Online Supplemental Table: Category descriptions

Category	Subcategory	Description
Partial seroconversion (PSC)		Developing, immature antibody response
	PSC1	The index sample was collected after symptoms of short duration (2 weeks or less), from a patient with a clinical syndrome compatible with Lyme disease, prior to effective antimicrobial administration. The antibody response is limited and immature, based on Western immunoblotting, and EIA index values are in the low-positive or high-negative range, compatible with active infection of short duration.
	PSC2	The index sample was collected after past treatment for erythema migrans. A new Lyme-compatible syndrome has been present for a short time (under 2

		weeks), but the new syndrome is non-specific and may not represent re-infection. The antibody response is blunted, with no IgM bands and a single IgG band. The WCS and C6 OD index values are just above cutoff. Taken together, the findings are compatible with past early-stage Lyme disease that was treated promptly enough to blunt the antibody response. Recent re-infection is possible, in addition.
	PSC3	The index sample was collected after recent past treatment for early disseminated Lyme disease, in the setting of persistent (post-treatment) signs and symptoms. The index sample yielded positive WCS and C6 EIAs and at least 2 IgM Western blot bands. Conventional two-tiered testing is considered negative because the symptoms were present for >1

		month at the time of index sample collection. The index sample findings are compatible with treated early Lyme disease, which was treated promptly enough to blunt the antibody response, limiting the antibody repertoire and isotype switching.
Partial Seroreversion (PSR)		Waning antibody response from past (inactive) infection
	PSR1	The index sample was collected after treatment for diagnosed 2nd- or 3rd-stage Lyme disease; no new Lyme-compatible syndrome is present, but persistent symptoms may be present. Based on Western immunoblotting, the antibody response is expanded and mature, with IgM to IgG isotype switching, indicating infection of weeks to months duration prior to treatment. However, the C6 OD index value

		is in the negative range (although usually with a value substantially higher than negative control values) and the WCS OD index value is in the positive range (often with a value substantially lower than the positive control values), suggesting a waning antibody response in the setting of successful treatment for Lyme disease (partial seroreversion).
	PSR2	The index sample was collected in setting of a new clinical syndrome of short duration, with a differential diagnosis including early disseminated Lyme disease. However, based on Western immunoblotting the antibody response is expanded and mature, with IgM to IgG isotype switching. The C6 OD index value is in the negative range (although usually with a value substantially higher than

		negative control values) and the WCS OD index value is in the positive range (often with a value substantially lower than the positive control values). Taken together, the serologic findings are unexpected if the current clinical syndrome is caused by early disseminated Lyme disease in a patient without previous exposure. Although a history of remote Lyme disease is not documented, the findings are most compatible with remote exposure with partial seroreversion, with or without a new (recent) exposure.
	PSR3	The index sample was collected after at least several weeks of symptoms with a syndrome potentially compatible with 2nd- or 3rd-stage Lyme disease, that

		had not yet been treated. There is an expanded and mature antibody response, with IgM to IgG isotype switching. However, the C6 OD index value is in the negative range (although usually with a value substantially higher than negative control values) and the WCS OD index value is in the positive range (often with a value substantially lower than the positive control values). Taken together the serologic findings are unexpected in a patient with active 2nd or 3rd stage Lyme disease, and are more compatible with remote exposure that has resolved, arguing against the idea that active Lyme disease explains the current clinical syndrome.

	PSR4	The index sample was collected in the setting of a new clinical syndrome compatible with late-stage Lyme disease. The IgG antibody response is expanded and mature. A past history of Lyme disease with appropriate treatment has been documented. The C6 OD index value is in the negative range (although usually with a value substantially higher than negative control values) and the WCS OD index value is in the positive range (often with a value substantially lower than the positive control values), likely indicating partial seroreversion, although a new, more recent re-infection cannot be ruled out. However, re-infection after treated *late* Lyme disease is rare.

	PSR5	The index sample was collected after 3-4 weeks of symptoms with a syndrome that was unlikely to have been caused by *B. burgdorferi* infection, although late-stage Lyme disease was considered on the differential diagnosis. An alternative diagnosis was ultimately established, and the patient was not given antimicrobial therapy directed at *B. burgdorferi*. There is an immuature antibody response, with 2 or 3 specific bands on the IgM Western blot, but IgM to IgG isotype switching is absent; the WCS OD index value is just above cutoff, and the C6 OD index value is negative with a value that is substantially higher than negative control values; this pattern is not consistent with late-stage Lyme disease. Taken together, the findings suggest past, early Lyme

		disease that was treated promptly enough to stunt an expanded antibody response with isotype switching; the IgM antibody response is unlikely to be related to the current clinical syndrome.
	PSR6	The index sample was collected after months of symptoms with a syndrome that was unlikely to have been caused by *B. burgdorferi* infection, although 2nd- or 3rd-stage Lyme disease was considered on the differential diagnosis. An alternative diagnosis was ultimately established, and the patient was not given antimicrobial therapy directed at *B. burgdorferi*. There are several specific IgG bands on the Western blot, with little or no IgM reactivity, but an expanded IgG antibody response is not present; a more expanded IgG response is expected in active Lyme

		disease of several months duration. The WCS and C6 EIA OD index values are well above cutoff. Taken together, the findings suggest past Lyme disease with partial seroreversion, although false-positive EIAs cannot be ruled out. Partial seroconversion is favored because the EIA index values are well above cutoff and several specific IgG bands are present on the IgG Western blot.
False positive MTTT (FPM)		Concurrently false positive WCS and C6 EIAs
	FPM1	The index sample was collected after months of symptoms with a syndrome potentially compatible with 2nd- or 3rd-stage Lyme disease. However, only 1 or two IgG Western blot bands are present, a pattern that is not consistent with Lyme disease of several months' duration. Furthermore, the WCS and C6 OD

		index values are just above cutoff. Taken together, the findings likely represent false-positive WCS and C6 EIAs, in a patient whose symptoms are not caused by Lyme disease.
	FPM2	The index sample was collected after weeks of symptoms with a syndrome that was unlikely to have been caused by *B. burgdorferi* infection, although late-stage Lyme disease was considered on the differential diagnosis. An alternative diagnosis was ultimately established, and the patient was not given antimicrobial therapy directed against *B. burgdorferi*. The established diagnosis is known to be associated with false-positive Lyme EIAs. A few IgG bands are present, but an expanded IgG antibody response is not present; this pattern is not consistent with active

		late-stage Lyme disease. The WCS and C6 EIA OD index values are well above cutoff. Taken together, the findings suggest false-positive EIAs, although past Lyme disease with partial seroreversion cannot be ruled out.
	FPM3	The index sample was collected after weeks of symptoms with a syndrome compatible with late-stage Lyme disease. An alternative diagnosis was ultimately established, and the patient was not given antimicrobial therapy directed against *B. burgdorferi*. A few IgG bands are present, but an expanded IgG antibody response is not present; this pattern is not consistent with active late-stage Lyme disease. The WCS and C6 EIA OD index values are well above cutoff. Taken together, the findings suggest false-

		positive EIAs, although past Lyme disease with partial seroreversion cannot be ruled out.
	FPM4	The index sample was collected after weeks to months of symptoms with a syndrome that was unlikely to have been caused by *B. burgdorferi* infection, although late-stage Lyme disease was considered on the differential diagnosis. An alternative diagnosis was ultimately established, and the patient was not given antimicrobial therapy directed against *B. burgdorferi*. Two or fewer IgG bands are present; this pattern is not consistent with active late-stage Lyme disease. The WCS and C6 EIA OD index values are just above cutoff. Taken together, the findings suggest false-positive EIAs.

	FPM5	The index sample was collected after a few weeks of symptoms with a syndrome potentially compatible with stage 2 Lyme disease. An alternative diagnosis was ultimately established, and the patient was not given antimicrobial therapy directed against *B. burgdorferi*. No IgM Western blot bands are present, and only 1 or two IgG Western blot bands are present. The WCS and C6 OD index values are just above cutoff. Taken together, the findings likely represent false-positive WCS and C6 EIAs, in a patient whose symptoms are not caused by Lyme disease. Past Lyme disease with partial seroreversion cannot be ruled out, to explain the seroreactivity.
False negative MTTT (FNM)		Falsely negative WCS and/or C6 EIAs

	FNM1	The index sample was collected after a few weeks of symptoms with a syndrome compatible with early disseminated Lyme disease, prior to treatment. The WCS EIA is strongly positive, and both the IgM and IgG Western blots are positive. The C6 EIA is negative, albeit with an index value substantially higher than negative control values. Taken together, the serologic findings support the diagnosis of early disseminated Lyme disease; the negative C6 EIA is an outlier and is likely falsely negative.
	FNM2	The index sample was collected after several weeks of symptoms with a syndrome compatible with late Lyme disease, prior to treatment, The WCS EIA is positive, and Western immunoblots demonstrate an expanded antibody response with IgM to IgG isotype

		switching. The C6 EIA is negative, albeit with an index value substantially higher than negative control values. Taken together, the serologic findings support the diagnosis of late Lyme disease; the negative C6 EIA is an outlier and is likely falsely negative.