STANDARD DIAGNOSTIC CULTURAL AND SEROLOGICAL PROCEDURES.

Participants of the Mycoplasma Workshop would like to go on record as formulating the following:

Recommendation I to the Mycoplasma Committee of the AAAP.

I. To establish a working committee or body which would provide:
   a) Testing and Standardization of commercial and other available antigens and reference serum and furnish endorsement thereof
   b) Determine mycoplasma colonies or isolates as being pure prior to their being used for F/A technique application
   c) Specific protocal for Cultural techniques and serological proceedures utilized for the diagnosis of mycoplasma.
   d) A method to determine a flock status after diagnostic procedures are applied, i.e. A- result + B result = positive or negative or suspect flock.

The above to be applied toward control and eradication of mycoplasma (MG and MS) in Parent, Grandparent and pedigree flocks.

Recommendation II.

That the specific protocal for cultural, serological and supplemental techniques be described in detail and made a part to the NPIP and NTIP published procedures.

Recommendation III.

Monies needed to support the above mentioned functions of the "working committee" be solicited from the turkey and chicken breeders within the industry.

Further Suggestions:

1. A "central reference lab" be established to disseminate the above mentioned testing procedures and endorsed reagent to recognized diagnostic laboratories.

2. A reporting system be worked out which would provide for the early dissemination of other than routine testing results to other disease control and eradication agencies which may offer assistance to the testing laboratory involved with the problem.

3. Telephone numbers and addresses of the respective Veterinary Services Regional Poultry Epidemiologist be incorporated in the plans for publication.

4. The Veterinary Services Diagnostic Laboratory at Ames, Iowa be developed as a reference and Centralized Laboratory.

5. It was further emphasized that serological and cultural problems encountered with various testing antigens and serum and reagents be well documented for future reference.

6. Stressor procedures be applied in controlled facilities to emphasize low virulent or weakly antigenic strains and mycoplasma in birds. i.e. aerosalization with NCD vaccine.

7. Some system be devised which would allow condemnation data to reflect more accurately the incidence of air sac lesions in processed poultry.

INFLUENCE OF STRAIN DIFFERENCES ON SEROLOGICAL, CULTURAL AND PATHOLOGICAL FINDINGS

1. Differences in virulence exist with MG, MS and MM.

2. If so, antibody response is poor. Culture more difficult. Large numbers. Repeated samples.

3. Differences in tissue tropism exist. MS MM?

4. Model for assesment of tissue tropism. (below)

5. Antigenic varients.
   a) SP test broad coverage.
   b) HI test more strain specific. Only documented with MG, especially occurs when sera are low titered.

6. Suggest addition of stress to a small group of banded individuals to enhance recovery of the organism.

7. Search should be made for antigenic varients, and reagents should be made available.

8. Research on the production and function of IgA in the respiratory system should be encouraged.

9. Criteria for identifying isolates:
   a) HA and HI
   b) Biochemical Reactions
   c) Growth Inhibition
   d) FA
   e) Complement Fixation
   f) Inoculation of susceptible chicks is final criteria.
      --Parenteral inoculation may give cross reactions.
      --Contact or eyedrop exposure preferred.
      --Follow serologically weekly 8 weeks.

TISSUE TROPISM

Group 1. Aerosol or air sac innoculation
Group 2. Same + stress i.e. IB vaccination. Stress 3-5 prior or simultaneous with mycoplasma exposure.
Group 3. Foot pad innoculation
Group 4. Controls, Innoculate with medium. Add the stressing agent. Use clinical signs and gross lesions as criteria.

CRITERIA FOR ANTIGENIC VARIENT:
--Select isolates from flock which gave atypical serological reactions.
--Plaque purify isolates.
--Infect mycoplasma free birds by non-parenteral routes.
--Follow weekly with standard antigen and antigens prepared from the isolate.
--If possible, follow birds exposed to "standard" isolate at the same time.
--Homologous reactions (especially HI) should be greater in both cases.

MYCOPLASMA ANTIGEN PREPARATION, TESTING AND SELECTION.

1. Isolation of antigen organism--5 single colony picks. FA identification. Use isolate that will detect antibodies for at least 5-6 different isolates.

2. Antisera prepared by intra-nasal instillation MG. Serum is harvested at 10 days (IgM) and 21 days (IgG). Negative serum from SPF birds.

3. Laboratory adapted strains are seeded into heart infusion + yeast 10% H.S. medium (MG) or Albimi PPLO broth + 10%-15% S.S. medium (MS). If destrose is used a 3 to 5% inoculum is seeded into the broth but harvest must be at 36 hours and not later than 50 hours.

4. Antigen standardized with a spectrophotometer (see attached). Smear antigen and fix with Bouin. Wash until yellow color disappears and stain with stock Giemsa 1 drop/ml for 1/2 hour. There should not be present extraneous debris. Large numbers of coccoid bodies and little else must be seen.

5. The antigen should agglutinate at dilutions of 1:2, 1:4 and 1:8. Box titration.

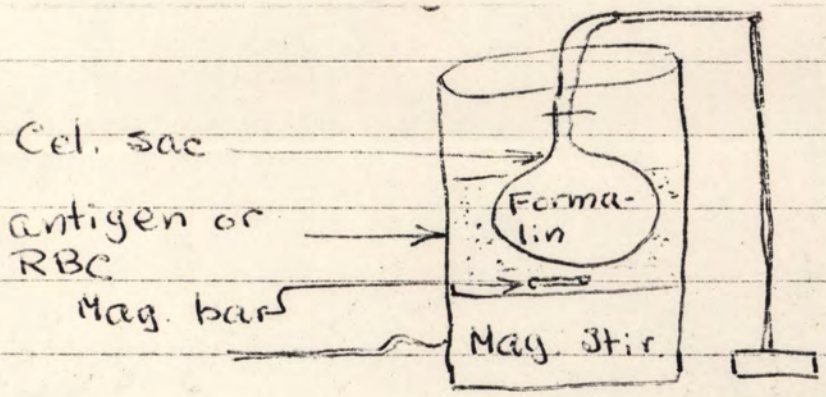
6. Preservation of the antigen seems to be most satisfactory with 1:10,000 merthiolate. Disperse antigen in small lots and use same batch until used completely. DO NOT FREEZE the antigen. For shipping of antigens from old stock (6 months +) add additional merthiolate to 1:15,000.

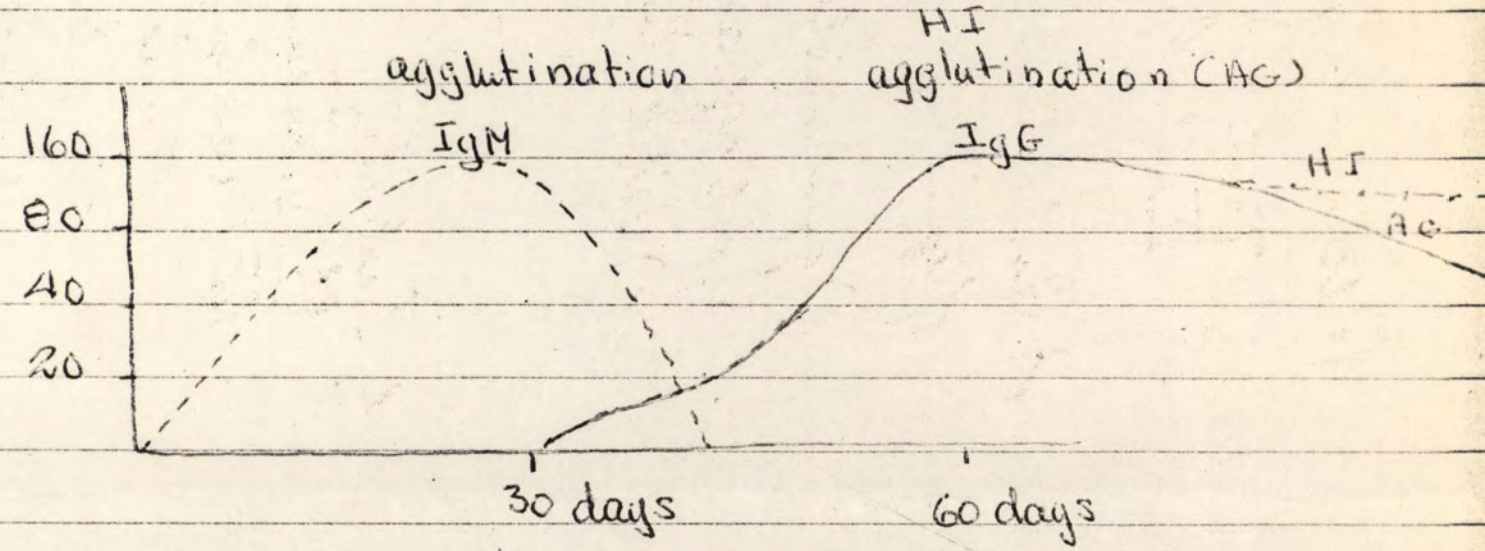
7. A quick gauge of antigen stability is to incubate at 37.8°--12 hours and check sensitivity. (Usually overnight).

8. Reading time of agglutination not beyond 3 minutes for chicken sera and 4 minutes for turkey sera.

9. A fomalin treated antigen and RBC can be made for MG but after storage the reactants must be washed to remove free formalin.

10. M.S. antigen can be prepared with a medium supplemented with Nicotinamide (NIC) in place of NAD using NIC adapted cultures. (WV 1843). Total man hours and medium cost is markedly reduced.





Contact infection with MG