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CONFERENCE ABSTRACTS

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Clinical biology

Prevalence of Sexually Transmitted Infections (STIs) in pregnant women and breastfeeding mothers in some rural communities in Abia State, Nigeria

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Introduction: Sexually transmitted infections (STIs) such as gonorrhoea, trichomoniasis, HIV, Hepatitis B, Syphilis and Hepatitis C are common infections in Nigeria, especially among women of childbearing age. Untreated infections in pregnancy and breastfeeding also pose a huge risk to the foetus and infant. STIs in pregnancy are associated with a high risk of miscarriages, premature births and babies with low birth weight. The objectives of the study are to determine the prevalence of some STIs and their associated risk factors among pregnant and nursing mothers that attend pre-natal and post-natal clinics in some rural communities in Abia State, Nigeria.

Methods: Socio-demographic parameters were obtained from 110 consenting participants via pre-tested structured questionnaires. Blood, urine and high vaginal swab samples were used to test for STIs using relevant microbiological techniques (such as biochemical and cultural characterization) and immunological tests (immunochromatographic test kits). The susceptibility of gonococcal isolates to anti-gonococcal antibiotics (ceftriaxone, cefotaxime, cefuroxime and cefixime) was tested using the Kirby-Bauer Disc Diffusion Assay.

Results: The results obtained from 43 pregnant women and 67 breastfeeding mothers revealed the presence of gonorrhoea (6.4%), trichomoniasis (10.9%), HIV (1.8%) and HBV (3.6%). Syphilis and HCV were not detected. All gonococcal isolates were resistant to cefuroxime, cefotaxime, cefixime and ceftriaxone. 43% and 29% of the gonococcal isolates showed intermediate and resistant phenotypes respectively to ofloxacin. Treatment with acridine dye did not improve susceptibility to these antibiotics showing that resistance observed may not be plasmid-mediated. Associated risk factors identified (p value <0.05) include a history of STI, urinary tract infection, miscarriage, stillbirth, employment status and level of education. Infection was most prevalent among unemployed married women aged between 18 and 29 years, with secondary school education as their highest level of education. The mean parity of women with infection was 2.42 ± 0.29. Variables independently associated with T. vaginalis and N. gonorrhoea infections in this study are the history of miscarriages (Odds Ratio (OR) = 0.32; p = <0.001), stillbirth (OR = 0.67; p = 0.025) and previous gonorrhoea or urinary tract infection (OR = 1.06; p = 0.031).

Conclusion: Our findings revealed a low prevalence of STIs among pregnant and nursing mothers despite low levels of education and lack of employment among the infected participants. Despite the low levels of infection, public enlightenment, awareness and pre-natal screening are still encouraged to reduce the burden of STIs among the vulnerable populations.

The impact of 100% reimbursed, prescription-free hiv testing in France: Strengthening patient access and the role of medical biologists

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Background: In France, HIV screening was historically limited by the need for a medical prescription, restricting early diagnosis. To address this, a pilot program was launched in July 2019 in Paris and Nice, offering fully reimbursed, prescription-free HIV testing in medical laboratories. Following its success, it was extended nationwide on January 1, 2022, enabling free testing without an appointment or advance payment. This aligns with France's goal to eradicate new HIV infections by 2030, yet 43% of diagnoses in 2023 were still at a late stage, highlighting the need for early detection and patient follow-up.

Purpose: This study evaluates the impact of this initiative on:
- HIV screening volume in medical laboratories.
- Early diagnoses.
- The new role of medical biologists, now responsible for announcing diagnoses and coordinating patient care.

Method: A retrospective analysis (2018-2023) examined national HIV screening data (Santé Publique France) and laboratory activity. Trends in testing volume, diagnostic timing, and patient follow-up were assessed. Medical biologists' perspectives were also collected to analyze their evolving role in HIV management.

Results:
- HIV screenings increased from 5.4M in 2020 to 6.5M in 2022 (+20%) and 7.5M in 2023 (+15%).
- The VIHTest program represented 5% of tests in 2022 and 15% in 2023, demonstrating its growing impact.
- Late-stage HIV diagnoses remain high (43% in 2023), requiring continued awareness efforts.
- Medical biologists now play a key role, managing:
  - o Diagnosis disclosure to patients.
  - o Confirmatory testing to validate results.
  - o Care coordination, ensuring patients secure follow-up in infectious disease units.

Conclusion: Nationwide prescription-free, 100% reimbursed HIV testing has significantly increased screening rates and earlier diagnoses, reinforcing the pivotal role of medical laboratories in public health. Beyond testing, biologists now actively manage patient care, enhancing continuity in the healthcare system. To further reduce late diagnoses, awareness campaigns and stronger lab integration in preventive medicine are needed. Expanding free-access programs to other STIs and systematizing patient follow-up could further improve outcomes.

Characterization of plasma vonoprazan and CYP3A activity using its endogenous marker and genetic variants in patients with digestive system disorders

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Introduction: Vonoprazan is a potassium-competitive acid blocker widely used for treating acid-related diseases such as gastroesophageal reflux disease and peptic ulcers. Vonoprazan has a superior efficacy in Helicobacter pylori eradication therapy compared to conventional proton pump inhibitors. In contrast, the patient factors that determine the clinical efficacy of vonoprazan have not been clearly identified, and its relationship with plasma vonoprazan concentration has not been evaluated. Also, vonoprazan is primarily metabolized by CYP3A, but the impact of CYP3A5 genetic polymorphisms on plasma vonoprazan concentration remains unclear. This study aimed to characterize plasma vonoprazan and CYP3A activity in patients with digestive system disorders.

Method: Fifty-three patients with digestive system disorder who were receiving vonoprazan for at least 3 days were enrolled. Blood samples were collected at steady-state conditions before vonoprazan administration on or after the fourth day of treatment. Plasma concentrations of vonoprazan and its major metabolite (ODA-VP) were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Plasma levels of 4β-hydroxycholesterol (4β-OHC), an endogenous marker of CYP3A activity, were also determined by LC-MS/MS, and plasma gastrin levels were measured using an enzyme-linked immunosorbent assay (ELISA). Genetic variants CYP3A5*3 (6986A > G) and ABCB1 3435C > T were analyzed using a TaqMan real-time polymerase chain reaction method. The contribution of CYP3A enzymes to vonoprazan metabolism was evaluated using recombinant human CYP3A4 and CYP3A5 enzymes in an in vitro metabolism assay.

Results: Plasma vonoprazan levels exhibited a large interindividual variation. The absolute plasma concentration of vonoprazan was correlated with its dose-normalized value

(r = 0.951, P < 0.001), and had a positive correlation with the inverse value of its metabolic ratio (r = 0.573, P < 0.001). A negative correlation was observed between plasma vonoprazan and 4β-OHC levels (r = -0.441, P = 0.001). The metabolic ratio of vonoprazan was positively correlated with the plasma 4β-OHC level (r = 0.450, P = 0.001). Genetic variants of CYP3A5 and ABCB1 were not associated with the plasma concentration of vonoprazan and its metabolic ratio. The plasma 4β-OHC level was higher in the *1 allele carrier group than the *3/*3 group (P = 0.037). In vitro metabolism assays demonstrated that vonoprazan was metabolized by CYP3A4, while CYP3A5 had little contribution to the conversion of vonoprazan to ODA-VP. Plasma gastrin levels were highly variable among patients but were not significantly correlated with plasma vonoprazan concentrations or CYP3A5/ABCB1 genotypes.

Conclusion: The correlation between absolute and dose-normalised plasma concentrations of vonoprazan suggested that the metabolism of vonoprazan to its major metabolite by metabolic processes at therapeutic doses may saturated. The CYP3A5/ABCB1 genotype did not associated with plasma vonoprazan concentration, while 4β-OHC, an endogenous marker of CYP3A activity, was negatively correlated with plasma vonoprazan concentration. In vitro metabolism studies confirmed that CYP3A4, but not CYP3A5, is involved in the metabolism of vonoprazan. CYP3A activity explained the variation in plasma vonoprazan in patients with digestive system disorders. Monitoring CYP3A activity using plasma 4β-OHC level could support optimizing vonoprazan treatment in clinical practice.

Development and validation of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of sitafloxacin in human plasma and bronchoalveolar lavage fluid

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Introduction: Sitafloxacin is indicated to treatment lower respiratory tract infections. It was demonstrated to be effective against antimicrobial resistant bacteria, including carbapenem-resistant Enterobacterales (CRE) and carbapenem-resistant A. baumannii (CRAB). The objective of this study was to develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for determining sitafloxacin concentrations in human plasma and bronchoalveolar lavage (BAL) fluid.

Method: We assay the amount of sitafloxacin by LCMS-8040 (serial no. 0105752; Shimadzu Corp.). The analytical method has been developed and validated according to the US Food and Drug Administration (USFDA) Bioanalytical Method Validation Guidance. A reverse-phase C18 column (ZORBAX SB C-18, 3.0 × 150 mm, 3.5 µm; Agilent Technologies, Inc.) and Poroshell 120 guard (SB-C18, 2.1 × 5 mm, 2.7 µm; Agilent Technologies, Inc.) were used for assay. Detection was performed in multiple reaction monitoring (MRM) mode by monitoring the transitions of m/z 410.10 to 393.15 for sitafloxacin and m/z 402.20 to 384.20 for moxifloxacin (IS).

Results: Quantification of sitafloxacin in plasma and BAL fluid was achieved with excellent linearity (R2=0.99) over concentration range of 0.0025-0.5 µg/mL. The lower limit of quantification (LLOQ) was 0.0025 µg/mL. Intra- and inter-day precisions and accuracy were found less than 15%. The extraction recovery ranged between 91.3% and 111.1% in plasma. The BAL fluid recoveries ranged between 95.0% and 103.0% at three concentration levels. The matrix factor ranged between 93% and 112%, with less than 14% relative error. The stability and dilution integrity of this method were also acceptable.

Conclusions: The developed method showed excellent accuracy and precision for the determination of sitafloxacin in plasma and BAL fluid. This simple and sensitive LC-MS/MS method can be applied in clinical practice for monitoring the drug concentrations to enhance therapeutic effects.

Elucidation of the mechanism of allergic sensitization enhanced by non-steroidal anti-inflammatory drugs in a rat model

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Background: Allergic reactions to foods are often caused by allergen-specific immunoglobulin E (IgE) under T-helper type (Th) 2 cell-dominant conditions. We have previously reported that non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin (IND), non-selective cyclooxygenase (COX)-1 and COX-2 inhibitors, facilitate the oral sensitization of egg-white allergen ovalbumin (OVA). However, the mechanisms underlying the NSAID-facilitated oral sensitization to OVA are unclear at present. Recently, epithelial-derived cytokines such as thymic stromal phosphoprotein (TSLP), interleukin (IL)-33 and IL-25 have been reported to promote IgE production by activating a Th2 cell-dominant responses to allergens in patients with asthma. These cytokines are released from the epithelial cells when

they are disrupted by exogenous factors such as protease. Epithelial-derived cytokines are also produced in the small intestinal epithelium. NSAIDs often cause gastrointestinal disorders by inhibiting COX-1. Therefore, we hypothesize that NSAIDs may facilitate oral sensitization to allergens by releasing epithelial-derived cytokines from the small intestinal epithelium after their disruption.

Purpose: In this study, we aimed to evaluate the contribution of TSLP to oral sensitization to OVA facilitated by dextran sulphate sodium salt (DSS) or IND in rats.

Method: Brown Norway rats (4-weeks old) were immunized with OVA by gavage every other day for 8 weeks. In the DSS-treated group, 1% DSS was administered via the drinking water 2 weeks before immunization until the end of the experiment. In the IND-treated group, IND was administered orally at a dose of 6 mg/kg 30 min before OVA administration using a stainless-steel feeding tube. Control rats were given each vehicle alone instead of DSS or IND solution. To evaluate the contribution of TSLP to oral OVA sensitization, anti-TSLP antibodies were administered intraperitoneally to each rat once a week for 8 weeks. Blood was collected from the jugular vein every 2 weeks after the first immunization to confirm plasma levels of OVA-specific IgE by ELISA. At the end of the experiment, the small intestine was isolated for histological analysis by hematoxylin-eosin staining.

Results: In the DSS-treated rats, plasma levels of OVA-specific IgE were significantly increased compared to the control rats, and were suppressed to the control levels by treatment with anti-TSLP antibodies. Histological analysis showed that DSS severely damaged the intestinal epithelium, causing villous atrophy and goblet cell hyperplasia. IND also facilitated the plasma levels of OVA-specific IgE, but anti-TSLP antibodies did not reduce their plasma levels facilitated by IND treatment.

Conclusion: TSLP released from the disrupted intestinal epithelium could facilitate oral sensitization to OVA. However, IND facilitated oral sensitization to OVA independently of TSLP. These results suggest that IND may facilitate oral sensitization to OVA via a different mechanism in epithelial-derived cytokines from disrupted intestinal epithelium. We are currently performing histological analysis to evaluate the effects of IND on the small intestinal epithelium. These findings may contribute to improved prevention and management strategies for food allergies.

Associations between serum tocilizumab level, interleukin-6, and endogenous markers of CYP3A activity in patients with rheumatoid arthritis

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Introduction: Tocilizumab, an anti-interleukin-6 (IL-6) receptor monoclonal antibody, is widely used for the treatment of rheumatoid arthritis (RA). Pharmacokinetic drug interactions between biologics and low molecular-weight drugs have been reported in clinical settings. Tocilizumab may alter the activity of cytochrome P450 (CYP) 3A by modulating the IL-6 signaling pathway in patients with inflammatory conditions. However, the association between serum tocilizumab level and CYP3A activity in RA patients has not been fully clarified. This study aimed to evaluate the relationships between serum tocilizumab levels, IL-6 and endogenous markers of CYP3A activity in RA patients with well-controlled disease activity.

Method: Thirty-five Japanese RA patients with well-controlled disease activity, who received intravenous or subcutaneous tocilizumab for more than 6 months at Hamamatsu University Hospital, were enrolled. Tocilizumab was administered intravenously at 8 mg/kg body weight every 4–5 weeks or subcutaneously at a fixed dose of 162 mg every 2 weeks. Serum tocilizumab levels were monitored just before dosing after reaching steady-state. Serum IL-6 levels, its soluble receptor (sIL-6R), and endogenous CYP3A activity markers, including serum 4β-hydroxycholesterol (4β-OHC) and 25-hydroxyvitamin D (25-OHD), were measured. CYP3A5 genotype was analyzed using a TaqMan real-time polymerase chain reaction assay. Patients with at least one CYP3A5*1 allele were classified into the CYP3A5*1 carrier group, whereas those without were classified into the CYP3A5*3/*3 group. Nonlinear correlations between 2 continuous variables were evaluated using Spearman's rank correlation test and the difference in serum markers of CYP3A activity between CYP3A5 genotypes was evaluated using the Mann-Whitney U test.

Results: Serum tocilizumab levels exhibited a large variability among the RA patients, with an interquartile range of 9.8–24.6 μg/mL. Tocilizumab treatment elevated levels of IL-6 (79.0 pg/mL [47.2, 120.2]) and sIL-6R (755 ng/mL [669, 884]). The serum tocilizumab levels had positive correlation with IL-

6 (rs = 0.379, P = 0.025), but not sIL-6R (rs = 0.252, P = 0.145). Among the tocilizumab-treated RA patients, the median serum levels of 4β-OHC and 25-OHD were 36.7 and 17.7 ng/mL, respectively. The serum tocilizumab level was not significantly correlated with the serum levels of 4β-OHC (rs = 0.266, P = 0.123) and 25-OHD (rs = 0.125, P = 0.481). CYP3A5 genotype was not also associated with the serum 4β-OHC level (P = 0.391). In the CYP3A5*1 carrier group, serum tocilizumab level was positively correlated with the serum level of 4β-OHC (rs = 0.557, P = 0.031), but not with that of 25-OHD (rs = 0.371, P = 0.191). Conversely, in the CYP3A5*3/*3 group, no associations were observed between the serum tocilizumab level and either serum levels of 4β-OHC (rs = 0.025, P = 0.920) or 25-OHD (rs = 0.089, P = 0.716).

Conclusion: Our study represents the first observation of a positive correlation between serum tocilizumab levels and IL-6. In the RA patients with functional CYP3A5 protein, the serum tocilizumab level may explain the interindividual variation in CYP3A activity.

Potential use of probiotic cell-free culture supernatants as novel antimicrobials against Gram-positive and Gram-negative bacteria

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Introduction: Probiotic supplements contain beneficial bacteria that help maintain or restore the host's natural gut microbiota. One of the key mechanisms by which probiotic bacteria can displace unwanted and potentially harmful bacterial species is through the production of bacteriocins, potent antimicrobial proteins that belong to a diverse family found among the major bacterial strains. Despite their potential, relatively few studies have investigated the use of probiotics as alternative antimicrobial therapies or as a source of novel antibiotics. Given the growing problem of antimicrobial resistance caused by the overuse and misuse of antibiotics, there is an urgent need for new therapeutic strategies. In this context, probiotic cell-free culture supernatants containing antimicrobial compounds are emerging as promising candidates for the development of novel antimicrobial agents.
The aim of our study was to investigate the antimicrobial effect of probiotic cell-free culture supernatants against the most important species of Gram-positive and Gram-negative bacteria that cause a range of infections in humans.

Method: Probiotic cell-free culture supernatants (CFS) were obtained after 48h cultivation of commercially available probiotic strains Lactobacillus helveticus Rosell-52, Lactobacillus rhamnosus Rosell-11, Bifidobacterium longum Rosell-175, and their mixtures in De Man–Rogosa–Sharpe broth (MRSb) or Lysogeny broth (Luria–Bertani broth, LB). The cultures were then centrifuged at 4000 rpm at 4° C for 30 min and sterilized by membrane filtration through a 0.22 µm filter. The antimicrobial activity of eight different CFS's was tested against four Gram-positive (Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis and Bacillus subtillis) and four Gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae. Salmonella enterica subsp. enterica serovar Abony and Pseudomonas aeruginosa) commonly found in the gut microbiota or as intestinal pathogens. The minimum inhibitory concentrations (MIC) of CFS were determined by modified broth microdilution test according to the EUCAST standard, with the addition of resazurin sodium salt as an indicator of metabolic activity in viable cells.

Results: The antimicrobial activity of CSFs was in the range of 8-66 %v/v. CSF from probiotic strains cultured in MRSb had two to four times higher inhibitory activity compared to CSF obtained after cultivation in LB. The lowest MIC (8-15 %v/v) was observed against Gram-positive bacteria S. aureus, S. epidermidis, and B. subtillis, and Gram-negative bacteria (15-33 %v/v) E. coli and K. pneumoniae. S. enterica subsp. enterica serovar Abony, P. aeruginosa and E. faecalis had MICs between 33-66 %v/v, or >66 %v/v. The highest inhibitory activity was observed in CSF from probiotic mixtures of all three probiotic strains.

Conclusion: The cell-free culture supernatants of commercially available probiotic lactobacilli and bifidobacteria strains demonstrate notable antibacterial activity against common intestinal pathogens. These findings support the beneficial role of probiotic supplements in managing or preventing intestinal bacterial colonization and infections, highlighting their potential in reducing the overuse of antibiotics.

Transplacental transmission and biomarkers for PFAS exposure: Findings from paired maternal and umbilical cord blood samples

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Introduction: Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals commonly used in industrial and consumer products due to their resistance to water, grease

and high temperatures. Their chemical stability allows persistence in the environment and bioaccumulation, contributing to global pollution of soil and water resources. PFAS are present in products such as Teflon, waterproof fabrics, food packaging, firefighting foam and pharmaceuticals. Human exposure occurs mainly through ingestion of contaminated food and water, inhalation and skin contact, with seafood, meat and dairy products being important sources. Contaminated drinking water and industrial waste also contribute to the risk of exposure.

Method: A comprehensive literature search was conducted to gather current knowledge on transplacental transmission of PFAS and the identification of biomarkers in paired maternal and cord blood samples. Key scientific databases were searched for relevant studies, focusing on PFAS exposure pathways, biomarker identification and health risks associated with prenatal exposure. This approach enabled the synthesis of findings from different studies to better understand the variability of exposure and potential health outcomes.

Results: It is known that PFAS cross the placental barrier, bind to plasma proteins and enter the fetal circulation. The concentrations in the umbilical cord blood often reflect the concentrations in the maternal blood, which indicates a considerable exposure of the fetus during development. In addition, PFAS can be transferred via breast milk, prolonging exposure after birth. Analysis of paired maternal and umbilical cord blood samples is an important method to evaluate the efficiency of transplacental transmission of PFAS. This approach helps to identify factors that influence variability, such as maternal age, diet and lifestyle habits, thus improving the understanding of exposure mechanisms and associated risks. Umbilical cord blood is an important biomarker source that provides direct insights into fetal exposure. Compared to maternal blood, it reflects intrauterine exposure more accurately and provides valuable data for the assessment of potential health effects. Its accessibility and minimal risk in sampling make it an ideal biological medium for such assessments. Research suggests that biomarkers in cord blood effectively represent exposure to environmental pollutants in utero. The most important biomarkers for assessing PFAS exposure include endocrine and metabolic indicators. Changes in thyroid hormones (T3, T4, TSH), steroid hormones (testosterone, oestradiol) and metabolic markers (glucose, insulin, leptin, adiponectin) can indicate possible endocrine and metabolic disorders due to prenatal exposure. In addition, immune biomarkers may indicate suppressed immune function, which is a potential long-term health risk for newborns.

Conclusion: These findings emphasise the importance of combining maternal blood and cord blood analyses to understand transplacental PFAS transmission and identify important biomarkers of exposure and potential health risks to newborns.

Impact of medical biologists in HPV screening: Enhancing cervical cancer prevention in France

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Background: Cervical cancer is primarily caused by persistent infection with the human papillomavirus (HPV). Screening, traditionally conducted by gynecologists or general practitioners, is essential for early detection of HPV infections and prevention of precancerous lesions. However, access to these professionals can be limited, particularly in rural areas. Since 2018, medical biologists (pharmacists and physicians) have been authorized to perform cervical samples for HPV screening in medical laboratories. This measure aims to improve access to screening and increase early detection rates of HPV infections, thus contributing to the prevention of cervical cancer.

Objective: This study evaluates the impact of involving medical biologists in the collection of vaginal samples and cervical smears for HPV screening in France. We seek to determine whether this development has led to an increase in the detection of HPV infections and better prevention of cervical cancer.*

Method: A retrospective study was conducted by analyzing national epidemiological data on HPV screening before and after medical biologists were authorized to perform cervical samples. Data sources include reports from the National Cancer Institute, public health databases, and scientific publications. The indicators analyzed include the total number of HPV tests conducted, the type of healthcare professionals performing the sampling, the positivity rate of tests, and the incidence of detected precancerous lesions.

Results: Since the involvement of medical biologists in 2018, there has been a significant increase in the number of HPV tests performed. In 2018, approximately 3 million HPV tests were conducted in France, with 70% of these tests performed by gynecologists or general practitioners. By 2022, this number rose to 4.5 million tests, with over 40% of these now being performed by medical biologists. This change has significantly improved screening accessibility, especially in rural and underserved areas.

The positivity rate of HPV tests has remained stable at around 7%, indicating consistent detection of the target population. Notably, the number of detected precancerous lesions increased by 15% from 2020 to 2022, suggesting that the increased availability of HPV testing through medical biologists may be contributing to earlier detection of HPV infections and cervical abnormalities.

Additionally, areas with higher concentrations of medical biologists have shown a 20% higher participation rate in screening compared to areas with lower concentrations of these professionals. This indicates a positive impact on screening uptake and better coverage of the target population.

Conclusion: The involvement of medical biologists in HPV screening has led to a marked increase in the number of tests performed, particularly in underserved areas, improving early detection of HPV infections and precancerous lesions. These findings support the continued expansion of this initiative and suggest it may play a crucial role in reducing cervical cancer incidence in France. Further evaluation of long-term impacts on cervical cancer mortality is recommended.