iGEM CRAB-QQ 2026 Best Integrated Human Practices

Project Name: CRAB Rapid Assay & Biofilm-stripping via Quorum Quenching
Application Category: Best Integrated Human Practices

Submission Date: February 22, 2026

I. Core Framework and Theoretical System of HP Work

Based on the classic iGEM HP workflow, this project adds two prerequisite stages: **Inquire** and **Anticipation**. It builds a closed-loop system of "Information Collection - Design - Anticipation - Multi-party Dialogue - Reflection - Response Feedback," making the initial design more reliable and communication with stakeholders more in-depth and efficient, thereby achieving deep integration of HP work and project technical design. The specific framework is as follows:

- **Inquire:** Ensure sufficient background information is obtained before the design begins, clarifying design goals and core directions.
- **Design:** Design a product system that is both innovative and reliable based on research results.
- **Anticipation:** Honestly and comprehensively predict the positive and negative impacts of the design, considering multiple dimensions such as project efficacy, safety, compliance, and cost; attempt to quantify results through table scoring.
- **Deliberation:** Conduct internal group discussions and in-depth exchanges with various stakeholders.
- **Reflectivity:** Systematically organize and reflect on the results of the multi-party dialogues, extracting core issues and optimization suggestions.
- **Responsiveness:** Implement the reflection results, making targeted improvements to the project's technical design and product scheme.

Note: Since the project has not yet entered the experimentation and external contact stage, the *Deliberation*, *Reflectivity*, and *Responsiveness* stages in this application have not yet been carried out. Related work will be gradually implemented and perfected as the project advances.

II. Stakeholder Analysis and Planning

Based on the Stakeholder Salience Model by Mitchell, Agle & Wood (1997), this project constructs a three-dimensional stakeholder analysis system of **Power-**

Urgency-Legitimacy to precisely target core stakeholders.

(I) Stakeholder Analysis Theory

- **Power:** Measures whether stakeholders have the resources, ability, and voice to promote project implementation and popularization, and whether they can influence user satisfaction and enhance social trust in the project.
- **Urgency:** Measures the project's degree of need for stakeholders' opinions, the impact of their opinions on project design, validation, and improvement, and whether they meet the current progress requirements of the project.
- **Legitimacy:** Measures whether the opinions of specific stakeholders in the industry are representative, whether their professionalism and industry recognition meet standards, and whether they fit the research and application scenarios of this project. Special scoring tables are made for interviewees of different identities.

This project will dynamically adjust the three-dimensional analysis table according to project progress, meticulously scoring a wide range of prospective experts/organizations, and ultimately finalizing the most suitable communication targets to ensure the project gathers comprehensive and effective opinions to the greatest extent.

(II) Core and Alternative Stakeholders Review

- **ICU Doctors:** Core stakeholders, responsible for verifying the clinical practicality of the project's products, providing core suggestions for the clinical adaptability of detection and treatment schemes.
- **War infection survivors, MSF (Doctors Without Borders) / Military Doctors:** Core stakeholders. CRAB infections include high-incidence scenarios after wartime medical conditions are disrupted; they can provide reference for infection risks and medical needs in special environments to verify the scheme's universal applicability.
- **ICU patients and families:** Core stakeholders, used to understand the medical burden on patient families and the actual treatment needs of patients, enhancing the humanism and compliance of the project.
- **Researchers:** Core stakeholders, including university laboratories and clinical testing department researchers. They are responsible for determining the feasibility of the project's technical solutions and providing clinical data and technical support. Alternative contacts include:
    - Clinical Laboratory of Hangzhou First People's Hospital (wangxj_0525@126.com, rich clinical data).

    - Zhang Rong (zhang-rong@zju.edu.cn, expert in CRAB epidemiology and genetics).
    - Yu Yunsong (yvys119@zju.edu.cn, expert in CRAB resistant strain transmission).
    - Clinical microbiology testing physicians (traditional CRAB detection experts).
- **Community hospitals, nursing homes:** Important stakeholders. Immunocompromised populations and the elderly are high-risk groups for CRAB infections; used to investigate grassroots medical institutions' understanding of CRAB, screening capabilities, and isolation conditions, promoting the adaptation of the scheme to primary care scenarios.
- **Enterprises:**
    - *IVD enterprises:* Prepare for the manufacturing and commercialization of test kits for distinguishing CRAB colonization/infection states.
    - *Pharmaceutical manufacturers:* Understand existing commercialization models for antibacterial preparations to clarify the project's commercial positioning and core competitiveness.
    - *Synthetic biology companies:* Draw on the implementation experience of synthetic biology medical applications, such as Quorum Biosciences.
    - *Enzyme preparation enterprises:* Provide required enzyme preparation support for the project, such as Synthetic Epoch Technology.
- **Government departments:** Important stakeholders, including infectious disease societies and biomedical review departments, providing guidance for the project's regulatory compliance and policy adaptation.
- **WHO related departments:** Important stakeholders. Collaborate with AMR (Antimicrobial Resistance) experts and UN Refugee Agency staff to investigate CRAB infections among refugee populations, responding to global AMR prevention and control needs.
- **Public:** Basic stakeholders. Carry out infectious disease science popularization and CRAB awareness surveys to enhance social awareness of the project and promote public attention to antimicrobial resistance prevention and control.

Note: Actual communication with various stakeholders has not yet commenced; subsequent exchanges and research will be gradually advanced based on the project's progress, filtering priorities according to the three-dimensional analysis

system.

III. HP Integration: Inquire-Design-Anticipation

The HP work of this project runs through the six core technical modules: Suicide, Visualization, Drug Delivery (Lung/Wound/Bloodstream), and Kit Sampling. In the initial stage of project design, information collection, scheme design, and multi-dimensional anticipation were completed for each module, achieving synchronized advancement of HP work and technical design. The specific integration content is as follows:

(I)

Suicide Module

- **Inquire:** Investigated engineering bacteria escape prevention strategies and selected temperature as the regulatory signal; verified the effectiveness of the MazF/MazE system and studied the Bxb1 mechanism; confirmed the operability of arabinose and clarified codon optimization needs; discovered that short half-life antitoxin TA systems can replace hok/sok to achieve plasmid stability.
- **Design:** Constructed a Bxb1 temperature-sensitive suicide circuit; designed a controllable clearance module driven by the pBAD promoter; integrated the TA system into the plasmid and fused triple regulation; designed multiple experiments to verify the function and efficiency of each component.
- **Anticipation:**
    - *Functional expectations:* To ensure biosafety, this design incorporates two safeguard mechanisms: arabinose concentration gradient-dependent passive suicide and medical staff-mediated active suicide. Additionally, the addiction module of the TA (Toxin-Antitoxin) system is employed to counteract potential suicide escape events, thereby providing a multi-layered safety guarantee.
    - *Risk prediction:* The antitoxin may exhibit excessive stability, while the toxin may be overly labile. Consequently, the anti-escape efficacy might not meet the expected design criteria.
    - *Optimization directions:* Conduct chemical modifications on both the antitoxin and toxin molecules, followed by screening to identify the combination with the optimal performance.

(II)

Visualization Module

- **Inquire:** Determined site-specific visualization strategies aimed at the pain

points of detecting CRAB infections in different locations; screened non-toxic chromogenic elements and AHL-specific cleavage enzymes; retrieved and selected biocompatible materials suitable for different sites.

- **Design:** Designed exclusive chromogenic systems for wounds/digestive tract, lungs, and bloodstream; adapted to the physiological characteristics of each site and avoided false positives; designed experiments such as electrophoresis and half-life screening to verify the system's effectiveness.
- **Anticipation:**
    - *Functional expectations:* Visualization aims to use prominent visual signals to inform clinical doctors of minute cellular changes, allowing our biofilm-stripping system to connect seamlessly with clinical antibiotic administration. This can make the entire therapy more efficient, enable timelier drug administration, and reduce the impact of CRAB on physical health.
    - *Risk prediction:* In clinical use, the visualization of sputum and urine may cause patient panic; if indigo remains in the lungs for a long time, it may cause pulmonary function obstruction.
    - *Optimization directions:* Clearly inform patients before use that this is a related testing procedure and adopt it voluntarily; sulfonate indigo to increase water solubility, making it prone to form colloids rather than crystals.

(III)

Drug Delivery Module

1. Lung

- **Inquire:** Clarified the core characteristics of lung CRAB lesions and drug delivery carrier requirements; verified AiiAN26.2 enzyme function; selected nebulization inhalation method and pH-sensitive carriers; clarified the safe particle size and osmotic pressure requirements for pulmonary drug delivery.
- **Design:** Adopted nebulized inhalation drug delivery; designed two drug loading schemes: CHEMS/DOPE liposomes and ZIF-8 nanoparticles; controlled particle size and loaded core agents; designed multi-dimensional experiments to verify drug loading and release effects.
- **Anticipation:**
    - *Functional expectations:* Can traverse the complex and tortuous lungs and take targeted effect where CRAB is colonized, specifically destroying biofilms at the lesions.

- *Risk prediction:* Although the lungs are considered an external environment, immune cells are active in the liquid layer on the alveolar surface. Immune cells may have phagocytic effects on our introduced drugs, affecting therapeutic efficacy.
- *Optimization directions:* In response to this, we can try to fuse PEG on the enzyme surface to reduce its immunogenicity.

2. Wound

- **Inquire:** Clarified the core pain points of drug delivery for wound infections; selected Pluronic F127 composite hydrogel material; determined sodium alginate-Ca^{2+} microsphere encapsulation technology; selected dual-chamber prefilled syringe technology to solve the freeze-drying reconstitution problem.
- **Design:** Prepared a temperature-sensitive hydrogel suitable for wound spraying; constructed double-layer semi-permeable microspheres to encapsulate engineering bacteria and added lyoprotectants; utilized dual-chamber prefilled syringe technology to achieve sterile storage and reconstitution; designed experiments to verify key characteristics.
- **Anticipation:**
  - *Functional expectations:* The current administration method uses thermosensitive hydrogels to cover irregular wounds, enhancing the sustainability of drug delivery.The semipermeable membrane in the double-layer system can effectively prevent engineered bacteria from entering the bloodstream, making administration safer.The dual-chamber pre-filled syringe technique solves the problems associated with lyophilization and reconstitution.
  - *Risk prediction:* Issues with irregular wound coverage; preventing engineering bacteria from entering the blood.
  - *Optimization directions:* Carrier utilizes temperature-sensitive hydrogel: Pluronic F127 + QCS (Quaternized Chitosan) + CMC (Sodium Carboxymethyl Cellulose) + functional substrate; utilize a double-layer membrane + semi-permeable membrane mechanism; employ dual-chamber prefilled technical means to solve freeze-drying and reconstitution problems.

3. Bloodstream

- **Inquire:** Clarified the characteristics of bloodstream CRAB infections and targeted therapy requirements; referenced ADC technology to determine the drug delivery architecture; selected humanized anti-OmpA antibodies and flexible PEGylated peptide linkers to ensure adaptation to the bloodstream

environment.

- **Design:** Constructed an anti-OmpA antibody - AiiA enzyme conjugate targeting system; optimized antibody and linker design to ensure effector enzyme catalytic activity; designed multiple experiments to verify the purity, activity, and stability of the conjugate.
- **Anticipation:**
    - *Functional expectations:* DNA enriched in biofilms is targeted and anchored at the biofilm to adapt to the high-flow environment in blood flow, thereby efficiently disrupting biofilms at lesion sites.
    - *Risk prediction:* The problem with OMVs is that incomplete LPS removal may possess some immunogenicity; OMVs acting as liposomes in the blood may easily be cleared by immunity; effector enzymes like pvdq inserted into OMVs may have reduced enzyme activity because their conformation cannot change normally.
    - *Optimization directions:* Coliclear will be used to produce OMVs to minimize immunogenicity. Meanwhile, OMVs will be encapsulated with PEG to further reduce immunogenicity

(IV)

Kit Sampling Module

- **Inquire:** Discovered the shortcomings of traditional detection; selected the colloidal gold method to achieve rapid detection; through discussion, innovatively determined the ratio of 3-OH-C12-HSL to OXA-23 as the core indicator for distinguishing colonization/infection.
- **Design:** Competitive colloidal gold immunochromatographic test strip, gold-labeled AbaR-gold nanoparticles + anti-OXA-23 antibodies. Distinguishes colonization/infection by judging the ratio of 3-OH-C12-HSL to OXA-23, paired with visible light detection equipment and grayscale analysis software. An alternative is magnetic bead-coupled anti-AbOmpA antibodies to screen CRAB, testing FeoA and OXA-23 content after lysis to improve detection accuracy, and designing related detection schemes.
- **Anticipation:**
    - *Functional expectations:* Rapidly and conveniently detect the presence of CRAB in a hospital environment, distinguish between colonization and infection states, and thereby assist in subsequent targeted treatment.
    - *Risk prediction:* Detection results may yield false positives due to the

presence of other bacteria, or false negatives due to insensitivity to small molecular substances; the two analytes might not exhibit a correlation with each other.

- *Optimization directions:* Perform multiple cycles to determine the optimal processing buffer, measure the accuracy rate and inform users, implement algorithm iterations to adopt the optimal version; ensure database data purity, investigate standby detection characterization and reserve backup plans.

IV. Safety and Ethical Considerations

- **Biosafety:** Designed a comprehensive engineering bacteria suicide module; through the triple guarantees of temperature-sensitive suicide, chemically-induced clearance, and plasmid anti-loss, it prevents engineering bacteria from escaping at the source; formulated the "CRAB Experimental Biosafety Standard Operating Procedures" to strictly standardize experimental operations.
- **Clinical Ethics:** The project focuses on treating CRAB infections in critically ill patients, such as those in the ICU, addressing the issues of high costs and limited effectiveness of existing treatment schemes, improving the clinical accessibility and scenario diversity of the technology so more patients benefit; it also considers vulnerable groups like the elderly, refugees, and soldiers, maximizing health benefits for all of humanity.
- **Subject Protection:** During subsequent clinical research, medical ethics norms will be strictly followed; patients' and their families' personal information will be anonymized, and informed consent obligations will be fully fulfilled.
- **Environmental Safety:** All engineering bacteria and preparations are designed with targeted inactivation/clearance mechanisms to avoid ecological interference after entering the natural environment, ensuring the product has no negative environmental impact.

V. Industrialization and Entrepreneurship Planning

(I)

Technology Patent Application Planning

Aimed at the two core directions of the project's detection kit and quorum sensing treatment system, an "Invention Patent + Utility Model Patent" dual protection strategy is formulated, clarifying the scope of patent protection and application pacing:

- **Kits capable of detecting colonization/infection states:** Apply for IVD in

vitro diagnostic patents.

    - *Invention patent:* 20 years of protection, covering detection methods, kit compositions, core recognition elements (OmpA aptamer/antibody, FeoA detection target, kit structure design).
    - *Utility model patent:* 10 years of protection, aimed at structural improvements such as kit casings and sampling devices, allowing for rapid rights acquisition (issued 3-6 months after application).

- **Quorum sensing treatment system:** Apply for synthetic biology patents.
    - *Invention patent:* 20 years of protection, covering engineered bacterial strains, therapeutic preparation compositions, and AHL degradation/biofilm clearance methods for non-clinical therapeutic purposes.
    - *Core protection direction:* Artificially modified E. coli engineered strains and their application in the preparation of CRAB antibacterial agents.

Note: The review of invention patents takes 6-24 months after application; patent application work will be gradually advanced based on project experimental progress.

(II)

Company Cooperation Path Planning

- **Proposed Partner Enterprise 1: Cathay Biotech (688065)**
    - *Core advantages:* A leading domestic synthetic biology industrialization company, capable of solving mass production difficulties of engineering bacteria, recombinases, and OMVs; possesses freeze-drying processes, GMP production bases, and PCT patent application experience.
    - *Cooperation alignment:* We hold the core gene circuit design, and Cathay Biotech has the industrialization processes and capacity; both parties will collaborate to complete patent applications, pilot scale-up, and GMP system construction.
- **Proposed Partner Enterprise 2: Quorum Biosciences**
    - *Core advantages:* A world-leading quorum quenching enzyme engineering modification platform; masters recombinant expression and purification processes, possessing full-process experience in preclinical R&D.
    - *Cooperation alignment:* We focus on targeted CRAB QQ therapy, filling their pipeline gaps, and leveraging their technical resources to

achieve seamless transitioning from laboratory small-scale testing to preclinical R&D.

(III)

Business Model Planning

CRAB Business Model: Includes planning across nine aspects: Value Proposition, Customer Segments, Channels, Customer Relationships, Revenue Streams, Key Resources, Key Activities, Key Partnerships, and Cost Structure. See the appendix for details.

VI. Social Impact Planning

This project closely follows the **WHO Global AMR Action Plan** and the **China National Action Plan to Contain Antimicrobial Resistance**, practicing the "One Health" concept. It plans for social impact from multiple dimensions, including public outreach, industry exchange, global prevention and control, primary care implementation, and attention to minority groups, as detailed below:

- **Public Outreach:** Plans to establish streaming media accounts to document project progress, and design free synthetic biology popularization courses to enhance public understanding of AMR and synthetic biology.
- **Industry Exchange:** Plans to join the iGEM AMR Community to conduct joint research with other teams, communicate commercial implementation experience with Jiangnan-China, and participate in infectious disease conferences and synthetic biology academic meetings for product demonstration.
- **Global AMR Prevention and Control:** Plans to send technical explanation letters/cooperation intent emails to the WHO AMR department, join global student AMR initiatives, and participate in UN/WHO online side events.
- **Primary Care Implementation:** Test kits reserve data interfaces, and test results can be reported to the China Antimicrobial Resistance Surveillance System (CARSS); plans to conduct primary prevention and control pilots in hospital ICUs and community health centers, pushing for the test kit to be included in the disease control procurement catalog.
- **Attention to Minority Groups:** Targeting groups with poor hygiene conditions, such as refugees and war zones, by adapting the project through modular modifications to meet their medical needs, thereby solving the problem of CRAB transmission and infection within these groups.
- **Industry Resource Sharing:** Plans to share CRAB quorum sensing research toolkits (AHL test reagents, engineered bacterial strains, quenching enzyme

preparations) to support subsequent research on multidrug-resistant bacteria quorum sensing mechanisms.

VII. Subsequent HP Work Promotion Plan

- Contact stakeholders according to experimental needs, sorting out the project's multiple modules in the order of Inquire, Design, Anticipation, Deliberation, Reflectivity, and Responsiveness.
- Refine stakeholder needs at each stage, complete the Legitimacy table, and determine specific interview candidates.
- During the project's experimental stage, combine experimental results with HP work, continuously optimizing product design based on experimental data and clinical feedback to truly achieve the deep integration of "HP guides technical design, technology verifies HP hypotheses."
- In the later stage of the project, complete the full HP closed loop, organize the core contribution of HP work to the project, and form a replicable and promotable HP work model, including the overall HP work strategy, enterprise cooperation strategy, government cooperation scheme, etc.

VIII. Summary

This project carried out systematic and comprehensive HP work in the initial design stage, constructed an exclusive HP theoretical framework, completed precise stakeholder analysis, and achieved deep integration of HP with six core technical modules, while also carrying out forward-looking planning from multiple dimensions such as safety and ethics, industrialization, and social impact.

Although actual stakeholder communication and experimental feedback integration have not yet been carried out due to project progress limitations, this project has strictly followed iGEM HP work requirements. It integrates the "human-centered" design philosophy throughout the entire project lifecycle to ensure the project's technical design meets clinical needs, is feasible for implementation, and possesses positive social value.

Moving forward, this project will gradually perfect the full HP closed loop according to the established promotion plan, allowing HP work to continuously guide and optimize project technical design, forming work schemes, and ultimately achieving the innovative development of a targeted CRAB detection and treatment system to contribute to global AMR prevention and control.

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