# Real-Time Pathogen Visualization: Fluorescence ROSE versus Kyoto Classification for H. pylori Infection Activity and Eradication Assessment

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**Abstract**

Objective To compare the diagnostic value of fluorescence rapid on-site evaluation (F-ROSE) and Kyoto classification-based endoscopic direct diagnosis in determining Helicobacter pylori (Hp) infection, distinguishing infection activity, and assessing post-eradication status, to clarify the differences between the two methods in low-bacterial-load infection, active infection, and residual infection after eradication, and to establish an integrated endoscopic precision diagnostic pathway for Hp covering qualitative, quantitative, activity and eradication assessment. Methods A total of 210 consecutive patients undergoing gastroscopy were enrolled and underwent both fluorescence ROSE and Kyoto classification endoscopic evaluation. ROSE used AO-EB dual fluorescent staining, and viable, non-viable and total bacteria were counted under ×400 high-power field (HPF). Kyoto classification classified patients as uninfected, currently infected, or past/eradicated infection based on regular arrangement of collecting venules (RAC), diffuse redness, atrophy, intestinal metaplasia, nodularity and other signs. Latent class analysis (LCA) combined with a Bayesian model was used to infer true infection and activity status. ROC curves, consistency tests and subgroup analyses were performed to compare the diagnostic performance of the two methods, focusing on low-bacterial-load infection, post-eradication residual infection, and endoscopic gray-zone cases. Results By model inference, among the 210 patients, 107 had true current infection, 68 had past/eradicated infection, and 35 were uninfected. The positive rate of fluorescence ROSE (50.5%) was significantly higher than that of Kyoto classification (44.3%, $P<0.05$). With true infection as reference: fluorescence ROSE showed sensitivity 92.5%, specificity 95.3%, Kappa=0.891, AUC=0.948; Kyoto classification showed sensitivity 79.4%, specificity 90.2%, Kappa=0.726, AUC=0.863. Among 57 patients with true low-bacterial-load infection (4-9 viable bacteria/HPF) inferred by the model, the detection rate of ROSE was 100.0%, while that of Kyoto classification was only 35.7%. In post-eradication cases, the sensitivity of ROSE in identifying viable residual/recurrent infection was 88.2%, significantly higher than 41.2% of Kyoto classification. Among 41 endoscopic gray-zone (suspicious infection) cases by Kyoto classification, 73.2% were confirmed as true viable infection by ROSE. Conclusions Kyoto classification relies on mucosal morphology to indirectly infer Hp status, which is prone to miss low-bacterial-load and post-eradication residual infection and cannot distinguish viable from dead bacteria. Fluorescence ROSE enables real-time visual

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identification of viable bacteria, accurate quantification, direct judgment of infection activity and eradication effect, breaking through the limitations of endoscopic morphology. It is an optimal technique for current infection, low-bacterial-load infection and post-eradication evaluation, and can complement Kyoto classification to form a dual-dimensional precision diagnosis system of morphology + pathogen.

**Key words:** fluorescence ROSE; Helicobacter pylori; Kyoto classification of gastritis; endoscopic direct diagnosis; infection activity; post-eradication evaluation; low-bacterial-load infection

## 1 Introduction

Helicobacter pylori (Hp) is a Gram-negative microaerophilic bacillus colonizing the gastric mucosa. It is closely related to chronic gastritis, peptic ulcer, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer, and has been classified as a Group I carcinogen by the WHO. The infection rate among Chinese adults is approximately 40%-50%. Accurate determination of infection status, infection activity, and eradication outcome is central to reducing the burden of gastric cancer [1-5].

Current endoscopic diagnosis of Hp mainly uses the Kyoto classification of gastritis [6-8], proposed by the Japan Gastroenterological Endoscopy Society in 2013. By integrating signs such as regular arrangement of collecting venules (RAC), diffuse redness, atrophy, intestinal metaplasia, and nodularity, it classifies gastric mucosa into Hp-uninfected, currently infected, and past (eradicated) infection, thus enabling infection and gastric cancer risk assessment based on mucosal morphology. However, it has clear limitations: ① indirect inference based on morphology, without direct visualization of the bacterium, leading to missed diagnosis in low-bacterial-load or focal infection; ② inability to distinguish viable from dead bacteria, hence cannot determine whether infection is active; ③ slow recovery of mucosal morphology after eradication, making it difficult to identify early residual/recurrent infection; ④ high subjectivity and low reproducibility, affected by endoscopist experience, equipment, and mucosal inflammation.

Fluorescence rapid on-site evaluation (F-ROSE) is a novel rapid pathogen diagnosis technique during endoscopy [9-11]. Using AO-EB dual fluorescent staining, it directly distinguishes viable (yellow-green) from dead (orange-red) bacteria and completes qualitative, quantitative, and activity assessment within 15-25 minutes, providing visual direct pathogen evidence for Hp infection.

In this study, under a no-gold-standard framework, we used LCA combined with a Bayesian model to infer true infection status [12-15] and systematically compared the diagnostic performance of fluorescence ROSE and Kyoto classification in five dimensions: Hp infection determination, activity differentiation, post-eradication assessment, low-bacterial-load identification, and endoscopic gray-zone interpretation.

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We further defined the complementary value of the two methods and constructed a new endoscopic precision diagnostic pathway for Hp. The study technical roadmap is shown in Figure 1.

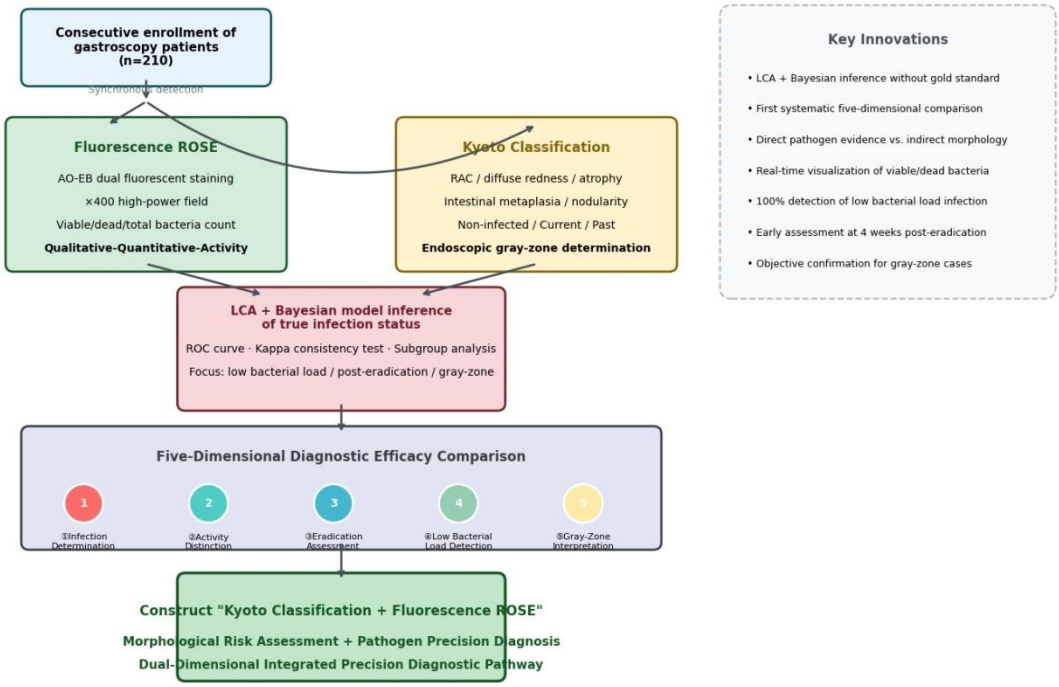


Figure 1. Study design and technical roadmap: Fluorescence ROSE versus Kyoto classification endoscopic diagnosis for H. pylori infection, activity, and eradication status

Figure legend: 210 consecutive patients undergoing gastroscopy were enrolled and underwent synchronous fluorescence ROSE (AO-EB dual staining, viable/dead/total bacteria count under ×400 HPF) and Kyoto classification endoscopic evaluation (RAC, diffuse redness, atrophy, intestinal metaplasia, nodularity). LCA combined with a Bayesian model was used to infer true infection status. ROC curves, Kappa consistency tests, and subgroup analyses were used to compare the two methods in five dimensions: infection determination, activity differentiation, eradication assessment, low-bacterial-load identification, and gray-zone interpretation. Finally, a "Kyoto classification + fluorescence ROSE" dual-dimensional integrated precision diagnostic pathway was constructed.

## 2 Materials and Methods

### 2.1 Study subjects

A total of 210 consecutive patients undergoing routine gastroscopy in our department of gastroenterology from November 2025 to April 2026 were enrolled. One standard biopsy specimen was taken from the gastric antrum in each case. Inclusion criteria: age 18-75 years; dyspeptic symptoms or health check-up requiring Hp screening; no use of antibiotics, bismuth salts, or PPIs for at least 4 weeks, and no $H_2$ receptor antagonists for 2 weeks before examination; no history of gastrectomy; no gastric cancer, severe dysplasia, or severe liver/renal dysfunction; signed informed consent; ability to complete both ROSE and Kyoto assessment. Exclusion criteria: previous gastric surgery; suspected malignancy; pregnancy or lactation; inability to tolerate

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gastroscopy; unsatisfactory specimen collection, preparation failure, or contamination.

2.2 Instruments and reagents

Main equipment: electronic gastroscope (Olympus GIF-H290); gastric mucosal biopsy forceps (Nanjing Micro-Tech Medical Co., Ltd.); fluorescence ROSE rapid detection kit for Hp (Jiangsu Nuo Biotech Co., Ltd.); AI intelligent high-definition fluorescence microscope analysis system (Jiangsu Nuo Biotech Co., Ltd.); $^{13}$C-urea breath test instrument and supporting reagents.

Main reagents: AO-EB dual fluorescent staining solution, methanol fixative, sterile saline, $^{13}$C-urea reagent, etc.

2.3 Fluorescence ROSE detection procedure

Biopsy specimen rinsed with saline → shredded and smeared → fixed with methanol for 5 minutes → stained with AO-EB dual dye in the dark for 10 minutes → rinsed and air-dried → five non-overlapping fields randomly selected under ×400 HPF to count viable, dead, and total bacteria. Criteria: viable bacteria appear green or yellow-green with intact rod, curved, or S-shaped morphology; dead bacteria appear orange-red or red, with morphology consistent with viable bacteria or partially coccoid and fragmented. The microscopic characteristics of fluorescence ROSE versus conventional histopathology and the identification of viable/dead bacteria are shown in Figure 2.

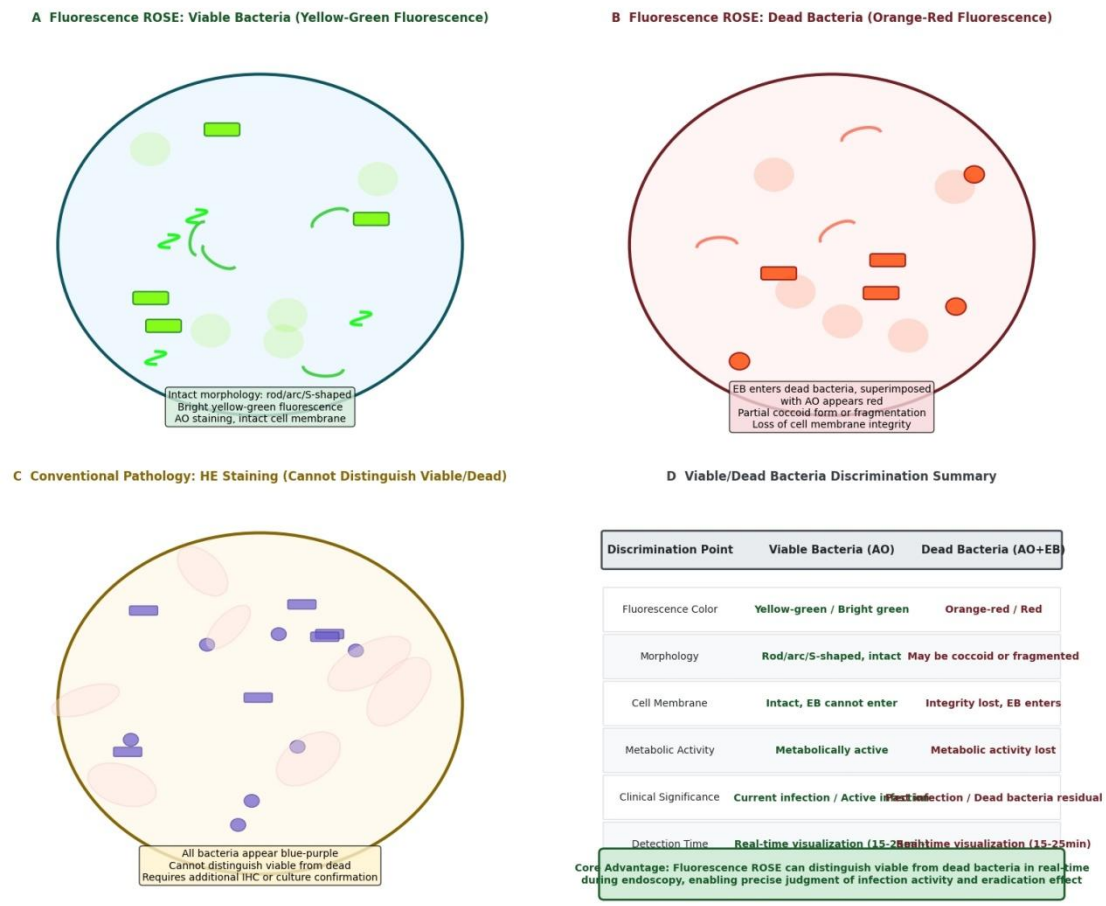

Figure 2. Fluorescence ROSE versus conventional histopathology: microscopic characteristics and viable/dead bacterial discrimination

Figure legend: A: Fluorescence ROSE shows viable bacteria as bright yellow-green fluorescent, intact morphology (rod/curved/S-shaped). AO staining, intact cell membrane prevents EB entry. B: Fluorescence ROSE shows dead bacteria as orange-red/red fluorescence; EB enters dead bacteria and overlaps with AO to produce red color, some coccoid or fragmented, loss of membrane integrity. C: Conventional histopathology (HE staining) shows all bacteria as blue-purple, cannot distinguish viable from dead, requiring additional immunohistochemistry or culture. D: Summary table of key points for viable/dead discrimination. The core advantage of fluorescence ROSE is real-time intra-endoscopic distinction between viable and dead bacteria, enabling precise judgment of infection activity and eradication efficacy.

### 2.4 Kyoto classification endoscopic diagnostic criteria

Two senior endoscopists performed blinded interpretation independently. Based on core sign scoring and classification: Uninfected: visible clear RAC, no diffuse redness, atrophy, intestinal metaplasia, or nodularity; Kyoto score 0. Current infection: diffuse redness, enlarged folds, nodularity, loss of RAC; Kyoto score ≥2. Past infection / post-eradication: presence of atrophy/intestinal metaplasia, disappearance of diffuse redness, map-like redness, no active inflammatory signs. Endoscopic gray zone: atypical signs, score 1, difficult to determine current vs. past infection. Figure 3 shows typical Hp-positive endoscopic signs according to Kyoto classification (nodularity “chicken-skin” appearance, enlarged folds with erosion, atrophy with redness and exudation) and typical Hp-negative findings (RAC/bird-claw vessels), along with corresponding fluorescence ROSE pathogen verification, visually demonstrating the consistency and complementarity of the two methods.

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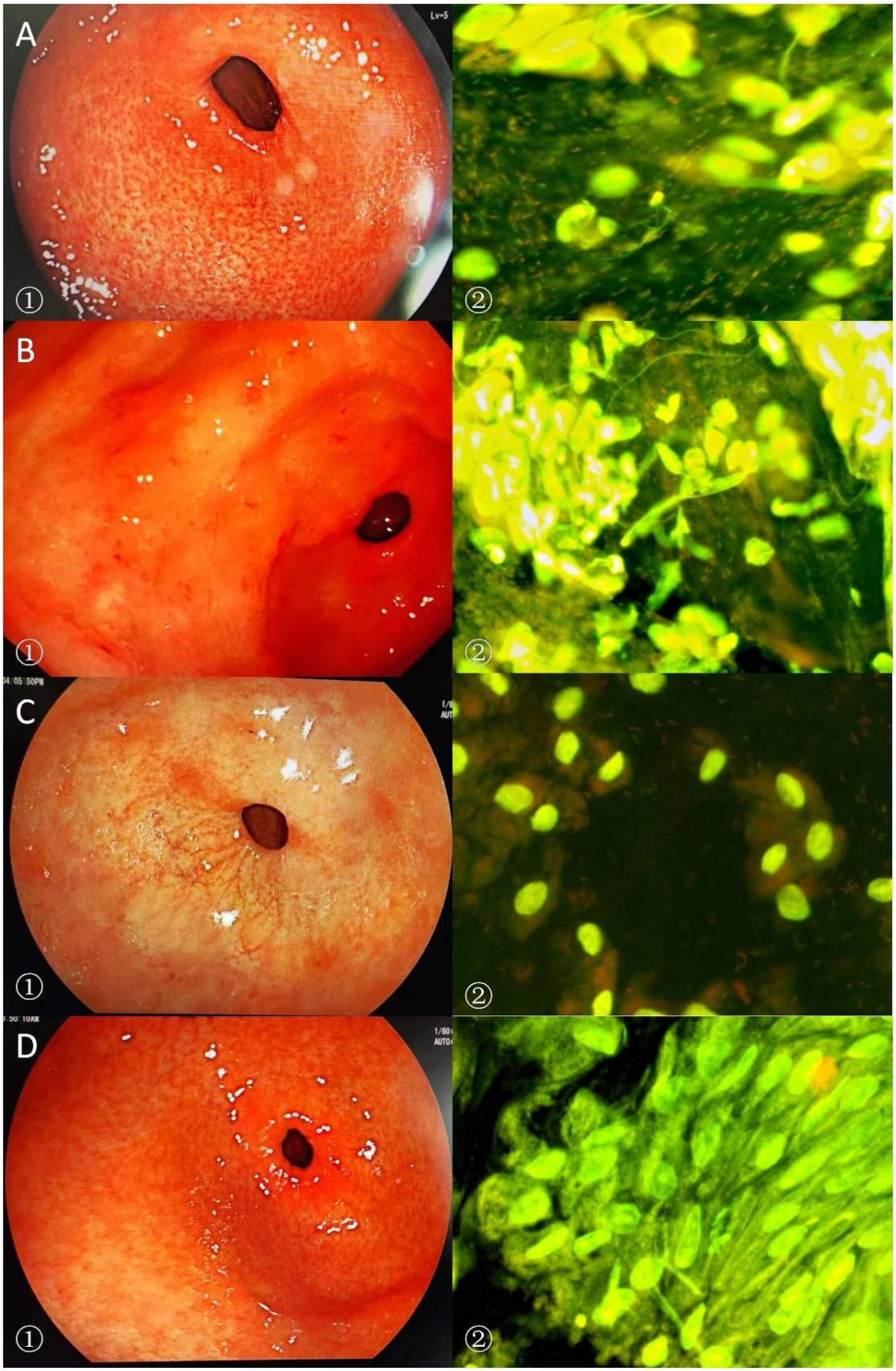


Figure 3. Typical endoscopic signs of Kyoto classification and fluorescence ROSE pathological verification

Figure legend: A: Nodularity (“chicken-skin” appearance). Endoscopy shows dense, uniform,

small white nodules in the antrum, a typical sign of current Hp infection. Corresponding fluorescence ROSE (A②) shows abundant yellow-green fluorescent viable bacteria, rod-shaped or curved, densely distributed, confirming active Hp infection. B: Enlarged folds with erosion. Endoscopy shows markedly thickened and swollen gastric body folds, with patchy erosions and diffuse redness; RAC absent. Corresponding fluorescence ROSE (B②) shows yellow-green fluorescent viable bacteria and red dead bacteria within tissue and mucus; abundant bacteria, confirming active Hp infection. C: Atrophy with redness and exudation. Endoscopy shows thin mucosa with visible vessels in the antrum and angulus, together with map-like redness and mild exudation, indicating current Hp infection with mucosal injury. Corresponding fluorescence ROSE (C②) shows scattered yellow-green fluorescent viable bacteria and dead bacteria; lower bacterial load, confirming persistent Hp infection. D: Superficial gastritis with typical RAC. Endoscopy shows regular arrangement of collecting venules (“bird-claw” or “tortoiseshell” pattern) in the lower gastric body, normal mucosal color, no diffuse redness, a typical Hp-uninfected appearance. Corresponding fluorescence ROSE (D②) shows no yellow-green fluorescent viable bacteria; background shows dark green autofluorescence, confirming Hp negativity.

### 2.5 Statistical methods

SPSS 26.0 and Mplus software were used. LCA combined with a Bayesian model inferred true infection status. Continuous variables were expressed as mean ± standard deviation; group comparisons used t-test. Categorical variables were expressed as n (%); group comparisons used $\chi^2$ test. ROC curves and subgroup analyses were used to compare diagnostic performance. $P<0.05$ was considered statistically significant. Cohen’s Kappa coefficient was used to evaluate consistency among fluorescence ROSE, Kyoto classification, and LCA-model-inferred true infection status. Kappa interpretation: ≤0.40 fair, 0.41-0.60 moderate, 0.61-0.80 substantial, 0.81-1.00 almost perfect. DeLong’s test was used to compare the AUCs of the two methods. This was a paired design diagnostic accuracy study; sample size was estimated using sensitivity comparison as the primary endpoint. Parameters: Kyoto classification sensitivity 79.4%, expected ROSE sensitivity 92.5%, difference $\delta=0.131$, $\alpha=0.05$, power=80%, prevalence 51.0%, dropout 10%. Using the formula for discordant pairs in a paired design, the planned sample size was approximately 238; 210 were actually enrolled, meeting the statistical requirements for primary and secondary endpoints. Post-hoc power analysis showed that detecting an AUC difference ≥0.05 had a power of 75%-85%. LCA modeling was performed with Mplus 8.0; 2-4 classes were tested, and the optimal number of classes was determined by the smallest Bayesian information criterion (BIC) and entropy >0.80 (in this study, the 3-class model had the smallest BIC and entropy=0.87). The Bayesian model used non-informative priors (Beta distribution $\alpha=\beta=1$). MCMC sampling used 4 chains, each with 20,000 iterations, with the first 5,000 as burn-in; Gelman-Rubin diagnostic factors were all <1.05, confirming convergence. Sensitivity analysis showed that posterior probabilities changed by <2% under different prior settings, indicating robust results.

## 3 Results

### 3.1 Comparison of positive rates and LCA-model-inferred true infection rates

Using latent class analysis (LCA) combined with a Bayesian model, the true infection status of the 210 subjects was inferred as follows: 107 true current infection, 68 post-eradication/past infection, and 35 uninfected, giving a true current infection rate of 51.0%, consistent with the epidemiological level of Hp infection in Chinese adults. Fluorescence ROSE results: 106 positive, 104 negative, positive rate 50.5%. Kyoto classification endoscopic results: 93 positive, 117 negative, positive rate 44.3%. The positive rate of fluorescence ROSE was significantly higher than that of Kyoto classification ($\chi^2$=7.08, P<0.05).

3.2 Overall diagnostic performance comparison

Using the model-inferred true infection status as the reference, the diagnostic performance of fluorescence ROSE and Kyoto classification was comprehensively evaluated, including sensitivity, specificity, positive predictive value, negative predictive value, Kappa consistency, and area under the ROC curve (AUC). Results are shown in Table 1. A comparison of diagnostic performance and ROC curve analysis is shown in Figure 4.

Table 1. Diagnostic performance comparison of the two methods for Helicobacter pylori infection

| Method | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Kappa | AUC |
|---|---|---|---|---|---|---|
| Fluorescence ROSE | 92. 5 | 95. 3 | 93. 4 | 94. 7 | 0. 891 | 0. 948 |
| Kyoto classification | 79. 4 | 90. 2 | 87. 1 | 83. 8 | 0. 726 | 0. 863 |

As shown in Table 1, fluorescence ROSE was significantly superior to Kyoto classification in sensitivity, NPV, diagnostic consistency (Kappa), and AUC, while specificities were similar, indicating that fluorescence ROSE has better overall ability to identify Hp infection.

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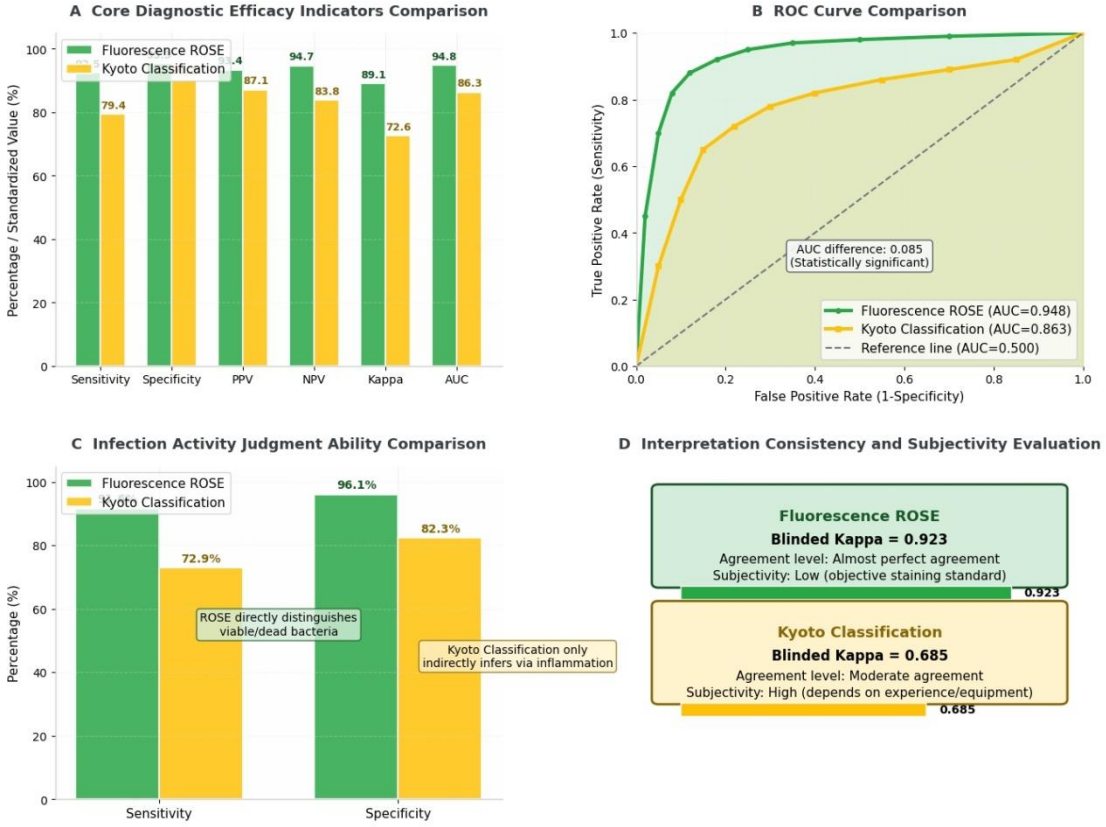


Figure 4. Diagnostic efficacy comparison and ROC curve analysis between fluorescence ROSE and Kyoto classification

Figure legend: A: Comparison of core diagnostic indicators (sensitivity, specificity, PPV, NPV, Kappa, AUC). Fluorescence ROSE is significantly superior to Kyoto classification in all core indicators. B: ROC curve comparison: fluorescence ROSE AUC=0.948, Kyoto classification AUC=0.863; AUC difference 0.085 (statistically significant). C: Comparison of infection activity judgment ability: fluorescence ROSE directly distinguishes viable/dead bacteria with sensitivity 91.6% and specificity 96.1%; Kyoto classification indirectly infers activity from inflammatory signs with sensitivity 72.9% and specificity 82.3%. D: Interobserver agreement and subjectivity: fluorescence ROSE double-blinded Kappa=0.923 (almost perfect agreement, low subjectivity); Kyoto classification double-blinded Kappa=0.685 (moderate agreement, high subjectivity).

### 3.3 Comparison of infection activity judgment ability

Using the model-determined “presence of viable bacteria” as the reference for true current/active infection, we compared the ability of the two methods to judge infection activity. Fluorescence ROSE, using AO-EB dual fluorescent staining, directly distinguished viable from dead bacteria, achieving a sensitivity of 91.6% and specificity of 96.1% for active infection. Kyoto classification could only indirectly infer active infection based on inflammatory signs such as diffuse redness and mucosal swelling, with a sensitivity of 72.9% and specificity of 82.3%, and it cannot distinguish viable from dead bacteria, leading to misjudgment in cases where residual inflammation persists after eradication.

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3.4 Subgroup analysis of low-bacterial-load infection

Low-bacterial-load infection was defined as: arithmetic mean of viable bacteria counted in five randomly selected non-overlapping fields under ×400 HPF, with 4-9 viable bacteria/HPF (inclusive). ≥10/HPF was defined as conventional bacterial load, and ≤3/HPF as very low bacterial load (not independently analyzed in this study). The LCA- and Bayesian-model-inferred true low-bacterial-load infection (4-9 viable bacteria/HPF) comprised 57 patients, representing the main challenging population for endoscopic diagnosis.

Using true low-bacterial-load infection as reference: fluorescence ROSE directly visualized and counted viable bacteria, successfully detecting all 57 cases (detection rate 100.0%). Kyoto classification, due to mild mucosal changes, incomplete disappearance of RAC, and atypical diffuse redness, detected only 20 cases (detection rate 35.7%). The difference was statistically significant ($P<0.001$). Low-bacterial-load infection was the main missed diagnosis type for Kyoto classification. In subgroup analysis, the power to detect a single-group rate difference (35.7% vs. 100%) in the low-bacterial-load group (n=57) was >99%; the power to detect the sensitivity difference (41.2% vs. 88.2%) in the post-eradication residual/recurrence subgroup (n=17) was 78%. Although the sample was limited, the results still provide valuable reference and require further validation in larger samples.

3.5 Post-eradication residual/recurrent infection assessment

A total of 68 patients who had received eradication therapy were included. Among them, 17 were inferred by the model to have viable bacterial residual/recurrence. This result was used as the reference to evaluate the ability of the two methods to identify post-eradication infection. The time interval from completion of eradication therapy to the current gastroscopy was: ≤4 weeks, 0 cases (drug interference excluded); 4-12 weeks, 29 cases; 12-24 weeks, 31 cases; >24 weeks, 8 cases. Subsequent analysis showed no significant effect of time interval on the accuracy of Kyoto classification ($P>0.05$). All post-eradication patients included were at least 4 weeks after the last eradication therapy (median 10 weeks, IQR 6-18 weeks) to exclude drug interference with ROSE detection.

Fluorescence ROSE directly observed whether viable bacteria were present in the mucosal tissue, correctly identifying 15 cases of residual/recurrent infection (sensitivity 88.2%). Because mucosal morphology recovery lags, with signs such as diffuse redness and fold abnormalities persisting for a long time, Kyoto classification correctly identified only 7 cases (sensitivity 41.2%). These findings indicate that fluorescence ROSE is significantly superior to Kyoto classification in early post-eradication assessment and identification of residual infection. The results of bacterial load stratification analysis for low-bacterial-load infection, post-eradication assessment, and endoscopic gray-zone cases are shown in Figure 5.

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Figure 5. Bacterial load stratification analysis: breakthrough in low-bacterial-load infection diagnosis and pathological positive threshold effect

Figure legend: A: Comparison of detection rates in low-bacterial-load infection (4-9 viable bacteria/HPF): fluorescence ROSE detection rate 100.0%, Kyoto classification only 35.7%; difference of 64.3 percentage points. B: Detection rate trends across different bacterial load strata: fluorescence ROSE maintains high detection rates at all load levels; Kyoto classification has a significant diagnostic blind spot at low load (4-9/HPF). C: Comparison of sensitivity for detecting post-eradication residual/recurrent infection: fluorescence ROSE directly observes viable bacteria and can be used as early as 4 weeks after therapy (sensitivity 88.2%); Kyoto classification relies on slow mucosal morphology recovery (sensitivity 41.2%). D: Pathogen verification results in endoscopic gray-zone cases: among 41 Kyoto-classified gray-zone cases, ROSE confirmed 73.2% (30 cases) as true low-bacterial-load infection and 26.8% (11 cases) as confirmed uninfected; ROSE provides objective confirmation.

### 3.6 Analysis of endoscopic gray-zone cases

According to Kyoto classification criteria, 41 cases (19.5% of total) were classified as endoscopic gray zone (atypical signs, suspicious infection). Pathogen verification with fluorescence ROSE showed: ROSE-positive in 30 cases (73.2%), all of which were true low-bacterial-load infection with 4-9 viable bacteria/HPF; ROSE-negative in 11 cases, completely consistent with the model-inferred uninfected status. These findings suggest that most endoscopic gray-zone cases of Kyoto classification

represent true low-viable-bacterial-load infection, and ROSE can provide objective confirmation.

3.7 Interobserver agreement and subjectivity

Two senior digestive endoscopists independently interpreted fluorescence ROSE images and Kyoto endoscopic images in a double-blinded manner, and the Kappa values for agreement were calculated. The double-blinded Kappa for fluorescence ROSE was 0.923, indicating almost perfect agreement, stable results, and low influence from subjective factors. The double-blinded Kappa for Kyoto classification was 0.685, indicating moderate agreement, with greater influence from endoscopist experience, equipment, mucosal preparation, and inflammation, reflecting higher subjectivity.

3.8 Kappa consistency test results (pairwise comparisons of the three methods)

Cohen's Kappa coefficients, standard errors (SE), and Z-test statistics are shown in Table 2.

Table 2. Kappa consistency test

| Comparison | Kappa | 95% CI | SE | Z | P | Agreement level |
|---|---|---|---|---|---|---|
| Fluorescence ROSE vs. LCA true status | 0.891 | 0.822–0.960 | 0.036 | 25.06 | <0.001 | High |
| Kyoto classification vs. LCA true status | 0.726 | 0.629–0.823 | 0.050 | 14.67 | <0.001 | Substantial |
| Fluorescence ROSE vs. Kyoto classification | 0.629 | 0.524–0.734 | 0.054 | 11.73 | <0.001 | Moderate |

The agreement between fluorescence ROSE and true status (Kappa=0.891) was significantly better than that between Kyoto classification and true status (Kappa=0.726); the difference was 0.165, indicating that ROSE diagnostic stability is significantly higher than endoscopic subjective interpretation.

3.9 DeLong test results (AUC comparison)

Since the two methods were paired (both tests performed on the same patients), the DeLong test was used to compare the areas under the ROC curves. Results are shown in Table 3.

Table 3. DeLong test results

| Method | AUC | 95%CI | AUC difference | Z | P |
|---|---|---|---|---|---|
| Fluorescence ROSE | 0.948 | 0.912–0.984 | — | — | — |
| Kyoto classification | 0.863 | 0.808–0.918 | — | — | — |
| Difference | — | — | 0.085 | 2.83 | 0.005 |

The AUC of fluorescence ROSE was significantly higher than that of Kyoto classification (P=0.005), indicating significantly better overall diagnostic performance.

## 4 Discussion

This study is the first to systematically compare fluorescence ROSE and Kyoto classification across multiple dimensions of Hp infection, activity, and eradication assessment, clarifying the advantages and limitations of each method and providing key evidence for constructing a dual-dimensional (morphology + pathogen) endoscopic precision diagnostic system.

### 4.1 Essential difference in diagnostic logic

Kyoto classification: indirect morphological inference, deducing Hp presence from secondary changes such as mucosal inflammation, atrophy, and intestinal metaplasia [16,17]; it is “inferring the cause from results” and cannot directly visualize the bacterium. Fluorescence ROSE: direct pathogen evidence, visualizing viable/dead Hp under the microscope with precise counting; it is “directly seeing the cause,” unaffected by mucosal morphology.

### 4.2 Infection activity judgment: Kyoto classification cannot distinguish viable from dead bacteria; ROSE achieves precise activity determination

Kyoto classification relies on inflammatory signs such as diffuse redness to judge active infection. After eradication, inflammation resolves slowly, which may lead to residual inflammation caused by dead bacteria being misinterpreted as active infection. Fluorescence ROSE, through AO-EB staining, directly distinguishes viable (yellow-green) from dead (orange-red) bacteria. It is the only technique that can determine whether infection is active in real time during endoscopy, providing direct evidence for precise eradication.

### 4.3 Low-bacterial-load infection: Kyoto classification shows significant missed diagnosis; ROSE 100% covers the diagnostic blind spot

Using model-inferred true low-bacterial-load infection (4-9 viable bacteria/HPF) as reference: fluorescence ROSE directly visualizes and accurately counts viable bacteria, achieving a detection rate of 100%. Kyoto classification, dependent on morphological changes, shows mild inflammation, persistent RAC, and atypical diffuse redness in low-bacterial-load infection, with a detection rate of only 35.7%; many cases are judged as negative or endoscopic gray zone. Fluorescence ROSE, by detecting at the pathogen level, completely breaks through the morphological limitation of endoscopy in diagnosing low-bacterial-load infection.

### 4.4 Post-eradication assessment: ROSE is the core tool for early identification of residual/recurrent infection

After eradication therapy, mucosal morphology (diffuse redness, fold swelling) takes months to recover. Kyoto classification cannot determine early whether true eradication has been achieved. Fluorescence ROSE directly visualizes whether viable bacteria are present and can accurately determine eradication efficacy as early as 4 weeks after therapy, identifying residual/recurrent infection early and avoiding blind

repeated treatment or delayed rescue therapy.

4.5 Endoscopic gray zone: ROSE provides objective confirmation

Approximately 19.5% of cases are classified as endoscopic gray zone by Kyoto classification, posing a clinical decision dilemma. This study demonstrated that 73.2% of these are true low-viable-bacterial-load infections; a traditional “watchful waiting” strategy would delay treatment. Fluorescence ROSE quickly provides a pathogen-based answer, enabling precise stratified management of gray-zone cases.

4.6 Objectivity and reproducibility: ROSE significantly superior to endoscopic subjective judgment

Kyoto classification is highly dependent on endoscopist experience, equipment conditions, and mucosal preparation, with moderate interobserver agreement (Kappa=0.685). Fluorescence ROSE uses standardized staining and objective interpretation criteria, achieving extremely high double-blinded agreement (Kappa=0.923), making it suitable for widespread implementation and quality control.

4.7 Complementary value: building a “Kyoto classification + fluorescence ROSE” dual-dimensional diagnostic system

Advantages of Kyoto classification: evaluating gastric cancer risk (extent and degree of atrophy and intestinal metaplasia), determining long-term outcomes of infection, guiding mucosal follow-up. Advantages of fluorescence ROSE: real-time pathogen diagnosis, determining infection activity, detecting low bacterial load, assessing eradication efficacy. Combined strategy: Kyoto classification evaluates mucosal background and gastric cancer risk; fluorescence ROSE confirms the presence of viable pathogen and activity. This integration achieves combined morphological risk assessment and precise pathogen diagnosis. The dual-dimensional Hp precision diagnostic pathway and complementary mechanism are shown in Figure 6.

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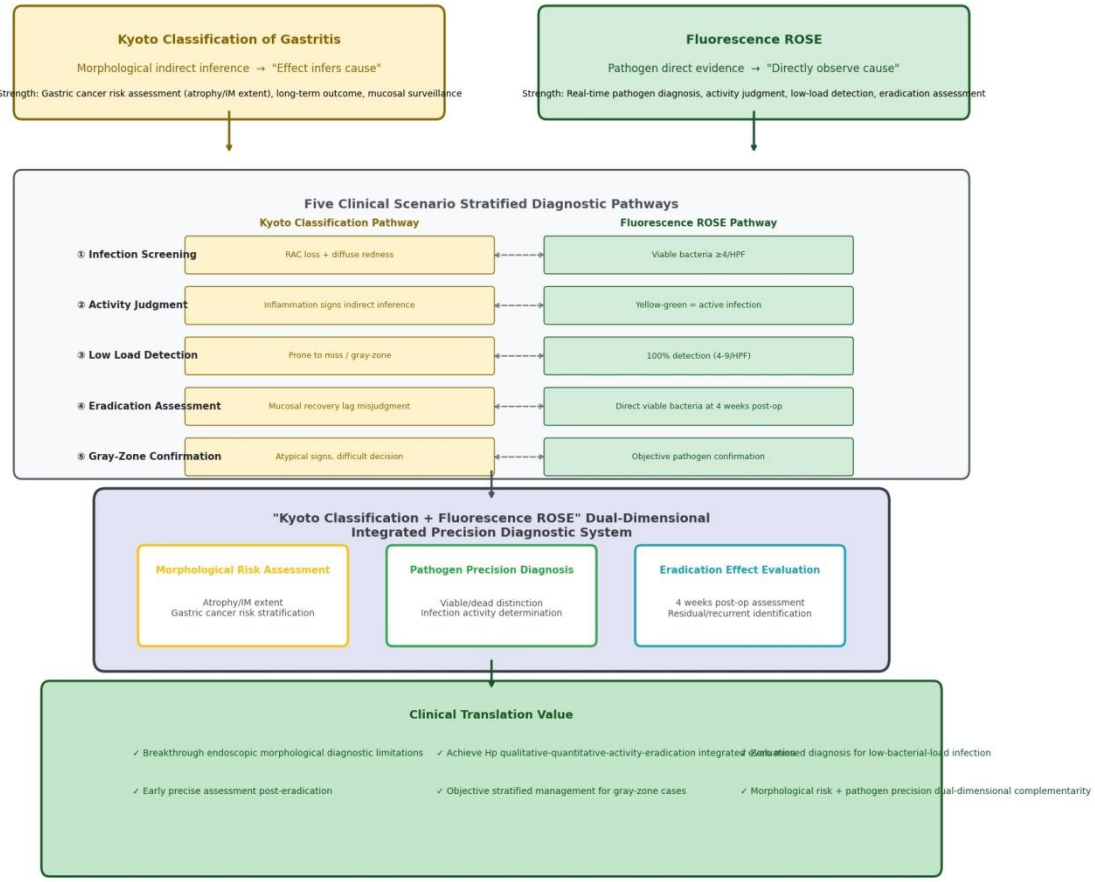


Figure 6. "Kyoto classification + fluorescence ROSE" dual-dimensional precision diagnostic pathway and complementary mechanism for H. pylori

Figure legend: Top: Essential differences and respective advantages of Kyoto classification (morphological indirect inference, "inferring cause from results") and fluorescence ROSE (direct pathogen evidence, "directly seeing the cause"). Middle: Stratified diagnostic pathways for five clinical scenarios (initial infection screening, activity judgment, low-bacterial-load identification, eradication assessment, gray-zone confirmation), demonstrating the complementary application of the two methods. Bottom: The "Kyoto classification + fluorescence ROSE" dual-dimensional integrated precision diagnostic system, integrating three modules: morphological risk assessment, precise pathogen diagnosis, and eradication efficacy evaluation. Far bottom: Summary of clinical translational value, achieving integrated endoscopic precision diagnosis of Hp covering qualitative, quantitative, activity, and eradication evaluation.

It should be noted that in clinical practice, non-invasive methods such as $^{13}$C-urea breath test and stool antigen test still have important value for initial Hp screening and post-eradication verification. The main role of fluorescence ROSE is not to replace these methods but to provide pathogen evidence during gastroscopy, especially suitable for patients who require simultaneous biopsy pathology or who cannot tolerate/cooperate with breath testing.

4.8 Study limitations

This was a single-center study with a limited sample size. Stratified comparisons

based on different degrees of atrophy/intestinal metaplasia were not performed. A parallel comparison between ROSE and conventional histopathology was not assessed. The conclusions need further validation in multicenter, large-scale, prospective studies.

## 5 Conclusions

Kyoto classification, based on mucosal morphology, enables assessment of Hp infection and gastric cancer risk, but it cannot directly visualize the bacterium, cannot distinguish viable from dead bacteria, has high missed diagnosis in low-bacterial-load and post-eradication settings, and is relatively subjective. Fluorescence ROSE enables real-time visualization, precise quantification, and distinction between viable and dead bacteria, and is superior to Kyoto classification in infection determination, activity assessment, low-bacterial-load detection, and post-eradication evaluation. Most endoscopic gray-zone cases under Kyoto classification are true low-viable-bacterial-load infections, and ROSE can provide objective confirmation. We recommend the dual-dimensional approach in clinical practice: Kyoto classification to assess mucosal background combined with fluorescence ROSE to confirm pathogen viability and activity, achieving precise diagnosis of Hp infection, individualized eradication, and comprehensive gastric cancer risk prevention. Fluorescence ROSE breaks through the limitations of endoscopic morphological diagnosis, effectively complements Kyoto classification, and represents a next-generation optimal technique for rapid and precise endoscopic Hp diagnosis, infection activity determination, and post-eradication assessment.

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