Supplementary Online Content

Hiemcke-Jiwa LS, ten Dam-van Loon NH, Leguit RJ, et al. Potential Diagnosis of Vitreoretinal Lymphoma by Detection of *MYD88* Mutation in Aqueous Humor With Ultrasensitive Droplet Digital Polymerase Chain Reaction. *JAMA Ophthalmol*. Published online July 19, 2018. doi:10.1001/jamaophthalmol.2018.2887

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. MYD88 p.(L265P) analysis (ddPCR) in treated VRL patients: all ocular fluids were negative after treatment.

	Before treatment		After treatment	
ddPCR	VF(%)	AH(%)	VF(%)	AH(%)
MYD88 p.(L265P) +	3(60)	1(20)[a]	0(0)	0(0)
MYD88 p.(L265P) -	2(40)	4(80)	5(100)	5(100)
Total(samples)	5	5	5	5

[a]This AH sample contained 104 wild-type droplets and 47 mutant droplets (mutation frequency 31%).
Abbreviations: AH: aqueous humor; ddPCR: droplet digital PCR; VF: vitreous fluid; VRL: vitreoretinal lymphoma; +: positive for MYD88 p.(L265P); - negative for MYD88 p.(L265P)

eTable 2. Etiology of investigated uveitis patients in this study. Etiology was based upon standard PCR and Goldmann-Witmer coefficient detection of infectious entities[1] and classified according to *the Standardization of Uveitis Nomenclature Working Group*[2].

Etiology	**No. of patients (%) (N=40)**
Non-infectious uveitis	4(10)
Infectious uveitis	36 (90)
-Toxoplasma	13(33)
-Varicella zoster virus	9(23)
-Herpes simplex virus	5(13)
-Cytomegalovirus	4(10)
-Rubella	4(10)
-Candida	1(3)

eTable 3. Ophthalmological findings and demographics of each of the investigated VRL patients (n=23).

Patient	Diagnosis	Available ocular fluid	Sex	Age	Race	Paired	Treated	Anterior chamber cells	Vitreous cells	Vitreous floaters	Retinal infiltrates
1	VRL	VF; AH	F	80	Caucasian	+		0	4+	3+	Retinal pigment changes
2	VRL	VF; AH	M	64	Caucasian	+		0	4+	3+	Retinal pigment changes
3	VRL	VF; AH	M	67	Caucasian	+		0.5	3+	1+	Retinal pigment changes
4	VRL	VF; AH (OD)	M	65	Caucasian	+		2+	4+	3+	-
4	VRL	VF; AH (OS)	M	65	Caucasian	+		1+	4+	3+	-
5	VRL	VF; AH	M	64	Caucasian	+		0.5	3+	3+	Retinal pigment changes
6	VRL	VF; AH	M	72	Caucasian	+		0	1+	2+	Retinal pigment changes
7	VRL	VF; AH	M	82	Caucasian	+		0.5	2+	4+	Retinal pigment changes
8	VRL	VF; AH	F	73	Caucasian	+		0	2+	2+	Yellow subretinal infiltrates
9	VRL	VF; AH	F	73	Caucasian	+		0	4+	3+	Retinal pigment changes
10	VRL	VF; AH	F	80	Caucasian		+	0	3+	3+	-
11	VRL	VF; AH	M	76	Caucasian	+		0	2+	3+	Retinal pigment changes
12	VRL	VF; AH	M	72	Caucasian		+	0	2+	0	Retinal pigment changes
13	VRL	VF; AH	F	75	Asian		+	0	2+	3+	Yellow subretinal infiltrates
14	VRL	VF; AH	F	77	Caucasian		+	2+	2+	4+	Retinal pigment changes/ Yellow subretinal infiltrates

Patient	Diagnosis	Available ocular fluid	Sex	Age	Race	Paired	Treated	Anterior chamber cells	Vitreous cells	Vitreous floaters	Retinal infiltrates
15	VRL	VF (OS); AH (OD)	M	71	Caucasian	Missing	Missing	0.5;0.5	2+;1+	3+;2+	-; Yellow subretinal infiltrates
16	VRL	VF; AH	F	63	Suriname	+		0.5	3+	4+	Yellow subretinal infiltrates
17	VRL	AH	M	65	Caucasian	Missing	Missing	0.5	2+	3+	-
18	VRL	VF (OD)	F	82	Caucasian	Missing	Missing	1+	2-3+	3+	Yellow subretinal infiltrates
18	VRL	VF; AH (OS)	F	82	Caucasian		+	1+	2-3+	3+	Yellow subretinal infiltrates
19	VRL	VF	M	83	Caucasian	Missing	Missing	0	1+	2+	-
20	VRL	VF	F	79	Caucasian	Missing	Missing	4+	Not visible due to corneal edema	Not visible due to corneal edema	Not visible due to corneal edema
21[a]	VRL	AH (OD)	M	56	Caucasian	Missing	Missing	0	0	0	Aspecific retinal lesions
21 [a]	VRL	AH (OS)	M	56	Caucasian	Missing	Missing	0	0	0	-
21 [a]	VRL	AH (OD)	M	56	Caucasian	Missing	Missing	0	0	0	Aspecific retinal lesions
21 [a]	VRL	AH (OD)	M	56	Caucasian	Missing	Missing	0	0	0	Aspecific retinal lesions
22	VRL	AH (OD)	M	66	Caucasian	Missing	Missing	0.5	1+	2+	-
22	VRL	AH (OS)	M	66	Caucasian	Missing	Missing	0.5	1+	2+	-
23	VRL	AH (OD)	F	75	Caucasian	Missing	Missing	0	4+	3+	-
23	VRL	AH (OS)	F	75	Caucasian	Missing	Missing	0	4+	3+	-

[a]Harvested during (Methotrexate) treatment. Abbreviations: AH: aqueous humor; OD: oculus dextra; OS: oculus sinistra; VF: vitreous fluid; -: no abnormalities detecte

eTable 4. Characteristics and test results of each of the investigated VRL patients (n=23).

Patient	Diagnosis	Available ocular fluid	Paired	Treated	Cytomorphology(VF)	Flow cytometry(VF)	MYD88 p.(L265P)(%FA)	
							VF	AH
1	VRL	VF; AH	+		Missing	-	+ (64)	+ (64)
2	VRL	VF; AH	+		+	+	+ (94)	+ (81)
3	VRL	VF; AH	+		-	+	+ (97)	+ (68)
4	VRL	VF; AH (OD)	+		-	+	-	-
4	VRL	VF; AH (OS)	+		+	+	-	-
5	VRL	VF; AH	+		-	Missing	+ (97)	+ (97)
6	VRL	VF; AH	+		-	-	-	-
7	VRL	VF; AH	+		-	+	+ (94)	+ (95)
8	VRL	VF; AH	+		-	+	+ (7)	-
9	VRL	VF; AH	+		+	+	+ (79)	+ (81)
10	VRL	VF; AH		+	-	+	-	-
11	VRL	VF; AH	+		-	-	+ (47)	+ (32)
12	VRL	VF; AH		+	-	+	+ (40)	-
13	VRL	VF; AH		+	-	Missing	+ (46)	-
14	VRL	VF; AH		+	-	-	-	+ (31)
15	VRL	VF (OS); AH (OD)	Missing	Missing	+	-	+ (33)	-
16	VRL	VF; AH	+		+	+	+ (41)	+ (40)
17	VRL	AH	Missing	Missing	Missing	Missing	Missing	-
18	VRL	VF (OD)	Missing	Missing	+	-	+ (47)	Missing
18	VRL	VF; AH (OS)		+	-	-	+ (35)	-
19	VRL	VF	Missing	Missing	-	Missing	+ (49)	Missing
20	VRL	VF	Missing	Missing	-	Missing	+ (87)	Missing

21[a]	VRL	AH (OD)	Missing	Missing	Missing	Missing	Missing	-
Patient	**Diagnosis**	**Available ocular fluid**	**Paired**	**Treated**	**Cytomorphology(VF)**	**Flow cytometry(VF)**	**MYD88 p.(L265P)(%FA)**	
							VF	**AH**
21 [a]	VRL	AH (OS)	Missing	Missing	NA	Missing	Missing	-
21 [a]	VRL	AH (OD)	Missing	Missing	NA	Missing	Missing	-
21 [a]	VRL	AH (OD)	Missing	Missing	NA	Missing	Missing	-
22	VRL	AH (OD)	Missing	Missing	NA	Missing	Missing	-
22	VRL	AH (OS)	Missing	Missing	-	Missing	Missing	-
23	VRL	AH (OD)	Missing	Missing	Missing	-	Missing	+ (20)
23	VRL	AH (OS)	Missing	Missing	Missing	-	Missing	+ (50)

[a]Harvested during (Methotrexate) treatment. Abbreviations: AH: aqueous humor; FA: fractional abundance (e.g. mutation frequency); OD: oculus dextra; OS: oculus sinistra; VF: vitreous fluid; +: positive test result; -: negative test result

eTable 4. Ophthalmological findings and demographics of each of the investigated VRL patients (n=23).

Patient	Diagnosis	Available ocular fluid	Sex	Age	Race	Paired	Treated	Anterior chamber cells	Vitreous cells	Vitreous floaters	Retinal infiltrates
1	VRL	VF; AH	F	80	Caucasian	+		0	4+	3+	Retinal pigment changes
2	VRL	VF; AH	M	64	Caucasian	+		0	4+	3+	Retinal pigment changes
3	VRL	VF; AH	M	67	Caucasian	+		0.5	3+	1+	Retinal pigment changes
4	VRL	VF; AH (OD)	M	65	Caucasian	+		2+	4+	3+	-
4	VRL	VF; AH (OS)	M	65	Caucasian	+		1+	4+	3+	-
5	VRL	VF; AH	M	64	Caucasian	+		0.5	3+	3+	Retinal pigment changes
6	VRL	VF; AH	M	72	Caucasian	+		0	1+	2+	Retinal pigment changes
7	VRL	VF; AH	M	82	Caucasian	+		0.5	2+	4+	Retinal pigment changes
8	VRL	VF; AH	F	73	Caucasian	+		0	2+	2+	Yellow subretinal infiltrates
9	VRL	VF; AH	F	73	Caucasian	+		0	4+	3+	Retinal pigment changes
10	VRL	VF; AH	F	80	Caucasian		+	0	3+	3+	-
11	VRL	VF; AH	M	76	Caucasian	+		0	2+	3+	Retinal pigment changes
12	VRL	VF; AH	M	72	Caucasian		+	0	2+	0	Retinal pigment changes
13	VRL	VF; AH	F	75	Asian		+	0	2+	3+	Yellow subretinal infiltrates
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16	VRL	VF; AH	F	63	Suriname	+		0.5	3+	4+	Yellow subretinal infiltrates
17	VRL	AH	M	65	Caucasian	Missing	Missing	0.5	2+	3+	-
18	VRL	VF (OD)	F	82	Caucasian	Missing	Missing	1+	2-3+	3+	Yellow subretinal infiltrates
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19	VRL	VF	M	83	Caucasian	Missing	Missing	0	1+	2+	-
20	VRL	VF	F	79	Caucasian	Missing	Missing	4+	Not visible due to corneal edema	Not visible due to corneal edema	Not visible due to corneal edema
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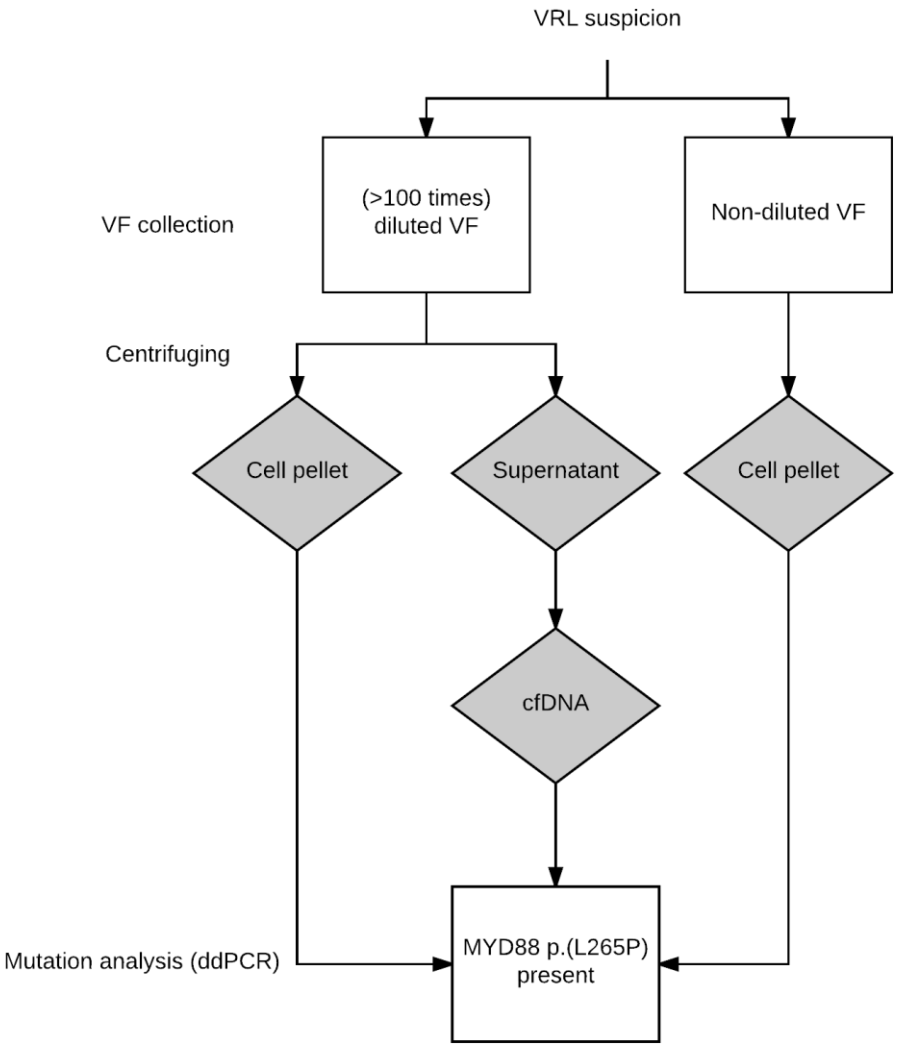
21 [a]	VRL	AH (OS)	M	56	Caucasian	Missing	Missing	0	0	0	-
21 [a]	VRL	AH (OD)	M	56	Caucasian	Missing	Missing	0	0	0	Aspecific retinal lesions
21 [a]	VRL	AH (OD)	M	56	Caucasian	Missing	Missing	0	0	0	Aspecific retinal lesions
22	VRL	AH (OD)	M	66	Caucasian	Missing	Missing	0.5	1+	2+	-
22	VRL	AH (OS)	M	66	Caucasian	Missing	Missing	0.5	1+	2+	-
23	VRL	AH (OD)	F	75	Caucasian	Missing	Missing	0	4+	3+	-
23	VRL	AH (OS)	F	75	Caucasian	Missing	Missing	0	4+	3+	-

[a]Harvested during (Methotrexate) treatment. Abbreviations: AH: aqueous humor; OD: oculus dextra; OS: oculus sinistra; VF: vitreous fluid; -: no abnormalities detected

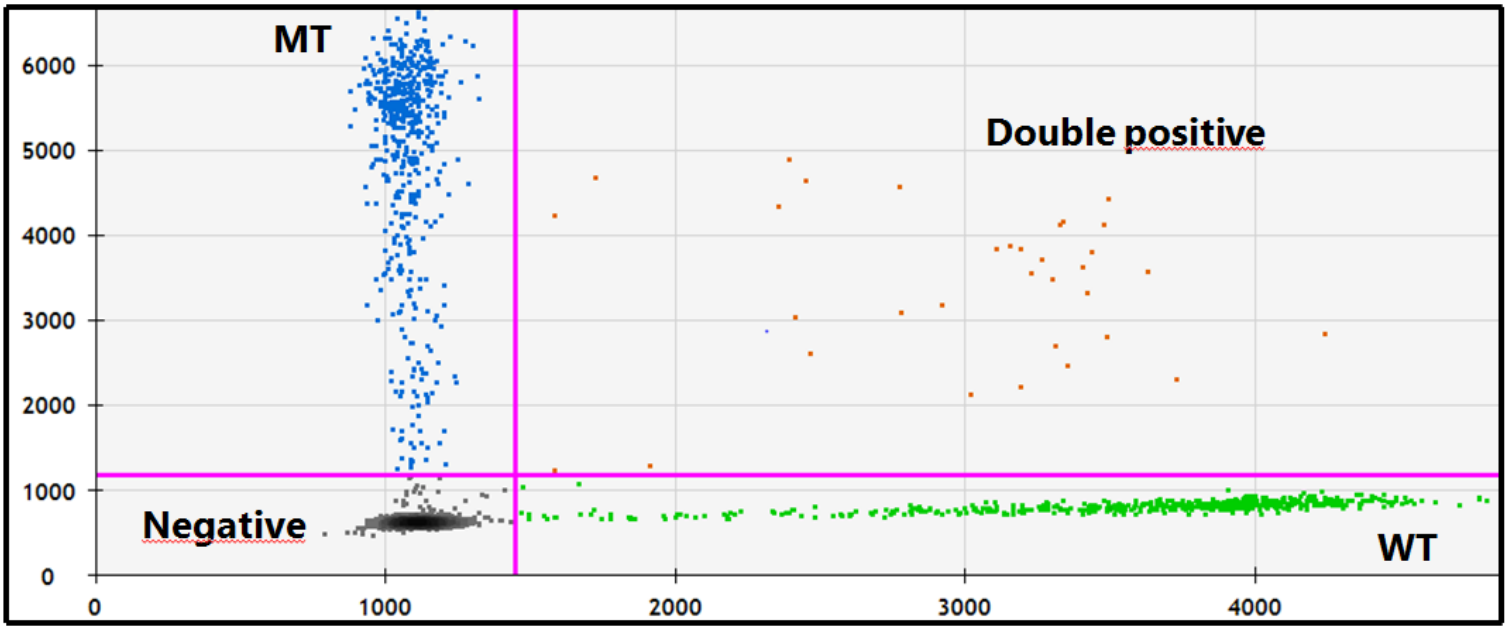
eTable 5. Sensitivity and specificity of *MYD88* analysis in VF (upper panel) and AH (lower panel) compared to routine laboratory tests in diagnostic work-up of VRL.

	Sensitivity [95%CI]			**Specificity [95%CI]**	
	Variable(%)	***MYD88* in VF(%)**		**Variable(%)**	***MYD88* in VF(%)**
Cytomorphology	30.0[10.0-50.0]	75.0[55.0-90.0]		100.0%[62.9-100.0]	100.0%[62.9-100.0]
Flow cytometry	58.8[35.3-82.4]	70.6[47.1-88.2]		100.0%[70.0-100.0]	100.0%[70.0-100.0]
	Variable(%)	***MYD88* in AH(%)**		**Variable(%)**	***MYD88* in AH(%)**
Cytomorphology	33.3[8.3-58.3]	58.3[33.3-83.3]		100.0%[5.5-100.0]	100.0%[5.5-100.0]
Flow cytometry	61.5[30.8-84.6]	69.2[46.2-92.3]		100.0%[20.0-100.0]	100.0%[20.0-100.0]

N=20 and n=18 patients for cytomorphology and flow cytometry on VF; n=18 patients for cytomorphology and flow cytometry on AH. Since cytomorphology and flow cytometry are (usually) not performed on AH samples, the performance of *MYD88* analysis in AH was compared to simultaneous evaluation of VF by these tests (lower panel). Abbreviations: AH: aqueous humor; VF: vitreous fluid



eFigure 1. Flow chart of MYD88 p.(L265P) analysis on cell pellet and cfDNA harvested from diluted and non-diluted VF of patients suspected with VRL. MYD88 p.(L265P) was detected in the cell pellet (diluted and non-diluted VF) as well as the cfDNA (diluted VF) from all patients. Abbreviations: cfDNA: cell-free DNA; ddPCR: droplet digital PCR; VF: vitreous fluid; VRL: vitreoretinal lymphoma



eFigure 2. ddPCR results of a MYD88 p.(L265P)-positive diluted VF sample (cfDNA; mutation frequency 48.0%). Wild-type (WT) droplets are depicted in green, mutant droplets (MT) in blue, double positive droplets (both WT and MT DNA) in orange and negative droplets in gray.

References

1.De Groot-Mijnes JD, Rothova A, Van Loon AM, et al. Polymerase chain reaction and Goldmann-Witmer coefficient analysis are complimentary for the diagnosis of infectious uveitis. *American journal of ophthalmology.* 2006;141(2):313-318.
2.Jabs DA, Nussenblatt RB, Rosenbaum JT. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. *American journal of ophthalmology.* 2005;140(3):509-516.