Fall/Winter 1997 Newsletter
(The formatting of this newsletter has been changed for electronic presentation.)

The Association of Veterinary Microbiologists

Message from the President

Dear Members of the AVM,

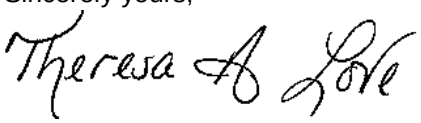
As a child, I had been fascinated by the way veterinarians were able to take care of sick animals. As I grew up, it became apparent that there were special hands working diligently behind the scenes giving these doctors their almost uncanny way of knowing what was wrong and how to cure it. Today, I can say I am very proud to be a part of "behind the scenes". We all work as a team. We are forever exchanging techniques and ideas, benefiting from the ideas and troubleshooting the problems. We complement each other.

There are many other organizations one can be a part of. When one enters a meeting of one of these organizations, what is gained? Technical advice for sure, but also a sense of detachment. When you leave one of those meetings, do you feel comfortable enough to give a member a call two or three weeks later and say for instance, "John, I have a problem with these reactions I got from this crazy instrument I ordered. What am I doing wrong?"

I want all of you to know how proud I am to be a member of the Association of Veterinary Microbiologists. Each of you are unique in your field, and gifted at your bench. We are all part of a family of caring individuals willing to share problems and ideas as well as handshakes. TOGETHER we can give our individual labs the best of the best.

Often, we never know how things always get done or accomplished, so there are some special thank you’s that must be given. I would like to thank Sherry Greer for always being there. Without her help and direction, this organization would not function. I commend her for all of her hard work. Judy Clapier, and Melody and Mike Parsley, thank you for such a wonderful time in Hot Springs; the meeting was a success and the accommodations were a delight. Rob Poston, thanks for putting together from cryptic notes a comprehensible scientific newsletter the Association can take pride in reading. My final thank you goes to John Black and Al Pursell for creating the Association of Veterinary Microbiologists, an organization with members dedicated to their work and to their colleagues.

Sincerely yours,



Theresa A. Love,
President, AVM
Jackson, Mississippi

AVM History and Philosophy

Drafted by Al Pursell, AVM Executive Advisor and Founding Member.

The idea for the Association of Veterinary Microbiologists was conceived in 1972 by Alfred R. Pursell of Tifton, Georgia and John W. Black of Hopkinsville, Kentucky. The concept was based upon an informal meeting of laboratory technicians and others interested in the laboratory diagnosis of animal diseases by virologic, bacteriologic, and serologic methods. The format for the meeting was to be a round table discussion on the problems and procedures of interest to the various areas.

At the 1973 regional meeting of the American Association of Veterinary Laboratory Diagnosticians in Nashville, Tennessee, representative of several diagnostic laboratories met with Pursell and Black to discuss the advantages of the proposed association. It was agreed that such an association would be beneficial to both the participants and their laboratories.

In 1975, with the encouragement of Dr. Wade Kadel, the Director of the Hopkinsville laboratory, John Black sent notices to the microbiologists at each of the veterinary diagnostic laboratories in the southeastern United States, announcing a "Symposium on Techniques of Diagnostic Veterinary Microbiology". This symposium was sponsored by the diagnostic laboratories of Tennessee, Kentucky, and Georgia. It was held at the Mountain View Hotel in Gatlinburg, Tennessee on May 22, 1976. A total of 33 people from eleven laboratories, representing eight states were present. John Black presided over the meeting and was elected the first president of the Association. Separate sessions on virology and bacteriology and a combined session of serology were held with a moderator selected to guide the discussion. An agenda served only as a guide and each participant was free to propose additional topics for discussion.

The primary participants were the people actually doing the work at the bench. They were encouraged to present any problems and to work together on possible solutions. To establish the informality required for such an exchange the use of titles was discouraged and everyone was on a first name basis.

The objectives of the association, which were set forth in the constitution adopted in 1978 are "...to promote scientific investigations and their applications to the advancement of knowledge in this field, and to provide mutual assistance to participating laboratories in solving problems".

AVM Membership Information

Who Can Join? Any person interested in the objectives of the AVM and who is active in the field of veterinary microbiology shall be eligible for membership.

Annual Meetings. The AVM annually conducts a Symposium on Techniques in Veterinary Microbiology. In addition to the business session, the general session, and guest speakers, the principle feature of the annual meeting is the discussion sessions, typically in the "round-table" format, on the problems and procedures of interest to the various areas and disciplines of veterinary microbiology. The southeastern United States has been divided into four geographic regions for the purpose of rotating the location of the annual meeting:
Region I: Kentucky and Tennessee
Region II: Delaware, Maryland, North Carolina, South Carolina, Virginia, West Virginia
Region III: Alabama, Florida and Georgia
Region IV: Arkansas, Louisiana and Mississippi

Membership Directory: A membership directory is published every year and mailed to each member. A membership directory and membership application form can be obtained by contacting the President or Secretary of the Association.

Newsletter: A newsletter is published each year, or biannually if resources permit. It contains information on meetings, training conferences, new techniques and other items of interest.

Exhibitors and Presentations: Commercial exhibits and presentations on topics of mutual interest are welcome; their incorporation into the meeting program are left to the discretion of the local meeting site arrangements committee.

AVM Fees: Membership dues (annually) $10.00, Meeting registration $15.00, Sustaining Membership (annually) $100.00, Exhibitor's fee, $300.00.

AVM Officers and Executive Board Members, 1997-1998

President Theresa Love, Jackson, MS Colonial States Representative
Vice President Judy Clapier, Raleigh, NC Marion Fowler, Dover, DE
Secretary/Treasurer Sherry Greer, Hopkinsville, KY Heartland Representative
Executive Advisor Al Pursell, Tifton, GA Tim Klinefelter, Ames, IA

Past President Rob Poston, Baton Rouge, LA Publications Chairperson
Past President Michael Justin, St. Joseph, MO Rob Poston, Baton Rouge, LA
Past President Melody Parsley, Little Rock, AR Meeting Site Chairperson
Historian Roxie Maddux, Hopkinsville, KY Kay Tipton, Nashville, TN

AVM Standing Committees

Publications Frank Austin, Tom Chang, Rob Poston, Dorothy Scott-Wright
Nominations Michael Justin, Melody Parsley, Rob Poston
By-Laws John Black, John Cole, Al Pursell
Audit Bill Cornell, Roxie Maddux
Meeting Site Judy Clapier, Kay Tipton

Program Sandy Blackwell, Theresa Love, Janet Mapp
Newsletter Advisory Sandy Baldwin, John Cole

Ad-Hoc Committees

Web Page Development Lea Dowd, Dexter Thompson
QA/QC Manual Marion Fowler, Beth Henricson, Steve Wessman

The AVM Colonial States Chapter

In 1985, The Colonial States Chapter was formed in Richmond, Virginia primarily from members belonging to Region II of the AVM. The CSC holds its annual meeting in Williamsburg, Virginia each November and usually sponsors a symposium on one of the microbiological disciplines (bacteriology, virology, immunology, serology, etc.) each spring. The pertinent membership information of the AVM also applies for the CSC. In years when the annual meeting of the AVM falls into Region II, the CSC annual meeting is usually held concurrently with the parent organization. CSC annual membership fees are $10, for sustaining membership, $50. Meeting registration is $15 and the exhibitor fee is $100. Those interested are invited to contact the chapter Secretary/Treasurer George Blackwell at 540/433-1638 or one of the other chapter officers.
Colonial States Chapter President Marion Fowler, Dover, DE
Colonial States Chapter Vice President Beth Henricson, Warrenton, VA
Colonial States Chapter Secretary/Treasurer George Blackwell, Harrisonburg, VA

The AVM Heartland Chapter

In 1995, The Heartland Chapter was formed, and is comprised of AVM members residing in the United States north-central region, which includes the states of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin. The Heartland Chapter typically holds organizational and scientific meetings and offers wet labs around the last weekend of April each year, in a format similar to the parent organization. Heartland Chapter annual dues are $5. Those interested are invited to contact the chapter Secretary/Treasurer Kathleen Strelow at 608/266-2465, or one of the other chapter officers.
Heartland Chapter President Tim Klinefelter, Ames, IA
Heartland Chapter Vice President Anne Parkinson, Reynoldsburg, OH
Heartland Chapter Secretary/Treasurer Kathleen Strelow, Madison, WI

AVM News, Events, and Items of Interest

Due to the chronic illness of AVM Secretary/Treasurer Sherry Greer, long-time AVM member Emilie Thost, currently retired from the Mississippi Board of Animal Health laboratory in Jackson, Mississippi, has consented to assist in the duties of the Secretary/Treasurer. Emilie, along with Sandy Blackwell and AVM President Theresa Love will conduct a significant portion of the AVM business affairs in advance of this summer's annual meeting. They can be contacted at the Mississippi Board of Animal Health, P.O. Box 4389, Jackson, Mississippi 39216, tel: 601/354-6089, fax: 601/354-6097.

AVM members are asked by the Program Committee to put on their thinking caps and consider possible discussion topics for this summer's meeting. If you have experienced difficulty with a particular technical procedure, been involved in some new discovery, developed a new improvement or wonder what works best in certain circumstances, make note of it and send, phone, or fax it to Janet Mapp, Sandy Blackwell, or Theresa Love at the Mississippi Board of Animal Health Laboratory, P.O. Box 4389, Jackson, Mississippi, 39216, tel: 601/354-6089, fax: 601/354-6097. To conveniently submit topics, please make use of the tear-off slip, found on the last page of this newsletter. Those members planning informal presentations at the discussion sessions are asked to assist organizational efforts by informing the program committee of the topic, and by providing a draft to the Publications committee to facilitate its inclusion in subsequent newsletters.

The AVM Colonial States Chapter has agreed to host the 1999 AVM meeting, planned for region II during that year. The dates, location and accommodations have yet to be decided, but further details will be announced at the national AVM annual meeting in 1998.

The AVM Colonial States Chapter is now accepting names toward nomination for chapter positions of Vice President and Secretary/Treasurer. To place names under consideration, or for information on the requirements and duties of chapter officers, AVM-CSC members are invited to contact current Chapter President Marion Fowler at 302/739-4811 ext. 275. Individuals put forth for consideration will be screened by the AVM-CSC's nomination committee, which selects the nominees for election at the AVM-CSC's annual meeting in November.

John Black announces that American BioResearch Laboratories have moved into a new 5000 sq. ft. facility. On Monday, March 2, 1998, ABR proudly began operating from a new, bigger, and more modern laboratory building located a short distance from the old facility. The new telephone number is: 423/908-8826; fax; 423/908-8738. The new addresses are:
Shipping: American BioResearch Mailing: American BioResearch
1419 Chapman Hwy P.O. Box 6609
Sevierville, TN 37876 Sevierville, TN 37864
Packages sent to the old address will be picked up there and delivered to the new building for approximately 60 days so that no shipments will be lost or returned. Phone calls to the old number will be automatically forwarded to the new lab for several months, as will FAXes. ABR invites any client to visit officially or unofficially at any time. ABR plans to soon have a new catalog, including pictures of improvements and personnel. ABR will be fully GLP compliant as soon as equipment has been validated and physical plant logs have been rewritten.

At the previous AVM Executive Board meeting, an Ad-Hoc Committee was formed and charged to solicit input from AVM members for a QA/QC guide, developed by Beth Henricson. The committee is comprised of Beth Henricson (Chairperson), Marion Fowler, and Steve Wessman. All AVM members are invited to participate in the review of this manual. Those interested in may contact Beth at 540/347-6385.

For an AVM Web Site development update, please see the Ad-Hoc Committee for Web Site Development report in this newsletter issue. The Ad Hoc Committee invites all AVM members to view the prototype AVM web site at http://www.gnat.net/~lea/avm/index.html. AVM members are asked to respond to the Web Site Questionaire, also included in this newsletter.
[NOTE: This web-site address is no longer valid as we now have our own site.]

AVM net-surfers are invited to check out VetMicroVirol E-mail list-serve, hosted by AVM sponsor VMRD, Inc. This is a forum for questions, answers and information relevant to the field of veterinary microbiology and virology. Members can subscribe at http://www.vmrd.com/vmv.htm/.

The weekly federal publication Morbidity and Mortality Weekly Report (MMWR) and the quarterly federal publication Emerging Infectious Diseases (EID) are accessible on the Internet at htpp://www.cdc.gov/.

Meetings of the AVM and its Chapters

18 April, 1998 (Saturday). The AVM Colonial States Chapter (CSC) Spring Symposium at the Boars Head Inn in Charlottesville, VA. The focus for this year's symposium will be on virology. Dr. Sandra S. Cloud of the University of Delaware Dept. of Animal and Food Sciences will speak on Avian Influenza Virus: Isolation, Characterization and Control; Dr. Anthony Castro of the Pennsylvania State University Animal Diagnostic Laboratory will speak on Optimization of Fluorescent Antibody and Immunohistochemical Techniques for Diagnosis of Animal Diseases, and Dr. Elizabeth Howerth of the University of Georgia School of Veterinary Medicine will speak on the Do's and Don'ts of Diagnosing Epizootic Hemorrhagic Disease. Mid-April typically features "Historic Garden Week" in Charlottesville and Albemarle County, when many scenic attractions and historic exhibits are at their peak for public viewing. Those wishing to attend will be responsible for their own overnight arrangements; a list of accommodations is available upon request, or by calling the regional Convention and Visitors Bureau at 804/977-1783. AVM-CSC members have been sent meeting information and registration forms. Anyone else desiring information or registration can contact Marion Fowler at 302/739-4811 ext. 275, Beth Henricson at 540/347-6385, or George Blackwell at 804/515-0462.

23 April, 1998 (Thursday). Mycotic Workshop, *Famous and Infamous Fungi: A "Hands On" Review of Medically Significant Fungi in Veterinary Practice*, co-sponsored by the AVM Heartland Chapter (HC) (in conjunction with its annual meeting, detailed below) and the National Laboratory Training Network, Midwest Office. Registration for the workshop will be $60.00, and is limited to 30 people. Participation qualifies for continuing education credit. HC members have already been sent application forms, which must be faxed or mailed to Barbara Henderson, NLTN, 2121 W. Taylor St., Chicago, IL 60612, fax: 312/793-3304. Anyone else interested in attending the workshop should contact Anne Parkinson at 614/728-6220.

24-25 April, 1998 (Friday and Saturday). The AVM Heartland Chapter Fourth Annual Meeting, hosted by the Ohio Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, 8995 E. Main St., Bldg. 6, Reynoldsburg, Ohio, 43068. In addition to the business meeting and round table discussions, Dr. Brenda Love will speak on Salmonella DT104 and susceptibility testing, and Dr. Sheila Grimes will speak on post-weaning multi-systemic syndrome in pigs. Reservations and accommodations will be provided by the Lenox Inn, P.O. Box 346, I-70 and St. Rd. 256, Reynoldsburg, Ohio 43068-0346, tel: 800/821-0007. Room rates will be $50 for single occupancy, $56 for double. To make reservations, ask for Group Block #2167 under the AVM. Reservation deadline is March 20. Meeting registration and membership dues are $45 and include a catered lunch on April 24th. Response forms have been mailed to all HC members; anyone else interested in attending should contact Kathy Strelow at 608/266-2465.

30 July to 1 August, 1998 (Thursday through Saturday). The AVM 23rd Annual Symposium on Techniques in Veterinary Microbiology at the Regal Maxwell

House in Nashville, Tennessee. Room rates for occupancy of one to four is anticipated to be $80 per night. The Regal Maxwell House is located just off of I-265, 1.5 miles north of downtown Nashville in the Metro Center office park. This location is convenient to downtown attractions such as the Historic Second Avenue district, featuring the Hard Rock Cafe, the Wild Horse Saloon, and Planet Hollywood. Meeting notices will be distributed to AVM members in the Spring of 1998. For information, contact Kaye Tipton at 615/837-5125. Individuals wanting advanced information on the amenities of Nashville and surrounding area are invited to call the tourism office at 615/259-4700.

12 November, 1998 (Thursday evening) AVM Colonial States Chapter (CSC) Executive Board meeting will be held in conjunction with the CSC annual meeting (see below). This year's CSC Board Members are: President Marion Fowler, Vice President Beth Henricson, Secretary/Treasurer George Blackwell, Past Presidents Earnest Wyant, Welford Harris, and Dorothy Scott-Wright, Advisors Lynn Lewis, Andy Myrup, Judy Clapier and Al Pursell.

13-14 November, 1998 (Friday and Saturday). The fourteenth annual meeting of the AVM Colonial States Chapter at the Ramada Inn and Conference Center in Williamsburg, Virginia. The meeting format will be similar to the AVM annual national meeting. Topic focus for the meeting has yet to be decided. Accommodations are expected to be available at a group discount rate. For meeting information, please contact Beth Henricson at 540/347-6385.

Meetings of the American Association of Veterinary Laboratory Diagnosticians
26-29 April 1998. The Northeast Branch of the American Association of Veterinary Laboratory Diagnosticians (AAVLD), Cornell University, Ithaca, New York. The tentative schedule includes a pre-meeting Fingerlakes winery region tour on the 26th. On the 27th, a Johne's and Rabies Symposium is planned. The next day (the 28th) will cover food safety, infectious disease reporting, QA/QC discussion, laboratory breakout sessions, enteric disease update (salmonellosis, including DT104), and the last day (the 29th) will cover equine topics. Contact: Don Lein, Cornell Diagnostic Lab, tel: 607/253-3903, e-mail: hmb5@cornell.edu; or Helen Bell, e-mail: hmb5@cornell.edu

30-31 May, 1998. The South Central Branch of the AAVLD Fourth Annual Meeting, New Mexico Veterinary Diagnostic Services, Albuquerque. Contact John Thilsted, tel: 505/841-2576, fax: 505/841-2518, e-mail: jpt@nmda.nmsu.edu

16-17 June 1998. The NCCVLD (the North Central Branch), Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia. Contact: Bill Van Alstine, tel: 765/494-7440, fax: 765-494-9181, e-mail: billvan@addl.purdue.edu

2-9 October, 1998. The 41st Annual Meeting of the AAVLD, Hilton Hotel, Minneapolis, Minnesota. Contact Art Bickford, AAVLD Secretary/Treasurer, CVDLS, P.O. Box 1522 Turlock, CA 95381, tel: 209/634-5837, fax; 209/667-4261, e-mail: abickfor@cvdls.ucdavis.edu, or an AAVLD representative at 314/882-6811, or visit the AAVLD web site: http://padls28.cas.psu.edu/ AAVLD/Future%20Meetings/

Meetings of the Veterinary Laboratory Association
9-12 December, 1999. The Veterinary Laboratory Association (VLA) 10th annual meeting at the Condado Plaza Hotel and Casino, San Juan, Puerto Rico. Contact: Carlos Ortiz at 787/753-2231 or Dick Kilburn at 630/983-5640. There may be additional regional VLA meetings during the Fall and Winter of 1998, and the Spring

of 1999; as details are established, they can be obtained from VLA newsletter coordinator Joni Ray at 940/241-2529, fax: 940/455-5025, e-mail: vlabjohn@aol.com, or from the Administrator of Proficiency Program Dr. Milton J. Becker at 773/764-1862.

Meetings of the American Society for Microbiology

27-28 March, 1998. Joint Annual Meeting of the ASM Missouri Branch, Missouri Valley Branch, and Midwest Microbiology Educators in Kansas City, Missouri. Contact: Nehad El-Sawi, President, Missouri Branch, Dept. of Microbiology, University of Health Sciences, College of Osteopathic Medicine, 2105 Independence Blvd., Kansas City, Missouri 64124; tel.: 816/283-2210; fax: 816/283-2357; E-mail, nelsawi@facl.uhs.edu.

17-21 May, 1998. The American Society for Microbiology (ASM) 98th general meeting, Atlanta, Georgia. Contact: ASM Meetings Dept., American Society of Microbiology, 1325 Massachusetts Ave, NW, Washington, D.C. 20005-4171; tel: 202/942-9248; fax: 202/942-9340; E-mail: meetingsinfo@asmusa.org; WWW http://www.asmusa.org/.

October-November 1998. Joint meeting of the Southeastern and South Central branches of the ASM, Gulf Shores, Alabama. No further details are available at this time, but they should be forthcoming in July.

Other Meetings

26-29 April, 1998. Clinical Virology Symposium and Annual Meeting, Pan American Society for Clinical Virology (14th). Clearwater Beach, Florida. Contact: Dr. Steven Specter, Dept. of Medical Microbiology and Immunology, Univ. of South Florida College of Medicine, 12901 N. Bruce B. Downs Blvd., Tampa, Florida 33612; fax: 813/974-4151; e-mail: sspecter@com1.med.usf.edu.

10-17 July, 1998. Rapid Methods and Automation in Microbiology Workshop (18th International). Manhattan, Kansas. Contact: Janice Nikkel, tel.: 785/532-5575; fax: 785/532-5637; E-mail: ksucon@dce.ksu.edu.

8-11 August, 1998. Southwestern Association of Clinical Microbiology (SWACM) Meeting. Austin, Texas. Contact: Suzanne S. Barth, Texas Dept. of Health Laboratory, 1100 W 49th Street, Austin, Texas 78756; tel.: 512/458-7214; fax: 512/458-7452; e-mail: sbarth@laba.tdh.state.tx.us.

Workshops Sponsored by the American Type Culture Collection

9-11 September, 1998. Cell Culture and Hybridomas: Quality Control and Cryopreservation.
23-25 September, 1998. Microscopy/Photomicrography.
16 October, 1998. Virus Propagation Seminar.
27-30 October, 1998. Freezing and Freeze-Drying of Microorganisms.
5-6 November, 1998. Anaerobic Bacteriology.
5-8 October, 1998. Hybridoma Technology and Monoclonal Antibody Product Development.
17-20 November, 1998. Polymerase Chain Reaction (PCR) Applications/Cycle DNA Sequencing.

For additional information, please call 800/638-6597; E-mail ATCC at workshops@atcc.org.

AVM Business Reports

Historian's Report and Archival Activities, 1996-97
Submitted by AVM Historian Roxanna Maddux.

AVM Directories 1976-1997. Protected in polyethylene storage bags; stored in drop-front archival box.

AVM Newsletters 1976-1997. Protected in polyethylene storage bags supported with bag stiffeners; stored in drop-front archival box.

AVM Programs 1976-1997. Protected in polyethylene storage bags; stored in drop-front archival box.

Photographs from 1992 and 1993 Meetings. Purchased 16 prints from Twin Lens Photo, Silver Springs, Maryland; protected in polyethylene Photo Guard ID pocket pages; stored in binder notebook.

Photographs from the Twentieth Annual Meeting, Gatlinburg, Tennessee. Protected in Photo Guard ID polypropylene pocket pages; stored in binder notebook.

Documents/Letters. Early documents and letters stored in protective sleeves and binder; request of early documents, letters, and photographs will be made during general meeting.
Budget Allowance, $300.00
Expenditures for 1996-1997
4/24/97 Light Impressions 121.50
4/01/97 Wal Mart 7.40
7/11/97 Light Impressions 36.20
7/11/97 Twin Lens Photo
7/ 97 Film

Minutes of the Business Session

The Twenty second Annual Meeting of the Association of Veterinary Microbiologists, The Arlington Resort Hotel and Spa, Hot Springs, Arkansas, Friday, August 1, 1997. The Business Session was called to order at 9:00 am by President Rob Poston, who welcomed everyone to the meeting and called for an introduction of all members.

President Poston introduced our exhibitors and thanked them for their support of our association. The exhibitors and the representatives were:
Accu Med International, Inc. Jennifer Lorbach 216/808-0000
Biolog, Inc. Debra Ann Gosling 408/842-7750
bioMerieux Vitek, Inc. Ray Turnley 800/638-4835
Centaur, Inc. Rod Hernandez 800/236-6180
Idexx, Inc Brenda Erickson 800/548-6733
Kirkegaard & Perry Laboratories Gwendolyn Campbell 301/948-7755
Synbiotics Corp. Howard Jones 800/841-1875
Viral Antigens, Inc. Robert Crandall 405/846-6003
VMRD, Inc. Thomas Kellner 509/334-5815

While the membership was reviewing the minutes from the twenty first annual meeting, held in Mobile, Alabama, President Poston made announcements regarding minor program changes, a questionnaire on the AVM Web Page under development, and a workshop on the identification of non-fermenting gram negative bacteria, sponsored by a subcommittee of the AAVLD.

The minutes from the business session of the twenty first annual AVM meeting were approved as written.
In the Treasurer's report, Secretary/Treasurer Greer reported income from August 1, 1996 through July 15, 1997 of $5145.00, with expenditures of $5290.12 for a balance on hand as of July 15, 1997 of $3991.54. The Treasurer's report was approved as read.

In the President's report, President Poston acknowledged and thanked the committee chairs and members, and others who have given their time and talent on behalf of the AVM, especially Meeting Site Chairperson Melody Parsley and Publications Chairperson Frank Austin for the superb results of their efforts. He told the membership that it was an honor and privilege to serve during the past year, and thanked the membership for the opportunity and their support.

Executive Board Report

President Poston continued with the Executive Board report. This consisted of:

1. Annual membership fees will remain at $10.00 per year.
2. Sustaining membership fees will remain at $100.00 per year.
3. The two-tiered registration fees policy was continued. This provided for a pre-registration fee of $15.00, and a regular registration fee of $25.00.
4. Exhibitor fees will remain at $300.00 per annual meeting.
5. Al Pursell was reappointed as Advisor to the Association.
6. An additional $200.00 was authorized to archive and preserve AVM memorabilia.
7. The By-Laws committee reported on proposed amendments to the AVM Constitution and By-Laws. The amendments will be published in the next newsletter and will be voted upon in the Business Session of the next AVM annual meeting.
8. Further considerations of the proposed Web Page, namely content, host, management and cost, were placed under the auspices of the Publication committee.
9. An Ad-Hoc committee was formed to solicit membership review of a QA/QC guide developed by Beth Henricson. The committee is comprised of Beth, Marion Fowler, Steve Wessman, and any other interested members.

The Meeting Site Committee report was given by Chairperson Melody Parsley. She introduced Roger Giddings, Superintendent of Hot Springs National Park. Mr Giddings presented a program on the history and amenities of Hot Springs, Arkansas.

The Colonial States Chapter report was given by Chapter President Marion Fowler. She discussed chapter activities during the past year, which included their annual meeting and a spring symposium. The next meeting, the fourteenth annual meeting of the CSC, will be held at the Ramada Inn and Conference Center, in Williamsburg, Virginia on November 7-8, 1997.

The Heartland Chapter report was given by Chapter President Tim Klinefelter. He discussed chapter activities during the past year, which included their annual meeting and a PCR wet lab on April 25-26, 1997 in Brookings, South Dakota. The next

meeting will be the fourth annual meeting of the HC, held on April 24-25, 1998 in Reynoldsburg, Ohio, hosted by the Ohio Animal Disease Diagnostic Laboratory.

The Audit Committee submitted a written report that the committee had looked at the books and the records of the Treasurer, and although a detailed financial audit was not done, according to the bank statements, checks drawn and receipts written, they appear in order.

President Poston solicited the membership for any unfinished or new business; at the time, there was none.

The Nominating Committee proposed Theresa Love as President and Judy Clapier as Vice President. They were elected by acclamation.

President Poston introduced Theresa Love, who as the newly installed AVM President, conducted the remainder of the business session.

President Love appointed Rob Poston, Frank Austin, Tom Chang, and Dorothy Scott-Wright to serve on the Publications Committee for the coming year.

John Cole, John Black and Al Pursell were reappointed to serve on the By-Laws Committee, and John Cole and Sandy Baldwin were reappointed to serve on the Newsletter Advisory Committee.

President Love appointed Sandy Blackwell and Janet Mapp to serve on the Program Committee.
The Nominating Committee for the coming year will be composed of Rob Poston, Mike Justin, Melody Parsley.

President Love appointed Judy Clapier to the Annual Meeting Site Committee, who reported that the twenty-third annual meeting of the AVM to be held in 1998 has been targeted for Memphis, Tennessee, depending on suitable arrangements.

President Love informed the members that she was looking forward to working with everyone in the coming year, and urged members to start making plans to attend the meeting next year.

There being no further business to conduct, the business meeting was adjourned.

Report of the Treasurer, July 15, 1997
Presented by AVM Secretary/Treasurer Sherry Greer to the general session of the AVM at the annual meeting in Mobile, Alabama, 1 August 1996.

BALANCE ON HAND, July 31, 1996 $4136.66

RECEIPTS:

Membership Fees	$1440.00
Registration fees:	
1996 Meeting	$ 990.00
1997 Meeting	$ 15.00
	$1005.00

Exhibitor Fees:

1996 Meeting:	$ 900.00	
1997 Meeting:	$1800.00	
	$2700.00	
TOTAL RECEIPTS:	$5145.00	

EXPENSES:

Annual Meeting 1996:

Holiday Inn, Mobile	$2169.88
Projector/Screen Rental	$ 110.00
Advisor Reimbursement	$ 240.00
Name Tags	$ 31.16
	$2551.04

Printing:	
Newsletter #1	$ 397.50
Program	$ 191.23
Newsletter #2 & Directory	$ 805.60
	$1394.33

Postage:	
Newsletter #1	$ 311.24
Program	$ 210.60
Newsletter #2 & Directory	$ 571.03
	$1092.87

Miscellaneous Expenses:	
Florist, J. Clapier	$ 40.44
Receipts, Ledger Sheets, Word Processor Ribbon	$ 53.74
Historian Supplies	$ 157.70
	$ 251.88
TOTAL EXPENSES:	$5290.12

BALANCE ON HAND July 15, 1997	$3991.54

Report of the By-Laws Committee

The following are proposed amendments to the AVM Constitution and By-Laws, put forth by the AVM By-Laws committee before the AVM Executive Board on July 31, 1997 in Hot Springs, Arkansas. According to Article X of the AVM By-Laws, the membership must be notified of proposed amendments by mail at least 30 days prior to the annual business meeting. Because the newsletter circulates to all AVM members in good standing, this page constitutes formal notification of the membership. It is the responsibility of all AVM members to review and consider the proposed amendments. A formal vote will occur during the next annual business meeting of the AVM this summer. The proposed changes are emphasized in italics.

Proposed Amendment to the AVM Constitution
ARTICLE I - TITLE, SECTION 3. Whenever a provision of these By-Laws is inconsistent with the provisions of the constitution, the provision in the constitution shall be controlling.

SECTION 3 IS TO BE DELETED
Proposed Amendments to the AVM By-Laws*.*
ARTICLE III - EXECUTIVE BOARD, SECTION 4. Two-thirds (2/3) of the voting members of the Executive Board shall constitute a quorum for the purpose of transacting business. At the discretion of the President, the Executive Board may transact business by mail*, fax, or electronic communication (e-mail).* At least two-thirds (2/3) of the voting members of the Board must participate in any action taken by *the above means of communication*.

PROPOSED LANGUAGE IN ITALICS IS TO BE ADDED
ARTICLE VI - COMMITTEES, SECTION 4. Annual Meeting Committee: The Annual Meeting Committee shall be composed of at least three (3) members residing in or near the city in which the Annual Meeting will be held. The Committee shall be responsible for the selection of a meeting site (city and hotel) and general supervision of the Annual Meeting, arrange the program of the scientific sessions, arrange for exhibits, demonstrations, *scientific papers,* and other activities associated with the Annual Meeting. The Chairperson shall be an ex-officio member of the Executive Board.

"SCIENTIFIC PAPERS" IS TO BE DELETED
ARTICLE VII - CHAPTERS, SECTION 2. B. That the petitioners will adopt the standard chapter By-Laws of the ASSOCIATION.
SECTION 2. B. TO BE CHANGED TO READ "That the petitioners shall establish standard By-Laws."
ARTICLE VIII - MEETINGS, SECTION 8. If necessary, voting on special issues may be conducted by mail ballot*, fax, or e-mail.* In the transaction of business by mail*, fax, e-mail,* fifty (50) percent of the membership shall constitute a quorum, provided that a reasonable time is allowed for members to submit their ballots.

PROPOSED LANGUAGE IN ITALICS IS TO BE ADDED
ARTICLE X - AMENDMENTS, SECTION 1. Amendments to the By-Laws shall be adopted by two-thirds (2/3) vote of the regular members present at the Annual Business Meeting of the ASSOCIATION.

SECTION 1 IS TO BE DELETED.
Amendments to the AVM By-Laws currently require a two-thirds (2/3) vote for approval; when approved, they become effective immediately. Once amendments to the AVM Constitution are approved by a majority vote during the annual business meeting, a written ballot is then sent to each member. To be valid, ballots must be returned within thirty five (35) days of mailing. A two-thirds (2/3) vote of the members submitting valid ballots is required for approval.

Report of the Ad-Hoc Committee on the AVM Web Page Development

Lea Dowd and Dexter Thompson
Greetings and Salutations for the World Wide Web,
Hope you have had a great fall and winter. Now for the business… We have been working with several ideas for the AVM's web presence. The first and foremost issue is where (what server) do we place it on? A permanent location is still being explored so we will have to get back to you at a later date. For now, temporary site has been

arranged. We prepared this a while back to just get something started. You can visit this first draft at:

http://www.gnat.net/~lea/avm/index.html
[Note: This is no longer a valid address. New address is www.avm.wso.net]

This is the first of the sample web pages. We are planning to put a link-up between the different examples, so that there are several different designs and types of web pages to be viewed. It will then be up to the members to make the decision as to the final look and content. Please check back at that address from time to time to take a look at our updates and additions. If you have any comments or suggestions, please jump right on in.

As stated, this is a start. We did not want to spend hours on this project until the general concept was agreed upon.

We also have discussed getting our own address (i.e., www.avm.net) and are considering the costs. Depending on what they are, attention will be given to finding a convenient and inexpensive host to locate the AVM web page. We are receptive to any thoughts or ideas regarding a potential host or sponsor.

Another factor carefully being considered is specialized web site functions for AVM members. Because one of the biggest advances of the Internet has been the rapid dissemination of information, several AVM members have inquired about the use of the web to pose questions. This would be similar to a newsgroup. While certain newsgroups are great, a newsgroup for a scientific organization will not work: Because newsgroups are open to the public, they are susceptible to erroneous information. We have been discussing a web bulletin board to which only AVM members would have access, a place for questions, answers and feedback that is private. We have also discussed placing a "Researchers" page up with the names and addresses and the areas of interest. These people could be listed under more than one topic; this also could be a private address. The first consideration though, would be the location of a web home.

Included in this newsletter is a questionnaire so that we can get a better feel of what you as AVM members would want or could use in a web page; responses from all members are welcome.

Another topic in discussion is the possibility of having an Internet workshop on the first day of the AVM Annual meeting this year. Some topics that would be discussed include:
I. How do I prepare to get on Internet? What will I need?
II. What software will I need?
III. What is the Internet?
IV. What can the Internet do for me?
V. What is E-mail? What is FTP?
VI. What are newsgroups? What is a web page? What is HTML?
We look forward to all comments and suggestions. Please contact us at:
Dexter Thompson, E-mail: alex@mail.misnet.com; WWW:
http://www2.misnet.com/~alex; tel: (601) 354-6089; fax: (601) 354-6097.
Lea Dowd, E-mail: lea@gnat.net.

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Internet Questionaire for AVM Members

Name: _____

Street Address: _____

City: _____

State/Province and Zip/Postal Code:_____

Phone: _____

1. How would you describe yourself as a Computer User (please indicate by checking box): Novice ____ Intermediate ___ Advanced ___
2. If you use a commercial online service, please specify it below.
3. CompuServe ____; America Online ____; Microsoft Network ____; Other ____
4. E-Mail Address: _____
5. Disk Operating System: Windows 95/NT ___; Windows 3.X ___ OS/2 ___ Other ___
6. If you use an Internet Browser, please specify it below.
7. Netscape ___; Internet Explorer ___; Mosaic ___; Other _____
8. Would you be interested in an Internet Workshop? Yes ___; No ___;
9. If you have a personal Web Site, please list below:
10. htpp://_____ _____

Please photocopy this page as necessary. After reviewing the AVM web site, please write-in any comments on this form. Please return completed forms to Dexter Thompson, Mississippi Board of Animal Health, Veterinary Diagnostic Lab, P.O. Box 4389, Jackson, Mississippi, 39216.

AVM Topic Discussion Session Notes

These notes were taken during the discussion sessions of the AVM 22nd Annual Symposium on Veterinary Microbiology held in Hot Springs, Arkansas on July 31-August 2, 1997. In advance of the meeting, discussion topics had been solicited by the Program Committee from members-at-large, who may have had unanswered questions on technical points surrounding these topics. In consideration of our topic submitters, additional information and references have been added where pertinent, in hopes that their questions will be more fully addressed. In the midst of coverage of certain published topics, discussions of other sub-topics unfolded. To ease information retrieval, some of these ancillary topics are presented under their own heading. Additionally, other miscellaneous information is placed where it would most likely be found, regardless of the heading under which it was actually discussed.

Avian Session

Moderated by Ching Ching Wu, Microbiology Department, Purdue University, West Lafayette, Indiana.

Reported by Kevin Cook, Missouri Department of Agriculture, Veterinary Diagnostic Laboratory, Springfield, Missouri; Rob Poston, Louisiana Veterinary Medical Diagnostic Laboratory, LSU School of Veterinary Medicine, Baton Rouge, Louisiana; Sara Rowe-Rossmanith, Charles S. Roberts Animal Disease Laboratory, Auburn, Alabama.

Laboratory Diagnosis of Avian and Ratite Adenoviruses. No one reported using established cell lines for poultry viral isolation. Laboratories performing avian virology typically generate their own Chick Embryo Fibroblast (CEF) cells. Eggs from a specific pathogen free flock (as from SPAFAS of Norwich, Connecticut or Hy-Line of Des Moines, Iowa) are recommended to establish CEF cultures.

The 1996 catalog of the American Type Culture Collection (ATCC), Rockville, Maryland lists a number of avian cell lines, including chicken embryo, chicken lymphoma (ALV-induced), goose, quail (fibrosarcoma QT6 and myoblast QM7), and turkey lymphoblast. Sandy Rodgers at OADDL, Stillwater, Oklahoma, was mentioned to have some success at developing an ostrich fibroblast cell line for viral work. [Rodgers S.J., et al, Preliminary studies of primary ostriches for the isolation of ratite viruses. Avian Dis. 1994 Oct-Dec. 38(4)866-72.]

Avian viral culture techniques may be derived from Isolation and Identification of Avian Pathogens, published by the American Association of Avian Pathologists (AAAP); a new edition is due to be released in October, 1997.

A report was mentioned of the successful isolation of an Adenovirus from an ostrich using CEF cells. [Please see Capua I. et al, Isolation of an adenovirus from an ostrich (Struthio camelus) causing pancreatitis in experimentally infected guinea fowl (Numida meleagris). Avian Dis. 1994 Jul-Sep. 38(3)642-6.]

Electron microscopic (EM) examination of homogenized tissues may reveal adenovirus if there is a large amount of virus in the specimen. EM is a relatively insensitive technique, requiring at least 10^6 viral particles per ml. Negative EM results have no meaning because virus could still be present in concentrations below the threshold of detection.

[On the significance of avian influenza in ratite species, please see Swayne D.E., et al, Assessment of the ability of ratite-origin influenza viruses to infect and produce disease in rheas and chickens. Avian Dis. 1996 Apr-Jun. 40(2). P 438-47.]

Diagnosis of Chlamydial Infection in Pet Birds by Fecal FA. None of the members present report using FA techniques to detect Chlamydial elementary bodies (EB) in bird droppings. To detect EB's, immune probes are typically directed against the major outer membrane protein (MOMP). As defined by MOMP analysis with monoclonal antibodies, at least three serovars of *Chlamydia psittaci* are recognized among pet and exotic birds, three in pigeons, and one in turkeys, with more being recognized with continued research. [Andersen, A.A. Serotyping of Chlamydia psittaci isolates using serovar-specific-monoclonal antibodies with the microimmunofluorescence test, J Clin Microbiol., 1991. Apr 29(4)707-711.]

To detect any possible avian serovars, the Chlamydial EB FA reagent should be directed against MOMP epitopes common to all significant avian serovars; if the reaction range of the FA conjugate is limited to one given serovar, some false negative reactions would be expected. Also, chlamydia-infected birds shed intermittently, and may go undetected if testing is performed on single samples. [For more information on the FA technique for Chl. psittaci in bird stool, please see Woods L.W., et al, A rapid monoclonal immunofluorescence assay for *Chlamydia psittaci* in fecal smears from psittacine birds, J Vet Diagn Invest., 1989, Apr 1(2)150-3.]

NVSL and numerous vendors of human diagnostic biologicals have available direct FA conjugates to detect group specific antigen of Chlamydia psittaci. These conjugates typically highlight the chlamydial inclusion or microcolony in the cytoplasm

of the infected cell, and will detect cells infected by most strains of Chlamydia. Theoretically, these conjugates are able to detect exfoliated, infected cells within a fecal smear, however false negatives would be anticipated when an infected bird fails to shed intact, infected cells in detectable amounts.

Some in attendance noted the inherent difficulty to culture Chlamydia from fecal samples due to bacterial contamination and low level intermittent shedding. [However, the bacterial content in feces from healthy pet birds is predominantly gram positive and may be controlled with aminoglycoside antibiotics (i.e., streptomycin, gentamicin, kanamycin, vancomycin, etc.) and low speed centrifugation.

The difficulty presented by low level intermittent shedding by asymptomatic or subclinically infected birds may be partially compensated by educating clientele on proper sampling protocol, which includes correct transport media, refrigerated storage, and serial collections. By following a multiple collection protocol, a negative culture result would carry a higher level of confidence than would come from single sample testing. Ideally, three separate negative culture attempts would give the greatest level of certainty that the bird was actually chlamydia-free. However, due to the biology of Chlamydia in pet birds, there always remains a slight probability that the bird may still be infected with Chlamydia, even with repeated negative cultures.] Avian chlamydial cultures were said to be performed by Arthur Andersen at NVSL, Ames, Iowa, and Rob Poston, LaVMDL, Baton Rouge, Louisiana. Although fecal sampling is popular in avian chlamydial diagnostics, oral and pharyngeal samples are useful because chlamydia also populate the linings of the respiratory tract and sinuses in cases of psittacosis. Prevalent culture techniques include the use of coverslip-shell vial cell cultures, using L92, Vero, or McCoy cell lines. Inoculum is centrifuged onto the monolayer, and low doses of cycloheximide is included in the post-inoculation medium. Infection is verified by Giemsa, Gimenez, or FA staining. Yolk sac inoculation of embryonated eggs remains a traditional method of Chlamydia culture.

The departure of the Kodak SureCell® Chlamydia ELISA leaves a vacuum in veterinary diagnostics; this kit had been popular in off-label pet bird use, but was removed from distribution due to inordinate amount of false positives in human testing.

Other commercial kits available to test for chlamydia infection are not licensed or approved for use in veterinary applications; relative sensitivities, specificity's, and reaction probabilities published for these human-targeted kits have no meaning for veterinary samples. In trying other rapid ELISA human test kits for diagnosis of Chlamydia infections in birds or animals, non-specific reactions are a potential problem. Although most kits target the group specific antigen of Chlamydia (so that they may react against other species of *Chlamydiae* and their subtypes), they differ by their specimen filtration and pretreatment steps, which are crucial in veterinary testing. Unlike human testing, animal specimens for chlamydial testing may be taken from a variety of origins (fecal, conjunctival, nasal, synovial, placental, pharyngeal, etc.), and tend to spend a lengthier time in transit, thus develop bacterial overgrowth, causing non-specific reactions.

According to a number of studies, ELISA test kits may not be as sensitive as culture in the detection of Chlamydia. [Stenberg K., et al, ELISA and immunofluorescence tests for the diagnosis of conjunctivitis caused by Chlamydia trachomatis in neonates and adults, APMIS, 1990 Jun. 98(6)514-20; Arizmendi F. and Grimes J.E., Comparison of the Gimenez staining method and antigen detection ELISA with culture for detecting chlamydiae in birds, J Vet Diagn Invest. 1995 Jul. 7(3)400-1;

Hewinson R.G., et al, Detection of Chlamydia psittaci DNA in avian clinical samples by polymerase chain reaction, Vet Microbiol. 1997 Feb. 54(2)155-66.]

[Readers interested in further discussion on Chlamydia ELISA test kits are invited to refer to the Fall/Winter 1996 edition of the AVM newsletter. Also, further discussion on Chlamydia, particularly on the TVMDL's EBA test for chlamydia antibody in pet birds, and on the development of a Chlamydia vaccine for use in pet birds, is found in the Virology Session notes of this issue under Chlamydia Update.]

False Positive Reactions by Avian Influenza (AI) AGID Serology. False positive reactions are suspected when testing reveals the presence of AI antibodies in a given bird, but attempts fail to culture the virus. However, discordant results between serology and culture may occur for biological reasons, i.e., the presence of antibody in the animal actively prevents the recovery of virus, or if the bird has been vaccinated (a practice prohibited in the U.S., according to Easterly, p. 599). [According to an old, undated version of an NVSL protocol for avian influenza viral antibody AGID testing, the following types of reactions are observed and interpreted: *Negative*, when the control lines continue into the test sample well without bending; *positive*, when control lines join with and form a continuous line with the line between the test serum and antigen; and *weak positive*, when the control lines bend slightly toward the antigen well and away from the positive control serum well, but do not form a complete line between antigen and test serum.]
Problems with serological technique are revealed when positive samples become negative upon repeated testing. With the AGID technique, possible causes of inconsistent reactions are cross- contamination of reagent wells, when leaks occur under the agar layer, similar to that occasionally seen in EIA viral antibody testing. A corrective suggestion was to change the base agar from Bacto-agar to agarose, which was said to give clearer, sharper lines. Caution is suggested when plugs are aspirated from wells; the agar areas surrounding the plate wells would be left undisturbed.

Most members in attendance reported using NVSL reagents without false reaction problems, despite variations in serotype. [For further information on Avian Influenza, please see Easterly, B. C., et al, Influenza, in Calnek, B.W., et al, eds., *Disease of Poultry*, 10th ed., Iowa State University Press, Ames, 1997, pp. 583-605.]

Mycoplasma Laboratory Diagnostics. The mycoplasma diagnostic protocol used by many members include the plate agglutination serology using antigen from Intervet, HI serology using antigen from NVSL, ELISA serology using commercial test kits, culture on Frey's media or PPLO broth with swine sera, and PCR methodology, either internally developed or from commercial test kits (as from IDEXX, costing $15 per sample). For regulatory purposes, the NPIP only recognizes results from culture.

A variety of culture media formulations and formats was reported to be in use by the AVM members. A procedure to culture avian mycoplasmas was distributed. [This protocol was provided by Annie P. Van der Lek, Department of Avian Pathology, UNC-VMDL, Columbia, Missouri, and will appear in the Spring/Summer 1998 edition of the AVM newsletter.] Routine practice of mycoplasma culture improves technique and increases the probability of successful recovery.

A multiplex PCR protocol to simultaneously detect several species of mycoplasmas was said to be in development by several research groups, including Lauerman (see below), Mark Jackwood at the University of Georgia, Athens, and David Ley and Ed Berkhoff at North Carolina State, Raleigh. PCR methods typically include computer analysis to identify species and strains by restriction fragment length polymorphism (RFLP) band patterns. Theoretically, PCR is more sensitive than culture, and is able

to render results in 48 hours. Continued use of PCR in Mycoplasma diagnostics is resulting in greater confidence in the technique.

Corroborating evidence is recommended in occasional instances when a PCR positive and culture negative tests are encountered; clinical evidence or serological data, as generated by ELISA or HI technology as prescribed by NPIP, are used to referee discordant data.

Lloyd Lauerman of the Charles S. Roberts Veterinary Diagnostic Laboratory, Auburn, Alabama (334/844-4987) offers PCR-based mycoplasma diagnostic service, satisfactorily used by some AVM members present. His technique uses RFLP analysis to increase specificity. [Notes on PCR and application of Lauerman's technique are found in the AVM Spring/Summer 1997 Newsletter.]
HI serology is still used as reference technology by some labs due to the number of suspicious, potentially false reactors found by ELISA, which may be too sensitive. Instances were mentioned of positive ELISA/negative HI serology reactors, from which culture attempts have always been negative. Labor and cost are still factors inhibiting greater use of ELISA testing.

It was noted that separate ELISA kits exist for chicken and turkey serum specimens: KPL has kits for chicken MG and MS, turkey MG, MS, and MM. IDEXX markets a chicken MS/MG combination kit and MG and MS kits separately, but has no serological kit to test turkey serum for mycoplasma antibodies.
Following infection by certain uncommon strains of Mycoplasmas, birds may remain negative by both HI and PCR, suggesting that these techniques, as currently practiced, may also lack sensitivity or be too specific for the common, known stains of Mycoplasma.

[For further information on PCR testing for mycoplasmas in birds, please see Wang H., et al, Multiplex PCR for avian pathogenic mycoplasmas, Mol Cell Probes. 1997. 11(3)211. Garcia M., et al, Use of species-specific oligonucleotide probes to detect *Mycoplasma gallisepticum, M. synoviae,* and *M. iowae* PCR amplification products. J Vet Diagn Invest. 1996 Jan. 8(1)56-63. Lauerman L.H., et al, Avian mycoplasma identification using polymerase chain reaction amplicon and restriction fragment length polymorphism analysis. Avian Dis. 1995 Oct-Dec. 39(4)804-11.]

Differentiation of Infectious Bronchitis Virus (IBV) Strains and Recognition of the New Variant Strain 072. The recognized genetically stable strains of IBV are Arkansas 99, Massachusetts, Connecticut, Florida, Georgia, and the 072. The Arkansas 99 strain was said to currently comprise 85% of the ongoing incidence of IBV infection. Another strain, which is a combination of Arkansas and Connecticut strains, also is occasionally seen.

IBV is a single stranded RNA virus of the *Coronaviridae* family. Dual infection by two different strains may give rise to phenotypic recombinant virus containing antigenic traits of both. Environmental selection and vaccination also may be forcing the creation of new IBV strains. PCR technology, as performed by Lauerman and Jackwood, is now extensively used to detect IBV and characterize the different strains, including the 072 strain.

Ovoculture, with subcultures, was said to be useful to increase the sensitivity and consistency of IBV detection by PCR in clinical samples. Lauerman reports the use of such an approach, now requiring only two subcultures, instead of the original three, before testing allantoic fluids for IBV by PCR.

An immunohistochemical procedure, using monoclonal antibody to detect IBV in tissues, was reported to have been developed by S.A. Naqi at Cornell University, Ithaca, New York. [Please see Naqi, S.A., Avian Diseases, 1990, Oct-Dec 34(4),893-8. A monoclonal antibody-based immunoperoxidase procedure for rapid detection of infectious bronchitis virus in infected tissues.]

[More information on IBV diagnostics is found in the avian session notes of the AVM Fall/Winter 1996 Newsletter, under the heading Methods and Techniques for Serotyping Avian Infectious Bronchitis Virus.]

Ornithobacterium rhinotrachaele (ORT) Serology. ORT is implicated in respiratory disease of turkeys and chickens. When ORT infection is clinically expressed, the signs are similar to those seen in Pasteurella multocida infection. ORT has also been discovered in asymptomatic infections, leading many to question whether ORT is a primary pathogen.

No members present reported the routine use of serological techniques to support ORT diagnosis. ORT serology was reported to be possible with a rapid plate agglutination method or an agar gel immunodiffusion (AGID) format. [On February 27, 1998, Dr. Dave Miller of the NVSL was contacted regarding a possible protocol to produce antigen for an AGID or slide agglutination serology test for ORT. He reports that no ORT serology is currently being performed at the NVSL, which uses cultural methods only to support diagnosis.]

For the diagnosis of ORT infection, many labs attempt culture, the reliability of which depends on proper technique and technical experience with the organism. ORT is recovered in greater frequency during cooler weather, and often appears in pure culture. ORT is cultured from the lung, trachea, and air sac on yeast blood plates in a 10% CO_2 environment. At 24 hours on primary culture, pinpoint colonies are observed. By 48 hours, colonies are about 1-2 mm in diameter, circular, opaque, somewhat iridescent, convex, gray with an entire edge. ORT does not grow on MacConkey agar; its isolation protocol does not require selective media. Smears made from growth reveal gram negative, highly pleomorphic rods. ORT is catalase negative and oxidase positive, does not react in standard tube biochemical tests, but is identified with the API 20NE and API ZYM systems. In the 20NE system, most ORT isolates are urease positive, and all are *B* D-galactosidase (PNPG) positive. [For further information, please see Chin, R.P, and Dronel, R., *Ornithobacterium Rhinotracheale* Infection, Calnek, B.W., et al, eds., Disease of Poultry, 10th ed., Iowa State University Press, Ames, 1997, pp.1012-1014.]

Laboratories working with ORT were recommended to obtain a reference culture for quality control of culture technique and to attempt antigen manufacture. ORT stock cultures are best stored frozen, at -20 C or lower, following harvest from the log phase of growth (24-48 hour old growth). Tissue culture grade fetal calf serum (FCS) and 10% dimethyl sulfoxide (DMSO) may be used as cryostabilizers. Another suitable cryostorage medium is made of BHI broth with 50% glycerol. Yolk sacs of embryonated eggs were said to make an excellent storage medium: Eggs of six day gestation are inoculated by the yolk sac route, then the yolk sacs are harvested after 48 hours incubation, aliquoted, and frozen.

Poultry Enteritis Mortality Syndrome (PEMS) of Turkeys. A greater incidence of enteritis was reported, but it is uncertain whether the increase in enteritis is comprised entirely of PEMS. Several different viruses (i.e., coronavirus, arenavirus, astrovirus, or the Stunting Syndrome Agent or SSA) have been implicated as possible causative agents of PEMS, which is now thought to have multiple etiologies.

Coronavirus is a suspected agent of PEMS, but it has not been able to completely reproduce a PEMS model infection in pathogen-free birds. Material from PEMS cases has been found to be FA negative for coronavirus, yet able to pass enteritis to other birds, which then shed coronavirus. The presence of coronavirus is still considered an indicator of PEMS and flock contamination.

To control enteritis in commercial flocks, conscientious industrial flock managers practice stringent biosecurity, eradicate coronavirus-infected birds, and attempt to control possible arthropod vectors, specifically flies and darkling beetle larvae. Diagnosis of PEMS includes FA for coronavirus on bursa or intestine. Despite this straight-forward diagnostic approach, other factors undoubtedly are involved in this disease. [For further information, please see Barnes, H. J. and Guy, J.S., Poult Enteritis-Mortality Syndrome ("Spiking Morality") of Turkeys, in Calnek, B.W., et al, eds., *Disease of Poultry*, 10th ed., Iowa State University Press, Ames, 1997, pp 1025-1030.]

Emerging Infectious Problems: Blackhead in Turkey Pullets. Certain turkey producers in Arkansas have seen the reemergence of blackhead (Histomoniasis) in baby chicks. Affected birds exhibit white, chalky lesions initially in the cecum, then in the liver. A characteristic initial lesion of Blackhead is the appearance of cecal cores, which develop around the eighth day of infection. As histomonads invade cecal tissue, the walls of the ceca become thickened and hyperemic; serosanguinous exudate overwhelms the mucosal surface and fills the cecal lumen. This forms a cheesy core that distends the cecal walls, leading to ulceration, perforation, and subsequent peritonitis. Active protozoa may be found in cecal cores, but only from fresh specimens. Protozoa are also seen histologically in acute cases. The clinical appearance of blackhead was said to be exacerbated by the presence of *E. coli* and *Salmonella spp*.

Effective treatment for Blackhead is somewhat limited, due to the removal of nitrofuralizone from usage; to treat Blackhead, it was said that some flock managers resort to the extra-label usage of Histostat or Flagyl. The pathological appearance of Blackhead is milder in chickens. Typically, blackhead is controlled by good management practices, minimizing the accumulation of litter, thereby suppressing ammonia levels.
[According to McDougald (cited below), Histomoniasis, also known as Blackhead or Infectious Enterohepatitis, is a parasitic infection involving the ceca and liver of gallinaceous birds. Histomonas meleagridis has a complex life cycle: Histomonas infect the nematode Heterakis gallinarum, which is transported by earthworms, which are then eaten by birds. Direct transmission is possible, but not likely because Histomonas is poorly viable outside of accessory hosts.

Blackhead is typically diagnosed by the characteristic gross appearance of the cecum and liver. Phase contrast microscopic examination of cecal contents reveals histomonads, which become active when the slide is gently warmed. With fresh specimens, in vitro culture is reported to be highly successful.

Climatic conditions and soil types favorable to Heterakis and earthworm proliferation are considered predisposing factors; birds kept on sunny, well-drained ranges have a greatly decreased disease incidence. Blackhead has been effectively treated and prevented with nitroimidazole feed additives, which are no longer available in the U.S. because of recent regulatory changes.

Chickens infected by Histomonas exhibit milder pathology than turkeys, and may be a source for continuous infestation of ranging grounds. Chiefly for this reason, cohabitation of turkeys and chickens is considered an unwise practice. For further

information, please see McDougald, L.R., Other Protozoal Diseases of the Intestinal Tract, in Calnek, B.W., et al, eds., *Disease of Poultry*, 10th ed., Iowa State University Press, Ames, 1997, p. 890.]

Increased Incidence of Marek's Disease (MD) and Leukosis. Ongoing, sporadic myeloblastosis has been seen primarily in the DelMarVa (Delaware, Maryland, and Virginia) region, but now was reported to be found in greater frequency in Arkansas. When myeloblastosis is discovered in chicks, as early as four weeks of age but up to 16 weeks old, it is generally attributed to Marek's Disease Virus (MDV). When myeloblastic disease is found in chickens older than 16 weeks, and involves the bursa, it is thought due to the J strain of Avian Leukosis Virus (ALV), which also occasionally manifests itself as encephalitis.
Despite the widespread practice of MDV vaccination in the poultry industry, MD occurs in occasional outbreaks, typically causing a lymphoproliferative disease. The continuous appearance of MD outbreaks may be attributable to the sporadic emergence of uncommonly virulent MDV strains, which are not completely controlled by the popular vaccine regimen.

The MDV group includes three serotypes; serotype I is MDV, serotype II includes non-pathogenic herpesviruses isolated from poultry, and serotype III is the Herpesvirus of Turkeys (HVT). Chickens are typically vaccinated against MD with HVT. To broaden the range of immunity, two other vaccine forms are now available; one is the HVT + SB-1, a bivalent vaccine of improved potency, attributable to synergism between serotypes II and III. The other is the Rispens vaccine, made from the mildly virulent CVI988 European strain, and only used in selected areas. [For further information on Marek's Disease Virus, see Calnek, B.W. and Witter, R.L., Marek's Disease, *in Diseases of Poultry*, 10th ed., B.W. Calnek, et al, eds., Iowa State Univ. Press, Ames, 1997.]

The J strain is more insidious than other strains of ALV due to its capacity for both vertical and lateral transmission. Commercial diagnostic test kits feature antigen capture ELISA technology, targeting the p27 group specific core protein of ALV, theoretically able to detect any ALV infection. There is concern whether the kits are too sensitive by their inadequacy to distinguish endogenous virus from field virus infection. The demonstration of lesions or ALV antibodies, for which commercial test kits are also available, helps to interpret antigen kit testing results. [According to Lisa Lemieux of IDEXX, presently there is no IDEXX kit test for Marek's Disease, but IDEXX is currently developing ELISA kits to specifically detect either antigen or antibody to J strain ALV. For further information on the Avian Leukosis/Sarcoma viruses, please see Payne, L.N. and Fadly, A.M., Leukosis/Sarcoma Group, in *Diseases of Poultry*, 10th ed., B.W. Calnek, et al, eds., Iowa State Univ. Press, Ames, 1997.]

Establishing an Specific Pathogen Free (SPF) Flock. Hy-Line International of West Des Moines, Iowa, and SPAFIS of Norwich, Connecticut are prominent reputable national sources of SPF eggs. AVM newsletter readers are invited to examine the quality assurance testing profiles from these firms to discern the extensive testing needed to establish and maintain an SPF flock. SPAFIS was mentioned to now guarantee chick anemia virus-free eggs. Some members expressed interest for a potential source of SPF turkey eggs for laboratory purposes.

Chick Anemia Rapid Diagnostic Test Kits. In April 1997, IDEXX unveiled its Chick Anemia test kit, which is a competitive serology assay that sells for $480 per kit, or about one dollar per well. Reactions are judged according to sample-to-negative (S/N) ratios.

KPL still offers its Chick Anemia testing service, which costs $75 per flock (20 samples per flock). KPL also intends to offer a test kit, pending USDA approval. The KPL kit is a direct ELISA assay, yielding antibody titer based on absorbance, and classifying reactions according to a sample-to-positive (S/P) ratio.
Testing and vaccination are inherently part of surveillance and control efforts. Vaccination may interfere with serodiagnosis, which requires paired sera.

The consensus of membership was that the Chick Anemia tests work well, and that not many positives are being found.

Increase of Larngyotracheitis (LT) Outbreaks: Field versus Vaccine Strains. LT has been recently reported in Arkansas and Georgia, but Alabama has not experienced any LT this past year. Genuine outbreaks seem to occur more frequently in winter, when poultry houses are shut.

Partially due to increasingly stringent export criteria, automatic control measures are triggered in certain states once a field strain of LTV is isolated. Laboratories may be pressured to discern LT viral isolates as "vaccine" strains to avoid export sanctions. This pressure was speculated to explain a recent increase in the isolation rate of vaccinal LTV.

Because of recent export testing stringencies instituted by Russia, certain criteria must now be met before an authentic outbreak of LT is reported; one criterion is that the affected flock must demonstrate a 10% or greater mortality. Once evidence of virus is found, reporting requirements now differ, depending on the strain of virus. The histopathological demonstration of specific lesions of LT remains the definitive method of verification.

It is believed that there are no longer any true outbreaks of LT, but disease incidence fluctuates in two to three year cycles. Because there exists a certain amount of background flock contamination with LTV, and that the practice of vaccination for LTV is not universal, industry-wide control or eradication measures are thought to be impractical.

Current conventional laboratory technology does not differentiate between vaccine strains and field strains. NVSL offers a good FA conjugate (not listed in their catalog) to detect any strain of LTV. Convenient differential technology is currently in development; PCR protocols to detect LTV have been published. [Please see Abbas F., et al, Development of a polymerase chain reaction and a nonradioactive DNA probe for infectious laryngotracheitis virus. Avian Dis. 1996 Jan-Mar. 40(1)56-62.]

In general, proper testing methodology should be selected to accommodate regulatory or industrial goals, whether for eradication or surveillance. Animal health authorities should make these goals clear to both producers and laboratories. Whenever eradication is the desired control approach, laboratory methods must be unambiguous and forensically defensible.

Ostrich Testing and the National Poultry Improvement Plan (NPIP). NPIP subpart F has been amended to include ostriches in Salmonella Pullorum/Typhimurium (PT) testing policy. However, no officially recognized standard methods or approved PT reagents are currently available to test ostriches. The use of poultry PT tube antigen on ostrich serum undoubtedly results in some, yet unknown degree of false indications. When an ostrich gives a positive PT plate reaction, it is unknown what action is necessary to sanction the animal, whether by the tube test, culture, necropsy, or other means.

For poultry, the consequences of positive reactions vary by the goals set by the animal health and regulatory authorities of different states: Certain states may call for eradication, while others mandate verification by one culture attempt from a cloacal swab. Testing with the current PT methodology could result in needless eradication. Without an indemnity program to compensate owners on the loss of stock, the owner of an ostrich found positive with current PT testing methodology may be unnecessarily forced to suffer a considerable economic loss.

Currently, PT testing of ratites is required in only certain states; many state ratite organizations have not addressed this issue. NPIP was changed to include ostriches, but not, as of yet, other ratite species; rhea and emu grower organizations have not yet advocated the inclusion of their species into the NPIP.
The difficulty demonstrated by this issue is a repeat of an old problem, when there are no officially approved reagents or methods for mandated testing.

Fluoroquinolone-resistant Salmonella and E. coli. Some concern is being expressed by public health and food safety groups, consumer interest groups, and the popular media for the appearance of antibiotic-resistant bacteria in the food supply. One potential source was suggested to be poultry processing effluent, because fluoroquinolones are known to be occasionally used in processing washes (of tanks and equipment). However, excluding incidental contamination, bacteria from slaughter house processing probably does not routinely contaminate live flocks; production and slaughter are typically two independent, compartmentalized operations that proceed in one direction, as product is taken to slaughter. Due to feed manufacturing methods, resistant bacteria are not likely to be disseminated in feed additives, however cattle may acquire resistant bacteria where poultry litter is permitted to be spread on cattle pastures. Those in attendance have not seen many poultry isolates demonstrating unusual antibiotic resistance patterns.

Antibiotic resistance by potentially pathogenic bacteria is thought to emanate in part from drug administration practices. When new antibiotics are developed, they are typically used first because they are thought to be the most effective. Antibiotics may be overly prescribed, principally by pediatricians, in the treatment of minor ailments as mild upper respiratory, sinus, or ear infections. By using newer antibiotics, some physicians attempt to avoid allergic reactions in their patients, who may have developed hypersensitivity to the older drugs. One thought was to mandate usage of older antibiotics in instances when they prove to be effective with sensitivity testing. Another suggestion was to establish a national database of antibiograms from food source zoonotic bacteria to determine if any industrial processes contribute to changes in antibiotic resistance patterns.

On the Cover...

Just another skunk and bull story, huh? On the morning of Thursday, August 7, 1997, a veterinarian practicing in south-central Louisiana treated an adult Charolais bull, pastured near Port Barre, for helminths and a possible bowel obstruction. Treatment was ineffective, so the bull was referred to the Large Animal Clinic of the LSU School of Veterinary Medicine. At presentation, the bull was anorexic and constantly strained to produce a bowel movement. The bull worsened overnight, became progressively ataxic, then exhibited generalized signs of posterior paresis. The bull went down, showed no prospect of improvement, so was euthanized.

On Friday afternoon, August 8th, the Virology Unit of the Louisiana Veterinary Medical Diagnostic Laboratory (VMDL) received brain tissue from the bull for rabies virus testing. Impression smears were made from the hippocampus, cerebellum, and

brain stem, and were fixed in acetone for one hour. The smears were stained with a direct fluorescent antibody conjugate against Rabies virus. The stained slides were incubated for 30 minutes, then rinsed for 10 minutes. Examination of the smears with fluorescent microscopy revealed apple-green fluorescence in a pattern characteristic of rabies virus: Antigen was deposited along axonal and dendritic processes, and occurred in small patches in infected cells. Occasionally, an entire neuron was found ladened with rabies virus antigen. FA staining was repeated twice more on additional smears, with positive reactions obtained each time. This procedure is typical of that performed by many State public health laboratories in their rabies virus testing programs.

Verification was provided by histopathological evaluation of brain tissue and rabies virus culture, which involved inoculation of suckling mice, understood to be the most sensitive lab host for the recovery of live rabies virus from clinical material. On August 12th, five weanling mice were inoculated intracranially with a homogenate of brain tissue. The first pair of mice died on August 29th, 17 days post-inoculation. The brain tissue of the mice was tested by FA and found to be positive for rabies virus. During the intervening period, histopathological evaluation of formalin-fixed brain tissue sections revealed lesions compatible with rabies virus infection.

Theoretically, all mammalian species are susceptible to rabies virus infection, but in the U.S., cattle, horses, dogs, cats, and humans are considered only incidental hosts for rabies. Natural host species for rabies virus include the bat (universal in North America), South Central Skunk, North Central and California Skunk, Atlantic Raccoon, Texas Gray Fox and Coyote, Arizona Gray Fox, and Arctic and Red Fox, all of which are reservoirs of rabies virus in nature. Compared to incidental hosts, rabies virus infection in its natural, biotypically homologous host is usually more protracted. Eventually, even the natural host is overcome by rabies virus infection, causing behavioral changes and ending in death.

Each natural host species has a unique rabies virus biotype, distributed according to the host's geographical habitat range and distinguished by monoclonal antibody analysis. In instances of rabies virus infection of unusual or incidental hosts, viral biotyping reveals the ultimate source of virus.

Rabies virus biotype is typically deduced from the pattern of reactions by a panel of monoclonal antibodies directed against epitopes on viral nucleocapsid and core proteins. In biotyping analysis, smears are made from either the original specimen, the mouse brain culture, or neuroblastoma cell line infected with the rabiesvirus isolate. The various monoclonals, as primary antibody in the IFA method, are separately reacted to fixed smears or infected cells. Anti-mouse Ig conjugate is applied as the secondary antibody. In brain smears, positive test reactions appear similar to the direct FA test. To discern the rabiesvirus biotype, the reaction pattern made by the various monoclonals is applied to an identification key, similar to the biochemical identification of bacteria.

The value of rabies biotyping is illustrated by the handful of human rabies victims annually, as reported in MMWR. Many human cases involve no identifiable exposure, bites, scratches or encounters with wild animals. Biotyping from these human cases almost invariably reveals a bat rabiesvirus biotype. With this knowledge, the victims' survivors sometimes remembers a startlingly casual, inconsequential (or so it was thought) contact involving a bat. For this reason, rabies prevention efforts target both children and adults, and advocate the avoidance of wild animals, especially sick animals, and all bats.

The mouse brain culture material from the bull rabiesvirus isolate was referred to the Texas State Public Health Laboratory in Austin for biotyping, which revealed a skunk biotype of rabiesvirus. This finding was not surprising; rabies testing records indicate that rabid skunks were occasionally found in the region where the bull had been pastured. This invites construction of a most probable scenario regarding the occurrence of transmission: The morbid skunk, perhaps partially blind due to the encroaching infection, wanders out onto a pasture in broad daylight. It sees something move, rages, attacks and bites. The recipient of the bite, one Charolais bull, may have subsequently kicked the skunk into oblivion, but the damage had been already done.

The appearance of the rabid bull resulted in the potential exposure of 29 students and staff of the LSU School of Veterinary Medicine. Of these, one had a pre-existing rabies titer and declined treatment. Sixteen had previously received pre-exposure vaccination and took a booster vaccination following this incident. The remaining twelve, who had not taken the pre-exposure rabies vaccination, received post-exposure treatment, which included the complete rabies vaccination series and immunoglobulin. Louisiana Workman's Compensation Corporation covered the costs associated with the rabies treatment, which was approximately $300 per boostered person, and $1200 per person for the complete vaccination series and immunoglobulin. CDC-published guidelines recommend that all animal health and care professionals and animal control personnel should receive pre-exposure rabiesvirus vaccination.

This article was submitted by Rob Poston, LaVMDL, LSU School of Veterinary Medicine, Baton Rouge, Louisiana, with contribution by Dr. Margie Gill, Veterinary Teaching Hospital and Clinic, LSU School of Veterinary Medicine, Baton Rouge, Louisiana. If any AVM member has an interesting case story, please consider writing an article and submitting it for publication in the AVM newsletter. Submissions are sent to the Publications Chairperson, currently Rob Poston, LaVMDL, P.O. Box 25070, Baton Rouge, Louisiana 70894, fax: 504/346-3390, E-mail: poston_r@vt8200.vetmed.lsu.edu.

Bacteriology Session

Moderated by Jim Gary, Purina Mills, Gray Summitt, Missouri.
Reported by Frank Austin, Mississippi State University College of Veterinary Medicine, Starkville, Mississippi; Angela Bridger and Kevin Cook, Missouri Department of Agriculture, Veterinary Diagnostic Laboratory, Springfield, Missouri; Dorothy Scott-Wright, Maryland Department of Agriculture Animal Health Laboratory, Frederick, Maryland; Kathy Strelow, Wisconsin Animal Health Laboratory, Madison, Wisconsin.

It was announced that the AAVLD subcommittee on Diagnostic Bacteriology, Mycology, and Mycoplasmology will sponsor a one day wet-lab workshop on a Practical Approach to the Identification of the Medically Important Glucose NonFermenting Gram-Negative Bacilli, to be held on Friday, October 17, 1997 at the School of Allied Health, Division of Clinical Laboratory Science, University of Louisville School of Medicine. The participation fee is $50. Those wanting further details, or a copy of the workshop program, are invited to contact Lloyd Lauerman of the Charles S. Roberts Veterinary Diagnostic Laboratory, Auburn, Alabama, at 334/844-4987.

New Kits and Reagents for Veterinary Microbiological Diagnostics. Sensititre plans to introduce a new plate for automated gram positive identifications, pending approval by federal authorities. [CHICAGO, Jan. 28 /PRNewswire/ AccuMed International, Inc. announced today that the U.S. Food and Drug Administration has granted the Company clearance to market its Sensititre Gram Positive Identification Panel for use in conjunction with the Sensititre Microbiology System for the automated identification of clinically significant Gram positive bacteria, according to Peter P. Gombrich, CEO and Chairman.]

IDS was said to now have identification test kits for the gram positive rods and gram negative cocci.

VMRD now offers a highly specific FA conjugate for testing of tissues for *Clostridium septicum*. The conjugate was reported to work well on frozen sections, in a technique requiring only two to three hours.

New Diagnostic Applications with PCR Technology. An increased reliance on PCR technology was reported by members, particularly for organisms that are difficult to recover and identify, such as *Leptospira interrogans* and *Actinobacillus pleuropneumoniae* (APP). Laboratories at Murray State University were said to be using PCR to identify Leptospira, and developing PCR to detect pathologically significant *E. coli*. Veterinary diagnostic facilities at Purdue University were reported to use PCR in the recognition of infections by Salmonella, Listeria, *Clostridium perfringens*, Avian mycoplasmas, *Bartonella henselae*, Poult enteritis (Coronavirus), *Lawsonia intracellularis*, and *Serpulina hyodysenteriae*. The Purdue labs charge $15 per assay, $30 for out-of-state requests. Facilities at Mississippi State University were said to perform PCR testing for IBV Arkansas, Connecticut, and Massachusetts strains. A PCR method was reported to be under development to detect *Listeria monocytogenes*. Animal testing laboratories in Virginia were said to be exploring the use of PCR to detect *Ehrlichia risticii* (the agent of Potomac Horse Fever); controls were described for this application.

In creating a PCR facility, great care and extensive controls were said to be necessary for early success. Controls should include a positive and negative DNA control, reagent controls, and a negative control from the uninoculated cell line, if cells were used elsewhere in the protocol. Southern blotting of reactants and controls should be attempted to confirm reaction products.

Compartmentalization of the PCR technique processes was said to be crucial to limiting problems with contamination: sample preparation, reactant mixture, PCR thermocycling, and electrophoretic resolution techniques should be performed in separate rooms. Other techniques to control contamination include filtered pipet tips, frequent glove changes, germicidal UV lighting, disinfection with bleach, and daily changes of absorptive paper counter matting.

The use of nested primers, which are separate small sequence primers bracketed inside a larger primer sequence, was said to increase sensitivity and specificity, and is a good confirmation technique. [For a general explanation on the PCR method, please see notes on a November 1996 presentation by Lloyd Lauerman before the AVM-CSC, published in the AVM Spring/Summer 1997 Newsletter.]

Anaerobic Susceptibility Testing. No one in attendance mentioned routinely performing susceptibility testing on anaerobes. Susceptibility testing is apparently reserved only for those anaerobic isolates considered to be clinically significant. The practice of anaerobic susceptibility testing remains a controversial subject due to

cost, medical significance, consistency, and difficulty in performance. A standardized disk-diffusion susceptibility test (Kirby-Bauer type) is not yet available for anaerobes. Dr. Jean Cooper of the Federal Food and Drug Administration, Rockville, Maryland, and a member of the National Committee on Clinical Laboratory Standards (NCCLS) was said to have some guidelines available. Anaerobic antibiotic sensitivity testing using an agar dilution method was reportedly done by Spencer Jang of the University of California at Davis.

Clostridium colinum Isolation Techniques. *Clostridia spp.* are anaerobic, typically gram positive rods possessing an endospore. Many are oxidase negative, catalase negative, and require enriched media for growth. *Cl. colinum* is responsible for ulcerative enteritis in gamed birds and fowl (quail disease), young chicks, and turkey pullets. Some members present were concerned about its successful isolation from quail and swine.

From the newest edition of Carter, *Cl. colinum* is best recovered from fresh specimens using thioglycolate containing 10% fetal bovine serum (FBS). Growth is subcultured to pre-reduced Columbia anaerobic blood agar (CNA) or Brucella agar. *Cl. colinum* typically makes small, gray, non-hemolytic colonies. It is a small rod that appears gram negative and produces no spores. A suspension is made from growth with characteristic colonial morphology and applied to an API 20 Anident strip. *Cl. colinum* is relatively inert metabolically, producing few biochemical reactions. Recovery can be made directly from fecal specimens within 24 hours of culture. Due to lack of other contaminating bacteria, liver tissue is an ideal post-mortem specimen. No FA conjugate was reported to be available for *Cl. colinum*. [For further information on staining Clostridia that are easily decolorized, please see Johnson M.J., et al, Techniques for controlling variability in gram staining of obligate anaerobes, J Clin Microbiol. 1995 Mar. 33(3)755-8.]

Laboratory diagnosis of enteropathic infection by *Cl. perfringens* in pigs focuses on the detection of the enterotoxin gene or gene product; not all strains of *Cl. perfringens* produce enterotoxin. Testing of intestinal contents using ELISA technology or cell culture toxicity methods is technically intense and expensive for routine veterinary application. It was reported that Songer (of the reference cited below) and Wu at Purdue University have developed a multiplex PCR to check for various genes capable of producing the many pathogenic toxins of *Cl. perfringens*.

The significance of *Cl. perfringens* in avian species was discussed. Among the members in attendance, it was unknown whether *Cl. perfringens* was significant in quail. Isolates from ratite sources should be analyzed for their toxin production capability, as are pig isolates. *Cl. perfringens* Type C toxin is acknowledged in chickens to cause necrotic enteritis, which is promoted by wheat in the bird diet. It is unknown whether wheat based diets have the same effect in other avians. [For further information on the Clostridia and necrotic enteritis in poultry, please see Gazdzinski P. and Julian R.J., Necrotic enteritis in turkeys, Avian Dis. 1992 Jul-Sep. 36(3)792-8. Branton S.L., et al., The effect of added complex carbohydrates or added dietary fiber on necrotic enteritis lesions in broiler chickens, Poult Sci. 1997 Jan. 76(1)24-8.]

Cl. difficile is recognized as a pathogenic agent in pigs and horses, especially foals. Toxins A and B and their precursors are responsible for inducing intestinal damage. Toxins are identified by using PCR methods. Although considered a significant pathogen in foals, *Cl. difficile* is inconsistently recovered from them. [As reported in the AVM Fall/Winter 1996 Newsletter, Oxoid markets a test kit detecting *Cl. difficile* toxin. For further information on *Cl. difficile*, please see Traub-Dargatz J.L. and Jones R.L., Clostridia-associated enterocolitis in adult horses and foals, Vet Clin North Am

Equine Pract. 1993 Aug. 9(2)411-21; Jones R.L., Diagnostic procedures for isolation and characterization of *Clostridium difficile* associated with enterocolitis in foals, J Vet Diagn Invest. 1989 Jan. 1(1)84-6.]

Cl. spiroforme was noted to be a serious pathogen in rabbits. Cases were described from St. Louis, Missouri and Tennessee. Rabbit pups were said to be predisposed to infection when they are prematurely weaned. Direct smears may reveal coil-spring spiroforme-like rods. To isolate the organism, CDC anaerobic blood plates are recommended. Colonies appear small and grayish; characteristic rods appear in stained smears. For heavily contaminated specimens, one recommendation was to decontaminate by pretreatment with a 1:1 dilution of alcohol, which selects sporulating organisms due to spore survival in alcohol. The surviving spores are plated and enriched for further testing. As such growth ages, colonies become sticky. With the Anident system, *Cl. spiroforme* will key as *Cl. ramosum*, the most closely related organism. [For further information, see Hara-Kudo Y., et al, Incidence of diarrhea with antibiotics and the increase of clostridia in rabbits, J Vet Med Sci. 1996 Dec. 58(12)1181-5; Butt M.T., et al, A cytotoxicity assay for *Clostridium spiroforme* enterotoxin in cecal fluid of rabbits, Lab Anim Sci. 1994 Feb. 44(1)52-4; Carman R.J and Wilkins T.D., In vitro susceptibility of rabbit strains of *Clostridium spiroforme* to antimicrobial agents, Vet Microbiol. 1991 Aug 30. 28(4)391-7.]

[A table featuring the common Clostridium species, their toxins and the diseases they produce was featured in the Bacteriology Session notes in the AVM Fall/Winter 1996 Newsletter. Those needing further information on the pathogenesis and isolation of *Clostridia spp*. are invited to review Songer, J.G., Clostridial enteric diseases of domestic animals. Clin Microbiol Rev. 1996; 9(2)216-234; Borriello S.P., Clostridial disease of the gut, Clin Infect Dis. 1995 Jun. 20 Suppl 2,S242-50; Alexander C.J., et al, Identification and antimicrobial resistance patterns of clinical isolates of *Clostridium clostridioforme, Clostridium innocuum,* and *Clostridium ramosum* compared with those of clinical isolates *Clostridium perfringens*, J Clin Microbiol. 1995 Dec. 33(12)3209-15.]

Diagnosis of Clostridium Infections by FA. Availability of Conjugates. As mentioned above, a highly specific FA conjugate for *Clostridium septicum* is now available from VMRD. This conjugate joins the VMRD's current battery of FA conjugates directed against *Clostridia spp*., chauveii, novii, and sordelli. These conjugates are distributed at working dilution (no dilution necessary) in 10 ml vials for $40 each, or in 1 ml vials for $8 each. VMRD also offers substrate slides to serve as positive controls for their Clostridial FA conjugates.

Diagnosis of Mycoplasma Infections by FA and DNA probes. The fluorescent antibody technique has been useful in the identification of Mycoplasmas from avian and cervine species. Mycoplasma has been successfully isolated with commercial media, such as from Remel. Cases of bovine mastitis, bovine lung, joint, and middle ear infection were reported to have yielded Mycoplasma, successfully identified as *M. bovis* using FA methods.

Among those in attendance, it was uncertain whether *M. bovis* has been associated with bovine abortion. Facilities at Purdue University have used FA methods to identify *M. hyorhinitis* and *M. hyopneumoniae*.

Consensus could not be achieved regarding isolation techniques for *M. dispar*, which causes pneumonia in calves. Commercial media, as available from Remel, can culture *M. pulmonis*, but its capability to grow *M. dispar* is unknown. Recovery of *M. agrenemia*, a non-pathogen, is not thought to be significant. [For information on culturing of mycoplasma from calves, please see ter Laak E.A., et al, Prevalence of

mycoplasmas in the respiratory tracts of pneumonic calves. Zentralbl Veterinarmed [B]. 1992 Oct. 39(8)553-62.]

Although cotton swabs work well to culture mycoplasma, dacron swabs were recommended for mycoplasma culture samples.

Mycoplasmas are also implicated in pathogenic conditions of small animals. Feline and canine mycoplasmas have been found in respiratory and genital infections, and associated with infertility and abortion. As in other domestic animals species, most of the accessible mucous membranes of dogs are colonized by mycoplasmas. At least 11 species, including *M. arginini, M. canis, M. cyno, M. edwardsii, M. feliminutum, M. gateae, M. maculosum,* and *M. opaiescens* have been found, of which *M. canis* and *M. cynos* are potentially pathogenic for the genitourinary and respiratory tracts, respectively. Although *M. canis* has been shown in experiments to produce orchitis, epididymitis, and purulent endometritis, a role for it in naturally occurring disease has not yet been convincingly established. However, its presence in pure culture in cases of urinary tract infection argues strongly for its involvement as a urinary tract pathogen. (Jang et al, 1984). *M. cynos* has been isolate from dogs with bronchitis and distemper pneumonia, in which it was probably a secondary invader (Rosendal, 1982). Mycoplasmas have also been isolated from canine cardiac lesions and from lesions of granulomatous colitis in boxers (Rosendal, 1982). [For more information on small animal mycoplasmas and their significance in disease, please see Jang, S.S., et al., Mycoplasmas as a cause of canine urinary tract infection. J Am Vet Med Assoc. 1984 Jul. 185(1)45-47.; Rosendal, S., Canine mycoplasmas. J Am Vet Med Assoc. 1982 May 130(10)1212-1214. For more information on contemporary techniques of Mycoplasma identification, please see Razin S., DNA probes and PCR in diagnosis of mycoplasma infections, Mol Cell Probes. 1994 Dec. 8(6)497-511.]

Sensitive Culture Techniques to Isolate *Salmonella spp.* A variety of protocols, incorporating basic microbiological technique, is reportedly used by members to culture Salmonella; it was felt that no single protocol was giving optimum sensitivity. One popular culture protocol uses a lactose pre-enrichment medium, followed by inoculation of Tetrathionate broth, then onto MacConkey and Brilliant Green plates. Some reportedly use XLD and XLT-4 plates.

Salmonella culture from horses was said to be difficult, requiring repeated sampling and rigorous homogenization; one of the protocols discussed recommended culturing one sample per day for five to six days, with enrichment.

There was much discussion regarding Salmonella rapid identification from food sources. Several formats were said to be commercially available, including Stratacon, IDEXX-Bind, and Neogen Reveal. Principles behind some of these kits are immunomagnetic bead reaction, bioluminescence, or chromatography. With kits of this type, members reported some difficulty, such as numerous positive reactors unconfirmed by culture.

[For further information on using rapid techniques to find Salmonella in food, please see Fierens H. and Huyghebaert A., Screening of Salmonella in naturally contaminated feeds with rapid methods, Int J Food Microbiol. 1996 Aug. 31(1-3)301-9; Quinn C., et al, A comparison of conventional culture and three rapid methods for the detection of Salmonella in poultry feeds and environmental samples. Lett Appl Microbiol. 1995 Feb. 20(2)89-91; Comments on the above article were made by Madden R., Lett Appl Microbiol. 1995 Dec. 21(6)406; For information on the recovery of Salmonella from food by culture, please see Warburton D.W., et al, A comparison of six different plating media used in the isolation of Salmonella. Int J Food Microbiol. 1994 Jun.22(4)277-89.]

Ratite Microbiology Update. Members now report few requests for microbiological work on ratites. This contrasts with the past few years, when culture requests on ostriches and emus were common.

[Recently, there have been reports of Serpulina infections of rheas. For more information, please see Jensen N.S., et al, Identification of the swine pathogen *Serpulina hyodysenteriae* in rheas (*Rhea americana*). Vet Microbiol. 1996. 52(3)259. For further information on infectious diseases of ratites, please see Tully T.N. and Shane S.M., Husbandry practices as related to infectious and parasitic diseases of farmed ratites. Rev Sci Tech. 1996 Mar. 15(1)73-89.]

Microbiology of Camelids (Camels and Llamas). Numerous articles have recently appeared on pathogenic microbiology of new world camelids. According to Thedford (cited below), from among the bacterial diseases, *Clostridium perfringens* enterotoxemia, caused by types C and D toxin, is considered the most significant infectious disease of North American camelids. Other bacterial infections of concern are tuberculosis, Johne's disease, anthrax, malignant edema, actinomycosis, and tetanus. Fungal infections include ringworm (*Trichophyton spp*.) and coccidioidomycosis, associated with the arid climate of southwestern U.S. Possible viral infections include rabies, ecthyma, and a recently described blindness neuropathy associated with equine herpesvirus type I. Successful experimental infections have been established with hoof-and-mouth disease virus and vesicular stomatitis virus. In camelids, much serological evidence exists to substantiate exposure to many viral agents (of unknown pathological significance) including bluetongue, parainfluenza type 3, bovine respiratory syncytial virus, bovine herpesvirus I, bovine viral diarrhea virus, influenza A virus, and rotavirus.

Reports of other potential bacterial pathogens in camelids include *Mycobacterium spp*. (including paratuberculosis and bovis), *Clostridium spp*., a proposed retrovirus responsible for juvenile llama immunodeficiency syndrome (JLIDS), necrotizing lymphadenitis associated with Rhodococcus equi, *Fasciola hepatica* (liver flukes), Salmonella, Coccidia, an adenovirus associated with pneumonia and hepatitis, listeria associated with abortions and ear infections, Nocardia, a herpesvirus similar to BHV-1, *Enterococcus spp*., *Escherichia coli*, *Actinobacillus spp., Klebsiella pneumoniae, Eimeria spp.,* and *Conidiobolus coronatus.*

According to Gionfriddo, Mycoplasmas should not be considered normal conjunctival flora of Camelids. [Gionfriddo J.R., et al, Bacterial and mycoplasmal flora of the healthy camelid conjunctival sac. Am J Vet Res. 1991 Jul. 52(7)1061-4.]

It was reported that llamas have been found infected with Ehrlichia. [Barlough J.E., et al, An Ehrlichia strain from a llama (*Lama glama*) and Llama-associated ticks (*Ixodes pacificus*). J Clin Microbiol. 1997 Apr. 35(4)1005-7.]

[For further information on bacterial diseases of llamas, please see Thedford T.R. and Johnson L.W., Infectious diseases of New-World camelids (NWC). Vet Clin North Am Food Anim Pract. 1989 Mar. 5(1)145-57; For further information on parasitic diseases of llamas, please see Rickard L.G., Update on llama medicine. Parasites. Vet Clin North Am Food Anim Pract. 1994 Jul. 10(2)239-47; Cheney J.M. and Allen G.T., Parasitism in llamas. Vet Clin North Am Food Anim Pract. 1989 Mar. 5(1)217-25.]

Diagnosis of Leptospirosis: Culture, FA and Serology. According to the members present, popular technology used in the diagnosis of leptospiral infections include FA, darkfield microscopic examination, culture using EMJH broth, and silver staining of

fixed tissue sections. Certain members reported difficulty using the NVSL FA conjugate, yet some said it was useful on frozen liver and kidney sections. FA on frozen necropsy tissues was said to be useful in instances when silver staining was inconclusive. Darkfield examination of fetal amnionic fluid or stomach contents, in addition to kidney, was said to be an efficacious method to detect Leptospira abortion, which may occur before the offending Leptospira migrate to the kidney.

To culture Leptospira, urine, stomach fluid, or tissue homogenized in PBS may be used as the specimen, which is inoculated with a pasture pipet 1/4 inch below the surface of EMJH broth, enriched with 20% rabbit serum. The growth is checked periodically by darkfield microscopy for characteristic morphology and motility.

[Recent reports suggest that PCR techniques compare favorably to culture in the detection of leptospira in clinical samples. Please see Zuerner R.L., et al, IS1533-based PCR assay for identification of *Leptospira interrogans* sensu lato serovars. J Clin Microbiol. 1995 Dec. 33(12)3284-9; Savio M.L., et al, Detection and identification of *Leptospira interrogans* serovars by PCR coupled with restriction endonuclease analysis of amplified DNA. J Clin Microbiol. 1994 Apr. 32(4)935-41.]

Serpulina Culture and Identification. The Serpulina are microaerophilic or anaerobic spirochetes producing varying degrees of hemolysis on blood plates. In pigs, *Serpulina hyodysenteriae* is known to cause severe colitis, a condition called swine dysentery. *S. hyodysenteriae* is a spirochete recognized by its strong beta-hemolysis when cultured on blood plates. Other weakly hemolytic enteric spirochetes are frequently recovered; such isolates were once thought to be non-pathogenic, referred to as *S. innocens*. Recently, certain weakly hemolytic spirochetes have been associated with colitis and are now speciated as group IV Serpulina or *S. pilosicoli*.

Fresh specimens and adequate specimen decontamination were thought to be crucial to successfully culture Serpulina from clinical specimens. In one specimen processing protocol, fecal samples from live animals or intestinal mucosal scrapings are diluted in BHI broth, then decontaminated with passage through a 0.45 um filter. The filtrate is plated onto non-selective anaerobic media.

Certain members attempt to culture Serpulina using in-house prepared media. At the time of this discussion, the commercial availability of special culture media was unknown. One culture protocol was described as using pre-reduced blood plates containing 200 ug/ml spectinomycin. Growth is enhanced by CO2 and optimum temperature is 42 C. Another used CVS agar (TSA base with colisten, vancomycin, and spectinomycin), which seems to work better than BJ media (CVS media with spiramycin and rifampin) in isolating the weakly hemolytic varieties. Another isolation protocol mentioned in this discussion makes use pre-reduced brucella agar or Schaedler blood agar. According to Carter, *S. hyodysenteriae* (formerly *Treponema hyodysenteriae*) grows well on freshly poured, pre-reduced, trypticase soy agar with 5% blood and 400 ug/ml of spectinomycin at 42 C for two to three days under anaerobic conditions. [Please see Carter, G.R., *Diagnostic Procedures in Veterinary Bacteriology and Mycology*, 4th ed., Charles C., Thomas, Springfield, Illinois, 1984, pp. 52-53. For a formulation of BJ media, please see Trampel, D.W., et al, Cecal spirochetosis in commercial laying hens. Avian Dis. 1994 Oct-Dec. 38(4). pp. 895-8.] From cases of swine dysentery, *S. hyodysenteriae* is identified on culture by its strong hemolysis. Darkfield examination of suspicious colonies reveal characteristic morphology: Cell sizes range from 0.3-0.4 um width and 6-8 um in length. Cells are loosely coiled with 2-4 coils and have tapered ends, and possess eight to twelve periplasmic flagella. Serpulina also may be found directly in gut mucosal scrapings or fecal material by examining wet mounts or smears stained with dilute carbol fuscin or Victoria blue 4-R.

In an identification scheme of Fellstrom (cited below), *Serpulina spp*. may be distinguished by the degree of hemolysis, and their indole and hippurate reactions. *S. hyodysenteriae* would be an example of a group I Serpulina, while *S. pilosicoli* falls into group IV (Table one).

As implied by Trott (cited below), *S. pilosicoli* may be under recognized in spirochetemic infection. *S. pilosicoli* is typically an agent of intestinal spirochetosis, but has been isolated from the blood of immunosuppressed or terminally ill human patients. By some unknown mechanism, *S. pilosicoli* may translocate from the large intestine to establish spirochetemia.

PCR identification of *Serpulina spp*. is becoming increasingly popular, but apparently not without problems. When Serpulina infection is suspected, one member was said to routinely refer testing requests to external PCR facilities, which has been returning unsatisfactory results.

[For further information on Serpulina culture and identification, please see Fellstrom C., et al, Identification of Serpulina species associated with porcine colitis by biochemical analysis and PCR. J Clin Microbiol. 1997 Feb. 35(2)462-7; Olson, L. D., Enhanced isolation of Serpulina hyodysenteriae by using sliced agar media. J Clin Microbiol. 1996 Dec. 34(12). p 2937-41; Achacha M. and Mittal K.R., Rapid identification of porcine Serpulina species by colony blot assay using a genus-specific monoclonal antibody. Vet Rec. 1996 Nov 30. 139(22)539-41; Trott D.J., et al, Identification and characterization of *Serpulina pilosicoli* isolates recovered from the blood of critically ill patients. J Clin Microbiol. 1997 Feb. 35(2)482-5.]

Canine Diarrhea. Toxins A and B, produced by *Cl. difficile* was said to be increasingly recognized as a cause of diarrhea in dogs. The use of a toxin kit was recommended to detect A and B toxins in clinical samples. Routine anaerobic culture should recover *Cl. difficile*. [There is evidence that *Cl. difficile* is asymptomatically carried by pet dogs and cats, which may serve as reservoir for the organism. Please see Riley T.V, et al, Gastrointestinal carriage of *Clostridium difficile* in cats and dogs attending veterinary clinics. Epidemiol Infect. 1991 Dec. 107(3)659-65.]

Other possible bacterial causes of diarrhea in dogs include *Cl. perfringens*, which may be found by gram stain to contain spores. *Cl. perfringens* was said to release its toxin, thus causing its pathogenic effect, at the time of sporulation. *Yersinia enterocolitica* has been implicated as an agent of enteritis in dogs; a weakly beta hemolytic spirochete has also been found in cases of puppy enteritis.

Vetlims Update. There was extensive discussion of this veterinary laboratory database computer program, and lab data systems in general. Nearly everyone present using Vetlims was unhappy with some aspects of the program in its current state. It was thought that customer support could be improved. Because of numerous experiences with fatal errors (computer crashes), the current version was not recommended to be used with the Windows 95 operating system. Less problems were said to occur in a Windows 3.1 or NT environment. Although its accounting function design seems good, retrieval of case data was said to be unsatisfactory. Data entry and retrieval problems were evidently cumulative, with a greater tendency for failure during high volume usage. Apparently within the design of Vetlims, data entry and retrieval were said to be opposing processes, if one is facilitated, the other is made difficult. The 32-bit version of Vetlims was reported to be available in 1998.

Certain laboratories were said to utilize off-the-shelf data base or spread sheet programs, such as D-Base, Quattro Pro and Lotus 1-2-3. The use of such software

may be tailored for handling lab data, especially in high volume and multiple samples, with the use of custom-written macros. Other labs were exploring the idea of in-house software development; customization permits each lab the fullest flexibility and accommodates the broadest variety of data types (i.e., different tests) at various levels of test volume. Whether adapting commercial software or designing an original program, patient and test information was suggested to be separate from accounting information.

Some of the discussed problems were of the type that would occur with any data base system, such as incorrect, inconsistent, or non-uniform data entry, attributable to instances when data entry tasks are the responsibility of several individuals, instead of a few. System users must be familiar with the manner of data entry to effectively query the database for lab results. As a mechanism to maintain the fidelity of data and flow of cases within the computer system, some labs manually verify pending cases that remain in the system longer than a specified time period (i.e., two weeks). In many such instances, cases have been found with an incorrect test request or other information, have been misdirected, or were canceled but not cleared from the system.

A group at Stanford, in cooperation with Roche, was said to be writing veterinary laboratory software. Vitek "datatrack" was said to be promising, but requires data to be entered twice.

For a low-cost information system, veterinary laboratories were suggested to consider a public domain integrated information system called the Decentralized Hospital Computer System (DHCP), designed and developed by the federal Department of Veterans Affairs for use in VA hospitals nationwide. A DHCP monograph is available to explain each of the more than 60 integrated DHCP applications, including Administrative and Clinical modules. Some examples include registration, admissions, scheduling, pharmacy, laboratory, order entry, health summary, medicine, MailMan, engineering, and procurement. DHCP was nominated for the 1995 Smithsonian Award for Best Use of Information Technology. This software was written in M[UMPS] or M language, (Massachusetts General Hospital Utility Multi-Programming System), and appears to be well supported [(http://www.hardhats.org/). For further information, please visit the web site http://www.va.gov/vama.htm/.]

Semiautomated Identification Systems for Gram Positive Organisms. Several different formats were said to be in use by membership, including Staph-Trac, Staph Ident, Biolog, IDS GP strip, Mini-tek, and API Rapid Strep. The BBL Crystal format for gram positives was reported to be made available soon. The Mini-tek system was said to be helpful to identify an unknown gram positive isolate when other systems are unable. Half of all members in attendance mentioned using the Staph Latex kit, which was said to give varying degrees of the service and satisfaction.

Much of the membership performs identifications on gram positives using manual methods, i.e., coagulation and mannitol salts reactions, or with Polymixin B or Novobiocin sensitivity (*S. aureus* is always resistant).

Identification of *Streptococcus spp*. is significant in certain medical conditions, such as milk culture for mastitis testing. The Streptococci may also be identified using popular semi-automated systems, such as from Remel, BioMerieux, Oxoid, or Burroughs Welcome. The cost of speciation may be prohibitive for veterinary specimens, costing an additional $3-4 per isolate. In lieu of expensive speciation techniques, some labs use antisera to identify isolates by serogroups (i.e., B, C, G,

etc.). Serogrouping requires very little incubation time (1-2 minutes). The Sensititre gram positive format was said to be worth consideration for the identification of *Streptococcus suis*.

Standard Operating Procedures (SOP) and Protocols. Several comprehensive SOP manuals were said to be available for the clinical microbiology laboratory. Manuals have been published by the Center for Disease Control (CDC), the American Association of Avian Pathologists (AAAP), the American Society for Microbiology (ASM), and the Office International Des Epizooties (OIE). Some published manuals have step-by-step instructions for general protocols, and other address specific applications in veterinary microbiology.

In establishing individualized laboratory SOP's, it is appropriate to exploit the published reference manuals. For each laboratory procedure in use, one of the popular reference manuals may be cited, then the protocol is outlined to include details and variables as actually practiced. Procedural outlines should be readily accessible in each respective lab section, and a comprehensive laboratory manual should be available at a central location. Standard operating procedures translate into consistency, while QA/QC efforts objectively demonstrate testing performance. Most labs report using both manuals and texts as reference material.

Johne's Disease Serology: Comparative Sensitivity, Specificity, Cost, and Automation. Laboratories in several states were said to have participated in the Johne's Disease serology check test. Laboratories at Illinois, Purdue University, Ohio, Wisconsin, and Maryland were said to use serological methods backed up by culture. The lab in Maryland was reported to have encountered false positive reactions with the AGID method. In addition to serology and culture, South Dakota and Kentucky laboratories were reported to use PCR technology in Johne's Disease diagnosis.

The AGID serology method was said to give a high rate of false positives. The IDEXX kit test on milk samples was said to work well, but problems with the accompanying software were cited. [According to Lisa Lemieux of IDEXX, an updated software version was said to be pending.] In current application, culture is more sensitive than PCR in detecting Johne’s bacillus.

Br. canis IFA Serology: Sensitivity and Specificity Relative to Other Techniques. For *Br. canis* serology testing, the AGID method is recognized as the standard reference technique, but it was said to have less than ideal sensitivity; it is believed that antibodies are not detected by AGID in an infected dog until four to six weeks post-infection. In absence of other technology, the tube agglutination test was said to be useful, but not as reliable as other methods. It was recommended to emphasize to clientele that medical decisions should not be based on the *Br. canis* tube test alone; other data, such as culture attempts or clinical signs, also should be considered. It was reported that Shin at Cornell University has independently developed a new slide agglutination test for *Br. canis* serology. The consensus belief among members present was that data from more than one testing method was necessary, and that a faster test format was needed. Two labs represented at this session reported to have participated in the NVSL field trials for *Br. canis* ELISA test.

For culture of *Br. canis*, heparinized blood or uteral swabs (post-mortem) are recommended specimens. Blood culture attempts were said to be optimized with at least two samples, taken two days apart, and enhanced if blood is frozen overnight, then thawed before plating. Blood agar is inoculated and grown aerobically, without CO_2. An isolator blood culture system also was said to be effective in recovering *Br. canis*.

[At the time of this writing, the *Br. canis* RSAT serology kit is now again available from Synbiotics. Please refer to the discussion in the virology session, under the heading Culture of Obligately Intracellular Microorganisms and Brucella canis for more information on *Br. canis*, including the IFA serology method. Also please see the Bacteriology Session notes from the 1996 AVM annual meeting in the AVM Fall/Winter 1996 Newsletter, and the Virology Session notes from the AVM-CSC 1996 annual meeting, published in the AVM 1997 Spring/Summer Newsletter.]

Office International Des Epizooties (OIE, or World Organisation for Animal Health): Manual of Standards of Diagnostic Tests and its Potential Impact on Laboratory Testing. The OIE manual, formally titled *Manual of Standards for Diagnostic Tests and Vaccines, Third Edition, 1996* (OIE publication reference number A-080) is a reference text of veterinary testing methods, particularly those important to international commerce within the European Economic Community. It is available directly from the publisher, the *Office International Des Epizooties* (12 rue de Prony, 75017 Paris, France, Tel: 33-(0)1 44 15 18 88, Fax: 33-(0)1 42 67 87, Telex: 642 285 EPIZOTI, E-mail: 100765.546@compuserv.com, web site: http://www.oie.org/), or in the U.S. from SMPF, Inc. (100 East 42nd Street, Suite 1002, New York, N.Y. 10017, Tel: 212/983-6278, Fax: 212/687-1407).

This manual has the potential to change veterinary diagnostic testing. Formidable guidelines are offered for certain testing, which will require considerable documentation for test validation, i.e., date of run, serology procedure and run parameters, control reactions, antigen lot, technician signature, etc. It was reported that AAVLD is considering its adoption. [For further information, please see this same topic heading in the Virology Session notes.]

Laboratory Diagnosis of *Tritrichomonas foetus*. Positive control stock cultures of *T. foetus* cannot be maintained for long periods on artificial media. *Tritrichomonas vaginalitis* is a common pathogen of humans, and can be obtained in culture from most hospital laboratories. *T. vaginalitis* may be substituted as a stock control for media quality and microscopic evaluation.

[Bovine trichomoniasis is a venereally transmitted infection caused by the flagellated protozoan, *T. foetus*. The principle manifestation of infection is early embryonic death or abortion. The cow shows few other signs of infection, and the bull shows none. The organism is infrequently found by direct microscopic examination of clinical specimens; serological methods are not thought to be reliable. Due to the lability of the organism and the floral content of preputial or vaginal samples, standard culture methods of propagation in liquid media (such as Diamond's Media) are prone to give false negative results. The use of commercially available *T. foetus* specimen collection systems (such as from BioMed Diagnostics of Santa Clara, California, 800/677-2885) greatly improve the success of culture.]

Virology Session

Moderated by Woody Fraser, Florida Animal Disease Diagnostic Laboratory, Kissimmee, Florida.
Reported by Tom Chang, Tennessee Animal Disease Diagnostic Laboratory, Nashville, Tennessee; Kevin Cook, Missouri Department of Agriculture, Veterinary Diagnostic Laboratory, Springfield, Missouri; Rob Poston, Louisiana Veterinary

Medical Diagnostic Laboratory, Louisiana State University School of Veterinary Medicine, Baton Rouge, Louisiana.

New Test Kits and Reagents. Laboratories must be members of the Veterinary Laboratory Association (VLA) to purchase quality control kits and to participate in their quality assurance program; laboratory membership dues are $25 annually. For information, contact Stacie Hotham at 800/565-0265.
Commercial representatives present at this session were asked to describe new additions to their product line, or improvements to their products.

KPL currently markets an ELISA to test for avian influenza antibodies. A kit to test for chicken anemia is due out in three months. An Infectious Bursal Disease (IBD) viral antigen test kit will be available soon. (Contributed by C. George.)

Meridian Cryptosporidium and Giardia antigen IFA kits, singly and in combination, are based on monoclonal antibody and are able to detect infection in any species.

Synbiotics At this time, there were no new additions to the product line since the last annual meeting of the AVM. In the near future, Synbiotics plans to market PCR primers for the diagnosis of *Bartonella henselae* (Cat Scratch Disease), *Chlamydia psittaci* (psittacosis), Feline Immunodeficiency Virus (FIV), Feline Infectious Peritonitis (FIP), Feline Leukemia Virus (FeLV), *Haemobartonella felis* (Feline Infectious Anemia, or FIA), *Mycobacterium spp*. (*M. avium, M. bovis,* and *M. tuberculosis*), Canine Parvovirus, Tick Multiplex (*Borrelia burgdorferi*, *Ehrlichia canis*, *Rickettsia rickettsii*), and *Yersinia pestis*. Rhone merieux has sold the rights of some of its veterinary diagnostics product line to Synbiotics, which will soon re-release some of these products. Synbiotics is also considering a kit to detect BVD viral antigen with ELISA technology. (Contributed by H. Jones).

Viral Antigen now has the Autolex PRV antibody screening test, which is a semi-automated assay using similar reagents as the manual PRV latex agglutination test. Autolex is run in 96-well flat bottom microtiter plates. A reader scans each well and records the light transmission at multiple points. Two readings are taken, the initial reading, which is the baseline reading following the addition of all reagents, sera, and controls, and the final reading, after 30 minutes of incubation. If a sample contains PRV antibodies, the latex agglutinates to allow additional light to pass through the well. If a sample is negative, the latex remains in suspension, with little change from the first reading in light transmission. Autolex software calculates break points by comparing the difference of the two readings for each sample with that of positive and negative controls. Autolex PRV applies principles similar to the Autolex Brucella Antibody Test Kit, a rapid semi-automated screening test that detects *Brucella abortus* antibody in cattle and pig serum. Viral Antigen also has been developing recombinant reagents for use in PRRS and Lyme Disease testing. (Contributed by R. Crandell).

VMRD has no new kits planned for release soon, but has some new monoclonals available. For details, AVM members are advised to check VMRD's web site (http://www.vmrd.com/vmv.htm). By the time of the next AVM annual meeting, VMRD may have two new diagnostic test kits. (Contributed by T. Keller).
[As reported in the AVM Fall/Winter 1996 Newsletter, Giardia and Cryptosporidium ELISA kits are available from Alexon of Sunnyvale, California. Wampole Laboratories offers a Latex Agglutination (LA) kit to test for cryptococcus antigen in serum or cerebrospinal fluid. According to Lisa Lemieux, IDEXX was said to be developing an ELISA antigen test kit for BVD, possibly available before 1999. Also, IDEXX is

developing ELISA-based antigen and antibody tests specifically for the J strain of avian leukosis virus.]

The general merits of monoclonal antibody (MAb) in diagnostic reagents were discussed. A cocktail or mixture of MAb's may perform better than single MAb's as an FA conjugate. Certain MAb's perform well with viral antigen, but some might be too specific for diagnostic testing.

Advances in Cell Culture Technology. The MDCK cell line, with the use of a special serum-free medium from Whittaker Bioproducts, Maryland, is popularly used in human influenza virus isolation. Serum-free medium does not inhibit the enzymatic action of trypsin, which is necessary for influenza viral growth. Maintenance of cell lines with serum-free media presents a problem when cells are to be dispersed; due to the lack of serum (and its antitrypsin properties), over-trypsinization is possible. Cells must be rinsed to remove excess trypsin before they are seeded into new flasks. Despite assurances by manufacturers of fetal bovine serum (FBS), BVD viral antibody is sometimes found in commercial source FBS, in neutralization titers of 1:2. With exception BVD antibody, and a rare IBR titer, FBS is usually antibody-negative for other bovine viruses but may still contain BVD viral contamination. Many session participants reported using heat inactivation of FBS as a method to reduce occurrence of viral contamination.

Some manufacturers assay their irradiated stock to insure it is BVD virus-free. John Black of American BioResearch of Seymour, Tennessee, reported that he has never found BVD virus in any irradiated serum. To check FBS for BVD viral contamination, bovine kidney cells (BVD-free) are grown in media containing the FBS for a week, then an FA for BVD virus is performed on the cells. To avoid contamination of bovine viral culture attempts with either virus or antibody from commercial FBS, equine serum is recommended as the serum additive in the post-inoculation media.

A certain strain of the Bovine Turbinate (BT) cell line, available from ATCC, is said to have been adapted to grow with equine serum, which usually performs unsatisfactorily on other cell lines. Typically, BT cells stick poorly to plastic or glass flasks when grown on media with horse serum.
Certain bovine cell lines may be maintained on media with goat serum, which is available from several vendors. JRH Biosciences of Lenexa, Kansas offers irradiated goat serum, but it's performance was reported to be less than ideal.

Donor Bovine Serum (DBS) from Harmon Technology of Gowrie, Iowa, is IBR and BVD virus and antibody free, however adult cattle serum usually still contains BRSV and PI-3 antibody. Certain specified Harmon DBS four-way negative lots (free of antibody to IBR, BVD, PI-3 and BRSV), and five-way negative lots (free of antibody to Bovine Adenovirus, also) contain serum from adult donors and were reported to possibly contain antibody to other bovine viruses.

Dr. Peter M. Boyt of Boyt Veterinary (18462 Redbud Road, Neosho, Missouri, 64850, 417/451-6369) offers donor calf serum reportedly free of IBR, BVD, PI-3, and BRSV antibodies. This serum was said to be produced by a single, closed, colostrum-deprived herd, and is possibly free of antibodies against many other bovine viruses. Boyt Veterinary also supplies high quality sterile defibrinated bovine red blood cells suitable for bacteriological plate medium.

[According to supplied literature, Boyt Veterinary is a start to finish bovine serum production facility located in southwest Missouri. Donor bovine animals are maintained in a bio-secure environment and all activities are under direct veterinary supervision. Boyt Veterinary has been in business since 1992 and has supplied

specific antibody negative donor bovine serum for use by major veterinary biological companies, veterinary diagnostic laboratories, and for human virology research.

The donor bovine serum is collected from colostrum-deprived calves ranging in age from one to six months. All donors are negative for BVD infection and antibody upon introduction to the facility. Being colostrum deprived, the donors should not have other viral antibodies and are confirmed negative for IBR, PI-3, and BRSV antibodies upon admittance. The donors are raised in groups and constantly monitored for seroconversion. The serum is considered negative for BVD, IBR, PI-3, and BRSV virus when all individuals in a group remain negative for antibody at least 30 days after collection. This utilization of donors for virus detection is a sensitive test system affording a higher degree of confidence than culture techniques.
Donor serum is sterile-filtered through a validated 0.2 um filter apparatus and bottled in a class 100 environment into standard PETG square bottles. Bottle sizes currently available are 100 ml, 500 ml, and 1000 ml.

Lot sizes typically are 10 to 20 liters in volume. Different sizes can be constructed as needed.
Pricing for the donor calf serum is as follows:
100 ml PETG bottle: $ 27.50
500 ml PETG bottle: $130.00
1000 ml PETG bottle: $250.00
Serum samples are available for customer testing upon request.

Donor serum has several advantages over fetal bovine serum. Donor serum has uniform characteristics from lot to lot with known health history of constituent animals. Both BVD virus and antibody can be excluded from the serum. There is also greater stability regarding availability and pricing.
Please address all inquiries to Peter M. Boyt DVM, MS, tel and fax: 417/451-6369.] BT cells easily become contaminated by BVD virus. To successfully grow and keep them free of BVD virus, cells are seeded in a relatively low density and allowed to grow for a week. A good growth medium may contain 5-10% DBS (BVD-free) and HEPES buffer. After cells have attached, maintenance media containing 0.5% DBS is added.

Inapparent mycoplasmal contamination of cell cultures may affect viral work or cause non-specific FA staining. Typically, mycoplasmas are sensitive to aminoglycoside antibiotics, but the continuous use of such does not necessarily insure mycoplasma-free cultures. Methods to inspect cell culture stocks for mycoplasma contamination include culture on PPLO media (broth and agar available from Difco), PCR method (kit available from ATCC, see note below), or the Hoechst's staining method (ICN, SIGMA, ATCC), which stains extranuclear nucleic acid to detect mycoplasma in the cell cytoplasm. [The use of Hoechst's stain was mentioned on page 41 of the AVM Spring/Summer 1997 Newsletter, under the heading "IFA and Protobacteria of Veterinary Significance" in Immunofluorescence Staining in Veterinary Diagnostic Virology.

The ATCC offers a PCR-based Mycoplasma detection kit (ATCC catalog No. 90-1001K) which provides 50 PCR reactions per kit and includes 1st stage (outer) primer mixture (50 ul), 2nd stage (inner) primer mixture (50 ul), positive control DNA #1 (50 ul), positive control DNA #2 (50 ul), storage box and instruction manual. This method can detect as few as 20-180 colony-forming units (CFU) per one milliliter of sample. It detects over 20 different mycoplasma species, including the eight most commonly encountered mycoplasma contaminants in cell culture: *M. arginini, M. fermentans, M. hominis, M. hyorhinis, M. orale, M. pirum, M. salivarium,* and *Acholeplasma laidlawii*.

As reported by ATCC literature, these eight organisms account for more than 95% of mycoplasma contaminants found in cell cultures. The kit price is $225 per kit.

The ATCC also offers a mycoplasma detection service, using ATCC's own PCR-based Mycoplasma detection kit. Positive and negative controls are included with each test and results are available each Friday. Samples can be further tested to determine if the positive DNA is one of the eight commonly encountered mycoplasma species.

Mycoplasma detection, PCR Method (Catalog No. 359-X) pricing:
One test sample.....$325
2-4 test samples.....$250 each
5-7 test samples.....$175 each
8-10 test samples .....$140 each
11+ samples .....$120 each

Speciation Option (if PCR is positive) (Catalog No. 361-X)..... $90 per sample
The ATCC is offering a workshop on Cell Culture and Hybridomas: Quality Control and Cryopreservation Techniques, to be held on September 9-11, 1998. A significant segment of this workshop includes PCR testing of cell cultures for mycoplasmal contamination.

The Cell Production Facility of the University of Nebraska Medical Center Department of Biochemistry and Molecular Biology currently offers a PCR-based mycoplasma contamination detection service. Testing includes a competitive internal control and a positive control with each test. The internal control will verify polymerase activity by serving as an alternative substrate for PCR primers. In the absence of mycoplasma contamination, amplification of the internal control will predominate, and a 420-base pair product will be visualized. In contaminated cell cultures, the mycoplasma template will competitively inhibit amplification of the internal control. The competitive internal control PCR protocol is an especially sensitive method for detecting mycoplasma contamination. The charge is $25 for each cell culture sample. This facility reports finding a 20% cell culture contamination rate. For information, contact Randall G. Davis or J.K. Vishwanatha, Microbial and Mammalian Cell Culture Facility, Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, 600 S. 42nd Street, Omaha, Nebraska 68198, Tel: 402/559-6663.

For further information on using PCR techniques to inspect cell culture stocks for Mycoplasmal contamination, please see Hu M., et al, Application of PCR for detection and identification of mycoplasma contamination in virus stocks, In Vitro Cell Dev Biol Anim. 1995 Oct. 31(9)710-5. Kobayashi H., et al, Rapid detection of mycoplasma contamination in cell cultures by enzymatic detection of polymerase chain reaction (PCR) products, J Vet Med Sci. 1995 Aug. 57(4)769-71. Rawadi G. and Dussurget O., Advances in PCR-based detection of mycoplasmas contaminating cell cultures. PCR Methods Appl. 1995 Feb. 4(4)199-208.]

New Diagnostic Applications with PCR Technology. Many laboratories have begun actively exploring the use of PCR methods in diagnostics. As mentioned above, Synbiotics will soon have PCR primers available for use in laboratory diagnosis of a number of common infectious agents of animals [listed above]. At the time of this meeting, the PCR primer to detect CPV is ready for use, suitable to detect infection using fecal samples. Additionally, Synbiotics may develop primers to detect *Ehrlichia equi*.

Loyd Sneed of TVMDL, College Station, Texas (409/845-3414) reports the use of PCR as an internal technique to back up to conventional technology for a number of

pathogenic microbial infections, such as BVD viral persistent infection using buffy coat or aborted fetal tissue, Bluetongue virus (BTV) in blood or tissue, BRSV, Mycoplasma (all species), Salmonella (all species), *Chlamydia psittaci*, and *Clostridium perfringens* (genotype). PCR technology has proven itself when other tests are deficient, such as detecting Leptospira (the seven principle species) and Campylobacter in fetal tissue. PCR is also used to diagnose avian polyomavirus and reticuloendotheliosis (IDEXX FlockChek).

Lloyd Lauerman of the Charles S. Roberts Veterinary Diagnostic Laboratory, Auburn, Alabama (334/844-4987) routinely performs diagnostic PCR for avian mycoplasmal infections (MG and MS, and any other known avian mycoplasma), infectious bronchitis virus (IBV), chicken anemia virus, psittacine polyomavirus, and circavirus. He also has developed PCR identification methods for BVD virus, *Campylobacter fetus, Campylobacter coli*, Chlamydia, *Clostridium perfringens*, Salmonella, and mycoplasmas of cattle, sheep, goat, pig, cat, and dog. In the past, he has offered to train technical personnel in PCR methods. Some AVM members present mentioned using his service. [Notes on the PCR technique may be found in the AVM Spring/Summer 1997 Newsletter, under the heading Current Applications of PCR in Veterinary Diagnostics.]

Availability of Nucleic Acid Hybridization Probes for Veterinary Diagnostics. Evidently, hybridization products are not yet routinely available. One value of such technology was mentioned to be in the retrospective diagnosis of lentivirus infections of cats and cattle (FIV and BIV, respectively), in a fashion similar to HIV in human AIDS fatalities. These viruses do not present much stable antigenic evidence, yet their presence may be genetically substantiated, particularly in fixed tissues. Insitu gene probes did not generate much interest in this session, because they are thought to be primarily a tool of histopathology.

Infectious Canine Hepatitis (ICH) FA. VMRD markets ICH FA conjugate and ICH viral substrate slides. Canine Parainfluenza (CPI) and Canine Adenovirus (CAdV) FA conjugates are made with pig-origin hyperimmune serum. The CAdV conjugate was said to react to both pathogenically significant canine adenoviruses, CAdV-1, or ICH virus, and CAdV-2, an agent of "kennel-cough". Those present report only a rare positive reactor using the ICH FA conjugate. ICH antibody titration and infection surveillance remain a concern of vaccine companies. [Hemagglutination inhibition (HI) is a traditional method used for serodiagnosis of ICH viral infections. This HI technique uses cell culture-adapted ICH virus, which agglutinates human-O RBC's. Due to the prevalence of vaccination, paired serum samples are necessary to demonstrate seroconversion.]

Direct FA for IBR, BVD, BRSV on Lung Tissues and Non-specific Fluorescence. Bovine lung tissue occasionally contains eosinophils, the granules of which often exhibit autofluorescence, a form of non-specific fluorescence. Eosinophilic granule autofluorescence cannot be prevented but can be recognized with experience by its more yellowish shade of green, relative to specific fluorescence. False positives occur when eosinophilic autofluoresce is misinterpreted as specific fluorescence by inexperienced observers. When examining viral FA stained slides, cells containing fluorescent granules should be disregarded; typically, granular cells are not virus-infected cells. Differentiation is aided with a quick examination of the slide with bright-field illumination; usually, anything standing out as fluorescent on white light is autofluorescence. Eosinophilic granules can also be recognized in bright-field examinations by their refractivity.

Another source of non-specific fluorescence in bovine lung is collagen, especially prominent in FA stained frozen sections. As smears or sections age, they tend to develop a distinct red-yellow background, another form of autofluorescence. Staphylococcal organisms in specimens, particularly in dog conjunctival smears, will give rise to non-specific staining due to protein A & G immunoglobulin binding. Tissues from patients on high doses of tetracycline are also found to exhibit autofluorescence.

When performing FA staining for BVD viral antigen, smears or sections should be fixed with pure, fresh acetone; much of the BVD antigen that would react in FA staining is evidently lost with other fixatives, resulting in a greater probability of false negative readings.

All well manufactured commercial conjugates have very little background fluorescence when used at the recommended working dilution. To diminish background fluorescence, some technicians make use of 0.025% Evans Blue in the FA stain itself, or as a counterstain. Specific fluorescence becomes less distinct with the use of background-diminishing counterstains, which would not be recommended when microphotography is intended.

Background problems are ameliorated by avoiding the use of water in the slide rinse; a buffered rinse should be used when available. Slides should be read soon after staining. Stained slides should be protected from bright light, especially fluorescent light. When examining slides on a darkfield fluorescent microscope, the proper filter package should be installed to suit the application. On Leitz microscopes, the H3 and particularly the H2 cube (the excitation filter block) may allow the passage of some non-specific fluorescence. FA reactions are not to be overly scrutinized; direct FA tests are best screened at 160-250X and verified at 400X. Fluorescent debris may be significant in FA-stained impression smears; whole, intact infected cells exhibiting specific viral fluorescence are not always observed.

Another form of non-specific fluorescence can be seen in rabies testing of cat brains. The stain appears to be absorbed non-specifically by the periphery of certain cells, which were said to correspond to condensed neurons seen in histological sections. Rabies conjugate is available from BBL (used at 1:80 to 1:160, but each lot should be titrated independently), Centocor, Chemicon, CDC, and possibly other commercial sources. CDC also may provide blocking reagents for rabies FA testing.

Laboratory Diagnosis of CAE/OPP by Virus Isolation. Isolation of these viruses from synovial fluid was reported to be routinely successful, with optimum sensitivity, only with the use of special cell cultures [such as ovine choroid plexus cells. Please see Virology Session notes in the AVM Fall/Winter 1996 Newsletter.] In attempts to manufacture direct conjugate to detect these viruses in tissue, hyperimmunized animals were found to be unable to produce antibody in sufficient titers for a serviceable conjugate. As with other lentivirus infections, serology was said to be the best diagnostic approach, either using AGID or IFA methods. An infected goat turbinate cell line serves as a suitable IFA substrate for CAE/OPP serology testing. AGID antigen preparations of these viruses typically contain components of both glycoprotein and structural protein. A positive serum sample may be expected to react with either one or both of the antigenic determinants present in the CAE/OPP AGID reagent antigen.

Bluetongue Serology Test Kits. The reading of AGID reactions in BTV serology is facilitated by using a larger AGID template, such as in EIA testing, rather than the small pattern template specifically made for BTV testing. The ELISA technique was said to be more sensitive in detecting antibody than AGID; ELISA was reported to

detect a BTV positive animal three to four days earlier than AGID. BTV ELISA is also more specific than AGID, which becomes positive when EHD viral antibodies are present the serum sample. Current commercial ELISA technology for BTV antibodies is specific for BTV; the kits do not erroneously detect EHD viral antibodies, but are able to detect all 24 serotypes of BTV.

Because EHD viral antibodies react in the BTV AGID test, European livestock importation authorities require an animal to be negative for EHD viral antibody by neutralization and BTV antibody by ELISA. The OIE manual contains protocol for EHD viral neutralization testing. In Canada, a competitive ELISA test kit to detect EHD viral antibodies was said to be available; it was uncertain at this time whether the kit will be made available to U.S. markets.

Isolation of Epizootic Hemorrhagic Disease and Bluetongue Viruses. To recover EHD virus from a potentially viremic deer, a heparin (green top) or citrate (blue top) anticoagulant blood tube is drawn. The buffy coat is separated with centrifugation and is harvested. Ideally, buffy coat cells are washed in Alsevers solution to remove any potential anti-EHD viral antibody. If pre-inoculated cells are grown and maintained on media containing bovine serum products, they should be rinsed with serum-free media before inoculation. A ten percent cell suspension is made from buffy coat cells in serum-free media, inoculated onto cell culture, and allowed to absorb overnight. EHD viral culture protocol typically recommends baby hamster kidney (BHK) cells, but embryonic bovine trachea cell line (EBTr, ATCC CCL 44) was reported to perform well in recovering EHD virus, as well as other bovine viruses. At the end of the adsorption period, leukocytes are rinsed off, and the inoculated cultures are replenished with post-inoculation media containing horse serum, or no serum at all. Inoculated cells are daily examined for the development of cytopathology, then at seven days post-inoculation cells are stained with EHD viral FA conjugate, available from NVSL.

Another member reports using lysed whole blood cells as inoculum for EHD viral isolation, similar to that prepared for BTV isolation (see below). In post-mortem testing, EHD virus may be recovered from the spleen of deer.

In search of the inapparent carrier infection in cattle, EHD virus is recovered from cattle only with great difficulty, with many samplings and isolation attempts. Virus probably appears in blood at lower levels than seen in deer, and then rapidly disappears when antibody forms. Cattle are thought to be a reservoir of EHD virus; partly for this reason, conscientious farm managers avoid co-mingling domesticated deer and cattle.

In BTV isolation, the red blood cell fraction of whole blood is processed as inoculum. Typically, whole blood is rinsed three times to remove BTV antibody. A portion of RBC's is then harvested and lysed by sonication, freeze-thaw, or hypotonicity (with deionized water, which must be returned to isotonicity with concentrated salt solution before inoculation). With lysed blood as inoculum, BHK cell culture was reported to be a suitable cell line for BTV isolation. Horse serum is used in the post-inoculation media. Inoculated cultures are incubated in a CO_2 environment.

[Embryonated chicken eggs are understood to be the most sensitive host for recovery of BTV from clinical specimens, and may be specified for BTV culture by importation authorities of certain countries. Eggs of 10-12 days gestation are inoculated intravenously with the lysed blood cell preparation. After seven days post-inoculation, tissues are harvested from all eggs, whether alive or dead, for subculture. Typically, embryonic tissues are freeze-thawed and centrifuged. The supernate is harvested, diluted, and inoculated intravenously into another set of

eggs. BTV causes specific egg death, recognized by the subcutaneous hemorrhaging of developing embryos. Field isolates of BTV may not be lethal to all eggs of primary culture, but usually kills most eggs upon subculture. Embryos displaying characteristic lesions are harvested, homogenized, and centrifuged. Diluted supernate is inoculated onto Vero cells, which may develop cytopathology within a week. The presence of BTV is verified with the use of BTV FA conjugate, available from NVSL.]

Herd Screening for Inapparent Persistent Infection (PI) by BVD Virus. From animals with persistent BVD infection, virus is easily isolated from antibody-negative serum samples. All animals persistently infected by BVD are thought to be serologically negative, yet some have been found with antibody. Such instances may not be true BVD viral persistence, but actually a sub-acute infection with virus carried by leukocytes.

A suggested protocol is to draw blood from ten to twenty animals [10% or less] in the suspected herd; BVD SN is run on serum from these samples. Specimens exhibiting low or no BVD antibody are chosen to perform virus isolation. The level of viremia in persistently infected animals is generally high; as long as serum samples are not heat-inactivated, the samples are suitable for virus isolation.
Mass herd screening, when large numbers of samples are to be tested, is facilitated with microtitration techniques. Virus isolation would be typically performed in 96-well microtiter plates. Twenty microliters (20 ul) of serum is inoculated onto a BVD susceptible cell line grown in each plate well. In lieu of FA, viral antigen may be detected in inoculated cells using an immunoperoxidase-labeled conjugate. Inoculated culture plates are fixed and stained two to three days after inoculation. Fixation of cells grown in plastic 96-well screening plates may require diluted acetone, to avoid dissolving the plastic. BVD viral antigen for FA testing does not fix well in anything but pure acetone, but it is uncertain how BVD antigen for immunoperoxidase staining is affected by diluted acetone, or acetone/methanol mixed fixatives. Once cells within microtiter plate wells are satisfactorily fixed, FA verification of BVD infection is possible with an inverted fluorescent microscope equipped with long working distance objectives.

BVD virus is also readily found in the buffy coat fraction, which contain a high percentage of infected white blood cells. To avoid interference by colostral antibody when testing calves less than three months old, rinsed buffy coat cells are used as inoculum, instead of serum. Recovery of virus is more efficacious when buffy coats are rinsed, which perhaps removes some low level heterotypic antibody. Buffy coat cells are washed once, then inoculated in 50 ul volumes onto a susceptible cell line. The buffy coat cell inoculum should be left on, not be removed by rinsing of the inoculated cell cultures.

In contrast, diagnosis of acute BVD infection with virus isolation must be attempted in a period three to ten days post-infection. In certain animals, viral recovery from blood may only be possible within a two to three day time frame during the course of infection. Only a transient viremia typically occurs in the acute infection, which is quickly followed by antibody production. The already slim time frame to detect viremia in acute BVD infection is even more narrow with specimens of serum, rather than whole blood. Whole blood is thought to be the best sample for isolation BVD virus when acute BVD virus is suspected, or when the course and duration of the suspected infection is uncertain.

In attempts to detect BVD infection from abortion materials, NVSL reportedly uses 100 ul of fetal fluid as inoculum.

Buffy coat cells for BVD FA. Detecting BVD antigen in cells of smears made from the buffy coat of blood is another prevalent technique to detect BVD infection. It is also particularly effective in detecting persistent infection, when much viral antigen appears in white blood cells.

For untrained observers, FA reactions are difficult to read; given the cell types in buffy coat smears, there is potential for non-specific fluorescence. The experienced observer interprets the greenish granular fluorescence as non-specific fluorescence, and recognizes the hard apple green perinuclear fluorescence characteristic of BVD viral antigen distribution. Attempts can be made to diminish non-specific fluorescence by counterstaining with Evans Blue, but this could inhibit or lessen specific viral fluorescence, especially when the titer is very low or the fluorescence is weak.

With the possibility of low-level antibody in the sample, BVD FA testing of buffy coats may be improved by rinsing the blood cells before the smears are made. The use of control slides in buffy coat FA staining may improve technique and interpretation. Once a good positive animal is discovered, a ten milliliter green top (heparin) blood tube is drawn. The cells are rinsed and smears are generated from the harvested buffy coat. The smears are fixed and stored. For labs without ready access to infected calves, there was a suggestion that BVD virus-infected bovine blood be made commercially available to serve as positive control.

Ultimately, FA techniques are inferior to viral culture because they cannot differentiate between cytopathic BVD and non-cytopathic virus. However, they do give diagnostic capability to those laboratories without cell culture facilities.

Serodiagnosis of Ovine Respiratory Syncytial Virus (ORSV) by ELISA. Only a low volume of sheep testing was reported by the members present. Antigenically, ORSV was said to be similar to bovine or human RSV. John Black reported that the BRSV FA conjugate made by American BioResearch will react to ORSV.

Canine Parvovirus Vaccines: Their Efficacy and Influence on Rapid Diagnostic Techniques. Apparently, not all vaccines function similarly. Among the six domestically available CPV vaccines, three were said to be more effective, and the other three were found to give little protection from infection. Recently licensed CPV vaccines generally are more efficacious than older versions. Currently, all commercial CPV vaccines are modified life vaccines (MLV). Dogs or pups receiving MLV CPV vaccine may shed virus for up to six days post-vaccination. Current diagnostic methods may detect some CPV vaccinal virus shedding. Pups may produce antibody and seroconvert within four to seven days post-vaccination. Due to its frequent appearance as a pathogen, CPV is now being considered an endemic disease of dogs.

CPV-1 infection, also known as Minute Virus of Canines (MVC), continues to be seen but in low frequency. MVC infected dogs may lack antibodies against the common CPV (CPV-2), yet may shed parvovirus in stool. During EM examination, MCV is morphologically indistinguishable from CPV.
A case of bloody diarrhea in a dog was briefly described. EM observation found particles morphologically similar to picornavirus or astrovirus, about 20-30 nm in diameter; no calicivirus was recognized in the sample. It was speculated that this virus, in a fashion similar to human echovirus, was responsible for the illness.

An incident of rotavirus infection of an adult dog was described. Protracted, chronic watery diarrhea was the only presenting sign; in all other respects, the dog was well. Laboratory tests were negative or inconclusive. EM analysis of stools revealed

rotavirus particles, the presence of which was verified by a commercial rotavirus antigen ELISA test kit.

On rare occasion, viral particles other than parvovirus, coronavirus, or rotavirus may be found in cases of small animal diarrhea. Calicivirus is rarely found in dogs. No one has been able to culture the putative canine picornavirus from dogs. EM analysis of cat stool may reveal the presence of astrovirus, which should be considered an authentic pathogen in instances of cat diarrhea. Many unclassified viruses discovered in stool by EM may in fact be bacteriophage.

Procedure to Characterize Viral Isolates That Do Not React with Any Available FA Conjugate. In the hands of skilled operators and experienced observers, electron microscopy (EM) has the capacity to identify viral unknowns to the level of its taxonometric family. If analysis of clinical materials or culture fluids are negative or inconclusive by negative contrast electron microscopy (NCEM), viral morphology may be found and recognized in epoxy ultra-thin, electron dense stained sections of tissues or infected cell cultures. However, certain viruses, such as BVD, do not have distinctive morphological features recognizable by EM.
In EM fecal examinations for the diagnosis of common enteropathic viruses as coronavirus or rotavirus, the phase of the disease when specimens are collected greatly determines whether or not virus is actually found. Viral shedding is highest at the first break of viral diarrhea, but quickly diminishes and becomes intermittent with time. Another factor of potentially adverse consequence is a limiting volume of specimen, as in instances when a specimen must be split with the parasitology lab. In human diagnostics, these factors are thought to be even more critical, because specimens typically are not clarified or concentrated by centrifugation.

One member reported receiving a testing request for rabbit hemorrhagic disease (RHD), caused by a calicivirus, for product export to Mexico. Other non-pathological caliciviruses of unknown significance also may be found in rabbits and mistaken for the agent of RHD.

Bovine Immunodeficiency Virus (BIV). Some members reported to have received occasional requests for BIV serology. IFA slides are commercially available from VMRD; control serum can be obtained from American BioResearch.

To produce suitable BIV antigen substrate slides for IFA, infected cells require at least three days of viral growth to allow for proper antigen development. The western blot technique for BIV antibodies reportedly is being used in research applications at NADC. Older animals are more likely to be found with antibodies to BIV, which is popularly viewed as a virus in search of a disease.

Feline Immunodeficiency Virus (FIV): ELISA vs IFA. Due to the lack of ownership of intellectual property rights, VMRD no longer markets FIV IFA substrate slides. Test kits based on ELISA technology, such as those marketed by IDEXX, are suitable for screening but ultimately may not be very reliable; rates on false positive reactions in ELISA testing may range as high as twenty percent.

[On occasion, practitioners experiencing equivocal or discordant test reactions from office kits will request western blot testing. Such service is available from National Veterinary Laboratories, Box 239, Franklin Lakes, New Jersey 07417, 201/891-2992.]

Viral Disease Testing of Camels and Llamas, Aquatic Mammals and Primates. Some members mentioned of occasional requests for testing on camels and llamas. Laboratories at Oregon State University at Corvallis, and Colorado State

University at Fort Collins were reported to have some capability to test llamas for infectious disease. NVSL was said to perform MCF testing on llamas.

[For further information on viral diseases in llamas, please see Mattson D.E., Update on llama medicine. Viral diseases. Vet Clin North Am Food Anim Pract. 1994 Jul. 10(2)345-51.]

Woody Fraser of the Florida Animal Disease Diagnostic Lab at Kissimmee reported using a dolphin kidney cell line in diagnostic virology applications for aquatic mammalian specimens. Oklahoma Animal Disease Diagnostic Lab (OADDL) was mentioned to offer aquatic Morbillivirus testing service. (A suggested resource for information was Sandy Rodgers at OADDL). Dolphins were said to have been found with titers to encephalitis virus. [The 1996 ATCC catalog lists a dolphin kidney cell line, Sp1K, ATCC No. CCL-78.]

Some laboratories represented by attending members routinely reject specimens of any kind for primate virology testing requests. Other labs only accept fixed tissues from primates. The Virus Reference Laboratory (7570 Louis Pasteur, San Antonio, Texas 78229, phone 210/614-7350, fax 210/614-7355) performs virological and serological assays on non-human primate specimens.

BL-3 containment was recommended to safely perform primate laboratory work. Primate specimen containers should be opened in some sort of biological containment, which was apparently not the custom at most labs. However, no one knew of instances, or reports of instances, when an illness had been contracted from opening samples. Most concern arises from incidents of leaking formalin containers; it was noted that some veterinarians still put biopsy tissues in prescription pill bottles.

USDA Certification of Fish Stocks for Export. Certain European countries have strict requirements for the importation of tropical fish. However, some of the testing mandated by import regulations seems unreasonable, such as instances when tropical fish must be tested for cold water fish infectious diseases. OIE has published a reference manual of procedures used in fish testing. Russia was reported to require FMD testing on trout. NVSL has tentative plans to become a reference laboratory for fish virus testing, due to an anticipated international trade demand for aquatic products. International testing requirements may be very elaborate, however it is hoped that eventually one agency will be responsible to test and issue certificates.

DiagXotics of Wilton, Connecticut continues to market the "Blue-Plate Special" ELISA test kit for BTV antibodies, but was reported to do so as sideline. Its main commercial interest is in fish diagnostics, which it supports with a line of FA conjugates against fish microorganisms.

Chlamydia Update. As mentioned in the AVM Fall/Winter 1996 Newsletter, the SureCell® Chlamydia test kit by Kodak is no longer available. It was reported that Johnson & Johnson had bought rights to the kit, but decided to drop the line after problems with false positives were discovered. A number of human Chlamydia antigen ELISA test kits are available, such as Clearview Chlamydia (Unipath Ltd., distributed by Fisher Scientific), QuickVue Chlamydia (Quidel Corp., San Diego, CA), Premier Chlamydia (Meridian Diagnostics, Cincinnati, OH), and TestPack Chlamydia (Abbott Laboratories, Abbott Park, IL). Facilities at Auburn University were reported to be using the Pathfinder® Direct Antigen Detection System, from Kallestad Diagnostics, to detect Chlamydia antigen.

Meridian markets a Chlamydia FA conjugate that performs well, but because it is a human diagnostic reagent, it is not available in concentrated form and is expensive

for veterinary application. The Chlamydia FA conjugate from NVSL was said to react well to group specific antigen contained within inclusions or microcolonies in the cytoplasm of infected cells. The NVSL conjugate was reported to be routinely used to verify Chlamydia isolates in culture attempts done in coverslip-vial cell cultures.

To serologically diagnose avian chlamydia infections, the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) detects antibody to elementary bodies (EB) in a micro-agglutination format (EBA). The EBA protocol was said to be derived from undocumented procedures reportedly in use at a University of Miami testing facility. The EBA test has replaced the old in-house generated latex agglutination (LA) test for chlamydia antibodies because EBA was found to become positive earlier in the course of infection than LA. A Texas turkey strain of Chlamydia, grown in roller bottle cell culture, serves as the antigen source for EBA. The antigen extraction process avoids the use of phenol, which tends to dissolve outer membrane proteins, a principle location of Chlamydia antigenicity. Except from cockatiels, serum from most avian species reacts equally well in the EBA; cockatiels fail to show much antibody detectable by EBA. When analyzing chicken serum, the appearance of EBA reactions may be affected by the inherent nature of chicken antibody, which tends to stick to other antibody.

IFA was said to be a suitable method to perform chlamydia serology on mammalian species, especially on cats and cattle. Substrate slides are made with a cell culture-adapted strain of *Chl. psittaci*, and display antibody reacting to group specific antigen contained within cytoplasmic inclusions.

An attempt to develop a vaccine against *Chl. psittaci* in pet birds was mentioned. Three different antigen formats were being investigated as a vaccine, an attenuated cell culture-adapted strain isolated from a cockatiel, a stable portion of the MOMP, and a stress-induced small adhesion protein. Results were inconclusive at this time: All inoculated birds, whether vaccinated or unvaccinated, show lesions compatible with *Chl. psittaci* infection, but reisolation from vaccinated birds was less than unvaccinated birds. A successful vaccine, if popularly used, may be expected to complicate serological diagnosis.

[For additional information on commercial products available for Chlamydial diagnostics and for an expanded discussion on the theory and practicality of applying commercial test kits targeting Chl. trachomatis to animal Chlamydia diagnostic, please see Bacteriology Session notes under the heading Kodak SureCell® Chlamydia Test Kit is No Longer Available: Alternatives in the AVM Fall/Winter 1996 Newsletter. Also, further discussion on Chlamydia is found in this issue, in the Avian Session notes, under Diagnosis of Chlamydial Infection in Pet Birds by Fecal FA.

Other products available for Chlamydial diagnostics include Chlamydiae Fluorescent Monoclonal Antibody Test For Detection In Smears, from American Micro Scan; Modified Imagen® Chlamydia Test, from Boots-Celltech Diagnostics, Inc.; Chlamydia-Check® Direct and Culture Confirmation Kit, from Diagnostic Technology, Inc.; Difco Chlamydia Direct Detection System and Cellmatics Chlamydia Monoclonal DFA Reagent, from Difco Laboratories; Pathfinder® Direct Antigen Detection System and Pathfinder® Chlamydia Culture Confirmation System, from Kallestad Diagnostics, A Division Of Erbamont, Inc.; Chlamydia Trachomatis Antigen Test, from Neogenex; Chlamyset Antigen, from Orion Corp.; Ortho* Chlamydia Direct Detection (FA) Test, from Ortho Diagnostic Systems, Inc., Monabrite Chlamydia Trachomatis Direct Specimen Test, from Serono Diagnostics, Inc.; Monofluor Chlamydia Trachomatis Direct Specimen Test Kit, from Synbiotics Corp.; Microtrak Chlamydia Trachomatis Direct Specimen Test, from the Syva Company; Fluorescein Conjugated Anti-Chlamydia Monoclonal Antibody, from Whittaker Bioproducts, Inc. For further

information on these products, please visit the web site http://www.neoforma-mi.com/cat-mi/p0a/p0asbddu.html/.]

Culture of Obligately Intracellular Microorganisms and Brucella canis. A few members in attendance reported attempting to culture for these type of organisms, particularly *Lawsonii intracellularis*, the agent of proliferative hemorrhagic enteritis.

Howard Jones of Synbiotics reported that the *Brucella canis* RSAT test kit may soon be available for release, with possible licensure due in October. [As of January 1998, the *Br. canis RSAT* kit is now available from Synbiotics.]

In the absence of the RSAT for *Br. canis* serology, some labs satisfactorily use IFA methodology to test for *Br. canis* antibody in dog serum. VMRD markets antigen substrate slides for IFA, and recommends a 1:50 screening dilution to detect weak reactors. Serum screening at additional dilutions of 1:100 and 1:200 was reported to compare favorably to results obtained by AGID at Cornell University. *Br. canis* AGID serology is also being performed in the veterinary bacteriology laboratory at the University of Tennessee at Knoxville and facilities at Michigan State University. [Dr. David Bemis of the University of Tennessee College of Veterinary Medicine in Knoxville cites the following article for technical information in their internally-made *Br. canis* AGID serology: Zoha S.J. and Carmichael, L.E., Serological responses of dogs to cell wall and internal antigens of *Brucella canis* (*B. Canis*). Vet Microbiol. 1982 Mar 7(1)35-50.]

Antigen used in the *Br. canis* AGID test is quite labile, and retains reactivity only for a couple of weeks in refrigerated storage. AGID reagent antigen of cytoplasmic origin was said to be more specific than antigens extracted from other cellular locations. The use of purified cell wall antigen as an AGID reagent may lead to an inordinate amount of false positive reactors.

Br. canis tube antigen is still available from NVSL [as of March 1998, and is made with a true *Br. canis* culture, not with *Br. ovis*]. To make an in-house *Br. canis* antigen, the organism is grown on blood agar surface; Roux bottles are good culture vessels, into which blood or Brucella agar is poured and allowed to set. Growth is harvested with large swabs, scrapers, or glass beads, then treated in a series of washes and extraction steps, depending on the cellular location of the selected target antigen.

New EIA Program Stringencies Regarding Trace-back Sampling, Horse Identification, and Disposition of Positive Reactors: A Comparison of State Regulations.

Arkansas A three year program, with new regulations, was recently put into effect. Horses are tested annually and will be destroyed if they test positive in the first of the year of the program. Wherever positive horses are found, all other horses pastured on adjacent land are also tested.

Kentucky Horses sold at sale barns were found to be occasionally released before test results are returned, so stringencies governing the documentation of testing and movement of horses have been instituted. EIA testing is performed at sale barn sites, and trace back testing done on other horses at the farm where the positive reactor had been originally boarded. Thoroughbred registry also requires EVA serology, which, if positive, would ban a horse from export to Europe, unless proof of vaccination exists or semen is demonstrated free of virus.

Louisiana The trace back and eradication policy has been vindicated by the reduction in the rate of occurrence of positive reactors from 2% to 0.9% in the three years the policy has been in effect. Horses are required to have permanent, indelible identification, such as a brand, tattoo, or microchip, which must be reflected on EIA documentation. Depending on a veterinarian's service charge, installation of a microchip typically costs about $75 to $100.

Oklahoma A strategy was devised to insure the testing of all horses at Oklahoma sale barns. Barn operators were made liable for any EIA positive horses passing through their facilities. Oklahoma sale barns have adopted the practice of requiring each horse to be EIA negative within a week of sale.
Texas Legislation enacting policy similar to Oklahoma was said to have been drafted but, due to the lobbying efforts of sale barn operators, was amended to become a voluntary program. The barn operator is still held liable if a horse is found positive within 60 days after sale; in such instances, the barn operator is required to buy back the horse.

Some labs were said to reject EIA serum samples if they appeared severely abused. The question was asked, at what point does a serum specimen become unsuitable for EIA testing? Antibody in uncontaminated serum may remain stable for months at room temperature, however AGID technology, the principle method of EIA testing, lacks sensitivity compared to other more recently developed technologies, as ELISA. Hemolysis of a given blood sample may be so severe that a weak positive AGID reaction is masked.

In EIA testing of horses and mules, a rare ELISA positive/AGID negative reactor is found. After two to three weeks, the same animal may become ELISA positive and AGID weak positive. From these observations, ELISA is believed to be able detect early infection better than AGID.

On rare occasion, precipitin lines of non-identity form between the test serum and reagent antigen in AGID testing; such lines would appear to intersect, rather than become contiguous with positive control lines, as is seen in a genuine positive reaction. One theoretical explanation is antibody made against horse serum albumin (HSA), induced by a previous blood transfusion containing albumin of a different isotype than the recipient. Anti-albumin antibodies could react with HSA, if included by the manufacturer in the reagent antigen as a protein stabilizer.

Certain lentivirus AGID antigen preparations give two specific lines; crude preparations may be treated with ether to remove the second line, but testing sensitivity is lost. EIA antigen made with recombinant technology produces no second line, but EIA antigen made from cell culture-grown virus requires ether treatment.

Another rare reaction seen in EIA AGID testing is the partial dissolution of the positive control lines adjacent to the test sample well. In a described instance of this observation, the serum, which appeared "bubbly and off color", was retested negative by ELISA. The fuzzy positive control line may be explained by the "prozone" effect, produced by an especially strong positive sample. Whenever a fuzzy line is seen, the sample should be diluted and the test repeated.

Although rare, false positive reactions occur in EIA ELISA serology. In the testing of mules, the probability for false positives is a bit higher than that of standard bred horses. It is thought that steroid treatment has the ability to suppress immune response of an animal, thus may affect EIA testing. [Although steroids have a profound effect on the inflammatory response, it is not clear whether antibody titers

are affected. Please see Halliwell, R.E.W. and Gorman, N.T., Anti-inflammatory drugs, immunosuppressive agents, and immunomodulators, in Veterinary Clinical Immunology, Halliwell and Gorman, eds., W.B. Saunders, Philadelphia, 1989, p. 494.]

Equivocal ELISA reactions are typically refereed by western blot technology, [available at NVSL, or from Dr. Chuck Issel or Sheila Wright, Gluck Equine Research Center, Department of Veterinary Sciences, University of Kentucky, Lexington, 606/257-1710.]

Office International Des Epizooties (OIE, also known as the World Organisation for Animal Health): Manual of Standards of Diagnostic Tests and Its Potential Impact on Laboratory Testing. The OIE has published an international standard operating procedures (SOP) manual for veterinary laboratory testing. Detailed procedures are given for many common laboratory test requests, particularly those mandated by regulations of international commerce. It was prepared and reviewed by the joint effort of animal health specialists of many countries. It covers internationally shared laboratory serology methods for disease diagnosis and requirements of the production and control of biological products. In the introductory chapters, it also includes topics of sampling, QA/QC, validation of diagnostic assays, tests for sterility and human safety in the veterinary microbiology laboratory. It is anticipated that OIE specifications will be adopted by many importing countries and become required for export testing. Laboratories with significant export demand will need to be update their SOP manuals to incorporate OIE specified methodology.

Certain procedures stipulated in the OIE manual were reported to differ from common practice in subtle ways. For example, in Vesicular Stomatitis Virus (VSV) antibody neutralization testing, the standardized virus infectivity is specified by the OIE manual to be 100 $TCID_{50}$, however the manual also states that, depending on the importing country, standard virus up to 1000 $TCID_{50}$ may be used. The OIE manual states that a serum is considered positive when it demonstrates neutralization at a 1:40 dilution, but the actual screening dilutions and significant titers also may vary between countries. Partially due to the variation in testing criteria, NVSL is seeking approval of a VSV ELISA antibody test method for international usage.

At the time of this meeting, an outbreak by the Indiana strain of VSV was underway. Two years ago, a VSV New Jersey strain outbreak involved livestock primarily in the "four-corners" region of the U.S., in Arizona, Colorado, New Mexico, and Utah. Possibly due to anamnestic response, primed by an exposure during the previous outbreak, a large range of titers to VSV in horses was being seen by testing agencies. [For further information, please see this same topic heading in the Bacteriology Session notes.]

Mycobacterium paratuberculosis (Johne's Disease) Antibody ELISA Test Kit. The current IDEXX test kit was said to depend on reagents produced exogenously by CLS. The agreement between IDEXX and CLS was said to have been discontinued. Although its current kit remains in good supply, IDEXX is now developing a new kit comprised entirely of internally manufactured reagents; its release is pending USDA approval, anticipated at the end of 1997. The current kit was said to be more sensitive and specific (>75%) than the universally accepted technology of complement fixation (CF). [According to Lisa Lemieux of IDEXX, the new kit was said to be 20% more sensitive than the current ELISA test kit.]

DNA probe technology for Johne's Disease diagnosis was said to be in routine use by labs in California, Florida, Pennsylvania, and Wisconsin. Although the specificity of

the PCR technique is very good, sensitivity is reportedly only 50%. PCR methods promise a much quicker turn-around than conventional culture. To be of greater use, Johne's PCR protocols should be refined to be able to detect low level shedding earlier in the course of infection.

[The sensitivity and specificity of the various diagnostic methods for Johne's Disease are compared in the Bacteriology Session notes from the 1996 AVM annual meeting, as published in the AVM Fall/Winter 1996 Newsletter.]

Case Accessions and Tracking, Data Retrieval. Some members reported intermittent case accession errors; specifically, specimens are being misdirected or having their documentation or identification swapped with that of other samples while mail is being opened and parcels sorted. Many labs have full time accession clerks, and some have personnel opening mail through the weekend. After all mailed-in specimens have been sorted, a good practice is to have a staff veterinarian review all cases before specimens are routed to their respective labs. Due to complexities of the task, opening and sorting of specimens should done by the same set of individuals every day, in lieu of rotating different employees through accessions on different days. To avoid misrouting specimens internally, some facilities use distinct secondary containers ("boats" or small paper trays such as those once used to serve french fries) for different specimen destinations.

Many laboratories represented at this session use computerized information systems to track case progress and manipulate laboratory data. Several commercially available system software packages are tailored for the veterinary medical laboratory, such as VetLIMS (Veterinary Medical Diagnostic Laboratory, University of Missouri, P.O. Box 6023, Columbia, Missouri, 65205) or VADDS (VetStar Animal Disease Diagnostic Systems, Advanced Technology Corporation, 79 N. Franklin Turnpike, Ramsey, New Jersey, 07446). Few users are completely satisfied with any single package. The length of time necessary to install a new computer system, or to update a current system, is frequently underestimated. To improve their usefulness, systems must evolve and change, but employees and clients suffer through temporary disruptions of service during updates. A good systems manager is critical to the function of the system; personality and ability to work with laboratory personnel is as important as knowledge of the computer and the information system.

Some members reported that their labs were experimenting with bar-code technology, scanning specimens into the computer system. Others reported the use of an automatically-advancing accession number stamp.
While protecting unauthorized access to data, security features on older systems are cumbersome, requiring some sort of personal access code. Popular system updates have treated security with some flexibility, allowing systems to evolve in order to permit veterinarians access to their patients' lab data through the internet. Most labs still telephone or fax lab results; some veterinarians have a pager service through which results may be sent. Each laboratory should include details on specimen accession operations within its quality assurance manual.

A question arose regarding the handling specimens within a chain of custody. It was reported that there were only two instances where a chain of custody would apply, for legal purposes or by institutions required to comply with the Code of Federal Regulations, title 21 (21 CFR), established by the FDA. John Black has an example of a standard form used to demonstrate chain of custody, which is not rigidly practiced by many veterinary diagnostic laboratories on routine basis.

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In the next issue of the AVM Newsletter...

Quality Assurance in Veterinary Clinical Labs, Heifer Project International, The Principles and Protocol of Mycoplasma Culture; The PCR Technique: Do's, Don'ts and Examples, Simplified Parasitological Protocols, and more!

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