

Performance of the CONFIRM™ anti-CD3 Rabbit Monoclonal Antibody (clone 2GV6)

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I. Intended Use:

This antibody is intended for *in vitro* diagnostic (IVD) use. Ventana Medical Systems' (Ventana) CONFIRM™ anti-CD3 (2GV6) Primary Antibody is a rabbit monoclonal antibody (IgG) directed against the nonglycosylated epsilon chain of the human CD3 molecule. This antibody is intended for use to qualitatively identify T-cells by light microscopy in sections of formalin fixed,

paraffin embedded tissue on a Ventana automated slide stainer. The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

II. Background:

CD3 bearing T-cells are readily found in the T-cell (e.g. paracortical region) regions of lymphoid organs (lymph nodes and spleen) and then distributed throughout all visceral organs except the brain. CD3 is a marker of the T-cell lineage and differentiation in T-cell neoplasms. Leukemias are the 5th leading cause of death due to cancer while lymphomas are the 8th leading cause of death due to cancer [1]. Evaluation of CD3 in lymphoid tissues is widely used to distinguish T-cell malignancies and B-cell malignancies [2].

The CD3 protein consists of five glycoprotein subunits that form a transmembrane protein complex. The five subunits (γ , δ , ϵ , ζ , η) form a signaling complex with the T-cell receptor (TCR)[3]. Upon recognition of antigen, a signal transduction cascade is initiated through tyrosine kinases and phospholipase C [4, 5]. Expression is initiated in the prothymocyte stage and continues through mature peripheral T-cells[2]. Cytoplasmic localization is lost during the differentiation process and localization is restricted to the plasma membrane in post-cortical T-cells.

The recent development of a stable immunoglobulin

secreting rabbit plasma-cytoma cell line, for use as a fusion partner, has led to a new generation of high affinity rabbit monoclonal antibodies (RMOAB). RMOABs have increased sensitivity compared to mouse monoclonal antibodies with no apparent loss of specificity [6]. Development of RMOABs results in antibodies against a greater variety of epitopes than antibodies generated in mice.

The generation of a RMOAB against CD3 utilized a synthetic 13mer peptide representing the cytoplasmic domain of human CD3 ϵ chain (20 kD) and was synthesized on a semi-automated peptide synthesizer.

This paper describes the performance of CONFIRM anti-CD3 (2GV6) Rabbit Monoclonal Primary Antibody. The data are derived from internal Ventana development studies and external studies [e.g. Cleveland Clinic Foundation (CCF)]. The data include: (a) tour of the body results to detail cross reactivity, (b) tour of all neoplasms again to detail cross reactivity, and (c) a test of 100+ previously characterized leukemia and lymphoma cases from the CCF to show equivalency to clone PS1 (mouse monoclonal).

III. Antibody Specificity

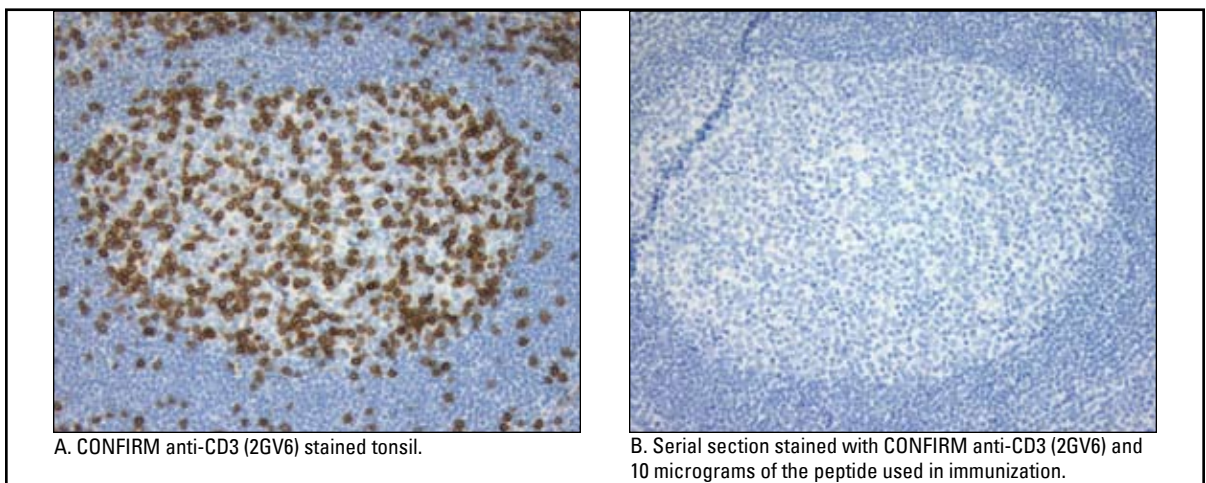
To show that the 2GV6 monoclonal antibody staining was specific to the CD3 peptide used for immunization, a peptide inhibition experiment was performed. In order to show inhibition, slides stained with a mixture of peptide and antibody

had to score 0. Three different peptide concentrations were tested. The following table depicts the staining intensity and background for each sample tested:

Table 1: CD3 Peptide Inhibition

Peptide Concentration	Stain Intensity	Background
10 µg	0	0
100 µg	0	0
1000 µg	0	0
Pos	4	0
Neg	0	0

Figure 1: Peptide Inhibition of 2GV6



III. Immunohistochemical Characteristics of anti-CD3 (2GV6)

Figure 2: Competitive Comparison

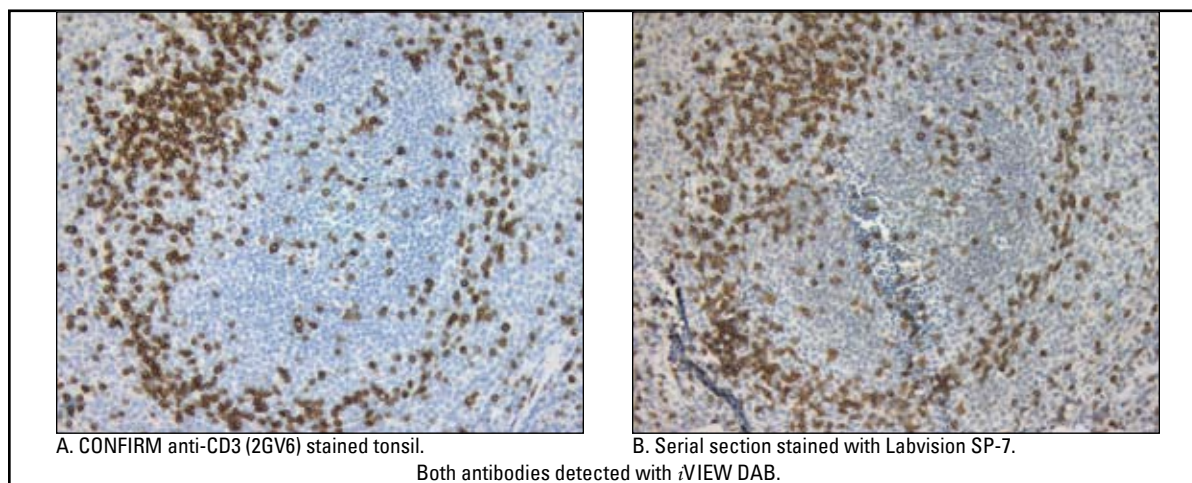


Figure 3: Comparison of *i*VIEW and *ultra*VIEW Detection

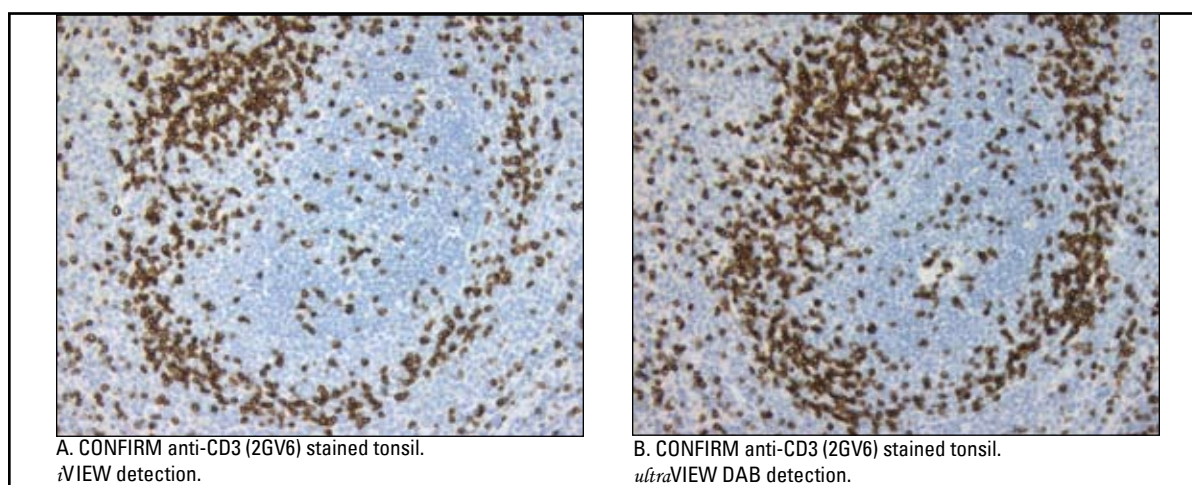


Figure 4: Competitive Comparison on a T-cell Lymphoma

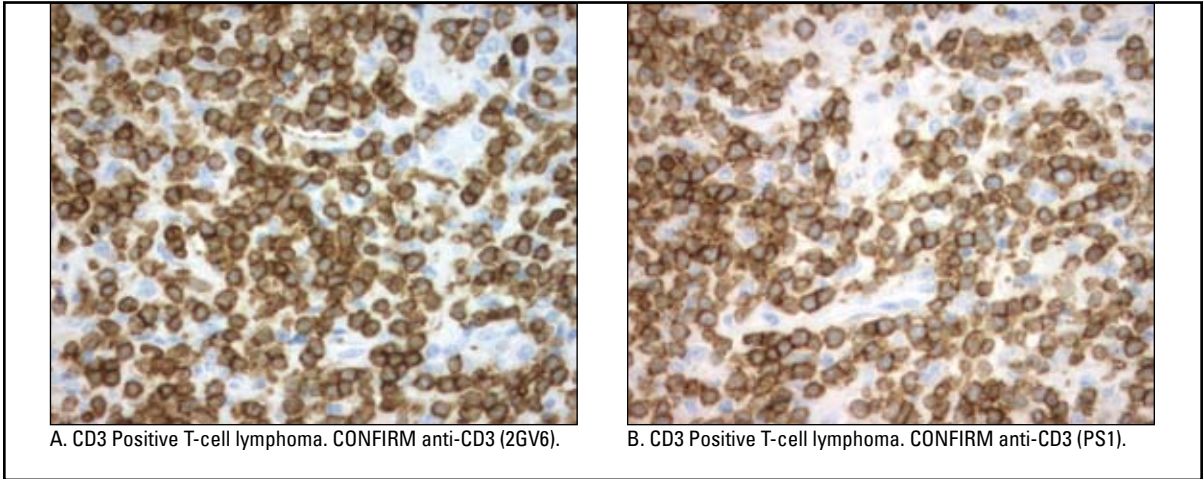


Figure 5: Competitive Comparison on an Acute Myelogenous Leukemia

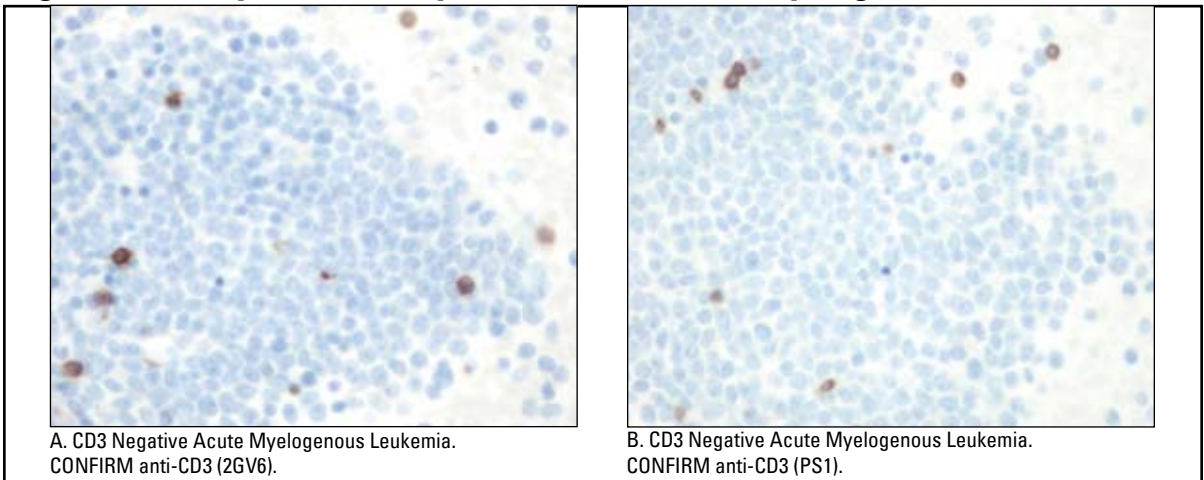
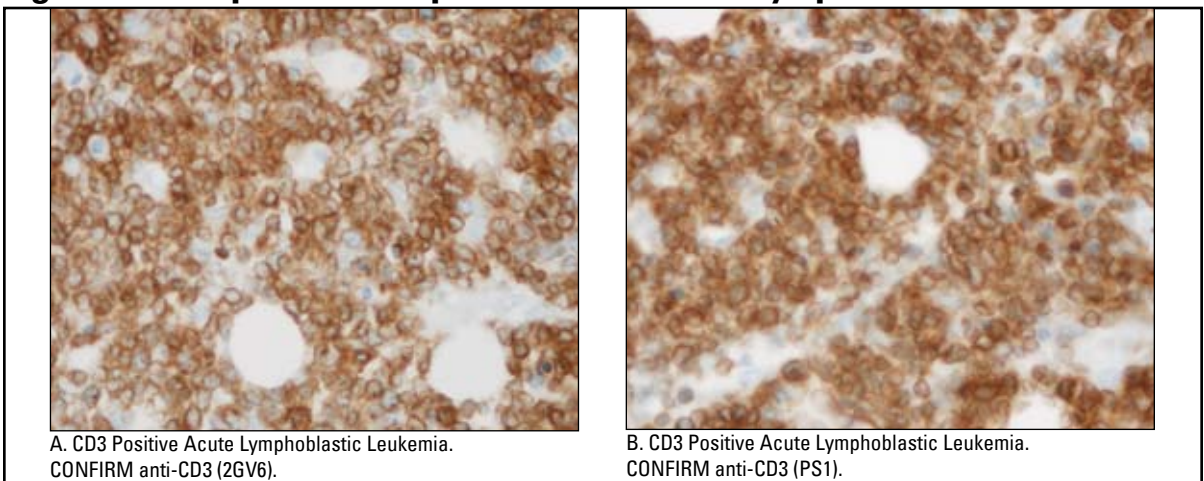


Figure 6: Competitive Comparison on an Acute Lymphoblastic Leukemia



V. Tour of Body

CONFIRM anti-CD3 (2GV6) specificity was tested across a 60 core normal tissue array that showed no specific cytoplasmic/membrane staining for the following normal tissues, however, infiltrating T-cells were noted in all tissues with the exception of the brain. Adrenal (0/3), brain (0/3), breast (0/3), colon

(0/2), fibroadipose tissue (0/1), heart (0/2), kidney (0/3), large intestine (0/1), liver (0/4), lung (0/4), ovary (0/4), pancreas (0/3), prostate (0/4), skin (0/2), small intestine (0/2), spleen (0/3), stomach (0/3), testis (0/3), thyroid (0/3), tonsil (0/3) and uterus (0/4).

VI. Tour of Tumors

CONFIRM anti-CD3 (2GV6) specificity was also tested across a 50 core neoplastic array that showed no specific cytoplasmic/membrane staining for the following neoplastic tissues, however, infiltrating T-cells were noted in all tissues with the exception of the teratoma tissues. Breast (0/4), carcinoids (0/2), colon (0/3), hepatocellular carcinoma (0/2), kidney (0/3), leiomyoma (0/2), liver (0/4), lung (0/2), non T-cell lymphoma (0/3), melanoma (0/2), ovary (0/2), pancreas

(0/3), prostate (0/3), renal cell carcinoma (0/2), sarcoma (0/2), skin (0/1), stomach (0/3), teratoma (0/2), thyroid (0/3), undifferentiated cancer (0/1), and vascular tissue (0/1). (0/3), colon (0/2), fibroadipose tissue (0/1), heart (0/2), kidney (0/3), large intestine (0/1), liver (0/4), lung (0/4), ovary (0/4), pancreas (0/3), prostate (0/4), skin (0/2), small intestine (0/2), spleen (0/3), stomach (0/3), testis (0/3), thyroid (0/3), tonsil (0/3) and uterus (0/4).

VII. External Validation

To validate the staining performance of 2GV6 relative to PS1, a study was conducted at the CCF in Dr. Eric Hsi's laboratory. In the 175 cohort containing leukemias and lymphomas, there was an overall agreement between 2GV6 and PS1 of 100%, with a sensitivity of 100% and specificity of 100%. These data

indicate that the two clones are substantially equivalent in their performance with leukemia and lymphoma samples (see Table 2). Specifically, both clones appropriately stained T-cell lymphomas and leukemias and not B-cell lymphomas and leukemias.

Table 2

		PS1 Assay			% Concordance
		Positive	Negative	Total	
2GV6 Assay	Positive	42	0	42	100%
	Negative	0	133	133	100%
	Total	42	133	175	100%

VIII. Conclusions

These results indicate that CONFIRM anti-CD3 (2GV6) Rabbit Monoclonal Primary Antibody has strong performance characteristics that are well suited for detection of normal and neoplastic T-cells. Additionally, 2GV6 demonstrates strong specificity and sensitivity in neoplastic tissues. This rabbit monoclonal antibody combined with BenchMark®

automation may enable the pathologist to detect CD3 in a standardized manner with excellent reproducibility. In very low level CD3 expressing cells, the CONFIRM anti-CD3 (2GV6) Rabbit Monoclonal Primary Antibody may be utilized with alternative Ventana detection chemistries (e.g. *ultraView™*) to achieve easily interpretable results.

IX. References

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