinction spectra for *Cryptosporidium parvum* indirectly stained with ab-FITC.

FIG. 3 shows extinction spectra for *Giardia lamblia* indirectly stained with ab-FITC.

FIG. 4 shows extinction spectra for *Cryptosporidium parvum* directly stained with ab-rhodamine.

FIG. 5 shows extinction spectra for *Giardia lamblia* directly stained with ab-rhodamine.

FIG. 6 is a weight-based particle size distribution of *C. parvum* and *C. baileyi*.

FIG. 7 is a weight-based particle size distribution of *G. lamblia* and *G. muris*.

FIG. 8 is a block diagram of the system configuration.

FIG. 9 is a flow chart of the data analysis method.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

A description of the preferred embodiments of the present invention will now be presented with reference to FIGS. 1-9.

The apparatus 10 and method of using same for detecting the presence of an organism in a sample of liquid are depicted in FIG. 8. In the particular embodiment to be treated herein for microorganisms, the protozoa *Cryptosporidium parvum* and *Giardia lamblia* in aqueous dispersion are detected through spectroscopic means over a predetermined wavelength range. In this embodiment, shown in FIG. 8 for the detection of the protozoa at a water treatment plant 30 having a filtration unit 40, a uv/vis extinction spectrum is collected with a pair of spectrophotometers/turbidimeters 20, one upstream of the filtration unit 40 and one downstream of the filtration unit 40 over the predetermined wavelength range is generally 400-820 nm. Three exemplary spectrophotometers that may be utilized comprise an Ocean Optics S-1000 miniature diode array spectrophotometer, a Hewlett-Packard 8452A diode array spectrophotometer, and a Perkin Elmer 3840 uv/vis spectrometer.

The measurements reported herein were conducted at room temperature in a 1-cm-path-length cell. Prior to collecting a spectrum, it should be determined whether the water sample is in a linear range of the spectrophotometer 20. If necessary, the water sample is diluted with a background solution to reach the linear range of the spectrophotometer 20. The data are then transmitted to a computer 50, which is configured in this exemplary embodiment to perform analytical tasks such as data processing, to issue alarms, and to issue signals for control action.

A spectrum obtained in this manner is then corrected for background. The average refractive index of the protozoa was approximated as 1.386 and was assumed constant as a

function of wavelength. The refractive index of water as a function of wavelength was calculated from previously published data.

Exemplary extinction spectra of *Giardia lamblia* and *Cryptosporidium parvum* are shown in FIG. 1. *C. parvum* is known to be spherical and to have a size of approximately 4-5  $\mu\text{m}$ ; *G. lamblia* is ovoid, with a length of approximately 10-15  $\mu\text{m}$  and a width of 6-10  $\mu\text{m}$ . These spectra are typical of purified samples diluted with formalin.

A tail trailing toward longer wavelengths can be seen in FIG. 1, indicating the presence of particulate matter. The greater intensity of the *G. lamblia* spectrum as compared with that of *C. parvum* is due to the greater scattering caused by the greater size of *G. lamblia*.

Comparison of the spectra in the wavelength region 200-400 nm indicates that there are spectral features that are usable for identification and classification purposes. It is known that staining a microorganism prior to collecting the spectrum can improve the sensitivity of spectroscopy measurements. Two stains that have been utilized on the protozoa under examination are ab-FITC complex and ab-rhodamine.

FIGS. 2 and 3 show the extinction spectra of *C. parvum* and *G. lamblia*, respectively, indirectly stained with ab-FITC complex, which fluoresces apple-green (the wavelength is in the general range of 300 nm; however, scattering and absorption will cause a shift in the pattern, making these numbers approximate). FITC has been known in the art for use by microscopists and can enable detection of fewer than ten organisms per milliliter from a purified stock. A comparison of the spectra in FIG. 1 with those in FIGS. 2 and 3 clearly indicates that staining with ab-FITC enhances the sensitivity of the spectroscopic measurements. In addition, the use of staining allows a direct comparison between the present results and previous microscopy analysis. It can also be noted that other features of the spectra are retained, with *G. lamblia* having a higher intensity in the visible range, due to its larger size.

FIGS. 4 and 5 show the extinction spectra of *C. parvum* and *G. lamblia* stained with ab-rhodamine. This stain, which is red (as above for ab-FITC, an approximate wavelength range is generally 400 nm), is known for use in microscopy studies. A comparison of FIGS. 1-5 indicates that the distribution of stains is different for the two protozoa and that, in addition to enhancing the signal, the use of stains greatly facilitates the identification and classification of microorganisms.

The collection of spectra such as shown in FIGS. 1-5 therefore permits an identification of microorganisms based on spectral signature; that is, the sample spectrum can be compared with a control spectrum for the organism to determine whether the organism is present in the water sample.

A spectrum collected as discussed above is then analyzed with the use of computation means 50, which comprises a computer having software for deconvoluting the spectrum to obtain a particle size distribution for the sample.

The present inventor has devised a technique for determining a discretized particle size distribution from turbidity spectra, shown diagrammatically in FIG. 9. The equations providing the theoretical framework are developed from a relation between the turbidity as a function of wavelength  $\tau(\lambda_0)$  and the normalized particle size distribution  $f(D)$ :

$$\tau(\lambda_0) = N_p(\pi/4) \int_0^{\infty} Q(\lambda_0, D) D^2 f(D) dD, \quad (1)$$

where  $D$  is the effective particle diameter,  $Q(\lambda_0, D)$  corresponds to the Mie scattering coefficient, and  $N_p$  is the number of particles per unit volume. Equation (1) can be written in matrix form by discretizing the integral with an appropriate quadrature approximation:

$$\tau = A f + \epsilon, \quad (2)$$

where  $\epsilon$  represents both experimental errors and errors due to the model and the discretization procedure. The regularized solution to Eq. (2) is given by:

$$f(\gamma) = (A^T A + \gamma H)^{-1} A^T \tau, \quad (3)$$

where  $H$  is a covariance matrix that essentially adaptively filters the experimental and the approximation errors ( $\epsilon$ ), and  $\gamma$  is the regularization parameter estimated using the generalized cross-validation technique. This technique requires the minimization of the following objective function with respect to  $\gamma$ :

$$V(\gamma) = m \{ [I - A(A^T A + \gamma H)^{-1}] \}^{-2} \tau^T \{ [I - A(A^T A + \gamma H)^{-1}] A^T \}^{-2} \tau \quad (4)$$

A simultaneous applications of Eqs. (3) and (4) to the measured turbidity spectra yields the discretized particle size distribution. The corrected scattering spectra can then be used for composition analysis and/or to fingerprint the absorption characteristics of particles.

Quantitative information, such as shown in FIGS. 6 and 7, may be obtained using the method of the present invention from the use of Eqs. (3) and (4) and spectra such as shown in FIG. 1.

FIG. 6 shows a weight-based particle size distribution (PSD) for *C. parvum* (peak 2) and *C. baileyi* (peaks 1 and 3). Peaks 2 and 3 are centered around 4.8  $\mu\text{m}$ , the size normally associated with *C. parvum*. The difference between the two samples is seen at peak 1, which is centered at 2.5  $\mu\text{m}$ . By deconvoluting the extinction spectra of the purified samples, the PSD was obtained. The abscissa represents the diameter of the microorganisms, and the ordinate, the weight-based density. Peak 1 is indicative of cell debris from broken oocysts and sporozoites; the *C. parvum* sample was purified by high-performance liquid chromatography, whereas the *C. baileyi* sample was not.

FIG. 7 shows the PSD for *G. muris* (peaks 1, 4, and 6), and *G. lamblia* (peaks 2, 3, and 5). Peaks 1 and 2, approximately located at 2.8  $\mu\text{m}$ , are indicative of cell debris resulting from the preparation of the samples. Peaks 3 and 4 are located at approximately 9.0  $\mu\text{m}$  and correspond to typical dimensions of both *G. lamblia* and *G. muris*. Peaks 5 and 6, centered at approximately 16  $\mu\text{m}$ , correspond to cell aggregates and/or cells oriented perpendicular to the incident light.

Utilizing the above method to calculate the particle size distribution, the PSD can then be compared with a control particle size distribution for the organism, and then one can determine from the comparison whether the organism to be detected is present in the sample.

These calculations can also be used to determine the quantity of the organism to be detected in the sample from the particle size distribution.

Another embodiment of the method of the present invention comprises determining the viability of the organism to be detected in the sample from the particle size distribution. In this embodiment staining would again be used, with the

stain uptake providing an indication of particle viability, for instance. This technique could also be performed on-line.

A particular use for the apparatus and method of the present invention is for on-line, real-time measurements to detect the presence of an organism in a flowing liquid. In order to accomplish this, a sample is taken from the flowing liquid, and an extinction spectrum is collected over a predetermined range of wavelengths of the sample of liquid, the spectrum being collected at the site of the sample collection. Staining may also be used as described above.

As previously the spectrum is deconvoluted to obtain a particle size distribution for the sample, and the spectrum and the particle size distribution are compared with, respectively, a control spectrum and a control particle size distribution for the organism. From these comparisons it can be determined whether the organism to be detected is present in the sample.

Also as previously, the quantity and the viability of the organism to be detected in the sample can be determined from the particle size distribution.

It is contemplated that such an on-site apparatus could be placed in communication with virtually any water line, including at an entrance to a dwelling. Should harmful particles be detected, an alarm could ring, as shown in FIG. 8 for a water treatment plant 30, indicating that the water flowing through the system was unsafe. Corrective action would then be taken, such as shutting down the water flow at the plant, and the water could be subjected to additional purification prior to proceeding downstream.

It may be appreciated by one skilled in the art that additional embodiments may be contemplated, including systems and methods for detecting other types of organisms and particles in a liquid, for studying and monitoring organism life cycles, for examining drug delivery mechanisms, for studying the mechanisms of infectious diseases, and for detecting abnormal cells.

In the foregoing description, certain terms have been used for brevity, clarity, and understanding, but no unnecessary limitations are to be implied therefrom beyond the requirements of the prior art, because such words are used for description purposes herein and are intended to be broadly construed. Moreover, the embodiments of the apparatus illustrated and described herein are by way of example, and the scope of the invention is not limited to the exact details of construction.

Having now described the invention, the construction, the operation and use of preferred embodiment thereof, and the advantageous new and useful results obtained thereby, the new and useful constructions, and reasonable mechanical equivalents thereof obvious to those skilled in the art, are set forth in the appended claims.

What is claimed is:

1. A method for detecting the presence of an microorganism in a sample of liquid, the method comprising the steps of:

collecting an extinction spectrum over a predetermined range of wavelengths of the sample of liquid;  
deconvoluting the spectrum to obtain a particle size distribution for the sample;

comparing the spectrum and the particle size distribution with, respectively, a control spectrum and a control particle size distribution for the microorganism; and  
determining from the comparisons whether the microorganism to be detected is present in the sample.

2. The method for detecting the presence of an microorganism recited in claim 1, further comprising the step of determining the quantity of the microorganism to be detected in the sample from the particle size distribution.

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3. The method for detecting the presence of an microorganism recited in claim 1, further comprising the step of determining the viability of the microorganism to be detected in the sample from the particle size distribution.

4. The method for detecting the presence of an microorganism recited in claim 1, wherein the collecting step comprises collecting an extinction spectrum over a wavelength range of 400 to 820 nm.

5. A method for spectrophotometrically detecting the presence of a microorganism in a water sample, the method comprising the steps of:

determining whether the water sample is in a linear range of the spectrophotometer;

diluting the water sample if necessary with a background solution to reach the linear range of the spectrophotometer;

using the spectrophotometer to collect a turbidity spectrum of the water sample over the wavelength range of 400 to 820 nm;

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correcting the spectrum for background;

calculating a particle size distribution from the corrected spectrum;

comparing the particle size distribution with a known particle size distribution for the microorganism to be detected; and

determining from the comparison whether the microorganism is present in the water sample.

6. The method recited in claim 5, further comprising the step of staining the microorganism prior to collecting the turbidity spectrum for improving sensitivity.

7. The method recited in claim 5, further comprising the step of quantifying, from the particle size distribution, the number of microorganisms present in the water sample.

8. The method recited in claim 5, further comprising the step of determining, from the particle size distribution, the viability of the microorganisms present in the water sample.

\* \* \* \* \*

SCHEDULE 2

PATENT POSITION

US Patent No. 5,616,457 dated April 1, 1997 under the title "Method and Apparatus for the Detection and Classification of Microorganisms in Water"

Schedule 2.01(I)

**SWT Unaudited Financial Statements**

**SAFE WATER TECHNOLOGIES, INC.**

**PLANT CITY, FLORIDA**

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**FINANCIAL STATEMENTS**

**AND ACCOUNTANTS' COMPILATION REPORT**

**FOR THE INTERIM PERIOD SEPTEMBER 13, 1999 TO SEPTEMBER 24, 1999**

**CONTENTS**

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Clifford Gene Myers  
Carole R. Mason

**MYERS, MASON & CO., P.A.**  
Certified Public Accountants

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To the Board of Directors of  
Safe Water Technologies, Inc.:

We have compiled the accompanying balance sheet of Safe Water Technologies, Inc. as of September 24, 1999 and the related statements of operations and retained earnings and cash flows for the interim period then ended, in accordance with Statements on Standards for Accounting and Review Services issued by the American Institute of Certified Public Accountants.

A compilation is limited to presenting in the effort of financial statements information that is the representation of management. We have not audited or reviewed the accompanying financial statements and, accordingly, do not express an opinion or any other form of assurance on them.

Tampa, Florida  
September 24, 1999

*Myers, Mason & Co., P.A.*  
- Certified Public Accountants

**SAFE WATER TECHNOLOGIES, INC****BALANCE SHEET  
SEPTEMBER 24, 1999****ASSETS****ASSETS**

Organization expense	\$ 600
Less accumulated amortization	<u>(10)</u>

**TOTAL ASSETS****\$ 590****LIABILITIES AND STOCKHOLDER'S EQUITY****LIABILITIES****None****STOCKHOLDER'S EQUITY:**

Common stock \$.001 per share; 10,000,000 shares authorized, 1,000 shares issued and outstanding	\$ 1
Additional paid in capital	599
Retained deficit	<u>(10)</u>

**Total stockholder's equity****590****TOTAL LIABILITIES AND STOCKHOLDER'S EQUITY****\$ 590**



**SAFE WATER TECHNOLOGIES, INC.****STATEMENT OF OPERATIONS AND RETAINED EARNINGS  
FOR THE INTERIM PERIOD SEPTEMBER 13, 1999 TO SEPTEMBER 24, 1999**

REVENUE	None
EXPENSES:	
Amortization	<u>\$ 10</u>
NET LOSS BEFORE INCOME TAX	(10)
PROVISION FOR INCOME TAX	<u>None</u>
NET LOSS	(10)
RETAINED EARNINGS, SEPTEMBER 13, 1999	<u>None</u>
RETAINED DEFICIT, SEPTEMBER 24, 1999	<u>\$ (10)</u>

**SAFE WATER TECHNOLOGIES, INC.****STATEMENT OF CASH FLOWS**  
**FOR THE INTERIM PERIOD SEPTEMBER 13, 1999 TO SEPTEMBER 24, 1999****CASH FLOWS FROM OPERATING ACTIVITIES:**

Net loss	\$ (10)
Adjustments to reconcile net loss to net cash provided by operating activities -	
Amortization	10
Increase in operating assets:	
Organization expense	<u>(600)</u>
Net cash used by operating activities	<u>(600)</u>

**CASH FLOWS FROM FINANCING ACTIVITIES:**

Proceeds from capital contributions	<u>600</u>
Net cash provided by investing activities	<u>600</u>

NET INCREASE IN CASH None

CASH AT SEPTEMBER 13, 1999 None

CASH AT SEPTEMBER 24, 1999 None

See Accountants' Compilation Report and Notes to Financial Statements.

Unaudited

Page 4

SAFE WATER TECHNOLOGIES, INC.

NOTES TO FINANCIAL STATEMENTS  
FOR THE INTERIM PERIOD SEPTEMBER 13, 1999 TO SEPTEMBER 24, 1999

NOTE 1 - SUMMARY OF SIGNIFICANT ACCOUNTING POLICIES:

**FORMATION -**

The Corporation was formed under the laws of the State of Florida on September 13, 1999 and is wholly owned by Utek Corporation.

**ACCOUNTING BASIS -**

The financial statements were prepared on the accrual basis of accounting.

**FEDERAL INCOME TAX -**

It is intended that the merger with CENTREX, Inc. qualify as a tax-free reorganization within the meaning of applicable provisions of the Internal Revenue Code.

**NOTE 2 - SUBSEQUENT EVENT:**

The Company has agreed to be merged with CENTREX, Inc. The merger agreement provides for the assets and business of the Company to be owned by CENTREX, Inc., all liabilities and obligations of the Company to become the liabilities and obligations of CENTREX, Inc., and all issued and outstanding shares of capital stock of the Company to be exchanged for common stock of CENTREX, Inc. The separate corporate existence of Safe Water Technologies, Inc. will cease and CENTREX, Inc. will continue as the surviving corporation.

Schedule 2.02(j)

**CENTREX Unaudited Financial Statements**

ICTRX 99-00  
Balance Sheet  
September 30, 1999

ASSETS

Current Assets	
Cash - Bank of Oklahoma	\$ 29.96
Prepaid Expenses	1,250.00
	<hr/>
Total Current Assets	1,279.96
Property and Equipment	
	<hr/>
Total Property and Equipment	0.00
Other Assets	
Licensed Technology	7,000.00
Sponsored Research	517,500.00
	<hr/>
Total Other Assets	524,500.00
	<hr/>
Total Assets	\$ 525,779.96
	<hr/> <hr/>

LIABILITIES AND CAPITAL

Current Liabilities	
Accounts Payable	\$ 2,050.00
Payable to IRC	7,224.50
Payable to University of Calif	105,000.00
Payable to Mabie	52,500.00
	<hr/>
Total Current Liabilities	166,774.50
Long-Term Liabilities	
Payable to University of Calif	235,000.00
Payable to Shareholders	125,000.00
	<hr/>
Total Long-Term Liabilities	360,000.00
	<hr/>
Total Liabilities	526,774.50
Capital	
Common Stock	7,000.00
Paid in Capital	<4,054.69>
Net Income	<3,939.85>
	<hr/>
Total Capital	<994.54>
	<hr/>
Total Liabilities & Capital	\$ 525,779.96
	<hr/> <hr/>