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Diagnostic performance and cutoff optimisation of the CTD Screen assay for ANA detection compared with HEp-2 IFA

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Abstract

Aim: Antinuclear antibodies (ANAs) are important in diagnosing systemic autoimmune rheumatic diseases (SARDs), and the HEp-2 cell indirect immunofluorescence assay (HEp-2 IFA) remains the gold standard for their detection. This study evaluates the utility and optimal cutoff of the EliA connective tissue disease Screen (CTD Screen), an enzyme immunoassay, as a complementary automated method for ANA detection.

Methods: A total of 694 samples (347 HEp-2 IFA positive and 347 HEp-2 IFA negative) were analysed using the CTD Screen assay. Samples that were positive by both methods were further tested using a line immunoassay (LIA) to detect specific ANAs. Diagnostic accuracy was assessed with a 95% confidence interval (95% CI) using sensitivity, specificity, predictive values, Cohen's kappa (κ), and receiver operating characteristic (ROC) analysis.

Results: With the manufacturer's cutoff, CTD Screen sensitivity and specificity were 73.9% (95% CI 69.0–78.3) and 92.6% (95% CI 89.2–95.0), respectively. The optimized in-house cutoff improved sensitivity to 81.5% (95% CI 77.1–85.3) and specificity to 85.6% (95% CI 81.4–89.0). The assay demonstrated substantial agreement with HEp-2 IFA (κ = 0.683) and an area under the ROC curve of 0.845 (95% CI 0.814–0.876). LIA confirmed the presence of antibodies in 89.9% of CTD Screen-positive samples. Disease-specific analysis demonstrated the highest sensitivity for systemic sclerosis (97.4%) and the best accuracy for mixed CTD [area under the curve (AUC) = 0.900].

Conclusions: CTD Screen shows substantial agreement with HEp-2 IFA and good diagnostic accuracy; however, it is best used within a tiered diagnostic approach that incorporates confirmatory assays and clinical correlation rather than as a standalone test.

Keywords

CTD Screen, antinuclear antibodies, systemic autoimmune rheumatic diseases, sensitivity, specificity

Introduction

Antinuclear antibodies (ANAs), also referred to as anti-cell antibodies, are hallmark biomarkers of systemic autoimmune rheumatic diseases (SARDs), a heterogeneous group of disorders characterised by immune-mediated inflammation and multisystem involvement, including systemic lupus erythematosus (SLE), Sjogren's syndrome (SjS), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), and inflammatory myopathies [1]. Besides the HEp-2 cell indirect immunofluorescence assay (HEp-2 IFA), which remains a gold standard screening test for ANA, several high-throughput, solid-phase alternatives, such as enzyme-linked immunosorbent assay (ELISA) and line immunoassay (LIA), are also widely used. However, because solid-phase assays detect only the antigens incorporated into the assay, they may fail to identify autoantibodies directed against antigens not included in the antigen panel [2, 3]. In contrast, HEp-2 IFA can identify a broad range of ANA patterns, including nuclear, cytoplasmic, and mitotic reactivities. Nevertheless, conventional HEp-2 IFA requires specialized expertise and is subject to operator-dependent interpretation. To address these limitations, several automated platforms incorporating automated image acquisition and artificial intelligence-assisted pattern recognition have recently been developed, including the Euroimmun EUROPAttern Classifier 2.4, Werfen NOVA View, Medipan AKLIDES/akiron NEO, AESKU HELIOS, and Immunoconcept Image Navigator.

In high-resource settings, multiplex and array-based platforms have further improved diagnostic precision by enabling simultaneous detection of multiple autoantibodies. In contrast, laboratories in resource-limited regions often face financial, infrastructural, and technical constraints that restrict testing to a single assay, potentially leading to missed or delayed diagnoses. In such settings, the availability of an efficient, cost-effective complementary tool for ANA screening could improve diagnostic access and facilitate the timely evaluation of patients suspected of SARDs [4].

The EliA CTD Screen is a fluorescence enzyme immunoassay (FEIA) designed to detect autoantibodies associated with SARDs and has demonstrated good diagnostic accuracy for ANA screening in multiple clinical studies [5–8]. Several clinical studies have demonstrated that the CTD Screen assay provides good diagnostic sensitivity and specificity, while its fully automated format offers advantages in standardization and laboratory workflow [8–11]. However, its diagnostic performance remains incompletely characterized, particularly when directly compared with HEp-2 IFA and LIA. Additionally, its performance relative to the International Consensus on Antinuclear Antibody Patterns (ICAP)-standardized HEp-2 IFA interpretation, as defined by the ICAP, has not been systematically evaluated. To our knowledge, no previous study has simultaneously evaluated diagnostic accuracy, disease-specific performance, and optimal cutoff determination of the CTD Screen assay against both HEp-2 IFA and LIA in a large Indian cohort. This study was therefore designed to address this gap.

Materials and methods

Study design

During the year 2023–2024, among the samples received in the Department of Immunopathology for HEp-2 IFA screening from patients clinically suspected of having SARDs, we selected 1,500 consecutive serum samples submitted for both HEp-2 IFA and LIA testing, irrespective of age and sex. These anonymised leftover samples were available in sufficient volume for testing, and informed consent for the research use of leftover material was obtained from the patients. Those samples with missing or insufficient clinical information, a referring diagnosis not suggestive of SARDs, inadequate volume, or unsuitable for testing (e.g., grossly haemolysed or lipemic) were excluded. Sample size was estimated to achieve a 95% confidence interval (95% CI) width ≤ 5% for sensitivity/specificity estimates based on prior CTD Screen data (expected sensitivity 75%, specificity 90%). Following HEp-2 IFA testing, 347 samples were positive and were subjected to the LIA and CTD Screen assays. For comparison, an equal number of HEp-2 IFA-negative samples (n = 347) were randomly selected from the HEp-2 IFA-negative pool using a computer-generated sequence and also tested with the CTD Screen. Samples that were positive by both HEp-2 IFA and CTD Screen underwent further testing with LIA to characterise autoantibody specificities (Figure 1). The

sample size, though determined pragmatically, provided adequate statistical precision for key diagnostic estimates. With 347 positive and 347 negative sera, the 95% CI for sensitivity and specificity remained within ± 2% to ± 4%, ensuring reliable comparative analysis. Demographic and clinical data were retrieved from the patient information card and laboratory records. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki (as revised in 2013). The Institutional Ethics Committee approved the study protocol, and written informed consent was obtained from all participants before enrolment.

Sample preparation

Before analysis, the serum was separated from the whole blood sample by centrifugation. Initially, HEp-2 IFA was performed, and after interpretation, the leftover samples were analysed using CTD Screen and LIA.

HEp-2 IFA

The HEp-2 IFA was performed following standard laboratory protocols. All reagents and serum samples were brought to room temperature (18–25°C) before testing. An initial 1:40 dilution was used to enhance sensitivity in screening settings, consistent with Indian diagnostic practice, and all positive samples were retested at ≥ 1:80 to confirm robustness. Patient sera were diluted in phosphate-buffered saline and applied (20–25 µL) to pre labeled HEp-2 slides (NOVA Lite, Inova Diagnostics, Inc., San Diego, CA 92131, USA), along with positive and negative controls. Slides were incubated for 30–35 minutes at 37°C in a humid chamber and rinsed.

CTD Screen

Residual serum samples were analyzed using EliA CTD Screen (Thermo Fisher Scientific/Phadia, Uppsala, Sweden) in a fully automated immunoassay analyzer (Phadia 250; Thermo Fisher Scientific, Germany) in accordance with the manufacturer's instructions. The system diluted samples at a 1:100 ratio and measured fluorescence signals after approximately 2.5 hours of incubation and processing. Results were interpreted according to the manufacturer's cut-off values as negative (< 0.7 ratio), equivocal (0.7–1.0 ratio), or positive (> 1.0 ratio).

LIA

Samples positive by both HEp-2 IFA and CTD Screen were further analyzed for specific autoantibodies using commercial LIA strips (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). Test strips were pre-soaked in sample diluent and incubated with diluted serum (1:101) for 30 minutes, followed by sequential washing and conjugate incubation steps. After substrate development and final washing, strips were air-dried, scanned, and interpreted using the EUROLineScan software system (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). Band intensities were scored on a 0–4+ scale by automated software and visually cross-checked. Tests lacking a control band were considered invalid.

Statistical analysis

Statistical analysis was performed using SPSS v26 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarise demographic and clinical characteristics, expressed as mean ± standard deviation or median (range) for continuous variables, and as frequencies and percentages for categorical variables. Associations were tested using Pearson's or Spearman's correlation as appropriate, and Chi-square tests were used for categorical comparisons. For ordinal data, the Mann–Whitney U and Kruskal–Wallis tests were employed. Diagnostic performance was evaluated by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), accuracy, Cohen's kappa (κ), and p-values. Receiver operating characteristic (ROC) curve analysis was performed, and the optimal cutoff value was identified using the Youden Index (J = Sensitivity + Specificity − 1) to maximize the combined sensitivity and specificity (Figure 1).

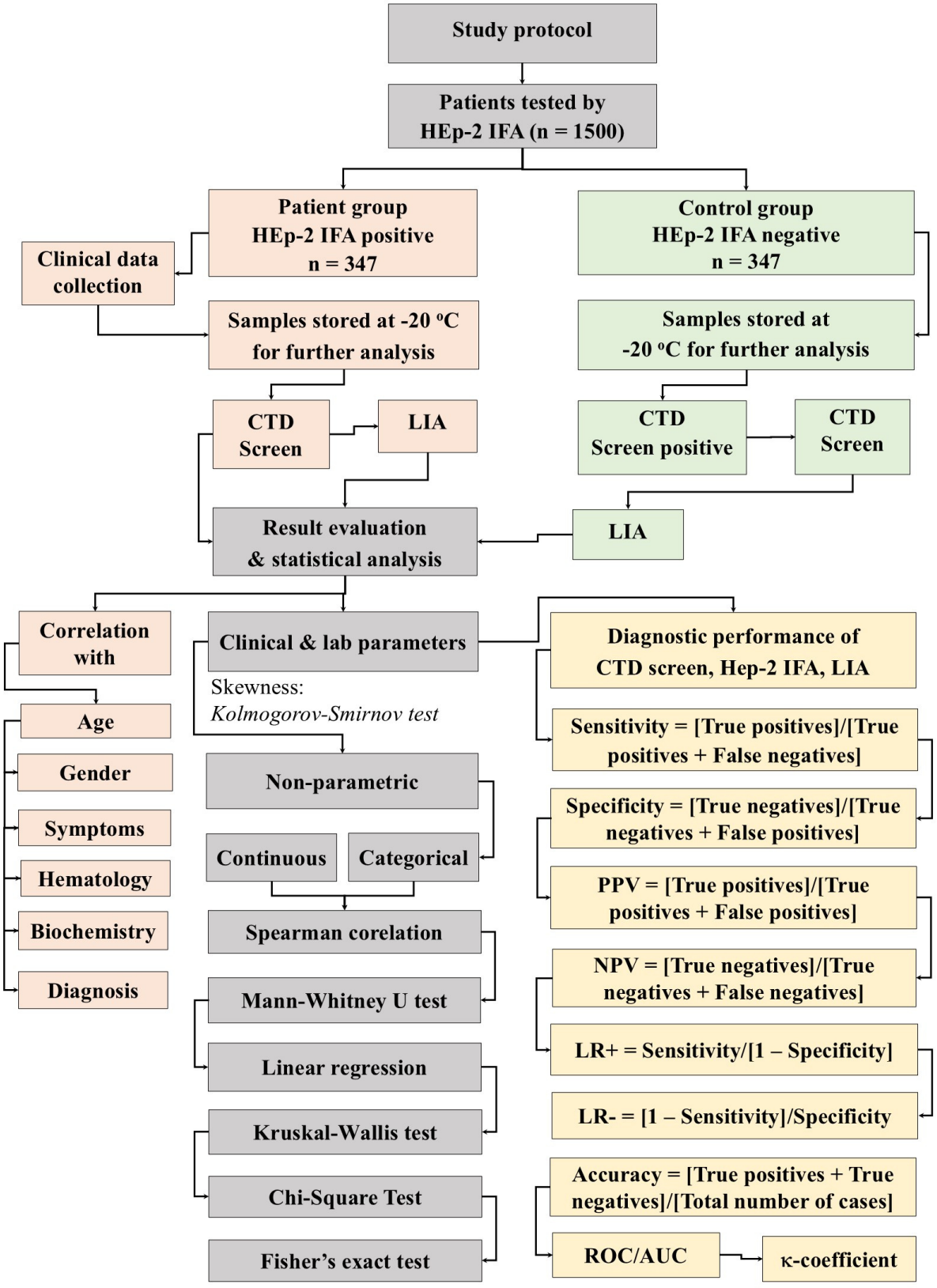

Figure 1. An outline of patient and sample selection, and serological and statistical analysis. AUC: area under the curve; CTD Screen: connective tissue disease Screen; HEp-2 IFA: HEp-2 cell indirect immunofluorescence assay; LIA: line immunoassay; LR: likelihood ratio; NPV: negative predictive value; PPV: positive predictive value; ROC: receiver operating characteristic; *κ*: Cohen's kappa.

Results

Patient characteristics

The patient cohort ranged in age from 1 to 81 years, with a median age of 40. The female-to-male ratio was 5.8:1. The study observed a diverse range of clinical presentations of SARDs (Table 1). Hematological parameters were deranged in a significant proportion of patients in the cohort [e.g., elevated ESR and C-reactive protein (CRP) levels in 87.8% and 80% of patients, respectively]. Similarly, abnormal biochemical findings were observed in varying numbers of cases, including elevated levels of urea (11.6%), creatinine (11.6%), and uric acid (12.1%). Liver enzymes, aspartate aminotransferase (AST) (22.5%), alanine aminotransferase (ALT) (12.7%), alkaline phosphatase (ALP) (12.1%), and gamma-glutamyl transferase (GGT) (5.5%), were also elevated, with abnormal total bilirubin (6.1%) and conjugated bilirubin levels (8.8%).

Table 1. Patient characteristics.

Total number		***N* = 347**	
Age		Range = 1–81 years	
		Median = 40 years	
		IQR = 30–51.5 years	
Male: Female ratio		1:5.8	
Clinical features			
Primary diagnosis		**Systems involved**	
SLE	36.0%	Musculoskeletal	62.0%
SjS	17.6%	Dermatological	47.0%
MCTD	14.9%	Cardiovascular	22.7%
LSSc	11.7%	Auditory/ocular	21.8%
DSSc	5.4%	Febrile illness	21.8%
UCTD	2.7%	Respiratory	20.0%
Dermatomyositis	0.3%	Endocrine	17.6%
Non-SARDs conditions	30%	Gastrointestinal/liver	16.1%
		Hematological	13.9%
		Renal	9.4%
		Central nervous system	8.8%
		Gynaecological system	7.3%
Laboratory findings (median values)			
Hemoglobin	11.3 g/dL	Blood urea	24 mg/dL
Anemia	56%	Uric acid	5.2 mg/dL
-Normocytic normochromic	74.5%	Serum creatinine	0.6 mg/dL
-Microcytic normochromic	23.5%	AST	25 IU/L
-Macrocytic	2.0%	ALT	27 IU/L
Leucocyte count	6.8 × 10^9/L	ALP	88 IU/L
-Leucocytosis	3.6%	GGT	148 IU/L
-Leukopenia	3.3%	Total bilirubin	0.8 mg/dL
Platelet count	224 × 10^9/L	Conjugated bilirubin	0.2 mg/dL
-Thrombocytosis	22%	Total protein	7.6 g/dL
-Thrombocytopenia	3.9%	Albumin	3.8 g/dL
ESR	36.5 mm/h	C3 (median)	124 mg/dL
-Raised	87.8%	C4 (median)	22 mg/dL
C-reactive protein	7 mg/L	LDH	293 U/L
-Raised	80%		

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; DSSc: diffuse systemic sclerosis; GGT: gamma-glutamyl transferase; IQR: inter-quartile range; LDH: lactate dehydrogenase; LSSc: limited systemic sclerosis;

MCTD: mixed connective tissue disease; SARDs: systemic autoimmune rheumatic diseases; SjS: Sjogren's syndrome; SLE: systemic lupus erythematosus; UCTD: undifferentiated connective tissue disease.

The total protein and albumin levels were reduced in 6.6% and 17.9% of cases, respectively. Altered C3 and C4 levels were noted in 20.5% and 26.9% of cases, respectively, and the lactate dehydrogenase (LDH) was elevated in 49.6% of patients. Among these, reduced hemoglobin, low complement C3 and C4, and increased ESR and ALP were found to be statistically significantly associated with SARDs ($p < 0.001$; $p = 0.029$ for ALP).

Autoimmune serologic profile

HEp-2 IFA results

Among patients tested with HEp-2 IFA, 64.8% exhibited nuclear staining pattern, 33.7% cytoplasmic pattern, and 1.4% showed a mitotic staining pattern. Patients with nuclear ANA patterns had a mean age of 39.9 years, while those with cytoplasmic patterns were slightly older, with a mean age of 42.5 years. Mitotic patterns were also observed in older individuals (mean age 52.4 years). Across all ANA pattern groups, there was a pronounced female predominance: 86.6% in the nuclear pattern group, 82.2% in the cytoplasmic group, and 100% in the mitotic pattern group. Of those who tested positive for HEp-2 IFA, 57.1% were clearly positive on the CTD Screen, 4.9% had equivocal results, and 38.0% were negative.

A significant correlation was found between the HEp-2 IFA pattern and the clinical diagnosis ($\chi^2 = 42.7$, $p < 0.001$). As shown in Table 2, the nuclear pattern was significantly associated with musculoskeletal ($\chi^2 = 16.7$, $p < 0.001$), cardiovascular ($\chi^2 = 26.7$, $p < 0.001$), endocrine ($\chi^2 = 21.9$, $p < 0.001$), auditory/eye ($\chi^2 = 11.1$, $p = 0.003$), and dermatological symptoms ($\chi^2 = 22.3$, $p < 0.001$). A higher intensity (3+ and 4+) was associated with SLE, SjS, limited systemic sclerosis (LSSc), MCTD, and undifferentiated connective tissue disease (UCTD), while rheumatoid arthritis (RA) predominated in 1+ ($\chi^2 = 53.5$, $p = 0.002$). Higher intensity was also associated with increased ESR ($\chi^2 = 10.2$, $p = 0.017$), decreased C3 ($\chi^2 = 15.5$, $p = 0.001$), C4 ($\chi^2 = 9.7$, $p = 0.021$), and elevated conjugated bilirubin ($\chi^2 = 9.3$, $p = 0.026$). Patients with cytoplasmic ANA patterns had higher total bilirubin ($\chi^2 = 16.7$, $p < 0.001$) and elevated liver enzymes, ALT ($\chi^2 = 14.5$, $p = 0.001$) and AST ($\chi^2 = 6.9$, $p = 0.031$). Mitotic patterns were significantly linked to elevated conjugated bilirubin ($\chi^2 = 20.2$, $p < 0.001$).

LIA results

Among HEp-2 IFA-positive cases, 228 (65.7%) were also positive by LIA. Among nuclear patterns, SSA60Kd, SSA52Kd, and U1RNP were the most common, while antibodies such as PM-Scl, Jo-1, and PCNA had lower prevalence. In the cytoplasmic group, dominant antibodies were SSA52Kd, SSA60Kd, and AMA-M2, while lower positivity was observed for PM-Scl, CENP-B, and PCNA. The mitotic patterns were few and primarily negative on LIA. Only one case showed positivity for Scl-70.

Multiple antibody positivity was most common (59.8%), followed by dual (36.9%) and single (3.3%) antibody positivity. SSA60Kd positivity was seen in 121 cases, accounting for 34.9% of the positives. Following closely was SSA52Kd, with 91 cases, making up 26.2%. U1RNP was identified in 54 (15.6%) cases, Sm in 42 (12.1%), SSB/La and Scl-70 in 30 (8.6%), Ribo-P in 27 (7.8%), AMA-M2 in 26 (7.5%), dsDNA in 23 (6.6%), CENP-B in 22 (6.3%), Nucleosome and Histone in 18 (5.2%) cases each, PCNA in 11 (3.2%), Jo-1 in 6 (1.7%), and PM-Scl in 4 (1.2%) of positive cases. The results of LIA were also used to correlate specific ANAs with different SARDs, showing high frequencies of Ribo-P, SSA52, SSA60, Scl-70, and CENP-B with SLE and MCTD, SjS, SSc, and UCTD, respectively (Table 2).

Statistical analysis revealed significant associations between specific antibodies and clinical symptoms: anti-nucleosome with renal symptoms ($\chi^2 = 15.031$, $p = 0.024$, Cramer's V = 0.21), anti-AMA-M2 with hematological symptoms ($\chi^2 = 6.664$, $p = 0.017$, V = 0.14), anti-dsDNA with fever of unknown origin ($\chi^2 = 6.800$, $p = 0.009$, V = 0.14), anti-Scl-70 and anti-CENP-B with cardiovascular symptoms ($\chi^2 = 4.777$, $p =$

Table 2. Disease association with different patterns in HEp-2 IFA-positive patients.

Diagnosis	Type of HEp-2 IFA patterns numbers (%)				Specific ANA found in LIA (%)														
	Nuclear	Cytoplasmic	Mitotic	Total	SSA52Kd	SSA60Kd	U1RNP	Sm	Ribo-P	Scl-70	SSB/La	CENP-B	dsDNA	AMA-M2	Histone	Nucleosome	PCNA	Jo-1	PM-Scl
SLE	58 (25.8)	21 (17.9)	1 (20)	80 (23.1)	9.5	11.1	11.1	15.9	20.6	0	1.6	1.6	14.3	0	6.3	7.9	0	0	0
SjS	21 (9.3)	17 (14.5)	1 (20)	39 (11.2)	38.2	34.4	3.1	0	0	0.8	13	2.3	0	3.8	1.5	0	1.5	1.5	0
MCTD	23 (10.2)	10 (8.5)	0	33 (9.5)	14.6	10.9	21.2	16.1	66	4.4	2.9	2.2	6.6	3.6	2.9	3.6	2.9	1.5	0
LSSc	26 (11.6)	0	0	26 (7.5)	8.8	8.8	5.9	0	2.9	44.1	2.9	20.6	0	0	0	2.9	0	0	2.9
DSSc	9 (4.0)	2 (1.7)	1 (20)	12 (3.5)	0	12.5	12.5	0	0	31.2	0	25	0	6.2	6.2	0	6.2	0	6.2
Overlap syndrome	15 (6.7)	11 (9.4)	0	26 (7.5)	23.2	23.2	5.8	7.2	4.3	2.9	8.7	2.9	4.3	5.8	8.7	4.3	0	1.4	2.9
UCTD	5 (2.2)	0	0	5 (1.4)	16.7	16.7	16.7	16.7	0	0	0	33.3	0	0	0	0	0	0	0
RA	16 (7.1)	5 (4.3)	0	21 (6.1)	36.4	27.3	9.1	0	0	9.1	0	0	0	0	0	9.1	0	0	0
Others	51 (22.7)	52 (44.4)	2 (40)	105 (30.3)	20	20	9.1	73	1.8	0	1.8	0	3.6	18.2	1.8	5.5	7.3	1.8	1.8

ANA: antinuclear antibody; DSSc: diffuse systemic sclerosis; HEp-2 IFA: HEp-2 cell indirect immunofluorescence assay; LIA: line immunoassay; LSSc: limited systemic sclerosis; MCTD: mixed connective tissue disease; RA: Rheumatoid arthritis; SjS: Sjogren's syndrome; SLE: systemic lupus erythematosus; UCTD: undifferentiated connective tissue disease.

0.029, V = 0.13), anti-PCNA with GIT/Liver symptoms (χ^2 = 12.441, p = 0.003, V = 0.19), and anti-PM-Scl with dermatological involvement (χ^2 = 4.572, p = 0.048, V = 0.12).

CTD Screen

Among HEp-2 IFA-positives, 215 (62%) were CTD Screen positive, including 17 (4.9%) samples with equivocal values (Table 3; Figure 2). HEp-2 IFA patterns were supported by the CTD Screen findings. Screen-positive patients predominantly exhibited nuclear fluorescence patterns, especially fine-speckled and homogeneous types. Cytoplasmic patterns were more common in screen-negative cases (Table 4). CTD Screen-positive individuals also demonstrated stronger HEp-2 IFA fluorescence intensity, with 60.6% showing 3+ and 15.2% showing 4+ intensity, compared to only 2.7% of CTD Screen-negative cases at 4+. Analysis of CTD Screen patterns revealed better performance for nuclear [area under the curve (AUC) = 50.736] than for cytoplasmic (AUC = 0.583) patterns, suggesting limited utility for cytoplasmic detection.

Table 3. A comparison of CTD Screen results with HEp-2 IFA and LIA.

HEp-2 IFA patterns	HEp-2 IFA	LIA		CTD Screen		
	Positive	Positive	Negative	Positive	Equivocal	Negative
Total	**347**	**228 (65.7%)**	**119 (34.3%)**	**198 (57.1%)**	**17 (4.9%)**	**132 (38.0%)**
Nuclear	225 (64.8%)	159 (45.8%)	67 (19.3%)	139 (40.1%)	8 (2.3%)	77 (22.2%)
Cytoplasmic	117 (33.7%)	66 (19.0%)	50 (14.4%)	56 (16.1%)	9 (2.6%)	53 (15.3%)
Mitotic	5 (1.4%)	3 (0.9%)	2 (0.6%)	3 (0.9%)	0	2 (0.6%)

CTD Screen: connective tissue disease Screen; HEp-2 IFA: HEp-2 cell indirect immunofluorescence assay; LIA: line immunoassay.

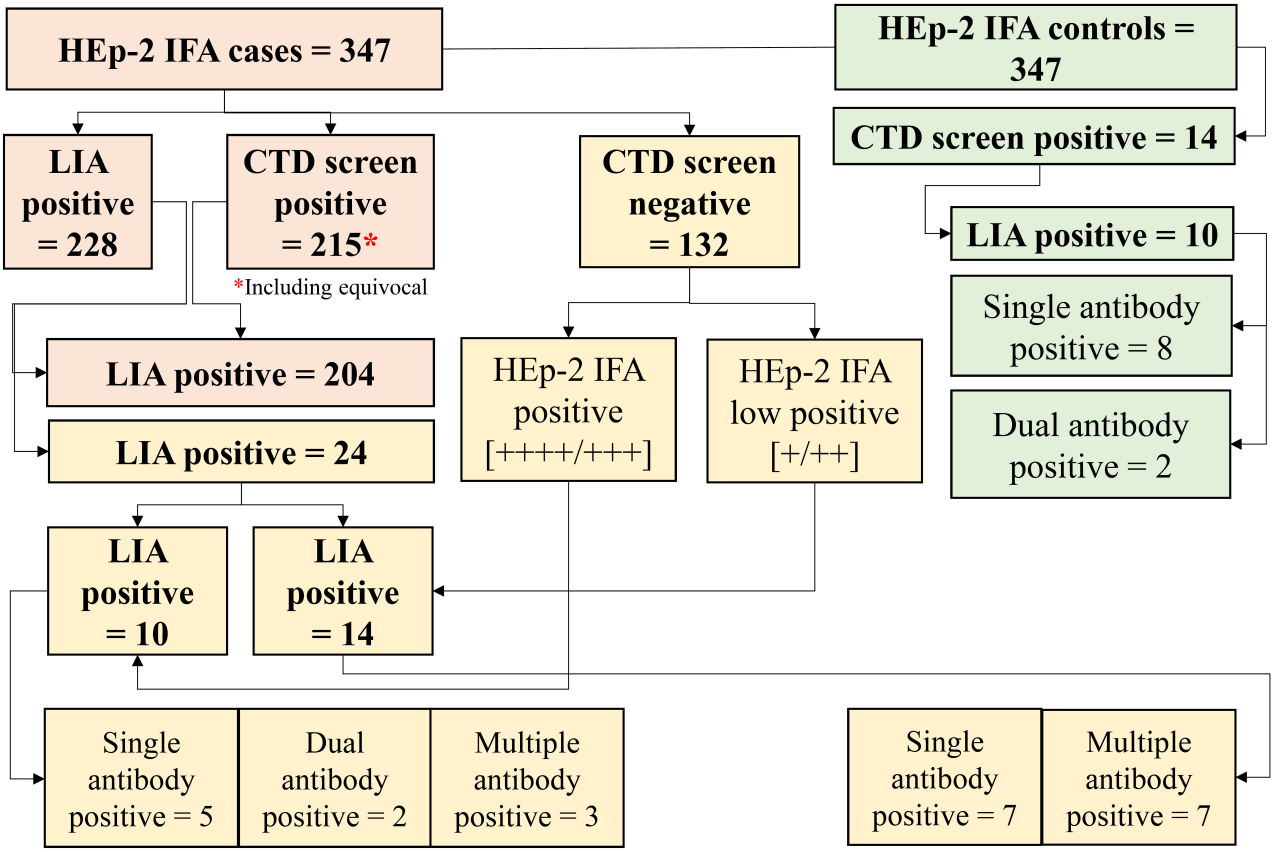

Figure 2. Inter-assay comparison. CTD Screen: connective tissue disease Screen; HEp-2 IFA: HEp-2 cell indirect immunofluorescence assay; LIA: line immunoassay.

Table 4. Distribution of HEp-2 IFA patterns and their concordance with CTD Screen results.

HEp-2 IFA				CTD Screen		
Type	Pattern	Intensity	Positives number (%)	Positive number (%)	Equivocal number (%)	Negative number (%)
Nuclear	Fine speckled	2+/3+/4+	89 (25.6%)	55 (15.9%)	4 (1.2%)	30 (8.6%)
(*n* = 225)	Homogeneous	2+/3+/4+	57 (16.4%)	31 (8.9%)	2 (0.6%)	24 (6.9%)
	Large speckled	2+/3+/4+	27 (7.8%)	25 (7.2%)	0	2 (0.6%)
	Centromere	2+/3+	16 (4.6%)	14 (4.0%)	0	2 (0.6%)
	Nucleolar homogeneous	2+/3+	12 (3.5%)	3 (0.9%)	2 (0.6%)	7 (2.0%)
	Dense fine speckled	2+/3+/4+	6 (1.7%)	2 (0.6%)	0	4 (1.2%)
	Topo I	3+	5 (1.4%)	5 (1.4%)	0	0
	Multiple discrete dots	3+/4+	4 (1.2%)	2 (0.6%)	0	2 (0.6%)
	Pleomorphic	2+/3+	3 (0.9%)	2 (0.6%)	0	1 (0.3%)
	Nucleolar punctate	3+	2 (0.6%)	0	0	2 (0.6%)

Table 4. Distribution of HEp-2 IFA patterns and their concordance with CTD Screen results. (*continued*)

HEp-2 IFA				CTD Screen		
Type	**Pattern**	**Intensity**	**Positives number (%)**	**Positive number (%)**	**Equivocal number (%)**	**Negative number (%)**
	Nucleolar clumpy	3+	2 (0.6%)	0	0	2 (0.6%)
	Envelope	2+	1 (0.3%)	0	0	1 (0.3%)
Cytoplasmic	Dense fine speckled	2+/3+/4+	32 (9.2%)	31 (8.9%)	1 (0.3%)	9 (2.6%)
(*n* = 117)	Fine speckled	2+/3+	4 (1.2%)	3 (0.9%)	1 (0.3%)	15 (4.3%)
	Discrete dots	2+/3+	5 (1.4%)	4 (1.2%)	1 (0.3%)	3 (0.9%)
	Fibrillary, filamentous	2+/3+	5 (1.4%)	4 (1.2%)	1 (0.3%)	8 (2.3%)
	Golgi	1+/2+	7 (2.0%)	5 (1.4%)	2 (0.6%)	2 (0.6%)
	Reticular	2+/3+/4+	10 (2.9%)	7 (2.0%)	3 (0.9%)	13 (3.7%)
	Fibrillary, linear	2+/3+	1 (0.3%)	1 (0.3%)	-	1 (0.3%)
	Rods & rings	2+/3+	1 (0.3%)	1 (0.3%)	-	2 (0.6%)
Mitotic	Centrosome	2+/4+	2 (0.6%)	2 (0.6%)	-	1 (0.3%)
(*n* = 5)	Intercellular bridge	2+	1 (0.3%)	1 (0.3%)	-	-
	Chromosomal	2+	-	-	-	1 (0.3%)

CTD Screen: connective tissue disease Screen; HEp-2 IFA: HEp-2 cell indirect immunofluorescence assay.

The diagnostic efficiency of the CTD Screen was also evaluated against LIA results, a second-line test used for detecting specific ANAs. LIA was positive in 65.7% of patients and negative in 34.3% (Table 3). Among the 132 HEp-2 IFA-positive but CTD Screen-negative patients, 24 were LIA-positive. Within this group, 8 patients had multi-antibody positivity, and 4 had dual antibody positivity, suggesting the possibility of false positivity and variability in antibody profiles, even with LIA, in cases with lower-intensity HEp-2 IFA positivity. Notably, among the 84 patients with low-intensity HEp-2 IFA but negative CTD Screen results, 14 were LIA-positive, split evenly between those with multiple antibody positivity and those with single antibody positivity. Among patients with HEp-2 IFA and CTD Screen dual positivity, 3.3% had single-antibody positivity, 36.9% had dual-antibody positivity, and 59.8% tested positive for three or more autoantibodies on LIA.

In the control cohort (HEp-2 IFA negative), 14 samples were CTD Screen positive. LIA performed on these 14 controls identified autoantibodies in 10 samples, with 8 showing single-antibody positivity and 2 showing dual-antibody positivity (Figure 2).

Notably, there was considerable discordance between HEp-2 IFA and LIA, with 34.3% (119/347) of HEp-2 IFA positives being LIA-negative, indicating limited LIA sensitivity due to the strip containing only a limited number of antigens. This was reflected in a very low coefficient of –0.025 (p = 0.286), suggesting minimal agreement between the two tests. The ROC analysis revealed that an HEp-2 IFA intensity of approximately 2.5 achieved an optimal balance between sensitivity and false-positive rate. When only 3+ and 4+ intensity results were considered positive, HEp-2 IFA showed a sensitivity of 73.7% and specificity of 71.1%.

It was found that 89.9% of patients who were CTD Screen positive also showed LIA positivity. Among HEp-2 IFA-positive but CTD Screen-negative/equivocal patients, 24 (16.1%) had a positive LIA. The most frequently detected antibodies in these patients were SSA52Kd (8.7%), SSA60Kd (7.4%), AMA-M2 (4.7%), followed by SM (4.0%), U1RNP (3.3%), Ribo-P (2.0%), SSB/La (2.0%), PCNA (1.3%), PM-Scl (1.3%), Scl-70 (0.8%), and Jo-1 (0.8%). Of these 24 patients, 12 (8.0%) had single, 4 (2.6%) had dual, and 8 (5.3%) showed multi-antibody positivity. This suggests that immunoblot testing can identify specific autoantibodies in HEp-2 IFA-positive patients who are negative on the CTD Screen, thereby highlighting its role in enhancing diagnostic specificity in the evaluation of SARDs. Among 84 (56.3%) patients with low-intensity HEp-2 IFA and negative CTD Screen, 14 (9.3%) tested positive, with half showing multiple antibody positivity and half showing single antibody positivity.

Among the 347 HEp-2 IFA negative controls, 10 were LIA-positive, and 14 were CTD Screen-positive. Each had single-antibody positivity for SSA60Kd, Scl-70, Jo-1, AMA-M2, dsDNA, and PCNA. Five were positive for SSA60Kd, and 2 samples had dual antibody positivity.

Diagnostic performance of CTD Screen

The diagnostic performance of the CTD Screen was evaluated using sensitivity, specificity, likelihood ratios (LRs), and ROC curve analysis, which identified an optimal cutoff of 0.6 (Figure 3). Diagnostic efficacy was compared between the manufacturer's recommended and an in-house modified cutoff. At the manufacturer's cutoff, sensitivity and specificity were 73.9% and 92.6%, respectively, with a positive LR (PLR) of 9.9 and a negative LR of 0.28. The modified cutoff, derived from ROC analysis, increased sensitivity to 81.5% with a slight drop in specificity to 85.6% [PLR = 5.6; negative likelihood ratio (NLR) = 0.22]. Corresponding PPV and NPV values were 82.8% and 87.9% for the manufacturer's cutoff, and 73.3% and 90.5% for the modified version. Overall diagnostic accuracy was 86.4% and 84.2%, with substantial agreement (κ) of 0.683 and 0.652, respectively. Both cutoffs showed comparable AUCs (0.845), confirming robust performance.

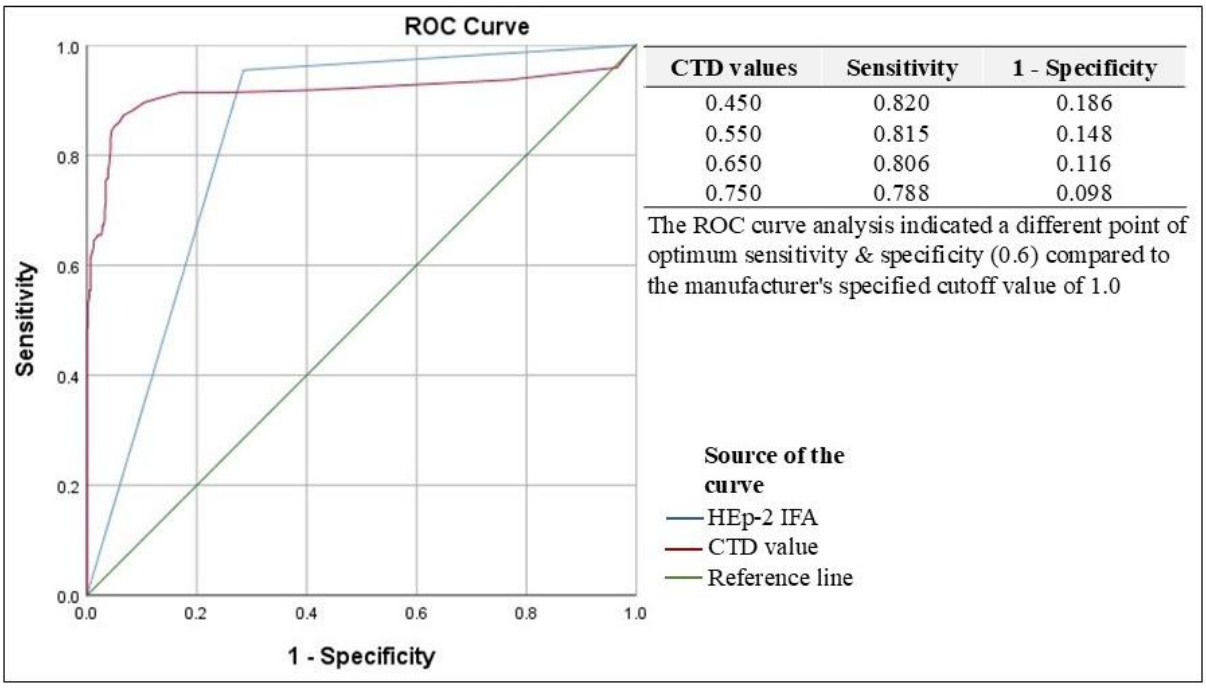

Figure 3. ROC curve analysis for CTD Screen. ANA: antinuclear antibody; CTD Screen: connective tissue disease Screen; HEp-2 IFA: Hep-2 cell indirect immunofluorescence assay; ROC: receiver operating characteristic.

Table 5 shows the test performance of the CTD Screen assay. Disease-specific analysis revealed the highest sensitivity in SSc (97.4%) and the highest specificity across SARDs (86.5%). MCTD and overlap syndrome (OS) demonstrated balanced profiles, with PPV = 50.9%, accuracy = 91.2%, and AUCs of 0.900 and 0.873, respectively. SSc also exhibited the lowest LR (0.03) and highest NPV (99.7%), indicating strong rule-out potential. Overall, the CTD Screen proved reliable and adaptable across SARD subtypes, and modifying the cutoff can enhance sensitivity and clinical applicability (Table 6).

Table 5. Test performance of the CTD Screen assay.

CTD Screen performance	With the manufacturer cutoff	With a modified cutoff	HEp-2 IFA
Sensitivity (95% CI)	73.9%	81.5%	73.7%
Specificity (95% CI)	92.6%	85.6%	71.1
LR+ (95% CI)	9.93	5.65	2.56

Table 5. Test performance of the CTD Screen assay. (*continued*)

CTD Screen performance	With the manufacturer cutoff	With a modified cutoff	HEp-2 IFA
LR– (95% CI)	0.28	0.22	0.37
PPV (95% CI)	82.8%	73.3%	77.2%
NPV (95% CI)	87.9%	90.5%	67.1%
Accuracy (95% CI)	86.4%	84.2%	72.6%
κ (95% CI)	0.683	0.652	0.455

95% CI: 95% confidence intervals; CTD Screen: connective tissue disease Screen; HEp-2 IFA: HEp-2 cell indirect immunofluorescence assay; LR: likelihood ratio; NPV: negative predictive value; PPV: positive predictive value; κ: Cohen's kappa.

Table 6. Diagnostic performance of the CTD Screen for common SARDs.

CTD Screen performance	SLE	SjS	SSc	MCTD	OS
Sensitivity (95% CI)	76.9%	72.5%	97.4%	87.9%	87.9%
Specificity (95% CI)	86.5%	86.5%	86.5%	86.5%	86.5%
LR+ (95% CI)	5.7	5.4	7.2	10.4	10.4
LR– (95% CI)	0.3	0.3	0.03	0.1	0.1
PPV (95% CI)	33.7%	49.6%	38.5%	50.9%	50.9%
NPV (95% CI)	97.7%	94.5%	99.7%	98.7%	98.7%
Accuracy (95% CI)	85.7%	84.3%	87.3%	91.2%	91.2%
κ (95% CI)	0.400	0.496	0.494	0.598	0.534
AUC (*p*-value)	0.855 (< 0.001)	0.774 (< 0.001)	0.957 (< 0.001)	0.900 (< 0.001)	0.873 (< 0.001)

95% CI: 95% confidence intervals; AUC: area under the curve; CTD Screen: connective tissue disease Screen; LR: likelihood ratio; MCTD: mixed connective tissue disease; NPV: negative predictive value; OS: overlap syndrome; PPV: positive predictive value; SARD: systemic autoimmune rheumatic disease; SjS: Sjogren's syndrome; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; κ: Cohen's kappa.

Discussion

The present study extends existing literature in several important ways. First, it represents one of the largest comparative evaluations of CTD Screen performed in a clinical cohort. Second, to our knowledge, it is the first study to establish an optimized, population-specific cutoff validated against both HEp-2 IFA and LIA. Third, our disease-specific analysis demonstrates differential assay performance across SARD subtypes, a dimension rarely assessed in prior reports. We found CTD Screen is a reliable adjunct to conventional HEp-2 IFA for the serological evaluation of SARDs. Although it cannot replace HEp-2 IFA, its high specificity, automation, and rapid turnaround time make it an attractive complementary screening assay, particularly in laboratories where fluorescence microscopy is unavailable or cost-prohibitive [10].

This study provides three key novel findings: 1) validation of CTD Screen performance in a large cohort, 2) derivation of an optimized diagnostic cutoff improving sensitivity by 7.6%, and 3) demonstration of disease-specific variability in assay performance. By directly comparing the manufacturer's cutoffs with an in-house-optimized cutoff, this study evaluated the diagnostic performance of the CTD Screen across a large, clinically diverse cohort. Using the manufacturer's cutoff, the assay achieved a sensitivity of 73.9% and a specificity of 92.6%. To maximize combined sensitivity and specificity, the Youden Index was used to determine the ROC-derived optimum cutoff of 0.6. Improved sensitivity may be beneficial in ANA screening workflows, as early detection of SARDs can prevent diagnostic and treatment delays. Its potential use as a screening threshold in clinically suspected cases is supported by the amended cutoff, which raised sensitivity from 73.9% to 81.5% while maintaining an acceptable specificity of 85.6%. Nevertheless, this improved threshold has not been validated externally and was obtained from a single-centre cohort. As a result, there is still uncertainty about its generalisability across various populations, ethnic groups, disease prevalence levels, and laboratory settings. To confirm this threshold's clinical usefulness and reproducibility, more multicentre prospective investigations are needed.

While comparing with previous reports, the diagnostic findings in our cohort closely align with them. Previous studies have reported sensitivities ranging from 72% to 100% for automated CTD screening assays [5, 6, 9, 10, 12–14]. The observed variability among studies may reflect differences in study populations, disease spectrum, assay antigen composition, inclusion criteria, and methodological approaches. The substantial concordance between CTD Screen and HEp-2 IFA in our data (κ = 0.683) and the high AUC (0.845) confirm robust diagnostic agreement consistent with international studies.

In our study, specificity was 92.6% using the manufacturer's cutoff and 85.6% using the in-house cutoff. These values are comparable with the specificity reported for the FEIA-based EliA CTD Screen assay in previous studies [9, 12]. Variation across studies may reflect demographic and clinical differences, including differences in the prevalence of overlapping autoimmune syndromes or in ethnicity-related antibody repertoires. It's also important to recognize that ANA may be positive in several non-rheumatologic conditions and is not limited to classical SARDs. Even in the absence of overt autoimmune disease, situations like chronic infections, cancer, aging populations, and chromosomal diseases like Klinefelter syndrome have been linked to increased ANA prevalence. These findings emphasize the importance of carefully considering clinical correlation when interpreting ANA screening results, particularly from highly sensitive automated platforms. Therefore, rather than being used as independent diagnostic markers, CTD Screen and HEp-2 IFA results should always be interpreted in conjunction with clinical observations, complementary serology, and pre-test disease probability [15].

Disease-specific analysis further supports the CTD Screen's diagnostic utility. SSc exhibited the highest sensitivity (97.4%) and NPV (99.7%), suggesting that a negative CTD Screen result effectively excludes this diagnosis in suspected cases. MCTD and OSs showed balanced diagnostic indices, likely attributable to their broader autoantibody spectrum. Interestingly, we observed an age-related pattern distribution: nuclear positivity predominated in younger individuals, while cytoplasmic and mitotic reactivities were more frequent in older patients. This may reflect immunosenescence-related antigenic drift or age-related changes in autoantibody expression.

Among CTD Screen-positive sera, nuclear antigen reactivity predominated (64.8), while cytoplasmic and mitotic patterns were less frequent, accounting for 33.7% and 1.4%, respectively. This distribution reflects the assay's emphasis on nuclear antigens and may explain its lower detection rate for cytoplasmic patterns, particularly reticular or fibrillary types. Median fluorescence values further supported this distinction, being higher for nuclear patterns than for cytoplasmic patterns (3.5 vs. 1.0).

The lower proportion of CTD Screen positivity in HEp-2 IFA cytoplasmic and mitotic patterns should be interpreted in light of the differing antigenic breadth of the two assays. HEp-2 IFA can detect autoantibodies against a broad spectrum of approximately 100 cellular antigens, whereas the CTD Screen used in this study includes only 17 defined antigens [3, 5]. Consequently, some HEp-2 IFA positive samples may contain antibodies against antigens not represented in the CTD Screen panel, limiting direct comparison of overall positivity rates between the two methods. A more appropriate evaluation involves correlating specific HEp-2 IFA patterns with their corresponding autoantibodies included in the CTD Screen panel, as partially addressed in Table 3. In addition, the diagnostic performance of solid-phase assays cannot be assumed to be uniform across all ANA specificities solely because they rely on similar technological platforms. While certain autoantibodies, such as anti-centromere antibodies (AC-3 pattern), demonstrate good concordance between solid-phase assays and HEp-2 IFA, others, including anti-fibrillarin antibodies (AC-9 pattern), may show substantially lower sensitivity in solid-phase assays due to differences in antigen composition, presentation, and conformational epitope recognition. Previous studies have similarly reported limited detection of antibodies such as fibrillarin and RNA polymerase II in assays lacking these target antigens [16–18]. These observations reinforce the continued complementary role of HEp-2 IFA in comprehensive ANA screening and pattern characterization, particularly for uncommon, cytoplasmic, or mitotic autoantibody reactivities. Operationally, the CTD Screen may offer advantages such as full automation, high throughput, reduced subjectivity, and faster reporting times [5, 19]. However, the HEp-2 IFA remains the gold standard for ANA screening. Hence, its use may be justified only in resource-limited settings where a fluorescence facility is unavailable or remains suspended due to logistical or technical issues [19].

However, using CTD Screen as an initial screening assay, followed by LIA confirmation of positive results, offers an efficient and cost-effective workflow, particularly for guiding the selection of subsequent individual ANA specificity testing. Nevertheless, HEp-2 IFA remains indispensable for pattern recognition and the detection of novel cytoplasmic or other reactivities not captured by solid-phase assays [19]. Therefore, a combined or tiered testing strategy may provide optimal diagnostic accuracy [20]. Our proposed algorithm (Figure 4) represents a pragmatic diagnostic workflow integrating automation with confirmatory testing, potentially improving laboratory efficiency without compromising diagnostic performance.

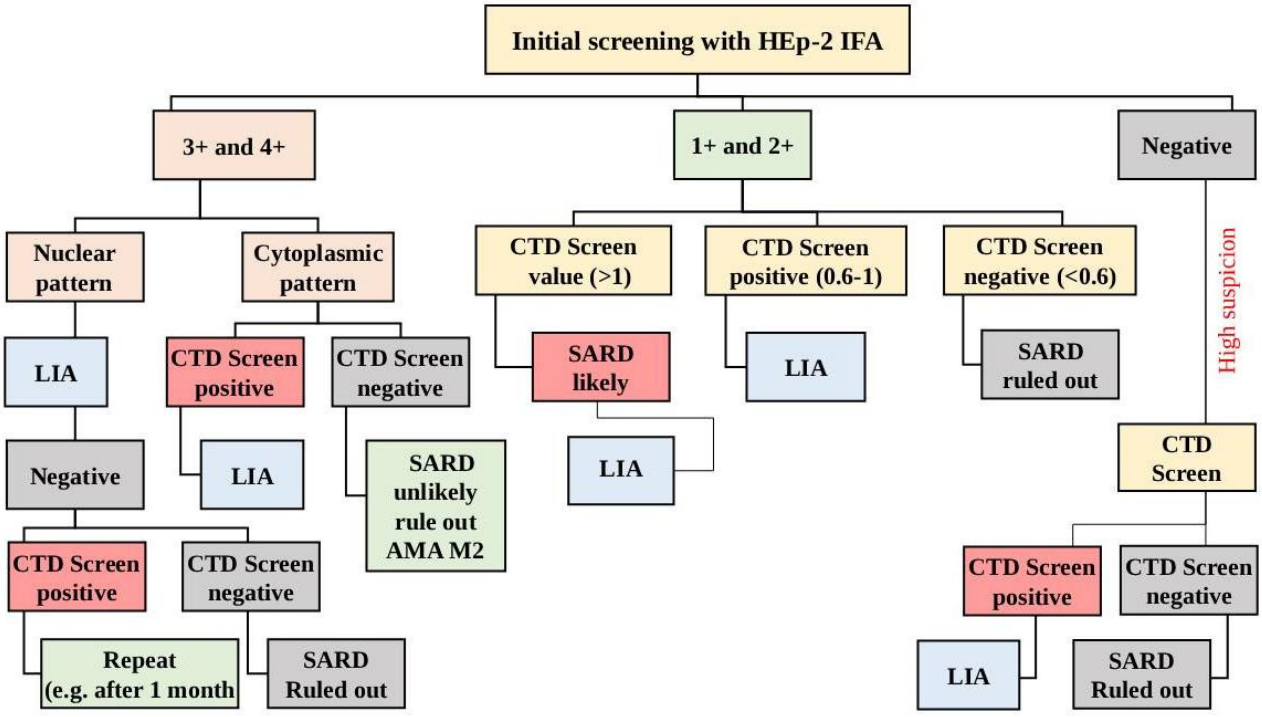

Figure 4. Proposed algorithm for ANA screening using HEp-2 IFA, CTD Screen, and LIA in SARDs. ANA: antinuclear antibody; CTD Screen: connective tissue disease Screen; HEp-2 IFA: HEp-2 cell indirect immunofluorescence assay; LIA: line immunoassay; SARD: systemic autoimmune rheumatic disease. Reference values mentioned for the CTD Screen are in-house cut off values.

While the study provides important insights, a few limitations merit consideration. An important methodological consideration is the initial use of a 1:40 HEp-2 IFA screening dilution, which is lower than the ≥ 1:80 threshold recommended by ICAP guidelines. Lower screening dilutions may improve analytical sensitivity and facilitate detection of weak antibody reactivities in early or evolving disease; however, they may also increase nonspecific staining and false-positive results, thereby reducing specificity. This may partly explain the discordance observed between HEp-2 IFA and CTD Screen/LIA results, particularly among low-intensity HEp-2 IFA-positive samples. To minimize this effect, all positive samples identified at 1:40 dilution were repeated at 1:80, and the results remained unchanged because faint fluorescence intensities had already been excluded during interpretation at 1:40. Nevertheless, using a lower screening dilution may limit direct comparability with studies that employ the internationally recommended ≥ 1:80 threshold. The 1:40 dilution was selected to reflect prevailing screening practices in many Indian laboratories, including ours, where higher analytical sensitivity is often prioritized in clinically suspected SARD cases. Another limitation is that LIA was not performed on double-negative samples, potentially leading to an underestimation of weak or rare autoantibody positivity. The inclusion of previously treated patients could have influenced antibody titers. The case-control design with equal numbers of HEp-2 IFA-positive and negative samples is another significant drawback, as it does not accurately reflect the prevalence of SARDs in standard clinical practice. As a result, prevalence-dependent metrics such as overall accuracy, PPV, and NPV may not be immediately applicable across different clinical contexts. To overcome

this, we also focused on LR and ROC-AUC values, which provide more reliable measures of diagnostic performance and are less affected by disease prevalence. Future multicentre, prospective studies should validate the optimized cutoff in clinically stratified cohorts, include cost-benefit analyses, and assess the integration of automated platforms into routine laboratory workflows.

In conclusion, this study represents one of the largest comparative cohort studies to validate a population-specific CTD Screen cutoff against both HEp-2 IFA and LIA. The CTD Screen demonstrated high diagnostic accuracy and substantial agreement with HEp-2 IFA, while offering advantages of automation, reproducibility, and efficiency. However, CTD Screen should not be used as a standalone diagnostic tool, particularly in low-prevalence settings, and must be interpreted in conjunction with clinical evaluation and confirmatory testing. A tiered diagnostic approach integrating CTD Screen with HEp-2 IFA and LIA may improve diagnostic efficiency, optimize resource utilization, and support early and accurate detection of SARDs.

Abbreviations

95% CI: 95% confidence interval

ALP: alkaline phosphatase

ALT: alanine aminotransferase

ANAs: antinuclear antibodies

AST: aspartate aminotransferase

AUC: area under the curve

CTD Screen: connective tissue disease Screen

ELISA: enzyme-linked immunosorbent assay

FEIA: fluorescence enzyme immunoassay

GGT: gamma-glutamyl transferase

HEp-2 IFA: HEp-2 cell indirect immunofluorescence assay

ICAP: International Consensus on Antinuclear Antibody Patterns

LIA: line immunoassay

LRs: likelihood ratios

LSSc: limited systemic sclerosis

MCTD: mixed connective tissue disease

NLR: negative likelihood ratio

NPV: negative predictive value

OS: overlap syndrome

PLR: positive likelihood ratio

PPV: positive predictive value

RA: rheumatoid arthritis

ROC: receiver operating characteristic

SARDs: systemic autoimmune rheumatic diseases

SjS: Sjogren's syndrome

SLE: systemic lupus erythematosus

SSc: systemic sclerosis

UCTD: undifferentiated connective tissue disease

κ: Cohen's kappa

Declarations

Author contributions

AK: Investigation, Writing—original draft. VD and SKS: Data curation. RWM: Supervision. MK and SC: Formal analysis. YK: Conceptualization, Methodology, Resources, Data curation, Supervision, Writing—original draft, Writing—review & editing. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The Institutional Ethics Committee approved this study (Approval No. INT/IEC/2022/SPL-1051).

Consent to participate

A prior written informed consent form was obtained from the parents/guardians of each subject.

Consent to publication

Not applicable.

Availability of data and materials

The datasets supporting the findings of this study are available from the corresponding author upon reasonable request.

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References

1. Agmon-Levin N, Damoiseaux J, Kallenberg C, Sack U, Witte T, Herold M, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis. 2014;73:17–23. [DOI] [PubMed]
2. Op De Beeck K, Vermeersch P, Verschueren P, Westhovens R, Mariën G, Blockmans D, et al. Detection of antinuclear antibodies by indirect immunofluorescence and by solid phase assay. Autoimmun Rev. 2011;10:801–8. [DOI] [PubMed]
3. Fritzler MJ, Choi MY. Antinuclear Antibody Testing: Gold Standard Revisited. J Appl Lab Med. 2022;7:357–61. [DOI] [PubMed]

4. Minz RW, Kumar Y, Saikia B, Anand S, Varma S, Singh S. Use of panel testing for detection of antinuclear antibody in a resource-limited setting: an appraisal. Postgrad Med. 2016;128:869–74. [DOI] [PubMed]
5. Yoon S, Moon HW, Kim H, Hur M, Yun YM. Clinical Performance of Two Automated Immunoassays, EliA CTD Screen and QUANTA Flash CTD Screen Plus, for Antinuclear Antibody Screening. Ann Lab Med. 2022;42:63–70. [DOI] [PubMed] [PMC]
6. Watanabe N, Nagatomo R, Okubo S, Yokota H, Ikeda H, Yatomi Y. Performance and clinical evaluation of antinuclear antibody test based on fluorescence enzyme immunoassay. Rinsho Byori. 2014;62: 315–23. Japanese. [PubMed]
7. Jeong S, Yang H, Hwang H. Evaluation of an automated connective tissue disease screening assay in Korean patients with systemic rheumatic diseases. PLOS ONE. 2017;12:e0173597. [DOI] [PubMed] [PMC]
8. González Rodríguez C, Fuentes Cantero S, Pérez Pérez A, Vázquez Barbero FJ, León Justel A. Comparison of the analytical and clinical performances of two different routine testing protocols for antinuclear antibody screening. J Clin Lab Anal. 2021;35:e23914. [DOI] [PubMed] [PMC]
9. van der Pol P, Bakker-Jonges LE, Kuijpers JHSAM, Schreurs MWJ. Analytical and clinical comparison of two fully automated immunoassay systems for the detection of autoantibodies to extractable nuclear antigens. Clin Chim Acta. 2018;476:154–9. [DOI] [PubMed]
10. Kapuczinski A, Parisis D, Kassab N, Smet J, Soyfoo M. Diagnosis of Systemic Rheumatic Disease Using the Connective Tissue Disease Screen. Antibodies. 2025;14:56. [DOI] [PubMed] [PMC]
11. Robier C, Amouzadeh-Ghadikolai O. The clinical significance of borderline results of the Elia CTD Screen assay. Clin Chem Lab Med (CCLM). 2018;56:2088–92. [DOI] [PubMed]
12. Orme ME, Andalucia C, Sjölander S, Bossuyt X. A comparison of a fluorescence enzyme immunoassay versus indirect immunofluorescence for initial screening of connective tissue diseases: Systematic literature review and meta-analysis of diagnostic test accuracy studies. Best Pract Res Clin Rheumatol. 2018;32:521–34. [DOI] [PubMed]
13. Bentow C, Lakos G, Rosenblum R, Bryant C, Seaman A, Mahler M. Clinical performance evaluation of a novel, automated chemiluminescent immunoassay, QUANTA Flash CTD Screen Plus. Immunol Res. 2014;61:110–6. [DOI] [PubMed]
14. Willems P, De Langhe E, Claessens J, Westhovens R, Van Hoeyveld E, Poesen K, et al. Screening for connective tissue disease-associated antibodies by automated immunoassay. Clin Chem Lab Med (CCLM). 2018;56:909–18. [DOI] [PubMed]
15. Bizzaro N, Brusca I, Previtali G, Alessio MG, Daves M, Platzgummer S, et al. The association of solid-phase assays to immunofluorescence increases the diagnostic accuracy for ANA screening in patients with autoimmune rheumatic diseases. Autoimmun Rev. 2018;17:541–7. [DOI] [PubMed]
16. Parker JC, Bunn CC. Sensitivity of the Phadia EliA connective tissue disease screen for less common disease-specific autoantibodies. J Clin Pathol. 2011;64:631–3. [DOI] [PubMed]
17. Keppeke GD, Landoni D, Kayser C, Matos P, Diogenes L, Keppeke J, et al. Anti-CENP-A/B reactivity in samples exhibiting the centromere HEp-2 pattern is associated with a lower frequency of interstitial lung disease in limited cutaneous systemic sclerosis patients. J Circ Biomark. 2025;14:54–63. [DOI] [PubMed] [PMC]
18. Keppeke GD, Satoh M, Kayser C, Matos P, Hasegawa T, Tanaka S, et al. A cell-based assay for detection of anti-fibrillarin autoantibodies with performance equivalent to immunoprecipitation. Front Immunol. 2022;13:1011110. [DOI] [PubMed] [PMC]
19. Robier C, Amouzadeh-Ghadikolai O, Stettin M, Reicht G. Comparison of the clinical utility of the Elia CTD Screen to indirect immunofluorescence on Hep-2 cells. Clin Chem Lab Med (CCLM). 2015;54: 1365–70. [DOI] [PubMed]

20. Jeong S, Hwang H, Roh J, Shim JE, Kim J, Kim GT, et al. Evaluation of an Automated Screening Assay, Compared to Indirect Immunofluorescence, an Extractable Nuclear Antigen Assay, and a Line Immunoassay in a Large Cohort of Asian Patients with Antinuclear Antibody-Associated Rheumatoid Diseases: A Multicenter Retrospective Study. J Immunol Res. 2018;2018:9094217. [DOI] [PubMed] [PMC]