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A novel XNA-based Luminex assay to detect UBA1 somatic mutations associated with VEXAS syndrome

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ABSTRACT

Objectives: Patients with VEXAS syndrome carry mutations of UBA1 gene coding for the E1 enzyme. The three most frequent mutations are p.M41T(122T > C), p.M41V (c.121A > G), and p.M41L (c.121A > C) in codon 41 of exon 3. Currently, sanger sequencing was mainly used to detect these mutations, which has low sensitivity and low throughput. There is a need of high sensitivity, simple and high throughput method to characterize patients with VEXAS syndrome.
Methods: Based on our proprietary XNA technology, we have developed a QClamp® Plex platform to detect eight mutations in a single reaction using the Luminex xMap technology. The assay sensitivity, specificity and precision were subsequently evaluated. Furthermore, the reference interval and clinical sensitivity/specificity were estimated using clinical healthy/positive DNA samples and the sanger sequencing method was used for comparison.
Results: With spiking synthetic mutant DNA in wildtype GM24385 cell line DNA, this assay can detect *UBA1* mutations with a detection limit of variant allele frequency (VAF) at 0.1–5%. Our assay shows 100% concordance with Sanger sequencing results when used for analyzing 15 positive and 19 negative clinical samples.
Conclusions: The QClamps® Plex *UBA1* Mutation Detection Assay is a quicker, simpler, and more sensitive assay that can accurately detect the *UBA1* mutations even at early stages with low mutation frequency.

1. Introduction

VEXAS syndrome (acronym for Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic) is a new adult-onset systemic autoinflammatory syndrome reported by Beck et al. in December 2020 [1]. Patients with VEXAS can have a wide range of inflammatory symptoms affecting multiple organs including skin, lung, joint, cartilaginous structures and vasculature [1]. Patients often show clinical manifestations of fever, extreme fatigue, chondritis, pulmonary infiltrates, cytopenia, vasculitis and neutrophilic dermatosis. Moreover, on many occasions patients with VEXAS present strong association with hematologic malignancy including myelodysplastic syndrome (MDS) and multiple myeloma [2].

VEXAS syndrome is a monogenic disease genetically characterized by somatic mutation of the UBA1 gene which encodes the

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ubiquitin like modifier activating enzyme 1 [1]. The three most frequent mutations of UBA1 gene are p.M41T(122T > C), p.M41V (c.121A > G), and p.M41L (c.121A > C) in codon 41 of exon 3 of transcript NM_153280 (4). Of the three M41 mutants, p.M41T is the most common mutation with 50% cases, followed by p.M41V at 26% and p.M41L at 19% and p.M41V is associated with the shortest median survival time [3]. Other mutations including splice site mutations at exon 3 (c.118-2A > C, c.118-1G > C and c.118-9_118-2del), p.S56F (c.167C > T) in codon 56 of exon 3, and p.S621C (c.1861A > T) in codon 621 of exon 16 have been reported [4–6] and accounts for 5% cases.

Due to the complexity of VEXAS syndrome, it is very important to diagnose and differentiate it from other diseases with similar symptoms by detecting the UBA1 mutations. Without accurate diagnosis, VEXAS patients were often put on various immunosuppressive medications which were found to be ineffective. Currently, the detection of UBA1 mutations is offered by a few academic research groups using next generation sequencing (NGS) or sanger sequencing [6,7]. The variant allele frequency (VAF) of UBA1 mutations is high in many cases. Vu reported a case having a missense mutation in UBA1 (c.122T > C, pMet41Thr) with a variant allele frequency of 53% [8]; Beck et al. identified 3 men with UBA1 p.Met41 variants exceeding 71% variant allele frequency [1]. Poulter stated that on average, the variant allele frequency (VAF) of the mutation was 44.1% (range:29.5%–59.3%) in his report [7]. Therefore, sanger sequencing can be used to detect UBA1 mutations even though it has a lower sensitivity of detection of approximately 15%–20% mutant allele frequency. Furthermore, sanger sequencing may also need careful human visualization of the chromatogram of the sequencing results to identify mutation. NGS is a much more sensitive, high throughput assay that has been used in VEXAS UBA1 mutation detection by some clinic laboratories and research groups, but it has a turnround time of 3 weeks and needs bioinformatics analysis support for reporting. Boerger et al. developed a droplet digital PCR (ddPCR) method to detect seven UBA1 mutations using four reactions and achieved an analytical sensitivity of 0.5% [9]. Recently, Duan et al. reported to reliably detect variant allele ratios of >0.1% (predicted 15.7 copies) from 80 ng of gDNA using ddPCR [10]. Although ddPCR has a higher sensitivity, it has a lower plex level and lower throughput.

VEXAS Syndrome patients benefit from early detection to separate from other auto-inflammation disorders and avoid ineffective treatment. With a case of c.121A > C mutation at 0.14% VAF reported [11], the UBA1 mutation VAF at early stage of VEXAS may be much lower than 15%, a quicker, simpler and more sensitive detection assay can help monitor patients with VEXAS for a full clinical spectrum of examination and in-time treatment.

Here, we describe a novel technology QClamp™ Plex to detect eight UBA1 mutations in a single multiplex reaction by combining XNA clamping and Luminex xMAP technology. The assay can reach as low as 0.1% VAF sensitivity with only 10 ng of genomic DNA input.

2. Materials and methods

2.1. Reference materials and clinical samples

Patient DNA samples with known UBA1 mutation status were provided as a courtesy by a CLIA-certified laboratory from an academic institution. Other UBA1 mutant negative blood samples are from employee volunteers. The use of deidentified leftover clinical samples was approved by the institutional review board (IRB) at UCSF (UCSF IRB #11–05207) as a no-subject contact study with waiver of consent and as exempt under category 4. Each mutant DNA reference is 200 base pairs of double strand DNA gBlock synthesized by Integrated DNA Technologies, Inc. (Coralville, Iowa, USA). Negative control DNA was extracted from GM24385 cell line using the blood & cell culture DNA Mini kit (Cat. No. / ID: 13323) from Qiagen (Redwood City, CA, USA). Other patients DNA from whole blood were extracted using vamPure® Blood Nucleic Acid Extraction kit (Catalog #: K5011720) from BioChain (Newark, CA USA). Different concentrations of mutant variants at 0.1%, 0.25%, 1%, 5% VAF were prepared by spiking synthetic gBlocks in 10 ng of wildtype DNA from GM24385 cell line.

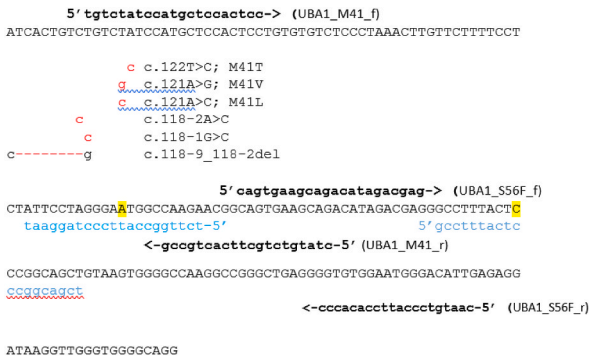

Fig. 1. A diagram showing the seven UBA1 mutations. Red letters are mutation bases with each labeled with mutation names at right; red dash line is the deleted region; blue region is the XNA sequences; yellow highlighted base "A" is the wild type base of mutation M41V and M41L and yellow highlighted base "C" is the wild type base of mutation S56F; bold letters are primer sequences.

2.2. Designs of PCR primers, ligation oligos and XNA oligos

The six UBA1 mutations of p.M41T(122T > C), p.M41V (c.121A > G), p.M41L (c.121A > C), c.118-2A > C, c.118-1G > C and c.118-9_118-2del are very close to each other (Fig. 1). The assay to detect them used the same PCR primer pair and one XNA oligo which inhibits wild type DNA amplification. The other two mutations have their own PCR primer pairs and XNA oligos. The ligation oligo pair for each mutation has one CP oligo and one LP oligo (Fig. 2). A CP oligo has unique tag sequence at 5 prime and mutant allele base at the most 3′ position while a LP oligo has 5′ phosphate group and 3' biotin group conjugated. The sequences of these PCR primers, ligation or XNA oligos are listed in (Supplementary Table 1). PCR primers and ligation oligos were purchased from Integrated DNA Technologies, Inc. (Coralville, Iowa, USA). XNA oligos were synthesized in-house at DiaCarta. ACTB is used as an internal control for sample quality control and has no XNA oligo binding to it.

2.3. The antiTags for multiplex assay and coupling to Luminex beads

Unique 25-mer antiTag sequences were designed to minimize secondary structure and cross hybridization among each other's reverse complementary sequences. Each was homology screened against current human, mouse, and rat genomes. The antiTag sequences with 5′-NH2-C6 linkers were synthesized by Integrated DNA Technologies, Inc. (Coralville, Iowa, USA) and covalently conjugated to the carboxylated fluorescent-encoded microspheres (Luminex, Austin, TX, USA). Briefly, 5×10^6 beads were resuspended in 100 µl of 0.1 M MES (pH 4.5) and incubated with 8 µM antiTags and 2 mg/ml 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Pierce, Rockford, IL, USA). Crosslinking efficiency and hybridization specificity of each bead were evaluated by hybridizing with individual antiTag-complementary biotinylated oligo to a mix of 9 oligo-coupled beads under the same conditions as used in the QClamp Plex assay.

2.4. QClamp plex assay

The PCR enrichment experiment was performed in 20 µl volume which contains 10 µl of NEBNext Ultra II Q5® Master Mix (NEB, Ipswich, MA, USA), 0.1uM of each of eight primers, 0.2uM of each XNA oligo and different VAF of mutant templates. 10 ng wildtype DNA and water were used as negative control. The PCR was run in ProFlex PCR system (ThermoFisher Scientific, Carlsbad, CA USA) with the conditions: 98 °C for 3 min, 30 cycles of [98 °C for 20 s, 62 °C for 30 s, 72 °C for 30 s], 72 °C for 5 min. The Ligation experiment was performed in 20 µl volume which contains 2ul 10x Taq ligase buffer, 10 unit of Taq ligase, 5 nM of ligation CP oligos and 50 nM of ligation LP oligos and 2 µl PCR products. The ligation was run in ABI machine with the conditions: 96 °C for 2 min, 30 cycles of [94 °C for 15 s, 50 °C for 60 s]. After ligation, the samples were denatured at 95 °C for 5 min, then 10 µl of ligation samples were mixed with pooled beads (1000 beads/target/assay) in 75 µl volume which contains 37.5 µl 2x hybridization buffer (DiaCarta) in a 96 well plate (Greiner 96-well microplate with Catalog: 655101) sealed with adhesive-backed foil (Greiner, Germany). The plate was incubated at 50 °C, 600 rpm for 1.5 h in a Thermal Shaker (DiaCarta). A hand-held magnet was used to wash the plate twice with 1x washing buffer (1xSSC, 0.03% lithium lauryl sulfate). Streptavidin-conjugated R-phycoerythrin (SAPE) was added and incubated at 37 °C for 30min. The beads were washed to remove unbound SAPE, followed by analysis with a Luminex MAGPIX system (Luminex).

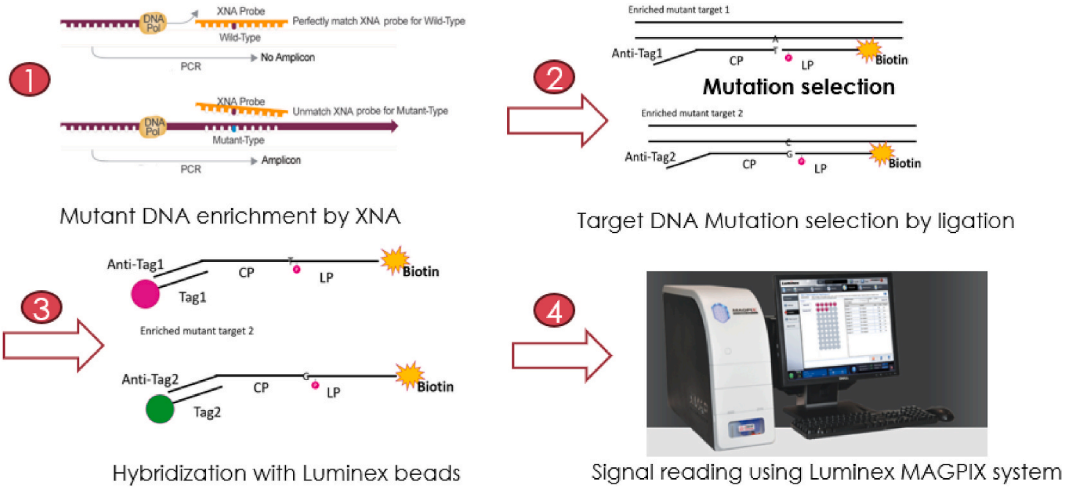

Fig. 2. Overview of the QClamp Plex assay. The four assay steps are: (1) XNA clamping PCR for mutant target enrichment; (2) allele specific ligation to label mutant target with biotin and Tag sequences; (3) capture of the specific mutant targets to their corresponding beads through Tag-antiTag hybridization; and (4) Hybridization with SAPE and analysis of beads intensity signal with Luminex system.

2.5. Data analysis and statistics

Twenty replicate assays (n = 20) were performed for all described experimental samples except where noted. All the multiplex data were reported from measuring median reporter fluorescence from 50 beads per target per well assayed and are represented as median fluorescence intensity (MFI). Background signals were determined from the assay with the wildtype DNA from cell line GM24385. The sensitivity of the assay for each target was evaluated by the limit of detection (LOD), defined as the signal/noise ratio (MFI of VAF / background signal) with the lowest concentration VAF greater than or equal to 3. The mutant positive call was determined by which mutant target has the largest sig/noise ratio among the eight targets and passed the LOD definition.

3. Results

3.1. Overview of the QClamp plex assay

To detect multiple DNA mutations with low variant allele frequency in a single reaction, this assay adapts XNA (xenonucleic acids) clamping PCR and allele specific ligation to the Luminex fluorescent bead-based xMAP platform. The assay briefly contains four steps as illustrated in Fig. 2. In the first step, PCRs are conducted with XNA probes that hybridize to the wild-type DNA sequences and therefore specifically amplify only pathogenic variant alleles whereas the wild-type alleles amplification is inhibited. In the second step, allele specific ligation produces products with 5′ having unique TAG sequences and 3' labeled with biotin. The 3rd step is to hybridize the ligation products to Luminex fluorescent magnet beads via Tag-antiTag interaction. Last, a hybridization with streptavidin phycoerythrin conjugate (SAPE) was preformed and the bead fluorescent color codes and SAPE intensity signals from all beads in the final hybridization mixture are reported using the Luminex system like MAGPIX, which maps each bead to a specific mutant target and provides a fluorescence measurement (MFI) of SAPE reporter associated with that bead.

3.2. Performance evaluation of the QClamp plex VEXAS syndrome UBA1 mutation detection assay

To determine the assay sensitivity of each UBA1 mutant target, 5%, 1%, 0.25%, 0.1% VAF samples of each target were tested with 20 replicates. The ratio of the signal of each replicate versus the maximal signal of 20 replicates of wild type DNA (SNR) was calculated. The sensitivity of the assay for each target was evaluated by the limit of detection (LOD), defined as that the SNR of 19 of 20 replicates is greater than or equal to 3. The LOD of 8 UBA1 mutant targets are listed in Table 1. UBA1 M41T and M41V assay have lowest sensitivity to detect 0.1% mutations while UBA1 c118-1G > C assay can detect 5% mutations.

To determine the cross-reactivity between mutant targets within the full panel, 5% VAF of each UBA1 mutant was individually analyzed with the bead array and probe/oligo sets for all 8 targets in the multiplex assay. The signal/noise ratio (SNR) was calculated for all mutant bead in the presence of each individual 5% VAF and graphed in Fig. 3. Cross-reactivity is expressed as the percentage of SNR generated by nontarget beads in the panel in relation to the intended target bead. Above LOD, M41T assay showed cross with M41V at 7.2%, M41L at 10.1%, S56F at 4%; M41V showed cross with M41T at 2.2%, M41L at 3%, S56F at 1%; c.118-2A > C assay showed cross with M41L at 8.6%.

Assay precision between different strip wells (intraplate), between assays performed on different days (interplate), between different PCR machines was assessed by calculating coefficients of variation (CVs) for each target MFI at 5% VAF gBlock mixture. Precision values for each mutant target were measured across 4 replicate strip wells. All the CVs are within 4–26% with an average CV of 11%. The average CV of 11% has been routinely obtained for all the data reported here.

3.3. Clinical sample validation

We tested saved leftover DNA samples extracted from whole blood previously collected from suspected VEXAS patients and tested at an academic CLIA laboratory by Sanger sequencing. The samples include 14 UBA1 mutation positive and 19 negative samples. Additionally, we also tested one known VEXAS patient's whole blood sample (#36 in Table 2) by extracting DNA, two volunteers' buffy coat DNA (#34, #35 in Table 2) and two mixed positive samples prepared from two DNA samples known to be positive for different UBA1 mutations. The DNA amount input in the test is 10 ng. The data was summarized in Table 3. Our assay detected all corresponding UBA1 mutations in the 14 positive patient samples, and did not detect UBA1 mutation in any of the 19 negative samples. The one patient's whole blood sample is positive for M41T in our assay, and the same mutation was also detected in this patient a few months earlier at another CLIA laboratory using Sanger sequencing. This indicated a 100% accuracy when compared to Sanger sequencing (Table 3). The clinical assay sensitivity, specificity and accuracy were summarized in Table 3 when compared with Sanger

Table 1
Assay sensitivity and assay background.

	M41T	M41V	M41L	c.118-2A > C	c118-1G > C	c.118- 9_118-2del	S56F	S621C
LOD (%VAF)	0.1%	0.1%	0.25%	1%	5%	1%	0.25%	1%
Maximal background (MFI)	15	21	22	7	9	7	44	17

*LOD definition: Signal/background of 19 of 20 replicates greater than or equal to 3.
*Background definition: the maximum of MFI in 20 replicates of wild type DNA.

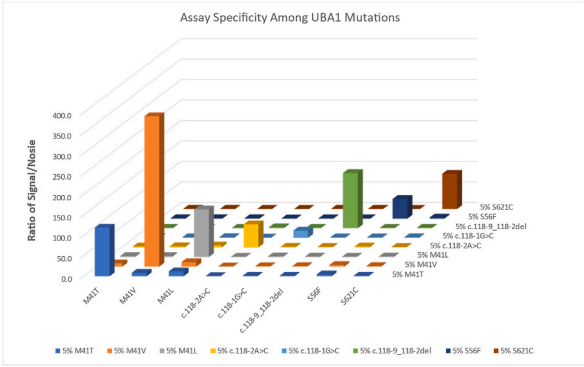

Fig. 3. Signal/noise ratio (SNR) of all mutant assay in the presence of each mutant synthetic DNA at 5%.

Table 2
Results of 36 clinical samples and two mixed DNA samples from the QClamp Plex assay.

Patient	M41T	M41V	M41L	c.118-2A > C	c.118-1G > C	c.118-9_118-2del	S56F	S621C
#1	–	–	–	–	–	–	–	–
#2	–	Pos	–	–	–	–	–	–
#3	Pos	–	–	–	–	–	–	–
#4	Pos	–	–	–	–	–	–	–
#5	Pos	–	–	–	–	–	–	–
#6	–	–	–	–	Pos	–	–	–
#7	–	–	Pos	–	–	–	–	–
#8	Pos	–	–	–	–	–	–	–
#9	Pos	–	–	–	–	–	–	–
#10	–	–	Pos	–	–	–	–	–
#11	–	–	–	–	Pos	–	–	–
#12	–	–	–	–	Pos	–	–	–
#13	Pos	–	–	–	–	–	–	–
#14	–	–	–	–	–	–	–	–
#15	–	–	–	–	–	–	–	–
#16	–	–	–	–	–	–	–	–
#17	–	–	–	–	–	–	–	–
#18	–	–	–	–	–	–	–	–
#19	–	–	–	–	–	–	–	–
#20	–	–	–	–	–	–	–	–
#21	–	–	–	–	–	–	–	–
#22	–	–	–	–	–	–	–	–
#23	–	–	–	–	–	–	–	–
#24	–	–	–	–	–	–	–	–
#25	–	–	–	–	–	–	–	–
#26	–	–	–	–	–	–	–	–
#27	–	–	–	–	–	–	–	–
#28	–	–	–	–	–	–	–	–
#29	–	–	–	–	–	–	–	–
#30	Pos	–	–	–	–	–	–	–
#31	–	–	–	–	–	–	–	–
#32	–	–	–	–	–	–	–	–
#33	–	–	Pos	–	–	–	–	–
#34	–	–	–	–	–	–	–	–
#35	–	–	–	–	–	–	–	–
#36	Pos	–	–	–	–	–	–	–
#2 + #3	Pos	Pos	–	–	–	–	–	–
#7 + #8	Pos	–	Pos	–	–	–	–	–

sequencing. The samples collected from two volunteers did not carry any of the eight UBA1 mutations. We also identified the two mutations in the two mixed samples correctly.

We subsequently developed an algorithm incorporating LOD and the specificity of each target and wrote an excel macro to automatically generate results (Table 2) from the output file of Luminex MAGPIX machine.

Table 3
The clinical sensitivity and specificity summary of QClamp® Plex VEXAS syndrome assay.

Assay performance		Standard of Care		
		Positive	Negative	Total
QClamp® Plex VEXAS Syndrome Test	**Positive**	15	0	15
	Negative	0	19	19
	Total	15	19	34
Sensitivity		100.00%	95% CI: 78.20%–100.00%	
Specificity		100.00%	95% CI: 82.35%–100.00%	
Positive predictive value		100.00%	95% CI: 78.20%–100.00%	
Negative predictive value		100.00%	95% CI: 82.35%–100.00%	
Accuracy		100.00%	95% CI: 89.72%–100.00%	

4. Discussion

VEXAS syndrome is a newly discovered chronic and progressive disease that is often misdiagnosed by hematologists, dermatologists and rheumatologists. Discovery of the UBA1 mutations in VEXAS syndrome patients helps risk stratification of the patients with autoimmune diseases who carry multiple symptoms in different organs. This allows the VEXAS syndrome patients to be properly treated as they are generally less likely to be responsive to immunosuppressive drugs [2].

The commonly used techniques in the past three years include Sanger sequencing, Next Generation Sequencing (NGS), and droplet digital PCR (ddPCR). As we have discussed in the Introduction section, these assays have both advantages and disadvantages.

We have used UBA1 mutation detection as an example to showcase a novel mutation detection assay platform, QClamp® Plex, which synergizes XNA clamping PCR, allele-specific ligation employing Taq DNA ligase, and Luminex xMAP technology. This amalgamation offers a straightforward, robust, and highly sensitive approach for detecting multiple mutation targets in a single run. The XNA clamping technique effectively curtails wild-type amplification and enriches mutant sequence amplification during PCR, enabling the detection of mutations at a very low variant allele frequency (VAF) of 0.1–0.5% [12]. Leveraging the high fidelity of Taq DNA ligase, which demonstrates remarkable discrimination for single-base mismatches on the 3′-side [13], the assay has further increased sensitivity to 0.1% VAF and high specificity for mutations even within the same codon.

The Luminex xMAP technology permits the simultaneous analysis of up to 100 targets, each uniquely encoded with color-coded microspheres [14,15]. Through the integration of our XNA technology and xMAP technology, we have successfully crafted the QClamp Plex, and used 9 color-coded microspheres for 9 targets for the UBA1 mutation analysis. The next step will be to increase the target number and make a larger QClamps Plex panel, up to 100 targets, which contains a collective of cancer gene targets for target therapy selection. If such a panel is successfully made, it will be a substantial improvement over qPCR or ddPCR with a limit of only several targets in a multiplex run. Furthermore, running such a panel in one day will significantly reduce the assay time compared to the 2–3 weeks of turnaround time of NGS.

The QClamp® Plex platform showcases remarkable utility in liquid biopsy samples, achieving a high sensitivity of 0.1% VAF. This heightened sensitivity positions the technology for applications in companion diagnostics and molecular residual disease (MRD) monitoring using liquid biopsy, facilitating personalized drug therapy guidance for cancer patients and monitoring treatment efficacy. Application of the QClamp Plex in VEXAS syndrome diagnosis not only detects the UBA1 mutations but can detect it at low frequencies when the disease is at an early stage. This is significant because early detection can help disease monitoring and treatment management. Other peer papers have used the combination of Sanger sequencing and ddPCR or deep amplicon sequencing to screen and monitor the early UAB1 mutation, respectively [4,10], which can be done by the QClamp Plex assay alone.

Among the eight UBA1 mutation targets evaluated, M41T and M41V exhibit a detection sensitivity of 0.1% VAF, surpassing the performance of the other targets (Table 2). Notably, M41T, M41V, and M41L, along with mutations at positions c.118-2A > C, c.118-1G > C, and c.118-9_118-2del, share a common XNA oligo. The spatial distribution of mutation bases in M41T, M41V, and M41L near the middle of the XNA sequences contributes to their superior resolution, as opposed to the three mutations occurring at the 3' end (Fig. 1). The S621C mutant, with a relatively lower sensitivity (LOD of 1%), may be attributed to the larger amplicon size of 170 bases compared to the ~90 bases amplicons of other mutations. When 40 PCR cycles are used, all the targets can reach a LOD of 0.1% except c.118-1G > C with a LOD of 1% (data not shown). Since the variant allele frequency of the UBA1 in VEXAS patients are very high with a range of 29.5%–59.3% (5) or even higher [1], 30 PCR cycles are selected to keep a shorter assay time.

Despite the high fidelity of Taq DNA ligase, its ability to ligate mismatches, especially T:G, T:T, and A:C mismatches, remains detectable [4]. This phenomenon explains the observed low cross-reactivity (<10%) among M41T, M41V, and M41L. The primers of S56F produced ~90 bases amplicon; its reverse primer UBA1_S56F_r and the forward primer UBA1_M41_f (Fig. 1) also produced a ~170 base amplicon. In the presence of M41T gBlocks of 200 bases which cover the 170base region (Fig. 1), some wild type S56F molecules could be generated due to the XNA inefficient blocking. Also the CP ligation oligo of S56F has a G::T mismatch with wild type DNA. This may explain why S56F has 4% cross with M41T synthetic DNA molecules (Fig. 3). Since all the crosses are low (<10%), we developed a very simple algorithm to make the positive call by giving a cutoff value for certain non-specific cross. We integrated this algorithm into our Excel macro, and we were able to identify the correct mutations of all the clinical samples and the two different mutations in the mixed samples (Table 3).

In summary, compared to traditional detection methods, the QClamps® Plex *UBA1* Mutation Detection Assay is a quicker, simpler,

and sensitive assay that can accurately detect the *UBA1* mutations even at early stages when the mutation frequency is still low. Compared to NGS, the assay is less time-consuming, lower price, and does not need bioinformatics expertise. The assay can reach the sensitivity that only deep NGS sequencing and ddPCR can achieve. To get early detection, regular Sanger sequencing is not very likely due to its lower sensitivity. Although qPCR and ddPCR can also be used for mutation detection, their lower multiplexity makes them harder to detect multiple UBA1 mutations, especially when the mutation list is still growing for VEXAS syndrome diagnosis. In addition, the QClamps® Plex platform can also be used to customize any target mutation detection panel as a faster and more economical alternative to NGS.

CRediT authorship contribution statement

Yunqing Ma: Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **ShianPin Hu:** Writing – review & editing, Validation, Resources, Data curation. **Rui Ni:** Validation. **Wei Liu:** Writing – review & editing. **Andrew Fu:** Resources. **Michael Sha:** Writing – review & editing. **Aiguo Zhang:** Project administration, Funding acquisition. **Chuanyi M. Lu:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2024.e00380.

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