Los Alamos

NATIONAL LABORATORY
TA-46, Bldg 42, MS J580
Los Alamos, NM 87545
Phone: 505-665-4186
FAX: 505-665-3911
E-mail: DBURNS@LANL.GOV
NIRman@AOL.COM

Date: Oct 9, 1997

To: Dr John Elling
MS J-580

Dora Vigil, Contract Administrator Assistant
MS P-274

FINAL REPORT

Subcontract #: C58660016-C

Subcontractor: Donald A Burns, dba NIR Resources
2 La Flora Court
Los Alamos, NM 87544

Work performed for: Dr John W Elling
MS J-580
Los Alamos National Laboratory
Los Alamos, NM 87545

Contract Title: Extraction Studies

Period of Effort: May 6, 1996 to Sept 30, 1997

SUMMARY

During the first week of this effort, an *Alpkem RFA-300* 4-channel automated chemical analyzer, used by me in the CMR Building while employed in the CLS Division, was transfered to the basement of building 42 at TA-46 for the purpose of performing extraction studies. Initially, this instrumentation was applied to soil samples known to contain DNA. Using the SFA (Segmented Flow Analysis) technique, several fluidic systems were evaluated to perform on-line filtration of several varieties of soil obtained from Cheryl Kuske and Kaysie Banton (TA-43, Bldg 1). Progress reports were issued monthly beginning May 15, 1996.

Early in 1997 there was a shift from the conventional 2-phase system (aqueous + air) to a 3-phase system (oil + aqueous + air) to drastically reduce sample size and reagent consumption. Computer animation was recorded on videotape for presentations to (a) University of California researcher Scott Lane in February, and (b) *Amgen* scientists in May. The time remaining on the subcontract was devoted to setting up existing equipment to incorporate the 3rd phase (a special fluorocarbon oil obtained from DuPont).

DISCLAIMER

Following termination of this contract, the position of *Guest Scientist* is expected so that this study can continue with the hope of (a) procuring a modest grant of funding and/or equipment from *The Hamilton Company* (or manufacturer of similar instrumentation), and (b) pursuing the study long enough to demonstrate the potential of a 3-phase microfluidic system for the automated analysis of viruses -- either as a sample preparation module, or as a stand-alone system. A successful demonstration of this could lead to a sizeable grant to LANL.

A copy of the full set of monthly reports follows this page, providing details of this summary.

Respectfuly submitted,

Donald A Burns

Donald A Burns, PhD
NIR Resources

L O S A L A M O S
NATIONAL LABORATORY

ESA-MT, TA-46, Bldg 42, MS J580
Los Alamos, NM 87545

Phone: 665-4186
E-mail: dburns@lanl.gov

Date: May 15, 1996

To: John Elling, J580

From: Don Burns

P R O J E C T R E P O R T

EXTRACTION STUDIES

HISTORY: About 50 hours of effort (required training, off-site conference [6,7], technical discussions, planning sessions, and demonstrations in the Life Science Building by Kaysie Banton) have already taken place. In addition to this, efforts have been underway for several weeks to put a contract in place in the name of "Donald A Burns dba NIR RESOURCES", and subcontract #C58660016-3C became effective on May 6, 1996. This document will be updated periodically and will serve as a chronology of the sub-contractor's activities on the subject project from today forward.

THIS WEEK the Alpkem RFA-300 automated chemical analyzer (which I brought with me from the CST Division) was inventoried to establish what accessories and spare parts might be required to fully utilize the instrumentation. Soil samples known to contain DNA were obtained from Kaysie, and similar material was taken from the ground near Building 42. General clogging of a standard filter prompted modifications in both the filter holder and syringe: drilling a larger conduit in the Luer end of the accompanying syringe, drilling completely through the Luer Lock fitting on the middle part of the 3-part polypropylene filter holder, and enlarging the space above the 25 mm filter element by the insertion of a second o-ring. Since a double o-ring is relatively unstable, a source is being sought for a larger or differently shaped o-ring (Len Stoval is looking through catalogs, and MSI [Micron Separations Inc.] has been queried via FAX). Subsequent experimentation with this modified filter holder strongly suggests that we're on the right track in this first of two approaches to sample cleanup prior to any chemical analysis.

The second approach may explore continuous decantation using the Alpkem continuous flow system. To this end, 3 sizes of glass beads have been obtained from Kaysie and some basic lab equipment (glassware, wash bottles, magnetic stirrer w/bars) has been ordered from Fisher Scientific. Also, continuous centrifugation is being explored via contact with various equipment manufacturers (e.g., Heraeus [8], Beckman, IEC), but this doesn't seem encouraging.

May 20-24: The required CPR class was attended. Having found many small, expensive parts missing from the Alpkem system, a search was made of the laboratory in the CMR building where the system originally operated. Two more boxes of accessories were discovered and they have been moved to the basement of building 42 for classifica-

tion/storage until needed. Except for items ordered from Fisher (some delivered this week, some circumvented by a trip to LANL's glass shop, and others destined for 10-15 days delivery), there was enough equipment to assemble an air-segmented analytical train. Initial attempt at continuous decantation with local dirt was unimpressive, possibly due to a lack of proper surfactant. E-mail from MSI on 5/23 provided phone/FAX for Micro Filtration Systems (supplier of the filter holder); query FAXed 5/24. Telephone discussions with Jacqueline Bower (Director, Sales & Technical Services, Advantec MFS, Inc. [correct name of company whose new address/phone is: 6691 Owens Drive, Pleasanton, CA 94588; phone 800-334-7132, FAX 510-225-0353]) addressed a (a) "removable" screen (but only in the 47mm version), (b) using it backwards (i.e., reversing inlet & outlet) after removing the screen), and (c) pointing the soil-filled syringe upward while transfering suspension into the filter. Literature forthcoming.

May 28-31: The required safety meeting was attended. Paul Mendoza and Evangeline Hodge quizzed re surfactant Brij-35 (needed for SFA); Evangeline came through with a quart bottle. Obtained more glass tubing (with smaller ID) from glass shop. Proceeded with continuous decantation studies. Received balance of materials from Fisher Scientific, and plumbed system with magnetic stirring input. Must optimize to avoid clogging. Meeting today with Kaysie and grad students to discuss everyone's involvement.

June 3-7: New badge obtained; it won't actuate palm reader; telephone tag initiated with badge office; eventual resolution: reprogrammed badge works OK. FAX of 5/31 to Millipore re female-to-female straight-thru connector evoked name of Cole-Parmer to whom a copy was sent on 6/3; their FAXed response included description/price of what we need. Replumbing of Alpkem system appears to produce reasonable segmented sample (dirt) pickup, debubble/rebubble after the pump, mixing/resuspension, and layering within a straight horizontal conduit. Decantation looks possible with some dirt, but not all samples.

June 10-14: Continuing problems with clogging of tubes has led to increasing the conduit IDs for fittings, pump tubes, and straight tube, after which decantation of LANL dirt looked quite good. However, when bead-beaten samples are used, pump tubes deteriorate rapidly, likely due to sharp edges of broken glass beads, but possibly due (in part, at least) to rather old pump tubes. [Microscopic examination of bead-beaten samples should confirm this.] Since the beads are used to lyse the cells, we must identify acceptable alternatives to this procedure that can be accomplished in continuous flow mode (e.g., the addition of an appropriate surfactant). If the subsequent extraction of DNA from the cell/dirt mixture can be accomplished by very efficient mixing (as in a standard mixing coil from Bran+Luebbe or Alpkem where repeated inversions of the liquid segment produce excellent extraction as the air-segmented stream worms its way through the coil), then the bead-beating step could be eliminated. Continuous decantation might then preclude the need for filtration and/or centrifugation (probably at the expense of sample dilution). This has been discussed briefly with Dante, and he will arrange a meeting for the week of June 24th to include anyone who might be able to contribute.

June 24-28: Following discussions with Dr Charles Patton (U S Geological Survey in Boulder CO) on the filtration of soils, contact was made with Whatman's Paul Bogner for samples of UNIPREP line of syringeless filters fitted with GDX stacked filter assemblies. These are arrays of graded filters, and Charlie has had great success with them, handling materials down to 0.2 micron without plugging. Samples have been shipped, should arrive next week, and will be evaluated along with those recently obtained by you and given to me June 27th.

July 1-5: Whatman filters don't look real appropriate for the multi-size particles in the soil samples. Cheryl won't deviate from using all 3 sizes of glass beads, so these and the larger dirt particles must be removed (by filtration?) prior to pumping anything through peristaltic tubing, if this is to be used as a front end to continuous decantation. A ringstand and clamps have been "borrowed" from room C220 at the MSL building, and can be used to properly orient a pistonless-syringe (used as a funnel) and a magnetic stirrer operating in a vertical plane. This configuration permits a small stirring bar to rotate in the syringe filled with sample. The inclusion of a series of graded filters should permit the continuous removal of troublesome particles prior to transfer of the remaining sample thru the pump and into a settling tube for eventual decantation.

July 8-12: Literature and note from Whatman's Paul Bogner describe a 25mm disposable filter funnel that can accommodate graded filters; it could well replace the pistonless syringe arrangement plumbed last week. Your "cloth screens" could be useful here.

July 15-19: Additional soil from Cheryl and 2 grades of cloth ready for continuing filtration studies. Lab-wide stand down curtails effort. Another sample filter funnel (Whatman #4) arrived from Paul Bogner; it accommodates a 25mm filter and can be fitted with a cloth screen and stirring bar. Initial run involved a 100 micron nylon net above a 5 micron membrane filter. With about 4 mL of water and a stirring bar in the funnel, one of Cheryl's "D" samples was poured in and its container washed with another couple of mL of water. The filtrate contained no particulates, was clear and lightly colored, and flowed easily at first. Plumbing included air-segmenting the filtrate, and the changing air/liquid ratio was an indication of the eventual clogging. Clearly, a stacked array of filters will be required.

July 22-26: The plumbing involved a total pull-thru of 0.947 mL/min with air addition at 0.385 mL/min. With a stack consisting of (top-to-bottom) 100 micron net, 8 micron membrane, and 5 micron membrane, Cheryl's bead-beaten sample "C" was poured into 4-5 mL water, with stirring, then its container washed with another 2 mL or so. Eventually all the liquid was pulled thru the filters. See Figure 1 on the next page. After pouring out most of the glass beads, the stack was separated into its original elements and these were examined at 100X under a microscope. The largest bead appeared to have a diameter of 0.0207"; the smallest 0.0017". Both the 180- and 100-mesh nets were examined and this data is available upon request. A few

pieces of broken glass were seen. The 5 micron membrane contained a checkerboard pattern (from its preceding net) of debris, as well as large beads (0.0038" diam) and small beads (0.0010" diam). Several more mesh sizes have been received for study: 80, 60, 40, 31, and 20.

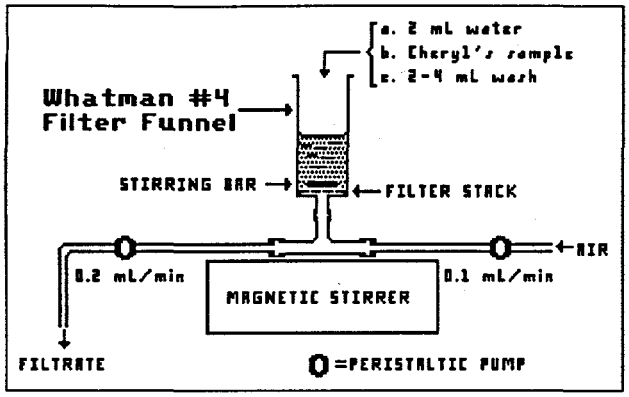


Figure 1

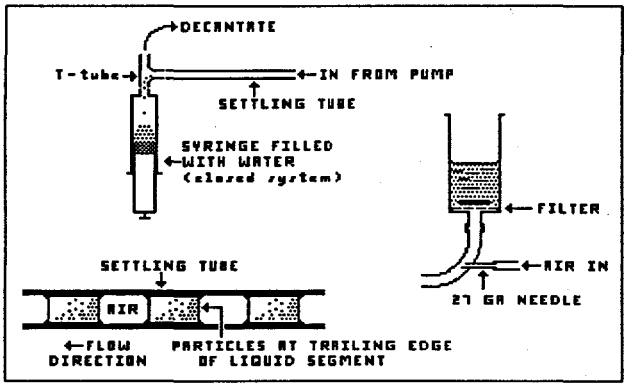
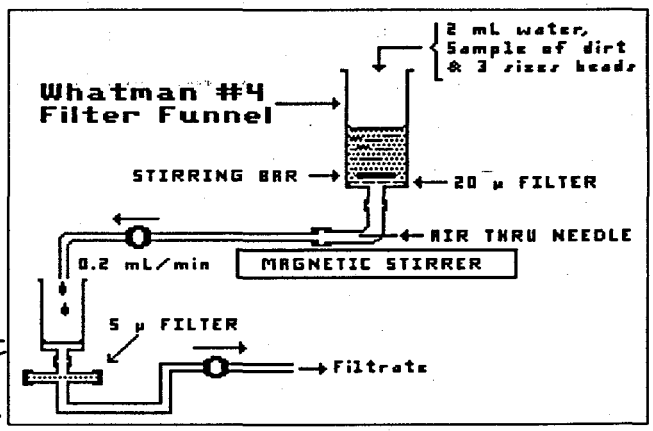


Figure 2

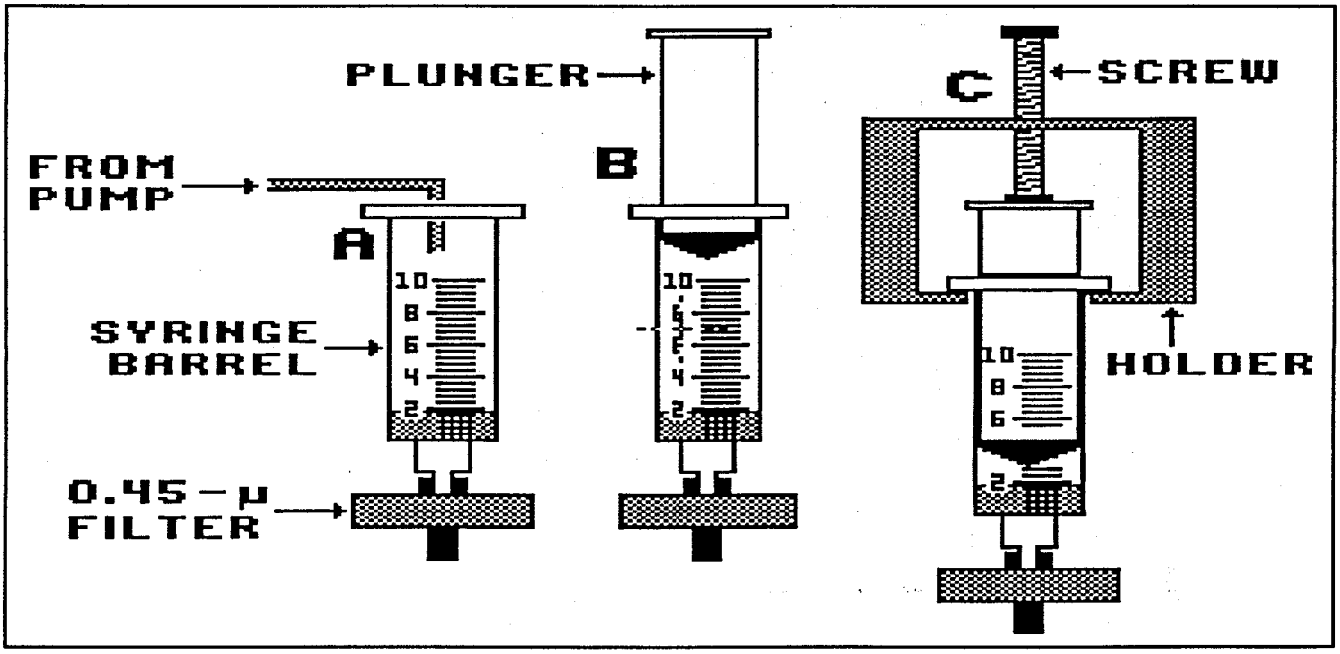
Using only the 100 micron net, sample "D", magnetic stirring, and air-segmentation below the funnel, filtration of about 7.5 mL seemed unhampered -- i.e., air/liquid ratio did not change over time. Air introduction is better using a 27 ga needle through the plastic tubing just below the funnel; air segmentation **only** following the pump leads to premature layering of solids and eventual clogging. Since particles <100 micron emerge from the filter, continuous decantation was employed to help clarify the flowing stream (see Figure 2). Rather than use a pump channel to pull off either the upper or lower stream from the T-tube, a closed system was arranged using a 10 mL syringe adjusted to about 5 mL. The larger particles dropped into the syringe as predicted, but unwanted pulsations in the segmented stream caused some of them to go upward instead of downward. This problem can be resolved with two changes: 1) insertion of a debubbler/rebubbler immediately after the pump, since a uniformly segmented stream passing thru the pump is frequently rendered random and contains many small bubbles, and 2) obtaining the correct size T-tube (those we have from Alpkem are too small, and those from Bran+Luebbe are too large).

July 29-31: Serial filtration as shown at the right was plumbed with considerable success. While not speedy, the resulting filtrate was clear and lightly colored. This was repeated with weighed bead-beaten samples, and a clear, slightly colored filtrate resulted. The addition of air pulses below the 2nd filter improved flow rate somewhat. Next, a DNA recovery study must be done to confirm that little or none is lost in the various conduits.



Aug 12-16: The previously described operations have been speeded up by directing the filtrate from the cloth filter into a syringe (barrel only; no plunger) fitted with a second filter holder containing a 5-micron element. Although this filtrate contained no visible particles (i.e., soil or glass beads), it was highly colored. When about 2 mL had been collected in the syringe, its plunger was inserted, compressed to about the 3-mL mark, and the second filtrate collected. This final filtrate was absolutely clear and had very little color. To avoid having to hold the syringe in this position for any appreciable time, Len Stoval was given some drawings (see Aug 14th memo) and asked to fabricate a syringe holder.

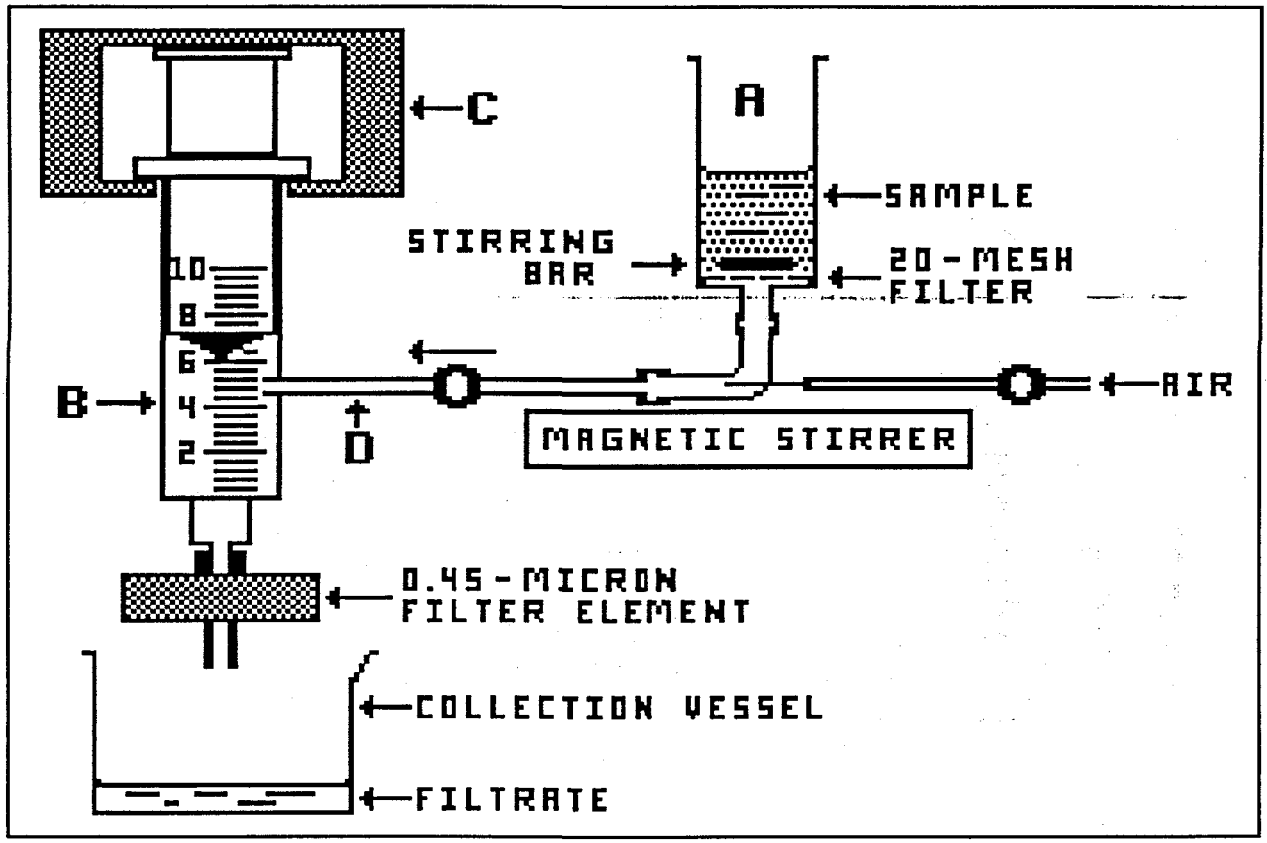
Aug 26-30: The drawings for the syringe holder were modified by Len to include an adjustment to accommodate any plunger position in a 10-mL syringe. The holder was completed and first tested on Aug 29th. Results with a simulated soil sample (i.e., dirt and 3 sizes of glass beads) were encouraging. With both a 5-micron and a 0.45-micron filter element for the second filtration, the 2 mL of highly colored effluent from the peristaltic pump could be rendered clear and almost colorless in about a minute. While not totally automated at this point, the operations require very little effort and should be highly reproducible. See drawing below to understand the modus operandi.



"A" shows an empty syringe receiving the first filtrate; about 2 mL is typically collected. "B" shows the addition of the syringe's plunger just after insertion. "C" shows the plunger pushed down to about the 3-mL mark, putting considerable pressure on the contents of the syringe. The plunger is held in this position by turning the screw on the syringe holder until its tip is properly positioned, thus maintaining pressure on the syringe's contents and forcing it through the 0.45-micron element in the filter without further operator effort.

Everything accomplished to date should be documented with photographs, photomicrographs, and videotape (with appropriate animation).

Sept 3-6: Digital camera obtained from Tracy Erkkila; batteries need recharging before it can be used. John Elling tracking down a lab-supplied video camera. A closed system was designed by drilling a hole in the side of a 10-mL syringe at about the 5-mL mark and inserting therein a nipple to which a conduit from a peristaltic pump could be attached. With the piston's tip adjusted to about the 6-mL mark, the syringe was positioned in a recently-designed holder whose screw was adjusted to maintain the syringe/piston geometry. Refer to the following diagram to understand how this is used.



Starting with a dry filter beneath "B", about 5 mL of water was put into stirred vessel "A". When this liquid reached "B", about 2 mL accumulated in the syringe before breaking through the 0.45-micron filter. The liquid moved through the filter even sooner when the filter was pre-wet with water.

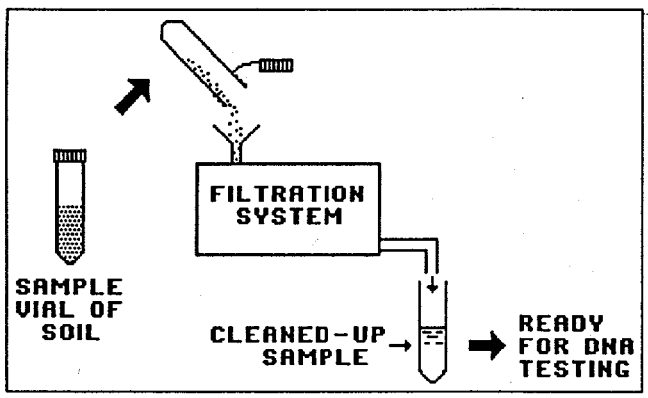
Using the correct ratio of 3 sizes of glass beads, soil, water, and buffer, a clear (almost colorless) filtrate was obtained. The original 2-mL sample (about 1:1 solid:liquid) was washed twice with 2-mL portions of water, providing a total liquid volume of about 5 mL. From this, about 3.2 mL of filtrate was collected without operator intervention. Then, after removing the connection at "D" and removing "B" from its holder "C", the piston was manually driven home to exude immediately another 0.7 mL of filtrate. A small amount of liquid remained in conduit "D".

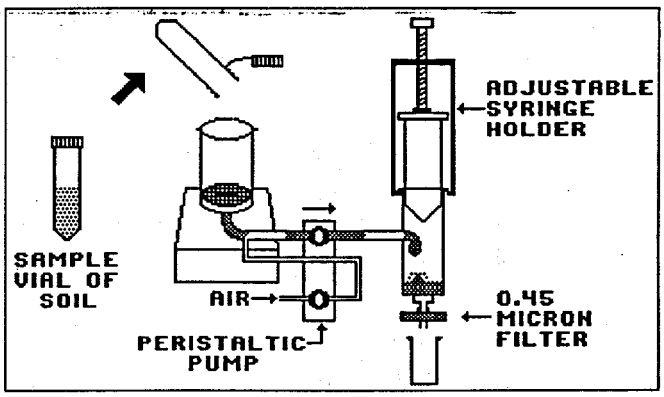
Extraction recovery and fragmentation study done on 4 real samples from Kaysie Banton: #1 run thru acetate filter; #2-4 run thru PVDF

filters. New filters used for each sample, all rinsed with 1-2 mL IXTENS buffer. All filtrates were considerably darker than previous runs where soil samples were dispersed in water, not buffer. However, there were no visible particles, and Kaysie seemed pleased with the appearance of the filtered samples. The proof of the pudding will come after she has analyzed them for DNA.

It appears that a fairly simple system can be fabricated using a relatively inexpensive 2-channel peristaltic pump together with the modules identified in this report. A larger pump and multiple magnetic stirrers would accommodate parallel sample processing.

Sept 9-13: A video program has been assembled from (a) about 28 minutes of videotaping of our automated filtration system, and (b) another few minutes of animation. Two of the composit scenes from animation sequences are shown below. The edited program runs about 8 minutes; you may want it reviewed by Kaysie and Cheryl for accuracy.

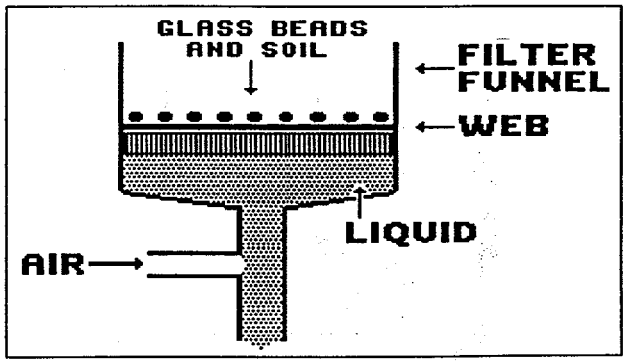




The left-hand figure above is a summary of what the video program includes -- i.e., a description of the various operations performed by the system between sample introduction and final clean sample. The right-hand figure is the result of several intermediate versions that identifies each module and how all modules work together to produce the clean sample for further testing.

Photomicrographs have been taken on 35mm color film in a Nikon camera designed to fit the microscope. The pictures will show individual webs with and without the various sizes of glass beads that they retain.

One additional animation sequence could depict the reverse flow where stirred material is periodically (every two seconds) lifted off the surface of the web to help prevent clogging of the filter. The animation for this introduces an air bubble that pushes the particles off the filter, then lets them settle on its surface before repeating the sequence.



Sep 16-20: The video program, intended to supplement the report which Cheryl will present at the end of the month, was completed this week. With the help of Torsten Staab, an IBM laptop computer was able to accept the Storyboard program and display the animation on its screen. This unit was taken over to CIC-9 where Fred Baker and Mike Kuchinski used their scan-converting camera to videotape the animation sequences off the laptop's screen. Results were disappointing: although scan lines disappeared, the colors and contrast left much to be desired. Specifically, the motion of the spinbar was all but invisible, and much of the animated flow was unimpressive. Accordingly, the video output from the laptop was run directly into a VCR, with overall better results. This is a tradeoff between slightly visible scan lines and far better color.

The photomicrographs were received, and are now part of the video program. Openings in both the 100- and 20-micron nylon web filters are seen, along with retained glass beads of all sizes. Also, the retention of the soil debris is apparent. Your narration, titles, and credits have been added to the video program, and the edit-master has been delivered to you.

Sep 23-27: Discussion with Len Stoval re alternative to commercial bead beater: he'll fabricate a device from a sabre saw and hair dryer to establish proof of principle. In the meantime, we've found 10 centrifuge tubes containing somewhat less than half a gram of various soils (2 each "C", "G", and clay; 4 "D"). Two of the type "D" soils were modified by adding 300 mg each of the 3 glass bead sizes and 1 mL buffer, then hand-shaken and run through our filtration system. Each tube was rinsed with 1 mL buffer and this was added to the stirred funnel. Filtrates were clear and lightly colored.

The remaining 8 samples were run as above, but with bead beating. Filtrates were particle-free, but the "C" and CLAY require some commentary. "C" soil samples required higher pressure (i.e., compression of the syringe) to exude filtrate, which was considerably darker. The CLAY, on the other hand, produced a much lighter colored filtrate. In an attempt to reduce loss due to the internal volume of the filter element, one of the two CLAY samples was directed into a LIDA #6499-06 0.45 micron PS filter, since its diameter was much smaller than the PVDF filters used for all the other samples. Although the first few drops of filtrate were very lightly colored, it required so much compression of the syringe that the filter element ruptured.

Since the 0.45 micron final filter retains about 1/2 mL of the filtrate (e.g., 3.01 g wet - 2.46 g dry), it seems prudent to rinse with <u>at least</u> that volume of buffer. Higher volumes are likely to provide greater recovery of the DNA, albeit at the expense of a lower concentration. An important question to be answered is "which is more important: final volume or final concentration?" And if the answer is final volume, then "what is the minimum acceptable volume?"

Discussion with Cheryl & Kaysie produced the following caveats: (1) soil samples not refrigerated need not be further analyzed for DNA, (2) to keep final volumes as small as possible, rinsing should not be done, and (3) similarly, filters should not be pre-wet with buffer, since this also dilutes the final sample. In mid October we'll likely receive more samples.

Further discussion with Cheryl revealed an interest in a filtration system capable of operation from a 12-volt battery. This would affect these modules: peristaltic pump, the magnetic stirrer, the bead beater, and the means of heating the sample. Len Stovall has been alerted to this potential requirement.

Oct 1996: Little has been accomplished the first three weeks of this month beyond (a) reviewing the "final" report on extraction, (b) playing "telephone tag" with personnel at the Life Science building regarding what samples to run and when, and (c) arranging for an office move [which is now on "hold" pending a decision by Tony Beugelsdijk and others].

On the 29th, several soil samples were prepared by Kaysie, half of which were passed through the automated filtration system. All samples and the resulting filtrates were kept on ice except while actually passing through the 2-stage filtration system. **Modus operandi**: cold bead-beaten samples were poured into new, stirred filter funnels containing a 20-micron web, and filtrate collected from a second new filter [0.45 micron]. One mL of buffer was then used to rinse the original sample vial. The rinse was passed through the system, and a second (final) filtrate collected in a separate container. For the subsequent DNA analysis, the operator had the option of combining filtrates or not.

In all cases, there was significant holdup in the second filter element; on one run **all** of the first filtrate remained in this module, even with manual manipulation of the syringe/plunger above the module. Since the diameter of this filter element is about one inch, it seems prudent to obtain smaller elements, e.g., some with a half-inch diameter and correspondingly smaller holdup volume. Alternately, the standard procedure could include a rinse consisting of 0.5-1.0 mL of buffer. Such a dilution, if tolerated, would only decrease the concentration by a factor of 1.5 or 2.0. Finally, foaming could account for some DNA loss. The addition of an appropriate anti-foaming agent might prevent this.

Verbal results relayed by Kaysie on Oct 31st revealed that (a) filtration efficiency was uniform across the 3 duplicate soil samples [6 runs, 12 containers], but (b) DNA yield was significantly less than with centrifugation. Cheryl will schedule a meeting with you/me/others as soon as possible to discuss any change in direction indicated by this latest study and/or the suggestions given above.

Nov 1996: Input from Cheryl (and Janet ?) leaves me with the impression that they believe a pumping scheme to be less desirable than a manual approach. This could be based upon

the observation that our final volumes are small due to holdup volumes retained inside the second filter. Accordingly, filter elements with smaller holdup volumes have been identified for you to order (as suggested in recent e-mail exchanges). These PVDF filters still have a 0.45 um pore size, will have an inside diameter of 13 mm (down from 25 mm), will have no prefilters (deemed unneeded in the light of our first stage filter), and will therefor hold up less filtrate. Cheryl has been alerted to our eventual need for more soil samples.

Foaming has been identified as a contributor to low final volume. Accordingly, a kit of six Fluorad surfactants has been obtained from The 3M Company. They are typically used at such levels as 10-200 ppm, and descriptions follow:

NAME	SOLUTION CONTAINS	pH
FC-120	25% sol'n of ammonium perfluoro-alkylsulfonates in a 50/50 blend of water and ethylene glycol monobutyl ether; anionic	8.5-9.5
FC-129	50% sol'n of potassium fluorinated alkyl carboxylates, 18% butyl cello-solve, 4% ehtanol; anionic	7-8
FC-135	34% isopropyl alcohol	3-4
FC-170-C	nonionic; polyoxyethylene ethanols	7-8
FC-171	fluorinated alkyl alkoxylates	
FC-430	nonionic fluorochemical	

Unless you/Cheryl/Kaysie provides some reason for not using any of these materials with the soil samples and its present buffer, they will be evaluated as antifoaming agents.

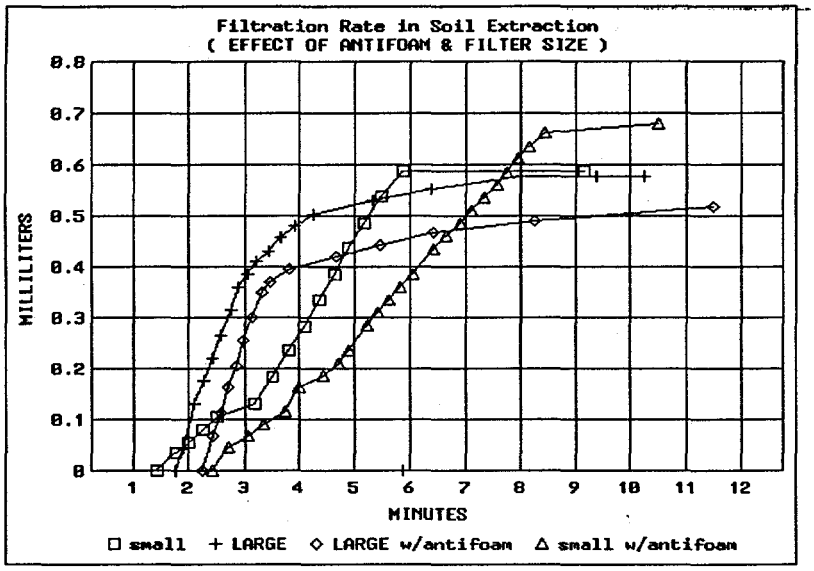
Dec 1996: At a meeting with Scott Lane on the 13th, a portion of a commercial video program (the TRAACS-800 system) was shown to describe the modus operandi of segmented flow analysis (SFA). This was followed by a brief slide presentation that showed the difference between SFA and the 3-phase system for zero carryover (from the presentation "Rapid Sample Transport System").

For the soil extraction project, smaller filters are now available. Kaysie has been asked to provide more soil samples so we can confirm our believe that larger volumes of filtrate can be obtained, but there has been no response to date. In the meantime, a request has been sent to Dow Corning for a sample of their newest silicone antifoam agent. This should have a significant impact on the foaming problem when filtrate emerges from the lower filter in our present system.

Jan 1997: Capability of interrogating the LANL computer from my home computer (to check e-mail sent to the lab) disappeared when the telephone lines to the "Coolworld" server were disconnected. Jim Whitfill provided a new phone number; it connects but I still can't read mail. Jim Laux has been alerted to the problem, but no solution has emerged yet.

Package of antifoam agent (1/2 lb. Dow Corning 1920 powdered antifoam) received, along with its MSDS. Cheryl has been asked (via e-mail; you were copied) to provide more soil samples. There being no response from her, and at your suggestion, I've worked with the existing package of soil.

The antifoam was dissolved in the usual buffer to make it 0.01 weight percent. The two smaller filter sizes are both about 10mm ID; the ODs are about 13mm (small) and 25mm (large). Four soil samples were prepared -- two each with and without antifoam. Results are graphed below:



The information was obtained by placing a 2-mL cup on the balance so that time vs. weight data could be generated in realtime. The clock was started when the soil/bead/buffer mixture was poured into the first (stirred) filter vessel. The first drop of filtrate emerged between 1 and 3 minutes, and pumping was stopped when the 2nd filter appeared to be empty.

Conclusions: (1) The volume of filtrate obtained with these smaller ID filters is significantly larger than with the ones used earlier, thereby avoiding the need for rinsing to obtain sufficient volume for later (DNA) testing, (2) the larger filter elements permit somewhat faster filtration and are nearly as efficient as the smaller ones (i.e., final volume is almost the same), and (3) incorporating the Dow-Corning antifoam not only doesn't eliminate foaming when manual pressure on the syringe is used to totally empty it, but actually slows the filtration a bit. Clearly, however, the new smaller filter elements are to be recommended over the original ones we used. Data sheet added to list of references.

Feb 1997: This month's effort was devoted to the computer animation of the 3-phase analytical system that is proposed as a means of automating virus determinations for Scott Lane. It is thought that about 80% of the project is completed, and it will be finished during the last two weeks in March.

The existing 5-minute program has been copied onto VHS video tape for your viewing and comments. The transfer results in colors that leave something to be desired, although on the computer they're fine. We must find some means of transfering (to video tape) that doesn't degrade the signal. One possibility, if the budget will stand it, is a computer/VCR interface card that's a step up from my personal equipment; cost: about $200.

To help in your evaluation of the first 5 minutes, I've also included (1) graphics of half a dozen video frames, and (2) a listing of the description of each scene and its corresponding narration.

Mar 1997: Vacation during early March has slowed progress on finishing the animation of the 3-phase system for automating an analysis for Scott Lane, pending your submission of an NIH proposal that will be accompanied by the video animation. Accordingly, the following has been accomplished: 1) additional video animation depicting the operation of the 3-phase system and its comparison with the inadequate 2-phase system, 2) at your recommendation, incorporation of reasonably smooth valve rotation, and 3) improved transfer of program material from computer to VCR. It is anticipated that all work will be completed during April.

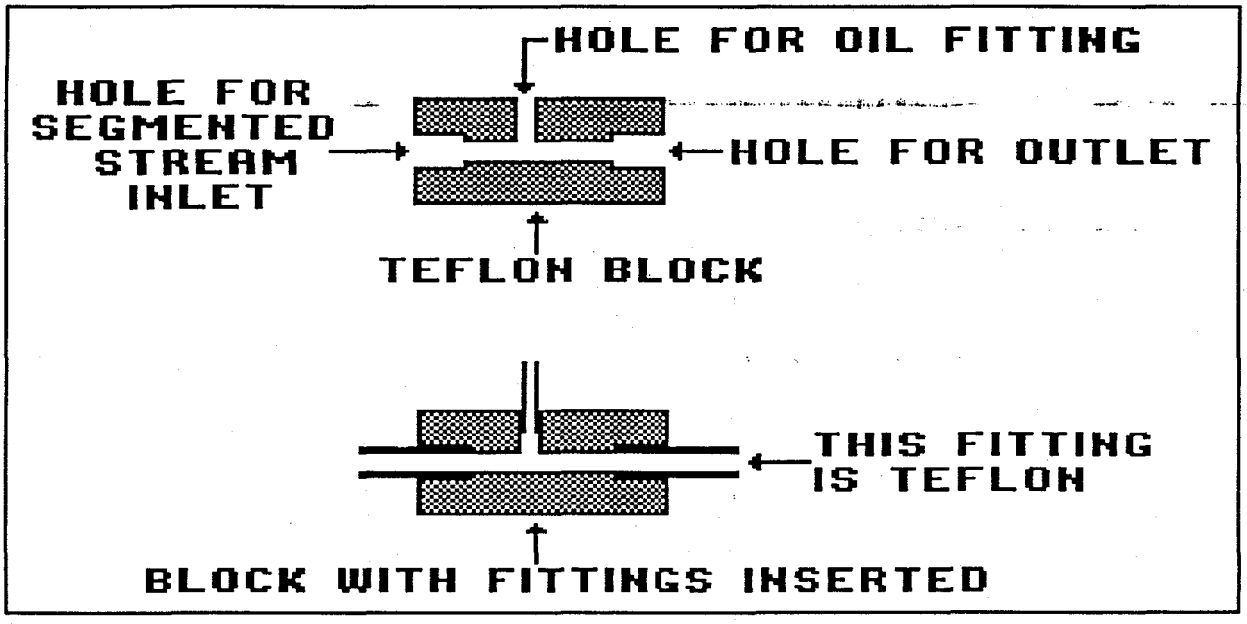
The following page of this report is a reproduction of selected frames from the video program. Left-to-right: #1 is the opening title, #2 is a still from the animated introduction of air into a flowing stream (i.e., the usual segmented flow), #3 adds a 3rd channel to depict the mixing of sample and reagent, #4 describes the unwanted liquid film that is ever present on the inside surface of conduits in an aqueous system, #5 is a cross-section of the Teflon fitting that produces the 3-phase system flow (i.e., fluorocarbon oil coating the inside of hydrophobic conduits to eliminate carryover), #6 is the usual HPLC valve, and #7 shows how a sample will be transfered from a volume-defining loop into the flowing stream between two air segments.

Apr 1997: No work performed, but sample of fluorocarbon oil (Krytox 157 FSL) was provided by DuPont.

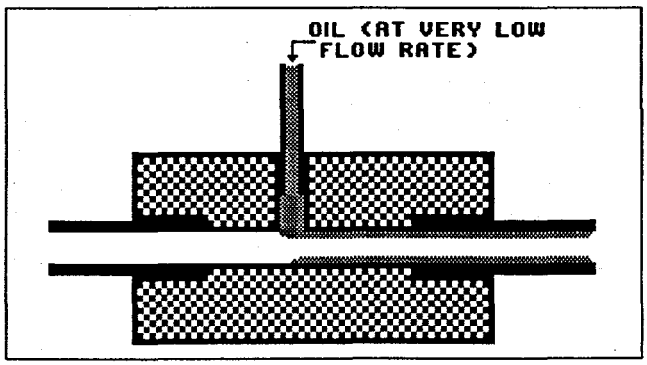
May 1997: Videotape completed in time for May 29th meeting with Amgen personnel. A demonstration was set up to show the hydraulics of segmented flow in action on the Alpkem system. Food coloring was used to simulate samples. The visual impact of carryover should make it clear to viewers that the 3-phase system has numerous advantages.

Sept 1997: No effort during June, July, or August. With the arrival of samples of DuPont's Krytox fluorocarbon oil, the 3-phase system was plumbed in September to demonstrate various capabilities of this version of Segmented Flow Analysis (SFA). Various sizes of teflon tubing (different IDs and wall thicknesses) are being evaluated to identify the optimum combination of tubing and flow rates for eventual analysis of (1) uL-size samples with fluorescent endpoint detection, and (2) somewhat larger samples containing living cells.

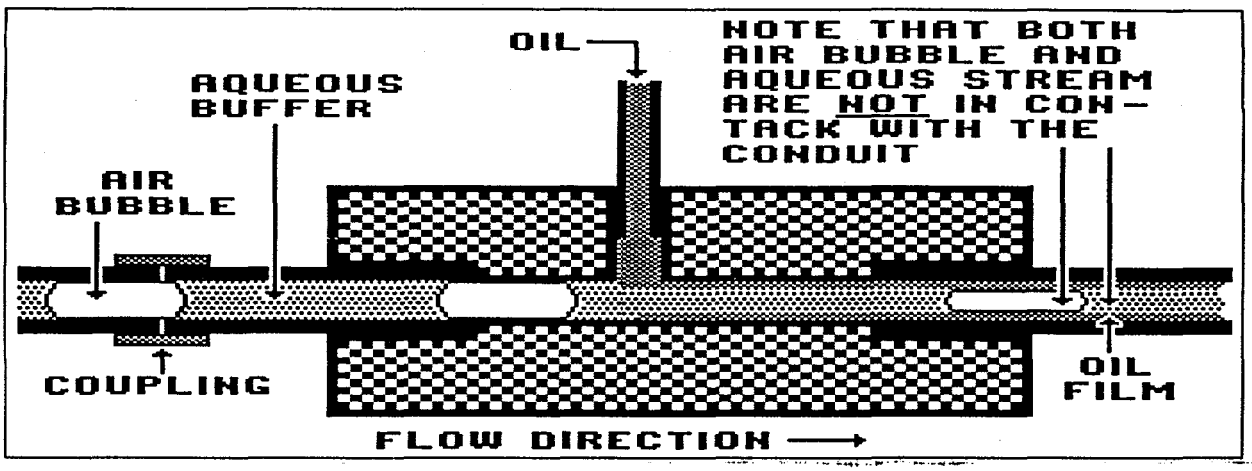
The heart of the 3-phase system is a special teflon fitting that maintains a thin film of fluorocarbon oil on the inside surface of a teflon conduit. A segmented aqueous stream entering this fitting will then pass through the conduit without ever coming in contact with it. The diagram below details its construction.



The diagram at the right shows how the fluorocarbon oil coats the teflon conduit. When the segmented aqueous stream enters the teflon fitting, it is forced into a smaller ID "liquid conduit", and neither it nor the segmenting bubbles ever come into contact with the inner walls of the teflon tubing.



Note the diagram below. When the air bubble reaches that part of the conduit containing the fluorocarbon oil, it acts like a paint brush, pushing the oil ahead of itself so that it coats the inner surface of the teflon tube (which, needless to say, it wets).



Unlike the conventional 2-phase system in which there is always a thin aqueous layer in contact with the conduit (leading to so-called "carryover" from one segment to another), there is no possibility of the contents of one segment contaminating those that follow it. Consequently, it is theoretically possible for each segment to represent one sample, leading to an enormous increase in efficiency of the analytical system.

There exists a video tape that shows, with animation, all of the effects that have been described in this report.

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*  THIS IS THE FINAL MONTHLY REPORT COVERED BY        *
*  THE CONTRACT. THE EFFORT WILL CONTINUE AS I        *
*  ASSUME THE ROLE OF "GUEST SCIENTIST" IN CON-       *
*  TRAST TO SUBCONTRACTOR.                            *
*                                                     *
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REFERENCES:

1. "The extraction and purification of microbial DNA from sediments" by Ogram, Sayler, & Barkey, J Microbiological Methods, 7, 57 (1987).

2. "Molecular Cloning - A Laboratory Manual" by Sambrook, Fritsch, & Maniatis, Pp 1.34 - 1.37, Cold Spring Harbor Laboratory Press (1989).

3. "Curent Protocols in Molecular Biology", Vol 1, Unit 2.4, Publ: Current Protocols [Wiley] (1994).

4. "Imaging detection methods for capillary isoelectric focusing" by Jiaqi Wu & Janusz Pawliszyn, Amer Lab (Oct), 48 (1994).

5. "Application of capillary and free-flow zone electrophoresis to the analysis and preparation of synthetic biopeptides" by Vaclav Kasicka & Zdenek Prusik, Amer Lab (Oct), 22 (1994).

6. "Automated DNA Sample Preparation" in Biochip arrayTechnologies, IBC Conference, Marina del Rey, CA, Mar 20, 1996.

7. "Advances in Amplification/PCR Technologies", ibid.

8. "Continuous Flow Centrifugation for Downstream Processing of Bio-products", brochure from Heraeus Instruments, So. Plainfield NJ.

9. Catalog from Perkin Elmer, "PCR Systems, Reagents & Consumables", contains a listing of several PCR publications, technical information, and a bibliography.

10. New Product Information sheet entitled "Dow Corning 1920 Powdered Antifoam" (1992).