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A National Reference Laboratory's interactions with veterinary diagnostic laboratories: example of Q fever, an abortive disease in ruminants and a zoonosis

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Abstract

To improve the control of targeted pathogens affecting animal and plant health, National Reference Laboratories (NRLs) have been mandated by the French Ministry of Agriculture. Their role involves ensuring that first-line departmental diagnostic laboratories are proficient in analytical methods. The NRL for Q Fever (QF-NRL) has developed measures contributing to the strong performance of methods within each laboratory's environment and on the network scale (national surveillance, epidemiological investigation). Following a survey of all (mandated and non-mandated) laboratories, the QF-NRL reports on the interactions between both parties and their interests, and outlines some prospects. Overall, the tools and exchanges (reference materials, validations, adoptions, control charts, inter-laboratory tests) are valued and provide means for determining the performance level of analytical methods and for proactively committing to further improvements.

Keywords

- ★ accreditation
- ★ analytical method
- ★ Control chart
- ★ ELISA
- ★ inter-laboratory comparison
- ★ method adoption
- ★ method validation
- ★ PCR
- ★ Q fever
- ★ reference materials

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Introduction

Caused by the bacteria *Coxiella burnetii*, Q fever is both a zoonosis and one of the main abortive diseases in domestic ruminants (OIE, 2015). It occurs worldwide and most species in the animal kingdom can be infected (Duron *et al.*, 2015; Lang, 1990; OIE, 2015). Ruminants are the primary reservoir for the bacteria causing human infection. It is especially at parturition and during abortions that infected animals can shed large bacterial loads into the environment (*via* placentas, vaginal secretions, faeces) (EFSA, 2010; de Crémoux *et al.*, 2012; Joulie *et al.*, 2015). Forms of the bacteria can survive in the environment and be dispersed (dust, aerosols). Transmission occurs primarily by air. Human cases of Q fever are typically sporadic. However, clustered cases regularly occur, often in *a priori* naïve populations in urban and peri-urban areas. For example, from 2007 to 2010, the Netherlands experienced the largest Q fever epidemic ever identified (more than 4000 human cases reported). The acute epidemic was controlled by drastic veterinary measures, such as a ban on the breeding and slaughter of gestating females and breeders, but the development of chronic forms of Q fever in exposed humans remains a problem for the coming decades (Van Asseldonk *et al.*, 2013). Risk factors for transmission to human populations are not completely understood. While the reservoirs and high-risk periods are known, these factors are harder to grasp. The emergence of human cases probably results from a combination of several factors such as the ambient bacterial load, the virulence of strains, the naïve immune status of people exposed, and especially factors favouring airborne diffusion (outdoor parturition, building cleaning, farm topography, dry and windy weather, etc.) (EFSA, 2010). In France, Q fever is not a notifiable disease. The actual number of cases in which treatment is sought is not known. Nonetheless, at least 200 hospitalisations related to Q fever are recorded each year according to the French Public Health Agency (Cazorla *et al.*, 2013).

In 2009, during the first wave of appointments of National Reference Laboratories (NRLs) in the field of veterinary public health and plant protection (Ministerial Order of 7 December 2016), ANSES's Sophia Antipolis Laboratory obtained the mandate of NRL for Q fever (QF-NRL). A long-standing activity was thus formalised. In fact, for the past 30 years, this laboratory has been organising inter-laboratory proficiency tests (ILPTs) for a large number of analytical laboratories for serological testing methods (Figure 1). Between 2007 and 2011, the QF-NRL also coordinated inter-laboratory tests for four counterpart agencies in EU Member States, in order to compare the performance of methods used in the areas of serology (ELISA and complement fixation), PCR detection (conventional and real-time) and molecular typing. It thus provided French and foreign diagnostic laboratories with the required reference materials (RMs) for ELISA serology and molecular biology. In 2013, the Sophia Antipolis Laboratory was recognised as the OIE Reference Laboratory for Q fever. With regards to research, it contributes to the development of tools and knowledge in order to better understand the epidemiology of animal Q fever, improve the control of infection, and thereby enhance the protection of public health. In France, Q fever is currently classified as a Category 3 health hazard for animal species, which means that no general-interest measures or collective mobilisation are provided for under the regulations. However, a State Note proposes a local organisational framework for the veterinary authorities in the event of clustered human cases with an investigation protocol for ruminant holdings and integrated management measures. Moreover, Q fever has been included as a priority topic for the National Epidemiological Surveillance Platform for Animal Health (ESA Platform; www.platforme-esa.fr) with the aim of better understanding the status of this disease in France. A pilot programme, paired with the surveillance plan for brucellosis, was implemented in 10 départements for three years (Gache *et al.*, 2017). With the creation of a network of 10 mandated laboratories for this programme, the QF-NRL reinforced its reference missions involving the standardisation and harmonisation of basic diagnostic methods: PCR and ELISA serology (Rousset & de Crémoux, 2013).

The pilot programme on Q fever ended in August 2015. This experiment contributed to the



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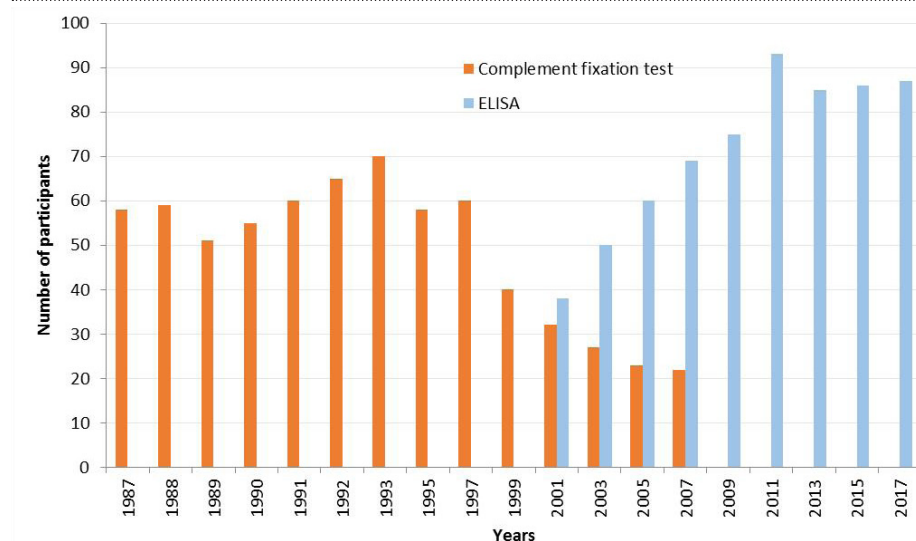
design of harmonised protocols for the differential diagnosis of abortions in ruminants and led to the deployment, in 2017, of a scheme called OSCAR (Observatory for Monitoring Causes of Abortions in Ruminants) aiming to collect, analyse and disseminate the results. Q fever is included in the differential diagnosis as a priority disease to be detected in both cattle and small ruminants. In this context, the QF-NRL undertook a survey of laboratories in the first quarter of 2016 involving a questionnaire with three sections: 1/ molecular biology and serology analyses, 2/ a proposed ILPT for PCR methods, and 3) the need for workshops with the NRL. The results of this survey were taken into account to draw up a review of the activities performed by the network of laboratories regarding Q fever and examine the NRL's actions to determine those to be improved and those to be extended to a larger number of laboratories.

Significance of the topic

Description of the network of mandated and recognised laboratories

The QF-NRL frequently addresses laboratories participating in ILPTs for serology (Figure 1), those developing new methods or amending the methods used (relative PCR vs quantitative PCR, other DNA extraction methods, modification of ELISA kits), and laboratories and kit manufacturers using reference materials (RMs). The deployment of the OSCAR scheme in 2017 may lead to an increase in requirements and related support actions, especially for PCR methods (Figure 2). The network thus comprises more than 60 public and private laboratories including 10 mandated laboratories in France and around 20 foreign laboratories (Figure 1). Non-mandated laboratories for which the NRL can have data or information on the quality of analyses are considered to be 'recognised' laboratories. This recognition of expertise by the NRL can favour a responsive mandate if necessary. The questionnaire was sent to the 83 French diagnostic laboratories. Despite a large number of questions (20, some of which were broken down), 44 questionnaires proved fully useable (53%, 44/83) while 10 were incomplete and could be used only for certain questions (65%, 54/83). The participation rate demonstrated laboratory interest in interactions with the dedicated NRL and indicated that the results were representative.

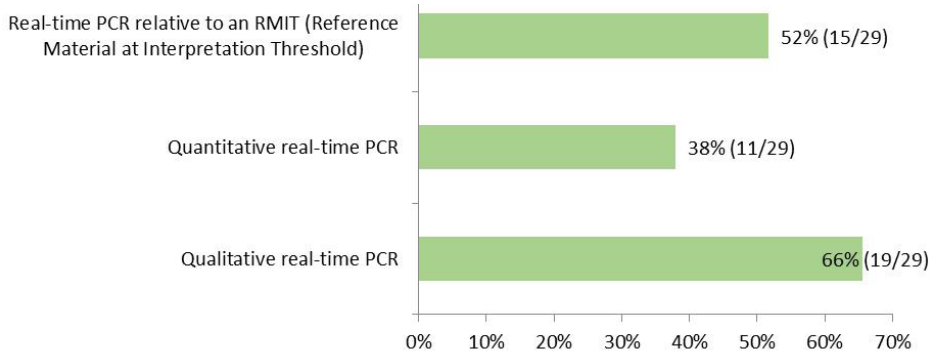
FIGURE 1/ Number of laboratories participating in inter-laboratory proficiency tests (ILPTs) for Q fever serology (complement fixation and ELISA) from 1987 to 2017.



Note: Unofficial tests for ELISA in 1997 and 1999; Transition between the two methods between 2001 and 2007 with four laboratories only for CF in 2007; Opening to foreign laboratories in 2009 (8, 21, 17, 22 and 22 from 2009 to 2017).

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FIGURE 2/ Proportion of laboratories using the various types of PCR.



Description of the Q fever analyses undertaken in French diagnostic laboratories

The survey confirmed that Q fever analyses are mainly performed in the context of diagnosing ruminant abortions. Serological testing from serum and PCR analyses from vaginal mucus or placentas are also used for certain epidemiological investigations. Serological testing from milk and PCR analyses from milk and faeces are seldom performed. The survey also highlighted a high proportion of laboratories using ELISA for serological testing (94%, 48/51), of which 82% participated in the ILPT organised by the NRL for this method (42/51). In addition, almost 80% of the respondents (42/54) perform or intend to implement PCR testing. In total, 72% of the laboratories can or wish to submit direct and indirect diagnostic results for Q fever (39/54).

Current contributions of the NRL

Support for the ISO 17025 accreditation process

The actions of the QF-NRL aim to ensure the quality of the results and interpretations produced by laboratories. One of the approaches consists in helping laboratories involved or wanting to be involved in an accreditation process. According to the survey, only 13% laboratories (7/54) are accredited for the two ranges of methods (ELISA and PCR). The main barriers to accreditation are an insufficient number of analyses, a lack of time, and/or budgetary constraints. The responses also show that ELISA methods are less often accredited (25% [12/48]) than PCR (45% [19/42]) (Figure 3).

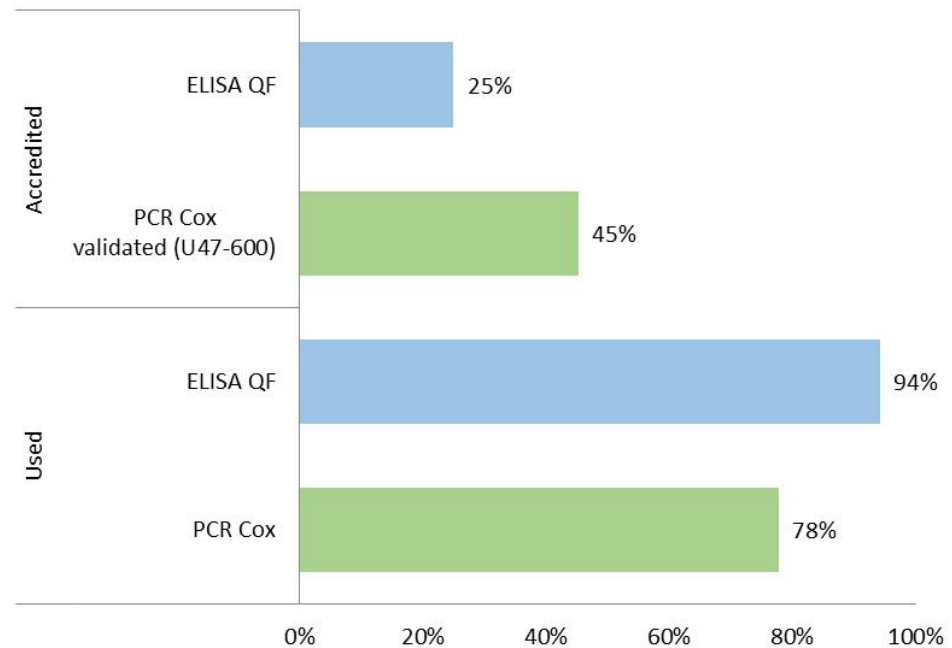
That said, ELISA methods have been used since the 1990s (Figure 1), whereas validated PCR methods have been implemented only more recently (Rousset *et al.*, 2012). The ELISA methods correspond to three commercial indirect ELISA kits, whose performance needs to be better evaluated (Rousset & de Crémoux, 2013; Horigan *et al.*, 2011; Emery *et al.*, 2012). Manufacturers wanting to validate these ELISA kits do not have a norm for serology like that for PCR; they also and most importantly do not have a sizeable collection of true negative and true positive sera (AFNOR, 2015; OIE, 2013). The logistics to obtain them are complicated and expensive for Q fever (complexity of experimental infections for ruminants in Biosafety Level 3 animal facilities, difficulties in qualifying the disease-free status of farms, etc.).

In the absence of a complete validation file, each diagnostic laboratory is required to undertake tests in order to confirm and provide evidence of the validity of the submitted results in relation to its own needs (confirmation file). A collection of characterised sera is required, even if it is more basic than for validation. Thus, there is a delay in terms of the standardisation of ELISA methods. Several actions are being pursued by the QF-NRL to better characterise the

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performance of existing serological methods: monitoring of batches of kits using a calibrating RM from the QF-NRL prior to the proposal of common acceptance criteria, and compared evaluation of ELISA kits by statistical modelling. PCR methods are also based on commercial kits.

FIGURE 3/ Proportion of accredited or unaccredited laboratories performing analytical methods for Q fever.



However, they were validated, according to French norm U47-600 and the performance stated by the QF-NRL, to be applied to the diagnosis of abortion in the framework of the 'pilot' programme deployed at the beginning of 2012 (Rousset & de Crémoux, 2013). The QF-NRL then supported the development of these methods (Rousset *et al.*, 2012). With duly validated methods, the laboratory only needs to verify their implementation, in its own environment, by referring to the criteria established to meet requirements for the diagnosis of abortion. Tests were first coordinated by the QF-NRL at the beginning of 2012, with the goal of mandating departmental laboratories from the pilot programme, and were then extended to other laboratories. The QF-NRL thus examined and certified the validation files of manufacturers and the verification test results of laboratories. Both of these probably influenced rapid changes in accreditation for PCR methods.

Needs for reference materials

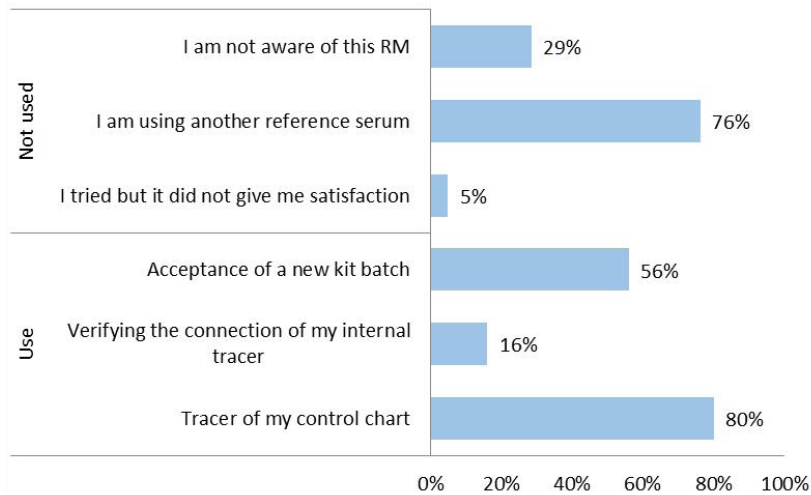
The survey's questions evaluated knowledge of RMs and their use by laboratories. Forty-five percent (19/42) of laboratories do not know about the RMs supplied for molecular biology methods; this figure is 13% (6/46) for that proposed for ELISA. Awareness-raising is still necessary. Nevertheless, many laboratories use or are planning to use the bacterial RM as a positive control and tracer for PCR methods (74%, 31/42). More than half of the laboratories already use the reference serum for ELISA analyses (54%, 25/46). Laboratories use it for one to three applications (Figure 4).

Eighty percent use it as a control chart tracer (20/25) and 56% for the acceptance of a new batch of kits (14/25). However, verification of the connection to an internal tracer seems to have less appeal (16%, 4/25). A high percentage of non-users use another reference serum (76%, 16/21). This serum may be an in-house material or be supplied by certain manufactu-

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users of ELISA kits or a group of laboratories. For PCR, to our knowledge, RMs are distributed only by the QF-NRL.

FIGURE 4/ Use of reference material (RM) for Q fever serology.



Changes in methods

Measurement uncertainty

The QF-NRL works to identify and encourage necessary changes in the methods used. For example, advice and quantitative data are specified in the latest ILPT reports on ELISA methods in order to promote the presentation of results with a level of uncertainty, inherent in any measurement method. This is a performance characteristic. The critical point is the measurement at the threshold level, which should be taken into account to identify results close to the threshold, to be distinguished from strictly positive or negative results. Knowledge of uncertainty at a method's threshold is enriched by inter-laboratory data from ILPTs and by initial verification data for the method or those from the control chart for a control calibrated to the threshold. The laboratories were surveyed regarding the reporting of results. The situation varies considerably between the two ranges of methods. For indirect diagnosis, 24% (11/46) specify measurement uncertainty on the results report. However, only one respondent, accredited for two types of PCR (quantitative and relative), gives this information for PCR results (2%, 1/42).

Relative (or semi-quantitative) PCR

Quantitative data were needed for the diagnosis of abortion in the pilot programme on Q fever. Real-time quantitative PCR (qPCR) was put into place but its financial cost is critical, especially due to the five quantification standards. The QF-NRL thus helped adapt the technique to make it more affordable. It recommended reporting results in relation to the diagnostic threshold for abortion, thus reducing the number of controls per series of analyses. The 'relative PCR in relation to a Reference material at interpretation threshold (RMIT)' type was taken into account in the revision of the PCR norm published in 2015 (AFNOR, 2015). Validations by manufacturers were undertaken and verified by the QF-NRL between mid-2016 and mid-2017. For the diagnosis of abortion, based on a clinical threshold, it proposed using either qPCR with a range or relative PCR in relation to an RMIT (rPCR). For the latter, the tracer is also the RMIT for the method. The number of controls for relative PCR is the same as for

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qualitative PCR and lower than for quantitative PCR. According to the survey results, at the beginning of 2016, the project for relative PCR interested half of the responding laboratories (21/42). Interest in this method is starting to increase, in particular among departmental laboratories volunteering to participate in the OSCAR scheme.

Future expectations

Inter-laboratory data for interactive monitoring

The QF-NRL wants to extend the use of similar inter-laboratory data to monitor the performance of PCR methods. The survey's results show strong laboratory interest in supporting these initiatives:

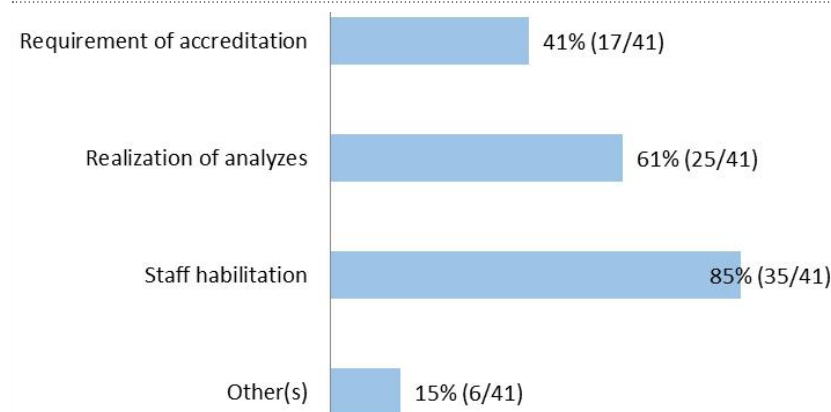
- 76% of laboratories (32/42) for initial verification results for the method according to a common experimental design,
- 74% of laboratories (31/42) for those of control charts based on a common assayed positive control (tracer/RMIT).

In the context of the network for the pilot programme, verification tests for methods, before their routine use, were a sort of ILPT. The collection of data from control charts enabled the responsive examination of problems encountered. It also made it possible to verify the reproducibility and accuracy of the qPCR results of the network of laboratories and thus determine whether they could be aggregated and used. These observations centralised by the QF-NRL seem useful to the laboratories, at least for the method's first two years of implementation.

Expectations for ILPTs

For the question asked about a first PCR-ILPT, 61% of laboratories are in favour (30/49). Both ILPTs were proposed for 2017, but the NRL will then need to organise ILPTs for Q fever on a biennial basis, alternating between ELISA and PCR. ILPTs contribute to the quality control process by enabling an external assessment. Participation is required by the French Accreditation Committee (COFRAC), but laboratory participation goes far beyond the accredited laboratories. Thus, every ILPT campaign for ELISA methods involves more than 60 French laboratories (Figure 1), showing that laboratories are concerned about verifying the quality of results, without necessarily wanting to be accredited for the method, and positioning themselves, in terms of results, in relation to other laboratories and the various ELISA methods available (Figure 5).

FIGURE 5/ Reasons for laboratory participation in inter-laboratory proficiency tests (ILPTs) for Q fever serology by ELISA.



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Need for a workshop with the NRL

The survey revealed an expectation involving workshops with the QF-NRL. For 83% (40/48) of the responding laboratories, an annual meeting is desirable; it should be open to kit manufacturers in particular.

Conclusion

The activity of NRLs is still relatively unknown. In France, NRLs are designated by Ministerial Order of the Minister of Agriculture. The tasks of NRLs and EURLs (European Union Reference Laboratories) are described in Regulation (EC) No 882/2004, recently replaced by Regulation (EU) No 625/2017 (Kremer & Carteau, 2017). With regards to veterinary laboratories, reference missions include the organisation of ILPTs, the preparation and distribution of RMs, method improvements, developments and validations, as well as scientific and technical monitoring, communication, and training. NRLs are also requested to provide support for the management of health crises in addition to expert appraisals for the health authorities. Reference activities are enhanced by knowledge of issues thanks to a series of interactions with various field stakeholders (analytical laboratories, veterinary practitioners, manufacturers of diagnostic kits and vaccines, health managers). The structured reference continuum also includes research work in collaboration with other scientific teams. Every theme generates specificities or special needs. A survey of 83 French laboratories conducted by the QF-NRL illustrated the various interactions and expectations of the network of over 60 'recognised' laboratories, including 10 mandated laboratories, which wants to be involved in better aligning its needs with the proposed reference activities. Overall, the survey showed that the responding laboratories are satisfied, and the normative framework driven and coordinated by the QF-NRL for the validation of PCR methods seems to provide a favourable context for accreditation in this area for diagnostic laboratories. For laboratories, the data and experiences shared via the QF-NRL are sources of information with which to respond to challenges and carry out improvements; overall, they build confidence in the quality of methods. For the QF-NRL, this knowledge of laboratories provides it with a responsive capacity to mandate new laboratories, or at the very least, recognise expertise for animal health managers, stakeholders in professional breeding sectors, and public health services.



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