The Association of Veterinary Microbiologists

Message from the President

Dear AVM members,

Thanks to all of you, the Nashville meeting in July was a success. Hats off to Kaye Tipton and her group who came up with the motel and "places of interest". For those of you who couldn't attend, well, we had a good time for you!!!

As you know, this has been a year of transition and confusion. Emily Thost, Theresa Love, and Dexter Thompson are working feverishly to get the secretary/treasurer position organized. Bear with them. They are super good people who will get things worked out.

To help the Mississippi group out, pre-register if you can. Be sure to fill out a membership application form completely, whether you pre-register or register at the meeting. Please use your work place address.

Don't forget to bring your problems, your solutions, your new procedure, etc., with you to Annapolis in July. Beth Henricson is doing a great job for the Colonial States Chapter, our host.

We're sad, but glad that John Cole has joined the retired list. He will still attend meetings, and he and the Tifton Lab are in charge of our Y2K meeting in Savannah.

GOOD NEWS! Sherry is doing beautifully. She's fixing her Mom's house up so that she can move into it. Our continued best wishes to her!!

See you in Annapolis!!!

Judy Clapier

Judith Clapier
AVM President

The AVM thanks all exhibitors and corporate representatives who participated at the AVM 23rd Annual Symposium on Veterinary Microbiology, Nashville, Tennessee, July 30-August 1, 1998. All members are invited and encouraged to contact these individuals with questions and suggestions on their products. The financial and scientific support from our benefactors help make possible AVM events and functions "to promote scientific knowledge of veterinary microbiology to stimulate scientific investigations and their application to the advancement of knowledge in the field of veterinary microbiology and provide mutual assistance to participating microbiologists in solving problems." The AVM is grateful for their support.

Tom Isett, Becton Dickinson Microbiology Systems		410/316-4000
Brain Sunkel, Biolog, Inc		510/785-2564
Rod Hernandez and Mark Metrokotsas, Centaur Inc		800/236-6180
Lisa Lemieux and Kathy Velek, IDEXX Laboratories, Inc	Livestock	800/548-9997
	Poultry	800/943-3999
Howard K. Jones, Synbiotics Corporation		800/841-1875
Vesna Eller, Trek Diagnostic Systems, Inc. (formerly Accumed International)		800/871-8909
Bob Crandall, Viral Antigens, Inc		409/690-3787
Tom Kellner, VMRD, Inc.		800/222-8673

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AVM History and Philosophy

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, representatives 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 in 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 on 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. An application form is found in the back of this newsletter.

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 "in advance" or $25.00 "at the door", Sustaining Membership (annually) $100.00, Exhibitor's fee, $300.00.

AVM Officers and Executive Board Members, 1998-1999

President	Judy Clapier, Monroe, NC
Vice President	Mike Parsley, Little Rock, AR
Secretary/Treasurer	Theresa Love, Jackson, MS
Executive Advisor	Al Pursell, Tifton, GA
Past President	Theresa Love, Jackson, MS
Past President	Rob Poston, Baton Rouge, LA
Past President	Jan Mapp, Jackson, MS
Founding Members	John Black, Al Pursell

Colonial States Representative
Beth Henricson, Warrenton, VA
Heartland Representative
Anne Parkinson, Reynoldsburg, OH
Publications Chairperson
Rob Poston, Baton Rouge, LA
Meeting Site Chairperson
Beth Henricson, Warrenton, VA

AVM Standing Committees

Publications	Anne Parkinson, Rob Poston, Sara Rowe-Rossmanith
Nominations	Theresa Love, Melody Parsley, Rob Poston
By-Laws	John Cole, Al Pursell
Audit	Bill Cornell, Roxie Maddux
Meeting Site	Judy Clapier, Marion Fowler, Beth Henricson
Program	Judy Clapier, Theresa Love, Rob Poston
Newsletter Advisory	Frank Austin, John Black
Historian	Roxie Maddux

Ad-Hoc Committees

Web Page Development	Lea Dowd, Dexter Thompson

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.

Chapter	**President**	Beth Henricson, Warrenton, VA
Officers	**Vice-president**	Suzy Trefsgar, Lynchburg, VA
1998-1999	**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.

Chapter	**President**	Anne Parkinson, Reynoldsburg, OH
Officers	**President-elect**	Greg Fritz, Galesburg, IL
1998-1999	**Secretary/Treasurer**	Kay Rathman, Madison, WI

AVM News, Events, and Items of Interest

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 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.

All AVM members are invited and encouraged to contribute protocols, articles, or news items for publication in future AVM newsletters. Please send contributions to the current Publications Chairperson, Rob Poston, LaVMDL, P.O. Box 25070, Baton Rouge, LA 70894, fax: 225/346-3390, E-mail: rposton@mail.vetmed.lsu.edu.

AVM members are encouraged to exercise your rights as members by casting your vote on the Constitutional amendment ballot enclosed within the information packet, and returning it by mail to Secretary/Treasurer Theresa Love by the 35-day deadline. For those members attending this summer's meeting, completed ballots will be accepted at the registration table. For further information, please refer to the ballot, or contact a member of the Executive Board or By-laws Committee.

The Ad-hoc Committee on Web Page Development invites all AVM members to view the updated AVM web site at **http://www.wso.net/avm**, installed on web-server space generously donated by Immune Technologies, Inc. To view the web-page, updated browsers are recommended, i.e. Netscape 4.05 and up or Internet Explorer 4.0 and up, and that the JAVA scripting capabilities are enabled for your browser. For further information, please see the report from the Web Page Development Committee on page 16, or contact Dexter Thompson at halflatin01@yahoo.com or Lea Dowd at lea@gnat.net.

Congratulations and good luck to the new officers of the Colonial States Chapter, elected at the AVM-CSC annual meeting on November 13, 1998 in Williamsburg, Virginia: President Beth Henricson, Vice President Suzy Trafgar, Secretary/Treasurer George Blackwell.

Also, congratulations and good luck to the new officers of the Heartland Chapter, elected at the AVM-HC annual meeting on April 23, 1999 in Galesburg, Illnois: President Greg Fritz, Vice President Linda Cox, Secretary/Treasurer Kay Rathman.

Sensititre users please note that Accumed International has become Trek Diagnostic Systems, Inc. Vesna Eller is the veterinary products representative (ph: 800/871-8909 x104). For Sensititre technical support, please call 800/642-7029.

In a matter to be considered at its upcoming annual meeting, the American Society of Microbiology (ASM) is contemplating the establishment of a new division in Animal Health Microbiology (Division Z), as proposed by Thomas Shrylock of Elanco Animal Health and Robert D. Walker of Michigan State University. A petition drive is underway among ASM members to bring about the creation of this division. According to the petition, "The purpose of the proposed Division would be to provide a forum for investigators whose interests encompass diseases of animals (e.g. companion, food, and exotic) and the control or treatment of these diseases using antimicrobial agents, vaccines, probiotics, and more. Current topics of interest include animal pathogen diagnostics, veterinary or zoonotic pathogen antimicrobial susceptibility testing, surveillance / epidemiological studies, new technologies to reduce on-farm zoonotic pathogens, immunology and pathogenesis." According to Shrylock, "The new division could provide representation in ASM for other groups as well as veterinary laboratory diagnosticians, veterinary school faculty, animal health company scientists, and government scientists." The petition can be viewed at WWW.asmusa.org/mbrsrc/divzpetition.htm. For information, please contact Merry Sloane at ASM Headquarters ph: 202/942-9303, fax: 202/942-9346, email msloane@asmusa.org.

Meetings of the AVM and its Chapters

The Association of Veterinary Microbiologists will hold its 24th Symposium on Techniques in Veterinary Microbiology in Annapolis, MD on July 22-25, 1999, at the Wyndham Gardens Hotel, hosted by the AVM Colonial States Chapter. In addition to the General Business session and the installation of new officers, there will be a keynote address by Dr. Niall Finnegan, Chief of the Government Relations Division of the AVMA, and an expert on bioterrorism. There will also be Avian, Virology and Bacteriology Scientific discussion sessions on current topics of Veterinary Microbiology that affect us all on a daily basis. Topics will range from implementation of quality assurance guidelines and procedures for newer bench techniques, to national and international regulations concerning veterinary diagnostics and policy about transport and maintenance of infectious substances. The Wyndham Gardens Hotel is located in picturesque Annapolis, the Maryland State Capital. The meeting site is five minutes away from historic East Port of Annapolis and the US Naval Academy, and less than 25 miles from either Baltimore-Washington International Airport or Reagan National Airport in Washington, DC. In DC, there is also historic Union Station, which will allow attendees to reach the meeting by rail. Hotel and meeting registration packets will be mailed in June, or obtained from AVM-Colonial States Chapter President Beth Henricson at BHenricson@AOL.com or from AVM National Secretary/Treasurer Theresa Love at 601/354-6089. Please join us in historic Annapolis.

The Heartland Chapter of the AVM plans to hold its sixth annual meeting on April 28-29, 2000 at the Ramada Inn in Manhattan, Kansas, hosted by Kansas State University. Tentatively scheduled speakers include Drs. Jerry and Nancy Jaax, former pathologists at The US Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick, Fredrick, Maryland. They will share some of their experiences of "Life in the Hot Zone" from their encounter with Ebola virus. Workshops for virology and bacteriology are also being planned. The subject of the virology workshop is anticipated to be Electron Microscopy and Intestinal Viruses.

For further information on next year's meeting or regarding chapter operations or practices, please contact Secretary/Treasurer Kay Rathman at 608/266-2465, or one of the chapter officers.

Meetings of the American Association of Veterinary Laboratory Diagnosticians

8-15 October, 1999, AAVLD 42nd Annual Meeting, Town and Country Hotel, San Diego, CA. Deadline for Abstracts is May 1, 1999, Contact: Dr. Bruce L. Akey (804) 786-9202

20-27 October, 2000, AAVLD 43rd Annual Meeting, Sheraton Birmingham, Birmingham, AL.

Meetings of the World Association of Veterinary Laboratory Diagnosticians (WAVLD)

2-5 June, 1999 MEETING IX International Symposium of Veterinary Laboratory Diagnosticians & OIE Seminar on Biotechnology, Memorial Student Center, Texas A&M University, College Station, TX. Co-hosted by the AAVLD and TVMDL. In addition to scientific sessions and presentations, there will be an OIE/WAVLD Biotechnology seminar on techniques to detect slow, persistent and latent infections, and a PCR Wet Lab, conducted by TVMDL personnel. Contact: Dr A.K. Eugster, P.O. Drawer 3040, College Station, TX 77841-3040 Fax: 409/845-1794.

The PURPOSE of the AAVLD: Dissemination of information relating to the diagnosis of animal diseases, Coordination of diagnostic activities of regulatory, research and service laboratories, Establishment of uniform diagnostic techniques, Improvement of existing diagnostic techniques, Development of new diagnostic techniques, Establishment of accepted guidelines for the improvement of diagnostic laboratory organizations relative to personnel qualifications and facilities, Consultant to the United States Animal Health Association on uniform diagnostic criteria involved in regulatory animal disease programs.

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 1999, and the Spring of 2000; as details are established, they can be obtained from VLA newsletter coordinator Joni Ray at 940/241-2529, fax: 940/455-5025, e-mail: johneray@flash.net, or from the Director of Regional Programming Dr. Milton J. Becker at 773/764-1862.

The VLA is an organization for veterinary diagnostic laboratories. This association was formed for the purpose of providing consistency throughout veterinary diagnostic laboratories, which ultimately improves animal welfare. The association does not limit membership to any geographical area nor denote that this association is specific for any type of laboratory, being commercial, federal, state, university, or other private ownership. The association was formed in Chicago, Illinois on February 10th and 11th, 1989. Membership is open to anyone interested in the field of veterinary laboratory medicine, to include technicians, technologists, veterinarians, veterinary pathologists, laboratory managers and directors, professional organizations and industry. Those interested in further information on the VLA are invited to contact the VLA President Joni Ray at 800/366-4184.

Meetings of the American Society for Microbiology (ASM) and affiliated groups

30 May-3 June 1999. ASM General Meeting (99th). Chicago, Illinois.

21-25 May 2000. ASM General Meeting (100th). Los Angeles, California.

ASM's General Meeting is held annually in the late spring and brings together microbiologists from diverse environments. ASM members in 25 topical divisions work together to create a program that is informative and educational in the following broad categories: diagnostic microbiology and epidemiology; pathogenesis and host response mechanisms; general and applied microbiology (includes environmental); and molecular microbiology, physiology, and virology. Workshops of special interest topics are offered immediately preceding the meeting for an additional fee. For further information, please contact the American Society for Microbiology, 1325 Massachusetts Ave., NW, Washington, DC 20005-4171; tel., 202-942-9248; fax, 202-942-9340; E-mail, MeetingsInfo@asmusa.org; WWW, http://www.asmusa.org.

Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC):

26-29 September 1999. (39th). San Francisco, California.

17-20 September, 2000. (40th) Toronto, Canada

The Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), held annually in the early fall, is ASM's preeminent meeting on infectious diseases. ICAAC programs stimulate the exchange of new information among over 10,000 scientists worldwide, including researchers and clinicians. Sessions are often developed in cooperation with other societies, both domestic and international. Invited presentations include over 30 symposia, 25 state-of-the-art minilectures, 20 meet-the-expert roundtables, 6 interactive sessions, 2 award lectures and an opening session providing an AIDS update. Workshops of special interest topics are offered immediately preceding ICAAC for an additional fee. For further information, please contact the American Society for Microbiology, 1325 Massachusetts Ave., NW, Washington, DC 20005-4171; tel., 202-942-9248; fax, 202-942-9340; E-mail, MeetingsInfo@asmusa.org; WWW, http://www.asmusa.org.

Conferences organized by ASM's Conference Cmte., Branches, or other ASM affiliated groups:

23-26 June, 1999. Yeast Genetics and Human Disease II. Vancouver, British Columbia. Contact: ASM Conferences, 1325 Massachusetts Ave., NW, Washington, DC 20005; tel., 202/942-9248; fax, 202/942-9340; E-mail, MeetingsInfo@asmusa.org; WWW, http://www.asmusa.org/mtgsrc/mtgs.htm.

1-5 September, 1999. Pseudomonas '99. Maui, Hawaii. Contact: ASM Conferences, 1325 Massachusetts Ave., NW, Washington, DC 20005; tel., 202/942-9248; fax, 202/942-9340; E-mail, MeetingsInfo@asmusa.org; WWW, http://www.asmusa.org/mtgsrc/mtgs.htm.

12-13 November, 1999. Clinical Microbiology in the Managed Care Environment-Eastern Pennsylvania Branch, ASM. Philadelphia, Pa. Contact: Kathleen Beavis, Thomas Jefferson Univ., tel., 215/955-2456; fax, 215/923-6039; E-mail, kathleen.beavis@mail.tju.edu.

For information about ASM meetings and conferences, please contact the American Society for Microbiology, 1325 Massachusetts Ave., NW, Washington, DC 20005-4171; tel., 202/942-9248; fax, 202/942-9340; E-mail, MeetingsInfo@asmusa.org; WWW, http://www.asmusa.org.

Other Meetings

13-18 June, 1999. Epizootic Foreign Animal Disease Training Course. Madison, WI. Contact: Dr. Christopher W. Olsen, Dept. of Pathobiological Sciences, School of Veterinary Medicine,

Univ. of Wisconsin-Madison, 2015 Linden Drive West, Madison, WI 53706; E-mail, olsenc@svm.vetmed.wisc.edu; WWW, http://www.vetmed.wisc.edu/pbs/courses/epizootic.html.

20-23 June, 1999. International Symposium on Animal and Zoonotic Diseases. Contact: Dr. Yoshihiro Kawaoka, Dept. of Pathobiological Sciences, School of Veterinary Medicine, Univ. of Wisconsin-Madison, 2015 Linden Drive West, Madison, WI 53706; E-mail, kawaokay@svm.vetmed.wisc.edu.

9-16 July, 1999. International Workshop and Mini-Symposium on Rapid Methods and Automation in Microbiology (19th). Manhattan, Kans. Contact: Dept. of Animal Sciences and Industry, Kansas State Univ., Manhattan, KS 66506; tel., 785/532-5654; fax, 785/532-5681; WWW, http://www.dec.ksu.edu/dce/conf/microbiology.

10-14 July, 1999. The American Society for Virology Annual Scientific Meeting (18th). Amherst, Mass. Contact: Dr. Sidney E. Grossberg, American Society for Virology, Dept. of Microbiology and Molecular Genetics, Medical Coll. of Wisc., 8701 Watertown Plank Road, Milwaukee, WI 53226-0509; tel., 414/456-8104; fax, 414/456-6566; E-mail, segrossb@mcw.edu.

18-23 July, 1999. Summer Update in Clinical Immunology, Microbiology, and Infectious Diseases. Jackson, WY. Contact: Jeannette Rejali, Dept. of Pathology, Division of Clinical Pathology, Univ. of Utah School of Medicine, Salt Lake City, UT 84132; tel., 801/581-5873; fax, 801/585-1265.

12-15 September, 1999. Virulence Mechanisms of Bacterial Pathogens. Ames, Iowa. Contact: Inst. for International Cooperation in Animal Biologics, Coll. of Veterinary Medicine, Ames, IA 50011; tel., 515/294-1850; fax, 515/294-8259; E-mail, iicab@iastate.edu; WWW, http://www.nadc.ars.usda.gov/virulencemechanisms.

14-16 October, 1999. South Central Association for Clinical Microbiology Fall Meeting. Akron, Ohio. Contact: Brenda McCurdy, tel., 313/576-1000 x 5071; WWW, http://pages.prodigy.com/scacm/.

8-12 July, 2000. The American Society for Virology Annual Scientific Meeting (19th). Fort Collins, Colo. Contact: Dr. Sidney E. Grossberg, American Society for Virology, Dept. of Microbiology and Molecular Genetics, Medical Coll. of Wisc., 8701 Watertown Plank Road, Milwaukee, WI 53226-0509; tel., 414/456-8104; fax, 414/456-6566; E-mail, segrossb@mcw.edu.

Heifer Project International has helped more than one million impoverished families throughout the world to become more self-reliant through the gift of livestock and training in their care. A non-profit organization rooted in the Christian tradition, HPI joins with people of faith everywhere to work for the dignity and well-being of all people. In 1994, HPI celebrated its 50th anniversary and commemorated its first shipment of animals, 18 heifers sent to struggling families in Puerto Rico, where malnourished children had never tasted cow's milk. Today, families and communities in approximately 110 countries and 35 US States are leading self-reliant lives as a result of HPI's efforts. HPI provides more than 20 types of food- and income-producing animals, as well as intensive training in animal husbandry, ecologically-sound, sustainable farming, and community development. HPI received the President's Award for

Voluntary Action in 1986 and the Presidential End Hunger Award in 1990. HPI is a member of InterAction and of International Service Agencies (#0315).

At the 1997 AVM Annual Meeting in Hot Springs, Arkansas, Robert K. Pelant gave a presentation on HPI activity, including the establishment of a rudimentary animal health testing facility in Southeast Asia. The AVM thanks Dr. Pelant for his time with us, and the HPI and all of its participants for their philanthropic endeavors. To participate, donate, or for further information, please contact HPI, P.O. Box 808, Little Rock, Arkansas, 72203, ph: 800/422-1311.

Report from the Business Session, 1998

The Twenty-third Annual Meeting of the Association of Veterinary Microbiologists, The Regal Maxwell House in Nashville, Tennessee, Friday, July 31, 1998.

The Business Session was called to order at 9:00 am by President Theresa Love, who welcomed everyone to the meeting and called for an introduction of all members.

President Love introduced our exhibitors and thanked them for their support of our association:

Becton-Dickinson	Tom Isett	410/316-4000
Biolog, Inc.	Brian Sunkel	510/785-2564
Centaur, Inc.	Rod Hernandez	800/236-6180
Idexx, Inc	Lisa Lemieux	800/548-6733
Synbiotics Corp.	Howard Jones	800/841-1875
Trek Diagnostic Systems, Inc.	Jennifer Lorbach	800/871-8909
Viral Antigens, Inc.	Robert Crandall	405/846-6003
VMRD, Inc.	Thomas Kellner	509/334-5815

The minutes from the business session of the twenty-second annual AVM meeting were approved as written.

In the President's report, President Love acknowledged and thanked the Committee Chairs and members, and others who have given their time and talent on behalf of the AVM. She 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.

President Love continued with the Executive Board report, which 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. The By-laws committee reported on proposed amendments to the AVM Constitution and By-Laws to be voted upon in the Business Session.

The Publications Committee reported that 325 copies of the *AVM 1997 Fall/Winter Newsletter* were printed by Southern Printing, Inc., of Hopkinsville, KY at a cost of $680.71 (or $2.10 per copy). Due to uncertain financing, there was not a Spring/Summer issue, nor was there an

updated AVM Directory for distribution. The Chairperson invited members to work on the Publications Committee or to contribute articles to the *AVM Newsletter*.

The By-laws Committee reported on the proposed amendments to the AVM Constitution and By-laws, as published in the *AVM Fall/Winter 1997 Newsletter*. The proposed Constitutional amendment (deletion of Section 3 in Article I) was affirmed by the membership body, thus will be put to the entire membership by ballot. It was announced that one of the proposed by-law changes had been incorrectly referenced in the newsletter: Section 2 had been cited for deletion, but it was actually Section 1 ("Proposed Amendments to the By-Laws shall be presented to the membership by mail at least (30) days prior to the Annual Business Meeting") that was being proposed for deletion. There was no objection to the announced correction. All of the proposed amendments to the AVM by-laws were ratified by the membership body without dissent, thus was made part of the by-laws.

The By-laws Committee then proposed changes to Article III Section 1 of the By-laws to read "The Executive Board shall consist of the President, Vice-president, Secretary/Treasurer, the three (3) active immediate Past Presidents, the Executive Advisor, the Site Committee Advisor, the Publications Committee Advisor, and a representative from each Chapter of the ASSOCIATION. The co-founding members (Mr. John Black and Mr. Al Pursell) shall be members of the board as long as they are active members of the ASSOCIATION." A proposal to replace "an ex-officio member" with "a voting member" in Article III Section 5 was made so these sections are consistent with the changes in Section 1. Because the pre-notification requirement was removed by previous action, these proposed By-laws changes were considered at this time; all were ratified by the membership body without dissent.

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 fifteenth annual meeting of the CSC, was announced for November 12-13, 1998 at the Ramada Inn and Conference Center in Williamsburg, Virginia.

The Heartland Chapter report was given by Chapter President Anne Parkinson. She discussed chapter activities during the past year, including their annual meeting and a fungal workshop. Their next meeting, the sixth annual meeting of the HC, was announced for April 23-24, 1999 in Galesburg, Illinois, hosted by the Illinois Department of Agriculture Animal Disease Laboratory.

The Ad-hoc Committee on Web Page Development thanked all members who responded to their questionnaire, and invited members to see and render feedback on the newly designed Web Page, demonstrated on a lap-top computer set up next to the meeting registration table.

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

The Nominating Committee proposed Judy Clapier as President, Mike Parsley as Vice-president, and Theresa Love as Secretary/Treasurer. They were elected by acclamation.

President Love introduced Judy Clapier, who as the newly installed AVM President, conducted the remainder of the business session. President Clapier appointed Rob Poston to chair the Publications Committee for the coming year. John Cole and Al Pursell were reappointed to serve on the By-Laws Committee, and John Black was appointed to serve on the Newsletter Advisory Committee. President Clapier appointed Theresa Love and Rob Poston to serve on the Program

Committee. The Nominating Committee for the coming year will be composed of Theresa Love, Rob Poston, and Melody Parsley.

President Clapier appointed Beth Henricson and Marion Fowler to the Annual Meeting Site Committee, who reported that the twenty-fourth annual meeting of the AVM will be held in Annapolis, Maryland in the summer of 1999.

President Clapier informed the membership 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.

Treasurer's Report

This partial report was compiled by Secretary/Treasurer Theresa Love in transition from acting Secretary/Treasurer Emilie Thost.

BALANCE ON HAND, February 13, 1998		$4,369.35
RECEIPTS:		
Membership/Registration Fees		
1998 Meeting	$3,321.32	
Exhibitor Fees, 1998 Meeting	2,600.00	
Hotel Reimbursement:	912.63	
TOTAL RECEIPTS:	$6,833.95	6,833.95
EXPENSES:		
Annual Meeting 1998:		
Regal Maxwell House, Nashville	$4,635.40	
Thursday Night Social	159.34	
Advisor Reimbursement	179.60	
Printing:		
Newsletter	680.71	
Program	66.98	
Postage:		
Newsletter	407.19	
Program	239.47	
Miscellaneous:		
Office Supplies	146.67	
Frame and Plaque	42.01	
Historian Supplies	88.46	
Corrspondence	60.00	
Checking Account Charge	41.00	
Total Expenses	$5,290.12	5,290.12
Balance on Hand, April 1, 1999		$4,460.47

Colonial States Chapter Report

With a mailing list of 126, the Colonial States Chapter continues to be an active part of the AVM. Participation for the 1998 Fall Meeting was 21, a slight decrease from the previous year. There will be no Spring Symposium. Nevertheless, total yearly participation is expected to rise

far above the usual 50 members, speakers and sponsors when the Colonial States Chapter hosts the AVM national meeting on July 23 and 24, 1999 in Annapolis, Maryland.

The 1998 Administrative Board Meeting was called to order by President Marion Fowler at 5:00 PM on November 12, 1998 at the Ramada Inn and Conference Center, Williamsburg, Virginia Copies of the Minutes of the 1997 Administrative Board Meeting were distributed by the Secretary/Treasurer. For details, please see "Minutes of the Administrative Board Meeting" November 12, 1998. Members present: Marion Fowler, President; Beth Henricson, Vice-president and Site Committee; George Blackwell, Secretary/Treasurer and Past President; Earnest Wyant, Past President; Alfred Pursell, Advisor. Member not present: Judy Clapier, Advisor and Past President; Dorothy Scott-Wright, Publications Committee and Past President; Lynn Lewis, Advisor, By-Laws Committee, and Past President; Andy Myrup, Advisor, By-Laws Committee, and Past President.

The 1998 Annual Meeting was held on November 13, 1998 at the Ramada Inn and Conference Center, Williamsburg, Virginia. At 8:30 AM President Marion Fowler welcomed everyone to the 14th Annual CSC-AVM Fall Symposium. President Fowler then gave a short history and overview of our meetings and concluded with a few procedural announcements.

Following her opening remarks, President Fowler called the Business Meeting to order and asked that everyone introduce themselves and state their place of employment. There were 21 present, including two speakers and two exhibitors. Following introductions, she called for the Secretary-Treasurer's Report. Secretary-Treasurer, George Blackwell read the minutes of the 1997 Business Meeting and the Treasurer's Report for 1997-98.

The President then gave the report of the Administrative Board following which she asked for the report of the regular committees:

Site/Meeting Committee - Beth Henricson

Publications Committee - Marion Fowler expressed Dorothy Scott-Wright's regrets that she would no longer be able to perform as the Committee Chair. Suzy Trefsgar volunteered to coordinate the compilation of materials for the upcoming AVM newsletter and asked members to submit their notes through her. It was noted the AVM would be publishing their newsletter as a web page on the Internet.

Constitution and By-Laws Committee - Read by Marion Fowler for Lynn Lewis. The following motions were proposed for consideration:

Motion 1: Article I, Section 3, be omitted. Current wording:

> Article I - TITLE, Section 3. Whenever a provision of this constitution is inconsistent with the provisions of the related By-Laws, the provision in the constitution shall be controlling.
>
> Rationale: The national organization is changing their constitution to remove this section.

Motion 2: Article III, Section 1, be reworded.

> Article III - MEMBERSHIP, Section 1. Active members in the Chapter shall be active members of the Association and shall be those individuals who are OR HAVE BEEN engaged in the field of Veterinary Microbiology. [Words in capital are to be added.]
>
> Rationale: We don't mean to exclude retired individuals from our organization!

Motion 3: Article IV, Section 2, be reworded.

Article IV - OFFICERS, Section 2. The Officers must be active members of the Chapter FOR AT LEAST 3 YEARS IMMEDIATELY PRIOR TO THEIR ELECTION. [Words in capital are to be omitted.]

Rationale: We often have difficulty finding officers who have been members for at least three years.

No By-Laws changes were proposed.

Nominating Committee - George Blackwell. The Nominating Committee selections were as follows: Beth Henricson for President, Suzy Trefsgar for the office of Vice-president, Shelly O'Brien for the office of Secretary/Treasurer. Suzy Trefsgar spoke up to say that Shelly O'Brien was not able to accept the nomination for Secretary/Treasurer. Her name was withdrawn.

President Fowler next called for any special announcements or unfinished business. Marion extended the chapter's congratulations and best wishes to Dr. Howard Jones of Synbiotics Corporation on his being made president of the Veterinary Laboratory Association. Rob Poston made a short announcement concerning the Heartland Chapter activities.

President Fowler's call for new business led to a vote on the above proposed Constitutional changes. All motions were carried by voice acclamation. Other new business included a discussion on whether to have a Spring Symposium in 1999. George Blackwell offered the Equine Medical Center in Leesburg, Virginia as a possible site. Suzy Trefsgar motioned that since the organization was hosting the 1999 AVM National Meeting, the Spring Meeting not be held. This motion was carried by voice acclimation. There was no other new business.

Elections concluded the Business Meeting with Marion Fowler presiding. The Nominating Committee selections were restated and there was a call for additional nominations from the floor. There were no nominations and no volunteers for the office of Secretary-Treasurer. George Blackwell agreed to continue in the position until a nominee could be found. It was suggested that Lisa McDonnell be approached for this position. There being no other nominations, a motion was made and seconded that nominations be closed. This was carried by voice acclamation and elections were conducted by voice ballot. Following the elections, Marion installed the newly re-elected officers by announcing that Beth Henricson and Suzy Trefsgar had been unanimously elected.

President Beth Henricson thanked everyone for their vote of confidence. She adjourned the Business Meeting at 9:15 AM

President Henricson then opened the General Session by presenting Secretary/Treasurer, George Blackwell, and Past President, Marion Fowler, with plaques congratulating each on a successful 1997-1998 term of office. Beth then announced her goals for 1999: 1) Hosting the 1999 AVM in Annapolis, Maryland in July. 2) Integrating our expertise into the veterinary community as a whole and 3) Increasing the CSC-AVM membership. After announcing the committee members and advisors for 1998-1999, the meeting was turned back to Marion Fowler. Marion introduced the first speaker, Beth Henricson, who gave a slide presentation on Wound Infection by Toxigenic *Corynebacterium diptheriae* in an Equine. Following a short break for exhibits,

George Blackwell spoke on *Salmonella typhimurium* DT104. The General Session adjourned around 11:45 AM.

Following lunch in Colonial Williamsburg, the membership clustered into a combined round-table session to discuss topics from the 24th Annual AVM Symposium in Nashville, Tennessee. Special thanks to Suzy Trefsgar and Rob Poston for moderating the combined Bacteriology and Virology Sessions. Members adjourned at 5:00 PM and reconvened at 8:30 AM on Saturday to complete the agenda.

FOR MAKING THE FALL MEETING POSSIBLE, THE AVM CSC THANKS:

Albert Wellstein of Biolog for their exhibit and support.
Dr. Howard Jones of Synbiotics Corporation for their exhibit and support.
Dr. Tom Kellner of VMRD and Michael Sims of VSR, Inc. for sponsoring the morning break and as Sustaining Members for 1998 -1999.
Kelly Bridges of Perdue, Inc. for sponsoring the continental breakfast.
Bob Studholme of Viral Antigens for sponsoring the Social Session.

The Heartland Chapter Report

On April 23,1998, immediately preceding its annual meeting, the Association of Veterinary Microbiologists-Heartland Chapter (AVM-HC) sponsored its first workshop, in cooperation with the National Laboratory Training Network and the Ohio Animal Disease Diagnostic Laboratory (OADDL). The topic was *Famous and Infamous Fungi: A "Hands On" Review of Medically Significant Fungi in Veterinary Practice* was conducted by Dr. Jim Harris, Bureau of Laboratories, Texas Department of Health, Austin, Texas. Chapter participants were Linda Cox, Troy Farrell, Connie Gates, Tim Klinefelter, Darlene Krogh, Annie van der Lek, Cindy Lindeman, Pam McKenney, Kathy Strelow, and Mary Beth Weisner. Assisting Dr. Harris were the OADDL's Brenda Love and Anne Parkinson, and special guest Marie Burleson from the Ohio Department of Health Laboratories.

The workshop provided participants with information on fungal identification through lectures and "hands-on" laboratory exercises. Topics included basic terminology, the fundamentals of specimen handling and culture techniques, selection of appropriate media, *Fusarium spp*. morphology and toxins, *Aspergillus spp*., *Penicillium spp*., differentiating zygomycetes, and the identification of systemic pathogens, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis*, and *Sporothrix schenkii*. Participants were also given the opportunity to share fungal isolates from their own laboratories for a workshop consultation.

This workshop was the first attempted by the Heartland Chapter, and was thought to be exceptionally successful. Many thanks to Dr. Harris for his efforts in tailoring a program to fit the Chapter's interests. Thanks and appreciation are due to Rosemary Humes, Valerie Johnson, and Barbara Henderson of the National Laboratory Training Network.

At 8:00 pm on Thursday, April 23, an executive committee meeting was held, attended by Al Pursell, AVM advisor; Greg Fritz, Site Committee; Cindy Lindeman, Audit Committee; Tim Klinefelter, President; John Landgraf, Nominating Committee; Anne Parkinson, President-elect; and Kathy Strelow, Secretary/Treasurer. It was decided that the annual dues would remain at $5, exhibitor fees at $100.00. A list of vendors will be obtained from the national organization. The

exhibitor fees will cover exhibiting space and attendance at the meeting for two representatives. Sustaining membership fees are $50. Dues and meeting registrations received later than two weeks prior to the 1999 meeting will incur a $5 additional fee. The possibility of hosting the AVM national annual meeting was discussed. Al Purcell advised that the chapter would need to apply for a request from the national organization in order to start the process to host a national meeting. The year 2002 was suggested as a possibility. The style and content of the newsletter was discussed. Anne Parkinson and Cindy Lindeman volunteered to be in charge of the newsletter. It was suggested that the business meeting notes be included along with letters from the out-going and in coming presidents. Anne and Cindy will work on other guidelines. It was suggested that the newsletter be mailed in the middle of January (if possible). Future meeting sites were discussed. The 1999 meeting will be held April 23 and 24 in Galesburg, Illinois (Illinois Department of Agriculture/Ramada Inn). The 2000 meeting will be in Manhattan, Kansas (KSU). The 2001 meeting will probably be in Minneapolis, MN. The Heartland Chapter Policy Manual was distributed to Committee members.

The Fourth Annual Meeting of the AVM-HC was held in Reynoldsburg, Ohio, on April 24 and 25, 1998. Forty-four persons and three exhibitors were present. A business meeting was held on the morning of April 24. Greg Fritz (Illinois) was elected President-elect to serve for one year. The following members were elected to serve on committees: Newsletter/Publication, Cindy Lindeman and Anne Parkinson; Nominating, John Landgraf, Tim Klinefelter, and Kay Rathman; Audit, Cindy Lindeman; Secretary/Treasurer, Kay Rathman; Program/Site, Greg Fritz; By-laws, Steve Wessman and Linda Schroeder-Tucker.

Dr. R. David Glauer, the Ohio state veterinarian, welcomed everyone to the meeting. General sessions in Virology and Bacteriology were opened by scientific presentations. Dr. Sheila Grimes discussed Circovirus in the virology session, and Dr. Brenda Love discussed *Salmonella typhimurium* DT 104 susceptibility trends in the bacteriology session. Tours of the new OADDL facilities were enjoyed by all. Informal round table discussions were held by virologists and bacteriologists.

The Heartland Chapter wishes to thank Anne Parkinson and all those who worked with her to make the Fourth Annual Heartland Chapter Meeting very successful and informative.

The Fifth Annual Meeting of the Heartland Chapter of the AVM meeting was held April 23-24, 1999 at the Ramada in Galesburg, Illinois. The meeting was hosted by the Illinois Department of Agriculture Animal Disease Laboratory, and attended by fifty people and six exhibitors, representing thirteen States. The program consisted of three excellent presentations entitled "PCR and Its Role in Diagnostic Medicine" by Carol Lichtensteiger DVM, Ph.D. of the University of Illinois; "Molecular Epidemiology of PRRSV in Illinois: Nature and Nurture in the World of Swine Viruses" by Tony L. Goldberg, Ph.D. of the University of Illinois; and "QA is Not a Four-Letter Word" by Rod Chitty of the NVSL, Ames, Iowa. Round table discussions and a tour of the Galesburg Animal Disease Lab were also included in the program. Newly-elected Heartland Chapter Officers include President Greg Fritz of Galesburg, IL, President-elect, Linda Cox of Manhattan, KS, and Secretary/Treasurer Kay Rathman of Madison, WI. Annual dues remain at $5, sustaining membership is $50, and the exhibitor's fee is $150.

Next year's meeting is scheduled for April 28 and 29, 2000 at the Ramada in Manhattan, Kansas, hosted by Kansas State University. Tentatively scheduled speakers include Drs. Jerry and Nancy

Jaax, former pathologists at USAMRIID, Fort Dietrich, Fredrick, Maryland. “Re-live the Hot Zone!” Workshops for virology and bacteriology are also being planned. The planned subject of the virology workshop is Electron Microscopy and Intestinal Viruses. For further information on next year’s meeting or regarding chapter operations or practices, please contact Secretary/Treasurer Kay Rathman at 608/266-2465, or one of the chapter officers.

Report from the Ad-hoc Committee on Web Page Development

The Web Site Committee is happy to announce the unveiling of the AVM Home Page. The site was donated to the AVM by Immune Technologies, Inc. It is located on a "State of the Art" web server to allow all of the "bells and whistles" that we may want or need. We hope that everyone will take a moment to visit the site and make notations of comments to improve upon what we have now. The pages are located at http://www.wso.net/avm. In order to view this site, updated browsers are recommended, i.e. Netscape 4.05 and up or Internet Explorer 4.0 and up. Make sure that the JAVA scripting capabilities are enabled for your browser.

We are excited! There are so many ways that it can be utilized to benefit our members and the field of veterinary microbiology. The site features the past 3 newsletters on-line and a search engine to help locate specific words or phrases. We will be distributing instructional brochures and other materials at the Annual Meeting in Maryland or on request to help our members navigate and utilize our site flawlessly.... After visiting the site, please send us any suggestion/corrections to either Dexter Thompson at halflatin01@yahoo.com or Lea Dowd at lea@gnat.net.

We look forward to seeing everyone at the meeting.

Historian's Report and Archival Activities, 1997-98

Photographs from 1997 AVM Meeting, Hot Springs, Arkansas: Photos received from John Black and added to TrueCore Binder Box Album

AVM Files from 1978-1999: Files received from Dr. John Cole. Letters and documents are being categorized and stored appropriately.

Budget Allowance: $200.00

Expenditures for 1997-1998:

7/01/97 Wal-Mart	Binders	$8.40
7/29/97 Rite-Aid	Film	6.22
5/26/98 Wal-Mart	Binder	4.97
7/13/98 Gaylord	Album Dividers	17.52
7/14/98 Light Impressions	Bag Stiffners	
	Binder Boxes	51.35
Total		$ 88.46

by Roxana Maddux, AVM Historian

E-mail Humor, Tales of the Technically Challenged (original author unknown)

I recently saw a distraught young lady weeping beside her car.

"Do you need some help?" I asked.

She replied, "I knew I should have replaced the battery in this remote door unlocker. Now I can't get into my car. Do you think they (pointing to a distant convenience store) would have a battery for this?"

"Hmmm, I dunno. Do you have an alarm, too?" I asked.

"No, just this remote 'thingy,'" she answered, handing it and the car keys to me.

As I took the key and manually unlocked the door, I replied, "Why don't you drive over there and check about the batteries...it's a long walk."

Mycoplasma Culture: Starting from Scratch

CULTURE OF MYCOPLASMA OR PPLO (PLEUROPNEUMONIA-LIKE ORGANISM)

contributed by Annie P. van der Lek
Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia

Mycoplasmas are the smallest free-living organisms. The elementary bodies released during multiplication will pass through a 0.150 micron filter. Mycoplasmas have no rigid cell wall and are remarkably pleomorphic. Their size ranges from 15 to 20 microns in diameter. An organism is considered a Mycoplasma if it does not revert to a bacterium upon passage into media free of inhibiting substances. Mycoplasmas are generally aerobic but an atmosphere containing some carbon dioxide assists the growth of some species. All but one (*Mycoplasma laidlawii*) require sterols for growth. The great majority of Mycoplasmas will grow on PPLO medium. Both liquid and solid media are used in culture attempts. Penicillin and Thallium acetate are usually added to the media to inhibit Gram-negative and Gram-positive contaminating bacteria. Cysteine hydrochloride is added to media as a reducing agent. NAD (Nicotinamide adenine dinucleotide) and heat-inactivated sera are added to culture media to enhance the growth:

Horse serum for *Mycoplasma meleagridis*
Swine serum for *Mycoplasma synoviae*
Swine or horse serum for *Mycoplasma gallisepticum*

Organ swabs or tissues, obtained aseptically, are cultured in Friis broth and on Friis' ("Dutch") agar for Mammalian Mycoplasmas. Frey's broth medium and Frey's (DA) agar (Difco Agar added to Frey's broth) is used for Avian Mycoplasmas (mainly MG, MS, and MM). All media used are modified to favor the growth of the particular type of Mycoplasma to be cultured and isolated.

SPECIMEN PREPARATION

Tissues. Under a laminar flow hood, approximately 1 cm^3 tissue is cut and minced with sterile scissors and forceps, and ground into sterile mortar and pestle with 4 ml of sterile broth medium. The ground tissue homogenate is placed in a sterile screw-cap tube, vortexed for 10 seconds and placed under the laminar flow hood for approximately 15 minutes (to allow pieces to settle).

Swabs made from tissue specimens. Tissues are swabbed, stabbed, and worked over with a culturette swab. The tissue swab is inserted in 4 ml of Mycoplasma broth and then gently vortexed for 10-15 seconds (to extract).

Swabs. Swabs are directly inserted in appropriate Mycoplasma culture broth and vortexed.

Procedure overview. The supernatant of vortexed tissue or swabs is picked up in a five ml syringe through a 18 x l" needle, which is then replaced by a 0.45 micron syringe filter. (If the swab is from bovine or porcine eye, or other site known to contain smaller-sized bacteria, a 0.22 micron filter may be used to reduce contaminating growth). Approximately eight drops of filtrate is passed through the filter and placed in a screw-cap tube with 4 ml of sterile broth medium. Serial dilutions of 10^{-1} to 10^{-3} are made in broth and are incubated at 37°C. A blood agar plate and a Mycoplasma agar plate (of appropriate type) are also directly inoculated with the filtered supernatant and incubated at 37°C (in a Whirl-pak bag with moist filter paper or in a humidified CO_2 incubator).

All cultures are checked twice daily for color change. Dilutions and passages in broth media and on agar media are made as needed. The culture tubes from each passage are checked for color change; those exhibiting color change are inoculated on appropriate Mycoplasma agar plates. The agar plate cultures are examined under 4X objective of an inverted light microscope after four to ten days of incubation. Evidence of growth (exhibited by a color change of broth or appearance of colonies on plates) appears in four days to six weeks after inoculation of culture medium.

Materials:

Sterile forceps and scissors
Mortar & pestle
Vortex mixer
Sterile Pasteur pipettes with droppers
Inoculating loop
Syringe filters, 0.45 micron
Syringe needles, 18 gauge x 1”
Deionized water
Inverted light microscope with 4X objective
CO_2 water jacket incubator, settings at 37°C, 1.9% CO_2, maximum humidity

Media:
- Frey's broth, four ml. in 16 x 125 mm. screw-cap tubes
- Friis broth, four ml. in 16 x 125 mm. screw-cap tubes
- Frey's agar plates
- Friis' agar plates
- Blood agar plates

Procedure:

1. Aseptically insert specimen, tissues or swabs, immediately into 4 ml of the appropriate type of Mycoplasma broth medium. Frey's medium is used for Avian Mycoplasmas, Friis' medium is used for Mammalian Mycoplasmas.
2. Gently vortex swabs inserted in broth medium. Tissues are minced and ground first, before vortexing in broth medium. Vortex for 10 seconds.
3. Let particles in broth tubes settle for approximately 15 minutes under a laminar flow hood.
4. Pick up supernatant through 18 gauge x 1” needle with a five ml syringe. Remove needle and filter supernate through a 0.45 micron syringe filter.

5. Inoculate tubes of appropriate Mycoplasma broth with eight drops of filtrate, which approximately gives a 10^{-1} dilution*. Make further serial dilutions to 10^{-3}, as needed.
6. Also inoculate filtrate on a Blood agar plate (to check for possible contaminants) and the appropriate Mycoplasma plate media (to detect early Mycoplasmas, L-forms, etc.) For better viewing of any developing Mycoplasma colonies, Mycoplasma agar plates are flooded or splashed with inoculum, in lieu of streak inoculation.
7. Incubate tubes at 37°C, checking daily for color change. Mycoplasma agar plates are kept in a moist environment and checked after a few days of incubation. Blood agar plates are checked daily for growth. (A CO_2 water-jacketed incubator is ideal. Incubator settings are 37°C, 1.9% CO_2, with maximum humidity.)
8. If color changes in broth culture, dilute 10^{-1} in sterile broth and subculture onto Mycoplasma plate medium. Check Mycoplasma plates after four and ten days incubation for Mycoplasma colonies under an inverted light microscope with a 4X objective. If growth is too dense, the broth culture is further diluted, and another series of plates are inoculated to see if colonies change in size and number. For questionable Mycoplasmas, one plate may be stained with Dienes' stain.
9. If no color change is noticed in broth cultures, subculture a dilution 10^{-1} of inoculated broth up to four consecutive times, once every three days. Hold cultures for up to six weeks. Plate each culture on appropriate Mycoplasma medium at least once a week, incubate at 37°C. Check these plates four and ten days after inoculation.
10. As soon as Mycoplasma cultures exhibit characteristic Mycoplasma colonies, cultures are reported as positive. Cultures not showing Mycoplasma colonies are held for six weeks before reporting as negative for Mycoplasmas.

*Estimated inoculum volume to make a 10^{-1} concentration: Approximately eight drops per four ml. broth or agar plate (volume of broth and volume of agar are each considered four mls). There are approximately 20 drops per ml., so four mls equals about 80 drops. 10^{-1} or 1:10 = 8:80, or about eight drops per four ml. This represents one drop inoculum into each nine drops of media, or eight drops inoculum into 72 drops Mycoplasma broth or one Mycoplasma agar plate (four ml. on both broth and agar)

MYCOPLASMA MEDIUM (FREY) AGAR (for Avian Mycoplasmas)

1. Prepare mixture A, sterilized by autoclaving 15 lbs., 120°C, for 15 minutes; cooled in 56°C waterbath.
2. Prepare mixture B, sterilized by filtration through 0.45 on 0.22 micron filter unit; warm to 56°C in waterbath.
3. Mix A with B, under Laminar flow hood. Return to 56°C waterbath until ready to pour plates.
4. Pour plates under a Laminar flow hood, 4 ml per 60 x 15 mm Petri dish.
5. Let cool and dry overnight under Laminar flow hood to avoid rapid molding.
6. Place in sleeve.
7. Store in 4°C refrigerator.

Mixture A

1. In a two liter Erlenmeyer flask with stir-bar on heat-stir plate, mix:

Deionized water	1	Liter
Mycoplasma broth base	22.6	grams
Dextrose	10	grams
Cysteine Hydrochloride	10.0	ml
Phenol red	0.025	grams

2. Check pH 7.7 to 7.9.
3. Stir to dissolve.
4. Add Agarose, Noble agar or Ion agar #2, 15 grams
5. Sterilize by autoclaving at 15 lbs., 120°C for 15 minutes.
6. Place in 56°C waterbath to cool.

Mixture B

1. Mix in 250 ml. Erlenmeyer:

NAD solution	10.0	ml.
Inactivated porcine serum	75	ml.
Inactivated horse serum	75	ml.
Penicillin-G solution	1000 units per	ml.
Thallium acetate 10% sol.	5	ml.

2. Sterilize by filtration through .45 on .22 micron filter unit.
3. Place in 56°C waterbath to warm up.

MYCOPLASMA MEDIUM (FREY) - BROTH (for Avian Mycoplasmas)

Materials:

Mycoplasma broth base (Gibco or Scott)	Erlenmeyer, 2 liter
Dextrose	Sterile Erlenmeyers, 250 ml.
Sterile porcine serum (inactivated before use)	Heat-stir plate
Sterile horse serum (inactivated before use)	Stir bar
Phenol red	Weigh boats
Penicillin-G potassium	Spatula
Thallium Acetate, 10% solution	Graduate cylinder, 100 ml.
Deionized water	Graduate cylinder, 10 ml.
Cysteine Hydrochloride 1% solution	Sterile screw-cap tubes, 16 x 125 mm
NAD, 1%	Freezer at -20°C
0.45 on 0.22 micron filter unit	Refrigerator at 4°C

Procedure:

1. Into 2 liter Erlenmeyer with stir bar on heat-stir plate weigh:

Deionized water	1	liter
Mycoplasma broth base	22.6	grams
Dextrose	10.0	grams
Cysteine Hydrochloride	10.0	ml.
Phenol red	0.025	grams

2. While stirring to dissolve, add:

1% NAD	10.0	ml.
Inactivated porcine serum	75.0	ml.
Inactivated horse serum	75.0	ml.
Penicillin-G solution	1000 units per	ml.
Thallium Acetate 10% sol.	5.0	ml.

3. Adjust pH to 7.8.
4. Pre-filter through a 0.45 micron filter unit, then filter through a 0.22 micron filter unit to sterilize.
5. Under a laminar flow hood, place media in 4 ml. volumes in sterile screw-cap tubes and 500 ml. sterile Erlenmeyer flasks with caps.
6. Store supplies in -20°C freezer and 4°C refrigerator, as needed.

STOCK SOLUTIONS FOR DA MEDIA: FREY'S AND FRIIS (Avian Mycoplasma Culture)

Materials:

Trypticase Peptone (BBL # 11921)	Pipette aid
NAD (B-Nicotinamide adenine dinucleotide)	Screw-cap tubes 12 x 75 mm. Falcon 2054
Thallium Acetate (Sigma T-8266)	17 x 100 mm. Falcon 2051
Penicillin-G Potassium, 500,000 units per ml.	16 x 125 mm. Falcon 2025
Sterile porcine serum	Syringe, 5 ml.
Sterile horse serum	Needle 18 gauge x 1”
Deionized water	Graduate cylinder, 250 ml
0.22 micron filter	Graduate cylinder, 100 ml
0.45 micron filter	Scale
Freezer at -20°C	Weighboats
Waterbath at 56°C	Spatula
Sterile pipettes, 5 ml.	Sterile pipettes, 10 ml.

5% Trypticase Peptone Solution: Dissolve 10 grams Trypticase Peptone in 200 ml of deionized water. Dispense 5 ml. per 12 x 75 mm screw-cap tube. Store at -20°C.

1% NAD Solution (B-Nicotinamide adenine dinucleotide): Dissolve one gram NAD in 95 ml of deionized water. Filter through layer of 0.45 and 0.22 micron filter to sterilize. Dispense 5 ml per 17 x 100 mm screw-cap tube. Store at -20°C.

10% Thallium Acetate Solution: Dissolve 25 grams Thallium Acetate in 250 ml deionized water. Dispense 10 ml per 16 x 125 mm. screw-cap tube. Store at -20°C.

Heat inactivate frozen sera. Thaw sera in refrigerator overnight. Bring to room temperature. Place in 56 °C waterbath for 30 minutes. To avoid clumping, swirl occasionally.

RE-ESTABLISMENT OF STORED MYCOPLASMA CULTURES, CULTURE STORAGE

1. Take from freezer and thaw under warm tap water (rapidly without heating over 110°F).
2. Warm culture and media to 37°C for one to two hours.
3. Following transfer, observe at least twice per day and transfer as needed (Acid formers are transferred when media starts to turn orange; most others are transferred at three days).
4. To store fresh growth, select tubes with slight change, wrap in paper towels and chill at -20°C for 24 hours, then transfer to -70°C for indefinite storage.

FRIIS MEDIUM (BROTH) (Mammalian Mycoplasma Culture)

Certain Mycoplasma sp., such as *Mycoplasma suipneumoniae* and *Mycoplasma flocculare,* have been notoriously difficult to cultivate. Penicillin-G and other benzyl derivatives of penicillin have an inhibitory effect on the replication of these Mycoplasmas. The basal medium is made from commercial dehydrated products. Pig serum is added and phenol red used as pH indicator. Various inorganic salts (Hank's balanced salt solution) are included and Penicillin-G replaced by Bacitracin and Methicillin as bacteriostatic agents. Half the volume of pig serum may be substituted with horse serum.

Materials:

Hank's balanced salt solution (modified)
Deionized water
Bacto brain heart infusion (Difco)
Bacto PPLO broth w/o Cystal Violet (Difco)
Yeast extract
0.5% phenol red solution
Bacitracin (see below)
Methicillin (see below)
Sterile 500 ml. bottles with screw-caps
Screw-cap tubes-16 x 125 mm. (Falcon 4-2025-2)
Thallium acetate concentration 1/10,000
Porcine serum (inactivated before use)
Horse serum (inactivated before use)
pH meter
2 ltr. Erlenmeyer
5 ltr. Erlenmeyer
Stir bar
Heat-stir plate
Freezer, -20°C

Procedure:

1. Mix in five liter Erlenmeyer with stir bar on heat-stir plate:

Hank's balanced salt solution (modified)	1000	ml.
Deionized water	1500	ml.

2. Add:

Bacto brain heart infusion	16.4	grams
Bacto PPLO broth w/o CV	17.4	grams

3. Dissolve
4. Autoclave at 121^oC, 15 lbs. for two to five minutes.
5. Cool in 56^oC waterbath.
6. Mix in two liter Erlenmeyer:

Yeast extract	120	ml.
phenol red solution	9.0	ml.
Bacitracin	500	mg.
Methicillin	500	mg.
Thallium acetate	0.2	grams
Pig serum	175	ml.
Horse serum	175	ml.

7. Adjust pH to 7.4
8. Warm to 56^oC in waterbath.
9. Mix contents of the two liter Erlenmeyer into the five liter Erlenmeyer.
10. Prepare -20^oC freezer supplies: 4 ml. per screw-cap tube, 500 ml. per screw-cap bottle.

Antibiotic suspensions:

Bacitracin: 1 gr. (1000 mg.) or 50,000 units into 10 ml., use 5 ml in above recipe.

Staphicillin (Methicillin, Sodium salt, 90% activity): 1 gr. (1000 mg.) = 900 mg. Methicillin, suspend into 4 ml., for 500 ml., use 2.22 ml.

MODIFIED HANK'S BALANCED SALT SOLUTION FOR FRIIS MEDIUM

(Mammalian Mycoplasma Culture)

1. To approximately 400 ml. deionized water, add:

Solution A (see below)	25	ml.
Solution B (see below)	25	ml.

2. Increase volume up to 500 ml. with deionized water

Solution A: In approximately 400 ml. of deionized water, dissolve:

NaCL	80.0	grams
KCL	4.0	grams
$MgS0_4 \cdot 7H_2O$	1.0	grams
$MgCL_2 \cdot 6H_2O$	1.0	grams
$CaCL_2$ (Anhydrous)	1.4	grams

Increase volume up to 500 ml. with deionized water.

Solution B: In approximately 400 ml. of deionized water, dissolve:

$Na_2HPO_4 \cdot 12H_2O$	1.5	grams
KH_2PO_4	0.6	grams

Increase volume up to 500 ml. with deionized water.

YEAST EXTRACT (SACCHAROMYCES CEREVISIAE)

Materials:

Waterbath at 37°C	50 ml. sterile screw-cap centrifuge tubes
Heat-stir plate	2 liter Erlenmeyer
Deionized water	Autoclave
Stir bar	Centrifuge

Fleischmann's pure dry yeast, type 2040, from Standard Brands, Inc., N.Y., N.Y.

Procedure:

1. Weigh 125 grams of yeast into 750 ml. deionized water.
2. Place suspension in 37°C waterbath for 20 minutes.
3. Heat and stir to 90 - 100°C for 5 minutes.
4. Autoclave supernatant at 120°C, 15 lbs. for 15 minutes.
5. Cool and centrifuge for 15 minutes at 2000 RPM, using sterile screw-cap tubes.

Centrifuged Yeast extract supernatant may be stored in 40 ml. quantities in 50 ml. screw-cap centrifuge tubes in -20 °C freezer for at least three months.

FRIIS MEDIUM ("DUTCH" AGAR PLATES) (Mammalian Mycoplasma Culture)

Materials:

Friis Medium (broth)	Petri dishes, 60 x 15 mm (Fisher Cat. # 8-757-13A)
Agarose ME (Kodak #EX - 1201680)	Pipette aid
Autoclave	Sterile pipette, 5 ml.
Waterbath 56°C	Laminar flow hood

Preparation:

1. Warm 500 ml. FRIIS MEDIUM (broth), taken from -20°C storage and put into 56°C water bath.
2. In one liter Erlenmeyer flask, place nine grams of Agarose, Ion agar #2 or Noble agar and 100 ml. distilled water. Autoclave at 120°C, 15 lbs. for 15 minutes. Cool to 56°C in water bath.
3. Under a laminar flow hood, while mixing thoroughly add warmed 500 ml. FRIIS broth to the contents of the Erlenmeyer flask.
4. Pour 4 ml. medium for each one 60 x 15 mm. Petri dish, while keeping medium warm at approximately 56°C.
5. Let plates cool and dry overnight under hood.
6. Repack Mycoplasma agar plates in sleeves, marked and dated appropriately.

7. Place in 4°C refrigerator storage till ready to use.

DIENES' STAIN

Staining solution:

Methylene blue	2.5	gms.
Maltose	10.0	gms.
Azure II	1.25	gms.
Sodium chloride	0.25	gms.
Deionized water	100.0	mls.

Staining of coverslips. A thin film of stain is applied to clean coverslips with a cotton swab. The film should be uniform and light. When dry, stained coverslips are ready for use and may be stored indefinitely.

Staining Procedure. A 1 cm^3 block is cut from an area containing suspected colonies and transferred to a microscope slide, colony side up. A treated coverslip is placed stain side down on the agar block. The staining reaction is complete within a few minutes; all colonies stain, but bacterial colonies decolorize in about 15 minutes, while the Mycoplasma colonies retain their color. The preparation is examined under low power or higher power using transmitted light.

Dienes' stain is commercially available (such as from Hyland Laboratories, Los Angeles, CA) and does not distinguish L-type colonies from Mycoplasma colonies.

Hemorrhagic Disease Update

According to Drs. Victor Nettles and David Stallknecht of the Southeastern Cooperative Wildlife Disease Study, several states have reported substantial hemorrhagic disease virus activity in 1998. Essentially all of the virus isolates have been epizootic hemorrhagic disease virus serotype 2 (EHDV-2). Deer die-offs were reported in Arkansas, Illinois, Iowa, Kansas, Kentucky, Missouri, Nebraska, South Dakota, Tennessee, Virginia, and Washington. EHDV-2 was isolated from Arkansas, Illinois, Kansas, Missouri, Oklahoma, Tennessee, and Virginia. Presence of EHDV-2 virus genetic material, detected by the polymerase chain reaction (PCR) test, was reported for Iowa and Missouri. Several other states have reported focal losses or evidence of convalescent cases (Alabama, Georgia, Indiana, Maryland, Oklahoma, and South Carolina). Outbreaks of deer adenovirus, which mimics Hemorrhagic Disease (HD), continues to be seen in California.

This year [1999] marks the 19th consecutive year for the HD survey, conducted by the Southeastern Cooperative Wildlife Disease Study. Awareness of the disease syndrome is increasing among wildlife managers; deer biologists will soon be factoring the impact of HD occurrences in their deer population and harvest models. In addition, HD now has the attention of people who are keeping white-tailed deer in enclosures due to heavy losses in HD-endemic areas. For more information on HD surveillance, please contact Victor F. Nettles at the Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, the University of Georgia, Athens, GA, 30602, ph: 706/542-1741, fax: 706/542-5865, e-mail vnettles@calc.vet.uga.edu.

Hemorrhagic Disease of Wildlife in the United States

contributed by Elizabeth "Buffy" Howerth, Department of Veterinary Pathology, the College of Veterinary Medicine, the University of Georgia, Athens

Etiology. Both Bluetongue virus (BTV) and Epizootic Hemorrhagic Disease virus (EHDV) cause hemorrhagic disease (HD). BTV and EHDV are closely related orbiviruses. Five serotypes of BTV (2, 10, 11, 13, and 17) exist in the US but only 10, 11, 13, and 17 are known to cause disease. Two serotypes of EHDV (1 and 2) exist in the US and both are known to cause disease.

Transmission. HD viruses are transmitted by *Culicoides sp. C. variipennis* is a confirmed vector of BTV and EHDV in the continental US but probably other species are involved*; C. insignis* is a confirmed vector of BTV. Disease generally occurs in late summer and early fall although early summer transmission has been reported.

Distribution. BTV and EHDV are widespread in the US. Disease in wildlife due to these viruses is also widespread and is enzootic in the southeastern US.

Epizootiology. Different patterns of EHDV and BTV transmission occur throughout the US. Their incidence ranges from cyclic to sporadic. Antibody prevalence to these viruses is highest in the southern part of the US where it can approach 100%. Antibody prevalence is age dependent, which should be considered in surveillance strategies. In the southeast, limited evidence suggests that the distribution of specific EHDV and BTV serotypes changes over time. The high

prevalence of EHDV and BTV antibody in wildlife species indicates that infection does not always result in disease.

Susceptible Wildlife Species in the US.

- BTV: Clinical disease in white-tailed deer, mule deer, black deer, pronghorn, and bighorn sheep; virus isolations from and/or antibodies in other wild ruminant species.
- EHDV: Clinical disease in white-tailed deer, mule deer, and pronghorn; clinical disease in bighorn sheep, bison, elk, perhaps mountain goat; virus isolations from and/or antibodies in other wild ruminant species.

White-tailed deer are one of the most susceptible species to infection with both viruses.

Clinical Signs.

- Depression, pyrexia, and hyperemia
- Oral lesions and lameness
- Animals may be found in or near water

Gross Pathology.

- Swelling of mucosal surfaces, tongue, lips and head and neck, hydropericardium, pulmonary edema (peracute form)
- Hemorrhagic diathesis, necrosis and ulceration (acute form)
- Atrophy and/or scarring of rumen, malnutrition, sloughing hooves (chronic form)

Histopathology.

- Endothelial cell swelling and mild perivascular infiltration, especially involving microvasculature of mucosal surfaces
- Hemorrhage and thrombosis
- Ulceration
- Myonecrosis

Electron Microscopy. Virus-associated microtubules, viral matrices, and 65 nm viral particles are found in endothelial cell cytoplasm.

Pathogenesis. Viral replication in microvasculature endothelium results in endothelial cell necrosis. Endothelial denudation exposes the basement membrane of blood vessels. Platelets and the clotting cascade are activated, resulting in thrombosis with concurrent fibrinolysis (disseminated intravascular coagulation). The final outcome of disease is influenced by varying degrees of viral immunosuppression.

Diagnosis.

- Histopathology: Examination should include buccal papillae, tongue, rumen, and pylorus, in addition to other major organs (including eye and brain).
- Serology: Paired sera is best. Acutely ill or dead animals may have detectable antibody levels.
- Viral isolation: Whole blood, spleen, lymph node are preferred samples. Although methods of isolation vary, efforts should be made to recover both EHDV and BTV from wild ruminant species.

Serology and virus isolation must be interpreted with caution in enzootic areas, especially in absence of classical disease syndrome.

Miller-Mallinson (MM) Medium:

An Improved Bacteriological Plate Media for the Isolation of Salmonella

Contributed by Ed Mallinson, the University of Maryland, College Park, MD

The Miller-Mallinson (MM) medium is said to perform similarly to Xylose-Lysine-Tergitol-4 (XLT4) agar in terms of specificity and sensitivity over Brilliant Green (BG), Hektoen (HEK), and Xylose-lysine-desoxycholate (XLD) medium. Further, MM medium may be superior to Xylose-Lysine-Tergitol 4 (XLT4) in the detection of weak and ultra-weak H_2S producing *Salmonellae*. Performance improvements are not limited to the surveillance for Salmonella in the poultry environment, but also can be seen with Salmonella detection in beef, pork, livestock manure, and clinical specimens.

Dr. Mallinson is Professor Emeritus at the Virginia-Maryland Regional College of Veterinary Medicine, Maryland Campus, the University of Maryland, College Park, MD, and is one of the co-developers of the XLT4 plate medium (available from Difco and BBL), commonly used in the isolation of *Salmonella spp*. Dr. Mallinson welcomes inquiries about the use of MM media, and invites feedback from members who have tried its use. Please contact him at the Avrum Gudelsky Veterinary Center, 8075 Greenmead Dr., College Park, MD 20742-3711, ph 301/935-6083 x114, fax: 301/935-6079. For further information, please see Tate, CR, et al, The isolation of *Salmonellae* from poultry environmental samples by several enrichment procedures using plating media with and without Novobiocin, Poultry Science 1990. 69 P 721-726, and Miller, RG, et al, Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*, Poultry Science 1991. 70 P 2429-2432, and Miller, RG, XLT4 agar - A highly selective plating medium for the isolation of *Salmonellae* and improvements to the original formulation, *Difco Culture Club News*, Spring 1994, vol 2.

MILLER-MALLINSON (MM) MEDIUM (US patent no. 5871944)

Formula for the isolation of non-typhi *Salmonella spp.* Amounts are given *per liter* of distilled-deionized water.

Ingredient	Amount	Ingredient	Amount
Sodium Thiosulfate	6.8 g	Agar, granulated	15.0 g
Ferric Ammonium Citrate	0.8 g	Sodium Chloride	3.0 g
Trizma Base*	0.7 g	X-gal*	0.1 g
Trizma Hydrochloride*	2.3 g	Yeast Extract	3.0 g
Alpha-Lactose	10.0 g	Beef Extract, desiccated	2.0 g
D(+) Cellobiose	5.0 g	Polypeptone Peptone	3.5 g
D-Mannitol	1.2 g	Niaproof 4	4.5 ml
D(+) Trehalose dihydrate	1.33 g	Distilled/Deionized Water	1000 ml

*Please see the full name on supply list.

Weigh all ingredients together except Niaproof 4 (N-4). Mix all solid ingredients in 1000 ml of distilled-deionized water to suspend. Pipette the 4.5 ml of N-4 into the blended MM solution, and bring to a boil until the agar is completely melted.

Cool to 45-50°C, and dispense about 20 ml of MM medium per 100 x 15 mm petri dish. Let hardened agar surface dry, and use immediately or refrigerate until needed. MM agar has a long shelf life. Plates can be stored for months if held refrigerated in well-sealed airtight plastic bags.

Colonies observed with non-pigmented peripheries and black (hydrogen sulfide or H_2S positive) centers are usually *Salmonella spp*. Those colonies showing green pigment with or without black centers are lactose fermenters. Most *Salmonella spp*. are not lactose fermenters.

Salmonella lactose fermentation is seen with strains of the more host specific *S. arizonae* or with strains (perhaps up to 10%) that have grown in the presence of lactose environment-type products generated mostly from the dairy industry. H_2S positive lactose fermenters appear as blue-green colonies with black centers.

H_2S-negative, umbonate, non-green pigmented colonies, with darkened brownish centers may be non-producing H_2S strains of *Salmonella*. This observation was especially noted with H_2S negative strains of *S. pullorum.* True H_2S negative colonies are white with umbonate edges and raised centers.

Ultra-weak H_2S producers (such as some strains of *S. dublin, enteritidis, pullorum*, etc.) may appear with a bright metallic silver sheen in the center of well-isolated colonies when incubated at room temperature or 35°C for an additional 24-48 hours.

The following sources and catalog numbers of the reagents necessary to prepare MM medium are provided as an aid; the reader may prefer other companies, except Biosynth International, the source of least cost for X-gal. Reagent quantities ordered will depend on your particular needs.

COMPONENT SOURCE LIST FOR MM MEDIUM

Item and Source		Catalog No.	Amount
Difco:	Beef extract, desiccated	0115-17-3	500 grams
	Yeast extract	0127-17-9	500 grams
BBL:	Polypeptone peptone	4311910	500 grams
	Agar, granulated	4311849	454 grams
Sigma:	D-(+) cellobiose	C-7252	100 grams
	Ferric ammonium citrate	F-5879	100 grams
	alpha lactose	L-3625	100 and 500 grams
	D-mannitol	M-4125	100 grams
	Niaproof 4	type 4	500 mL
	Sodium chloride	S-5886	500 grams
	Sodium thiosulfate	S-1648	250 grams
	D(+) trehalose dihydrate	T-0167	25 grams
	trizma base (tris(hydroxymethyl) aminomethane)	T-1503	25 grams
	trizma hydrochloride (tris(hydroxymethyl) aminomethane hydrochloride)	T-3253	100 grams
	X-gal (5-bromo-4-chloro-3-Indolyl-beta-D-galactopyranoside)		
	from Sigma	B4252	
	from Biosynth International	B715	10 grams
	(800/270-2436)	B7150	1 gram

INDEPENDENT EVALUATION OF MM AGAR FOR IDENTIFICATION OF *SALMONELLA* FROM VARIOUS CLINICAL SOURCES

June de Graft-Hanson, the University of Maryland, Eastern Shore, Princess Anne, MD

Samples analyzed included poultry and bovine tissues. Samples from pullorum-typhoid reactor birds were composite viscera, blended in nutrient broth and tetrathionate broth, Hajna (TTH) and intestines (blended in TTH). All other poultry tissues, including livers, spleens, guts, yolk sacs and cecal tonsils were simply cut into pieces and added to tubes of TTH and/or selenite F broth. Yolk swabs were added to broth tubes. Intestinal samples enriched in TTH were incubated at 41°C. All other broth cultures, agar plates and biochemical tests were incubated and performed at 37°C. Agar plates and biochemical tests were incubated for 48 hours before being discarded.

Three agar plates were used to compare the efficacy of MM in identifying *Salmonella.* Four suspect colonies from each agar plate were aseptically transferred to TSI agar slants. Suspects showing typical reactions were transferred to LIA agar slants, purified and then used to run a battery of biochemical tests and Salmonella serogrouping for complete identification. The BioMerieux API 20E kit identified cultures that did not give definitive biochemical reactions and could not be serogrouped.

Salmonella colonies on MM agar were intensely black or black with tan peripheries when they produced H_2S. Non-H_2S producers were moist, entire tan colonies. All *Salmonellae* were easily picked with a needle. Some non-*Salmonellae* H_2S producers were a dirty black color and usually non-discrete. These non-*Salmonellae* were extremely tenacious and difficult to pick; the whole colony invariably came off and left a perfect imprint on the agar surface. Most dirty, black tenacious colonies turned out to be *Citrobacter freundii.*

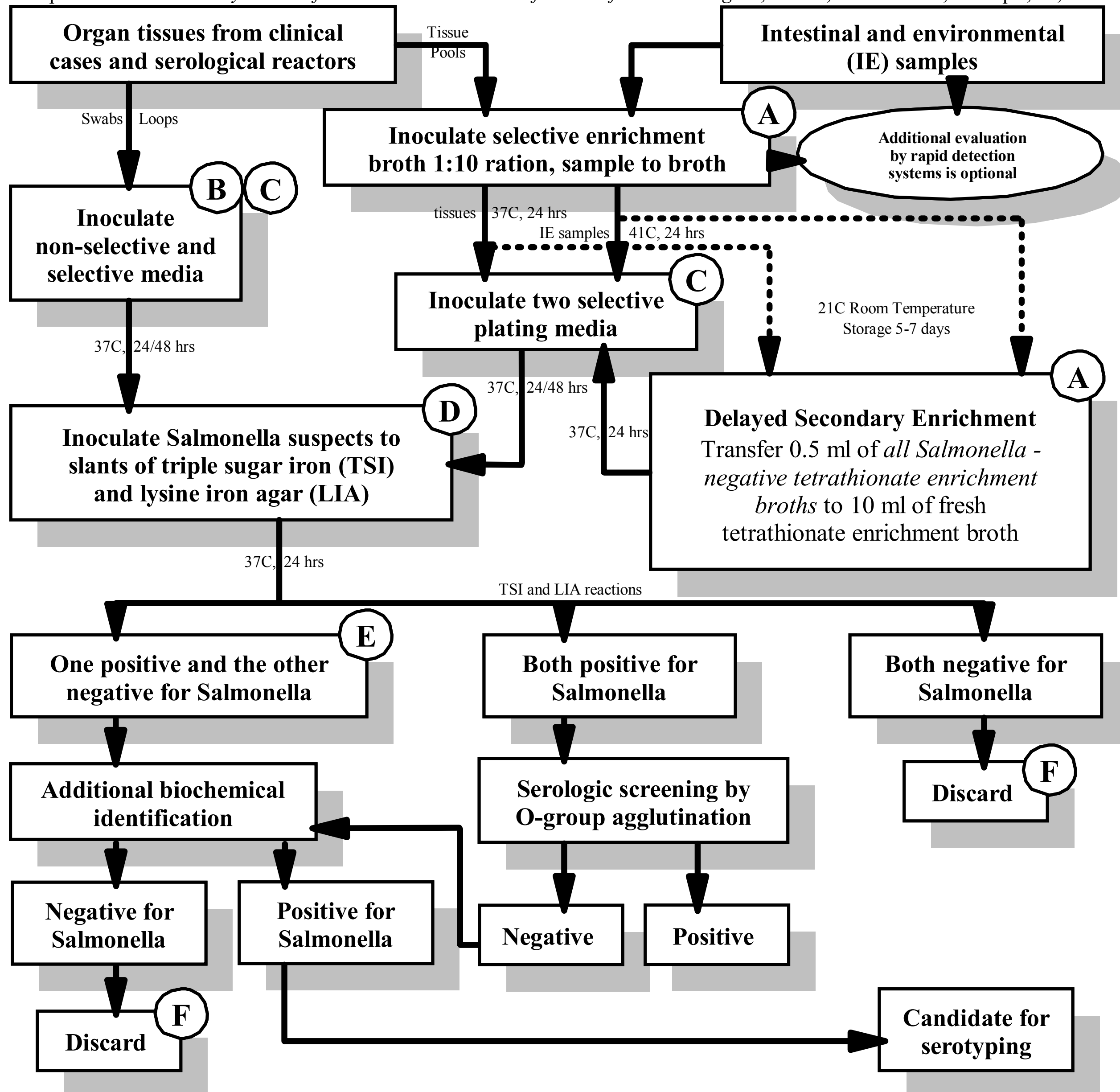
All types of colonies were picked and analyzed; after a time, growth was identifiable by its gross appearance. Primarily only the *Enterobacteriaceae* and *Pseudomonads* had the capability to grow on MM agar. The *Pseudomonads* produced very large tan "fuzzy" (irregular edges, not entire) colonies that turned light brown after 48 hours of incubation. *E. coli* colonies were either blue, green to deep green or turquoise and entire; most turquoise colonies had a metallic sheen. On occasion, *E. coli* colonies were fuzzy (not entire) but distinguishable from the *Pseudomonad* colonies by their turquoise color. *Hafnia alvei* colonies were pale green to turquoise in color, but smooth and entire. *E. cloacae* colonies appeared dirty green in color. On only one occasion, a Gram-positive organism was picked; its colonies were tan with a metallic sheen, instead of the turquoise metallic sheen of *E. coli.*

There was a difference in colonial morphology between the *Salmonellae* and other members of the *Enterobacteriaceae.* Most *Salmonella* colonies were smooth, entire and slightly raised. Other *Enterobacteriaceae* typically formed smooth, entire colonies that had deep depressions or craters in their centers. All *Salmonella* isolates appeared black or tan. Only two morphological types were seen for *Salmonella.* All other *Enterobacteriaceae* were shades of green and turquoise. The only other organisms isolated from this agar medium were *Pseudomonads* and one unidentified Gram-positive organism.

Considering the fact that samples came from various animals, tissues and organs, and were cultured in three different broth media at two different incubation temperatures, the MM media compared extremely favorably with both BGN and XLT4.

General Salmonella Isolation and Identification Flowchart

adapted from *A Laboratory Manual for the Isolation and Identification of Avian Pathogens*, 3rd ed., Kendall/Hunt, Dubuque, IA, 1989.



A. Hajna TT, or Mueller Kauffmann tetrathionate enrichment broths. For Rappaport Vassiliadis (RV) broth, follow special inoculation and pre-enrichment instructions of manufacturer.

B. Beef extract, veal infusion, or comparable non-selective media. A broth tube helps detect low Salmonella levels in live birds.

C. Brilliant green (BG) agar supplemented with novobiocin (BGN) in combination with xylose-lysine-tergitol (Niaproof) 4 (XLT4) agar is preferred. MM medium performs similarly to XLT4 and is more sensitive in detecting Salmonella with weak to ultra-weak H_2S production.

D. Colony lift immunoassays significantly increases the reliability detecting Group D Salmonella (e.g. *S. enteritidis, S. pullorum,* etc.) on plating agars.

E. If TSI and LIA slants and additional identification media yield mixed results, and O-group screening procedures are inconclusive, re-streak original colony onto selective plating media to check for purity.

F. Reevaluate if epidemiologic, necropsy, or other information strongly suggests the presence of an unusual strain of Salmonella.

The Avian Session

Moderated by **Ching Ching Wu**, Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, Indiana.

Reported by **Sara Rowe-Rossmanith**, Charles S. Roberts Animal Disease Laboratory, Auburn, Alabama, and **Diane Trinkle**, Diagnostic Laboratory, Perdue Farms, Washington, Illinois.

Attended by:

Sara Rowe-Rossmanith	CS Roberts Diagnostic Laboratory, Auburn, AL	344/844-4987
Amy Beckett	Perdue Diag Lab, Washington, IL	812/254-2440
Diane Trinkle	Perdue Diag Lab, Washington, IL	812/254-2440
Ching Ching Wu	ADDL, Purdue University, West Lafayette, IN	765/494-7459
Billy Williams	retired	205/384-0670
Tom Chang	CE Kord Ani Disease Diag Lab, Nashville, TN	615/837-5257
Gene Knight	Cuddy Farms Diag Lab, Polkton, NC	704/272-7261
Janet Harper	Cobb-Vantress, Siloam Springs, AR	501/549-6039
Lloyd Keck	Keck & Associates	501/761-3246
Melody Parsley	Arkansas Livestock and Poultry, Little Rock, AR	501/225-3630
Earnie Wyant	BUTA, Lewisburg, WV	304/647-4312
Lyn Perry	Purina Mills Res Ctr, Inc., Gray Summitt, MO	314/768-4400
Kathy Velek	IDEXX, Inc., Westbrook, ME	207/856-0300
Monica Kiker	Cuddy Farms, Polkton, NC	704/272-2761
Lester Buckner, Jr.	MSU Breathitt Vet Ctr, Hopkinsville, KY	502/886-3959
Susan Krampe	MSU Breathitt Vet Ctr, Hopkinsville, KY	502/886-3959
Kaye Tipton	Tennessee Dept Ag, Nashville, TN	615/837-5221

Availability of conjugates to detect avian viruses. Session members were unaware of any current commercial source of direct anti-viral conjugates for the detection of avian viruses. Researchers and diagnosticians working with avian viruses often share self-made conjugates and other reagents not available through the market. One cited example is R.S. Resurreccion at the Georgia Poultry Laboratory in Oakwood, GA, who makes a conjugate for infectious laryngotracheitis virus (LTV) to support internal research. Although not listed in its catalog, the National Veterinary Services Lab (NVSL) of Ames, IA is said to have direct LTV conjugate for distribution to reputable labs. SPAFAS is a commercial source of specific pathogen free (SPF) eggs, avian viruses, and antibody against avian viruses, the latter of which may be applied in IFA methods.

PCR techniques to detect Avian Infectious Bronchitis Virus (IBV) and other avian infectious pathogens are being performed at several facilities, including the Department of Avian Medicine, College of Veterinary Medicine, University of Georgia, Athens (M. Jackwood), the Department of Animal Science and Agricultural Biochemistry, College of Agricultural Sciences, University of Delaware, Newark (J. Gelb), and Charles S. Roberts Veterinary Diagnostic Laboratory, Alabama Department of Agriculture and Industries, Auburn, Alabama (L. Li).

IDEXX reportedly does not sell anti-viral conjugates, but offers a PCR test kit for the detection of Infectious Bursal Disease Virus (IBDV) Gumboro strain.

For an analysis of laboratory techniques used for the detection of avian viruses, please see Goodwin MA., et al, Comparison of histopathology to the direct immunofluorescent antibody test for the diagnosis of infectious laryngotracheitis in chickens. Avian Dis. 1991 Apr-Jun. 35(2). P 389-91.

What is the percentage of positive samples seen in Mycoplasma ELISA (IDEXX MG / MS combo and individual MG or MS, and KPL test kits)? With the MG/MS combo ELISA antibody test kits from IDEXX and KPL, some members have experienced positive samples that subsequently test negative with plate agglutination and hemagglutination inhibition (HI) methods. It was said that false positives are not seen as frequently with the ELISA test kits that individually test for MG and MS antibody, but when they occur, false positives are seen more so with the MS kit than with MG. Many of these false positive reactions are just above the cut-off threshold. To adjudicate discordant reactions, the specimens in question have been referred to the test kit manufacturers, which reportedly used higher cut-offs in their re-testing of samples by ELISA.

False positive reactions are believed to occur when samples are collected within three weeks of a previous administration of medication or vaccination, or during concurrent Staphylococcus infection. For more information on possible causes of false positive reactions in MS/MG testing, please contact Janet Harper or Melody Parsley.

The method of sample storage is thought to affect testing results. Although IDEXX has been successfully storing serum samples for approximately 20 years, sera is best stored at -20°C in small aliquots (to avoid re-freezing) for no longer than a three week period. Serum stored at refrigerated temperature (4°C) was thought to be stable only for one week. If possible, serum is tested soon after collection. Frost-free freezers are more detrimental to samples than storage in non-self defrosting freezers. Each laboratory should perform their own in-house testing to determine serum degradation rates for their particular storage methods. For more information on this suggestion, please contact Melody Parsley.

When a positive reaction is seen against one or both agents in the combo ELISA, typically the sample is re-tested by plate agglutination. If the sample remains positive, the sample is then heat-inactivated and re-run by the plate test using dilutions of 1:5 and 1:10. If the serum remains positive, the sample is re-tested with HI or the individually-reacting ELISA test kits. Ultimately, an attempt to recover the organism is made with culture or PCR. For more information on the arbitration of positive or discordant reactions experienced in MS/MG ELISA testing, please contact Melody Parsley or Earnie Wyant.

If MG or MS ELISA titers are consistently found on an asymptomatic flock, but are confirmed as seronegative by other technologies or facilities, then a search for the organism with PCR or culture is appropriate.

For QA/QC documentation, many laboratories subscribe to the check test offered by the Poultry Disease Research Center (PDRC). The check test costs $150 for three sets of sera, and demonstrates proficiency for the plate, HI, or ELISA methods. For more information on this check test, please contact Dr. Stan Klevens or Ms Joan King of the PDRC, Dept. of Avian Medicine, University of Georgia, 153 College Station Road, Athens, GA 30605, ph: 706/542-5639.

In conclusion, the manufacturers are reportedly aware of the potential for false positive reactions with their ELISA test kits. (For specificity and sensitivity data on the IDEXX MG/MS test kit, please contact Kathy Velek.) Some of these problems are avoided by scheduling samples before, or at least three weeks after the administration of medications or vaccinations. Isolation is the final test for ruling out whether a sample is indeed a false positive or a true positive after preliminary testing by plate, HI or ELISA methods.

For information on culture methods of Mycoplasmas, please see **Mycoplasma Culture: Starting from Scratch** in this issue of the *AVM Newsletter*.

NPIP regulations--update on changes. The following changes were instituted at the 1998 National Poultry Improvement Program (NPIP) meeting: *Salmonella typhimurium* is no longer being monitored by the NPIP. ELISA technology is now recognized for the serological testing of avian influenza (AI). The KPL AI ELISA test kit was said to be in need of greater specificity. IDEXX is currently developing an AI ELISA test kit, which is expected to have 99.8% specificity. (For more information on the IDEXX AI test kit, please contact Kathy Velek.) For AI certification, the NPIP now requires serum testing at both 90 and 180 days. Although ratites have been added to NPIP surveillance efforts, currently no approved antigen exists to test ratite serum for *Salmonella pullorum* antibody. For further information on these and other NPIP changes, please contact Melody Parsley.

The NVSL of Ames, IA performs serotyping for *Salmonella spp*. isolates, and reportedly does not charge a fee for this service provided that the accompanying documentation (the NVSL provides a specific form) has been filled out completely. Certain facilities attempt to perform their own Salmonella serotyping; for experiences in such, please contact Earnie Wyant. Single-factor antiserum for Salmonella serotyping is commercially available; for possible sources, please contact Janet Harper. Certain facilities limit their referral serotyping to only "B" and "D" groups, for information on the advantages of this practice, please contact Melody Parsley.

Fluoroquinolone-resistant *E. coli*. Representatives of some poultry testing facilities have noticed an increased incidence of Sarafloxacin-resistant *E. coli*, but not up to the level seen for Enrofloxacin. Currently, it is thought that up to 95% of all *E. coli* remains sensitive to fluoroquinolone antibiotics. For more information on the evolution of *E. coli* with increased antibiotic resistance, please contact Janet Harper or Gene Knight.

In an attempt to analyze this phenomenon with more precision, some facilities subject resistant organisms (as determined by the standard disk diffusion Kirby-Bauer method) to minimum inhibitory concentration (MIC) determinations, which are performed on semi-automated equipment as Sensititre; for experiences in such, please contact Susan Krampe. For information on MIC determinations using Sensititer equipment, please contact Vesna Eller of Trek Diagnostic Systems, 800/871-8909.

***Listeria monocytogenes* in chickens.** No discussion was offered on this topic at this session. The current belief, reflected in the scientific literature cited below, seems to be that *L. monocytogenes* is not a natural contaminant of poultry products, but is introduced into products by the processing plant environment. The incidence of *L. monocytogenes* is reduced with improved hygiene. For further information on this topic, please see Salvat G., et al, Effects of AvGard treatment on the microbiological flora of poultry carcasses. Br Poult Sci. 1997 Dec. 38(5). P 489-98, Foster EM. Historical overview of key issues in food safety. Emerg Infect Dis. 1997 Oct-Dec. 3(4). P 481-2,

Ojeniyi B., et al, *Listeria monocytogenes* in poultry and poultry products: epidemiological investigations in seven Danish abattoirs. J Appl Bacteriol. 1996 Apr. 80(4). P 395-401., and Lawrence LM., et al, Characterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. Appl Environ Microbiol. 1995 Jun. 61(6). P 2139-44.

Tilmicosin for MG treatment in chickens (experimental). No discussion was offered on this topic at this session. Tilmicosin appears to be somewhat successful in the control of mycoplasmal infections of poultry. For further information, please see Kempf I., et al, Efficacy of tilmicosin in the control of experimental *Mycoplasma gallisepticum* infection in chickens. Avian Dis. 1997 Oct- Dec. 41(4). P 802-7, Jordan FT and Horrocks BK. The minimum inhibitory concentration of tilmicosin and tylosin for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and a comparison of their efficacy in the control of *Mycoplasma gallisepticum* infection in broiler chicks. Avian Dis. 1996 Apr-Jun. 40(2). P 326-34, and Shryock TR., et al, Effect of bentonite incorporated in a feed ration with tilmicosin in the prevention of induced *Mycoplasma gallisepticum* airsacculitis in broiler chickens. Avian Dis. 1994 Jul-Sep. 38(3). P 501-5.

IBV problems in Mississippi & elsewhere. Variant recombinant strains of IBV have been detected with PCR in greater frequency on the US East Coast. Jack Gelb of the Department of Animal Science and Agricultural Biochemistry, University of Delaware at Newark will be speaking on emerging IBV variants at the Northcentral Avian Disease Conference in Indianapolis, IN on September 27-29, 1998.

IBV seems to peak in January and February. The Arkansas 99 strain is the most common strain now seen. With PCR detection of virus in the allantoic fluid of inoculated eggs, successful isolation of IBV only requires one passage in embryonated eggs. For further information on IBV strains and their recognition in clinical specimens, please see **Methods and Techniques for Serotyping Avian Infectious Bronchitis Virus** in the *AVM Fall/Winter 1996 Newsletter*, **Current Applications of PCR in Veterinary Diagnostics** in the *AVM Spring/Summer 1997 Newsletter*, and **Differentiation of Infectious Bronchitis Virus (IBV) Strains and Recognition of the New Variant Strain 072** and **New Diagnostic Applications with PCR Technology** in the *AVM Fall/Winter 1997 Newsletter*, or contact Sarah Rowe or Ching Ching Wu.

J virus. The incidence of J virus is on the increase primarily due to inadequate early detection methods, and that the virus is transmitted both horizontally between chickens and vertically from the breeder stock. J virus causes myeloid tumors or lymphoid leukosis in chickens. Due to the increased incidence of J virus, IDEXX currently is working on an ELISA test kit to recognize J virus in breeders, and to distinguish J virus from other avian leukosis viruses. (For information on this test kit, please contact Kathy Velek.) Work continues at the CS Roberts Diagnostic Laboratory in Auburn, AL for a PCR method to detect J virus. For further information on the J strain of avian leukosis virus, please see Payne, LN. and Fadly, AM., Leukosis/Sarcoma Group, in *Diseases of Poultry*, 10th ed., BW. Calnek, et al, eds., Iowa State Univ. Press, Ames, 1997, and **Increased Incidence of Marek's Disease (MD) and Leukosis** in the *AVM Fall/Winter 1997 Newsletter*, or contact Janet Harper of Cobb-Vantress or Lanquing Li of the CS Roberts Diagnostic Laboratory, Auburn, AL.

Salmonella surveillance: Drag swabs? Farm to table. BUTA now uses “boot swabs”, rather than drag swabs to monitor for the presence of *Salmonella spp*. in turkey pens. Boot swabs

consist of individual plastic boot covers with cloth soles. Poultry caretakers collect environmental samples as they work within the bird pens. Boot swab/shoe covers are removed and taken to the lab for testing within 5-10 minutes of collection. Boot swab samples are rinsed in sterile buffered peptone water (BPW), which is subcultured to Tetrathionate broth and incubated at 41°C for up to six days, then later plated to differential and selective media.

Bayer markets Avi-Gard for competitive exclusion (CE) of pathogens. Some poultry interests (as BUTA) make their own CE substrate with the use of an in-house fermentation process. All odor associated with the process is removed by a double water trap on the fermenter discharge; the water traps contain chlorinated water that is periodically changed.

According to BW. Chalnek (in *Diseases of Poultry*, 10th ed., BW. Calnek, et al, eds., Iowa State Univ. Press, Ames, 1997, pp. 111) CE "treatments involve administering defined or undefined bacterial culture to poultry in order to diminish intestinal colonization by Salmonellae." Intestinal or fecal material from healthy, mature birds are given to chicks and poults to reduce intestinal colonization by various paratyphoid Salmonellae and prevent subsequent invasion of intestinal tissues. CE treatments are administered via crop lavage, vent lip application, in drinking water, on alginate beads in feed, or to the air cell *in ovo*.

Tetrathionate (HAJNA) has been the traditionally recognized medium for the enrichment of Salmonella culture attempts. A recent Salmonella workshop conducted by Dr. Doug Waltman at his Oakwook, GA facility revealed that other tetrathionate formulations are being explored. According to workshop notes, "The recovery of Salmonella may not be as high with selenite as with tetrathionate, especially with environmental samples. Selenite F (Stokes and Osborne) is deficient in inhibiting *Proteus* and only delays the grow of coliforms. Salmonella die rapidly after they reach full growth in Selenite while non-Salmonellae like *Proteus* increase growth in prolonged incubation. If you let incubation go beyond 24 hours, you get a major die-off of Salmonella especially at the high tempreatures as 43°C. The half-life of selenite is shorter than tetrathionate and the effectiveness is weakened at elevated temperatures. Selenite is also a potential mutagen."

ADDL at Purdue University uses 24-48 hour tetrathionate broth culture to selectively enhance growth for Salmonella, then plates to BGN and XLT4 media. They also use PCR methods to identify Salmonella. For more information on the traditional culture methods for Salmonella, please contact Ching Ching Wu.

IDEXX has developed a pre-enrichment media for drag swabs, and uses a "super cooler" with broth media to determine the presence or absence of Salmonella within eight hours. The hands-on time is two to four hours; the cost of testing is about $3-4 per sample. For more information on the development of this process, contact Janet Harper. For information on the IDEXX product, please contact Kathy Velek.

BUTA has greatly reduced their incidence of Salmonella by 75% using CE, surveillance and monitoring, biosecurity, vaccination of breeders, and antibiotic treatment. For further information on monitoring and controlling *Salmonella spp.* in poultry pens, please contact Earnie Wyant.

In summary, boot swabs are replacing drag swabs in *Salmonella* monitoring efforts. Several *Salmonella* pre-enrichment methods are practiced at different labs. Most laboratories are using XLT4 as their differential/selective plate media. The practice of CE has caused a significant

decrease of *Salmonella* in poultry flocks. For more information on the culture and identification of *Salmonella*, please see *Miller-Mallinson (MM) Medium: An Improved Bacteriological Plate Media for the Isolation of Salmonella*, as well as related topics in the Bacteriology Session notes, all in this issue of the *AVM Newsletter*.

New Diagnostic Tests for Poultry PCR. The CS Roberts Diagnostic Laboratory of Auburn, AL currently offers PCR diagnostic services for potential infections by IBV, ALV-J virus, Chick Anemia Agent (CAA), Avian Polyomavirus, and the Mycoplasmas. For more information, please contact Sarah Rowe or Lanquing Li.

IDEXX markets a PCR-based test kit for the detection of IBV. For further information on this and other IDEXX poultry products, please contact Kathy Velek.

Breathitt Veterinary Center in Hopkinsville, KY has the ability to perform PCR to detect ALV-J virus, IBD virus and MG. For more information, please contact Susan Krampe.

ADDL at Purdue University performs PCR for the detection of Turkey Coronavirus (TCV) and IBD virus. For more information about PCR testing at ADDL, please contact Ching Ching Wu.

General biohazard protection for your employees at your laboratory (vaccinations?). Some facilities require employees to receive rabiesvirus and tetanus vaccines. Certain facilities bank employees' serum, which is drawn annually from each employee and stored to provide a baseline titer to help recognize possible lab-contracted infectious illnesses.

Incoming specimen parcels and mail are opened only in a dedicated receiving area, in which specimens are sorted and routed to appropriate laboratories for testing. Ideally, receiving areas are managed under an established biosecurity policy.

The US Postal Service, the US Dept. of Transportation (49 CFR), the Occupational Safety and Health Administration (OSHA) (29 CRF part 1910 and 1030), the Center for Disease Control and Prevention (CDC) (42 CFR part 72), the United Nations, the International Air Transport Association (IATA), and the International Civil Aviation Organization (ICAO) all have established procedures for the packaging, identification, and transport of potentially hazardous material. Regulations mandate crush-resistance of primary containers, and dictate whether secondary or tertiary leak-proof containers are necessary (such as in the case of shipping *Salmonella* isolates). Typically, the shipper is held responsible for violations, however laboratories have a role in edifying their clientele on appropriate shipping protocol. For information or reference materials on regulations governing the shipment of hazardous substances, please contact O. Berk, International, Union, NJ (800/577-7624, 908/687-7720) or the *Six Point Two News* ™ of Kenosha, WI (414/653-0433).

Most animal testing labs do not have the expertise, reagents, nor facilities (i.e., access to BL-3 lab space) for primate diagnostics. Where resources do not exist for testing on non-human primate species, specimens either are rejected for testing and returned to sender, or referred to facilities specializing in primate testing, such as Yerkes Primate Research Center, Emory University, Atlanta, GA 30322 (404/727-7724), Tulane Delta Regional Primate Research Center, Tulane University, Covington, LA (504/892-2040), or Virus Reference Laboratory, Inc., San Antonio, TX (210/614-7350). Most unfixed primate tissue specimens probably qualify as potentially hazardous material. Testing facilities often receive non-human primate samples that

have been improperly packaged and labeled either from ignorance or by the shipper's intent to "sneak" specimens through.

Awareness and emphasis are being invested in the proper handling, shipping, and transport of potential biohazardous substances. Increasingly, violating parties receive fines and sanctions. All facilities either sending or receiving biological specimens must become aware of regulations, and advocate compliance to their clientele to prevent interruption or delay of requested test services. For more information, please see related topics in Bacteriology and Virology session notes in this issue of the *AVM Newsletter*.

Chlamydia testing update. Due to the potential for human infection, certain facilities represented at this session offer Chlamydia testing only on a referral basis (such as to the NVSL). However, there are several facilities represented in the AVM that routinely perform Chlamydia diagnostics on animal specimens. Except for large volumes or aerosolized infectious material, *Chlamydia psittaci* can be handled safely in BL-2 facilities, according to CDC's *Biosafety in Microbiological and Biomedical Laboratories*. For more information, please see **Kodak SureCell *Chlamydia* Test Kit is No Longer Available: Alternatives** in the *AVM Fall/Winter 1996 Newsletter*, **Diagnosis of Chlamydial Infection in Pet Birds by Fecal FA** and **Chlamydia Update** in the *AVM Fall/Winter 1997 Newsletter*, and related topics the Virology session notes in this issue of the *AVM newsletter*.

Infectious neuropathies secondary to bacterial, fungal, parasitic, and viral etiologies (e.g. *Listeria monocytogenes, Staphylocococcus aureus, Enterococcus spp.*) No discussion was offered on this topic at this session. For some insight on instances when avian systemic viral infections generate neurological manifestations, please see Hooper PT., et al, Observations on the relationship in chickens between the virulence of some avian influenza viruses and their pathogenicity for various organs. Avian Dis. 1995 Jul-Sep. 39(3). P 458-64, and Barton JT., et al, Avian paramyxovirus type 1 infections in racing pigeons in California. I. Clinical signs, pathology, and serology. Avian Dis. 1992 Apr-Jun. 36(2). P 463-8.

E-mail Humor, Montana Grizzly Bear Warnings [original author unknown]

In light of the rising frequency of human/grizzly bear conflicts, the Montana Department of Fish and Game is advising hikers, hunters, and fishermen to take extra precautions and keep alert for bears while in the field.

We advise that outdoorsmen wear noisy little bells on their clothing so as not to startle bears that aren't expecting them. "We also advise outdoorsmen to carry pepper spray with them in case of an encounter with a bear.

"It is also a good idea to watch out for fresh signs of bear activity. Outdoorsmen should recognize the difference between black bear and grizzly bear poop.

"Black bear poop is smaller and contains lots of berries and squirrel fur. Grizzly bear poop has little bells in it and smells like pepper."

The Bacteriology Session

Moderated by **John Cole**, Georgia Diagnostic and Investigatory Laboratory, Tifton, Georgia.

Reported by **Judy Clapier**, North Carolina Dept of Agr, Monroe, North Carolina, **Linda Cox**, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, **Marlo Pacoli**, CS Roberts Diagnostic Laboratory, Auburn, Alabama, and **Anne Parkinson**, Ohio Animal Disease Diagnostic Laboratory, Reynoldsburg, Ohio.

Attended by:

Name	Affiliation	Phone
Beth Henricson	Virginia Dept of Agr, Warrenton, VA	540/347-6385
Marcia Browning	Florida Ani Disease Diag Lab, Kissimmee, FL	407/846-5200
Lynn Keach	Peterson & Smith Eq Vet Laboratory, Ocala, FL	352/237-6153
Tim Klinefelter	Iowa State Univ Vet Diag Lab, Ames, IA	515/294-1950
Connie Gates	SDSU Vet Res & Diag Lab, Brookings, SD	605/688-5689
Mary Jean Bryant	U of Tenn College of Vet Med, Knoxville, TN	423/974-5639
Jim Gary	Purina Mills Res Ctr, Gray Summitt, MO	314/768-4400
Dexter Thompson	Mississippi Vet Diag Lab, Jackson, MS	601/354-6089
Theresa Love	Mississippi Vet Diag Lab, Jackson, MS	601/354-6089
Judy Clapier	North Carolina Dept of Agr, Monroe, NC	704/283-1158
Shri Singh	MSU Breathitt Vet Ctr, Hopkinsville, KY	502/886-3959
Kristi P. Pool	MSU Breathitt Vet Ctr, Hopkinsville, KY	502/886-3959
Lisa Nash	MSU Breathitt Vet Ctr, Hopkinsville, KY	502/886-3959
Roxanna Maddux	MSU Breathitt Vet Ctr, Hopkinsville, KY	502/886-3959
Michele Mariano	Merck & Co., Rahway, NJ	732/594-3008
Gail Clifton	Georgia Diag and Invest Lab, Tifton, GA	912/386-3340
Sharon Chitwood	Athens Diag Lab, Univ of Georgia, Athens GA	706/542-5568
Pam Currin	Athens Diag Lab, Univ of Georgia, Athens GA	706/542-5913
Vesna Eller	Trek Diagnostic Systems, Inc., Westlake, OH	800/871-8909
Jennifer Lorbach	Trek Diagnostic Systems, Inc., Westlake, OH	800/871-8909
Linda Cox	Kansas State University, CVM, Manhattan, KS	785/532-4468
Anne Parkinson	Ohio Ani Dis Diag Lab, Reynoldsburg, OH	614/728-6220
Randal Buren	Bayer Ani Health, Worthington, MN	507/372-6115
Marlo Pacoli	CS Roberts Diagnostic Laboratory, Auburn, AL	344/844-4987
Bonita Vera	ADDL, Purdue University, West Lafayette, IN	765/494-7457
Margaret Gehlhausen	SIPAC, Purdue University	812/678-3401
Julie Bright	Alabama State Vet Diag Lab, Hanceville, AL	256/352-8037
Suzanne E. Taber	New Bolton Ctr., Kennett Square, PA	610/444-5800
Marion T. Fowler	Delaware Dept of Agr, Dover, DE	302/789-4811
Brian Sunkel	Biolog, Inc.	510/785-2564
Leslie Greiman	Illinois Dept of Agr, Centralia, IL	618/495-2550
John Abell	Maryland Dept of Agr, College Park, MD	301/935-6074

Laura Smith	Maryland Dept of Agr, College Park, MD	301/935-6074
Mary Proctor	VAMC, Louisville, KY	502/895-3401
John Cole	Georgia Diag and Invest Lab, Tifton, GA	912/386-3340
Susan Krampe	MSU Breathitt Vet Ctr, Hopkinsville, KY	502/886-3959
Earnie Wyant	BUTA, Lewisburg, WV	304/647-4312
Ching Ching Wu	ADDL, Purdue University, West Lafayette, IN	765/494-7459
Selvin Harrell	Clemson Animal Diag Lab, Columbia, SC	803/788-2260
Monica Kiker	Cuddy Farms Diag Lab, Polkton, NC	704/272-2761
Peter M. Boyt	Boyt Veterinary Lab, Neosho, MO	417/451-6369
Lyn Perry	Purina Mills Res Ctr, Inc., Gray Summitt, MO	314/768-4400
Sara Rowe-Rossmanith	CS Roberts Diagnostic Laboratory, Auburn, AL	344/844-4987
Ray Hines	Clemson Animal Diag Lab, Columbia, SC	803/788-2260
Gene Knight	Cuddy Farms Diag Lab, Polkton, NC	704/272-7261
Ron Wilson	CE Kord Ani Disease Diag Lab, Nashville, TN	615/837-5125
Pam McKenney	Grand Labs, Inc., Larchwood, IA	800/454-3424
Rob Poston	Louisiana Vet Med Diag Lab, Baton Rouge, LA	225/346-3193

General Biohazard Precautions for Employees of Veterinary Laboratories. Most laboratories have some type of rabies vaccination program in place, whether voluntary or mandatory. A blood serum titer of greater than or equal to five provides adequate protection against exposure. Also, certain labs practice blood-banking, which involves the collection of baseline serum samples from employees wishing to be vaccinated against zoonotic diseases. However, according to Shri Singh, AAVLD recommends that veterinary laboratory personnel do not handle human samples due to liability issues and disposal concerns. A copy of the protocol addressing biosafety in the veterinary laboratory can be obtained from John Cole. [*Veterinary Diagnostic Laboratory Guidelines for Radiation, Chemical, and Biological Safety*, 2nd Edition, 1996, published by the Laboratory Safety Committee of the AAVLD. Each AAVLD participating facility may receive one free copy of this manual by contacting the AAVLD Secretary/Treasurer, currently Art Bickford, CVDLS, SVM, UC Davis, P.O. Box 1522, Turlock, CA 95381, e-mail: abickfor@cvdls.ucdavis.edu] John also suggested that blood samples be collected from all new lab employees and stored at -70^{0}C to be used as a baseline for comparison if an employee ever becomes ill. The Georgia lab discontinued the vaccination of personnel for EEE virus because the vaccine must be administered by a medical doctor. The lab at Iowa State requires all employees receive physical examinations every five years.

About a fourth of the session participants handle all tissues in biological safety cabinets. Marion Fowler said that all exotic avian necropsies are performed in a biological safety cabinet at her laboratory, due to the death of a veterinarian from psittacosis. Dexter Thompson strongly recommended that any primate necropsies should be done under the protection of a biological safety cabinet. Most laboratories agreed that tissues and fluids transported from necropsy areas into the laboratory should be covered to prevent aerosols and protected from leakage. Culturing methods for *Bacillus anthracis, Brucella spp., Mycobacterium spp.* and *Leptospira spp.* should be performed in a biosafety cabinet.

Dexter Thompson asked how laboratories are dealing with biohazardous wastes generated in the laboratory. Ideally, all biohazardous waste should be rendered harmless by incinerated, however

some labs use alternative methods, the most popular of which is autoclaving. Appropriate disposal of biohazardous waste is often determined by State and local laws and ordinances. For further information on laboratory safety, please see related topics in the Avian and Virology Session notes in this issue of the *AVM newsletter*.

***Aspergillus spp*. in dog urine**. Consensus was that this is a rare occurrence. One participant reported seeing this once in 12 years. John Abell also recalled seeing it once, and said that the veterinarian claimed it was a common occurrence in German Shepherds, but had no reference to prove the fact. For further information on this topic, please see Kelly SE., et al, Long-term survival of four dogs with disseminated *Aspergillus terreus* infection treated with itraconazole. Aust Vet J. 1995 Aug. 72(8). P 311-3, Watt PR., et al, Disseminated opportunistic fungal disease in dogs: 10 cases (1982-1990). J Am Vet Med Assoc. 1995 Jul 1. 207(1). P 67-70, Simpson KW., et al, Systemic mycosis caused by *Acremonium sp* in a dog. J Am Vet Med Assoc. 1993 Nov 1. 203(9). P 1296-9, and Dallman MJ., et al, Disseminated aspergillosis in a dog with diskospondylitis and neurologic deficits. J Am Vet Med Assoc. 1992 Feb 15. 200(4). P 511- 3.

Recovery of *Salmonella spp*. from equine feces. A few laboratories are experiencing difficulties isolating *Salmonella spp.* from fecal samples due to inadequate sample submissions. For reliable testing results, the session consensus held that five consecutive fecal samples are needed, one every day for five days. Some labs are holding broth enrichment cultures for 72 hours, others hold for five days, and yet others are plating broth cultures every 24 hours for five days to increase recovery rates, which seem to vary between laboratories. Marlo Pacoli has isolated *Salmonella spp.* from a single swab. Lynn Keach has recovered *Salmonella spp.* from one or two samples from a single animal, but never from all five samples. This further demonstrates the need to culture a series of samples to confirm an animal's status.

Choices for enrichment broths varied between laboratories. Most labs are using tetrathionate broth, while some are using selenite or Rappaport's broth. Jim Gary mentioned that the use of selenite still seems to be of concern because of disposal problems and potential carcinogenicity. For further information on Salmonella culture, please see the article ***Miller-Mallinson (MM) Medium: An Improved Bacteriological Plate Media for the Isolation of Salmonella*** in this issue of the *AVM newsletter*. Also, please see **Salmonella surveillance: Drag swabs? Farm to table** in the Avian Session Notes of this issue of the *AVM Newsletter*.

In a related topic, Mary Jean Bryant isolated a mucoid isolate from a foal's bronchial tubes that was identified as *Salmonella spp.* on both the Vitek and API formats. The cultural appearance of the isolate was similar to *Klebsiella spp. Rhodococcus sp*. was also isolated from the foal. The foal's dam later died, and the same unusual isolate was recovered from its intestines. The isolate was submitted to NVSL for evaluation and results were pending at meeting time.

Salmonella serotyping. Tim Klinefelter reported that Difco had had a bad lot of C1 antisera. BBL's antisera seems to be non-specific. Most laboratories seem to be using the Difco product. Several participants mentioned non-specific reactions with *Citrobacter spp*., or other isolates that are biochemically similar to either *Citrobacter spp.* or *Salmonella spp.*, and cross-react with several serogroups. One participant thought that API strips could unequivocally separate them, but others disagreed. Roxie Maddux described a nifty "Sherry Greer" method to serotype *Salmonella spp*. using a disposable Kirby-Bauer (150 X 15mm) petri dish: The plate is scored with a wax pencil for up to 12 tests and is autoclaved for disposal.

Salmonella DT104. Ray Hines reported the isolation of a DT104 phage-type from pen-raised quail and a macaw from a pet shop. Anne Parkinson mentioned that her laboratory has isolated DT104 from bovine specimens. NVSL reportedly is performing phage typing on suspicious *Salmonella typhimurium* isolates. For more information on the culture and identification of Salmonella, please see *Miller-Mallinson (MM) Medium: An Improved Bacteriological Plate Media for the Isolation of Salmonella*, as well as related topics in the Avian Session notes, all in this issue of the *AVM Newsletter*.

***Bordetella spp*. in cats.** Many laboratories are isolating *Bordetella spp*. from the lungs, eye swabs and transtracheal washings of cats. Margaret Gehlhausen stated that her facilities are experiencing an increased number of *B. bronchiseptica* versus *B. avium* recovered from turkeys. Judy Clapier said that the same is true in North Carolina; about half of their *Bordetella spp.* isolates are *B. bronchiseptica* rather than *avium*.

***Haemophilus spp*. isolations.** Shri Singh reported that his laboratory had isolated a "*Haemophilus*-like" organism from the throat of a cat. Colonies of this organism exhibited a satellite growth pattern. A similar isolate has been recovered by facilities in Georgia. Judy Clapier reports encountering an isolate of *H. parasuis* that did not display satellite growth. To speciate unusual isolates, Beth Henricson uses the API-Zyme strip, which helps differentiate *Haemophilus* from other organisms. Some laboratories use chocolate agar only, while others use blood plates with a *Staphylococcus spp.* nurse streak. Session participants recommended both approaches, with Columbia agar-base blood plate suggested as an alternative. Linda Cox said that if a sample is suspect for *H. parasuis*, they use a *Staphylococcus* nurse streak on both the blood and chocolate agar. With this method, she reports recovering some isolates that grew as satellites on chocolate agar, but failed to grow on the blood plate.

RGP (Rapid Gram Positive) from Crystal (BBL). Theresa Love likes the anaerobic product, but is reserving judgment on RGP's performance on gram positive cocci. She reports that the RGP seems to work well for gram positive rods such as *Corynebacterium spp*. and *Rhodococcus spp*., yielding results in four hours. Shri Singh suggested that a heavier inoculum be used if *Streptococcus spp*. are suspected because they will not grow rapidly enough to identify in four hours. Tim Klinefelter said that his facilities did not like the RGP for anaerobes. Sharon Chitwood uses the RGP to backup conventional identification methods. The RGP was viewed more as a tool for equine and small animal isolates

***Pasteurella multocida* in equine joints.** *P. multocida* is recognized as a cause of lameness in adult horses. John Abell uses the CDC manual entitled *Identification of Unusual Pathogenic Gram-Negative Aerobic and Facultatively Anaerobic Bacteria, Second Edition* to identify *Pasteurella spp*. other than *P. multocida* from horses. This reference manual is also useful for unusual non-fermenters occasionally seen in all species. The manual is available from Williams & Wilkins at 800/787-5938. [*Identification of unusual pathogenic gram-negative aerobic and facultatively anaerobic bacteria*: US Dept. of Health and Human Services, Public Health Service, Centers for Disease Control; Atlanta, Ga., For sale by the Supt. of Docs., [US GPO, 1984] Description: iii, 383 p. ill. (some col.), 1 port. ; 28 cm.]

Marcia Browning also reported isolating a *Pasteurella*-like organism from a hippopotamus.

***Pseudomonas spp*. antimicrobial resistance.** Bacteria isolated from canine ears are displaying an increasing degree of antibiotic resistance. John Cole reported that an article on recommended

treatment for canine ears was printed in the AVM newsletter several years ago. The article recommended an acetic acid/EDTA wash to change the pH of the ear rather than use antibiotic therapy as curative. The same treatment is employed for *Malassezia pachydermatis* infections. It was reported that many *Pseudomonas spp*., as well as increasing numbers of *Enterococcus spp*. and *E. coli*, are becoming resistant to enrofloxacin (Baytril). NCCLS guidelines state that enrofloxacin is to be used in dogs and for bovine respiratory symptoms only. Sarafloxacin is approved for use in poultry. The new NCCLS document M31-A, entitled *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals* is expected to be released in April, 1999. Beth Henricson pointed out that susceptibility comparisons cannot be made between different generations of antibiotics because effective antimicrobial activity may not be the same from one generation to the next.

Dermatophyte Test Medium (DTM). Some labs have used a 14-day card-like plate test from Bio-Med [Intray-DM from Bio Med Diagnostic of San Jose, CA, ph: 408/451-0400] and have given it mixed reviews. Some felt that the card dried out too fast and that other organisms turned the card red. Connie Gates prefers Sabourauds and Mycosel media. Some labs are using Potato Dextrose Agar; according to Connie, if the growth of spores is a problem, Potato Dextrose will support sporulation in a couple of days. Most laboratories that have tried Trichophyton media would not recommend it, even though it was reportedly more species specific. For a critique of the Bio-Med dermatophyte plate test, please contact Connie Gates, Linda Cox, or Theresa Love.

***Corynebacterium spp*. in caprines.** Many laboratories frequently isolate *Corynebacterium pseudotuberculosis* from abscesses on goats with caseous lymphadenitis. Other facilities have recovered the organism from camelid lymph nodes. For more information, please see Dercksen DP., et al, Eradication programme for caseous lymphadenitis in goats in The Netherlands. Vet Rec. 1996 Mar 9. 138(10). P 237, Lindsay HJ. and Lloyd S., Diagnosis of caseous lymphadenitis in goats. Vet Rec. 1991 Jan 26. 128(4). P 86, and Holstad G., *Corynebacterium pseudotuberculosis* infection in goats. IX. The effect of vaccination against natural infection. Acta Vet Scand. 1989. 30(3). P 285-93.

***Francisella tularensis* in species other than leporines.** John Cole reported that there had been a major outbreak of *F. tularensis* in felines about ten years ago in Georgia. According to John Abell, the Maryland laboratory has also recovered *F. tularensis* from cats. Bonita Vera said that their laboratory has isolated *F. tularensis* from an otter. Identification is typically based on antiserum agglutination (Difco) and growth on cysteine blood agar. Remel of Lexena, KS, (ph: 800/255-6730) supplies cysteine heart agar via overnight shipment. For primary isolation, Mary Jean Bryant recommends CDC blood agar with cysteine and CO_2 incubation. For further information, please see Woods JP., et al, Tularemia in two cats. J Am Vet Med Assoc. 1998 Jan 1. 212(1). P 81-3, Waggie KS., et al, Diagnostic exercise: illness, cutaneous hemorrhage, and death in two squirrel monkeys (*Saimiri sciureus*). Lab Anim Sci. 1997 Dec. 47(6). P 647-9, Junhui Z., et al, Detection of *Francisella tularensis* by the polymerase chain reaction. J Med Microbiol. 1996 Dec. 45(6). P 477-82., and Morner T., The ecology of tularemia. Rev Sci Tech. 1992 Dec. 11(4). P 1123-30.

Campylobacter spp. To culture ***Campylobacter spp.***, a variety of transport media is in popular use. Some laboratories report using Clark's media for transport, while Anne Parkinson and Connie Gates use Weybridge media. Beth Henricson uses Cary-Blair or Amies media for transport. Anne Parkinson also said that if specimens for darkfield examination and culture are

not submitted in transport media, they must arrive at the lab within six hours of collection. Brenda Love of the Ohio Animal Disease Diagnostic Lab in Reynoldsburg is conducting a "mini-study" on the different types of transport media, comparing standard saline, Amies, Cary-Blair, and Weybridge media; results will be published in a future issue of the *AVM-HC newsletter*. Shri Singh has performed such an evaluation; his facility now uses DEM, a Brucella base medium with antibiotics.

Although most laboratories regularly perform testing on bull studs, its not always clear which governing agency sets the standards for such testing. In some export situations, the *Office International des Epizooties* (OIE) or USDA, APHIS would be the governing body. Domestically, test protocols approved by AAVLD or NVSL are acceptable. AAVLD was said to be drafting specific guidelines for the collection and transport of specimens for the culture of *Campylobacter spp.*

John Abell submitted an abstract that reports a potentially new species of *Campylobacter* isolated from waterfowl. The organism was recovered from the intestines at a culture temperature of 42^0C, but failed to grow at 25^0C. It was first recovered in low numbers from birds that had apparently died of *Pasteurella multocida* infection. Since its discovery, this organism has been found in other birds with increasing frequency. For more information on this new isolate, or for copies of the abstract, please contact John Abell.

ASM. The American Society for Microbiology is considering the creation of an Animal Health Microbiology – Division Z. For further information, please see the paragraph under **AVM News, Events, and Items of Interest**. Jim Gary had copies of a *Symposium Update on Gram-Positive Cocci: Taxonomy, Identification, and Clinical Significance,* from the 98th General Meeting of ASM in May. For additional copies, please contact Jim Gary.

Clostridium perfringens. John Cole reported that zoos are experiencing problems with *Cl. perfringens* in cheetahs being fed raw meat. No fluorescent antibody reagent has been successfully made to *Cl. perfringens* due to its cross-reactivity with other *Clostridium spp*. Mouse protection tests are no longer routinely performed by the labs represented at this session. Dr. Songer at the University of Arizona, Dr. Wu at Purdue University, Dr. Singh at Breathitt, and Dr. Hennings at South Dakota State University are all doing toxin typing with PCR methodology on *Cl. perfringens* isolates. Fees for this service vary between laboratories, so interested parties are advised to contact individual laboratories for their fee schedule.

Moraxella bovis* vs. *Branhamella ovis. *M. bovis* is extremely difficult to recover and differentiate from ***B. ovis***. To optimize isolation attempts for *M. bovis*, samples must be plated to blood agar as soon as possible from "wet-faced" calves. Pam McKenney reported that Grand Labs experiences a good rate of recovery from swabs shipped overnight, then planted on blood agar plates. Tim Klinefelter said that their laboratory is using an indirect fluorescent antibody test to identify *M. bovis*; interested parties are invited to contact him for a copy of the protocol. Shri Singh reported on the development of an FA reagent from a commercially available monoclonal antibody; the reagent is not working as well as expected although the antibody seems to be very specific. For differentiation, Connie Gates relies on a positive gelatin test for *M. bovis*, negative for *B. ovis*. Jim Gary mentioned using the three-phase litmus milk test, and Roxie Maddux relies on nitrate and casein agar reactions for identification. It was said that both *M. bovis* and *B. ovis*

can be nitrate positive; their utilization of casein agar is not necessarily a definitive differential criterion.

Mycoplasma isolation in finches. The National Wildlife Disease Laboratory in Madison, Wisconsin has a protocol for isolating *Mycoplasma spp*. from the eyes of finches. For a copy, please contact Beth Henricson. For further information on Mycoplasma culture, please see the article **Mycoplasma Culture: Starting from Scratch** in this issue. Also, please see Luttrell MP., et al, Natural *Mycoplasma gallisepticum* infection in a captive flock of house finches. J Wildl Dis. 1998. 34(2). P 289, Frasca S Jr., et al, Mycoplasmal conjunctivitis in a European starling. J Wildl Dis. 1997 Apr. 33(2). P 336-9, Fischer JR., et al, Mycoplasmal conjunctivitis in wild songbirds: the spread of a new contagious disease in a mobile host population. Emerg Infect Dis. 1997 Jan-Mar. 3(1). P 69-72, Ley DH., et al, *Mycoplasma gallisepticum* isolated from house finches (*Carpodacus mexicanus*) with conjunctivitis. Avian Dis. 1996 Apr-Jun. 40(2). P 480-3, Luttrell MP., et al, Field investigation of *Mycoplasma gallisepticum* infections in house finches (*Carpodacus mexicanus*) from Maryland and Georgia. Avian Dis. 1996 Apr-Jun. 40(2). P 335-41.

***Yersinia spp*. identification.** John Abell asked if anyone is experiencing problems identifying *Yersinia spp*. with the API 20E strip at different incubation temperatures. At 28^0C a certain *Yersinia sp.* is identified as *Yersinia intermedia*, while at 37^0C, its identification is *Yersinia enterocolitica*. The API 20E kit instructions state that 28^0C is the accepted temperature, while representatives of API told John that 35^0C is the proper incubation temperature. Beth Henricson pointed out that some enzymatic responses by *Yersinia spp*. are temperature dependent, i.e., pathogenicity is not expressed at 28^0C, but is at 37^0C. This property directly relates to nature's reservoir for *Y. pestis*, the flea. The flea maintains a body temperature of 28^0C, but exhibits no illness. However a potential host, which maintains a body temperature of 37^0C or higher, develops clinical disease after being bitten by the infected flea.

***E. coli* toxin detection.** Pathogenicity of *E. coli* is based on toxin production. Tim Klinefelter said that Harley Moon of ISU is using a DNA probe to detect toxins. Suzanne Taber says that her laboratory is toxin-testing for a fee of $27.50 per sample. Shri Singh reported that Breathitt is also performing a PCR multiplex for toxin typing, but separately tests for attaching factors. Although Ray Hines said that his laboratory is using the FDA protocol to recover and identify O157:H7, most veterinary laboratories do not performing tests for the detection of O157:H7 because of the legal implications of such testing. The USDA is in the process of creating guidelines for the detection of *E. coli* O157:H7. Theresa Love pointed out that there is no legal protection for diagnostic laboratories, unlike meat inspection agencies. The group consensus was that animal disease testing and food quality testing are unique microbiological operations; each use distinctly different and specialized protocols, and should be conducted in separate facilities with proper equipment and trained personnel.

Brian Sunkel pointed out that Biolog makes Rainbow Agar, which is used to screen potentially pathogenic *E. coli* isolates for serotyping. He mentioned that serotypes O111 and O26 are also toxinogenic and are recovered on Rainbow Agar.

A potential challenge to any type of standardized testing is the variety of acceptable protocols in use. For the recovery of *E. coli*, several protocols have been drafted by various authorities,

including the Association of Official Analytical Chemists (AOAC), the FDA's Bacteriological Analytical Manual (BAM), and the USDA.

PCR and DNA Probes for Johne's Disease. Many of the represented laboratories using the IDEXX probe are dissatisfied with the kit due to its lack of sensitivity. Shri Singh reported that their laboratory is using an "in-house" probe with 83% sensitivity, which is not as good as culturing, but better that the IDEXX probe. Ching Ching Wu said that their laboratory cultures for four to five weeks, then performs PCR on the growth taken from the agar. She reported a 90% sensitivity with this method. John Cole mentioned that his laboratory plants cultures, incubate them for a few weeks, then performing PCR on fluid from the agar slants. This seems to increase the numbers of organisms available for detection.

Participants from Breathitt reported an unusual case involving a seven-month-old calf with clinical signs of Johne's Disease. The calf was both serologically and DNA probe positive for *Mycobacterium paratuberculosis*. Cultures were still in progress at the time of this session. It is uncommon for animals under 18 months to contract Johne's Disease.

A question was raised on what medium seemed to work the best for the isolation of *M. paratuberculosis*. Anne Parkinson reported that their laboratory ran a side-by-side comparison of their prepared "in-house" medium with the commercially prepared media from Remel. Although the Remel media did support growth with adequate numbers of colonies, their "in-house" medium was far superior to Remel's preparation, probably because the "in-house" medium had been more recently prepared, and possibly to the source of eggs used in the "in-house" preparation. Dr. Bob Whitlock of the University of Pennsylvania has protocols for media preparation and culture of *M. paratuberculosis*. Connie Gates mentioned that BBL is developing a Herrold's media, available for purchase through BBL distributors.

Lyme's Disease. Lyme's Disease is mostly seen in the eastern US seaboard and midwest where there are populations of white tailed deer. Ching Ching Wu reported that a Fort Dodge Laboratories representative had said one or two cases have been seen in Indiana, but no species had been named. Suzanne Taber occasionally finds a positive equine sera. John Abell stated that an IFA serology testing format is being used in Maryland; reactions are being confirmed with a Western blot technique. Most of their positives are coming from equids pastured in the Chesapeake Bay area.

***Mycobacterium spp.* in fish.** A new species of *Mycobacterium* has been described in striped bass according to John Abell. The original isolation was made with tissue culture, but subcultures of the organism grow on blood agar. Most rapid-growing *Mycobacterium spp*. will grow on blood agar, but other *Mycobacterium spp.* require Middlebrook H7 or Lowenstein–Jensen media to support growth. Marcia Browning and John Cole reported that their laboratories are using Dubos Broth to cultivate *Mycobacterium spp*. Marcia pointed out that *Mycobacterium spp*. sometimes are difficult to recover from birds; organisms are found in acid fast-stained smears, but attempts fail to culture the organism.

A common fish pathogen, *M. marinum*, is being recognized as a zoonotic agent associated with "fish tank" granuloma, septic arthritis, and superficial cutaneous lesions on the forearms of humans; such lesions often resist conventional antibiotic treatment. *M. marinum* infection is typically diagnosed by biopsy or culture on blood agar. For further information, please see Ryan JM. and Bryant GD., Fish tank granuloma--a frequently misdiagnosed infection of the upper

limb. J Accid Emerg Med. 1997 Nov. 14(6). P 398-400; Knibb W., et al, Detection and identification of a pathogenic marine Mycobacterium from the European seabass *Dicentrarchus labrax* using polymerase chain reaction and direct sequencing of 16S rDNA sequences. Mol Mar Biol Biotechnol. 1993 Aug. 2(4). P 225-32; and Hedrick RP, et al. Mycobacteriosis in cultured striped bass from California. J Wildl Dis. 1987 Jul; 23(3):391-5.

Leptospira spp. John Cole reported that *L. interrogans* serovar *grippotyphosa* is a serious problem in dogs. His laboratory has found the organism in every kennel tested. The *grippotyphosa* serovar can cause acute or chronic renal failure in dogs. He attributes the spread of this serovar to the movement of dogs all over Europe and the US eastern seaboard. It has been seen in foxhounds from England. Marcia Browning said their laboratory has detected the organism in Rottweilers imported from Germany. Ching Ching Wu said that their laboratory found high titers in two dogs for *L. interrogans* serovars *pomona*, *grippotyphosa,* and *bratislava*. Coincidentally, these dogs were located on a farm with pigs, the usual hosts for the *bratislava* serovar. Most laboratories using the FA technique were unhappy with results due to the large amounts of artifacts present. Shri Singh said that his laboratory is using a DNA probe to detect Leptospirosis. The test cannot speciate, but is able to distinguish pathogenic and non-pathogenic species; pathogenic leptospires are then speciated with the familiar MAT test.

QA/QC and SOP's. An AAVLD workgroup from the subcommittee in Bacteriology, Mycoplasmology and Mycoplasmology (chaired by CC Wu) has generated a general guideline document on QA/QC. Although it is not in print or available from the AAVLD, it has been forwarded through the Bacteriology steering committee to the Executive Board for possible policy development. [A draft outline of this report was published in the *AVM Heartland Chapter 1997-1998 Newsletter*.] Preliminary thought was that compliance ultimately may require too many standards for most participating laboratories to easily support. The effort requires a financial commitment and dedication of human resources that most veterinary labs may not be able to afford. However, consensus was that all laboratories need some form of SOP's on record.

According to Beth Henricson, another AAVLD subcommittee is compiling work group surveys on testing protocols and other elements of an SOP manual. It is hoped that this endeavor will provide a uniform body of information and protocols for use by veterinary diagnostic laboratories. The new QA/QC effort, introduced to the AAVLD and managed by Beth, includes the solicitation of input from both AVM and AAVLD members and labs. This effort evolved into the survey to discern the most favorable uniform format for test protocols. It is intended to generate a compendium of standardized protocols for AAVLD accredited labs. Beth reports initial resistance to having input from the bench level scientists on these protocols. She believes that bench level input is essential for efficiency and compliance, to assure that guidelines are practical, that they can be accomplished on a daily basis, and that they fit in with existing work flow. In this way, a QA program actually helps labs to be more cost effective, not less.

Beth believes that movement toward adequate QA/QC should be voluntary. Labs should be encouraged to move toward more complete compliance rather than be punished for non-compliance. However, QA/QC does require a commitment to higher standards.

Beth reports that it is the intent to post the results of this survey on the AAVLD web-site (WWW.AAVLD.ORG). According to Beth, preliminary feedback from this survey has indicated that there has been a great increase in the number of labs that have formulated their own SOP's;

some labs have borrowed SOP's from other labs and adapted them to their own situation. The establishment of one overall SOP document format for so many different laboratory situations is expected to be a difficult undertaking.

For further information, please see the column **Quality Corner** by Belinda Lawler-Goff in the January and April 1999 issues of the *AAVLD Newsletter*, and **Activities in quality assurance (QA) and quality control (QC) in animal testing** under Virology session topic notes in this issue of the *AVM newsletter*, or contact Beth Henricson or Ching Ching Wu.

Growth of Fastidious Organisms. Some laboratories using the Sensititre System are experiencing difficulties growing fastidious organisms for susceptibilities. Jennifer Lorbach and Vesna Eller reported that Sensititre is now offering Mueller-Hinton Broth (MHB) with lysed horse blood for the growth of fastidious organisms. This broth has a six-month shelf life. Tim Klinefelter reported that their laboratory was obtaining good growth by most isolates in MHB with lysed horse blood. Some of the more stubborn organisms still required the addition of 100 ul of both bovine serum and NAD to the broth. Sensititre is developing a new Fastidious Test Media (FTM) that is being recommended by NCCLS for the propagation of fastidious organisms in susceptibility testing.

Discussion of fastidious organisms continued with comments on NCCLS standards. John Cole reported that at the NCCLS meeting, Dr. Clyde Thornsberry stated that susceptibilities are invalid if incubated longer than 24-36 hours. This is based on the diffusion rate of antibiotics into agar versus the organism growth rate. Antibiotics diffuse into the agar faster than fastidious organisms can grow, resulting in erroneous zone patterns. Due to insufficient growth for zone interpretation, the NCCLS does not recommend susceptibility testing on the slow-growing *Arcanobacterium pyogenes*. Participants from Indiana reported that FTM supports the growth of *Haemophilus parasuis* in only about 60% of attempts; other laboratories have had less success. *Actinobacillus pleuropneumoniae* seems to grow well in FTM. Dr. Jean Cooper of the FDA was mentioned to be a good source on information on antibiotic susceptibility testing of fastidious organisms; she serves on the NCCLS committee.

Performance of QA/QC on antibiotic susceptibilities varies between laboratories. Tim Klinefelter says their laboratory performs QA/QC every two weeks, while Ching Ching Wu and Beth Henricson reported that their laboratories test every week.

Actinobacillus pleuropneumoniae – biotype II. Linda Cox and Tim Klinefelter reported that their laboratories are recovering isolates of this organism, which is non-NAD dependent and difficult to separate from *Actinobacillus lignieresii*. The clinical appearance of biotype II infection is similar to that of typical *A. pleuropneumoniae* (APP). Apparently not even DNA testing can separate the two. Dr. Brad Fenwick at Kansas State performs an APP-specific PCR, followed by a RAPD (random amplification of polymorphic DNA) to determine the serotype of suspicious isolates. For information on recent developments in the recognition of *A. pleuropneumoniae* biotype and serotypes, please see Chevallier B., et al, Chromosome sizes and phylogenetic relationships between serotypes of *Actinobacillus pleuropneumoniae*. FEMS Microbiol Lett. 1998 Mar 15. 160(2). P 209-16, Gagne A., et al, Development of an immunomagnetic method for selective isolation of *Actinobacillus pleuropneumoniae* serotype 1 from tonsils. J Clin Microbiol. 1998 Jan. 36(1). P 251-4, and Haesebrouck F., et al,

Actinobacillus pleuropneumoniae infections in pigs: the role of virulence factors in pathogenesis and protection. Vet Microbiol. 1997 Nov. 58(2-4). P 239-49.

Brucella canis. Marcia Browning reported that Florida is using the card test for *Br. canis* serological screening. Evidently, antigen for the tube agglutination test is hard to come by, is difficult to prepare, and gives inconsistent results. Most laboratories performing tube agglutination are using antigen available from NVSL. John Cole says that Georgia makes their own tube antigen. The same antigen cannot be used for both the tube test and the slide agglutination test. Confirmation of *Br. canis* cannot be made solely with the slide agglutination test; other methods, such as tube agglutination, AGID, IFA, or culture, are often used to validate card test positive reactors. Beth Henricson mentioned that VMRD has *Br. canis* antigen substrate slides to run IFA, and that her laboratory successfully uses this format. NVSL is said to be developing an ELISA test for *Br. canis* antibody, but no one present had any information on the status of this project.

The RB51 vaccine for *Br. abortus* reportedly produces fewer problems from vaccine reactors with the Bang's test (card agglutination for *Br. abortus* antibody) than the old strain 19 vaccine, however session participants had insufficient information to be able to make a statement on its effectiveness.

For further information on Br. canis testing, please see **Canine Brucella Testing** in the *AVM Spring / Summer 1997 Newsletter*, **Br. canis IFA Serology: Sensitivity and Specificity Relative to Other Techniques** and **Culture of Obligately Intracellular Microorganisms and Brucella canis** in the *AVM Fall / Winter 1997 Newsletter*, and ***Brucella canis*** **ELISA: Availability and Experience** in this issue of the *AVM Newsletter*.

Listeriosis in Caprines. John Cole reported that several Kikos, Boers, and goat crossbreeds have died of listeriosis in Georgia, but the ultimate source of infection had not been found. Clinical manifestations during the outbreak included reproductive and neurologic signs. In this instance, a carrier state is suspected. An autogenous bacterin was employed to curtail the outbreak. Biochemically, the isolate appeared atypical, possibly of a new type. The isolate was forwarded to Cornell and was speciated as an atypical *Listeria monocytogenes.*

E-mail Humor, "Vet's Second Opinion", original author unknown

A man runs into the vet's office carrying his dog, screaming for help. The vet rushes him back to an examination room and has him put his dog down on the examination table. The vet examines the still, limp body and after a few moments tells the man that his dog, regrettably, is dead. The man, clearly agitated and not willing to accept this, demands a second opinion.

The vet goes into the back room and comes out with a cat and puts the cat down next to the dog's body. The cat sniffs the body, walks from head to tail poking and sniffing the dog's body and finally looks at the vet and meows. The vet looks at the man and says, "I'm sorry, but the cat thinks that your dog is dead too."

The man is still unwilling to accept that his dog is dead. The vet brings in a black labrador. The lab sniffs the body, walks from head to tail, and finally looks at the vet and barks. The vet looks at the man and says, "I'm sorry, but the lab thinks your dog is dead too."

The man, finally resigned to the diagnosis, thanks the vet and asks how much he owes.

The vet answers, "$650."

"$650 to tell me my dog is dead?" exclaimed the man....

"Well," the vet replies, "I would only have charged you $50 for my initial diagnosis. The additional $600 was for the cat scan and lab tests."

The Virology Session

Moderated by **Gary Gustasfin**, National Veterinary Services Laboratory, Ames, Iowa, and **Mike Parsley**, Arkansas Livestock and Poultry, Little Rock, Arkansas.

Reported by **Rob Poston**, Louisiana Veterinary Medical Diagnostic Laboratory, Louisiana State University School of Veterinary Medicine, Baton Rouge, Louisiana, and **Mary Woodruff**, Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, Indiana.

Attended by:

Mike Parsley	Arkansas Livestock and Poultry, Little Rock	501/225-3630
Gary Gustafson	National Veterinary Services Lab, Ames, IA	515/239-8551
Rod Hernandez	Centaur, Inc., Overland Park, KS	800/236-6180
Kurt Peterson	Centaur, Inc., Overland Park, KS	800/236-6180
Tim Doeden	Bayer Animal Health, Worthington, MN	507/372-6172
A.C. Ellis	Auburn, AL	334/844-4987
Charles Burcaw	Maryland Dept Agr, Frederick, MD	301/663-9528
Lisa Lemieux	IDEXX, Inc., Westbrook, ME	800/551-0998
Kent Whitaker	MSU Breathitt Vet Ctr, Hopkinsville, KY	502/886-3959
Laura Clark	MSU Breathitt Vet Ctr, Hopkinsville, KY	502/886-3959
Sandra Petty	Tennessee Dept Agr, Nashville, TN	615/837-5221
Lisa Moss	Tennessee Dept Agr, Nashville, TN	615/837-5221
J. Read Hodges	Tennessee Dept Agr, Nashville, TN	615/837-5221
Selvin Harrell	Clemson Animal Diag Lab, Columbia, SC	803/788-2260
Bob Crandall	Viral Antigens, Inc., College Station, TX	409/690-3787
Mark Metrokotsas	Centaur, Inc., Overland Park, KS	800/236-6180
Tom Hutchings	Animal Disease Lab, Centralia, IL	618/532-6701
Howard K. Jones	Synbiotics Corp., Kansas City, MO	800/841-1875
Beverly A. Branch	Quest Diagnostics, Nashville, TN	615/255-5786
Peter M. Boyt	Boyt Veterinary Lab, Neosho, MO	417/451-6369
Ron Sanders	Synbiotics Corp., Kansas City, MO	619/451-3771
Tom Kellner	VMRD Inc, Pullman, WA	509/334-5815
Melissa Kennedy	U of Tenn College of Vet Med, Knoxville, TN	523/974-5643
Sonia Doss	U of Tenn College of Vet Med, Knoxville, TN	523/974-5643
Vina Diderrich	U of Tenn College of Vet Med, Knoxville, TN	523/974-5643
Pam McKenney	Grand Labs, Inc., Larchwood, IA	800/454-3424
Mary Woodruff	ADDL, Purdue Univ, West Lafayette, IN	765/494-7480
Kay Rathman	Wisconsin Ani Health Lab, Madison, WI	608/266-2466
Marge Muenzenberger	Kansas State Univ Diag Med/Path, Manhattan, KS	785/532-5650

Steve Wessman	National Vet Services Lab, Ames, IA	515/239-8656
Misty S. Moore	American BioResearch, Sevierville, TN	423/908-8826
Michele D. Hall	Georgia Vet Diag & Invest. Lab, Tifton, GA	912/836-3340
Kristie Goins	Georgia Vet Diag & Invest. Lab, Tifton, GA	912/836-3340
Donna Lester	Iowa State Univ., Ames, IA	515/294-1950
Margie Richardson	American BioResearch, Sevierville, TN	423/908-8826
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Rosangela Navarro	Maryland Dept Agr, College Park, MD	301/483-8456
Kaye Tipton	Tennessee Dept Ag, Nashville, TN	615/837-5221
Lyn Perry	Purina Mills, Inc.	314/768-4400
John Black	American BioResearch, Sevierville, TN	423/908-8826
Bill Cornell	MSU BVC, Hopkinsville, KY	502/886-3959
Al Pursell	retired, Tifton, GA	912/382-7971
Rob Poston	Louisiana Vet Med Diag Lab, Baton Rouge, LA	225/346-3193
Tom Chang	CE Kord Ani Disease Lab, Nashville, TN	615/837-5257
Ray Hines	Clemson Ani Diag Lab, Columbia, SC	803/788-2260

Respiratory disease by Bovine/Porcine Coronaviruses. As infectious agents of animals, coronaviruses are known principally by their localized enteric infections, whether asyptomatic, subclinical, or acute and severe, as occasionally seen in neonates. In humans, coronaviruses are viewed primarily as upper respiratory tract pathogens. Coronaviruses now are recognized as a potentially significant contributor to bovine respiratory disease complex (BRDC), also known as "shipping fever" syndrome. BRDC is understood to be initiated by stressful conditions, which promote viral respiratory infections, then together predispose cattle to secondary pneumonic infections by other agents as *Pasteurella spp*.

As other “shipping fever” viruses, Bovine Respiratory Coronavirus (BRCorV) is sought in cells of turbinate or tracheal impressions or scrapings, and in lung sections or impressions by FA using the available bovine coronavirus conjugate. Respiratory coronaviruses have been successfully cultured from clinical material with a “G-clone” of the human rectal tumor (HRT-18g) cell line. Cells of this polarized cell line contain receptors for coronavirus attachment on their apical aspect. Viral attachment is thought to be related to the acetylesterase function on one of the peplomers of coronavirus.

In the laboratory diagnosis of enteric infections, coronaviruses are found with EM: Particles appear dense and cavitated, with a characteristic halo or “corona”. Inexperienced observers may have difficulty recognizing coronavirus particles in EM preparations. A few members attending this session mentioned verification attempts with immune EM (IEM). Coronavirus infection is confirmed by FA on exfoliated infected cells: A fecal sample is smeared on a slide, acetone fixed, then stained with coronavirus conjugate. A direct ELISA test kit to detect coronavirus antigen in bovine fecal samples was said to be available in Europe from Cypress Diagnostics of Leuven, Belgium (Fax:++32 16 44 77 62).

In the diagnosis of Hemagglutinating Encephalomyelitis Virus (HEV, a coronavirus of pigs) the swine testicle (ST) cell line was said to be susceptible to infection by virus in clinical material. HEV also infects in PK-15 cells, but is reportedly non-cytopathic in this cell line.

For information on the significance of coronaviruses as respiratory pathogens in cattle, please see Storz, J., et al., Coronavirus isolation from nasal swab samples in cattle with signs of respiratory tract disease after shipping. JAVMA 1996. 208(9). P 1452, and Kapil, S and Basaraba, RJ, Infectious bovine rhinotracheitis, parainfluenza-3, and respiratory coronavirus. Vet Clin North Am Food Anim Pract 1997. Nov 13(3). P 455-469. For a critique on coronavirus diagnostic techniques, please see Dar, AM, et al, Comparison of immunohistochemistry, electron microscopy, and direct fluorescent antibody test for the detection of bovine coronavirus. J Vet Diagn Invest 1998. Apr 10(2). P 152-157. For further technical information on the lab diagnosis of respiratory coronavirus infections, please contact Rob Poston.

Bovine Leukemia Virus (BLV). For domestic purposes, any technology may be used to detect BLV antibody, but for export purposes, testing must be done with a USDA-approved regulatory method. The IFA works well in detecting BLV antibody, but because it is not officially recognized, it cannot be used for export testing.

Quality assurance (QA) is an important aspect of testing for export purposes. With QA, the animal health authorities of the exporting country are able to assure the international market that the laboratories it authorizes to perform regulatory testing can do so reliably. The USDA accomplishes this by certifying the manufacture of uniform testing reagents and methods, and by certifying test performance in recognized facilities with its check test program.

Animal testing regulations of the importing country are another important aspect of regulatory testing. Importing countries will specify the technology, sometimes in detail, that must be used in the testing of animals entering their jurisdiction. Typically, it is the responsibility of the exporter to request lab testing of the appropriate type of technology, depending on the regulations of the destination country. The USDA plays a role in encouraging the use of common technology most readily accepted by international authorities.

Synbiotics BLV AGID kit is now off the market. How do you deal with turn-around time with BLV ELISA? The BLV ELISA test kit currently available has a three-hour run time to screen samples, then requires another run of three hours to verify positive reactors. When technical personnel have additional duties, a complete same-day run and report of BLV testing is difficult. In instances when an assay run cannot be completed in one work day, an overnight refrigerated incubation step was suggested. For more information on this approach, please contact Kay Rathman.

During the recent BLV serology check test, about half of the participating labs used the AGID test method, which will soon no longer be commercially available. These labs will be asked to resubmit check tests so that all participating labs are approved to use the ELISA test kit. While it remains available, the BLV AGID test still is acceptable for export. Currently, no in-house generated technology is sanctioned for use in regulatory and export BLV testing. For more information on regulatory BLV testing and its federal quality assurance program, please contact Gary Gustafson. [Synbiotics reports that additional lots of the standard BLV AGID test kit recently have become available, and will be distributed while supplies last. For information, please contact Howard Jones.]

Both Synbiotics and VMRD are developing new BLV ELISA test kits. Synbiotics currently has reagents available, but they are not yet approved for export use. VMRD has trials underway for

their new BLV ELISA test format. For more information about these and other future products, please contact Howard Jones of Synbiotics and Tom Kellner of VMRD.

The ELISA test is expected to be recognized as the standard technology for regulatory use, and is thought to be more sensitive than AGID in detecting BLV antibody. The new ELISA test kits under development will permit testing of a few samples at a time and will not require the use of a plate reader.

BLV testing export requirements usually depend on the recipient country, which specifies the technology, and sometimes the manufacturer of the technology.

According to Lisa Lemieux, IDEXX has changed the format of its BLV antibody ELISA test kit; it now contains both antigen and "normal host" coated strip wells to accommodate small volume verification testing. By testing with the old format IDEXX test kit, Mike Parsley and Kay Rathman have found a 60-65% seropositive incidence among Southern cattle and 30-35% among northern cattle.

***Brucella canis* ELISA: Availability and Experience.** The current laboratory diagnostic scheme, practiced by most labs represented at this session, is to screen serum samples with the Rapid Slide Agglutination Test (RSAT) kit and verify with IFA technology (using commercially prepared slides), if AGID technology is unavailable. Wayne Roberts of Athens, GA was said to be able to routinely grow *Br. canis* and produce IFA substrate slides. *Br. canis* slides made by Lee Fuller 714/525-7660, marketed through VMRD, were said to perform well. A few attendees reported using tube agglutination testing to verify reactors.

For those considering alternative technology, NVSL was said to be developing an ELISA for *Br. canis* serology. The Diagnostic Lab at Cornell University (607/253-3900) makes AGID reagents and performs AGID testing for a closed clientele. The AGID reagents were said to be unavailable to the public, but a protocol is provided to those interested in making their own reagents. For further information, please see **Canine Brucella Testing** in the Spring/Summer 1997 issue of the *AVM Newsletter*, **Br. canis IFA Serology: Sensitivity and Specificity Relative to Other Techniques** and **Culture of Obligately Intracellular Microorganisms and Brucella canis** in the Fall/Winter 1997 issue of the *AVM Newsletter*, and related topics under the Bacterilogy Session notes in this issue of the *AVM Newsletter*.

Bovine Herpesviruses type 4 (BHV-4) and type 5 (BHV-5). BHV-4 has been isolated from respiratory specimens, serum (as a contaminant in fetal bovine serum) and aborted tissues. Often characterized as "slow IBR", BHV-4 isolates usually grow well in cell culture. An occasional serum sample is found positive for BHV-4 antibody, the significance of which is unknown. The putative agent causing feline uroliths, previously identified as feline herpesvirus type 2 (FHV-2), has since been identified as the bovine herpesvirus DN599, a strain of BHV-4 which occasionally contaminates fetal calf serum and cell culture. Please see Egyed, L. Replication of BHV-4 in human cells in vitro. 1998. J Clin Microb 36(7). P 2109-2111, and Kruger, JM, et al, Genetic and serologic analysis of feline cell-associated herpesvirus-induced infection of the urinary tract in conventionally reared cats. 1989. AJVR 50(12). P 2023-7.

BHV-5, also known as bovine encephalitis herpesvirus or neurotropic IBR virus, is currently classified as an alpha herpesvirus, similar to IBR. Differentiation reportedly is done in facilities at Colorado State University, Fort Collins. A sixth bovine herpesvirus may have been discovered

and associated to disease in cattle; if officially recognized, this new herpesvirus may be designated as BHV-6, now that the goat herpesvirus, once BHV-6, has been designated as caprine herpesvirus type one. This putative new bovine herpesvirus was said to be among the lymphotrophic herpesviruses.

According to Field's *Virology*, BHV-4 (Movar strain) is an unclassified member of the *Gammaherpesvirinae* subfamily. BHV-5 is in the *Varicellovirus* genus of the subfamily *Alphaherpesvirinae*. What was once BHV-3 or Malignant Catarrhal fever (MCF) virus of cattle is now referred to alcelaphine (wildebeest) herpesvirus type 1 (AHV-1). For further taxonometric information on bovine herpesviruses, please see Murphy, FA, Virus Taxonomy, in *Virology*, 3rd ed., Fields, BN, et al, eds., Lippincott-Raven, Philadelphia, 1996.

Source for Dynex (Dynatec) Replacement Parts. Serial dilutor handles, transfer plate parts, and other parts for microtiter devices are reportedly difficult to obtain, or no longer available. Com-Disco of Canton, MA (617/575-0777, 800/545-0540) was said to vend Dynatech plate reader parts. Including microtiter equipment, Com-Disco buys and sells other types of used technical equipment. Disposable microtiter droppers were said to be available through VWR. [According to Laura Clark, the Dynex Co. markets its disposable droppers through the Fisher Co.: Disposable Pipette, .025 ml - Fisher # 14245225, Disposable Pipette, .050 ml - Fisher # 14245226. DYNEX Technologies' Corporate Headquarters Contact telephone: 800/336-4543, in VA 703/631-7800, Fax: 703/631-7816, Postal address: 14340 Sullyfield Circle, Chantilly, VA, USA 20151-1683, E-mail: webmaster@dynextechnologies.com]

Availability of conjugates to detect avian viruses. No one present at the session had knowledge of a commercial source of direct fluorescent conjugates to detect avian viruses. KPL of Gaithersburg, Maryland and SPAFAS of Norwich, Connecticut were given as a possible sources. Fluorescent detection of avian viruses in infected tissues or inoculated host systems is often done with indirect FA using anti-viral monoclonal antibodies, for which there are several sources. KPL offers goat-origin conjugates reacting to chicken, duck, parrot, and turkey Ig's. NVSL has available direct fluorescent conjugates against Avian Reovirus, Duck Viral Enteritis Virus (DVE), Infectious Laryngotracheitis Virus (ILTV), and Psittacine Herpesvirus.

General Biohazard Precautions for Employees of Veterinary Laboratories. In those facilities that routinely perform Rabies testing, employees generally are vaccinated for Rabiesvirus, and possibly the Equine Encephalitis viruses. To facilitate recognition of possible lab-derived illnesses, some labs practice "serum banking", which involves the collection and storage of a small aliquot of serum from each employee; the serum provides a baseline titer for serodiagnosis if a lab employee's illness is suspected to be associated with workplace activity. It remains the policy of some veterinary labs to avoid handling any human serum. Many labs do not accept fresh primate samples, which represent a potential source for zoonotic infections in veterinary testing facilities. Due to optimal climatic conditions in certain regions in the country, the potential for the occurrence of anthrax was reported to be greater this year. In these areas, vaccination of lab employees for *B. anthrasis* was considered justifiable. Please see related topics in the Avian and Bacteriology Session notes in this issue of the *AVM newsletter*.

New susceptible cell lines for viral isolation. The isolation of certain respiratory strains of coronavirus was said to be possible with a polarized, cloned cell line derived from human rectal tumor (HRT-18g or "g-clone"). Porcine Respiratory and Reproductive Syndrome (PRRS) virus is

recovered from the blood or lung of infected pigs with Porcine Alveolar Macrophage (PAM) cell culture or with the MA-104 cell line [or better, the MARC-145 cell clone. Please see Kim, HS, et al, Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homogeneous subpopulation of MA-104 cell line. Arch Virol 1993. 133. P 477-483.] No other common pig cell lines have been reported to be useful in the primary isolation of PRRS virus.

Animal disease testing labs in Wisconsin and Kansas were reported to have routine access to primary kidney cell culture for routine isolation of viruses. For information on such, please contact Kay Rathman or Marge Muenzenberger. With proper technique, infectious Chlamydia is recoverable with Vero, Buffalo Green Monkey, or McCoy cell lines (the latter of which may be out-of-stock at the ATCC at the time of this session.)

PRRS: Significance of titer, serology vs. FA, technology for low volume testing. Porcine Respiratory and Reproductive Syndrome (PRRS) virus is isolated from serum despite the presence of antibody; the first antibody responding to infection is typically non-neutralizing. Laboratory diagnosis of PRRS virus infection is done by isolating virus from serum and tissues, although PRRS virus is not easily recovered from fetal tissues. In isolation attempts, viral cytopathology develops two days after inoculation of MA-104 cells. PAM cells, also useful to isolate PRRS virus, are short-lived and cannot be split; typically, they are started from cryogenic storage for each use. PRRS viral infection in culture (or possibly in tissue) is substantiated by direct FA, the reagent for which is available for $300/ml from Rural Technologies, Inc., 414 Seventh St., Brookings, SD 57006, ph: 605/692-6953 fax: 605/692-7963.

PRRS viral antibody is detected or titrated with Neutralization, IFA, and ELISA technology, which cannot easily distinguish vaccine response from that of actual infection, although an OD reading >2 probably indicates infection. For information on the interpretation of PRRS ELISA serology, please see **Use and Interpretation of PRRS Viral Antibody ELISA** in the *1996 Fall/Winter AVM Newsletter*, or contact Gene Erickson of the Rollins Animal Disease Diagnostic Laboratory in Raleigh, North Carolina. Also, please see Zimmerman, J, et al, *1998 PRRS Compendium*, published by the National Pork Producers Council, fax: 515/223-2646, e-mail pork@nppc.org. For further information on PRRS laboratory diagnosis, please contact Kent Whitaker or Tim Doeden.

Infectious bovine rhinotrachietis (IBR), Bovine viral diarrhea (BVD), and Parainfluenza type 3 (PI-3) viruses: ELISA vs. SN? IDEXX offers an ELISA kit to detect IBR viral antibody in serum, plasma, or milk (IDEXX HerdChek® IBR antibody ELISA). An ELISA kit to detect antibodies to BVD virus is available in Europe [such as from Cypress Diagnostics, Leuven, Belgium (Fax:++32 16 44 77 62)]. Syracuse Bioanalytical, Inc. of Ithaca, New York (607/226-0609) markets an ELISA kit in the US for BVD viral antigen detection in serum.

Domestically, typical in-house technology to assay for IBR and BVD antibodies is based on neutralization or IFA, both of which are used in screening and endpoint determinations. Many labs do not receive paired samples to demonstrate seroconversion, which has the capability to recognize acute infection in a single affected animal. Identification of infectious abortion in a herd is made from a representative screening of animals in an affected herd; the demonstration of seroconversion is not necessary. An initial, serum-screening dilution of 1:50 is recommended in IFA testing.

Antibody measured by IFA does not necessarily parallel the serum neutralization response. IFA titers have been seen to rise faster than neutralization antibody. Because the IFA antibody response is closer in time to the actual infection than the neutralization response, IFA may be better than neutralization in diagnostic applications. For further information on IFA versus neutralization in determining antibody titers against IBR or BVD viruses, please contact John Black. For information on IBR or BVD antibody ELISA test kits, please contact Lisa Lemieux.

Circovirus. A circovirus of pigs has been tentatively associated with Post-weaning Multisystemic Wasting Syndrome (PMWS) in swine. PMWS was first reported in western Canada and California, but now also has been identified elsewhere in the USA, and in Spain and France. Pigs 6-14 week of age exhibit weight loss and breathing difficulty. Usually 5-10% of post-weaning are affected, although up to 50% have been reported in some PMWS herds. Most affected pigs display enlarged lymph nodes, especially noticeable in the inguinal area. Some pigs develop diarrhea and jaundice due to severe liver disease. Consistent post-mortem findings include diffuse interstitial pneumonia and enlarged lymph nodes. Microscopically, the most common lesions are granulomatous inflammation with multinucleated giant cells and inclusions in lymphoid tissues. PMWS usually is diagnosed by histopathological lesions, based on the identification of intranuclear inclusion bodies found in macrophages.

Conjugates and/or monoclonals reportedly are now available to detect porcine circovirus (PCV) in clinical specimens. Certain Canadian labs were said to be currently performing diagnostics for PCV infection using monoclonals and PCR [The Virology Laboratory, Veterinary Service Branch, Manitoba Agriculture]. Anti-circovirus serum (rabbit origin) reportedly is available from Prem Paul or Igor Morosov of Iowa State University (ph: 515/294-8385, e-mail: igor@iastate.edu) The Georgia animal testing laboratory was also said to have probes to detect circovirus. Because so little virus is present in clinical samples and because of its small size, circovirus is difficult to detect directly in clinical material with most current conventional techniques. Most clones of the PK-15 cell line are endogenously infected with PCV, thus can be used as antigen substrate in an IFA format to detect PCV antibody. NVSL was said to have available PCV-free PK-15 cell culture; these cells and the ST cell line are reportedly susceptible to the growth of field PCV. Cell culture pretreatment, as with dextran or glucosamine, seems to be effective to increase viral propagation.

Perhaps because of the paucity of genetic information, PCV is heavily dependent on the cell's replicative metabolism. Replication of PCV seems to occur only in the mitotic cell phase. In PCV infected cells, the first antigen appears in the nucleus; as infection progresses within the cell, antigen appears in both the cytoplasm and nucleus. In older infected cells (6-7 days post-inoculation) PCV antigen is found predominantly in the cytoplasm, giving the infected cell a stippled appearance. Because only dividing cells in an infected culture are susceptible to PCV, infection advances slowly through the cell monolayer; several blind subcultures may be required before any notable CPE develops.

For further information on porcine circovirus, please see Ellis, J, et al, Isolation of circovirus from lesions of pigs with PMWS. 1998. Can Vet J 39:44-51, Allan GM., et al. Isolation of porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. J Vet Diagn Invest. 1998. Jan 10(1). P 3-10, Harding JCS., et al, Postweaning multisystemic wasting syndrome: Epidemiology and clinical presentation. Swine Health Prod. 1998. 6. P 249-254, Nayar GP., et al, Detection and characterization of porcine circovirus associated with

postweaning multisystemic wasting syndrome in pigs. Can Vet J. 1997. 38:385, and Tescher, I., et al, A very small porcine virus with circular, single-stranded DNA. 1982. Nature 295:64-66. For information on the appearance of PCV in infected cultures, please contact John Black. For information on PCV-free PK-15 cell line, please contact John Langraf at NVSL, DVL, Ames, IA, ph: 515/239-8551.

Viral encephalitis testing of horses. Although Eastern Equine Encephalitis (EEE) virus grows in many cell lines (such as Vero cells), other zoonotic encephalitic viruses may not be so easily recovered in isolation attempts with common established cell lines. To broaden the range of recovery for encephalitis viruses, isolation attempts are made in embryonated eggs or with intracranial inoculation of laboratory mice.

The demand for EEE diagnostics is lessened by decreased interest in ratite husbandry, and where climatic changes have led to cooler, drier seasons. Encephalitis surveillance and diagnostics remain an ongoing concern in wet, freeze resistant locations such as in the delta regions of the southeast US, where mosquito and migratory bird populations endure the winter. Typically, Eastern and Western Equine Encephalitis, St. Louis Encephalitis, and California Encephalitis viruses are monitored during the summer months by public health and mosquito control authorities. ELISA techniques are used to detect antibody in captured and sentinel birds and to detect antigen in trapped mosquito pools. Also, PCR is being used with greater popularity to detect encephalitis viruses in mosquito pools. Techniques used in encephalitis surveillance are adaptable to animal diagnostic applications.

Astrovirus. Astroviruses have been associated with diarrhea in bovine, porcine, canine, and feline species, and generally are not culturable in common laboratory host systems. Astroviral particle morphology is similar to that of caliciviruses, but slightly smaller. Astrovirus genetic information is contained on genomic RNA. Although much of her recent published effort has been on Rotaviruses, Linda Saif was said to be a possible source of information on astroviruses in animals; she is with the Ohio Agricultural Research and Development Center, Department of Food Animal Health Research at Wooster, Ohio. For further information, please see Carter MJ. and Willcocks MM. The molecular biology of astroviruses. Arch Virol Suppl. 1996. 12. P 277-85.

New or improved diagnostic kits or reagents.

Synbiotics: Equine Infectious Anemia (EIA) viral antibody ELISA test kit manufacture and certification is in progress, but one deterring consideration is that some States do not yet recognize the ELISA format for EIA testing. [According to the VLA Newsletter of October 1998, Hawaii, Maine, New Jersey, New Mexico, and Pennsylvania do not accept ELISA testing for EIA antibody.] Like the AGID, antibodies to EIAV p26 are targeted by the ELISA kit under development. The RSAT test kit for *Br. canis* antibody is now available. For more information on these products, please contact Howard Jones.

VMRD: A direct ELISA BLV antibody test kit, using gp51-absorbed strip wells, will soon be available for regulatory testing. A competitive ELISA test kit to detect Caprine arthritis-encephalitis (CAE) viral antibody is in development and may be available in two years. The CAE-only kit reportedly is more specific than the Ovine progressive pneumonia (OPP) virus/CAE virus dual test kit. Veterinary Diagnostics is currently the only source for testing and reagents to certify CAE-free herds. A competitive ELISA test kit is in development to detect

antibodies to *Anaplasma marginale*. It, too, is expected to be in the strip well format; reactions will be judged on percent color inhibition, the determination of which initially may require a plate reader. For more information on VMRD products, please contact Tom Kellner.

IDEXX: A direct ELISA test kit in strip-well format is now available to detect antibodies to *Neospora canium* and Johnes Disease bacillus (*Mycobacterium paratuberculosis*). While the current format remains available, the *Mycobacterium paratuberculosis* DNA probe test kit will be redeveloped for improved performance. The development of a direct ELISA test kit to detect antibody to Swine influenza virus and *Mycoplasma hyopneumoniae* is currently in progress. For more information on these products, please contact Lisa Lemieux.

Boyt Veterinary: Donor calf serum free of IBR, BVD, PI-3, and Bovine respiratory syncytial virus (BRSV) antibody is available for cell culture applications. This serum is produced in a single, closed, colostrum-deprived herd, and is probably 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. For more on these products, please contact Peter M. Boyt.

Activities in quality assurance (QA) and quality control (QC) in animal testing. In labs performing viral isolation to detect BVD virus infection, a major concern is to keep cell line stocks free of non-cytopathic BVD, which adversely affects the susceptibility of cell lines for BVD field strains. BVD virus is a constant threat to cell stocks because it is occasionally found contaminating certain lots of commercial fetal bovine serum (FBS). A clean source of BVD-free serum is a vital quality assurance component in a veterinary virology laboratory. When viral antibody-free and BVD virus-free bovine serum is unavailable or unsuitable to cell lines, goat serum has been used as a substitute for FBS in the maintenance of susceptible bovine cell lines. However, donor goat serum from most sources often is found with CAE virus or antibody. CAE-free goat serum as once provided by Central Biomedia is no longer available.

In the absence of certified virus and antibody free supplement serum, one method to maintain BVD-free cell lines is to replace the FBS in the growth and maintenance media with a mixture of FBS and adult bovine serum (ABS), which usually contains antibody to BVD and other bovine viruses. The BVD antibody in the ABS neutralizes any potential BVD virus in the FBS. Most bovine cell lines are unaffected by this media component change. Before inoculation of clinical material, the maintenance media must be removed and rinsed from the cells, or else antibody in the ABS will interfere with the isolation of bovine viruses. Replacement post-inoculation media is supplemented with horse serum.

QA/QC efforts in veterinary viral diagnostic facilities include certain basic components: (1) Written protocols are in place for the frequently-used basic techniques. The protocols have been approved by the appropriate authorities. (2) Positive or negative control samples are routinely used. They are either from a reputable commercial source or are field samples that have been independently verified by other laboratories. In either case, control verification is documented in testing records. (3) Ingredients or reagents used in more complicated procedures are from certified sources, or must be internally certified. An example of this is antibody and virus-free FBS cited above. If FBS is from a suspected source, then cell lines are periodically screened to insure their BVD-free status. Ingredient and reagent certification is documented in the testing records. (4) Check-tests, when available, are periodically run and independently evaluated, as in

the case of the federally-regulated testing programs for EIA, BLV and Bluetongue virus (BTV). (5) Quality assurance also includes documentation of personnel training. In addition to providing resumes and academic records, new employees are asked to document their formal orientation to laboratory operations in their immediate work environment, such as equipment operation, biohazardous disposal procedures, and bio-containment and safety training. Established employees certify their continuing education efforts by earning CE credits, periodic in-house training, and attending seminars, technical workshops, and meetings of professional associations. (6) Laboratory facilities periodically undergo independent review of their overall operations and receive accreditation to operate from peer-recognized professional organizations, such as the AAVLD.

Unlike human clinical laboratories, which must maintain strict QA/QC documentation to fulfill federal and state requirements, state-affiliated veterinary testing facilities are under little external pressure to comply with the tenets of sound QA/QC practices. However, most quality animal testing labs probably exercise the components of a QA/QC program, but they probably have not adequately documented the effort. To rectify, some labs designate a quality assurance officer, an existing employee who dedicates a portion of work time to gather QA/QC documentation and to periodically refresh the file.

QA/QC measures will become an increasingly important laboratory operation in animal diagnostic facilities. The AAVLD has established a committee for QA/QC practices, which undoubtedly will become more prominent in future accreditation reviews.

How is PCR comparing to conventional techniques? Currently, few labs report using PCR techniques in routine diagnostics. When employed in diagnostics, PCR protocols tend to be simple; complex nested procedures have been avoided.

Bluetongue virus infection diagnosis and surveillance is one possible application where PCR techniques have shown their potential. BTV PCR reactions tend to remain positive after BTV is no longer detected by culture of blood from an infected animal. A multiplex PCR, developed by Donna Johnson of NVSL, Ames, Iowa, is available to detect and differentiate BTV strains common in the US.

Despite its technical complexity, viral isolation remains the standard, internationally accepted procedure to detect BTV. Culture is able to detect more viruses than just the one targeted by the PCR primer. Although much effort is being put toward their development, most PCR techniques have been inadequately proven for routine diagnostic use. For an overview on PCR as a diagnostic technique, and in comparison to conventional techniques, please contact Gary Gustafson.

Synbiotic markets primers for the laboratory diagnosis of small animal infections by PCR techniques. Although sales reportedly are slow, Synbiotics remains a credible commercial source of PCR primers. For further information on the line of available PCR primers, please contact Howard Jones.

Laminar flow was said to be crucial for successful PCR processing, especially so in nested primer protocols, which are prone to carryover contamination. Specimen extraction, reagent mixing, and thermocycling often are performed in separate rooms. Other methods of contamination control are the use of dedicated, hooded countertop workspaces, commercial

decontaminants (containing proteases and nucleases), separate pipettors for each virus system, filtered pipette tips, and disposable gloves. For further information on contamination control in PCR procedures, please contact Marge Muenzenberger.

Any new FA testing available? Although no FA reagents exist to assist in the laboratory diagnosis of virus infections, Becton-Dickinson (B-D) markets the Directigen Flu-A viral antigen capture ELISA test kit. With two separate monoclonals, this kit targets the group-specific nucleocapsid (NC) antigen in an eight-step immunomembrane antigen capture format. The kit has a 15 minute run time, but requires a high number of particles for detection; a culture passage in the allantoic sac of embryonated eggs amplifies the amount of virus, thus increases sensitivity of detection. The kit costs about $250 for 20 tests. For further information on this kit, please contact B-D (410/316-4000). [The web site for American Diagnostics of Pendleton, IN, ph: 800/730-0018, lists the test kit Directigen Influenzae A, 20 Tests, Product No. 8560-20, $403.20.]

American BioResearch (ABR) and VMRL are developing immune probes for the detection of Equine viral arteritis (EVA) virus in clinical material. A monoclonal cocktail against EVA virus and an anti-mouse conjugate are a promising combination to detect EVA virus-infected cells scraped from the linings of large blood vessels of infected, aborted foals. A direct FA conjugate for EVA virus may evolve from this effort. In the fluorescent detection of BVD virus, the direct FA conjugate made from hyperimmune pig serum is said to yield the broadest range of reaction against the many strains of BVD virus. An indirect system is being formulated to detect BVD virus in cell culture with greater sensitivity. The primary reagent will be polyclonal of goat origin and the secondary reagent will be fluorescein-conjugated monoclonal anti-goat IgG. Immune probes for the detection of Porcine Circovirus also are currently under development at ABR. For further information on FA reagents from American BioResearch, please contact John Black.

To assist in the laboratory diagnosis of Canine parvovirus (CPV) infections when intestine is not available, CPV FA testing on frozen sections of tongue basal epithelium is a suitable alternative. Thymus is an excellent tissue in which to search for CPV antigen by FA. However, thymic tissue is difficult to locate in older puppies, and may be FA positive for a period of time after modified live viral vaccine has been given.

Is anybody seeing suspicious BVD cases (based on clinical, gross, and histopathology) which are negative on virus isolation and FA? In the diagnosis of BVD viral infection, pathologists seem to find gross or histological lesions compatible with BVD in greater frequency than the virology lab is able to verify these same cases as BVD. To explain this phenomenon, two possibilities exist: Either the current BVD viral diagnostic techniques lack sensitivity, or alternative etiologies exist that cause lesion patterns similar to BVD viral infection.

On the question of testing sensitivity, different cell line types have different susceptibilities for the culture of BVD virus field isolates. For example, the BT cell line seems more sensitive than MDBK cells. The EBTr cell line was also said to be suitably susceptible for culturing BVD field isolates. Once BVD virus has been cultured on one cell line, it is adapted easily to other lines. The culture system of choice for primary isolation seems to be BVD-free primary bovine fetal lung or kidney cells, which are burdensome to establish and maintain for routine use.

Testing sensitivity also depends on the reactivity of the FA probes used on tissues or inoculated cell cultures. Depending on the method of preparation, anti-BVD antibody or conjugate seems to

react better to their homologous viral biotype than the heterologous types. Testing facilities must be confident that the BVD immune probe has a broad scope of reaction, equally detecting the different biotypes. As part of their quality assurance effort, labs are invited to contact reagent vendors for such certification.

Another factor influencing testing sensitivity is sample selection. The location of virus during the course of infection is dictated by the bovine pestiviral (BVD-MD) pathogenesis, which is complex and may vary between cases, depending on whether infection was derived in utero, as a neonate or adult, or whether there was suprainfection by another strain of pestivirus. During active viremic or carrier infections, BVD virus is recovered readily by culture from the serum or buffy coat cells. On post-mortem cases with little background or history, there are no obvious tissues from which BVD virus may be cultured consistently in all types of BVD infection. For virology testing, lung, spleen, and lesion samples from mucosal surfaces are popular post-mortem specimens, but evidence suggests that BVD virus appears only fleetingly in these tissues, depending on the type and course of infection. According to immunohistochemistry and PCR data, BVD virus seems to persist longer in tonsils and mandibular lymph nodes. Obviously, a better knowledge of bovine pestiviral pathogenesis, careful specimen selection, and newer PCR technology could render improved detection rates of BVD virus. For further information on tissue selection and the pathogenesis of BVD, please see Greiser-Wilke I., et al, Distribution of cytopathogenic and noncytopathogenic bovine virus diarrhea virus in tissues from a calf with experimentally induced mucosal disease using antigenic and genetic markers. Arch Virol Suppl. 1993. 7. P 295-302.

As of yet, there is no hard evidence of other etiologic agents producing Mucosal Disease (MD) signs and lesions in cattle. However, except for “classic BVD”, the clinical and pathological presentation of bovine pestivirus infections is multifactorial, thus signs and lesions may vary somewhat between cases. Diathesis is an underestimated property of BVD viral infection, in which tissues are predisposed to react adversely to other foreign stimuli, causing the body to become more susceptible to certain diseases. Such as in immunosuppressive infections, BVD-infected animals are prone to develop serious illnesses from secondary etiologies, such as in Pasteurella pneumonia. It is theoretically possible that an undiscovered agent or combination of agents contributes to the appearance of clinical BVD-MD in nature. One arbitrative exercise would be to attempt to reconcile the rate of BVD histological diagnosis with BVD serology testing. If the rate of histological diagnoses runs well above the rate of seropositive cattle in a given area, there may be other factors generating signs and lesions similar in appearance to BVD viral infection. AVM members are advised to consult their local pathologists and continue to review literature reporting on new developments in the understanding of BVD-MD.

Lymphocystis in fish. No comment was offered on this topic during the session. Lymphocystis is caused by a highly contagious Iridovirus capable of infecting several species of fish, predominantly saltwater, game varieties. An infected fish displays growths over its external surfaces. The usually non-fatal infection is self-limiting and peaks in incidence approximately every three years. Diagnosis typically is made with histopathological examination. This fish disease is of lesser concern in the US than in Europe, where it is a potential threat to the mariculture industry. For information, please see Bernard J. and Bremont M., Molecular biology of fish viruses: a review. Vet Res. 1995. 26(5-6). P 341-51, or Plumb JA. Major diseases of striped bass and redfish. Vet Hum Toxicol. 1991. 33 Suppl 1. P 34-9, or contact John P. Hawke,

Louisiana Aquatic Animal Disease Diagnostic Laboratory, LSU School of Veterinary Medicine, Baton Rouge, LA 70803, ph: 225/346-3281.

NVSL Fee Structure. NVSL fees stem from a mandate that USDA programs strive toward self sufficiency, where feasible. Funds generated by permits, reagents and testing help cover the increasing cost of consumables and the operational overhead of quality assurance programs. Program funds are then freed to offer market-competitive salaries to attract and retain quality managerial personnel.

The fate of Brucellosis testing programs. Because many States, or regions within States are certified as Brucellosis-free, the testing and surveillance program, funded through the Food Safety Inspection Service (FSIS), is being considered for termination. Funding for private testing is no longer available, and once States are certified as Brucellosis-free by December, they may have their federal program funding cut. The increased use of the new RB-51 strain of *Br. abortus* vaccine promotes this trend; unlike the old strain 19 vaccine, the RB-51 vaccine does not generate antibodies that react in conventional screening tests for Brucellosis. Surveillance programs no longer need to invest in additional technology to determine whether positive reactors stem from actual infection or vaccination.

Chlamydia serology and culture. Popular Chlamydial culture techniques involve the use of coverslip-shell vial cell cultures with L-92, Vero, or McCoy cell lines. Specimen homogenates or swab rinses are decontaminated with aminoglycoside antibiotics and low speed (300 x g) centrifugation. An aliquot of the supernate is centrifuged onto the cell culture monolayer at about 300 x g for 30 minutes at 35-37°C. Post-inoculation medium includes 2 ug/ml of cycloheximide. Infection is verified three days post-inoculation with Giemsa, Gimenez, or FA staining. For greater sensitivity, at least one blind subculture is recommended. Yolk sac inoculation of five to seven day old embryonated eggs remains a traditional method of Chlamydia culture. For further information on the lab diagnosis of Chlamydial infections of birds and animals, please see **Kodak SureCell *Chlamydia* Test Kit is No Longer Available: Alternatives** in the *1996 Fall/Winter AVM Newsletter*, **Diagnosis of Chlamydial Infection in Pet Birds by Fecal FA** and **Chlamydia Update** in the *1997 Fall/Winter AVM Newsletter.* Also, please see Butler, JC and Whitney, CG, Compendium of measures to control Chlamydia psittaci infection among humans (psittacosis) and pet birds (avian chlamydiosis), 1998, MMWR 47/No. RR-10, July 10, 1998. For additional practical insight on the culture of *Chlamydia psittaci* from veterinary clinical specimens, please contact Rob Poston.

Veterinary laboratory testing information systems. Some members reported that their facilities are changing their computerized databasing systems. No severe problems, other than the anticipated functional disruption, have been noted by certain members when their laboratories changed to 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). In one instance, a change to the Unix-based VetStar system reportedly was made with great difficulty, and has led to continued troubles from its relative incompatibility with existing Microsoft/Windows operating systems.

In the selection of database systems, customer support during and after installation is as crucial, if not more, than the functional features of the software. A generally held consensus among

session participants was that computer programmers, analysts, managers or technicians have a greater capacity to affect laboratory operations than the actually computer system or software. Most computer personnel do not have the background, training, or experience in veterinary laboratory data management, thus seem relatively inflexible in addressing laboratory needs. For further information on veterinary laboratory databasing, please see **Vetlims Update** and **Case Accessions and Tracking, Data Retrieval** in the *1997 Fall/Winter AVM Newsletter*, or contact Marge Muenzenberger or Mike Parsley.

Pipetting technique. One member noted that automated micropipettes draw up a metered amount of fluid then dispense, but are incapable of reverse pipetting, that is to draw up an excess of fluid, then dispense the metered amount. The latter approach is believed to lead to greater precision. However, for most microbiological techniques, volumetric precision is not critical to the success of the procedure. By allowing samples to come to room temperature before pipetting, and by pre-wetting pipette tips, greater precision is gained with existing micropipetting equipment. For a critique of common micropipetting techniques, please contact Kay Rathman.

Micropipetting and repetitive motion. Occupational safety is a growing concern, demonstrated by the reported appointment of an Ergonomics officer at laboratory facilities in North Dakota. As supported by the testimony of some of the members present, repetitive motion injuries have been associated with intensive, continuous micropipetting. Hand-stretching exercises can be performed to reduce discomfort and injury. To relieve the mechanical stress on the hand, Rainin offers Pipet-Plus® Latch-Mode™ Pipette, a new micropipet model designed to lessen the risk of repetitive motion injuries.

Rainin offers the following hints to reduce fatigue, errors, and the risk of Repetitive Motion Injuries (RMI's) or Cumulative Trauma Disorders (CTD's). 1) Limit pipetting intervals to 30 minutes or less, taking frequent short pauses between pipettings. 2) Rotate pipetting activities between laboratory tasks, hands, and different people. 3) Use thin-wall pipette tips that fit correctly and are easy to eject. 4) Use minimal force when applying pipette tips. 5) Keep samples and instruments within reach. 6) Use an adjustable stool when sitting at a lab bench. 6) Work with your arms close to the body. 7) Keep your head, wrist, and shoulders in a "neutral" position. 8) Support the upper body when leaning forward. 9) Use a latch-mode or an electronic pipette for repetitive pipetting tasks. (source: http://www.rainin.com/ergo.html).

Polyomavirus. Some European laboratory workers have been discovered with antibody titers to SV-40 virus, a common contaminant of cell culture and serum products. In Europe, a correlation between polyomavirus antibody and leukemia has been suggested. SV-40 has also been found as a contaminant of the original Salk Poliovirus killed vaccine once used in the 1950's in the US. The use of this contaminated vaccine has yet to be associated with any serious health problems. Many scientists question the significance of the SV-40 antibody and its alleged link to leukemia. For more information on this story, please contact John Black.

Nanobacterium. First recognized in Europe, this bacterium has been found as a contaminant of filtered fetal bovine serum and tissue culture. It possesses a relatively slow five to six day growth cycle, sticks to glass surfaces, and calcifies by absorbing the divalent cation from the medium. It evades sterilization efforts by being gamma radiation and heat resistant, and passes through filters. Because it stains poorly, it is not easy to detect. For further information, please see Ciftcioglu, N, et al, A New Potential Threat in Antigen and Antibody Products: Nanobacteria. in

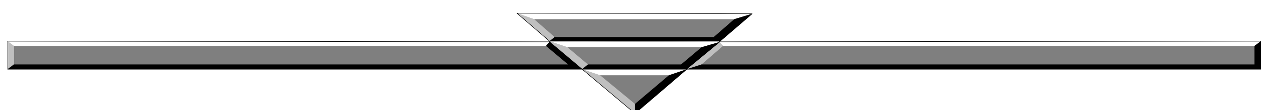
Vaccines 97, Brown et al, eds., Cold Spring Harbor Laboratory Press, New York, 1997; Ciftcioglu, N, et al, Apoptotic effect of Nanobacteria on cultured mammalian cells. Mol. Biol. Cell. Suppl. 1996. 7. P 517a; Kajander, EO, et al, Fatal (fetal) bovine serum: Discovery of Nanobacteria. Mol. Biol. Cell. 1996. Suppl., 7. P 517a; or please contact John Black.

[*Nanobacterium sanquineum* is a newly discovered organism recoverable from mammalian blood and blood products. Nanobacteria are able to pass sterilizing filters, thus occasionally are isolated from supposedly sterile commercial cell culture serum. Pooled sera and cell culture products often are found contaminated by Nanobacteria, which are cytotoxic to cultured fibroblasts. Nanobacteria have the ability to penetrate several types of cultured cells, but their pathogenicity has yet to be elucidated. Contaminated cell culture products or blood and tissues may taint research data and may invite risk and liability if the contaminated products are put to therapeutic use. All standard sterility testing methods fail to detect Nanobacteria, which stain poorly, multiply slowly, are present at low numbers, and do not grow on standard bacteriological media.

An ELISA-based test kit to detect *Nanobacterium sanquineum* has been developed by ABCELL Corp. of Kuopio, Finland and marketed in the US by BioWorld, ph: 800/860-9729, 614/792-8680, fax: 614/792-8685. Assay specificity is based on two monoclonal antibodies recognizing separate epitopes on Nanobacteria surface. The epitopes have not been found in other microbes or animal or human tissues. ABCELL's ELISA system produces a result within five hours. After sample incubation, the test wells are washed and incubated with enzyme-labeled tracer. After washing and adding the substrate, the result is read in 15 min. The entire procedure is performed with the reagents included in the kit, which contains serum for standardization and coefficients for estimating the actual particle number in the sample. The Nanobacteria ELISA Kit is semi-quantitative, able to detect as few as 200 particles per ml in biological fluid or cell culture. For information on this kit, please see http://www.ispex.ca/chemicals/ABCELL.html.]

Source of Ehrlichia IFA substrate slides. Lee Fuller (714/525-7660), who manufacturers *Ehrlichia canis* substrate slides for VMRD, also has IFA substrate slides to test for antibody against other Ehrlichia species. ProtoTek International (ph: 612/466-5391, fax: 412/644-6831) remains a popular source of IFA substrate slides for Ehrlichia and Babesia antibody testing. Integrated Diagnostics Inc. of Baltimore, MD (ph: 410/737-8500, fax 410/536-1212) markets IFA substrate slides and some direct FITC conjugates for certain Ehrlichia and Rickettsia spp. [Integrated Diagnostics has been a past sponsor of the AVM-CSC.] Although MRL Diagnostics (formerly Hillcrest) of Cypress, CA (800/838-4548) currently carries only *E. chaffeensis* in IFA substrate slides for Ehrlichia testing, it offers an entire line of IFA slides to test for antibodies against a number of vector-borne infectious agents of both humans and animals, such as Rocky Mountain Spotted Fever *(Rickettsia rickettsii*), Lyme's disease (*Borrellia burgdorferi*), and Q Fever (*Coxsiella burnetii*). MRL also markets slides for *Bartonella spp* IFA testing and microimmunofluorescence (MIF) antibody testing for *Chlamydia spp*. The Louisiana Veterinary Medical Diagnostic Lab has resources for the internal production of substrate slides for IFA testing of several Ehrlichia species of veterinary importance.

Illumination problems with FA microscopes. One member reported a problem of illumination dimming associated with powerline fluctuation. Longer life can be given to aging transformers with the use of surge protectors or uniform power supplies (UPS). One incidence of illumination flicker and loss has been associated with lamp contact corrosion, caused by microscope operation



in a humid environment; replacement of the lamp base remedied the problem. Segermark Microscope Repair Co. of Morton Grove, Illinois (ph: 847/966-3492) and Cancienne's Microscope Service of Baton Rouge, Louisiana (ph: 225/474-3040 fax: 225/473-9011) are firms handling maintenance, service and repairs of FA microscopes. For further information, please contact Mary Woodruff or Rob Poston.

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Theresa Love, AVM Secretary/Treasurer
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P.O. Box 4389
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AVM Meeting Discussion Topics

I would like the following topics placed on the program for the next AVM Annual Symposium on Techniques in Veterinary Microbiology for discussion in the (circle) Avian, Serology, Bacteriology, Virology Session:

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Please fill in your name, professional affiliation, address, and phone number in the space provided in the membership application above, and mail the entire page to Theresa Love, AVM Secretary/Treasurer, as indicated above.

Notes