VDx: Unlocking Complex Diagnostics

VDx now offers PARR testing in-house on formalin-fixed tissue

Complicated Case?

Is this cat's chronic lymphocytic enteritis really chronic IBD or is this early small cell lymphoma? Is this dog's splenic nodule or enlarged lymph node benign or really indolent lymphoma?

We're here to help.

PCR for Antigen Receptor Rearrangement (aka PARR) is a tool which has greatly increased the sensitivity in diagnosing lymphoma in difficult cases, helping diagnose lymphoma earlier and with less invasive sampling. VDx is currently the only private, non-academic laboratory in the U.S. to offer PARR testing on formalin-fixed tissue and cytology samples.

Why choose VDx Veterinary Diagnostics for PARR testing?

PARR analysis can be run on biopsy or cytology specimens, including those already evaluated at other labs. PARR testing at VDx is rendered with a full evaluation by a pathologist, which includes review of the biopsy/cytology specimen and any IHC/ICC or flow cytometry data. Turnaround time is 7-9 working days from sample receipt at the laboratory.

At VDx, we specialize in diagnostics and our reputation for outstanding quality and service proves it. The board-certified pathologists at VDx Veterinary Diagnostics offer exceptional biopsy/cytology service to Oncologists and Internal Medicine Specialists in Northern California – Now we'd like to extend the same service to you.

Our goal is to provide you with a personalized service not available at any other laboratory. We believe when it comes to providing comprehensive evaluations, especially for your complex cases, experience does matter.

Whether you wish to send a sample directly to us or would like another laboratory to forward case materials to VDx for a second opinion, we're here to help.

Choice is good.

VDx allows you and your staff to focus on what matters most – caring for your patients.

No two laboratories are alike and that's the way it should be. Sending a sample to VDx Veterinary Diagnostics is easy, please call 1-877-753-4285 and a laboratory assistant will be more than happy to provide you with submission information and pricing.

Questions?

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VDx®
Veterinary Diagnostics

PARR testing offered at VDx

There are numerous indications for using PARR testing in feline or canine lymphoid diseases, especially when the morphological, cytological or IHC/ICC properties of a lymphoid cell population are inconclusive. VDx Veterinary Diagnostics offers PARR as a tool for increased sensitivity in diagnosing lymphoma.

- In addition to formalin-fixed tissue, PARR testing at VDx can also be run on cytology samples.
- Results are typically available within 7-9 working days of PARR request and/or sample receipt at VDx.

VDx offers two forms of PARR testing;

- Stand-alone testing on biopsy or cytology samples previously submitted to VDx.
- PARR Profile which includes full biopsy or cytology evaluation (including microscopic description, diagnosis and comments) plus PARR testing. In some difficult lymphoma cases IHC/ICC may be required in conjunction with PARR to arrive at an accurate diagnosis. In these cases IHC/ICC will be run at a nominal charge and only with authorization.

VDx also offers 2nd opinions, including PARR testing, on biopsy or cytology samples previously evaluated at other laboratories.

Materials sent from other laboratories will be consumed during testing, in whole or in part, and cannot be returned to the originating laboratory. To submit samples previously evaluated elsewhere please request the following from the originating laboratory:

Cytology: All stained and unstained cytology slides. VDx will review and chose the best.
Biopsy: Paraffin block(s) (preferred), or at least 10 unstained recuts on positively charged slide from each block.

Biopsy and cytology samples not initially analyzed at VDx must be evaluated by a VDx pathologist prior to PARR evaluation – All 2nd opinion submissions will be run as a PARR Profile.

Why VDx?

Since 2001, the pathologists and staff of VDx Veterinary Diagnostics have focused upon what veterinarians have told us they want out of a laboratory – Accurate results delivered on time, pathologists who don't "fence sit" and are readily available for consultations, plus a customer service staff that actually does what it says it is going to do. Don't your patients deserve results you trust?

"I receive accurate and thorough reports on samples I send to VDx. I value the accessibility and expertise of VDx pathologists and their personal interest in each of my cases. Prompt, courteous and professional service makes it easy for me to do business with VDx. I encourage my colleagues to send their biopsies to VDx."

-Marcia Smith, DVM, DACVIM (Small Animal)

VDx® Veterinary Diagnostics

PARR Testing
For The Diagnosis of Lymphoma in Dogs and Cats
FREQUENTLY ASKED QUESTIONS

WHAT IS PARR / CLONALITY PCR?

PCR for Antigen Receptor Rearrangement (aka PARR or Clonality PCR) is a tool which has greatly increased the sensitivity of diagnosing lymphoma in difficult cases, helping diagnose lymphoma earlier and with less invasive sampling.

This technique is based on the fact that during maturation, lymphocytes undergo a series of genetic alterations (VDJ rearrangements) which are unique from cell to cell, and ultimately culminate in almost unlimited variety among the antigen receptors on B and T lymphocytes. By using PCR primers to amplify the area encoding diversity of the immunoglobulin heavy chain (IgH) of B cells and the gamma subunit of the T cell receptor of T cells (TCRy), DNA from clinical specimens can be analyzed to determine whether the lymphocytes in a specimen share identical antigen receptors (ie are "clonal"), or whether they are genetically different (ie "polyclonal"). As suggested by the clonal theory of cancer, a clonal expansion is very highly suggestive of neoplasia.

WHEN IS PARR TESTING APPROPRIATE?

PARR testing is especially useful when the results of biopsy or cytology evaluation are inconclusive or are suggestive, but insufficient for a definitive diagnosis, of lymphoma.

PARR should be conducted as part of a stepwise process, which begins with clinical assessment (presentation, history and other lab work) along with microscopic evaluation of tissue (cytology or biopsy) and in some cases immunohisto/cytochemistry.

It can be tempting to view PARR as a shortcut to diagnosis of lymphoma. However, when steps are omitted (eg biopsy or cytology, immunohistochemistry), misdiagnosis or errors in interpretation may sometimes occur. To prevent this, PARR evaluation at VDx always includes morphologic review by a pathologist.

WHAT SAMPLES ARE SUITABLE FOR PARR?

VDx can perform PARR analysis on the following types of canine and feline specimens:

- Cytology samples: including air dried FNAs and fluid or blood smears (stained or unstained).
- Biopsy samples: including formalin-fixed tissue, as well as paraffin-embedded tissue from unstained recut slides or paraffin blocks.

We can also analyze biopsy and cytology samples processed at other labs. Biopsy and cytology samples not initially evaluated at VDx will also be evaluated by a VDx pathologist in conjunction with PARR evaluation. To submit samples previously evaluated elsewhere, please request the following from the originating lab:

- Cytology: All stained and unstained cytology slides. VDx will review and chose the best.
- Biopsy: Paraffin block(s) (preferred), or at least ten unstained recuts on positively charged slides.

Note: Material sent from other labs will be consumed during testing, in whole or in part, and cannot be returned to the originating lab/party.

HOW IS PARR DIFFERENT FROM IMMUNOHISTOCHEMISTRY AND IMMUNOCYTOCHEMISTRY?

Immunohistochemistry (aka IHC, for biopsies) and immunocytochemistry (aka ICC, for cytologies) are techniques utilizing antibodies to specific cellular components (such as parts of the B and T cell antigen receptors) to determine the phenotype or lineage of cells in a specimen. IHC/ICC allows determination of the types of cells that are present but cannot directly differentiate neoplasia vs. reactive change (ie it cannot determine whether a population of T or B cells is genetically identical).

However, IHC/ICC is used as an adjunct to evaluation of cellular morphology and assessment of tissue architecture (ie cytology and biopsy evaluation) and to assist in the interpretation of PARR results. In some difficult lymphoma diagnoses, IHC/ICC must also be performed and interpreted in conjunction with PARR to arrive at an accurate diagnosis. The pathologist will advise if this is the case. Omitting IHC/ICC can lead to increased risk of misinterpretation of PARR results.

HOW IS PARR DIFFERENT FROM FLOW CYTOMETRY?

Flow cytometry (or "flow"), is similar to IHC, but performed on fluid samples or cell suspensions. Like IHC/ICC, flow also uses antibodies to detect various cell surface markers, but because flow is performed on unfixed cells, a greater array of cell markers can be evaluated, allowing more fine differentiation among various classes of lymphocytes.

Certain patterns are very strongly associated with various lymphoid malignancies. However, unlike IHC, which allows evaluation of phenotype in conjunction with tissue architecture, flow does not allow assessment of tissue architecture or cell morphology, unless biopsy or cytology are also performed, thus flow results should not be interpreted in a vacuum. For proper interpretation, flow results must be evaluated in conjunction with other clinical variables, including presentation, history and morphologic assessment.

IS ALL PARR TESTING EQUIVALENT?

No. Different labs analyze different types of specimens, use different primer sets and different detection technologies with differing sensitivities. Thus, PARR results may occasionally differ between labs.
Not all labs have the ability to perform PARR analysis on formalin-fixed paraffin-embedded biopsy tissue and not all labs include review by a pathologist. VDx does!

At VDx, we work closely with Dr. Peter Moore and the Leukocyte Biology Laboratory at UC Davis and utilize an advanced set of primers that allow increased sensitivity over earlier published reports. Our primer set is continually undergoing refinement, improving the sensitivity and specificity of the assay**.**

WHAT ARE THE SENSITIVITY AND SPECIFICITY OF PARR?

Dog	Cat
T cell: ~95% sensitivity.	T cell: >90% sensitivity.
B cell: ~80% sensitivity.	B cell: ~50-60% sensitivity

The specificity of this test is excellent, especially when performed with rigorous quality control (all assays at VDx are performed in duplicate or triplicate) and in conjunction with morphologic assessment, IHC/ICC and pathologist review.

ARE THERE FALSE NEGATIVES AND FALSE POSITIVES WITH PARR?

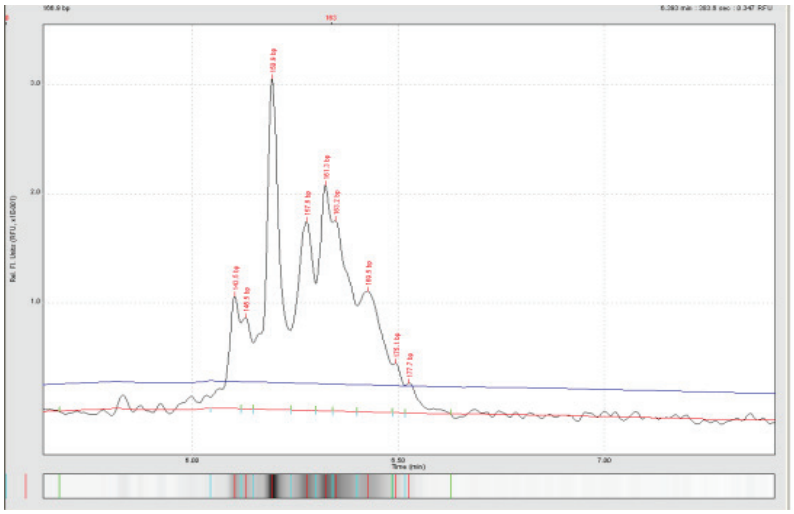
Yes. No lab test is perfect. False negative results may occur for one or more of the following reasons:

1. Clonal rearrangements that fall outside of the areas assessed by primers. The canine and feline genomic structure is complex and VDJ recombinations may occur at numerous sites, not all of which are detected by current primer sets.
2. Mutations or deletions at the primer binding sites, which block primer binding and prevent amplification. This is particularly common with B cell lymphomas, due to the fact that B cells undergo somatic hypermu - tation as part of the affinity maturation of the immune response (and the reason that sensitivity of the B cell assay is lower).
3 Large numbers of reactive lymphocytes in a specimen, which may drown out the presence of a subtle neo plastic population. This is a particular risk in lymphoid tissues (lymph nodes, spleen, tonsil, GALT, etc…), inflamed processes (eg inflamed lymphoma) and cases of early emerging lymphoma where there is a heavy background of reactive or preneoplastic disease (eg early lymphoma arising out of IBD).

False positive results may occur for one or more of the following reasons:

1. Rarely, very highly focused immunologic responses may present with a clonal pattern, ie "reactive clonal expansion". Eg, lymphocyte clonality has been observed with E. canis infection, with idiosyncratic drug reactions, and in the T cell infiltrates in regressing histiocytomas (PF Moore, pers comm).
2. Very low numbers of B or T lymphocytes in a specimen.
3. Additionally, while clonality patterns correlate strongly with lineage (clonal IgH rearrangements are generally associated with B cell neoplasia, TCRy with T cell neoplasia), some lymphomas may exhibit rearrangement of both IgH and TCRy (aka "cross-lineage rearrangement), complicating diagnosis. Thus, PARR can lead to erroneous results if used as a sole means of lineage determination.

These are reasons why morphology (path review) and IHC/ICC are critical parts of a complete workup. When the full workup is employed, the risk of false positives is very low.



Feline TCRy assay: Clonal spikes among a polyclonal background = lymphoma among a background of inflammation / IBD.

1-877-753-4285

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