

Newsletter - Summer 2018

Message from the Interim Director

We hope your summer is off to a great start and appreciate you taking time to catch up with WVDL in our quarterly newsletter. We are excited to welcome Ailam Lim, Lorelei Clarke, and Jaime Miller to the WVDL family this summer. Dr. Lim will be our new Virology Section Head and Dr. Clarke and Dr. Miller are joining our team of boardcertified diagnostic pathologists.

Other important information you will find in this edition focuses on new test development and test improvement with our Salmonella real-time PCR diagnostics. Our team member spotlight is Todd McCoy, who has worked at both WVDL laboratories and now is a supervisor at our Barron location.



Please let us know if you our your company is interested in attending our annual Bovine Genetics meeting. This meeting is geared towards regulatory testing for export of bovine genetics and is free of charge. We will have updates from the testing and client services sections, updates and roundtable discussion with DATCP and USDA-APHIS, and a guest speaker to review bovine Salmonella infection from her review chapter in VCNA, Dr. Chelsea Holschbach. The meeting is Thursday, August 2nd. Contact us for details or to RSVP.

Enjoy the warm weather, please contact us at any time.

Keith Poulsen Interim Director

UPDATE: Salmonella Dublin real-time PCR

Testing improvements at WVDL

Upon recent examination of the gene target for the *Salmonella enterica* subspecies *enterica* serotype Dublin real-time PCR, the WVDL discovered that this gene can now be harbored in *E. coli, Enterobacter*, and *Citrobacter*. At this time, the WVDL does not know the prevalence of this gene in non-*Salmonella* ser. Dublin bacteria. The WVDL has suggestive evidence that the prevalence of this gene, in our submitting population, is low (3-6% of tests on fecal and tissues samples per year). Currently, we have obtained two *E. coli* isolates carrying this gene and additional testing of samples submitted since 6/27/2018 have not yielded any additional isolates testing positive.

Because Salmonella ser. Dublin diagnosis on a farm has a high consequence, the WVDL will continue to offer the Salmonella ser. Dublin via rtPCR as it still has significant clinical utility. We have investigated a conventional PCR to confirm our current rtPCR, but due to sensitivity and specificity issues, we are unable to utilize these assays. We will continue to develop a new rtPCR assay. However, effective 7/16/2018, the WVDL will change the name of the Salmonella ser Dublin rtPCR to the VagC rtPCR. VagC is the gene target for

the Salmonella ser. Dublin PCR that is no longer specific to Salmonella ser. Dublin. This PCR is run as a multiplex with the Salmonella species PCR and results will be reported for both the Salmonella species and ser. Dublin.

 Negative Salmonella ser, Dublin results will be reported as negative, as this cross reaction does not affect negative results.

Positive VagC PCR results that do not have a positive Salmonella species PCR results will be reported as positive, but should be interpreted as a possible cross-reaction with a non-Salmonella ser. Dublin

- C_T values <35 will be automatically set up for culture and sensitivity, as is our current practice (see below for further information)
- Correlate this result to clinical history on the farm and for the animal sampled
 Consider confirmatory testing with ELISA

- Consider testing additional animals necropsy and culture of tissues is the best sample to recover Salmonella ser. Dublin
- Samples testing positive for both Salmonella species and VagC gene target should be interpreted as positive in conjunction with the clinical history of the herd and of the animal tested.
 - C_T values <35 will be automatically set up for culture and sensitivity, as is our current practice (see below for further information)
- We do not recommend making management decisions for a herd or an animal on a single PCR test result. Clinical signs of the animal tested and history of the herd are very important factors to be considered. Further testing may be in the best interest of the herd owner.
- Preliminary results will be reported and, as always, available 24/7 on our portal at www.wvdl.wisc.edu.

Any pending *Salmonella* ser. Dublin rtPCR from 6/27/18 to 7/13/18 will be reported as inconclusive. As stated above, a *Salmonella* ser. Dublin rtPCR positive results with a positive *Salmonella* species rtPCR result should be interpreted as positive in conjunction with the clinical history of the herd and of the animal tested.

Since 2015, the WVDL has been collecting data comparing our PCR and culture. Currently, the WVDL automatically cultures all samples that obtain a 35 or lower C_T (cycle threshold) on the Salmonella species PCR. For 2017, we found that 80.7% of samples with a 35 or lower C_Tvalue cultured Salmonella of any serotype (Table 1). Interestingly, we also observed that if both the *Salmonella* species rtPCR and the *Salmonella* ser. Dublin (now to be called by the target name, VagC) rtPCR are less than or equal to 35, we cultured *Salmonella* ser. Dublin 57.3% (Table 2). Therefore, we will be automatically culturing samples that obtain a 35 or less C_Tvalue for VagC rtPCR, as we have previously done for the *Salmonella* species rtPCR, as our recovery rate is remarkably high. Previous reports demonstrate much lower culture rates for Salmonella ser. Dublin (0-25%; Nielsen et al., 2013). We believe this to be best practice to confirm the VagC rtPCR result by using culture when there is a high likelihood of recovery. The figures below demonstrate our culture rates for *Salmonella* post *Salmonella* species and *Salmonella* ser. Dublin rtPCRs.

Table 1: Culture rate for Salmonella species post multiplex rtPCR for 2017.

| Salmonella species PCR C _T Value | Number of Tests Examined | Culture Rate | |
|---|--------------------------|--------------|--|
| ≤ 35 | 693 | 80.7% | |
| ≤ 30 | 479 | 93.9% | |
| ≤ 25 | 164 | 97.6% | |

Table 2: Culture rate for Salmonella ser. Dublin post multiplex rtPCR for 2017.

| Salmonella ser. Dublin PCR C _T Value | Number of Tests Examined | Culture Rate for Salmonella ser. Dublin (Group D1) |
|--|--------------------------|--|
| ≤ 35 | 118 | 57.3% |
| ≤ 30 | 94 | 76.0% |
| ≤ 25 | 54 | 95.0% |

Note: Culture rate does not take into account animals that were treated with antibiotics prior to submission.

We apologize for the delay and any inconvenience this may create for you and your clients. We appreciate your business and continued patronage as we work to improve this test. If you have any questions, please see our website or contact us at any time.

Barron

Dr. Taylor is Back! - Dr. Holly Taylor returned to the lab on May 29, following her maternity leave! We are full speed ahead in our pathology department.

New Necropsy Floor - During Dr. Taylor's leave, the necropsy floor was re-done (see below). It was completed in 3 days with an additional 24 hours of curing. Our Veterinary Technician Kris Garza applied a fresh coat of paint and it looks great!!



Submitting Samples - Just a reminder that submitting specimens in gloves (especially milk) likely will result in rejection of the sample because of contamination and poor reliability of results. Please see our <u>Submission Guidelines</u> page on our website (<u>www.wvdl.wisc.edu</u>) for information on the proper way to submit samples.

Microbiology

Johne's Testing - After 28 years in the business of servicing the Johne's disease testing needs of veterinarians and animal owners across the country, the Johne's Testing Center (JTC) housed at the School of Veterinary Medicine under the direction of Dr. Michael Collins is closing. However, the JTC has arranged to continue the same high quality services at roughly the same fees, by merging its business with the Wisconsin Veterinary Diagnostic Laboratory. Although we here at WVDL are sad to see the JTC close, we welcome the new business opportunities as well as the continued opportunity to partner with the expertise of the JTC staff. The merger will take affect September 1, 2018.

Beginning July 1st 2018, WVDL instituted several price changes related to Johne's Disease testing to better serve our clients.

- 1. No out of state surcharge on Johne's ELISA and Johne's direct PCR assays.
- 2. Johne's ELISA on bovine and caprine serum has been reduced to \$6/sample.

Mycoplasma bovis Testing - WVDL has completed validation of a *Mycoplasma bovis* ELISA for bovine serum samples. The test will be offered weekly (Monday) at the Madison location. Samples must arrive by Friday in order to be tested on Monday. Cost per sample is \$10 for in-state and \$15 for out-of-state clients.

WVDL is also now offering *Mycoplasma bovis* testing on semen samples by PCR. This test is currently being run on Tuesdays and samples must be received no later than Friday the week before. The cost is \$30/sample for in-state clients and \$45/sample for out-of-state clients. Raw and extended semen are acceptable sample types for this assay.

Tritrichomonas foetus PCR Testing - The WVDL is proud to offer a pooling option for *Tritrichomonas foetus* real-time PCR testing. Although the feasibility of pooling has been previously published for this assay, WVDL conducted a small validation to make sure pooling is a viable option for our clients. We validated the pools to contain up to 5 samples per pool. As with any PCR, pooling does decrease the sensitivity of the assay due to a dilution factor of the sample. During our validation we found a decrease of 1.5-3 C_T s per pool containing one positive sample and 4 negative samples. During our initial validation of our real-time PCR assay, we tested 25 positive field samples and the highest C_T detected from those animals was 34. With this information in mind, we feel that the loss of up to 3 C_T s will not affect the detection rate of our assay for its purpose. Beginning August 1, 2018, WVDL will offer a pooling option for *Tritrichomonas foetus* rtPCR testing with a cost of \$27.46/pool for in-state clients and \$41.19 for out-of-state clients. The WVDL will pool the 5 samples for our clients. Please do not pool your samples prior to submission. We will automatically unpool positive samples, so as to determine which sample(s) are causing the positive result. Remember that preputial scraping/genital washing should be submitted in TF InPouches and will be incubated for 24 hours prior to rtPCR. This test is offered Tuesdays and Thursdays. This pooling option is only for real time PCR and does not affect culture submissions.

Strangles Testing - WVDL is offering *Streptococcus equi equi* and *Streptococcus equi zooepidemicus* real time PCR confirmation on bacterial isolates. The test cost per isolate is \$25 for in-state clients and \$37.50 for out-of-state clients. Well isolated colonies on bacterial culture plates are the preferred samples for submission. Please circle colonies if a pure culture is not submitted. This test is offered on Tuesday and Thursdays.

Barron Laboratory to offer NPIP Salmonella ser. Pullorum-Typhoid reactor birds or birds from a Salmonella ser. Enteritidis-positive environment necropsy - For the same price as an avian necropsy, the Barron Laboratory will be performing NPIP- approved necropsies for Pullorum-Typhoid reactor birds or birds

from a *Salmonella* ser. Enteritidis-positive environment. Please submit whole birds and indicate to staff their status, so as the proper necropsy can be performed.

New human cases of Salmonellosis caused by Multidrug Resistant (MDR) Salmonella ser. Heidelberg since December 2017 - As the weather warms, there has been an increase in bovine cases of MDR Salmonella enterica subspecies enterica serotype/serovars Heidelberg. In addition, the CDC has reopened their investigation into the multidrug resistant Salmonella ser. Heidelberg due to new human cases. Since December 2017, the WVDL has isolated this MDR Salmonella ser. Heidelberg strain 10 times from bovine fecal or tissues samples. This is a gross under-representation of the amount of disease. From these isolates and isolates from previous years, we have noticed a regional difference between the two dominate PFGE patterns. The JF6X01.0523 strain is most commonly associated with the Midwest or animals originating from the Midwest. This strain is the more virulent strain and more common, whereas the JF6X01.0590 strain resides in the southeast and has demonstrated less mortality. There is ongoing research to understand the virulence differences between these two PFGE patterns as they both contain the same MDR genes. The WVDL continues to work with our state and federal counterparts to understand this outbreak, which has been occurring since 2015.

Center for Disease Control and Prevention (CDC)
https://www.cdc.gov/salmonella/heidelberg-11-16/index.html
Wisconsin Department of Health Services
https://www.dhs.wisconsin.gov/salmonella/heidelberg/index.htm
Wisconsin Department of Agriculture, Trade and Consumer Protection
https://datcp.wi.gov/Pages/News_Media/2016.11.29_SalmonellaHeidelberg.aspx
Wisconsin Veterinary Diagnostic Laboratory
https://www.wvdl.wisc.edu/index.php/salmonella/

Quantification of Salmonella enterica subspecies enterica Serotypes/Serogroups Identified at the WVDL in 2017 - In light of the outbreak that is going on with Salmonella ser. Heidelberg, the WVDL would like to keep our clients aware of the serotypes and serogroups identified at the WVDL in 2017. Salmonella enterica subspecies enterica contains greater than 2,500 different serotypes, also called serovars. Each serotype is identified at the WVDL using various sera that bind and agglutinate if a particular molecular pattern is present on the surface of the Salmonella bacterium in question. These agglutination reactions are run and positive reactions are used to first group (Group A, B, C1, C2, D1, E1, and G) the Salmonella isolate and then serotype it to identify a specific serotype name such as Salmonella ser. Heidelberg is in the Group B along with Salmonella ser. Typhimurium.

At the WVDL, not all *Salmonella* are serotyped fully as to save money for our clients. Often for bovine cases, only one isolate from an animal or sample will be fully serotyped and the other *Salmonella* isolates will be serogrouped. As long as the other *Salmonella* isolates serogroup (e.g. serogroup B for *Salmonella* ser. Heidelberg) and look the same on the agar plate as the other *Salmonella* colonies that have been serogrouped then no further serotyping is performed and the serogroup of the isolate is reported. Therefore in the table below, more serogroups have been identified than serotypes as a single case may have many *Salmonella* serogrouped, but only one *Salmonella* isolate that was serotyped. As an example, a group of 10 feces submitted for *Salmonella* culture might obtained 5 *Salmonella* isolates (5 of the 10 animals had *Salmonella* isolated), where only one will be serotyped and the other four will be serogrouped (e.g. 1 serotype reported and 4 serogroups reported). The WVDL deals with a variety of *Salmonella* serotypes obtained from various species and samples types. Table 1 demonstrates the variety that the WVDL identified in 2015.

The WVDL identified a total of 2,158 *Salmonella* isolates in 2017. This table does not include isolates that could not be fully serotyped by ourselves or the National Veterinary Services Laboratories (NVSL) or monophasic *Salmonella* except for 1,4,[5],12:i:-. Of those isolates 67% were bovine, 30% were avian, 0.3% were equine and 3% were canine, porcine, caprine and feline in origin. Fifty-one serotypes were identified from 10 serogroups. Access this table here: All *Salmonella enterica* subspecies *enterica* serotypes/serogroups identified in 2017'.

Staff Spotlight: Todd McCoy, Barron Microbiology Supervisor



You have worn many hats at WVDL, How long have you been a 'WVDLite' and tell us about all your expertise and the jobs you've had here?

I have been with the WVDL for almost 15 years, beginning in the TSE lab as a lab technician, advancing on to a project microbiologist that moved up in the lab (one flight of stairs) to Virology and Molecular Diagnostics as a microbiologist senior. After working a few years in the rarified air that is the 3rd floor of the WVDL, I decided to move farther up in the world (North) to Barron to work in Serology and now I have recently been hired as the Microbiology Supervisor.

Where are you from? What high school and college did you graduate from? What are your degree(s) in?

I grew up in northern Illinois in a little town called Woodstock, no not that one but populated by people that didn't complete the journey east. I attended the private high school in town and after graduation I went to UW Green Bay and majored in Ecology. I also received an honorary degree of a B.S. in T.C.B!

What would your dream job be? To dream up jobs for other people!

As an expert in this field, what are your top five 'flavors' of beers or microbrews?

I have always been a fan of a Bitter Woman, but it is always a good idea to have a Scapegoat Ale around when the Moose Drool is flowing. Sometimes when you are stuck with a Polygamy Porter, it is best to leave those behind and ship out on an Edmund Fitzgerald Porter!

What is one thing on your 'Bucket List'?

Every time I look on my back, someone puts a sign that says "kick me".

If you could time travel, where would you go and why? Probably last Thursday. I haven't seen my wallet since then!

How does a Bears' (& Cubs') fan survive this long in Wisconsin? Next question!

What's the best piece of advice you've received? Don't spit into the wind!

Virology

Diagnostic / Surveillance testing

BVD PCR retests - Since the inception of the BVD PCR program, BVD re-tests (any POS BVD PCR result) have been offered free of charge. This was done to encourage business/submissions for the program and for WVDL to obtain more information on BVD (EDTA, ear notch, serum and nasal swabs were required to be resubmitted). Below is a brief summary of the results:

- A pooling strategy followed by individual testing using a highly optimized extraction and rRT-PCR protocol facilitates a more cost effective high-throughput process.

 Ct values for pools are not predictive of whether any of the samples within that pool came from PI
- animals.
- Ct values for individual samples are predictive of PI status.

 - Individual samples with Ct levels 30 or greater usually cleared by 4 weeks
 The lower the Ct below 30, the more strongly suggestive of a PI animal.
 An animal testing positive and retesting positive ≥ 4 weeks later is strongly indicative of a confirmed PI animal.
- o A strict regimen for cleaning the ear notcher is required for this program to be cost effective. On some
- farms, whole blood samples were easier to keep uncontaminated.

 Nasal swabs are a noninvasive sample which does not rely on a tool that has to be cleaned between animals. Nasal swab samples can be arrayed in 96-well format which makes it amenable to high through

NOTE: Beginning August 1, 2018, BVD PCR retests will no longer be free of charge and will be billed back to the client for the following reasons:

- The BVD PCR testing program is well established; WVDL now has the data that it needed at the time of the program inception.
- Through customer education (Dr. Kurth, Dr. Sockett and others); the number of PI animals has decreased.
- Charging for BVD re-tests will allow WVDL to be compensated for the work performed.

Current prices: BVD PCR Pool: \$31.20 BVD IND PCR: \$8.84

West Nile Virus (WNV) – Since 1999, health officials began a surveillance program to monitor the mortality due to WNV in avian species, testing primarily members of the Corvidae family (crows, ravens and blue jays). Dead birds throughout Wisconsin are submitted to WVDL via public health departments. Virology personnel remove skin samples from dead birds and test for WNV by PCR. A county is closed after one bird is determined to be positive and no other birds will be tested from that county after that result. As of 7/3/18:

- 24 birds tested from 19 counties (21 American Crows, 2 Blue Jays, 1 Common Raven)
- 19 of the birds tested negative.
- 5 of the birds (1 Blue Jay & 4 American Crows) have tested positive.
 5 of the counties are closed (Columbia, Washington, Racine, Sauk, Green Lake)

Oral Fluid Porcine Assays – In February 2018, WVDL developed oral fluid porcine assays at the request of DATCP and WPPA. Tests include PRRS ELISA, PRRS PCR and SECD PCR testing on oral fluids. SECD PCR tests include porcine epidemic diameter virus (PEDV), transmissible gastroenteritis virus (TGEV) and swine delta corona virus (SDCoV). *Only rope extracted oral fluids will be accepted as an appropriate sample type for these tests.* these tests. This is what you need to know:

- The WVDL requests that veterinarians send in oral fluids samples (min. of 5 ml) from rope testing kits.
- These rope-testing kits are currently available via email to supplyroom@wvdl.wisc.edu
 Testing will be done on Tuesdays (PCR) and Wednesdays (ELISA) with 24-hour turnaround. Samples need to be at WVDL by noon prior to testing day. The 24-hour turnaround time may be extended if samples require repeat testing.
- PRRS vaccinated animals will most likely be antibody POSITIVE.

 Please see DATCP website for up to date information on testing regulations https://datcp.wi.gov/Pages/Programs Services/SwineMovement.aspx

| Test | Sample Type | Cost (in state) | Test set up day | Result Available |
|---------------------------------|-------------|-----------------|-----------------|------------------|
| PRRS Ab ELISA* | Oral Fluid | \$8 | Wednesday | Within 24 hrs. |
| PRRS PCR (NA and EU strains) | Oral Fluid | \$45 | Tuesday | Within 24 hrs. |
| SECD PCR Panel** | Oral Fluid | \$65 | Tuesday | Within 24 hrs. |
| Porcine Oral Fluid Panel*** | Oral Fluid | \$80 | Tuesday | Within 24 hrs. |

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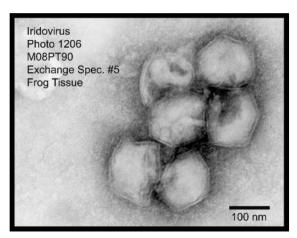
NAHLN Exercise

On May 8-10, 2018, WVDL participated in a FMD outbreak scenario exercise. The exercise was designed to introduce an escalating outbreak of foot-and-mouth disease (FMD) in North America and to drive play to include resource management and incident command at the county, state, and federal levels.

 Many sections at WVDL were involved, including Sample Receiving, IT and Virology.
 The exercise specifically tested the automatic messaging of results from WVDL UVIS to the NAHLN system, and confirmed the importance of clear, accurate and timely communication within WVDL and resource agencies.

A current hurdle is the inability to test milk. This is in the process of being approved by NAHLN, and poses logistical and implementation challenges for WVDL and resource agencies. WVDL testing load (ex. how would FMD affect the export industry) would greatly affect our testing capacity (available microbiologists, laboratory space for testing, etc.).

WVDL Electron Microscopy Services



Wisconsin Veterinary Diagnostic Laboratories Electron Microscopy services offer negative staining of fecal, lesions and tissue culture isolation fluids for viral identification. Price is \$37.50 per sample in state and \$56.63 out of state per sample.

Pathology Sciences

The WVDL Welcomes Two New Pathologists - Dr. Lorelei Clarke (left) and Dr. Jaimie Miller (below) are the newest additions to the WVDL Pathology Team. Dr. Clarke was born and raised in Hawaii and earned her DVM (2012) from Colorado State University. She recently completed a combined residency-PhD program in anatomic pathology at the University of Georgia, becoming board certified in 2016 and earning her doctorate later this year. Her doctoral work focused on arboviral diseases of white-tailed deer, and she has broader interests in infectious diseases of wildlife and livestock. Outside of the lab, Dr. Clark enjoys hiking, camping, marathon running and water sports. Dr. Miller has both a Bachelor of Science (Zoology) and a Bachelor of Arts (Classics) degree from the University of Florida (2005). Before veterinary school, she worked at a parasitology lab and wildlife disease lab, where her interest in pathology began. She earned her DVM from UF in 2012 before traveling north to the University of Wisconsin SVM where she completed her anatomic pathology residency (2015) and stayed on as a Clinical Instructor, passing the American College of Veterinary



Pathologists (ACVP) certifying exam in 2017. Dr. Miller and her husband, Seth, have a two-year old son Jack and will welcome their second son in September.

TSE Testing at WVDL Transmissible Spongiform
Encephalopathy (TSE) Lab at the
WVDL was formed in 2002 in
response to the discovery of
Chronic Wasting Disease (CWD) in
the wild deer population in



Southwest Wisconsin. We have been conducting CWD testing in partnership with the Wisconsin Department of Natural Resources every year since and have now tested 209,946 animals from all around the state. During the 2017 testing season, we tested 9,882 samples and identified CWD in 600. Five counties had their first cases of CWD: Lincoln (1 of 47), Oneida (1 of 199), Eau Claire (1 of 89), Milwaukee (1 of 63) and Dodge (1 of 166). Of the 72 counties in Wisconsin, 25 currently have CWD in its wild deer herd populations. Of the 25 counties, 18 are in the southern farmland zone, four are in the central forest or central farmland zones and 3 are in the northern forest zone. The WI DNR reports that the disease is slowly geographically spreading from the southern farmland zone. For more information about CWD test results, please visit the WI DNR website at https://dnr.wi.gov/topic/wildlifehabitat/results.html.

In May, we identified the first case of Chronic Wasting Disease in a reindeer in North America. We also tested samples from an Iowa County captive deer farm that were collected in a collaborative effort between the United States Department of Agriculture (USDA) and the Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP). We tested 79 adult animals and 21 tested positive for CWD. Interestingly, of the 21 CWD positive animals, 20 were male and only 1 was female. In addition, we performed the testing that identified CWD in several other captive deer and elk farms in Wisconsin. The farms with CWD positive animals are located in Dane, Forest, Richland, Sauk and Washington counties. The findings were confirmed by the National Veterinary Services Laboratory in Ames, Iowa.

In addition to CWD, we also test for other types of TSE's including; Bovine Spongiform Encephalopathy (BSE/Mad Cow Disease) and Scrapie as part of the national surveillance programs. Over the last 12 months we have tested 10,206 cattle for BSE, 24,938 deer and elk for CWD and 2,028 sheep and goats for Scrapie.

Please Remember When Submitting Forensic and/or Insurance Claim Submissions - We have a new submission form that <u>MUST</u> be used for all legal, potentially legal or insurance claim cases -- <u>Forensic and Insurance Claim Submission Form</u>. Submissions that involve possible litigation require a significant investment of time and resources by all sections at WVDL in order to provide results of sufficient detail for legal proceedings (including but not limited to chain of custody documentation, photodocumentation, in-depth interpretation, radiography, etc.). All other pathology cases can be submitted using the <u>General Submission</u> Form.

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