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MICROBIAL LOAD MONITOR

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Prepared by:
R. S. Caplin
Engineer
Biomedical Engineering

Approved by:
S. F. Gibson
Microbiologist
Aerospace Medicine

E. R. Royer
Senior Engineer
Biomedical Engineering

Approved by:
A. V. Montgomery M.D., Ph.D.
Acting Manager
Aerospace Medicine

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MCDONNELL DOUGLAS ASTRONAUTICS COMPANY - ST. LOUIS
Saint Louis, Missouri 63166 (314) 232-0232
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1.0 SUMMARY

Work under Contract NAS 9-11877 to provide a total design of a Microbial Load Monitor (MLM) system flight engineering model, continues as scheduled. Activities during this contractual quarter include assembly and testing of Sample Receiving and Card Loading Devices (SRCLDs), operator related software, and testing of biological samples in the MLM. Progress in these areas is summarized below.

Progress has been made in assembling SRCLDs with minimal leaks and which operate reliably in the Sample Loading System.

Seven operator commands are now in use to control various aspects of the MLM such as calibrating and reading the incubating reading head, setting the clock and reading time, and status of Card - which will be modified to include the tentatively set threshold values.

Testing of the instrument, both in hardware and biologically, was performed. Hardware testing concentrated on SRCLDs. Biological testing covered 66 clinical and seeded samples. Tentative thresholds have been set and media performance listed.
2.0 TECHNICAL DISCUSSION

Major items for this reporting period are assembly and operation of hardware, operation of Microbial Load Monitor software (as seen by the operator of the instrument), and testing of the instrument with biological samples.

2.1 Hardware - During the last three month period, three hardware items have been under close scrutiny. They are the fifth incubating reading head, SRCLD fabrication, and operation of the Sample Loading System. In the past, some of the arrays have been borrowed from the fifth reading head and used in the other four heads. When the three spare array pairs have been fabricated, this will no longer be necessary. The fifth incubating reading head currently has three good array pairs.

Sample Receiving and Card Loading Device fabrication concentrated on part inspection and quality assembly during this period. Problems relating to SRCLD can be categorized under three headings:

a. Improper fill due to septum material clogging needles.

b. Improper fill due to magnet impellor not rotating at moment of fill.

c. Leaky SRCLDs due to incomplete ultrasonic weld on bottom piece.

Needle clogging has been reduced greatly by drilling the septum entrance hole for the double-ended Card injection needle to a diameter of 0.060 inch after the septum material has cured. This cleans the hole area of overflow septum material and prevents it being forced into the needle by side pressure of the small hole.

Magnet impellor rotation is greatly improved by two things: Quality assembly of SRCLDs which yield freely rotating magnets, and spacing of the solid sample plunger. Quality assembly yields magnets which are centered in the impellor plastic and have a snug enough fit so that they do not come loose and rub the chamber wall. Proper alignment during welding also assures freely moving pivot points. When the rubber tipped solid sample plunger is inserted completely, it projects into the mixing chamber. During rotation the magnetic impellor can rub the rubber tip, thereby preventing it from rotating freely. Spacers will be added to the
upper end of the plunger to prevent improper insertion. Results to date look quite good. The software LOAD subroutine has been written to further reduce the magnetic impeller problem. It is described in 2.2.1 and 2.2.2.

Assembly of SRCLDs to prevent leaks was approached in two ways: Very critical alignment of pieces during ultrasonic welding and cementing the leaks with #1 pleximent containing ethylene dichloride solvent. Table 1 lists the results of the ultrasonic welding.

<table>
<thead>
<tr>
<th>Type</th>
<th>Start</th>
<th>Visual Reject</th>
<th>Pressure Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RTV Fail</td>
</tr>
<tr>
<td>N</td>
<td>47</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>S1</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Type N SRCLDs were normal ultrasonic welding. Type S1 was a modified impeller and fairly close alignment. Type S was near-perfect horn alignment during weld. Type N therefore had less than 50 percent good parts and Type S had over 90 percent good parts. These good parts were then packaged, marked as to type and sterilized with electron beam sterilization. These were then used in biological testing. The results are reported in Section 2.3.2. From an assembly standpoint, it is worth taking extreme care in ultrasonically welding the SRCLDs.

Attempts were made to seal the leakers with #1 pleximent. Of the 22 attempted 13 still had leaks 48 hours later. An attempt was then made to use vacuum to pull the pleximent into the leak on a different set of SRCLDs. Of 25 attempted, 19 were good after 48 hours and only 14 after approximately a week. Periodic examination will continue to determine long term effects, but pleximent will not be used on delivered items if the Type S welds hold up during continued biological testing.

A portion of the Sample Loading System was modified and adjusted to accommodate the extended length of the solid sample plunger with installed spacers. The SLS chamber lever which pushes the air/vacuum plunger interfered with the extended
solid sample plunger. The lever was removed from the chamber and machined to clear
the top of the solid sample plunger. It was then reinstalled and tested satisfac-
torily.

2.2 Microbial Load Monitor Software - The Microbial Load Monitor instrument
software during this period concentrated on the operator interface. Some details
of this software and its use is given below.

2.2.1 Operator Commands - The TIMMON supervisory program has the purpose of
acquiring data, transferring the data to magnetic tape, keeping track of initial
data values, and making decisions concerning organism growth, sensitivity and
enumeration. The operator interfaces with the program by various four letter
keyboard commands after the program has been started. Seven commands are now in
use. They are: TIME, ITIM, IDAT, CALH, READ, STAT, and LOAD. The purpose of each
is explained below.

TIME: This is a request by the operator to have the date and time displayed
and printed. It is displayed in 24 hour format. Example: 0123 1540. This would
be the first month (January), 23rd day, 15th hour (3 p.m.) and 40 minutes past the
hour.

ITIM: Initialize Time. This is a request to change or initialize the hour and
minute shown by a TIME request. This command is used when the time is not correct,
but should not be used unless none of the incubating reading heads have an active
Card.

IDAT: Initialize Date. This is a request to change or initialize the month
and day shown by a time request. The same purpose and restriction apply as for
ITIM. The operation of TIME, ITIM, and IDAT is described in Section 2.2.3.

CALH: Calibrate Head. This is a request to recalibrate the LED current
crives of a particular incubating reading head. The subroutine prompts with the
symbol # thereby requesting a one digit head number from 1 to 5. This should be
used before starting any incubating reading head.
READ: Read data from incubating reading head. This is a request to initialize control parameters and start data gathering for a particular head. Subroutine prompting is the same as for CALH.

STAT: Status of the Card in a particular incubating reading head. Prompting for head number is the same as for CALH and READ. An abbreviated status of that particular Card is displayed. Addition of threshold values will make this command meaningful.

LOAD: Load Card from SRCLDs. This command activates the subroutine that controls the Sample Loading System (SLS) and loads the Card. This subroutine is interactive with the operator, and is described in Section 2.2.2.

2.2.2 Sample Loading System Software - The software subroutine which controls the Sample Loading System (SLS) is started with the command LOAD. At this time the SRCLDs should be in the SLS, the Card positioned in the holder, the holder lowered and the lid latched.

The subroutine starts the magnetic impellors by ramping up the speed of the drive motor. The display then requests - IS SPEED OK?-. If it is, answer with a letter Y for yes and the subroutine continues with the fill. A letter Q for quit exits the subroutine, and any other key causes a restart of the motor. A letter Y is answered only if all magnetic impellors are rotating. The air/vacuum plunger is pushed in and the chamber evacuated. Next the chamber pressure is measured and if it is not below approximately 3.75 psia the error message - NO VACUUM - is displayed and the SLS is shut down. The load procedure must be repeated after the vacuum source is checked. An unlatched lid can also cause a vacuum error.

Assuming proper vacuum, the subroutine will evacuate for six minutes after the pressure reaches nearly zero. During this six minutes the display indicates the length of time into the cycle and the chamber pressure. If any impellor stops rotating it can be restarted by any of three single letter commands:

M - restart motor at same speed
D - restart motor at slightly less speed
U - restart motor at slightly higher speed.
If impellor still refuses to reliably rotate, the letter Q for quit can be used for an orderly shut down and subsequent test replacement. Two critical times for impellor rotation are onset of vacuum and during Card filling.

At six minutes, the Card is pushed connecting it to the impellor chamber. The SLS chamber is then connected to atmospheric pressure through a slow leak valve and the Card begins a slow fill. The display now indicates only chamber pressure. When the chamber pressure reaches approximately 3.75 psi, a valve opens and fast fill begins. At 3.75 psi the Card should be nearly full with small bubbles which rapidly shrink as the SLS chamber reaches atmospheric pressure. Time is allowed for the pressure to stabilize, then the Card and SRCLDs separate. The display now says LOAD COMPLETE. The Card and SRCLD can be removed and another Card prepared for loading by the same sequence of events.

2.2.3 Microbial Load Monitor Clock - The timekeeping function in the MLM is a hardware clock integrated circuit which is initialized and read by software subroutines. Available to the operator are three commands: TIME, ITIM, IDAT. These were described in Section 2.2.1. To accurately set the clock the commands should be used in the following manner, after noting the clock needs to be changed, by using the TIME command.

First the month and day should be set by keying in IDAT. The MLM display clears and prompts with: MONTH 01/12-. A two digit number (with leading zero as shown by 01) representing the month should be keyed in. The MLM then prompts with: DAY 01 TO 31-. Likewise a two digit number is to be keyed in. Any character other than a proper decimal number and in the proper range for two characters generates ERROR INPUT on the display. If a return character is input from the keyboard, the subroutine will restart with display of the month message. Any character other than return causes an abort and return to the main program. If all information has been entered correctly, the hardware clock will be set to the input time. For month and day this can take a maximum of 43 seconds, since updating is done with a one second clock drive. When updated, the display will show the time and date as if the TIME command had also been used.

Next the hour and minute are set with the ITIM command. If very accurate time is wanted (almost to the second) the ITIM command should be used twice. The
first update should be with the correct hour but with the minutes about two minutes slow. This can take up to 84 seconds before time is displayed. Now when the correct time is keyed in for the second update, it will take only two seconds. By keying in the last digit of minutes when a second sweep hand approaches zero, the clock will quickly update and be only a few seconds off. The prompting messages are HOUR 01 TO 24- and MIN 00 TO 59-. Input errors are handled identical to IDAT.

2.2.4 Magnetic Tape Software - The MLM magnetic tape software writes and reads two different types of records with the main difference being length. Data records are six heading words plus 35 words of reading data. Calibration records are 6 heading words and 65 words of calibration data. The six heading words are listed below:

1st word  0002  Start Character
2nd word  XXON  TITLE (includes Head Number)
3rd word  XXXX
4th word  XXXX
5th word  XXXX
6th word  NNNN  Length of additional record portion

Where X is unused but may be used in the future and N is a hexadecimal number.

The magnetic tape recorder is a simple replacement for paper tape and has no capability to go in reverse without operator intervention. This is not significant as long as everything runs properly. Problems arise when the magnetic tape used is damaged, such as bent, creased, oxide missing from the recording surface, etc. More sophisticated software is being written to bypass records with errors created by damaged tape (hard errors). Soft errors can usually be corrected by rereading the tape. The software will be able to detect a dropout of information and bypass the record by going to the next interrecord gap which was created by stopping and restarting the recorder between recording the records.

Preliminary software to develop the proper bypass techniques has been written which allows the operator to bypass a bad record near the beginning of the tape. This is where 95% of tape errors have occurred. This will be adapted for the MLM
plot program, which will allow automatic bypassing of bad records with error indications to the operator. The error indications will be given only if the error indicates loss of data for the individual head.

2.2.5 Diagnostic Software - Diagnostic software can take many forms. In every case, its purpose is to make problem diagnoses and repair of the instrument easier. One form could be the error messages designed to prevent wrong operation of the Microbial Load Monitor. Two important subroutines have been written for diagnostic or setup use: one is called DVM and the other is CHEXAM.

DVM stands for digital voltmeter. In hardware it is an analog to digital convertor (commonly called ADC) the input of which can be switched by a multiplexor. The DVM subroutine limits this input to incubating reading head temperatures and the Sample Loading System chamber pressure. Inputs controlled by other subroutines are the LED-phototransistor pairs. The controlling keys are the "line feed" for next input and "return" to exit the subroutine. The inputs loop around starting with chamber pressure, ending with the fifth head temperatures and looping back to chamber pressure.

In isolated cases where one particular channel or group of channels gives unstable readings, a subroutine called CHEXAM can be used. This subroutine allows control of the basic head reading routines from the keyboard. Normally this would be used in conjunction with an oscilloscope to view the waveforms in the head electronics. Light emitting diodes (LED) which do not light can be seen visually during a calibration test with the head open. Phototransistors which also have no detected output are also easily determined if the LED is working. Unstable or oscillatory conditions need to be viewed with an oscilloscope. The CHEXAM subroutine stands for "channel exam." Using the keyboard CHEX starts the routine which then prompts "#" for the head number.

The subroutine then starts at channel one. The letter N (for next) changes to the next channel. A set current drive is used at first. The letter U (for up) will increase the current in predetermined steps and the letter D (for down) will decrease it. Any other character will cause an exit from the program. By using these three commands the effects of nearly any current for any channel can be continually viewed and analyzed on an oscilloscope.
2.3 Microbial Load Monitor Testing - Testing of the MLM during this period included both hardware and biological types. Hardware testing centered on the Sample Loading System and its related SRCLDs. Biological testing entered on media performance with clinical and seeded samples, and the related threshold values for determination of positives.

2.3.1 Biological Tests

2.3.1.1 Test Samples - To date 66 samples have been tested with the MLM. These include 42 clinical urines and 24 seeded urines.

2.3.1.2 Thresholds - Tentative threshold values have been set for the following broths:

- E. coli 50%
- Klebsiella-Enterobacter 30%
- C. freundii 30%
- Proteus sp. 25%
- S. aureus 15%
- Grp D enterococcus 30%
- P. aeruginosa 10%
- Serratia sp. 40%
- Yeast 20%
- Positive Control 15%
- Enumeration
  - $<10^5$/ml = 0-4 wells positive
  - $\geq10^5$/ml = 5 wells positive, 15% threshold

2.3.1.3 Media Performance - Performance results of the media listed above are presented in Table 2. Performance is shown as overall percent correlation to standard tests.

Two false positives due to C. freundii occurred in the E. coli broth. Both were identified in the C. freundii broth as well as E. coli medium.

One false positive noted in the C. freundii well was later identified as an E. coli.
### Table 2
**Media Performance Results**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Total No. Samples</th>
<th>True Positives</th>
<th>False Positives</th>
<th>True Negatives</th>
<th>False Negatives</th>
<th>% Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>62</td>
<td>18</td>
<td>2*</td>
<td>41</td>
<td>1</td>
<td>95%</td>
</tr>
<tr>
<td>Klebsiella-Enterobacter</td>
<td>62</td>
<td>8</td>
<td>0</td>
<td>52</td>
<td>2</td>
<td>97%</td>
</tr>
<tr>
<td>C. freundii</td>
<td>62</td>
<td>3</td>
<td>1</td>
<td>58</td>
<td>0</td>
<td>98%</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>66</td>
<td>12</td>
<td>0</td>
<td>52</td>
<td>2</td>
<td>97%</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>62</td>
<td>2</td>
<td>0</td>
<td>59</td>
<td>1</td>
<td>98%</td>
</tr>
<tr>
<td>Serratia sp.</td>
<td>62</td>
<td>3</td>
<td>1</td>
<td>58</td>
<td>0</td>
<td>98%</td>
</tr>
<tr>
<td>S. aureus</td>
<td>62</td>
<td>4</td>
<td>1</td>
<td>57</td>
<td>0</td>
<td>98%</td>
</tr>
<tr>
<td>Group D enterococcus</td>
<td>62</td>
<td>13</td>
<td>0</td>
<td>49</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Yeast</td>
<td>62</td>
<td>2</td>
<td>0</td>
<td>59</td>
<td>1</td>
<td>98%</td>
</tr>
<tr>
<td>Positive Control ≥ 10^2/ml</td>
<td>62</td>
<td>52</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>93.5%</td>
</tr>
</tbody>
</table>

**Enumeration**

<table>
<thead>
<tr>
<th></th>
<th>Total No.</th>
<th>True Positives</th>
<th>False Positives</th>
<th>True Negatives</th>
<th>False Negatives</th>
<th>% Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Wells 15% = ≥ 10^5/ml</td>
<td>62</td>
<td>53</td>
<td>2 (MLM &gt; 10^5)</td>
<td>6 (MLM &lt; 10^5)</td>
<td>1 (MLM &lt; 10^5)</td>
<td>97%</td>
</tr>
<tr>
<td>(Std &lt; 10^5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

% Correlation = \frac{\text{True pos} + \text{true neg}}{\text{total}}

*C. freundii*
A false positive in the Serratia medium occurred in a mixed sample of $10^8$ CFU/ml including C. freundii, Klebsiella sp. and Proteus sp.

The false positive in S. aureus medium was due to a large number of Proteus sp. No false positives have been noted to date with the Klebsiella-Enterobacter, Proteus, P. aeruginosa, Grp D enterococcus or yeast media.

False negative results totaled 12. An E. coli ($1 \times 10^4$/ml) was not detected within the 12 hour time period. Two Klebsiella sp. ($10^4$/ml and $10^6$/ml) were not detected. Two Proteus sp. ($10^3$/ml and $10^5$/ml) and a C. albicans ($10^3$/ml) were not detected. The positive control well failed to detect $10^2$/ml a total of four times for an overall correlation of 93.5%.

Enumeration tests indicated an overall 97% correlation to standard plate counts.

There were three discrepancies noted for 62 total tests. In two cases, the MLM enumeration method indicated $>10^5$/ml total organisms present, whereas standard plate counts indicated $<10^5$/ml.

2.3.1.4 Additional Media - Work continues on Group A Beta streptococci medium with various formulations currently under testing. To date, a successful freeze-dried formulation is not available for Card testing.

2.3.2 Sample Receiving and Card Loading Device Test Results - Initially, both main channel and the enumeration channel SRCLDs were loaded with a combined total for sample and diluent of 5 ml. Eighty percent of the time a reasonable fill would result. On the other 20%, air would be introduced near the end of the fill as if not enough fluid was available to fill the Card. Increasing the total fluid for main channel filling to 5.5 ml alleviated this marginal condition.

Previously, in Section 2.1, mention was made of special care taken in assembling the SRCLDs. The comparison of the S type assembled SRCLDs (extreme care) with previous methods is presented in Table 3. The data show that repair of SRCLDs is not worth the time and effort involved, and that extreme care should be taken in
original ultrasonic welding. This care will improve both leakers and magnetic impellor reliability.

**TABLE 3**

**SRCLD ASSEMBLY METHOD COMPARISON**

<table>
<thead>
<tr>
<th>Type</th>
<th>Card Loadings</th>
<th></th>
<th>SRCLD Leaks</th>
<th>Plunger Broken</th>
<th>Magnet Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>6</td>
<td></td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>R</td>
<td>11</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>N</td>
<td>22</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

*S = extreme care taken on assembly  
N1 = reasonable care taken  
N = assembled before problem was noticed  
R = repaired SRCLDs and tested good once  

All solid sample plungers have been spaced.
3.0 PROGRAM STATUS

The MLM System Engineering model reached the following levels at the end of this contractual quarter. Spare array pairs are in the process of fabrication and checkout. Software has reached the stage where the MLM can be controlled by simple commands even if the form or length of the programs change. Testing has resulted in tentative threshold values and media performance will continue to occupy a major portion of the remaining contract effort.

A contract review by the contract Monitor, Dr. J. K. Ferguson, occurred in December. The review included all parts of the MLM system: Media Pumping System, Card Taper, two runs of the MLM instrument, and the operator interface. Also included was a tour of the commercial facilities of the newly formed VITEK Corporation.