



MVP Laboratories, Inc.

5404 Miller Avenue • Ralston, NE 68127

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Research Roundup

MVP Laboratories strives to stay current in the latest developments in veterinary microbiology. In this quarterly publication, we will bring you topics of interest that may have application in your practice situation. Some of the research discussed here is generated in our own laboratories and some is summarized from recent journals. We will occasionally address “frequently-asked-questions” that we get from our customers by phone or e-mail. We welcome ideas from you for future issues. Our contact information can be found on the back of this newsletter.

Inside this issue:

The value of diagnostic microbiology

***Clostridium perfringens* field isolates study**

Contact MVP

Tissue Submission Tips

Select Animals that are:

- Acutely ill
- Not treated with antibiotics
- Representative of disease situation (submit samples from 3-5 animals)

Send tissues using:

- Overnight courier
- Ice packs

Getting The Right “Bug” Or The Value in Quality Bench-Level Diagnostic Microbiology

In the last issue, we mentioned the value of PCR in diagnostic veterinary microbiology with regard to strain differentiation and characterization. PCR’s role cannot be underestimated, yet molecular techniques will probably never fully replace the routine bench-level microbiology that goes into working up diagnostic cases. Correct diagnostic results are especially important when an autogenous vaccine is needed. When MVP’s diagnostic department receives tissues or cultures for autogenous bacterin production, we need to ensure that isolates are appropriate to use in a bacterin. Here are some questions we’ve been asked about our diagnostic procedures:

Q: How do you know that the animals from which samples originated are representative of the disease?

A: We don’t, but you do! One reason that autogenous vaccine manufacturers are required to have a veterinarian involved in every case is to ensure that appropriate animals are sampled for the disease situation. The referring veterinarian should select animals that are the correct age, exhibiting typical clinical signs and have not been treated with antibiotics. Ideally, samples from three to five animals should be submitted, to maximize the chances of recovering clinically significant bacterial isolates.

Q: How do you tell which microorganism on a plate is causing the problem?

A: We depend on the submitting veterinarian to provide us with the information we need (history, clinical signs, vaccination status, percent morbidity/mortality, etc.) to formulate a list of differentials. From there, an experienced veterinary microbiologist will know what pathogens to be looking for, and what those colonies look like on blood agar and selective or enriched medias. It’s relatively easy to determine that an organism is a true pathogen when it is isolated from multiple animals in pure or nearly-pure culture with large numbers of colonies (and it fits the clinical picture). The more usual scenario we encounter in the lab is looking for pathogens from a number of different colony types. In those cases we must pick colonies that resemble suspected pathogens and restreak them to new plates for isolation and further testing for identification and characterization.

Q: Can every microorganism we isolate be manufactured into a vaccine?

A: No. Some isolates belonging to particularly fastidious species do not grow well in artificial media and conditions. An example is *Haemophilus parasuis*. Occasionally, some *H. parasuis* isolates will only grow in the presence of “nurse colonies” of *Staphylococcus*, as this organism provides nutrients that the agar media does not. Obviously, such an isolate would not be a candidate for vaccine production. Fortunately this is rare, and most *H. parasuis* isolates grow well in the lab.

Further, not every organism isolated from tissue is appropriate to use in a vaccine. We report all diagnostic findings, but rely on experience, common sense, and input from the referring veterinarian to determine which individual isolates, if any, should be incorporated into a vaccine. For example, lung isolates of respiratory pathogens, such as *Actinobacillus pleuropneumoniae* or *Mycoplasma hyopneumoniae* would likely be useful in a vaccine. While it’s not uncommon to recover *Escherichia coli* or other enteric bacteria from lung, especially if the tissue is autolyzed, these organisms are not primary respiratory pathogens and would be useless in a vaccine designed to prevent respiratory disease.

A retrospective study of the prevalence of *cpa*, *cpb*, *cpe*, and *cpb2* toxin genes among *Clostridium perfringens* field isolates obtained from pigs with gastrointestinal diseases using PCR genotyping

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Clostridium perfringens plays an important role in causing gastrointestinal (GI) diseases in pigs. The virulence of this pathogen comes from its ability to produce toxins. The *C. perfringens* isolates of pig origin have been found to be able to produce at least four different toxins (i.e., the alpha toxin, beta toxin, enterotoxin, and beta 2 toxin). The knowledge about the prevalence of these four toxin genes among the field strains of *C. perfringens* is very limited. In this study, a total of 113 field strains were isolated from pigs with GI diseases and examined with PCR genotyping. Of these, 46.0% were PCR positive for the *cpb2* gene, 18.6% were positive for the *cpe* gene, 98.2% were positive for the *cpa* gene, and 31.0% were positive for the *cpb* gene. Of the 113 field isolates, 69.0% were type A, 29.2% were type C, and 1.8% were untypeable due to missing the *cpa* gene. The isolates that only carried the *cpa* gene (29.2%) and those that carried both *cpa* and *cpb2* genes (29.2%) were most prevalent and were followed by those that carried both *cpa* and *cpb* genes (11.5%). Only a small portion of the 113 isolates carried both *cpe* and *cpb2* genes (7.1%). Based on the results obtained by PCR genotyping, a subtyping system was established to help distinguish the difference due to the presence of *cpe* and *cpb2* genes among field strains that are traditionally classified as type A or type C.

Figure 1. A single PCR assay for identifying *cpa*, *cpb*, *cpe*, and *cpb2* toxin genes of the *C. perfringens* field isolates. Lane 1: DNA ladder; lane 2: PCR assay for *cpa* gene; lane 3: PCR assay for *cpb* gene; lane 4: PCR assay for *cpe* gene; lane 5: PCR assay for *cpb2* gene.



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