CareDx Perspective on
Questions for CAC on the use of Molecular Diagnostic Testing to identify acute rejection in Lung Transplant recipients

1. Is there sufficient evidence to identify the patient population that the molecular diagnostic test could be used? (e.g., risk level, ethnic/cultural demographics, repeat transplant recipients, etc.)

Yes. The Keller JHLT 2022a (Keller et al., J Heart Lung Transplant) clinical utility study publication describes a multi-center study in which AlloSure lung was validated in all lung transplant recipients 30 days to 3 years post-transplant.

2. Is there sufficient evidence on the clinical context (i.e., for-cause vs. surveillance) in which the molecular diagnostic test could be used?

The evidence clearly indicates that AlloSure can be applied for both for-cause and surveillance use. The first two validity publications (Sayah 2020, Khush 2021), as well as the clinical utility publication (Keller 2022a) all tested patients regardless of biopsy indication (surveillance or for-cause).

3. In the existing evidence, what is the level of confidence (or certainty) regarding test performance data reported without any confidence intervals?

There is high confidence in AlloSure Lung, based on the analytical validation studies demonstrating high precision and the reproducibility of the optimal cutoff for distinguishing rejection from no-rejection. The published optimal thresholds are all very similar, at 0.87%, 0.85%, and 0.91% (Sayah 2020, Khush 2021, Keller 2022a).

4. Is there sufficient evidence to support the utility of surveillance (i.e., not for-cause) testing in lung transplant recipients?

There is sufficient evidence to support the utility of surveillance testing in lung transplant recipients.

The Keller 2022a multicenter clinical utility study has a primary focus on surveillance use and demonstrates reduction in biopsies by 82% compared to a historical control in which patients underwent invasive surveillance biopsies. When used for surveillance, AlloSure was able to identify subclinical/asymptomatic acute lung allograft dysfunction (ALAD) - defined as a composite outcome

of ACR, AMR, or infection - in ~8% of visits where AlloSure was assessed. There is variability in surveillance among transplant centers and testing schedules/intervals are set by a physicians based on medical necessity and their assessment of the patient needs.

4b. If yes, what is the appropriate testing schedule based on the published evidence?

The testing schedule used in the Keller 2022a clinical utility study was based a consensus agreement among the centers participating. The clinicians agreed among themselves to use AlloSure monthly in the first year post-transplant and quarterly after 1 year.

5. Is there sufficient evidence on the ability of the molecular diagnostic test to discriminate rejection (acute cellular, antibody-mediated, chronic lung allograft dysfunction) from quiescence?

Yes, there is evidence to demonstrate the ability of AlloSure to differentiate rejection from stable transplant recipients. The published AUC of an ROC analysis for each of the three primary studies was 0.72, 0.67, and 0.82 (Sayah 2020, Khush 2021, and Keller 2022a). The clinical utility study, where the data were produced in a real-world use with strict adjudication of every sample illustrates the best discrimination.

6. Is there sufficient evidence to indicate that in patients **without signs and symptoms** of rejection, use of the molecular diagnostic test would preclude the need for transbronchial biopsy?

Yes, the evidence in the Keller 2022 clinical utility publication demonstrated that without surveillance biopsy, AlloSure identified subclinical rejection in ~8% (23/290). No adverse outcomes were identified or reported. The majority (82%; 237/290) of dd-cfDNA drawn for surveillance were <1%, and only 9 (3.8%) were categorized as having ALAD within 1 month of the dd-cfDNA draw. Furthermore, subsequent analysis of these results was presented by Shah at ISHLT 2021 and showed that outcomes in terms of pulmonary function testing, development of donor specific antibodies, and hospitalizations, were not worse at 6 months later than those of comparable patients managed by a biopsy-based surveillance protocol.

7. Is there sufficient evidence to indicate that in patients **with signs and symptoms** of rejection, use of the molecular diagnostic test would preclude the need for transbronchial biopsy?

Three publications have been published (Sayah 2020, Khush 2021, Keller 2022a) that included a mixed population of surveillance and for-cause biopsies to which AlloSure was compared. In the clinical utility study described in Keller et al 2022, the clinicians used a lower threshold of 0.5% to preclude biopsy in surveillance. Currently, due to the high risk nature of lung transplantation, clinicians are unlikely to forego a biopsy in a patient with clinical signs and symptoms of rejection regardless of the AlloSure result.

8. Is there sufficient evidence on the ability of the molecular diagnostic test to guide clinical management without transbronchial biopsy?

Yes. In surveillance use in Keller 2022a, stable patients were managed without biopsy.

8b. If "Yes" for any of the above, what aspect of your clinical management would be influenced by the test result?

Per the clinical utility study published by Keller 2022a, patients were successfully managed without a biopsy if their results were below 0.5%. For patients with results >1%, they received full workup, including biopsy. Asymptomatic patients with results between 0.5% and 1% did not receive a biopsy, but received a repeat AlloSure test within 1-2 weeks and if the repeat result was <1%, did not receive a biopsy.

9. Would you perform a transbronchial biopsy if the molecular diagnostic test indicates rejection, but the patient exhibits no signs and symptoms of rejection?

Yes. In the clinical utility study described by Keller 2022a, asymptomatic patients with a result greater than the upper threshold received a full workup, including biopsy to confirm rejection and define the type to advise treatment. Notably, ALAD was diagnosed in 43.4% (23/53) of asymptomatic patients with surveillance AlloSure results ≥1%.

10. How confident are you in the evidence that, for this test, an elevation in donor-derived cell-free DNA indicates rejection?

Fairly confident. Rejection is identified by elevated AlloSure. Since dd-cfDNA detects graft damage, AlloSure also identifies other injuries, especially in lung transplantation where infection is quite common. Therefore, an AlloSure greater than the upper threshold has high confidence for ALAD (a composite outcome that includes ACR, AMR and infection), but would also require additional workup to determine the cause of injury and the appropriate treatment.