Virus Detection Device for Honey Bees

Project No.:   **07-POLL9-Wick**

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Interpretive Summary:

BVS, Inc. made a request to the Almond Board of California that funded, in part, the purchase of the Integrated Virus Detection System (IVDS) providing the opportunity for BVS to collaborate with the beekeeping industry and with key researchers to bring this new and innovative technology to bear on issues related to Colony Collapse Disorder. BVS has been successful in purchasing the instrument and obtaining a license directly from the Army as well as a research agreement with the Army lab at Edgewood Chemical and Biological Center (ECBC). There were delays in the start of operations, but these were overcome and operations commenced in June. In cooperation with Bee Alert Technologies, who has collected and logged all the bee samples we have processed, nearly 400 samples from beekeepers. This has been a unified effort that can and has combined data, which we expect will provide a more complete picture of bee health as a management tool.

We are assembling a database for detecting viruses in bees. The database contains information that will be offered to beekeepers in the form of reports. These data appear to be unique and will offer information to beekeepers that has not previously been available.

This information, which can be formatted into reports to beekeepers, is just getting underway with the recent completion of data formatting. The following charts are

examples of this effort. The IVDS data reflect the size of the virus detected in nanometers (nm). While this is a very accurate and consistant method, we still do not have the names on the individual virus and size correlation. However, the detections are there and appear to be relative to bee health. Our next step, if we have the funding, is to work with the Army, Bee Alert, and others to identify the detected size with the virus name.

Objectives:

The primary objective was to fund (in conjunction with the Montana Beekeers, the Idaho Beekeepers, California State Beekeepers, California Queen Breeders, and Project Apis m) the purchase of an IVDS instrument and to place it in operation. Both have been accomplished. The extras that have come from this are an Army License, an Army Research Agreement, and a database of bee virus screening.

Materials and Methods:

Set up of the IVDS Lab
- Bee grinding station and course filtration
- Centrifuge stations
- Ultra filtration Stations
- IVDS station

Methods:
- Bees were collected at the direction of Bee Alert Technologies and delivered to BVS for processing.
- 6 grams of bees for each sample, if sample did not have enough bees the whole sample is processed. Each sample is blended with 100ml of Reverse Osmosis (RO) water and coarse filtered through single layer cheesecloth.
- 30ml of the sample is centrifuged for 60 minutes at 19,000 X g.
- The supernatant is recovered and Ultrafilterd through a 100,000 Dalton hollow fiber filtration system and a 300ml RO wash and reduced to ~ 3ml.
- The solution is prepped for IVDS by a 1:10 and 1:100 dilutions with Ammonium Acetate (AA).
- IVDS scans are a 5 scan average and saved in the IVDS database.
- Charts and tables are created from the exported data from IVDS.

Results and Discussion:

Setting up the lab showed us many limitations that were not seen in the initial design for a production lab. The first issue we found was in the ultra filtration stage. It took too long to process a single sample. The initial fix was to add filtration stations. One station was added which doubled output, by adding another increased production by a third. Projcet Apis m has recently funded 3 more filtration stations and they are currently in the process of being set up. In addition to the new filtration stations we have changed

the filtration cartridge to one tested by ECBC that has a higher throughput which should result in more samples processed per filtration station per day. The second issue was with our centrifuges. We were sold a pair of centrifuges that could not stand the number of samples we were running per day even though we did not exceed the continous rating of the centrifuges. I talked with the manufacturer after having three replacements for one and two replacements for the other (all under warranty) and Themofisher (at their expense) has sent a single centrifuge of higher capacity and rating, they said is the right tool for the job. Good for them! It arrived today, that will solve the second problem. After a steep learning curve on IVDS, I am pleased to say it is working exactly as expected.

Screening for viruses has generated the included data for overall detections showing detections from 17.5 nm to 46.1 nm as the apparent virus window of interest. For the purpose of this report particle detection in IVDS will be considered to be a virus, based on the sample preparation and nature of viruses in solutions. The sample set that we have used was generated at the BVS, Inc lab in Missoula that generated the 324 samples used for this report.

BVS, Inc. has processed nearly 400 samples total at this date. The data that we have prepared for this report is from 324 of the samples processed at BVS. The distribution chart of viruses shows us that out of 324 we had a low of one virus only being detected in one sample out of the 324. The virus that was detected in the most samples was 32.2nm and was detected in 126 of the samples.

Data Charts:

A series of charts that will help show the results of the time and work invested in the project.

Figure 1 shows that 31 viruses have been detected in honey bees that include the frequency of detections and size in nanometers (nm) detected.
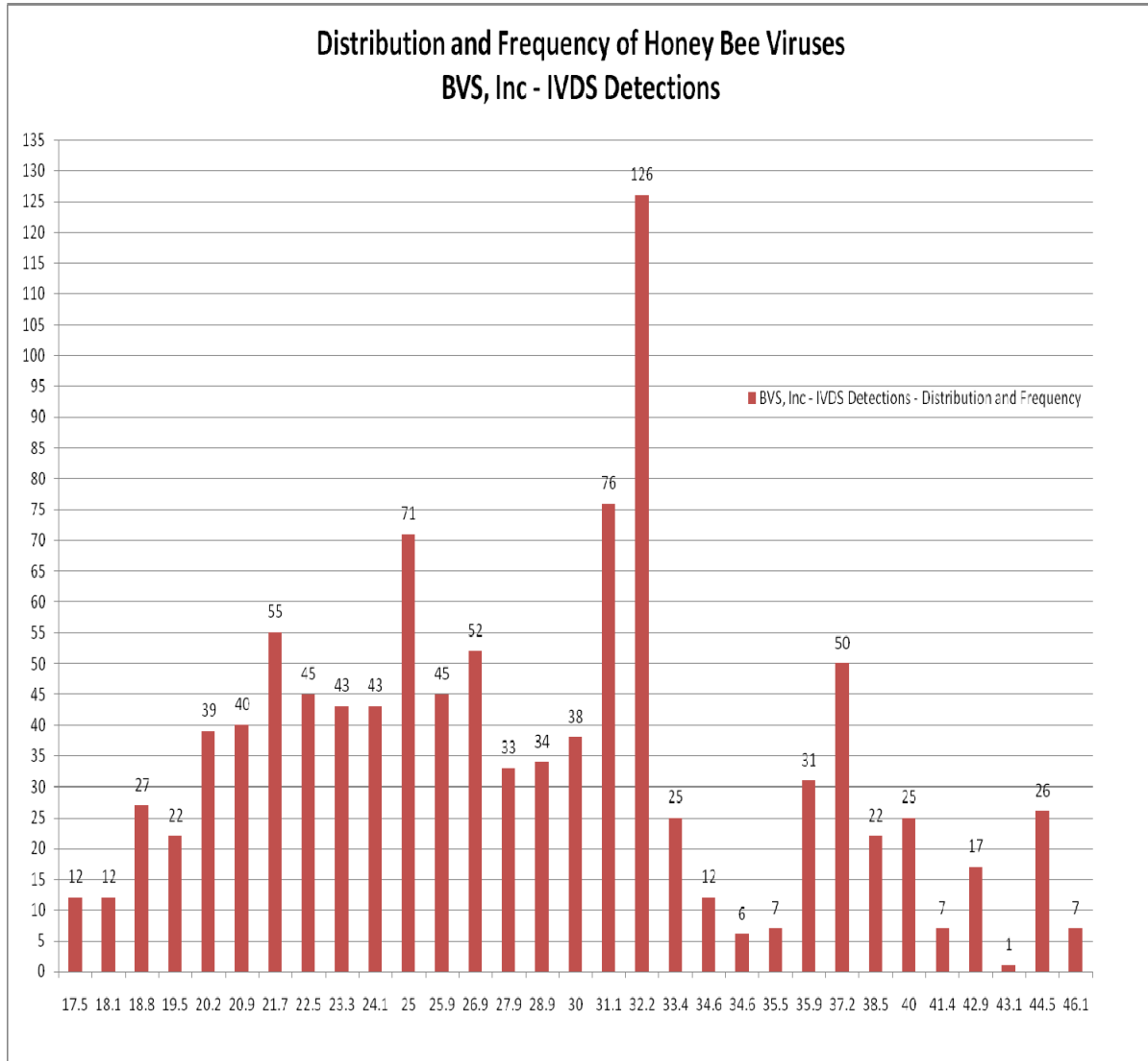
Figure 1: Distribution and Frequency of Honey Bee Viruses. 31 Viruses detected in honey bees from 17.5 nm to 46.1nm. These data represent 324 samples. The numbers above the columns represent the number of samples with that virus size detected.

All of the detection data in this report comes from the basic IVDS detection chart for each sample processed. The chart shows individual peaks representing detections at a specific size. This information is then moved to tables and combined with other samples to create the analytical charts.

Figure 2 Chart showing all ranges with high, low and median counts of honey bee viruses from 17 -46 as observedin 324 samples. Some samples had no detections and some had high detections, the median shows that viruses are possible in all the samples and the database shows some had none.

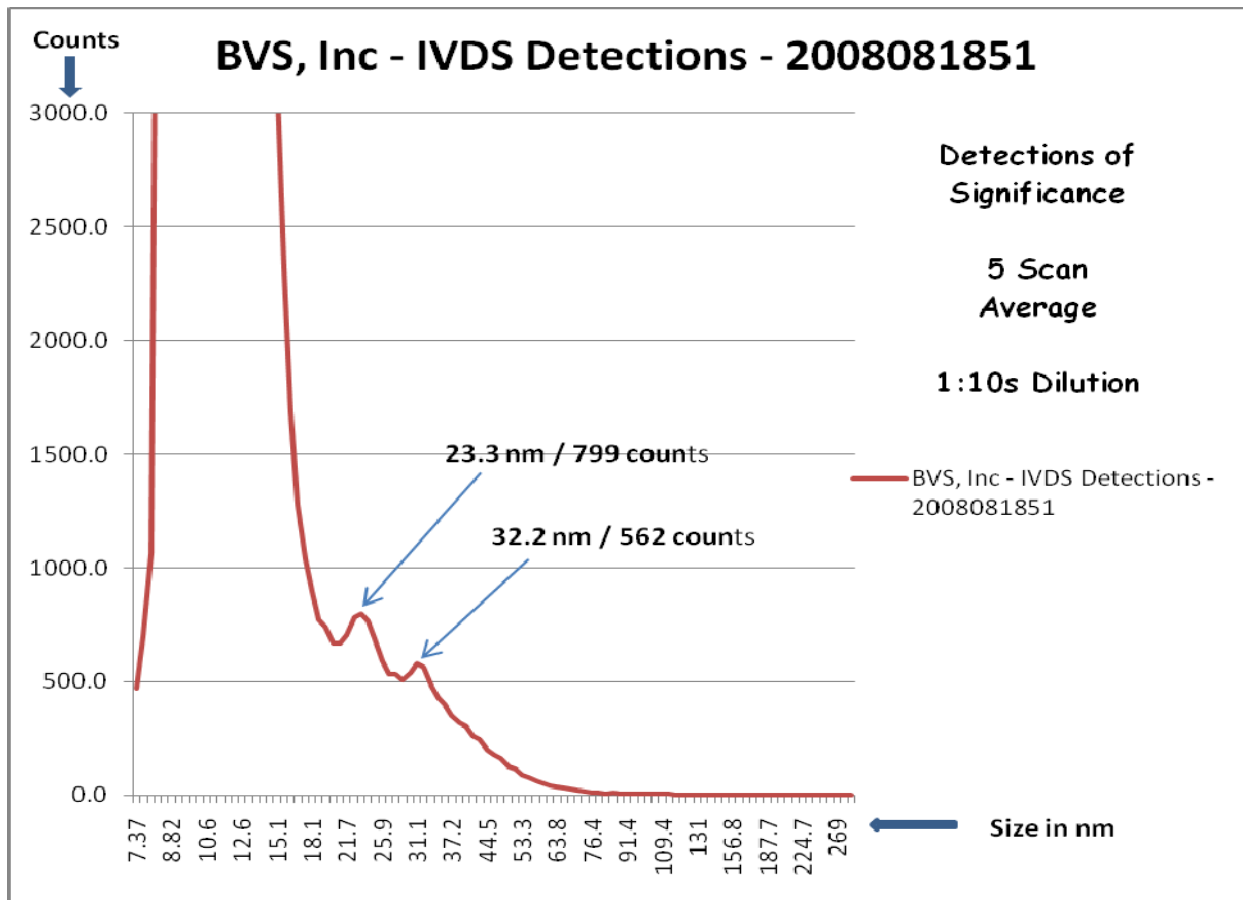Figure 3-5 shows the combination of detections for the highest counts, the median counts, and lowest counts. The high counts skew the charts and make them difficult to cross reference to other data in the chart. This series of charts show the different scales that give a different perspective on the same data. Above or below the median is reference that could relate to bee health or can give a reference point for viral load comparisons. These data are new and the development of that correlation is underway.

High, Median, and Low virus counts for each virus size
BVS, Inc - IVDS Detections

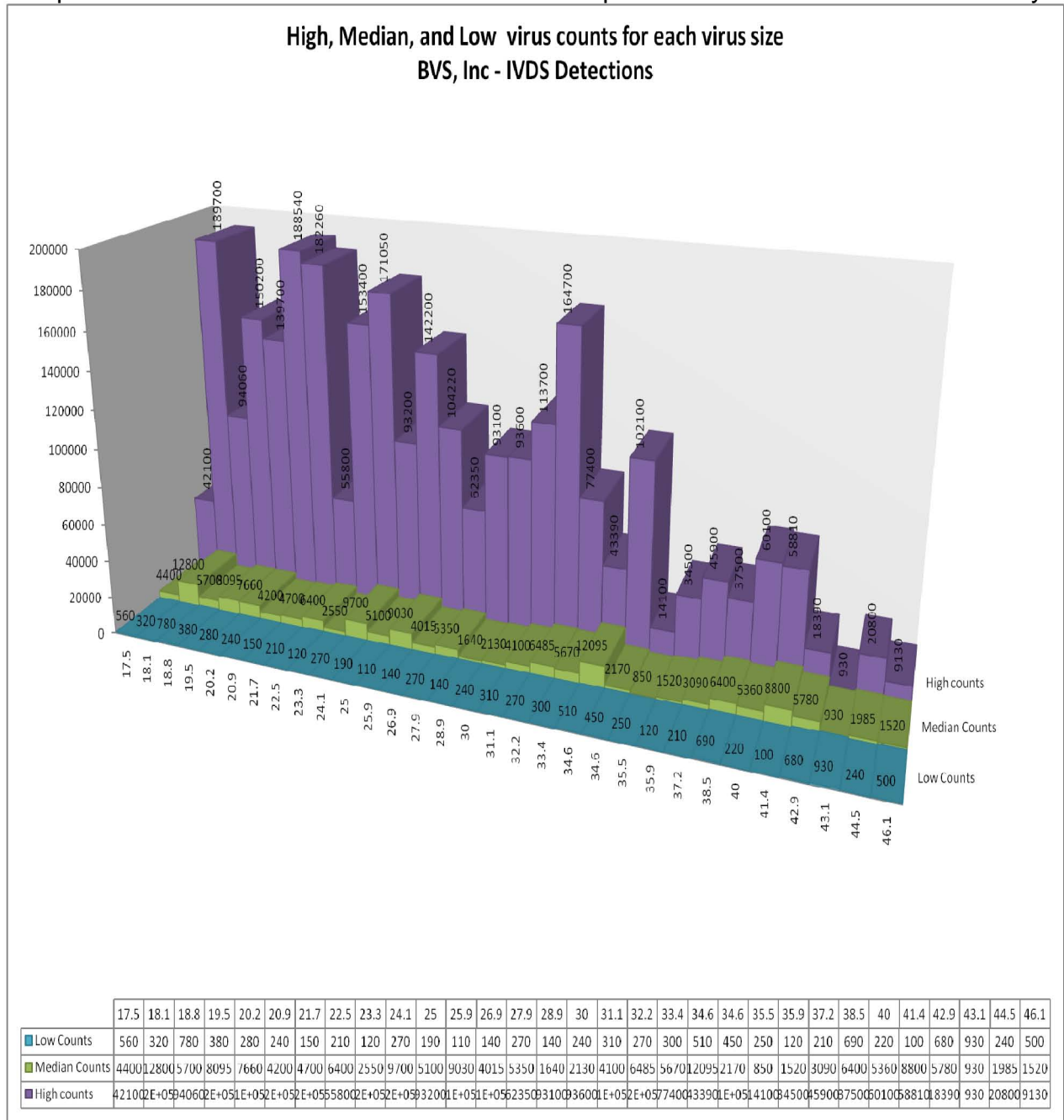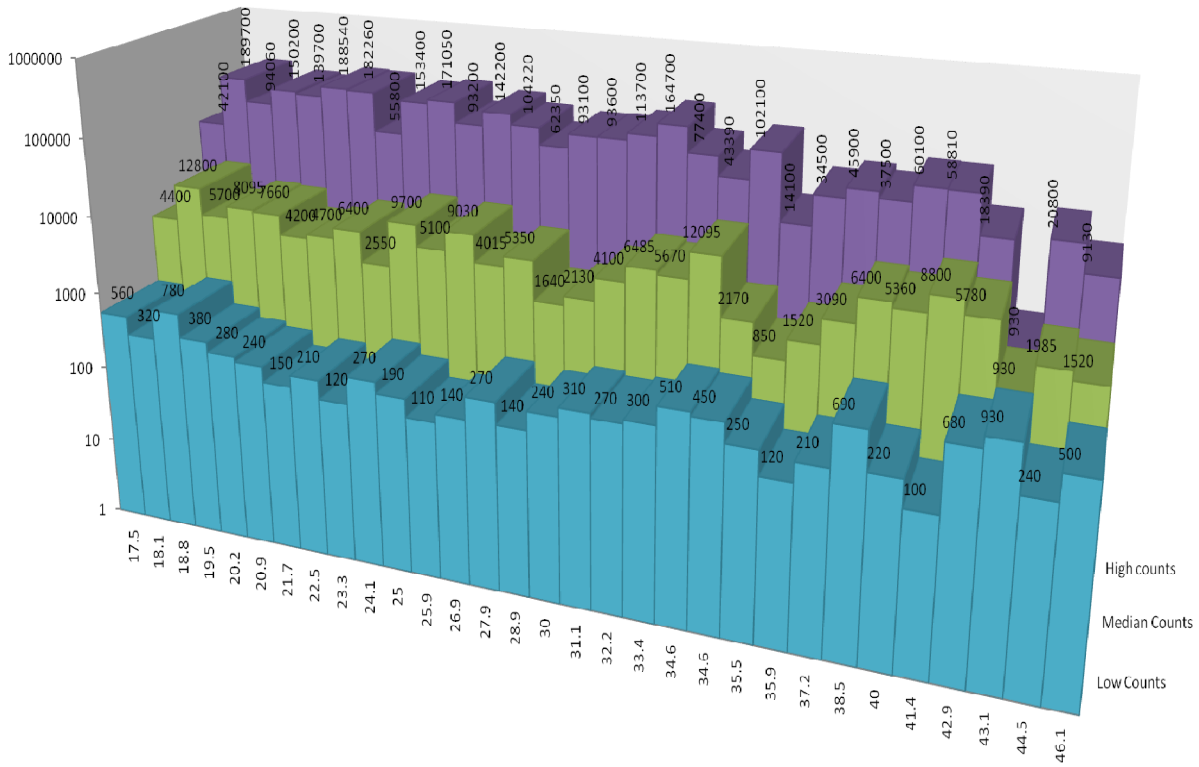|	17.5	18.1	18.8	19.5	20.2	20.9	21.7	22.5	23.3	24.1	25	25.9	26.9	27.9	28.9	30	31.1	32.2	33.4	34.6	34.6	35.5	35.9	37.2	38.5	40	41.4	42.9	43.1	44.5	46.1
Low Counts	560	320	780	380	280	240	150	210	120	270	190	110	140	270	140	240	310	270	300	510	450	250	120	210	690	220	100	680	930	240	500
Median Counts	4400	12800	5700	8095	7660	4200	4700	6400	2550	9700	5100	9030	4015	5350	1640	2130	4100	6485	5670	12095	2170	850	1520	3090	6400	5360	8800	5780	930	1985	1520
High counts	42100	2E+05	94060	2E+05	1E+05	2E+05	2E+05	2E+05	55800	2E+05	2E+05	93200	1E+05	1E+05	62350	93100	93600	1E+05	2E+05	77400	43390	1E+05	14100	34500	45900	37500	60100	58810	18390	930	2080	9130

Figure 3. These data in purple (the back columns) represent the high counts but without scaling the low and median are flat and difficult to evaluate. The data table at the bottom shows the acuual counts for each size.
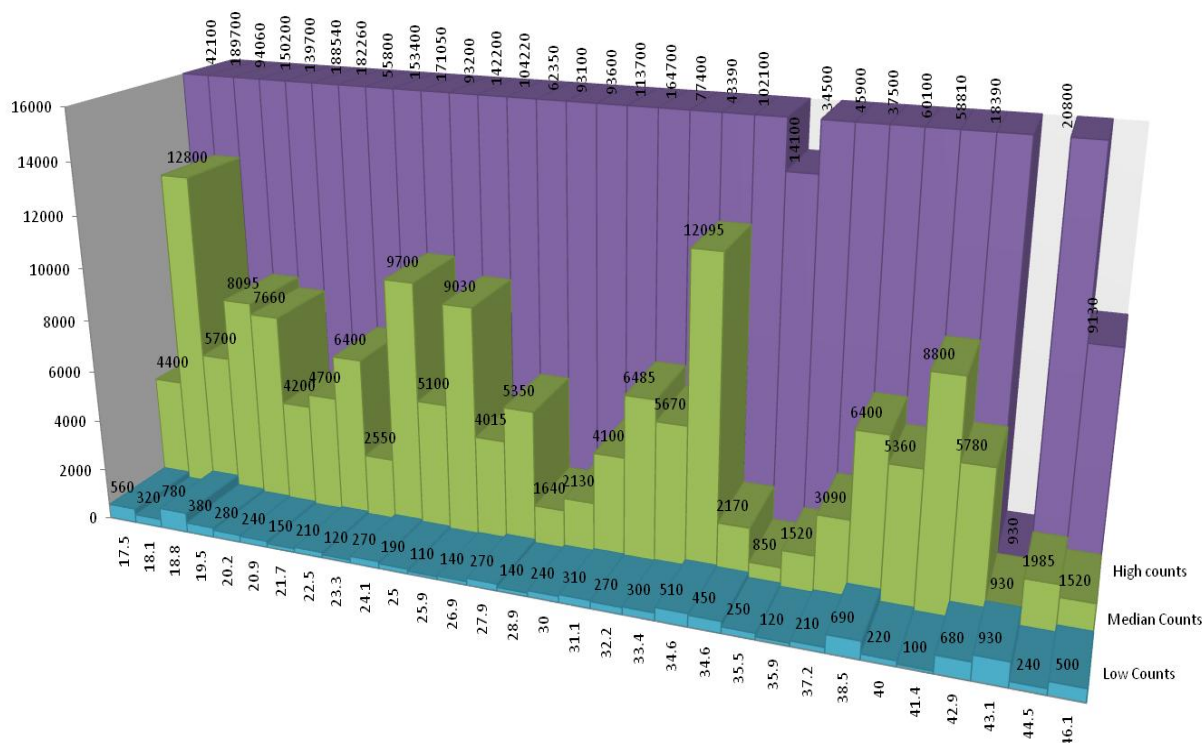
High, Median, and Low Logarithmic Scale for virus counts for each virus size
BVS, Inc - IVDS Detections

	17.5	18.1	18.8	19.5	20.2	20.9	21.7	22.5	23.3	24.1	25	25.9	26.9	27.9	28.9	30	31.1	32.2	33.4	34.6	34.6	35.5	35.9	37.2	38.5	40	41.4	42.9	43.1	44.5	46.1
Low Counts	560	320	780	380	280	240	150	210	120	270	190	110	140	270	140	240	310	270	300	510	450	250	120	210	690	220	100	680	930	240	500
Median Counts	4400	12800	5700	8095	7660	4200	4700	6400	2550	9700	5100	9030	4015	5350	1640	2130	4100	6485	5670	12095	2170	850	1520	3090	6400	5360	8800	5780	930	1985	1520
High counts	42100	2E+05	94060	2E+05	1E+05	2E+05	2E+05	55800	2E+05	2E+05	93200	1E+05	1E+05	62350	93100	93600	1E+05	2E+05	77400	43390	1E+05	14100	34500	45900	37500	60100	58810	18390	930	20800	9130

Figure 4. High, Median, and Low Logarithmic Scale. This data format presents the material so the count fluxuations of each band of relative data (high, median and low) easily stand out.

High, Median, and Low Fixed Scale - limited at 16000 counts for each virus size
BVS, Inc - IVDS Detections

	17.5	18.1	18.8	19.5	20.2	20.9	21.7	22.5	23.3	24.1	25	25.9	26.9	27.9	28.9	30	31.1	32.2	33.4	34.6	34.6	35.5	35.9	37.2	38.5	40	41.4	42.9	43.1	44.5	46.1
Low Counts	560	320	780	380	280	240	150	210	120	270	190	110	140	270	140	240	310	270	300	510	450	250	120	210	690	220	100	680	930	240	500
Median Counts	4400	12800	5700	8095	7660	4200	4700	6400	2550	9700	5100	9030	4015	5350	1640	2130	4100	6485	5670	12095	2170	850	1520	3090	6400	5360	8800	5780	930	1985	1520
High counts	42100	2E+05	4060	2E+05	1E+05	2E+05	2E+05	5580	2E+05	2E+05	93200	1E+05	1E+05	62350	93100	9360	1E+05	2E+05	77400	43390	1E+05	14100	34500	45900	37500	60100	58810	18390	930	20800	9130

Figure 5. High, Median, and Low Fixed Scale. The high data set is capped and only shows data up to 16,00 counts, although each size has the actual count labed.

The samples run show that we have the ability to compare an individual colony to the all of the samples we have processed. A comparison of this nature will show the increase or decrease in viral loads over time in any particular colony or apiary. The reports can bring together several combinations of viral load data that would be relevant to any one beekeeper. The following charts in Figures 6-9 show a series of colony data to the all the data, to a single colony over time and multiple colonies in a single apiary.

Single colony to all the data

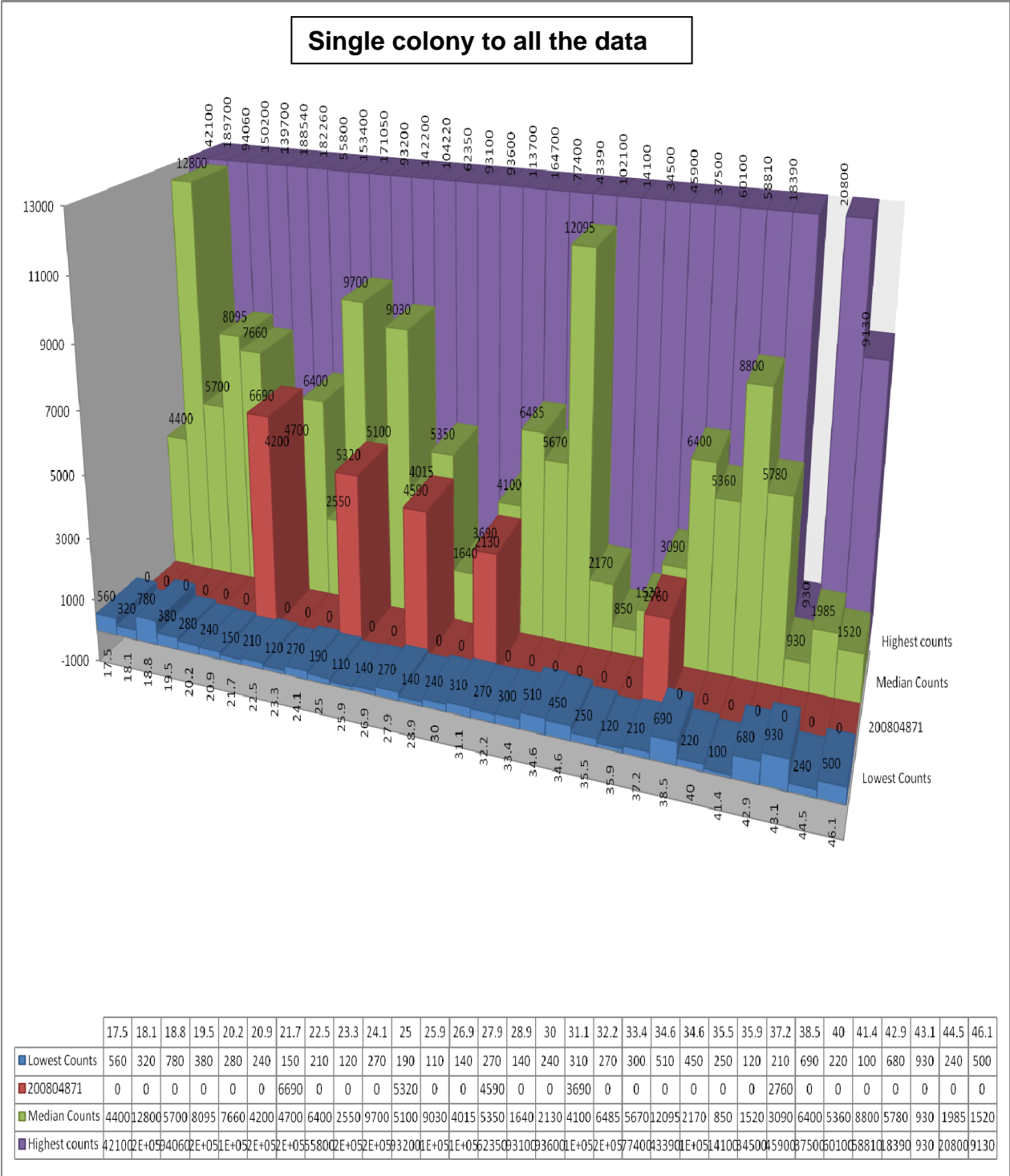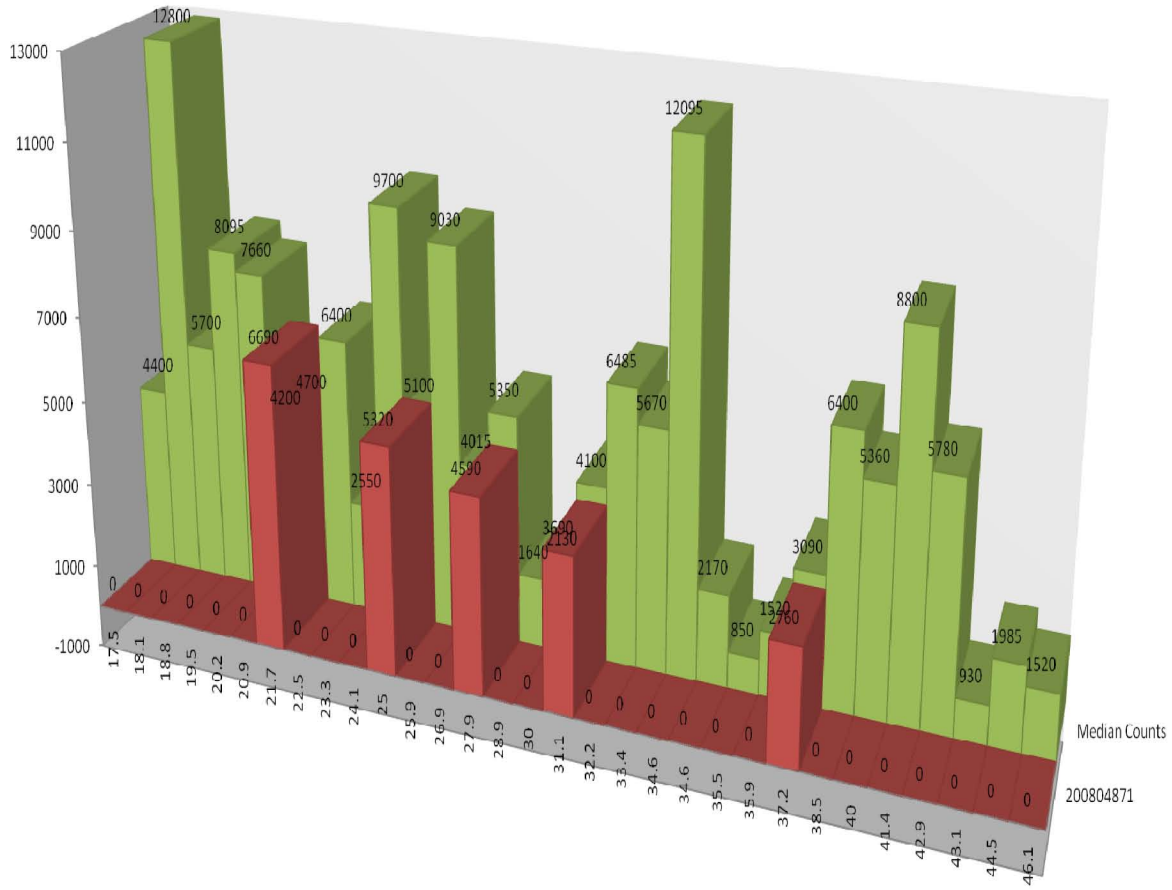|	17.5	18.1	18.8	19.5	20.2	20.9	21.7	22.5	23.3	24.1	25	25.9	26.9	27.9	28.9	30	31.1	32.2	33.4	34.6	35.5	35.9	37.2	38.5	40	41.4	42.9	43.1	44.5	46.1
■ Lowest Counts	560	320	780	380	280	240	150	210	120	270	190	110	140	270	140	240	310	270	300	510	450	250	120	210	690	220	100	680	930	240	500
■ 200804871	0	0	0	0	0	0	6690	0	0	0	5320	0	0	4590	0	0	3690	0	0	0	0	0	0	2760	0	0	0	0	0	0	0
■ Median Counts	4400	12800	5700	8095	7660	4200	4700	6400	2550	9700	5100	9030	4015	5350	1640	2130	4100	6485	5670	12095	2170	850	1520	3090	6400	5360	8800	5780	930	1985	1520
■ Highest counts	42100	2E+05	94060	2E+05	1E+05	2E+05	2E+05	55800	2E+05	2E+05	93200	1E+05	1E+05	62350	93100	93600	1E+05	2E+05	77400	43390	1E+05	14100	34500	45900	37500	60100	58810	18390	930	20800	9130

Figure 6 Single colony to all the data. The account number listed shows data from a single colony and how the viral loads compare to the median and low data as well as the diversity of viruses detected in a colony.
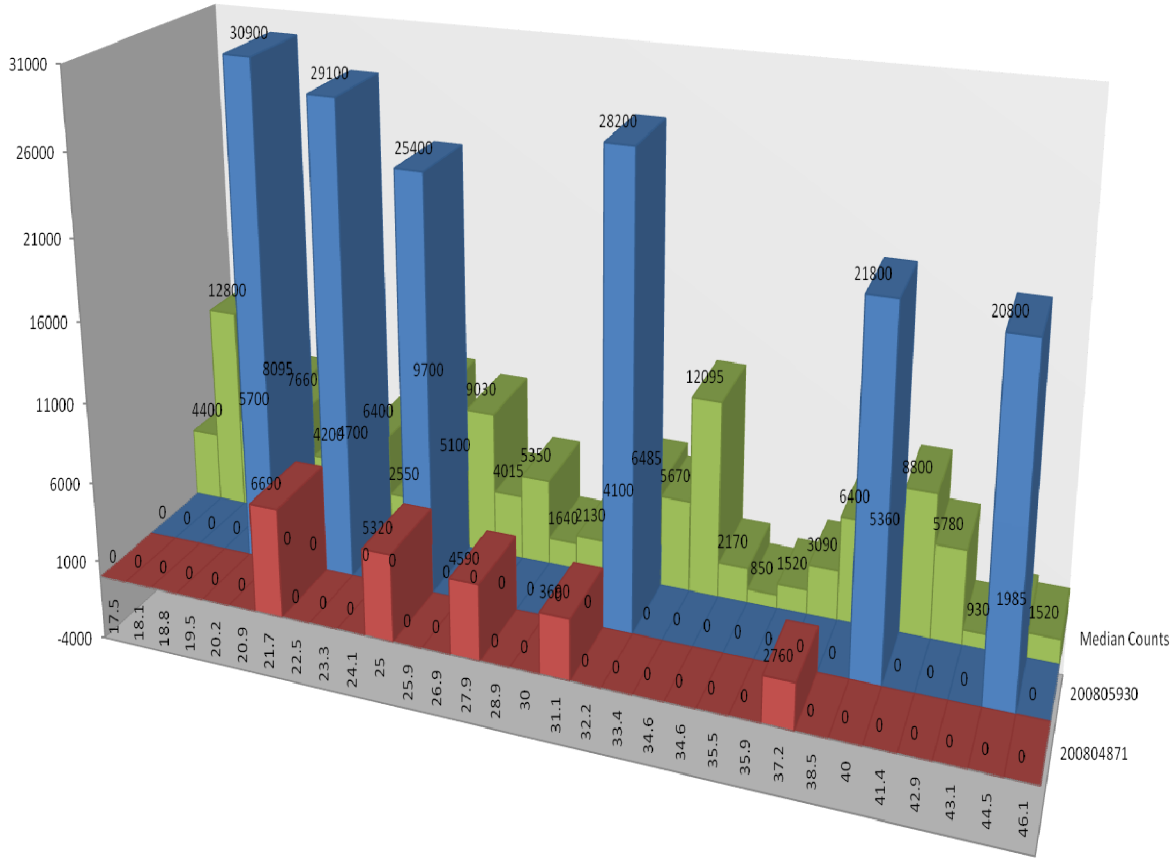
Single colony compared to the median values only

	17.5	18.1	18.8	19.5	20.2	20.9	21.7	22.5	23.3	24.1	25	25.9	26.9	27.9	28.9	30	31.1	32.2	33.4	34.6	34.6	35.5	35.9	37.2	38.5	40	41.4	42.9	43.1	44.5	46.1
20080487/1	0	0	0	0	0	0	6690	0	0	0	5320	0	0	4590	0	0	3690	0	0	0	0	0	0	2760	0	0	0	0	0	0	0
Median Counts	4400	12800	5700	8095	7660	4200	4700	6400	2550	9700	5100	9030	4015	5350	1640	2130	4100	6485	5670	12095	2170	850	1520	3090	6400	5360	8800	5780	930	1985	1520

Figure 7 Single colony compared to the median values only. The data can be simplified to show only selected data from the whole that will give a quick comparison of viral loads that are relative to bee health.

Multiple colonies and the median values (note the scale change for the virus counts)

	17.5	18.1	18.8	19.5	20.2	20.9	21.7	22.5	23.3	24.1	25	25.9	26.9	27.9	28.9	30	31.1	32.2	33.4	34.6	35.5	35.9	37.2	38.5	40	41.4	42.9	43.1	44.5	46.1
200804871	0	0	0	0	0	0	6690	0	0	0	5320	0	0	4590	0	0	3690	0	0	0	0	0	0	2760	0	0	0	0	0	0	0
200805930	0	0	0	0	30900	0	0	29100	0	0	25400	0	0	0	0	0	0	28200	0	0	0	0	0	0	0	21800	0	0	0	20800	0
Median Counts	4400	12800	5700	8095	7660	4200	4700	6400	2550	9700	5100	9030	4015	5350	1640	2130	4100	6485	5670	12095	2170	850	1520	3090	6400	5360	8800	5780	930	1985	1520

Figure 8. Multiple colonies and the median values. The ability to compare colonies side by side for viral loads and diversity for following the spread of infections from on colony or apiary to another is a useful tool.

The next chart shows a comparison of the same colony with a two week interval between samples. The second set has noticeably lower counts and fewer peak detections (again the median values are in the background for comparison);

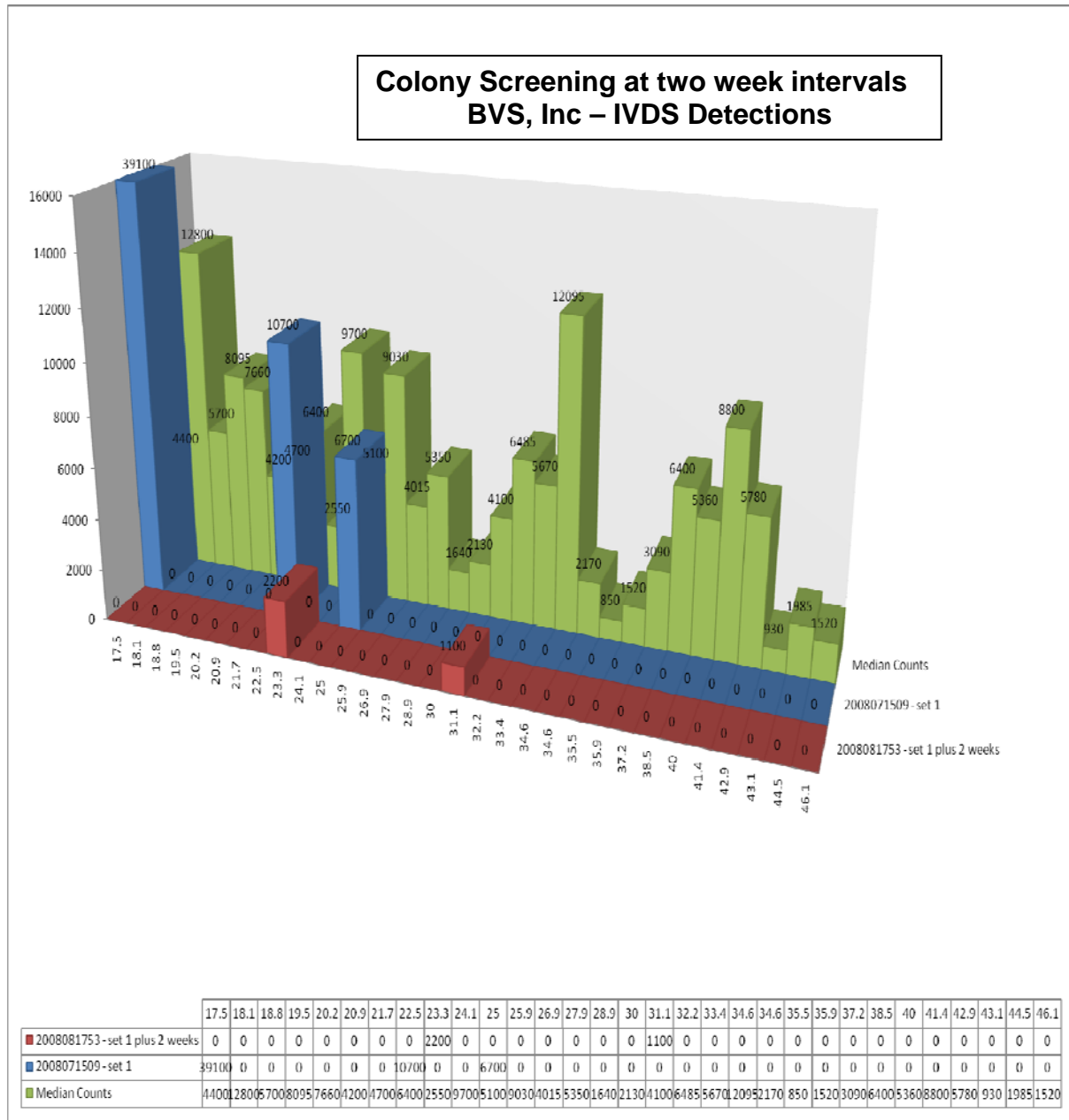|	17.5	18.1	18.8	19.5	20.2	20.9	21.7	22.5	23.3	24.1	25	25.9	26.9	27.9	28.9	30	31.1	32.2	33.4	34.6	34.6	35.5	35.9	37.2	38.5	40	41.4	42.9	43.1	44.5	46.1
■ 2008081753 - set 1 plus 2 weeks	0	0	0	0	0	0	0	0	2200	0	0	0	0	0	0	0	1100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
■ 2008071509 - set 1	39100	0	0	0	0	0	0	10700	0	0	6700	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
■ Median Counts	4400	12800	5700	8095	7660	4200	4700	6400	2550	9700	5100	9030	4015	5350	1640	2130	4100	6485	5670	1205	2170	850	1520	3090	6400	5360	8800	5780	930	1985	1520

Figure 9 Colony Screening at Two Week Intervals. The data show the diversity and counts of viruses changing over a two week period. These data show a change in viruses in two weeks, there are only two and they are different.

The data and the related charts produced from the IVDS instrument is what we were looking for and a success in the form of a useful tool to the beekeepers. BVS, Inc would like to acknowledge The Almond Board of California for their help in making this type of

data possible. A new way of looking a viral loads and virus management is here because of vision and support from beekeepers and industry.

Virus analysis to the beekeepers has been difficult because of the cost and difficulty in detection. We can now work to find solutions to viral management and beekeeper practices in relation to viral loads. Correlation of these data with transportation, feeding, neighbors bee health, honey flow, wintering, and other beekeeping data will provide a significant tool on what to do with viruses and how to manage them.

The next step is to do the research and comparative work that will put virus names on the peaks. In addition, we are ready to screen bees for viruses for beekeepers and to have them send samples. This will be initiated by direct contact, sending out letters to them directly, and by publications in journals, newsletters and emails offering our services.

Recent Publications:

UM plays role in understanding Colony Collapse Disorder
Montana Kaimin - Missoula,MT,USA
The common symptom of **CCD** is a hive that is missing most of its **bees**, although no dead **bees** can be found in or around the hive. Using new technology created **...**

From Edgewood Chemical and Biological Center (ECBC):

Scientists Discover New Virus Invading US Honeybees

Aberdeen Proving Ground, MD ─ Scientists at the Edgewood Chemical Biological Center (ECBC), located in Edgewood, MD, working with scientists at the University of Montana and industry partners Bee Alert Technology, Inc. and BVS, Inc. have discovered in U.S. honeybees a virus only before identified in European honeybees.

The invading bee virus newly discovered in the U.S. is called Varroa Destructor Virus -1 (VDV-1). First definitively identified in Europe in 2006, VDV-1 is carried by both honeybees and the tiny varroa mites that affect them. VDV-1 is related to a family of paralytic viruses that causes a breakdown of some membranes. In silkworms the virus causes flaccid disease, which causes the worms to digest themselves internally.

The virus was discovered using a technology developed for battlefield detection of viruses. This technology, called Integrated Virus Detection System / Proteomic Mass Spectrometry, reveals virus by size and peptide information contained in a sample and compares that information against known genetic sequences. This approach may provide important clues to scientists around the world working to find the cause of Colony Collapse Disorder -- a mysterious malady that has caused rapid depopulation of beehives around the globe.

This is the first detection of this virus in North America and will allow beekeepers in the U.S. the possibility of early control and quarantine of affected colonies.

For more information, please contact Joan Michel (410.436.3610; 410.652.3912 – mobile).