

Supplementary Table 1. Number of individuals positive with EuroLine for each specificity among HBD ($n=236$), SLE discovery ($n=282$) and SLE replication ($n=228$) cohorts, pSS ($n=116$) and SSc ($n=57$). SI values for the specificities are presented in median SI units and range.

Specificity	Cohort	Positive individuals, (n)	Signal intensity (SI), median (range)
Scl-70	HBD	2	12.5 (12–13)
	SLE: Discovery	3	16 (12–42)
	SLE: Replication	4	17 (14–58)
	pSS	0	
	SSc	12	76.5 (11–116)
CENP-A	HBD	1	23 (23–23)
	SLE: Discovery	17	23 (11–135)
	SLE: Replication	17	22 (11–142)
	pSS	12	122 (12–145)
	SSc	12	139.5 (48–150)
CENP-B	HBD	4	16.5 (12–37)
	SLE: Discovery	8	19.5 (11–122)
	SLE: Replication	7	36 (13–133)
	pSS	11	106 (14–142)
	SSc	14	141 (28–153)
RNA Pol III 11kDa	HBD	4	35.5 (24–40)
	SLE: Discovery	2	12 (11–13)
	SLE: Replication	6	14.5 (13–40)
	pSS	1	15 (15–15)
	SSc	12	99 (15–130)
RNA Pol III 155kDa	HBD	4	25 (16–37)
	SLE: Discovery	2	20 (16–24)
	SLE: Replication	5	16 (13–29)
	pSS	0	
	SSc	15	78 (15–131)
Fibrillarin	HBD	4	16 (13–53)
	SLE: Discovery	2	15.5 (14–17)
	SLE: Replication	6	14 (11–88)
	pSS	0	
	SSc	2	21 (17–25)
NOR90	HBD	5	13 (11–52)
	SLE: Discovery	14	37.5 (14–94)
	SLE: Replication	4	19.5 (13–31)
	pSS	5	30 (11–138)
	SSc	3	13 (13–95)

Th/To	<i>HBD</i>	14	14.5 (11–60)
	<i>SLE: Discovery</i>	4	13 (11–26)
	<i>SLE: Replication</i>	9	15 (11–38)
	<i>pSS</i>	3	19 (16–21)
	<i>SSc</i>	3	24 (14–27)
PM-Scl100			
	<i>HBD</i>	9	23 (11–62)
	<i>SLE: Discovery</i>	4	14 (11–30)
	<i>SLE: Replication</i>	7	13 (11–29)
	<i>pSS</i>	3	12 (12–36)
	<i>SSc</i>	9	56 (13–86)
PM-Scl75			
	<i>HBD</i>	16	14.5 (11–52)
	<i>SLE: Discovery</i>	9	16 (11–93)
	<i>SLE: Replication</i>	3	26 (12–145)
	<i>pSS</i>	2	13 (11–15)
	<i>SSc</i>	8	62 (11–142)
Ku			
	<i>HBD</i>	10	13.5 (12–19)
	<i>SLE: Discovery</i>	13	20 (11–122)
	<i>SLE: Replication</i>	16	26 (12–132)
	<i>pSS</i>	2	67 (30–104)
	<i>SSc</i>	6	32.5 (11–90)
PDGFR			
	<i>HBD</i>	0	
	<i>SLE: Discovery</i>	0	
	<i>SLE: Replication</i>	0	
	<i>pSS</i>	0	
	<i>SSc</i>	0	

Supplementary Table 2. Comparisons between immunoassays. All samples achieving positive test results with EuroLine (reference assay) were evaluated with BlueDot and EliA™. Results are shown in total and for each specificity in signal intensity (SI) intervals. Highest agreement of positive results was found at SI units >50, and for anti-CENP-B followed by anti-CENP-A.

		Number of positive antibodies and agreement with EuroLine, n (%)	Signal intensity (SI), units		
			11–25	26–50	>50
Total	<i>EuroLine</i>	266	164	49	53
	<i>BlueDot</i>	48 (18)	4 (2)	5 (10)	39 (74)
	<i>EuroLine</i>	97	61	19	17
	<i>EliA</i>	18 (19)	2 (3)	3 (16)	13 (76)
Scl-70					
	<i>EuroLine</i>	9	7	1	1
	<i>BlueDot</i>	0	0	0	0
	<i>EliA</i>	1 (11)	0	0	1
CENP-A					
	<i>EuroLine</i>	44	20	7	17
	<i>BlueDot</i>	13 (30)	0	0	13
CENP-B					
	<i>EuroLine</i>	30	12	5	13
	<i>BlueDot</i>	17 (57)	2	2	13
	<i>EliA</i>	16 (53)	2	2	12
RNA Pol III 11kDa					
	<i>EuroLine</i>	13	9	4	0
	<i>BlueDot*</i>	1 (8)	1	0	0
	<i>EliA*</i>	0	0	0	0
RNA Pol III 155kDa					
	<i>EuroLine</i>	11	7	4	0
	<i>BlueDot*</i>	0	0	0	0
	<i>EliA*</i>	0	0	0	0
Fibrillarin					
	<i>EuroLine</i>	11	9	0	2
	<i>BlueDot</i>	1 (9)	1	0	0
	<i>EliA</i>	0	0	0	0
NOR90					
	<i>EuroLine</i>	27	14	8	5
	<i>BlueDot</i>	3 (11)	0	0	3
Th/To					
	<i>EuroLine</i>	30	24	5	1
	<i>BlueDot</i>	0	0	0	0
PM-Scl100					
	<i>EuroLine</i>	23	17	5	1
	<i>BlueDot</i>	4 (17)	0	3	1
	<i>EliA</i>	1 (4)	0	1	0
PM-Scl75					
	<i>EuroLine</i>	30	22	4	4
	<i>BlueDot</i>	4 (13)	0	0	4
Ku					
	<i>EuroLine</i>	38	23	6	9
	<i>BlueDot</i>	5 (13)	0	0	5

* In contrast to the EuroLine assay, the BlueDot and EliA™ assays detect antibodies against the entire RNA polymerase III complex.

Supplementary Table 3. Concordance of EuroLine results (reference assay) versus (vs.) BlueDot and EliA™ results in patients with SSc. For most specificities, BlueDot and EliA™ showed a high level of concordance with the EuroLine results.

	EuroLine (n=57)	BlueDot (n=57)	EuroLine (n=56)	EliA™ (n=56)	Concordance: EuroLine vs. BlueDot	Concordance: EuroLine vs. EliA™
All positive and negative results					94.4%	96.1%
+	96	63	62	49		
-	531	529	274	274		
ScI-70					98.2%	100.0%
+	12	11	12	12		
-	45	45	44	44		
CENP-A					98.2%	
+	12	11				
-	45	45				
CENP-B					96.5%	98.2%
+	14	12	14	13		
-	43	43	42	42		
RNA Pol III 11 kDa					94.7%*	94.6%*
+	12	9	11	8		
-	45	45	45	45		
RNA Pol III 155 kDa					89.5%*	89.3%*
+	15	9	14	8		
-	42	42	42	42		
Fibrillarin					96.5%	96.4%
+	2	0	2	0		
-	55	55	54	54		
NOR90					93.0%	
+	3	0				
-	54	53				
Th/To					94.7%	
+	3	0				
-	54	54				
PM-ScI100					94.7%	98.2%
+	9	7	9	8		
-	48	47	47	47		
PM-ScI75					91.2%	
+	8	3				
-	49	49				
Ku					91.2%	
+	6	1				
-	51	51				

* Comparisons were done between antibody results of the entire RNA polymerase III complex (BlueDot and EliA™) vs. the 11 and 155 kDa antigens (EuroLine), respectively.

Supplementary Table 4. Diagnostic performance with RP as outcome based on data from individuals with SLE and HBD. Sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) are detailed including 95% confidence intervals (in parentheses).

	Sensitivity	Specificity	Accuracy	PPV	NPV
IF-ANA	0.62 (0.53–0.70)	0.61 (0.56–0.66)	0.70 (0.67–0.74)	0.34 (0.28–0.41)	0.83 (0.78–0.87)
Ro52/SSA	0.33 (0.26–0.42)	0.80 (0.76–0.84)	0.80 (0.76–0.82)	0.36 (0.28–0.45)	0.79 (0.74–0.82)
Scl-70	0.01 (0–0.05)	0.99 (0.97–1)	0.85 (0.83–0.88)	0.20 (0.01–0.62)	0.75 (0.71–0.79)
CENP-A	0.04 (0.02–0.09)	0.97 (0.95–0.98)	0.85 (0.82–0.87)	0.31 (0.14–0.56)	0.75 (0.71–0.79)
CENP-B	0.02 (0–0.06)	0.98 (0.95–0.99)	0.85 (0.82–0.87)	0.18 (0.05–0.48)	0.75 (0.71–0.79)
RNA Pol III 11kDa	0.02 (0–0.06)	0.99 (0.97–1)	0.86 (0.83–0.88)	0.33 (0.10–0.70)	0.75 (0.71–0.79)
RNA Pol III 155kDa	0.01 (0–0.05)	0.99 (0.97–0.99)	0.85 (0.83–0.88)	0.17 (0.01–0.56)	0.75 (0.71–0.79)
Fibrillarin	0.01 (0–0.05)	0.99 (0.97–0.99)	0.85 (0.83–0.88)	0.17 (0.01–0.56)	0.75 (0.71–0.79)
NOR90	0.02 (0–0.06)	0.96 (0.93–0.97)	0.84 (0.81–0.86)	0.12 (0.03–0.34)	0.75 (0.71–0.78)
Th/To	0.02 (0–0.06)	0.96 (0.94–0.98)	0.84 (0.81–0.86)	0.12 (0.03–0.36)	0.75 (0.71–0.79)
PM-Scl100	0.03 (0.01–0.08)	0.98 (0.96–0.99)	0.85 (0.83–0.87)	0.33 (0.14–0.61)	0.75 (0.71–0.79)
PM-Scl75	0.04 (0.02–0.09)	0.95 (0.92–0.97)	0.84 (0.81–0.86)	0.21 (0.09–0.40)	0.75 (0.71–0.79)
Ku	0.03 (0.01–0.08)	0.96 (0.93–0.97)	0.84 (0.81–0.86)	0.20 (0.08–0.42)	0.75 (0.71–0.79)
PDGFR	0 (0–0.03)	1 (0.99–1)	0.86 (0.83–0.88)	–	0.75 (0.71–0.79)
≥1 antibody specificity	0.71 (0.62–0.78)	0.41 (0.36–0.46)	0.55 (0.50–0.59)	0.28 (0.24–0.34)	0.81 (0.75–0.86)